Industria y Comercio

SUPERINTENDENCIA

P.C.T.

Delegatura de propiedad Industrial

División de Nuevas Creaciones

SOLICITUD

PATENTE DE INVENCIÓN

21 **EXPEDIENTE No.** 06019270

54 **TÍTULO** CONSTRUCCIONES DE UNION Y METODOS PARA USO DE ELLAS

51 **CLASIFICACIÓN INTERNACIONAL** C12N 15/00 A61K 39/395 C07K 16/00 A61/37/100, 29/00.

71 **SOLICITANTE** TROBION PHARMACEUTICALS INC

DOMICILIO Seattle WA Estados Unidos

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22 **BOGOTÁ, D. C.,** FEBRERO 27/06.

Pendiente cambio 378 -> 379

(FORMA P 10)

PETITORIO

Industria y Comercio
SUPERINTENDENCIA

SUPERINTENDENCIA Y COMERCIO
Radicacion : 06015270 00000000 Folios: 1001
Fecha (AMD): 2006-02-27 16:51:10
Tramite : 011 PCT-PATENT 370 FASE NACI 411 PRESENTAC
Remitente: SUPO DIVISION DE NUEVOS SERVICIOS

FORMULARIO ÚNIC DE S LIC PCT

ENTRADA EN FASE NACIONAL 26-02-2006

1) SOLICITUD DE		
☒ Patente de invención	☐ Patente de Modelo de Utilidad	
☐ Capitulo I	☒ Capitulo II	
2) SOLICITANTE (71)	**Nombre** TRUBION PHARMACEUTICALS , INC **Dirección** 2401 Fourth Avenue, Suite 1050, Seattle, WA 98121, Estados Unidos **Nacionalidad o domicilio** Seattle, WA, Estados Unido s **Lugar de constitución** WA, Estados Unidos	**IDENTIFICACIÓN** C C ☐ NIT ☐ C E ☐ OTRO ☐ Cual _____ NUMERO _____
	Teléfono Fax **E. Mail**	
3) REPRESENTANTE APODERADO	Nombre DILIA MARÍA RODRÍGUEZ D ALEMAN Dirección Carrera 11 N° 86-53 Piso 6 Bogotá D C Teléfono 6181088 Fax 6350824 E Mail info@clarkemodet com co	**IDENTIFICACIÓN** C C ☒ NIT ☐ C E ☐ OTRO ☐ **NÚMERO** 41 654 882 TP 20824 CSJ
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5) PCT (86) Solicitud Internacional No PCT/US2003/041600 Fecha DIC 24, 2003 Publicación Internacional No WO2005/017148 A1 Fecha Feb 24, 2005		
6)Titulo (54) CONSTRUCCIONES DE UNIÓN Y MÉTODOS PARA USO DE ELLAS		
7) Clasificación Internacional C12N 15/00, A61K 39/395,C07K 16/00 , A61P37/00, 29/00		

8) PRIORIDAD SI ☒ NO ☐	**33) País de Origen**	**(32) Fecha**	**(31) Numero de Solicitud**
	Estados Unidos	Juli 26, 2003	10/627,556

9) Comprobante de pago N° 06-7,292 **Fecha** Enero 27, 2006

Instrucciones para el diligenciamiento del presente formulario Ver reverso de esta pagina

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SUPERINDUSTRIA Y COMERCIO
Radicacion : 06019270 00000000 Folios. 1001
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Dependencia: 2020 DIVISION DE NUEVAS CREACIONES

SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO

NIT 800176089-2

RECIBO OFICIAL DE CAJA 06 - 7,292
FECHA ENERO 27 DE 2006

C O N S I G N A C I O N

DEPOSITANTE	TIPO PAGO	BANCO	CUENTA	No PAGO	FECHA PGO	Vr PAGO
CLARKE MODET Y CO	COLOMBCONSIGNACION	BANCO POPULAR	050-00110-6	49044331	27/01/2006	3,364,000 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	463,000 00
			TOTAL $	463,000 00

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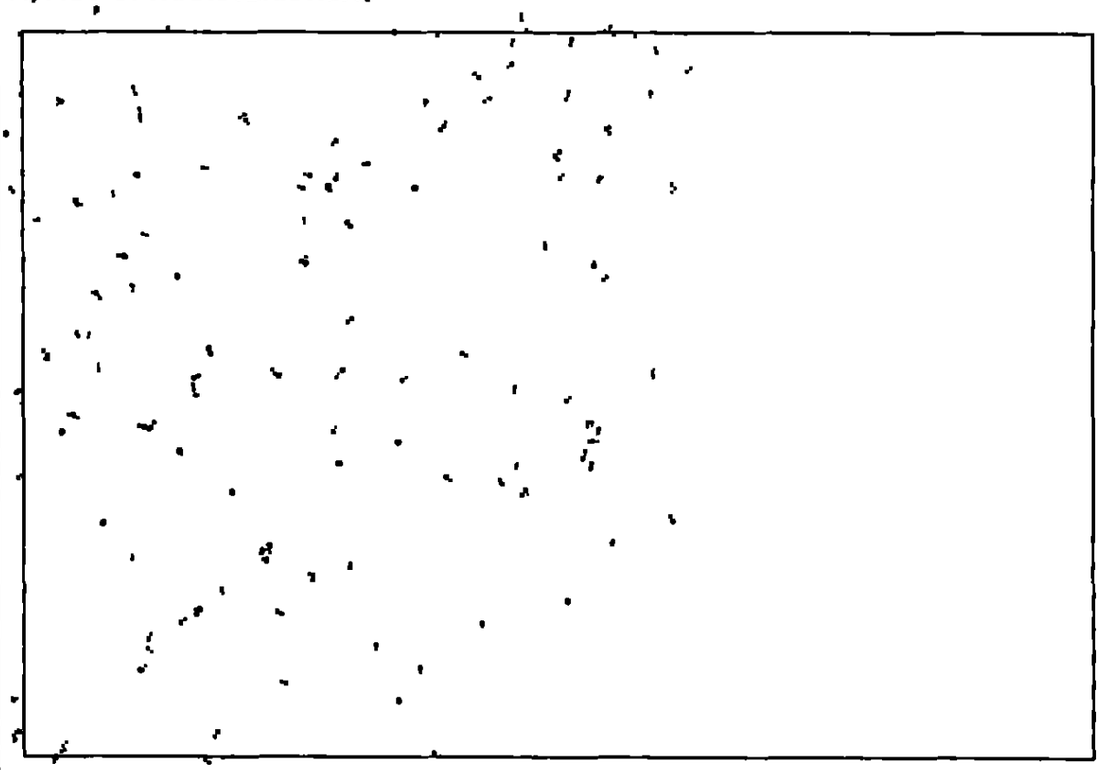
RESPONSABLE _____

RECIBO DE CAJA APLICADO AL EXPEDIENTE No _____

10) ANEXOS

- [x] Comprobante de pago de la tasa de presentación de la solicitud
- [ ] Comprobante de pago por reivindicación de prioridad
- [ ] Documento que acredita la existencia y representación legal cuando el solicitante sea persona Jurídica
- [ ] Poderes si fuera el caso
- [ ] Documento de cesión del inventor al solicitante o a su causante
- [ ] Declaraciones
- [x] Copia de la solicitud Internacional (Resumen, descripción, reivindicación, Dibujos)
- [x] Traducción de la solicitud internacional al castellano (Resumen descripción reivindicación Dibujos)
- [ ] Copia 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 12 x 12 y 6 x 6 cm
- [ ] De ser el caso información sobre otras solicitudes de patente o títulos obtenidos en el extranjero por el mismo titular o su causante relacionados parcial o totalmente con la invención de esta solicitud
- [x] Otros
  1. Reporte Internacional de búsqueda
  2. Opinión escrita
  3. Solicitud PCT
  4. Solicitud PCT Capitulo II

11) FIGURA CARACTERÍSTICA.



12) Solicitud concesión de la patente,

NOMBRE DILIA RODRÍGUEZ D ALEMAN

FIRMA

C C 41 654 882 de Bogota TP 20824 CSJ

Instrucciones para el diligenciamiento del presente formulario Ver reverso de esta pagina

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

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
24 February 2005 (24.02.2005)

PCT

(10) International Publication Number
WO 2005/017148 A1

(51) International Patent Classification⁷: C12N 15/00, A61K 39/395, C07K 16/00

(21) International Application Number: PCT/US2003/041600

(22) International Filing Date: 24 December 2003 (24.12.2003)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
10/627,556 26 July 2003 (26.07.2003) US

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(81) Designated States (*national*): AE, AG AL, AM, AT AU AZ, BA, BB, BG BR, BY BZ, CA, CH, CN CO, CR, CU CZ, DE, DK DM DZ, EC, EE, ES, FI GB GD, GE, GH GM, HR, HU ID, IL, IN IS JP KE, KG KP KR, KZ, LC, LK, LR, LS LT LU LV MA, MD, MG MK, MN MW MX, MZ, NI NO, NZ, OM, PG PH, PL, PT RO, RU SC, SD, SE, SG, SK, SL, SY TJ TM TN TR TT TZ, UA, UG US, UZ, VC, VN YU ZA, ZM, ZW

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

Published
— *with international search report*

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: BINDING CONSTRUCTS AND METHODS FOR USE THEREOF

(57) Abstract: The invention relates to novel binding domain-immunoglobulin fusion proteins that feature a binding domain for a cognate structure such as an antigen, a counterreceptor or the like, a wild-type IgG 1CH or IgE hinge-acting region, *i.e.* IgE CH2, region polypeptide or a mutant IgG1 hinge region polypeptide having either zero, one or two cysteine residues, and immunoglobulin CH2 and CH3 domains, and that are capable of ADCC and/or CDC while occurring predominantly as polypeptides that are compromised in their ability to form disulfide-linked multimers. The fusion proteins can be recombinantly produced at high expression levels. Also provided are related compositions and methods, including cell surface forms of the fusion proteins and immunotherapeutic applications of the fusion proteins and of polynucleotides encoding such fusion proteins.

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BINDING CONSTRUCTS AND METHODS FOR USE THEREOF

All applications from which this application takes priority are incorporated herein by reference in their entirety

FIELD OF THE INVENTION

The present invention relates generally to compounds having various utilities including uses for research, diagnostics, and therapy, for example, immunotherapy Compounds of the invention include immunologically active proteins and protein conjugates. Such proteins include recombinant or engineered binding proteins such as, for example, binding domain-immunoglobulin fusion proteins, which may include single chain Fv-immunoglobulin fusion proteins and compounds containing single chain Fv-immunoglobulins. The present invention also relates to compositions and methods for treating conditions, diseases and disorders that would improved, eased, or lessened from the administration of, for example, polypeptide and/or nucleic acid constructs of the invention, including, for example, malignant conditions and B cell disorders, including diseases characterized by autoantibody production and/or inflammation.

BACKGROUND OF THE INVENTION

The immune system is one of the most complex of the body's many intricate systems. A vast and complicated arrangement made up of many different types of cells and involving many different kinds of molecules, the human immune system allows the body to respond to foreign invaders such as bacteria, viruses, and other infectious agents, as well as foreign material such as pollen. In general, the human immune system is divided into two main parts, antibody-mediated immunity (also called "humoral" or "circulating" immunity) and cell-mediated immunity, both of which are managed by lymphocytes Lymphocytes are one of the five kinds of white blood cells (leukocytes) circulating in the blood. There are several kinds of lymphocytes, each with different functions to perform. The most common types of lymphocytes are B lymphocytes (B cells), which are responsible for making antibodies, and T lymphocytes (T cells) Cells of the immune system not only include T cells and B cells, but also Natural Killer Cells, granulocytes (or polymorphonuclear (PMN) leukocytes), macrophages, and dendritic cells. The humoral system is managed by B cells with help from T cells and deals with infectious agents in the

blood and tissues of the body The cell-mediated system is managed by T cells and deals with cells of the body that have been infected.

An antigen is a substance, usually macromolecular, that stimulates or induces an immune response. Because of its complex macromolecular structure, a single microorganism consists of multiple antigens (*e.g.*, surface structures such as cell wall components, fimbriae, flagella, *etc.*, or extracellular proteins, such as toxins or enzymes produced by the microorganism) The coat proteins and some of the envelope proteins of animal viruses are also usually antigenic. A host is generally able to respond specifically to antigens that come into contact with components of its immune system. Both the antibody-mediated immunity and cell-mediated immunity systems involve complex interrelationships that allow them to mount immune reactions to almost any antigen. In other words, the immune system is able to recognize foreign substances (antigens) that stimulate the system to produce antibody-mediated immunity, cell-mediated immunity, or both.

The immune system complex is constituted by a variety of different cell types and organs disseminated throughout the body These include the primary and secondary lymphoid organs. The primary lymphoid organs are the bone marrow and the thymus. All the cells of the immune system are initially derived from the bone marrow in a process called hematopoiesis During hematopoiesis bone marrow-derived stem cells differentiate into either mature cells of the immune system ("B" cells) or into precursors of cells that migrate out of the bone marrow to mature in the thymus ("T" cells) In addition to red blood cells, platelets, and B cells, the bone marrow also produces Natural Killer cells, granulocytes, and immature thymocytes. The function of the thymus is to produce mature T cells. Immature thymocytes, also known as prothymocytes, leave the bone marrow and migrate into the thymus where they mature and are then released into the bloodstream. The immune system complex also includes secondary lymphoid organs, *e.g.*, the spleen, the lymph nodes, *etc.*, as well as a circulatory system that is separate from blood vessels

The spleen, made up of B cells, T cells, macrophages, dendritic cells, Natural Killer cells, and red blood cells, is an immunologic filter of the blood Migratory macrophages and dendritic cells capture antigens from blood that passes through the spleen. Migratory macrophages and dendritic cells also bring antigens to the spleen via the

bloodstream. An immune response is initiated in the spleen when macrophages or dendritic cells present the antigen to the appropriate B or T cells, and B cells become activated and produce large amounts of antibody

Lymphatic vessels and lymph nodes are the parts of a special circulatory system that carries lymph. Lymph is a transparent fluid containing white blood cells, chiefly lymphocytes. Lymph bathes the tissues of the body, and is then collected in lymphatic vessels. Lymph nodes dot a network of lymphatic vessels and, when afferent lymph ducts bring lymph-containing antigens into the node, function as an immunologic filter for lymph. Composed mostly of T cells, B cells, dendritic cells, and macrophages, the lymph nodes drain fluid from most tissues. Antigens are filtered out of the lymph in the lymph node before the lymph is returned to the circulation. Macrophages and dendritic cells that capture antigens also present these foreign materials to T and B cells in the lymph nodes, resulting in the stimulation of B cells to develop there into antibody-secreting plasma cells. Antibodies leave the lymph node by the efferent ducts that empty into the blood stream. Lymphocytes can also leave the node by the efferent duct and travel to other sites in the lymphatic system or enter into the blood circulation. A single lymphocyte completes a circuit through the circulating blood and lymphatic systems once every 24 hours.

Tonsils, adenoids, Peyer's patches, and the appendix are also lymphoid tissues. Peyer's patches (masses of lymphocytes) are similar to the tonsils and are found throughout the body, especially in the mucous linings of the digestive and respiratory tracts It is the function of the phagocytic cells found in Peyer's patches and other lymphatic aggregate follicles to defend the body against, for example, inadequately digested food particles crossing the gut wall and entering the blood, and to attack unwanted foreign invaders while they are still in the bowel

The major function of B cells is the production of antibodies in response to foreign proteins of bacteria, viruses, and tumor cells T cells are usually divided into two major groups, namely, the cytotoxic T lymphocytes ("Tc" cells or CTLs) and the helper T cells ("Th" cells or T helper cells) Th cells, also referred to as CD4+ T cells, function to augment or potentiate immune responses by the secretion of specialized factors that activate other white blood cells to fight off infection. They enhance the production of antibodies by B cells Tc cells, also called CD8+ T cells, can directly kill certain tumor

cells, viral-infected cells, and sometimes parasites. Tc cells are also important in down-regulation of immune responses. Both types of T cells often depend on the secondary lymphoid organs (the lymph nodes and spleen) as sites where activation occurs, but they are also found in other tissues of the body, including the liver, lung, blood, and intestinal and reproductive tracts.

Natural Killer cells, often referred to as NK cells, represent another type of lymphocyte and are similar to the Tc cell subset. They function as effector cells that directly kill certain tumors such as melanomas and lymphomas, and viral-infected cells. They are called "natural" killers because, unlike cytotoxic T cells, they do not need to recognize a specific antigen before carrying out their function. While NK cells, unlike the Tc cells, kill their targets without prior activation in the lymphoid organs, NK cells activated by Th cell secretions will kill tumor or viral-infected targets more effectively NK cells target tumor cells and protect against a wide variety of infectious microbes In several immunodeficiency diseases, including AIDS, Natural Killer cell function is abnormal. Natural Killer cells may also contribute to immunoregulation by secreting high levels of influential lymphokines.

Some NK cells have surface receptors (FcγRIII, also called CD16) for the Fc portion of the IgG antibody They bind to target cells through receptors for the Fc portion of an antibody that has reacted with antigen on a target cell This type of cell-mediated immunity is called antibody-dependent cell-mediated cytotoxicity (ADCC) NK cells may also have receptors for the C3 component of complement, another immune defense system, and therefore recognize cells that are coated with C3 as targets ADCC is thought to be an important defense against a variety of parasitic infections caused, for example, by protozoa and helminths.

Although small lymphocytes look identical, they can be distinguished by molecules carried on their cell surface. Not only do such markers distinguish between B cells and T cells, they distinguish among various subsets of cells that behave differently Every mature T cell, for instance, carries a marker known as T3 (or CD3) In addition, most helper T cells carry a T4 (CD4) marker, a molecule that recognizes Class II major histocompatibility complex ("MHC") antigens. A molecule known as T8 (CD8), which recognizes Class I MHC antigens, is found on many suppressor/cytotoxic T cells.

Another group of white blood cells collectively referred to as granulocytes, or polymorphonuclear leukocytes (PMNs), is composed of three cell types These cells, neutrophils, eosinophils, and basophils are important in the removal of bacteria and parasites from the body Neutrophils migrate through capillary walls and into infected tissue where they kill invaders (*e.g*, bacteria) and then engulf the remnants by phagocytosis. Eosinophils are cytotoxic, releasing the contents of their granules on an invader Basophils leave the blood and accumulate at the site of an infection or other inflammation and discharge the contents of their granules, releasing a variety of mediators such as histamine, serotonin, prostaglandins and leukotrienes that, for example, increase blood flow to the area. Mediators released by basophils also play an important part in some allergic responses such as hay fever and anaphylactic responses to insect stings.

Monocytes are large phagocytic white blood cells released from the bone marrow into the blood circulation. When a monocyte enters tissue, it develops into a macrophage Macrophages are also large, phagocytic cells that engulf foreign material (antigens) that enter the body, as well as dead and dying cells of the body Macrophages are important in the regulation of immune responses, and are often referred to as scavengers, or antigen-presenting cells (APCs) because they pick up and ingest foreign materials and present these antigens to other cells of the immune system such as T cells and B cells This is one of the important first steps in the initiation of an immune response. Stimulated macrophages exhibit increased levels of phagocytosis and also secrete Interleukin-1 (IL-1), a product that helps to activate B cells and T cells.

Dendritic cells also originate in the bone marrow and function as APCs. They are usually found in the structural compartment of lymphoid organs such as the thymus, lymph nodes and spleen, but are also found in the bloodstream and other tissues It is believed that dendritic cells capture antigen or bring it to the lymphoid organs where an immune response is initiated.

Important features of the immunological system relevant to host defense and/or immunity to pathogenic microorganisms include specificity, memory, and tolerance It is understood, for example, that an antibody or reactive T cell will react specifically with the antigen that induced its formation, it will not react with other antigens Generally, this specificity is of the same order as that of enzyme-substrate specificity or receptor-ligand specificity, although cross-reactivity is possible. The specificity of the immune response is

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explained by clonal selection. During the primary immune response, a specific antigen selects a pre-existing clone of specific lymphocytes and stimulates its activation, proliferation and differentiation. It is also understood that once the immune system has responded to produce a specific type of antibody or reactive T cell, it is capable of producing more of the antibody or activated T cell more rapidly and in larger amounts, thus is called the secondary (or memory) response. It is also recognized that an animal generally does not undergo an immunological response to its own (potentially-antigenic) components. The animal is said to be tolerant, or unable to react to its own potentially antigenic components. This ensures that under normal conditions, an immune response to "self" antigens (called an autoimmune response) does not occur Tolerance is brought about in a number of ways, but in essence the immune system is able to distinguish "self" components from "non-self" (foreign) antigens, it will respond to "non-self" but not to "self" Sometimes in an animal, tolerance can be "broken", which may result in an autoimmune disease.

The biological activities of the antibody-mediated and cell-mediated immune responses are different and vary from one type of infection to another There are several classes or types of antibodies (and subclasses of various types) involved in antibody-mediated immunity All of the classes of antibodies that are produced in response to a specific antigen react stereochemically with that antigen and not with other (different) antigens. The host has the genetic capacity to produce specific antibodies to thousands of different antigens, but does not do so until there is an appropriate (specific) antigenic stimulus Due to clonal selection, the host produces only the homologous antibodies that will react with that antigen which, as noted above, are found in blood (plasma), lymph, and many extravascular tissues. Once the antibody-mediated immunity response occurs following interaction of B lymphocytes with antigen and their differentiation into antibody-secreting plasma cells, the secreted antibody binds to the antigen which, in turn, results in its neutralization or elimination from the body

Cell-mediated immunity, on the other hand, is mediated by specific subpopulations of T-lymphocytes called effector T cells that exist in precursor form as "resting T cells" (pT cells) These cells bear receptors for specific antigens and recognize these antigens on the surfaces of other cells. Stimulation with that antigen results in T cell activation. T cells enlarge, enter into a mitotic cycle, reproduce and develop into effector T

cells whose activities are responsible for this type of immunity They also develop into clones of identical reactive T-cells called memory T cells As noted above, most of the T cells in the body belong to one of two subsets and are distinguished by the presence on their surface of one or the other of two glycoproteins designated CD4 and CD8 Which of these molecules is present determines the types of cells to which the T cell can bind T cells bearing CD8 ($CD8^+$ T cells) always recognize antigen in association with Class I MHC proteins and typically function as cytotoxic T cells. Almost all the cells of the body express Class I MHC molecules T cells bearing CD4 ($CD4^+$ T cells) always recognize antigens in association with Class II MHC proteins on the surfaces of other cells. Only specialized antigen-presenting cells express Class II MHC molecules, including dendritic cells, phagocytic cells such as macrophages, and B cells. $CD4^+$ T lymphocytes generally function as T helper cells

T helper cells, which include Th1 cells and Th2 cells, respond to antigen with the production of lymphokines. Th1 and Th2 cells can be distinguished based on their lymphokine profiles. Like all T cells, Th cells arise in the thymus. When they are presented with an antigen by antigen-presenting dendritic cells they begin to proliferate and become activated. There are two kinds of dendritic cell, DC1 cells (descended from monocytes) and DC2 cells (which appear to be derived from lymphocytes)

Th1 cells (inflammatory Th1 cells involved in the elimination of pathogens residing intracellularly in vesicular compartments) are produced when DC1-type dendritic cells present antigen to the T cell receptor for antigen (TCR) and secrete Interleukin 12 (IL-12) This paracrine stimulation activates Th1 cells to secrete their own lymphokines, in particular, Tumor-Necrosis Factor-beta (TNF-β) (also known as lymphotoxin) and Interferon-gamma (IFN-γ) These lymphokines stimulate macrophages to kill bacteria they have engulfed by phagocytosis and they recruit other leukocytes to the site producing inflammation. Th1 cells are essential for cell-mediated immunity and for controlling intracellular pathogens such as, for example, *Listeria* and *Mycobacterium tuberculosis*

Th2 cells ("true" helper Th2 cells, which are required for antibody production by B cells) are produced when DC2-type dendritic cells present antigen to the T cell receptor for antigen and, presumably, one or more paracrine stimulants. The major lymphokines secreted by Th2 cells are Interleukin 4 (IL-4), which stimulates class-switching in B cells and promotes their synthesis of IgE antibodies, acts as a positive-

feedback device promoting more pre-Th cells to enter the Th2 pathway, and blocks expression of the IL-12 receptor thereby inhibiting pre-Th cells in the thymus from entering the Th1 pathway IL-4 also causes B cells to proliferate and differentiate into antibody-secreting plasma cells and memory B cells IL-4 activates only B cells in the vicinity which themselves have bound the antigen, and not others, so as to sustain the specificity of the immune response. Th2 cells also produce Interleukin 5 (IL-5, which attracts and activates eosinophils), Interleukin 10 (IL-10, which inhibits IL-12 production by DCs and prevents maturation of pre-Th cells to Th1 cells), and Interleukin 13 (IL-13, which also promotes the synthesis of IgE antibodies).

Activation of the Th2 cell also causes it to begin to produce Interleukin 2 (IL-2), and to express a membrane receptor for IL-2 The secreted IL-2 autostimulates proliferation of Th2 cells. For example, IL-2 binds to IL-2 receptors on other T cells (which have bound the antigen) and stimulates their proliferation. In addition to IL-2, stimulated Th2 cells also produce IFN-γ and Interleukin 6 (IL-6), which mediate various aspects of the immune response IFN-γ activates Natural Killer cells to their full cytolytic potential, and is an activator of macrophages and thus increases their antitumor activities. If the macrophages are infected by intracellular parasites, it activates macrophages, which in turn destroy the parasites. IFN-γ also reinforces the antitumor activities of the cytotoxic lymphocytes, increases the nonspecific activities of NK-cells, and is one of the factors that controls the differentiation of B cells and increases the secretion of immunoglobins. IL-6 stimulates several types of leukocytes, as well as the production of Acute Phase Proteins in the liver It is particularly important in inducing B cells to differentiate into antibody forming (plasma) cells. Thus, Th2 cells provide help for B cells and are essential for antibody-mediated immunity

Cytotoxic T lymphocytes are able to kill cells that show a new or foreign antigen on their surface (for example, virus-infected cells, or tumor cells, or transplanted tissue cells) The $CD8^+$ CTLs also come in two subsets Tc1 that, like Th1 cells, secrete IFN-γ, and Tc2 that, like Th2 cells, secrete IL-4

The cell-mediated immunity response also plays a role in destruction of tumor cells and in rejection of tissue transplants in animals. A major problem in tissue transplantation is rejection, which is often based on cell-mediated immunity response to "foreign" cells (because they are not a perfect antigenic match) Because tumor cells

contain specific antigens not seen on normal cells they also may be recognized as foreign and destroyed by the forces of cell-mediated immunity If tumor cells develop on a regular basis in animals, it may be cell-mediated immunity that eliminates them or holds them in check The increase in the incidence of many types of cancer (tumors) in humans with advancement of age may be correlated with a decline in the peak efficiency of the immune system that occurs about 25 years of age.

A summary of the types of cells involved in the expression of cell-mediated immunity follows. Tc lymphocytes kill cells bearing foreign antigen on surface in association with Class I MHC and can kill cells that are harboring intracellular parasites (either bacteria or viruses) as long as the infected cell is displaying a microbial antigen on its surface. Tc cells kill tumor cells and account for rejection of transplanted cells. Tc cells recognize antigen-Class I MHC complexes on target cells, contact them, and release the contents of granules directly into the target cell membrane that lyses the cell. Th lymphocytes produce lymphokines that are "helper" factors for development of B cells into antibody-secreting plasma cells They also produce certain lymphokines that stimulate the differentiation of effector T lymphocytes and the activity of macrophages Th1 cells recognize antigen on macrophages in association with Class II MHC and become activated (by IL-1) to produce lymphokines including IFN-γ that activates macrophages and NK cells. These cells mediate various aspects of the cell-mediated immunity response including delayed-type hypersensitivity reactions Th2 cells recognize antigen in association with Class II MHC on an APC and then produce interleukins and other substances that stimulate specific B cell and T cell proliferation and activity Macrophages are important as antigen-presenting cells that initiate T cell interactions, development, and proliferation Macrophages are also involved in expression of cell-mediated immunity because they become activated by IFN-γ produced in a cell-mediated immunity response Activated macrophages have increased phagocytic potential and release soluble substances that cause inflammation and destroy many bacteria and other cells. Natural Killer cells are cytotoxic cells that lyse cells bearing new antigen regardless of their MHC type and even lyse some cells that bear no MHC proteins NK cells are defined by their ability to kill cells displaying a foreign antigen (*e.g.*, tumor cells) regardless of MHC type and regardless of previous sensitization (exposure) to the antigen NK cells can be activated by IL-2 and IFN-γ, and lyse cells in the same manner as cytotoxic T lymphocytes Some NK cells have

receptors for the Fc domain of the IgG antibody and are thus able to bind to the Fc portion of IgG on the surface of a target cell and release cytolytic components that kill the target cell via antibody-dependent cell-mediated cytotoxicity

Extracellular factors that affect cell proliferation and differentiation have been defined as cytokines. These include the lymphokines, which are proteins produced by T-lymphocytes that have effects on the differentiation, proliferation, and activity of various cells involved in the expression of cell-mediated immunity In general, lymphokines function by (1) focusing circulating leukocytes and lymphocytes into the site of immunological encounter; (2) stimulating the development and proliferation of B cells and T cells, (3) stimulating and preparing macrophages for their phagocytic tasks, (4) stimulating Natural Killer cells, and (5) providing antiviral cover and activity A summary of various important lymphokines follows. Initially referred to as lymphocyte activation factor, IL-1 is mainly a product of macrophages, and has a variety of effects on various types of cells It acts as a growth regulator of T cells and B cells, and it induces other cells such as hepatocytes to produce proteins relevant to host defense. IL-1 forms a chemotactic gradient for neutrophils and serves as an endogenous pyrogen that produces fever Thus, IL-1 plays an important role in both the immune responses and in the inflammatory response. IL-2 stimulates the proliferation of T cells and activates NK cells. IL-3 regulates the proliferation of stem cells and the differentiation of mast cells. IL-4 causes B cell proliferation and enhanced antibody synthesis IL-6 (also referred to as Interferon-beta2, hybridoma growth factor, B-cell differentiation factor, and hepatocyte stimulatory factor) has effects on B cell differentiation and on antibody production and on T cell activation, growth, and differentiation, and probably has a major role in the mediation of the inflammatory and immune responses initiated by infection or injury IL-8 is a chemotactic attractant for neutrophils IL-13 shares many of the properties of IL-4, and is a potent regulator of inflammatory and immune responses. IFN-γ is produced by T cells and may be considered a lymphokine. It is sometimes called "immune interferon" (Interferon-alpha being referred to as "leukocyte interferon" and Interferon-beta being referred to as "fibroblast interferon") IFN-γ has several antiviral effects including inhibition of viral protein synthesis in infected cells. It also activates macrophages and NK cells, and stimulates IL-1, IL-2, and antibody production. Lymphotoxins include the Tumor Necrosis Factors. T cells produce TNF-beta, while TNF-alpha is produced by T

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cells as well as other types of cells. TNFs function to kill cells, including tumor cells (at a distance). There are several Colony Stimulating Factors (CSFs), including granulocyte macrophage colony stimulating factor (GMCSF), which cause phagocytic white cells of all types to differentiate and divide.

The nature of the membrane receptors for antigen on B cells and T cells is fairly well understood. Each B cell has approximately 10^5 membrane-bound antibody molecules (IgD or IgM) that correspond in specificity to the antibody the cell is programmed to produce (these receptors being referred to as BCRs) CD32 (FcγRII) on B cells are receptors for the Fc region of IgG CD21 and CD35 on B cells are receptors for complement components. Each T cell has about 10^5 molecules of a specific antigen-binding T cell receptor (a TCR) exposed on its surface. The TCR is similar, but not identical, to an antibody There are two types of T cells that differ in their TCRs, alpha/beta (αβ) T cells and gamma/delta (γδ) T cells The TCR of alpha/beta T cells binds a bimolecular complex displayed by a Class I MHC molecule at the surface of an antigen-presenting cell. As noted above, most Th cells express CD4, whereas most Tc cells express CD8

Both BCRs and TCRs are similar in that they are integral membrane proteins, they are present in thousands of identical copies exposed at the cell surface, they are made before the cell ever encounters an antigen, they are encoded by genes assembled by the recombination of segments of DNA, they have a unique binding site that binds through non-covalent forces to a portion of the antigen called an epitope (or antigenic determinant) that depends on complementarity of the surface of the receptor and the surface of the epitope, and successful binding of the antigen receptor to the epitope, if accompanied by additional signals, results in stimulation of the cell to leave G_0 and enter the cell cycle and repeated mitosis that leads to the development of a clone of cells bearing the same antigen receptor, *i.e.*, a clone of cells of the identical specificity BCRs and TCRs differ in their structure, the genes that encode them, and the type of epitope to which they bind.

Induction of a primary immune response begins when an antigen penetrates epithelial surfaces It will eventually come into contact with macrophages or certain other classes of antigen presenting cells, including B cells, monocytes, dendritic cells, Langerhans cells, and endothelial cells Antigens, such as bacterial cells, are internalized

by endocytosis and "processed" by APCs, then "presented" to immunocompetent lymphocytes to mitiate the early steps of the immunological response. Processing by a macrophage (for example) results in attaching antigenic materials to the surface of the membrane in association with Class II MHC molecules on the surface of the cell. The antigen-class II MHC complex is presented to a T-helper (Th2) cell, which is able to recognize processed antigen associated with a Class II MHC molecule on the membrane of the macrophage. This interaction, together with stimulation by IL-1 from secreted by the macrophage, will activate the Th2 cell.

As indicated above, B cells themselves behave as APCs. Cross-linked antigens bound to antibody receptors on the surface of a B cell cause internalization of some of the antigen and expression on the B cell membrane together with Class II MHC molecules. The Th2 cell recognizes the antigen together with the Class II MHC molecules, and secretes the various lymphokines that activate the B cells to become antibody-secreting plasma cells and memory B cells. Even if the antigen cannot cross-link the receptor, it may be endocytosed by the B cell, processed, and returned to the surface in association with Class II MHC where it can be recognized by specific Th2 cells which will become activated to initiate B cell differentiation and proliferation. In any case, the overall B cell response leads to antibody-mediated immunity

The antigen receptors on B cell surfaces are thought to be the specific types of antibodies that they are genetically programmed to produce. Hence, there are thousands of sub-populations of B cells distinguished by their ability to produce a unique antibody molecule. B cells can also react with a homologous antigen on the surface of the macrophage, or with soluble antigens When a B cell is bound to antigen, and simultaneously is stimulated by IL-4 produced by a nearby Th2 cell, the B cell is stimulated to grow and divide to form a clone of identical B cells, each capable of producing identical antibody molecules The activated B cells further differentiate into plasma cells which synthesize and secrete large amounts of antibody, and into memory B cells. The antibodies produced and secreted by the plasma cells will react specifically with the homologous antigen that induced their formation. Many of these reactions lead to host defense and to prevention of reinfection by pathogens Memory cells play a role in secondary immune responses Plasma cells are relatively short-lived (about one week) but produce large amounts of antibody during this period Memory cells, on the other hand,

are relatively long-lived and upon subsequent exposure to antigen they become quickly transformed into antibody-producing plasma cells

Generation of cell mediated immunity begins when, for example, a cytotoxic T cell recognizes a processed antigen associated with Class I MHC on the membrane of a cell (usually an altered self cell, but possibly a transplanted tissue cell, or a eukaryotic parasite) Under stimulation by IL-2 produced by Th2 cells, the Tc cell becomes activated to become a cytotoxic T lymphocyte capable of lysing the cell which is showing the new foreign antigen on its surface, a primary manifestation of cell-mediated immunity The interaction between an antigen-presenting macrophage and a Th cell stimulates the macrophage to produce and secrete a Interleukin-1 that acts locally on the Th cell, stimulating the Th-cell to differentiate and produce its own cytokines (which may here be called lymphokines because they arise from a lymphocyte) These lymphokines have various functions. IL-4 has an immediate effect on nearby B cells. IL-2 has an immediate effect on T cells as described above.

Leucocytes also express adhesion-promoting receptors that mediate cell-cell and cell-matrix interactions. These adhesive interactions are crucial to the regulation of haemopoiesis and thymocyte maturation, the direction and control of leucocyte traffic and migration through tissues, and the development of immune and non-immune inflammatory responses. Several families of adhesion receptors have been identified. The leucocyte integrin family comprises three alpha-beta heterodimeric membrane glycoproteins that share a common beta subunit, designated CD18 The alpha subunits of each of the three members, lymphocyte function associated antigen-1 (LFA-1), macrophage antigen-1 (Mac-1) and p150, are designated CD11a, b, and c respectively These adhesion molecules play a critical part in the immune and inflammatory responses of leucocytes. The leucocyte integrin family is, in turn, part of the integrin superfamily, members of which are evolutionally, structurally and functionally related Another integrin subfamily found on leucocytes is the VLA group, so-called because the "very late activation antigens" VLA-1 and VLA-2 were originally found to appear late in T-cell activation. Members of this family function mainly as extracellular matrix adhesion receptors and are found both on haemopoietic and non-haemopoietic cells They play a part in diverse cellular functions including tissue organization, lymphocyte recirculation and T-cell immune responses Another integrin subfamily, the cytoadhesins, are receptors on platelets and endothelial

cells that bind extracellular matrix proteins. A second family of adhesion receptors is the immunoglobulin superfamily, members of which include CD2, LFA-3, and ICAM-1, which participate in T-cell adhesive interactions, and the antigen-specific receptors of T and B cells, CD4, CD8, and the MHC Class I and II molecules. Another recognized family of adhesion receptors is the selectins, characterized by a common lectin domain. Leucocyte adhesion molecule-1 (LAM-1), which is the human homologue of the murine homing receptor, MEL-14, is expressed on leucocytes, while endothelial leucocyte adhesion molecule-1 (ELAM-1) and granule membrane protein (GMP-140) are expressed on stimulated endothelial cells and activated platelets

Activation of an immune response requires physical cell-cell contact in addition to cytokines Thus, for example, development of B and T cell precursors require intimate contact with stromal cells. At least three critical cell-cell contact events are required for the generation of immune responses. The first is initial contact of a specific antigen with a naive T cell. Because of the requirement for MHC presentation, this is an obligate cell contact event. In normal situations the critical antigen presenting cell is the dendritic cell In addition to the MHC/peptide-TCR interaction there are other non-antigen specific membrane bound ligand-receptor pairs that are important for the dendritic cell-T cell interaction. The principal one is the association of the CD28 molecule on the T cell with either of two ligands, B7 1 (CD80) and B7 2 (CD86), on the dendritic cell These molecules are termed accessory molecules and it is understood that the CD28 molecule delivers an essential second signal to the T cell without which the T cell does not become activated.

A second essential cell-cell contact is between the activated T cell and an antigen-specific B cell. Most antigens are T cell-dependent, that is, an antibody response to the antigen absolutely requires T cell help This help is delivered both by cytokines and by cell-cell contact. Cells bind specific antigen via surface Ig, then internalize, process, and present it on Class II MHC molecules This enables them to be recognized by T cells specific for helper epitopes from the specific antigen. This cell-cell interaction also requires CD28 binding to B7 on the B cell In addition, a molecule called CD40 ligand or CD154, the expression of which is induced upon T cell activation, binds to CD40 on B cells CD40 crosslinking promotes B cell proliferation, prevents apoptosis of germinal-center B cells, and promotes immunoglobulin isotype switching. The CD28-B7 and CD40-

CD40L receptor ligand interactions are both essential for the dialogue between B and T cells that causes their mutual activation.

A third cell-cell interaction that is essential in immune responses is the binding of activated B cells (which have migrated into a specialized structure in lymphoid organs called germinal centers) to follicular dendritic cells (FDCs) FDCs are specialized stromal cells that hold intact, *i.e.*, unprocessed, antigen on their surface in the form of long-lived immune complexes. Among other molecules, FDCs express CD23, which binds to germinal center B cells via a CR2 receptor and stimulates differentiation to plasma cells

Time is required before a primary immune response is effective as a host defense Antigens have to be recognized, taken up, digested, processed, and presented by APCs. A few select Th cells must react with antigen and respond, preexisting B or T lymphocytes must encounter the antigen and proliferate and differentiate into effector cells (plasma cells or Tc cells) In the case of antibody-mediated immunity, antibody level has to build up to an effective physiological concentration to render its host resistant. It may take several days or weeks to reach a level of effective immunity, even though this immunity may persist for many months, or years, or even a lifetime, due to the presence of the antibodies In natural infections, the inoculum is small, and even though the antigenic stimulus increases during microbial replication, only small amounts of antibody are formed within the first few days, and circulating antibody is not detectable until about a week after infection.

With regard to induction of a secondary immune response, it is understood that on re-exposure to microbial antigens (secondary exposure to antigen), there is an accelerated immunological response, *i.e.*, the secondary or memory response. Larger amounts of antibodies are formed in only 1-2 days This is due to the activities of specific memory B cells or memory T cells which were formed during the primary immune response. These memory cells, when stimulated by homologous antigen, "remember" having previously seen the antigen, and are able to rapidly divide and differentiate into effector cells. Stimulating memory cells to rapidly produce very high (effective) levels of persistent circulating antibodies is the basis for giving "booster"-type vaccinations to humans and pets Thus, following the first exposure to an antigen the immune response (as evidenced by following the concentration of specific antibody in the serum) develops gradually over a period of days, reaches a low plateau within 2-3 weeks, and usually begins

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to decline in a relatively short period of time. When the antigen is encountered a second time, a secondary (memory) response causes a rapid rise in the concentration of antibody, reaching a much higher level in the serum, which may persist for a relatively long period of time. A protective level of antibody may be reached by primary exposure alone, but usually to ensure a high level of protective antibody that persists over a long period of time, it is necessary to have repeated antigenic stimulation of the immune system.

An immunoglobulin molecule (abbreviated Ig), is a multimeric protein composed of two identical light chain polypeptides and two identical heavy chain polypeptides (H_2L_2) that are joined into a macromolecular complex by interchain disulfide bonds, *i.e.*, covalent bonds between the sulfhydryl groups of neighboring cysteine residues. There are various classes of human antibody proteins, each of which is produced by a specific clone of plasma cells. Five human immunoglobulin classes are defined on the basis of their heavy chain composition, and are named IgG, IgM, IgA, IgE, and IgD. The IgG-class and IgA-class antibodies are further divided into subclasses, namely, IgG1, IgG2, IgG3, and IgG4, and IgA1 and IgA2. Intrachain disulfide bonds join different areas of the same polypeptide chain, which results in the formation of loops that, along with adjacent amino acids, constitute the immunoglobulin domains. At the amino-terminal portion (also called the "NH_2-terminus" or the "N-terminus"), each light chain and each heavy chain has a single variable region that shows considerable variation in amino acid composition from one antibody to another. The light chain variable region, V_L, associates with the variable region of a heavy chain, V_H, to form the antigen binding site of the immunoglobulin, called the Fv.

In addition to variable regions, each of the antibody chains has one or more constant regions. Light chains have a single constant region domain. Thus, light chains have one variable region and one constant region. Heavy chains have several constant region domains. The heavy chains in IgG, IgA, and IgD antibodies have three constant region domains, which are designated CH1, CH2, and CH3, and the heavy chains in IgM and IgE antibodies have four constant region domains, CH1, CH2, CH3 and CH4. Thus, heavy chains have one variable region and three or four constant regions. Immunoglobulin structure and function are reviewed, for example, in Harlow *et al.*, Eds., *Antibodies A Laboratory Manual*, Chapter 14, Cold Spring Harbor Laboratory, Cold Spring Harbor (1988).

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The heavy chains of immunoglobulins can also be divided into three functional regions the Fd region (a fragment comprising V_H and CH1, *i.e.*, the two N-terminal domains of the heavy chain), the hinge region, and the Fc region (the "fragment crystallizable" region, derived from constant regions and formed after pepsin digestion) The Fd region in combination with the light chain forms an Fab (the "fragment antigen-binding") Because an antigen will react stereochemically with the antigen-binding region at the amino terminus of each Fab the IgG molecule is divalent, *i.e.*, it can bind to two antigen molecules. The Fc contains the domains that interact with immunoglobulin receptors on cells and with the initial elements of the complement cascade. Thus, the Fc fragment is generally considered responsible for the effector functions of an immunoglobulin, such as complement fixation and binding to Fc receptors. Pepsin sometimes also cleaves before the third constant domain (CH3) of the heavy chain to give a large fragment F(abc) and a small fragment pFcb These terms are also used for analogous regions of the other immunoglobulins. The hinge region, found in IgG, IgA, and IgD class antibodies, acts as a flexible spacer, allowing the Fab portion to move freely in space In contrast to the constant regions, the hinge domains are structurally diverse, varying in both sequence and length among immunoglobulin classes and subclasses

For example, the length and flexibility of the hinge region varies among the IgG subclasses. The hinge region of IgG1 reportedly encompasses amino acids 216-231 and because it is freely flexible, the Fab fragments can rotate about their axes of symmetry and move within a sphere centered at the first of two inter-heavy chain disulfide bridges. IgG2 has a shorter hinge than IgG1, reportedly 12 amino acid residues and four disulfide bridges The hinge region of IgG2 lacks a glycine residue, it is relatively short and contains a rigid poly-proline double helix, stabilized by extra inter-heavy chain disulfide bridges. These properties restrict the flexibility of the IgG2 molecule IgG3 differs from the other subclasses by its unique extended hinge region (about four times as long as the IgG1 hinge), and is reported to contain 62 amino acids (including 21 prolines and 11 cysteines), forming an inflexible poly-proline double helix In IgG3 the Fab fragments are relatively far away from the Fc fragment, giving the molecule a greater flexibility The elongated hinge in IgG3 is also responsible for its higher molecular weight compared to the other subclasses The hinge region of IgG4 is shorter than that of IgG1 and its flexibility is intermediate between that of IgG1 and IgG2 The flexibility of the hinge region reportedly

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decreases in the order IgG3>IgG1>IgG4>IgG2. The four IgG subclasses also differ from each other with respect to their effector functions. This difference is related to differences in structure, including with respect to the interaction between the variable region, Fab fragments, and the constant Fc fragment. Nevertheless, aside from glycosylation within the CH2 region, for example, and in spite of this knowledge there are no set rules or conventions regarding means or methods to change features, including sequences, of these subclasses of molecule to change, control, add, or remove different functions, for example, ADCC, CDC, and other functions.

According to crystallographic studies, the immunoglobulin hinge region can be further subdivided functionally into three regions: the upper hinge region, the core region, and the lower hinge region. Shin *et al*, 1992 *Immunological Reviews* 130 87 The upper hinge region includes amino acids from the carboxyl end of CH1 to the first residue in the hinge that restricts motion, generally the first cysteine residue that forms an interchain disulfide bond between the two heavy chains. The length of the upper hinge region correlates with the segmental flexibility of the antibody The core hinge region contains the inter-heavy chain disulfide bridges, and the lower hinge region joins the amino terminal end of the CH2 domain and includes residues in CH2 *Id* The core hinge region of human IgG1 contains the sequence Cys-Pro-Pro-Cys which, when dimerized by disulfide bond formation, results in a cyclic octapeptide believed to act as a pivot, thus conferring flexibility The hinge region may also contain one or more glycosylation sites, which include a number of structurally distinct types of sites for carbohydrate attachment For example, IgA1 contains five glycosylation sites within a 17 amino acid segment of the hinge region, conferring resistance of the hinge region polypeptide to intestinal proteases, considered an advantageous property for a secretory immunoglobulin

Conformational changes permitted by the structure and flexibility of the immunoglobulin hinge region polypeptide sequence may also affect the effector functions of the Fc portion of the antibody Three general categories of effector functions associated with the Fc region include (1) activation of the classical complement cascade, (2) interaction with effector cells, and (3) compartmentalization of immunoglobulins The different human IgG subclasses vary in the relative efficacies with which they fix complement, or activate and amplify the steps of the complement cascade. *See, e.g*, Kruchfink, 2001 *Immunol. Rev* 180 177, Chakraborti *et al*, 2000 *Cell Signal* 12 607,

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Kohl *et al*, 1999 *Mol. Immunol.* 36 893, Marsh et al., 1999 *Curr Opin. Nephrol. Hypertens* 8.557, Speth *et al*, 1999 *Wien Klin. Wochenschr* 111.378

Complement-dependent cytotoxicity (CDC) is believed to be a significant mechanism for clearance of specific target cells such as tumor cells. CDC is a stream of events that consists of a series of enzymes that become activated by each other in a cascade fashion. Complement has an important role in clearing antigen, accomplished by its four major functions (1) local vasodilation, (2) attraction of immune cells, especially phagocytes (chemotaxis), (3) tagging of foreign organisms for phagocytosis (opsonization), and (4) destruction of invading organisms by the membrane attack complex (MAC attack) The central molecule is the C3 protein. It is an enzyme that is split into two fragments by components of either the classical pathway or the alternative pathway Antibodies, especially IgG and IgM, induce the classical pathway while the alternative pathway is nonspecifically stimulated by bacterial products like lipopolysaccharide (LPS) Briefly, the products of the C3 split include a small peptide C3a that is chemotactic for phagocytic immune cells and results in local vasodilation by causing the release of C5a fragment from C5 The other part of C3, C3b coats antigens on the surface of foreign organisms and acts to opsonize the organism for destruction. C3b also reacts with other components of the complement system to form an MAC consisting of C5b, C6, C7, C8 and C9

In general, IgG1 and IgG3 most effectively fix complement, IgG2 is less effective, and IgG4 does not activate complement. Complement activation is initiated by binding of C1q, a subunit of the first component C1 in the cascade, to an antigen-antibody complex Even though the binding site for C1q is located in the CH2 domain of the antibody, the hinge region influences the ability of the antibody to activate the cascade For example, recombinant immunoglobulins lacking a hinge region are reportedly unable to activate complement. Shin *et al*, 1992 Without the flexibility conferred by the hinge region, the Fab portion of the antibody bound to the antigen may not be able to adopt the conformation required to permit C1q to bind to CH2 *See id.* Hinge length and segmental flexibility have been reported to correlate with complement activation, however, the correlation is not absolute. Human IgG3 molecules with altered hinge regions that are as rigid as IgG4, for example, can still effectively activate the cascade.

The absence of a hinge region, or a lack of a functional hinge region, can also affect the ability of certain human IgG immunoglobulins to bind Fc receptors on immune effector cells. Binding of an immunoglobulin to an Fc receptor facilitates antibody-dependent cell-mediated cytotoxicity, which as noted above is presumed to be an important mechanism for the elimination of tumor cells. The human IgG Fc receptor (FcR) family is divided into three groups, FcγRI (CD64), which is capable of binding IgG with high affinity, and FcγRII (CD32) and FcγRIII (CD16), both of which are lower affinity receptors. The molecular interaction between each of the three receptors and an immunoglobulin has not been defined precisely, but experimental evidence indicates that residues in the hinge proximal region of the CH2 domain may be important to the specificity of the interaction between the antibody and the Fc receptor IgG1 myeloma proteins and recombinant IgG3 chimeric antibodies that lack a hinge region are reportedly unable to bind FcγRI, perhaps because accessibility to CH2 is decreased. Shin *et al*, 1993 *Intern. Rev Immunol.* 10 177, 178-79

Unusual and apparently evolutionarily unrelated exceptions to the H_2L_2 structure of conventional antibodies occur in some isotypes of the immunoglobulins found in camelids (camels, dromedaries and llamas, Hamers-Casterman *et al.*, 1993 *Nature* 363.446; Nguyen *et al*, 1998 *J Mol. Biol* 275 413), nurse sharks (Roux *et al*, 1998 *Proc. Nat. Acad. Sci. USA* 95 11804), and in the spotted ratfish (Nguyen, *et al*, "Heavy-chain antibodies in Camelidae; a case of evolutionary innovation," 2002 *Immunogenetics* 54(1).39-47) These antibodies can apparently form antigen-binding regions using only heavy chain variable region, *i.e*, these functional antibodies are homodimers of heavy chains only (referred to as "heavy-chain antibodies" or "HCAbs") In both species, these variable regions often contain an extended third complementarity determining region (CDR3) that may help compensate for the lack of a light chain variable region, and there are frequent disulfide bonds between CDR regions that presumably help to stabilize the binding site Muyldermans *et al*, 1994 *Prot. Engineer* 7 1129, Roux *et al*, 1998 However, the precise function of the heavy chain-only "antibodies" is unknown, and the evolutionary pressure leading to their formation has not been identified See, *e.g*, Nguyen, *et al*, 2002, *supra* Camelids, including camels, llamas, and alpacas, also express conventional H_2L_2 antibodies, and the heavy chain-only antibodies thus do not appear to be present in these animals simply as an alternative antibody structure.

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Variable regions (V_HH) of the camelid heavy chain-only immunoglobulins and conventional (H_2L_2) heavy chain variable regions contain amino acid differences, including differences at several positions that may be involved in the interface between conventional V_H and V_L domains. Nguyen *et al*, 1998 *J Mol. Biol* 275 413, Muyldermans *et al*, 1994 *Prot Engineer* 7 1129 Camelid V_HH reportedly recombines with IgG2 and IgG3 constant regions that contain hinge, CH2, and CH3 domains and lack a CH1 domain. Hamers-Casterman *et al*., 1993 *Nature* 363 446. Interestingly, V_HH are encoded by a chromosomal locus distinct from the V_H locus (Nguyen *et al*, 1998, *supra*), indicating that camelid B cells have evolved complex mechanisms of antigen recognition and differentiation. Thus, for example, llama IgG1 is a conventional (H_2L_2) antibody isotype in which V_H recombines with a constant region that contains hinge, CH1, CH2 and CH3 domains, whereas the llama IgG2 and IgG3 are heavy chain-only isotypes that lack CH1 domains and that contain no light chains.

The classes of immunoglobulins have different physical and chemical characteristics and they exhibit unique biological properties Their synthesis occurs at different stages and rates during an immune response and/or during the course of an infection. Their importance and functions in host resistance (immunity) are different.

Immunoglobulin G (IgG), a protein with a molecular weight of about 150,000 daltons (150kD), is the predominant Ig in the serum. It makes up about 80% of the total antibody found in an animal at any given time, being 75% of the total serum antibody It can diffuse out of the blood stream into the extravascular spaces and it is the most common Ig found there Its concentration in tissue fluids is increased during inflammation, and it is particularly effective at the neutralization of bacterial extracellular toxins and viruses. It also has opsonizing ability and complement-fixing ability The polypeptide composition of the Fc region of all IgG1 antibody molecules is relatively constant regardless of antibody specificity; however, as noted above, the Fab regions always differ in their exact amino acid sequences depending upon their antigenic specificity Specific amino acid regions of the Fc portion of the molecule are recognized by receptors on phagocytes and certain other cells, and the Fc domain contains a peptide region that will bind to and activate complement, which is often required for the manifestation of antibody-mediated immunity Because the IgG molecule is divalent, it can cross-link antigen molecules, which may lead to precipitation or agglutination of

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antigens, if IgG is bound to antigen on a microbial cell or surface, its Fc region may provide an extrinsic ligand that will be recognized by specific receptors on phagocytes Microbial cells or viruses coated with IgG molecules are opsonized for phagocytosis, and opsonized pathogens are taken up and destroyed much more readily by phagocytes than their non-opsonized counterparts. IgG, as well as IgM and IgA, will neutralize the activity of toxins, including bacterial exotoxins. Furthermore, cross-linked IgG molecules on the surface of a cell can bind and activate complement from the serum and set off a cascade of reactions that can lead to destruction of the cell.

IgM is the first immunoglobulin to appear in the blood stream during the course of an infection. It is mainly confined to the bloodstream and provides protection against blood-borne pathogens. IgM makes up about 10% serum immunoglobulins, and is arranged to resemble a pentamer of five immunoglobulin molecules (having a molecular weight of about 900kD) tethered together at by their Fc domains. In addition to covalent linkages between the monomeric subunits, the pentamer is stabilized by a 15kd polypeptide called J chain. IgM, therefore, has a theoretical "valence" of ten (*i.e.*, it has ten exposed Fab domains) Probably, the most important role of IgM is its ability to function early in the immune responses against blood-borne pathogens given its efficiency in agglutinating particulate antigens IgM binds also complement strongly and IgM antibodies bound to a microbial surface act as opsonins, rendering the microbe more susceptible to phagocytosis. In the presence of complement and IgM whole microbial cells may be killed and lysed. As noted above, IgM also appears on the surfaces of mature B cells as a transmembranous monomer where it functions as an antigen receptor, capable of activating B cells when bound to antigen.

Gene rearrangement at the immunoglobulin loci during lymphoid development generates a repertoire of B lymphocytes that express a diversity of antigen receptors The gene rearrangement, which is catalysed by the rearrangement-activating gene ("RAG") recombinase, integrates the immunoglobulin V, D and J gene segments to yield productively rearranged immunoglobulin genes that encode the heavy and light chains of IgM antibodies. The diversity of IgM antibodies in this primary repertoire is achieved through combinatorial mechanisms (the choice of V, D and J gene segments utilized in a particular antibody), as well as junctional diversity The joining of V, D and J gene segments is somewhat imprecise, and nucleotides may be inserted at the junction in a

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non-templated manner There is therefore a very high degree of resultant diversity at the V-D-J borders. This contributes in a major way to the structural diversity of the third complementarity determining region of the antibody, a region that often plays a critical role in antigen recognition. The primary repertoire of IgM antibodies comprises a few million different structures. The size of this repertoire means that any incoming antigen is likely to encounter an antibody that recognizes it with acceptable affinity A high-affinity binding site is unlikely to be available for most incoming antigens (the repertoire is not large enough), but the affinity of the available IgM antibodies in the primary repertoire will vary from antigen to antigen. If an epitope is re-iterated at high density on the surface of the antigen (*e.g.*, a repeated structure on the surface of a virus or bacterium), then an IgM antibody may nevertheless be effective in mediating clearance of the organism, despite the low affinity of the individual interaction between antigenic epitope and immunoglobulin combining site. The density of the epitopes may allow multivalent interactions with IgM, leading to a high-avidity interaction, providing that a suitable spacing of antigenic epitopes can occur Nevertheless, to ensure an effective and specific response, especially when the concentration of antigen is low (as may occur when the body is faced with a very small number of infecting viral particles), it would be preferable if high-affinity antibodies were available for neutralizing, for example, an infecting organism. The size of the primary repertoire militates against the likelihood of such high-affinity antibodies being present in this repertoire The immune system therefore operates using a two-stage strategy The primary repertoire of IgM antibodies is generated by a process of gene rearrangement and takes place prior to antigen encounter during early lymphocyte development. However, once foreign antigen has been encountered, those B cells in the primary repertoire that encode suitable (albeit low-affinity) antibodies are selectively expanded and subjected to an iterative alternation of directed hypermutation and antigen-mediated selection. This allows a significant maturation in affinity of the antigen-specific antibodies that are produced. Antigen triggering also drives isotype switch recombination. Thus, in the absence of external antigen stimulation and any maternally derived immunoglobulin, the serum will only contain a diversity of unmutated IgM molecules that have been generated by gene rearrangement. This repertoire shifts with age as a result of continuous antigen exposure, such that the majority of the serum immunoglobulin in older animals is

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composed of mutated IgG (and IgA) molecules whose specificities have developed as a consequence of antigen selection.

IgA exists as a H_2L_2 monomer of about 160kD in serum and, in secretions, as a dimer of the H_2L_2 monomer of about 400kD As with IgM, polymerization (dimerization) is via a J-chain. IgA has two subclasses based on different heavy chains, IgA1 and IgA2 IgA1 is produced in bone marrow and makes up most of the serum IgA. Both IgA1 and IgA2 are synthesized in GALT (gut associated lymphoid tissues) to be secreted onto the mucosal surfaces. Because IgA may be synthesized locally and secreted in the seromucous secretions of the body, it is sometimes referred to as secretory antibody or sIgA. Quantitatively, IgA is synthesized in amounts greater than IgG, but it has a short half life in serum (6 days), and it is lost in secretory products. The concentration of IgA in serum is about 15% of the total antibody Secretion of dimeric IgA is mediated by a 100kD glycoprotein called secretory component. It is the addition of the secretory piece to IgA molecules that accounts for their ability to exit the body to mucosal surfaces via the exocrine glands IgM can be transported similarly and makes up a small proportion of secretory antibodies. Secretory IgA is the predominant immunoglobulin present in gastrointestinal fluids, nasal secretions, saliva, tears and other mucous secretions of the body IgA antibodies are important in resistance to infection of the mucosal surfaces of the body, particularly the respiratory, intestinal and urogenital tracts IgA acts as a protective coating for the mucous surfaces against microbial adherence or mutual colonization. It can also neutralize toxin activity on mucosal surfaces Fc receptors for IgA-coated microorganisms found on monocytes and neutrophils derived from the respiratory mucosa suggest that IgA may have a role in the lung, at least, in opsonization of pathogens Secretory IgA is also transferred via the milk, *i e*, the colostrum, from a nursing mother to a newborn, which provides passive immunity to many pathogens, especially those that enter by way of the GI tract.

IgE is an immunoglobulin of about 190kD that accounts for about 0 002% of the total serum immunoglobulins It is produced by plasma cells below the respiratory and intestinal epithelia. The majority of IgE is bound to tissue cells, especially mast cells. If an infectious agent succeeds in penetrating the IgA barrier, it comes up against the next line of defense, the MALT (mucosa-associated lymphoid tissues) system that is managed by IgE IgE is bound very firmly to specific IgE Fc receptors on mast cells. Contact with

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antigen leads to release of mediators of inflammation from the mast cells, which effectively recruits various agents of the immune response including complement, chemotactic factors for phagocytes, T cells, *etc* Although a well-known manifestation of this reaction is a type of immediate hypersensitivity reaction called atopic allergy (*e.g* , hives, asthma, hay fever, *etc*), the MALT responses act as a defense mechanism because they amplify the inflammatory response and may facilitate rejection of a pathogen.

IgD is a molecule of about 175kd that resembles IgG in its monomeric form. IgD antibodies are found for the most part on the surfaces of B lymphocytes. The same cells may also carry IgM antibody As noted above, it is thought that IgD and IgM function as mutually interacting antigen receptors for control of B cell activation and suppression. Hence, IgD may have an immunoregulatory function.

In addition to opsonization, activation of complement, and ADCC, antibodies have other functions in host defense including steric hindrance, toxin neutralization, agglutination, and precipitation. With regard to steric hindrance, it is understood that antibodies combine with the surfaces of microorganisms and may block or prevent their attachment to susceptible cells or mucosal surfaces. Antibody against a viral component can block attachment of the virus to susceptible host cells and thereby reduce infectivity Secretory IgA can block attachment of pathogens to mucosal surfaces Toxin-neutralizing antibodies (antitoxins) can also react with a soluble bacterial toxin and block the interaction of the toxin with its specific target cell or substrate. Antibodies can also combine with the surfaces of microorganisms or soluble antigens and cause them to agglutinate or precipitate. This reduces the number of separate infectious units and makes them more readily phagocytosed because the clump of particles is larger in size. Phagocytes may remove floccules or aggregates of neutralized toxin.

Antibodies have been proposed for use in therapy Animals, including humans and mice have the ability to make antibodies able to recognize (by binding to) virtually any antigenic determinant and to discriminate between similar epitopes. Not only does this provide the basis for protection against disease organisms, but it also makes antibodies attractive candidates to target other types of molecules found in the body, such as receptors or other proteins present on the surface of normal cells and molecules present uniquely on the surface of cancer cells Thus the remarkable specificity of antibodies makes them promising agents for human therapy

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Initial antibody preparations available for use, such as intravenous gammaglobulins, included animal and human antisera that were used *in vivo* to destroy bacteria (tetanus, pneumococcus) and neutralize virus (hepatitis A and B, rabies, cytomegalovirus, and varicella zoster) in the blood of infected individuals. Possibly the most important early application was the use of endotoxins. However, there are problems associated with the use of antibodies in human therapy because the response of the immune system to any antigen, even the simplest, is "polyclonal," *i.e.*, the system manufactures antibodies of a great range of structures both in their binding regions as well as in their effector regions. Polyclonal antibody treatment was also associated with unwanted side effects. In addition to the polyclonal nature of these antibody preparations, there was the risk of infection from contaminating viruses. Serum sickness and anaphylaxis were also considered limiting factors. Furthermore, even if one were to isolate a single antibody-secreting cell, and place it in culture, it would die out after a few generations because of the limited growth potential of all normal somatic cells.

Until the late 1970s, polyclonal antibodies obtained from the blood serum of immunized animals provided the only source of antibodies for research or treatment of disease. Isolation of specific antibodies was essentially impossible until Kohler and Milstein discovered how to make "monoclonal antibodies" that would have a single specificity, that would all be alike due to manufacture by a single clone of plasma cells and that could be grown indefinitely. This technique was described in a 1975 publication (*Nature* 256.495-97), and Köhler and Milstein received the 1984 Nobel Prize in Medicine for their work.

The first step in Kohler and Milstein's technique for production of monoclonal antibodies involves immunizing an experimental animal with the antigen of interest. In most of their experiments, Kohler and Milstein injected a mouse with sheep red blood cells. The mouse's body initiates an immune response and begins producing antibodies specific to the antigen. The mouse's spleen is then removed and B cells producing the antibody of interest are isolated. Tumor-producing cells that have been grown in culture are then fused with the B lymphocytes using polyethylene glycol in order to produce a "hybridoma." Only hybridomas resulting from the fusion will survive. The spleen lymphocyte has a limited life span, so any B cells that did not fuse with a myeloma will die in the culture. Additionally, those cells that lack the antibody-producing aspect of

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the B cell will not secrete the enzyme HGPRT, which is required for growth in the hypoxanthine-aminopterin-thymidine (HAT) medium. The HAT medium, on which the cells are grown, inhibits the pathway for nucleotide synthesis. Cells that produce HGPRT can bypass this pathway and continue to grow By placing the fused cells in a HAT medium, true hybridomas can be isolated. The isolated hybridoma cells are then screened for specificity to the desired antigen. Because each hybridoma descends from one B cell, it makes copies of only one antibody The hybridoma that produces the antibody of interest is grown in culture to produce large amounts of monoclonal antibodies, which are then isolated for further use. The technique is called somatic cell hybridization, and the resulting hybridoma (selected for both immortality and production of the specific antibody of interest) may be cultured indefinitely, *i.e.*, it is a potentially immortal cell line.

Monoclonal antibodies are now widely used as diagnostic and research reagents. However, their introduction into human therapy has been much slower One principal difficulty is that mouse antibodies are "seen" by the human immune system as foreign. The human patient mounts an immune response against them, producing HAMA ("human anti-mouse antibodies") These not only cause the therapeutic antibodies to be quickly eliminated from the host, but also form immune complexes that cause damage to the kidneys

Two approaches have been used in an attempt to reduce the problem of HAMA. The first is the production of chimeric antibodies in which the antigen-binding part (variable regions) of a mouse monoclonal antibody is fused to the effector part (constant region) of a human antibody using genetic engineering. In a second approach, rodent antibodies have been altered through a technique known as complementarity determining region (CDR) grafting or "humanization." In this process, the antigen binding sites, which are formed by three CDRs of the heavy chain and three CDRs of the light chain, are excised from cells secreting rodent mAb and grafted into the DNA coding for the framework of the human antibody Because only the antigen-binding site CDRs, rather than the entire variable domain of the rodent antibody are transplanted, the resulting humanized antibody (a second generation or "hyperchimeric" antibody) is reportedly less immunogenic than a first generation chimeric antibody This process has been further improved to include changes referred to as "reshaping" (Verhoeyen, *et al*, "Reshaping human antibodies grafting an anti-lysozyme activity," 1988 *Science* 239 1534-1536;

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Riechmann, *et al*, "Reshaping human antibodies for therapy," 1988 *Nature* 332 323-337, Tempest, *et al*, "Reshaping human monoclonal antibody to inhibit respiratory syncitial virus infection in vivo," *Bio/Technol* 1991 9.266-271), "hyperchimerization" (Queen, *et al*, "A humanized antibody that binds to the human interleukin 2 receptor," 1989 *Proc Natl Acad Sci USA* 86 10029-10033, Co, *et al*, "Humanized antibodies for antiviral therapy," 1991 *Proc Natl Acad Sci USA* 88:2869 –2873, Co, *et al*, "Chimeric and humanized antibodies with specificity for the CD33 antigen," 1992 *J Immunol* 148 1149-1154), and "veneering" (Mark, *et al*., "Derivation of therapeutically active humanized and veneered anti-CD18 antibodies. In. Metcalf BW, Dalton BJ, eds. Cellular adhesion molecular definition to therapeutic potential New York. Plenum Press, 1994 291-312)

In the reshaping process on the basis of homology, the rodent variable region is compared with the consensus sequence of the protein sequence subgroup to which it belongs. Similarly, the selected human constant region accepting framework is compared with its family consensus sequence. Guasowal, *et al*, "Humanization of monoclonal antibodies," 1991 *Meth Enzymol* 203 99-121 The sequence analyses identify residues, which may have undergone mutation during the affinity maturation procedure and may therefore be idiosyncratic Inclusion of the more common human residues is said to lessen immunogenicity problems by replacing human acceptor idiosyncratic residues Further, the reshaping process is said to allow comparison of human and rodent consensus sequences to identify any systematic "species" differences. RSV19 antibodies were humanized by employing this procedure Taylor *et al*, "Humanized monoclonal antibody to respiratory syncitial virus," 1991 *Lancet* 337 1411-1412, Tempest, *et al*, "Reshaping a human monoclonal antibody to inhibit human respiratory syncitial virus infection in vivo," 1991 *Bio/Technol* 9:266-271

Hyperchimerization is an alternative method of identifying residues outside CDR regions that are likely to be involved in the reconstitution of binding activity In this method, the human sequences are compared with murine variable region sequences and the one with highest homology is selected as the acceptor framework As in the reshaping procedure, the "idiosyncratic" residues are replaced by more commonly occurring human residues The non-CDR residues that may be interacting with the CDR sequences are identified. Finally, it is determined which one of these residues is to be included in the variable region framework. Humanized antibodies against CD33 antigen were reportedly

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developed by this method. Co, *et al*, "Chimeric and humanized antibodies with specificity for the CD33 antigen," 1992 *J Immunol* 148 1149-154 See also Carter, *et al*, "Humanization of an anti-p185 HER2 antibody for human cancer therapy," 1992 *Proc Natl Acad Sci USA* 89 4285-4289

The displayed surface of the protein is the primary determinant of its immunogenicity A humanized murine antibody can thus be made less immunogenic by replacing exposed residues that differ from those commonly found in human antibodies. This method of humanization is referred to as "veneering." Appropriate replacement of the outer residues may have little or no impact on the inner domains or interdomain framework. Veneering is a two-step process. In the first step, the most homologous human variable regions are selected and compared by each single residue to the corresponding mouse variable regions. In the second step, the residues present in the human homologue replace the mouse framework residues, which differ from its human homologue This replacement involves only those residues that are on the surface and at least partially exposed.

Nevertheless, it took more than a quarter century of research for monoclonal antibody technology and genetic engineering methods to result in the development of immunoglobulin molecules for treatment of human diseases. Indeed, it was not until the past five years that monoclonal antibodies became an expanding class of therapeutics See Glennie MJ and van de Winkel JG, *Drug Discov Today* 2003 Jun 1,8(11):503-10, Souriau C and Hudson PJ, "Recombinant antibodies for cancer diagnosis and therapy," 2003 *Expert Opin Biol Ther* 3(2) 305-18 See also Pendley C, *et al*, "Immunogenicity of therapeutic monoclonal antibodies," 2003 *Curr Opin Mol Ther* 5(2):172-9

All the same, an average of less than one therapeutic antibody per year has been introduced to the market beginning in 1986, eleven years after the publication of monoclonal antibodies Five murine monoclonal antibodies were introduced into human medicine over a ten year period from 1986-1995, including "muromonab-CD3" (OrthoClone OKT3®), which binds to a molecule on the surface of T cells and was launched in 1986 to prevent acute rejection of organ transplants, "edrecolomab" (Panorex®), launched in 1995 for treatment of colorectal cancer; "oduhimomab" (Antilfa®), launched in 1997 for use in transplant rejection, and, "ibritumomab" (Zevalin® yiuxetan), launched in 2002 for use in non-Hodgkin's lymphoma. Additionally, one

monoclonal Fab, "abciximab" (ReoPro®), was launched in 1995. It inhibits the clumping of platelets by binding the receptors on their surface that normally are linked by fibrinogen and may be helpful in preventing reclogging of the coronary arteries in patients who have undergone angioplasty. Three chimeric monoclonal antibodies were also launched "rituximab" (Rituxan®), in 1997, which binds to the CD20 molecule found on most B cells and is used to treat B cell lymphomas, "basiliximab" (Simulect®), in 1998 for transplant rejection, and "infliximab" (Remicade®) which binds to tumor necrosis factor-alpha (TNF-a), in 1998 for treatment of rheumatoid arthritis and Crohn's disease. Additionally, "abciximab" (ReoPro®), a 47.6 kD Fab fragment of the chimeric human-murine monoclonal antibody 7E3 that binds to the glycoprotein (GP) IIb/IIIa receptor of human platelets, was launched in 1995 as an adjunct to percutaneous coronary intervention for the prevention of cardiac ischemic complications in patients undergoing percutaneous coronary intervention. Finally, seven "humanized" monoclonals were launched from 1997-2003: "daclizumab" (Zenapax®) in 1997, which binds to part of the IL-2 receptor produced at the surface of activated T cells and is used to prevent acute rejection of transplanted kidneys, "palivizumab" (Synagis®) in 1998 for RSV, "trastuzumab" (Herceptin®) in 1998, which binds HER-2, a growth factor receptor found on breast cancers cells, "gemtuzumab" (Mylotarg®) in 2000, which is a conjugate of a monoclonal antibody that binds CD33, a cell-surface molecule expressed by the cancerous cells in acute myelogenous leukemia (AML) but not found on the normal stem cells needed to repopulate the bone marrow; and "alemtuzumab" (MabCampath®) in 2001, which binds to CD52, a molecule found on white blood cells and has produced temporary remission of chronic lymphocytic leukemia, "adalimumab" (Humira® (D2E7)), a human anti-TNF monoclonal containing human-derived heavy chain and light chain variable regions and human IgG$_1$κ constant regions was launched in 2002 for the treatment of rheumatoid arthritis, and, "omalizumab" (Xolair®), which binds to IgE and prevents it from binding to mast cells was approved in 2003 for the treatment of adults and adolescents over 12 years of age with moderate to severe persistent asthma who have a positive skin test or *in vitro* reactivity to a perennial aeroallergen and whose symptoms are inadequately controlled with inhaled corticosteroids.

Thus, protein engineering has been applied in an effort to diminish problems related to immunogenicity of administered recombinant immunoglobulin polypeptides and

to try to alter antibody effector functions However, problems remain. For example, the majority of cancer patients treated with rituximab relapse, generally within about 6-12 months, and fatal infusion reactions within 24 hours of rituximab infusion have been reported. These fatal reactions followed an infusion reaction complex that included hypoxia, pulmonary infiltrates, acute respiratory distress syndrome, myocardial infarction, ventricular fibrillation or cardiogenic shock. Acute renal failure requiring dialysis with instances of fatal outcome has also been reported in the setting of tumor lysis syndrome following treatment with rituximab, as have severe mucocutaneous reactions, some with fatal outcome. Additionally, high doses of rituximab are required for intravenous injection because the molecule is large, approximately 150 kDa, and diffusion is limited into the lymphoid tissues where many tumor cells reside.

Trastuzumab administration can result in the development of ventricular dysfunction and congestive heart failure, and the incidence and severity of cardiac dysfunction has been reported to be particularly high in patients who received trastuzumab in combination with anthracyclines and cyclophosphamide. Trastuzumab administration can also result in severe hypersensitivity reactions (including anaphylaxis), infusion reactions, and pulmonary events

Patients receiving daclizumab immunosuppressive therapy are at increased risk for developing lymphoproliferative disorders and opportunistic infections, and it is not known whether daclizumab use will have a long-term effect on the ability of the immune system to respond to antigens first encountered during daclizumab-induced immunosuppression.

Hepatotoxicity, including severe hepatic veno-occlusive disease (VOD), has also been reported in association with the use of gemtuzumab as a single agent, as part of a combination chemotherapy regimen, and in patients without a history of liver disease or hematopoietic stem-cell transplant (HSCT) Patients who receive gemtuzumab either before or after HSCT, patients with underlying hepatic disease or abnormal liver function, and patients receiving gemtuzumab in combinations with other chemotherapy may be at increased risk for developing severe VOD Death from liver failure and from VOD has been reported in patients who received gemtuzumab, and it has been cautioned that even careful monitoring may not identify all patients at risk or prevent the complications of hepatotoxicity

Hepatotoxicity was also reported in patients receiving alemtuzumab Serious and, in some rare instances fatal, pancytopenia/marrow hypoplasia, autoimmune idiopathic thrombocytopenia, and autoimmune hemolytic anemia have occurred in patients receiving alemtuzumab therapy Alemtuzumab can also result in serious infusion reactions as well as opportunistic infections

In patients treated with adalimumab, serious infections and sepsis, including fatalities, have been reported, as has the exacerbation of clinical symptoms and/or radiographic evidence of demyelinating disease, and patients treated with adalimumab in clinical trials had a higher incidence of lymphoma than the expected rate in the general population.

Serious adverse reactions in clinical studies with omalizumab have included malignancies and anaphylaxis, in which the observed incidence of malignancy among omalizumab-treated patients (0.5%) was numerically higher than among patients in control groups (0 2%)

Smaller immunoglobulin molecules have been constructed in an effort to overcome various problems associated with whole immunoglobulin therapy Single chain immunoglobulin variable region fragment polypeptides (scFvs) are made of an immunoglobulin heavy chain variable domain joined via a short linker peptide to an immunoglobulin light chain variable domain. Huston *et al*, 1988 *Proc. Natl Acad. Sci. USA*, 85 5879-83 It has been suggested that the smaller size of scFv molecules may lead to more rapid clearance from plasma and more effective penetration into tissues than whole immunoglobulins *See, e.g*, Jain, 1990 *Cancer Res* 50 814s-819s An anti-tumor scFv was reported to show more rapid tumor penetration and more even distribution through the tumor mass than the corresponding chimeric antibody Yokota *et al*, *Cancer Res.* 52 3402-08 (1992)

Despite advantages that scFv molecules may have with regard to serotherapy, drawbacks to this therapeutic approach also exist. For example, rapid clearance of scFv may prevent delivery of a minimum effective dose to the target tissue. Additionally, manufacturing adequate amounts of scFv for administration to patients has been challenging due to difficulties in expression and isolation of scFv that adversely affect yields. During expression, scFv molecules lack stability and often aggregate due to pairing of variable regions from different molecules. Furthermore, production levels of scFv

molecules in mammalian expression systems are reportedly low, which may limit the potential for efficient manufacturing of scFv molecules for therapy Davis *et al*, 1990 *J Biol. Chem.* 265 10410-18, Traunecker *et al*, 1991 *EMBO J* 10 3655-59 Strategies for means to improve production have been explored, and reportedly include the addition of glycosylation sites to variable regions. *See, e.g*, U.S Patent No 5,888,773, Jost *et al*, 1994 *J Biol. Chem.* 269.26267-73 Another disadvantage to the use of scFvs for therapy is the lack of effector function. An scFv that lacks the cytolytic functions, ADCC, and complement dependent-cytotoxicity may be less effective or ineffective for treating disease. Even though development of scFv technology began over 12 years ago, there are currently no scFv products approved for therapy

Alternatively, it has been proposed that fusion of an scFv to another molecule, such as a toxin, could take advantage of the specific antigen-binding activity and the small size of an scFv to deliver the toxin to a target tissue. Chaudary *et al*., 1989 *Nature* 339 394, Batra *et al*, 1991 *Mol Cell Biol* 11.2200 Conjugation or fusion of toxins to scFvs has thus been offered as an alternative strategy to provide potent, antigen-specific molecules, but dosing with such conjugates or chimeras can be limited by excessive and/or non-specific toxicity due to the toxin moiety of such preparations Toxic effects may include supraphysiological elevation of liver enzymes and vascular leak syndrome, and other undesired effects. In addition, immunotoxins are themselves highly immunogenic upon administration to a host, and host antibodies generated against the immunotoxin limit potential usefulness for repeated therapeutic treatments of an individual.

Fusion proteins in which immunoglobulin constant region polypeptide sequences are present and nonimmunoglobulin sequences are substituted for the antibody variable regions have also been investigated. For example, CD4, the T cell surface protein recognized by HIV, was recombinantly fused to an immunoglobulin Fc effector domain, and an IL-2-IgG1 fusion protein reportedly effected complement-mediated lysis of IL-2 receptor-bearing cells. *See* Sensel *et al*, *Chem. Immunol.* 65 129-158 (1997) An extensive introduction as well as detailed information about all aspects of recombinant antibody technology can be found in the textbook "Recombinant Antibodies" (John Wiley & Sons, NY, 1999). A comprehensive collection of detailed antibody engineering lab

Protocols can be found in R. Kontermann and S Dübel (eds), "The Antibody Engineering Lab Manual" (Springer Verlag, Heidelberg/New York, 2000).

Diseases and disorders thought to be amenable to some type of immunoglobulin therapy include cancer and immune system disorders. Cancer includes a broad range of diseases, affecting approximately one in four individuals worldwide. Rapid and unregulated proliferation of malignant cells is a hallmark of many types of cancer, including hematological malignancies Although patients with a hematologic malignant condition have benefited from advances in cancer therapy in the past two decades, Multani *et al* , 1998 *J Clin. Oncology* 16 3691-3710, and remission times have increased, most patients still relapse and succumb to their disease. Barriers to cure with cytotoxic drugs include, for example, tumor cell resistance and the high toxicity of chemotherapy, which prevents optimal dosing in many patients.

Nevertheless, patients have been treated with immunotherapeutics that target malignant cells, *i.e* , to antigens expressed on tumor cells With regard to the selection of tumor cell surface antigens suitable for use as immunotherapy targets, it is preferable that such a target antigen is not expressed by normal tissues, particularly where the preservation of such tissue is important to host survival. In the case of hematologic malignancy, malignant cells express many antigens that are not expressed on the surfaces of stem cells or other essential cells Treatment of a hematologic malignant condition using a therapeutic regimen that depletes both normal and malignant cells of hematological origin has been acceptable where regeneration of normal cells from progenitors can occur after therapy has ended. Additionally, the target antigen is desirably expressed on all or virtually all clonogenic populations of tumor cells, and it is best that expression persists despite selective pressure from immunoglobulin therapy Strategies that employ selection of a cell surface idiotype (*e.g* , a particular idiotope) as a target for therapy of B cell malignancy have been limited by the outgrowth of tumor cell variants with altered surface idiotype expression, even where the antigen exhibits a high degree of tumor selectivity Meeker *et al* , 1985 *N Engl. J Med* 312 1658-65 The selected antigen should also traffic properly after an immunoglobulin binds to it. Shedding or internalization of a cell surface target antigen after an immunoglobulin binds to the antigen may allow tumor cells to escape destruction, thus limiting the effectiveness of serotherapy Finally, binding of an immunoglobulin to cell surface target antigens that transmit or transduce cellular activation

signals may result in improved functional responses to immunotherapy in tumor cells, and can lead to growth arrest and/or apoptosis. While all of these properties are important, the triggering of apoptosis after an immunoglobulin binds to the target antigen may also be a critical factor in achieving successful serotherapy

Antigens that have been tested as targets for serotherapy of B and T cell malignancies include Ig idiotype (Brown *et al*, 1989 *Blood* 73:651-61), CD19 (Hekman *et al*, 1991 *Cancer Immunol Immunother* 32.364-72), Vlasveld *et al*, 1995 *Cancer Immunol. Immunother* 40 37-47), CD20 (Press *et al*, 1987 *Blood* 69 584-91), Maloney *et al*, 1997 *J Clin. Oncol* 15 3266-74), CD21 (Schenberg *et al*, 1990 *J Clin. Oncol* 8 792-803), CD5 (Dillman *et al*, 1986 *J Biol. Respn. Mod.* 5 394-410), and CD52 (CAMPATH) (Pawson *et al*, 1997 *J Clin Oncol.* 15 2667-72) Of these, greater benefit for therapy of B cell lymphomas has been obtained using molecules that target CD20 Other targets have been limited by biological properties of the antigen. For example, surface idiotype can be altered through somatic mutation, allowing tumor cell escape. CD5, CD21, and CD19 are rapidly internalized after monoclonal antibody binding, allowing tumor cells to escape destruction unless monoclonal antibodies are conjugated with toxin molecules. CD22 is expressed on only a subset of B cell lymphomas, thereby limiting its usefulness, while CD52 is expressed on both T cells and B cells and may therefore generate counterproductive immunosuppression by depletion.

Treatment of patients with low grade or follicular B cell lymphoma using a chimeric CD20 monoclonal antibody has been reported to induce partial or complete responses in patients McLaughlin *et al*, 1996 *Blood* 88 90a (abstract, suppl. 1), Maloney *et al*, 1997 *Blood* 90:2188-95 However, as noted above, tumor relapse commonly occurs within six months to one year Further improvements in serotherapy are needed to induce more durable responses, for example, in low grade B cell lymphoma, and to allow effective treatment of high-grade lymphoma and other B cell diseases.

Another approach has been to target radioisotopes to B cell lymphomas using monoclonal antibodies specific for CD20 While the effectiveness of therapy is reportedly increased, associated toxicity from the long *in vivo* half-life of the radioactive antibody increases also, sometimes requiring that the patient undergo stem cell rescue. Press *et al*, 1993 *N Eng J Med.* 329 1219-1224, Kaminski *et al*, 1993 *N Eng.J Med* 329.459-65 Monoclonal antibodies to CD20 have also been cleaved with proteases to

yield $F(ab')_2$ or Fab fragments prior to attachment of radioisotope. This has been reported to improve penetration of the radioisotope conjugate into the tumor and to shorten the *in vivo* half-life, thus reducing the toxicity to normal tissues However, these molecules lack effector functions, including complement fixation and/or ADCC.

CD20 was the first human B cell lineage-specific surface molecule identified by a monoclonal antibody It is a non-glycosylated, hydrophobic 35 kDa B cell transmembrane phosphoprotein that has both amino and carboxy ends situated in the cytoplasm. Einfeld *et al*, 1988 *EMBO J* 7:711-17 CD20 is expressed by all normal mature B cells, but is not expressed by precursor B cells. Natural ligands for CD20 have not been identified, and the function of CD20 in B cell biology is still incompletely understood.

Anti-CD20 monoclonal antibodies affect the viability and growth and growth of B cells. Clark *et al*, 1986 *Proc. Natl. Acad. Sci. USA* 83 4494-98 Extensive cross-linking of CD20 can induce apoptosis in B lymphoma cell lines, Shan *et al*, 1998 *Blood* 91 1644-52, and cross-linking of CD20 on the cell surface has been reported to increase the magnitude and enhance the kinetics of signal transduction, for example, as detected by measuring tyrosine phosphorylation of cellular substrates Deans *et al*, 1993 *J Immunol.* 146 846-53 Therefore, in addition to cellular depletion by complement and ADCC mechanisms, Fc-receptor binding by CD20 monoclonal antibodies *in vivo* may promote apoptosis of malignant B cells by CD20 cross-linking, consistent with the theory that effectiveness of CD20 therapy of human lymphoma in a SCID mouse model may be dependent upon Fc-receptor binding by the CD20 monoclonal antibody Funakoshi *et al*, 1996 *J Immunotherapy* 19 93-101 The presence of multiple membrane spanning domains in the CD20 polypeptide (Einfeld *et al*, 1988 *EMBO J* 7 711-17, Stamenkovic *et al*, 1988 *J Exp Med.* 167 1975-80, Tedder *et al*, 1988 *J Immunol.* 141 4388-4394), prevent CD20 internalization after antibody binding, and thus was recognized as an important feature for therapy of B cell malignancies when a murine CD20 monoclonal antibody, 1F5, was injected into patients with B cell lymphoma, resulting in significant depletion of malignant cells and partial clinical responses Press *et al*, 1987 *Blood* 69 584-91

Because normal mature B cells also express CD20, normal B cells are depleted by anti-CD20 antibody therapy Reff, M E. *et al*, 1994 *Blood* 83 435-445 After treatment is completed, however, normal B cells can be regenerated from CD20 negative B

cell precursors, therefore, patients treated with anti-CD20 therapy do not experience significant immunosuppression. Depletion of normal B cells may also be beneficial in diseases that involve inappropriate production of autoantibodies or other diseases where B cells may play a role. A chimeric monoclonal antibody specific for CD20, consisting of heavy and light chain variable regions of mouse origin fused to human IgG1 heavy chain and human kappa light chain constant regions, reportedly retained binding to CD20 and the ability to mediate ADCC and to fix complement. Liu *et al*, 1987 *J Immunol.* 139 3521-26 The mechanism of anti-tumor activity of rituximab, discussed above, is thought to be a combination of several activities, including ADCC, complement fixation, and triggering of signals that promote apoptosis in malignant B cells, although the large size of rituximab prevents optimal diffusion of the molecule into lymphoid tissues that contain malignant B cells, thereby limiting these anti-tumor activities.Autoimmune diseases include autoimmune thyroid diseases, which include Graves' disease and Hashimoto's thyroiditis In the United States alone, there are about 20 million people who have some form of autoimmune thyroid disease. Autoimmune thyroid disease results from the production of autoantibodies that either stimulate the thyroid to cause hyperthyroidism (Graves' disease) or destroy the thyroid to cause hypothyroidism (Hashimoto's thyroiditis) Stimulation of the thyroid is caused by autoantibodies that bind and activate the thyroid stimulating hormone (TSH) receptor Destruction of the thyroid is caused by autoantibodies that react with other thyroid antigens Current therapy for Graves' disease includes surgery, radioactive iodine, or antithyroid drug therapy Radioactive iodine is widely used, since antithyroid medications have significant side effects and disease recurrence is high. Surgery is reserved for patients with large goiters or where there is a need for very rapid normalization of thyroid function. There are no therapies that target the production of autoantibodies responsible for stimulating the TSH receptor Current therapy for Hashimoto's thyroiditis is levothyroxine sodium, and therapy is usually lifelong because of the low likelihood of remission. Suppressive therapy has been shown to shrink goiters in Hashimoto's thryoiditis, but no therapies that reduce autoantibody production to target the disease mechanism are known.

Rheumatoid arthritis (RA) is a chronic disease characterized by inflamation of the joints, leading to swelling, pain, and loss of function. RA affects an estimated 2 5 million people in the United States RA is caused by a combination of events including an

mutual infection or injury, an abnormal immune response, and genetic factors While autoreactive T cells and B cells are present in RA, the detection of high levels of antibodies that collect in the joints, called rheumatoid factor, is used in the diagnosis of RA. Current therapy for RA includes many medications for managing pain and slowing the progression of the disease. No therapy has been found that can cure the disease. Medications include nonsteroidal antiinflamatory drugs (NSAIDS), and disease modifying antirheumatic drugs (DMARDS). NSAIDS are useful in benign disease, but fail to prevent the progression to joint destruction and debility in severe RA. Both NSAIDS and DMARDS are associated with significant side effects Only one new DMARD, Leflunomide, has been approved in over 10 years. Leflunomide blocks production of autoantibodies, reduces inflamation, and slows progression of RA. However, this drug also causes severe side effects including nausea, diarrhea, hair loss, rash, and liver injury

Systemic Lupus Erythematosus (SLE) is an autoimmune disease caused by recurrent injuries to blood vessels in multiple organs, including the kidney, skin, and joints. SLE is estimated to affect over 500,000 people in the United States In patients with SLE, a faulty interaction between T cells and B cells results in the production of autoantibodies that attack the cell nucleus. These include anti-double stranded DNA and anti-Sm antibodies. Autoantibodies that bind phospholipids are also found in about half of SLE patients, and are responsible for blood vessel damage and low blood counts Immune complexes accumulate the kidneys, blood vessels, and joints of SLE patients, where they cause inflamation and tissue damage No treatment for SLE has been found to cure the disease. NSAIDS and DMARDS are used for therapy depending upon the severity of the disease. Plasmapheresis with plasma exchange to remove autoantibodies can cause temporary improvement in SLE patients. There is general agreement that autoantibodies are responsible for SLE, so new therapies that deplete the B cell lineage, allowing the immune system to reset as new B cells are generated from precursors, would offer hope for long lasting benefit in SLE patients

Sjogren's syndrome is an autoimmune disease characterized by destruction of the body's moisture-producing glands. Sjogren's syndrome is one of the most prevelant autoimmune disorders, striking up to an estimated 4 million people in the United States About half of people stricken with Sjogren's syndrome also have a connective tissue disease, such as RA, while the other half have primary Sjogren's syndrome with no other

concurrent autoimmune disease. Autoantibodies, including anti-nuclear antibodies, rheumatoid factor, anti-fodrin, and anti-muscarinic receptor are often present in patients with Sjogren's syndrome Conventional therapy includes corticosteroids, and additional more effective therapies would be of benefit.

Immune thrombocytopenic purpura (ITP) is caused by autoantibodies that bind to blood platelets and cause their destruction. Drugs cause some cases of ITP, and others are associated with infection, pregnancy, or autoimmune disease such as SLE About half of all cases are classified as "idiopathic", meaning the cause is unknown. The treatment of ITP is determined by the severity of the symptoms. In some cases, no therapy is needed although in most cases immunosuppressive drugs, including corticosteroids or intravenous infusions of immune globulin to deplete T cells, are provided Another treatment that usually results in an increased number of platelets is removal of the spleen, the organ that destroys antibody-coated platelets. More potent immunosuppressive drugs, including cyclosporine, cyclophosphamide, or azathioprine are used for patients with severe cases. Removal of autoantibodies by passage of patients' plasma over a Protein A column is used as a second line treatment in patients with severe disease Additional more effective therapies are desired.

Multiple sclerosis (MS) is also an autoimmune disease. It is characterized by inflamation of the central nervous system and destruction of myelin, which insulates nerve cell fibers in the brain, spinal cord, and body Although the cause of MS is unknown, it is widely believed that autoimmune T cells are primary contributors to the pathogenesis of the disease. However, high levels of antibodies are present in the cerebral spinal fluid of patients with MS, and some theories predict that the B cell response leading to antibody production is important for mediating the disease. No B cell depletion therapies have been studies in patients with MS, and there is no cure for MS Current therapy is corticosteroids, which can reduce the duration and severity of attacks, but do not affect the course of MS over time. New biotechnology interferon (IFN) therapies for MS have recently been approved but additional more effectiver therapies are desired.

Myasthenia Gravis (MG) is a chronic autoimmune neuromuscular disorder that is characterized by weakness of the voluntary muscle groups MG effects about 40,000 people in the united states MG is caused by autoantibodies that bind to acetylcholine receptors expressed at neuromuscular junctions The autoantibodies reduce

or block acetylcholine receptors, preventing the transmission of signals from nerves to muscles. There is no known cure for mg. Common treatments include immunosuppression with corticosteroids, cyclosporine, cyclophosphamide, or azathioprine. Surgical removal of the thymus is often used to blunt the autoimmune response Plasmapheresis, used to reduce autoantibody levels in the blood, is effective in mg, but is short-lived because the production of autoantibodies continues. Plasmapheresis is usually reserved for severe muscle weakness prior to surgery New and effective therapies would be of benefit.

Psoriasis affects approximately five million people, and is characterized by autoimmune inflammation in the skin. Psoriasis is also associated with arthritis in 30% (psoriatic arthritis) Many treatments, including steroids, uv light retenoids, vitamin d derivatives, cyclosporine, methotrexate have been used but it is also plain that psoriasis would benefit from new and effective therapies. Scleroderma is a chronic autoimmune disease of the connective tissue that is also known as systemic sclerosis Scleroderma is characterized by an overproduction of collagen, resulting in a thickening of the skin, and approximately 300,000 people in the United States have scleroderma, which would also benefit from new and effective therapies.

There is a clear need for improved compositions and methods to treat malignacies, including B cell malignancies and disorders including autoimmune diseases, disorders, and conditions, as well as other diseases, disorders, and conditions, The compositions and methods of the present invention described and claimed herein provide such improved compositions and methods as well as other advantages.

SUMMARY OF THE INVENTION

It is an aspect of the present invention to provide a binding domain-immunoglobulin fusion protein, comprising a binding domain polypeptide that is fused or otherwise connected to an immunoglobulin hinge or hinge-acting region polypeptide, which in turn is fused or otherwise connected to a region comprising one or more native or engineered constant regions from an immunoglobulin heavy chain, other than CH1, for example, the CH2 and CH3 regions of IgG and IgA, or the CH3 and CH4 regions of IgE. The binding domain-immunoglobulin fusion protein further comprises a region that comprises, consists essentially of, or consists of, a native or engineered immunoglobulin heavy chain CH2 constant region polypeptide (or CH3 in the case of a construct derived in

whole or in part from IgE) that is fused or otherwise connected to the hinge region polypeptide and a native or engineered immunoglobulin heavy chain CH3 constant region polypeptide (or CH4 in the case of a construct derived in whole or in part from IgE) that is fused or otherwise connected to the CH2 constant region polypeptide (or CH3 in the case of a construct derived in whole or in part from IgE). Such binding domain-immunoglobulin fusion proteins are capable of at least one immunological activity selected from the group consisting of antibody dependent cell-mediated cytotoxicity and complement fixation. Such binding domain polypeptides are also capable of binding or specifically binding to a target, for example, a target antigen.

In certain embodiments, for example, the binding domain polypeptide comprises at least one immunoglobulin variable region polypeptide that is selected from a native or engineered immunoglobulin light chain variable region polypeptide and/or a native or engineered immunoglobulin heavy chain variable region polypeptide. In certain further embodiments the binding domain-immunoglobulin fusion protein comprises a native or engineered immunoglobulin heavy chain variable region polypeptide, wherein the heavy chain variable region polypeptide is an engineered human immunoglobulin heavy chain variable region polypeptide (or an engineered immunoglobulin heavy chain variable region polypeptide from a non-human species) comprising a mutation, substitution, or deletion of an amino acid(s) at a location corresponding to any one or more of amino acid positions 9, 10, 11, 12, 108, 110, and/or 112 Mutations, substitutions, or deletions of an amino acid(s) at a location corresponding to any one or more of amino acid positions 9, 10, 11, 12, 108, 110, and/or 112 in a heavy chain variable region may be included within a construct such as the construct corresponding to, for example, SEQ ID NO ___ In certain other further embodiments the fusion protein comprises a polypeptide having a sequence selected from SEQ ID NOS __ or SEQ ID NO __ In certain embodiments the immunoglobulin variable region polypeptide is derived from, for example, a human immunoglobulin, and in certain other embodiments the immunoglobulin variable region polypeptide comprises a humanized immunoglobulin polypeptide sequence. In certain embodiments the immunoglobulin variable region polypeptide, whether or not humanized, is derived from a murine immunoglobulin, or is derived from an immunoglobulin from another species, including, for example a rat, a pig, a monkey, or a camelid

According to certain embodiments of the present invention, the binding domain polypeptide comprises, consists essentially of, or consists of, (a) at least one native or engineered immunoglobulin light chain variable region polypeptide; (b) at least one native or engineered immunoglobulin heavy chain variable region polypeptide, and (c) at least one linker polypeptide that is fused or otherwise connected to the polypeptide of (a) and to the polypeptide of (b) In certain further embodiments the native or engineered immunoglobulin light chain variable region and heavy chain variable region polypeptides are derived or constructed from human immunoglobulins, and in certain other further embodiments the linker polypeptide comprises at least one polypeptide including or having as an amino acid sequence Gly-Gly-Gly-Gly-Ser [SEQ ID NO __] In other embodiments the linker polypeptide comprises at least two or three repeats of a polypeptide having as an amino acid sequence Gly-Gly-Gly-Gly-Ser [SEQ ID NO __] In other embodiments the linker comprises a glycosylation site, which in certain further embodiments is an asparagine-linked glycosylation site, an O-linked glycosylation site, a C-mannosylation site, a glypiation site or a phosphoglycation site. In another embodiment at least one of a native or engineered immunoglobulin heavy chain CH2 constant region polypeptide and a native or engineered immunoglobulin heavy chain CH3 constant region polypeptide is derived from an IgG or IgA human immunoglobulin heavy chain. In another embodiment at least one of a native or engineered immunoglobulin heavy chain CH3 constant region polypeptide and a native or engineered immunoglobulin heavy chain CH4 constant region polypeptide is derived from an IgE human immunoglobulin heavy chain.

An immunoglobulin hinge region polypeptide may comprise, consist essentially or, or consist of, for example, any of (1) any hinge or hinge-acting peptide or polypeptide that occurs naturally for example, a human immunoglobulin hinge region polypeptide including, for example, a wild-type human IgG hinge or a portion thereof, a wild-type human IgA hinge or a portion thereof, a wild-type human IgD hinge or a portion thereof, or a wild-type human IgE hinge-acting region, *i.e.*, IgE CH2, or a portion thereof, a wild-type camelid hinge region or a portion thereof (including a IgG1 llama hinge region or portion thereof, a IgG2 llama hinge region or portion thereof, and a IgG3 llama hinge region or portion thereof), a nurse shark hinge region or portion thereof, and/or a spotted ratfish hinge region or a portion thereof; (2) a mutated or otherwise altered or engineered hinge region polypeptide that contains no cysteine residues and that is derived or

constructed from a wild-type immunoglobulin hinge region polypeptide having one or more cysteine residues, (3) a mutated or otherwise altered or engineered hinge region polypeptide that contains one cysteine residue and that is derived from a wild-type immunoglobulin hinge region polypeptide having one or more cysteine residues, (4) a hinge region polypeptide that has been mutated or otherwise altered or engineered to contain or add one or more glycosylation sites, for example, an asparagine-linked glycosylation site, an O-linked glycosylation site, a C-mannosylation site, a glypiation site or a phosphoglycation site; (5) a mutated or otherwise altered or engineered hinge region polypeptide in which the number of cysteine residues is reduced by amino acid substitution or deletion, for example, a mutated or otherwise altered or engineered IgG1 or IgG4 hinge region containing for example zero, one, or two cysteine residues, a mutated or otherwise altered or engineered IgG2 hinge region containing for example zero, one, two or three cysteine residues, a mutated or otherwise altered or engineered IgG3 hinge region containing for example zero, one, two, three, or from four to ten cysteine residues, or a mutated or otherwise altered or engineered human IgA1 or IgA2 hinge region polypeptide that contains zero or only one or two cysteine residues (*e.g.*, an "SCC" hinge), a mutated or otherwise altered or engineered IgD hinge region containing no cysteine residues, or a mutated or otherwise altered or engineered human IgE hinge-acting region, *i.e.*, IgE CH2 region polypeptide that contains zero or only one, two, three or four cysteine residues, or (6) any other connecting region molecule described or referenced herein or otherwise known or later discovered as useful for connecting adjoining immunoglobulin domains such as, for example, a CH1 domain and a CH2 domain. For example, a hinge region polypeptide may be selected from the group consisting of (i) a wild-type human IgG1 immunoglobulin hinge region polypeptide, for example, (ii) a mutated or otherwise altered or engineered human IgG1 or other immunoglobulin hinge region polypeptide that is derived or constructed from a wild-type immunoglobulin hinge region polypeptide having three or more cysteine residues, wherein said mutated human IgG1 or other immunoglobulin hinge region polypeptide contains two cysteine residues and wherein a first cysteine of the wild-type hinge region is not mutated, (iii) a mutated or otherwise altered or engineered human IgG1 or other immunoglobulin hinge region polypeptide that is derived from a wild-type immunoglobulin hinge region polypeptide having three or more cysteine residues, wherein said mutated human IgG1 or other immunoglobulin hinge

region polypeptide contains no more than one cysteine residue, and (iv) a mutated or otherwise altered or engineered human IgG1 or other immunoglobulin hinge region polypeptide that is derived from a wild-type immunoglobulin hinge region polypeptide having three or more cysteine residues, wherein said mutated or otherwise altered or engineered human IgG1 or other immunoglobulin hinge region polypeptide contains no cysteine residues In certain embodiments, for example, the immunoglobulin hinge region polypeptide is a mutated or otherwise altered or engineered hinge region polypeptide and exhibits a reduced ability to dimerize, relative to a wild-type human immunoglobulin G or other wild type hinge region or hinge-acting polypeptide.

The immunoglobulin heavy chain constant region polypeptides may be, for example, native or engineered CH2 and CH3 domains of an isotype that is human IgG or human IgA. The immunoglobulin heavy chain constant region polypeptides may also be, for example, native or engineered immunoglobulin heavy chain constant region CH3 and CH4 polypeptides of an isotype that is human IgE.

In certain other embodiments the target or target antigen may be, for example, CD19 (B-lymphocyte antigen CD19, also referred to as B-lymphocyte surface antigen B4, or Leu-12), CD20 (B-lymphocyte antigen 20, also referred to as B-lymphocyte surface antigen B1, Leu-16, or Bp35), CD22 (B-cell receptor CD22, also referred to as Leu-14, B-lymphocyte cell adhesion molecule, or BL-CAM), CD37 (leukocyte antigen CD37), CD40 (B-cell surface antigen CD40, also referred to as Tumor Necrosis Factor receptor superfamily member 5, CD40L receptor, or Bp50), CD80 (T lymphocyte activation antigen CD80, also referred to as Activation B7-1 antigen, B7, B7-1, or BB1), CD86 (T lymphocyte activation antigen CD86, also referred to as Activation B7-2 antigen, B70, FUN-1, or BU63), CD137 (also referred to as Tumor Necrosis Factor receptor superfamily member 9), CD152 (also referred to as cytotoxic T-lymphocyte protein 4 or CTLA-4), CD45 (Leukocyte common antigen, also referred to as L-CA, T200, and EC 3 1.3 48), CD45RA (an isoform of CD45, and an antigen expressed on naive or immature lymphocytes), CD45RB (an isoform of CD45), CD45RO (an isoform of CD45, and a common leukocyte antigen expressed on memory B and T cells), L6 (Tumor-associated antigen L6, also referred to as Transmembrane 4 superfamily member 1, Membrane component surface marker 1, or M3S1), CD2 (T-cell surface antigen CD2, also referred to as T-cell surface antigen T11/Leu-5, LFA-2, LFA-3 receptor, Erythrocyte receptor, or

Rosette receptor), CD28 (T-cell-specific homodimer surface protein CD28, also referred to as Tp44), CD30 (lymphocyte activation antigen CD30, also referred to as Tumor Necrosis Factor receptor superfamily member 8, CD30L receptor, or Ki-1), CD50 (also referred to as Intercellular adhesion molecule-3 (ICAM3), or ICAM-R), CD54 (also referred to as Intercellular adhesion molecule-1 (ICAM1), or Major group rhinovirus receptor), B7-H1 (ligand for an immunoinhibitory receptor expressed by activated T cells, B cells, and myeloid cells, also referred to as PD-L1, see Dong, *et al*, "B7-H1, a third member of the B7 family, co-stimulates T-cell proliferation and interleukin-10 secretion," 1999 *Nat. Med.* 5 1365-1369), CD134 (also referred to as Tumor Necrosis Factor receptor superfamily member 4,OX40, OX40L receptor, ACT35 antigen, or TAX-transcriptionally activated glycoprotein 1 receptor), 41BB (4-1BB ligand receptor, T-cell antigen 4-1BB, or T-cell antigen ILA), CD153 (also referred to as Tumor Necrosis Factor ligand superfamily member 8, CD30 ligand, or CD30-L), CD154 (also referred to as Tumor Necrosis Factor ligand superfamily member 5, CD40 ligand, CD40-L, TNF-related activation protein, TRAP, or T cell antigen Gp39), ICOS (Inducible Costimulator), CD3 (one or more of the delta, epsilon, gamma, eta and/or zeta chains, alone or in combination), CD4 (T-cell surface glycoprotein CD4, also referred to as T-cell surface antigen T4/Leu-3), CD25 (also referred to as Interleukin-2 receptor alpha chain, IL-2 receptor alpha subunit, p55, or Tac antigen), CD8α (T-cell surface glycoprotein CD8 alpha chain, also referred to as T-lymphocyte differentiation antigen, T8/Leu-2, and Lyt-2), CD11b (also referred to as Integrin alpha-M, Cell surface glycoprotein MAC-1 alpha subunit, CR-3 alpha chain, Leukocyte adhesion receptor Mo1, or Neutrophil adherence receptor), CD14 (Monocyte differentiation antigen CD14, also referred to as Myeloid cell-specific leucine-rich glycoprotein or LPS receptor), CD56 (also referred to as Neural cell adhesion molecule 1), or CD69 (also referred to as Early T-cell activation antigen p60, Gp32/28, Leu-23, MLR-3, Activation inducer molecule, or AIM), and TNF factors (for example TNF-α) The above list of construct targets and/or target antigens is exemplary only and is not exhaustive.

In another aspect, the invention includes a binding construct (or a polynucleotide encoding such a construct) that comprises a CD154 extracellular domain, or desired functional portion thereof In one embodiment of this aspect of the invention, for example, the binding construct comprises a CD154 extracellular domain fused or otherwise connected to a second binding domain The second binding domain, for example, may

comprise, consist essentially of, or consist of at least one immunoglobulin variable region polypeptide. The at least one immunoglobulin variable region polypeptide may be a native or engineered scFv The native or engineered scFv may be a native or engineered scFv disclosed or described herein The second binding domain, including a native or engineered scFv, may be one that binds, for example, to any of the targets, including target antigens, disclosed or described herein, including but not limited to, for example, any of B7-H1, ICOS, L6, CD2, CD3, CD8, CD4, CD11b, CD14, CD19, CD20, CD22, CD25, CD28, CD30, CD37, CD40, CD45, CD50, CD54, CD56, CD69, CD80, CD86, CD134, CD137, CD152, CD153, or CD154

In another embodiment the binding domain polypeptide comprises a CTLA-4 extracellular domain, or desired functional portion thereof, and in further embodiments at least one of the immunoglobulin heavy chain constant region polypeptides selected from a CH2 constant region polypeptide and a CH3 constant region polypeptide is a human IgG1 constant region polypeptide, either native or engineered

In another further embodiment at least one of the immunoglobulin heavy chain constant region polypeptides selected from a CH2 constant region polypeptide and a CH3 constant region polypeptide is a human IgA constant region polypeptide, either native or engineered.

In another further embodiment at least one of the immunoglobulin heavy chain constant region polypeptides selected from a CH3 constant region polypeptide and a CH4 constant region polypeptide is a human IgE constant region polypeptide, either native or engineered.

Turning to another embodiment, the present invention provides a binding domain-immunoglobulin fusion protein, comprising, consisting essentially of, or consisting of, (a) a binding domain polypeptide that is fused or otherwise connected to an immunoglobulin hinge region polypeptide, (b) a native or engineered immunoglobulin heavy chain CH2 (or IgE Ch3) constant region polypeptide that is fused or otherwise connected to the hinge region polypeptide, and (c) a native or engineered immunoglobulin heavy chain CH3 (or IgE CH4) constant region polypeptide that is fused or otherwise connected to the CH2 (or IgE CH3) constant region polypeptide, wherein (1) the binding domain polypeptide comprises a CTLA-4 extracellular domain, or a portion thereof, that is capable of binding or specifically binding to at least one CTLA-4 ligand selected from the

group consisting of CD80 and CD86, (2) the immunoglobulin hinge region polypeptide may be as described above or herein, and may comprise, consist essentially of, or consist of, for example, a polypeptide that is selected from the group consisting of a native or engineered human IgA hinge region polypeptide, a native or engineered human IgG1 hinge region polypeptide, and a native or engineered human IgE CH2 region polypeptide (3) a immunoglobulin heavy chain constant region polypeptide that comprises, consists essentially of, or consists of, a polypeptide that is selected from the group consisting of a native or engineered human IgA heavy chain CH2 constant region polypeptide, a native or engineered human IgG1 heavy chain CH2 constant region polypeptide, and a native or engineered human IgE heavy chain CH3 constant region polypeptide (4) a immunoglobulin heavy chain constant region polypeptide that comprises, consists essentially of, or consists of, a polypeptide that is selected from the group consisting of a native or engineered human IgA heavy chain CH3 constant region polypeptide, a native or engineered human IgG1 heavy chain CH3 constant region polypeptide, and a native or engineered human IgE heavy chain CH4 constant region polypeptide, and (5) the binding domain-immunoglobulin fusion protein is capable of inducing at least one immunological activity selected from the group consisting of antibody dependent cell-mediated cytotoxicity, CDC, and complement fixation. In a further embodiment, the binding domain-immunoglobulin fusion protein is capable of inducing two immunological activities selected from the group consisting of antibody dependent cell-mediated cytotoxicity, CDC, and complement fixation

In another embodiment the present invention provides a binding domain-immunoglobulin fusion protein, comprising, consisting essentially of, or consisting of (a) a binding domain polypeptide that is fused or otherwise connected to an immunoglobulin hinge region polypeptide, wherein said hinge region polypeptide may be as described above or herein, and may comprise, consist essentially of, or consist of, for example, a native or engineered human IgE hinge-acting region, *i.e.*, a IgE CH2 region polypeptide, (b) a first native or engineered immunoglobulin heavy chain constant region polypeptide that is fused or otherwise connected to the hinge region polypeptide, wherein said native or engineered constant region polypeptide comprises, consists essentially of, or consists of, a native or engineered human IgE CH3 constant region polypeptide; and (c) a second native or engineered immunoglobulin heavy chain constant region polypeptide that is fused or

otherwise connected to the first native or engineered constant region polypeptide, wherein said native or engineered second constant region polypeptide comprises, consists essentially of, or consists of, a native or engineered human IgE CH4 constant region polypeptide and wherein (1) the binding domain-immunoglobulin fusion protein is capable of inducing at least one immunological activity selected from antibody dependent cell-mediated cytotoxicity and induction of an allergic response mechanism, and (2) the binding domain polypeptide is capable of binding or specifically binding to an antigen. In a further embodiment the antigen is a tumor antigen.

In certain other embodiments the present invention provides a binding domain-immunoglobulin fusion protein, comprising, consisting essentially of, or consisting of, (a) a binding domain polypeptide that is fused or otherwise connected to an immunoglobulin hinge region polypeptide, wherein the binding domain polypeptide is capable of binding or specifically binding to at least one antigen that is present on an immune effector cell and wherein the hinge region polypeptide may be as described above or herein, and may comprise, consist essentially of, or consist of, for example, a polypeptide selected from the group consisting of a native or engineered human IgA hinge region polypeptide, a native or engineered human IgG hinge region polypeptide, and a native or engineered human IgE hinge-acting region, *i.e.*, IgE CH2 region polypeptide; (b) a first native or engineered immunoglobulin heavy chain constant region polypeptide that is fused or otherwise connected to the hinge region polypeptide, wherein said first native or engineered constant region polypeptide comprises, consists essentially of, or consists of, a polypeptide selected from the group consisting of a native or engineered human IgA CH2 constant region polypeptide, a native or engineered human IgG CH2 constant region polypeptide, and a native or engineered human IgE CH3 constant region polypeptide; (c) a second native or engineered immunoglobulin heavy chain constant region polypeptide that is fused or otherwise connected to the first constant region polypeptide, wherein said second constant region polypeptide comprises, consists essentially of, or consists of, a polypeptide selected from the group consisting of a native or engineered human IgA CH3 constant region polypeptide, a native or engineered human IgG CH3 constant region polypeptide, and a native or engineered human IgE CH4 constant region polypeptide, and (d) a native or engineered plasma membrane anchor domain polypeptide. In one example of this embodiment, the plasma membrane anchor domain polypeptide links to a membrane

via a native or engineered glycosyl-phosphatidylinositol-linkage. In a further embodiment the plasma membrane anchor domain polypeptide comprises, consists essentially of, or consists of, a native or engineered transmembrane domain polypeptide. In another further embodiment the membrane anchor domain polypeptide comprises, consists essentially of, or consists of, a native or engineered transmembrane domain polypeptide and a native or engineered cytoplasmic tail polypeptide. In a still further embodiment the cytoplasmic tail polypeptide comprises, consists essentially of, or consists of, a native or engineered apoptosis signaling polypeptide sequence, which in a still further embodiment is derived or constructed from a native or engineered receptor death domain polypeptide, a death domain, or a functional portion of either In a further embodiment the native or engineered death domain polypeptide comprises, consists essentially of, or consists of, for example, a native or engineered polypeptide selected from an ITIM domain (immunoreceptor Tyr-based inhibition motif), an ITAM domain (immunoreceptor Tyr-based activation motif), TRAF, RIP, CRADD, FADD (Fas-associated death domain), TRADD (Tumor Necrosis Factor receptor type 1 associated DEATH domain protein), RAIDD (also referred to as RAID), CD95 (Tumor Necrosis Factor receptor superfamily member 6, also referred to as FASL receptor, Apoptosis-mediating surface antigen FAS, FAS and Apo-1 antigen), TNFR1, and/or DR5 (death receptor-5). In another embodiment the native or engineered apoptosis signaling polypeptide sequence comprises, consists essentially of, or consists of, for example, a polypeptide sequence derived from a native or engineered caspase polypeptide that is caspase-3 or caspase-8 or caspase-10, including caspase 8/FLICE/MACH/Mch5 and caspase 10/Flice2/Mch4. In another embodiment the plasma membrane anchor domain polypeptide comprises, consists essentially of, or consists of, for example, a native or engineered glycosyl-phosphatidylinositol-linkage polypeptide sequence. In another embodiment the antigen that is present on an immune effector cell is, for example, CD2, CD16, CD28, CD30, CD32, CD40, CD50, CD54, CD64, CD80, CD86, B7-H1, CD134, CD137, CD152, CD153, CD154, ICOS, CD19, CD20, CD22, CD37, L6, CD3, CD4, CD25, CD8, CD11b, CD14, CD56, or CD69 In another embodiment the human IgG is a native or engineered human IgG1 These binding domain-immunoglobulin fusion proteins may be capable of inducing, for example, at least one immunological activity selected from antibody dependent cell-mediated cytotoxicity and/or complement fixation and/or CDC, and are capable of binding or specifically binding to a target,

including, for example, a target antigen. In other embodiments, the binding domain-immunoglobulin fusion proteins may be capable of inducing, for example, two immunological activities selected from antibody dependent cell-mediated cytotoxicity and/or complement fixation and/or CDC, and are capable of binding or specifically binding to a target. Immune effector cells include, for example, granulocytes, mast cells, monocytes, macrophages, dendritic cells, neutrophils, eosinophils, basophils, NK cells, T cells (including Th1 cells, Th2 cells, Tc cells, memory T cells, null cells, and large granular lymphocytes, *etc*), and B cells This embodiment of the invention further includes the use of such proteins for therapy, and, for example, the use of such vectors for *in vivo* and *ex vivo* gene therapy The above lists of construct components and targets are not exhaustive and may include any desired target or component that may function as, or be useful for the purposes, described herein.

In another embodiment, the invention provides a protein having a first protein motif that comprises, consists essentially of, or consists of, (1) a native or engineered immunoglobulin hinge region or hinge-acting region (*e.g*, IgE CH2) polypeptide that is fused or otherwise connected to (2) a native or engineered CH2 constant region polypeptide (or native or engineered IgE CH3 constant region polypeptide) Said first protein motif may be fused or otherwise connected to one or more other such first protein motifs to form a second protein motif, the second protein motif being fused or otherwise connected to (3) a native or engineered CH3 constant region (or a native or engineered IgE CH4 constant region) to form a third protein motif. Said first, second or third protein motifs may be fused or otherwise connected to one or more of the herein-described native or engineered plasma membrane anchor domain polypeptides, including, for example, a native or engineered transmembrane domain polypeptide, and a native or engineered transmembrane domain polypeptide and a native or engineered cytoplasmic tail polypeptide, such as for example, a native or engineered apoptosis signaling polypeptide sequence, which may be derived or constructed from a native or engineered receptor death domain polypeptide, a death domain, or a functional portion of either Thus, a protein or polynucleotide within this aspect of the invention may be, for example, a Hinge-CH2-CH3-TransmembraneDomain-DeathDomain construct. It may also be, for example, a (Hinge-CH2)$_X$-CH3- TransmembraneDomain-DeathDomain construct, where X is from 2 to about 5, or such other number as may be needed to achieve a desired length or Fc

receptor binding and/or complement fixation function(s). This embodiment of the invention also includes polynucleotides encoding such proteins, vectors including such polynucleotides, and host cells containing such polynucleotides and vectors This embodiment of the invention further includes the use of such proteins for therapy, and, for example, the use of such polynucleotides and/or vectors for *in vivo* and *ex vivo* gene therapy The invention provides, in another embodiment, a binding domain-immunoglobulin fusion protein, comprising, consisting essentially of, or consisting of, (a) a binding domain polypeptide that is fused or otherwise connected to an immunoglobulin hinge region polypeptide, wherein the binding domain polypeptide is capable of binding or specifically binding to at least one antigen that is present on a cancer cell surface and wherein the hinge region polypeptide may be as described above or herein, and may comprise, consist essentially of, or consist of, for example, a polypeptide selected from the group consisting of a native or engineered human IgA hinge region polypeptide, a native or engineered human IgG hinge region polypeptide, and a native or engineered human IgE hinge-acting region, *i e*, IgE CH2, region polypeptide; (b) a first native or engineered immunoglobulin heavy chain constant region polypeptide that is fused or otherwise connected to the hinge region polypeptide, wherein the first constant region polypeptide comprises, consists essentially of, or consists of, a polypeptide that is a native or engineered human IgA CH2 constant region polypeptide, a native or engineered human IgG CH2 constant region polypeptide, or a native or engineered human IgE CH3 constant region polypeptide; and (c) a second native or engineered immunoglobulin heavy chain constant region polypeptide that is fused or otherwise connected to the first constant region polypeptide, wherein the second constant region polypeptide comprises, consists essentially of, or consists of, a polypeptide that is a native or engineered human IgA CH3 constant region polypeptide, a native or engineered human IgG CH3 constant region polypeptide, or a native or engineered human IgE CH4 constant region polypeptide. In a further embodiment the human IgG polypeptides are native or engineered human IgG1 polypeptides.

In another embodiment the present invention provides a binding domain-immunoglobulin fusion protein, comprising, consisting essentially of, or consisting of, (a) a binding domain polypeptide that is fused or otherwise connected to an immunoglobulin hinge region polypeptide, wherein said hinge region polypeptide may be as described

above or herein, and may comprises, consist essentially of, or consist of, for example, a wild-type or engineered human IgA hinge region polypeptide; (b) a native or engineered immunoglobulin heavy chain CH2 constant region polypeptide that is fused or otherwise connected to the hinge region polypeptide, wherein said native or engineered CH2 constant region polypeptide comprises, consists essentially of, or consists of, a native or engineered human IgA CH2 constant region polypeptide; and (c) a native or engineered immunoglobulin heavy chain CH3 constant region polypeptide that is fused or otherwise connected to the native or engineered CH2 constant region polypeptide, wherein the native or engineered CH3 constant region polypeptide comprises, consists essentially of, or consists of, a polypeptide that is (i) a wild-type human IgA CH3 constant region polypeptide or other IgA region, preferably human or humanized, that is capable of associating with J Chain, (ii) a mutated, altered or otherwise engineered human IgA CH3 constant region polypeptide that is, for example, incapable of associating with a J chain, wherein (1) the binding domain-immunoglobulin fusion protein is capable of at least one immunological activity selected from the group consisting of antibody dependent cell-mediated cytotoxicity, CDC, and complement fixation, and (2) the binding domain polypeptide is capable of binding or specifically binding to a target such as, for example, an antigen. In certain further embodiments the mutated human IgA CH3 constant region polypeptide that is incapable of associating with a J chain is (i) a polypeptide comprising, consisting essentially of, or consisting of, an amino acid sequence as set forth in SEQ ID NO __ or (ii) a polypeptide comprising, consisting essentially of, or consisting of, an amino acid sequence as set forth in SEQ ID NO __ In other embodiments, the IgA hinge region polypeptide is a native or engineered IgA1 hinge region polypeptide or a native or engineered IgA2 hinge region polypeptide In still other embodiments, the IgA hinge region polypeptide is different from a wild-type IgA1 or IgA2 hinge region polypeptide by, for example, the alteration, substitution, or deletion of one or more of the cysteine residues within said wild-type hinge region.

In certain other embodiments the present invention provides a binding domain-immunoglobulin fusion protein, comprising, consisting essentially of, or consisting of (a) a binding domain polypeptide that is fused or otherwise connected to an immunoglobulin hinge region polypeptide; (b) a native or engineered immunoglobulin heavy chain CH2 constant region polypeptide that is fused or otherwise connected to the

hinge region polypeptide, wherein the native or engineered CH2 constant region polypeptide comprises, consists essentially of, or consists of, a native or engineered llama CH2 constant region polypeptide that is a native or engineered llama IgG1 CH2 constant region polypeptide, a native or engineered llama IgG2 CH2 constant region polypeptide, or a native or engineered llama IgG3 CH2 constant region polypeptide, and (c) a native or engineered immunoglobulin heavy chain CH3 constant region polypeptide that is fused or otherwise connected to the native or engineered CH2 constant region polypeptide, wherein said native or engineered CH3 constant region polypeptide comprises, consists essentially of, or consists of, a native or engineered llama CH3 constant region polypeptide that is selected from the group consisting of a native or engineered llama IgG1 CH3 constant region polypeptide, a native or engineered llama IgG2 CH3 constant region polypeptide and a native or engineered llama IgG3 CH3 constant region polypeptide wherein (1) the binding domain-immunoglobulin fusion protein is capable of at least one immunological activity selected from the group consisting of antibody dependent cell-mediated cytotoxicity, fixation of complement and CDC, and (2) the binding domain polypeptide is capable of binding or specifically binding to a target, for example a target antigen. In a further embodiment the immunoglobulin hinge region polypeptide, the native or engineered llama CH2 constant region polypeptide and the native or engineered llama CH3 constant region polypeptide comprise sequences derived from a native or engineered llama IgG1 polypeptide and the fusion protein does not include a native or engineered llama IgG1 CH1 domain. In certain embodiments the invention provides any of the above described binding domain-immunoglobulin fusion proteins wherein the hinge region polypeptide is mutated, engineered, or otherwise altered to contain a glycosylation site, which in certain further embodiments is an asparagine-linked glycosylation site, an O-linked glycosylation site, a C-mannosylation site, a glypiation site or a phosphoglycation site.

In certain embodiments the invention, there are provided any of the above or herein described binding constructs, including binding domain-immunoglobulin fusion proteins, wherein a binding region or binding domain polypeptide comprises two or more binding domain polypeptide sequences wherein each of the binding domain polypeptide sequences is capable of binding or specifically binding to a target(s) such as an antigen(s), which target(s) or antigen(s) may be the same or may be different. A native, for more

preferably an engineered, IgD hinge is a desired connecting region between binding domains of a bispecific molecule of the invention, *i.e.*, one with two or more binding domains, preferably two The wild type human IgD hinge has one cysteine that forms a disulfide bond with the light chain in the native IgD structure. It is desirable to mutate or delete this cysteine in the human IgD hinge for use as a connecting region between binding domains of, for example, a bispecific molecule Other amino acid changes or deletions or alterations in an IgD hinge that do not result in undesired hinge inflexibility are within the scope of the invention. Native or engineered IgD hinge regions from other species are also within the scope of the invention, as are humanized native or engineered IgD hinges from non-human species The present invention also provides, in certain embodiments, a binding domain-immunoglobulin fusion protein, comprising, consisting essentially of, or consisting of (a) a binding domain polypeptide that is fused or otherwise connected to an immunoglobulin hinge region polypeptide, wherein the hinge region polypeptide may be as described above or herein, and may comprise, consist essentially of, or consist of, for example, an alternative hinge region polypeptide sequence, (b) a first native or engineered immunoglobulin heavy chain constant region, such as an IgG or IgA CH2 constant region polypeptide (or an IgE CH3 constant region polypeptide) that is fused or otherwise connected to the hinge region polypeptide, and (c) a second native or engineered immunoglobulin heavy chain constant region, such as an IgG or IgA CH3 constant region polypeptide (or an IgE CH4 constant region polypeptide) that is fused or otherwise connected to the first constant region polypeptide, wherein (1) the binding domain-immunoglobulin fusion protein is capable of at least one immunological activity selected from the group consisting of antibody dependent cell-mediated cytotoxicity, CDC, and complement fixation, and (2) the binding domain polypeptide is capable of binding or specifically binding to a target, such as an antigen

Turning to another embodiment there is provided a binding domain-immunoglobulin fusion protein, comprising, consisting essentially of, or consisting of (a) a binding domain polypeptide that is fused or otherwise connected to an immunoglobulin hinge region polypeptide, wherein the binding domain polypeptide is capable of binding or specifically binding to at least one target, such as an antigen, that is present on a cancer cell surface and wherein the hinge region polypeptide may be as described above or herein, and may comprise, consist essentially of, or consist of, for example, an alternative hinge region

polypeptide sequence; (b) a first native or engineered immunoglobulin heavy chain constant region polypeptide that is fused or otherwise connected to the hinge region polypeptide, wherein said native or engineered constant region polypeptide comprises, consists essentially of, or consists of, a polypeptide selected from the group consisting of a native or engineered human IgA CH2 constant region polypeptide, a native or engineered human IgG CH2 constant region polypeptide, and a native or engineered human IgE CH3 constant region polypeptide; and (c) a second immunoglobulin heavy chain constant region polypeptide that is fused or otherwise connected to the first constant region polypeptide, wherein the second constant region polypeptide comprises, consists essentially of, or consists of, a polypeptide that is a native or engineered human IgA CH3 constant region polypeptide, a native or engineered human IgG CH3 constant region polypeptide, or a native or engineered human IgE CH4 constant region polypeptide. In certain further embodiments the alternative hinge region polypeptide sequence comprises, consists essentially of, or consists of, a polypeptide sequence of at least ten continuous amino acids that are present in a sequence selected from SEQ ID NOS __-__

In certain embodiments the present invention provides polynucleotides or vectors (including cloning vectors and expression vectors) or transformed or transfected cells, including isolated or purified or pure polynucleotides, vectors, and isolated transformed or transfected cells, encoding or containing any one of the above or herein described polypeptide or protein constructs of the invention, for example, including binding domain-immunoglobulin fusion proteins. Thus, in various embodiments the invention provides a recombinant cloning or expression construct comprising any such polynucleotide that is operably linked to a promoter

In other embodiments there is provided a host cell transformed or transfected with, or otherwise containing, any such recombinant cloning or expression construct. Host cells include the cells of a subject undergoing *ex vivo* cell therapy including, for example, *ex vivo* gene therapy

In a related embodiment there is provided a method of producing a polypeptide or protein or other construct of the invention, for example, including a binding domain-immunoglobulin fusion protein, comprising the steps of (a) culturing a host cell as described or provided for herein under conditions that permit expression of the construct, for example, a binding domain-immunoglobulin fusion protein, and (b) isolating the

construct, for example, the binding domain-immunoglobulin fusion protein from the host cell or host cell culture.

In another embodiment there is provided a pharmaceutical composition comprising any one of the above or herein described polypeptide or protein or other constructs of the invention, for example (including, for example, binding domain-immunoglobulin fusion proteins), in combination with a physiologically acceptable carrier

In another embodiment the invention provides a pharmaceutical composition comprising, for example, an isolated, purified, or pure polynucleotide encoding any one of the polypeptide or protein constructs of the invention, for example (including, for example, binding domain-immunoglobulin fusion proteins), in combination with a physiologically acceptable carrier, or for example, in combination with, or in, a gene therapy delivery vehicle or vector

In another embodiment the invention provides a method of treating a subject having or suspected of having a malignant condition or a B cell disorder, comprising administering to a patient a therapeutically effective amount of any of the pharmaceutical compositions described or claimed herein.

In certain further embodiments the malignant condition or B cell disorder is a B cell lymphoma or B cell leukemia, or a disease characterized by autoantibody production, and in certain other further embodiments the B cell disorder is, for example, rheumatoid arthritis, myasthenia gravis, Grave's disease, type I diabetes mellitus, multiple sclerosis or an autoimmune disease. In certain other embodiments the malignant condition is, for example, melanoma, myeloma, glioma, astrocytoma, lymphoma, leukemia, carcinoma, or sarcoma, and so on.

It is another aspect of the present invention to provide a binding domain-immunoglobulin fusion protein, comprising, consisting essentially or, or consisting of, (a) a binding domain polypeptide that is fused or otherwise connected to an immunoglobulin hinge region polypeptide, wherein said hinge region polypeptide is as described herein, and may be selected from the group consisting of (i) a mutated, engineered or otherwise altered hinge region polypeptide that contains no cysteine residues and that is derived from a wild-type immunoglobulin hinge region polypeptide having one or more cysteine residues, (ii) a mutated, engineered or otherwise altered hinge region polypeptide that contains one cysteine residue and that is derived from a wild-type immunoglobulin hinge region

polypeptide having two or more cysteine residues, (iii) a wild-type human IgA hinge region polypeptide, (iv) a mutated, engineered or otherwise altered human IgA hinge region polypeptide that contains no cysteine residues, (v) a mutated, engineered or otherwise altered human IgA hinge region polypeptide that contains one cysteine residue and (vi) a mutated, engineered or otherwise altered human IgA hinge region polypeptide that contains two cysteine residues, (b) a native or engineered immunoglobulin heavy chain CH2 constant region polypeptide that is fused or otherwise connected to the hinge region polypeptide, and (c) a native or engineered immunoglobulin heavy chain CH3 constant region polypeptide that is fused or otherwise connected to the CH2 constant region polypeptide, wherein (1) the binding domain-immunoglobulin fusion protein is capable of at least one immunological activity selected from the group consisting of antibody dependent cell-mediated cytotoxicity and complement fixation, and (2) the binding domain polypeptide is capable of binding or specifically binding to an antigen. In one embodiment the immunoglobulin hinge region polypeptide is a mutated hinge region polypeptide, for example, and the resulting construct exhibits a reduced ability to dimerize, relative to a construct containing a wild-type human immunoglobulin G hinge region polypeptide. In another embodiment the binding domain polypeptide comprises, consists essentially of, or consists of, at least one native or engineered immunoglobulin variable region polypeptide that is a native or engineered immunoglobulin light chain variable region polypeptide and/or a native or engineered immunoglobulin heavy chain variable region polypeptide. In a further embodiment the native or engineered immunoglobulin variable region polypeptide is derived from a human immunoglobulin and, for example, may be humanized.

In another embodiment, the invention provides a binding domain-immunoglobulin fusion protein includes a binding domain polypeptide that comprises, consists essentially of, or consists of, (a) at least one native or engineered immunoglobulin light chain variable region polypeptide, (b) at least one native or engineered immunoglobulin heavy chain variable region polypeptide, and (c) at least one linker peptide that is fused or otherwise connected to the polypeptide of (a) and to the polypeptide of (b) In a further embodiment the native or engineered immunoglobulin light chain variable region and the native or engineered heavy chain variable region polypeptides are derived from human immunoglobulins and may, for example, be humanized

In another embodiment at least one of the native or engineered immunoglobulin heavy chain CH2 (or IgE CH3) constant region polypeptide and the native or engineered immunoglobulin heavy chain CH3 (or IgE CH4) constant region polypeptide is derived or constructed from a human immunoglobulin heavy chain. In another embodiment the native or engineered immunoglobulin heavy chain constant region CH2 and CH3 polypeptides are of, or are derived or otherwise prepared or constructed from, an isotype selected from human IgG and human IgA. In another embodiment the target, for example, the target antigen is selected from the group consisting of CD16, CD19, CD20, CD37, CD40, CD45RO, CD80, CD86, CD137, CD152, and L6. In certain further embodiments of the above described fusion protein construct, the binding domain comprises, consists essentially of, or consists of, an scFv and the scFv contains a linker polypeptide that comprises, consists essentially of, or consists of, at least one polypeptide comprising or having as an amino acid sequence Gly-Gly-Gly-Gly-Ser [SEQ ID NO __], and in certain other embodiments the linker polypeptide comprises, consists essentially of, or consists of, at least three repeats of a polypeptide having as an amino acid sequence Gly-Gly-Gly-Gly-Ser [SEQ ID NO:__] In certain embodiments the immunoglobulin hinge region polypeptide comprises, consists essentially of, or consists of, a native or engineered human IdG, IgA, IgD hinge region polypeptide, or a native or engineered IgE CH2 region polypeptide. In certain embodiments the binding domain polypeptide comprises, consists essentially of, or consists of, a native or engineered CD154 extracellular domain. In certain embodiments the binding domain polypeptide comprises, consists essentially of, or consists of, a native or engineered CD154 extracellular domain and at least one a native or engineered immunoglobulin variable region polypeptide.

In other embodiments the invention provides an isolated polynucleotide encoding any of the constructs of the invention, for example, protein or polypeptide constructs of the invention including binding domain-immunoglobulin fusion proteins, and in related embodiments the invention provides a recombinant expression construct comprising such a polynucleotide, and in certain further embodiments the invention provides a host cell transformed or transfected with, or otherwise containing, such a recombinant expression construct In another embodiment the invention provides a method of producing a construct of the invention, for example, a protein or polypeptide construct of the invention such as a binding domain-immunoglobulin fusion protein, comprising the

steps of (a) culturing a host cell that has been transformed or transfected with, or otherwise made to contain, a polynucleotide construct of the invention under conditions that permit expression of the construct, for example, a construct encoding a binding domain-immunoglobulin fusion protein, and (b) isolating the construct, for example, the binding domain-immunoglobulin fusion protein, from the host cell culture.

The inventions described and claimed herein include novel molecules useful, for example, as therapeutics and other purposes including diagnostic and research purposes. Such molecules have, for example, antigen binding or other binding function(s) and one or more effector functions DNA constructs of the invention are useful in, for example, gene therapies, including *in vivo* and *ex vivo* gene therapies

In one aspect, various constructs of the molecules of the invention include molecules comprising a "binding region", a "tail" region, and a "connecting" region that joins a binding region and a tail region.

Binding regions within the molecules of the invention may comprise, for example, binding domains for desired targets, including antigen-binding targets Binding domains for antigen-binding targets may comprise, for example, single chain Fvs and scFv domains In certain embodiments, molecules of the invention may comprise a binding region having at least one immunoglobulin variable region polypeptide, which may be a light chain or a heavy chain variable region polypeptide In certain embodiments, molecules of the invention may comprise at least one such light chain V-region and one such heavy chain V-region and at least one linker peptide that connects the V-regions. ScFvs useful in the invention also include those with chimeric binding or other domains or sequences. Other ScFvs useful in the invention also include those with humanized binding or other domains or sequences In such embodiments, all or a portion of an immunoglobulin binding or other sequence that is derived from a non-human source may be "humanized" according to recognized procedures for generating humanized antibodies, *i.e.*, immunoglobulin sequences into which human Ig sequences are introduced to reduce the degree to which a human immune system would perceive such proteins as foreign.

Example of scFvs useful in the invention, whether included as murine or other scFvs (including human scFvs), chimeric scFvs, or humanized scFvs, in whole or in part, include anti-human CD20 scFvs (for example, "2H7" scFvs), anti-human CD37 scFvs (for example, "G28-1" scFvs), anti-human CD40 scFvs (for example, "G28-5" scFvs and

"40.2.220" scFvs), anti-carcinoma-associated antigen scFvs (for example, "L6" scFvs), anti-CTLA-4 (CD152) scFvs (for example, "10A8" scFvs), anti-human CD28 scFvs (for example, "2E12" scFvs), anti-murine CD3 scFvs (for example, "500A2" scFvs), anti-human CD3 scFvs (for example, G19-4 scFvs), anti-murine 4-1BB scFvs (for example, "1D8" scFvs), anti-human 4-1BB scFvs (for example, "5B9" scFvs), anti-human CD45RO (for example, "UCHL-1" scFvs), and anti-human CD16 (for example, "Fc2" scFvs)

scFvs useful in the invention also include scFvs, including chimeric and humanized scFvs, having one or more amino acid substitutions. A preferred amino acid substitution is at amino acid position 11 in the variable heavy chain (the V_H). Such a substitution may be referred to herein as "XxxV_H11Zxx" Thus, for example, where the normally occurring amino acid at position V_H11 is a Leucine, and a Serine amino acid residue is substituted therefore, the substitution is identified as "L V_H11S" or "Leu V_H11Ser" Other preferred embodiments of the invention include molecules containing scFvs wherein the amino acid residue normally found at position V_H11 is deleted. Still other preferred embodiments of the invention include molecules containing scFvs wherein the amino acid residues normally found at positions V_H10 and/or V_H11 and/or V_H12 are substituted or deleted

Other binding regions within the molecules of the invention may include domains that comprise sites for glycosylation, for example, covalent attachment of carbohydrate moieties such as monosaccharides or oligosaccharides.

Still other binding regions within molecules of the invention include polypeptides that may comprise proteins or portions thereof that retain the ability to specifically bind another molecule, including an antigen. Thus, binding regions may comprise or be derived from hormones, cytokines, chemokines, and the like, cell surface or soluble receptors for such polypeptide ligands, lectins, intercellular adhesion receptors such as specific leukocyte integrins, selectins, immunoglobulin gene superfamily members, intercellular adhesion molecules (ICAM-1, -2, -3) and the like, histocompatibility antigens, and so on. Binding regions derived from such molecules generally will include those portions of the molecules necessary or desired for binding to a target.

Certain constructs include binding regions that comprise receptor or receptor-binding domains Receptor domains useful for binding to a target include, for example, a CD154 extracellular domain, or a CTLA-4 extracellular domain. In another

example, the binding domain may include a first portion comprising, consisting essentially or, or consisting of, a CD154 extracellular domain and a second portion comprising, consisting essentially or, or consisting of, at least one immunoglobulin variable region polypeptide, said second portion including, for example, an scFv or a V_H. Examples of other cell surface receptors that may comprise, consist essentially or, or consist of, or a portion of which may provide, a binding region or binding domain polypeptide, include, for example, HER1, HER2, HER3, HER4, epidermal growth factor receptor (EGFR), vascular endothelial cell growth factor, vascular endothelial cell growth factor receptor, insulin-like growth factor-I, insulin-like growth factor-II, transferrin receptor, estrogen receptor, progesterone receptor, follicle stimulating hormone receptor (FSH-R), retinoic acid receptor, MUC-1, NY-ESO-1, Melan-A/MART-1, tyrosinase, Gp-100, MAGE, BAGE, GAGE, any of the CTA class of receptors including in particular HOM-MEL-40 antigen encoded by the SSX2 gene, carcinoembryonic antigen (CEA), and PyLT Additional cell surface receptors that may be sources of binding regions or binding domain polypeptides include, for example, CD2, 4-1BB, 4-1BB ligand, CD5, CD10, CD27, CD28, CD152/CTLA-4, CD40, interferon-γ (IFN-γ), interleukin-4 (IL-4), interleukin-17 (IL-17) and interleukin-17 receptor (IL-17R) Still other cell surface receptors that may be sources of binding regions and/or binding domain polypeptides include, for example, CD59, CD48, CD58/LFA-3, CD72, CD70, CD80/B7 1, CD86/B7 2, B7-H1/B7-DC, IL-17, CD43, ICOS, CD3 (*e.g.*, gamma subunit, epsilon subunit, delta subunit), CD4, CD25, CD8, CD11b, CD14, CD56, CD69 and VLA-4 ($\alpha_4\beta_7$) The following cell surface receptors are typically associated with B cells CD19, CD20, CD22, CD30, CD153 (CD30 ligand), CD37, CD50 (ICAM-3), CD106 (VCAM-1), CD54 (ICAM-1), interleukin-12, CD134 (OX40), CD137 (41BB), CD83, and DEC-205 These lists are not exhaustive. Binding regions such as those set forth above may be connected, for example, by a native or engineered IgD hinge region polypeptide, preferably a human or humanized native or engineered IgD hinge region polypeptide. The invention thus further provides constructs that comprise, consist essentially of, or consist of, two binding regions, for example, an scFv and a cell surface receptor (or portion thereof), connected by a third molecule, for example, an IgD hinge region polypeptide as described herein.

Various molecules of the invention described and claimed herein include a connecting region joining one end of the molecule to another end. Such connecting

regions may comprise, for example, immunoglobulin hinge region polypeptides, including any hinge peptide or polypeptide that occurs naturally A connecting region may also include, for example, any artificial peptide or other molecule (including, for example, non-peptide molecules, partial peptide molecules, and peptidomimetics, *etc*) useful for joining the tail region and the binding region. These may include, for example, alterations of molecules situated in an immunoglobulin heavy chain polypeptide between the amino acid residues responsible for forming intrachain immunoglobulin-domain disulfide bonds in CH1 and CH2 regions Naturally occurring hinge regions include those located between the constant region domains, CH1 and CH2, of an immunoglobulin. Useful immunoglobulin hinge region polypeptides include, for example, human immunoglobulin hinge region polypeptides and llama or other camelid immunoglobulin hinge region polypeptides Other useful immunoglobulin hinge region polypeptides include, for example, nurse shark and spotted ratfish immunoglobulin hinge region polypeptides. Human immunoglobulin hinge region polypeptides include, for example, wild type IgG hinges including wild-type human IgG1 hinges, human IgG-derived immunoglobulin hinge region polypeptides, a portion of a human IgG hinge or IgG-derived immunoglobulin hinge region, wild-type human IgA hinge region polypeptides, human IgA-derived immunoglobulin hinge region polypeptides, a portion of a human IgA hinge region polypeptide or IgA-derived immunoglobulin hinge region polypeptide, wild-type human IgD hinge region polypeptides, human Ig-D derived immunoglobulin hinge region polypeptides, a portion of a human IgD hinge region polypeptide or IgD-derived immunoglobulin hinge region polypeptide, wild-type human IgE hinge-acting region, *i.e.*, IgE CH2 region polypeptides (which generally have 5 cysteine residues), human IgE-derived immunoglobulin hinge region polypeptides, a portion of a human IgE hinge-acting region, *i e*, IgE CH2 region polypeptide or IgE-derived immunoglobulin hinge region polypeptide, and so on. A polypeptide "derived from" or that is "a portion or fragment of" an immunoglobulin polypeptide chain region regarded as having hinge function has one or more amino acids in peptide linkage, for example 15-115 amino acids, preferably 95-110, 80-94, 60-80, or 5-65 amino acids, preferably 10-50, more preferably 15-35, still more preferably 18-32, still more preferably 20-30, still more preferably 21, 22, 23, 24, 25, 26, 27, 28 or 29 amino acids. Llama immunoglobulin hinge region polypeptides include, for example, an IgG1 llama hinge. The connecting region may comprise a stretch of

consecutive amino acids from an immunoglobulin hinge region. For example, the connecting region can comprise at least five consecutive hinge region amino acids, at least ten consecutive hinge region amino acids, at least fifteen consecutive hinge region amino acids, at least 20 consecutive hinge region amino acids, and at least twenty five or more consecutive hinge region amino acids from human IgG hinge, human IgA hinge, human IgE hinge, camelid hinge region, IgG1 llama hinge region, nurse shark hinge region, and spotted ratfish hinge region, including for example an IgG_1 hinge region, a IgG_2 hinge region, a IgG_3 hinge region, an IgG_3 hinge region, and an IgG_4 hinge region.

Such connecting regions also include, for example, mutated or otherwise altered or engineered immunoglobulin hinge region polypeptides A mutated or otherwise altered or engineered immunoglobulin hinge region polypeptide may comprise, consist essentially of, or consist of, a hinge region that has its origin in an immunoglobulin of a species, of an immunoglobulin isotype or class, or of an immunoglobulin subclass that is the same or different from that of any included native or engineered CH2 and CH3 domains Mutated or otherwise altered or engineered immunoglobulin hinge region polypeptides include those derived or constructed from, for example, a wild-type immunoglobulin hinge region that contains one or more cysteine residues, for example, a wild-type human IgG or IgA hinge region that naturally comprises three cysteines In such polypeptides the number of cysteine residues may be reduced by amino acid substitution or deletion or truncation, for example. These polypeptides include, for example, mutated human or other IgG1 or IgG4 hinge region polypeptides containing zero, one, or two cysteine residues, and mutated human or other IgA1 or IgA2 hinge region polypeptides that contain zero, one, or two cysteine residues. Mutated or otherwise altered or engineered immunoglobulin hinge region polypeptides include those derived or constructed from, for example, a wild-type immunoglobulin hinge region that contains three or more cysteine residues, for example, a wild-type human IgG2 hinge region (which has 4 cysteines) or IgG4 hinge region (which has 11 cysteines) Mutated or otherwise altered or engineered immunoglobulin hinge region polypeptides include those derived or constructed from, for example, an IgE CH2 wild-type immunoglobulin region that generally contains five cysteine residues. In such polypeptides the number of cysteine residues may be reduced by one or more cysteine residues by amino acid substitution or deletion or truncation, for example. Also included are an altered hinge region polypeptides in which cysteine

residues in the hinge region are substituted with serine or one or more other amino acids that are less polar, less hydrophobic, more hydrophilic, and/or neutral Such mutated immunoglobulin hinge region polypeptides include, for example, mutated hinge region polypeptides that contain one cysteine residue and that are derived from a wild-type immunoglobulin hinge region polypeptide having two or more cysteine residues, such as a mutated human IgG or IgA hinge region polypeptide that contains one cysteine residue and that is derived from a wild-type human IgG or IgA region polypeptide. Connecting region polypeptides include immunoglobulin hinge region polypeptides that are compromised in their ability to form interchain, homodimeric disulfide bonds

Mutated immunoglobulin hinge region polypeptides also include mutated hinge region polypeptides that exhibit a reduced ability to dimerize, relative to a wild-type human immunoglobulin G hinge region polypeptide, and mutated hinge region polypeptides that allow expression of a mixture of monomeric and dimeric molecules. Mutated immunoglobulin hinge region polypeptides also include hinge region polypeptides engineered to contain a glycosylation site. Glycosylation sites include, for example, an asparagine-linked glycosylation site, an O-linked glycosylation site, a C-mannosylation site, a glypiation site, and a phosphoglycation site.

Specific connecting regions useful in molecules of the invention described and claimed herein include, for example, the following 18 amino acid sequences, DQEPKSCDKTHTCPPCPA, DQEPKSSDKTHTSPPSPA, and DLEPKSCDKTHTCPPCPA. Other specific connecting regions include, for example, the mutant hinges within the sequences referred to herein as "2H7 scFv (SSS-S)H WCH2 WCH3" and "2H7 scFv (CSS)H WCH2 WCH3", and the human IgA-derived hinge referred to herein as "2H7 scFv IgAH WCH2 WCH3"

Tail regions within the molecules of the invention may include heavy chain constant region immunoglobulin sequences Tail regions may thus include, for example, a polypeptide having at least one of an immunoglobulin heavy chain CH2 constant region polypeptide and an immunoglobulin heavy chain CH3 constant region polypeptide. At least one of the immunoglobulin heavy chain CH2 constant region polypeptide and the immunoglobulin heavy chain CH3 constant region polypeptide may be derived from a human immunoglobulin heavy chain. Thus, for example, CH2 and/or CH3 polypeptides may be derived from human IgG, human IgA, or human IgD molecules. Tail regions may

also include, for example, a polypeptide having at least one of an immunoglobulin heavy chain CH3 constant region polypeptide and an immunoglobulin heavy chain CH4 constant region polypeptide. At least one of the immunoglobulin heavy chain CH3 constant region polypeptide and the immunoglobulin heavy chain CH4 constant region polypeptide may be derived from a human immunoglobulin heavy chain. Thus, for example, CH3 and/or CH4 polypeptides may be derived from human IgE. An immunoglobulin heavy chain CH2 region polypeptide included within a molecule of the invention may, for example, be from the IgG1, IgG2, IgG3 and/or IgG4 subclasses. An immunoglobulin heavy chain CH3 region polypeptide included within a molecule of the invention may also, for example, be from the IgG1, IgG2, IgG3 and/or IgG4 subclasses Additionally, both the immunoglobulin heavy chain CH2 region polypeptide and the immunoglobulin heavy chain CH2 region polypeptide included within a molecule of the invention may, for example, be from the IgG1, IgG2, IgG3 and/or IgG4 subclasses. In other molecules of the invention at least one of the immunoglobulin heavy chain constant region polypeptides selected from a CH2 constant region polypeptide and a CH3 constant region polypeptide is a human IgA constant region polypeptide. An immunoglobulin heavy chain CH2 region polypeptide included within a molecule of the invention may, for example, be from the IgA1 and/or IgA2 subclasses An immunoglobulin heavy chain CH3 region polypeptide included within a molecule of the invention may also, for example, be from the IgA1 and/or IgA2 subclasses Additionally, both the immunoglobulin heavy chain CH2 region polypeptide and the immunoglobulin heavy chain CH2 region polypeptide included within a molecule of the invention may, for example, be from the IgA1 and/or IgA2 subclasses In still other molecules of the invention, the tail region may comprise or consist essentially of a CH2 and/or CH3 constant region polypeptide comprising a polypeptide from human IgA and/or human IgE. In other embodiments, for example, the tail region within a molecule of the invention may include an immunoglobulin heavy chain CH2 and/or CH3 constant region polypeptide that is a mutated (for example, a mutated IgA CH3 constant region polypeptide that is incapable of associating with a J chain in which, for example, the IgA CH3 constant region polypeptide is of human origin) The tail region may also comprise, consist essentially of, or consist of an extracellular portion of a protein from the TNF superfamily, for example, CD154

For molecules of the invention intended for use in humans, these regions will typically be substantially or completely human to minimize potential human immune responses against the molecules and to provide appropriate effector functions. In certain embodiments of the invention, for example, the tail region includes a human IgG1 CH3 region sequence, a wild-type IgA heavy chain constant region polypeptide sequence that is capable or incapable of associating with J chain.

In preferred embodiments of the invention, a CH1 domain is not included in the tail region of the molecule, and the carboxyl end of the binding region is joined to the amino terminus of a CH2 portion of a tail region either directly or indirectly A binding region may be indirectly joined to a tail region, for example via a connecting region polypeptide or other connecting molecule

The invention also includes molecules that have mutated CH2 and/or CH3 sequences within a tail region. For example, a molecule of the invention may include a mutated Fc domain that has one or more mutations introduced into the CH2, CH3 and/or CH4 domains In certain embodiments of the invention, molecules may include an IgA CH3 constant region polypeptide such as a human IgA CH3 constant region polypeptide in which two or more residues from the C-terminus have been deleted to yield a truncated CH3 constant region polypeptide. In other embodiments of the invention, molecules include a mutated human IgA CH3 constant region polypeptide that is incapable of associating with a J chain that comprises a C-terminal deletion of either four or 18 amino acids However, the invention need not be so limited, such that molecules containing the mutated IgA CH3 constant region polypeptide may comprise a deletion of 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21-25, 26-30 or more amino acids, so long as the fusion protein is capable of specifically binding an antigen and capable of at least one immunological activity such as ADCC, CDC or complement fixation. The invention also includes molecules containing a tail region that comprises a mutated IgA CH3 constant region polypeptide that is incapable of associating with a J chain by virtue of replacement of the penultimate cysteine, or by chemical modification of that amino acid residue, in a manner that prevents interchain disulfide bond formation.

Various molecules of the invention include, for example, a binding domain scFv- fusion protein having a binding domain polypeptide comprising, consisting essentially of, or consisting of, (a) at least one immunoglobulin light chain variable region

polypeptide, (b) at least one immunoglobulin heavy chain variable region polypeptide, and at least one linker peptide that joins the polypeptide of (a) and the polypeptide of (b) Such polypeptides may, for example, be derived from human immunoglobulins or non-human immunoglobulins.

Thus, in one aspect, the invention includes a non-naturally occurring single chain protein and/or V_H protein and/or V_L protein, or a desired portion of any of the above, including a first polypeptide comprising a binding domain polypeptide capable of binding to a target molecule, a second polypeptide comprising a flexible or other desired linker attached to said first polypeptide, a third polypeptide comprising a tail region, for example, an N-terminally truncated immunoglobulin heavy chain constant region polypeptide (or desired portion thereof) attached to the second polypeptide. The flexible linker may comprise, consist essentially of, or consist of, an immunoglobulin hinge region or portion thereof that has been mutated or otherwise altered or engineered, for example, one that contains a number of cysteine residues that is less than the number of cysteine residues present in the wild type immunoglobulin hinge region or portion (for example, zero, one, or two cysteines in the case of IgG1 or IgG4), and wherein said non-naturally occurring single-chain protein is capable of at least one immunological activity, for example, ADCC, CDC, and/or complement fixation. The single chain protein may be capable of two immunological activities including, for example, ADCC, CDC, and/or complement fixation. This protein may include a binding domain polypeptide that is a single chain Fv Additionally, this protein may include a binding domain polypeptide that is a single chain Fv wherein the heavy chain variable region of the single chain Fv has an amino acid deletion or substitution at one or more of amino acid positions 9, 10, 11, 12, 108, 110, and 112 The protein may also include a binding domain polypeptide that is a single chain Fv wherein the light chain variable region of the single chain Fv has an amino acid deletion or substitution at one or more of amino acid positions 12, 80, 81, 83, 105, 106, and 107

In another aspect, the invention includes a non-naturally occurring V_H protein, or a desired portion thereof, that comprises, consists essentially of, or consists of, alone or in combination with any other molecule or construct, a V_H region or portion thereof that has an amino acid deletion or substitution at one or more of amino acid positions 9, 10, 11, 12, 108, 110, and 112 of said V_H region. Amino acids may be

substituted with either naturally occurring or non-naturally occurring amino acids, or any other desired useful molecule.

Also described and claimed are uses of V_H proteins, or desired portions thereof, that comprise, consist essentially of, or consist of, alone or in combination with any other molecule or construct, a V_H region or portion thereof that has an amino acid deletion or substitution at one or more of amino acid positions 9, 10, 11, 12, 108, 110, and 112 of said V_H region. Such uses include uses in phage display, yeast display, and ribosome display systems and methods.

In yet another aspect, the invention includes a non-naturally occuring V_L protein, or a desired portion thereof, that comprises, consists essentially of, or consists of, alone or in combination with any other molecule, a V_L region or portion thereof that has an amino acid deletion or substitution at one or more of amino acid positions 12, 80, 81, 83, 105, 106, and 107 of said V_L region. Amino acids may be substituted with either naturally occurring or non-naturally occurring amino acids, or any other desired useful molecule.

Also described and claimed are uses of V_L proteins, or desired portions thereof, that comprises, consists essentially of, or consists of, alone or in combination with any other molecule, a V_L region or portion thereof that has an amino acid deletion or substitution at one or more of amino acid positions 12, 80, 81, 83, 105, 106, and 107 of said V_L region. Such uses include uses in phage display, yeast display, and ribosome display systems and methods.

In yet another aspect, the invention includes a molecule comprising, consisting essentially of, or consisting of, (1) a V_H protein, or a desired portion thereof, wherein the V_H protein or portion thereof has an amino acid deletion or substitution at one or more of amino acid positions 9, 10, 11, 12, 108, 110, and 112, and (2) a non-naturally occurring V_L protein, or a desired portion thereof, alone or in combination with any other molecule, wherein the V_L protein or portion thereof has an amino acid deletion or substitution at one or more of amino acid positions 12, 80, 81, 83, 105, 106, and 107 Amino acids may be substituted with either naturally occurring or non-naturally occurring amino acids, or any other desired useful molecule.

Also described and claimed are uses of a molecule comprising, consisting essentially of, or consisting of, (1) a V_H protein, or a desired portion thereof, wherein the V_H protein or portion thereof has an amino acid deletion or substitution at one or more of

amino acid positions 9, 10, 11, 12, 108, 110, and 112, and (2) a non-naturally occurring V_L protein, or a desired portion thereof, alone or in combination with any other molecule, wherein the V_L protein or portion thereof has an amino acid deletion or substitution at one or more of amino acid positions 12, 80, 81, 83, 105, 106, and 107 Such uses include uses in phage display, yeast display, and ribosome display systems and methods

The invention also includes molecular constructs wherein the binding domain is a single chain Fv and the heavy chain variable region of said single chain Fv has an amino acid substitution at amino acid position 11 The amino acid substituted for the amino acid at position of 11 of the single chain Fv heavy chain variable region may be selected from the group consisting of serine, threonine, tyrosine, asparagine, glutamine, aspartic acid, glutamic acid, lysine, arginine, and histidine. The invention thus includes, for example, a construct wherein the binding domain is a single chain Fv and the heavy chain variable region of said single chain Fv has a serine amino acid substitution at amino acid position 11 Other amino acid position changes, substitutions, and deletions, are noted herein.

The invention also includes, for example, a construct wherein the binding domain is a single chain Fv and the amino acid at position 10 and/or 11 of the heavy chain variable region of said single chain Fv has been deleted.

In another aspect, the invention includes constructs wherein the binding region binds to a tumor or tumor-associated antigen. The binding region of a construct of the invention may bind, for example, to a cancer cell antigen. Cancer cell antigens to which constructs of the invention bind include cancer cell surface antigens and intracellular cancer cell antigens

In yet another aspect, the invention includes a construct wherein the binding region binds to an antigen on an immune effector cell.

In another aspect, the invention includes a construct wherein the binding region binds to a B cell antigen including, for example, a B cell antigen selected from the group consisting of CD19, CD20, CD22, CD37, CD40, CD80, and CD86 Constructs of the invention that bind to such B cell antigens include, for example, binding regions comprising an single chain Fv Examples of such single chain Fv binding regions include molecules comprising or consisting essentially of single chain Fvs selected from the group consisting of HD37 single chain Fv, 2H7 single chain Fv, G28-1 single chain Fv, and

4.4.220 single chain Fv Other examples include a binding region comprising, consisting essentially of, or consisting of, an extracellular domain of CTLA-4.

In another aspect, the invention includes a construct wherein the binding region binds to a B cell differentiation antigen. B cell differentiation antigens include, for example, CD19, CD20, CD21, CD22, CD23, CD37, CD40, CD45RO, CD80, CD86, and HLA class II

In another aspect, the invention includes a construct wherein the binding region binds to a target selected from the group consisting of CD2, CD3, CD4, CD5, CD6, CD8, CD10, CD11b, CD14, CD19, CD20, CD21, CD22, CD23, CD24, CD25, CD28, CD30, CD37, CD40, CD43, CD50 (ICAM3), CD54 (ICAM1), CD56, CD69, CD80, CD86, CD134 (OX40), CD137 (41BB), CD152 (CTLA-4), CD153 (CD30 ligand), CD154 (CD40 ligand), ICOS, L6, B7-H1, and HLA class II.

The invention also includes protein constructs having a binding region, a tail region, and a connecting region, wherein the protein construct is capable of existing in solution as a monomer or in substantially monomeric form.

The invention also includes protein constructs having a binding region, a tail region, and a connecting region, wherein the protein construct is capable of forming a complex comprising two or more of said protein constructs including, for example, wherein said complex is a dimer

In another aspect, constructs of the invention are capable of participating in or inducing or eliciting or helping to induce or elicit, directly or indirectly, at least one immunological activity selected from the group consisting of antibody dependent cell-mediated cytotoxicity, complement-dependent cytotoxicity (or complement-mediated lysis), complement fixation, induction of apoptosis, induction of one or more biologically active signals, induction of one or more immune effector cells, activation of cellular differentiation, cellular activation, release of one or more biologically active molecules, and neutralization of an infectious agent or toxin.

In another aspect, binding constructs of the invention are capable of induction of biologically active signals by activation or inhibition of one or more molecules selected from the group consisting of protein kinases, protein phosphatases, G-proteins, cyclic nucleotides or other second messengers, ion channels, and secretory pathway components Such biologically active molecules are, for example, proteases.

Other biologically active molecules are, for example, cytokines, including by way of example monokines, lymphokines, chemokines, growth factors, colony stimulating factors, interferons, and interleukins

In another aspect, constructs of the invention are capable of induction, or participation in the induction, of one or more immune effector cells selected from the group consisting of NK cells, monocytes, macrophages, B cells, T cells, mast cells, neutrophils, eosinophils, and basophils

In another aspect, constructs of the invention are capable of induction, or participation in the induction, of one or more immune effector cells that results in antibody dependent cell-mediated cytotoxicity or the release of one or more biologically active molecules.

In another aspect, constructs of the invention are capable of participating in and/or initiating apopotosis within target cells, for example, by activating one or more signalling mechanisms or molecules.

In another aspect, constructs of the invention are capable of induction, or participation in the induction, of cellular activation, wherein said activation leads to changes in cellular transcriptional activity In one embodiment, cellular transcriptional activity is increased. In another embodiment, cellular transcriptional activity is decreased.

In another aspect, constructs of the invention having tail regions comprising, consisting essentially of, or consisting of, constant regions from IgA or IgE molecules, are capable of induction, or participation in the induction, of degranulation of neutrophils and/or mast cells

In another aspect, constructs of the invention are capable of promotion, or participation in the promotion, of neutralization of an infectious agent, wherein said infectious agent is, for example, a bacterium, a virus, a parasite, or a fungus

In another aspect, constructs of the invention are capable of promoting, or participating in the promotion of, neutralization of a toxin, wherein said toxin is selected from the group consisting of endotoxins and exotoxins Such toxins include, for example, exotoxins selected from the group consisting of anthrax toxin, cholera toxin, diphtheria toxin, pertussis toxin, *E. coli* heat-labile toxin LT, *E coli* heat stable toxin ST, shiga toxin *Pseudomonas* Exotoxin A, botulinum toxin, tetanus toxin, *Bordetella pertussis* AC toxin, and *Bacillus anthracis* EF toxin. Other toxins include, for example, saxitoxins,

tetrodotoxin, mushroom toxins (amatoxins, gyromitrin, orellanine, *etc*), aflatoxins, pyrrolizidine alkaloids, phytohemagglutinins, and grayanotoxins

In another aspect, constructs of the invention are capable of binding to an intracellular target to, for example, effect (or participate in effecting) a cellular function. Such constructs include, for example, constructs that include a tail region comprising, consisting essentially of, or consisting of, a native or engineered IgA CH2 domain region and a native or engineered IgA CH3 domain region, said tail region being capable of binding J chain. Such a tail region is found, for example, in the 2H7 scFv IgAH WIgACH2 WCH3 + JChain construct. Thus, the invention includes constructs having, for example, an "Anti-Intracellular Target" binding domain (for example, and "Anti-Intracellular Target" scFv), a connecting region, and a native or engineered IgA constant region capable of binding J chain (for example, WIgACH2 WCH3)

In still another aspect, constructs of the invention include a molecule wherein an N-terminally immunoglobulin heavy chain constant region polypeptide comprises an IgG CH2 constant region polypeptide attached to an immunoglobulin heavy chain IgG CH3 constant region polypeptide.

In yet another aspect, the invention includes a method of reducing a target cell population in a subject comprising administering to said subject a therapeutically effective amount of a protein molecule that is less than about 120kK, or less than about 150kD, as measured, for example, by HPLC and non-reducing gels and consists essentially of (a) a first protein or peptide molecule that is capable of binding to cells within said target cell population, and (b) a second protein or peptide molecule that is capable of (i) binding to an Fc receptor and/or (ii) inducing target cell apoptosis, and/or (iii) fixing complement, wherein said first protein or peptide molecule is directly connected to said second protein or peptide molecule, or, optionally, said first protein or peptide molecule and said second protein or peptide molecule are linked by a third protein or peptide molecule, wherein said protein molecule is not an antibody, a member of the TNF family or the TNF receptor family, and is not conjugated with a bacterial toxin, a cytotoxic drug, or a radioisotope

In another aspect, the invention also includes single chain proteins comprising, consisting essentially of, or consisting of, (i) a first polypeptide having a binding domain polypeptide capable of binding to a target molecule, (ii) a second

polypeptide comprising a connecting region attached to the C-terminus of said first polypeptide; and (iii) a third polypeptide comprising an N-terminally truncated immunoglobulin heavy chain constant region polypeptide attached to the C-terminus of said second polypeptide, wherein said single-chain protein is capable of at least one immunological activity, and provided that (a) when the connecting region polypeptide comprises an IgG hinge region polypeptide having no cysteine residues, the binding domain polypeptide target is not CD20 or L6, or (b) when the connecting region polypeptide comprises an IgG hinge region polypeptide having no cysteine residues, the single chain protein is not a 1F5 scFv capable of binding to CD20 The invention also includes single chain proteins comprising, consisting essentially of, or consisting of, (i) a first polypeptide having a binding domain polypeptide capable of binding to a target molecule; (ii) a second polypeptide comprising a connecting region attached to the C-terminus of said first polypeptide; and (iii) a third polypeptide comprising an N-terminally truncated immunoglobulin heavy chain constant region polypeptide attached to the C-terminus of said second polypeptide, wherein said single-chain protein is capable of binding to a target molecule on or in a target cell and decreasing the number of target cells *in vivo* and/or depleting a population of target cells *in vivo* The invention also includes single chain proteins comprising, consisting essentially of, or consisting of, (i) a first polypeptide having a binding domain polypeptide capable of binding to a target molecule; (ii) a second polypeptide comprising a connecting region attached to the C-terminus of said first polypeptide; and (iii) a third polypeptide comprising an N-terminally truncated immunoglobulin heavy chain constant region polypeptide attached to the C-terminus of said second polypeptide, wherein said single-chain protein is capable of inducing antibody-dependent cell-mediated cytotoxicity and complement fixation The invention also includes single chain proteins comprising, consisting essentially of, or consisting of, (i) a first polypeptide having a binding domain polypeptide capable of binding to a target molecule; (ii) a second polypeptide comprising a connecting region attached to the C-terminus of said first polypeptide, and (iii) a third polypeptide comprising an N-terminally truncated immunoglobulin heavy chain constant region polypeptide attached to the C-terminus of said second polypeptide, wherein said single-chain protein is capable of (1) inducing antibody-dependent cell-mediated cytotoxicity and complement fixation, and (2) binding to a target molecule on or in a target cell and decreasing the number of target cells

in vivo and/or depleting a population of target cells *in vivo* The invention also includes single chain proteins comprising, consisting essentially of, or consisting of, (i) a first polypeptide having a binding domain polypeptide capable of binding to a target molecule, (ii) a second polypeptide comprising a connecting region attached to the C-terminus of said first polypeptide, and (iii) a third polypeptide comprising an N-terminally truncated immunoglobulin heavy chain constant region polypeptide attached to the C-terminus of said second polypeptide, wherein when said connecting region comprises an IgG hinge region polypeptide having at least first, second, and third cysteine residues, said first cysteine being N-terminal to said second cysteine and said second cysteine being N-terminal to said third cysteine, one or both of said second and third cysteine residues is substituted or deleted, and wherein said single-chain protein is capable of at least one immunological activity The invention also includes single chain proteins comprising, consisting essentially of, or consisting of, (i) a first polypeptide having a binding domain polypeptide capable of binding to a target molecule; (ii) a second polypeptide comprising a connecting region attached to the C-terminus of said first polypeptide, and (iii) a third polypeptide comprising an N-terminally truncated immunoglobulin heavy chain constant region polypeptide attached to the C-terminus of said second polypeptide, wherein when said connecting region comprises an IgG hinge region polypeptide having at least first, second, and third cysteine residues, said first cysteine being N-terminal to said second cysteine and said second cysteine being N-terminal to said third cysteine, one or both of said second and third cysteine residues is substituted or deleted, and wherein said single-chain protein is capable of inducing at least one immunological activity selected from (a) antibody-dependent cell-mediated cytotoxicity and (b) complement fixation The invention also includes single chain proteins comprising, consisting essentially of, or consisting of, (i) a first polypeptide having a binding domain polypeptide capable of binding to a target molecule; (ii) a second polypeptide comprising a connecting region attached to the C-terminus of said first polypeptide, and (iii) a third polypeptide comprising an N-terminally truncated immunoglobulin heavy chain constant region polypeptide attached to the C-terminus of said second polypeptide, wherein when said connecting region comprises an IgG hinge region polypeptide having at least first, second, and third cysteine residues, said first cysteine being N-terminal to said second cysteine and said second cysteine being N-terminal to said third cysteine, one or both of said second and third cysteine residues is

substituted or deleted, and wherein said single-chain protein is capable of antibody-dependent cell-mediated cytotoxicity and complement fixation. The invention also includes single chain proteins comprising, consisting essentially of, or consisting of, (i) a first polypeptide having a binding domain polypeptide capable of binding to a target molecule, (ii) a second polypeptide comprising a connecting region attached to the C-terminus of said first polypeptide, and (iii) a third polypeptide comprising an N-terminally truncated immunoglobulin heavy chain constant region polypeptide attached to the C-terminus of said second polypeptide, wherein when said connecting region comprises an IgG hinge region polypeptide having at least first, second, and third cysteine residues, said first cysteine being N-terminal to said second cysteine and said second cysteine being N-terminal to said third cysteine, one or both of said second and third cysteine residues is substituted or deleted, and wherein said single-chain protein is capable of binding to a target molecule on or in a target cell and decreasing the number of target cells *in vivo* and/or depleting a population of target cells *in vivo* The invention also includes single chain proteins comprising, consisting essentially of, or consisting of, (i) a first polypeptide having a binding domain polypeptide capable of binding to a target molecule, (ii) a second polypeptide comprising a connecting region attached to the C-terminus of said first polypeptide; and (iii) a third polypeptide comprising an N-terminally truncated immunoglobulin heavy chain constant region polypeptide attached to the C-terminus of said second polypeptide, wherein when said connecting region comprises an IgG hinge region polypeptide having at least first, second, and third cysteine residues, said first cysteine being N-terminal to said second cysteine and said second cysteine being N-terminal to said third cysteine, one or both of said second and third cysteine residues is substituted or deleted, and wherein said single-chain protein is capable of inducing at least one immunological activity selected from antibody-dependent cell-mediated cytotoxicity and complement fixation, and wherein said single-chain protein is capable of binding to a target molecule on or in a target cell and decreasing the number of target cells *in vivo* and/or depleting a population of target cells *in vivo* The invention also includes single chain proteins comprising, consisting essentially of, or consisting of, (i) a first polypeptide having a binding domain polypeptide capable of binding to a target molecule, (ii) a second polypeptide comprising a connecting region attached to the C-terminus of said first polypeptide; and (iii) a third polypeptide comprising an N-terminally truncated

immunoglobulin heavy chain constant region polypeptide attached to the C-terminus of said second polypeptide, wherein when said connecting region comprises an IgG hinge region polypeptide having at least first, second, and third cysteine residues, said first cysteine being N-terminal to said second cysteine and said second cysteine being N-terminal to said third cysteine, one or both of said second and third cysteine residues is substituted or deleted, and wherein said single-chain protein is capable of inducing antibody-dependent cell-mediated cytotoxicity and complement fixation, and wherein said single-chain protein is capable of binding to a target molecule on or in a target cell and decreasing the number of target cells *in vivo* and/or depleting a population of target cells *in vivo* The invention also includes single chain proteins comprising, consisting essentially of, or consisting of, (i) a first polypeptide having a binding domain polypeptide capable of binding to a target molecule, said binding domain polypeptide comprising a heavy chain variable region wherein leucine at position 11 in the first framework region of the heavy chain variable region is deleted or substituted with another amino acid, (ii) a second polypeptide comprising a connecting region attached to the C-terminus of said first polypeptide; and (iii) a third polypeptide comprising an N-terminally truncated immunoglobulin heavy chain constant region polypeptide attached to the C-terminus of said second polypeptide, wherein said single-chain protein is capable of at least one immunological activity, and provided that when the binding domain polypeptide is capable of binding to CD20 said connecting region comprises three cysteine residues wherein one or two of said three cysteine residues is substituted or replaced with another amino acid. The invention also includes single chain proteins comprising, consisting essentially of, or consisting of, (i) a first polypeptide having a binding domain polypeptide capable of binding to a target molecule, said binding domain polypeptide comprising a heavy chain variable region wherein leucine at position 11 in the first framework region of the heavy chain variable region is deleted or substituted with another amino acid, (ii) a second polypeptide comprising a connecting region attached to the C-terminus of said first polypeptide; and (iii) a third polypeptide comprising an N-terminally truncated immunoglobulin heavy chain constant region polypeptide attached to the C-terminus of said second polypeptide, wherein said single-chain protein is capable of inducing at least one immunological activity, provided that when binding domain polypeptide is a 2H7 scFv capable of binding to CD20 and said connecting region comprises an IgG hinge region

polypeptide having at least first, second, and third cysteine residues, said first cysteine being N-terminal to said second cysteine and said second cysteine being N-terminal to said third cysteine, one or two of said cysteine residues is substituted or deleted. The invention also includes single chain proteins comprising, consisting essentially of, or consisting of, (i) a first polypeptide having a binding domain polypeptide capable of binding to a target molecule on or in a target cell, said binding domain polypeptide comprising a heavy chain variable region wherein leucine at position 11 in the first framework region of the heavy chain variable region is deleted or substituted with another amino acid, (ii) a second polypeptide comprising a connecting region attached to said first polypeptide, and (iii) a third polypeptide comprising an N-terminally truncated immunoglobulin heavy chain constant region polypeptide attached to the second polypeptide, wherein said single-chain protein is capable of at least one immunological activity, provided that said single chain protein does not comprise, or consist essentially of, a binding domain polypeptide capable of binding to CD20 and a connecting region that comprises (a) an IgG hinge having three cysteine residues or (b) an IgG hinge comprising three serine (or like amino acid) residues that have been substituted for cysteine residues. There are many possible variations and variants of these single chain proteins. For example, the binding domain polypeptide may be a single chain antibody or scFv including naturally occurring and/or non-naturally occurring V_H and V_L polypeptides, and the binding domain polypeptide may bind any of a number of targets. Non-naturally occurring V_H polypeptides include, by way of example and not limitation, human heavy chain variable region polypeptide comprising a mutation, substitution, or deletion of an amino acid(s) at a location corresponding to any one or more of amino acid positions 9, 10, 11, 12, 108, 110, and/or 112. Non-naturally occurring V_L polypeptides include, by way of example and not limitation, human light chain variable region polypeptides comprising a mutation, substitution, or deletion of an amino acid(s) at a location corresponding to any one or more of amino acid positions 12, 80, 81, 83, 105, 106, and 107 Targets include, by way of example and not limitation, CD19, CD20, CD28, CD30, CD37, CD40, L6, HER2, epidermal growth factor receptors (EGFRs), vascular endothelial cell growth factors (VEGFs), tumor necrosis factors (*e.g.*, TNF-alpha), as well as other targets described or referred to herein or otherwise useful, whether now known or later discovered. Additionally, the connecting region polypeptide may be any of a number of molecules, both naturally occurring and non-naturally occurring. Connecting region

polypeptides include, by way of example and not limitation, naturally occurring and non-naturally occurring immunoglobulin hinge region polypeptides. Naturally occurring immunoglobulin hinge region polypeptides include wild-type immunoglobulin hinge region polypeptides such as, by way of example and not limitation, human IgG1 hinge region polypeptides, human IgA hinge region polypeptides, human IgD hinge region polypeptides, human IgE hinge-acting regions (*e.g.*, IgE CH2), camelid immunoglobulin hinge regions, or any other naturally occurring hinge region peptides described or referred to herein or otherwise useful useful, whether now known or later discovered. Non-naturally occurring immunoglobulin hinge region polypeptides include, by way of example and not limitation, mutated naturally occurring immunoglobulin hinges, including immunoglobulin hinge region polypeptides that contain less than the wild-type number of cysteines, for example, mutated naturally occurring immunoglobulin hinge region polypeptides that contain zero, one, or two cysteines, and any other connecting region molecule described or referenced herein or otherwise useful or now known or later discovered as useful for connecting joining, for example, immunoglobulin domains such as a CH1 domain and a CH2 domain. N-terminally truncated immunoglobulin heavy chain constant region polypeptides include naturally occurring and non-naturally occurring N-terminally truncated immunoglobulin heavy chain constant region polypeptides that, with or without other portions of the single chain protein, provide one or more effector functions such as those described herein. Naturally occurring N-terminally truncated immunoglobulin heavy chain constant region polypeptides include, by way of example and not limitation, CH2CH3 constant region polypeptides, including CH2CH3 constant region polypeptides taken, separately or together, from human IgGs, human IgAs, and human IgE, and any other immunoglobulin heavy chain constant region polypeptide described or referenced herein or otherwise now known or later discovered to be useful Non-naturally occurring N-terminally truncated immunoglobulin heavy chain constant region polypeptides include, by way of example and not limitation, any mutated naturally occurring heavy chain constant region polypeptide described or referred to herein or otherwise now known or later discovered to be useful

Various specific constructs of the invention include, by way of example only, the following:

1 2H7 scFv VH L11S (CSC-S) H WCH2 WCH3

2 2H7 scFv VH L11S IgE CH2 CH3 CH4

3 2H7 scFv VH L11S mIgB CH2 CH3 CH4

4 2H7 scFv VH L11S mIgAH WIgACH2 T4CH3

5 2H7 scFv VH L11S (SSS-S) H K322S CH2 WCH3

6 2H7 scFv VH L11S (CSS-S) H K322S CH2 WCH3

7 2H7 scFv VH L11S (SSS-S) H P331S CH2 WCH3

8 2HU scFv VH L11S (CSS-S) H P331S CH2 WCH3

9 2H7 scFv VH L11S (SSS-S) H T256N CH2 WCH3

10 2H7 scFv VH L11S (SSS-S) H RTPE/QNAK (255-258) CH2 WCH3

11 2H7 scFv VH L11S (SSS-S) H K290Q CH2 WCH3

12 2H7 scFv VH L11S (SSS-S) H A339P CH2 WCH3

13 G28-1 scFv (SSS-S) H WCH2 WCH3

14 G28-1 scFv IgAH WCH2 WCH3

15 G28-1 scFv VH L11S (SSS-S) H WCH2 WCH3

16 G28-1 scFv VH L11S (CSS-S) H WCH2 WCH3

17 G28-1 scFv VH L11S (CSC-S) H WCH2 WCH3

18 G28-1 scFv VH L11S (SSC-P) H WCH2 WCH3

19 CTLA4 (SSS-S) H P238SCH2 WCH32

20 CTLA4 (CCC-P) WH WCH2 WCH3

21 FC2-2 scFv (SSS-S) H WCH2 WCH3

22 FC2-2 scFv VHL11S (SSS-S) H WCH2 WCH3

23 UCHL-1 scFv (SSS-S) H WCH2 WCH3

24 UCHL-1 scFv VHL11S (SSS-S) H WCH2 WCH3

25 5B9 scFv (SSS-S) H WCH2 WCH3

26 5B9 scFv VHL11S (SSS-S) H WCH2 WCH3

27 2H7 scFv (SSS-S) H WCH2 WCH3

28 2H7 scFv (SSS-S) H P238SCH2 WCH3

29 2H7 scFv IgAH WCH2 WCH3

30 2H7 scFv IgAH WIgACH2 T4CH3

31 2H7 scFv IgAH WIgACH2 WCH3 + JChain

32 2H7 scFv (CCC-P) WH WCH2 WCH3

33 2H7 scFv (SSS-S) H WCH2 F405YCH3

34 2H7 scFv (SSS-S) H WCH2 F405ACH3

35 2H7 scFv (SSS-S) H WCH2 Y407ACH3

36 2H4 scFv (SSS-S) HWCH2 F405A, Y407ACH3

37 2H7 scFv (CSS-S) H WCH2 WCH3

38 2H7 scFv (SCS-S) H WCH2 WCH3

39 2H7 scFv (SSC-P) H WCH2 WCH3

40 2H7 scFv (CSC-S) H WCH2 WCH3

41 2H7 scFv (CCS-P) H WCH2 WCH3

42 2H7 scFv (SCC-P) H WCH2 WCH3

43 2H7 scFv VH L11S (SSS-S) H WCH2 WCH3

44 2H7 scFv VH L11S (CSS-S) H WCH2 WCH3

45 G28-1 scFv VH L11S (SCS-S) H WCH2 WCH3

46 G28-1 scFv VH L11S (CCS-P) H WCH2 WCH3

47 G28-1 scFv VH L11S (SCC-P) H WCH2 WCH3

48 G28-1 scFv VH L11S mIgE CH2 CH3 CH4

49 G28-1 scFv VH L11S mIgAH WIgACH2 T4CH3

50 G28-1 scFv VH L11S hIgE CH2 CH3 CH4

51 G28-1 scFv VH L11S hIgAH WIgACH2 T4CH3

52 HD37 scFv IgAH WCH2 WCH3

53 HD37 scFv (SSS-S) H WCH2 WCH3

54. HD37 scFv VH L11S (SSS-S) H WCH2 WCH3

55 L6 scFv IgAH WCH2 WCH3

56 L6 scFv VHL11S (SSS-S) H WCH2 WCH3

57 2H7 scFv-llama IgG1

58 2H7 scFv-llama IgG2

59 2H7 scFv-llama IgG3

60 CD16-6 low (ED)(SSS-S) H P238SCH2 WCH3

61 CD16-9 high (ED)(SSS-S) H P238SCH2 WCH3

62 2e12 scFv (SSS-s)H P238SCH2 WCH3—hCD80TM/CT

63 10A8 scFv (SSS-s)H P238SCH2 WCH3—hCD80TM/CT

64 40.2 36 scFv (SSS-s)H P238SCH2 WCH3—hCD80TM/CT

65 2H7 scFv (SSS-s)H P238SCH2 WCH3—hCD80TM/CT

66 G19-4 scFv (SSS-s)H P238SCH2 WCH3—hCD80TM/CT

67 2e12 scFv (SSS-s)H WCH2 WCH3—hCD80TM/CT

68 2e12 scFv IgAH IgACH2 T4CH3—hCD80TM/CT

69 2e12 scFv IgE CH2CH3CH4—hCD80TM/CT

70 2e12 scFv (SSS-s)H P238SCH2 WCH3—mFADD-TM/CT

71 2e12 scFv (SSS-s)H WCH2 WCH3—mFADD-TM/CT

72 2e12 scFv (SSS-s)H WCH2 WCH3—mcasp3-TM/CT

73 2e12 scFv (SSS-s)H P238SCH2 WCH3—mcasp3-TM/CT

74. 2e12 scFv (SSS-s)H WCH2 WCH3—mcasp8--TM/CT

75 2e12 scFv (SSS-s)H P238SCH2 WCH3—mcasp8-TM/CT

76. 2e12 scFv (SSS-s)H WCH2 WCH3—hcasp3--TM/CT

77 2e12 scFv (SSS-s)H P238SCH2 WCH3—hcasp3-TM/CT

78 2e12 scFv (SSS-s)H WCH2 WCH3—hcasp8--TM/CT

79 2e12 scFv (SSS-s)H P238SCH2 WCH3—hcasp8-TM/CT

80 1D8 scFv--hIgG1 (SSS-s)H P238SCH2 WCH3—hCD80TM/CT

81 1D8 scFv--hIgG1 (SSS-s)H WCH2 WCH3—hCD80TM/CT

82. 1D8 scFv—mIgAT4—hCD80TM/CT

83 1D8 scFv--hIgE—hCD80TM/CT

84. 1D8 scFv--hIgG1 (SSS-s)H P238SCH2 WCH3—mFADD-TM/CT

85 1D8 scFv--hIgG1 (SSS-s)H WCH2 WCH3—mFADD-TM/CT

86 1D8 scFv--hIgG1 (SSS-s)H WCH2 WCH3—mcasp3-TM/CT

87 1D8 scFv--hIgG1 (SSS-s)H P238SCH2 WCH3—mcasp3-TM/CT

88 1D8 scFv--hIgG1 (SSS-s)H WCH2 WCH3—mcasp8--TM/CT

89 1D8 scFv--hIgG1 (SSS-s)H P238SCH2 WCH3—mcasp8-TM/CT

90 1D8 scFv--hIgG1 (SSS-s)H WCH2 WCH3—hcasp3--TM/CT

91 1D8 scFv--hIgG1 (SSS-s)H P238SCH2 WCH3—hcasp3-TM/CT

92 1D8 scFv--hIgG1 (SSS-s)H WCH2 WCH3—hcasp8--TM/CT

93 1D8 scFv--hIgG1 (SSS-s)H P238SCH2 WCH3—hcasp8-TM/CT L6 scFv (SSS-S) H WCH2 WCH3

94 2H7 scFv CD154 (L2)

95 2H7 scFv CD154 (S4)

96 CTLA4 IgAH IGACH2CH3

97 CTLA4 IgAH IgACH2 T4CH3

98 2H7 scFv IgAH IgACH2CH3

99 2H7 scFv IgAH IgAHCH2 T18CH3

100 2H&-40.2.220 scFv (SSS-S) H WCH2 WCH3 (bispecific anti-ccd20-anti-cd40)

101 2H7 scFv IgAH IgACH2 T4CH3-hCD89 TM/CT

102 G19-4 scFv (CCC-P) WH WCH2 WCH3-hCD89 TM/CT

103 2e12 scFv (CCC-P) WH WCH2 WCH3-hCD89 TM/CT

These and other aspects of the present invention will become further apparent upon reference to the following detailed description and attached drawings. As noted herein, all referenced patents, articles, documents, and other materials disclosed or identified herein are hereby incorporated by reference in their entireties as if each was incorporated individually

Brief Description of the Drawings

Figure 1 shows DNA and deduced amino acid sequences [SEQ ID NOS __] of 2H7scFv-Ig, a binding domain-immunoglobulin fusion protein capable of specifically binding CD20

Figure 2 shows production levels of 2H7 scFv-Ig by transfected, stable CHO lines and generation of a standard curve by binding of purified 2H7 scFv-Ig to CHO cells expressing CD20

Figure 3 shows SDS-PAGE analysis of multiple preparations of isolated 2H7scFv-Ig protein.

Figure 4 shows complement fixation (Fig. 4A) and mediation of antibody-dependent cellular cytotoxicity (Fig. 4B) by 2H7scFv-Ig.

Figure 5 shows the effect of simultaneous ligation of CD20 and CD40 on growth of normal B cells.

Figure 6 shows the effect of simultaneous ligation of CD20 and CD40 on CD95 expression and induction of apoptosis in a B lymphoblastoid cell line

Figure 7 shows DNA and deduced amino acid sequences [SEQ ID NOS __] of 2H7scFv-CD154 L2 (Fig. 7A, SEQ ID NOS __) and 2H7scFv-CD154 S4 (Fig. 7B, SEQ ID NOS __) binding domain-immunoglobulin fusion proteins capable of specifically binding CD20 and CD40

Figure 8 shows binding of 2H7scFv-CD154 binding domain-immunoglobulin fusion proteins to CD20+ CHO cells by flow immunocytofluorimetry

Figure 9 shows binding of Annexin V to B cell lines Ramos, BJAB, and T51 after binding of 2H7scFv-CD154 binding domain-immunoglobulin fusion protein to cells

Figure 10 shows effects on proliferation of B cell line T51 following binding of 2H7scFv-CD154 binding domain-immunoglobulin fusion protein.

Figure 11 depicts schematic representations of the structures of 2H7ScFv-Ig fusion proteins [SEQ ID NOS __] referred to as CytoxB or CytoxB derivatives CytoxB-MHWTG1C (2H7 ScFv, mutant hinge, wild-type human IgG1 Fc domain), CytoxB-MHMG1C (2H7 ScFv, mutant hinge, mutated human IgG1 Fc domain) and CytoxB-IgAHWTHG1C (2H7 ScFv, human IgA-derived hinge [SEQ ID NO __], wild-type human IgG1 Fc domain) Arrows indicate position numbers of amino acid residues believed to contribute to FcR binding and ADCC activity (heavy arrows), and to complement fixation (light arrows) Note absence of interchain disulfide bonds

Figure 12 shows SDS-PAGE analysis of isolated CytoxB and 2H7scFv-CD154 binding domain-immunoglobulin fusion proteins

Figure 13 shows antibody dependent cell-mediated cytotoxicity activity of CytoxB derivatives

Figure 14 shows complement dependent cytotoxicity of CytoxB derivatives

Figure 15 shows serum half-life determinations of CytoxB-MHWTG1C in macaque blood samples.

Figure 16 shows effects of CytoxB-MHWTG1C on levels of circulating CD40+ B cells in macaque blood samples

Figure 17 shows production levels of HD37 (CD19-specific) ScFv-Ig by transfected mammalian cell lines and generation of a standard curve by binding of purified HD37 ScFv-Ig to cells expressing CD19

Figure 18 shows production levels of L6 (carcinoma antigen) ScFv-Ig by transfected, stable CHO lines and generation of a standard curve by binding of purified L6 ScFv-Ig to cells expressing L6 antigen.

Figure 19 shows antibody dependent cell-mediated cytotoxicity activity of binding domain-immunoglobulin fusion proteins 2H7 ScFv-Ig, HD37 ScFv-Ig and G28-1 (CD37-specific) ScFv-Ig.

Figure 20 shows antibody dependent cell-mediated cytotoxicity activity of L6 ScFv-Ig fusion proteins.

Figure 21 shows SDS-PAGE analysis of L6 ScFv-Ig and 2H7 ScFv-Ig fusion proteins

Figure 22 shows SDS-PAGE analysis of G28-1 ScFv-Ig and HD37 ScFv-Ig fusion proteins

Figure 23 presents a sequence alignment of immunoglobulin hinge and CH2 domains of human IgG1 (SEQ ID NO __) with the hinge and CH2 domains of llama IgG1 (SEQ ID NO __), IgG2 (SEQ ID NO __), and IgG3 (SEQ ID NO __)

Figure 24 illustrates migration of purified 2H7 scFv llama IgG fusion proteins in a 10% SDS polyacrylamide gel. Purified fusion proteins (5 µg per sample) were prepared in non-reducing sample buffer (lanes 2-5) and in reducing sample buffer (lanes 6-9) Lane 1 molecular weight markers (non-reduced), lanes 2 and 6 2H7 scFv–llama IgG1 (SEQ ID NO __); Lanes 3 and 7 2H7 scFv-llama IgG2 (SEQ ID NO __) lanes 4 and 8 2H7 scFv-llama IgG3 (SEQ ID NO __), and Lanes 5 and 9 Rituximab (chimeric anti-CD20 antibody (human IgG1 constant region))

Figure 25 shows binding of 2H7 scFv–llama IgG1 (SEQ ID NO __), 2H7 scFv-llama IgG2 (SEQ ID NO __), and 2H7 scFv-llama IgG3 (SEQ ID NO __) to CD20+ CHO cells detected by flow immunocytofluorimetry

Figure 26 depicts CDC activity of 2H7 scFv llama IgG fusion proteins, 2H7 scFv–llama IgG1 (SEQ ID NO __), 2H7 scFv-llama IgG2 (SEQ ID NO __), and 2H7 scFv-llama IgG3 (SEQ ID NO __), and 2H7 scFv human IgG1 (2H7 scFv IgG WTH WTCH2CH3) (SEQ ID NO __) against BJAB cells in the presence of rabbit complement. Rituximab was included as a control.

Figure 27 shows antibody dependent cell-mediated cytotoxicity activity of 2H7 scFv llama IgG fusion proteins, 2H7 scFv–llama IgG1 (SEQ ID NO __), 2H7 scFv-llama IgG2 (SEQ ID NO __), and 2H7 scFv-llama IgG3 (SEQ ID NO __) Effector cells (human PBMC) were combined with target cells (BJAB cells) at three different ratios,

1·25, 1 50, and 1 100 Rituximab was included as a control. Each data point represents three separate measurements

Figure 28 shows antibody dependent cell-mediated cytotoxicity activity of 2H7 scFv llama IgG fusion proteins, 2H7 scFv–llama IgG1 (SEQ ID NO __), 2H7 scFv-llama IgG2 (SEQ ID NO __), and 2H7 scFv-llama IgG3 (SEQ ID NO __) Effector cells (llama PBMC) were combined with target cells (BJAB cells) at three different ratios, 1 25, 1 50, and 1 100 Rituximab was included as a control. Each data point represents three separate measurements.

Figure 29 depicts complement dependent cytotoxicity activity of Reh cells (acute lymphocytic leukemia) expressing scFv-Ig fusion proteins on the cell surface. Reh cells were transfected with constructs encoding scFv antibodies specific for human costimulatory molecules, CD152, CD28, CD40, and CD20, fused to human IgG1 wild-type hinge-CH2-CH3, which was fused to human CD80 transmembrane and cytoplasmic tail domains. Complement dependent cytotoxicity activity was measured in the presence and absence of rabbit complement (plus C' and no C', respectively) The data represent the average of duplicate samples. Reh anti-hCD152 scFvIg Reh cells transfected with polynucleotide 10A8 scFv IgG MTH (SSS) MT CH2CH3 (SEQ ID NO __), Reh anti-hCD28scFvIg: 2E12 scFv IgG MTH (SSS) MT CH2CH3 (SEQ ID NO __), Reh anti-hCD40scFvIg: 4.2.220 scFv IgG MTH (SSS) MT CH2CH3 (SEQ ID NO __), and Reh anti-hCD20scFvIg. 2H7 scFv IgG MTH (SSS) MT CH2CH3 (SEQ ID NO __)

Figure 30 presents antibody dependent cell-mediated cytotoxicity activity of Reh cells that were transfected with constructs encoding scFv antibodies specific for human costimulatory molecules, CD152, CD28, CD40, and CD20, as described for Figure 29, and for murine CD3, fused to human mutant IgG1 hinge and mutant CH2 and wild type CH3 (Reh anti-mCD3scFv designating Reh cells transfected with polynucleotide 500A2 scFv IgG MTH (SSS) MTCH2WTCH3 SEQ ID NO __)), which was fused to human CD80 transmembrane and cytoplasmic tail domains The data represent the average of quadruplicate samples.

Figure 31 lists immunoglobulin constant region constructs that were used in experiments illustrated in subsequent figures.

Figure 32 depicts complement dependent cytotoxicity activity of CTLA-4 Ig fusion proteins, CTLA-4 IgG WTH (CCC) WTCH2CH3 (SEQ ID NO __) (2 µg/ml)

and CTLA-4 IgG MTH MTCH2WTCH3 (SEQ ID NO __) (2 μg/ml), in the presence and absence of rabbit complement (plus C' and no C', respectively) The target cells were Reh cells and Reh cells transfected with CD80 (Reh CD80 10)

Figure 33 shows antibody dependent cell-mediated cytotoxicity activity of CTLA-4 Ig fusion proteins, CTLA-4 IgG WTH (CCC) WTCH2CH3 (SEQ ID NO __) (2 μg/ml) and CTLA-4 IgG MTH MTCH2WTCH3 (SEQ ID NO __) (2 μg/ml) Effector cells, human PBMC, were added to target cells, Reh or Reh CD80 1, at the ratios indicated. Figure 33A presents the level of natural killing in Reh CD80 1 cells in the absence of any Ig fusion protein. Figure 33B presents antibody dependent cell-mediated cytotoxicity mediated by CTLA-4 IgG MTH MTCH2WTCH3, and Figure 33C presents antibody dependent cell-mediated cytotoxicity mediated by CTLA-4 IgG WTH (CCC) WTCH2CH3 Each data point represents the average percent specific killing measured in four sample wells.

Figure 34 illustrates binding of 2H7 (anti-CD20) scFv Ig fusion proteins to (CD20+) CHO cells by flow immunocytofluorimetry

Figure 35 presents an immunoblot of 2H7 scFv IgG and IgA fusion proteins COS cells were transiently transfected with various 2H7 scFv Ig fusion protein constructs. The expressed polypeptides were immune precipitated with protein A, separated in a non-reducing SDS polyacrylamide gel, and then transferred to a polyvinyl fluoride membrane. Proteins were detected using an anti-human IgG (Fc specific) horseradish peroxidase conjugate. Lane 1 vector only; lane 2 2H7 scFv IgG WTH (CCC) WTCH2CH3 (SEQ ID NO __), lane 3 2H7 scFv IgG MTH (CSS) WTCH2CH3 (SEQ ID NO __), lane 4 2H7 scFv IgG MTH (SCS) WTCH2CH3 (SEQ ID NO __), lane 5 2H7 scFv IgAH IgG WTCH2CH3 (SEQ ID NO __); and lane 6 2H7 scFv IgG MTH (SSS) WTCH2CH3 (SEQ ID NO __)

Figure 36 illustrates binding of 2H7 scFv IgAH IgACH2CH3 polypeptide (SEQ ID NO __) and 2H7 scFv IgAH IgAT4 (SEQ ID NO __) to (CD20+) CHO cells by flow immunocytofluorimetry The source of the polypeptides was culture supernatants from transiently transfected COS cells COS cells transfected with a plasmid comprising a sequence encoding 2H7 scFv IgAH IgACH2CH3 were co-transfected with a plasmid containing nucleotide sequence encoding human J chain.

Figure 37 illustrates antibody dependent cell-mediated cytotoxicity activity of anti-CD20 (2H7) scFv Ig fusion proteins against BJAB target cells using whole blood as the source of effector cells Purified 2H7 scFv Ig fusion proteins were titrated and combined with ^{51}Cr-labeled BJAB cells (5 x 10^4) and whole blood (1 4 final dilution) Each data point represents the average percent specific killing measured in four sample wells

Figure 38 demonstrates antibody dependent cell-mediated cytotoxicity activity of 2H7 scFv Ig fusion proteins (5 µg/ml) against ^{51}Cr-labeled BJAB cells at 0 25, 0 125, and 0 625 dilutions of whole blood. Each data point represents the average percent specific killing measured in four sample wells

Figure 39 shows a comparison of antibody dependent cell-mediated cytotoxicity activity of 2H7 scFv IgG MTH (SSS) WTCH2CH3 (5 µg/ml) and 2H7 scFv IgAH IgACH2CH3 (5 µg/ml) when human PBMC are the source of effector cells (Figure 39A) and when human whole blood is the source of effector cells (Figure 39B)

Figure 40 presents an immunoblot of 2H7 scFv IgG fusion proteins COS cells were transiently transfected with various 2H7 scFv Ig fusion protein constructs. Culture supernatants containing the expressed polypeptides were separated in a non-reducing SDS polyacrylamide gel, and then were transferred to a polyvinyl fluoride membrane. Proteins were detected using an anti-human IgG (Fc specific) horseradish peroxidase conjugate Lanes 1-5 purified 2H7 scFv IgG MTH (SSS) WTCH2CH3 at 40 ng, 20 ng, 10 ng/ 5 ng, and 2.5 ng per lane, respectively Culture supernatants were separated in lanes 6-9 Lane 6 2H7 scFv IgG WTH (CCC) WTCH2CH3, lane 7 2H7 scFv IgG MTH (CSS) WTCH2CH3, lane 8 2H7 scFv IgG MTH (SCS) WTCH2CH3, and lane 9 2H7 scFv VHSHR11 IgG MTH (SSS) WTCH2CH3 The molecular weight (kDal) of marker proteins is indicated on the left side of the immunoblot.

Figure 41 illustrates cell surface expression of 1D8 (anti-murine 4-1BB) scFv IgG WTH WTCH2CH3-CD80 fusion protein on K1735 melanoma cells by flow immunofluorimetry (Fig. 41A) The scFv fusion protein was detected with phycoerythrin-conjugated F(ab')$_2$ goat anti-human IgG Fig. 41B depicts growth of tumors in naïve C3H mice transplanted by subcutaneous injection with wild type K1735 melanoma cells (K1735-WT) or with K1735 cells transfected with 1D8 scFv IgG WTH WTCH2CH3-CD80 (K1735-1D8) Tumor growth was monitored by measuring the size of the tumor

Fig. 41C demonstrates the kinetics of tumor growth in naive C3H mice injected intraperitoneally with monoclonal antibodies to remove $CD8^+$, $CD4^+$, or both $CD4^+$ and $CD8^+$ T cells prior to transplantation of the animals with K1735-1D8 cells

Figure 42 demonstrates therapy of established K1735-WT tumors using K1735-1D8 as an immunogen. Six days after mice were transplanted with K1735-WT tumors, one group (five animals) was injected subcutaneously with K1735-1D8 cells (open circles) or irradiated K1735-WT cells (solid squares) on the contralateral side. A control group of mice received PBS (open squares) Treatments were repeated on the days indicated by the arrows.

Figure 43 shows the growth of tumors in animals that were injected subcutaneously with 2×10^6 K1735-WT cells (solid squares) and the growth of tumors in animals that were injected subcutaneously with 2×10^6 K1735-WT cells plus 2×10^5 K1735-1D8 cells (open triangles)

Figure 44 presents a flow cytometry analysis of antigen104 murine sarcoma tumor cells transfected with 1D8 scFv IgG WTH WTCH2CH3-CD80 isolated after repeated rounds of panning against anti-human IgG Transfected cells expressing 1D8 scFv IgG WTH WTCH2CH3-CD80 were detected with fluoroisothiocyanate (FITC)-conjugated goat anti-human IgG (depicted in black). Untransfected cells are shown in gray

Figure 45 illustrates migration of various 2H7 scFv Ig fusion proteins in a 10% SDS-PAGE gel. 2H7 was the anti-CD20 scFv and 40.2.220 was the anti-CD40 scFv Lane 1 Bio-Rad prestained molecular weight standards, lane 2 anti-CD20 scFv IgG MTH (SSS) MTCH2WTCH3, lane 3 anti-CD20 scFv IgG MTH (SSS) WTCH2CH3, lane 4 2H7 scFv IgAH IgG WTCH2CH3, lane 5 anti-CD20-anti-CD40 scFv IgG MTH (SSS) MTCH2WTCH3, lane 6 Rituximab, lane 7 Novex Multimark® molecular weight standards.

Figure 46 illustrates effector function as measured in an antibody dependent cell-mediated cytotoxicity assay of 2H7 Ig fusion proteins that contain a mutant CH2 domain or wild type CH2 domain The percent specific killing of BJAB target cells in the presence of human PBMC effector cells by 2H7 scFv IgG MTH (SSS) MTCH2WTCH3 (diamonds) was compared to 2H7 scFv IgG MTH (SSS) WTCH2CH3 (squares) and 2H7 scFv IgAH IgG WTCH2CH3 (triangles) and Rituximab (circles)

Figure 47 shows cell surface expression of an anti-human CD3 scFv IgG WTH WTCH2CH3-CD80 (SEQ ID NO __) fusion protein on Reh cells (Fig. 47A) and T51 lymphoblastoid cells (Fig. 47B) by measuring the linear fluorecent equivalent (LFE) using flow immunocytofluorimetry

Figure 48 presents the percent specific killing of untransfected Reh and T51 cells and the percent specific killing of Reh cells (Reh anti-hCD3) (Fig. 48A) and T51 cells (T51 anti-hCD3) (Fig. 48B) that were transfected with a construct encoding scFv antibodies specific for human CD3, fused to human IgG1 wild-type hinge-CH2-CH3, which was fused to human CD80 transmembrane and cytoplasmic tail domains (anti-human CD3 scFv IgG WTH WTCH2CH3-CD80 (SEQ ID NO __) Human PBMC (effector cells) were combined with BJAB target cells at the ratios indicated.

Figure 49 illustrates binding of 5B9, an anti-murine CD137 (4-1BB) monoclonal antibody, and a 5B9 scFv IgG fusion protein (5B9 scFv IgG MTH (SSS) WTCH2CH3 (SEQ ID NO ___) to stimulated human PBMC Binding of the 5B9 scFv IgG fusion protein was detected by flow immunocytofluorimetry using FITC conjugated goat anti-human IgG Binding of the 5B9 monoclonal antibody was detected with FITC conjugated goat anti-mouse IgG

Figure 50 illustrates the effect of the LV_H11S mutation on the expression of 2H7 LV_H11S scFv WCH2 WCH3 ("CytoxB scFv Ig", SEQ ID NO __) in CHO cell lines.

Figure 51 shows a semi-quantitative SDS-PAGE analysis examining the expression of 2H7 LV_H11S scFv WCH2 WCH3 (SEQ ID NO __) when transiently transfected in CHO cells Lanes 2-5 are various amounts of 2H7 LV_H11S scFv WCH2 WCH3 Lanes 6-10 are 10μl samples from five different clones expressing 2H7 LV_H11S scFv WCH2 WCH3

Figure 52 shows differences in binding capacity between a G28-1 LV_H11S scFv Ig construct (SEQ ID NO __) and a G28-1 wild type scFv Ig binding domain fusion protein construct (SEQ ID NO __), both obtained from transiently transfected COS cells. Binding to Ramos cells was determined using flow cytometry The data illustrates a significant increase in binding of the V_H11S protein to CD37+Ramos cells

Figure 53 illustrates increased levels of expression of a G28-1 LV_H11S scFv Ig construct (SEQ ID NO __) compared to a G28-1 wild type scFv Ig construct in COS Protein levels were compared using immunoblot analysis Both immunoblot gels

have quantitated amounts of purified a G28-1 scFv Ig (SSS-S) H WCH2 WCH3 contruct of the invention in lanes 1-4. Lanes 5-9 of the first immunoblot represent five different clones each transfected with G28-1 scFv (SSS-S) H WCH2 WCH3, while lanes 5-9 of the second immunoblot represent five different clones transfected with G28-1 LV_H11S scFv (SSS-S) H WCH2 WCH3 The immunoblots illustrate that the LV_H11S form causes the G28-1 scFv Ig construct to express at very high levels.

Figure 54 illustrates the binding of 2H7 scFv Ig derivatives with altered hinges (SEQ ID NOs __,__,__,__,__,__,__,__) to CHO cells expressing CD20 (CD20+ CHO) by flow cytometry, and indicates that these altered connecting region hinge constructs (including (SSS-S), (CSS-S), (SCS-S) and (CSC-S) hinge regions) retain binding function to CD20

Figure 55 shows the ability to mediate antibody dependent cell-mediated cytotoxicity of various constructs against Bjab targets (A) 2H7 scFv Ig constructs of the invention that contain connecting regions comprising (CSS-S), (SCS-S), (CSC-S), and (SSS-S) hinges (SEQ ID NOs __,__,__,__,__,__,__,__) and (B) 2H7 scFv constructs of the invention with various connecting regions and tail regions (SEQ ID NOs __,__,__,__,__,__) Percent specific killing is compared to total killing induced by a detergent. The controls are natural killing in target cells with effectors added and a 2H7 construct with an IgA hinge connecting region and IgA-derived tail region that does not bind PBMC effectors

Figure 56 illustrates the ability of various 2H7 scFv Ig constructs of the invention (SEQ ID NO __,__,__,__,__,__,__) that include connecting regions having various hinge regions (*e.g.*, (CSC-S), (SSS-S), (SCS-S), and (CSS-S)) to mediate complement activity in Ramos cells Percent specific killing is measured against the control of complement only, and 100% killing was determined by exposure of cells to detergent.

Figure 57 illustrates the shows the binding of 2H7 scFv Ig constructs of the invention containing different tail regions (SEQ ID NO __,__,__) to CD20+ CHO using immunocytofluroimetry The different proteins were detected using FITC conjugated to anti-IgG, anti-IgA, and anti-IgE

Figure 58A shows the binding of 2H7 V_H L11S scFv IgECH2CH3CH4, purified using Hydrophobic charge induction chromatography (HCIC) and eluted at different pHs 4 0 and 3 5, (SEQ ID NO __) in CD20+ CHO cells by flow cytometry,

indicating that the proteins bound CD20 whether eluted at pH 4.0 or 3 5 Figure 58B is a data graph indicating the ability of these 2H7 VH L11S scFv IgE constructs of the invention to mediate, for example, ADCC in Bjab target cells

Figure 59 shows the binding capacity of G28-1 VH L11S mIgECH2CH3CH4 (SEQ ID NO __) (A) to Bjab and Ramos target cells and (B) to CD20+ CHO cells by flow cytometry

Figure 60 shows the High Performance Liquid Chromatography (HPLC) profiles of various protein constructs of the invention (A) 2H7 scFv (SSS-S) H (P238S)CH2 WCH3 (SEQ ID NO __) (B) 2H7 scFv (CSS-S) H WCH2 WCH3, (SEQ ID NO· __) (C) 2H7 scFv (SCS-S) H WCH2 WCH3, (SEQ ID NO __) and (D) 2H7 scFv (SSS-S) H WCH2 (Y407A)CH3 (SEQ ID NO· __), indicating that construct A has apparent molecular weight forms of 100kD and 75kD and that, by introducing certain changes a predominant 75kD molecular weight form is obtained, as seen in constructs B, C, and D See Example 40

Figure 61 shows the HPLC profiles of various protein constructs of the invention (A) 2H7 scFv (SSS-S) H WCH2 WCH3, (SEQ ID NO __) (B) 2H7 scFv (CSC-S) H WCH2 WCH3, (SEQ ID NO __) (C) 2H7 scFv (CCC-P) H WCH2 WCH3, (SEQ ID NO __) and (D) 2H7 scFv IgAH WCH2 WCH3 (SEQ ID NO __), indicating that construct A has apparent molecular weight forms of 100kD and 75kD and that, by introducing certain changes a predominant 75kD molecular weight form is obtained, as seen in constructs B and C, while construct D (which has an IgA tail region) has an apparent molecular weight of 150kD See Example 40

Figure 62 shows the HPLC profiles of various protein constructs of the invention (A) 2H7 scFv (SSS-S) H WCH2 WCH3, (SEQ ID NO __) (B) 2H7 scFv (SCS-S) H WCH2 WCH3, (SEQ ID NO __) (C) 2H7 scFv IgA 3TCH2 WCH3, (SEQ ID NO· __) and (D) 2H7 scFv (SSS-S) H WCH2 (F405A Y407A)CH3 (SEQ ID NO __), indicating that construct A has two forms with apparent molecular weights at 100kD and 75kD, construct B has a predominant form with an apparent molecular weight of 75kD, while construct C with a T4 mutation leads to three forms with apparent molecular weights near 600kD and construct D with a double point mutation in the CH3 region leads to a predominant form having an apparent molecular weight less than 44kD A T4 mutation here refers to a truncation of four amino acids from a CH3 region See Example 40

Figure 63 compares the effect on binding CD20+CHO cells by 2H7 V_H L11S scFv Ig constructs (SEQ ID NOs __,__), with and without F405A and Y407A alterations in the CH3 region, by flow cytometry, indicating a loss of binding capability with this double amino acid change. See Example 41

Figure 64 shows the binding capacity of FITC conjugated 2H7 V_H L11S scFv Ig derivatives (SEQ ID NOs __,__,__,__) to CH20+ CHO cells by flow cytometry, indicating that these constructs do not lose binding capacity when conjugated to a florescent marker See Example 41

Figure 65 shows a nonreducing SDS-PAGE analysis examining 10 μg (per lane) of various purified 2H7 V_H L11S scFv Ig constructs of the invention (SEQ ID NOs __,__,__,__,__,__), indicating an apparent molecular weight for each construct in reference to a standard molecular weight marker in lane 1 See Example 41

Figure 66 compares the CH2 domain sequences of four different human IgG regions, hIgG1, hIgG2, hIgG3, hIgG4, hIgG4, and one rat region, rIgG2b Point mutations affecting ADCC and CDC are labeled with arrows. See Example 52

Figure 67 demonstrates the ability of various 2H7 V_H L11S scFv Ig constructs (SEQ ID NOs __,__) to mediate ADCC in CHO and Lec13 CHO transiently transfected cells, indicating that constructs expressed in Lec 13 CHO cells had a 20% increase in specific killing over the same construct expressed in regular CHO cells. See Example 42

Figure 68 shows SDS-PAGE analysis, both reduced and nonreduced, of high and low affinity alleles of soluble CD 16(ED) (SSS-S) H P283S CH2 WCH3 (SEQ ID NOs __,__) See Example 43

Figure 69 demonstrates the different binding capabilities of the high and low affinity CD16 fusion proteins (SEQ ID NOs __,__) to 2H7 V_H L11S scFv (CSC-S) WCH2 WCH3 (SEQ ID NO __) or 2H7 V_H L11S scFv (SSS-S) WCH2 WCH3 (SEQ ID NO: __), and indicates a loss of high and low affinity allele binding using P238S CH2 constructs See Example 43

Figure 70 shows a diagram of (A) an assay used to detect changes in Fc receptor binding using FITC conjugated CD16 extracellular domain Ig fusion protein with a mutated tail, which eliminates self-association and B) a mammalian display system using cell surface expression of constructs See Example 44

Figure 71 shows the induction of apoptosis in Bjab and Ramos cells by various mAbs (SEQ ID NOs __,__,__,__,__,__,__) and scFvIg constructs of the invention (SEQ ID NOs __,__,__,__,__,__) See Example 45

Figure 72 illustrates the ability of 2H7 scFv (SSS-S) H WCH2 WCH3 and 2H7 scFv (SSS-S) H P238CH2 WCH3 constructs to induce apoptosis in Ramos cells that is mediated by activation of caspase 3 The results indicate that under these conditions, both constructs bind CD20 and induced apoptosis

Figure 73 illustrates the ability of 2H7 scFv (SSS-S) H WCH2 WCH3 and 2H7 scFv (SSS-S) H P238CH2 WCH3 constructs to mediate CDC activity in CD20 positive Bjab target cells The results indicate both constructs had the ability to mediate CDC

Figure 74 compares the ability of 2H7 scFv (SSS-S) H WCH2 WCH3 and 2H7 scFv (SSS-S) H P238CH2 WCH3 constructs to mediate ADCC in Bjab target cells The results indicate the 2H7 scFv (SSS-S) H WCH2 WCH3 construct was very effective and induced high levels of specific killing, while the of 2H7 scFv (SSS-S) H P238CH2 WCH3 construct was not effective in mediating ADCC

Figure 75 compares the ability of 2H7 scFv (SSS-S) H WCH2 WCH3 and 2H7 scFv (SSS-S) H P238CH2 WCH3 constructs to bind soluble CD16 (high affinity and low affinity forms) in CD20 positive CHO cells. Results indicate that 2H7 scFv (SSS-S) H WCH2 WCH3 was able to bind CD16 (both forms), while 2H7 scFv (SSS-S) H P238CH2 WCH3 was not able to bind CD16 (either form)

Figure 76 compares the ability of 2H7 scFv (SSS-S) H WCH2 WCH3 and 2H7 scFv (SSS-S) H P238CH2 WCH3 constructs to bind CD64 positive U937 cells The results indicate that both constructs had high affinity for FcγRI, and that the P238S mutation selectively reduced binding to FcγRIII.

Figure 77 illustrates an *in vivo* experiment in macaques to measure the effect of where 2H7 scFv (SSS-S) H WCH2 WCH3 and 2H7 scFv (SSS-S) H P238CH2 WCH3 constructs on B cell depletion. The constructs were administered one week apart and B cells were measures on days –7, 0, 1, 3, 7, 8, 10, 14, 28 and 43 using complete blood counts and two-color flow cytometry The results indicate that the 2H7 scFv (SSS-S) H WCH2 WCH3 construct resulted in rapid and complete depletion of B cells, which lasted up to 28 days after the second injection. Conversely the 2H7 scFv (SSS-S) H P238CH2

WCH3 construct resulted in a slow reduction in B cells to about 50% in the first 2 weeks, however, B cells rapidly began to return to regular level shortly after this This suggests that ADCC mediated by CD16 interaction is likely necessary for rapid and sustained B cell depletion.

Figure 78 illustrates the SEC profiles of G28-1 VL11S scFv (SSS) H WCH2 WCH3 and G28-1 VL11S scFv (SSC) H WCH2 WCH3 constructs. The construct with an SSS hinge generated a single uniform peak at approximately 75-100 Kd, while the construct with a SSC hinge generated a smaller form and other heterogeneous forms, including one at greater than 200 Kd.

Figure 79 illustrates the binding ability of G28-1 VL11S scFv (SSS) H WCH2 WCH3 and G28-1 VL11S scFv (SSC) H WCH2 WCH3 constructs in B cell lymphoma cells: Bjab, Ramos, WIL-2, Namalwa and Raji The results in (a) indicate that G28-1 VL11S scFv (SSS) H WCH2 WCH3 bound to Bjab and Ramos cells, and moderately to WIL-2 cells, and at lower levels to Namalwa and Raji cells. The results in (b) indicate the G28-1 VL11S scFv (SSC) H WCH2 WCH3 bound to Bjab cells, and moderately to and Ramos and WIL-2 cells, and at lower levels to Namalwa and Raji cells.

Figure 80 illustrates annexin and v-propidium iodide binding in Ramos cells incubated with G28-1 VL11S scFv (SSS) H WCH2 WCH3 and G28-1 VL11S scFv (SSC) H WCH2 WCH3 constructs overnight. The results indicate that both constructs induced apoptosis, however, the construct with the (SSC) hinge induced more apoptosis than the construct with the (SSS) hinge

Figure 81 illustrates the ability of G28-1 VL11S scFv (SSS) H WCH2 WCH3 and G28-1 VL11S scFv (SSC) H WCH2 WCH3 constructs to inhibit proliferation of Ramos cells The results indicate that both constructs inhibited proliferation.

Figure 82 illustrates the ability of G28-1 VL11S scFv (SSS) H WCH2 WCH3 and G28-1 VL11S scFv (SSC) H WCH2 WCH3 and 2H7 scFv (CSS) H WCH2 WCH3 constructs to induce apoptosis in Ramos B cells, alone and in different combinations The results shown in this experiment indicate that the both G28-1 constructs were more efficient than the 2H7 construct. The results also indicate that the G28-1 VL11S scFv (SSS) H WCH2 WCH3 construct was more efficient than the G28-1 VL11S scFv (SSC) H WCH2 WCH3 construct However, the amount of apoptosis was greatest when the 2H7 and the G28-1 constructs were used in combination.

Figure 83 compares the ability of G28-1 VL11S scFv (SSS) H WCH2 WCH3, G28-1 VL11S scFv (SSC) H WCH2 WCH3 and 2H7 scFv (CSS) H WCH2 WCH3 constructs to mediate CDC in Ramos B cells. The results indicate that all the constructs mediated CDC The 2H7 construct was the most efficient, followed by G28-1 VL11S scFv (SSC) H WCH2 WCH3, then G28-1 VL11S scFv (SSS) H WCH2 WCH3

Figure 84 compares the ability of the G28-1 VL11S scFv (SSS) H WCH2 WCH3, G28-1 VL11S scFv (SSC) H WCH2 WCH3 and 2H7 scFv (CSS) H WCH2 WCH3 constructs to mediate ADCC in Ramos B cells. The results indicate that the G28-1 VL11S scFv (SSS) H WCH2 WCH3 and G28-1 VL11S scFv (SSC) H WCH2 WCH3 constructs were able to mediate ADCC, while the 2H7 scFv (CSS) H WCH2 WCH3 construct ability to mediate ADCC was lower, but still higher than the level of natural killing.

Detailed Description of the Invention

The present invention is directed to novel molecules useful, for example, as therapeutics, as well as for other purposes including diagnostic and research purposes Such molecules have, for example, antigen binding or other binding function(s) and, for example, one or more effector functions. The invention includes molecular constructs, including binding domain-immunoglobulin fusion proteins, and related compositions and methods, which will be useful in immunotherapeutic and immunodiagnostic applications, and in research methods, and which offer certain advantages over antigen-specific compounds and polypeptides of the prior art. The constructs, including fusion proteins, of the present invention are preferably single polypeptide chains that comprise, in pertinent part, the following fused or otherwise connected domains or regions a binding region construct, such as a binding domain or polypeptide, a connecting region construct including, for example, a native or engineered immunoglobulin hinge region polypeptide, and a tail region construct, including, for example, a construct that may comprise, consist essentially of, or consist of, a native or engineered immunoglobulin heavy chain CH2 constant region polypeptide and a native or engineered immunoglobulin heavy chain CH3 constant region polypeptide. According to certain embodiments that are particularly useful for gene therapy, the constructs, including fusion proteins, of the present invention may further comprise a native or engineered plasma membrane anchor domain. According to certain other preferred embodiments the constructs, including fusion proteins, of the present invention may further include a tail region having a native or engineered

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immunoglobulin heavy chain CH4 constant region polypeptide. In particularly preferred embodiments, the binding regions, such as polypeptide domains, of which the constructs, including binding domain-immunoglobulin fusion proteins, are comprised are, or are derived from, polypeptides that are the products of human gene sequences, but the invention need not be so limited and may in fact relate to constructs, including binding domain-immunoglobulin fusion proteins, as provided herein that are derived from any natural or artificial source, including genetically engineered and/or mutated polypeptides.

The present invention relates in part to the surprising observation that the novel constructs, including binding domain-immunoglobulin fusion proteins, described herein are capable of immunological activity More specifically, these proteins retain the ability to participate in well known immunological effector activities including, for example, antibody dependent cell mediated cytotoxicity (*e.g*, subsequent to antigen binding on a cell surface, engagement and induction of cytotoxic effector cells bearing appropriate Fc receptors, such as Natural Killer cells bearing FcRγIII, under appropriate conditions) and/or complement fixation in complement dependent cytotoxicity (*e.g*, subsequent to antigen binding on a cell surface, recruitment and activation of cytolytic proteins that are components of the blood complement cascade) despite having structures not be expected to be capable of promoting such effector activities or to promotion of such activities as described herein. For reviews of ADCC and CDC see, *e.g*, Carter, 2001 *Nat Rev Canc.* 1 118, Sulica *et al*, 2001 *Int. Rev Immunol* 20.371, Maloney *et al*, 2002 *Semin. Oncol.* 29 2, Sondel *et al*, 2001 *Hematol Oncol Clin North Am* 15(4) 703-21, Maloney 2001 *Anticanc Drugs* 12 SuppL2 1-4 IgA activation of complement by the alternative pathway is described, for example, in Schneideman *et al*, 1990 *J Immunol* 145:233 As described in greater detail below, ADCC, complement fixation, and CDC are unexpected functions for constructs, including fusion proteins, comprising for example immunoglobulin heavy chain regions and having the structures described herein, and in particular for immunoglobulin fusion proteins comprising, for example, immunoglobulin hinge region polypeptides that are compromised in their ability to form interchain, homodimeric disulfide bonds.

Another advantage afforded by the present invention is constructs, including binding domain-immunoglobulin fusion polypeptides, of the invention that can be produced in substantial quantities that are typically greater than those routinely attained

with single-chain antibody constructs of the prior art, for example. In preferred embodiments, constructs, including the binding domain-immunoglobulin fusion polypeptides, of the present invention are recombinantly expressed in mammalian or other desired and useful expression systems, which offer the advantage of providing polypeptides that are stable *in vivo* (*e.g.*, under physiological conditions) According to non-limiting theory, such stability may derive in part from posttranslational modifications, and specifically glycosylation. Production of the constructs, including binding domain-immunoglobulin fusion protein constructs, of the invention via recombinant mammalian expression has been attained in static cell cultures at a level of greater than 50 mg protein per liter culture supernatant and has been routinely observed in such cultures at 10-50 mg/liter, such that preferably at least 10-50 mg/liter may be produced under static culture conditions, also contemplated are enhanced production, in whole or in part, of the protein constructs of the invention using art-accepted scale-up methodologies such as "fed batch" (*i.e.*, non-static) production, where yields of at least 5-500 mg/l, and in some instances at least 0 5-1 gm/l, depending on the particular protein product, are obtained.

A construct, including a binding domain polypeptide, according to the present invention may be, for example, any polypeptide that possesses the ability to specifically recognize and bind to a cognate biological molecule or complex of more than one molecule or assembly or aggregate, whether stable or transient, of such a molecule. Such molecules include proteins, polypeptides, peptides, amino acids, or derivatives thereof; lipids, fatty acids or the like, or derivatives thereof; carbohydrates, saccharides or the like or derivatives thereof; nucleic acids, nucleotides, nucleosides, purines, pyrimidines or related molecules, or derivatives thereof, or the like; or any combination thereof such as, for example, glycoproteins, glycopeptides, glycolipids, lipoproteins, proteolipids, or any other biological molecule that may be present in a biological sample Biological samples may be provided, for example, by obtaining a blood sample, biopsy specimen, tissue explant, organ culture, biological fluid or any other tissue or cell or other preparation from a subject or a biological source. The subject or biological source may, for example, be a human or non-human animal, a primary cell culture or culture adapted cell line including but not limited to genetically engineered cell lines that may contain chromosomally integrated or episomal recombinant nucleic acid sequences, immortalized or immortalizable cell lines, somatic cell hybrid cell lines, differentiated or differentiatable

cell lines, transformed cell lines and the like, *etc* In certain preferred embodiments of the invention, the subject or biological source may be suspected of having or being at risk for having a disease, disorder or condition, including a malignant disease, disorder or condition or a B cell disorder, which in certain further embodiments may be an autoimmune disease, and in certain other embodiments of the invention the subject or biological source may be known to be free of a risk or presence of such disease, disorder or condition.

A binding region, including a binding domain polypeptide, for example, may be any naturally occurring, synthetic, semi-synthetic, and/or recombinantly produced binding partner for a biological or other molecule that is a target structure of interest, herein sometimes referred to as an "antigen" but intended according to the present disclosure to encompass any target biological or other molecule to which it is desirable to have the subject invention, for example, a fusion protein, bind or specifically bind. Constructs of the invention, including binding domain-immunoglobulin fusion proteins, are defined to be "immunospecific" or capable of binding to a desired degree, including specifically binding, if they bind a desired target molecule such as an antigen as provided herein, at a desired level, for example, with a K_a of greater than or equal to about 10^4 M^{-1}, preferably of greater than or equal to about 10^5 M^{-1}, more preferably of greater than or equal to about 10^6 M^{-1} and still more preferably of greater than or equal to about 10^7 M^{-1} Affinities of even greater than about 10^7 M^{-1} are still more preferred, such as affinities equal to or greater than about about 10^7 M^{-1}, about 10^8 M^{-1}, and about 10^9 M^{-1}, and about 10^{10} M^{-1} Affinities of binding domain-immunoglobulin fusion proteins according to the present invention can be readily determined using conventional techniques, for example those described by Scatchard *et al*, 1949 *Ann. N Y Acad. Sci.* 51 660 Such determination of fusion protein binding to target antigens of interest can also be performed using any of a number of known methods for identifying and obtaining proteins that specifically interact with other proteins or polypeptides, for example, a yeast two-hybrid screening system such as that described in U S Patent No 5,283,173 and U S Patent No 5,468,614, or the equivalent.

Preferred embodiments of the subject invention constructs, for example, binding domain-immunoglobulin fusion proteins, comprise binding regions or binding domains that may include, for example, at least one native or engineered immunoglobulin

variable region polypeptide, such as all or a portion or fragment of a native or engineered heavy chain and/or a native or engineered light chain V-region, provided it is capable of binding or specifically binding an antigen or other desired target structure of interest at a desired level of binding and selectivity In other preferred embodiments the binding region or binding domain comprises, consists essentially of, or consists of, a single chain immunoglobulin-derived Fv product, for example, and scFv, which may include all or a portion of at least one native or engineered immunoglobulin light chain V-region and all or a portion of at least one native or engineered immunoglobulin heavy chain V-region, and a linker fused or otherwise connected to the V-regions, preparation and testing such constructs are described in greater detail herein. Other preparation and testing methods are well known in the art.

As described herein and known in the art, immunoglobulins comprise products of a gene family the members of which exhibit a high degree of sequence conservation. Amino acid sequences of two or more immunoglobulins or immunoglobulin domains or regions or portions thereof (*e.g.*, V_H domains, V_L domains, hinge regions, CH2 constant regions, CH3 constant regions) may be aligned and analyzed. Portions of sequences that correspond to one another may be identified, for instance, by sequence homology Determination of sequence homology may be determined with any of a number of sequence alignment and analysis tools, including computer algorithms well known to those of ordinary skill in the art, such as Align or the BLAST algorithm (Altschul, 1991 *J Mol Biol.* 219 555-565, Henikoff and Henikoff, 1992 *Proc. Natl. Acad Sci. USA* 89 10915-10919), which is available at the NCBI website (http://www/ncbi.nlm.nih.gov/cgi-bin/BLAST) Default parameters may be used.

Portions, for example, of a particular immunoglobulin reference sequence and of any one or more additional immunoglobulin sequences of interest that may be compared to a reference sequence. "Corresponding" sequences, regions, fragments or the like, may be identified based on the convention for numbering immunoglobulin amino acid positions according to Kabat, *Sequences of Proteins of Immunological Interest*, (5th ed Bethesda, MD Public Health Service, National Institutes of Health (1991)) For example, according to this convention, the immunoglobulin family to which an immunoglobulin sequence of interest belongs is determined based on conservation of variable region polypeptide sequence invariant amino acid residues, to identify a particular numbering

system for the immunoglobulin family, and the sequence(s) of interest can then be aligned to assign sequence position numbers to the individual amino acids which comprise such sequence(s) Preferably at least about 70%, more preferably at least about 80%-85% or about 86%-89%, and still more preferably at least about 90%, about 92%, about 94%, about 96%, about 98% or about 99% of the amino acids in a given amino acid sequence of at least about 1000, more preferably about 700-950, more preferably about 350-700, still more preferably about 100-350, still more preferably about 80-100, about 70-80, about 60-70, about 50-60, about 40-50 or about 30-40 consecutive amino acids of a sequence, are identical to the amino acids located at corresponding positions in a reference sequence such as those disclosed by Kabat (1991) or in a similar compendium of related immunoglobulin sequences, such as may be generated from public databases (*e.g*, Genbank, SwissProt, *etc*) using sequence alignment tools such as, for example, those described above. In certain preferred embodiments, an immunoglobulin sequence of interest or a region, portion, derivative or fragment thereof is greater than about 95% identical to a corresponding reference sequence, and in certain preferred embodiments such a sequence of interest may differ from a corresponding reference at no more than about 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 amino acid positions.

For example, in certain embodiments the present invention is directed to a construct, including a binding domain-immunoglobulin fusion protein, comprising in pertinent part a human or other species immunoglobulin heavy chain variable region polypeptide comprising a mutation, alteration or deletion at an amino acid at a location or locations corresponding to one or more of amino acid positions 9, 10, 11, 12, 108, 110, 111, and 112 in, for example, SEQ ID NO __, which comprises, for example, a murine V_H-derived sequence. At a relatively limited number of immunoglobulin V_H sequence positions, for example, including position 11, amino acid conservation is observed in the overwhelming majority of V_H sequences analyzed across mammalian species lines (*e.g*, Leu11, Val37, Gly44, Leu45, Trp47, Nguyen *et al*, 1998 *J Mol. Biol* 275.413) Various such amino acid residues, and hence their side chains, are located at the surface of the variable domain (V_H) They may contact residues of the C_H1 (*e.g*, Leu11) and the V_L domains (*e.g*, Val37, Gly44, Leu45, and Trp47) and may, in the absence of light chains, contribute to stability and solubility of the protein (*see, e.g*, Chothia *et al*, 1985 *J Mol. Biol* 186 651, Muyldermans *et al*, 1994 *Prot Engineer* 7 1129, Desmyter *et al*, 1996

Nat. Struct. Biol. 3 803, Davies *et al*, 1994 *FEBS Lett.* 339:285) In certain embodiments, for example, the present invention is also directed to a construct, including a binding domain-immunoglobulin fusion protein, comprising in pertinent part a human immunoglobulin light chain variable region polypeptide, or an immunoglobulin light chain variable region polypeptide from another species, comprising a mutation, alteration or deletion at an amino acid at a location or locations corresponding to one or more of amino acid positions 12, 80, 81, 82, 83, 105, 106, 107 and 108 In still other certain embodiments, for example, the present invention is directed to a construct, including a binding domain-immunoglobulin fusion protein, comprising in pertinent part (1) a human immunoglobulin heavy chain variable region polypeptide, or an immunoglobulin light chain variable region polypeptide from another species, comprising, consisting essentially of, or consisting of, said heavy chain sequence having a mutation, alteration or deletion at a location or locations corresponding to one or more of amino acid positions 9, 10, 11, 12, 108, 110, 111, and 112, and (2) a human immunoglobulin light chain variable region polypeptide, or an immunoglobulin light chain variable region polypeptide from another species, comprising, consisting essentially of, or consisting of, said light chain sequence having a mutation, alteration or deletion at a location or locations corresponding to one or more of amino acid positions 12, 80, 81, 82, 83, 105, 106, 107 and 108

As another example, by reference to immunoglobulin sequence compendia and databases such as those cited above, for example, the relatedness of two or more immunoglobulin sequences to each other can readily and without undue experimentation be established in a manner that permits identification of the animal species of origin, the class and subclass (*e.g.*, isotype) of a particular immunoglobulin or immunoglobulin region polypeptide sequence. Any immunoglobulin variable region polypeptide sequence, including native or engineered V_H and/or V_L and/or single-chain variable region (sFv) sequences or other native or engineered V region-derived sequences or the like, may be used as a binding region or binding domain. Engineered sequences includes immunoglobulin sequences from any species, preferably human or mouse, for example, that include, for example, a mutation, alteration or deletion at an amino acid at a location or locations corresponding to one or more of amino acid positions 9, 10, 11, 12, 108, 110, 111, and 112 in a heavy chain variable region sequence or an scFv, and/or a mutation, alteration or deletion at a location or locations corresponding to one or more of amino acid

positions 12, 80, 81, 82, 83, 105, 106, 107 and 108 in a light chain variable region sequence or an scFv

Various preferred embodiments include, for example, native or engineered immunoglobulin V region polypeptide sequences derived, for example, from antibodies including monoclonal antibodies such as murine or other rodent antibodies, or antibodies or monoclonal antibodies derived from other sources such as goat, rabbit, equine, bovine, camelid or other species, including transgenic animals, and also including human or humanized antibodies or monoclonal antibodies. Non-limiting examples include variable region polypeptide sequences derived from monoclonal antibodies such as those referenced herein and/or described in greater detail in the Examples below, for instance, CD20-binding or specific murine monoclonal antibodies (*e.g.*, 2H7), other CD20-binding or specific murine monoclonal antibodies that are not 1F5 antibodies, monoclonal antibody L6 (specific for a carbohydrate-defined epitope and available from American Type Culture Collection, Manassas, VA, as hybridoma HB8677), and monoclonal antibodies that bind to or are specific for CD28 (*e.g.*, monoclonal antibody 2E12), CD40, CD80, CD137 (*e.g.*, monoclonal antibody 5B9 or monoclonal antibody 1D8 which recognizes the murine homologue of CD137, 41BB) and CD152 (CTLA-4)

Other binding regions, including binding domain polypeptides, may comprise any protein or portion thereof that retains the ability to bind or specifically bind to an antigen as provided herein, including non-immunoglobulins Accordingly the invention contemplates constructs, including fusion proteins, comprising binding region or binding domain polypeptides that are derived from polypeptide ligands such as hormones, cytokines, chemokines, and the like, cell surface or soluble receptors for such polypeptide ligands, lectins, intercellular adhesion receptors such as specific leukocyte integrins, selectins, immunoglobulin gene superfamily members, intercellular adhesion molecules (ICAM-1, -2, -3) and the like, histocompatibility antigens, *etc*

Examples of cell surface receptors useful in the preparation of, or as, binding regions, or that may provide a binding domain polypeptide, and that may also be selected as a target molecule or antigen to which a construct, including for example, a binding domain-Ig fusion protein of the present invention desirably binds, include the following, or the like HER1 (*e.g.*, GenBank Accession Nos U48722, SEG_HBGFRHXS, K03193), HER2 (Yoshino *et al*, 1994 *J Immunol.* 152 2393, Dinn *et al*, 1994 *Canc. Res*

54 16, see also, *e.g.*, GenBank Acc Nos. X03363, M17730, SEG_HUMHER20), HER3 (*e.g.*, GenBank Acc. Nos U29339, M34309), HER4 (Plowman *et al.*, 1993 *Nature* 366 473, see also *e.g*, GenBank Acc. Nos L07868, T64105), epidermal growth factor receptor (EGFR) (*e.g*, GenBank Acc Nos. U48722, SEG_HEGFREXS, KO3193), vascular endothelial cell growth factor (*e.g*, GenBank No M32977), vascular endothelial cell growth factor receptor (VEGF) (*e.g.*, GenBank Acc. Nos. AF022375, 1680143, U48801, X62568), insulin-like growth factor-I (*e.g*, GenBank Acc Nos X00173, X56774, X56773, X06043, *see also* European Patent No GB 2241703), insulin-like growth factor-II (*e.g*, GenBank Acc Nos X03562, X00910, SEG_HUMGFIA, SEG_HUMGFI2, M17863, M17862), transferrin receptor (Trowbridge and Omary, 1981 *Proc. Nat Acad. USA* 78 3039, see also *e.g*, GenBank Acc. Nos X01060, M11507), estrogen receptor (*e.g*, GenBank Acc. Nos. M38651, X03635, X99101, U47678, M12674), progesterone receptor (*e.g*, GenBank Acc. Nos. X51730, X69068, M15716), follicle stimulating hormone receptor (FSH-R) (*e.g*, GenBank Acc. Nos. Z34260, M65085), retinoic acid receptor (*e.g*, GenBank Acc. Nos L12060, M60909, X77664, X57280, X07282, X06538), MUC-1 (Barnes *et al*, 1989 *Proc. Nat. Acad. Sci. USA* 86 7159, see also *e.g*, GenBank Acc. Nos SEG_MUSMUCIO, M65132, M64928) NY-ESO-1 (*e.g*, GenBank Acc Nos AJ003149, U87459), NA 17-A (*e.g*, European Patent No WO 96/40039), Melan-A/MART-1 (Kawakami *et al*, 1994 *Proc. Nat. Acad Sci. USA* 91 3515, see also *e.g*, GenBank Acc. Nos. U06654, U06452), tyrosinase (Topalian *et al*, 1994 *Proc. Nat Acad. Sci USA* 91 9461, see also *e.g*, GenBank Acc. Nos. M26729, SEG_HUMTYR0, *see also* Weber *et al*, *J Clin. Invest* (1998) 102 1258), Gp-100 (Kawakami *et al*, 1994 *Proc. Nat. Acad. Sci. USA* 91.3515, see also *e.g.*, GenBank Acc No S73003, *see also* European Patent No EP 668350, Adema *et al*, 1994 *J Biol. Chem* 269:20126), MAGE (van den Bruggen *et al*, 1991 *Science* 254 1643, see also *e.g* GenBank Acc. Nos U93163, AF064589, U66083, D32077, D32076, D32075, U10694, U10693, U10691, U10690, U10689, U10688, U10687, U10686, U10685, L18877, U10340, U10339, L18920, U03735, M77481), BAGE (*e.g*, GenBank Acc. No U19180, see also U S Patent Nos. 5,683,886 and 5,571,711), GAGE (*e.g*, GenBank Acc Nos AF055475, AF055474, AF055473, U19147, U19146, U19145, U19144, U19143, U19142), any of the CTA class of receptors including in particular HOM-MEL-40 antigen encoded by the SSX2 gene (*e.g*, GenBank Acc. Nos X86175, U90842, U90841, X86174), carcinoembyonic antigen (CEA, Gold and Freedman,

1985 *J Exp. Med.* 121 439, *see also e.g.*, GenBank Acc Nos. SEG_HUMCEA, M59710, M59255, M29540), and PyLT (*e.g.*, GenBank Acc. Nos. J02289, J02038)

Additional cell surface receptors that may be sources of binding region or binding domain polypeptides or portions thereof, or that may be targets, including target antigens, include the following, or the like: CD2 (*e.g.*, GenBank Acc. Nos. Y00023, SEG_HUMCD2, M16336, M16445, SEG_MUSCD2, M14362), 4-1BB (CDw137, Kwon *et al.*, 1989 *Proc. Nat Acad. Sci. USA* 86 1963, 4-1BB ligand (Goodwin *et al.*, 1993 *Eur J Immunol* 23:2361, Melero *et al.*, 1998 *Eur J Immunol.* 3 116), CD5 (*e.g.*, GenBank Acc Nos X78985, X89405), CD10 (*e.g.*, GenBank Acc Nos M81591, X76732) CD27 (*e.g.*, GenBank Acc Nos M63928, L24495, L08096), CD28 (June *et al.*, 1990 *Immunol. Today* 11.211, see also, *e.g.*, GenBank Acc Nos. J02988, SEG_HUMCD28, M34563), CD152/CTLA-4 (*e.g.*, GenBank Acc Nos. L15006, X05719, SEG_HUMIGCTL), CD40 (*e.g.*, GenBank Acc. Nos. M83312, SEG_MUSC040A0, Y10507, X67878, X96710, U15637, L07414), interferon-γ (IFN-γ, see, *e.g.*, Farrar *et al* 1993 *Ann. Rev Immunol* 11 571 and references cited therein, Gray *et al* 1982 *Nature* 295 503, Rinderknecht *et al* 1984 *J Biol. Chem* 259 6790, DeGrado *et al* 1982 *Nature* 300 379), interleukin-4 (IL-4, see, e.g., *53rd Forum in Immunology*, 1993 *Research in Immunol* 144 553-643, Banchereau *et al.*, 1994 in *The Cytokine Handbook*, 2nd ed., A. Thomson, ed., Academic Press, NY, p 99, Keegan *et al.*, 1994 *J Leukocyt Biol.* 55 272, and references cited therein), interleukin-17 (IL-17) (*e.g.*, GenBank Acc. Nos. U32659, U43088) and interleukin-17 receptor (IL-17R) (*e.g.*, GenBank Acc Nos U31993, U58917) Notwithstanding the foregoing, the present invention expressly does not encompass any immunoglobulin fusion protein that is disclosed in U S 5,807,734, or U S 5,795,572

Additional cell surface receptors that may be sources of binding region or binding domain polypeptides or portions thereof, or that may serve as targets including target antigens or binding sites include the following, or the like CD59 (*e.g.*, GenBank Acc Nos SEG_HUMCD590, M95708, M34671), CD48 (*e.g.*, GenBank Acc. Nos M59904), CD58/LFA-3 (*e.g.*, GenBank Acc. No A25933, Y00636, E12817, see also JP 1997075090-A), CD72 (*e.g.*, GenBank Acc. Nos AA311036, S40777, L35772), CD70 (*e.g.*, GenBank Acc. Nos Y13636, S69339), CD80/B7 1 (Freeman *et al.*, 1989 *J Immunol* 43:2714, Freeman *et al.*, 1991 *J Exp Med.* 174 625, see also *e.g.*, GenBank Acc. Nos U33208, I683379), CD86/B7 2 (Freeman *et al.*, 1993 *J Exp. Med.* 178 2185, Borriello *et*

al., 1995 *J Immunol.* 155 5490, see also, *e.g.*, GenBank Acc. Nos. AF099105, SEG_MMB72G, U39466, U04343, SEG_HSB725, L25606, L25259), B7-H1/B7-DC (*e.g.*, Genbank Acc. Nos NM_014143, AF177937, AF317088, Dong *et al.*, 2002 *Nat. Med.* Jun 24 [epub ahead of print], PMID 12091876, Tseng *et al.*, 2001 *J Exp. Med.* 193 839, Tamura *et al.*, 2001 *Blood* 97 1809, Dong *et al.*, 1999 *Nat Med* 5 1365), CD40 ligand (*e.g.*, GenBank Acc. Nos SEG_HUMCD40L, X67878, X65453, L07414), IL-17 (*e.g.*, GenBank Acc Nos U32659, U43088), CD43 (*e.g.*, GenBank Acc. Nos X52075, J04536), ICOS (*e.g.*, Genbank Acc No AH011568), CD3 (*e.g.*, Genbank Acc. Nos. NM_000073 (gamma subunit), NM_000733 (epsilon subunit), X73617 (delta subunit)), CD4 (*e.g.*, Genbank Acc. No NM_000616), CD25 (*e.g* Genbank Acc No NM_000417), CD8 (*e.g.*, Genbank Acc No.M12828), CD11b (*e.g.*, Genbank Acc No J03925), CD14 (*e.g.*, Genbank Acc. No XM_039364), CD56 (*e.g.*, Genbank Acc No U63041), CD69 (*e.g* Genbank Acc No NM_001781) and VLA-4 ($\alpha_4\beta_7$) (*e.g.*, GenBank Acc. Nos L12002, X16983, L20788, U97031, L24913, M68892, M95632) The following cell surface receptors are typically associated with B cells. CD19 (*e.g.*, GenBank Acc. Nos SEG_HUMCD19W0, M84371, SEG_MUSCD19W, M62542), CD20 (*e.g.*, GenBank Acc. Nos. SEG_HUMCD20, M62541), CD22 (*e.g.*, GenBank Acc Nos. I680629, Y10210, X59350, U62631, X52782, L16928), CD30 (*e.g.*, Genbank Acc. Nos. M83554, D86042), CD153 (CD30 ligand, *e.g.*, GenBank Acc Nos L09753, M83554), CD37 (*e.g.*, GenBank Acc. Nos SEG_MMCD37X, X14046, X53517), CD50 (ICAM-3, *e.g.*, GenBank Acc No. NM_002162), CD106 (VCAM-1) (*e.g.*, GenBank Acc. Nos X53051, X67783, SEG_MMVCAM1C, *see also* US Patent No 5,596,090), CD54 (ICAM-1) (*e.g.*, GenBank Acc Nos X84737, S82847, X06990, J03132, SEG_MUSICAM0), interleukin-12 (see, *e.g.* Reiter *et al*, 1993 *Crit. Rev Immunol.* 13 1, and references cited therein), CD134 (OX40, *e.g.*, GenBank Acc No AJ277151), CD137 (4IBB, *e.g.*, GenBank Acc No L12964, NM_001561), CD83 (*e.g.*, GenBank Acc Nos AF001036, AL021918), DEC-205 (*e.g.*, GenBank Acc Nos AF011333, U19271)

Constructs, including binding domain-immunoglobulin fusion proteins, of the present invention comprise, for example, a binding domain, such as a binding domain polypeptide that, according to certain particularly preferred embodiments, is capable of binding or specifically binding at least one target, for example, a target antigen or other binding site that is present on an immune effector cell. According to non-limiting theory,

such constructs, including for example binding domain- immunoglobulin fusion proteins, may advantageously recruit desired immune effector cell function(s) in a therapeutic context, where it is well known that immune effector cells having different specialized immune functions can be identified or distinguished from one another on the basis of their differential expression of a wide variety of cell surface antigens, including many of the antigens described herein to which constructs of the invention including binding domain polypeptides can specifically bind. As noted herein, immune effector cells include any cell that is capable of directly mediating an activity that is a component of immune system function, including cells having such capability naturally or as a result of genetic engineering.

In certain embodiments an immune effector cell comprises a cell surface receptor for an immunoglobulin or other peptide binding molecule, such as a receptor for an immunoglobulin constant region and including the class of receptors commonly referred to as "Fc receptors" ("FcR"s) A number of FcRs have been structurally and/or functionally characterized and are well known in the art, including FcR having specific abilities to interact with a restricted subset of immunoglobulin heavy chain isotypes, or that interact with Fc domains with varying affinities, and/or which may be expressed on restricted subsets of immune effector cells under certain conditions (*e.g.*, Kiyimoto-Ochichai *et al.*, 2002 *Cell Mol. Life Sci.* 59:648, Davis *et al.*, 2002 *Curr Top Microbiol. Immunol.* 266 85, Pawankar, 2001 *Curr Opin. Allerg Clin. Immunol.* 1 3, Radaev *et al.*, 2002 *Mol Immunol.* 38 1073, Wurzburg *et al.*, 2002 *Mol. Immunol.* 38 1063, Suhca *et al.*, 2001 *Int Rev Immunol.* 20.371, Underhill *et al.*, 2002 *Ann. Rev Immunol.* 20 825, Coggeshall, 2002 *Curr Dir Autoimm* 5 1, Mimura *et al.*, 2001 *Adv Exp Med. Biol* 495 49, Baumann *et al.*, 2001 *Adv Exp Med. Biol* 495 219, Santoso *et al.*, 2001 *Ital. Heart J* 2 811, Novak *et al.*, 2001 *Curr Opin. Immunol.* 13 721, Fossati *et al.*, 2001 *Eur J Clin. Invest* 31 821)

Cells that are capable of mediating ADCC are preferred examples of immune effector cells according to the present invention. Other preferred examples include Natural Killer cells, tumor-infiltrating T lymphocytes (TILs), cytotoxic T lymphocytes, and granulocytic cells such as cells that comprise allergic response mechanisms Immune effector cells thus include, but are not limited to, cells of hematopoietic origins including cells at various stages of differentiation within myeloid

and lymphoid lineages and which may (but need not) express one or more types of functional cell surface FcR, such as T lymphocytes, B lymphocytes, NK cells, monocytes, macrophages, dendritic cells, neutrophils, basophils, eosinophils, mast cells, platelets, erythrocytes, and precursors, progenitors (*e.g.*, hematopoietic stem cells), as well as quiescent, activated, and mature forms of such cells. Other immune effector cells may include cells of non-hematopoietic origin that are capable of mediating immune functions, for example, endothelial cells, keratinocytes, fibroblasts, osteoclasts, epithelial cells, and other cells. Immune effector cells may also include cells that mediate cytotoxic or cytostatic events, or endocytic, phagocytic, or pinocytotic events, or that effect induction of apoptosis, or that effect microbial immunity or neutralization of microbial infection, or cells that mediate allergic, inflammatory, hypersensitivity and/or autoimmune reactions.

Allergic response mechanisms are well known in the art and include an antigen (*e.g.*, allergen)-specific component such as an immunoglobulin (*e.g.*, IgE), as well as the cells and mediators which comprise sequelae to allergen-immunoglobulin (*e.g.*, IgE) encounters (*e.g.*, Ott *et al.*, 2000 *J. Allerg. Clin. Immunol.* 106:429, Barnes, 2000 *J. Allerg. Clin. Immunol.* 106:5, Togias, 2000 *J. Allerg. Clin. Immunol.* 105:S599, Akdis *et al.*, 2000 *Int. Arch. Allerg. Immunol.* 121:261, Beach, 2000 *Occup. Med.* 15:455). Particularly with regard to constructs, including binding domain-immunoglobulin fusion proteins, of the present invention that interact with FcR, certain embodiments of the present invention contemplate constructs including fusion proteins that comprise one or more IgE-derived domains including, for example, those that are capable of inducing an allergic response mechanism that comprises IgE-specific FcR, or portions thereof, which IgE-specific FcRs include those noted above and described or identified in the cited articles. Without wishing to be bound by particular theory or mechanism, and as disclosed herein, constructs, including fusion proteins, of the present invention may comprise portions of IgE heavy chain Fc domain polypeptides, for example, native or engineered IgE CH3 and CH4 domains, whether provided or expressed as cell surface proteins (*e.g.*, with a plasma membrane anchor domain) or as soluble or otherwise not cell-bound proteins (*e.g.*, without a plasma membrane anchor domain). Further according to non-limiting theory, recruitment and induction of an allergic response mechanism (*e.g.*, an FcR-epsilon expressing immune effector cell) may proceed as the result of either or both of the presence of an IgE Fc domain or portion thereof as described herein (*e.g.*, one that is capable of triggering an

allergic mechanism by FcR crosslinking) and the presence of a target such as a antigen to which the binding region, for example a binding domain, binds or specifically binds. The present invention therefore exploits induction of allergic response mechanisms in heretofore unappreciated contexts, such as treatment of a malignant condition or a B cell disorder, including those described or referenced herein

An immunoglobulin hinge region polypeptide includes any hinge peptide or polypeptide that occurs naturally, as an artificial peptide or as the result of genetic engineering and that is situated, for example, in an immunoglobulin heavy chain polypeptide between the amino acid residues responsible for forming intrachain immunoglobulin-domain disulfide bonds in CH1 and CH2 regions Hinge region polypeptides for use in the present invention may also include a mutated or otherwise altered hinge region polypeptide. Accordingly, for example, an immunoglobulin hinge region polypeptide may be derived from, or may be a portion or fragment of (i.e., one or more amino acids in peptide linkage, typically about 15-115 amino acids, preferably about 95-110, about 80-94, about 60-80, or about 5-65 amino acids, preferably about 10-50, more preferably about 15-35, still more preferably about 18-32, still more preferably about 20-30, still more preferably about 21, 22, 23, 24, 25, 26, 27, 28 or 29 amino acids) an immunoglobulin polypeptide chain region classically regarded as having hinge function, including those described herein, but a hinge region polypeptide for use in the instant invention need not be so restricted and may include one or more amino acids situated (according to structural criteria for assigning a particular residue to a particular domain that may vary, as known in the art) in an adjoining immunoglobulin domain such as a CH1 domain and/or a CH2 domain in the cases of IgG, IgA and IgD (or in an adjoining immunoglobulin domain such as a CH1 domain and/or a CH3 domain in the case of IgE), or in the case of certain artificially engineered immunoglobulin constructs, an immunoglobulin variable region domain.

Wild-type immunoglobulin hinge region polypeptides include any known or later-discovered naturally occurring hinge region that is located between the constant region domains, CH1 and CH2, of an immunoglobulin, for example, a human immunoglobulin (or between the CH1 and CH3 regions of certain types of immunoglobulins, such as IgE). For use in constructing one type of connecting region, the wild-type immunoglobulin hinge region polypeptide is preferably a human

immunoglobulin hinge region polypeptide, preferably comprising a hinge region from a human IgG, IgA, or IgD immunoglobulin (or the CH2 region of an IgE immunoglobulin), and more preferably, for example, a hinge region polypeptide from a wild-type or mutated human IgG1 isotype as described herein.

As is known to the art, despite the tremendous overall diversity in immunoglobulin amino acid sequences, immunoglobulin primary structure exhibits a high degree of sequence conservation in particular portions of immunoglobulin polypeptide chains, notably with regard to the occurrence of cysteine residues which, by virtue of their sulfyhydryl groups, offer the potential for disulfide bond formation with other available sulfydryl groups. Accordingly, in the context of the present invention wild-type immunoglobulin hinge region polypeptides for use as connecting regions include those that feature one or more highly conserved (*e.g.*, prevalent in a population in a statistically significant manner) cysteine residues, and in certain preferred embodiments a connecting region may comprise, or consist essentially of, or consist of, a mutated hinge region polypeptide may be selected that contains less than the number of naturally-occurring cysteines, for example, zero or one or two cysteine residue(s) in the case of IgG1 and IgG4 hinge regions, and that is derived or constructed from (or using) such a wild-type hinge region sequence.

In certain preferred embodiments wherein the connecting region is a hinge region polypeptide and the hinge region polypeptide is a mutated, engineered or otherwise altered human IgG1 immunoglobulin hinge region polypeptide that is derived or constructed from (or using) a wild-type hinge region sequence, it is noted that the wild-type human IgG1 hinge region polypeptide sequence comprises three non-adjacent cysteine residues, referred to as a first cysteine of the wild-type hinge region, a second cysteine of the wild-type hinge region and a third cysteine of the wild-type hinge region, respectively, proceeding along the hinge region sequence from the polypeptide N-terminus toward the C-terminus. This can be referred to herein as a "CCC" hinge (or a "WTH", *i.e.*, a wild-type hinge). Examples of mutated or engineered hinge regions include those with no cysteines, which may be referred to herein as an "XXX" hinge (or, for example, as "MH-XXX," referring to a mutant or engineered hinge with three amino acids or other molecules in place of naturally occurring cysteines, such as, for example, "MH-SSS", which refers to a mutant hinge with three serine residues in place of the naturally occurring cysteine

residues It will be understood that the term "mutant" refers only to the fact that a different molecule or molecules is present, or no molecule, at the position of a naturally occurring residue and does not refer to any particular method by which such substitution, alteration, or deletion has been carried out. Accordingly, in certain embodiments of the present invention, the connecting region may be a hinge region polypeptide and the hinge region polypeptide is a mutated human IgG1 immunoglobulin hinge region polypeptide that contains two cysteine residues and in which the first cysteine of the wild-type hinge region has not changed or deleted, for example This can be referred to as a "MH-CXX" hinge, for example, a "MH-CSC" hinge, in which case the cysteine residue has been replaced with a serine residue. In certain other embodiments of the present invention the mutated human IgG1 immunoglobulin hinge region polypeptide contains no more than one cysteine residue and include, for example, a "MH-CSS" hinge or a "MH-SSC" hinge or a "MH-CSC" hinge, and in certain other embodiments the mutated human IgG1 immunoglobulin hinge region polypeptide contains no cysteine residues such as, for example, a "MH-SSS" hinge.

The constructs, including binding domain-immunoglobulin fusion proteins, of the present invention expressly do not contemplate any fusion protein that is disclosed in U.S Patent No 5,892,019 U S Patent No 5,892,019 refers to a human IgG1 hinge region in which the first IgG1 hinge region cysteine residue has been changed or deleted, but retains both of the second and third IgG1 hinge region cysteine residues that correspond to the second and third cysteines of the wild-type IgG1 hinge region sequence. The patent states that the first cysteine residue of the wild-type IgG1 hinge region is replaced to prevent interference by the first cysteine residue with proper assembly of the polypeptide described therein into a dimer The patent requires that the second and third cysteines of the IgG1 hinge region be retained to provide interchain disulfide linkage between two heavy chain constant regions to promote dimer formation so that the molecule contains has effector function such as the ability to mediate ADCC

By contrast and as described herein, the constructs, including the binding domain-immunoglobin fusion proteins, of the present invention, various of which are capable of ADCC, CDC and/or complement fixation, for example, are not so limited and may comprise, in pertinent part, for example, (i) a wild-type immunoglobulin hinge region polypeptide, such as a wild-type human immunoglobulin hinge region polypeptide, for

example, a human IgG1 immunoglobulin hinge region polypeptide, (ii) a mutated or otherwise altered immunoglobulin hinge region polypeptide, such as a mutated or otherwise altered human immunoglobulin hinge region polypeptide, for example, a mutated or otherwise altered human IgG1 immunoglobulin hinge region polypeptide that, for example, is or has been derived or constructed from (or using) a wild-type immunoglobulin hinge region polypeptide or nucleic acid sequence having three or more cysteine residues, wherein the mutated or otherwise altered human IgG1 immunoglobulin hinge region polypeptide contains two cysteine residues and wherein a first cysteine of the wild-type hinge region is not mutated or deleted, (iii) a mutated or otherwise altered immunoglobulin hinge region polypeptide, such as a mutated or otherwise altered human immunoglobulin hinge region polypeptide, for example, a mutated or otherwise altered human IgG1 immunoglobulin hinge region polypeptide that, for example, is or has been derived or constructed from (or using) a wild-type immunoglobulin hinge region polypeptide or nucleic acid sequence having three or more cysteine residues, wherein the mutated or otherwise altered human IgG1 immunoglobulin hinge region polypeptide contains no more than one cysteine residue, or (iv) a mutated or otherwise altered immunoglobulin hinge region polypeptide, such as a mutated or otherwise altered human immunoglobulin hinge region polypeptide, for example, a mutated or otherwise altered human IgG1 immunoglobulin hinge region polypeptide that is or has been derived or constructed from (or using) a wild-type immunoglobulin hinge region polypeptide or nucleic acid sequence having three or more cysteine residues, wherein the mutated or otherwise altered (for example, by amino acid change or deletion) human IgG1 immunoglobulin hinge region polypeptide contains no cysteine residues The present invention offers unexpected advantages associated with retention by the constructs, including the fusion proteins, described herein of the ability to mediate ADCC and/or CDC and/or complement fixation notwithstanding that the ability to dimerize via IgG1 hinge region interchain disulfide bonds is ablated or compromised by the removal or replacement of one, two or three hinge region cysteine residues, and even in constructs where the first cysteine of an IgG1 hinge region, for example, is not mutated or otherwise altered or deleted

A connecting region may comprise a mutated or otherwise altered immunoglobulin hinge region polypeptide, which itself may comprise a hinge region that

has its origin in an immunoglobulin of a species, of an immunoglobulin isotype or class, or of an immunoglobulin subclass that is different from that of the tail region, for example, a tail region comprising, or consisting essentially or, or consisting of, CH2 and CH3 domains (or IgE CH3 and CH4 domains) For instance, in certain embodiments of the invention, a construct, for example, a binding domain-immunoglobulin fusion protein, may comprise a binding region such as a binding domain polypeptide that is fused or otherwise connected to an immunoglobulin hinge region polypeptide comprising, or consisting essentially of, or consisting of, a wild-type human IgA hinge region polypeptide, or a mutated or otherwise altered human IgA hinge region polypeptide that contains zero or only one or more cysteine residues (but less than the wild-type number of cysteines), as described herein, or a wild-type human IgG hinge, such as an IgG1 hinge, region polypeptide, or a wild-type human IgE hinge-acting region, *i.e.*, IgE CH2 region polypeptide, or a mutated or otherwise altered human IgG hinge, such as an IgG1 hinge, region polypeptide that is or has been mutated or otherwise altered to contain zero, one or two cysteine residues wherein the first cysteine of the wild-type hinge region is not mutated or altered or deleted, as also described herein. Such a hinge region polypeptide may be fused or otherwise connected to, for example, a tail region comprising, or consisting essentially of, or consisting of, an immunoglobulin heavy chain CH2 region polypeptide from a different Ig isotype or class, for example an IgA or an IgD or an IgG subclass (or a CH3 region from an IgE subclass), which in certain preferred embodiments will be the IgG1 or IgA or IgE subclass and in certain other preferred embodiments may be any one of the IgG2, IgG3 or IgG4 subclasses.

For example, and as described in greater detail herein, in certain embodiments of the present invention a connecting region may be selected to be an immunoglobulin hinge region polypeptide, which is or has been derived from a wild-type human IgA hinge region that naturally comprises three cysteines, where the selected hinge region polypeptide is truncated or otherwise altered or substituted relative to the complete and/or naturally-occurring hinge region such that only one or two of the cysteine residues remain (*e.g.*, SEQ ID NOS 35-36) Similarly, in certain other embodiments of the invention, the construct may be binding domain-immunoglobulin fusion protein comprising a binding domain polypeptide that is fused or otherwise connected to an immunoglobulin hinge region polypeptide comprising a mutated or otherwise altered hinge region polypeptide in which the number of cysteine residues is reduced by amino acid

substitution or deletion, for example a mutated or otherwise altered IgG1 hinge region containing zero, one or two cysteine residues as described herein, or an IgD hinge region containing zero cysteine residues.

A mutated or otherwise altered hinge region polypeptide may thus be derived or constructed from (or using) a wild-type immunoglobulin hinge region that contains one or more cysteine residues. In certain embodiments, a mutated or otherwise altered hinge region polypeptide may contain zero or only one cysteine residue, wherein the mutated or otherwise altered hinge region polypeptide is or has been derived from a wild type immunoglobulin hinge region that contains, respectively, one or more or two or more cysteine residues In the mutated or otherwise altered hinge region polypeptide, the cysteine residues of the wild-type immunoglobulin hinge region are preferably deleted or substituted with amino acids that are incapable of forming a disulfide bond In one embodiment of the invention, a mutated or otherwise altered hinge region polypeptide is or has been derived from a human IgG wild-type hinge region polypeptide, which may include any of the four human IgG isotype subclasses, IgG1, IgG2, IgG3 or IgG4. In certain preferred embodiments, the mutated or otherwise altered hinge region polypeptide is or has been derived from (or using) a human IgA or IgD wild-type hinge region polypeptide. By way of example, a mutated or otherwise altered hinge region polypeptide that is or has been derived from a human IgG1 or IgA wild-type hinge region polypeptide may comprise mutations, alterations, or deletions at two of the three cysteine residues in the wild-type immunoglobulin hinge region, or mutations, alterations, or deletions at all three cysteine residues

The cysteine residues that are present in a wild-type immunoglobulin hinge region and that are removed or altered by mutagenesis or any other techniques according to particularly preferred embodiments of the present invention include cysteine residues that form, or that are capable of forming, interchain disulfide bonds Without wishing to be bound by particular theory or mechanism of action, the present invention contemplates that mutation, deletion, or other alteration of such hinge region cysteine residues, which are believed to be involved in formation of interchain disulfide bridges, reduces the ability of the subject invention binding domain-immunoglobulin fusion protein to dimerize (or form higher oligomers) via interchain disulfide bond formation, while surprisingly not ablating or undesireably compromising the ability of a fusion protein or other construct to promote

ADCC, and/or CDC and/or to fix complement. In particular, the Fc receptors that mediate ADCC (*e.g.*, FcRIII, CD16) exhibit low affinity for immunoglobulin Fc domains, supporting the idea that functional binding of Fc to FcR requires avidity stabilization of the Fc-FcR complex by virtue of the dimeric structure of heavy chains in a conventional antibody, and/or FcR aggregation and cross-linking by a conventional antibody Fc structure. Sondermann *et al*, 2000 *Nature* 406:267, Radaev *et al*, 2001 *J Biol. Chem* 276 16469; Radaev *et al*, 2001 *J Biol. Chem* 276 16478, Koolwijk *et al*, 1989 *J Immunol.* 143 1656, Kato *et al*, 2000 *Immunol Today* 21.310 Hence, the constructs, including for example binding domain-immunoglobulin fusion proteins, of the present invention provide the advantages associated with single-chain constructs including single-chain immunoglobulin fusion proteins while also unexpectedly retaining one or more immunological activities. Similarly, the ability to fix complement is typically associated with immunoglobulins that are dimeric with respect to heavy chain constant regions such as those that comprise Fc, while various constructs, including binding domain-immunoglobulin fusion proteins, of the present invention, which may, due to the replacement or deletion of hinge region cysteine residues or due to other structural modifications as described herein, for example, have compromised or ablated abilities to form interchain disulfide bonds, exhibit the unexpected ability to fix complement. Additionally, according to certain embodiments of the present invention wherein a construct, including, for example, a binding domain-immunoglobulin fusion protein, may comprise a connecting region and tail region comprising, or consisting essentially of, or consisting of, one or more of a human IgE hinge-acting region, *i.e.*, a IgE CH2 region polypeptide, a human IgE CH3 constant region polypeptide, and a human IgE CH4 constant region polypeptide, the invention constructs including fusion proteins unexpectedly retain the immunological activity of mediating ADCC and/or of inducing an allergic response mechanism

Selection of an immunoglobulin hinge region polypeptide as a connecting region according to certain embodiments of the subject invention constructs, such as binding domain-immunoglobulin fusion proteins, may relate to the use of an "alternative hinge region" polypeptide sequence, which includes a polypeptide sequence that is not necessarily derived from any immunoglobulin hinge region sequence *per se* Instead, an alternative hinge region refers to a hinge region polypeptide that comprises an amino acid

sequence, or other molecular sequence, of at least about ten consecutive amino acids or molecules, and in certain embodiments at least about 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21-25, 26-30, 31-50, 51-60, 71-80, 81-90, or 91-110 amino acids or molecules that is present in a sequence selected from any one of SEQ ID NOS __-__ , for example a polypeptide sequence that is or has been derived from a region located between intrachain disulfide-generated immunoglobulin-like loop domains of immunoglobulin gene superfamily members such as CD2 (*e.g.*, Genbank Acc. No NM_001767), CD4 (*e.g.*, Genbank Acc. No NM_000616), CD5 (*e.g.*, Genbank Acc. No BC027901), CD6 (*e.g.*, Genbank Acc. No NM_006725), CD7 (*e.g.*, Genbank Acc Nos. XM_046782, BC009293, NM_006137) or CD8 (*e.g.*, Genbank Acc. No M12828), or other Ig superfamily members. By way of non-limiting example, an alternative hinge region used as a connecting region, for example, may provide a glycosylation site as provided herein, or may provide a human gene-derived polypeptide sequence for purposes of enhancing the degree of "humanization" of a fusion protein, or may comprise, or consist essentially of, or consist of, an amino acid sequence that eliminates or reduces the ability of a construct of the invention, such as a fusion protein, to form multimers or oligomers or aggregates or the like. Certain alternative hinge region polypeptide sequences, including those described herein, may be derived or constructed from (or using) the polypeptide sequences of immunoglobulin gene superfamily members that are not actual immunoglobulins *per se*. For instance and according to non-limiting theory, certain polypeptide sequences that are situated between intrachain disulfide-generated immunoglobulin loop domain of immunoglobulin gene super-family member proteins may be used in whole or in part as alternative hinge region polypeptides as provided herein, or may be further modified for such use.

As noted above, the constructs of the invention, including binding domain-immunoglobulin fusion proteins, are believed, according to non-limiting theory, to be compromised in their ability to dimerize via interchain disulfide bond formation, and further according to theory, this property is a consequence, in whole or in part, of a reduction in the number of cysteine residues that are present in an immunoglobulin hinge region polypeptide selected for inclusion in the construction of the construct, such as a fusion protein construct. Determination of the relative ability of a polypeptide to dimerize is well within the knowledge of the relevant art, where any of a number of established

methodologies may be applied to detect protein dimerization (see, *e.g.*, Scopes, *Protein Purification Principles and Practice*, 1987 Springer-Verlag, New York) For example, biochemical separation techniques for resolving proteins on the basis of molecular size (*e.g.*, gel electrophoresis, gel filtration chromatography, analytical ultracentrifugation, *etc.*), and/or comparison of protein physicochemical properties before and after introduction of sulfhydryl-active (*e.g.*, iodoacetamide, N-ethylmaleimide) or disulfide-reducing (*e.g.*, 2-mercaptoethanol, dithiothreitol) agents, or other equivalent methodologies, may all be employed for determining a degree of polypeptide dimerization or oligomerization, and for determining possible contribution of disulfide bonds to such potential quarternary structure. In certain embodiments, the invention relates to a construct, for example a binding domain-immunoglobulin fusion protein, that exhibits a reduced (i.e., in a statistically significant manner relative to an appropriate IgG-derived control, for example) ability to dimerize, relative to a wild-type human immunoglobulin G hinge region polypeptide as provided herein. Those familiar with the art will be able readily to determine whether a particular fusion protein displays such reduced ability to dimerize.

Compositions and methods for preparation of immunoglobulin fusion proteins, for example, are well known in the art. See, *e.g.*, U S Patent No 5,892,019, which reports recombinant proteins that are the product of a single encoding polynucleotide but which are not constructs, including binding domain-immunoglobulin fusion proteins, according to the present invention.

For a construct, for example, in an immunoglobulin fusion protein of the invention that is intended for use in humans, any included Ig constant regions will typically be of human sequence origin, or humanized, to minimize a potential anti-human immune response and to provide appropriate and/or desired effector functions. Manipulation of sequences encoding antibody constant regions is referenced in the PCT publication of Morrison and Oi, WO 89/07142 In particularly preferred embodiments, a tail region is prepared from an immunoglobulin heavy chain constant region in which the CH1 domain is or has been deleted (the CH1 and CH2 regions in the case of IgE) and the carboxyl end of the binding domain, or where the binding domain comprises two immunoglobulin variable region polypeptides, the second (i.e., more proximal to the C-terminus) variable region is joined to the amino terminus of CH2 through one or more connecting regions, such as a hinge or altered region. A schematic diagram depicting the structures of two

exemplary binding domain-immunoglobulin fusion proteins is shown in FIG 11 In particularly preferred embodiments no interchain disulfide bonds are present, and in other embodiments a restricted number of interchain disulfide bonds may be present relative to the number of such bonds that would be present if wild-type hinge region polypeptides were instead present. In other embodiments a construct of the invention, such as for example, a fusion protein, comprises, or consists essentially of, or consists of, a mutated or otherwise altered hinge region polypeptide that exhibits a reduced ability to dimerize, relative to a wild-type human IgG hinge region polypeptide. Thus, an isolated polynucleotide molecule coding for such a single chain construct, such as an immunoglobulin fusion protein, has a binding region, for example, a domain that provides specific or otherwise desired binding affinity and selectivity for a target, such as a target antigen.

The invention also contemplates, for example, in certain embodiments, constructs including binding domain-immunoglobulin fusion proteins that comprise fused or otherwise connected polypeptide sequences or portions thereof derived or prepared from a plurality of genetic sources, for example, according to molecular "domain swapping" paradigms. Those having familiarity with the art will appreciate that selection of such polypeptide sequences for assembly into a coinstruct, such as a binding domain-immunoglobulin fusion protein, for example, may involve determination of appropriate portions of each component polypeptide sequence, for example, based on structural and/or functional properties of each such sequence (see, *e.g.*, Carayannopoulos *et al.*, 1996 *J Exp. Med.* 183 1579, Harlow *et al.*, Eds., *Antibodies A Laboratory Manual*, Cold Spring Harbor Laboratory, Cold Spring Harbor (1988)) The component polypeptide sequences of which the construct, such as a fusion protein, is comprised or prepared may therefore comprise intact or full-length binding domain, immunoglobulin, linker and/or plasma membrane anchor domain polypeptide sequences, or truncated versions or variants thereof such as those provided herein. According to these and related embodiments of the invention, any two or more of the candidate component polypeptides of which the subject invention constructs, for example, fusion proteins, may be comprised will be derived or prepared from independent sources, such as from immunoglobulin sequences of differing allotype, isotype, subclass, class, or species of origin (*e.g.*, xenotype). Thus, as a non-limiting example, a binding domain polypeptide (or its constituent polypeptides such as

one or more variable region polypeptides and/or a linker polypeptide), a hinge region polypeptide, immunoglobulin heavy chain CH2 and CH3 constant region polypeptides and optionally an immunoglobulin heavy chain CH4 constant region polypeptide as may be obtained from an IgM or IgE heavy chain, and a plasma membrane anchor domain polypeptide may all be separately obtained from distinct genetic sources and engineered into a chimeric or fusion protein using well known techniques and according to methodologies described herein, for example.

Accordingly, a construct of the invention, for example a binding domain-immunoglobulin fusion protein according to certain embodiments of the present invention, may also therefore comprise in pertinent part an immunoglobulin heavy chain CH3 constant region polypeptide that is a wild-type IgA CH3 constant region polypeptide, or alternatively, that is a mutated or otherwise altered or substituted or truncated IgA CH3 constant region polypeptide that is incapable of associating with a J chain, or that will not associate to an undesired degree with a J chain, preferably the IgA CH3 constant region polypeptides used in a tail region portion of a construct are of human origin or are humanized. By way of brief background, IgA molecules are known to be released into secretory fluids by a mechanism that involves association of IgA into disulfide-linked polymers (*e.g* dimers) via a J chain polypeptide (*e.g*, Genbank Acc. Nos XM_059628, M12378, M12759, Johansen *et al*, 1999 *Eur J Immunol.* 29 1701) and interaction of the complex so formed with another protein that acts as a receptor for polymeric immunoglobulin, and which is known as transmembrane secretory component (SC, Johansen *et al*, 2000 *Sc. J Immunol.* 52.240; see also, *e.g*, Sorensen *et al*, 2000 *Int. Immunol.* 12 19, Yoo *et al*, 1999 *J Biol. Chem* 274 33771, Yoo *et al*, 2002 *J Immunol. Meth* 261 1, Corthesy, 2002 *Trends Biotechnol.* 20 65, Symersky *et al*, 2000 *Mol Immunol.* 37 133, Crottet *et al*, 1999 *Biochem.* J 341 299) Interchain disulfide bond formation between IgA Fc domains and J chain is mediated through a penultimate cysteine residue in an 18-amino acid C-terminal extension that forms part of the IgA heavy chain constant region CH3 domain polypeptide (Yoo *et al*, 1999, Sorensen *et al*, 2000) Certain embodiments of the subject invention constructs, including for example, fusion proteins, therefore contemplate inclusion of the wild-type IgA heavy chain constant region polypeptide sequence, which is capable of associating with J chain. Certain other embodiments of the invention, however, contemplate fusion proteins that comprise a

mutated or otherwise altered, substituted, or truncated IgA CH3 constant region polypeptide that is incapable of associating with a J chain. According to such embodiments, for example, two or more residues from the C-terminus of an IgA CH3 constant region polypeptide such as a human IgA CH3 constant region polypeptide may be deleted to yield a truncated CH3 constant region polypeptide as provided herein. In preferred embodiments and as described in greater detail herein, a mutated human IgA CH3 constant region polypeptide that is incapable of associating with a J chain comprises such a C-terminal deletion of either four or 18 amino acids However, the invention need not be so limited, such that the mutated IgA CH3 constant region polypeptide may comprise a deletion of about 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20,, 21-25, 26-30 or more amino acids, so long as the construct, for example, the fusion protein, is capable of specifically binding an antigen and of at least one immunological activity as provided herein. Alternatively, the invention also contemplates constructs, for example, fusion proteins, having a tail region that comprises a mutated IgA CH3 constant region polypeptide that is incapable of associating with a J chain by virtue of replacement of the penultimate cysteine, or by chemical modification of that amino acid residue, in a manner that prevents, or inhibits an undesired level of, interchain disulfide bond or multimer formation. Methods for determining whether a construct, for example a fusion protein, can associate with a J chain will be known to those having familiarity with the art and are described or referenced herein

As also described herein and according to procedures known in the art, the construct, for example a fusion protein, may further be tested routinely for immunological activity, for instance, in ADCC or CDC assays As an illustrative example, a construct, for example a fusion protein, according to such an embodiment may comprise a binding domain polypeptide derived or constructed from (or using) a native or engineered human heavy chain variable region polypeptide sequence, a native or engineered human IgA-derived immunoglobulin hinge region polypeptide sequence, a native or engineered human IgG1 immunoglobulin heavy chain CH2 constant region polypeptide sequence, a native or engineered human IgG2 immunoglobulin heavy chain CH3 constant region polypeptide sequence, and optionally a native or engineered human IgE immunoglobulin heavy chain CH4 constant region polypeptide sequence and/or a native or engineered human TNF-α receptor type 1 (TNFR1) plasma membrane anchor domain polypeptide sequence that

comprises a cytoplasmic tail polypeptide which is capable of apoptotic signaling or otherwise promoting apoptosis. The invention therefore contemplates these and other embodiments according to the present invention in which two or more polypeptide sequences that are present in a construct, for example a fusion protein, have independent genetic origins.

As noted above, in certain embodiments the construct, for example a binding protein-immunoglobulin fusion protein, comprises at least one native or engineered immunoglobulin variable region polypeptide, which may be a native or engineered light chain or a native or engineered heavy chain variable region polypeptide, and in certain embodiments the fusion protein comprises at least one such native or engineered light chain V-region and one such native or engineered heavy chain V-region and at least one linker peptide that is fused or otherwise connected to to each of the native or engineered V-regions Construction of such binding domains, for example single chain Fv domains, is known in the art and is described in greater detail in the Examples below, and has been described, for example, in various documents cited herein, selection and assembly of single-chain variable regions and of linker polypeptides that may be fused or otherwise connected to each of a heavy chain-derived and a light chain-derived V region (*e.g.*, to generate a binding region, such as a binding domain that comprises a single-chain Fv polypeptide) is also known to the art and described herein. See, *e.g.*, U S Patent Nos. 5,869,620, 4,704,692, and 4,946,778 In certain embodiments all or a portion or portions of an immunoglobulin sequence that is derived from a non-human source may be "humanized" according to recognized procedures for generating humanized antibodies, *i.e.*, immunoglobulin sequences into which human Ig sequences are introduced to reduce the degree to which a human immune system would perceive such proteins as foreign (see, *e.g.*, U S Patent Nos 5,693,762, 5,585,089, 4,816,567, 5,225,539, 5,530,101, and documents cited therein)

Constructs of the invention, including binding domain-immunoglobulin fusion proteins, as described herein may, according to certain embodiments, desirably comprise sites for glycosylation, *e.g.*, covalent attachment of carbohydrate moieties such as, for example, monosaccharides or oligosaccharides Incorporation of amino acid sequences that provide substrates for polypeptide glycosylation is within the scope of the relevant art, including, for example, the use of genetic engineering or protein engineering

methodologies to obtain a polypeptide sequence containing, for example, the classic Asn-X-Ser/Thr site for N-(asparagine)-linked glycosylation, or a sequence containing Ser or Thr residues that are suitable substrates for O-linked glycosylation, or sequences amenable to C-mannosylation, glypiation/glycosylphosphatidylinositol modification, or phosphoglycation, all of which can be identified according to art-established criteria (*e.g.*, Spiro, 2002 *Glycobiology* 12.43R) Without wishing to be bound by any particular theory or mechanism, glycosylated constructs such as fusion proteins having particular amino acid sequences may beneficially possess attributes associated with one or more of improved solubility, enhanced stability in solution, enhanced physiological stability, improved bioavailability including in vivo biodistribution, and superior resistance to proteases, all in a statistically significant manner, relative to constructs, including fusion proteins, having the same or highly similar amino acid sequences but lacking glycosyl moieties. In certain preferred embodiments the subject invention constructs, such as fusion protein constructs, may comprise a glycosylation site that is present in a linker as provided herein, and in certain other preferred embodiments the subject invention construct, for example, a fusion protein, comprises a glycosylation site that is present in a connecting region, such as a hinge region polypeptide sequence as provided herein.

In certain preferred embodiments of the present invention, such as those useful for gene therapy applications or in display systems or assays, such as screening assays (including library display systems and library screening assays), the construct, for example, a binding domain-immunoglobulin fusion protein, is a protein or glycoprotein that is capable of being expressed by a host cell such that it localizes to the cell surface. Constructs, such as binding domain-immunoglobulin fusion proteins, that localize to the cell surface may do so by virtue of having naturally present or artificially introduced structural features that direct the fusion protein to the cell surface (*e.g.*, Nelson *et al* 2001 *Trends Cell Biol* 11.483, Ammon *et al*, 2002 *Arch. Physiol Biochem* 110 137, Kasai *et al*, 2001 *J Cell Sci* 114 3115, Watson *et al*, 2001 *Am J Physiol. Cell Physiol* 281 C215, Chatterjee *et al*, 200 *J Biol Chem* 275 24013) including by way of illustration and not limitation, secretory signal sequences, leader sequences, plasma membrane anchor domain polypeptides and transmembrane domains such as hydrophobic transmembrane domains (*e.g.*, Heuck *et al*, 2002 *Cell Biochem Biophys* 36 89; Sadiiah *et al*, 2002 *Biochem J* 364 777, Phoenix *et al*, 2002 *Mol. Membr Biol.* 19 1, Minke *et al*, 2002 *Physiol. Rev*

82.429) or glycosylphosphatidylinositol attachment sites ("glypiation" sites, *e.g.*, Chatterjee *et al.*, 2001 *Cell Mol. Life Sci.* 58 1969, Hooper, 2001 *Proteomics* 1 748, Spiro, 2002 *Glycobiol.* 12 43R), cell surface receptor binding domains, extracellular matrix binding domains, or any other structural feature that causes at least a desired portion of the fusion protein population to localize, in whole or in part, to the cell surface. Particularly preferred are fusion protein constructs that comprise a plasma membrane anchor domain which includes a transmembrane polypeptide domain, typically comprising a membrane spanning domain which includes a hydrophobic region capable of energetically favorable interaction with the phospholipid fatty acyl tails that form the interior of the plasma membrane bilayer. Such features are known to those of ordinary skill in the art, who will further be familiar with methods for introducing nucleic acid sequences encoding these features into the subject expression constructs by genetic engineering, and with routine testing of such constructs to verify cell surface localization of the product.

According to certain further embodiments, a plasma membrane anchor domain polypeptide comprises such a transmembrane domain polypeptide and also comprises a cytoplasmic tail polypeptide, which refers to a region or portion of the polypeptide sequence that contacts the cytoplasmic face of the plasma membrane and/or is in contact with the cytosol or other cytoplasmic components. A large number of cytoplasmic tail polypeptides are known that comprise the intracellular portions of plasma membrane transmembrane proteins, and discrete functions have been identified for many such polypeptides, including biological signal transduction (*e.g.*, activation or inhibition of protein kinases, protein phosphatases, G-proteins, cyclic nucleotides and other second messengers, ion channels, secretory pathways), biologically active mediator release, stable or dynamic association with one or more cytoskeletal components, cellular differentiation, cellular activation, mitogenesis, cytostasis, apoptosis and the like (*e.g.*, Maher *et al.*, 2002 *Immunol. Cell Biol.* 80 131, El Far *et al.*, 2002 *Biochem J.* 365 329, Teng *et al.*, 2002 *Genome Biol.* 2REVIEWS 3012, Simons *et al.*, 2001 *Cell Signal* 13 855, Furie *et al.*, 2001 *Thromb. Haemost.* 86 214, Gaffen, 2001 *Cytokine* 14 63, Dittel, 2000 *Arch. Immunol. Ther. Exp.* (Warsz.) 48 381, Parnes et al., 2000 *Immunol. Rev.* 176 75, Moretta *et al.*, 2000 *Semin. Immunol.* 12 129, Ben Ze'ev, 1999 *Ann. N.Y. Acad. Sci.* 886 37, Marsters *et al.*, *Recent Prog. Horm. Res.* 54 225)

Figure 70 illustrates the binding, for example, of fluorecsne-conjugated FcRIII (CD16) soluble fusion proteins to 2H7 scFv-binding domain constructs that are attached to CD20 expressed by cells, CHO cells in this example. CD16 binding to a construct of the invention, for example, scFv-binding domain construct, provides one example of a screening tool that may be used to detect and/or quantitate changes in CD16 binding to altered constructs of the invention, including scFv-binding domain constructs, that contains targeted or site-specific mutations, substitutions, deletions, or other alterations. Changes in CD16 binding properties may be reflected, for example, by changes in binding of either CD16 high affinity protein (158V) or CD16 low affinity protein (158 F) or both.

A schematic representation of one example of such a screening process is diagrammed in the second drawing in Figure 70, in which scFv-binding domain constructs are displayed on the cell surface of mammalian cells. scFv-binding domain molecules in this example are displayed on the cell surface through a molecule that serves as a transmembrane domain anchor These molecules may represent, for example, a single scFv-binding domain construct or may be introduced into a population of mammalian cells as a library of such molecules Transfected cells with altered binding properties can then, for example, be panned, sorted, or otherwise isolated from other cells by altering the stringency of the selection conditions and using CD16 fusion proteins as a binding probe. Cells that express scFv-Ig molecules with altered binding to either CD16 high affinity allele (158V) or CD16 low affinity allele (158F) or both, for example, can be isolated.

This display system can be used, for example, to create a library of constructs of the invention with mutated or otherwise altered tail regions with short stretches of CH2 sequence replaced with randomized oligonucleotides or, for example, randomization of a single residue with all possible amino acid substitutions, natural or unnatural, including synthetic amino acids Once such a library is constructed, it can be transfected into appropriate cells, for example, COS cells, by methods known in the art. Transfectants can then be bound to, for example, labeled CD16 constructs, and panned or sorted based on their relative or desired binding properties to multiple allelotypes/isoforms Desired cells may be harvested, and the DNA, for example, plasmid DNA, isolated and then transformed into, for example, bacteria. This process may be repeated iteratively multiple times until desire single clones are isolated from the mammalian host cells See

Seed B and Aruffo A, *Pro. Nat'l Acad Sci USA* 1987 84.3365-3369, Aruffo A and Seed B, *Pro. Nat'l Acad Sci USA* 1987 84 8573-8577

One such use of this type of screening system, for example, is for the identification and/or isolation of constructs of the invention having tail regions, or tail regions, that bind equally well to both the high and low affinity alleles of CD16 with the goal of improving effector functions mediated by scFv-binding domain constructs in multiple subpopulations of patients Constructs of the invention having tail regions, or tail regions with altered binding properties to other Fc receptors can also be selected using such a display system, for example, the display system described. Other display systems that do not glycosylate proteins, for example, those that use bacteriophage or yeast, are not generally desired for selection of constructs of the invention having Ig-based tail regions, or Ig-based tail regions, with altered FcR binding properties Most non-mammalian systems, for example, do not glycosylate proteins.

Expression of constructs of the invention, for example, scFv-binding domain constructs, expressed on the surface of a mammalian cell by incorporation of an appropriate molecule into the construct, for example, by incorporation of a transmembrane domain or a GPI anchor signal, also have utility in other display systems that are useful, for example, for selection of constructs of the invention, for example, altered scFv-binding domain molecules that will be produced at higher or other desired levels In one such an embodiment, cells that are useful in the production of glycosylated proteins, for example, mammalian cells such as COS cells, are transfected with a library of scFv- binding domain constructs in a plasmid that directs their expression to the cell surface. Cells, such as COS cells, that express the highest or other desired level of the scFv-binding domain molecules are selected by techniques known in the art (for example panning, sterile cell sorting, magnetic bead separation, *etc*), and DNA, for example, plasmid DNA, is isolated for transformation into other cells, for example, bacteria. After one or more rounds of selection single clones are isolated that encode scFv-binding domain molecules capable of a high or other desired level of expression. The isolated clones may then be altered to remove the membrane anchor and expressed in an appropriate cells system, for example, a mammalian cell system, wherein the scFv- binding domain constructs will be produced, for example, by secretion into the culture fluid at desired levels Without being bound by any particular mechanism or theory, this is believed to result from the common requirement of

secreted glycoproteins and cell surface glycoproteins for a signal peptide and processing through the golgi for expression. Thus, selection for a molecule that shows an improvement expression levels on a cell surface will also result in the identification of a molecule having an improvement in levels of secreted protein.

These display systems utilizing a construct of the invention may also be used for screening and/or identifying and/or isolating affinity variants of the binding domain within a construct.

Particularly preferred are such display and/or screening systems, for example, that include or use constructs that include (1) an immunoglobulin variable region polypeptide sequence, including native or engineered V_H and/or V_L and/or single-chain variable region (sFv) sequences, and which include, for example, a mutation, alteration or deletion at an amino acid at a location or locations corresponding to one or more of amino acid positions 9, 10, 11, 12, 108, 110, 111, and 112, in a V_H region sequence (including in a V_H region sequence within an scFv or other construct), and/or (2) an immunoglobulin variable region polypeptide sequence, including native or engineered V_H and/or V_L and/or single-chain variable region (sFv) sequences, and which include, for example, a a mutation, alteration or deletion at a location or locations corresponding to one or more of amino acid positions 12, 80, 81, 82, 83, 105, 106, 107 and 108 in a light chain variable region sequence (including in a V_L region sequence within an scFv or other construct) Especially preferred are such display and/or screening systems that include or use constructs that include an engineered V_H sequence (whether or not associated with one or more other sequences, including immunoglobulin-derived and other sequences contained, for example, within an sFv or scFv-containing construct), which includes a mutation, alteration or deletion at an amino acid at a location or locations corresponding to amino acid position 11 The V_H11 amino acid, if substituted, may be substituted with another amino acid as described herein, or by another molecule as desired.

In the context of other methods of using constructs of the invention, including binding domain-immunoglobulin fusion proteins, for the treatment of a malignant condition or a B cell disorder(s) as provided herein, including, for example, by one or more of a number of gene therapy methods and related construct delivery techniques, the present invention also contemplates certain embodiments wherein a construct, for example, a binding domain-immunoglobulin fusion protein that comprises a

plasma membrane anchor domain polypeptide is expressed (or capable or expression) at a cell surface and may further comprise a cytoplasmic tail polypeptide which comprises an apoptosis signaling polypeptide sequence. A number of apoptosis signaling polypeptide sequences are known to the art, as reviewed, for example, in *When Cells Die. A Comprehensive Evaluation of Apoptosis and Programmed Cell Death* (R.A. Lockshin *et al*, Eds., 1998 John Wiley & Sons, New York; see also, *e.g*, Green *et al*, 1998 *Science* 281 1309 and references cited therein, Ferrara *et al*, 2002 *Clin. Canc. Res* 8.2024, Gurumurthy *et al*, 2001 *Cancer Metastas Rev* 20 225, Kanduc *et al*., 2002 *Int. J Oncol.* 21 165) Typically an apoptosis signaling polypeptide sequence comprises all or a portion of, or is derived or constructed from, a receptor death domain polypeptide, for instance, FADD (*e.g*, Genbank Acc Nos U24231, U43184, AF009616, AF009617, NM_012115), TRADD (*e.g*, Genbank Acc. No NM_003789), RAIDD (*e.g*, Genbank Acc. No U87229), CD95 (FAS/Apo-1, *e.g*, Genbank Acc. Nos X89101, NM_003824, AF344850, AF344856), TNF-α-receptor-1 (TNFR1, *e.g*, Genbank Acc Nos. S63368, AF040257), DR5 (*e.g* Genbank Acc No. AF020501, AF016268, AF012535), an ITIM domain (*e.g*, Genbank Acc. Nos AF081675, BC015731, NM_006840, NM_006844, NM_006847, XM_017977, see, *e.g* Billadeau *et al*, 2002 *J Clin. Invest* 109 161), an ITAM domain (*e.g*, Genbank Acc. Nos. NM_005843, NM_003473, BC030586, see, *e.g*, Billadeau *et al*, 2002), or other apoptosis-associated receptor death domain polypeptides known to the art, for example, TNFR2 (*e.g* Genbank Acc No L49431, L49432), caspase/procaspase-3 (*e.g* Genbank Acc No XM_54686), caspase/procaspase-8 (*e.g*, AF380342, NM_004208, NM_001228, NM_033355, NM_033356, NM_033357, NM_033358), caspase/procaspase-2 (*e.g*, Genbank Acc. No AF314174, AF314175), *etc*

Cells in biological samples that are suspected of undergoing apoptosis may be examined for morphological, permeability or other changes that are indicative of an apoptotic state. For example by way of illustration and not limitation, apoptosis in many cell types may cause altered morphological appearance such as plasma membrane blebbing, cell shape change, loss of substrate adhesion properties or other morphological changes that can be readily detected by a person having ordinary skill in the art, for example by using light microscopy As another example, cells undergoing apoptosis may exhibit fragmentation and disintegration of chromosomes, which may be apparent by microscopy and/or through the use of DNA-specific or chromatin-specific dyes that are

known in the art, including fluorescent dyes. Such cells may also exhibit altered plasma membrane permeability properties as may be readily detected through the use of vital dyes (*e.g.*, propidium iodide, trypan blue) or by the detection of lactate dehydrogenase leakage into the extracellular milieu. These and other means for detecting apoptotic cells by morphologic criteria, altered plasma membrane permeability, and related changes will be apparent to those familiar with the art.

In another embodiment of the invention wherein a construct, such as a binding domain-immunoglobulin fusion protein, that is expressed at a cell surface comprises a plasma membrane anchor domain having a transmembrane domain and a cytoplasmic tail that comprises an apoptosis signaling polypeptide, cells in a biological sample may be assayed for translocation of cell membrane phosphatidylserine (PS) from the inner to the outer leaflet of the plasma membrane, which may be detected, for example, by measuring outer leaflet binding by the PS-specific protein annexin. Martin *et al.*, *J Exp. Med.* 182 1545, 1995, Fadok *et al.*, *J Immunol.* 148 2207, 1992. In still other related embodiments of the invention, including embodiments wherein a construct, such as a binding domain-immunoglobulin fusion protein, is expressed at the cell surface and comprises a plasma membrane anchor domain having an apoptosis signaling polypeptide and also including embodiments wherein the construct, such as a binding domain-immunoglobulin fusion protein, is a soluble protein that lacks a membrane anchor domain and that is capable of inducing apoptosis, a cellular response to an apoptogen is determined by an assay for induction of specific protease activity in any member of a family of apoptosis-activated proteases known as the caspases (see, *e.g.*, Green *et al.*, 1998 *Science* 281 1309). Those having ordinary skill in the art will be readily familiar with methods for determining caspase activity, for example by determination of caspase-mediated cleavage of specifically recognized protein substrates. These substrates may include, for example, poly-(ADP-ribose) polymerase (PARP) or other naturally occurring or synthetic peptides and proteins cleaved by caspases that are known in the art (*see, e.g.*, Ellerby *et al.*, 1997 *J Neurosci.* 17 6165). The synthetic peptide Z-Tyr-Val-Ala-Asp-AFC (SEQ ID NO__), wherein "Z" indicates a benzoyl carbonyl moiety and AFC indicates 7-amino-4-trifluoromethylcoumarin (Kluck *et al.*, 1997 *Science* 275 1132, Nicholson *et al.*, 1995 *Nature* 376 37), is one such substrate. Other non-limiting examples of substrates include nuclear proteins such as U1-70 kDa and DNA-PKcs (Rosen and Casciola-Rosen, 1997 *J*

Cell. Biochem. 64 50, Cohen, 1997 *Biochem. J* 326 1) Cellular apoptosis may also be detected by determination of cytochrome c that has escaped from mitochondria in apoptotic cells (*e.g.*, Liu *et al.*, *Cell* 86 147, 1996) Such detection of cytochrome c may be performed spectrophotometrically, immunochemically or by other well established methods for determining the presence of a specific protein. Persons having ordinary skill in the art will readily appreciate that there may be other suitable techniques for quantifying apoptosis

Particularly preferred embodiments of constructs useful for gene therapy applications, including those constructs that include a plasma membrane anchor domain and/or cytoplasmic tail polypeptide (including, for example, an apoptosis signaling sequence), are such constructs that include (1) an immunoglobulin variable region polypeptide sequence, including native or engineered V_H and/or V_L and/or single-chain variable region (sFv) sequences, and which include, for example, a mutation, alteration or deletion at an amino acid at a location or locations corresponding to one or more of amino acid positions 9, 10, 11, 12, 108, 110, 111, and 112, in a V_H region sequence (including in a V_H region sequence within an scFv or other construct), and/or (2) an immunoglobulin variable region polypeptide sequence, including native or engineered V_H and/or V_L and/or single-chain variable region (sFv) sequences, and which include, for example, a a mutation, alteration or deletion at a location or locations corresponding to one or more of amino acid positions 12, 80, 81, 82, 83, 105, 106, 107 and 108 in a light chain variable region sequence (including in a V_L region sequence within an scFv or other construct) Especially preferred are constructs that include an engineered V_H sequence (whether or not associated with one or more other sequences, including immunoglobulin-derived and other sequences contained, for example, within an sFv or scFv-containing construct), which includes a mutation, alteration or deletion at an amino acid at a location or locations corresponding to amino acid position 11 The V_H11 amino acid, if substituted, may be substituted with another amino acid as described herein, or by another molecule as desired.

Once a construct, such as for example a binding domain-immunoglobulin fusion protein, as provided herein has been designed, polynucleotides including DNAs encoding the construct, where it or a relevant portion of it is a polypeptide, may be synthesized in whole or in part via oligonucleotide synthesis as described, for example, in Sinha *et al.*, *Nucleic Acids Res.*, 12 4539-4557 (1984), assembled via PCR as described, for

example in Innis, Ed., *PCR Protocols*, Academic Press (1990) and also in Better *et al J Biol. Chem* 267 16712-16118 (1992); cloned and expressed via standard procedures as described, for example, in Ausubel *et al*, Eds, *Current Protocols in Molecular Biology*, John Wiley & Sons, New York (1989) and also in Robinson *et al*, *Hum. Antibod. Hybridomas*, 2 84-93 (1991), and tested for desired activity, for example, binding to a target, or specific antigen binding activity, as described, for example, in Harlow *et al*, Eds., *Antibodies A Laboratory Manual*, Chapter 14, Cold Spring Harbor Laboratory, Cold Spring Harbor (1988) and Munson *et al*, *Anal. Biochem*, 107:220-239 (1980)

The preparation of single polypeptide chain binding molecules of the Fv region, single-chain Fv molecules, is known in the art. See, *e.g*, U S Pat. No 4,946,778 In the present invention, single-chain Fv-like molecules that may be included in constructs of the invention may be synthesized by encoding a first variable region of the heavy or light chain, followed by one or more linkers to the variable region of the corresponding light or heavy chain, respectively The selection of various appropriate linker(s) between the two variable regions is described in U S Pat. No 4,946,778 (see also, *e.g*, Huston *et al*, 1993 *Int Rev Immunol* 10 195) An exemplary linker described herein is (Gly-Gly-Gly-Gly-Ser)$_3$, but may be of any desired length. The linker is used to convert the naturally aggregated but chemically separate heavy and light chains into the amino terminal antigen binding portion of a single polypeptide chain, for example, wherein this antigen binding portion will fold into a structure similar to the original structure made of two polypeptide chains, or that otherwise has the ability to bind to a target, for example a target antigen. For those constructs that include an scFv as a binding region, a native or engineered immunoglobulin hinge as a connecting region, and one or more native or engineered heavy chain constant regions as a binding region, nucleotide sequences encoding the variable regions of native or engineered heavy and light chains, joined by a sequence encoding a linker, are joined to a nucleotide sequence encoding native or engineeredantibody constant regions, as desired. The constant regions may be those that permit the resulting polypeptide to form interchain disulfide bonds to form a dimer, and which contain desired effector functions, such as the ability to mediate ADCC, CDC, or fix complement, although native or engineered constant regions that do not favor dimer or other multimer formation or aggregation are preferred For a construct, such as an immunoglobulin-like molecule, of the invention that is intended for use in humans, the

included sequences having constant regions and/or desired constant regions function(s) will typically be human or substantially human or humanized to minimize a potential anti-human immune response and to provide appropriate or desired effector functions. Manipulation of sequences encoding antibody constant regions is referenced in the PCT publication of Morrison and Oi, WO 89/07142. In preferred embodiments, the CH1 domain is deleted in whole or in part from a tail region that includes, or consists essentially of, or consists of, a native or engineered immunoglobulin constant region(s) (for example, native or engineered CH2 and/or CH3 constant region(s), or native or engineered CH2 and/or CH3 and/or CH4 constant region(s)), and the carboxyl end of the binding region, for example, a binding domain polypeptide such as an immunoglobulin variable region polypeptide, is joined to the amino terminus of, for example, a CH2 via a connecting region, for example, a native or engineered hinge region polypeptide as provided herein.

As described above, the present invention provides recombinant expression constructs capable of directing the expression of constructs of the invention, including binding domain-immunoglobulin fusion proteins, as provided herein. The amino acids, which occur in the various amino acid sequences referred to herein, are identified according to their well known three-letter or single-letter abbreviations. The nucleotides, which occur in the various DNA sequences or fragments thereof referred herein, are designated with the standard single letter designations used routinely in the art. A given amino acid sequence may also encompass similar but changed amino acid sequences, such as those having only minor changes, for example by way of illustration and not limitation, covalent chemical modifications, insertions, deletions and substitutions, which may further include conservative substitutions or substitutions with non-naturally-occuring amino acids Amino acid sequences that are similar to one another may share substantial regions of sequence homology In like fashion, nucleotide sequences may encompass substantially similar nucleotide sequences having only minor changes, for example by way of illustration and not limitation, covalent chemical modifications, insertions, deletions and substitutions, which may further include silent mutations owing to degeneracy of the genetic code. Nucleotide sequences that are similar to one another may share substantial regions of sequence homology

The presence of a malignant condition in a subject refers to the presence of dysplastic, cancerous, and/or transformed cells in the subject, including, for example,

neoplastic, tumor, non-contact inhibited, or oncogenically transformed cells, or the like (*e.g.* melanoma, carcinomas such as adenocarcinoma, squamous cell carcinoma, small cell carcinoma, oat cell carcinoma, *etc*, sarcomas such as chondrosarcoma, osteosarcoma, *etc*) which are known to the art and for which criteria for diagnosis and classification are established. In preferred embodiments contemplated by the present invention, for example, such cancer cells are malignant hematopoietic cells, such as transformed cells of lymphoid lineage and in particular, B cell lymphomas and the like, cancer cells may in certain preferred embodiments also be epithelial cells such as carcinoma cells. The invention also contemplates B cell disorders, which may include certain malignant conditions that affect B cells (*e.g.*, B cell lymphoma) but which is not intended to be so limited, and which is also intended to encompass autoimmune diseases and in particular, diseases, disorders and conditions that are characterized by autoantibody production, for example.

Autoantibodies are antibodies that react with self-antigens. Autoantibodies are detected in several autoimmune diseases (i.e. a disease, disorder or condition wherein a host immune system generates an inappropriate anti-"self" immune reaction) where they are involved in disease activity Current treatments for various autoimmune diseases include immunosuppressive drugs that require continuing administration, lack specificity, and cause significant side effects New approaches that can eliminate autoantibody production with minimal toxicity will address an unmet medical need for a spectrum of diseases that affect many people Constructs of the subject invention, including binding domain-immunoglobulin fusion proteins, are designed, for example, for improved penetration into lymphoid tissues Depletion of B lymphocytes interrupts the autoantibody production cycle, and allows the immune system to reset as new B lymphocytes are produced from precursors in the bone marrow

A number of diseases, disorders, and conditions have been identified for which beneficial effects are believed, according to non-limiting theory, to result from B cell depletion therapy Such diseases disorders, and conditions include, but are not limited to, Grave's disease, Hashimoto's disease, rheumatoid arthritis, systemic lupus erythematosus, Sjogrens Syndrome Immune Thrombocytopenic purpura, multiple sclerosis, myasthenia gravis, scleroderma, psoriasis, Inflamatory Bowel Disease including Crohn's disease and ulcerative colitis Inflamatory Bowel Disease including Crohn's disease and Ulcerative colitis, are autoimmune diseases of the digestive system.

The present invention further relates to nucleotide constructs encoding constructs of the invention, for example, binding domain-immunoglobulin fusion proteins, and in particular to methods for administering recombinant constructs encoding such proteins for gene therapy applications that may be expressed, for example, as fragments, analogs and derivatives of such polypeptides.

The terms "fragment," "derivative" and "analog" when referring to constructs of the invention including, for example, binding domain-immunoglobulin fusion polypeptides or fusion proteins, refers to any construct, such as a binding domain-immunoglobulin fusion polypeptide or fusion protein, that retains essentially the same biological function or activity as such polypeptide. Thus, an analog includes a pro- or prepro- form of a construct, for example, a pro-protein that can be activated by cleavage of the pro-protein portion to produce an active construct, such as a binding domain-immunoglobulin fusion polypeptide.

A fragment, derivative or analog of a construct of the invention, for example, a binding domain-immunoglobulin fusion polypeptide or fusion protein, including binding domain-immunoglobulin fusion polypeptides or fusion proteins encoded by the cDNAs referred to herein, may be (i) one in which one or more of the amino acid residues are substituted with a conserved or non-conserved amino acid residue (preferably a conserved amino acid residue) and such substituted amino acid residue may or may not be one encoded by the genetic code, or (ii) one in which one or more of the amino acid residues includes a substituent group, or (iii) one in which additional amino acids are fused or otherwise connected to the construct, *e.g.*, a binding domain-immunoglobulin fusion polypeptide, including amino acids that are employed for detection or specific functional alteration of the construct, including such constructs as a binding domain-immunoglobulin fusion polypeptide or a proprotein sequence. Such fragments, derivatives and analogs are deemed to be within the scope of those skilled in the art from the teachings herein.

The constructs, including polypeptide constructs, of the present invention include, for example, binding domain-immunoglobulin fusion polypeptides and fusion proteins having binding regions such as binding domain polypeptide amino acid sequences that are identical or similar to sequences known in the art, or fragments or portions thereof For example by way of additional illustration and not limitation, a human CD154 molecule extracellular domain [SEQ ID NO __] is contemplated for use according to the instant

invention, as are portions of such polypeptides and/or polypeptides having at least about 70% similarity (preferably greater than a 70% identity) and more preferably about 90% similarity (more preferably greater than a 90% identity) to the reported polypeptide and still more preferably about 95% similarity (still more preferably greater than a 95% identity) to the reported polypeptides and to portions of such polypeptides, wherein such portions of a binding domain-immunoglobulin fusion polypeptide, for example, generally contain at least about 30 amino acids and more preferably at least about 50 amino acids. Extracellular domains include, for example, portions of a cell surface molecule, and in particularly preferred embodiments cell surface molecules that are integral membrane proteins or that comprise a plasma membrane spanning transmembrane domain, that are constructed to extend beyond the outer leaflet of the plasma membrane phospholipid bilayer when the molecule is expressed at a cell surface, preferably in a manner that exposes the extracellular domain portion of such a molecule to the external environment of the cell, also known as the extracellular milieu Methods for determining whether a portion of a cell surface molecule comprises an extracellular domain are well known to the art and include, for example, experimental determination (*e.g* direct or indirect labeling of the molecule, evaluation of whether the molecule can be structurally altered by agents to which the plasma membrane is not permeable such as proteolytic or lipolytic enzymes) or topological prediction based on the structure of the molecule (*e.g*, analysis of the amino acid sequence of a polypeptide) or other methodologies.

As used herein, an "amino acid" is a molecule having the structure wherein a central carbon atom (the alpha (α)-carbon atom) is linked to a hydrogen atom, a carboxylic acid group (the carbon atom of which is referred to herein as a "carboxyl carbon atom"), an amino group (the nitrogen atom of which is referred to herein as an "amino nitrogen atom"), and a side chain group, R. When incorporated into a peptide, polypeptide, or protein, an amino acid loses one or more atoms of its amino and carboxylic groups in the dehydration reaction that links one amino acid to another As a result, when incorporated into a protein, an amino acid may also be referred to as an "amino acid residue." In the case of naturally occurring proteins, an amino acid residue's R group differentiates the 20 amino acids from which proteins are typically synthesized, although one or more amino acid residues in a protein may be derivatized or modified following incorporation into protein in biological systems (*e.g*, by glycosylation and/or by the formation of cystine

through the oxidation of the thiol side chains of two non-adjacent cysteine amino acid residues, resulting in a disulfide covalent bond that frequently plays an important role in stabilizing the folded conformation of a protein, *etc.*) As those in the art will appreciate, non-naturally occurring amino acids can also be incorporated into proteins, particularly those produced by synthetic methods, including solid state and other automated synthesis methods Examples of such amino acids include, without limitation, α-amino isobutyric acid, 4-amino butyric acid, L-amino butyric acid, 6-amino hexanoic acid, 2-amino isobutyric acid, 3-amino propionic acid, ornithine, norleucine, norvaline, hydroxproline, sarcosine, citralline, cysteic acid, t-butylglycine, t-butylalanine, phenylglycine, cyclohexylalanine, β-alanine, fluoro-amino acids, designer amino acids (*e.g.*, β-methyl amino acids, α-methyl amino acids, Nα-methyl amino acids) and amino acid analogs in general In addition, when an α-carbon atom has four different groups (as is the case with the 20 amino acids used by biological systems to synthesize proteins, except for glycine, which has two hydrogen atoms bonded to the α carbon atom), two different enantiomeric forms of each amino acid exist, designated D and L. In mammals, only L-amino acids are incorporated into naturally occurring polypeptides. The instant invention envisions proteins incorporating one or more D- and L- amino acids, as well as proteins comprised of just D- or L- amino acid residues.

Herein, the following abbreviations may be used for the following amino acids (and residues thereof) alanine (Ala, A), arginine (Arg, R), asparagine (Asn, N), aspartic acid (Asp, D), cysteine (Cys, C), glycine (Gly, G), glutamic acid (Glu, E), glutamine (Gln, Q), histidine (His, H), isoleucine (Ile, I), leucine (Leu, L), lysine (Lys, K), methionine (Met, M), phenylalanine (Phe, F), proline (Pro, P), serine (Ser, S), threonine (Thr, T), tryptophan (Trp, W), tyrosine (Tyr, Y), and valine (Val, V) Non-polar (hydrophobic) amino acids include alanine, leucine, isoleucine, valine, proline, phenylalanine, tryptophan, and methionines Neutral amino acids include glycine, serine, threonine, cysteine, tyrosine, asparagine, and glutamine. Positively charged (basic amino acids include arginine, lysine and histidine. Negatively charged (acidic) amino acids include aspartic acid and glutamic acid.

"Protein" refers to any polymer of two or more individual amino acids (whether or not naturally occurring) linked via a peptide bond, and occurs when the carboxyl carbon atom of the carboxylic acid group bonded to the α-carbon of one amino

acid (or amino acid residue) becomes covalently bound to the amino nitrogen atom of amino group bonded to the α-carbon of an adjacent amino acid. The term "protein" is understood to include the terms "polypeptide" and "peptide" (which, at times, may be used interchangeably herein) within its meaning. In addition, proteins comprising multiple polypeptide subunits or other components will also be understood to be included within the meaning of "protein" as used herein. Similarly, fragments of proteins, peptides, and polypeptides are also within the scope of the invention and may be referred to herein as "proteins."

In biological systems (be they *in vivo* or *in vitro*, including cell-free, systems), the particular amino acid sequence of a given protein (*i.e.*, the polypeptide's "primary structure," when written from the amino-terminus to carboxy-terminus) is determined by the nucleotide sequence of the coding portion of a mRNA, which is in turn specified by genetic information, typically genomic DNA (which, for purposes of this invention, is understood to include organelle DNA, for example, mitochondrial DNA and chloroplast DNA). Of course, any type of nucleic acid which constitutes the genome of a particular organism (*e.g.*, double-stranded DNA in the case of most animals and plants, single or double-stranded RNA in the case of some viruses, *etc.*) is understood to code for the gene product(s) of the particular organism. Messenger RNA is translated on a ribosome, which catalyzes the polymerization of a free amino acid, the particular identity of which is specified by the particular codon (with respect to mRNA, three adjacent A, G, C, or U ribonucleotides in the mRNA's coding region) of the mRNA then being translated, to a nascent polypeptide. Recombinant DNA techniques have enabled the large-scale synthesis of proteins and polypeptides (*e.g.*, human insulin, human growth hormone, erythropoietin, granulocyte colony stimulating factor, *etc.*) having the same primary sequence as when produced naturally in living organisms. In addition, such technology has allowed the synthesis of analogs of these and other proteins, which analogs may contain one or more amino acid deletions, insertions, and/or substitutions as compared to the native proteins. Recombinant DNA technology also enables the synthesis of entirely novel proteins.

In non-biological systems (*e.g.*, those employing solid state synthesis), the primary structure of a protein (which also includes disulfide (cystine) bond locations) can be determined by the user. As a result, polypeptides having a primary structure that

duplicates that of a biologically produced protein can be achieved, as can analogs of such proteins In addition, completely novel polypeptides can also be synthesized, as can protein incorporating non-naturally occurring amino acids

As is known in the art, "similarity" between two polypeptides may be determined by comparing the amino acid sequence (including conserved amino acid substitutes therein) of one polypeptide to the sequence of a second polypeptide. Fragments or portions of the nucleic acids encoding polypeptides of the present invention may be used to synthesize full-length nucleic acids of the present invention. As used herein, "% identity" refers to the percentage of identical amino acids situated at corresponding amino acid residue positions when two or more polypeptide are aligned and their sequences analyzed using, for example, a gapped BLAST algorithm (*e.g.*, Altschul *et al*, 1997 *Nucl. Ac. Res* 25 3389, Altschul *et al.*, 1990 *J Mol. Biol.*, 215 403-410) which weights sequence gaps and sequence mismatches according to the default weightings provided by the National Institutes of Health/ NCBI database (Bethesda, MD, *see* www.ncbi.nlm.nih.gov/cgi-bin/BLAST/nph-newblast) Other alignment methods include BLITZ (MPsrch) (Sturrock & Collins, 1993), and FASTA (Pearson and Lipman, 1988 *Proc. Natl. Acad. Sci USA* 85 2444-2448)

The term "isolated" means, in the case of a naturally occuring material, that the material is or has been removed from, or is no longer associated with, its natural or original environment. For example, a naturally occurring nucleic acid or protein or polypeptide present in a living animal is not isolated, but the same nucleic acid or polypeptide, separated from some or all of the co-existing materials in the natural system, is isolated Such nucleic acids could be part of a vector and/or such nucleic acids or polypeptides could be part of a composition, and still be isolated in that such vector or composition is not part of its natural environment. The term "isolated", in the case of non-naturally occuring material, such as a recombinantly manufactured construct of the invention, includes material that is substantially or essentially free from components which normally accompany it during manufacture, such as, for example, proteins and peptides that have been purified to a desired degree, preferably, for example, so that they are at least about 80% pure, more preferably at least about 90%, and still more preferably at least about 95% as measured by techniques known in the art

The term "gene" means a segment of DNA involved in producing a polypeptide chain, it may also include regions preceding and following a polypeptide coding region, for example, a "leader and trailer" as well as intervening sequences (introns) between relevant individual coding segments (exons)

As described herein, the invention provides constructs, including binding domain-immunoglobulin fusion proteins, that may be encoded in whole or in part by nucleic acids that have a binding region coding sequence such as, for example, a binding domain coding sequence fused or otherwise connected in frame to an additional native or engineered immunoglobulin domain encoding sequence to provide for expression of, for example, a binding domain polypeptide sequence fused or otherwise connected to an additional functional polypeptide sequence that permits, for example by way of illustration and not limitation, detection, functional alteration, isolation and/or purification of the fusion protein Such fusion proteins may permit functional alteration of a binding domain by containing additional immunoglobulin-derived polypeptide sequences that influence behavior of the fusion product, for example (and as described above) by reducing the availability of sulfhydryl groups for participation in disulfide bond formation, and by conferring the ability to potentiate ADCC and/or CDC and/or fix complement.

Modification of a polypeptide may be effected by any means known to those of skill in this art. The preferred methods herein rely on modification of DNA encoding, for example, a fusion protein and expression of the modified DNA. DNA encoding one of the constructs of the invention, for example, one of the binding domain-immunoglobulin fusions discussed herein, for example, may be altered or mutagenized using standard methodologies, including those described below For example, cysteine residues that may otherwise facilitate multimer formation or promote particular molecular conformations can be deleted from a polypeptide or replaced, *e.g.*, cysteine residues that are responsible for or participate in aggregate formation. If necessary, for example, the identity of cysteine residues that contribute to aggregate formation may be determined empirically, by deleting and/or replacing a cysteine residue and ascertaining whether the resulting protein aggregates in solutions containing physiologically acceptable buffers and salts. In addition, fragments of, for example, binding domain-immunoglobulin fusions may be constructed and used. As noted above, counterreceptor/ligand binding domains for many candidate binding domain-immunoglobulin fusion have been delineated, such that

one having ordinary skill in the art may readily select appropriate polypeptide domains for inclusion in encoded products of the instant expression constructs.

Conservative substitutions of amino acids are well known and may be made generally without altering the biological activity of the resulting binding domain-immunoglobulin fusion protein molecule. For example, such substitutions are generally made by interchanging within the groups of polar residues, charged residues, hydrophobic residues, small residues, and the like. If necessary, such substitutions may be determined empirically merely by testing the resulting modified protein for the ability to bind to the appropriate cell surface receptors in *in vitro* biological assays, or to bind to appropriate antigens or desired target molecules.

The present invention further relates to nucleic acids which hybridize to constructs of the invention, including for example, binding domain-immunoglobulin fusion protein encoding polynucleotide sequences as provided herein, or their complements, as will be readily apparent to those familiar with the art, if there is at least about 70%, preferably at least about 80-85%, more preferably at least about 90%, and still more preferably at least about 95%, 96%, 97%, 98% or 99% identity between the sequences.

The present invention particularly relates to nucleic acids that hybridize under stringent conditions to, for example, the binding domain-immunoglobulin fusion encoding nucleic acids referred to herein. As used herein, to "hybridize" under conditions of a specified stringency is used to describe the stability of hybrids formed between two single-stranded nucleic acid molecules Stringency of hybridization is typically expressed in conditions of ionic strength and temperature at which such hybrids are annealed and washed The term "stringent conditions" refers to conditions that permit hybridization between polynucleotides Stringent conditions can be defined by salt concentration, the concentration of organic solvent (*e.g*, formamide), temperature, and other conditions well known in the art. In particular, stringency can be increased by reducing the concentration of salt, increasing the concentration of organic solvents (*e.g*, formamide), or raising the hybridization temperature. For example, stringent salt concentration will ordinarily be less than about 750 mM NaCl and 75 mM trisodium citrate, preferably less than about 500 mM NaCl and 50 mM trisodium citrate, and most preferably less than about 250 mM NaCl and 25 mM trisodium citrate. Low stringency hybridization can be obtained in the absence of organic solvent, *e g*, formamide, while high stringency hybridization can be obtained in

the presence of an organic solvent (*e.g.*, at least about 35% formamide, most preferably at least about 50% formamide). Stringent temperature conditions will ordinarily include temperatures of at least about 30°C, more preferably of at least about 37°C, and most preferably of at least about 42°C Varying additional parameters, for example, hybridization time, the concentration of detergent, *e.g.*, sodium dodecyl sulfate (SDS), and the inclusion or exclusion of carrier DNA, are well known to those skilled in the art. Various levels of stringency are accomplished by combining these various conditions as needed, and are within the skill in the art. Other typical "high", "medium" and "low" stringency encompass the following conditions or equivalent conditions thereto high stringency: 0.1 x SSPE or SSC, 0.1% SDS, 65°C, medium stringency: 0.2 x SSPE or SSC, 0.1% SDS, 50°C, and low stringency: 1.0 x SSPE or SSC, 0.1% SDS, 50°C As known to those having ordinary skill in the art, variations in stringency of hybridization conditions may be achieved by altering the time, temperature and/or concentration of the solutions used for prehybridization, hybridization and wash steps, and suitable conditions may also depend in part on the particular nucleotide sequences of the probe used, and of the blotted, proband nucleic acid sample Accordingly, it will be appreciated that suitably stringent conditions can be readily selected without undue experimentation where a desired selectivity of the probe is identified, based on its ability to hybridize to one or more certain proband sequences while not hybridizing to certain other proband sequences

As used herein, preferred "stringent conditions" generally refer to hybridization that will occur only if there is at least about 90-95% and more preferably at least about 97% identity between the sequences The nucleic acid constructs which hybridize to, for example, binding domain-immunoglobulin fusion encoding nucleic acids referred to herein, in preferred embodiments, encode polypeptides which retain substantially the same biological function or activity as, for example, the binding domain-immunoglobulin fusion polypeptides encoded by the cDNAs.

The nucleic acids of the present invention, also referred to herein as polynucleotides, may be in the form of RNA, for example, mRNA, or in the form of DNA, which DNA includes cDNA (also called "complementary DNA", which is a DNA molecule that is complementary to a specific messenger RNA), genomic DNA, and synthetic DNA. The DNA may be double-stranded or single-stranded, and if single stranded may be the coding strand or non-coding (anti-sense) strand. A coding sequence

which encodes a construct of the invention, for example, a binding domain-immunoglobulin fusion polypeptide for use according to the invention may contain portions that are identical to the coding sequence known in the art or described herein for portions thereof , or may be a different coding sequence, which, as a result of the redundancy or degeneracy of the genetic code, encodes the same construct or portion thereof, including all or a portion of a binding domain-immunoglobulin fusion polypeptide.

The nucleic acids which encode constructs of the invention, for example, binding domain-immunoglobulin fusion polypeptides, for use according to the invention may include, but are not limited to only the coding sequence for the construct, such as a binding domain-immunoglobulin fusion polypeptide, the coding sequence for the construct, such as a binding domain-immunoglobulin fusion polypeptide and additional coding sequence; the coding sequence for the construct, such as a binding domain-immunoglobulin fusion polypeptide (and optionally additional coding sequence) and non-coding sequence, such as introns or non-coding sequences 5' and/or 3' of the coding sequence for the binding domain-immunoglobulin fusion polypeptide or a portion(s) thereof, which for example may further include but need not be limited to one or more regulatory nucleic acid sequences that may be a regulated or regulatable promoter, enhancer, other transcription regulatory sequence, repressor binding sequence, translation regulatory sequence or any other regulatory nucleic acid sequence Thus, the term "nucleic acid encoding" or "polynucleotide encoding" a construct, for example, a binding domain-immunoglobulin fusion protein, encompasses a nucleic acid which includes only coding sequence for, for example, a binding domain-immunoglobulin fusion polypeptide as well as a nucleic acid which includes additional coding and/or non-coding sequence(s)

Nucleic acids and oligonucleotides for use as described herein can be synthesized by any method known to those of skill in this art (*see, e.g* , WO 93/01286, U.S Application Serial No 07/723,454, U S Patent No 5,218,088, U.S Patent No 5,175,269, U S Patent No 5,109,124) Identification of various oligonucleotides and nucleic acid sequences also involves methods known in the art. For example, the desirable properties, lengths and other characteristics of oligonucleotides useful for cloning are well known. In certain embodiments, synthetic oligonucleotides and nucleic acid sequences may be designed that resist degradation by endogenous host cell nucleolytic enzymes by containing such linkages as phosphorothioate, methylphosphonate, sulfone, sulfate, ketyl,

phosphorodithioate, phosphoramidate, phosphate esters, and other such linkages that have proven useful in antisense applications. See, *e.g.*, Agrwal *et al*, *Tetrahedron Lett.* 28 3539-3542 (1987), Miller *et al*, *J Am. Chem. Soc.* 93 6657-6665 (1971), Stec *et al*, *Tetrahedron Lett* 26 2191-2194 (1985), Moody *et al*, *Nucl Acids Res* 12.4769-4782 (1989), Uznanski *et al*, *Nucl Acids Res.* (1989), Letsinger *et al*, *Tetrahedron* 40 137-143 (1984), Eckstein, *Annu. Rev. Biochem* 54.367-402 (1985), Eckstein, *Trends Biol Sci.* 14 97-100 (1989), Stein In. *Oligodeoxynucleotides Antisense Inhibitors of Gene Expression*, Cohen, Ed, Macmillan Press, London, pp 97-117 (1989), Jager *et al*, *Biochemistry* 27 7237-7246 (1988)

In one embodiment, the present invention provides truncated components (*e.g*, binding domain polypeptide, hinge region polypeptide, linker, *etc*) for use in a construct of the invention, for example, a binding domain-immunoglobulin fusion protein. In another embodiment the invention provides nucleic acids encoding a construct of the invention, for example, a binding domain-immunoglobulin fusion protein having such truncated components. A truncated molecule may be any molecule that comprises less than a full length version of the molecule of interest. Truncated molecules provided by the present invention may include truncated biological polymers, and in preferred embodiments of the invention such truncated molecules may be truncated nucleic acid molecules or truncated polypeptides. Truncated nucleic acid molecules have less than the full length nucleotide sequence of a known or described nucleic acid molecule, where such a known or described nucleic acid molecule may be a naturally occurring, a synthetic, or a recombinant nucleic acid molecule, so long as one skilled in the art would regard it as a full length molecule. Thus, for example, truncated nucleic acid molecules that correspond to a gene sequence contain less than the full length gene where the gene comprises coding and non-coding sequences, promoters, enhancers and other regulatory sequences, flanking sequences and the like, and other functional and non-functional sequences that are recognized as part of the gene. In another example, truncated nucleic acid molecules that correspond to a mRNA sequence contain less than the full length mRNA transcript, which may include various translated and non-translated regions as well as other functional and non-functional sequences

In other preferred embodiments, truncated molecules are polypeptides that comprise less than the full-length amino acid sequence of a particular protein or

polypeptide component. As used herein "deletion" has its common meaning as understood by those familiar with the art, and may refer to molecules that lack one or more portions of a sequence from either terminus or from a non-terminal region, relative to a corresponding full length molecule, for example, as in the case of truncated molecules provided herein Truncated molecules that are linear biological polymers such as nucleic acid molecules or polypeptides may have one or more of a deletion from either terminus of the molecule and/or one or more deletions from a non-terminal region of the molecule, where such deletions may be deletions of from about 1-1500 contiguous nucleotide or amino acid residues, preferably about 1-500 contiguous nucleotide or amino acid residues and more preferably about 1-300 contiguous nucleotide or amino acid residues, including deletions of about 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, 31-40, 41-50, 51-74, 75-100, 101-150, 151-200, 201-250 or 251-299 contiguous nucleotide or amino acid residues In certain particularly preferred embodiments truncated nucleic acid molecules may have a deletion of about 270-330 contiguous nucleotides. In certain more preferred embodiments, truncated polypeptide molecules may have a deletion, for example, of about 80-140 contiguous amino acids

The present invention further relates to variants of the herein referenced nucleic acids that encode fragments, analogs and/or derivatives of a construct of the invention, for example, a binding domain-immunoglobulin fusion polypeptide. The variants of the nucleic acids encoding constructs of the invention, for example, binding domain-immunoglobulin fusion proteins, may be naturally occurring allelic variants of one or more portions of the the nucleic acid sequences included therein, or non-naturally occurring variants of such sequences or portions or sequences, including sequences varied by molecular engineering using, for example, methods know in the art for varying sequence. As is known in the art, an allelic variant is an alternate form of a nucleic acid sequence which may have at least one of a substitution, a deletion or an addition of one or more nucleotides, any of which does not substantially or undesireably alter the function of the encoded binding domain-immunoglobulin fusion polypeptide.

Variants and derivatives of constructs of the invention, for example, binding domain-immunoglobulin fusion proteins, may be obtained by mutations of nucleotide sequences encoding, for example, binding domain-immunoglobulin fusion polypeptides or any portion thereof Alterations of the native amino acid sequence may be accomplished

by any of a number of conventional methods. Mutations can be introduced at particular loci, for example, by synthesizing oligonucleotides containing a mutant sequence, flanked by restriction sites enabling ligation to fragments of the native sequence. Following ligation, the resulting reconstructed sequence encodes an analog having the desired amino acid insertion, substitution, or deletion.

Alternatively, for example, oligonucleotide-directed site-specific mutagenesis procedures can be employed to provide an altered gene wherein predetermined codons can be altered by substitution, deletion or insertion. Exemplary methods of making such alterations are disclosed by Walder *et al*, 1986 *Gene* 42 133, Bauer *et al*, 1985 *Gene* 37 73, Craik, January 1985 *BioTechniques* 12-19; Smith *et al*, January 1985 *Genetic Engineering Principles and Methods BioTechniques* 12-19, Costa GL, *et al*, "Site-directed mutagenesis using a rapid PCR-based method," 1996 *Methods Mol Biol* 57 239-48, Rashtchian A., "Novel methods for cloning and engineering genes using the polymerase chain reaction," 1995 *Curr Opin Biotechnol* 6(1) 30-6, Sharon J, et al., "Oligonucleotide-directed mutagenesis of antibody combining sites," 1993 *Int Rev Immunol* 10(2-3) 113-27, Kunkel, 1985 *Proc. Natl Acad. Sci. USA* 82 488, Kunkel *et al*, 1987 *Methods in Enzymol* 154 367, and, U S Patent Nos. 4,518,584 and 4,737,462

As an example, modification of DNA may be performed by site-directed mutagenesis of DNA encoding a protein combined with the use of DNA amplification methods using primers to introduce and amplify alterations in the DNA template, such as PCR splicing by overlap extension (SOE) Site-directed mutagenesis is typically effected using a phage vector that has single- and double-stranded forms, such as M13 phage vectors, which are well known and commercially available. Other suitable vectors that contain a single-stranded phage origin of replication may be used. See, *e.g*, Vera *et al*, 1987 *Meth Enzymol* 15 3 In general, site-directed mutagenesis is performed by preparing a single-stranded vector that encodes the protein of interest (*e.g*, all or a component portion of a given binding domain-immunoglobulin fusion protein) An oligonucleotide primer that contains the desired mutation within a region of homology to the DNA in the single-stranded vector is annealed to the vector followed by addition of a DNA polymerase, such as *E. coli* DNA polymerase I (Klenow fragment), which uses the double stranded region as a primer to produce a heteroduplex in which one strand encodes the altered sequence and the other the original sequence. The heteroduplex is introduced into

appropriate bacterial cells and clones that include the desired mutation are selected. The resulting altered DNA molecules may be expressed recombinantly in appropriate host cells to produce the modified protein.

Equivalent DNA constructs that include code for additions or substitutions of amino acid residues or sequences, or deletions of terminal or internal residues or sequences not needed or desired for biological activity, for example, are also encompassed by the invention For example, and as discussed above, sequences encoding Cys residues that are not desirable or essential for biological activity can be altered to cause the Cys residues to be deleted or replaced with other amino acids, for example, thus preventing formation of incorrect or undesired intramolecular disulfide bridges upon synthesis or renaturation.

A "host cell" or "recombinant host cell" is a cell that contains a vector, *e.g*, an expression vector, or a cell that has otherwise been manipulated by recombinant techniques to express a protein of interest Host organisms include those organisms in which recombinant production of constructs of the invention, for example, binding domain-immunoglobulin fusion products encoded by the recombinant constructs of the present invention may occur, such as bacteria (for example, *E. coli*), yeast (for example, *Saccharomyces cerevisiae* and *Pichia pastoris*), insect cells, and mammalian cells, including *in vitro* and *in vivo* expression. Host organisms thus may include organisms for the construction, propagation, expression or other steps in the production of the compositions provided herein. Hosts include subjects in which immune responses take place, as described herein. Presently preferred host organisms for production of constructs of the invention that produce glycosylated proteins are mammalian cells or other cells systems that permit the expression and recovery of glycosylated proteins. Other cell lines include inbred murine strains and murine cell lines, and human cellsand cell lines.

A DNA construct encoding a desired construct of the invention, for example, a binding domain-immunoglobulin fusion protein is introduced into a vector, for example, a plasmid, for expression in an appropriate host. In preferred embodiments, the host is a mammalian host, for example, a mammalian cell line. The sequence encoding the ligand or nucleic acid binding domain is preferably codon-optimized for expression in the particular host. Thus, for example, if a construct, for example, is a human binding domain-immunoglobulin fusion and is expressed in bacteria, the codons may be optimized for

bacterial usage. For small coding regions, the gene can be synthesized as a single oligonucleotide. For larger proteins, splicing of multiple oligonucleotides, mutagenesis, or other techniques known to those in the art may be used. The sequences of nucleotides in plasmids or other vectors that are regulatory regions, such as promoters and operators, are operationally associated with one another for transcription. The sequence of nucleotides encoding a binding domain-immunoglobulin fusion protein may also include DNA encoding a secretion signal, whereby the resulting peptide is a precursor protein. The resulting processed protein may be recovered from the periplasmic space or the fermentation medium.

In preferred embodiments, the DNA plasmids may also include a transcription terminator sequence. As used herein, a "transcription terminator region" is a sequence that signals transcription termination. The entire transcription terminator may be obtained from a protein-encoding gene, which may be the same or different from the inserted binding domain-immunoglobulin fusion encoding gene or the source of the promoter. Transcription terminators are optional components of the expression systems herein, but are employed in preferred embodiments.

The plasmids or other vectors used herein include a promoter in operative association with the DNA encoding the protein or polypeptide of interest and are designed for expression of proteins in a suitable host as described above (*e.g.*, bacterial, murine, or human) depending upon the desired use of the plasmid (*e.g.*, administration of a vaccine containing binding domain-immunoglobulin fusion encoding sequences). Suitable promoters for expression of proteins and polypeptides herein are widely available and are well known in the art. Inducible promoters or constitutive promoters that are linked to regulatory regions are preferred. Such promoters include, for example, but are not limited to, the T7 phage promoter and other T7-like phage promoters, such as the T3, T5 and SP6 promoters, the trp, lpp, and lac promoters, such as the lacUV5, from *E. coli*, the P10 or polyhedrin gene promoter of baculovirus/insect cell expression systems (*see*, *e.g.*, U.S. Patent Nos. 5,243,041, 5,242,687, 5,266,317, 4,745,051, and 5,169,784) and inducible promoters from other eukaryotic expression systems. For expression of the proteins such promoters are inserted in a plasmid in operative linkage with a control region such as the lac operon.

Preferred promoter regions are those that are inducible and functional in mammalian cells, for example. Examples of suitable inducible promoters and promoter regions for bacterial expression include, but are not limited to the *E. coli* lac operator responsive to isopropyl β-D-thiogalactopyranoside (IPTG; see Nakamura *et al*, 1979 *Cell* 18 1109-1117), the metallothionein promoter metal-regulatory-elements responsive to heavy-metal (*e.g*, zinc) induction (see, *e.g*, U.S Patent No 4,870,009), the phage T7lac promoter responsive to IPTG (see, *e.g*, U S Patent No 4,952,496, and Studier *et al*, 1990 *Meth. Enzymol.* 185 60-89) and the TAC promoter Depending on the expression host system to be used, plasmids may optionally include a selectable marker gene or genes that are functional in the host. Thus, for example, a selectable marker gene includes any gene that confers a phenotype on bacteria that allows transformed bacterial cells to be identified and selectively grown from among a vast majority of untransformed cells. Suitable selectable marker genes for bacterial hosts, for example, include the ampicillin resistance gene (Amp^r), tetracycline resistance gene (Tc^r) and the kanamycin resistance gene (Kan^r) The kanamycin resistance gene is presently preferred for bacterial expression.

In various expression systems, plasmids or other vectors may also include DNA encoding a signal for secretion of the operably linked protein. Secretion signals suitable for use are widely available and are well known in the art. Prokaryotic and eukaryotic secretion signals functional in *E. coli* may be employed. Depending on the expression systems, presently preferred secretion signals may include, but are not limited to, those encoded by the following *E coli* genes ompA, ompT, ompF, ompC, beta-lactamase, and alkaline phosphatase, and the like (von Heijne, *J Mol. Biol* 184 99-105, 1985) In addition, the bacterial pelB gene secretion signal (Lei *et al*, *J Bacteriol.* 169.4379, 1987), the phoA secretion signal, and the cek2 functional in insect cell may be employed. The most preferred secretion signal for certain expression systems is the *E. coli* ompA secretion signal Other prokaryotic and eukaryotic secretion signals known to those of skill in the art may also be employed (*see, e.g*, von Heijne, *J Mol Biol 184* 99-105, 1985) Using the methods described herein, one of skill in the art can substitute secretion signals that are functional, for example, in yeast, insect or mammalian cells to secrete proteins from those cells

Preferred plasmids for transformation of *E coli* cells include the pET expression vectors (*e.g* pET-11a, pET-12a-c, pET-15b, *see* U S Patent No 4,952,496,

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available from Novagen, Madison, WI). Other preferred plasmids include the pKK plasmids, particularly pKK 223-3, which contains the tac promoter (Brosius *et al*, 1984 *Proc. Natl. Acad. Sci.* 81 6929, Ausubel *et al*, *Current Protocols in Molecular Biology*; U S Patent Nos 5,122,463, 5,173,403, 5,187,153, 5,204,254, 5,212,058, 5,212,286, 5,215,907, 5,220,013, 5,223,483, and 5,229,279) Plasmid pKK has been modified by replacement of the ampicillin resistance gene with a kanamycin resistance gene. (Available from Pharmacia, obtained from pUC4K, see, *e.g*, Vieira *et al* (1982 *Gene* 19 259-268, and U S Patent No 4,719,179) Baculovirus vectors, such as pBlueBac (also called pJVETL and derivatives thereof), particularly pBlueBac III (see, *e.g*, U S Patent Nos. 5,278,050, 5,244,805, 5,243,041, 5,242,687, 5,266,317, 4,745,051, and 5,169,784, available from Invitrogen, San Diego) may also be used for expression of the polypeptides in insect cells Other plasmids include the pIN-IIIompA plasmids (*see* U.S Patent No 4,575,013, see also Duffaud *et al*, *Meth. Enz. 153* 492-507, 1987), such as pIN-IIIompA2

Preferably, if one or more DNA molecules is replicated in bacterial cells, the preferred host is *E. coli* The preferred DNA molecule is such a system also includes a bacterial origin of replication, to ensure the maintenance of the DNA molecule from generation to generation of the bacteria. In this way, large quantities of the DNA molecule can be produced by replication in bacteria. In such expression systems, preferred bacterial origins of replication include, but are not limited to, the f1-ori and col E1 origins of replication. Preferred hosts for such systems contain chromosomal copies of DNA encoding T7 RNA polymerase operably linked to an inducible promoter, such as the lacUV promoter (see U S Patent No 4,952,496) Such hosts include, but are not limited to, lysogens *E coli* strains HMS174(DE3)pLysS, BL21(DE3)pLysS, HMS174(DE3) and BL21(DE3) Strain BL21(DE3) is preferred The pLys strains provide low levels of T7 lysozyme, a natural inhibitor of T7 RNA polymerase.

The DNA molecules provided may also contain a gene coding for a repressor protein. The repressor protein is capable of repressing the transcription of a promoter that contains sequences of nucleotides to which the repressor protein binds. The promoter can be derepressed by altering the physiological conditions of the cell. For example, the alteration can be accomplished by adding to the growth medium a molecule that inhibits the ability to interact with the operator or with regulatory proteins or other regions of the DNA or by altering the temperature of the growth media. Preferred

repressor proteins include, but are not limited to the *E. coli* lacI repressor responsive to IPTG induction, the temperature sensitive λ cI857 repressor, and the like. The *E. coli* lacI repressor is preferred

In general, recombinant constructs of the subject invention will also contain elements necessary for transcription and translation. In particular, such elements are preferred where the recombinant expression construct containing nucleic acid sequences encoding binding domain-immunoglobulin fusion proteins is intended for expression in a host cell or organism In certain embodiments of the present invention, cell type preferred or cell type specific expression of a cell binding domain-immunoglobulin fusion encoding gene may be achieved by placing the gene under regulation of a promoter The choice of the promoter will depend upon the cell type to be transformed and the degree or type of control desired Promoters can be constitutive or active and may further be cell type specific, tissue specific, individual cell specific, event specific, temporally specific or inducible. Cell-type specific promoters and event type specific promoters are preferred. Examples of constitutive or nonspecific promoters include the SV40 early promoter (U S Patent No 5,118,627), the SV40 late promoter (U S Patent No. 5,118,627), CMV early gene promoter (U S Patent No 5,168,062), and adenovirus promoter In addition to viral promoters, cellular promoters are also amenable within the context of this invention. In particular, cellular promoters for the so-called housekeeping genes are useful. Viral promoters are preferred, because generally they are stronger promoters than cellular promoters Promoter regions have been identified in the genes of many eukaryotes including higher eukaryotes, such that suitable promoters for use in a particular host can be readily selected by those skilled in the art.

Inducible promoters may also be used. These promoters include MMTV LTR (PCT WO 91/13160), inducible by dexamethasone, metallothionein promoter, inducible by heavy metals, and promoters with cAMP response elements, inducible by cAMP By using an inducible promoter, the nucleic acid sequence encoding a binding domain-immunoglobulin fusion protein may be delivered to a cell by the subject invention expression construct and will remain quiescent until the addition of the inducer This allows further control on the timing of production of the gene product.

Event-type specific promoters are active or up regulated only upon the occurrence of an event, such as tumorigenicity or viral infection. The HIV LTR is a well-

known example of an event-specific promoter The promoter is inactive unless the *tat* gene product is present, which occurs upon viral infection. Some event-type promoters are also tissue-specific

Additionally, promoters that are coordinately regulated with a particular cellular gene may be used. For example, promoters of genes that are coordinately expressed may be used when expression of a particular binding construct of the invention, for example, a binding domain-immunoglobulin fusion protein-encoding gene is desired in concert with expression of one or more additional endogenous or exogenously introduced genes. This type of promoter is especially useful when one knows the pattern of gene expression relevant to induction of an immune response in a particular tissue of the immune system, so that specific immunocompetent cells within that tissue may be activated or otherwise recruited to participate in the immune response.

In addition to the promoter, repressor sequences, negative regulators, or tissue-specific silencers may be inserted to reduce non-specific expression of binding domain-immunoglobulin fusion protein encoding genes in certain situations, such as, for example, a host that is transiently immunocompromised as part of a therapeutic strategy Multiple repressor elements may be inserted in the promoter region. Repression of transcription is independent on the orientation of repressor elements or distance from the promoter One type of repressor sequence is an insulator sequence. Such sequences inhibit transcription (Dunaway *et al*, 1997 *Mol Cell Biol* 17 182-9, Gdula *et al*, 1996 *Proc Natl Acad Sci USA* 93 9378-83, Chan *et al*, 1996 *J Virol* 70 5312-28, Scott and Geyer, 1995 *EMBO J* 14 6258-67, Kalos and Fournier, 1995 *Mol Cell Biol* 15 198-207, Chung *et al*, 1993 *Cell 74* 505-14) and will silence undesired background transcription.

Repressor elements have also been identified in the promoter regions of the genes for type II (cartilage) collagen, choline acetyltransferase, albumin (Hu *et al*, 1992 *J Cell Growth Differ* 3(9) 577-588), phosphoglycerate kinase (PGK-2) (Misuno *et al*., 1992 *Gene* 119(2)·293-297), and in the 6-phosphofructo-2-kinase/fructose-2, 6-bisphosphatase gene. (Lemaigre *et al*, *Mol Cell Biol.* 11(2) 1099-1106) Furthermore, the negative regulatory element Tse-1 has been identified in a number of liver specific genes, and has been shown to block cAMP response element(CRE)-mediated induction of gene activation in hepatocytes (Boshart *et al*, 1990 *Cell* 61(5) 905-916,)

In preferred embodiments, elements that increase the expression of the desired product are incorporated into the construct Such elements include internal ribosome binding sites (IRES, Wang and Siddiqui, 1995 *Curr Top. Microbiol. Immunol* 203.99, Ehrenfeld and Semler, 1995 *Curr Top Microbiol Immunol.* 203 65, Rees *et al.*, 1996 *Biotechniques* 20 102, Sugimoto et al , 1994 *Biotechnology* 12 694) IRES increase translation efficiency As well, other sequences may enhance expression For some genes, sequences especially at the 5' end inhibit transcription and/or translation. These sequences are usually palindromes that can form hairpin structures. Any such sequences in the nucleic acid to be delivered are generally deleted Expression levels of the transcript or translated product are assayed to confirm or ascertain which sequences affect expression. Transcript levels may be assayed by any known method, including Northern blot hybridization, RNase probe protection and the like. Protein levels may be assayed by any known method, including ELISA, western blot, immunocytochemistry or other well-known techniques.

Other elements may be incorporated into the constructs of the invention, for example, into binding domain-immunoglobulin fusion protein encoding constructs of the present invention. In preferred embodiments, the construct includes a transcription terminator sequence, including a polyadenylation sequence, splice donor and acceptor sites, and an enhancer Other elements useful for expression and maintenance of the construct in mammalian cells or other eukaryotic cells may also be incorporated (*e.g.*, origin of replication) Because the constructs are conveniently produced in bacterial cells, elements that are necessary for, or that enhance, propagation in bacteria are incorporated. Such elements include an origin of replication, a selectable marker and the like.

As provided herein, an additional level of controlling the expression of nucleic acids encoding constructs of the invention, for example, binding domain-immunoglobulin fusion proteins, delivered to cells for gene therapy, for example, may be provided by simultaneously delivering two or more differentially regulated nucleic acid constructs The use of such a multiple nucleic acid construct approach may permit coordinated regulation of an immune response such as, for example, spatiotemporal coordination that depends on the cell type and/or presence of another expressed encoded component. Those familiar with the art will appreciate that multiple levels of regulated gene expression may be achieved in a similar manner by selection of suitable regulatory

sequences, including but not limited to promoters, enhancers and other well known gene regulatory elements

The present invention also relates to vectors, and to constructs prepared from known vectors that include nucleic acids of the present invention, and in particular to "recombinant expression constructs", including any of various known constructs, including delivery constructs, useful for gene therapy, that include any nucleic acids encoding, for example, binding domain-immunoglobulin fusion proteins and polypeptides according to the invention as provided herein, to host cells which are genetically engineered with vectors and/or other constructs of the invention and to methods of administering expression or other constructs comprising nucleic acid sequences encoding, for example, binding domain-immunoglobulin fusion polypeptides and fusion proteins of the invention, or fragments or variants thereof, by recombinant techniques.

Various constructs of the invention, including for example, binding domain-immunoglobulin fusion proteins, can be expressed in virtually any host cell, including *in vivo* host cells in the case of use for gene therapy, under the control of appropriate promoters, depending on the nature of the construct (*e.g.*, type of promoter, as described above), and on the nature of the desired host cell (*e.g.*, whether postmitotic terminally differentiated or actively dividing; *e.g.* whether the expression construct occurs in host cell as an episome or is integrated into host cell genome)

Appropriate cloning and expression vectors for use with prokaryotic and eukaryotic hosts are described, for example, by Sambrook, *et al*, *Molecular Cloning A Laboratory Manual*, Second Edition, Cold Spring Harbor, NY, (1989), as noted herein, in particularly preferred embodiments of the invention, recombinant expression is conducted in mammalian cells that have been transfected or transformed with the subject invention recombinant expression construct. See also, for example, Machida, CA., "Viral Vectors for Gene Therapy- Methods and Protocols", Wolff, JA, "Gene Therapeutics. Methods and Applications of Direct Gene Transfer" (Birkhauser 1994), Stern, U and Walther, W (eds.P, "Gene Therapy of Cancer- Methods and Protocols" (Humana Press 2000), Robbins, PD (ed.), "Gene Therapy Protocols" (Humana Press 1997), Morgan, JR (ed.), "Gene Therapy Protocols" (Humana Press 2002), Meager, A (ed.), "Gene Therapy Technologies, Applications and Regulations From Laboratory to Clinic" (John Wiley & Sons Inc 1999), MacHida, CA and Constant, JG, "Viral Vectors for Gene Therapy- Methods and Protocols"

(Humana Press 2002),"New Methods Of Gene Therapy For Genetic Metabolic Diseases NIH Guide," Volume 22, Number 35, October 1, 1993 See also recent U S patents relating to gene therapy, including vaccines, which include U S Pat. Nos. 6,384,210 ("Solvent for biopolymer synthesis, solvent microdroplets and methods of use"), 6,384,203 ("Family of immunoregulators designated leukocyte immunoglobulin-like receptors (LIR)"), 6,384,202 ("Cell-specific active compounds regulated by the cell cycle"), 6,384,018 ("Polynucleotide tuberculosis vaccine"), 6,383,814 ("Cationic amphiphiles for intracellular delivery of therapeutic molecules"), 6,383,811 ("Polyampholytes for delivering polyions to a cell"), 6,383,795 ("Efficient purification of adenovirus"), 6,383,794 ("Methods of producing high titer recombinant adeno-associated virus"), 6,383,785 ("Self-enhancing, pharmacologically controllable expression systems"), 6,383,753 ("Yeast mammalian regulators of cell proliferation"), 6,383,746 ("Functional promoter for CCR5"), 6,383,743 ("Method for serial analysis of gene expression"), 6,383,738 ("Herpes simplex virus ORF P is a repressor of viral protein synthesis"), 6,383,737 ("Human oxalyl-CoA Decarboxylase"), 6,383,733 ("Methods of screening for pharmacologically active compounds for the treatment of tumour diseases"), 6,383,522 ("Toxicity reduced composition containing an anti-neoplastic agent and a shark cartilage extract"), 6,383,512 ("Vesicular complexes and methods of making and using the same"), 6,383,481 ("Method for transplantation of hemopoietic stem cells"), 6,383,478 ("Polymeric encapsulation system promoting angiogenesis"), 6,383,138 ("Method for transdermal sampling of analytes"), 6,380,382 ("Gene encoding a protein having diagnostic, preventive, therapeutic, and other uses"), 6,380,371 ("Endoglycan. a novel protein having selectin ligand and chemokine presentation activity"), 6,380,369 ("Human DNA mismatch repair proteins"), 6,380,362 ("Polynucleotides, polypeptides expressed by the polynucleotides and methods for their use"), 6,380,170 ("Nucleic acid construct for the cell cycle regulated expression of structural genes"), 6,380,169 ("Metal complex containing oligonucleoside cleavage compounds and therapies"), 6,379,967 ("Herpesvirus saimiri as viral vector"), 6,379,966 ("Intravascular delivery of non-viral nucleic acid protease proteins, and uses thereof")

Typically, for example, expression constructs are derived from plasmid vectors. One preferred construct is a modified pNASS vector (Clontech, Palo Alto, CA), which has nucleic acid sequences encoding an ampicillin resistance gene, a

polyadenylation signal and a T7 promoter site. Other suitable mammalian expression vectors are well known (*see, e.g.*, Ausubel *et al.*, 1995, Sambrook *et al.*, *supra*, see also, *e.g.*, catalogues from Invitrogen, San Diego, CA, Novagen, Madison, WI, Pharmacia, Piscataway, NJ, and others) Presently preferred constructs may be prepared that include a dihydrofolate reductase (DHFR) encoding sequence under suitable regulatory control, for promoting enhanced production levels of the binding domain-immunoglobulin fusion protein, which levels result from gene amplification following application of an appropriate selection agent (*e.g.*, methotrexate)

Generally, recombinant expression vectors will include origins of replication and selectable markers permitting transformation of the host cell, and a promoter derived from a highly-expressed gene to direct transcription of a downstream structural sequence, as described above. The heterologous structural sequence is assembled in appropriate phase with translation initiation and termination sequences Thus, for example, the binding domain-immunoglobulin fusion protein encoding nucleic acids as provided herein may be included in any one of a variety of expression vector constructs as a recombinant expression construct for expressing a binding domain-immunoglobulin fusion polypeptide in a host cell In certain preferred embodiments the constructs are included in formulations that are administered *in vivo* Such vectors and constructs include chromosomal, nonchromosomal and synthetic DNA sequences, *e.g.*, derivatives of SV40, bacterial plasmids, phage DNA, yeast plasmids; vectors derived from combinations of plasmids and phage DNA, viral DNA, such as vaccinia, adenovirus, fowl pox virus, and pseudorabies, or replication deficient retroviruses as described below However, any other vector may be used for preparation of a recombinant expression construct, and in preferred embodiments such a vector will be replicable and viable in the host.

The appropriate DNA sequence(s) may be inserted into a vector, for example, by a variety of procedures In general, a DNA sequence is inserted into an appropriate restriction endonuclease site(s) by procedures known in the art. Standard techniques for cloning, DNA isolation, amplification and purification, for enzymatic reactions involving DNA ligase, DNA polymerase, restriction endonucleases and the like, and various separation techniques are those known and commonly employed by those skilled in the art. A number of standard techniques are described, for example, in Ausubel

et al (1993 *Current Protocols in Molecular Biology*, Greene Publ. Assoc. Inc & John Wiley & Sons, Inc., Boston, MA), Sambrook *et al* (1989 *Molecular Cloning*, Second Ed., Cold Spring Harbor Laboratory, Plainview, NY), Maniatis *et al* (1982 Molecular Cloning, Cold Spring Harbor Laboratory, Plainview, NY), Glover (Ed.) (1985 *DNA Cloning Vol I and II*, IRL Press, Oxford, UK), Hames and Higgins (Eds.), (1985 *Nucleic Acid Hybridization*, IRL Press, Oxford, UK), and elsewhere.

The DNA sequence in the expression vector is operatively linked to at least one appropriate expression control sequence(s) (*e.g.*, a constitutive promoter or a regulated promoter) to direct mRNA synthesis. Representative examples of such expression control sequences include promoters of eukaryotic cells or their viruses, as described above. Promoter regions can be selected from any desired gene using CAT (chloramphenicol transferase) vectors or other vectors with selectable markers. Eukaryotic promoters include CMV immediate early, HSV thymidine kinase, early and late SV40, LTRs from retrovirus, and mouse metallothionein-I. Selection of the appropriate vector and promoter is well within the level of ordinary skill in the art, and preparation of certain particularly preferred recombinant expression constructs comprising at least one promoter or regulated promoter operably linked to a nucleic acid encoding an binding domain-immunoglobulin fusion polypeptide is described herein.

Transcription of the DNA encoding proteins and polypeptides included within the present invention by higher eukaryotes may be increased by inserting an enhancer sequence into the vector. Enhancers are *cis*-acting elements of DNA, usually about from 10 to 300 bp that act on a promoter to increase its transcription. Examples including the SV40 enhancer on the late side of the replication origin bp 100 to 270, a cytomegalovirus early promoter enhancer, the polyoma enhancer on the late side of the replication origin, and adenovirus enhancers.

Gene therapy is the use of genetic material to treat disease. It comprises strategies to replace defective genes or add new genes to cells and/or tissues, and is being developed for application in the treatment of cancer, the correction of metabolic disorders and in the field of immunotherapy. Gene therapies of the invention include the use of various constructs of the invention, with or without a separate carrier or delivery vehicle or constructs, for treatment of the diseases, disorders, and/or conditions noted herein. Such constructs may also be used as vaccines for treatment or prevention of the diseases,

disorders, and/or conditions noted herein. DNA vaccines, for example, make use of polynucleotides encoding immunogenic protein and nucleic acid determinants to stimulate the immune system against pathogens or tumor cells Such strategies can stimulate either acquired or innate immunity or can involve the modification of immune function through cytokine expression. *In vivo* gene therapy involves the direct injection of genetic material into a patient or animal model of human disease Vaccines and immune modulation are systemic therapies With tissue-specific *in vivo* therapies, such as those that aim to treat cancer, localized gene delivery and/or expression/targeting systems are preferred Diverse gene therapy vectors have been designed to target specific tissues, and procedures have been developed to physically target specific tissues, for example, using catheter-based technologies, all of which are contemplated herein. Ex vivo approaches to gene therapy are also contemplated herein and involve the removal, genetic modification, expansion and re-administration of a patient's own cells Examples include bone marrow transplantation for cancer treatment or the genetic modivation of lymphoid progenitor cells *Ex vivo* gene therapy is preferably applied to the treatment of cells that are easily accessible and can survive in culture during the gene transfer process (such as blood or skin cells)

Useful gene therapy vectors include adenoviral vectors, lentiviral vectors, Adeno-associated virus (AAV) vectors, Herpes Simplex Virus (Hsv) vectors, and retroviral vectors Gene therapies may also be carried out using "naked DNA," liposome-based delivery, lipid-based delivery (including DNA attached to positively charged lipids), and electroporation.

As provided herein, in certain embodiments, including but not limited to gene therapy embodiments, the vector may be a viral vector such as, for example, a retroviral vector Miller *et al*, 1989 *BioTechniques* 7:980, Coffin and Varmus, 1996 Retroviruses, *Cold Spring Harbor Laboratory Press*, NY For example, retroviruses from which the retroviral plasmid vectors may be derived include, but are not limited to, Moloney Murine Leukemia Virus, spleen necrosis virus, retroviruses such as Rous Sarcoma Virus, Harvey Sarcoma virus, avian leukosis virus, gibbon ape leukemia virus, human immunodeficiency virus, adenovirus, Myeloproliferative Sarcoma Virus, and mammary tumor virus

Retroviruses are RNA viruses that can replicate and integrate into the genome of a host cell via a DNA intermediate. This DNA intermediate, or provirus, may

be stably integrated into the host cell DNA. According to certain embodiments of the present invention, an expression construct may comprise a retrovirus into which a foreign gene that encodes a foreign protein is incorporated in place of normal retroviral RNA. When retroviral RNA enters a host cell coincident with infection, the foreign gene is also introduced into the cell, and may then be integrated into host cell DNA as if it were part of the retroviral genome. Expression of this foreign gene within the host results in expression of the foreign protein.

Most retroviral vector systems that have been developed for gene therapy are based on murine retroviruses. Such retroviruses exist in two forms, as free viral particles referred to as virions, or as proviruses integrated into host cell DNA. The virion form of the virus contains the structural and enzymatic proteins of the retrovirus (including the enzyme reverse transcriptase), two RNA copies of the viral genome, and portions of the source cell plasma membrane containing viral envelope glycoprotein. The retroviral genome is organized into four main regions: the Long Terminal Repeat (LTR), which contains *cis*-acting elements necessary for the initiation and termination of transcription and is situated both 5' and 3' of the coding genes, and the three coding genes *gag*, *pol*, and *env*. These three genes *gag*, *pol* and *env* encode, respectively, internal viral structures, enzymatic proteins (such as integrase), and the envelope glycoprotein (designated gp70 and p15e) which confers infectivity and host range specificity of the virus, as well as the "R" peptide of undetermined function.

Separate packaging cell lines and vector producing cell lines have been developed because of safety concerns regarding the uses of retroviruses, including their use in expression constructs as provided by the present invention. Briefly, this methodology employs the use of two components, a retroviral vector and a packaging cell line (PCL). The retroviral vector contains long terminal repeats (LTRs), the foreign DNA to be transferred and a packaging sequence (y). This retroviral vector will not reproduce by itself because the genes that encode structural and envelope proteins are not included within the vector genome. The PCL contains genes encoding the *gag*, *pol*, and *env* proteins, but does not contain the packaging signal "y". Thus, a PCL can only form empty virion particles by itself. Within this general method, the retroviral vector is introduced into the PCL, thereby creating a vector-producing cell line (VCL). This VCL

manufactures virion particles containing only the retroviral vector's (foreign) genome, and therefore has previously been considered to be a safe retrovirus vector for therapeutic use.

"Retroviral vector construct" refers to an assembly that is, within preferred embodiments of the invention, capable of directing the expression of a sequence(s) or gene(s) of interest, such as binding domain-immunoglobulin fusion encoding nucleic acid sequences Briefly, the retroviral vector construct must include a 5' LTR, a tRNA binding site, a packaging signal, an origin of second strand DNA synthesis and a 3' LTR. A wide variety of heterologous sequences may be included within the vector construct, including for example, sequences which encode a protein (*e.g*, cytotoxic protein, disease-associated antigen, immune accessory molecule, or replacement gene), or which are useful as a molecule itself (*e.g*, as a ribozyme or antisense sequence)

Retroviral vector constructs of the present invention may be readily constructed from a wide variety of retroviruses, including for example, B, C, and D type retroviruses as well as spumaviruses and lentiviruses (see, *e.g* RNA Tumor Viruses, Second Edition, Cold Spring Harbor Laboratory, 1985) Such retroviruses may be readily obtained from depositories or collections such as the American Type Culture Collection ("ATCC", Rockville, Maryland), or isolated from known sources using commonly available techniques Any of the above retroviruses may be readily utilized in order to assemble or construct retroviral vector constructs, packaging cells, or producer cells of the present invention given the disclosure provided herein, and standard recombinant techniques (*e.g*, Sambrook *et al*, *Molecular Cloning A Laboratory Manual*, 2d ed., Cold Spring Harbor Laboratory Press, 1989, Kunkle, 1985 *PNAS 82* 488)

Suitable promoters for use in viral vectors generally may include, but are not limited to, the retroviral LTR, the SV40 promoter; and the human cytomegalovirus (CMV) promoter described in Miller, *et al*, 1989 *Biotechniques* 7 980-990, or any other promoter (*e.g*, cellular promoters such as eukaryotic cellular promoters including, but not limited to, the histone, pol III, and β-actin promoters) Other viral promoters that may be employed include, but are not limited to, adenovirus promoters, thymidine kinase (TK) promoters, and B19 parvovirus promoters The selection of a suitable promoter will be apparent to those skilled in the art from the teachings contained herein, and may be from among either regulated promoters or promoters as described above.

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

The producer cell line generates infectious retroviral vector particles that include the nucleic acid sequence(s) encoding the binding domain-immunoglobulin fusion polypeptides or fusion proteins Such retroviral vector particles then may be employed, to transduce eukaryotic cells, either *in vitro* or *in vivo* The transduced eukaryotic cells will express the nucleic acid sequence(s) encoding the binding domain-immunoglobulin fusion polypeptide or fusion protein. Eukaryotic cells which may be transduced include, but are not limited to, embryonic stem cells, as well as hematopoietic stem cells, hepatocytes, fibroblasts, circulating peripheral blood mononuclear and polymorphonuclear cells including myelomonocytic cells, lymphocytes, myoblasts, tissue macrophages, dendritic cells, Kupffer cells, lymphoid and reticuloendothelia cells of the lymph nodes and spleen, keratinocytes, endothelial cells, and bronchial epithelial cells.

As another example of an embodiment of the invention in which a viral vector is used to prepare, for example, a recombinant binding domain-immunoglobulin fusion expression construct, in one preferred embodiment, host cells transduced by a recombinant viral construct directing the expression of binding domain-immunoglobulin fusion polypeptides or fusion proteins may produce viral particles containing expressed binding domain-immunoglobulin fusion polypeptides or fusion proteins that are derived from portions of a host cell membrane incorporated by the viral particles during viral budding

In another aspect, the present invention relates to host cells containing the herein described nucleic acid constructs, such as, for example, recombinant binding domain-immunoglobulin fusion expression constructs Host cells are genetically engineered (transduced, transformed or transfected) with the vectors and/or expression

constructs of this invention that may be, for example, a cloning vector, a shuttle vector, or an expression construct. The vector or construct may be, for example, in the form of a plasmid, a viral particle, a phage, *etc*. The engineered host cells can be cultured in conventional nutrient media modified as appropriate for activating promoters, selecting transformants or amplifying particular genes such as genes encoding binding domain-immunoglobulin fusion polypeptides or binding domain-immunoglobulin fusion fusion proteins. The culture conditions for particular host cells selected for expression, such as temperature, pH and the like, will be readily apparent to the ordinarily skilled artisan.

The host cell for production or expression of a construct of the invention, for example, can be a higher eukaryotic cell, such as a mammalian cell, or a lower eukaryotic cell, such as a yeast cell, or the host cell can be a prokaryotic cell, such as a bacterial cell. Representative examples of appropriate host cells according to the present invention include, but need not be limited to, bacterial cells, such as *E. coli, Streptomyces Salmonella typhimurium*, fungal cells, such as yeast; insect cells, such as *Drosophila S2* and *Spodoptera Sf9*; animal cells, such as CHO, COS or 293 cells, adenoviruses, plant cells, or any suitable cell already adapted to *in vitro* propagation or so established *de novo*. The selection of an appropriate host is deemed to be within the scope of those skilled in the art from the teachings herein.

Various mammalian cell culture systems can also be employed to express recombinant protein. Examples of mammalian expression systems include the COS-7 lines of monkey kidney fibroblasts, described by Gluzman, 1981 *Cell* 23 175, and other cell lines capable of expressing a compatible vector, for example, the C127, 3T3, CHO, HeLa and BHK cell lines. Mammalian expression vectors will comprise an origin of replication, a suitable promoter and enhancer, and also any necessary ribosome binding sites, polyadenylation site, splice donor and acceptor sites, transcriptional termination sequences, and 5' flanking nontranscribed sequences, for example as described herein regarding the preparation of binding domain-immunoglobulin fusion expression constructs. DNA sequences derived from the SV40 splice, and polyadenylation sites may be used to provide the required nontranscribed genetic elements. Introduction of the construct into the host cell can be effected by a variety of methods with which those skilled in the art will be familiar, including but not limited to, for example, calcium phosphate transfection, DEAE-

Dextran mediated transfection, or electroporation (Davis *et al*, 1986 *Basic Methods in Molecular Biology*)

The present invention constructs, for example, binding domain-immunoglobulin fusion proteins, or compositions comprising one or more polynucleotides encoding same as described herein (for example, to be administered under conditions and for a time sufficient to permit expression of a binding domain-immunoglobulin fusion protein in a host cell *in vivo* or *in vitro*, for gene therapy, for example, among other things), may be formulated into pharmaceutical compositions for administration according to well known methodologies Pharmaceutical compositions generally comprise one or more recombinant expression constructs, and/or expression products of such constructs, in combination with a pharmaceutically acceptable carrier, excipient or diluent. Such carriers will be nontoxic to recipients at the dosages and concentrations employed. For nucleic acid-based formulations, or for formulations comprising expression products of the subject invention recombinant constructs, about 0 01 μg/kg to about 100 mg/kg body weight will be administered, for example, typically by the intradermal, subcutaneous, intramuscular or intravenous route, or by other routes A preferred dosage, for example, is about 1 μg/kg to about 1 mg/kg, with about 5 μg/kg to about 200 μg/kg particularly preferred. It will be evident to those skilled in the art that the number and frequency of administration will be dependent upon the response of the host. "Pharmaceutically acceptable carriers" for therapeutic use are well known in the pharmaceutical art, and are described, for example, in *Remingtons Pharmaceutical Sciences*, Mack Publishing Co (A.R. Gennaro edit. 1985) For example, sterile saline and phosphate-buffered saline at physiological pH may be used. Preservatives, stabilizers, dyes and even flavoring agents may be provided in the pharmaceutical composition For example, sodium benzoate, sorbic acid and esters of *p*-hydroxybenzoic acid may be added as preservatives. *Id* at 1449 In addition, antioxidants and suspending agents may be used. *Id*

"Pharmaceutically acceptable salt" refers to salts of the compounds of the present invention derived from the combination of such compounds and an organic or inorganic acid (acid addition salts) or an organic or inorganic base (base addition salts). The compounds of the present invention may be used in either the free base or salt forms, with both forms being considered as being within the scope of the present invention.

The pharmaceutical compositions that contain one or more nucleic acid constructs of the invention, for example, binding domain-immunoglobulin fusion protein encoding constructs (or their expressed products) may be in any form that allows for the composition to be administered to a patient. For example, the composition may be in the form of a solid, liquid or gas (aerosol) Typical routes of administration include, without limitation, oral, topical, parenteral (*e.g.*, sublingually or buccally), sublingual, rectal, vaginal, and intranasal. The term parenteral as used herein includes subcutaneous injections, intravenous, intramuscular, intrasternal, intracavernous, intrathecal, intrameatal, intraurethral injection or infusion techniques The pharmaceutical composition is formulated so as to allow the active ingredients contained therein to be bioavailable upon administration of the composition to a patient. Compositions that will be administered to a patient take the form of one or more dosage units, where for example, a tablet may be a single dosage unit, and a container of one or more compounds of the invention in aerosol form may hold a plurality of dosage units.

For oral administration, an excipient and/or binder may be present. Examples are sucrose, kaolin, glycerin, starch dextrins, sodium alginate, carboxymethylcellulose and ethyl cellulose. Coloring and/or flavoring agents may be present A coating shell may be employed.

The composition may be in the form of a liquid, *e.g.*, an elixir, syrup, solution, emulsion or suspension The liquid may be for oral administration or for delivery by injection, as two examples When intended for oral administration, preferred compositions contain, in addition to one or more binding domain-immunoglobulin fusion construct or expressed product, one or more of a sweetening agent, preservatives, dye/colorant and flavor enhancer In a composition to be administered by injection, one or more of a surfactant, preservative, wetting agent, dispersing agent, suspending agent, buffer, stabilizer and isotonic agent, for example, may be included.

A liquid pharmaceutical composition as used herein, whether in the form of a solution, suspension or other like form, may include one or more of the following adjuvants sterile diluents such as water for injection, saline solution, preferably physiological saline, Ringer's solution, isotonic sodium chloride, fixed oils such as synthetic mono or diglycerides which may serve as the solvent or suspending medium, polyethylene glycols, glycerin, propylene glycol or other solvents, antibacterial agents such

as benzyl alcohol or methyl paraben, antioxidants such as ascorbic acid or sodium bisulfite; chelating agents such as ethylenediaminetetraacetic acid, buffers such as acetates, citrates or phosphates and agents for the adjustment of tonicity such as sodium chloride or dextrose The parenteral preparation can be enclosed in ampoules, disposable syringes or multiple dose vials made of glass or plastic. Physiological saline is a preferred adjuvant. An injectable pharmaceutical composition is preferably sterile.

It may also be desirable to include other components in the preparation, such as delivery vehicles including but not limited to aluminum salts, water-in-oil emulsions, biodegradable oil vehicles, oil-in-water emulsions, biodegradable microcapsules, and liposomes Examples of immunostimulatory substances (adjuvants) for use in such vehicles include N-acetylmuramyl-L-alanine-D-isoglutamine (MDP), lipopoly-saccharides (LPS), glucan, IL-12, GM-CSF, gamma interferon and IL-15

While any suitable carrier known to those of ordinary skill in the art may be employed in the pharmaceutical compositions of this invention, the type of carrier will vary depending on the mode of administration and whether a sustained release is desired. For parenteral administration, such as subcutaneous injection, the carrier preferably comprises water, saline, alcohol, a fat, a wax or a buffer For oral administration, any of the above carriers or a solid carrier, such as mannitol, lactose, starch, magnesium stearate, sodium saccharine, talcum, cellulose, glucose, sucrose, and magnesium carbonate, may be employed Biodegradable microspheres (*e.g.*, polylactic galactide) may also be employed as carriers for the pharmaceutical compositions of this invention. Suitable biodegradable microspheres are disclosed, for example, in U S Patent Nos 4,897,268 and 5,075,109 In this regard, it is preferable that the microsphere be larger than approximately 25 microns.

Pharmaceutical compositions may also contain diluents such as buffers, antioxidants such as ascorbic acid, low molecular weight (less than about 10 residues) polypeptides, proteins, amino acids, carbohydrates including glucose, sucrose or dextrins, chelating agents such as EDTA, glutathione and other stabilizers and excipients Neutral buffered saline or saline mixed with nonspecific serum albumin are exemplary appropriate diluents Preferably, product is formulated as a lyophilizate using appropriate excipient solutions (*e g*, sucrose) as diluents

As described above, the subject invention includes compositions capable of delivering nucleic acid molecules encoding binding domain-immunoglobulin fusion

proteins Such compositions include recombinant viral vectors (e.g , retroviruses (*see* WO 90/07936, WO 91/02805, WO 93/25234, WO 93/25698, and WO 94/03622), adenovirus (see Berkner, 1988 *Biotechniques* 6 616-627, Li *et al* , 1993 *Hum. Gene Ther* 4 403-409, Vincent *et al* , *Nat. Genet* 5 130-134, and Kolls *et al* , 1994 *Proc. Natl. Acad. Sci. USA* 91.215-219), pox virus (*see* U S Patent No 4,769,330, U S Patent No 5,017,487, and WO 89/01973)), recombinant expression construct nucleic acid molecules complexed to a polycationic molecule (*see* WO 93/03709), and nucleic acids associated with liposomes (*see* Wang *et al* , 1987 *Proc. Natl Acad. Sci. USA* 84 7851) In certain embodiments, the DNA may be linked to killed or inactivated adenovirus (*see* Curiel *et al* , 1992 *Hum. Gene Ther* 3 147-154, Cotton *et al* , 1992 *Proc Natl. Acad. Sci. USA 89*·6094) Other suitable compositions include DNA-ligand (*see* Wu *et al* , 1989 *J Biol. Chem. 264* 16985-16987) and lipid-DNA combinations (see Felgner *et al* , 1989 *Proc. Natl. Acad. Sci. USA* 84 7413-7417)

In addition to direct *in vivo* procedures, *ex vivo* procedures may be used in which cells are removed from a host, modified, and placed into the same or another host animal It will be evident that one can utilize any of the compositions noted above for introduction of constructs of the invention, for example, binding domain-immunoglobulin fusion proteins or of binding domain-immunoglobulin fusion protein encoding nucleic acid molecules into tissue cells in an *ex vivo* context. Protocols for viral, physical and chemical methods of uptake are well known in the art.

Accordingly, the present invention is useful for treating a patient having a B cell disorder or a malignant condition, or for treating a cell culture derived from such a patient As used herein, the term "patient" refers to any warm-blooded animal, preferably a human A patient may be afflicted with cancer or a malignant condition, such as B cell lymphoma, or may be normal (*i* e., free of detectable disease and infection) A "cell culture" includes any preparation amenable to *ex vivo* treatment, for example a preparation containing immunocompetent cells or isolated cells of the immune system (including, but not limited to, T cells, macrophages, monocytes, B cells and dendritic cells) Such cells may be isolated by any of a variety of techniques well known to those of ordinary skill in the art (*e g* Ficoll-hypaque density centrifugation) The cells may (but need not) have been isolated from a patient afflicted with a B cell disorder or a malignant condition, and may be reintroduced into a patient after treatment.

A liquid composition intended for either parenteral or oral administration should contain an amount of a construct of the invention, for example, a binding domain-immunoglobulin fusion protein encoding construct or expressed product, such that a suitable dosage will be obtained Typically, this amount is at least 0 01 wt% of a binding domain-immunoglobulin fusion construct or expressed product in the composition. When intended for oral administration, this amount may be varied to be between 0 1 and about 70% of the weight of the composition. Preferred oral compositions contain between about 4% and about 50% of binding domain-immunoglobulin fusion construct or expressed product(s) Preferred compositions and preparations are prepared so that, for example, a parenteral dosage unit contains between 0 01 to 1% by weight of active compound.

The pharmaceutical composition may be intended for topical administration, in which case the carrier may suitably comprise a solution, emulsion, ointment, or gel base. The base, for example, may comprise one or more of the following: petrolatum, lanolin, polyethylene glycols, beeswax, mineral oil, diluents such as water and alcohol, and emulsifiers and stabilizers. Thickening agents may be present in a pharmaceutical composition for topical administration. If intended for transdermal administration, the composition may include a transdermal patch or iontophoresis device. Topical formulations may contain a concentration of a construct of the invention, for example, a binding domain-immunoglobulin fusion construct or expressed product, of from about 0 1 to about 10% w/v (weight per unit volume)

The composition may be intended for rectal administration, in the form, *e.g.*, of a suppository that will melt in the rectum and release the drug. The composition for rectal administration may contain an oleaginous base as a suitable nonirritating excipient. Such bases include, without limitation, lanolin, cocoa butter and polyethylene glycol.

In the methods of the invention, a construct of the invention, for example, a binding domain-immunoglobulin fusion encoding constructs or expressed product(s), may be administered through use of insert(s), bead(s), timed-release formulation(s), patch(es) or fast-release formulation(s)

Constructs of the invention, for example, antigen-binding constructs of the invention, may be administered or co-administered to an animal or patient in combination with, or at the same or about the same time, as other compounds In one aspect, one or more constructs, including for example one or more antigen-binding constructs, are

administered to an animal or patient in conjunction with one or more chemotherapeutic compounds such as alkylating agents, nucleoside analogues, and the like. The administration or co-administration of one or more constructs, including one or more antigen-binding constructs, of the invention and one or more chemotherapeutic agents can be used for the treatment of tumors or cancer in an animal or patient. Exemplary cancers include, but are not limited to, head and neck cancer, breast cancer, colorectal cancer, gastric cancer, hepatic cancer, bladder cancer, cervical cancer, endometrial cancer, lung cancer (non-small cell), ovarian cancer, pancreatic cancer, prostate cancer; choriocarcinoma (lung cancer), hairy cell leukemia, chronic lymphotic leukemia, acute lymphocytic leukemia (breast & bladder), acute myelogenous leukemia, meningeal leukemia, chronic myelogenous leukemia, erythroleukemia. More commonly the cancers treated include non-Hodgkin's lymphoma (osteogenic sarcoma, adult soft tissue sarcoma), T-cell lymphoma, chronic lymphocytic leukaemia, slowly growing non-Hodgkin's lymphomas, Hodgkin's lymphoma and ovarian cancer

Examples of an alkylating agents that can be co-administered with one or more constructs, including one or more antigen-binding constructs, of the invention include mechlorethamine, chlorambucil, ifosfamide, melphalan, busulfan, carmustine, lomustine, procarbazine, dacardazine, cisplatin, carboplatin, mitomycin C, cyclophosphamide, isosfamide, , hexamethylmelamine, thiotepa, , and dacarbazine, and analogues thereof See for example U S Pat. No 3,046,301 describing the synthesis of chlorambucil, U S Pat. No 3,732,340 describing the synthesis of ifosfamide, U S Pat. No 3,018,302 for the synthesis of cyclophosphamide, U S Pat. No 3,032,584 describing the synthesis of melphalan, and Braunwald et al , "Harrison's Principles of Internal Medicine," 15th Ed , McGraw-Hill, New York, NY, pp 536-544 (2001) for clinical aspects of cyclophosphamide, chlorambucil, melphalan, ifosfamide, procarbazine, hexamethylmelamine, cisplatin, and carboplatin. Examples of nucleoside analogues, include, but are not limited to, fludarabine pentostatin, methotrexate, fluorouracil, fluorodeoxyuridine, CB3717, azacitidine, cytarabine, floxuridine, mercaptopurine, 6-thioguanine, cladribine, and analogues thereof One example is the combination of constructs, including antigen-binding constructs that bind CD20 This construct acts as a chemosensitising agent and works together with chemotherapeutic agents, such that less chemotherapeutic agents are necessary to achieve anti-tumor or anti-cancer effects For

example, U S Pat. No 3,923,785 describing the synthesis of pentostatin, U S Pat. No 4,080,325 describing the synthesis of methotrexate, U S Pat. No 2,802,005 describing the synthesis of fluorouracil, and Braunwald et al., "Harrison's Principles of Internal Medicine," 15th Ed , McGraw-Hill, New York, NY, pp 536-544 (2001) for clinical aspects of methotrexate, 5-fluorouracil, cytosine arabinoside, 6-mercaptopurine, 6-thioguanine, and fludarabine phosphate.

In another aspect, one or more constructs, including one or more antigen-binding constructs, of the invention can be administered or co-administered compounds that inhibit topoisomerase II or compounds that otherwise interact with nucleic acids in cells Such compounds include, for example, doxorubican, epirubicin, etoposide, teniposide, mitoxantrone, and analogues thereof. In one example, this combination is used in treatment to reduce tumor cell contamination of peripheral blood progenitor cells (PBSC) in conjunction with high-dose chemotherapy and autologous stem cell support (HDC-ASCT) See U S Patent 6,586,428 to Gerom *et al*

In anther aspect, one or more constructs, including one or more antigen-binding constructs, of the invention can be administered or co-administered with therapeutic drugs For example, Viruhizin (Lorus Therapeutics), which is believed to stimulate the release of tumour necrosis factor, TNF-alpha, by tumour cells in vitro and stilumalate activation of macrophage cells This can be used in combination with one or more constructs, including one ore more antigen-binding constructs, of the invention to increase cancer cell apoptosis and treat various types of cancers including Pancreatic Cancer, Malignant Melanoma, Kaposi's Sarcoma (KS), Lung Cancer, Breast Cancer, Uterine, Ovarian and Cervical Cancer Another example is CpG 7909 (Coley Pharmaceutical Group), which is believed to activate NK cells and monocytes and enhance ADCC This drug can be used in combination with cancer or tumor specific constructs, including antigen-binding constructs, of the invention, such as an anti-CD20 construct, to treat non-Hodgkin's lymphoma and other cancers

One or more constructs, including one or more antigen-binding constructs, of the invention can also be combined with angiogenesis inhibitors to increase anti-tumor effects Angiogenisis is the growth of new blood vessels This process allows tumors to grow and metastasize Inhibiting angiogenesis can help prevent metastasis, and stop the spread of tumors cells Angiogenisis inhibitors include, but are not limited to, angiostatin,

endostatin, thrombospondin, platelet factor 4, Cartilage-derived inhibitor (CDI), retinoids, Interleukin-12, tissue inhibitor of metalloproteinase 1, 2 and 3 (TIMP-1, TIMP-2, and TIMP-3) and proteins that block the angiogenesis signaling cascade, such as anti-VEGF (Vascular Endothelial Growth Factor) and IFN-alpha. Angiogenesis inhibitors can be administered or co-administered with tumor specific constructs, including antigen-binding constructs capable of mediating, for example, ADCC and/or complement fixation or chemotherapy-conjugated antigen-binding of the invention to combat various types of cancers, for example, solid tumor cancers such as lung and breast cancer

In another aspect, one or more constructs, including one or more antigen-binding constructs, of the invention can be administered or co-administered with disease modifying anti-rheumatic agents (DMAR agents) for the treatment of rheumatoid arthritis, psoriasis, ulcerative colitis, systemic lupus erythematosus (SLE), Crohn's disease, ankylosing spondylitis, and various inflammatory disease processes. In such treatment, the constructs, for example, antigen-binding constructs, of the invention are commonly administered in conjunction with compounds such as azathioprine, cyclosporin, gold, hydroxychloroquine, methotrexate, penicillamine, sulphasalazine, and the like

In another aspect, one or more constructs, including one or more antigen-binding constructs, of the invention can be administered or co-administered with agents or compounds that counteract the biological effects of interleukin-1, including for example interleukin-1 inhibitors and interleukin-1 receptor antagonist. It is thought that interleukin-1 has a role in the generation of rheumatoid arthritis (RA), inflammation, and the destruction of joints IL-1 inhibitors can also be used in conjunction with the constructs, including antigen-binding constructs, of the invention to treat arthritis, inflammatory bowel disease, sepsis and septic shock, ischemic injury, reperfusion, ischemic brain injury such as cerebral palsy and multiple sclerosis See U S Patent No 6,159,460 to Thompson *et al* In another aspect, for example, one or more constructs, including one or more antigen-binding constructs, of the invention can be administered or co-administered to an animal or patient in conjunction with one or more glucocorticoids for example, methylprednisolone, dexamethasone, hydrocortisone, and the like Glucocorticoids have been used to induce apoptosis and inhibit growth, independent of ADCC and CDC These compounds can be combined with constructs, including antigen-binding constructs, of the invention capable of inducing apoptosis in cancer cells In one example is the anti-CD20, and anti-CD40

antigen-binding constructs, which can be used to induce apoptosis in B-cells, are combined with glucocorticoids to treat B-cell non-Hodgkin's lymphoma (NHL).

In another aspect, one or more constructs, including one or more antigen-binding constructs, of the invention can be administered or co-administered with p38 inhibitors or antagonists The p38 mitogen-activated protein kinase pathway is involved in a number of cellular processes instrumental to the development of rheumatoid arthritis. For example, the activation and infiltration of leukocytes as well as the production of inflammatory cytokines are p38-dependent processes

In another aspect, one or more constructs, including one or more antigen-binding constructs, of the invention are administered or co-administered with compounds that promote the differentiation and proliferation of B-cells Cytokines such as \ interleukin-4 (IL-4) and interleukin-6 (IL-6), in additional to other biological activities, have been shown to stimulate antibody synthesis and secretion by activated B lympocytes. In a particular aspect of the invention, constructs, including antigen-binding constructs that recognize and bind CD20 are co-administered with one or more of interleukin-4 (IL-4) and interleukin-6 (IL-6)

In another aspect one or more constructs, including one or more antigen-binding constructs, of the invention can be administered or co-administered with Interleukin-2 (IL-2) Interleukin 2 (IL-2) is a lymphokine that increases production of effector cells, such as CD4+ T-helper cells, CD8 cytotoxic cells, antibody producing B cells, natural killer cells (NK), and monocytes/macrophages. IL-2 helps produce T-cells, which in turn secrete more of the IL-2 (an "autocrine loop"). IL-2 can be used to augment antibody-dependent cell-mediated cytotoxicity (ADCC) and immunotherapies associated with constructs of the invention In one example, an anti-CD20 construct of the invention and IL-2 are used to treat patients with relapsed or refractory follicular non-Hodgkin's lymphoma. In another example IL-2 is administered or co-administered with HIV immunotherapies to help with T cell recovery

In another aspect one or more constructs, including one or more antigen-binding constructs, of the invention can be administered or co-administered with Interleukin-12 (IL-12) IL-12 is know to enhance cytolytic T-cell responses, promote the development of helper T cells, enhance the activity of natural killer (NK) cells, and induces the secretion of IFN-γ in T and NK cells IL-12 also increases many helper and effector

cells that mediate apoptosis In another aspect of the invention, one or more constructs, including one or more antigen-binding constructs, are administered or co-administeredwith IL-12 in the treatment of an animal or patient with a tumor or cancer For example, a construct, including an antigen-binding construct, of the invention that binds CD20 combined with IL-2 for thetreatment of a patient with B-cell non-Hodgkin's lymphoma (NHL)

One or more constructs, including one or more antigen-binding constructs, of the invention can also be combined with immunomodulators to boost the efficacy of the antigen-binding constructs of the invention. Immunomodulators include, but are not limited to, Colony Stimulating Factors (CSF), Tumor necrosis Factors (TNF), and Interferons (IFN)

CSFs can include granulocyte-macrophage CSF (GM-CSF), granulocyte-CSF (G-CSF), and macrophage CSF (M-CSF) GM-CSF is thought to regulate the development of neutrophils, macrophages, monocytes and eosinophils. G-CSF has been shown to induce neutrophil production, and M-CSF production. M-CSF has been shown to stimulate macrophages and monocytes. The use of CSFs to treat neutropenia in cancer patients has been long established. In one example, constructs, including antigen-binding constructs, of the invention can be combined with GM-CSF, G-CSF or combinations thereof in order to accelerate recovery from neutropenia in patients after bone marrow trans-plantation and chemotherapy Neutrophils play a major role in fighting microbes such as bacterial, fungi and parasites Patients with neutropenia are particularly susceptible to bacterial and wide spread fungal infections In another example, a construct, including an antigen binding construct, of the invention can be combined with GM-CSF-treated neutrophils, monocytes and macrophages to increase activity against bacteria, fungi, etc, including the dreaded Pneumocystis carinii

An example of an IFN is interferon alpha (IFN-α) IFN-α is made naturally by some types of white blood cell as part of the immune response when the body reacts to cancers or viral infections It has two main modes of attack, interfering with growth and proliferation of cancer cells and it boosting the production of killer T cells and other cells that attack cancer cells Interferon is also thought to facilitate cancer cells to put out chemical signals that make them better targets for the immune system, and has been used in recent years for several different types of cancer, particularly kidney cancer, melanoma,

multiple myeloma, and some types of leukemia. It is also used to treat viral infections such as hepatitis Interferon-alpha2a, for example, enhances ADCC and can be combined with one or more constructs, including antigen-binding constructs, of the invention to increase the efficiency of ADCC activity associated with the construct. In another example, one or more constructs, including one or more antigen-binding constructs of the invention are administered or co-administered to an animal or patient with interferon-gamma (IFN-γ), which has been show to increase the number of anti-CD20 antigens on B cells and bone marrow plasma cells (BMPC) This is particularly useful for the treatment of patients with multiple myelomas, which have a reduced expression of CD20 in their B cells and bone marrow plasma cells (BMPC) Accordingly, the treatment of multiple myeloma patients with constructs, including antigen-binding constructs of the invention, in particular constructs that bind CD20, may be usefully co-administered in conjunction with IFN-γ

TNF is a class of natural chemicals with anticancer properties One example of a TNF is TNF- alpha. TNF-alpha has also been shown to have synergistic effects with IFN-gamma and IL-12 In another example, TNF can be administered or co-administered with one or more tumor specific constructs, including one or more antigen–binding constructs, of the invention, and include chemotherapy-conjugated antigen binding constructs of the invention, together with IFN-gamma, IL-12 or various combinations thereof. TNF is also known to be an inflammatory regulation molecule TNF-alpha antibodies or antagonist(s) can be combined with anti-T cell constructs, including antigen-binding constructs, of the invention to treat patients with rheumatoid arthritis, psoriasis, ulcerative colitis, systemic lupus erythematosus (SLE), Crohn's disease, ankylosing spondylitis, and various inflammatory disease processes.

In another aspect, one or more constructs, including one or more antigen-binding constructs, of the invention can be administered or co-administered with another antibody or antigen-binding construct of the invention. One example is a construct, for example, an antigen-binding construct of the invention capable of binding CD20 combined with a construct capable of binding CD22, CD19 or combinations thereof This combination is effective as a treatment for indolent and aggressive forms of B-cell lymphomas, and acute and chronic forms of lymphatic leukemias. See U S Patent 6,306,393 to Goldberg. In another example, constructs, including antigen-binding constructs, of the invention are co-administered with other constructs such as antigen-

binding constructs of the invention that aid in mediating apoptosis. For example, a combination of one or more constructs, including one or more antigen-binding constructs of the invention capable of binding CD28, CD3, CD20 or a combination thereof The combination of anti-CD28 and CD3 provides a method for prolonged proliferation of T-cells See U S Patent No 6,352,694 to June et al Thus prolonged T-cell proliferation increases the efficiency immune dependent cytotoxicity, particularly those associated with anti-CD20

In another aspect, constructs, including antigen-binding constructs, of the invention can be administered or co-administered with one or more T-cell regulatory molecules One example is a combination with interleukin-12 (IL-12) The IL-12 cytokine stimulates cell-mediated immunity, has angiostatic activity, and possesses significant anti-tumor effects in a variety of tumor models. IL-12 has also been shown to stimulate the production of interferon-gamma (IFN-γ) Accordingly, the treatment of multiple myeloma patients with one or more constructs, including one or more antigen-binding constructs, of the invention, in particular those that bind CD20, is expected to be more efficacious when co-administered in conjunction with IL-12 In another example, one or more constructs, including one or more antigen-binding constructs, of the invention can be administered or co-administered with a binding-domain construct of the invention other protein capable of binding CTLA-4 to enhance the anti-tumor immune response, by inhibiting the downregulation of T-cell activation

In another aspect, one or more constructs, including one or more antigen-binding constructs, of the invention can be combined with gene therapies In one example, a chemotherapy-conjugated construct of the invention is administered or co-administered with the Bcl-2 antisense oligonucleotide Bcl-2 is associated with tumor resistance to anti-cancer therapies, and its believed to blocking chemotherapy-induced cell death In another example one or more constructs, including one or more antigen-binding constructs, of the invention is administered or co-administered with an adenovirus for delivery of a "suicide gene." The adenovirus inserts the gene directly into the tumor cells, which makes these cells sensitive to an otherwise ineffective drug. Drug treatment then destroys the tumor cells, while leaving healthy cells untouched. However, once therapy is complete stray cancer cells that escaped therapy can reestablish and metastasize. Combining gene therapy

with one or more constructs, including one or more antigen-binding constructs, will help kill stray cancer cells and minimize cancer reoccurrence

A similar combination can be used with palliative (non-radical) operations to surgically remove tumors In this example one or more constructs, including one or more antigen-binding constructs, of the invention can be administered before and after surgical extractions of tumors in order to increase the immune response and reduce the likelihood of reoccurrence by killing any cancer cells that were not removed during the surgery

Another aspect combines a cancer or antigen vaccine and T-cell regulator molecules For example, the binding portion, for example, an antigen-binding portion, of a construct can be specific for a cancer cell or antigen, or a protein fragment from a cancer cell or antigen. This can help mediate an immune response against a particular tumor or antigen. Such constructs can be combined with T-cell regulators to increase the efficiency of the immune response.

In another example, one or more constructs, including one or more antigen-binding constructs, of the invention is administered or co-administered with retinoids Retinoids include Vitamin A and its derivatives, which have the ability to stop cells from dividing and cause them to differentiate. Vitamin A is combined with an anti-cancer construct(s), including antigen-binding construct(s), of the invention to combat various forms of cancer

The terms "binding construct" and "antigen-binding construct" as used herein may refer to, for example, engineered polypeptides, recombinant polypeptides, synthetic, semi-synthetic or other fusion proteins that are capable of binding a target, for example, an antigen Antigen-binding constructs of the invention may be used in various applications, including those within the variety of uses to which antibodies or related immunoglobulin-type constructs may be put. Constructs, including antigen-binding constructs of the invention can be used in *in vivo* and *in vitro* experiments for therapeutic, diagnostic, research, and other purposes Such uses include, for example, the following.

Constructs, including antigen-binding constructs of the invention may be used for immunohistochemistry applications For example, they may be used for immunolocalization of a particular antigen or group of antigens in a tissue. Tissue can be fixed and incubated with antigen-binding constructs of interest. These constructs can then

be localized using a secondary antibody or binding construct of the invention coupled to a label, for example, to a gold particle or an enzyme that gives a chemical reaction, like horseradish peroxidase or beta-galactosidase. A secondary antibody or binding construct is frequently made that is reactive against, for example, a portion of the primary binding construct. Thus, for example, if the primary binding construct has a human tail portion, the secondary antibody or binding construct could be, for example, a rabbit anti-mouse antibody or antigen-binding construct that has been linked to beta-galactosidase. Alternatively the antibody or binding construct of the invention can be purified and then conjugated to another molecule to produce a fluorescent antibody or binding construct.

Constructs, including antigen-binding contructs of the invention can also be used to detect the location of an antigen or antigens on the surface of cells or to detect the location of intracellular materials using, for example, Immunoelectron Microscopy Electron dense materials such as ferritin or colloidal gold, for example, can be conjugated to an antigen-binding construct Scanning electron microscopy can be used to detect the localization of the antigen/binding construct complex

Constructs, including antigen-binding constructs of the invention may also be used to quantitate the presence of an antigen or antigens using one of a variety of immunoassay formats, for example, a radioimmunoassay (RIA) format or an enzyme-linked immunosorbent assay (ELISA) format. There are many variants of these approaches, but those are based on a similar idea. For example, if an antigen can be bound to a solid support or surface, or is in solution, it can be detected by reacting it with a specific antigen-binding construct of the invention. The presence or amount of the construct can then be detected or quantitated by reacting it with, for example, either a secondary antibody or a second antigen-binding construct of the invention by incorporating a label directly into the primary antibody Alternatively, for example, an antigen-binding polypeptide of the invention can be bound to a solid surface and the antigen added. A second antibody or antigen-binding polypeptide(s) of the invention that recognizes a distinct epitope on the antigen can then be added and detected. This technique is commonly referred to as a "sandwich assay", which is frequently used to avoid problems of high background or non-specific reactions, among other reasons.

Because the binding constructs of the invention can have high affinity/affinities and/or selectivity/selectivities for a particular epitope or epitopes, they

can also be used as affinity reagents, for example, in protein or antigen purification. In one example of such a process, antigen-binding constructs of the invention are immobilized on a suitable support, for example, Sephadex resin or filter paper The immobilized construct is exposed to a sample containing, or suspected of containing, a target protein(s) or antigen(s) The support is rinsed with a suitable buffer that will remove unwanted materials. The support is washed with another buffer that will release the bound protein(s) or antigen(s)

Because particular binding constructs of the invention can bind to proteins or other antigens with high affinity and selectivity they can also be used as a criterion for the importance of a particular enzyme or other macromolecule in a particular reaction. If an antigen-binding construct of the invention can interfere with a reaction in a solution, this will indicate that the construct may be binding specifically to a protein or other antigenic material involved in that reaction.

Constructs, including antigen-binding constructs of the invention can also be used as receptor blockers or inhibitors or antagonists

Constructs, including antigen-binding contructs of the invention can also be used in identifying and studying the function(s) of proteins. If an antigen-binding construct of the invention reacts with a specific protein, for example, that protein can subsequently be precipitated from solution, for example Precipitation is typically performed by using a secondary antibody or antigen-binding construct of the invention that links primary complexes together Alternatively, the complex can be removed by reacting the solution with either protein A or, for example, depending on the construct, an anti-Fc antibody, for example, which has been attached to beads, for example, so that can be easily removed form the solution.

Constructs, including antigen-binding constructs of the invention can also be used in conjunction with gel-shift experiments to identify specific nucleic acid-binding proteins such as DNA-binding proteins For example, DNA-binding proteins can be assayed by their ability to bind with high affinity to a particular oligonucleotide. The mobility of an oligonucleotide associated with the protein is far different than the mobility of a free oligonucleotide and results in a gel migration pattern and signal that is commonly referred to as a gel shift The addition of the construct to the binding assay can have either of two effects If the construct binds to a region of a protein not involved in DNA binding

it can result in a complex that has even a slower mobility and is detected as a greater shift in mobility (a super-shift) Alternatively, if the construct binds to a region of the protein involved in recognizing the DNA then it can disrupt the binding and eliminate the shift. In either case, the data from these experiments can serve as a criterion to identify a DNA-binding protein, for example

It is also possible to use constructs, including antigen-binding constructs of the invention to detect a protein by western blotting after fractionation by SDS-PAGE, for example Once fractionated proteins are transferred to a membrane such as a nitrocellulose sheet, they are exposed to a particular antigen-binding construct of the invention that specifically recognizes, or recognizes to a desired degree of selectivity, proteins immobilized to the blot. Thus allows particular proteins to be identified. This approach is particularly useful if the mobility of the protein changes during an experiment. For example, incorporation of a phosphate or a carbohydrate, or cleavage of the protein, results in a change in mobility that can be followed in straightforward manner by western analysis With appropriate controls, this approach can be used to measure the abundance of a protein in response to experimental manipulations

The combination of SDS gels and immunoprecipitation can also be extremely effective. If a particular protein can be immunoprecipitated in a solution, both supernatant and precipitated fractions can be separated on an SDS gel and studied using an antigen-binding construct(s) of the invention.

Sometimes a binding construct of the invention directed against one protein will also precipitate a second protein that interacts with the first protein. The second protein, as well as the first, can then be seen by staining the gel or by autoradiography This relationship is frequently the first indication that a protein functions as part of a complex and it can also be used to demonstrate a physical interaction of two proteins that are hypothesized to interact on the basis of other evidence (*e.g*, a two hybrid screen or a supressor mutation) This approach can be combined with western blotting analysis in several extremely effective ways

Thus, for example, antigen-binding constructs of the invention can be used in a combination of immunoprecipitation and western analysis in the study, for example, of signal transduction and protein processing For example, an immunoprecipitated protein can be subsequently studied by western analysis using a different antibody or antigen-

binding construct of the invention that binds to the protein. The most useful of are those that are directed against particular structural determinants that may be present in a protein. Thus, an antibody or antigen-binding construct of the invention directed against a region of the protein that undergoes proteolytic processing can be useful to follow proteolytic processing. Additionally, a construct of the invention or a mixture of antigen-binding constructs of the invention that recognize phophorylated peptides (*e.g.*, anti PY (phosphorylated tyrosine) can be used to follow the extent of phosphorylation of a protein (using western analysis) after it is precipitated, or *visa versa* Glycosylation reactions can also be followed by antigen-binding constructs of the invention directed against a carbohydrate epitope (or by lectins, *i.e.*, proteins that recognize carbohydrates) Likewise, some antigen-binding constructs of the invention can be made that specifically recognize a phosphorylated epitope, for example, that will recognize a tyrosine or a serine residue after phosphorylation, but will not bind (or detectably bind) the epitope in the absence of phosphate. This approach can be used to determine the phosphorylation state of a particular protein For example, the phosphorylation of CREB (the cAMP response element binding protein) can be followed by an antibody that specifically recognizes an epitope in a way that is dependent on the phosphorylation of serine 133

Constructs, including antigen-binding constructs of the invention can also be used to screen expression libraries to isolate candidate polynucleotides that express or present a particular epitope, or that have a particular affinity or expression characteristic

Constructs, including antigen-binding constructs of the invention that bind to a cell surface can also be used as a marker to quantitate the fraction of cells expressing that marker using flow cytometry If different antigen binding constructs of the invention / fluorescent dye combinations are used, for example, the fraction of cells expressing several antigens can be determined

Constructs, including antigen-binding constructs of the invention that function like anti-idiotype antibodies, *i.e.*, antibodies against the binding domain of another antibody, can be used in any of a number of methods in which is would be desireable or useful to mimic the structure of an antigen. Such uses include, for example, uses as cancer vaccines (including antigen binding constructs of the invention that incorporate a molecular adjuvant), as probes for receptors, as receptor agonists, as receptor antagonists, as receptor blockers or inhibitors, and so on

In another aspect, constructs, including antigen-binding constructs of the invention may bispecific and thus capable of binding to two distinct epitopes, which may be present on the same or different cell types

In vivo uses of constructs of the invention, including antigen-binding constructs, include therapy, alone or in combination with one or more other therapies, for various diseases including cancers as well as B-cell disorders including autoimmune diseases In some cases the constructs of the invention are administered to a patient. In other cases, the construct may be coupled to another molecule by techniques known in the art, for example, a fluorescent molecule to aid in imaging a target, or a therapeutic drug and/or a toxin to aid in killing a target.

For example, a labeling molecule or atom can be conjugated or otherwise linked to the antigen-binding construct of the invention to aid in imaging or as a diagnostic agent. These include, but are not limited to enzymatic labels, radioisotopes or radioactive compounds or elements, fluorescent compounds or metals, chemiluminescent compounds and bioluminescent compounds Thus, binding contructs or antigen-binding constructs of the invention can be conjugated to a drug, which allows specific drug targeting and increased efficiency once the drug reaches the target. This facilitates drug therapy while reducing systemic toxicity and side effects. This allows use of drugs that would otherwise be unacceptable when administered systemically Dosage will depend on the potency of the drug and the efficiency of the carrier construct. Other examples of *in vivo* uses include the use of binding constructs or antigen-binding constructs of the invention in which a toxin is chemically linked or conjugated to an polypeptide of the invention to form, for example, molecules that may be termed "immunoconjugates" or "immunotoxins." Typically, for example, such a toxin may include one ore more radioisotopes (for example, Iodine-131, Yttrium-90, Rhenium-186, Copper-67, and/or Bismuth-212), natural toxins, chemotherapy agents, biological response modifiers, or any other substance that is capable of assisting in damaging or killing a target cell, inhibiting target cell replication, or is effective in disrupting a desired cellular function in a target cell.

The toxin portion of the immunotoxin can be derived form various sources. Toxins are commonly derived from plants or bacteria, but toxins of human origin or synthetic toxins can be used as well, for example Examples of toxins derived from bacteria or plants include, but are not limited to, abrin, α-sarcin, diptheria toxin, ricin,

saporin, and *pseudomonas* exotoxin. Examples of mammalian enzymes include, but are not limited to, ribonucleases (RNAse) and deoxyribonucleases. Numerous immunotoxins that may be used with one or more constructs of the invention have been described in the art. See, for example, U S Pat No 4,753,894 to Frankel *et al*, U S Pat. No 6,099,842 to Pastan *et al*, Nevelle, *et al*, 1982 *Immunol Rev* 62 75-91, Pastan *et al*, 1992 *Ann Rev Biochem* 61 331-354, Chaudary *et al*, 1989 *Nature* 339 394, and Batra *et al*, 1991 *Mol. Cell. Biol* 11 2200 Modified toxins described herein and those described in the various publications are also within the scope of the instant invention.

Generally, the immunotoxins and other therapeutic agents of this invention are administered at a concentration that is therapeutically effective to treat or prevent a particular disease, disorder, or condition, such as for the treatment of tumors and malignancies, the treatment of autoimmune diseases, allergies and inflammation, *etc* This effective dosage and mode of administration will depend on the animal or patient being treated, the disease or condition being treated, the strength of the immunoconjugates or immunotoxins and the efficiency of the conjugate. To accomplish this goal, the immunotoxins may be formulated using a variety of acceptable formulations and excipients known in the art Typically, for example, the immunotoxins are administered by injection, either intravenously or intraperitoneally Methods to accomplish this administration are known to those of ordinary skill in the art. It another aspect, the invention includes topically or orally administered compositions such as an aerosol or cream or patch that may be capable of transmission across mucous membranes

Formulants may be added to an immunoconjugates or immunotoxins of the invention before administration to a patients being treated. A liquid formulation is most common, but other formulations are within the scope of the invention. The formulants may include for example oils, polymers, vitamins, carbohydrates, amino acids, salts, buffers, albumin, surfactants, or bulking agents Carbohydrates can include sugar or sugar alcohols such as mono, di, or polysaccharides, or water-soluble glucans The saccharides or glucans can include for example fructose, dextrose, lactose, glucose, mannose, sorbose, xylose, maltose, sucrose, dextran, pullulan, dextrin, alpha and beta cyclodextrin, soluble starch, hydroxethyl starch and carboxymethylcellulose, or mixtures thereof. "Sugar alcohol" may be defined as a C_4 to C_8 hydrocarbon having an -OH group and includes, for example, galactitol, inositol, mannitol, xylitol, sorbitol, glycerol, and arabitol These sugars or sugar

alcohols mentioned above may be used individually or in combination. There is no fixed limit to the amount used as long as the sugar or sugar alcohol is soluble in the aqueous preparation. In one aspect, the sugar or sugar alcohol concentration is between 0 5 w/v % and 15 w/v %, typically between 1 0 w/v % and 7 0 w/v %, more typically between 2 0 and 6.0 w/v %

Exemplary amino acids include levorotary (L) forms of carnitine, arginine, and betaine, however, other amino acids may be added Commonly used polymers include polyvinylpyrrolidone (PVP) with an average molecular weight between 2,000 and 3,000, for example, or polyethylene glycol (PEG) with an average molecular weight between 3,000 and 5,000, for example. A buffer can be used in the composition to minimize pH changes in the solution before lyophilization or after reconstitution. Any physiological buffer may be used, but citrate, phosphate, succinate, and glutamate buffers or mixtures thereof are more commonly utilized The concentration can be, for example, from 0 01 to 0.3 molar Higher or lower concentrations may be used.

Immunotoxins of the invention can be chemically modified by covalent conjugation to a polymer to increase their circulating half-life, for example Exemplary polymers and methods to attach them to peptides are referenced in U S Pat. Nos 4,766,106 to Katre *et al*, 4,179,337 to Davis *et al*, 4,495,285 to Shimizu *et al*, and 4,609,546 to Hiratani

The following Examples are offered by way of illustration and not by way of limitation.

EXAMPLE 1

CLONING OF THE 2H7 VARIABLE REGIONS AND CONSTRUCTION AND SEQUENCING OF 2H7SCFV-IG

This Example illustrates the cloning of cDNA molecules that encode the heavy chain and light chain variable regions of the monoclonal antibody 2H7 This Example also demonstrates the construction, sequencing, and expression of 2H7scFv-Ig.

Prior to harvesting, cells expressing 2H7 monoclonal antibody that specifically bound to CD20 were kept in log phase growth for several days in RPMI 1640 media Invitrogen/Life Technologies, Gaithersburg, MD) supplemented with glutamine, pyruvate, DMEM non-essential amino acids, and penicillin-streptomycin. Cells were pelleted by centrifugation from the culture medium, and 2 x 10^7 cells were used to prepare

RNA. RNA was isolated from the 2H7-producing hybridoma cells using the Pharmingen (San Diego, CA) total RNA isolation kit (Catalog # 45520K) according to the manufacturer's instructions accompanying the kit One microgram (1 μg) of total RNA was used as template to prepare cDNA by reverse transcription. The RNA and 300 ng random primers were combined and denatured at 72 °C for 10 minutes prior to addition of enzyme. Superscript II reverse transcriptase (Life Technologies) was added to the RNA plus primer mixture in a total volume of 25 μl in the presence of 5X second strand buffer and 0 1 M DTT provided with the enzyme The reverse transcription reaction was allowed to proceed at 42°C for one hour

The 2H7 cDNA generated in the randomly primed reverse transcriptase reaction and V region specific primers were used to amplify by PCR the variable regions for the light and heavy chain of the 2H7 antibody The V region specific primers were designed using the published sequence (Genbank accession numbers M17954 for V_L and M17953 for V_H) as a guide The two variable chains were designed with compatible end sequences so that an scFv could be assembled by ligation of the two V regions after amplification and restriction enzyme digestion.

A $(Gly_4Ser)_3$ peptide linker to be inserted between the two V regions was incorporated by adding the extra nucleotides to the antisense primer for the V_L of 2H7 A Sac I restriction site was also introduced at the junction between the two V regions. The sense primer used to amplify the 2H7 V_L, that included a HindIII restriction site and the light chain leader peptide was 5'-gtc *aag ctt* gcc gcc atg gat ttt caa gtg cag att ttt cag c-3' (SEQ ID NO __) The antisense primer was 5'-gtc gtc *gag ctc* cca cct cct cca gat cca cca ccg ccc gag cca ccg cca cct ttc agc tcc agc ttg gtc cc-3' (SEQ ID NO __) The reading frame of the V region is indicated as a bold, underlined codon. The Hind III and SacI sites are indicated by underlined italicized sequences

The V_H domain was amplified without a leader peptide, but included a 5' SacI restriction site for fusion to the V_L and a BclI restriction site at the 3' end for fusion to various tails, including the human IgG1 Fc domain and the truncated forms of CD40 ligand, CD154 The sense primer was 5'-gct gct *gag ctc* tca ggc tta tct aca gca agt ctg g-3' (SEQ ID NO __) The SacI site is indicated in italicized and underlined font, and the reading frame of the codon for the first amino acid of the V_H domain is indicated in bold, underlined type. The antisense primer was 5'-gtt gtc *tga tca* gag acg gtg acc gtg gtc cc-3'

(SEQ ID NO __) The BclI site is indicated in italicized, underlined type, and the last serine of the V_H domain sequence is indicated in bold, underlined type.

The scFv-Ig was assembled by inserting the 2H7 scFv HindIII-BclI fragment into pUC19 containing the human IgG1 hinge, CH2, and CH3 regions, which was digested with restriction enzymes, HindIII and BclI. After ligation, the ligation products were transformed into DH5α bacteria. Positive clones were screened for the properly inserted fragments using the SacI site at the V_L-V_H junction of 2H7 as a diagnostic site. The 2H7scFv-Ig cDNA was subjected to cycle sequencing on a PE 9700 thermocycler using a 25-cycle program by denaturing at 96 °C for 10 seconds, annealing at 50 °C for 30 seconds, and extending at 72°C for 4 minutes. The sequencing primers were pUC forward and reverse primers and an internal primer that annealed to the CH2 domain human in the IgG constant region portion Sequencing reactions were performed using the Big Dye Terminator Ready Sequencing Mix (PE-Applied Biosystems, Foster City, CA) according to the manufacturer's instructions Samples were subsequently purified using Centrisep columns (Catalog # CS-901, Princeton Separations, Adelphia, N J), the eluates dried in a Savant vacuum dryer, denatured in Template Suppression Reagent (PE-ABI), and analyzed on an ABI 310 Genetic Analyzer (PE-Applied Biosystems) The sequence was edited, translated, and analyzed using Vector Nti version 6 0 (Informax, North Bethesda, MD) Figure 1 shows the cDNA and predicted amino acid sequence of the 2H7scFv-Ig construct.

EXAMPLE 2

EXPRESSION OF 2H7 SCFV-IG IN STABLE CHO CELL LINES

This Example illustrates expression of 2H7scFv-Ig in a eukaryotic cell line and characterization of the expressed 2H7scFv-Ig by SDS-PAGE and by functional assays, including ADCC and complement fixation

The 2H7scFv-Ig HindIII-XbaI (~1 6 kb) fragment with correct sequence was inserted into the mammalian expression vector pD18, and DNA from positive clones was amplified using QIAGEN plasmid preparation kits (QIAGEN, Valencia, CA) The recombinant plasmid DNA (100 μg) was then linearized in a nonessential region by digestion with AscI, purified by phenol extraction, and resuspended in tissue culture media, Excell 302 (Catalog # 14312-79P, JRH Biosciences, Lenexa, KS) Cells for transfection, CHO DG44 cells, were kept in logarithmic growth, and 10^7 cells harvested for each

transfection reaction Linearized DNA was added to the CHO cells in a total volume of 0 8 ml for electroporation.

Stable production of the 2H7 scFv-Ig fusion protein (SEQ ID NO 10) was achieved by electroporation of a selectable, amplifiable plasmid, pD18, containing the 2H7 scFv-Ig cDNA under the control of the CMV promoter, into Chinese Hamster Ovary (CHO) cells (all cell lines from American Type Culture Collection, Manassas, VA, unless otherwise noted) The 2H7 expression cassette was subcloned downstream of the CMV promoter into the vector multiple cloning site as a ~1 6 kb HindIII-XbaI fragment. The pD18 vector is a modified version of pcDNA3 encoding the DHFR selectable marker with an attenuated promoter to increase selection pressure for the plasmid. Plasmid DNA was prepared using Qiagen maxiprep kits, and purified plasmid was linearized at a unique AscI site prior to phenol extraction and ethanol precipitation. Salmon sperm DNA (Sigma-Aldrich, St Louis, MO) was added as carrier DNA, and 100 µg each of plasmid and carrier DNA was used to transfect 10^7 CHO DG44 cells by electroporation. Cells were grown to logarithmic phase in Excell 302 media (JRH Biosciences) containing glutamine (4mM), pyruvate, recombinant insulin, penicillin-streptomycin, and 2X DMEM nonessential amino acids (all from Life Technologies, Gaithersburg, Maryland), hereafter referred to as "Excell 302 complete" media. Media for untransfected cells also contained HT (diluted from a 100X solution of hypoxanthine and thymidine) (Invitrogen/Life Technologies) Media for transfections under selection contained varying levels of methotrexate (Sigma-Aldrich) as selective agent, ranging from 50 nM to 5 µM Electroporations were performed at 275 volts, 950 µF Transfected cells were allowed to recover overnight in non-selective media prior to selective plating in 96 well flat bottom plates (Costar) at varying serial dilutions ranging from 125 cells/well to 2000 cells/well Culture media for cell cloning was Excell 302 complete, containing 100 nM methotrexate. Once clonal outgrowth was sufficient, serial dilutions of culture supernatants from master wells were screened for binding to CD20-CHO transfected cells The clones with the highest production of the fusion protein were expanded into T25 and then T75 flasks to provide adequate numbers of cells for freezing and for scaling up production of the 2H7scFvIg. Production levels were further increased in cultures from three clones by progressive amplification in methotrexate containing culture media. At each successive passage of cells, the Excell 302 complete

media contained an increased concentration of methotrexate, such that only the cells that amplified the DHFR plasmid could survive.

Supernatants were collected from CHO cells expressing the 2H7scFv-Ig, filtered through 0 2 μm PES express filters (Nalgene, Rochester, NY) and were passed over a Protein A-agarose (IPA 300 crosslinked agarose) column (Repligen, Needham, MA) The column was washed with PBS, and then bound protein was eluted using 0 1 M citrate buffer, pH 3 0 Fractions were collected and eluted protein was neutralized using 1M Tris, pH 8 0, prior to dialysis overnight in PBS Concentration of the purified 2H7scFv-Ig (SEQ ID NO __) was determined by absorption at 280 nm An extinction coefficient of 1 77 was determined using the protein analysis tools in the Vector Nti Version 6 0 Software package (Informax, North Bethesda, MD) This program uses the amino acid composition data to calculate extinction coefficients

Production levels of 2H7scFv-Ig by transfected, stable CHO cells were analyzed by flow cytometry Purified 2H7scFv-Ig to CHO cells was allowed to bind to CHO cells that expressed CD20 (CD20 CHO) and analyzed by flow cytometry using a fluorescein-conjugated anti-human IgG second step reagent (Catalog Numbers H10101 and H10501, CalTag, Burlingame, CA) Figure 2 (top) shows a standard curve generated by titration of 2H7scFv-Ig binding to CD20 CHO At each concentration of 2H7scFv-Ig, the mean brightness of the fluorescein signal in linear units is shown. Supernatants collected from T flasks containing stable CHO cell clones expressing 2H7scFv-Ig were then allowed to bind to CD20 CHO and the binding was analyzed by flow cytometry The fluorescein signal generated by 2H7scFv-Ig contained in the supernatants was measured and the 2H7scFv-Ig concentration in the supernatants was calculated from the standard curve (Figure 2, bottom)

Purified 2H7scFv-Ig (SEQ ID NO __) was analyzed by electrophoresis on SDS-Polyacrylamide gels Samples of 2H7scFv-Ig, purified by independent Protein A Agarose column runs, were boiled in SDS sample buffer without reduction of disulfide bonds and applied to SDS 10% Tris-BIS gels (Catalog # NP0301, Novex, Carlsbad, CA) Twenty micrograms of each purified batch was loaded on the gels The proteins were visualized after electrophoresis by Coomassie Blue staining (Pierce Gel Code Blue Stain Reagent, Catalog #24590, Pierce, Rockford, IL), and destaining in distilled water Molecular weight markers were included on the same gel (Kaleidoscope Prestained

Standards, Catalog # 161-0324, Bio-Rad, Hercules, CA) The results are presented in Figure 3 The numbers above the lanes designate independent purification batches. The molecular weights in kilodaltons of the size markers are indicated on the left side of the figure Further experiments with alternative sample preparation conditions indicated that reduction of disulfide bonds by boiling the protein in SDS sample buffer containing DTT or 2-mercaptoethanol caused the 2H7scFv-Ig to aggregate.

Any number of other immunological parameters may be monitored using routine assays that are well known in the art. These may include, for example, antibody dependent cell-mediated cytotoxicity (ADCC) assays, secondary *in vitro* antibody responses, flow immunocytofluorimetric analysis of various peripheral blood or lymphoid mononuclear cell subpopulations using well established marker antigen systems, immunohistochemistry or other relevant assays. These and other assays may be found, for example, in Rose *et al* (Eds), *Manual of Clinical Laboratory Immunology 5th Ed* , 1997 American Society of Microbiology, Washington, DC

The ability of 2H7scFv-Ig to kill CD20 positive cells in the presence of complement was tested using B cell lines Ramos and Bjab Rabbit complement (Pel-Freez, Rogers, AK) was used in the assay at a final dilution of 1/10 Purified 2H7scFv-Ig was incubated with B cells and complement for 45 minutes at 37 °C, followed by counting of live and dead cells by trypan blue exclusion. The results in Figure 4A show that in the presence of rabbit complement, 2H7scFv-Ig lysed B cells expressing CD20

The ability of 2H7scFv-Ig to kill CD20 positive cells in the presence of peripheral blood mononuclear cells (PBMC) was tested by measuring the release of ^{51}Cr from labeled Bjab cells in a 4-hour assay using a 100 1 ratio of PBMC to Bjab cells The results shown in Figure 4B indicated that 2H7scFv-Ig can mediate antibody dependent cellular cytotoxicity (ADCC) because the release of ^{51}Cr was higher in the presence of both PBMC and 2H7scFv-Ig than in the presence of either PBMC or 2H7scFv-Ig alone.

EXAMPLE 3

EFFECT OF SIMULTANEOUS LIGATION OF CD20 AND CD40 ON GROWTH OF NORMAL B CELLS, AND ON CD95 EXPRESSION, AND INDUCTION OF APOPTOSIS

This Example illustrates the effect on cell proliferation of cross-linking of CD20 and CD40 expressed on the cell surface

Dense resting B cells were isolated from human tonsil by a Percoll step gradient and T cells were removed by E-rosetting. Proliferation of resting, dense tonsillar B cells was measured by uptake of ^{3}[H]-thymidine during the last 12 hours of a 4-day experiment Proliferation was measured in quadruplicate cultures with means and standard deviations as shown. Murine anti-human CD20 monoclonal antibody 1F5 (anti-CD20) was used alone or was cross-linked with anti-murine κ monoclonal antibody 187 1 (anti-CD20XL) CD40 activation was accomplished using soluble human CD154 fused with murine CD8 (CD154) (Hollenbaugh *et al* , *EMBO J* 11 4212-21 (1992)), and CD40 cross-linking was accomplished using anti-murine CD8 monoclonal antibody 53-6 (CD154XL) Thus procedure allowed simultaneous cross-linking of CD20 and CD40 on the cell surface. The results are presented in Figure 5

The effect of CD20 and CD40 cross-linking on Ramos cells, a B lymphoma cell line, was examined. Ramos cells were analyzed for CD95 (Fas) expression and percent apoptosis eighteen hours after treatment (no goat anti-mouse IgG (GAM)) and/or cross-linking (+GAM) using murine monoclonal antibodies that specifically bind CD20 (1F5) and CD40 (G28-5). Control cells were treated with a non-binding isotype control (64 1) specific for CD3

Treated Ramos cells were harvested, incubated with FITC-anti-CD95, and analyzed by flow cytometry to determine the relative expression level of Fas on the cell surface after CD20 or CD40 cross-linking. Data is plotted as mean fluorescence of cells after treatment with the stimuli indicated (Figure 6A)

Treated Ramos cells from the same experiment were harvested and binding of annexin V was measured to indicate the percentage apoptosis in the treated cultures Apoptosis was measured by binding of Annexin V 18 hours after cross-linking of CD20 and CD40 using 1F5 and G28-5 followed by cross-linking with GAM. Binding of Annexin V was measured using a FITC-Annexin V kit (Catalog # PN-IM2376, Immunotech, Marseille, France,) Annexin V binding is known to be an early event in progression of cells into apoptosis Apoptosis, or programmed cell death, is a process characterized by a cascade of catabolic reactions leading to cell death by suicide In the early phase of apoptosis, before cells change morphology and hydrolyze DNA, the integrity of the cell membrane is maintained but cells lose the asymmetry of their membrane phospholipids, exposing negatively -charged phospholipids, such as

phosphatidylserine, at the cell surface. Annexin V, a calcium and phospholipid binding protein, binds preferentially and with high affinity to phosphatidylserine. Results demonstrating the effect of cross-linking both CD20 and CD40 on expression of the FAS receptor (CD95) are presented in Figure 6B The effect of cross-linking of both CD20 and CD40 on Annexin V binding to cells is shown in Figure 6B

EXAMPLE 4

CONSTRUCTION AND CHARACTERIZATION OF 2H7 SCFV-CD154 FUSION PROTEINS

To construct a molecule capable of binding to both CD20 and CD40, cDNA encoding the 2H7 scFv was fused with cDNA encoding CD154, the CD40 ligand. The 2H7 scFv cDNA encoded on the HindIII-BclI fragment was removed from the 2H7 scFvIg construct, and inserted into a pD18 vector along with a BamHI-XbaI cDNA fragment encoding the extracellular domain of human CD154 The extracellular domain is encoded at the carboxy terminus of CD154, similar to other type II membrane proteins

The extracellular domain of human CD154 was PCR amplified using cDNA generated with random primers and RNA from human T lymphocytes activated with PHA (phytohemagglutinin) The primer sets included two different 5' or sense primers that created fusion junctions at two different positions within the extracellular domain of CD154 Two different fusion junctions were designed that resulted in a short or truncated form (form S4) including amino acids 108 (Glu)-261 (Leu) + (Glu), and a long or complete form (form L2) including amino acids 48 (Arg) -261 (Leu) + (Glu), of the extracellular domain of CD154, both constructed as BamHI-XbaI fragments The sense primer that fuses the two different truncated extracellular domains to the 2H7scFv includes a BamHI site for cloning. The sense primer for the S4 form of the CD154 cDNA is designated SEQUENCE ID NO 11 or CD154BAM108 and encodes a 34 mer with the following sequence 5'-gtt gtc gga tcc aga aaa cag ctt tga aat gca a-3' , while the antisense primer is designated SEQUENCE ID NO 12 or CD154XBA and encodes a 44 mer with the following sequence 5'-gtt gtt tct aga tta tca ctc gag ttt gag taa gcc aaa gga cg-3'

The oligonucleotide primers used in amplifying the long form (L2) of the CD154 extracellular domain encoding amino acids 48 (Arg)-261 (Leu) + (Glu), were as follows The sense primer designated CD154 BAM48 (SEQUENCE ID NO 13) encoded a 35-mer with the following sequence 5'-gtt gtc gga tcc aag aag gtt gga caa gat aga ag-3' The antisense primer designated or CD154XBA (SEQUENCE ID NO __) encoded the 44-

mer- 5'-gtt gtt tct aga tta tca ctc gag ttt gag taa gcc aaa gga cg-3' Other PCR reaction conditions were identical to those used for amplifying the 2H7 scFv (see Example 1) PCR fragments were purified by PCR quick kits (QIAGEN, San Diego, CA), eluted in 30 µl ddH_2O, and digested with BamHI and XbaI (Roche) restriction endonucleases in a 40 µl reaction volume at 37 °C for 3 hours Fragments were gel purified, purified using QIAEX kits according to the manufacturer's instructions (QIAGEN), and ligated along with the 2H7 HindIII-BclI fragment into the pD18 expression vector digested with HindIII+XbaI. Ligation reactions were transformed into DH5-alpha chemically competent bacteria and plated onto LB plates containing 100 µg/ml ampicillin. Transformants were grown overnight at 37 °C, and isolated colonies used to inoculate 3 ml liquid cultures in Luria Broth containing 100 µg/ml ampicillin Clones were screened after mini-plasmid preparations (QIAGEN) for insertion of both the 2H7 scFv and the CD154 extracellular domain fragments.

The 2H7scFv-CD154 construct cDNAs were subjected to cycle sequencing on a PE 9700 thermocycler using a 25-cycle program that included denaturating at 96 °C, 10 seconds, annealing at 50 °C for 5 seconds, and extension at 60°C, for 4 minutes The sequencing primers used were pD18 forward (SEQ ID NO __ 5'-gtctatataagcagagctctggc-3') and pD18 reverse (SEQ ID NO __ 5'-cgaggctgatcagcgagctctagca-3') primers In addition, an internal primer was used that had homology to the human CD154 sequence (SEQ ID NO __ 5'-ccgcaatttgaggattctgatcacc-3') Sequencing reactions included primers at 3 2 pmol, approximately 200 ng DNA template, and 8 µl sequencing mix Sequencing reactions were performed using the Big Dye Terminator Ready Sequencing Mix (PE-Applied Biosystems, Foster City, CA) according to the manufacturer's instructions Samples were subsequently purified using Centrisep columns (Princeton Separations, Adelphia, NJ) The eluates were dried in a Savant speed-vacuum dryer, denatured in 20 µl template Suppression Reagent (ABI) at 95°C for 2 minutes, and analyzed on an ABI 310 Genetic Analyzer (PE Applied Biosystems) The sequence was edited, translated, and analyzed using Vector Nti version 6 0 (Informax, North Bethesda, MD) The 2H7scFv-CD154 L2 cDNA sequence and predicted amino acid sequence is presented in Figure 7A, and 2H7scFv-CD154 S4 cDNA sequence and predicted amino acid sequence is presented in Figure 7B

The binding activity of the 2H7 scFv-CD154 fusion proteins (SEQ ID NO __ and __) to CD20 and CD40 simultaneously was determined by flow cytometry The assay used CHO cell targets that express CD20 After a 45-minute incubation of CD20 CHO cells with supernatants from cells transfected with the 2H7 scFv-CD154 expression plasmid, the CD20 CHO cells were washed twice and incubated with biotin-conjugated CD40-Ig fusion protein in PBS/2% FBS After 45 min, cells were washed twice and incubated with phycoerythrin (PE)-labeled streptavidin at 1 100 in PBS/2% FBS (Molecular Probes, Eugene OR) After an additional 30 min incubation, cells were washed 2X and were analyzed by flow cytometry The results show that the 2H7 scFv-CD154 molecule was able to bind to CD20 on the cell surface and to capture biotin-conjugated CD40 from solution (Figure 8)

To determine the effect of the 2H7scFv-CD154 on growth and viability of B lymphoma and lymphoblastoid cell lines, cells were incubated with 2H7scFv-CD154 L2 (SEQ ID NO __) for 12 hours and then examined for binding of Annexin V Binding of Annexin V was measured using a FITC-Annexin V kit (Immunotech, Marseille, France, Catalog # PN-IM2376) B cell lines were incubated in 1 ml cultures with dilutions of concentrated, dialyzed supernatants from cells expressing secreted forms of the 2H7scFv-CD154 fusion proteins The results are presented in Figure 9

The growth rate of the Ramos B lymphoma cell line in the presence of 2H7scFv-CD154 was examined by uptake of ^{3}H-thymidine for the last 6 hours of a 24-hour culture The effect of 2H7scFv-CD154 on cell proliferation is shown in Figure 10

EXAMPLE 5

CONSTRUCTION AND CHARACTERIZATION OF CYTOXB ANTIBODY DERIVATIVES

CytoxB antibodies were prepared using 2H7 scFv-IgG polypeptide. The 2H7 scFv (see Example 1) was linked to the human IgG1 Fc domain via an altered hinge domain (see Figure 11) Cysteine residues in the hinge region were substituted with serine residues by site-directed mutagenesis and other methods known in the art. The mutant hinge was fused either to a wild-type Fc domain to create one construct, designated CytoxB-MHWTG1C, or was fused to a mutated Fc domain (CytoxB-MHMG1C) that had additional mutations introduced into the CH2 domain. Amino acid residues in CH2 that are implicated in effector function are illustrated in Figure 11 Mutations of one or more of these residues may reduce FcR binding and mediation of effector functions. In this

Example, the leucine residue 234 known in the art to be important to Fc receptor binding, was mutated in the 2H7 scFv fusion protein, CytoxB-[MG1H/MG1C] In another construct, the human IgG1 hinge region was substituted with a portion of the human IgA hinge, which was fused to wild-type human Fc domain (CytoxB-IgAHWTHG1C) (See Figure 11) This mutated hinge region allows expression of a mixture of monomeric and dimeric molecules that retain functional properties of the human IgG1 CH2 and CH3 domains Synthetic, recombinant cDNA expression cassettes for these molecules were constructed and polypeptides were expressed in CHODG44 cells according to methods described in Example 2

Purified fusion protein derivatives of CytoxB-scFvIg molecules were analyzed by SDS-PAGE according to the methods described in Example 2. Polyacrylamide gels were run under non-reducing and reducing conditions Two different molecule weight marker sets, BioRad prestained markers, (BioRad, Hercules, CA) and Novex Multimark molecular weight markers were loaded onto each gel The migration patterns of the different constructs and of Rituximab™ are presented in Figure 12

The ability of the different derivatives of CytoxB-scFvIg molecules to mediate ADCC was measured using the Bjab B lymphoma cells as the target and freshly prepared human PBMCs as effector cells (See Example 2) Effector to target ratios were varied as follows 70 1, 35 1, and 18 1, with the number of Bjab cells per well remaining constant but the number of PBMCs were varied. Bjab cells were labeled for 2 hours with ^{51}Cr and aliquoted at a cell density of 5×10^4 cells/well to each well of flat-bottom 96 well plates Purified fusion proteins or rituximab were added at a concentration of 10 µg/ml to the various dilutions of PBMCs Spontaneous release was measured without addition of PBMC or fusion protein, and maximal release was measured by the addition of detergent (1% NP-40) to the appropriate wells Reactions were incubated for 4 hours, and 100 µl of culture supernatant was harvested to a Lumaplate (Packard Instruments) and allowed to dry overnight prior to counting cpm released The results are presented in Figure 13

Complement dependent cytotoxicity (CDC) activity of the CytoxB derivatives was also measured Reactions were performed essentially as described in Example 2 The results are presented in Figure 14 as percent of dead cells to total cells for each concentration of fusion protein.

EXAMPLE 6

In Vivo Studies in Macaques

Initial *in vivo* studies with CytoxB derivatives have been performed in nonhuman primates. Figure 15 shows data characterizing the serum half-life of CytoxB in monkeys. Measurements were performed on serum samples obtained from two different macaques (J99231 and K99334) after doses of 6 mg/kg were administered to each monkey on the days indicated by arrows. For each sample, the level of 2H7scFvIg present was estimated by comparison to a standard curve generated by binding of purified CytoxB-(MHWTG1C)-Ig fusion protein to CD20 CHO cells (see Example 2). The data are tabulated in the bottom panel of the Figure 15.

The effect of CytoxB-(MHWTG1C)Ig fusion protein on levels of circulating CD40+ cells in macaques was investigated. Complete blood counts were performed at each of the days indicated in Figure 16. In addition, FACS (fluorescence activated cell sorter) assays were performed on peripheral blood lymphocytes using a CD40-specific fluorescein conjugated antibody to detect B cells among the cell population. The percentage of positive cells was then used to calculate the number of B cells in the original samples. The data are graphed as thousands of B cells per microliter of blood measured at the days indicated after injection (Figure 16).

Example 7

Construction and Expression of an Anti-CD19 scFv-Ig Fusion Protein

An anti-CD19 scFv-Ig fusion protein was constructed, transfected into eukaryotic cells, and expressed according to methods presented in Examples 1, 2, and 5 and standard in the art. The variable heavy chain regions and variable light chain regions were cloned from RNA isolated from hybridoma cells producing antibody HD37, which specifically binds to CD19. Expression levels of a HD37scFv-IgAHWTG1C and a HD37scFv-IgMHWTG1C were measured and compared to a standard curve generated using purified HD37 scFvIg. The results are presented in Figure 17.

Example 8

Construction and Expression of an Anti-L6 scFv-Ig Fusion Protein

An scFv-Ig fusion protein was constructed using variable regions derived from an anti-carcinoma monoclonal antibody, L6. The fusion protein was constructed, transfected into eukaryotic cells, and expressed according to methods presented in

Examples 1, 2, and 5 and standard in the art. Expression levels of L6 scFv-IgAH WCH2 CH3 and L6 scFv-(SSS-S)H WCH2 WCH3 were measured and compared to a standard curve generated using purified L6 scFvIg The results are presented in Figure 18

EXAMPLE 9

CHARACTERIZATION OF VARIOUS SCFV-IG FUSION PROTEINS

In addition to the scFv-Ig fusion protein already described, G28-1 (anti-CD37) scFv-Ig fusion proteins were prepared essentially as described in Examples 1 and 5 The variable regions of the heavy and light chains were cloned according to methods known in the art. ADCC activity of 2H7-MHWTG1C, 2H7-IgAHWTG1C, G28-1-MHWTG1C, G28-1 IgAHWTG1C, HD37- MHWTG1C, and HD37- IgAHWTG1C was determined according to methods described above (see Example 2) Results are presented in Figure 19 ADCC activity of L6scFv-IgAHWTG1C and L6scFv-IgMHWTG1C was measured using the 2981 human lung carcinoma cell line The results are presented in Figure 20 The murine L6 monoclonal antibody is known not to exhibit ADCC activity

The purified proteins were analyzed by SDS-PAGE under reducing and non reducing conditions Samples were prepared and gels run essentially as described in Examples 2 and 5 The results for the L6 and 2H7 scFv-Ig fusion proteins are presented in Figure 21 and the results for the G28-1 and HD37 scFv-Ig fusion proteins are presented in Figure 22

EXAMPLE 10

CONSTRUCTION AND EXPRESSION OF ANTI-CD20 SCFV-LLAMA IG FUSION PROTEINS

This Example illustrates the cloning of llama IgG1, IgG2, and IgG3 constant region domains and the construction of immunoglobulin fusion proteins with each of the three constant regions and anti-CD20 scFv

The constant regions of llama IgG1, IgG2, and IgG3 immunoglobulins were cloned and inserted into mammalian vector constructs containing an anti-CD20 single chain Fv, 2H7 scFv Total RNA was isolated from peripheral blood mononuclear cells (PBMC) from llama blood (Triple J Farms, Bellingham, WA) by lysing the lymphocytes in TRIzol® (Invitrogen Life Technologies, Carlsbad, CA) according to the manufacturer's instructions One microgram (1 μg) of total RNA was used as template to prepare cDNA by reverse transcription. The RNA and 200 ng random primers were combined and

denatured at 72 °C for 10 minutes prior to addition of enzyme. Superscript II reverse transcriptase (Invitrogen Life Technologies) was added to the RNA plus primer mixture in a total volume of 25 µl in the presence of 5X second strand buffer and 0 1 M DTT provided with the enzyme The reverse transcription reaction was allowed to proceed at 42 °C for one hour The cDNA was amplified by PCR using sequence specific primers The 5' primers were designed according to published sequences for the V_{HH} and V_H domains of camelids The 3' primer, which was used to amplify all three isotypes, was designed using mammalian CH3 domain sequences as a guide The following specific primers were used. The BcI and XbaI sites are indicated by underlined italicized sequences

5' primer for llama IgG1 constant region

LLG1-5'bgl 5'-gtt gt*t gat c*aa gaa cca cat gga ggt tgc acg tg-3'

(SEQ ID NO ___)

5' primer for llama IgG2 constant region

LLG2-5'bgl 5'-gtt gt*t gat c*aa gaa ccc aag aca cca aaa cc-3'

(SEQ ID NO ___)

5' primer for llama IgG3 constant region

LLG3-5'bgl 5'-gtt gt*t gat c*aa gcg cac cac agc gaa gac ccc-3'

(SEQ ID NO ___)

3' primer for llama IgG1, IgG2, and IgG3 constant regions

LLG123-3'X 5'-gtt gtt *tct aga* tta cta ttt acc cga aga ctg ggt gat gga-3'

(SEQ ID NO ___)

PCR fragments of the expected size were cloned into TOPO® cloning vectors (Invitrogen Life Technologies) and then were sequenced. The sense sequencing primer, LLseqsense, had the sequence 5'-ctg aga tcg agt tca gct g-3' (SEQ ID NO ___), and the antisense primer, LLseqAS, had the sequence 5'-cct cct ttg gct ttg tct c-3' (SEQ ID NO ___) Sequencing was performed as described in Example 1 Figure 23 compares the amino acid sequence of the three isotype llama constant regions containing the hinge, CH2, and CH3 domains with the amino acid sequence of human IgG1 hinge, CH2, and CH3 domains

After verifying the sequence, the amplified PCR products were digested with restriction enzymes BclI and Xba1 to create compatible restriction sites. The digested fragments were then gel-purified, and the DNA was eluted using a QIAquick Gel

Extraction Kit (QIAGEN, Valencia, CA) The 2H7scFv-Ig pD18 mammalian expression vector construct (see Example 2) was digested with BclI and XbaI to remove the human IgG hinge, CH2, and CH3 domains The pD18 vector is a modified derivative of pCDNA3 that contains an attenuated DHFR gene, which serves as a selectable marker for mammalian expression (Hayden *et al*, *Tissue Antigens* 48 242-54 (1996)) The purified llama IgG1, IgG2, and IgG3 constant region PCR products were ligated by T4 DNA ligase (Roche Molecular Biochemicals, Indianapolis, IN) into the double-digested 2H7 scFv-pD18 vector at room temperature overnight according to the manufacturer's instructions. After ligation, the ligation products were transformed into *E. coli* DH5α bacteria (BD Biosciences, Palo Alto, CA) and plated according to standard molecular biology procedures and manufacturer's instructions Isolated colonies were chosen to screen for transformants containing the correct inserts

For expression of the encoded polypeptides, plasmid DNA from positive clones was transiently transfected into COS-7 cells using DEAE-dextran (Hayden *et al*, *Ther Immunol* 1 3-15 (1994)) COS-7 cells were seeded at approximately 3 x 10^6 cells per 150 mm plate and grown overnight until the cells were about 75% confluent. Cells were then washed once with serum-free DMEM (Invitrogen Life Technologies, Grand Island, NY) Transfection supernatant (10 ml) containing 400 μg/ml DEAE-dextran, 0 1 mM chloroquine, and 5 μg/ml of the DNA constructs were added to the cells, which were then incubated at 37 °C for 3-4 hrs After incubation, cells were pulsed with 10 ml of 10% dimethyl sulfoxide (DMSO) in 1x PBS at room temperature for 2 minutes Cells were then placed back into fully supplemented DMEM/10% FBS (1% L-glutamine, 1% penicillin/streptomycin, 1% sodium pyruvate, 1% MEM essential amino acids) (Invitrogen Life Technologies) After 24 hours, the media was replaced with serum-free fully supplemented DMEM (Invitrogen Life Technologies), and the cells were maintained up to 21 days with media changes every 3-4 days

Ig-fusion proteins were purified by passing COS cell culture supernatants through Protein A Agarose (Repligen, Cambridge, MA) columns After application of the culture supernatant, the Protein A columns were then washed with 1x PBS (Invitrogen Life Technologies) Bound Ig-fusion proteins were eluted with 0 1 M citric acid (pH 2 8), and the collected fractions were immediately neutralized with Tris base (pH 10 85) The fractions containing protein were identified by measuring the optical density (A_{280}) and

then were pooled, dialyzed against 1x PBS, (Invitrogen Life Technologies) and filtered through a 0 2 µm filter

The purified Ig-fusion proteins were analyzed by SDS-PAGE. Aliquots of 2H7 scFv-llama IgG1, 2H7 scFv-llama IgG2, 2H7 scFv-llama IgG3, and Rituxan® (Rituximab, anti-CD20 antibody, Genentech, Inc. and IDEC Pharmaceuticals Corp) (5 µg protein) were combined with 25 µl 2x NuPAGE® SDS Sample Buffer (Invitrogen Life Technologies) (non-reduced samples) Samples of each protein were also prepared in reducing sample buffer containing 5% 2-mercaptoethanol (Sigma-Aldrich, St. Louis, MO) Molecular weight markers (Invitrogen Life Technologies) were applied to the gels in non-reducing buffer only The proteins were fractionated on NuPAGE® 10% Bis-Tris gels (Invitrogen Life Technologies) After electrophoresis (approximately 1 hour), the gels were washed three times, five minutes each, with Distilled Water (Invitrogen Life Technologies) and then stained in 50 ml Bio-Safe Coomassie Stain (BioRad, Hercules, CA) overnight at room temperature After a wash in Distilled Water, the gels were photographed The migration pattern of each Ig-fusion protein is presented in Figure 24.

The ability of the 2H7 scFv-llama Ig fusion proteins to bind to cells expressing CD20 was demonstrated by flow cytometry Serial dilutions starting at 25 µg/ml of purified 2H7 scFv-llama IgG1, 2H7 scFv-llama IgG2, and 2H7 scFv-llama IgG3 were prepared and incubated with CD20-transfected (CD20+) CHO cells (from the laboratory of Dr S Skov, Institute of Medical Microbiology and Immunology, Copenhagen Denmark in 1% FBS 1x PBS media (Invitrogen Life Technologies) for one hour on ice After the incubation, the cells were then centrifuged and washed with 1% FBS in 1x PBS To detect bound 2H7 scFv-llama Ig, the cells were incubated for one hour on ice with fluorescein-conjugated goat anti-camelid IgG (heavy and light chain) (1 100) (Triple J Farms) The cells were then centrifuged and resuspended in 1% FBS-1x PBS and analyzed using a Coulter Epics XL cell sorter (Beckman Coulter, Miami, FL) The data (percent of maximum brightness) are presented in Figure 25

EXAMPLE 11

EFFECTOR FUNCTION OF ANTI-CD20 SCFV-LLAMA IG FUSION PROTEINS

This Example demonstrates the ability of anti-CD20 llama IgG1, IgG2, and IgG3 fusion proteins to mediate complement dependent cytotoxicity (CDC) and antibody dependent cell-mediated cytotoxicity (ADCC)

The ability of the 2H7 scFv-llama IgG fusion proteins to kill CD20 positive cells in the presence of complement was tested using the BJAB human B cell line. Rabbit complement was obtained from 3-4 week old rabbits (Pel-Freez, Brown Deer, WI) BJAB cells (2 x 10^6 cells/ml) were combined with rabbit complement (final dilution 1 10) and purified 2H7 Ig fusion proteins 2H7 scFv-llama IgG1, 2H7 scFv-llama IgG2, 2H7 scFv-llama IgG3, and 2H7 scFv-human IgG1 wild type hinge-CH2-CH3) (Example 1) were added at 1 3 serial dilutions beginning at a concentration of 30 µg/ml. After one hour at 37 °C, cell viability was determined by counting live and dead cells by trypan blue exclusion (0 4%) (Invitrogen Life Technologies) using a hemacytometer (Bright-line, Horsham, PA) The percent killing was calculated by dividing the number of dead cells by the number of total cells (dead + live cells) The data presented in Figure 26 show that all Ig fusion proteins had CDC activity

The ADCC activity of the 2H7 scFv-llama IgG fusion proteins was determined using BJAB cells as target cells and human or llama peripheral blood monotnuclear cells (PBMC) as effector cells BJAB cells were pre-incubated for approximately 2 hours with ^{51}Cr (100 µCi) (Amersham Biosciences, Piscataway, NJ) in fully supplemented IMDM (Invitrogen Life Technologies) containing 15% FBS The cells were mixed intermittently during the pre-incubation period Fresh, resting human PBMC were purified from whole blood using Lymphocyte Separation Media (LSM) (ICN Pharmaceuticals, New York, NY) PBMC were combined with labeled BJAB cells (5 x 10^4 cells per well of 96 well tissue culture plate) at ratios of 25 1, 50 1, and 100 1 To each combination was added 10 µg/ml of purified 2H7 scFv-llama IgG1, 2H7 scFv-llama IgG2, 2H7 scFv-llama IgG3, Rituximab, or no anti-CD20 antibody The mixtures were incubated for 6 hours at 37 °C Supernatant from each reaction containing ^{51}Cr released from lysed cells was collected onto a LumaPlate-96 filter plate (Packard, Meriden, CT), which was dried overnight The amount of ^{51}Cr was measured by a TopCount NXT plate reader (Packard) Figure 27 shows that the 2H7 scFv-llama IgG2 fusion protein was the most effective llama fusion protein in mediating ADCC Each data point represents the average measurement of triplicate wells

ADCC activity was affected by the source of effector cells. Llama PBMC were isolated from llama blood (Triple J Farms) using LSM Llama effector cells were added at the same ratios to BJAB target cells as described for the ADCC assay using

human effector cells The cells were combined with 10 µg/ml of purified 2H7 scFv-llama IgG1, 2H7 scFv-llama IgG2, 2H7 scFv-llama IgG3, Rituximab, or no anti-CD20 antibody The results are presented in Figure 28

EXAMPLE 12

CONSTRUCTION AND CHARACTERIZATION OF scFv Ig FUSION PROTEINS EXPRESSED ON THE CELL SURFACE

This Example describes a retroviral transfection system for ectopic surface expression of genetically engineered cell surface receptors composed of scFvs that bind costimulatory receptors. The Example also demonstrates the effector function of these various scFv Ig fusion proteins expressed on the surface of target cells.

The heavy and light chain variable regions were cloned from murine monoclonal antibodies specific for various costimulatory receptors, and single chain Fv constructs were prepared essentially as described in Example 1 Antibodies included 2H7, anti-human CD20, 40.2 220, anti-human CD40, 2E12, anti-human CD28, 10A8, anti-human CD152 (anti-CTLA-4), and 500A2, anti-murine CD3 The heavy chain and light chain variable regions of each antibody were cloned according to standard methods for cloning immunoglobulin genes and as described in Example 1 Single chain Fv constructs were prepared as described in Example 1 by inserting a nucleotide sequence encoding a $(gly_4ser)_3$ peptide linker between the VL region nucleotide sequence of 40.2 220, 2E12, 10A8, and 500A2, respectively (SEQ ID NOs __, respectively) and the VH region nucleotide sequence of 40 2 220, 2E12, 10A8, and 500A2, respectively (SEQ ID NOs __, respectively) The polypeptide sequence for VL of 40 2 220, 2E12, 10A8, and 500A2 are set forth in SEQ ID NOs ___, respectively, and the polypeptide sequence for VH of 40.2 220, 2E12, 10A8, and 500A2 are set forth in SEQ ID NOs ___, respectively Each scFv polynucleotide (SEQ ID NOs __ for 40 2 220, 2E12, 10A8, and 500A2, respectively) was then fused to human IgG1 mutant hinge (CCC→SSS) and mutant CH2 (proline to serine mutation at residue 238 (238 numbering according to EU nomenclature, Ward *et al*, 1995 *Therap Immunol* 2 77-94, residue 251 according to Kabat *et al*) and wild type CH3 domains according to the methods described in Example 5 and 11 Each scFv mutant IgG1 fusion polynucleotide sequence was then fused in frame to sequences encoding the transmembrane domain and cytoplasmic tail of human CD80 (SEQ ID NO __), such that when the fusion protein was expressed in the transfected cell, CD80 provided an anchor for

surface expression of the scFv Ig fusion protein. cDNAs encoding the scFv-IgG-CD80 fusion proteins (SEQ ID NOs __ for 40 2 220-, 2E12-, 10A8-, and 500A2-scFv-IgG-CD80, respectively) were inserted into the retroviral vector pLNCX (BD Biosciences Clontech, Palo Alto, CA) according to standard molecular biology procedures and vendor instructions. The scFv-Ig-CD80 cDNA was inserted between the 5'LTR-neomycin resistance gene-CMV promoter sequences and the 3'LTR sequence The retroviral constructs were transfected into Reh, an acute lymphocytic leukemia cell line (ATCC CRL-8286) Transfected cells were screened to select clones that were expressing scFv-Ig fusion proteins on the cell surface

CDC and ADCC assays were performed with the transfected Reh cells to determine if expression of the scFv-Ig polypeptides on the cell surface augmented effector cell function. Reh cells expressing anti-human CD152 scFv-mutant IgG-CD80 (SEQ ID NO __), Reh anti-human CD28 scFv- mutant IgG-CD80 (SEQ ID NO __), Reh anti-human CD28 scFv- mutant IgG-CD80 (SEQ ID NO __), Reh anti-human CD40 scFv-mutant IgG-CD80 (SEQ ID NO __), Reh anti-human CD20 scFv- mutant IgG-CD80 (SEQ ID NO __) were combined with human PBMC (see Example 11) and rabbit complement (10 µg/ml) for one hour at 37 °C. Untransfected Reh cells were included as a control Viability of the cells was determined by trypan blue exclusion, and the percent of killed cells was calculated (see Example 11) Figure 29 shows the effectiveness of the scFv-IgG-CD80 fusion proteins when expressed on the cell surface of tumor cells to mediate complement dependent cytotoxicity

The same transfected Reh cells tested in the CDC assay plus Reh cells transfected with the polynucleotide construct that encodes anti-murine CD3-scFv-Ig-CD80 (SEQ ID NO __) were analyzed for ADCC activity (see Example 11) Untransfected and transfected Reh cells were pre-labeled with ^{51}Cr (100 µCi) (Amersham) for two hours at 37 °C Human PBMC served as effector cells and were added to the Reh target cells (5 x 10^4 cells per well of 96 well plate) at ratios of 5 1, 2 5 1, and 1.25 1 After five hours at 37 °C, culture supernatants were harvested and analyzed as described in Example 11 Percent specific killing was calculated according to the following equation. ((experiment release minus spontaneous release)/(maximum release minus spontaneous release)) x 100 The data are presented in Figure 30 Each data point represents the average of quadruplicate samples

Using the same procedures described above, the same results with other binding domains were obtained using the following monoclonal antibodies monoclonal antibodies as sources of sFv for CD20, 1F5 (Genbank AY 058907 and AY058906), for CD40, 2 36 and G28 5, for CD28, 9 3

Cell surface expression of antibody binding domains is accomplished by fusing antibody scFvs to IgA hinge and constant regions and IgE hinge-acting region, *i.e.*, IgE CH2, and constant regions Polynucleotides encoding an anti-4-1BB scFv, 5B9 (anti-human 4-1BB) scFv, and 2e12 (anti-human CD40) fused to IgAH IgA T4 (four terminal CH3 residues deleted) fused to the CD80 transmembrane and cytoplasmic domains and IgE Fc regions are shown in SEQ ID NOs ___ The encoded polypeptides are shown in SEQ ID NOs ___

EXAMPLE 13

CONSTRUCTION AND SEQUENCE OF HUMAN IG HINGE-CH2-CH3 MUTANTS AND 2H7 VARIABLE REGION MUTANTS

This Example describes construction of scFv fusion proteins containing mutant human IgG1 and IgA constant regions This Example also describes construction of a 2H7 scFv mutant with a single point mutation in the variable heavy chain region. Mutations were introduced into variable and constant region domains according to methods described herein and known in the molecular biology arts. Figure 31 presents nomenclature for the Ig constant region constructs

The human IgG1 hinge region of the 2H7 scFv human IgG1 hinge-CH2-CH3 fusion proteins was mutated to substitute cysteine residues that in a whole immunoglobulin are involved in forming disulfide bonds between two heavy chain molecules One mutant, 2H7 scFv fused to a human IgG1 hinge region in which all three cysteine residues were mutated to serine residues (MTH (SSS)), was prepared as described in Example 5 (designated in Example 5 as CytoxB-MHWTG1C (includes wild type IgG1 CH2 and CH3 domains)) (now referred to as 2H7 scFv MTH (SSS) WTCH2CH3) and comprises the polynucleotide sequence SEQ ID NO __ encoding the polypeptide as set forth in SEQ ID NO__ The polynucleotide sequence encoding this mutant (SEQ ID NO __) was used as a template to create mutant hinge regions in which the first two cysteine residues were substituted with serine residues (IgG MTH (SSC)) An oligonucleotide was designed to substitute the third serine residue with a cysteine and had

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the following sequence 5'-gtt gtt gat cag gag ccc aaa tct tct gac aaa act cac aca tct cca ccg tgc cca gca cct g-3' (HuIgGMHncs3, SEQ ID NO ___) A second mutant was prepared in which the mutant hinge had serine residues substituting the first and third cysteine residues (IgG MTH (SCS)) The sequence of the oligonucleotide to create this mutant was as follows 5'-gtt gtt gat cag gag ccc aaa tct tct gac aaa act cac aca tgc cca ccg-3' (HuIgGMHncs2, SEQ ID NO ___) A third mutant was prepared with cysteine residues substituted at the second and third positions (IgG MTH (CSS)), also using the IgG MTH (SSS) mutant as template, and an oligonucleotide having the sequence, 5'-gtt gtt gat cag gag ccc aaa tct tgt gac aaa act cac-3' (HuIgGMHncs1, SEQ ID NO ___)

The oligonucleotides introducing the mutations into the hinge region were combined with template and a 3' oligonucleotide containing an XbaI site (underlined and italicized) (5'-gtt gtt *tct aga* tca ttt acc cgg aga cag gga gag gct ctt ctg cgt gta g-3' (SEQ ID NO ___)) to amplify the mutant hinge-wild type (WT)-CH2-CH3 sequences by PCR. The IgG MTH CSS and IgG MTH SCS mutant sequences were amplified for 25 cycles with a denaturation profile of 94 °C, annealing at 52 °C for 30 seconds, and extension at 72 °C for 30 seconds The IgG MTH SSC mutant sequence was amplified under slightly different conditions denaturation profile of 94 °C, annealing at 45 °C for 30 seconds, and extension at 72 °C for 45 seconds The amplified polynucleotides were inserted into the TOPO® cloning vector (Invitrogen Life Technologies) and then were sequenced as described in Example 1 to confirm the presence of the mutation. pD18 vector containing 2H7 scFv was digested to remove the constant region sequences essentially as described in Example 10 The mutant hinge-wild type CH2-CH3 regions were inserted in frame into the digested vector DNA to obtain vectors comprising 2H7 scFv MTH (CSS) WTCH2CH3 encoding DNA (SEQ ID NO ___), 2H7 scFv MTH (SCS) WTCH2CH3 encoding DNA (SEQ ID NO ___), and 2H7 scFv MTH (SSC) WTCH2CH3 encoding DNA (SEQ ID NO ___)

A mutation of leucine to serine at position 11 in the first framework region of the heavy chain variable region (numbering according to Kabat *et al*, *Sequences of Proteins of Immunological Interest*, 5th ed. Bethesda, MD Public Health Service, National Institutes of Health (1991)) was introduced into the 2H7 scFv MTH (SSS) WTCH2CH3 fusion protein (SEQ ID NO __) The wild type leucine residue was substituted with serine by site-directed mutagenesis using the oligonucleotide Vhser11 5'-gga ggt ggg agc tct cag gct tat cta cag cag tct ggg gct gag tcg gtg agg cc-3' (SEQ ID NO ___)(this sequence, or an

amino acid sequence that it encodes, may be optionally excluded from particular claimed embodiments of the instant invention) The 3'-primer for PCR was huIgG1-3' having the sequence 5'-gtc *tct aga* cta tca ttt acc cgg aga cag-3' (SEQ ID NO___) (XbaI site underlined and italicized) After PCR amplification, the fragments were inserted into the TOPO® cloning vector and sequenced to confirm the presence of the VH11 leucine to serine mutation. The 2H7 scFv-IgG MTH (SSS) WTCH2CH3 encoding DNA was shuttled into the PSL1180 cloning vector (Pharmacia Biotech, Inc., Piscataway, NJ) The construct PSL1180-2H7 scFv-IgG MTH (SSS) WTCH2CH3 was digested with Sac and XbaI to remove the wild type VH domain and the hinge and CH2 and CH3 domains. The PCR product comprising the VH11 mutant was digested with Sac and XbaI and then inserted into the digested PSL1180 construct according to standard molecular biology procedures The construct was then digested with Hind III and XbaI, and inserted into the mammalian expression vector pD18 (see methods described in Example 1 and Example 10) The mutant is designated 2H7 scFv VH11SER IgG MTH (SSS) WTCH2CH3 (Figure 31). The polynucleotide sequence is provided in SEQ ID NO__, and the encoded polypeptide sequence is provided in SEQ ID NO___ (these sequences may be optionally excluded from particular claimed embodiments of the instant invention).

Four constructs containing IgA constant region domains were prepared. One construct contained wild type IgA hinge fused to human IgG1 CH2 and CH3 (IgAH IgG WTCH2CH3) (Figure 31) Sequential PCR amplifications were performed to substitute the human IgG1 hinge of the 2H7 scFv construct with nucleotide sequences encoding the IgA hinge The 5' oligonucleotide primer (huIgA/Gchm5) for the first PCR reaction had the sequence, 5'-cca tct ccc tca act cca cct acc cca tct ccc tca tgc gca cct gaa ctc ctg-3' (SEQ ID NO___) The primer (huIgAhg-5') for the second PCR reaction to add more IgA specific hinge sequence and add a BclI restriction enzyme site (italicized and underlined) had the sequence, 5'-gtt *gtt gat cag* cca gtt ccc tca act cca cct acc cca tct ccc caa ct-3' (SEQ ID NO___) The 3' primer for both amplification steps was huIgG1-3' having the sequence, 5'-gtc tct aga cta tca ttt acc cgg aga cag-3' (SEQ ID NO___). The sequence of the PCR product was confirmed by TOPO ® cloning as described above The gel-purified fragment was digested with BclI and XbaI and then inserted into the 2H7 scFv-pD18 vector that had been digested BclI and XbaI to remove all the IgG1 constant region domains Ligation was performed as described in Example 10 to provide a

mammalian expression vector comprising the nucleotide sequence (SEQ ID NO ___) encoding a 2H7 scFv IgA hinge-IgG1 CH2-CH3 polypeptide (SEQ ID NO ___).

A second pD18 mammalian expression vector was constructed that had a polynucleotide sequence (SEQ ID NO ___) that encoded a 2H7 scFv fused to wild type IgA hinge, CH2, and CH3 domains (SEQ ID NO ___) Human IgA constant regions sequences were obtained by using random primers to reverse transcribe total RNA isolated from human tonsil followed by PCR amplification of the cDNA using sequence specific primers, essentially as described in Example 10 Human IgA hinge-CH2-CH3 nucleotide sequence (SEQ ID NO ___) encoding the IgA-CH2-CH3 polypeptide (IgAH IgACH2CH3, Figure 31) (SEQ ID NO ___) was amplified using the 5' oligonucleotide huIgAhg-5' (SEQ ID NO (*same as above*___) and a 3' oligonucleotide huIgA3' having the sequence, 5'-gtt gtt tct aga tta tca gta gca ggt gcc gtc cac ctc cgc cat gac aac-3' (SEQ ID NO ___). Secretion of a 2H7-IgA hinge-IgA CH2-CH3 polypeptide from transfected mammalian cells required co-expression of human J chain that covalently binds to two IgA CH3 domains via disulfide bonds Total RNA was isolated from tonsil B cells and was reversed transcribed to generate cDNA as described above. PCR amplification of the nucleotide sequence encoding the J chain was performed with J chain specific primers The 5' PCR primer, HUJCH5nl, had the sequence, 5'-gtt gtt aga tct caa gaa gat gaa agg att gtt ctt-3' (SEQ ID NO ___), and sequence of the 3' primer, HUJCH3, was 5'-gtt gtt tct aga tta gtc agg ata gca ggc atc tgg-3' (SEQ ID NO ___) The cDNA was cloned into TOPO® for sequencing as described in Example 10 J chain encoding cDNA (SEQ ID NO ___) was then inserted into pD18 and pCDNA3-Hygro (+) (Invitrogen Life Technology) vectors for co-transfection with 2H7 scFv IgA hinge-CH2-CH3 constructs The J chain has the predicted amino acid sequence set forth in SEQ ID NO ___

Secretion of an scFv IgA constant region construct in the absence of J chain was accomplished by engineering a truncated CH3 domain with a deletion of the four carboxy terminal amino acids (GTCY, SEQ ID NO ___) (IgAH IgA-T4, Figure 31), which include a cysteine residue that forms a disulfide bond with the J chain The IgA hinge-CH2-CH3 nucleotide sequence containing the deletion in CH3 (SEQ ID NO ___) was prepared using a 5' PCR primer (huIgAhg-5') having the sequence 5'-gtt *gtt gat cag* cca gtt ccc tca act cca cct acc cca tct ccc tca act-3' (SEQ ID NO ___) (BclI site is underlined and italicized), and a 3' PCR primer (HUIGA3T1) having the sequence 5'-gtt gtt tct aga tta tca

gtc cac ctc cgc cat gac aac aga cac-3' (SEQ ID NO:___) This mutated IgA constant region nucleotide sequence was inserted into a 2H7 scFv pD18 vector as described for the generation of the previous 2H7 scFv-Ig constructs (see Example 1 and this Example) that comprises the polynucleotide sequence (SEQ ID NO ___) encoding a 2H7 IgAH IgA-T4 polynucleotide (SEQ ID NO ___)

A fourth construct was prepared that encoded a 2H7 scFv-IgA constant region fusion protein with a deletion of 14 additional amino acids, most of which are hydrophobic residues, from the carboxy terminus of IgA CH3 The 2H7 scFv-IgAH IgA-T4 encoding polynucleotide was used as template to engineer a deletion of the nucleotide sequence encoding PTHVNVSVVMAEVD (SEQ ID NO ___). The 5' oligonucleotide primer had the sequence 5'-gtt *gtt gat cag* cca gtt ccc tca act cca cct acc cca tct ccc tca act-3' (SEQ ID NO ___) (BclI site shown as underlined and italicized) The 3' oligonucleotide sequence was 5'-gtt gtt tct aga tta tca ttt acc cgc caa gcg gtc gat ggt ctt-3' (SEQ ID NO ___) The deleted IgA CH3 region was amplified by using the above oligonucleotides to amplify the IgA constant region from RNA isolated from human tonsil such that the cDNA contained the deleted carboxyl terminal encoding region for the 18 amino acids The IgAH IgA-T18 constant region was inserted into a 2H7 scFv pD18 vector that comprises the polynucleotide sequence (SEQ ID NO ___) encoding a 2H7 IgAH IgA-T18 polynucleotide (SEQ ID NO ___) as described above.

EXAMPLE 14

EFFECTOR FUNCTION OF CTLA-4 IGG FUSION PROTEINS

The Example compares the effector functions of CTLA-4 Ig fusion proteins in CDC and ADCC assays

Two CTLA-4 IgG fusion proteins were constructed One fusion protein comprises the extracellular domain of CTLA-4 fused to human IgG1 wild type hinge, CH2, and CH3 domains and is designated CTLA-4 IgG WTH (CCC) WTCH2CH3 (SEQ ID NO __) A pD18 mammalian expression vector comprising a polynucleotide sequence encoding CTLA-4 IgG WTH (CCC) WTCH2CH3 (SEQ ID NO ___) was prepared by fusing in frame the nucleotide sequence encoding the extracellular domain of CTLA-4 (SEQ ID NO __) (see U S Patent No 5,844,095) to the nucleotide sequence encoding IgG WTH (CCC) WTCH2CH3 (SEQ ID NO ___) according to the methods described in Examples 1 and 10 The extracellular domain nucleotide sequence also comprises a BclI

restriction enzyme site at the 3' end, and a leader peptide nucleotide sequence (SEQ ID NO __) that encodes an oncoM leader peptide (SEQ ID NO ___) A second CTLA-4 IgG fusion protein, designated CTLA-4 IgG MTH (SSS) MTCH2WTCH3, contained the extracellular domain of CTLA-4 (plus the oncoM leader peptide sequence) fused to a mutant IgG hinge in which all three cysteine residues were replaced with serine residues. The hinge region was fused to a mutant IgG1 CH2 domain that had a mutation at isotype position 238 (EU numbering, Ward *et al*, *supra*, (position 251 using numbering according to Kabat *et al*, *supra*, position 209 where numbering commences with first residue of IgG1 CH1, *i e*, PAPELLDGPS (SEQ ID NO __) of wild type IgG1 CH2 is modified to PAPELLDGSS (SEQ ID NO __)), which was fused to IgG1 wild type CH3 (U S Patent No 5,844,095). The CTLA-4 IgG MTH (SSS) MTCH2WTCH3 polynucleotide comprises the nucleotide sequence in SEQ ID NO ___ and the deduced amino acid sequence comprises the sequence provided in SEQ ID NO __ CTLA-4 fusion proteins were also prepared using CTLA-4 extracellular membrane encoding sequences without the leader peptide (SEQ ID NO __)

To measure CDC activity, purified CTLA-4 IgG WTH (CCC) WTCH2CH3 (2 µg/ml) or CTLA-4 IgG MTH (SSS) MTCH2WTCH3 (2 µg/ml) was added to Reh cells (see Example 12) and to Reh cells transfected with the costimulatory molecule CD80 such that CD80 was expressed on the cell surface (Reh CD80 10, see Doty *et al*, 1998 J *Immunol* 161 2700, Doty *et al*, 1996 *J Immunol* 157 3270), in the presence or absence of rabbit complement (10 µg/ml) Purified CTLA Ig fusion proteins were prepared from culture supernatants of transiently transfected COS cells according to methods described in Example 10 The assays were performed essentially as described in Example 11 and 12 The data presented in Figure 32 show that only CD80-transfected Reh cells were killed in the presence of complement and CTLA-4 IgG WTH (CCC) WTCH2CH3 fusion protein.

The purified CTLA-4 Ig fusion proteins were also tested in ADCC assays. Human PBMC, serving as effector cells, were added to Reh or Reh CD80 1 target cells at a ratio of 1 25 1, 2 5 1, 5 0 1, and 10 1 Cells were labeled and the assays performed essentially as described in Examples 11 and 12 The results are presented in Figure 33 Each data point represents the average of four independent culture wells at each effector-target cell ratio The data show that only CTLA-4 IgG WTH (CCC) WTCH2CH3 mediated significant ADCC of Reh CD80 10 cells.

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EXAMPLE 15

EFFECTOR FUNCTION OF CTLA-4 IGA FUSION PROTEINS

CTLA-4 IgA fusion proteins are prepared as described for the IgG fusion proteins (see Examples 1, 13, and 14) CTLA-4 extracellular domain nucleotide sequence (SEQ ID NO ___) is fused in open reading frame to nucleotides encoding IgAH IgACH2CH3 (SEQ ID NO ___) to provide the nucleotide sequence (SEQ ID NO ___) encoding a CTLA-4 IgAH IgACH2CH3 fusion protein (SEQ ID NO ___) The fusion protein is transiently expressed in COS cells (see Example 10) or stably expressed in CHO cells (see Example 1) Secretion of the CTLA-4 IgAH IgACH2CH3 fusion protein requires co-transfection with a construct containing a polynucleotide sequence (SEQ ID NO ___) that encodes human J chain (SEQ ID NO ___) The CTLA-4 IgAH IgACH2CH3 fusion protein is isolated as described in Examples 10 and 14 To express a CTLA-4 IgA construct without the presence of J chain, a CTLA-4 IgAH IgA-T4 construct is prepared and transfected into mammalian cells In a similar manner as described for the CTLA-4 extracellular fragment fused to wild type IgA hinge-CH2CH3, the CTLA-4 extracellular domain nucleotide sequence (SEQ ID NO ___) is fused in open reading frame to a nucleotide sequence (SEQ ID NO ___) encoding a IgAH IgA-T4 polypeptide (SEQ ID NO ___) to provide a nucleotide sequence comprising SEQ ID NO ___ encoding a CTLA-4 IgAH IgA-T4 polypeptide (SEQ ID NO ___) Effector function of each construct is evaluated by CDC and ADCC as described in Example 14

EXAMPLE 16

BINDING OF ANTI-CD20 SCFV HUMAN IG FUSION PROTEINS TO CHO CELLS EXPRESSING CD20

This Example describes binding of 2H7 scFv Ig fusion proteins to CHO cells that express CD20 The analysis was performed by flow cytometry Culture supernatants were collected from transiently transfected COS cells expressing 2H7 scFv IgG WTH (CCC) WTCH2CH3 (SEQ ID NO __), 2H7 scFv IgG MTH (CSS) WTCH2CH3 (SEQ ID NO __), 2H7 scFv IgG MTH (SCS) WTCH2CH3 (SEQ ID NO __), and 2H7 scFv VHSHR11 WTH WTCH2CH3 (SEQ ID NO __), and two-fold serial dilutions were prepared. Serial two-fold dilutions of purified 2H7 scFv IgG MTH (SSC) WTCH2CH3 (SEQ ID NO __) were prepared starting at a concentration of 5 µg/ml The culture supernatants and purified fusion protein samples were incubated with (CD20+) CHO cells

for one hour on ice The cells were washed twice and then incubated with 1 100 FITC-conjugated goat anti-human IgG (CalTag) for 40 minutes The unbound conjugate was then removed by washing the cells and flow cytometry analysis was performed using a Coulter Epics XL cell sorter Results are presented in Figure 34

EXAMPLE 17

IMMUNOBLOT ANALYSIS OF ANTI-CD20 SCFV HUMAN IGG AND IGA FUSION PROTEINS

This Example describes immunoblot analysis of 2H7 scFv IgG and 2H7 scFv IgA fusion proteins that were immunoprecipitated from transfected cell culture supernants

COS cells were transiently transfected with plasmids comprising nucleotide sequences for 2H7 scFv IgG WTH (CCC) WTCH2CH3 (SEQ ID NO), 2H7 scFv IgG MTH (CSS) WTCH2CH3 (SEQ ID NO), 2H7 scFv IgG MTH (SCS) WTCH2CH3 (SEQ ID NO), 2H7 scFv IgA H IgG WTCH2CH3 (SEQ ID NO), and scFv IgG MTH (SSS) WTCH2CH3 (SEQ ID NO·) essentially according to the method described in Example 10 Cells were also transfected with vector only After 48-72 hours at 37°C, cell culture supernatants were harvested and combined with protein A-agarose beads (Repligen) for one hour at 4 °C The beads were centrifuged and washed several times in TNEN [20 mM Tris base, 100 mM NaCl, 1 mM EDTA, and 0 05% NP-40, pH 8 0). The immunoprecipitates were combined with 25 µl 2x NuPAGE® SDS Sample Buffer (Invitrogen Life Technologies) (non-reduced samples) The proteins were fractionated on NuPAGE® 10% Bis-Tris gels (Invitrogen Life Technologies) After electrophoresis (approximately 1 hour), the proteins were transferred from the gel onto a Immobilon P polyvinylidene fluoride (PVDF) membrane (Millipore, Bedford, MA) using a semi-dry blotter (Ellard Instrumentation, Monroe, WA) The PVDF membrane was blocked in PBS containing 5% nonfat milk and then probed with HRP-conjugated goat anti-human IgG (Fc specific) (CalTag) After washing the immunoblot several times in PBS, the blot was developed using ECL (Amersham Biosciences) The results are shown in Figure 35

EXAMPLE 18

BINDING OF ANTI-CD20 SCFV HUMAN IGA FUSION PROTEINS TO CD20+ CHO CELLS

This Example describes flow immunocytofluorimetry analysis of binding of 2H7 scFv IgAH IgACH2CH3 (SEQ ID NO ___) and 2H7 scFv IgAH IgAT4 (SEQ ID NO ___) fusion proteins to (CD20+) CHO cells.

COS cells were transiently co-transfected as described in Example 10 with plasmid DNA comprising a polynucleotide sequence (SEQ ID NO ___) encoding 2H7 scFv IgAH IgACH2CH3 polypeptide (SEQ ID NO ___) and with a separate plasmid comprising a polynucleotide sequence (SEQ ID NO ___) encoding a human J chain polypeptide (SEQ ID NO ___) COS cells were also transfected with a polynucleotide sequence (SEQ ID NO ___) encoding a 2H7 scFv IgA fusion protein that had a deletion of four amino acids at the carboxy terminus of CH3 (2H7 scFv IgAH IgA-T4, SEQ ID NO ___) The transfections were performed as described in Example 10 Culture supernatants from transfected COS cells were combined with (CD20+) CHO cells (see Example 1) and incubated for one hour on ice. The cells were washed twice with PBS-2%FBS and then combined with FITC-conjugated goat anti-human IgA chain (CalTag) (1 100) for 40 minutes The cells were again washed and then analyzed by flow cytometry using a Coulter Epics XL cell sorter Figure 36 shows that co-transfection with J chain was not required for secretion of 2H7 scFv IgAH IgAT4, the 2H7 IgA fusion protein with the truncated CH3 carboxy end (SEQ ID NO ___)

EXAMPLE 19

EFFECTOR FUNCTION OF ANTI-CD20 SCFV HUMAN IGA FUSION PROTEINS

This Example illustrates ADCC activity of 2H7 IgG and IgA fusion proteins against cells that express CD20 BJAB cells were prelabeled with ^{51}Cr (100 µCi) (Amersham) for two hours at 37 °C Effector cells were obtained from fresh, resting human whole blood, which was diluted in an equal volume of Alsever's solution to prevent coagulation 2H7 scFv IgG MTH (SSS) WTCH2CH3 (SEQ ID NO __), 2H7 scFv IgG MTH (SCS) WTCH2CH3 (SEQ ID NO __), 2H7 scFv IgG WTH (CCC) WTCH2CH3 (SEQ ID NO __), and 2H7 scFv IgAH IgACH2CH3 (SEQ ID NO __) fusion proteins were purified from transiently transfected COS cell supernatants (100-200 ml) by protein A chromatography as described in Example 10 COS cells transfected with the plasmid encoding 2H7 scFv IgAH IgACH2CH3 were co-transfected with a plasmid encoding human J chain as described in Example 18 Two-fold serial dilutions of the purified 2H7 Ig fusion proteins starting at 5 µg/ml were added to the labeled BJAB cells (5×10^4 cells

per well of 96 well tissue culture plate) in the presence of whole blood (100 µl of whole blood diluted 1 1 in Alsever's solution, final dilution 1 4) and incubated for five hours at 37 °C Culture supernatants were harvested and analyzed as described in Example 11 Percent specific killing was calculated according to the following equation. ((experiment release minus spontaneous release)/(maximum release minus spontaneous release)) x 100 The data are presented in Figure 37 Each data point represents the average of quadruplicate samples

In a second ADCC assay, the number of labeled BJAB target cells was held constant in each sample, and whole blood was added at dilutions of 0.25, 0 125, and 0 0625 Purified 2H7 IgG and IgA fusion proteins were added at a concentration of 5 µg/ml The BJAB cells, whole blood, and fusion proteins were incubated, the supernatants harvested, and the percent specific killing was calculated as described above Percent specific killing for each of the 2H7 fusion proteins is presented in Figure 38

The ADCC activity of purified 2H7 scFv IgG MTH (SSS) WTCH2CH3 (5 µg/ml) and of purified 2H7 scFv IgAH IgACH2CH3 (5 µg/ml) was compared in the presence of different effector cell populations PBMC were isolated from whole blood as described in Examples 11 and 12 PBMC were combined with labeled BJAB target cells (5 x 10^4 per well of 96 well tissue culture plate) at ratios of 50 1, 25 1, and 12 5 1 The assay was performed and the data analyzed as described above. Figure 39A shows that only the 2H7 scFv IgG MTH (SSS) WTCH2CH3 fusion protein had ADCC activity when PBMC served as the effector cells. Figure 39B shows that both 2H7 scFv IgG MTH (SSS) WTCH2CH3 and 2H7 scFv IgAH IgACH2CH3 exhibit ADCC activity when whole blood was the source of effector cells (as illustrated in Figure 38)

EXAMPLE 20

EXPRESSION LEVEL OF 2H7 SCFV VH11SER IGG MTH (SSS) WTCH2CH3 FUSION PROTEIN

This Example compares the expression level of 2H7 scFv VH11Ser IgG MTH (SSS) WTCH2CH3 fusion protein (SEQ ID NO__) with other 2H7 scFv IgG constructs that do not contain the mutation in the variable heavy chain domain. The mammalian expression vector pD18 comprising nucleotide sequences 2H7 scFv IgG MTH (SSS) WTCH2CH3 (SEQ ID NO), 2H7 scFv IgG MTH (CSS) WTCH2CH3 (SEQ ID NO), 2H7 scFv IgG MTH (SCS) WTCH2CH3 (SEQ ID NO), 2H7 scFv IgG WTH

(CCC) WTCH2CH3 (SEQ ID NO), and 2H7 scFv VHSER11 IgG MTH (SSS) WTCH2CH3 (see Examples 1 and 13) were transiently transfected into COS cells as described in Example 10 After 72 hours at 37 °C, culture supernatants were harvested, and 1 µl of each supernatant was combined with non-reducing sample buffer (see method described in Example 10) The culture supernatant samples and aliquots of purified 2H7 scFv IgG MTH (SSS) WTCH2CH3 (40 ng, 20 ng, 10 ng/ 5 ng, and 2 5 ng) were fractionated on 10% Bis-Tris (MOPS) NuPAGE® gels (Invitrogen Life Technologies) Multimark® protein standards (Invitrogen Life Technologies) were also separated on the gel. The proteins were transferred to a PDVF membrane and immunoblotted as described in Example 17 The immunoblot is presented in Figure 40 The amounts of the fusion proteins were quantified by densitometry analysis of the blots using the ScionImage for Windows software and comparison with the standard curve The 2H7 scFv IgG WTH (CCC) WTCH2CH3 construct produced approximately 12 ng/ul or 12 micrograms/ml, the 2H7 scFv IgG MTH (CSS) WTCH2CH3 produced approximately 10 ng/ul or 10 micrograms/ml, the 2H7 scFv IgG MTH (SCS) WTCH2CH3 construct produced approximately 1 ng/ul or 1 microgram/ml, and the 2H7 scFv VHSER11 IgG MTH (SSS) WTCH2CH3 construct produced approximately 30 ng/ml or 30 micrograms/ml. In particular claimed embodiments the instant invention, an amino acid sequence of 2H7 scFv VHSER11 IgG MTH (SSS) WTCH2CH3, or a polynucleotide sequence that encodes 2H7 scFv VHSER11 IgG MTH (SSS) WTCH2CH3 may be optionally excluded from the instant invention Similarly, an amino acid sequence of 2H7 scFv VHSER11 IgG WTH (CCC) WTCH2CH3, or a polynucleotide sequence that encodes 2H7 scFv VHSER11 IgG WTH (CCC) WTCH2CH3 may be optionally excluded from particular claimed embodiments of the instant invention Additionally, an amino acid substitution of a leucine at position 11 to serine in the variable heavy chain domain, or polynucleotides that encode an amino acid substitution of a leucine at position 11 to serine in the variable heavy chain domain, may be optionally excluded from particular claimed embodiments of the instant invention

EXAMPLE 21

CONSTRUCTION OF A 2H7 SCFV IGG FUSION PROTEIN WITH A MUTANT CH3 DOMAIN

Amino acid mutations were introduced into the CH3 domain of a 2H7 IgG fusion protein The pD18 vector comprising 2H7 scFv IgG MTH (SSS) WTCH2CH3

(SEQ ID NO __) was digested with BclI and XbaI to remove the MTH WTCH2CH3 (SEQ ID NO __) fragment, which was then subcloned into pShuttle vector (BD Biosciences Clontech, Palo Alto, CA) that was double-digested with BclI and XbaI. Subcloning was performed in a kanamycin resistant vector because the ampicillin resistance gene has an XmnI site, which is required for this cloning procedure. Five constructs were prepared with the following substitutions (1) a phenylalanine residue at position 405 (numbering according to Kabat *et al supra*) was substituted with tyrosine using the oligonucleotide CH3Y405, (2) the phenylalanine position at 405 was substituted with an alanine residue using the oligonucleotide CH3A405, (3) the tyrosine residue at position 407 was substituted with an alanine using the oligonucleotide CH3A407, (4) both wild type amino acids at positions 405 and 407 were substituted with tyrosine and alanine, respectively using the oligonucleotide CH3Y405A407, and (5) both wild type amino acids at positions 405 and 407 were substituted with alanine using the oligonucleotide CH3A405A407 The oligonucleotides were the 3' primers for PCR amplification of a portion of the CH3 domain. The nucleotide sequences for each 3' oligonucleotide were as follows.

CH3Y405 5'-gtt gtt gaa gac gtt ccc ctg ctg cca cct gct ctt gtc cac ggt gag ctt gct gta gag gta gaa gga gcc-3' (SEQ ID NO ___)

CH3A405 5'-gtt gtt gaa gac gtt ccc ctg ctg cca cct gct ctt gtc cac ggt gag ctt gct gta gag ggc gaa gga gcc-3' (SEQ ID NO ___)

CH3A407 5'-gtt gtt gaa gac gtt ccc ctg ctg cca cct gct ctt gtc cac ggt gag ctt gct ggc gag gaa gaa gga gcc-3' (SEQ ID NO ___)

CH3Y405A407 5'-gtt gtt gaa gac gtt ccc ctg ctg cca cct gct ctt gtc cac ggt gag ctt gct ggc gag gta gaa gga gcc-3' (SEQ ID NO ___)

CH3A405A407 5'-gtt gtt gaa gac gtt ccc ctg ctg cca cct gct ctt gtc cac ggt gag ctt gct ggc gag ggc gaa gga gcc-3' (SEQ ID NO ___)

The template was the mutant hinge MHWTCH2CH3 human IgG1 The 5' PCR oligonucleotide primer was huIgGMHWC, [SEQ ID NO __] The amplified products were TOPO® cloned and sequenced as described in Examples 1 and 10 DNA from the clones with the correct sequence was digested with BclI and XmnI and transferred to pShuttle containing the MTH WTCH2CH3 sequence, which was also digested with the

same restriction enzymes The mutated IgG sequences were then removed by digestion with BclI and XbaI and inserted into a pD18 vector containing 2H7 scFv that was also digested with BclI and XbaI The polynucleotide sequences for mutated the CH3 domains, MTCH3 Y405, MTCH3 A405, MTCH3 A407, MTCH3 Y405A407, and MTCH3 A405A407 are shown in SEQ ID NOs ____, respectively, and the polypeptide sequences for each are shown in SEQ ID NOs ____, respectively The polynucleotide sequences for the 2H7 scFv MTH WTCH2 MTCH3 Y405, 2H7 scFv MTH WTCH2 MTCH3 A405, scFv MTH WTCH2 MTCH3 A407, scFv MTH WTCH2 MTCH3 Y405A407, and scFv MTH WTCH2 MTCH3 A405A407, respectively, and the deduced amino acid sequences are shown in SEQ ID NOs ___, respectively

EXAMPLE 22

CONSTRUCTION OF 2H7 SCFV IGG FUSION PROTEINS WITH HINGE MUTATIONS

A 2H7 scFv IgG fusion protein was constructed with the third cysteine residue in the IgG1 hinge region substituted with a serine residue. The template for introduction of the mutations was a polynucleotide encoding 2H7 scFv WTH WTCH2CH3 (SEQ ID NO ___) The oligonucleotide introducing the mutations was a 5' PCR primer oligonucleotide HIgGMHcys3 having the sequence 5'-gtt gtt gat cag gag ccc aaa tct tgt gac aaa act cac aca tgt cca ccg tcc cca gca cct-3' The oligonucleotide introducing the mutation into the hinge region was combined with template and a 3' oligonucleotide containing an XbaI site (underlined and italicized) (5'-gtt gtt *tct aga* tca ttt acc cgg aga cag gga gag gct ctt ctg cgt gta g-3' (SEQ ID NO ___)) to amplify the mutant hinge-wild type (WT)-CH2-CH3 sequences by PCR The IgG MTH CCS mutant sequence was amplified for 30 cycles with a denaturation profile of 94 °C, annealing at 50 °C for 30 seconds, and extension at 72 °C for 30 seconds The amplified polynucleotides were inserted into the TOPO® cloning vector (Invitrogen Life Technologies) and then were sequenced as described in Example 1 to confirm the presence of the mutation pD18 vector containing 2H7 scFv was digested to remove the constant region sequences essentially as described in Example 10 The mutant hinge-wild type CH2-CH3 regions were inserted in frame into the digested vector DNA to obtain vectors comprising 2H7 scFv MTH (CCS) WTCH2CH3 encoding DNA (SEQ ID NO ___) The deduced polypeptide sequence is shown in SEQ ID NO __

EXAMPLE 23

CONSTRUCTION OF ANTI-CD20 IGE FUSION PROTEINS

A binding domain is fused to IgE constant region sequences such that the expressed polypeptide is capable of inducing an allergic response mechanism. The single chain Fv nucleotide sequence of 40 2.220 (SEQ ID NO __), an anti-CD40 antibody, is fused to IgE CH2-CH3-CH4 according to methods described for other scFv immunoglobulin constant region constructs (see Examples 1, 5, 10, and 13) To PCR amplify the IgE CH2-CH3-CH4 domains, a 5' oligonucleotide primer, hIgE5Bcl, having the sequence 5'-gtt gtt gat cac gtc tgc tcc agg gac ttc acc cc-3', and a 3' oligonucleotide primer, hIgE3stop, having the sequence 5'- gtt gtt tct aga tta act ttt acc ggg att tac aga cac cgc tcg ctg g-3' are used.

The retroviral transfection system for ectopic surface expression of genetically engineered cell surface receptors composed of scFvs that bind costimulatory receptors described in Example 12 is used to construct a 40 2.220 scFv IgE-CD80 fusion protein. The 40 2 220 scFv IgE fusion polynucleotide sequence is fused in frame to sequences encoding the transmembrane domain and cytoplasmic tail of human CD80 (SEQ ID NO __), such that when the fusion protein is expressed in the transfected cell, CD80 provided an anchor for surface expression of the scFv Ig fusion protein cDNA encoding the anti-CD40 scFv-IgE-CD80 fusion proteins (SEQ ID NO __) is inserted into the retroviral vector pLNCX (BD Biosciences Clontech) according to standard molecular biology procedures and vendor instructions The 40.2.220 scFv-Ig-CD80 cDNA is inserted between the 5'LTR-neomycin resistance gene-CMV promoter sequences and the 3'LTR sequence. The retroviral constructs are transfected into a carcinoma cell line, and transfected cells are screened to select clones that are expressing the 40.2.220 scFv-Ig-CD80 fusion protein on the cell surface

EXAMPLE 24

CONSTRUCTION OF IGA-T4 MUTANTS THAT ARE EXPRESSED ON THE CELL SURFACE

The retroviral transfection system for ectopic surface expression of genetically engineered cell surface receptors composed of scFvs that bind costimulatory receptors described in Example 12 is used to construct a 2H7 scFv IgA hinge IgA-T4-CD80 fusion protein The 2H7 scFv IgAH IgA-T4 fusion polynucleotide sequence (SEQ ID NO __) is fused in frame to sequences encoding the transmembrane domain and cytoplasmic tail of human CD80 (SEQ ID NO __), such that when the fusion protein is

expressed in the transfected cell, CD80 provided an anchor for surface expression of the scFv Ig fusion protein cDNA encoding the 2H7 scFv IgAH IgA-T4-CD80 fusion protein (SEQ ID NO __) is inserted into the retroviral vector pLNCX (BD Biosciences Clontech) according to standard molecular biology procedures and vendor instructions The 2H7 scFv IgAH IgA-T4-CD80 cDNA is inserted between the 5'LTR-neomycin resistance gene-CMV promoter sequences and the 3'LTR sequence The retroviral construct is transfected into Reh, an acute lymphocytic leukemia cell line (ATCC CRL-8286) Transfected cells are screened to select clones that are expressing 2H7 scFv-Ig fusion proteins on the cell surface

EXAMPLE 25

CHARACTERIZATION OF AN ANTI-4-1BB SCFV IG-CD80 FUSION PROTEIN EXPRESSED ON THE CELL SURFACE OF TUMOR CELLS AND GROWTH OF THE TUMOR CELLS *IN VIVO*

This Example describes construction of an anti-murine 4-1BB (CD137) scFv fusion protein that has an IgG wild type hinge and CH2 and CH3 domains that is fused to the CD80 transmembrane and cytoplasmic domains The Example also illustrates the effect of the cell surface expression of the anti-4-1BB scFv IgG CD80 polypeptide when the transfected tumor cells are transplanted into mice.

The heavy and light chain variable regions of a rat anti-4-1BB (CD137) monoclonal antibody (1D8) were cloned, and a single chain Fv construct was prepared essentially as described in Example 1 The heavy chain and light chain variable regions of each antibody were cloned according to standard methods for cloning immunoglobulin genes and as described in Example 1 Single chain Fv construct was prepared as described in Example 1 by inserting a nucleotide sequence encoding a $(gly_4ser)_3$ peptide linker between the VL region nucleotide sequence of 1D8 (SEQ ID NO __) and the VH region nucleotide sequence of 1D8 (SEQ ID NO __) The polypeptide sequence for 1D8 VL is shown in SEQ ID NO ___, and the polypeptide sequence for the VH domain is shown in SEQ ID NO ___ The scFv polynucleotide (SEQ ID NO __) was then fused to human IgG1 wild-type hinge-CH2-CH3 domains according to the methods described in Example 1 The scFv IgG1 fusion polynucleotide sequence was then fused in frame to sequences encoding the transmembrane domain and cytoplasmic tail of human CD80 (SEQ ID NO __) essentially as described in Example 12, such that when the fusion protein was expressed in the transfected cell, CD80 provided an anchor for surface expression of the

scFv Ig fusion protein. cDNA encoding the scFv-IgG-CD80 fusion protein (SEQ ID NO:__) was inserted into the retroviral vector pLNCX (BD Biosciences Clontech) according to standard molecular biology procedures and vendor instructions. The scFv-Ig-CD80 cDNA was inserted between the 5'LTR-neomycin resistance gene-CMV promoter sequences and the 3'LTR sequence

The retroviral constructs were transfected into the metastatic M2 clone of K1735, a melanoma cell line, provided by Dr I Hellstrom, FNRI, Seattle, WA. Transfected cells were screened to select clones that were expressing scFv-Ig fusion proteins on the cell surface To demonstrate that the 1D8 scFv IgG-CD80 construct was expressed on the cell surface of the tumor cells, the transfected cells were analyzed by flow immunocytofluorimetry Transfected cells (K1735-1D8) were incubated for one hour on ice in phycoerythrin-conjugated $F(ab')_2$ goat anti-human IgG The unbound conjugate was then removed by washing the cells and flow cytometry analysis was performed using a Coulter Epics XL cell sorter Results are presented in Figure 41A.

The growth of K1735-1D8 transfected cells was examined *in vivo* K1735-WT cells grew progressively when transplanted subcutaneously (s c.) in naïve C3H mice. Although the same dose of K1735-1D8 cells initially formed tumors of an approximately 30 mm^2 surface area, the tumors started to regress around day 7 and had disappeared by day 20 as shown in Figure 41B. Tumor cells that were transfected with a similarly constructed vector encoding a non-binding scFv, a human anti-CD28 scFv construct, grew as well as tumor cells that had not been transfected. The presence of a foreign protein, that is, human IgG1 constant domains or rat variable regions, did not make transfected K1735-WT cells immunogenic, the growth of the K1735-1D8 cells in C3H mice was identical to that of K1735-WT cells (untransfected)

To investigate the roles of $CD4^+$ and $CD8^+$ T lymphocytes and NK cells in the regression of K1735-1D8 tumors, naïve mice were injected intraperitoneally (i p) with monoclonal antibodies (monoclonal antibodies, typically 50 µg in a volume 0 1 ml) to remove $CD8^+$, $CD4^+$ or both $CD4^+$ and $CD8^+$ T cells, or were injected with anti-asialo-GM1 rabbit antibodies to remove NK cells Twelve days later, when flow cytometry analysis of spleen cells from identically treated mice showed that the targeted T cell populations were depleted, K1735-1D8 cells were transplanted s.c to each T cell-depleted

group K1735-1D8 had similar growth kinetics in mice that had been injected with the anti-CD8 MAb or control rat IgG while removal of $CD4^+$ T cells resulted in the growth of K1735-1D8 with the same kinetics as K1735-WT This failure to inhibit tumor growth after CD4+ T cell removal was observed regardless of the presence or absence of CD8+ T cells K1735-1D8 grew in all NK-depleted mice, although more slowly than in the CD4-depleted group The results are presented in Figure 41C

EXAMPLE 26

THERAPEUTIC EFFECT OF TUMOR CELLS EXPRESSING ANTI-4-1BB SCFV IGG-CD80 FUSION PROTEIN

This Example examines the ability of K1735-1D8 transfected tumor cells expressing an anti-CD137 scFv on the cell surface to generate a sufficient immune response in mice to mediate rejection of established, untransfected wild type tumors. C3H mice were transplanted with K1735-WT tumors ($2x10^6$ cells/animal) and grown for approximately six days Experiments were performed using mice with established K1735-WT tumors of 30 mm^2 surface area. Mice were vaccinated by s c. injection of K1735-1D8 or irradiated K1735-WT cells on the contralateral side. Identical injections were repeated at the time points indicated in Figure 42 One group of animals was given four weekly injections of K1735-1D8 cells According to the same schedule, another group was given irradiated (12,000 rads) K1735-WT cells, and a third group was injected with PBS The data are plotted in Figure 42 The WT tumors grew progressively in all control mice and in all mice that received irradiated K1735-WT cells. In contrast, the tumors regressed in 4 of the 5 mice treated by immunization with K1735-1D8. The animals remained tumor-free and without signs of toxicity when the experiment was terminated 3 months later In the fifth mouse, the tumor nodule decreased in size as long as the mouse received K1735-1D8 cells, but the tumor grew back after therapy was terminated.

In another experiment with 5 mice/group, mice were injected intravenously (i v) with 3 x 10^5 K1735-WT cells to initiate lung metastases Three days later, K1735-1D8 cells were transplanted s c This procedure was repeated once weekly for a month, control mice were injected with PBS The experiment was terminated when one mouse in the control group died, 37 days after receiving the K1735-WT cells At that time, lungs of the control mice each had >500 metastatic foci In contrast, less than 10 metastatic foci were present in the lungs of the immunized mice.

In a third experiment, mixtures of K1735-WT cells and K1735-1D8 cells were injected into immunocompetent syngeneic C3H mice Mice were injected subcutaneously with K7135-WT cells alone or with a mixture of $2x10^6$ K1735-WT cells and $2x10^5$ K1735-1D8 cells Tumor growth was monitored at 5-day intervals

EXAMPLE 27

EXPRESSION OF ANTI-4-1BB scFv IgG-CD80 FUSION PROTEIN ON THE CELL SURFACE OF SARCOMA CELLS

This Example demonstrates expression of an anti-CD137 scFv on the cell surface of a second type of tumor cell by transfecting a murine sarcoma cell line with an anti-CD137 scFv IgG-CD80 construct.

The 1D8 scFv IgG WTH WTCH2CH3-CD80 polynucleotide (SEQ ID NO __) was transferred from the pLNCX vector into pCDNA3-hygro vector using restriction enzyme digestion and ligation steps according to standard molecular biology methods The constuct was cut with HindIII + Cla1 and thesFv fragment was filled in with Klenow (Roche) and the blunt-ended fragment was ligated into EcoR5 site of pcDNA3 Ag104 murine sarcoma tumor cells were transfected with the pCDNA3-hygro vector containing the 1D8 scFv IgG CD80 fusion protein Hygromycin-resistant clones were screened by flow cytometry using a FITC anti-human IgG antibody to detect expression of the transgene. Only approximately 15% of the resistant clones had detectable fusion protein initially Positive cells identified by flow cytometry were repeatedly panned on flasks coated with immobilized anti-human IgG (10 µg/ml) according to standard methods Panning was performed by incubating cells on the coated plates for 30 min at 37C, the plates were then washed 2-3x in versene or PBS After each round, cells were tested for IgG expression by FACS The histogram in Figure 44 shows the staining pattern after four rounds of panning against anti-human IgG (black) Untransfected cells were stained and are indicated in gray All of the cells in the population were positive

EXAMPLE 28

CONSTRUCTION AND CHARACTERIZATION OF A BISPECIFIC scFv IG FUSION PROTEIN AND scFv IG FUSION PROTEINS WITH A MUTATION IN THE IgG1 CH2 DOMAIN

An anti CD20 (2H7) scFv IgG fusion protein was constructed that had a mutant hinge (MT (SSS)) and a mutant CH2 domain in which the proline at residue

(position number 238 according to Ward *et al*, *supra*) was substituted with a serine. The 2H7 scFv IgG MTH (SSS) MTCH2WTCH3 encoding polynucleotide (SEQ ID NO __) was constructed essentially according to methods described in Examples 1, 5, and 13 The IgG mutant hinge-mutant CH2-wild type CH3 domains were also fused to an anti-CD20 (2H7)-anti-CD40 (40 2 220) bispecific scFv The anti-CD20-anti-CD40 scFv IgG MTH (SSS) MTCH2WTCH3 encoding polynucleotide sequence is shown in SEQ ID NO ___ and the encoded polypeptide is shown in SEQ ID NO ___

COS cells were transiently transfected with vectors comprising the polynucleotide sequences encoding 2H7 scFv IgG MTH (SSS) MTCH2WTCH3 (SEQ ID NO __), anti-CD20-anti-CD40 scFv IgG MTH (SSS) MTCH2WTCH3 (SEQ ID NO __), 2H7 scFv IgG MTH (SSS) WTCH2CH3 (SEQ ID NO __), and 2H7 scFv IgAH IgG WTCH2CH3 (SEQ ID NO __) as described in Example 10 Culture supernatants were collected and the fusion proteins were purified by protein A chromatography (see Example 10) The purified polypeptides were fractionated by SDS-PAGE according to the method described in Example 10 Rituximab (anti-CD20 monoclonal antibody), and Bio-Rad prestained molecular weight standards (Bio-Rad, Hercules, CA), and Multimark® molecular weight standards (Invitrogen Life Technologies were also applied to the gel. The results are presented in Figure 45

The 2H7 scFv Ig fusion protein that contains a mutation in the CH2 domain was compared to fusion proteins that have the wild type CH2 domain in an ADCC assay The assays were performed essentially as described in Examples 11 and 19 Fresh resting PBMC (effector cells) were added to ^{51}Cr-labeled BJAB cells (target cells) at the ratios indicated in Figure 46 Purified 2H7 scFv IgG MTH (SSS) MTCH2WTCH3, 2H7 scFv IgG MTH (SSS) WTCH2CH3, 2H7 scFv IgAH IgG WTCH2CH3, and Rituximab, each at 10 µg/ml were added to the effector/target cell mixtures and incubated for five hours at 37 °C Supernatants were harvested and the amount of chromium released was determined as described in Examples 11 and 19 Percent specific killing by each fusion protein is presented in Figure 46

EXAMPLE 29

TUMOR CELL SURFACE EXPRESSION OF AN ANTI-HUMAN CD3 SCFV IGG FUSION PROTEIN

An anti-human CD3 scFv Ig CD80 fusion protein was prepared essentially as described in Examples 1 and 12 The fusion protein comprised an anti-human CD3 scFv fused to wild type IgG1 hinge (SEQ ID NO __) and wild type CH2 (SEQ ID NO __) and CH3 (SEQ ID NO __) domains, fused to CD80 transmembrane and cytoplasmic domains (SEQ ID NO __) to enable cell surface expression of the anti-CD3 scFv The anti-human CD3 scFv IgG WTH WTCH2CH3-CD80 polynucleotide (SEQ ID NO __) encoding the polypeptide (SEQ ID NO __) was transfected in Reh cells and into T51 cells (lymphoblastoid cell line) Expression of the anti-human CD3 scFv IgG fusion protein was detected by flow cytometry using FITC conjugated goat anti-human IgG (see methods in Examples 4, 10, 16, 18) Figure 47A illustrates expression of the anti-human CD3 fusion protein on the cell surface of Reh cells, and figure 47B shows expression of the fusion protein on T41 cells

ADCC assays were performed with the transfected Reh and T51 cells to determine if expression of the scFv-Ig polypeptides on the cell surface augmented effector cell function. Untransfected and transfected Reh cells and untransfected and transfected T51 cells were pre-labeled with ^{51}Cr (100 µCi) (Amersham) for two hours at 37 °C. Human PBMC served as effector cells and were added to the target cells (5×10^4 cells per well of 96 well plate) at ratios of 20 1, 10 1, 5 1, and 2 5 1 After four hours at 37 °C, culture supernatants were harvested and analyzed as described in Examples 11 and 12 Percent specific killing was calculated as described in Example 12 The results are presented in Figure 48

EXAMPLE 30

INDUCTION OF CYTOKINE EXPRESSION IN TUMOR CELLS EXPRESSING ANTI-CD28 SCFV ON THE CELL SURFACE

This Example describes the effect of cell surface expressed scFv on cytokine mRNA induction in stimulated lymphocytes co-cultured with tumor cells transfected with an anti-human CD28 scFv IgG-CD80 fusion protein

Real time PCR analysis was performed on RNA samples from human PBMC stimulated with Reh, Reh-anti-CD28 (2e12) (see Example 12 for construction of 2e12 scFv IgG WTH WHTCH3CH2-CD80 and transfection of Reh cells), and Reh-CD80 (see Example 14) in order to measure the effects of the surface expressed scFv on cytokine production by the PBMC effector cells For the real-time PCR assay, SYBR Green

(QIAGEN) (Morrison *et al*, *Biotechniques* 24 954-8, 960, 962 (1998)) was used and measured by an ABI PRISM® 7000 Sequence Detection System (Applied Biosystems, Foster City, CA) that measures the formation of PCR product after each amplification cycle Cells were harvested from cultures and total RNA prepared using QIAGEN RNA kits, including a QIA shredder column purification system to homogenize cell lysates, and RNeasy® mini-columns for purification of RNA. cDNA was reverse transcribed using equal amounts of RNA from each cell type and Superscript II Reverse Transcriptase (Life Technologies) SYBR Green real-time PCR analysis was then performed using the prepared cDNA as template and primer pairs specific for cytokine gene products. The average length of the PCR products that were amplified ranged from 150-250 base pairs The cDNA levels for many activation response molecules including IFNγ, TNFα, GM-CSF, IL-4, IL-5, IL-6, IL-8, IL-10, IL-12, IL-15, ICOSL, CD80 and CD86 were assayed. Control reference cDNAs for constitutively expressed genes, including GAPDH, β-actin, and CD3□ were measured in each assay The most significant induction of specific mRNA was observed for IFN-γ, and more modest induction was observed for CTLA-4 and ICOS

EXAMPLE 31

CLONING OF AN ANTI-HUMAN 4-1BB ANTIBODY AND CONSTRUCTION OF AN ANTI-HUMAN 4-1BB SCFV IG FUSION PROTEIN

A hybridoma cell line expressing a mouse anti-human monoclonal antibody (designated 5B9) was obtained from Dr Robert Mittler, Emory University Vaccine Center, Atlanta, GA The variable heavy and light chain regions were cloned according to known methods for cloning of immunoglobulin genes and as described herein Cells were grown in IMDM/15% FBS (Invitrogen Life Technologies) media for several days. Cells in logarithmic growth were harvested from cultures and total RNA prepared using QIAGEN RNA kits, including a QIA shredder column purification system to homogenize cell lysates, and RNeasy® mini-columns for purification of RNA according to manufacturer's instructions cDNA was reverse transcribed using random hexamer primers and Superscript II Reverse Transcriptase (Invitrogen Life Technologies)

cDNA was anchor-tailed using terminal transferase and dGTP PCR was then performed using an anchor-tail complementary primer and a primer that annealed specifically to the antisense strand of the constant region of either mouse Ck (for

amplification of VL) or the appropriate isotype mouse CH1 (for amplification of VH) The amplified variable region fragments were TOPO® cloned (Invitrogen Life Technologies), and clones with inserts of the correct size were then sequenced Consensus sequence for each variable domain was determined from sequence of at least four independent clones The 5B9 VL and VH polynucleotide sequences are shown in SEQ ID NOs __ and __, respectively, and the deduced amino acid sequences are shown in SEQ ID NOs __ and ___ The scFv was constructed by a sewing PCR method using overlapping primers containing a synthetic $(Gly_4Ser)_3$ linker domain inserted between the light and heavy chain variable regions (see Example 1) The 5B9 scFv polypeptide (SEQ ID NO __) is encoded by the polynucleotide sequence comprising SEQ ID NO ___

5B9 scFv polynucleotide sequence was fused in frame to the polynucleotide sequence encoding the human IgG1 mutant hinge and wild type CH2 and CH3 (MTH (SSS) WTCH2CH3, SEQ ID NO ___) according to methods described in Examples 5, 10, and 13 COS cells were transiently transfected with a vector comprising the 5B9 scFv IgG MTH (SSS) WTCH2CH3 polynucleotide sequence (SEQ ID NO ___). Supernatant was collected and binding of the 5B9 scFv IgG MTH (SSS) WTCH2CH3 polypeptide (SEQ ID NO ___) was measured by flow immunocytofluorimetry essentially as described in Examples 4, 10, 16, and 18 Culture supernatant from the 5B9 hybridoma cell line was also included in the binding assay Fresh human PBMC were incubated in the presence of immobilized anti-CD3 for four days prior to the binding experiment to induce expression of CD137 on the surface of activated T cells Stimulated PBMC were washed and incubated with COS or hybridoma culture supernatant containing the 5B9 scFv IgG fusion protein or 5B9 murine monoclonal antibody, respectively, for 1 hour on ice Binding of 5B9 scFv IgG fusion protein or 5B9 murine monoclonal antibody was detected with FITC conjugated anti-human IgG or anti-mouse IgG, respectively The results are presented in Figure 49

EXAMPLE 32

CONSTRUCTION OF 2H7 SCFV IGG FUSION PROTEINS WITH HINGE MUTATIONS

2H7 scFv IgG fusion proteins are constructed with the first cysteine residue and the second cystein in the IgG1 hinge region substituted with a serine residue to provide MTH (SCC) and MTH (CSC) The template for introduction of the mutations is a polynucleotide encoding 2H7 scFv WTH WTCH2CH3 (SEQ ID NO ___) The

oligonucleotide introducing the mutations are 5' PCR primer oligonucleotides HIgGMHcys1 (SEQ ID NO __) and HIgGMHcys2 (SEQ ID NO __) The constructs are prepared as described in SEQ ID NO __) The encoding polynucleotides of the mutants are presented in SEQ ID NOs __) and the polypeptide sequences are provided in SEQ ID NO ___)

Example 33

CONSTRUCTION OF 2H7 V_HL11S scFv (SSS-S) H WCH2 WCH3

A change from leucine to serine at position 11 in the heavy chain variable region (numbering according to Kabat et al, *Sequences of Proteins of Immunological Interest*, 5th ed. Bethesda, MD Public Health Service, National Institutes of Health (1991)) was introduced into the 2H7 scFv MTH (SSS) WTCH2CH3 fusion protein (SEQ ID NO __) The wild type leucine residue was substituted with serine by site-directed mutagenesis using the oligonucleotide Vhser11 5'-gga ggt ggg agc tct cag gct tat cta cag cag tct ggg gct gag tcg gtg agg cc-3' (SEQ ID NO ___) The 3'-primer for PCR was huIgG1-3' having the sequence 5'-gtc *tct aga* cta tca ttt acc cgg aga cag-3' (SEQ ID NO ___) (XbaI site underlined and italicized) After PCR amplification, the fragments were inserted into the TOPO® cloning vector and sequenced to confirm the presence of the VH11 leucine to serine mutation. The 2H7 scFv-IgG (SSS-S) H WCH2 WCH3 encoding DNA was shuttled into the PSL1180 cloning vector (Pharmacia Biotech, Inc, Piscataway, NJ) The construct PSL1180-2H7 scFv-IgG (SSS-S) H WCH2 WCH3 was digested with Sac and XbaI to remove the wild type VH domain and the connecting region and CH2 and CH3 domains The PCR product comprising the VH11 mutant was digested with Sac and XbaI and then inserted into the digested PSL1180 construct according to standard molecular biology procedures. The construct was then digested with Hind III and XbaI, and inserted into the mammalian expression vector pD18 (see methods described in Example 1 and Example 10) The mutant is designated 2H7 scFv VH L11S IgG (SSS-S) H WCH2 WCH3 The polynucleotide sequence is provided in SEQ ID NO __, and the encoded polypeptide sequence is provided in SEQ ID NO ___

EXAMPLE 34

EXPRESSION AND OF 2H7 scFv VH L11S (SSS-S) H WCH2 WCH3 IN STABLE CHO LINES

CHO DG44 cells were transfected by electroporation with approximately 150 micrograms of linearized expression plasmid encoding the 2H7 VH L11S scFv (SSS-S) H WCH2 WCH3 Cultures were plated in selection media containing 100 nM methotrexate, in 96 well, flat bottom tissue culture plates at various numbers of cells/well, ranging from 125 to 2000 Methotrexate resistant clones were selected and culture supernatants were screened for the highest expressors of the fusion protein using a CD20CHO binding assay similar to that described for Figure 1 Clones were amplified after the initial selection in gradually increasing doses of methotrexate. Cells were passaged for two passages in the higher concentration prior to adjusting the concentration to the next higher dose Clones were amplified to a final concentration of 1 micromolar methotrexate.

Figure 50B illustrates the production levels of 2H7 VH L11S scFv (SSS-S)H WCH2 WCH3 Spent supernatants from amplified CHO cells expressing this molecule and growing in stationary T25 flasks were tested for quantitative binding to CD20 CHO cells by flow cytometry The activity was converted to protein concentration by generation of a standard curve using the same molecule purified from supernatants with Protein A affinity chromatography (Figure 50A) The concentration of the purified protein was determined by A280 using an extinction coefficient provided by the amino acid composition of the recombinant protein (Vector NTI) Although levels of production varied between clones tested, multiple clones produced over 1 mg/ml This level of protein expression is over 10-fold higher than the identical molecule except for the amino acid change in V_H

Figure 51 illustrates the production levels of 2H7 VH L11S scFv (SSS-S) H WCH2 WCH3 by semi-quantitative analysis on SDS-PAGE Ten microliters of spent supernatant from amplified CHO cells expressing this molecule and growing in stationary T25 flasks were mixed with 10 microliters 2X non-reducing SDS sample buffer, run on SDS-PAGE gels, and stained with coomassie blue

EXAMPLE 35

CONSTRUCTION AND BINDING CAPACITY OF G28-1 SCFV IG CONSTRUCTS

Construction of the G28-1 (anti-CD37) scFv was performed using total RNA isolated from the G28-1 hybridoma using Trizol (Invitrogen) reagent according to manufacturer's instructions cDNA was prepared using random primers and the protocol

described previously for 2H7 cloning in Example 1 The variable domains of the scFv was cloned using one of two methods the first method used a family of degenerate 5' oligonucleotides specific for each V region gene family and a single 3' primer specific for the constant region of either the light or heavy chain using methods and primers described in (Ig-Prime Kit Mouse Ig-Primer Set, Novagen) The second approach used the anchor-tailing methods and primers described in (Gilliland LK et al, Tissue Antigens 47 1-20 (1995) In either case, PCR amplified products were cloned into the TOPO cloning vector (Invitrogen) The clones were digested with EcoRI and screened for inserts of the proper size. Positive clones were sequenced as previously described in Example 1

Specific primers were then designed for each V region, one with the leader sequence and one without Primers were also designed to include desired linkers and/or restriction sites at the primer ends PCR reactions were performed on the TOPO cloned DNA using a 25 cycle program with the following profile 94C, 30 sec, 55C, 30 sec, 72C, 30 sec, followed by a final extension at 72C for 8 minutes PCR products were gel purified and fragments recovered using a QIAQUICK gel extraction kit (QIAGEN, Valencia, CA) Fragments were diluted 1 50 and 1 microliter used for SEWING PCR reactions according to the methods described in Example 1 The following oligonucleotides were used for the secondary PCR reactions of the V_L domain for the G28-1 scFv

5' primers with SalI site without leader 5'-
GTTGTTGTCGACATCCAGATGACTCAGTC
TCCA-3' (SEQ ID NO__)

5' primer with HindIII site and leader sequence 5'-
GTCAAGCTTGCCGCCATGGTATCCA
CAGCTCAGTTCCTTGG-3' (SEQ ID NO__)

3' primer 5'-
GCCACCCGACCCACCACCGCCCGAGCCACCGCCACCTTTGATCTCCA
GTTCGGTG CC-3' (SEQ ID NO__)

The primers used for the V_H domain are shown below

5' sense primer
TCGGGCGGTGGTGGGTCGGGTGGCGGCGGATCGTCAGCGGTCCAGCTGCA
GCAGTCTGGA-3' (SEQ ID NO__)

3' antisense primer with BclI site 5'-
TCAGTGCTGATCAGAAGAGACGGTGACTGAGGTTCCTTG-3' (SEQ ID NO__)

A change from leucine to serine at position 11 in the heavy chain variable region (Kabat numbering) was introduced in into the G28-1 scFv by site-directed mutagenesis. The wild type form of the G28-1 scFv was initially constructed by sewing/overlap PCR to insert a (gly4ser)3 linker between the VL and VH domains as described above However, no Sac I site was introduced as a part of this fusion of the variable domains, so alternative, nearby restriction sites (HaeII and PvuII) near leucine 11 were used to synthesize the VL+mutated VH domain. Primers were designed to contain one of these sites and the DNA sequence including the L to S change, followed by 12 wild type base pairs Several attempts at this strategy failed, so an alternative strategy using the Genetailor (Invitrogen) method of site directed mutagenesis was used to introduce the desired mutation The mutagenesis was carried out according to manufacturer's instructions Briefly the procedure involves methylation of the plasmid DNA with DNA methylase, amplification of the DNA in a mutagenesis reaction with two overlapping primers, one of which contains the target mutations, trasformation of the plasmid into wild type E coli which digests all methylated DNA and leaves only the unmethylated, mutated amplification product. Both primers are approximately 30 nucleotides in length (not including the mutation site on the mutagenic primer, with an overlapping region at the 5' ends of 15-20 nucleotides, for efficient end joining of the mutagenesis product The template for the mutagenesis reaction was 100 ng of a plasmid containing the wild type G28-1 scFvIg construct, and the primers used for the G28-1 VH mutagenesis are as follows

Forward primer
5'-GCAGCAGTCTGGACCTGAGTCGGAAAAGCCTG-3' (SEQ ID NO__)
Reverse Primer
5'-CTCAGGTCCAGACTGCTGCAGCTGGACCGC-3' (SEQ ID NO__)

PCR reactions were performed using the 15 ng methylated template, the primers above, and the usual reaction components as previously described A 20 cycle program with the following profile was used for amplification 94C, 30 sec, 55C, 30 sec, 68C, 8 min, followed by a final 68C extension step for 10 minutes. PCR products were transformed into wild type bacteria, and colonies screened by sequencing Clones with

only the desired mutation were isolated and plasmid prepared as previously described Example 33 The mutant is designated G28-1 scFv VH L11S (SSS-S) H WCH2 WCH3 The polynucleotide sequence is provided in SEQ ID NO __, and the encoded polypeptide sequence is provided in SEQ ID NO ___

The expression level of G28-1 fusion proteins was confirmed using Immunblot analysis according to the methods described in Example 17 Figure 53 illustrates a large increase in protein expression in the VH L11S mutant G28-1 fusion proteins compared to the G28-1 fusion protein without the mutation.

The G28-1 scFv Ig fusion proteins were transiently transfected and expressed in COS cells according to methods described in Example 10 Figure 52 illustrates the capacity of the G28-1 scFv Ig fusion proteins from the COS supernatants to bind CD37 Ramos and BJAB cells both express human CD37, and were therefore used to screen the G28-1 supernatants for functional activity Binding of G28-1 scFv (SSS-S) H WCH2 WCH3 and G28-1 scFv VH L11S (SSS-S) H WCH2 WCH3 to CD37+ Ramos cells was measured by flow cytometry according to methods described in Example 2 Each point on the graph represents the mean of five replicate transfections The graph illustrates that the G28-1 scFv VH L11S (SSS-S) H WCH2 WCH3 is able to bind CD37+ Ramos cells

Addition constructs were made with different connecting regions The pD18 G28-1 scFv VHL11S (SSS-S) H WCH2 WCH3 vector was digested with BclI and XbaI to remove the connecting region, CH2 and CH3 These were replaced with each different connecting region, CH2 and CH3 according to the methods described in Example 13 The new constructs were designated G28-1 scFv VHL11S (CSS-S) H WH2 WH3 (SEQ ID NO__), G28-1 scFv VHL11S (CSC-S) H WH2 WH3 (SEQ ID NO__), G28-1 scFv VHL11S (CSS-S) H WH2 WH3 (SEQ ID NO__), G28-1 scFv VHL11S (SSC-P) H WH2 WH3 (SEQ ID NO__), G28-1 scFv VHL11S (SCS-S) H WH2 WH3 (SEQ ID NO__), G28-1 scFv VHL11S (CCS-P) H WH2 WH3 (SEQ ID NO__), and G28-1 scFv VHL11S (SCC-P) H WH2 WH3 (SEQ ID NO__)

The G28-1 scFv was also attached to an IgA connecting region, CH2, CH3 and an IgE CH2, CH3, CH4 The pD18 G28-1 scFv VHL11S (SSS-S) H WCH2 WCH3 plasmid was digested using methods above to remove the connecting region CH2, and CH3 The IgA regions were inserted using methods described in Example 13 The

construct was designated G28-1 scFv VHL11S IgAH IgACH2 T4CH3 (SEQ ID NO__) The IgE CH2 CH3 CH4 region was inserted into the digested pD18 vector above using methods described in Example 39 The construct was designated G28-1 scFv VHL11S IgECH2 CH3 CH4 (SEQ ID NO__)

EXAMPLE 36

Characterization of 2H7 scFv Ig Mutant Fusion Proteins

Figure 54 illustrates the binding capacity of purified 2H7 scFv Ig constructs to CD20+ CHO cells The proteins were transfected into stable CHO cells according to methods described in Example 2 Binding was determined using flow cytometry according to the methods described in Example 2 The graph in Figure 54 illustrates that these proteins retain binding function to CD20 with altered connecting regions Comparative results were obtained in each of the 2H7 scFv VHL11S mutants with each type of altered connecting region (results omitted)

The ability of 2H7scFv-Ig constructs with mutated connecting regions to kill CD20 positive cells in the presence of peripheral blood mononuclear cells (PBMC) through ADCC was tested by measuring the release of ^{51}Cr from labeled BJAB cells in a 4 hr assay using 100 1 ratio of PBMC to BJAB cells The results shown in Figure 55 indicate that 2H7scFv-Ig mutants can mediate antibody dependent cellular cytotoxicity (ADCC), since the release of ^{51}Cr was significantly higher in the presence of both PBMC and 2H7scFv-Ig than in the presence of either PBMC or 2H7scFv-Ig alone. Comparative results were obtained in each of the 2H7 scFv VHL11S mutants with each type of altered connecting region (results omitted)

The ability of 2H7scFv-Ig mutant fusion proteins to kill CD20 positive cells in the presence of complement was tested using B cell lines Ramos target cells Rabbit complement was purchased from Pel-Freez (Rogers, AK), and was used in the assay at a final concentration of 1/10 Purified 2H7scFv-Ig was incubated with B cells and complement for 45 minutes at 37°C, followed by counting of live and dead cells by trypan blue exclusion. The results in Figure 56 show that 2H7scFv-Ig mutants in the presence of rabbit complement lysed B cells expressing CD20

EXAMPLE 37

COMPARATIVE BINDING OF IGA, IGG, AND IGE 2H7 SCFV CONSTRUCTS

Binding capacity of Ig constructs IgA, IgG and IgG were measured using flow cytometry according to the methods described in Example 2, using a commercially available (Caltag) second step specific for each Ig tail The results in Figure 57 show that all the IgE constructs were able to bind CD20+ CHO cells comparable to the binding abilities of IgG and IgA. These results also demonstrate that the IgE constructs were detected with the IgE second step, but not the IgA or IgG second step

EXAMPLE 38

CONSTRUCTION AND CHARACTERIZATION OF 2H7 VH L11S IGE CONSTRUCTS

IgE tail RNA was isolated from SKO-007 cells(ATCC) using QIAGEN QIAshredder homogenization and RNA minikits Random-primed cDNA was generated according to the usual protocol, with 4 microliters RNA eluted from the QIAGEN columns Human IgE from the beginning of CH1 through CH4 (approximately 1 2 kb) was isolated by PCR amplification of 5 microliters cDNA, with an amplification profile of 94C, 60 sec, 72C, 2 minutes for 35 cycles, and the following primers

5' primer 5'-ggatccacccgctgctgcaaaaacattcctccaatgccacctccgtgac-3 (SEQ ID NO__)

3' primer 5'-tcatttaccgggatttacagacaccgctcgctggacggtctgtgaggggctcgctgc-3' (SEQ ID NO__)

PCR fragments were ligated into PCR 2 1-TOPO vector, and transformants screened for inserts of the correct size by digestion with EcoRI according to the methods described in Example 1 One of the clones with the correct sequence from was used as template to amplify the CH2-CH4 domains with appropriate restriction sites attached for subcloning as soluble or cell surface (ORF) forms The following primers were used with an amplification profile of 94C, 60 sec, 55C, 60 sec, 72C, 2 min, for 35 cycles to amplify a fragment of approximately 950 bp

5' primer (attaches BclI site to 5' end of CH2 domain of IgE)

5'-gttgttgatcacgtctgctccagggacttcacc-3' (SEQ ID NO__)

3' primer (attaches stop codon and XbaI site to 3' end of CH4 of IgE)

5'-gttgtttctagattatcatttaccaggatttacagacaccgctcgctg-3' (SEQ ID NO__)

3' primer (attaches SfuI and BamHI to 3' end of CH4 without a stop codon)

5'-gttgtttcgaaggatccgctttaccagatttacagacaccgctcgctg-3' (SEQ ID NO__)

The IgE CH2CH3CH4 tail with a stop codon was digested with BclI and XbaI and inserted into a pD18 vector that contains 2H7 VHL11S scFv This construct was designated 2H7 IgECH2CH3CH4 The polynucleotide sequence is provided in SEQ ID NO __, and the encoded polypeptide sequence is provided in SEQ ID NO ___ The IgE CH2CH3CH4 tail with no stop codon (ORF) was digested with BclI and SfuI and inserted in into a pD18 vector that contains 2H7 VHL11S scFv This construct was designated 2H7 IgECH2CH3CH4(ORF) The polynucleotide sequence is provided in SEQ ID NO __, and the encoded polypeptide sequence is provided in SEQ ID NO ___

The human IgE was also amplified as a fragment missing both CH1 and CH2 domains, with only the CH3 and CH4 domains attached to the human IgG1 hinge. Sequential PCR reactions using overlapping 5' oligonucleotides were used to attach the IgG1 hinge to the CH3 domain of human IgE Primers for the first step of the PCR reaction

5' Primer 5'-actcacacatcccaccgtccccagcatccaacccgagaggggtgagc-3' (SEQ ID NO__)

Primers for the second step of the PCR reaction

5' primer 5'-tctgatcaggagcccaaatcttctgacaaaactcacacatcccaccg-3' (SEQ ID NO__)

3' primer 5'-gttgtttctagattatcatttaccaggatttacagacaccgctcgctg-3' (SEQ ID NO__)

The PCR product was digested with EcoRI and sequenced according to the methods described in Example 1 Positive clones were inserted into pD18 plasmid containing 2H7 VHL11S scFv (SSS-S) H. The construct was designated 2H7 VHL11S scFv (SSS-S) H IgE CH3CH4 The polynucleotide sequence is provided in SEQ ID NO __, and the encoded polypeptide sequence is provided in SEQ ID NO ___

Binding capacity of 2H7 scFv VH L11S IgECH2 CH3 CH4, was measured using flow cytometry, essentially according to Example 2. The protein was purified using MEP HyperCel, (Cipergen, Catalog # 12035-010, Lot# 200920/0271) chromatography resin and Hydrophobic Charge Induction Chromatography (HCIC) HCIC absorbent is a high capacity, high selectivity, absorbent designed for capture and purification of monoclonal and polyclonal antibodies from various sources including cell culture supernatants Columns were packed with a 10 ml bead volume of MEP Hypercel, and equilibrated with PBS, pH 7.4 containing 0 1% NaN3 Approximately 1 liter of 2H7 scFv VHL11S IgE CH2CH3CH4 CHO culture supernatant was then run over the column. A series of citrate buffers ranging from pH 3-6 were prepared for elution of the fusion

protein The column was washed in PBS Protein was eluted in fifteen 1 ml fractions at pH6, 5, and 4 A final 15 ml fraction was collected at pH 3 5 Aliquots from each fraction were analyzed for A280 and were also subjected to SDS-PAGE, loading roughly 10 micrograms/well based on the A280 reading. The results of these two analyses indicated that the bulk of the protein did not elute in citrate buffers at the higher pH, but eluted at pH4, and the post elution wash at pH3 5 also contained significant amounts of protein.

The ability of these 2H7 VH L11S IgE purified proteins to bind CD20+ CHO cells was determined using flow cytometry according to the methods described in Example 2 using FITC-conjugated goat-anti-human IgE. Figure 58A illustrates that both purified proteins are able to bind CD20+ CHO cells

The ability of these 2H7 VH L11S IgE purified proteins to mediate ADCC against BJAB target cells with PBMC effectors was measured according to the methods described in Example 2 Figure 58B illustrates that both proteins were able to mediate ADCC at similar levels

EXAMPLE 39

CONSTRUCTION AND BINDING CAPACITY OF scFv VH L11S MUTANTS WITH MOUSE IGA AND IGE TAIL REGIONS

Murine IgA was cloned from murine spleen RNA using essentially the same methods used to clone the human IgE tails in Example 38 The PCR reactions were performed with a 94C 60 sec, 52C 60 sec, 72C 2 min amplification profile for 35 cycles The PCR primers used to clone CH1-CH4 regions were

5' primer 5'-atctgttcicctcctactactcctcctccacct-3' (SEQ ID NO__)

3' primer 5'-tcagtagcagatgccatctccctctgacatgatgacagacacgct-3' (SEQ ID NO__)

PCR primers used to delete the CH1 region

5' primer 5'-gttgttgatcacaicigttctcctcctactactcctcctccacct-3' (SEQ ID NO__)

3' primer with a stop codon, XbaI site at end of Ig tail, and the T4 mutation in CH3 region

5'-gttgtttctagattatcaatctccctctgacatgatgacagacac-3' (SEQ ID NO__)

3' primer for the ORF, a SfuI and BamHI sites, and T4 mutation in the CH3 region

5'-gttcttcgaaggatccgcatctccctctgacatgatgac-3' (SEQ ID NO__)

The mouse IgACH2 T4CH3 tail with a stop codon was digested with BclI and XbaI and inserted into a pD18 vector that contains 2H7 VHL11S scFv and the IgAH. This construct was designated 2H7 VHL11S scFv IgAH mIgACH2 T4CH3 The

polynucleotide sequence is provided in SEQ ID NO __, and the encoded polypeptide sequence is provided in SEQ ID NO ___

The mouse IgACH2 T4CH3 tail with no stop codon (ORF) was digested with BclI and SfuI and inserted in mto a pD18 vector that contains 2H7 VHL11S scFv and IgAH Thus construct was designated 2H7 VHL11S scFv IgAH mIgACH2 T4CH3 (ORF) The polynucleotide sequence is provided in SEQ ID NO __, and the encoded polypeptide sequence is provided in SEQ ID NO ___

Murine IgE was cloned murine IgE La2 (ATCC) RNA essentially according the methods described in Example 38 The PCR reactions were performed with a 94C 60 sec, 52C 60 sec, 72C 2 min amplification profile for 35 cycles Initial PCR primers used to clone the CH1-CH4

5' primer 5'-tctatcaggaaccctcagctctacccctttgaagccctg-3' (SEQ ID NO__)

3' primer 5'-gttgtttctagattatcaggatggacggagggaggtgttaccaaggct-3' (SEQ ID NO__)

PCR primers to remove the CH1 region

5' primer 5'-gttgttgatcacgttcgacctgtcaacatcactgagccccacc-3' (SEQ ID NO__)

3' primer with stop codon and XbaI site

5'-gttgtttctagattatcaggatggacggagggaggtgttaccaaggct-3' (SEQ ID NO__)

3' primer ORF, Sful and Bam HI 5'-gttgttttcgaaggatccgcggatggacggagggaggtgtta-3' (SEQ ID NO__)

The mouse IgE CH2CH3CH4 tail with a stop codon was digested with BclI and XbaI and inserted into a pD18 vector that contains 2H7 VHL11S scFv Thus construct was designated 2H7 VHL11S mIgECH2CH3CH4 The polynucleotide sequence is provided in SEQ ID NO __, and the encoded polypeptide sequence is provided in SEQ ID NO ___

The mouse IgE CH2CH3CH4 tail with no stop codon (ORF) was digested with BclI and SfuI and inserted in into a pD18 vector that contains 2H7 VHL11S scFv Thus construct was designated 2H7 VHL11S scFv mIgECH2CH3CH4(ORF) The polynucleotide sequence is provided in SEQ ID NO __, and the encoded polypeptide sequence is provided in SEQ ID NO ___

Binding capacity of 2H7 VH L11S mIgE and mIgA (with mouse tail regions) to CD20+ CHO cells were also measured by flow cytometry according to the methods described in Example 2 using commercially available IgE or IgA second step

reagents (Caltag) Each point in Figure 59 represents the mean of a population with brightness corrected by subtracting the binding of the second step alone. This figure illustrates that these constructs have the ability to bind CD20+ CHO cells.

EXAMPLE 40

HPLC PROFILES OF 2H7 SCFV IG MUTANT FUSION PROTEINS

HPLC analysis of purified 2H7 scFv-Ig mutant fusion proteins with altered connecting and CH3 regions Each protein was purified by Protein A affinity chromatography from supernatants of transfected COS or CHO cells. Twenty-five to fifty micrograms of each sample was run at 1 ml/min in PBS on a TSK-GEL G3000SW$_{XL}$ 30 cm column (Tosoh Biosep, Stuttgart, Germany) The arrow near the beginning of each profile represents the sample injection point. Gel filtration standards (Bio-Rad) included thryoglobulin (670 kDa), gamma globulin (158 kDa), ovalbumin (44 kDa), myoglobin (12.5 kDa), and vitamin B-12 (1 35 kDa) Standards were run at the beginning and end of each experiment. Migration positions of standards are shown and did not vary between experiments (Figures 60-62)

EXAMPLE 41

BINDING CAPACITY OF 2H7 VH L11S MUTANT FUSION PROTEINS

Binding effects of the CH3 mutant were compared to the non-mutated CH3 fusion protein The constructs were transfected into COS cells and purified from the supernatant using protein A column purification techniques described in Example 2 Figure 63 illustrates the differential effects of CH3 mutations on binding using flow cytometry according to the methods described in Example 2 This figure illustrates that some binding ability is lost when the double point mutation is introduced in the CH3 region

Binding of fluorescein isothiocyanate (FITC) conjugated 2H7 VH L11S mutant fusion proteins was determined using flow cytometry A 1 mg/ml solution of FITC was prepared in DMSO Fusion proteins were dialyzed in pH 9 3 bicarbonate buffer overnight at 4C in a volume of 2 liters Concentration of the protein was adjusted to 1-5 mg/ml prior to conjugation A series of Falcon 5 ml tubes was set up with varying FITC to protein ratios, ranging from 15-60, but minimally ratios of 20 and 40 Conjugation reactions were incubated at 37C for 30 minutes protected from light Fluorescein labeled

protein was separated from free fluorescein on a 2 ml Sephadex G-25 column equilibrated with PBS, 0 5 M NaCl, and 1% NaN3 The fluorescein labeled protein eluted from the column first and was collected in a 5ml tube The degree of labeling was determined by measuring the absorbance of the diluted conjugate at 280 and 494 nm, and utilizing the formulas provided by technical services at Molecular Probes (Eugene, OR) Data has been corrected for FITC protein ratio Figure 64 illustrates that these constructs do not lose binding capacity when conjugated to a fluorescent marker

Purified 2H7 VH L11S constructs were run on a non-reducing SDS gel according to the methods described in Example 2 The migration patterns are presented in Figure 65

EXAMPLE 42

CHARACTERIZATION OF 2H7 SCFV VH L11S (CSC-S) H WCH2 WCH3 IN LEC13 CHO CELLS

2H7 scFv VH L11S (CSC-S) H WCH2 WCH3 were transiently transfected and expressed in Lec13 CHO cells Lec13CHO cells were used as the mammalian cell hosts for either the 2H7 scFv VHS11 hIgG1 (CSS-S)H WCH2 WCH3 and (CSC-S)H WCH2 WCH3 expressing plasmids in side-by-side transfections All transfections were performed in 100 mm tissue culture dishes Cells were transfected when approximately 90% confluent using lipofectamine 2000 (Invitrogen, Catalog # 11668-027, 0 75 ml), following manufacturer's instructions Both cell lines were grown in the presence of serum to promote and maintain adherence to the cell culture dishes, simplifying transfection manipulations, washes, and supernatant harvests DNA lipofectamine complexes were allowed to form in the absence of serum and antibiotics, following the suggested protocol /conditions recorded in the product insert. Culture supernatants were harvested 72 hours after transfection, and then again 72 hours after the first harvest Fusion protein from the two CHO sources was isolated by protein A purification as previously described and used in CD20 binding and ADCC assays

The ability of mutated fusion protein to mediate ADCC in CD20 positive cells was determined using the methods described in Example 2 Constructs expressed in Lec13 CHO cells exhibited better binding to CD20 CHO target cells and also showed significantly improved activity in ADCC assays relative to the CHO DG44 derived proteins at equivalent concentrations as illustrated in Figure 67

EXAMPLE 43

CONSTRUCTION OF HIGH AND LOW AFFINITY CD16 ALLELES

The low(V) and high(F) affinity alleles at position 158 of the human CD16 extracellular domain were cloned from cDNA derived from human PBMC using PCR assay PCR reactions used random primed cDNA made from PBMC stimulated for 3 days with immobilized anti-CD3 antibody (64 1) prior to harvest. PCR reactions included 2, 4, 6 or 8 microliters of cDNA, each primer at 25 pmol, and an amplification profile of 94C 60 sec; 55C 60 sec, 72C 2 min, for 35 cycles The PCR primers are listed below

5' primer - no leader peptide 5'-
GTTGTTACCGGTGCAATGCGGACTGAAGATCTCCC
AAAGGCTGTG-3' (SEQ ID NO__)

3'antisense primer 5'-
GTTGTTTGATCAGCCAAACCTTGAGTGATGGTGATGTTCACA-3' (SEQ ID NO__)

Positive clones were sequenced, and inserted into a vector containing an efficient leader peptide and the (SSS-S)H P238SCH2 WCH3 human IgG tail. Two different versions of the CD16 ED fusion proteins were expressed. The first contained the F158 (high affinity) and the second contained the V158 (low affinity) allele. Constructs were cloned into a (SSS-S)H P238S CH2 WCH3 pD18 plasmid and expressed in COS and CHO cells as previously described in examples 1 and 10 CHO clones were screened for expression using an IgG sandwich ELISA to determine relative expression levels of the fusion proteins in the culture supernatant using the following protocol Immulon 1V plates were coated at 4C with 0 4 microgram/ml goat anti-human IgG (mouse Adsorbed), (CalTag, Catalog # H10500) in PBS buffer Plates were then blocked in PBS/1 5% nonfat milk at 4C overnight Plates were washed three times in PBS/0 1% Tween 20, then incubated with 100 microliters dilution series from CHO clone culture supernatants at room temperature for 3 hours Four dilutions per clone were added to successive wells, diluting in 5 fold increments from 1 5 to 1 375 In addition a standard curve was derived using CTLA4 hIgG1 (SSS-S)H P238SCH2 WCH3 as a concentration standard. The dilution series utilized 5 fold dilutions starting at 0 5 micrograms/ml, a second set of 8 wells was used to make a 2-fold dilution series starting at 0 34 micrograms/ml Plates were washed 3 times in PBS/0 1% Tween 20, and incubated with goat anti-human IgG, conjugated to horseradish peroxidase (GAH IgG-HRP) at 1 5000, in PBS/0 5% BSA for 1

hour Plates were washed four times with PBS/0 1% Tween 20, then TMB chromagen substrate (BD-Pharmingen) was added for 10 minutes, and reactions stopped by addition of 100 microlitres 1N sulfuric acid. Plates were then read at 415nm on a SpectraCount plate reader Concentrations of fusion protein were estimated by comparison of the ODs in the linear range to the CTLA4Ig standard curve run on each plate.

Proteins were purified using Protein A purification and were directly conjugated to fluorescein isothiocyanate (FITC) as described in Example 42 These proteins were run out on SDS gels under reduced and nonreduced conditions according to the methods described in Example 2 The migration of these proteins is presented in Figure 68

The ability of the high and low CD16 alleles to bind 2H7 VH111S (CSC-S) H WCH2 WCH3 or bind 2H7 VH111S (SSS-S) H (P238S)CH2 WCH3 expressed on the cell surface of CD20+ CHO target cell is determined using flow cytometry according to the methods described in Example 2 The results in Figure 69 demonstrate both the high and low affinity alleles were able to bind 2H7 VHL11S (CSC-S)H WCH2 WCH3 (SEQ ID NO__) and lost some binding capabilities when the P238S mutation was introduced into the CH2 region of the construct (SEQ ID NO__)

EXAMPLE 44

MAMMALIAN DISPLAY SYSTEM

Figure 70A diagrams how FITC conjugates of FcRIII (CD16) soluble fusion proteins bind to 2H7 scFv-Ig constructs that are attached to CD20 expressed by CHO cells The CD16 binding to a scFv-Ig provides a screening tool for detecting changes in CD16 binding to an altered scFv-Ig constructs containing targeted or site-specific mutations Changes in CD16 binding properties may be changes in binding of either CD16 high affinity protein (158F) or CD16 low affinity protein (158 V) or both.

A schematic representation of such a screening process is diagrammed in Figure 70B, where scFv-Ig constructs are displayed on the cell surface of mammalian cells The scFv-Ig molecules in this Example are displayed on the cell surface because they contain a transmembrane domain anchor These molecules may represent a single scFv-Ig construct or may be introduced into a population of mammalian cells as a library of such molecules Transfected cells with altered binding properties can then be panned, sorted, or otherwise isolated from other cells by altering the stringency of the selection conditions

and using CD16 fusion proteins as the binding probe Cells that express scFv-Ig molecules with altered binding to either CD16 high affinity allele (158F) or CD16 low affinity allele (158V) or both can be isolated For example, this display system can be used to create a library of mutated Ig tails with short stretches of CH2 sequence replaced with randomized oligonucleotides or possibly randomization of a single residue with all possible amino acid substitutions, including synthetic amino acids Once such a library is constructed, it can be transfected into COS cells by methods well known in the art. Transfectants can then be bound to the labeled CD16 constructs, and panned or sorted based on their relative binding properties to multiple allelotypes/isoforms Panned cells are harvested, and the plasmid DNA is isolated and then transformed into bacteria. This process may be repeated iteratively multiple times until single clones are isolated from the mammalian host cells (see Seed B and Aruffo A, PNAS 84 3365-3369 (1987) and Aruffo A and Seed B, PNAS 84 8573-8577 (1987)) One such use of this type of screening system would be to isolate Ig tails which bind equally well to both the high and low affinity alleles of CD16 with the goal of improving effector functions mediated by scFv-Ig constructs in multiple subpopulations of patients Ig tails with altered binding properties to other Fc receptors can also be selected using the display system described Other display systems for example those that use bacteriophage or yeast are not suitable for selection of Ig tails with altered FcR binding properties because of the requirement for glycosylation in the Ig CH2 domain that would not occur in non-mammalian systems

This system is also useful for selection of altered scFv-Ig molecules that will be produced at higher levels In this Example, mammalian cells such as COS cells can be transfected with a library of scFv-Ig constructs in a plasmid that directs their expression to the cell surface COS cells that express the highest levels of the scFv-Ig molecules can be selected by techniques well known in the art (for example panning, sterile cell sorting, magnetic bead separation, etc), and plasmid DNA is isolated for transformation into bacteria After several rounds of selection single clones are isolated that encode scFv-Ig molecules capable of high level expression When the isolated clones are altered to remove the membrane anchor and then expressed in mammalian cells, the scFv-Ig constructs will be secreted into the culture fluid in high levels This reflects the common requirement of secretory glycoproteins and cell surface glycoproteins for a signal peptide and processing through the golgi for expression, so that selection for a molecule that

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illustrates an improvement in expression levels on the cell surface will also select for a molecule that illustrates an improvement in levels of secreted protein.

EXAMPLE 45

CHARACTERIZATION OF G28-1 MABS AND SCFVS

Ability of G28-1 mAbs and scFvs to induce apoptosis was measured by binding Annexin V, using the methods described in Example 3 The results in Figure 71 demonstrate that the Annexin V binding of G28-1 antibodies and scFv is increased when treated together with 2I antibodies and scFv constructs.

EXAMPLE 46

CONSTRUCTION OF FC2-2 SCFV CONSTRUCTS

Construction of the FC2-2 (anti-CD16) scFv was performed using total RNA isolated from the FC2-2 hybridoma and cloned using methods described in Example 35 The polynucleotide sequence is provided in SEQ ID NO__, and the encoded polypeptide sequence is provided in SEQ ID NO___ The specific primers for the 5' second ry PCR reactions listed bellow The following are primers for the light chain variable region

5' primer with HindIII site with no leader 5'-
GTTGITAAGCTTGCCATGGATTCAC
AGGCCAGGTTCTT (SEQ ID NO__)

5' primer with SnaI site with leader 5'-
GTTGTGTCGACAGATGTCACAGTCTCC
ATCCTCCCTGTCTCT ID NO__)

3' primer
5'-TCTGTGCGATCTAAGGAGACGGTGACTGAGGTTCCTT-3' (SEQ ID NO__)

The following are primers for the heavy chain variable region

5' primer
TCGGCCGGCCATGGCCCGGGTGGCGGCGGATCGTCACAGGTGCAGTTG
AAGGAGTCTGGTCCTG IQ ID NO__)

3' primer 5
ACCCCTACCTCCTCCCCGAGCCACCGCCACCTTTTATTTCCAGCTTG
GTGCCACCTCCGA (SEQ ID NO__)

chang rom leucine to serine at position 11 in the heavy chain variable region (Kabat number) was introduced in into the FC2-2 scFv by site-directed mutagenesis a uding the methods described in Example 33 The scFv was attached to the (SSS S) H CH2 113 IgG tail according to methods described in Example 33 The mutant des ed FC 2 scFv VH L11S (SSS-S) H WCH2 WCH3 The polynucleotide sequenc is pr ed Q ID NO __, and the encoded polypeptide sequence is provided in SEQ ID NO

EXAMPLE 47

CONSTRUCTION OF 5B9 SCFV CONSTRUCTS

onstru on of the 5B9 (anti-CD137) scFv was performed using total RNA is olate in th 5B9 hybridoma and cloned using methods described in Example 35 The po ucleo sequence is provided in SEQ ID NO __, and the encoded polypeptide ence provided in SEQ ID NO ___ The specific primers for the second rv PC re ucle e listed bellow The following are primers for the light chain variable regio

5' prim idil with no leader 5 -
GTTG TA TC CCATGAGGTTCT
CTGC G CTG (SEQ ID NO__)
5' prim ll si leader 5'-
GTTG GIC CA TTGATGACGCAGGCTG
CATT CCA 3-3 Q ID NO__)
3' prim 5' G GATCAGAGGAGGACGGTGACTGAGGTTCCTTG-3' (SEQ ID NO

ie D are primers for the heavy chain variable region.

5' pri 5
CGGC GT G GGGTGGCGGCGGATCGTCACAGGTGCAGCTGA
AGCA CA A-3 Q ID NO__)
3' pri
CCCG C CAC CCGAGCCACCGCCACCCTTCAGCTCCAGCTTG
GTGC GC C-3' Q ID NO__)

ch m leucine to serine at position 11 in the heavy chain variable region (Kab num) was introduced in into the 5B9 scFv by site-directed

mutagenesis l attac o (SSS-S) H WCH2 WCH3 according to the methods described
in Exa ple This istruct was designated 5B9 scFv VHL11S (SSS-S) H WCH2
WCH3 The , olynuc ide sequence is provided in SEQ ID NO __, and the encoded
polypeptide seq ence ovided in SEQ ID NO ___

EXAMPLE 48

C NSTRUCTION OF UCHL1 scFv CONSTRUCTS

Constr on of the UCHL1 (anti-CD45RO) scFv was performed using
total RNA iso d f he UCHL1 hybridoma and cloned using methods described in
Examp le 35 l pol eotide sequence is provided in SEQ ID NO __, and the encoded
polypeptide s ience , ovided in SEQ ID NO ___ The following are primers for the
light ch n ole n
5' prim r with l id 5'-
GTTG TAAC TTC CATGAAGTTGCCTGTTAGGCTG
TTGC GC (S NO__)
3' prin s
AGAC C CC CCAGATCCACCACCGCCCGAGCCAC
CGCC TCT G \GCTTGGT-3' (SQ ID NO__)
e re primers for the heavy chain variable region.
5' prin r
TTTC GAGCTCCCACCTCCTCCAGATCCACCACCGC
CCGA (S D)
3' prin r 5 \ GATCATGCAGAGAGACAGTGACCAGAGTCCC-3' (SEQ
ID NO)

ch in leucine to serine at position 11 in the heavy chain variable
region was introduced in into the 5B9 scFv by site-directed
mutag s c d to a(SSS-S) HWCH2 WCH3 according to the methods
descri ed in the mutant is designated UCHL1 scFv VH L11S (SSS-S) H
WCH WCF r ucleotide sequence is provided in SEQ ID NO __, and the
encod oo tide e is provided in SEQ ID NO ___

EXAMPLE 49

\ IH 11S scFv (SSS-S) H WCH2 WCH3

\ ch ... from leucine to serine at position 11 in the heavy chain variable region (... numb ... \ was introduced in into the L6 scFv (SSS-S) H WCH2 WCH3 (constr ... ...ond... ...ethods described in Example 106) by site-directed mutagenesis accord ... to ... ... described in Example 33 The L6scFvIg (SSS-S) H WCH2 WCH3 ... ... s used as template. Positive clones were inserted into the pD18 plasmid ... ...ing (...S) H WCH2 WCH3 according to methods described in Example 33 ... ...ated L6 scFv VH L11S (SSS-S) H WCH2 WCH3 The polynu... ... ... seq... ... provided in SEQ ID NO__, and the encoded polypeptide sequen... ... ...e ... ID NO___

PCR p... ... ...lo... ...

5' Pri... ... ... site 5'-ggcgg... ... ...cgtctggacctgagtcgaagaagcct gggag... ... ...

3'Prim... ... ...cacc-3' (SEQ ID NO__)

EXAMPLE 50

CONSTRUCTION OF HD37 scFv VHL11S CONSTRUCT

... ...m leucine to serine at position 11 in the heavy chain variable region ... ...was introduced in into the HD37 scFv by site-directed mutag... ... ...ic methods described in Example 33 The HD37 scFv (SSS-S)H W... H2 ... H3 ... ...3 plasmid was used as a template. Positive clones were inserted into th... ... ...ning (SSS-S) H WCH2 WCH3 according to methods described ... ... The mutant is designated HD37 scFv VH L11S (SSS-S) H WCH2 WCH3 ... ...leotide sequence is provided in SEQ ID NO__, and the encod... ... ...ce is provided in SEQ ID NO___ PCR primers are listed below

5' pri... ... ...gtctggggctgagtcggtgaggcctgg-3' (SEQ ID NO__)

3' pri... ... ...tcagagtggc-3' (SEQ ID NO__)

EXAMPLE 51

2H7 scFv VHL11S CONSTRUCTS

... ...ldi... ...H7 VHL11S constructs were made with different connecting region ... ...s VHL11S (SSS-S) H WCH2 WCH3 vector was digested with

BclI [illegible] to rem[illegible] connecting region, CH2 and CH3 Theses were replaced with each [illegible] connection, CH2 and CH3 according to the methods described in Exam[illegible] 13 The [illegible]ructs were designated. 2H7scFv VHL11S (CSS-S) H WH2 WH3 [illegible] O) NO [illegible] 7scFv VHL11S (CSC-S) H WH2 WH3 (SEQ ID NO__) 2H7 scFv [illegible] was [illegible]ched to an IgA connecting region, CH2, CH3 and an IgE CH2, CH3, [illegible]4 The [illegible]7 scFv VHL11S (SSS-S) H WCH2 WCH3 plasmid was digest[illegible] su[illegible], meth[illegible] to remove the connecting region CH2, and CH3 The IgA region [illegible] insert[illegible] methods described in Example 13 The construct was design[illegible]7 scFv VHL11S IgAH IgACH2 T4CH3 (SEQ ID NO__). The IgE CH2 CH3 [illegible] on [illegible]d into the digested pD18 vector above using methods descri[illegible] [illegible] example [illegible] construct was designated 2H7 scFv VHL11S IgECH2 CH3 CH4 ([illegible] NO_

EXAMPLE 52

21 [illegible]v VH L11S (CSC-S) H WCH2 WCH3

This [illegible] is a 2H7 (anti-CD20) single chain Fv binding region with a [illegible] point [illegible] [illegible] [illegible] residue 11 in the heavy chain variable region, where the leucine [illegible]een ch[illegible] serine, as described in Example 33 This binding region is [illegible] connec[illegible] human [illegible] connecting region, CH2 and CH3 region, where in the second [illegible] cystein[illegible] [illegible] pro[illegible] connecting region have been changed to serines (SSS-S) as descril[illegible] [illegible] The polynucleotide sequence is provided in SEQ ID NO __, and th[illegible] [illegible] sequence is provided in SEQ ID NO ___

EXAMPLE 53

[illegible]v VH L11S IgE CH2 CH3 CH4

This [illegible] is a 2H7 (anti-CD20) single chain Fv binding region with a point [illegible] [illegible] residue 11 in the heavy chain variable region, where the leucine [illegible] serine, as described in Example 33 This binding region is attache[illegible] [illegible] constant region containing CH2, CH3 and CH4 as described in Exam[illegible] [illegible] The [illegible]otide sequence is provided in SEQ ID NO __, and the encod[illegible] [illegible] provided in SEQ ID NO ___

EXAMPLE 54

[illegible]V VH L11S mIgE CH2 CH3 CH4

This c s 2H7 (anti-CD20) single chain Fv binding region with a point m at residue 11 in the heavy chain variable region, where the leucine b n ch serine, as described in Example 33 This binding region is attache mouse c nit region containing CH2, CH3 and CH4 as described in Exampl 9 Tl leotide sequence is provided in SEQ ID NO __, and the encoded polypeptide provided in SEQ ID NO ___

EXAMPLE 55

S \ H L11S MIGAH WIGACH2 T4CH3

This s 2H7 (anti-CD20) single chain Fv binding region with a point m at residue 11 in the heavy chain variable region, where the leucine l en ch ine, as described in Example 33 This binding region is attache from human IgA as desribed in Example 5 This connec gion mouse IgA constant region consisting of a wild type CH2 re nd a m region where there is a truncation of 4 amino acid residues prior t stop described in Example 39 The polynucleotide sequence is provid SEQ ID he encoded polypeptide sequence is provided in SEQ ID NO __

EXAMPLE 56

2 _ S H I 11S (SSS-S) H K322S CH2 WCH3

This 2H7 (anti-CD20) single chain Fv binding region with a point at n at idue 11 in the heavy chain variable region, where the leucine is been ch ine, as described in Example 33 This binding region is connec l to a mut G connecting region where all of the cysteines and one proline ive been s (SSS-S) according to methods described in Example 5 Tl onnectin ed ted to a mutated IgG CH2 region and a wild type IgG CH3 r n The in the CH2 region is at a residue 322, where a Lysine has be b nged overlapping PCR assay An (SSS-S) H WCH2 WCH3 IgG1 plate in r was used for PCR amplification, to create (SSS-S) H derv es contain utations PCR reactions used a cycling profile of 94C, 30 sec 5C 30 for 37 cycles to complete the reactions This IgG1 derv vas using sequential PCR reactions with overlapping

oligom eotides in nd secondary reactions The primary amplification primers introduced ( ) but deleted one end of the Fc domain. Secondary reactio primers re c ic nds using overlapping primers The first overlapping primer as added e be g of the PCR, the reactions allowed to proceed for 12 cycles, used and the d overlapping primer added to the reactions followed by 25 mor cycles to c rlap extension PCR reactions

Primers or the firs t

5' Prin r 5'-ggag gctgggagggctttgttggagaccgagcacttgtactcc-3'(SEQ ID NO)

3' prin 5 ggaca -3' (SEQ ID NO)

PCT ned into TOPO vector and sequenced for verific on. Positiv sed as templates for the second overlapping PCR reactio

5' prim r 5'ccgtctc ig atctctgacaaaactcacacatcccccaccgtccccagc-3' (SEQ ID NO)

5'prim overlappin

tccccac tccccagcac gatcgtcagtcttcctcttccccc

caaaac 3' (SEQ ID)

3' prin r 5' cagg g SEQ ID NO)

PCR cloned into TOPO vector and sequenced. The polynu otide seq d in SEQ ID NO__, and the encoded polypeptide sequen is p d

__

EXAMPLE 57

2 l 115 (CSS-S) H K322S CH2 WCH3

This l 7 (anti-CD20) single chain Fv binding region with a point n tation a 11 in the heavy chain variable region, where the leucine has be ch as described in Example 33 This binding region is attached to a m n 3 n region, where the second and third cysteines have been ch nged to ic has been changed to serine (CSS-S), according to methods desc e connecting region is attached to a mutated IgG CH2 r 3 region. The K322S mutation in the CH2 region is at a resid 322 l re n changed to a serine using overlapping PCR assay

246

region s attach d to CH2 region and a wild type IgG CH3 region. The
mutation in the CH2 r g l by overlapping PCR reaction essentially according
to Exan ple 57 h 2 WHC3 IgG1 pD18 vector as a template in the first
PCR r tion u l du p the second PCR reaction, which are listed below
5'prim r 5 - gle ttcttgtgacaaaactcacacatccccaccgtccccagc-3' (SEQ ID
NO__)
5'over lapping ner
tcccac tcgtcagtcttcctcttcccccaaaacc-3'
3' prin SEQ ID NO)
oned into the TOPO vector and sequenced. The
polynu leotide equer in SEQ ID NO__, and the encoded polypeptide
sequen is pr d __

EXAMPLE 58

211 S (SSS-S) H P331S CH2 WCH3

ns c 7 (anti-CD20) single chain Fv binding region with a
point n tion 11 in the heavy chain variable region, where the
leucine s is described in Example 33 This binding region is
connec l connecting region where all of the cysteines and one
prolin e SSS-S) according to methods described in Example
5 The connec a mutated IgG1 CH2 region and a wild type IgG1
CH3 r tion in the CH2 region, where the proline at residue
331 h s incorporated using a single PCR reaction, using a
(SSS-S te and a cycling profile of 94C, 30 sec, 55C, 30 sec,
72C, 3 ic primers for reaction are listed below
5' prim ttctgacaaaactcacacatccccaccgtccccagc-3' (SEQ ID
NO)
3' 5'-
gcaggt gctttggagatggttttctcgatggaggctgggagg-3' (SEQ ID
NO)
p uence is provided in SEQ ID NO__, and the
encod e ed in SEQ ID NO ___

EXAMPLE 59

211 S (CSS-S) H P331S CH2 WCH3

'is b ttached to a mutant IgG connecting region, where
the sec hird (been changed to serines and the proline has been
changed (fCS) methods described in Example13 The connecting
region ' ' to ' ' 12 region and a wild type IgG CH3 region. The
mutatio ' mut ' egion, where the proline at residue 331 has been
changed e, using a single PCR reaction, using a (CSS-S) H
WCH2)1 ' cling profile of 94C, 30 sec, 55C, 30 sec, 72C, 30
sec, fo ' rs for reaction are listed below
5' prim ctct ttgtgacaaaactcacacatcccccaccgtccccagc-3' (SEQ ID
NO)
3' Prim
gcagg ' gcttggagatggttttctcgatggaggctgggagg-3' (SEQ ID
NO)

' pc uence is provided in SEQ ID NO__, and the
encode ' ide ' led in SEQ ID NO ___

EXAMPLE 60

211 (SSS-S) H T256N CH2 WCH3

' his c (anti-CD20) single chain Fv binding region with a
point m ' m in the heavy chain variable region, where the
leucine ' han described in Example 33 This binding region is
connec ' ulate ' ecting region where all of the cysteines and one
prolin ' ' ' S S) according to methods described in Example
5 The ' re ' ' mutated IgG CH2 region and a wild type IgG
CH3 r ' ' CH2 region, the threonine at residue 256 has been
change ' ' ' ' ipping PCR methods described in Example 56
The s ' ' '
' ' ' eaction
5' pru ' tc tcctcatgatctcccggaaccctgaggtcac-3' (SEQ ID
NO)
3' pru ' ' SEQ ID NO)

CR) OPO vector and sequenced This product was
used as late in reaction. Primers for the second PCR reaction.
5' prim tctctg c tctgacaaaactcacacatcccaccgtccccagc-3' (SEQ ID
NO)
5 5'over imer
tcccca(ce gtcagtcttcctcttccccccaaaacc-3' (SEQ ID NO)
3' prim gaaac Q ID NO)
e pr ence is provided in SEQ ID NO__, and the
encod tide s ed in SEQ ID NO ___

10 EXAMPLE 61
Fv V) H RTPE/QNAK (255-258) CH2 WCH3
his co (anti-CD20) single chain Fv binding region with a
point t it am l in the heavy chain variable region, where the
leucine chai is described in Example 33 This binding region is
15 connec utal necting region where all of the cysteines and one
proline n (' C S) according to methods described in Example
5 Tl . ng r mutated IgG CH2 region and a wild type IgG
CH3 R l in the CH2 region, where residues 255-258 have
been m a proline, glutamic acid to glutamine, asparagines,
20 alanine c, re overlapping PCR reactions described in Example
56 Th prim
PCR p the f
5' pri tctt accctcatgatctcccagaacgctaaggtcacatgc-3' (SEQ
ID NC
25 3' prin cagt Q ID NO)
he PC l into TOPO vector, sequenced and used as a
templ seco e primers for the second PCR reaction
5 prin tctg caaaactcacacatcccaccgtccccagc-3' (SEQ ID
NO)
30 5'over imer
tcccca cacc tgtcttcctcttccccccaaaacc-3' (SEQ ID NO)
3' pri tgaa ID NO)

[illegible]ie pol[illegible]nce is provided in SEQ ID NO __, and the encode[illegible]tide sc[illegible]in SEQ ID NO ___

[illegible]MPLE 62

2H7 [illegible]SS-S) H K290Q CH2 WCH3

his co[illegible](anti-CD20) single chain Fv binding region with a point m[illegible]t amin[illegible]in the heavy chain variable region, where the leucine [illegible] chang[illegible]described in Example 33 This binding region is connect[illegible]nutated [illegible]ting region where all of the cysteines and one prolin[illegible]n chan[illegible]S) according to methods described in Example 5 The [illegible]g rec[illegible]utated human IgG1 CH2 region and a wild type human [illegible]3 rec[illegible]ilation in the CH2 region, where the Lysine at residu[illegible]ocen c[illegible]ic, using a single PCR reaction according to the metho[illegible]ed in [illegible]pecific primers used in this reaction are listed below

5' prim[illegible]ctctg[illegible]caaaactcacacatccccaccgtccccagc-3' (SEQ ID NO)

3' prim[illegible]ccgc[illegible]Q ID NO)

[illegible]CR p[illegible]to TOPO and sequenced. The polynucleotide sequen[illegible]ided [illegible]d the encoded polypeptide sequence is provided in SEQ [illegible]

[illegible]MPLE 63

2H7 [illegible]S-S) H A339P CH2 WCH3

his co[illegible]nti-CD20) single chain Fv binding region with a point [illegible]amino[illegible]the heavy chain variable region, where the leucin[illegible]chang[illegible]cribed in Example 33 This binding region is connec[illegible]tated[illegible]ting region where all of the cysteines and one prolin[illegible]een c[illegible]n[illegible])ccording to methods described in Example 5 Th[illegible]re[illegible]utated human IgG1 CH2 region and a wild type [illegible]' CH3[illegible]mutation in the CH2 region, where the alanine at res[illegible]bee[illegible], using a single PCR reaction according to the

method I in Ex ific primers used in this reaction are listed below

5' prim tctgat icaaaactcacacatcccccaccgtccccagc-3' (SEQ ID NO)

3' Prim gtggg ctcgggggctgcccttgggtttggagatgg-3' (SEQ ID NO)

R prod nto TOPO and sequenced. The polynucleotide sequen d in f the encoded polypeptide sequence is provided in SEQ

MPLE 64

-S) H WCH2 WCH3

is con (anti-CD37) single chain Fv binding region made i o meth mple 35 This binding region is connected to a mut IgG1 where all of the cysteines and one proline have been c same to methods described in Example 5 Thus connec n is e type human IgG1 CH2 and CH3 region as descri nple has previously been referred to as G28-1- MHW G28 the same sequence as the abouve construct The p d se SEQ ID NO __, and the encoded polypeptide sequer ded in

MPLE 65

H WCH2 WCH3

is con (anti-CD37) single chain Fv binding region made to meth mple 35 This binding region is connected to a hum nectin pe human IgG CH2 and CH3 constant regions as des Examp t has previously been referred to as G28-1- IgAH the p e is provided in SEQ ID NO __, and the encod SEQ ID NO ___

MPLE 66

G2 (SSS-S) H WCH2 WCH3

us co nti-CD37) single chain Fv binding region with
a poi in the heavy chain variable region, where the
leucin scribed in Example 35 This binding region is
conne cting region where all of the cysteines and one
prolu S) according to methods described in Example
5 T ild type human IgG1 CH2 and CH3 region as
descri e sequence is provided in SEQ ID NO __, and
the en ed in SEQ ID NO ___

MPLE 67

C (CSS-S) H WCH2 WCH3

us co nti-CD37) single chain Fv binding region with
a poi in the heavy chain variable region, where the
leucin scribed in Example 35 This binding region is
attach g region, where the second and third cysteines
have oline has been changed to serine (CSS-S),
accor 3 ' is connecting region is attached to wild
type described in Example 1 The polynucleotide
sequ l the encoded polypeptide sequence is provided
in SE

MPLE 68

C2 (CSC-S) H WCH2 WCH3

s co nti-CD37) single chain Fv binding region with
a poi in the heavy chain variable region, where the
leucin cribed in Example 35 This binding region is
attach n where the second cysteine and the
prolin g to methods described in Example
32 T ld ty human IgG1 CH2 and CH3 region as
desc e sequence is provided in SEQ ID NO __, and
the en in SEQ ID NO ___

MPLE 69

G2 SC-S) H WCH2 WCH3

47

T [illegible] con
a point mutation [illegible]
leucin [illegible] beca [illegible]
attach [illegible] [illegible] [illegible] int h
have [illegible]e cha[illegible]ed to
Thus connecting gio
described in Ex[illegible]ple
the en[illegible]d pol[illegible]pt

Thus construct [illegible] the
This b[illegible]ndi[illegible]g re[illegible]on i
the ex[illegible]ne
descri[illegible]nple
region and a wi[illegible]ype
residue 239 v[illegible]hran
were [illegible]erf[illegible]rme[illegible]d in
PCR [illegible] [illegible] [illegible]
72C [illegible] [illegible]or [illegible]ycle
fragments were [illegible]PCR
sequenced as de[illegible]ibe
5' pri[illegible]er 5 -
gtgtt[illegible] [illegible]eman'
accgt[illegible] [illegible] (SE(
3' pri[illegible]er [illegible]g [illegible]
[illegible] [illegible]
MTC [illegible] [illegible] vhi
sequc [illegible] ded i [illegible]
in SE[illegible] [illegible]

iti-CD37) single chain Fv binding region with
n t[illegible] heavy chain variable region, where the
[illegible]ed in Example 35 Thus binding region is
region, where the first and second cysteines
ording to methods described in Example 13
[illegible] human IgG1 CH2 and CH3 region as
[illegible]uce is provided in SEQ ID NO __, and
SEQ ID NO ___

[illegible] 70

P[illegible]SCH2 WCH3

[illegible] [illegible]ng region as described in Example 14
[illegible]man IgG1 connecting region where all of
[illegible] to serines (SSS-S) according to methods
[illegible]ttached to a mutated human IgG1 CH2
[illegible]e P238S mutation, where a proline at
[illegible] using a PCR assay PCR reactions
[illegible]ured from human tonsil B cell RNA.
of 94C 4 min, [94C 1 min, 55C 1 min,
[illegible]nsion step for 6 minutes at 72C PCR
[illegible] [illegible] inserts of approximately 800 bp were
[illegible]d for the PCR are listed below

cgtccccagcacctgaactcctgggtgg

[illegible]Q ID NO)
[illegible] [illegible]red to as CTLA-4 IgG MTH (SSS)
[illegible]ne above construct. The polynucleotide
[illegible]coded polypeptide sequence is provided

CH2 WCH3

us construct
This bind ig r on is attac
described in E. mple 1
CH2 nd 11 -gion is d
refram ' ' LA-4 IgG W
above o trus The polv
encoded p p ide s ic

ig region as described in Example 14.
an IgG1 connecting region (CCC-P) as
connected to a wild type human IgG1
This construct has previously been
H3, which has the same sequence as the
provided in SEQ ID NO __, and the
D NO ___

FC

us construct
methods c c d in ' xa
IgG1 connect regi /
serine S s ccor b
connc ld t pe l
The p k s
seque ed

2

VCH2 WCH3

016) single chain Fv made according to
-gion is connected to a mutated human
and one proline have been changed to
Example 5 This connecting region is
H3 region as described in Example 1
) NO __, and the encoded polypeptide

FC2-2

s c sm
mutat ' ' ' ' r
been c ' ser
mutat ' ' ' G1 n
been ' ser S
conne ' is
descr ol
the e t

3

H WCH2 WCH3

CD16) single chain Fv with a point
n variable region, where the leucine has
6 This binding region is connected to a
of the cysteines and one proline have
hods described in Example 5 This
man IgG1 CH2 and CH3 region as
nce is provided in SEQ ID NO __, and
Q ID NO ___

74

((
s c to
accor ' ll od
mutat ' gG1 n

WCH2 WCH3

nti-CD45RO) single chain Fv made
This binding region is connected to a
of the cysteines and one proline have

been c seri ethods described in Example 5 This
connec in is human IgG1 CH2 and CH3 region as
descri' iple ience is provided in SEQ ID NO __, and
the en ncpti SEQ ID NO ___

5

UC S) H WCH2 WCH3
his co CD45RO) single chain Fv with a point
mutat o acic in variable region, where the leucine has
been s seri 8 This binding region is connected to a
10 mutat IgG1 l of the cysteines and one proline have
been s scri thods described in Example 5 This
conne is c in IgG1 CH2 and CH3 constant region
as dc xamj equence is provided in SEQ ID NO __,
and tl olyp in SEQ ID NO ___

15

76
CH2 WCH3
is co 37) single chain Fv made according to
metho d in F gion is connected to a mutated human
IgG1 s regio s and one proline have been changed to
20 serine iccon Example 5 This connecting region is
conne ild CH3 constant region as described in
Exan co: 'erred to as 5B9 scFv IgG MTH (SSS)
WTC ich J above construct. The polynucleotide
seque 'ed i coded polypeptide sequence is provided
25 in SI —

7
5 'I WCH2 WCH3
is cor 37) single chain Fv with a point mutation
at am sidue ble region, where the leucine has been
30 chang ne i This binding region is connected to a
mutat gG1 of the cysteines and one proline have

been [illegible] serin [illegible] ethods described in Example 5 Thus
conne [illegible] n is c[illegible] an IgG1 CH2 and CH3 constant region
as de[illegible] xamp[illegible] equence is provided in SEQ ID NO __,
and th polypc [illegible] n SEQ ID NO ___

5

[illegible]

CH2 WCH3

[illegible]us co [illegible]20) single chain Fv binding region as
descri [illegible]mple [illegible]connected to a mutated human IgG1
conne [illegible]n whe [illegible]c proline have been changed to serines
10 (SSS [illegible]ng to [illegible]mple 5 This connecting region is
conne [illegible]vild ty [illegible]nit region as described in Example 1
This c [illegible]s pre [illegible]7-MHWTG1C, CytoxB-(MHWTG1C)
– Ig scFv [illegible]3, CytoxB-MHWTG1C, 2H7 scFv-
huma d typ scFv IgG MTH (SSS) WTCH2CH3,
15 whicl [illegible]c sar [illegible]ruct The polynucleotide sequence is [illegible]F
provi [illegible]ID N[illegible] [illegible]ide seqnence is provided in SEQ ID [illegible]
NO _

P238SCH2 WCH3

20 [illegible]s co [illegible]0) single chain Fv binding region as
descri [illegible]mple [illegible]connected to a mutated human IgG1
conne [illegible]n wl proline have been changed to serines
(SSS [illegible]s, to [illegible]5 This binding region is connected to
a mu [illegible]1 IgG [illegible]of the cysteines and one proline have
25 been [illegible]ser [illegible]hods described in Example 5 This
hmge [illegible]ach [illegible]H2 region and a wild type human IgG1
CH3 P2 [illegible]residue 238 was changed to a serine,
was [illegible]ccc [illegible]n Example 70 Thus construct has
previ [illegible]ref [illegible]' (SSS) MTCH2WTCH3, anti-CD20
30 scFv [illegible]SS [illegible]'IMG1C which all have the same

seque abo ide sequence is provided in SEQ ID NO _ nce ided in SEQ ID NO ___

d

12 WCH3

us c 0) single chain Fv binding region as descri impl onnected to a human IgA connecting regio ype nstant regions as described in Example 5 T t h re as 2H7 scFv IgAH IgG WTCH2CH3, 2H7 ing -IgAHWTHG1C, which all have the same d o cleotide sequence is provided in SEQ ID N e ei rovided in SEQ ID NO ___

1

12 T4CH3

us c 0) single chain Fv binding region as descri nn attached to a connecting region from hum scri ing region is attached to a human IgA const cons g ion and a mutated CH3 region where there ion to the 3' stop codon as described in Exam erred to as 2H7 scFv IgAH IgAT4, whic ue t The polynucleotide sequence is prov D ide sequence is provided in SEQ ID NO _

7 S CH3 + JCHAIN

single chain Fv binding region as descri imp attached to a wild type human IgA conn n as onnecting region is attached to a wild type CH ng to methods described in Example 13 as described in Example 13 The polyn __, and the encoded polypeptide sequ e

C1I2 WCH3

[illegible]his c [illegible]) single chain Fv binding region as
descri [illegible]amp [illegible]ttached to a wild type human IgG1
conne [illegible]on (C[illegible] [illegible]. This connecting region is attached
to wil [illegible]nan [illegible]ons as described in Example 1. This
consti [illegible]vion [illegible]g WTH (CCC) WTCH2CH3, 2H7
scFv l [illegible]VTC [illegible]h both have the same sequence as the
above [illegible]l' [illegible]ovided in SEQ ID NO __, and the
encoc [illegible]ptide [illegible]NO ___

2 1405YCH3

[illegible]his c [illegible]) single chain Fv binding region as
descr [illegible]xamp [illegible]onnected to a mutated human IgG1
conne [illegible]on wi [illegible]roline have been changed to serines
(SSS- [illegible]g to [illegible]. This connecting region is attached
to a [illegible]umn [illegible]n IgG1 CH3 region. The F405Y
mutat [illegible]thi [illegible]been changed to a tyrosine, was
introc [illegible]rdin [illegible]21. This construct has previously
been [illegible]as [illegible]405, which has the same sequence
as the [illegible]sti[illegible] [illegible]is provided in SEQ ID NO __, and
the e[illegible]pep [illegible]NO ___

2 1405ACH3

[illegible]his [illegible]) single chain Fv binding region as
descr [illegible]amp [illegible]nected to a mutated human IgG1
conne [illegible]n w [illegible]roline have been changed to serines
(SSS [illegible]ng t[illegible] [illegible]. This connecting region is attached
to a [illegible]hum [illegible]IgG1 CH3 region. The F405A
mutat [illegible]l [illegible]been changed to an alanine, was
introc [illegible]u [illegible]. This construct has previously

been r[illegible]s 21 [illegible]405, which has the same sequence as the [illegible]struc[illegible] provided in SEQ ID NO __, and the en[illegible]cepti[illegible]) NO ___

[illegible]107ACH3

[illegible]s c[illegible]ngle chain Fv binding region as descri[illegible]mp[illegible]cted to a mutated human IgG1 conne[illegible]n w[illegible]ne have been changed to serines (SSS-[illegible]g to [illegible]This connecting region is attached to a w[illegible]uma[illegible] IgG1 CH3 region. The Y407A mutat[illegible]hc[illegible]ed to an alanine, was introduced accor[illegible]ho[illegible]s construct has previously been referr[illegible]h has the same sequence as the above [illegible]ided in SEQ ID NO __, and the encod[illegible]d.)___

[illegible], Y407ACH3

[illegible]ngle chain Fv binding region as desc[illegible]un[illegible]ected to a mutated human IgG1 conn[illegible]n[illegible]line have been changed to serines (SSS[illegible]g[illegible]Thus connecting region is attached to a [illegible]mu[illegible]gG1 CH3 region. The F405A and Y40[illegible]5 has been changed to an alanine and t[illegible]t[illegible]lanine, was introduced according to m[illegible]rib[illegible]as previously been referred to as sc[illegible]e[illegible]he same sequence as the above cons[illegible]po[illegible]SEQ ID NO __, and the encoded polyp[illegible]ci[illegible]

[illegible] WCH3

259

[illegible] is c ngle cham Fv binding region as
descri[illegible] [illegible] iched to a mutant human IgG1
conne[illegible] n, w have been changed to serines and
the p[illegible] bee[illegible] ording to methods described in
Exam[illegible] is co[illegible] type human IgG1 CH2 and CH3
const[illegible] as c[illegible] ct has previously been referred to
as 2[illegible] [illegible] e same sequence as the above
consti[illegible] poly SEQ ID NO __, and the encoded
polyp[illegible] ience

WCH3

[illegible] is ngle cham Fv binding region as
descri[illegible] xamp iched to a mutant human IgG1
conne[illegible] n, wl e been changed to serines and the
prolin[illegible] chai methods described in Example13
This [illegible] re[illegible] n IgG1 CH2 and CH3 constant
region[illegible] [illegible]een eviously been referred to as 2H7
scFv [illegible](SC sequence as the above construct
The [illegible] de s __, and the encoded polypeptide
seque[illegible] ide

WCH3

[illegible] his [illegible] ngle cham Fv binding region as
descr[illegible] amp iched to a mutant human IgG1
conne[illegible] n, s have been changed to serines
(SSC[illegible] ine 13 This connecting region is
attac[illegible] [illegible]vo -gions as described in Example
1 T[illegible] [illegible] h scFv MTH (SSC) WTCH2CH3,
whic[illegible] [illegible] The polynucleotide sequence is
provi[illegible] [illegible]QID sequence is provided in SEQ ID
NO _

260

\WCH3

his c … igle chain Fv binding region as
descri… xamp… hed to a mutant human IgG1
conne… n, w… line has been changed to serine
(CSC-… ling … 2 This connecting region is
attach… type … regions as described in Example
1 Th… ct h… scFv MTH (CSC) WTCH2CH3,
which… seque… The polynucleotide sequence is
provi… ID … sequence is provided in SEQ ID
NO _

\WCH3

This … gle chain Fv binding region as
descri… xamp… hed to a mutant human IgG1 …
conne… n, … o a serine (CCS-P), according to …
metho… bed … n is attached to wild type human
IgG1 … CH … xample 1 This construct has
previ… re… TCH2CH3, which has the same
sequ… ib… equence is provided in SEQ ID
NO _ … ner… in SEQ ID NO ___

\WCH3

… his … igle chain Fv binding region as
descr… xam… ched to a mutant human IgG1
conne… on, … to a serine (SCC-P), according to
metho… bed … n is attached to wild type human
IgG1 … Cl … xample 1 This construct has
prev… CH2CH3, which has the same
sequ… … quence is provided in SEQ ID
NO _ … ne… in SEQ ID NO ___

H2 WCH3

his le chain Fv binding region with a

point ıt chain variable region, where the

leuci ı ch ımple 33 This binding region is

conn ıul vhere all of the cystemes and one

prolı ıt ı o methods described in Example

5 1 ıı ı ın IgG1 CH2 and CH3 constant

regıo ue ɔreviously been referred to as 2H7

scFv ı scFv ıd 2H7 scFv VHSER11 WTH

WTC h ıs the above construct. The

polyı ɑ, _, and the encoded polypeptide

sequ ıd

CH2 WCH3

his e chain Fv binding region with a

point hain variable region, where the

leucı cl ımple 33 This binding region is

attacl tar ɔre the second and third cystemes

have ıgɔ' ɔn changed to serine (CSS-S),

accor ho ecting region is attached to wild

type ı described in Example 1 The

polyı ɑ, _, ınd the encoded polypeptide

sequ ıd

C H2 WCH3

ıs ıgle chain Fv binding region with

a po ı ı chain variable region, where the

leuci ch ımple 35 This binding region is

attacl tar ıere the first and third cystemes

have ɔd ɔɔ ı changed to serine (SCS-S),

acco thc nnecting region is attached to wild
type sl described in Example 1 The
poly sequ __, and the encoded polypeptide
sequ vided

5

WCH2 WCH3

his igle chain Fv binding region with
a po n at chain variable region, where the
leuc n ch mple 35 This binding region is
10 attac mut where third cysteine has been
chan em described in Example 22 This
conn m CH2 and CH3 constant regions as
desc mp provided in SEQ ID NO __, and
the e pe O ___

15

WCH2 WCH3

his gle chain Fv binding region with
a po at chain variable region, where the
leuc n ch mple 35 This binding region is
20 attac utant first cysteine has been changed
to a C-P mple 32. This connecting
regio n constant regions as described in
Exa Q ID NO __, and the encoded
poly; e

25

WCH3 CH4

his gle chain Fv binding region with
a po at ch in variable region, where the
leuc l cl mple 35 This binding region is
30 attac lt t n using methods described in

Exa[illegible] he p[illegible] in SLQ ID NO __, and the encoded
poly[illegible] uen[illegible]

C[illegible] ACH2 [illegible]4CH3

This [illegible] ingle chain Fv binding region with
a poi[illegible] n it[illegible] y cha[illegible] variable region, where the
leuc[illegible] [illegible] ch[illegible] xample 35 This binding region is
attacl[illegible] onn[illegible] desc[illegible]bed in Example 39 Thus
conn[illegible] ion [illegible] [illegible]n consisting of a wild type
CH2 [illegible]'a n[illegible] [illegible]m [illegible]o [illegible]cid truncation at residues
as de[illegible] Ex[illegible] [illegible]ce [illegible] provided in SEQ ID NO __,
and t[illegible] 'po[illegible] Q ID O___

[illegible] [illegible] 2CH [illegible] CH4

his [illegible] ingle [illegible]in Fv binding region with
a poi[illegible] n it[illegible] [illegible] v ch[illegible] [illegible] variable region, where the
leucin[illegible] [illegible] cha[illegible] [illegible]mp[illegible] 35 This binding region is
attach[illegible] [illegible]ld t[illegible] [illegible]n[illegible] [illegible]ng CH2, CH3 and CH4 as
descri[illegible] [illegible]mp[illegible] [illegible] [illegible]rovided in SEQ ID NO __,
and th[illegible] [illegible]pol[illegible] [illegible] ___

C[illegible] \CH2 [illegible]CH3

his [illegible] [illegible] [illegible]ain Fv binding region with
a poi[illegible] [illegible]t[illegible] [illegible] [illegible] variable region, where the
leucin[illegible] [illegible] ch[illegible] [illegible] p [illegible]5 This binding region is
attach[illegible] onne[illegible] desc[illegible]bed in Example 5 This
conne[illegible] [illegible]n [illegible] consisting of a wild type
CH2 r[illegible] [illegible] n [illegible] of 4 amino acid residues
prior [illegible] [illegible] 'ynucleotide sequence is
prov[illegible] ID [illegible]e is provided in SEQ ID
NO _

EXAMPLE 103

HD37 scFv IgAH WCH2 WCH3

The HD37 scFv was cloned from the HD37 hybridoma using the Novagen – Ig family primer sets, TOPO cloning and sequencing and sewing PCR assay For the mutal PCR reactions prior to sewing, the TOPO clone templates HD37 VH C-1 and HD37 KVL B-9 were used at 1 100 with an amplification profile of 94C 30 sec, 55C, 30 sec,72C, 30 seconds for 25 cycles. To provide templates for the secondary SEWING PCR reactions, primary reaction products were gel purified, QIAQUICK purified, and the eluates diluted 50 fold One microliter each VL and VH overlapping templates were added to PCR reactions with the following amplification profile 94C, 60 sec, 55C, 60 sec, 72C, 60 sec, for 30 cycles. After two cycles, the machine was paused, and the flanking 5'VL and 3'VH primers were added to the reactions at 25 pmol each, and the PCR reactions resumed PCR products were checked on a gel for the presence of an 750-800 bp fragment, and the reactions products QIAQUICK purified and digested with the appropriate restriction enzymes for insertion into pD18 Ig expression vectors

PCR of VL domain with native leader peptide and part of glyser linker

5' primer 5'-gttgttaagcttgccgccatggagacagacacactcctgctatgg-3' (SEQ ID NO)

3' primer 5'gccaccgacccaccaccgcccgagccaccgccaccttigatttccagcttggtgcctcc-3' (SEQ ID NO)

PCR of VL domain without leader peptide (SalI site) and part of glyser linker

5' primer 5'-gttgttgtcgacattgtgctgacccaatctcca-3' (SEQ ID NO)

3' prismr 5'-gccaccgacccaccaccgcccgagccaccgccaccttigatttccagcttggtgcctcc-3' (SEQ ID NO)

PCR of VH domain with part of glyser linker and BclI site for fusion to –Ig tails

5'primer 5'-tcgggcggtggtgggtcgggtggcggcggatcgtcacaggttcagctgcagcagtctgg-3' (SEQ ID NO)

3' primer 5'-tcagtgctgatcagaggagacggtgactgaggttccttg-3' (SEQ ID NO)

This binding region is connected to a wild type human IgA connecting region and wild type human IgG CH2 and CH3 constant regions as described in Example 5 This connecting region is attached to wild type human IgG1 CH2 and CH3 constant

regions as described in Example 1 This construct has previously been referred to as HD37 scFv-IgAHWTG1C and HD37- IgAHWTG1C, which both have the same sequence as the above construct. The polynucleotide sequence is provided in SEQ ID NO __, and the encoded polypeptide sequence is provided in SEQ ID NO ___

EXAMPLE 104

HD37 scFv (SSS-S) H WCH2 WCH3

This construct has a HD 37 single chain Fv as described in Example 103 This binding region is connected to a mutated human IgG1 connecting region where all of the cysteines and one proline have been changed to serines (SSS-S) according to methods described in Example 5 This connecting region is attached to wild type human IgG1 CH2 and CH3 constant regions as described in Example 1 This construct has previously been referred to as HD37- MHWTG1C and HD37 scFv-IgMHWTG1C, which both have the same sequence as the above construct. The polynucleotide sequence is provided in SEQ ID NO __, and the encoded polypeptide sequence is provided in SEQ ID NO ___

EXAMPLE 105

HD37 scFv VH L11S (SSS-S) H WCH2 WCH3

This construct has a HD 37 single chain Fv with a mutation in the heavy chain variable region at amino acid residue 11, where leucine has been changed to serine according to the methods described in Example 50 This binding region is connected to a mutated human IgG1 connecting region where all of the cysteines and one proline have been changed to serines (SSS-S) according to methods described in Example 5 This connecting region is attached to wild type human IgG1 CH2 and CH3 constant regions as described in Example 1 The polynucleotide sequence is provided in SEQ ID NO __, and the encoded polypeptide sequence is provided in SEQ ID NO ___

EXAMPLE 106

L6 scFv IGAH WCH2 WCH3

The L6 scFv was cloned from the L6 hybridoma (I Hellstrom) using the anchor-tailing method described in the Tissue Antigens Paper from 1996 The PCR profile was 94C, 1 min, 50C, 2 min, 72C, 2 min, for 35 cycles Once consensus sequence was obtained for VL and VH regions from at least 4 TOPO clones, primers were ordered for PCR reactions prior to SEWING PCR reactions as follows For the initial PCR reactions

prior to sewing, the TOPO cloned templates L6VK and L6VH were used at 1 100 with an amplification profile of 94C 30 sec; 55C, 30 sec;72C, 30 seconds for 25 cycles. To provide templates for the secondary SEWING PCR reactions, primary reaction products were gel purified, QIAQUICK purified, and the eluates diluted 50 fold. One microliter each VL and VH overlapping templates were added to PCR reactions with the following amplification profile 94C, 60 sec, 55C, 60 sec, 72C, 60 sec, for 30 cycles. After two cycles, the machine was paused, and the flanking 5'VL and 3'VH primers were added to the reactions at 25 pmol each, and the PCR reactions resumed. PCR products were checked on a gel for the presence of an 750-800 bp fragment, and the reactions products QIAQUICK purified and digested with the appropriate restriction enzymes for insertion into pD18 Ig expression vectors

PCR of VL domain with native leader peptide and part of glyser linker

L6VLHindIII 5'-gttgttaagcttgccgccatggattttcaagtgcagattttcagcttc-3' (SEQ ID NO)

L6VLLK3 5'-gccaccccgacccaccaccgcccgagccaccgccaccagagagctctttcagctccagcttggt-3' (SEQ ID NO)

PCR of VL domain without leader peptide (SalI site) and part of glyser linker

5' primer 5'-gttgttgtcgacattgttctctcccagtctccagcaatcctgtctg-3' (SEQ ID NO)

3' primer 5'-gccaccccgacccaccaccgcccgagccaccgccaccagagagctctttcagctccagcttggt-3' (SEQ ID NO)

PCR of VH domain with part of glyser linker and BclI site for fusion to –Ig tails

5' 5'-tcgggcggtggtgggtcgggtggcggcggatctctgcagatccagttggtgcagtct-3' (SEQ ID NO)

3'Bcl 5'-tcagtgctgatcagaggagactgtgagagtggtgccttg-3' (SEQ ID NO)

This binding region is connected to a human IgA connecting region and wild type human IgG1 CH2 and CH3 constant regions as described in Example 5 This connecting region is attached to wild type human IgG1 CH2 and CH3 constant regions as described in Example 1 This construct has previously been referred to as L6 scFv-IgAHWTG1C, which HAS the same sequence as the above construct. The polynucleotide sequence is provided in SEQ ID NO __, and the encoded polypeptide sequence is provided in SEQ ID NO ___

EXAMPLE 107

L6 scFv VHL11S (SSS-S) H WCH2 WCH3

This construct has a L6 single chain Fv with a mutation in the heavy chain variable region at amino acid residue 11, where leucine has been changed to serine according to the methods described in Example 49 This binding region is connected to a mutated human IgG1 connecting region where all of the cysteines and one proline have been changed to serines (SSS-S) according to methods described in Example 5 This connecting region is attached to wild type human IgG1 CH2 and CH3 constant regions as described in Example 1 The polynucleotide sequence is provided in SEQ ID NO __, and the encoded polypeptide sequence is provided in SEQ ID NO ___

EXAMPLE 108

2H7 scFv-LLAMA IgG1

This construct has a 2H7 (anti-CD20) single chain Fv binding region as described in Example 1 This binding region is attached to a llama IgG1 hinge, CH2 and CH3 regions according to the methods described in Example 10 The polynucleotide sequence is provided in SEQ ID NO __, and the encoded polypeptide sequence is provided in SEQ ID NO ___

EXAMPLE 109

2H7 scFv-LLAMA IgG2

This construct has a 2H7 (anti-CD20) single chain Fv binding region as described in Example 1 This binding region is attached to a llama IgG2 hinge, CH2 and CH3 regions according to the methods described in Example 10 The polynucleotide sequence is provided in SEQ ID NO __, and the encoded polypeptide sequence is provided in SEQ ID NO ___

EXAMPLE 110

2H7 scFv-LLAMA IgG3

This construct has a 2H7 (anti-CD20) single chain Fv binding region as described in Example 1 This binding region is attached to a llama IgG3 hinge, CH2 and CH3 regions according to the methods described in Example 10 The polynucleotide sequence is provided in SEQ ID NO __, and the encoded polypeptide sequence is provided in SEQ ID NO ___

EXAMPLE 111

CD16 LOW (ED)(SSS-S) H P238SCH2 WCH3

This construct has the extra cellular, CD16 low affinity allele binding domain as described in Example 43 This binding region is connected to a mutated human IgG1 connecting region where all of the cysteines and one proline have been changed to serines (SSS-S) according to methods described in Example 5 This hinge region is attached to a mutated human IgG1 CH2 region and a wild type human IgG1 CH3 region The P238S mutation, where a proline at residue 238 was changed to a serine, was introduced according to methods described in Example 70 The polynucleotide sequence is provided in SEQ ID NO __, and the encoded polypeptide sequence is provided in SEQ ID NO ___

EXAMPLE 112

CD16-9 HIGH (ED)(SSS-S) H P238SCH2 WCH3

This construct has the extra cellular, CD16 high affinity allele binding domain as described in Example 43 This binding region is connected to a mutated human IgG1 connecting region where all of the cysteines and one proline have been changed to serines (SSS-S) according to methods described in Example 5 This hinge region is attached to a mutated human IgG1 CH2 region and a wild type human IgG1 CH3 region. The P238S mutation, where a proline at residue 238 was changed to a serine, was introduced according to methods desribed in Example 70 The polynucleotide sequence is provided in SEQ ID NO __, and the encoded polypeptide sequence is provided in SEQ ID NO ___

Example 113

2e12 scFv (SSS-S)H P238SCH2 WCH3—hCD80TM/CT

This construct has a 2e12 (anti-CD28) single chain Fv binding region described in Example 12 This binding region is connected to a mutated human IgG1 connecting region where all of the cysteines and one proline have been changed to serines (SSS-S) according to methods described in Example 5 This connecting region is attached to a mutated human IgG1 CH2 region and a wild type human IgG1 CH3 region The P238S mutation, where a proline at residue 238 was changed to a serine, was introduced according to methods described in Example 70 The CH3 region is attached to a human

CD80 transmembrane and cytoplasmic tail region (hCD80 TM/CT). The hCD80 TM/CT was cloned using random primed cDNA derived from the BJAB cell line according to methods described in Example 12 This TM/CT region was attached to a Ig CH3 region with an open reading frame (ORF) Open reading frame versions of scFvIg constructs of interest were created by replacement of the soluble versions of each –Ig tail with ORF (open reading frame versions) of these tails. PCR primers were designed for the existing clones of soluble –Ig tails which delete the stop codon and add one or more restriction sites to the 3' end of the new –Ig cassettes. The desired transmembrane and cytoplasmic tail sequences can then be subcloned downstream of these new –Ig cassettes. Each construct utilized the existing available 5' BCLI oligonucleotide used in amplifying the soluble version of the tails for the PCR reactions The 3' oligonucleotides replace the stop codon with out of frame restriction sites fused to the coding region for protein domains involved in regulation of apoptosis

The PCR amplifications were carried out with 25 pmol of each primer, standard PCR reagents, and varying volumes of either cloned domains or cDNA obtained from PBMC, spleen, or thymus RNA. The reactions used a cycling profile of 94C, 60sec, 55C, 60 sec, 72C, 2 min, for 35 cycles The primers for the IgG ORF are listed below

5' primer 5'-
gttgtagatcaggagcccaaatcttctgacaaaactcacacatctccaccgtccccagcacctgaactcctggg
ggaccgtcagtcttcc-3'(SEQ ID NO)

3' primer 5'-gttgttttcgaaggatccgctttacccgggagcagggagaggctcttctgcgtgtagtg-3' (SEQ ID NO)

The polynucleotide sequence is provided in SEQ ID NO__, and the encoded polypeptide sequence is provided in SEQ ID NO ___

EXAMPLE 114

10A8 scFv (SSS-S)H P238SCH2 WCH3—hCD80TM/CT

This construct has a 10A8 (anti-CD2152) single chain Fv binding region described in Example 12 This binding region is connected to a mutated human IgG1 connecting region where all of the cysteines and one proline have been changed to serines (SSS-S) according to methods described in Example 5 This hinge region is attached to a mutated human IgG1 CH2 region and a wild type human IgG1 CH3 region. The P238S mutation, where a proline at residue 238 was changed to a serine, was introduced according

to methods described in Example 70 This CH3 region is attached to a hCD80 TM/CT according to methods described in Example 113 The polynucleotide sequence is provided in SEQ ID NO __, and the encoded polypeptide sequence is provided in SEQ ID NO ___

EXAMPLE 115

40.2.220 scFv (SSS-S)H P238SCH2 WCH3—hCD80TM/CT

This construct has a 40 2.220 (anti-CD40) single chain Fv binding region described in Example 12 This binding region is connected to a mutated human IgG1 connecting region where all of the cysteines and one proline have been changed to serines (SSS-S) according to methods described in Example 5 This hinge region is attached to a mutated human IgG1 CH2 region and a wild type human IgG1 CH3 region. The P238S mutation, where a proline at residue 238 was changed to a serine, was introduced according to methods described in Example 70 This CH3 region is attached to a hCD80 TM/CT according to methods described in Example 113 The polynucleotide sequence is provided in SEQ ID NO __, and the encoded polypeptide sequence is provided in SEQ ID NO ___

EXAMPLE 116

2H7 scFv (SSS-S)H P238SCH2 WCH3—hCD80TM/CT

This construct has a 2H7 (anti-CD20) single chain Fv binding region as described in Example 1 This binding region is connected to a mutated human IgG1 connecting region where all of the cysteines and one proline have been changed to serines (SSS-S), according to methods described in Example 5 This hinge region is attached to a mutated human IgG1 CH2 region and a wild type human IgG1 CH3 region. The P238S mutation, where a proline at residue 238 was changed to a serine, was introduced according to methods described in Example 70 This CH3 region is attached to a hCD80 TM/CT according to methods described in Example 113 This construct has previously been referred to as The polynucleotide sequence is provided in SEQ ID NO __, and the encoded polypeptide sequence is provided in SEQ ID NO ___

EXAMPLE 117

G19-4 scFv (SSS-S)H P238SCH2 WCH3—hCD80TM/CT

This construct has a G19 (anti-CD3) single chain Fv binding region described in Example 29 This binding region is connected to a mutated human IgG1 connecting region where all of the cysteines and one proline have been changed to serines

(SSS-S) according to methods described in Example 5 This hinge region is attached to a mutated human IgG1 CH2 region and a wild type human IgG1 CH3 region The P238S mutation, where a proline at residue 238 was changed to a serine, was introduced according to methods described in Example 70 This CH3 region is attached to a hCD80 TM/CT according to methods described in Example 113 The polynucleotide sequence is provided in SEQ ID NO __, and the encoded polypeptide sequence is provided in SEQ ID NO ___

EXAMPLE 118

2E12 scFv (SSS-S)H WCH2 WCH3—hCD80TM/CT

This construct has a 2e12 (anti-CD28) single chain Fv binding region described in Example 12 This binding region is connected to a mutated human IgG1 connecting region where all of the cysteines and one proline have been changed to serines (SSS-S) according to methods described in Example 5 This connecting region is attached to wild type human IgG1 CH2 and CH3 constant regions as described in Example 1 This CH3 region is attached to a hCD80 TM/CT according to methods described in Example 113 This construct has previously been referred to as 2e12 scFv IgG WTH WHTCH3CH2-CD80, which has the same sequence as the above construct. The polynucleotide sequence is provided in SEQ ID NO __, and the encoded polypeptide sequence is provided in SEQ ID NO ___

EXAMPLE 119

2E12 scFv IgAH IgACH2 T4CH3—hCD80TM/CT

This construct has a 2e12 (anti-CD28) single chain Fv binding region described in Example 12 This binding region is attached to a connecting region from human IgA as described in Example 5 This connecting region is attached to a human IgA constant region consisting of a wild type CH2 region and a mutated CH3 region where there is a truncation of 4 amino acid residues prior to the 3' stop codon as described in Example 13 This CH3 region is attached to a hCD80 TM/CT according to methods described in Example 113 The specific primers used to create an IgA ORF are listed below

5'primer 5'-gttgttgatcagccagttccctcaactccacctacc-3' (SEQ ID NO)

3' primer 5'-gttgtttcgaaggatccgcgtccacctccgccatgacaacaga (SEQ ID NO)

The polynucleotide sequence is provided in SEQ ID NO__, and the encoded polypeptide sequence is provided in SEQ ID NO ___

EXAMPLE 120

2E12 scFv IgE CH2CH3CH4—hCD80TM/CT

This construct has a 2e12 (anti-CD28) single chain Fv binding region described in Example 12 This binding region is attached to a human IgE constant region containing CH2, CH3 and CH4 as described in Example 38 This CH4 region is attached to a hCD80 TM/CT essentially according to methods described in Example 113 The specific primers used to create an IgE ORF are listed below

5' primer 5'-gttgttgatcacgtctgctccagggacttcacc-3' (SEQ ID NO)

3'primer: 5'-gttgttttcgaaggatccgctttaccagatttacagacaccgctcgctg-3' (SEQ ID NO)

The polynucleotide sequence is provided in SEQ ID NO__, and the encoded polypeptide sequence is provided in SEQ ID NO ___

EXAMPLE 121

2E12 scFv (SSS-S)H P238SCH2 WCH3—mFADD-TM/CT

This construct has a 2e12 (anti-CD28) single chain Fv binding region described in Example 12 This binding region is connected to a mutated human IgG1 connecting region where all of the cysteines and one proline have been changed to serines (SSS-S) according to methods described in Example 5 This hinge region is attached to a mutated human IgG1 CH2 region and a wild type human IgG1 CH3 region. The P238S mutation, where a proline at residue 238 was changed to a serine, was introduced according to methods described in Example 70 The CH3 region is attached to a mouse FADD transmembrane and cytoplasmic tail region (mFADD TM/CT) This region is cloned using essentially the same methods described in Example 113 The domain was PCR amplified from randomly primed cDNA from mouse spleen RNA. The specific primers are listed below

5' primer 5'-gttgtggatccttcgaacccattcctggtgctgctgcactcgctg-3' (SEQ ID NO)

3' primer 5'-gttgttatcgatctcgagtcagggtgtttctgaggaagacacagt-3' (SEQ ID NO)

The specific primers used to create a mouse IgG ORF are listed below

5'primer 5'-gttgtagatctggagcccagagggcccacaatcaagccctctcctccaagcaaaagccca-3' (SEQ ID NO)

3'primer 5'-gttgttttcgaaggatccgctttacccggagtccgggagaag-3' (SEQ ID NO)

The polynucleotide sequence is provided in SEQ ID NO__, and the encoded polypeptide sequence is provided in SEQ ID NO___

EXAMPLE 122

2E12 scFv (SSS-S)H WCH2 WCH3—mFADD-TM/CT

This construct has a 2e12 (anti-CD28) single chain Fv binding region described in Example 12. This binding region is connected to a mutated human IgG1 connecting region where all of the cysteines and one proline have been changed to serines (SSS-S) according to methods described in Example 5 This connecting region is attached to wild type human IgG1 CH2 and CH3 constant regions as described in Example 1 The CH3 region was attached to a mFADD TM/TM region according to methods described in Example 113 and 121 The polynucleotide sequence is provided in SEQ ID NO__, and the encoded polypeptide sequence is provided in SEQ ID NO___

EXAMPLE 123

2E12 scFv (SSS-S)H WCH2 WCH3—mCASP3-TM/CT

This construct has a 2e12 (anti-CD28) single chain Fv binding region described in Example 12 This binding region is connected to a mutated human IgG1 connecting region where all of the cysteines and one proline have been changed to serines (SSS-S) according to methods described in Example 5 This connecting region is attached to wild type human IgG1 CH2 and CH3 constant regions as described in Example 1 The CH3 region is attached to a mouse casp3 transmembrane and ctyoplasmic tail region according to methods described in Examples 113 and 121 The specific primers used to isolate the mcasp3 TM/CT region are listed below

5' primer 5'-gttgttggatccttcgaacatggagaacaacaaaacctcagtggattca-3' (SEQ ID NO)

3' primer 5'-gttgttatcgatctcgagctagtgataaaagtacagttctttcgt-3' (SEQ ID NO)

The polynucleotide sequence is provided in SEQ ID NO__, and the encoded polypeptide sequence is provided in SEQ ID NO___

EXAMPLE 124

2E12 scFv (SSS-S)H P238SCH2 WCH3—mCASP3-TM/CT

This construct has a 2e12 (anti-CD28) single chain Fv binding region described in Example 12 This binding region is connected to a mutated human IgG1

connecting region where all of the cysteines and one proline have been changed to serines (SSS-S) according to methods described in Example 5 This hinge region is attached to a mutated human IgG1 CH2 region and a wild type human IgG1 CH3 region The P238S mutation, where a proline at residue 238 was changed to a serine, was introduced according to methods described in Example 70 The CH3 region is connected to a mcasp3 TM/CT region according to methods described in Examples 113, 121 and 123 The polynucleotide sequence is provided in SEQ ID NO __, and the encoded polypeptide sequence is provided in SEQ ID NO ___

EXAMPLE 125

2E12 scFv (SSS-S)H WCH2 WCH3—MCASP8–TM/CT

This construct has a 2e12 (anti-CD28) single chain Fv binding region described in Example 12 This binding region is connected to a mutated human IgG1 connecting region where all of the cysteines and one proline have been changed to serines (SSS-S) according to methods described in Example 5 This connecting region is attached to wild type human IgG1 CH2 and CH3 constant regions as described in Example 1 The CH3 region is attached to a mouse casp8 transmembrane and cytoplasmic tail region (mcasp8 TM/CT) essentially according to methods described in Example 113 and 121 The specific primers used to clone the mcasp8 TM/CT region are listed below

5' primer 5'-gttgtttcgaacatggatttccagagttgtcttttatgctattgctg-3' (SEQ ID NO)

3' primer 5'-gttgttatcgatctcgagtcattagggagggaagaagagcttcttccg-3' (SEQ ID NO)

The polynucleotide sequence is provided in SEQ ID NO __, and the encoded polypeptide sequence is provided in SEQ ID NO ___

EXAMPLE 126

2E12 scFv (SSS-S)H P238SCH2 WCH3—MCASP8-TM/CT

This construct has a 2e12 (anti-CD28) single chain Fv binding region described in Example 12 This binding region is connected to a mutated human IgG1 connecting region where all of the cysteines and one proline have been changed to serines (SSS-S) according to methods described in Example 5 This hinge region is attached to a mutated human IgG1 CH2 region and a wild type human IgG1 CH3 region. The P238S mutation, where a proline at residue 238 was changed to a serine, was introduced according to methods described in Example 70 The CH3 region was attached to a mcasp8 TM/CT

region according to methods described in Examples 113, 121 and 125 The polynucleotide sequence is provided in SEQ ID NO __, and the encoded polypeptide sequence is provided in SEQ ID NO ___

EXAMPLE 127

2E12 scFv (SSS-S)H WCH2 WCH3—HCASP3–TM/CT

This construct has a 2e12 (anti-CD28) single chain Fv binding region described in Example 12 This binding region is connected to a mutated human IgG1 connecting region where all of the cysteines and one proline have been changed to serines (SSS-S) according to methods described in Example 5 This connecting region is attached to wild type human IgG1 CH2 and CH3 constant regions as described in Example 1 The CH3 region was attached to a human casp3 transmembrane and cytoplasmic tail region (hcasp3 TM/CT) essentially according to methods describe in Examples 113 The specific primers used to clone the hcasp3 TM/CT region are listed below

5' Primer 5'-gttgtggatccttcgaacatggagaacactgaaaactcagtggat-3' (SEQ ID NO)

3' Primer 5'-gttgttatcgatctcgagttagtgataaaaatagagttctttttgtgag-3' (SEQ ID NO)

The polynucleotide sequence is provided in SEQ ID NO __, and the encoded polypeptide sequence is provided in SEQ ID NO ___

EXAMPLE 128

2E12 scFv (SSS-S)H P238SCH2 WCH3—HCASP3-TM/CT

This construct has a 2e12 (anti-CD28) single chain Fv binding region described in Example 12 This binding region is connected to a mutated human IgG1 connecting region where all of the cysteines and one proline have been changed to serines (SSS-S) according to methods described in Example 5 This hinge region is attached to a mutated human IgG1 CH2 region and a wild type human IgG1 CH3 region. The P238S mutation, where a proline at residue 238 was changed to a serine, was introduced according to methods described in Example 70 The CH3 region is attached to a hcasp3 TM/CT region according to methods described in Examples 113 and 127 The polynucleotide sequence is provided in SEQ ID NO __, and the encoded polypeptide sequence is provided in SEQ ID NO ___

EXAMPLE 129

2E12 scFv (SSS-S)H WCH2 WCH3—HCASP8–TM/CT

This construct has a 2e12 (anti-CD28) single chain Fv binding region described in Example 12 This binding region is connected to a mutated human IgG1 connecting region where all of the cysteines and one proline have been changed to serines (SSS-S) according to methods described in Example 5 This connecting region is attached to wild type human IgG1 CH2 and CH3 constant regions as described in Example 1 The CH3 region is attached to a human casp8 transmembrane and cytoplasmic tail region (hcasp8 TM/CT) according to methods described in Example 133 The specific primers used to clone the hcasp8 TM/CT region are listed below

5' Primer 5'-gttgtggatccttcgaacatggacttcagcagaaatctttatgat-3' (SEQ ID NO)

3' Primer 5'-gttgttatcgatgcatgctcaatcagaagggaagacaagtttttttct-3 (SEQ ID NO)

The polynucleotide sequence is provided in SEQ ID NO__, and the encoded polypeptide sequence is provided in SEQ ID NO ___

EXAMPLE 130

2E12 scFv (SSS-S)H P238SCH2 WCH3—HCASP8-TM/CT

This construct has a 2e12 (anti-CD28) single chain Fv binding region described in Example 12 This binding region is connected to a mutated human IgG1 connecting region where all of the cysteines and one proline have been changed to serines (SSS-S) according to methods described in Example 5 This hinge region is attached to a mutated human IgG1 CH2 region and a wild type human IgG1 CH3 region. The P238S mutation, where a proline at residue 238 was changed to a serine, was introduced according to methods described in Example 70 The CH3 region is attached to a hcasp8 TM/CT according to methods described in Examples 113 and 129 The polynucleotide sequence is provided in SEQ ID NO__, and the encoded polypeptide sequence is provided in SEQ ID NO ___

EXAMPLE 131

1D8 scFv (SSS-S)H P238SCH2 WCH3—HCD80TM/CT

This construct has a 1D8 (anti-4-1BB) single chain Fv binding region described in Example 25 This binding region is connected to a mutated human IgG1 connecting region where all of the cysteines and one proline have been changed to serines (SSS-S) according to methods described in Example 5 This hinge region is attached to a mutated human IgG1 CH2 region and a wild type human IgG1 CH3 region The P238S

mutation, where a proline at residue 238 was changed to a serine, was introduced according to methods described in Example 70 The CH3 region is attached to a hCD80 TM/CT region according to methods described in Example 113 The polynucleotide sequence is provided in SEQ ID NO:__, and the encoded polypeptide sequence is provided in SEQ ID NO ___

EXAMPLE 132

1D8 scFv (SSS-S)H WCH2 WCH3—hCD80TM/CT

This construct has a 1D8 (anti-4-1BB) single chain Fv binding region described in Example 25 This binding region is connected to a mutated human IgG1 connecting region where all of the cysteines and one proline have been changed to serines (SSS-S) according to methods described in Example 5 This connecting region is attached to wild type human IgG1 CH2 and CH3 constant regions as described in Example 1 The CH3 region is attached to a hCD80 TM/CT region according to methods described in Example 113 This construct has previously been refered to as 1D8 scFv IgG WTH WTCH2CH3-CD80, which has the same sequence as the above construct The polynucleotide sequence is provided in SEQ ID NO __, and the encoded polypeptide sequence is provided in SEQ ID NO ___

EXAMPLE 133

1D8 scFv—mIgAH WIgA CH2 T4CH3—hCD80TM/CT

This construct has a 1D8 (anti-4-1BB) single chain Fv binding region described in Example 25 This binding region is attached to a connecting region from human IgA as described in Example 5 This connecting region is attached to a mouse IgA constant region consisting of a wild type CH2 region and a mutated CH3 region where there is a truncation of 4 amino acid residues prior to the 3' stop codon as described in Example 39 The CH3 region can be attached to a hCD80 TM/CT region according to methods described in Examples 113 using primers that create an IgA ORF

EXAMPLE 134

1D8 scFv IgE CH2CH3CH4 -hCD80TM/CT

This construct has a 1D8 (anti-4-1BB) single chain Fv binding region described in Example 25 This binding region is attached to a human IgE constant region containing CH2, CH3 and CH4 as described in Example 38 The CH4 region is attached to

a hCD80 TM/CT according to methods described in Examples 113 and 120 The polynucleotide sequence is provided in SEQ ID NO __, and the encoded polypeptide sequence is provided in SEQ ID NO ___

EXAMPLE 135

1D8 scFv (SSS-S)H P238SCH2 WCH3—mFADD-TM/CT

This construct has a 1D8 (anti-4-1BB) single chain Fv binding region described in Example 25 This binding region is connected to a mutated human IgG1 connecting region where all of the cysteines and one proline have been changed to serines (SSS-S) according to methods described in Example 5 This hinge region is attached to a mutated human IgG1 CH2 region and a wild type human IgG1 CH3 region. The P238S mutation, where a proline at residue 238 was changed to a serine, was introduced according to methods described in Example 70 The CH3 region was attached to a mFADD TM/TM region according to methods described in Example 113 and 121 The polynucleotide sequence is provided in SEQ ID NO __, and the encoded polypeptide sequence is provided in SEQ ID NO ___

EXAMPLE 136

1D8 scFv (SSS-S)H WCH2 WCH3—mFADD-TM/CT

This construct has a 1D8 (anti-4-1BB) single chain Fv binding region described in Example 25 This binding region is connected to a mutated human IgG1 connecting region where all of the cysteines and one proline have been changed to serines (SSS-S) according to methods described in Example 5 This connecting region is attached to wild type human IgG1 CH2 and CH3 constant regions as described in Example 1 The CH3 region was attached to a mFADD TM/TM region according to methods described in Example 113 and 121 The polynucleotide sequence is provided in SEQ ID NO __, and the encoded polypeptide sequence is provided in SEQ ID NO ___

EXAMPLE 137

1D8 scFv (SSS-S)H WCH2 WCH3—mcasp3-TM/CT

This construct has a 1D8 (anti-4-1BB) single chain Fv binding region described in Example 25 This binding region is connected to a mutated human IgG1 connecting region where all of the cysteines and one proline have been changed to serines (SSS-S) according to methods described in Example 5 This connecting region is attached

to wild type human IgG1 CH2 and CH3 constant regions as described in Example 1 The CH3 region was attached to a mcasp3 TM/TM region according to methods described in Example 113, 121 and 123 The polynucleotide sequence is provided in SEQ ID NO __, and the encoded polypeptide sequence is provided in SEQ ID NO ___

EXAMPLE 138

1D8 scFv (SSS-S)H P238SCH2 WCH3—MCASP3-TM/CT

This construct has a 1D8 (anti-4-1BB) single chain Fv binding region described in Example 25 This binding region is connected to a mutated human IgG1 connecting region where all of the cysteines and one proline have been changed to serines (SSS-S) according to methods described in Example 5 This hinge region is attached to a mutated human IgG1 CH2 region and a wild type human IgG1 CH3 region. The P238S mutation, where a proline at residue 238 was changed to a serine, was introduced according to methods described in Example 70 The CH3 region was attached to a mcasp3 TM/TM region according to methods described in Example 113, 121 and 123 The polynucleotide sequence is provided in SEQ ID NO __, and the encoded polypeptide sequence is provided in SEQ ID NO ___

EXAMPLE 139

1D8 scFv (SSS-S)H WCH2 WCH3—MCASP8-TM/CT

This construct has a 1D8 (anti-4-1BB) single chain Fv binding region described in Example 25 This binding region is connected to a mutated human IgG1 connecting region where all of the cysteines and one proline have been changed to serines (SSS-S) according to methods described in Example 5 This connecting region is attached to wild type human IgG1 CH2 and CH3 constant regions as described in Example 1 The CH3 region was attached to a mcasp8 TM/TM region according to methods described in Example 113, 121 and 125 The polynucleotide sequence is provided in SEQ ID NO __, and the encoded polypeptide sequence is provided in SEQ ID NO ___

EXAMPLE 140

1D8 scFv (SSS-S)H P238SCH2 WCH3—MCASP8-TM/CT

This construct has a 1D8 (anti-4-1BB) single chain Fv binding region described in Example 25 This binding region is connected to a mutated human IgG1 connecting region where all of the cysteines and one proline have been changed to serines

(SSS-S) according to methods described in Example 5 This hinge region is attached to a mutated human IgG1 CH2 region and a wild type human IgG1 CH3 region. The P238S mutation, where a proline at residue 238 was changed to a serine, was introduced according to methods described in Example 70 The CH3 region was attached to a mcasp8 TM/TM region according to methods described in Example 113, 121 and 125 The polynucleotide sequence is provided in SEQ ID NO __, and the encoded polypeptide sequence is provided in SEQ ID NO ___

EXAMPLE 141

1D8 scFv (SSS-S)H WCH2 WCH3—HCASP3--TM/CT

This construct has a 1D8 (anti-4-1BB) single chain Fv binding region described in Example 25 This binding region is connected to a mutated human IgG1 connecting region where all of the cysteines and one proline have been changed to serines (SSS-S) according to methods described in Example 5 This connecting region is attached to wild type human IgG1 CH2 and CH3 constant regions as described in Example 1 The CH3 region is attached to a hcasp3 TM/CT according to methods described in Examples 113 and 127 The polynucleotide sequence is provided in SEQ ID NO __, and the encoded polypeptide sequence is provided in SEQ ID NO ___

EXAMPLE 142

1D8 scFv (SSS-S)H P238SCH2 WCH3—HCASP3-TM/CT

This construct has a 1D8 (anti-4-1BB) single chain Fv binding region described in Example 25 This binding region is connected to a mutated human IgG1 connecting region where all of the cysteines and one proline have been changed to serines (SSS-S) according to methods described in Example 5 This hinge region is attached to a mutated human IgG1 CH2 region and a wild type human IgG1 CH3 region. The P238S mutation, where a proline at residue 238 was changed to a serine, was introduced according to methods described in Example 70 The CH3 region is attached to a hcasp3 TM/CT according to methods described in Examples 113 and 127 The polynucleotide sequence is provided in SEQ ID NO __, and the encoded polypeptide sequence is provided in SEQ ID NO ___

EXAMPLE 143

1D8 scFv (SSS-S)H WCH2 WCH3—HCASP8--TM/CT

This construct has a 1D8 (anti-4-1BB) single chain Fv binding region described in Example 25 This binding region is connected to a mutated human IgG1 connecting region where all of the cysteines and one proline have been changed to serines (SSS-S) according to methods described in Example 5 This connecting region is attached to wild type human IgG1 CH2 and CH3 constant regions as described in Example 1 The CH3 region is attached to a hcasp8 TM/CT according to methods described in Examples 113 and 129 The polynucleotide sequence is provided in SEQ ID NO __, and the encoded polypeptide sequence is provided in SEQ ID NO ___

EXAMPLE 144

1D8 scFv (SSS-S)H P238SCH2 WCH3—HCASP8-TM/CT

This construct has a 1D8 (anti-4-1BB) single chain Fv binding region described in Example 25 This binding region is connected to a mutated human IgG1 connecting region where all of the cysteines and one proline have been changed to serines (SSS-S) according to methods described in Example 5 This hinge region is attached to a mutated human IgG1 CH2 region and a wild type human IgG1 CH3 region. The P238S mutation, where a proline at residue 238 was changed to a serine, was introduced according to methods described in Example 70 The CH3 region is attached to a hcasp8 TM/CT according to methods described in Examples 113 and 129 The polynucleotide sequence is provided in SEQ ID NO __, and the encoded polypeptide sequence is provided in SEQ ID NO ___

EXAMPLE 145

L6 scFv (SSS-S) H WCH2 WCH3

This construct has a L6 scFv binding domain as described in Example 105 This binding region is connected to a mutated human IgG1 connecting region where all of the cysteines and one proline have been changed to serines (SSS-S) according to methods described in Example 5 This connecting region is attached to wild type human IgG1 CH2 and CH3 constant regions as described in Example 1 This construct has previously been refered to as L6 scFv-IgMHWTG1C, which has the same sequence as the above construct. The polynucleotide sequence is provided in SEQ ID NO __, and the encoded polypeptide sequence is provided in SEQ ID NO ___

EXAMPLE 146

2H7 scFv CD154 (L2)

This construct has a 2H7 (anti-CD20) single chain Fv binding region as described in Example 1 This binding region has been attached to CD154 extracellular domain according to methods described in Example 4 The polynucleotide sequence is provided in SEQ ID NO __, and the encoded polypeptide sequence is provided in SEQ ID NO ___

EXAMPLE 147

2H7 scFv CD154 (S4)

This construct has a 2H7 (anti-CD20) single chain Fv binding region as described in Example 1 This binding region has been attached to CD154 extracellular domain according to methods described in Example 4, such that methods of attachment resulted in a truncated version compared to the construct describe in Example 146 The polynucleotide sequence is provided in SEQ ID NO __, and the encoded polypeptide sequence is provided in SEQ ID NO ___

EXAMPLE 148

CTLA4 IgAH IgACH2CH3

This construct has the extra cellular CTLA-4 binding region as described in Example 14. This binding region is attached to a wild type human IgA connecting region as described in Example 5 This connecting region is attached to a wild type human IgA CH2 and CH3 constant region according to methods described in Example 13 This constant region is attached to a J-chain region as described in Example 13 This construct has previously been refered to as CTLA-4 IgAH IgACH2CH3, which has the same sequence as the above construct. The polynucleotide sequence is provided in SEQ ID NO __, and the encoded polypeptide sequence is provided in SEQ ID NO ___

EXAMPLE 149

CTLA4 IgAH IgACH2 T4CH3

This construct has the extra cellular CTLA-4 binding region as described in Example 14 This binding region is attached to a connecting region from human IgA as described in Example 5 This connecting region is attached to a human IgA constant region consisting of a wild type CH2 region and a mutated CH3 region where there is a truncation of 4 amino acid residues prior to the 3' stop codon as described in Example 13 This

construct has previously been refered to as CTLA-4 IgAH IgA-T4, which has the same sequence as the above construct. The polynucleotide sequence is provided in SEQ ID NO __, and the encoded polypeptide sequence is provided in SEQ ID NO ___

EXAMPLE 150

2H7 scFv IGAH IGACH2CH3

This construct has a 2H7 (anti-CD20) single chain Fv binding region as described in Example 1 This binding region is attached to a wild type human IgA connecting region as described in Example 5 This connecting region is attached to a wild type human IgA CH2 and CH3 constant region according to methods described in Example 13 The polynucleotide sequence is provided in SEQ ID NO __, and the encoded polypeptide sequence is provided in SEQ ID NO ___

EXAMPLE 151

2H7 scFv IGAH IGAHCH2 T18CH3

This construct has a 2H7 (anti-CD20) single chain Fv binding region as described in Example 1 This binding region is attached to a connecting region from human IgA as desribed in Example 5 This connecting region is attached to a human IgA constant region consisting of a wild type CH2 region and a mutated CH3 region where there is a truncation of 18 amino acid residues prior to the 3' stop codon as described in Example 13 The polynucleotide sequence is provided in SEQ ID NO __, and the encoded polypeptide sequence is provided in SEQ ID NO ___

EXAMPLE 152

2H7-40.2.220 scFv (SSS-S) H WCH2 WCH3

A bispecific fusion protein was constructed between 2H7 scFv (anti-CD20) and 40 2 220 (anti-CD40), which both target B cell receptors The 2H7 scFv hIgG1 (SSS-S)H WCH2 WCH3 construct in the expression vector pD18 was passaged through dam bacteria in order to permit cleavage at the BclI site Cleaved plasmid was treated with alkaline phosphatase prior to ligation to a BclI cut linker-CD40 scFv fragment. This fragment was synthesized from the existing 40 2 220 scFv by successive PCR reactions with overlapping primers The linker attached is a previously patented (BMS patent issued) helical type linker with a high number of lysine and glutamic acid residues The scFv for CD40 was PCR amplified without the leader peptide as a SalI-BclI fragment, but

tail included in an existing scFvIg construct for CD20 (2H7 scFv hIgG1 constructs) The 3 end was similar to the other scFv VH molecules with an out of frame BclI site fused to the VTVSS type sequence at the end of the VH domain.

PCR oligos

40.2.220 scFv

5' primer--40.2.220S5 5'-gttgttgtcgacattgttctgactcagtctccagccaccctgtc-3' (SEQ ID NO)

3' primer--40 2 220Bcl3 5'-gttgttgatcagagacagtgaccagtgtccttgg-3' (SEQ ID NO)

Linker Primers

BclI-SalI fragment created by annealing complementary oligonucleotides This fragment is then ligated into BclI digested vector along with a SalI-BclI scFv to create the (linker-scFv) BclI fragment desired for shuttling.

5' primer 5'-gatcaatccaactctgaagaagcaaagaaagaggaggccaaaaaggaggaagccaagaaatctaacagcg-3' (SEQ ID NO)

3' primer 5 -tcgacgctgttagatttcttggcttcctcctttttggcctccctctttcttgcttcttcagagttggatt-3' (SEQ ID NO)

This BclI fragment was then ligated downstream of the 2H7 scFv in pD18-Ig. Transformants were screened for the presence of a 2.4 kb HindIII-Xba insert and positive clones sequenced prior to further studies COS cell transient transfections were performed with this construct and culture supernatants screened for the presence of protein of the predicted size and for binding to CD20 and to CD40 transfected CHO cells

New bispecific constructs can be created by designing hinge type linkers which incorporate one or preferably two restriction sites at either end of the linker, facilitating asymmetric digests and transfer of (linker-scFv) or (scFv-linker) cassettes between different constructs These constructs will also incorporate the VH L11S and other V region substitutions, which presumably facilitate proper folding and result in increased expression of the molecules in which they are inserted.

This binding region is connected to a mutated human IgG1 connecting region where all of the cysteines and one proline have been changed to serines (SSS-S) according to methods described in Example 5 This connecting region is attached to wild type human IgG1 CH2

same sequence as the above construct. The polynucleotide sequence is provided in SEQ ID NO __, and the encoded polypeptide sequence is provided in SEQ ID NO ___

EXAMPLE 153

2H7 scFv IGAH IGACH2 T4CH3-HCD80 TM/CT

This construct has a 2H7 (anti-CD20) single chain Fv binding region as described in Example 1 This binding region is attached to a connecting region from human IgA as described in Example 5 This connecting region is attached to a human IgA constant region consisting of a wild type CH2 region and a mutated CH3 region where there is a truncation of 4 amino acid residues prior to the 3' stop codon as described in Example 13 This CH3 region is attached to a hCD80 TM/CT according to the methods described in Examples 113 and 119 This construct has previously been referred to as 2H7 scFv IgA hinge IgA-T4-CD80 and 2H7 scFv IgAH IgA-T4-CD80, which both have the same sequence as the above construct. The polynucleotide sequence is provided in SEQ ID NO __, and the encoded polypeptide sequence is provided in SEQ ID NO ___

EXAMPLE 154

G19-4 scFv (CCC-P) WH WCH2 WCH3-HCD80 TM/CT

This construct has a G19 (anti-CD3) single chain Fv binding region described in Example 29 This binding region is attached to a wild type human IgG1 connecting region (CCC-P) as described in Example 1 This connecting region is attached to wild type human IgG1 CH2 and CH3 constant regions as described in Example 1 This CH3 region is attached to a hCD80 TM/CT according to the methods described in Examples 113 The polynucleotide sequence is provided in SEQ ID NO __, and the encoded polypeptide sequence is provided in SEQ ID NO ___

EXAMPLE 155

2E12 scFv (CCC-P) WH WCH2 WCH3-HCD80 TM/CT

This construct has a 2e12 (anti-CD28) single chain Fv binding region described in Example 12 This binding region is attached to a wild type human IgG1 connecting region (CCC-P) as described in Example 1 This connecting region is attached

to wild type human IgG1 CH2 and CH3 constant regions as described in Example 1 This CH3 region is attached to a hCD80 TM/CT according to the methods described in Examples 113 The polynucleotide sequence is provided in SEQ ID NO __, and the encoded polypeptide sequence is provided in SEQ ID NO ___

EXAMPLE 156

2H7 VHL11S scFv (SSS-S) IgE.CH3CH4

This construct has a 2H7 (anti-CD20) single chain Fv binding region with a point mutation at amino acid residue 11 in the heavy chain variable region, where the leucine has been changed to a serine, as described in Example 33 This binding region is connected to a mutated human IgG1 connecting region where all of the cysteines and one proline have been changed to serines (SSS-S) according to methods described in Example 5 This connecting region is attached to human IgE CH3 and CH4 constant region. This truncated constant region was created according to the methods described in Example 38 The polynucleotide sequence is provided in SEQ ID NO __, and the encoded polypeptide sequence is provided in SEQ ID NO ___

EXAMPLE 157

IGD HINGE

An alternative hinge region can be isolated from human IgD immunoglobulin hinge region by using PCR assay to isolate the desired region. The PCR reaction is the same used in Example 1 This hinge was truncated by 6 amino acid residues at the 3' end. The primers used in this PCR reaction are listed below

5" Primer 5'-GTGGATCCAGGTTCGAAGTCTCCAAAGGCACAGGCC-3' (SEQ ID NO)

3' primer 5'-GTTGTCGACTGCACCGGTCTTTGTCTCTCTCTCTTC-3' (SEQ ID NO)

The polynucleotide sequence is provided in SEQ ID NO:__, and the encoded polypeptide sequence is provided in SEQ ID NO ___

EXAMPLE 158

HCD28 TM/CT

For some of the cell surface ORF constructs, the transmembrane domain of CD80 was substituted with the transmembrane domain of human CD28 because it forms a

dimer on the cell surface rather than a monomer as the CD80 does Several of the molecules which drive the apoptotic program require oligomerization/trimerization to form a signaling complex, therefore, it is important to be able to control initiation of signaling by controlling the degree of oligomerization of these recombinant receptors on the cell surface

The primers used in PCR amplification of the CD28 tail are given below

5' Primer 5'-gttgtggatcctcgaaccccttttgggtgctggtggtggttggtggga-3' (SEQ ID NO)

3' primer 5'-gttgttatcgatctcgagtcaggagcgataggctgcgaagtc-3' (SEQ ID NO)

The polynucleotide sequence is provided in SEQ ID NO __, and the encoded polypeptide sequence is provided in SEQ ID NO ___

EXAMPLE 159

2H7 scFv VH L11S (SSS-S) H K322L CH2 WCH3

This construct has a 2H7 (anti-CD20) single chain Fv binding region with a point mutation at amino acid residue 11 in the heavy chain variable region, where the leucine has been changed to a serine. as described in Example 33 This binding region is connected to a mutated human IgG connecting region where all of the cysteines ,and one proline have been changed to serines (SSS-S) according to methods described in Example 5 The connecting region is attached to a mutated IgG CH2 region and a wild type IgG CH3 region. The K322L mutation in the CH2 region is at a residue 322, where a Lysine has been changed to a leucine using overlapping PCR described in Example 56, but with different primers for the first PCR reaction, which are listed below

5' primer 5'ttctctttccccccaaaacccaaggacaccctcatgatctcccggaaccctgaggtcac-3' (SEQ ID NO)

3' primer 5'-ggacagtgggagtggcacc-3' (SEQ ID NO)

PCR product was cloned into TOPO vector and sequenced. The polynucleotide sequence is provided in SEQ ID NO __, and the encoded polypeptide sequence is provided in SEQ ID NO ___

EXAMPLE 160

2H7 scFv VH L11S (CSS-S) H K322L CH2 WCH3

This construct has a 2H7 (anti-CD20) single chain Fv binding region with a point mutation at amino acid residue 11 in the heavy chain variable region, where the

leucine has been changed to a serine, as described in Example 33 This binding region is attached to a mutant IgG connecting region, where the second and third cysteines have been changed to serines and the proline has been changed to serine (CSS-S), according to methods described in Example13 The connecting region is attached to a mutated IgG CH2 region and a wild type IgG CH3 region. The K322L mutation in the CH2 region is at a residue 322, where a Lysine has been changed to a leucine using overlapping PCR described in Example 56, using primers from Example 159 in the first PCR reaction and primers from Example 57 for the second PCR reaction.

PCR products were cloned into the TOPO vector and sequenced. The polynucleotide sequence is provided in SEQ ID NO__, and the encoded polypeptide sequence is provided in SEQ ID NO___

EXAMPLE 161

IN VIVO B CELL DEPLETION BY 2H7 scFv (SSS-S) H WCH2 WCH3 AND 2H7 scFv (SSS-S) H P238SCH2 WCH3

This Example describes experiments relating to ADCC effector mechanisms in the depletion of B cells through comparison of the activities of an anti-CD20 construct (2H7 scFv (SSS-S) H WCH2 WCH3) and a anti-CD20 construct with a proline to serine amino acid substitution in the CH2 domain (2H7 scFv (SSS-S) H P238SCH2 WCH3) Figure 72 shows the results of an experiment on apoptosis induction by these constructs The results indicated that both molecules bind CD20 and induce apoptosis that is mediated by activation of caspase 3

Figure 73 illustrates that anti-CD20 constructs mediate CDC activity towards CD20 positive target cells A 2H7 scFv (SSS-S) H WCH2 WCH3 construct, a 2H7 scFv (SSS-S) H P238SCH2 WCH3 construct, or Rituximab were incubated at increasing concentrations with 10^4 Bjab Target Cells and a 1 10 dilution of rabbit complement (PelFreez) in a volume of 100 microliters for sixty minutes Aliquots were stained with trypan blue (Invitrogen), and counted using a hemacytometer to determine the percentage of the cell population killed during treatment Negative controls with cells and only one reagent were also included Figure 74 illustrates that the 2H7 scFv (SSS-S) H WCH2 WCH3 construct was effective in ADCC with peripheral blood mononuclear cells ADCC activity of 2H7 scFv (SSS-S) H WCH2 WCH3 or Rituximab was measured *in vitro* against BJAB B

lymphoma cell line as the target cells, and using fresh human PBMC as effector cells Effector to target ratios were varied as follows 100 1, 50 1, and 25 1, with the number of BJAB cells per well remaining constant but varying the number of PBMC. BJAB cells were labeled for 2 hours with ^{51}Cr and aliquoted at a cell density of $5x10^4$ cells/well to each well of flat-bottom 96 well plates Purified fusion proteins or Rituximab were added at a concentration of 10 μg/ml, and PBMC were added at 1.25 x 10^6 cells /well (25 1), 2 5 x 10^6 cells/well (50·1), or 5 x 10^6 cells/well (100 1), in a final volume of 200 μl. Natural killing was measured at each effector·target ratio by omission of the construct or MAb Spontaneous release was measured without addition of PBMC or fusion protein, and maximal release was measured by the addition of detergent (1% NP-40) to the appropriate wells. Reactions were incubated for 5 hours, and 100 μl culture supernatant harvested to a Lumaplate (Packard Instruments) and allowed to dry overnight prior to counting cpm released on a Packard Top Count NXT Microplate Scintillation Counter Figure 75 illustrates that the 2H7 scFv (SSS-S) H WCH2 WCH3 construct bound soluble CD16 fusion protein (constructed from either high or low affinity alleles (158V/F)), while the 2H7 scFv (SSS-S) H P238SCH2 WCH3 construct did not show detectable binding. CD20 CHO cells (10^6) were incubated with saturating amounts of 2H7 scFv (SSS-S) H WCH2 WCH3 or 2H7 scFv (SSS-S) H P238SCH2 WCH3 (10 ug/ml) for one hour on ice in PBS/2% FBS Cells were washed in PBS/2% FBS and incubated with serial dilutions of 0 5 mg/ml FITC-CD16 for one hour on ice. Cells were washed and specific binding measured by flow cytometry using a Beckman-Coulter Epics C machine. Results were analyzed using Expo analysis software and normalized fluorescence units graphed as a function of concentration. Figure 76 illustrates an experiment on the ability of 2H7 scFv (SSS-S) H WCH2 WCH3 and 2H7 scFv (SSS-S) H P238SCH2 WCH3 constructs to bind U937 cells. U937 cells (10^6) expressing CD64 were incubated in PBS/2%FBS for one hour on ice with 2H7 scFv (SSS-S) H WCH2 WCH3 or 2H7 scFv (SSS-S) H P238SCH2 WCH3 Cells were washed and incubated for one hour on ice with FITC-goat anti-human IgG1 (Fc specific) (Caltag) at a final dilution of 1 100 Cells were washed and fluorescence analyzed on a Beckman-Coulter EpicsC flow cytometer Data was analyzed using Expo analysis software, and fluorescence intensity graphed as a function of the concentration of 2H7 scFv (SSS-S) H WCH2 WCH3 or 2H7 scFv (SSS-S) H P238SCH2 WCH3 This figure shows that both the 2H7 scFv (SSS-S) H WCH2 WCH3 construct and

the 2H7 scFv (SSS-S) H P238SCH2 WCH3 construct bound to U937 cells with equivalent titration curves. The results indicated that the 2H7 scFv (SSS-S) H P238SCH2 WCH3 construct was not impaired in its binding to the high affinity Fc receptor FcγRI (CD64) The fact that binding to high affinity FcγRI on U937 cells was similar for the two molecules in this experiment indicated that the P238S amino acid change selectively reduced the binding to FcγRIII. Figure 77 illustrates B cell depletion in macaques mediated by a anti-CD20 construct (2H7 scFv (SSS-S) H WCH2 WCH3) and a anti-CD20) scFv with a CH2 domain mutation. 2H7 scFv (SSS-S) H WCH2 WCH3 or 2H7 scFv (SSS-S) H P238SCH2 WCH3 were administered to macaques (*M fascicularis*) by intravenous injection at 6 mg/kg, with two infusions given one week apart. The effect on circulating B cells was measured by detection of CD40 positive B cells in peripheral blood. Blood samples were drawn from injected animals at days 7,0,1,3,7,8,10,14, 28, and 43 B cell depletion was estimated by performing CBC (complete blood counts) and two-color flow cytometry analysis on monkey blood FITC or PE conjugates of antibodies against CD40, CD19, CD20, IgG, CD3, CD8 were used in various combinations Data are plotted as the number of CD40 positive blood B cells tabulated in thousands of cells per microliter over time relative to the initial pre-injection time point level of B cells (maximum) This experiment showed that 2H7 scFv (SSS-S) H WCH2 WCH3 scFv injection resulted in rapid and complete depletion of circulating B cells that lasted longer than 28 days following the second injection. Injection of 2H7 scFv (SSS-S) H P238SCH2 WCH3 scFv did not completely deplete B cells even though CD20 epitopes were saturated A slow reduction in B cells to approximately 50% of initial levels was observed during the first 2 weeks, but B cells rapidly returned to starting levels in these animals These results indicate that the ability to bind high affinity FcγRI and to mediate complement dependent cytotoxicity is not sufficient for a CytoxB20G molecule to completely deplete circulating B cells, and that ADCC mediated by interaction with CD16 is likely necessary for rapid and sustained B cell depletion.

These experiments indicated that the 2H7 scFv (SSS-S) H WCH2 WCH3 scFvs are able to trigger B cell depletion functions through three different mechanisms of action (1) induction of apoptosis, (2) CDC effector mechanisms, and (3) ADCC effector mechanisms All three of these mechanisms probably contribute to depletion of B cells *in vivo* The data indicate that complete and sustained depletion of B cells requires intact ADCC effector

mechanisms However, the partial depletion obtained with an ADCC negative mutant also indicates that apoptosis and CDC mechanisms contribute to mediating a portion of the total B cell depletion effects. The results support the use of 2H7 scFv (SSS-S) H WCH2 WCH3 scFv in patients with a CD20 positive B cell malignancy or autoimmune disease.

EXAMPLE 162

INDUCTION OF APOPTOSIS IN B CELLS BY ANTI-CD37 GS-1 VL11S SCFV (SSS) H WCH2WCH3 CONSTRUCTS

In this Example, anti-CD37 GS-1 VL11S scFv (SSS) H WCH2WCH3 constructs were evaluated for their ability to bind target cells and for their ability to induce apoptosis The constructs were also physically characterized by size-exclusion chromatography (SEC) Figure 78 illustrates the SEC profile of G28-1 (anti-CD37) constructs having SSC hinge domain forms (GS-1 VL11S scFv (SSC) H WCH2WCH3) G28-1 (anti-CD37) constructs were purified from CHO culture supernatants by Protein A affinity chromatography Purified aliquots of 10-25 mg were subjected to HPLC over a Tosoh Biosep, Inc TSK 3000 SWXL HPLC column, pore size 5 mm. The flow rate was 1ml/min, in PBS, pH 7 2 running buffer Migration rates of molecular weight standards are indicated below the tracing The GS-1 VL11S scFv (SSS) H WCH2WCH3 construct is indicated in blue, while the GS-1 VL11S scFv (SSC) H WCH2WCH3 construct is indicated in red. The GS-1 VL11S scFv (SSS) H WCH2WCH3 construct generated a uniform peak of approximately 75-100 kDa, while the GS-1 VL11S scFv (SSC) H WCH2WCH3 construct generated a smaller form and other heterogeneous forms, including a high molecular weight form greater than 200 kDa. Figure 79 illustrates the binding of G28-1 (anti CD37) constructs to B cell lymphoma cell lines Serial dilutions of purified GS-1 VL11S scFv (SSS) H WCH2WCH3 or GS-1 VL11S scFv (SSC) H WCH2WCH3 were incubated with 10^6 cells of each cell type for 60 minutes on ice in PBS/2%FBS Samples were washed twice, and incubated with a mixture of FITC goat anti-human IgG and FITC goat anti-human IgG F(ab')2 (CalTag) at 1 100 each, on ice for 45 minutes Samples were washed and analyzed by flow cytometry using a FACsCalibur (Becton-Dickinson) The results of this experiment show that both GS-1 VL11S scFv (SSS) H WCH2WCH3 and GS-1 VL11S scFv (SSC) H WCH2WCH3 constructs bound to BJAB and Ramos cells, and moderately to WIL-2 cells. G28-1 (anti-CD37) (SSS)H G scFv or G28-1 (SSC)H G scFv constructs molecules bound Raji and Namalwa cells at

lower levels in these experiments. Binding to all lines was significant because background fluorescence has been subtracted from the data shown in Figure 79 Figure 80 illustrates the binding of annexin and v-propidium iodide to Ramous cells after overnight incubation with GS-1 VL11S scFv (SSS) H WCH2WCH3 or GS-1 VL11S scFv (SSC) H WCH2WCH3 forms of scFvs AnnexinV-PI staining of Ramos Cells incubated for 24 hours with G28-1 (anti-CD37) scFvs Ramos B cells at 10^6 cells/ml were incubated in 12 well dishes for 24 hours with G28-1 (anti-CD37) scFvs at 10 mg/ml, in a total volume of 2 ml. Cells were stained with annexinV and propidium iodide with a kit obtained from Immunotech, according to manufacturer's instructions Samples were analyzed by two-color flow cytometry using a FACsCalibur flow cytometer (Becton-Dickinson) The % of the total cells in each quadrant is indicated next to each dot plot Figure 83 shows the binding of Annexin V-Propidium Iodide to cells after overnight incubation with GS-1 VL11S scFv (SSS) H WCH2WCH3 or GS-1 VL11S scFv (SSC) H WCH2WCH3 The results indicated that both GS-1 VL11S scFv (SSS) H WCH2WCH3 and GS-1 VL11S scFv (SSC) H WCH2WCH3 constructs were able to induce apoptosis, but that the SSC form induced more apoptosis than the SSS form. Figure 81 illustrates the inhibition of proliferation of Ramos cells grown in the presence of G28-1 (anti-CD37) constructs. Ramos B cells were incubated with serial dilutions of purified G28-1 (anti-CD37) constructs containing either the IgG1 hinge identified as (SSS)H or (SSC)H Cultures were incubated in 96 well flat bottom tissue culture dishes (Costar) at 37°C, 5%CO_2 for 36 hours prior to pulsing with ^{3}H-thymidine for the last 12 hours of a 48 hour incubation (0 75 mCi/well) Cells were harvested onto 96-well GFC plates using a Packard harvester, dried, and 25 ml Microscint scintillation fluid added to each well prior to counting on a TopCount NXT microplate (Packard) scintillation counter Data are plotted as cpm incorporated versus protein concentration. Each construct showed increasing inhibition of proliferation with increasing protein concentration Figure 82 illustrates the induction of apoptosis in Ramos B cells cultured in the presence of and 2H7 (anti-CD20) and G28-1 (anti-CD37) constructs Ramos B cells were incubated with CD20 and/or CD37 targeted constructs (10 mg/ml) in solution for 20 hours The cells were then harvested, washed, and incubated in annexinV and propidium iodide using a staining kit from Immunotech prior to two color flow cytometry using a FACsCalibur flow cytometer (Becton-Dickinson) The graph shows the percentage of annexin V positive cells identified by their staining in the

right quadrants of the dot plots The results of this experiment indicate that the both G28-1 constructs were more efficient than the 2H7 construct and that the G28-1 VL11S scFv (SSS) H WCH2 WCH3 construct was more efficient than the G28-1 VL11S scFv (SSC) H WCH2 WCH3 construct However, the amount of apoptosis of Ramos cells was greatest when the 2H7 and the G28-1 constructs were used in combination. Figure 83 illustrates the killing of Ramos cells by complement dependent cytotoxicity (CDC) induced by 2H7 (anti-CD20) constructs and G28-1 (anti-CD37) constructs with hinge region mutations 2H7 scFv (CSS-S) H WCH2 WCH3 (anti-CD20), G28-1 scFv (SSS)H G, G28-1 (SCS)H (anti-CD37-scFv), G28-1 (CSS)H (anti-CD37-scFv), or G28-1 (SSC)H (anti-CD37-scFv) were incubated at 10 mg/ml with 10^4 Ramos Target Cells and a 1 10 dilution of rabbit complement (PelFreez) in a volume of 150 ml for 90 minutes Aliquots were stained with trypan blue (Invitrogen), and counted using a hemacytometer to determine the percentage of the cell population killed during treatment Negative controls with cells and only one reagent were also included. Both G28-1 (SSS) (anti-CD37 scFv) and G28-1 (SSC) (anti-CD37 scFv) rapidly killed Ramos cells in the presence of rabbit complement in this experiment The activity of G28-1 (SSC) (anti-CD37 scFv) was higher than that of G28-1 (SSS) (anti-CD37 scFv), and was nearly as potent in this assay as the CD20-directed 2H7 scFv (CSS-S) H WCH2 WCH3 scFv molecule. Figure 84 illustrates the induction of ADCC of Ramos cells incubated with G28-1 (anti-CD37) scFvs G28-1 (anti-CD37) constructs at 10 mg/ml were incubated in flat-bottom 96 well plates with 10^4 ^{51}Cr-labeled Ramos cells and resting human PBMCs at different effector:target ratios ranging from 0 to 100 All incubations were performed in triplicate at each effector:target ratio Natural killing was measured at each effector:target ratio by omission of the constructs Spontaneous release was measured without addition of PBMC or fusion protein, and maximal release was measured by the addition of detergent (1% NP-40) to the appropriate wells. Reactions were incubated for 6 hours, and 100 ml culture supernatant harvested to a Lumaplate (Packard Instruments) and allowed to dry overnight prior to counting cpm released on a Packard Top Count NXT Microplate Scintillation Counter Results from these experiments indicated that the G28-1 (anti-CD37) constructs bind to target cells, that they induce apoptosis in the B cell lines, and that they mediate effector functions including CDC and ADCC

Additional non-limiting representative constructs or sequences within, or useful within, the present inventions are as follows

HuIgG1 wild type hinge, CH2, CH3 (nucleotide sequence) (SEQ ID NO ___)

tctgatcaggagcccaaatcttgtgacaaaactcacacatgcccaccgtgcccagcacctgaactcctgggggggaccgtcagtctt
cctcttccccccaaaacccaaggacaccctcatgatctcccggacccctgaggtcacatgcgtggtggtggacgtgagccacgaa
gaccctgaggtcaagttcaactggtacgtggacggcgtggaggtgcataatgccaagacaaagccgcgggaggagcagtacaa
cagcacgtaccgtgtggtcagcgtcctcaccgtcctgcaccaggactggctgaatggcaaggagtacaagtgcaaggtctccaac
aaagccctcccagcccccatcgagaaaacaatctccaaagccaaagggcagccccgagaaccacaggtgtacaccctgcccca
atcccgggatgagctgaccaagaaccaggtcagcctgacctgcctggtcaaaggcttctatcccagcgacatcgccgtggagtgg
gagagcaatgggcagccggagaacaactacaagaccacgcctcccgtgctggactccgacggctccttcttcctctacagcaag
ctcaccgtggacaagagcaggtggcagcaggggaacgtcttctcatgctccgtgatgcatgaggctctgcacaaccactacacgc
agaagagcctctccctgtctccgggtaaatgatctaga

HuIgG1 wild type hinge, CH2, CH3 (amino acid sequence) (SEQ ID NO ___)

SDQEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHE
DPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCK
VSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAV
EWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALH
NHYTQKSLSLSPGK

Llama IgG1 hinge, CH2, CH3 (nucleotide sequence) (SEQ ID NO ___)

tgatcaagaaccacatggaggatgcacgtgcccncagtgcccncaatgcccngcnccngaactnccaggaggcccttctgtcttt
gtcttccccccgaaacccaaggacgtcctctccatttttggaggccgagtcacgtgcgttgtagtggacgtcggaaagaaagaccc
cgaggtcaatttcaactggtatattgatggcgttgaggtgcgaacggccaatacgaagccaaaagagggaacagttcaacagcacg
taccgcgtggtcagcgtcctgcccatccagcaccaggactggctgacggggaaggaattcaagtgcaaggtcaacaacaaagct
ctcccggcccccatcgagaggaccatctccaaggccaaagggcagacccgggagccgcaggtgtacaccctggcccacacc
gggaagaactggccaaggacaccgtgagcgtaacatgcctggtcaaaggcttctacccagctgacatcaacgttgagtggcaga
ggaacggtcagccggagtcagagggcacctacgccaacacgccgccacagctggacaacgacgggacctacttcctctacagc
aagctctcggtgggaaagaacacgtggcagcggggagaaaccttaacctgtgtggtgatgcatgaggccctgcacaaccactac
acccagaaatccatcacccagtcttcgggtaaatagtaatctaga

Llama IgG1 hinge, CH2, CH3 (in figure 23 as Llama IgG1) (amino acid sequence) (SEQ ID NO ___)

EPHGGCTCPQCPAPELPGGPSVFVFPPKPKDVLSISGRPEVTCVVVDVGKEDPEVN
FNWYIDGVEVRTANTKPKEEQFNSTYRVVSVLPIQHQDWLTGKEFKCKVNNKAL
PAPIERTISKAKGQTREPQVYTLAPHREELAKDTVSVTCLVKGFYPADINVEWQRN
GQPESEGTYANTPPQLDNDGTYFLYSRLSVGKNTWQRGETLTGVVMHEALHNHY
TQKSITQSSGK

Llama IgG2 (nucleotide sequence) (SEQ ID NO ___)

tgatcaagaacccaagacaccaaaaccacaaccacaaccacaaccacaacccaatcctacaacagaatccaagtgtcccaaatgt
ccagccctgagctcctgggagggccctcagtcttcatcttcccccccgaaacccaaggacgtcctctccatttctgggaggcccga
ggtcacgtgcgttgtggtagacgtgggccaggaagaccccgaggtcagttcaactggtacattgatggcgtgaggtgcgaacg
gccaacacgaggccaaaagaggaacagttcaacagcacgtaccgcgtggtcagcgtcctgcccatccagcaccaggactggct
gacggggaaggaattcaagtgcaaggtcaacaacaaagctctcccggcccccatcgagaagaccatctccaaggccaaagggc
agaccccgggagccgcaggtgtacaccctggcccccaccgggaagagctggccaaggacaccgtgagcgtaacatgcctggt
caaaggcttctacccacctgatatcaacgtggagtggcagaggaatgggcagccggagtcagagggcacytacgccaccacgc
cacccagctggacaacgacgggacctacttcctctacagcaagctctcggtgggaaagaacacgtggcagcagggagaaacc
ttcacctgtgtggtgatgcacgaggccctgcacaaccactacacccagaatccatcacccagtcttcgggtaaatagtaatctaga.

Llama IgG2 (amino acid sequence) (SEQ ID NO ___)

DQEPKTPKPQPQPQPQPNPTTESKCPKCPAPELLGGPSVFIFPPKPKDVLSISGRPEV
TCVVVDVGQEDPEVSFNWYIDGAEVRTANTRPKEEQFNSTYRVVSVLPIQHQDWL
TGKEFKCKVNNKALPAPIEKTISKAKGQTREPQVYTLAPHREELAKDTVSVTCLVK
GFYPPDINVEWQRNGQPESEGTYATTPPQLDNDGTYFLYSKLSVGKNTWQQGETF
TCVVMHEALHNHYTQKSITQSSGK

Llama IgG3 Fc (nucleotide sequence) (SEQ ID NO ___)

tgatcaagcgcaccacagcgaagaccccagctccaagtgtcccaaatgcccaggccctgaactccttggagggcccacggtctt
catcttcccccccgaaagccaaggacgtcctctccatcacccgaaaacctgaggtcacgtgcttgtggtggacgtgggtaaagaag
accctgaggtcgagttcaagctggtccgtggatgacacagaggtacacacggctgagacaaagccaaaggaggaacagttcaac

agcacgtaccgcgtggtcagcgtcctgcccatccagcaccaggactggctgacggggaaggaattcaagtgcaaggtcaacaa
caaagctctcccagcccccatcgagaggaccatctccaaggccaaagggcagaccccgggagccgcaggtgtacaccctggcc
ccacacccgggaagagctggccaaggcaccgtgagcgtaacctgcctggtcaaaggcttcttcccagctgacatcaacgttgagt
ggcagaggaatgggcagccggagtcagagggcacctacgccaacacgccgccacagctggacaacgacgggacctacttcct
ctacagcaaactctccgtgggaaagaacacgtggcagcagggagaagtcttcacctgtgtggtgatgcacgaggctctacacaat
cactccacccagaaatccatcacccagtcttcgggtaaatagtaatctagagggccc

Llama IgG3 Fc (amino acid sequence) (SEQ ID NO ___)
DQAHHSEDPSSKCPKCPGPELLGGPTVFIFPPKAKDVLSITRKPEVTCLWWTWVKK
TLRSSSSWSVDDTEVHTAETKPKEEQFNSTYRVVSVLPIQHQDWLTGKEFKCKVN
NKALPAPIERTISKAKGQTREPQVYTLAPHREELAKDTVSVTCLVKGFFPADINVE
WQRNGQPESEGTYANTPPQLDNDGTYFLYSKLSVGKNTWQQGEVFTCVVMHEA
LHNHSTQKSITQSSGK

HuIgG1 wild type hinge (nucleotide sequence) (SEQ ID NO ___)
gatcaggagcccaaatcttgtgacaaaactcacacatgcccaccgtgcccagca

HuIgG1 wild type hinge (amino acid sequence) (SEQ ID NO ___)
DQEPKSCDKTHTCPPCPA

HuIgG1 H2, wild type hinge with leu at second position (nucleotide sequence) (SEQ ID NO ___)
gatctggagcccaaatcttgtgacaaaactcacacatgcccaccgtgcccagca

HuIgG1 H2, wild type hinge with leu at second position (amino acid sequence) (SEQ ID NO ___)
DLEPKSCDKTHTCPPCPA

NTHuIgG1 wild type CH2 (nucleotide sequence) (SEQ ID NO ___)

cctgaactcctgggggggaccgtcagtcttcctcttccccccaaaacccaaggacaccctcatgatctcccggacccctgaggtcac
atgcgtggtggtggacgtgagccacgaagaccctgaggtcaagttcaactggtacgtggacggcgtggaggtgcataatgccaa
gacaaagccgcgcgggaggagcagtacaacagcacgtaccgtgtggtcagcgtcctcaccgtcctgcaccaggactggctgaatg
gcaaggagtacaagtgcaaggtctccaacaaagccctcccagccccatcgagaaaaccatctccaaagccaaa

HuIgG1 wild type CH2(amino acid sequence) (SEQ ID NO ___)

PELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHN
AKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAK

HuIgG1 wild type CH3 (nucleotide sequence) (SEQ ID NO ___)

gggcagccccgagaaccacaggtgtacaccctgcccccatcccgggaggagatgaccaagaaccaggtcagcctgacctgcct
ggtcaaaggcttctatcccagcgacatcgccgtggagtgggagagcaatgggcagccggagaacaactacaagaccacgcctc
ccgtgctggactccgacggctccttcttcctctatagcaagctcaccgtggacaagagcaggtggcagcaggggaacgtcttctca
tgctccgtgatgcatgaggctctgcacaaccactacacgcagaagagcctctccctgtccccgggtaaatga

HuIgG1 wild type CH3 (amino acid sequence) (SEQ ID NO ___)

GQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPP
VLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

HuIgG1 mutated hinge (C-C-C→S-S-S) (nucleotide sequence) (SEQ ID NO ___)

gatcaggagcccaaatcttctgacaaaactcacacatccccaccgtccccagca

HuIgG1 mutated hinge (C-C-C→S-S-S) (amino acid sequence) (SEQ ID NO ___)

DQEPKSSDKTHTSPPSPA

HIgG1MTH WTCH2CH3 (mutant hinge with wild type CH2 and CH3 (reads from the hinge+Ig tail) (nucleotide sequence) (SEQ ID NO ___)

tgatcaccccaaatcttctgacaaaactcacacatctccaccgtcctcagcacctgaactcctgggtggaccgtcagtcttcctcttcc
ccccaaaacccaaggacaccctcatgatctcccggacccctgaggtcacatgcgtggtggtggacgtgagccacgaagaccctg
aggtcaagttcaactggtacgtggacggcgtggaggtgcataatgccaagacaaagccgcgggaggagcagtacaacagcacg
taccgtgtggtcagcgtcctcaccgtcctgcaccaggactggctgaatggcaaggagtacaagtgcaaggtctccaacaaagccc
tcccagccccatcgagaaaacaatctccaaagccaaagggcagccccgagaaccacaggtgtacaccctgccccatcccgg
gatgagctgaccaagaaccaggtcagcctgacctgcctggtcaaaggcttctatcccagcgacatcgccgtggagtgggagagc
aatgggcagccggagaacaactacaagaccacgcctcccgtgctggactccgacggctccttcttcctctacagcaagctcaccg
tggacaagagcaggtggcagcaggggaacgtcttctcatgctccgtgatgcatgaggctctgcacaaccactacacgcagaaga
gcctctccctgtctccgggtaaatgataatctaga

Mutant hinge, but wild type CH2 and CH3 (amino acid sequence) (SEQ ID NO ___)

DHPKSSDKTHTSPPSSAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDP
EVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVS
NKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEW
ESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNH
YTQKSLSLSPGK

2H7 scFv llama IgG1 (nucleotide sequence) (SEQ ID NO ___)

aagcttgccgccatggatttttcaagtgcagattttcagcttcctgctaatcagtgcttcagtcataattgccagaggacaaattgttctct
cccagtctccagcaatcctgtctgcatctccaggggagaaggtcacaatgacttgcagggccagctcaagtgtaagttacatgcact
ggtaccagcagaagccaggatcctccccaaaccctggatttatgccccatccaacctggcttctggagtcccttgcttcagttcagtg
gcagtgggtctgggacctcttactctctcacaatcagcagagtggaggctgaagatgctgccacttattactgccagcagtggagttt
taacccacccacgttcggtgctgggaccaagctggagctgaaagatggcggtggctcgggcggtggtggatctggaggaggtg
ggagctctcaggcttatctacagcagtctggggctgagctggtgaggcctgggacctcagtgaagatgtcctgcaaggcttctggc
tacacatttaccagttacaatatgcactgggtaaagcagacacctagacagggcctggaatggattggagctatttatccaggaaat
ggtgatacttcctacaatcagaagttcaagggcaaggccacactgactgtagacaaatcctccagcacagcctacatgcagctcag
cagcctgacatctgaagactctgcggtctatttctgtgcaagagtggtgtactatagtaactcttactggtacttcgatgtctggggcac
agggaccacggtcaccgtctcttctgatcaagaaccacatggaggatgcacgtgcccncagtgcccncaatgcccngcnccng
aactnccaggaggcccttctgtctttgtcttcccccccgaaacccaaggacgtcctctccatttttggaggccgagtcacgtgcgttgt
agtggacgtcggaaagaaagacccccgaggtcaatttcaactggtatattgatggcgttgaggtgcgaacggccaatacgaagcca
aaagaggaacagttcaacagcacgtaccgcgtggtcagcgtcctgcccatccagcaccaggactggctgacggggaaggaatt

caagtgcaaggtcaacaacaaagctctcccggcccccatcgagaggaccatctccaaggccaaagggcagacccgggagccg
caggtgtacaccctggccccacaccgggaagaactggccaaggacaccgtgagcgtaacatgcctggtcaaaggcttctaccca
gctgacatcaacgttgagtggcagagggaacggtcagccggagtcagagggcacctacgccaacacgccgccacagctggaca
acgacgggacctacttcctctacagcaagctctcggtgggaaagaacacgtggcagcggggagaaaccttaacctgtgtggtgat
gcatgaggccctgcacaaccactacacccagaaatccatcacccagtcttcgggtaaatagtaatctaga

2H7 scFv llama IgG1 (amino acid sequence) (SEQ ID NO ___)

MDFQVQIFSFLLISASVIIARGQIVLSQSPAILSASPGEKVTMTCRASSSVSYMHWY
QQKPGSSPKPWIYAPSNLASGVPARFSGSGSGTSYSLTISRVEAEDAATYYCQQWS
FNPPTFGAGTKLELKDGGGSGGGGSGGGGSSQAYLQQSGAELVRPGASVKMSCK
ASGYTFTSYNMHWVKQTPRQGLEWIGAIYPGNGDTSYNQKFKGKATLTVDKSSS
TAYMQLSSLTSEDSAVYFCARVVYYSNSYWYFDVWGTGTTVTVSSDQEPHGGCT
CPQCPAPELPGGPSVFVFPPKPKDVLSIFGGRVTCVVVDVGKKDPEVNFNWYIDGV
EVRTANTKPKEEQFNSTYRVVSVLPIQHQDWLTGKEFKCKVNNKALPAPIERTISK
AKGQTREPQVYTLAPHREELAKDTVSVTCLVKGFYPADINVEWQRNGQPESEGTY
ANTPPQLDNDGTYFLYSKLSVGKNTWQRGETLTCVVMHEALHNHYTQKSITQSS
GK

2H7 scFv llama IgG2 (nucleotide sequence) (SEQ ID NO ___)

aagcttgccgccatggattttcaagtgcagattttcagcttcctgctaatcagtgcttcagtcataattgccagaggacaaattgttctct
cccagtctccagcaatcctgtctgcatctccaggggagaaggtcacaatgacttgcagggccagctcaagtgtaagttacatgcact
ggtaccagcagaagccaggatcctcccccaaaccctggatttatgccccatccaacctggcttctggagtccctgctcgcttcagtg
gcagtgggtctgggacctcttactctctcacaatcagcagagtggaggctgaagatgctgccacttattactgccagcagtggagttt
taacccacccacgttcggtgctgggaccaagctggagctgaaagatggcggtggctcgggcggtggtggatctggaggaggtg
ggagctctcaggcttaatctacagcagtctggggctgagctggtgaggcctggggcctcagtgaagatgtcctgcaaggcttctggc
tacacatttaccagttacaatatgcactgggtaaagcagacacctagacagggcctggaatggattggagctatttatccaggaaat
ggtgatacttcctacaatcagaagttcaagggcaaggccacactgactgtagacaaatcctccagcacagcctacatgcagctcag
cagcctgacatctgaagactctgcggtctatttctgtgcaagagtggtgtactatagtaactcttactggtacttcgatgtctggggcac
agggaccacggtcaccgtctcttctgatcaagaacccaagacaccaaaaccacaaccacaaccacaaccacaaccacaaccctac
aacagaatccaagtgtcccaaatgtccagccccctgagctcctgggagggccctcagtcttcatcttcccccccgaaacccaaggac
gtcctctccattctgggaggcccgaggtcacgtgcgttgtggtagacgtgggccaggaagaccccgaggtcagtttcaactggta

cattgatggcgctgaggtgcgaacggccaacacgaggccaaaagaggaacagttcaacagcacgtaccgcgtggtcagcgtcc
tgcccatccagcaccaggactggctgacggggaaggaattcaagtgcaaggtcaacaacaaagctctcccggccccccatcgag
aagaccatctccaaggccaaagggcagaccccgggagccgcaggtgtacaccctggccccacaccgggaagagctggccaag
gacaccgtgagcgtaacatgcctggtcaaaggcttctaccccacctgatatcaacgttgagtggcagaggaatgggcagccggagt
cagagggcacytacgccaccacgccacccccagctggacaacgacgggacctacttcctctacagcaagctctcggtgggaaag
aacacgtggcagcagggagaaaccttcacctgtgtggtgatgcacgaggccctgcacaaccactacacccagaagtccatcacc
cagtcttcgggtaaatagtaatctaga

AA2H7 scFv llama IgG2 (amino acid sequence) (SEQ ID NO ___)

MDFQVQIFSFLLISASVIIARGQIVLSQSPAILSASPGEKVTMTCRASSSVSYMHWY
QQKPGSSPKPWIYAPSNLASGVPARFSGSGSGTSYSLTISRVEAEDAATYYCQQWS
FNPPTFGAGTKLELKDGGGSGGGGSGGGGSSQAYLQQSGAELVRPGASVKMSCK
ASGYTFTSYNMHWVKQTPRQGLEWIGAIYPGNGDTSYNQKFKGKATLTVDKSSS
TAYMQLSSLTSEDSAVYFCARVVYYSNSYWYFDVWGTGTTVTVSSDQEPKTPKP
QPQPQPQPNPTTESKCPKCPAPELLGGPSVFIFPPKPKDVLSISGRPEVTCVVVDVG
QEDPEVSFNWYIDGAEVRTANTRPKEEQFNSTYRVVSVLPIQHQDWLTGKEFKCK
VNNKALPAPIEKTISKARGQTREPQVYTLAPHREELAKDTVSVTCLVKGFYPPDIN
VEWQRNGQPESEGTYATTPPQLDNDGTYFLYSKLSVGKNTWQQGETFTCVVMHE
ALHNHYTQKSITQSSGK

NT2H7 scFv llama IgG3 (nucleotide sequence) (SEQ ID NO ___)

aagcttgccgccatggattttcaagtgcagattttcagcttcctgctaatcagtgcttcagtcataattgccagaggacaaattgttctct
cccagtctccagcaatcctgtctgcatctccaggggagaaggtcacaatgacttgcagggccagctcaagtgtaagttacatgcact
ggtaccagcagaagccaggatcctccccaaaccctggatttatgccccatccaacctggcttctggagtccctgctcgcttcagtg
gcagtgggtctgggacctcttactctctcacaatcagcagagtggaggctgaagatgctgccacttattactgccagcagtggagttt
tacccaccccacgttcggtgctgggaccaagctggagctgaaagatggcggtggctcgggcggtggtgggtctgggggaggtg
ggaggctctcaggcttatctacagcagtctggggctgagctggtgaggcctggggcctcagtgaagatgtcctgcaaggcttctggc
tacacatttaccagttacaatatgcactgggtaaagcagacacctagacagggcctggaatggattggagctatttatccaggaaat
ggtgatacttcctacaatcagaagttcaagggcaaggccacactgactgtagacaaatcctccagcacagcctacatgcagctcag
cagcctgacatctgaagactctgcggtctatttctgtgcaagagtggtgtactatagtaactcttactggtacttcgatgtctggggcac
agggaccacggtcaccgtctcttctgatcaagcgaaccacagcgaagaccccagctccaagtgtcccaaatgcccaggccctga
actcctggagggcccacggtcttcatcttccccccgaaagccaaggacgtcctctccatcacccgaaaacctgaggtcacgtgctt
gtggtggacgtgggtaaagaagaccctgagatcgagttcaagctggtccgtggatgacacagaggtacacacggctgagacaaa

gccaaaggaggaacagttcaacagcacgtaccgcgtggtcagcgtcctgcccatccagcaccaggactggctgacggggaag
gaattcaagtgcaaggtcaacaacaaagccctcccagccccatcgagaggaccatctccaaggccaaagggcagaccccggga
gccgcaggtgtacaccctgggccccacacccgggaagagctggccaaggacaccgtgagcgtaacctgcctggtcaaaggcttctt
cccagctgacatcaacgttgagtggcagaggaatgggcagccggagtcagagggcacctacgccaacacgccgccacagctg
gacaacgacgggacctacttcctctacagcaaactctccgtgggaaagaacacgtggcagcagggagaagtcttcacctgtgtgg
tgatgcacgaggctctacacaatcactccacccagaaatccatcacccagtcttcgggtaaatagtaatctagagggccc

2H7 scFv llama IgG3 (amino acid sequence) (SEQ ID NO ___)

MDFQVQIFSFLLISASVIIARGQIVLSQSPAILSASPGEKVTMTCRASSSVSYMHWY
QQKPGSSPKPWIYAPSNLASGVPARFSGSGSGTSYSLTISRVEAEDAATYYCQQWS
FNPPTFGAGTKLELKDGGGSGGGGSGGGGSSQAYLQQSGAELVRPGASVKMSCK
ASGYTFTSYNMHWVKQTPRQGLEWIGAIYPGNGDTSYNQKFKGKATLTVDKSSS
TAYMQLSSLTSEDSAVYFCARVVYYSNSYWYFDVWGTGTTVTVSSDQAHSHSED
PSSKCPKCPGPELLGGPTVFIFPPKAKDVLSITRKPEVTCLWWTWVKKTLRSSSSW
SVDDTEVHTAETKPKEEQFNSTYRVVSVLPIQHQDWLTGKEFKCKVNNKALPAPI
ERTISKAKGQTREPQVYTLAPHREELAKDTVSVTCLVKGFFPADINVEWQRNGQP
ESEGTYANTPPQLDNDGTYFLYSKLSVGKNTWQQGEVFTCVVMHEALHNHSTQK
SITQSSGK

2H7 scFv WTH WTCH2CH3 (nucleotide sequence)

aagcttgccgccatggatttttcaagtgcagatttttcagcttcctgctaatcagtgcttcagtcataattgccagaggacaaaattgttctct
cccagtctccagcaatcctgtctgcatctccaggggagaaggtcacaatgacttgcagggccagctcaagtgtaagttacatgcact
ggtaccagcagaagccaggatcctcccccaaaccctggatttatgccccatccaacctggcttctggagtccctgctcgcttcagtg
gcagtgggtctgggacctcttactctctcacaatcagcagagtggaggctgaagatgctgccacttattactgccagcagtggagttt
tacccacccacgttcggtgctgggaccaagctggagctgaaagatggcggtggctcgggcggtggtgggatctggaggaggtg
ggagctctcaggcttatctacagcagtctggggctgagctggtgaggcctggggcctcagtgaagatgtcctgcaaggcttctggc
tacacatttaccagttacaatatgcactgggtaaagcagacacctagacagggcctggaatggattggagctatttatccaggaaat
ggtgatacttcctacaatcagaagttcaagggcaaggccacactgactgtagacaaatcctccagcacagcctacatgcagctcag
cagcctgacatctgaagactctgcggtctattttgtgcaagagtggtgtactatagtaactcttactggtacttcgatgtctggggcac
agggaccacggtcaccgtctcttctgatcaggagcccaaatcttgtgacaaaactcacacatgcccaccgtgcccagcacctgaac
tcctgggggggaccgtcagtcttcctcttccccccaaaacccaaggacaccctcatgatctcccggacccctgaggtcacatgcgtg

gtggtggacgtgagccacgaagaccctgaggtcaagttcaactggtacgtggacggcgtggaggtgcataatgccaagacaaag
ccgcgggaggagcagtacaacagcacgtaccgtgtggtcagcgtcctcaccgtcctgcaccaggactggctgaatggcaagga
gtacaagtgcaaggtctccaacaaagccctcccagcccccatcgagaaaacaatctccaaagccaaagggcagccccgagaac
cacaggtgtacaccctgcccccatcccgggatgagctgaccaagaaccaggtcagcctgacctgcctggtcaaaggcttctatcc
cagcgacatcgccgtggagtgggagagcaatgggcagccggagaacaactacaagaccacgcctcccgtgctggactccgac
ggctccttcttcctctacagcaagctcaccgtggacaagagcaggtggcagcaggggaacgtcttctcatgctccgtgatgcatga
ggctctgcacaaccactacacgcagaagagcctctccctgtctccgggtaaatgatctaga

2H7 scFv WTH WTCH2CH3 (amino acid sequence) (SEQ ID NO ___)

MDFQVQIFSFLLISASVIIARGQIVLSQSPAILSASPGEKVTMTCRASSSVSYMHWY
QQKPGSSPKPWIYAPSNLASGVPARFSGSGSGTSYSLTISRVEAEDAATYYCQQWS
FNPPTFGAGTKLELKDGGGSGGGGSGGGGSSQAYLQQSGAELVRPGASVKMSCK
ASGYTFTSYNMHWVKQTPRQGLEWIGAIYPGNGDTSYNQKFKGKATLTVDKSSS
TAYMQLSSLTSEDSAVYFCARVVYYSNSYWYFDVWGTGTTVTVSSDQEPKSCDK
THTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWY
VDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPI
EKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPE
NNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSL
SPGK

NTCD80 transmembrane domain and cytoplasmic tail (+restriction sites) (nucleotide sequence) (SEQ ID NO ___)

gcggatccttcgaacctgctcccatcctgggccattaccttaatctcagtaaatggaatttttgtgatatgctgcctgacctactgctttg
ccccaagatgcagagagaggaggaaggaggaatgagagattgagaagggaaagtgtacgccctgtataaatcgat

CD80 transmembrane domain and cytoplasmic tail (amino acid sequence) (SEQ ID NO ___)

ADPSNLLPSWAITLISVNGIFVICCLTYCFAPRCRERRRNERLRRESVRPV

40.2.220 VL (anti-human CD40 scFv #1--VL) (nucleotide sequence) (SEQ ID NO ___)

aagcttatggattttcaagtgcagattttcagcttcctgctaatcagtgcttcagtcataatgtccagaggagtcgacattgttctgactc
agtctccagccaccctgtctgtgactccaggagatagagtctctctttcctgcagggccagccagagtattagcgactacttacactg
gtatcaacaaaaatcacatgagtctccaaggcttctcatcaaatatgcttcccattccatctctgggatcccctccaggttcagtggca
gtggatcagggtcagatttcactctcagtatcaacagtgtggaacctgaagatgttggaatttattactgtcaacatggtcacagcttc
cgtggacgttcggtggaggcaccaagctggaaatcaaacgg

40.2.220 VL (anti-human CD40 scFv #1--VL) (amino acid sequence) (SEQ ID NO ___)

MDFQVQIFSFLLISASVIMSRGVDIVLTQSPATLSVTPGDRVSLSCRASQSISDYLHW
YQQKSHESPRLLIKYASESISGIPSRFSGSGSGSDFTLSINSVEPEDVGIYYCQHGHSF
PWTFGGGTKLEIKR

40.2.220 VH (for anti-human CD40 scFv #1--VH) (nucleotide sequence) (SEQ ID NO ___)

cagatccagttggtgcaatctggacctgagctgaagaagcctggagagacagtcaggatctcctgcaaggcttctgggtatgccti
cacaactactggaatgcagtgggtgcaagaggatgccaggaaagggtttgaagtggattggctggataaacaccccactctggagt
gccaaaatatgtagaagacttcaaggacggtttgccttctctttggaacctctgccaacactgcatatttacagataagcaacctcaa
agatgaggacacggctacgtatttctgtgtgaggatccgggaatggtaactatgacctggcctactttgcttactggggccaagggac
actggtcactgtctctgatca

40.2.220 VH (for anti-human CD40 scFv #1--VH) (amino acid sequence) (SEQ ID NO ___)

QIQLVQSGPELKKPGETVRISCKASGYAFTTTGMQWVQEMPGKGLKWIGWINTPL
WSAKICRRLQGRFAFSLETSANTAYLQISNLKDEDTATYFCVRSGNGNYDLAYFA
YWGQGTLVTVS

40.2.220 scFv (anti-human CD40 scFv #1) (nucleotide sequence) (SEQ ID NO ___)

aagcttatggattttcaagtgcagattttcagcttcctgctaatcagtgcttcagtcataatgtccagaggagtcgacattgttctgactc
agtctccagccaccctgtctgtgactccaggagatagagtctctctttcctgcagggccagccagagtattagcgactacttacactg
gtatcaacaaaaatcacatgagtctccaaggcttctcatcaaatatgcttcccattccatctctgggatcccctccaggttcagtggca
gtggatcagggtcagatttcactctcagtatcaacagtgtggaacctgaagatgttggaatttattactgtcaacatggtcacagcttc

cgtggacgttcggtggaggcaccaagctggaaatcaaacgggtggcggtggctcgggcggaggtgggtcgggtggcggcg
gatctcagatccagttggtgcaatctggacctgagctgaagaagcctggagagacagtcaggatctcctgcaaggcttctgggtat
gccttcacaactactggatgcagtgggtgcaagagatgccaggaaagggtttgaagtggattggctggataaacaccccactctg
gagtgccaaaatatgtagaagacttcaaggacggtttgccttctctttggaaacctctgccaacactgcatatttacagatcagcaacc
tcaaagatgaggacacggctacgtatttctgtgtgagatccgggaatggtaactatgacctggcctacttttgcttactggggccaag
ggacactggtcactgtctctgatca

40.2 220 scFv (anti-human CD40 scFv #1) (amino acid sequence) (SEQ ID NO ___)

MDFQVQIFSFLLISASVIMSRGVDIVLTQSPATLSVTPGDRVSLSCRASQSISDYLHW
YQQKSHESPRLLIKYASHSISGIPSRFSGSGSGSDFTLSINSVEPEDVGIYYCQHGHSF
PWTFGGGTKLEIKRGGGGSGGGGSGGGGSQIQLVQSGPELKKPGETVRISCKASG
YAFTTTGMQWVQEMPGKGLKWIGWINTPLWSAKICRRLQGRFAFSLETSANTAY
LQISNLKDEDTATYFCVRSGNGNYDLAYFAYWGQGTLVTVS

2e12 VL (with L6 VK leader peptide) (nucleotide sequence) (SEQ ID NO ___)

atggatttcaagtgcagattttcagcttcctgctaatcagtgcttcagtcataatgtccagaggagtcgacattgtgctcacccaatctc
cagcttctttggctgtgtctctaggtcagagagccaccatctcctgcagagccagtgaaagtgttgaatattatgtcacaagtttaatgc
agtggtaccaacagaaaccaggacagccacccaaactcctcatctctgctgcatccaacgtagaatctggggtccctgccaggttt
agtggcagtgggtctgggacagacttcagcctcaacatccatcctgtggaggaggatgatattgcaatgtatttctgtcagcaaagta
ggaaggttccttggacgttcggtggaggcaccaagctggaaatcaaacgg

2e12 VL (with L6 VK leader peptide) (amino acid sequence) (SEQ ID NO ___)

MDFQVQIFSFLLISASVIMSRGVDIVLTQSPASLAVSLGQRATISCRASESVEYYVTS
LMQWYQQKPGQPPKLLISAASNVESGVPARFSGSGSGTDFSLNIHPVEEDDIAMYF
CQQSRKVPWTFGGGTKLEIKR

2e12 VH (no leader peptide) (nucleotide sequence) (SEQ ID NO ___)

caggtgcagctgaaggagtcaggacctggcctggtggcgccctcacagagcctgtccatcacatgcaccgtctcagggttctcatt
aaccggctatggtgtaaactgggttcgccagcctccaggaaagggtctggagtggctgggaatgatatgggggtgatggaagcac
agactataattcagtctcaaatccagactgagcatcaccaaggacaactccaagagccaagttttcttaaaaatgaacagtctgcaa

actgatgacacagccagatactactgtgccagagatggttatagtaactttcattactatgttatggactactggggtcaaggaacctc
agtcaccgtctcctca(gatctg)

2e12 VH (amino acid sequence) (SEQ ID NO ___)

QVQLKESGPGLVAPSQSLSITCTVSGFSLTGYGVNWVRQPPGKGLEWLGMIWGDG
STDYNSALKSRLSITKDNSKSQVFLKMNSLQTDDTARYYCARDGYSNFHYYVMD
YWGQGTSVTVSS

2e12scFv(+Restriction sites) (nucleotide sequence) (SEQ ID NO ___)

aagcttatggattttcaagtgcagattttcagcttcctgctaatcagtgcttcagtcataatgtccagaggagtcgacattgtgctcaccc
aatctccagcttctttggctgtgtctctaggtcagagagccaccatctcctgcagagccagtgaaagtgttgaatattatgtcacaagtt
taatgcagtggtaccaacagaaaccaggacagccacccaaactcctcatctctgctgcatccaacgtagaatctggggtccctgcc
aggtttagtggcagtgggtctgggacagacttcagcctcaacatccatcctgtggaggaggatgatattgcaatgtatttctgtcagc
aaagtaggaaggttccttggacgttcggtggaggcaccaagctggaaatcaaacggggtggcggtggctcgggcggaggtggg
tcgggtggcggcggatctcaggtgcagctgaaggagtcaggacctggcctggtggcgccctcacagagcctgtccatcacatgc
accgtctcagggttctcattaaccggctatggtgtaaactgggttcgccagcctccaggaaagggtctggagtggctgggaatgat
atggggtgatggaagcacagactataattcagctctcaaatccagactgagcatcaccaaggacaactccaagagccaagttttctt
aaaaatgaacagtctgcaaactgatgacacagccagatactactgtgccagagatggttatagtaactttcattactatgttatggact
actggggtcaaggaacctcagtcaccgtctcctct(gatcag)

2e12scFv (amino acid sequence) (SEQ ID NO ___)

MDFQVQIFSFLLISASVIMSRGVDIVLTQSPASLAVSLGQRATISCRASESVEYYVTS
LMQWYQQKPGQPPKLLISAASNVESGVPARFSGSGSGTDFSLNIHPVEEDDIAMYF
CQQSRKVPWTFGGGTKLEIKRGGGGSGGGGSGGGGSQVQLKESGPGLVAPSQSLS
ITCTVSGFSLTGYGVNWVRQPPGKGLEWLGMIWGDGSTDYNSALKSRLSITKDNS
KSQVFLKMNSLQTDDTARYYCARDGYSNFHYYVMDYWGQGTSVTVSS

10A8 is anti-CD152 (CTLA-4)

10A8 VL (with L6 VK leader peptide) (nucleotide sequence) (SEQ ID NO ___)

306

atggattttcaagtgcagattttcagcttcctgctaatcagtgcttcagtcataatgtccagaggagtcgacatccagatgacacagtct
ccatcctcactgtctgcatctctgggaggcaaagtcaccatcacttgcaaggcaagccaagacattaagaagtatataggttggtac
caacacaagcctggaaaaggtcccaggctgctcatatattacacatctacattacagccaggcatcccatcaaggttcagtggaagt
gggtctgggagagattattccctcagcatcagaacctggagcctgaagatattgcaacttattattgtcaacagtatgataatcttcc
attgacgttcggctcggggacaaagttggaataaaacgg

10A8 VL (amino acid sequence) (SEQ ID NO ___)
MDFQVQIFSFLLISASVIMSRGVDIQMTQSPSSLSASLGGKVTITCKASQDIKKYIG
WYQHKPGKGPRLLIYYTSTLQPGIPSRFSGSGSGRDYSLSIRNLEPEDIATYYCQQY
DNLPLTFGSGTKLEIKR

10A8 VH (no leader peptide) (nucleotide sequence) (SEQ ID NO ___)
gatgtacagcttcaggagtcaggacctggcctcgtgaaacctctcagtctctgtctctcacctgctctgtcactggctactccatcac
cagtggtttctactggaactggatccgacagtttccgggaaacaaactggaatggatgggctacatagctacgacggtagcaat
aactacaacccatctctcataaatcgaatctccatcactcgtgacacatctaagaaccagttttcctgaagttgagtctgtgactactg
aggacacagctacatatttctgtgcaagacactacggtagtagcgggagctatggactactggggtcaaggaacctcagtcaccgtc
tcctctgatca

10A8 VH (amino acid sequence) (SEQ ID NO ___)
DVQLQESGPGLVKPSQSLSLTCSVTGYSITSGFYWNWIRQFPGNKLEWMGHISHD
GRNNYNPSLINRISITRDTSKNQFFLKLSSVTTEDTATYFCARHYGSSGAMDYWGQ
GTSVTVSS

10A8 SCFV (nucleotide sequence) (SEQ ID NO ___)
aagcttatggattttcaagtgcagattttcagcttcctgctaatcagtgcttcagtcataatgtccagaggagtcgacatccagatgaca
cagtctccatcctcactgtctgcatctctgggaggcaaagtcaccatcacttgcaaggcaagccaagacattaagaagtatataggtt
ggtaccaacacaagcctggaaaaggtcccaggctgctcatatattacacatctacattacagccaggcatcccatcaaggttcagtg
gaagtgggtctgggagagattattccctcagcatcagaacctggagcctgaagatattgcaacttattattgtcaacagtatgataat
cttccattgacgttcggctcggggacaaagttggaataaaacggggtggcggtggctcgggcggtggtgggtcgggtggcggc
ggatctgatgtacagcttcaggagtcaggacctggcctcgtgaaacctctcagtctctgtctctcacctgctctgtcactggctactc

catcaccagtggttctactggaactggatccgacagttccgggaaacaaactggaatggatgggccacataagccacgacggta
ggaataactacaacccatctctcataaatcgaatctccatcactcgtgacacatctaagaaccagttttcctgaagttgagttctgtga
ctactgaggacacagctacatattttctgtgcaagacactacggtagtagcggagctatggactactgggggtcaaggaacctcagtc
accgtctcctctgatca

10A8 SCFV (amino acid sequence) (SEQ ID NO ___)

MDFQVQIFSFLLISASVIMSRGVDIQMTQSPSSLSASLGGKVTITCKASQDIKKYIG
WYQHKPGKGPRLLIYYTSTLQPGIPSRFSGSGSGRDYSLSIRNLEPEDIATYYCQQY
DNLPLTFGSGTKLEIKRGGGGSGGGGSGGGGSDVQLQESGPGLVKPSQSLSLTCSV
TGYSITSGFYWNWIRQFPGNKLEWMGHISHDGRNNYNPSLINRISITRDTSKNQFFL
KLSSVTTEDTATYFCARHYGSSGAMDYWGQGTSVTVSSD

40.2.220-hmtIgG1-hCD80 (nucleotide sequence) (SEQ ID NO ___)

aagcttatggattttcaagtgcagattttcagcttcctgctaatcagtgcttcagtcataatgtccagaggagtcgacattgttctgactc
agtctccagccaccctgtctgtgactccaggagtagagtctttctttcctgcagggccagccagagtattagcgactacttacactg
gtatcaacaaaaatcacatgagtctccaaggcttctcatcaaatatgcttcccatccatctctgaggatccccttccaggttcagtggca
gtggatcagggtcagatttcactctcagtatcaacagtgtggaacctgaagatgttggaatttattactgtcaacatggtcacagcttc
cgtggacgttcggtggaggcaccaagctggaaatcaaacgggatggcggtggctcgaggcggaggtgggtcgggtggcggcgg
gatctcagatccagttggtgcaatctggacctgagctgaagaagcctggagagacagtcaggatctcctgcaaggcttctgggtat
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ggggatcgtcagtcttcctcttcccccaaaacccaaggacaccctcatgatctcccggacccctgaggtcacatgcgtggtggtg
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tcttcctctacagcaagctcaccgtggacaagagcaggtggcagcaggggaacgtcttctcatgctccgtgatgcatgaggctctg
cacaaccactacacgcagaagagcctctccctgtctccgggtaaagcggatccttcgaacctgctccatcctgggccattacctta

atctcagtaaatggaatttttgtgatatgctgcctgacctactgctttgccccaagatgcagaggaggaaggaggaatgagaggatga
gaagggaaagtgtacgccctgtataaatcgat

40.2.220-hmtIgG1-hCD80 (amino acid sequence) (SEQ ID NO ___)

MDFQVQIFSFLLISASVIMSRGVDIVLTQSPATLSVTPGDRVSLSCRASQSISDYLHW
YQQKSHESPRLLIKYASHSISGIPSRFSGSGSGSDFTLSINSVEPEDVGIYYCQHGHSF
PWTFGGGTKLEIKRGGGGSGGGGSGGGGSQIQLVQSGPELKKPGETVRISCKASG
YAFTTTGMQWVQEMPGKGLKWIGWINTPLWSAKICRRLQGRFAFSLETSANTAY
LQISNLKDEDTATYFCVRSGNGNYDLAYFAYWGQGTLVTVSDLEPKSSDKTHTSP
PSPAPELLGGSSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVE
VHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISK
AKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKT
TPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGKA
DPSNLLPSWAITLISVNGIFVICCLTYCFAPRCRERRRNERLRRESVRPV

2e12scFv- hmtIgG1-CD80 fusion protein (nucleotide sequence) (SEQ ID NO ___)

agcttatggatttcaagtgcagatttcagcttcctgctaatcagtgcttcagtcataatgtccagaggagtcgacattgtgctcaccc
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taatgcagtggtaccaacagaaaccaggacagccacccaaactcctcatctctgctgcatccaacgtagaatctggggtccctgcc
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ccccgagaaccacaggtgtacaccctgccccatcccgggatgagctgaccaagaaccaggtcagcctgacctgcctggtcaaa

ggcttctatcccagcgacatcgccgtggagtgggagagcaatgggcagccggagaacaactacaagaccacgcctcccgtgct
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gtgatgcatgaggctctgcacaaccactacacgcagaagagcctctccctgtctccgggtaaagcggatccttcgaacctgctccc
atcctgggccattaccttaatctcagtaaatggaatttttgtgatatgctgcctgacctactgctttgccccaagatgcagagagagaa
ggaggaatgagagattgagaagggaaagtgtacgccctgtataaatcgat

2e12scFv-hmtIgG1-CD80 fusion protein (amino acid sequence) (SEQ ID NO ___)

MDFQVQIFSFLLISASVIMSRGVDIVLTQSPASLAVSLGQRATISCRASESVEYYVTS
LMQWYQQKPGQPPKLLISAASNVESGVPARFSGSGSGTDFSLNIHPVEEDDIAMYF
CQQSRKVPWTFGGGTKLEIKRGGGGSGGGGSGGGGSQVQLKESGPGLVAPSQSLS
ITCTVSGFSLTGYGVNWVRQPPGKGLEWLGMIWGDGSTDYNSALKSRLSITKDNS
KSQVFLKMNSLQTDDTARYYCARDGYSNFHYYVMDYWGQGTSVTVSSDLEPKS
SDKTHTSPPSPAPELLGGSSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFN
WYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALP
APIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQ
PENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSL
SLSPGKADPSNLLPSWAITLISVNGIFVICCLTYCFAPRCRERRRNERLRRESVRPV

10A8 scFv-hmtIgG1-CD80 (nucleotide sequence) (SEQ ID NO ___)

aagcttatggattttcaagtgcagattttcagcttcctgctaatcagtgcttcagtcataatgtccagaggagtcgacatccagatgaca
cagtctccatcctcactgtctgcatctctgggaggcaaagtcaccatcacttgcaaggcaagccaagacattaagaagtatataggtt
ggtaccaacacaagcctggaaaaggtcccaggctgctcatatattacacatctacattacagccaggcatcccatcaaggttcagtg
gaagtgggtctgggagagattattccctcagcatcagaaacctggagcctgaagatattgcaacttattattgtcaacagtatgataat
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catcaccagtggtttctactggaactggatccgacagttccgggaaacaaactggaatggatgggccacataagccacgacggta
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accgtctcctctgatctggagcccaaatcttctgacaaaactcacacatcccccaccgtcccccagcacctgaactcctgggggggatc
gtcagtcttcctcttcccccaaaacccaaggacaccctcatgatctcccggaccccctgaggtcacatgcgtggtggtggacgtga
gccacgaagaccctgaggtcaagttcaactggtacgtggacggcgtggaggtgcataatgccaagacaaagccgcgggaggg

gcagtacaacagcacgtaccgtgtggtcagcgtcctcaccgtcctgcaccaggactggctgaatggcaaggagtacaagtgcaa
ggtctccaacaaagccctcccagccccatcgagaaaaccatctccaaagccaaagggcagccccgagaaccacaggtgtaca
ccctgccccatcccgggatgagctgaccaagaaccaggtcagcctgacctgcctggtcaaaggcttctatcccagcgacatcgc
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cactacacgcagaagagcctctccctgtctccgggtaaagcggatccttcgaacctgctcccatcctgggccattacctaatctca
gtaaatggaatttttgtgatatgctgcctgacctactgctttgccccaagatgcagaggaggaaggaggaatgagagattgagaagg
gaaagtgtacgccctgtataaatcgat

10A8 scFv-hmtIgG1-CD80 (amino acid sequence) (SEQ ID NO ___)

MDFQVQIFSFLLISASVIMSRGVDIQMTQSPSSLSASLGGKVTITCKASQDIKKYIG
WYQHKPGKGPRLLIYYTSTLQPGIPSRFSGSGSGRDYSLSIRNLEPEDIATYYCQQY
DNLPLTFGSGTKLEIKRGGGGSGGGGSGGGGSDVQLQESGPGLVKPSQSLSLTCSV
TGYSITSGFYWNWIRQFPGNKLEWMGHISHDGRNNYNPSLINRISITRDTSKNQFFL
KLSSVTTEDTATYFCARHYGSSGAMDYWGQGTSVTVSSDLEPKSSDKTHTSPPSP
APELLGGSSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVH
NAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAK
GQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPP
VLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGKADPS
NLLPSWAITLISVNGIFVICCLTYCFAPRCRERRRNERLRRESVRPV

500A2-hmtIgG1-CD80 (nucleotide sequence) (SEQ ID NO ___)

atgttgtatacatctcagctccttgggcttttactcttctggatttcagcctccagaagtgacatagtgctgactcagactccagccactc
tgtctctaattcctggagaaagagtcacaatgacctgtaaggccagtcagaatattggcacaatcttacactggtatcaccaaaaacc
aaaggaggctccaagggctctcatcaagtatgcttcgcagtccattcctgggatcccctccagattcagtggcagtggttcggaaac
agatttcactctcagcatcaataacctggagcctgatgatatcggaatttattactgtcaacaaagtagaagctggcctgtcacgttcg
gtcctggcaccaagctggagataaaacggggtggcggtggctcgggcggaggtgggtcgggtggcggcggatctcaggtcaa
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gattctgccatctattactgtgcaagaaggccggtagcgacgggccatgctatggactactggggtcaggggatccaagttaccgt

ctcctctgatctggagcccaaatcttctgacaaaactcacacatcccccaccgtccccagcacctgaactcctgggggggatcgtcagt
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aagaccctgaggtcaagttcaactggtacgtggacggcgtggaggtgcataatgccaagacaaagccgcgggaggagcagtac
aacagcacgtaccgtgtggtcagcgtcctcaccgtcctgcaccaggactggctgaatggcaaggagtacaagtgcaaggtctcca
acaaagccctcccagccccccatcgagaaaaccatctccaaagccaaagggcagccccgagaaccacaggtgtacaccctgccc
ccatcccgggatgagctgaccaagaaccaggtcagcctgacctgcctggtcaaaggcttctatcccagcgacatcgccgtggagt
gggagagcaatgggcagccggagaacaactacaagaccacgcctcccgtgctggactccgacggctccttcttcctctacagca
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gcagaagagcctctccctgtctccgggtaaagcggatccttcgaacctgctcccatcctgggccattacctaatctcagtaaatgga
attttgtgatatgctgcctgacctactgctttgccccaagatgcagagagagaaggaggaatgagagattgagaagggaaagtgta
cgccctgtataaatcgat

500A2-hmtIgG1-CD80 (amino acid sequence) (SEQ ID NO ___)

MLYTSQLLGLLLFWISASRSDIVLTQTPATLSLIPGERVTMTCKTSQNIGTILHWYH
QKPKEAPRALIKYASQSIPGIPSRFSGSGSETDFTLSINNLEPDDIGIYYCQQSRSWPV
TFGPGTKLEIKRGGGGSGGGGSGGGGSQVKLQQSGSELGKPGASVKLSCKTSGYIF
TDHYISWVKQKPGESLQWIGNVYGGNGGTSYNQKFQGKATLTVDKISSTAYMEL
SSLTSEDSAIYYCARRPVATGHAMDYWGQGIQVTVSSDLEPKSSDKTHTSPPSPAP
ELLGGSSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNA
KTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQ
PREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVL
DSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGKADPSNL
LPSWAITLISVNGIFVICCLTYCFAPRCRERRRNERLRRESVRPV

2H7 scFv MTH(SSS)WTCH2CH3 (nucleotide sequence) (SEQ ID NO ___)

aagcttgccgccatggattttcaagtgcagattttcagcttcctgctaatcagtgcttcagtcataattgccagaggacaaattgttctct
cccagtctccagcaatcctgtctgcatctccaggggagaaggtcacaatgacttgcagggccagctcaagtgtaagttacatgcact
ggtaccagcagaagccaggatcctccccaaaccctggatttatgccccatccaacctggcttctggagtccctgctcgcttcagtg
gcagtgggtctgggacctcttactctctcacaatcagcagagtggaggctgaagatgctgccacttattactgccagcagtggagttt
tacccacccacgttcggtgctgggaccaagctggagctgaaagatggcggtggctcgggcggtggtggatctggaggaggtg
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ggtgatacttcctacaatcagaagttcaagggcaaggccacactgactgtagacaaatcctccagcacagcctacatgcagctcag
cagcctgacatctgaagactctgcggtctatttctgtgcaagagtggtgtactatagtaactcttactggtacttcgatgtctggggcac
agggaccacggtcaccgtctcttctgatcaggagcccaaatcttctgacaaaactcacacatcccccaccgtcccagcacctgaac
tcctgggggggaccgtcagtcttcctcttccccccaaaacccaaggacaccctcatgatctcccggacccctgaggtcacatgcgtg
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cacaggtgtacaccctgcccccatcccgggatgagctgaccaagaaccaggtcagcctgacctgcctggtcaaaggcttctatcc
cagcgacatcgccgtggagtgggagagcaatgggcagccggagaacaactacaagaccacgcctcccgtgctggactccgac
ggctccttcttcctctacagcaagctcaccgtggacaagagcaggtggcagcaggggaacgtcttctcatgctccgtgatgcatga
ggctctgcacaaccactacacgcagaagagcctctccctgtctccgggtaaatgatctaga

2H7 scFv MTH(SSS)WTCH2CH3 (amino acid sequence) (SEQ ID NO ___)

MDFQVQIFSFLLISASVIIARGQIVLSQSPAILSASPGEKVTMTCRASSSVSYMHWY
QQKPGSSPKPWIYAPSNLASGVPARFSGSGSGTSYSLTISRVEAEDAATYYCQQWS
FNPPTFGAGTKLELKDGGGSGGGGSGGGGSSQAYLQQSGAELVRPGASVKMSCK
ASGYTFTSYNMHWVKQTPRQGLEWIGAIYPGNGDTSYNQKFKGKATLTVDKSSS
TAYMQLSSLTSEDSAVYFCARVVYYSNSYWYFDVWGTGTTVTVSSDQEPKSSDK
THTSPPSPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYV
DGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIE
KTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPEN
NYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLS
PGK

2H7 scFv IgAH IGG WT CH2CH3 (2H7 scFv with IgA hinge and WT CH2 and CH3) (nucleotide sequence) (SEQ ID NO ___)

aagcttgccgccatggattttcaagtgcagattttcagcttcctgctaatcagtgcttcagtcataattgccagaggacaaattgttctct
cccagtctccagcaatcctgtctgcatctccaggggagaaggtcacaatgacttgcagggccagctcaagtgtaagttacatgcact
ggtaccagcagaagccaggatcctcccccaaaccctggatttatgccccatccaacctggcttctggagtccctgctcgcttcagtg
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tacccacccacgttcggtgctgggaccaagctggagctgaaagatggcggtggctcgggcggtggtggatctggaggaggtg
ggagctctcaggcttatctacagcagtctggggctgagctggtgaggcctggggcctcagtgaagatgtcctgcaaggcttctggc
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tgccaagacaaagccgcgcgggaggagcagtacaacagcacgtaccgtgtggtcagcgtcctcaccgtcctgcaccaggactggc
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ctggactccgacggctccttcttcctctacagcaagctcaccgtggacaagagcaggtggcagcaggggaacgtcttctcatgctc
cgtgatgcatgaggctctgcacaaccactacacgcagaagagcctctccctgtctccgggtaaatgatctaga

2H7 scFv IgAH 1GG WT CH2CH3 (amino acid sequence) (SEQ ID NO ___)

MDFQVQIFSFLLISASVIIARGQIVLSQSPAILSASPGEKVTMTCRASSSVSYMHWY
QQKPGSSPKPWIYAPSNLASGVPARFSGSGSGTSYSLTISRVEAEDAATYYCQQWS
FNPPTFGAGTKLELKDGGGSGGGGSGGGGSSQAYLQQSGAELVRPGASVKMSCK
ASGYTFTSYNMHWVKQTPRQGLEWIGAIYPGNGDTSYNQKFKGKATLTVDKSSS
TAYMQLSSLTSEDSAVYFCARVVYYSNSYWYFDVWGTGTTVTVSDQPVPSTPPTP
SPSTPPTPSPSCAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFN
WYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALP
APIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQ
PENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSL
SLSPGK

2H7 scFv IgAH IgACH2CH3 (2H7 scFv IgAhinge and IgA CH2 and CH3) (nucleotide sequence) (SEQ ID NO ___)

aagcttgccgccatggattttcaagtgcagattttcagcttcctgctaatcagtgcttcagtcataattgccagaggacaaattgttctct
cccagtctccagcaatcctgtctgcatctccaggggagaaggtcacaatgacttgcagggccagctcaagtgtaagttacatgcact

ggtaccagcagaagccaggatcctccccaaaccctggatttatgccccatccaacctggcttctggagtcctgctcgcttcagtg
gcagtgggtctgggacctcttactctctcacaatcagcagagtggaggctgaagatgctgccacttattactgccagcagtggagttt
taacccacccacgttcggtgctgggaccaagctggagctgaaagatggcggtggctcgggcggtggtggatctggaggaggtg
ggagctctcaggcttatctacagcagtctggggctgagctggtgaggcctgggacctcagtgaagatgtcctgcaaggcttctggc
tacacatttaccagttacaatatgcactgggtaaagcagacacctagacagggcctggaatggattggagctatttatccaggaaat
ggtgatacttcctacaatcagaagttcaagggcaaggccacactgactgtagacaaatcctccagcacagcctacatgcagctcag
cagcctgacatctgaagactctgcggtctatttctgtgcaagagtggtgtactatagtaactcttactggtacttcgatgtctggggcac
agggaccacggtcaccgtctcttctgatcagccagttcctcaactccacctaccccatctccctcaactccacctaccccatctccc
tcatgctgccacccccgactgtcactgcaccgaccggccctcgaggacctgctcttaggttcagaagcgatcctcacgtgcacact
gaccggcctgagagatgcctcaggtgtcaccttcacctggacgccctcaagtgggaagagcgctgttcaaggaccacctgaccgt
gacctctgtggctgctacagcgtgtccagtgtcctgccgggctgtgccgagccatggaaccatgggaagaccttcacttgcactgc
tgcctaccccgagtccaagaccccgctaaccgccaccctctcaaaatccggaaacacattccggcccgaggtccacctgctgccg
ccgccgtcggaggagctggccctgaacgagctggtgacgctgacgtgcctggcacgtggcttcagccccaaggatgtgctggtt
cgctggctgcaggggtcacaggagctgccccgcgagaagtacctgacttgggcatcccggcaggagcccagccagggcacca
ccaccttcgctgtgaccagcatactgcgcgtggcagccgaggactggaagaagggggacaccttctcctgcatggtgggccatcg
aggccctgccgctggccttcacacagaagaccatcgaccgcttggcgggtaaacccacccatgtcaatgtgtctgtgtcatggcg
gaggtggacggcacctgctactgataatctaga

2H7 scFv IgAH IgACH2CH3 (2H7 scFv IgA hinge and IgA CH2 and CH3) (amino acid sequence) (SEQ ID NO ___)

MDFQVQIFSFLLISASVIIARGQIVLSQSPAILSASPGEKVTMTCRASSSVSYMHWY
QQKPGSSPKPWIYAPSNLASGVPARFSGSGSGTSYSLTISRVEAEDAATYYCQQWS
FNPPTFGAGTKLELKDGGGSGGGGSGGGGSSQAYLQQSGAELVRPGASVKMSCK
ASGYTFTSYNMHWVKQTPRQGLEWIGAIYPGNGDTSYNQKFKGKATLTVDKSSS
TAYMQLSSLTSEDSAVYFCARVVYYSNSYWYFDVWGTGTTVTVSSDQPVPSTPPT
PSPSTPPTPSPSCCHPRLSLHRPALEDLLLGSEAILTCTLTGLRDASGVTFTWTPSSG
KSAVQGPPDRDLCGCYSVSSVLPGCAEPWNHGKTFTCTAAYPESKTPLTATLSKS
GNTFRPEVHLLPPPSEELALNELVTLTCLARGFSPKDVLVRWLQGSQELPREKYLT
WASRQEPSQGTTTFAVTSILRVAAEDWKKGDTFSCMVGHEALPLAFTQKTIDRLA
GKPTHVNVSVVMAEVDGTCY

IgA hinge-CH2-CH3 (Human IgA tail, full length) (nucleotide sequence) (SEQ ID NO ___)

tgatcagccagttccctcaactccacctaccccatctccctcaactccacctaccccatctcctcatgctgccaccccccgactgtca
ctgcaccgaccggcccctcgaggacctgctcttaggttcagaagcgatcctcacgtgcacactgaccggcctgagagatgcctcag
gtgtcaccttcacctggacgccctcaagtgggaagagcgctgttcaaggaccacctgaccgtgacctctgtggctgctacagcgtg
tccagtgtcctgccgggctgtgccgagccatggaaccatgggaagaccttcacttgcactgctgcctaccccgagtccaagaccc
cgctaaccgccaccctctcaaaatccggaaacacattccggccgaggtccacctgctgccgccgtcggaggagctggccc
tgaacgagctggtgacgctgacgtgcctggcacgtggcttcagccccaaggatgtgctggttcgctggctgcaggggtcacagg
agctgccccgcgagaagtacctgacttgggcatcccggcaggagccagccagggcaccaccaccttcgctgtgaccagcata
ctgcgcgtggcagccgaggactggaagaagggggacaccttctcctgcatggtgggccacgaggccctgccgctggccttcac
acagaagaccatcgaccgcttggcgggtaaacccacccatgtcaatgtgtctgttgtcatggcggaggtggacggcacctgctact
gataatctaga

IgA hinge-CH2-CH3 (Human IgA tail, full length) (amino acid sequence) (SEQ ID NO ___)

DQPVPSTPPTPSPSTPPTPSPSCCHPRLSLHRPALEDLLLGSEAILTCTLTGLRDASGV
TFTWTPSSGKSAVQGPPDRDLCGCYSVSSVLPGCAEPWNHGKTFTCTAAYPESKT
PLTATLSKSGNTFRPEVHLLPPPSEELALNELVTLTCLARGFSPKDVLVRWLQGSQ
ELPREKYLTWASRQEPSQGTTTFAVTSILRVAAEDWKKGDTFSCMVGHEALPLAF
TQKTIDRLAGKPTHVNVSVVMAEVDGTCY

Human J Chain (nucleotide sequence) (SEQ ID NO ___)

agatctcaagaagatgaaaggattgttcttgttgacaacaaatgtaagtgtgcccggattacttccaggatcatccgttcttccgaaga
tcctaatgaggacattgtggagagaaacatccgaattattgttcctctgaacaacagggagaataictctgatcccacctcaccattga
gaaccagatttgtgtaccatttgtctgacctcagctgtaaaaaatgtgatcctacagaagtggagctggatnatcagatagttactgct
acccagagcaatatctgtgatgaagacagtgctacagagacctgctacacttatgacagaaacaagtgctacacagctgtggtccc
actcgtatatggtggtgagaccaaaatggtggaaacagccttaaccccagatgcctgctatcctgactaatctaga

Human J Chain (amino acid sequence) (SEQ ID NO ___)

RSQEDERIVLVDNKCKCARITSRIIRSSEDPNEDIVERNIRIIVPLNNRENISDPTSPLR
TRFVYHLSDLSCKKCDPTEVELDNQIVTATQSNICDEDSATETCYTYDRNKCYTAV
VPLVYGGETKMVETALTPDACYP

4 carboxy terminal amino acids deleted from IgA CH3 (amino acid sequence) (SEQ ID NO ___)
GTCY

IgAH IgAT4 (human IgA tail, truncated (3T1)-(missing last 4 amino acids from carboxy terminus) (nucleotide sequence) (SEQ ID NO ___)
tgatcagccagttccctcaactccacctaccccatctccctcaactccacctaccccatctcctcatgctgccacccccgactgtca
ctgcaccgaccggccctcgaggacctgctcttaggttcagaagcgatcctcacgtgcacactgaccggcctgagagatgcctcag
gtgtcaccttcacctggacgccctcaagtgggaagagcgctgttcaaggaccacctgaccgtgacctctgtggctgctacagcgtg
tccagtgtcctgccgggctgtgccgagccatggaaccatgggaagaccttcactgcactgctgcctaccccgagtccaagaccc
cgctaaccgccacccctctcaaaatccggaaacacattccggcccgaggtccacctgctgccgccgccgtcgtgaggagctggccc
tgaacgagctggtgacgctgacgtgcctggcacgtggcttcagccccaaggatgtgctggttcgctggctgcagggctcacagg
agtatgcccgcgagaagtacctgacttgggcatcccggcaggagcccagccagggcaccaccaccttcgctgtgaccagcata
ctgcgcgtggcagccgaggactggaagaagggggacaccttctcctgcatggtgggccacgaggccctgccgctggccttcac
acagaagaccatcgaccgcttggcgggtaaacccacccatgtcaatgtgtctgttgtcatggcggaggtggactgataatctaga

IgAH IgAT4 (amino acid sequence) (SEQ ID NO ___)
DQPVPSTPPTPSPSTPPTPSPSCCHPRLSLHRPALEDLLLGSEAILTCTLTGLRDASGV
TFTWTPSSGKSAVQGPPDRDLCGCYSVSSVLPGCAEPWNHGKTFTCTAAYPESKT
PLTATLSKSGNTFRPEVHLLPPPSEELALNELVTLTCLARGFSPKDVLVRWLQGSQ
ELPREKYLTWASRQEPSQGTTTFAVTSILRVAAEDWKKGDTFSCMVGHEALPLAF
TQKTIDRLAGKPTHVNVSVVMAEVD

2H7 scFv IgAH IgAT4 (2H7 scFv IgA 3T1 construct; truncates the CH3 domain at the 3'end) (nucleotide sequence) (SEQ ID NO ___)

aagcttgccgccatggattttcaagtgcagattttcagcttcctgctaatcagtgtttcagtcataattgtccagaggacaaattgttctct
cccagtctccagcaatcctgtctgcatctccaggggagaaggtcacaatgacttgcagggccagctcaagtgtaagttacatgcact
ggtaccagcagaagccaggatcctcccccaaaccctggatttatgccccatccaacctggcttctggagtccctgctcgcttcagtg
gcagtgggtctgggacctcttactctctcacaatcagcagagtggaggctgaagatgctgccactiattactgccagcagtggagtit
tacccacccacgttcggtgctgggaccaagctggagctgaaagatggcggtggctcgggcggtggtggatctggaggaggtg
ggagctctcaggcttatctacagcagtctggggctgagctggtgaggcctggggcctcagtgaagatgtcctgcaaggcttctggc
tacacatttaccagttacaatatgcactgggtaaagcagacacctagacagggcctggaatggattggagctatttatccaggaaat
ggtgatacttcctacaatcagaagttcaagggcaaggccacactgactgtagacaaatcctccagcacagcctacatgcagctcag
cagcctgacatctgaagactctgcggtctatttctgtgcaagagtggtgtactatagtaactcttactggtacttcgatgtctggggcac
agggaccacggtcaccgtctcttctgatcagccagttcctcaactccacctaccccatctcctcaactccacctaccccatctccc
tcatgctgccacccccgactgtcactgcaccgaccggccctcgaggacctgctcttaggttcagaagcgatcctcacgtgcacact
gaccggcctgagagatgcctcaggtgtcaccttcacctggacgcccctcaagtgggaagagcgctgttcaaggaccacctgaccgt
gacctctgtggctgctacagcgtgtccagtgtcctgccgggctgtgccgagccatggaaccatgggaagacctcacttgcactgc
tgcctaccccgagtccaagaccccgctaaccgccacctctcaaaatccggaaacacattccggccgaggtccacctgctgccg
ccgccgtcggaggagctggccctgaacgagctggtgacgctgacgtgcctggcacgtggcttcagccccaaggatgtgctggtt
cgctggctgcaggggtcacaggagctgccccgcgagaagtacctgacttgggcatcccggcaggagcccagccagggcacca
ccaccttcgctgtgaccagcatactgcgcgtggcagccgaggactggaaggaggggacaccttctcctgcatggtgggccacg
aggccctgccgctggccttcacacagaagaccatcgaccgcttggcgggtaaacccacccatgtcaatgtgtctgttgtcatggcg
gaggtggactgataatctaga

2H7 scFv IgAH-T4 (amino acid sequence) (SEQ ID NO ___)

MDFQVQIFSFLLISASVIIARGQIVLSQSPAILSASPGEKVTMTCRASSSVSYMHWY
QQKPGSSPKPWIYAPSNLASGVPARFSGSGSGTSYSLTISRVEAEDAATYYCQQWS
FNPPTFGAGTKLELKDGGGSGGGGSGGGGSSQAYLQQSGAELVRPGASVKMSCK
ASGYTFTSYNMHWVKQTPRQGLEWIGAIYPGNGDTSYNQKFKGKATLTVDKSSS
TAYMQLSSLTSEDSAVYFCARVVYYSNSYWYFDVWGTGTTVTVSSDQPVPSTPPT
PSPSTPPTPSPSCCHPRLSLHRPALEDLLLGSEAILTCTLTGLRDASGVTFTWTPSSG
KSAVQGPPDRDLCGCYSVSSVLPGCAEPWNHGKTFTCTAAYPESKTPLTATLSKS
GNTFRPEVHLLPPPSEELALNELVTLTCLARGFSPKDVLVRWLQGSQELPREKYLT
WASRQEPSQGTTTFAVTSILRVAAEDWKKGDTFSCMVGHEALPLAFTQKTIDRLA
GKPTHVNVSVVMAEVD

14 amino acids deleted from IgAH-T4 (so that total of 18 amino acids deleted from wild type IgA CH3) (amino acid sequence) (SEQ ID NO ___)

PTHVNVSVVMAEVD

IgAH IgA-T18 (human IgA Tail truncated, 3T2) (nucleotide sequence) (SEQ ID NO ___)

tgatcagccagttccctcaactccacctaccccatctcctcaactccacctaccccatctcctcatgctgccaccccccgactgtca
ctgcaccgaccggcctcgaggacctgctcttaggttcagaagcgatcctcacgtgcacactgaccggcctgagagatgcctcag
gtgtcaccttcacctggacgccctcagtgggaagagcgctgttcaaggaccacctgaccgtgacctctgtggctgctacagcgtg
tccagtgtcctgccgggctgtgccgagccatggaaccatgggaagaccttcacttgcactgctgcctaccccgagtccaagaccc
cgctaaccgccaccctctcaaaatccggaaacacattccggcccgaggtccacctgctgccgccgccgtcggaggagctggccc
tgaacgagctggtgacgctgacgtgcctggcacgtggcttcagccccaaggatgtgctggttcgctggctgcaggggtcacagg
agctgccccgcgagaagtacctgactgggcatccggcaggagcccagccagggaccaccaccttcgctgtgaccagcata
ctgcgcgtggcagccgaggactggaagaggggacaccttctcctgcatggtgggccacgaggccctgccgctggccttcac
acagaagaccatcgaccgcttggcgggtaaa

IgAH IgA-T18 (amino acid sequence) (SEQ ID NO ___)

DQPVPSTPPTPSPSTPPTPSPSCCHPRLSLHRPALEDLLLGSEAILTCTLTGLRDASGV
TFTWTPSSGKSAVQGPPDRDLCGCYSVSSVLPGCAEPWNHGKTFTCTAAYPESKT
PLTATLSKSGNTFRPEVHLLPPPSEELALNELVTLTCLARGFSPKDVLVRWLQGSQ
ELPREKYLTWASRQEPSQGTTTFAVTSILRVAAEDWKKGDTFSCMVGHEALPLAF
TQKTIDRLAGK

2H7 scFv IgAH IgAT18 (human IgA Tail truncated, 3T2) (nucleotide sequence) (SEQ ID NO ___)

aagcttgccgccatggatttttcaagtgcagattttcagcttcctgctaatcagtgcttcagtcataattgccagaggacaaattgttctct
cccagtctccagcaatcctgtctgcatctccaggggagaaggtcacaatgacttgcagggccagctcaagtgtaagttacatgcact
ggtaccagcagaagccaggatcctcccccaaaccctggatttatgccccatccaacctggcttctggagtccctgctcgcttcagtg
gcagtgggtctgggacctcttactctctcacaatcagcagagtggaggctgaagatgctgccacttattactgccagcagtggagttt
taacccacccacgttcggtgctgggaccaagctggagctgaaagatggcggtggctcgggcggtggtggatctggaggaggtg

ggagctctcaggcttatctacagcagtctggggctgagctggtgaggcctggggcctcagtgaagatgtcctgcaaggcttctggc
tacacatttaccagttacaatatgcactgggtaaagcagacacctagacagggcctggaatggattggagctatttatccaggaaat
ggtgatacttcctacaatcagaagttcaagggcaaggccacactgactgtagacaaatcctccagcacagcctacatgcagctcag
cagcctgacatctgaagactctgcggtctattttctgtgcaagagtggtgtactatagtaactcttactggtacttcgatgtctggggcac
agggaccacggtcaccgtctcttctgatcagccagttccctcaactccacctaccccatctccctcaactccacctaccccatctccc
tcatgctgccaccccccgactgtcactgcaccgaccggccctcgaggacctgtcttaggttcagaagcgatcctcacgtgcacact
gaccggcctgagagatgcctcaggtgtcaccttcacctggacgccctcaagtgggaagagcgctgttcaaggaccacctgaccgt
gacctctgtggctgctacagcgtgtccagtgtcctgccgggctgtgccgagccatggaaccatgggaagaccttcacttgcactgc
tgcctaccccgagtccaagaccccgctaaccgccaccctctcaaaatccggaaacacattccggcccgaggtccacctgctgccg
ccgccgtcggaggagctggccctgaacgagctggtgacgctgacgtgcctggcacgtggcttcagcccaaggatgtgctggtt
cgctggctgcaggggtcacaggagctgccccgcgagaagtacctgacttgggcatcccggcaggagcccagccagggcacca
ccaccttcgctgtgaccagcatactgcgcgtggcagccgaggactggaagaagggggacacctctcctgcatggtgggccacg
aggccctgccgctggccttcacacagaagaccatcgaccgcttggcgggtaaa

2H7 scFv IgAH IgAT18 (amino acid sequence) (SEQ ID NO __)

MDFQVQIFSFLLISASVIIARGQIVLSQSPAILSASPGEKVTMTCRASSSVSYMHWY
QQKPGSSPKPWIYAPSNLASGVPARFSGSGSGTSYSLTISRVEAEDAATYYCQQWS
FNPPTFGAGTKLELKDGGGSGGGGSGGGGSSQAYLQQSGAELVRPGASVKMSCK
ASGYTFTSYNMHWVKQTPRQGLEWIGAIYPGNGDTSYNQKFKGKATLTVDKSSS
TAYMQLSSLTSEDSAVYFCARVVYYSNSYWYFDVWGTGTTVTVSSDQPVPSTPPT
PSPSTPPTPSPSCCHPRLSLHRPALEDLLLGSEAILTCTLTGLRDASGVTFTWTPSSG
KSAVQGPPDRDLCGCYSVSSVLPGCAEPWNHGKTFTCTAAYPESKTPLTATLSKS
GNTFRPEVHLLPPPSEELALNELVTLTCLARGFSPKDVLVRWLQGSQELPREKYLT
WASRQEPSQGTTTFAVTSILRVAAEDWKKGDTFSCMVGHEALPLAFTQKTIDRLA
GK

CTLA-4 IgG WTH WTCH2CH3 (human-oncoMLP-CTLA4EC-hIgGWT)
(nucleotide sequence) (SEQ ID NO ___)

gcaacctacatgatggcggaatgagttgacctcctagatgattccatctgcacgggcacctccagtggaaatcaagtgaacctcact
atccaaggactgaggggccatggacacgggactctacatctgcaaggtggagctcatgtacccaccgccatactacctgggcatag
gcaacggaacccagatttatgtaattgatccagaaccgtgcccagattctgatcaacccaaatcttgtgacaaaactcacacatgcc

caccgtgcccagcacctgaactcctgggggaccgtcagtcttcctcttccccccaaaacccaaggacaccctcatgatctcccgg
acccctgaggtcacatgcgtggtggtggacgtgagccacgaagaccctgaggtcaagttcaactggtacgtggacggcgtggag
gtgcataatgccaagacaaagccgcgggaggagcagtacaacagcacgtaccgtgtggtcagcgtcctcaccgtcctgcaccag
gactggctgaatggcaaggagtacaagtgcaaggtctccaacaaagccctcccagccccatcgagaaaacaatctccaaagcc
aaagggcagccccgagaaccacaggtgtacaccctgcccccatcccgggatgagctgaccaagaaccaggtcagcctgacctg
cctggtcaaaggcttctatcccagcgacatcgccgtggagtgggagagcaatgggcagccggagaacaactacaagaccacgc
ctcccgtgctggactccgacggctccttcttcctctacagcaagctcaccgtggacaagagcaggtggcagcaggggaacgtctt
ctcatgctccgtgatgcatgaggctctgcacaaccactacacgcagaagagcctctccctgtctccgggtaaatga

CTLA-4 IgG WTH WTCH2CH3 (amino acid sequence) (SEQ ID NO ___)

MGVLLTQRTLLSLVLALLFPSMASMAMHVAQPAVVLASSRGIASFVCEYASPGKA
TEVRVTVLRQADSQVTEVCAATYMMGNELTFLDDSICTGTSSGNQVNLTIQGLRA
MDTGLYICKVELMYPPPYYLGIGNGTQIYVIDPEPCPDSDQPKSCDKTHTCPPCPAP
ELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNA
KTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQ
PREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVL
DSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

Human OncoM leader Peptide+CTLA4 EC (BclI) (nucleotide sequence) (SEQ ID NO ___)

atggggtactgctcacacagaggacgctgctcagtctggtccttgcactcctgtttccaagcatggcgagcatggcaatgcacgt
ggcccagcctgtgtggtactggccagcagccgaggcatcgccagctttgtgtgtgagtatgcatctccaggcaaagccactgag
gtccgggtgacagtgcttcggcaggctgacagccaggtgactgaagtctgtgcggcaacctacatgatggggaatgagttgacct
tcctagatgattccatctgcacgggcacctccagtggaaatcaagtgaacctcactatccaaggactgagggccatggacacggg
actctacatctgcaaggtggagctcatgtacccaccgccatactacctgggcataggcaacggaacccagatttatgtaattgatcc
agaaccgtgcccagatttctgatcaa

Human OncoM leader Peptide+CTLA4 EC (amino acid sequence) (SEQ ID NO ___)

MGVLLTQRTLLSLVLALLFPSMASMAMHVAQPAVVLASSRGIASFVCEYASPGKA
TEVRVTVLRQADSQVTEVCAATYMMGNELTFLDDSICTGTSSGNQVNLTIQGLRA
MDTGLYICKVELMYPPPYYLGIGNGTQIYVIDPEPCPDSDQ

Human OncoM leader (nucleotide sequence) (SEQ ID NO ___)

atggggtactgctcacacagaggacgctgctcagtctggtccttgcactcctgttccaagcatggcgagcatg

Human OncoM leader (amino acid sequence) (SEQ ID NO ___)

MGVLLTQRTLLSLVLALLFPSM

Human CTLA4 EC (no LP) (nucleotide sequence) (SEQ ID NO ___)

gcaatgcacgtggcccagcctgctgtggtactggccagcagccgaggcatcgccagctttgtgtgtgagtatgcatctccaggca
aagccactgaggtccgggtgacagtgcttcggcaggctgacagccaggtgactgaagtctgtgcggcaacctacatgacgggga
atgagttgaccttcctagatgattccatctgcacgggcacctccagtggaaatcaagtgaacctcactatccaaggactgagggcca
tggacacgggactctacatctgcaaggtggagctcatgtacccaccgccatactacctgggcataggcaacggaacccagatttat
gtaattgatccagaaccgtgcccagattct

Human CTLA4 EC (no LP) (amino acid sequence) (SEQ ID NO ___)

AMHVAQPAVVLASSRGIASFVCEYASPGKATEVRVTVLRQADSQVTEVCAATYM
TGNELTFLDDSICTGTSSGNQVNLTIQGLRAMDTGLYICKVELMYPPPYYLGIGNG
TQIYVIDPEPCPDS

Human CTLA4 IgG MTH (SSS) MTCH2CH3 (nucleotide sequence) (SEQ ID NO ___)

atggggtactgctcacacagaggacgctgctcagtctggtccttgcactcctgttccaagcatggcgagcatggcaatgcacgt
ggcccagcctgctgtggtactggccagcagccgaggcatcgccagctttgtgtgtgagtatgcatctccaggcaaagccactgag
gtccgggtgacagtgcttcggcaggctgacagccaggtgactgaagtctgtgcggcaacctacatgatggggaatgagttgacct
tcctagatgattccatctgcacgggcacctccagtggaaatcaagtgaacctcactatccaaggactgagggccatggacacggg
actctacatctgcaaggtggagctcatgtacccaccgccatactacctgggcataggcaacggaacccagatttatgtaattgatcc
agaaccgtgcccagattctgatcaacccaaatcttctgacaaaactcacacatcccccaccgtcccccagcacctgaactcctgggggg
gatcgtcagtcttcctcttcccccccaaaacccaaggacaccctcatgatctcccggacccctgaggtcacatgcgtggtggtggac
gtgagccacgaagaccctgaggtcaagttcaactggtacgtggacggcgtggaggtgcataatgccaagacaaagccgcgggga
ggagcagtacaacagcacgtaccgtgtggtcagcgtcctcaccgtcctgcaccaggactggctgaatggcaaggagtacaagtg

caaggtctccaacaaagccctcccagccccatcgagaaaacaatctccaaagccaaagggcagccccgagaaccacaggtgt
acaccctgcccccatcccgggatgagctgaccaagaaccaggtcagcctgacctgcctggtcaaaggcttctatcccagcgacat
cgccgtggagtgggagagcaatgggcagccggagaacaactacaagaccacgcctcccgtgctggactccgacggctccttctt
cctctacagcaagctcaccgtggacaagagcaggtggcagcaggggaacgtcttctcatgctccgtgatgcatgaggctctgcac
aaccactacacgcagaagagcctctccctgtctccgggtaaatga

Human CTLA4 IgG MTH (SSS) MTCH2CH3 (amino acid sequence) (SEQ ID NO ___)

MGVLLTQRTLLSLVLALLFPSMASMAMHVAQPAVVLASSRGIASFVCEYASPGKA
TEVRVTVLRQADSQVTEVCAATYMMGNELTFLDDSICTGTSSGNQVNLTIQGLRA
MDTGLYICKVELMYPPPYYLGIGNGTQIYVIDPEPCPDSDQPKSSDKTHTSPPSPAP
ELLGGSSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNA
KTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQ
PREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVL
DSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

CTLA-4 IgAH IgACH2CH3 (human-oncoMLP-CTLA4EC-IgA) (nucleotide sequence) (SEQ ID NO ___)

atggggtactgctcacacagaggacgctgctcagtctggtccttgcactcctgtttccaagcatggcgagcatggcaatgcacgt
ggcccagcctgctgtggtactggccagcagccgaggcatcgccagctttgtgtgtgagtatgcatctccaggcaaagccactgag
gtccgggtgacagtgcttcggcaggctgacagccaggtgactgaagtctgtgcggcaacctacatgatggggaatgagttgacct
tcctagatgattccatctgcacgggcacctcagtggaaatcaagtgaacctcactatccaaggactgagggccatggacacggg
actctacatctgcaaggtggagctcatgtacccaccgccatactacctgggcataggcaacggaacccagatttatgtaattgatcc
agaaccgtgcccagattctgatcagccagtccctcaactccacctacccctctccctcaactccacctaccccatctccctcatgct
gccaccccccgactgtcactgcaccgaccggccctcgaggacctgctcttaggttcagaagcgatcctcacgtgcacactgaccgg
cctgagagatgcctcaggtgtcaccttcacctggacgccctcagtgggaagagcgctgttcaaggaccacctgaccgtgacctct
gtggctgctacagcgtgtccagtgtcctgccgggctgtgccgagccatggaaccatgggaagaccttcacttgcactgctgcctac
cccgagtccaagaccccgctaaccgccaccctctcaaaatccggaaacacattccggcccgaggtccacctgctgccgccgccg
tcggaggagctggccctgaacgagctggtgacgctgacgtgcctggcacgtggcttcagccccaaggatgtgctggttcgctgg
ctgcaggggtcacaggagctgcccccgcgagaagtacctgacttgggcatcccggcaggagcccagccagggcaccaccacctt
cgctgtgaccagcatactgcgcgtggcagccgaggactggaagaggggacacctttctcctgcatggtgggccacgaggccc

tgccgctggccttcacacagaagaccatcgaccgcttggcgggtaaacccacccatgtcaatgtgtctgttgtcatggcggaggtg
gacggcacctgctactgataatctaga

CTLA-4 IgAH IgACH2CH3 (amino acid sequence) (SEQ ID NO ___)

MGVLLTQRTLLSLVLALLFPSMASMAMHVAQPAVVLASSRGIASFVCEYASPGKA
THVRVTVLRQADSQVTEVCAATYMMGNELTFLDDSICTGTSSGNQVNLTIQGLRA
MDTGLYICKVELMYPPPYYLGIGNGTQIYVIDPEPCPDSDQPVPSTPPTPSPSTPPTP
SPSCCHPRLSLHRPALEDLLLGSEAILTCTLTGLRDASGVTFTWTPSSGKSAVQGPP
DRDLCGCYSVSSVLPGCAEPWNHGKTFTCTAAYPESKTPLTATLSKSGNTFRPEVH
LLPPPSEELALNELVTLTCLARGFSPKDVLVRWLQGSQELPREKYLTWASRQEPSQ
GTTTFAVTSILRVAAEDWKKGDTFSCMVGHEALPLAFTQKTIDRLAGKPTHVNVS
VVMAEVDGTCY

CTLA-4 IgAH IgA-T4 (human-oncoMLP-CTLA4EC-IgA3T1) (nucleotide sequence), (SEQ ID NO ___)

atggggtactgctcacacagaggacgctgctcagtctggtccttgcactcctgttccaagcatggcgagcatggcaatgcacgt
ggcccagcctgctgtggtactggccagcagccgaggcatcgccagctttgtgtgtgagtatgcatctccaggcaaagccactgag
gtccgggtgacagtgcttcggcaggctgacagccaggtgactgaagtctgtgcggcaacctacatgatggggaatgagttgacct
tcctagatgattccatctgcacgggcacctccagtggaaatcaagtgaacctcactatccaaggactgagggccatggacacggg
actctacatctgcaaggtggagctcatgtacccaccgccatactacctgggcataggcaacggaacccagatttatgtaattgatcc
agaaccgtgcccagattctgatcagccagttcctcaactccacctacccatctccctcaactccacctaccccatctccctcatgct
gccacccccgactgtcactgcaccgaccggcctcgaggacctgctcttaggttcagaagcgatcctcacgtgcacactgaccgg
cctgagagatgcctcaggtgtcaccttcacctggacgccctcaagtgggaagagcgctgttcaaggaccacctgaccgtgacctct
gtggctgctacagcgtgtccagtgtcctgccgggctgtgccgagccatggaaccatgggaagaccttcacttgcactgctgcctac
cccgagtccaagaccccgctaaccgccacccctctcaaaatccggaaacacattccggcccgaggttcacctgctgccgccgccg
tcggaggagctggccctgaacgagctggtgacgctgacgtgcctggcacgtggcttcagccccaaggatgtgctggttcgctgg
ctgcaggggtcacaggagctgccccgcgagaagtacctgacttgggcatcccggcaggagcccagccagggcaccaccacctt
cgctgtgaccagcatactgcgcgtggcagccgaggactggaagaagggggacaccttctcctgcatggtgggccacgaggccc
tgccgctggccttcacacagaagaccatcgaccgcttggcgggtaaacccacccatgtcaatgtgtctgttgtcatggcggaggtg
gactgataatctaga

CTLA-4 IgAH IgA-T4 (amino acid sequence) (SEQ ID NO ___)

MGVLLTQRTLLSLVLALLFPSMASMAMHVAQPAVVLASSRGIASFVCEYASPGKA
TEVRVTVLRQADSQVTEVCAATYMMGNELTFLDDSICTGTSSGNQVNLTIQGLRA
MDTGLYICKVELMYPPPYYLGIGNGTQIYVIDPEPCPDSDQPVPSTPPTPSPSTPPTP
SPSCCHPRLSLHRPALEDLLLGSEAILTCTLTGLRDASGVTFTWTPSSGKSAVQGPP
DRDLCGCYSVSSVLPGCAEPWNHGKTFTCTAAYPESKTPLTATLSKSGNTFRPEVH
LLPPPSEELALNELVTLTCLARGFSPKDVLVRWLQGSQELPREKYLTWASRQEPSQ
GTTTFAVTSILRVAAEDWKKGDTFSCMVGHEALPLAFTQKTIDRLAGKPTHVNVS
VVMAEVD

human IgG1 CH2 with 238 mutation pro→ser (nucleotide sequence) (SEQ ID NO ___)

cctgaactcctgggggsatcgtcagtcttcctcttccccccaaaacccaaggacaccctcatgatctcccggacccctgaggtcac
atgcgtggtggtggacgtgagccacgaagacccctgaggtcaagttcaactggtacgtggacggcgtggaggtgcataatgccaa
gacaaagccgcgggaggagcagtacaacagcacgtaccgtgtggtcagcgtcctcaccgtcctgcaccaggactggctgaatg
gcaaggagtacaagtgcaaggtctccaacaaagccctcccagccccatcgagaaaaccatctccaaagccaaag

human IgG1 CH2 with 238 mutation pro→ser (amino acid sequence) (SEQ ID NO ___)

PELLGGSSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHN
AKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAK

Amino acids surrounding Pro to Ser in CH2 (amino acid sequence) (SEQ ID NO ___)

PAPELLGGPS

Amino acids surrounding Pro to Ser in CH2 (amino acid sequence) (SEQ ID NO ___)

PAPELLGGSS

hIgE3BB (leaves an open reading frame at end of gene to read into transmembrane and cytoplasmic tail domain attached at either the BamHI or SfuI sites) (nucleotide sequence) (SEQ ID NO ___)

gtt gtt ttc gaa gga tcc gct ttn ccg gga ttt aca gac acc gct cgc tgg

human IgE Fc (CH2-CH3-CH4) ORF (nucleotide sequence) (SEQ ID NO ___)

tgatcacgtctgctccagggacttcaccccgcccaccgtgaagatcttacagtcgtcctgcgacggcggcgggcacttccccccg
accatccagctcctgtgcctcgtctctgggtacaccccagggactatcaacatcacctggctggaggacgggcaggtcatggacg
tggacttgtccaccgcctctaccacgcaggagggtgagctggcctccacacaaagcgagctcaccctcagccagaagcactggc
tgtcagaccgcacctacacctgccaggtcacctatcaaggtcacaccttgaggacagcaccaagaagtgtgcagattccaaccc
gagaggggtgagcgcctacctaagccggcccagcccgttcgacctgttcatccgcaagtcgcccacgatcacctgtctggtggtg
gacctggcacccagcaaggggaccgtgaacctgacctggtcccgggccagtgggaagcctgtgaaccactccaccagaaagg
aggagaagcagcgcaatggcacgttaaccgtcacgtccaccctgccggtgggcacccgagactggatcgagggggagacctа
ccagtgcagggtgacccaccccccacctgcccagggccctcatgcggtccacgaccaagaccagcggcccgcgtgctgccccg
gaagtctatgcgtttgcgacgccggagtggccggggagccgggacaagcgcaccctcgcctgcctgatccagaacttcatgcct
gaggacatctcggtgcagtggctgcacaacgaggtgcagctcccggacgcccggcacagcacgacgcagccccgcaagacc
aagggctccggcttcttcgtcttcagccgcctggaggtgaccagggccgaatgggagcagaaagatgagttcatctgccgtgcag
tccatgaggcagcgagccccctcacagaccgtccagcgagcggtgtctgtaaatcccggtaaagcggatccttcgaa

human IgE Fc (CH2-CH3-CH4) ORF (amino acid sequence) (SEQ ID NO ___)

DHVCSRDFTPPTVKILQSSCDGGGHFPPTIQLLCLVSGYTPGTINITWLEDGQVMDV
DLSTASTTQEGELASTQSELTLSQKHWLSDRTYTCQVTYQGHTFEDSTKKCADSN
PRGVSAYLSRPSPFDLFIRKSPTITCLVVDLAPSKGTVNLTWSRASGKPVNHSTRKE
EKQRNGTLTVTSTLPVGTRDWIEGETYQCRVTHPHLPRALMRSTTKTSGPRAAPE
VYAFATPEWPGSRDKRTLACLIQNFMPEDISVQWLHNEVQLPDARHSTTQPRKTK
GSGFFVFSRLEVTRAEWEQKDEFICRAVHEAASPSQTVQRAVSVNPGKADPS

IFhIgGwtBcl5 (nucleotide sequence) (SEQ ID NO ___)

gtt gtt tga tca gga gcc caa atc ttg tga caa aac tca cac atg ccc acc gtg ccc agc acc (63 mer)

HuIgGMHWC (sense, 5' primer for mutating wild type hinge CCC to mutant SSS) (nucleotide sequence) (SEQ ID NO ___)

gtt gtt gat cag gag ccc aaa tct tct gac aaa act cac aca tct cca ccg tcc cca gca cct gaa ctc ctg ggt gga ccg tca gtc ttc c

1D8 VH (nucleotide sequence) (SEQ ID NO ___)

caggtgcagctgaaggaggcaggacctggcctggtgcaaccgacacagaccctgtccctcacatgcactgtctctgggttctcatt aaccagcgatggtgtacactggattcgacagcctccaggaaagggtctggagtggatgggaataatatattatgatggaggcaca gattataattcagcaattaaatccagactgagcatcagcagggacacctccaagagccaagtttttcttaaaaatcaacagtctgcaaa ctgatgacacagccatgtattactgtgccagaatccactttgattactggggccaaggagtcatggtcacagtctcctct

1D8 VH (no leader) (amino acid sequence) (SEQ ID NO ___)

QVQLKEAGPGLVQPTQTLSLTCTVSGFSLTSDGVHWIRQPPGKGLEWMGIIYYDG GTDYNSAIKSRLSISRDTSKSQVFLKINSLQTDDTAMYYCARIHFDYWGQGVMVT VSS

1D8 VL (no leader) (nucleotide sequence) (SEQ ID NO ___)

gacattgtgctcactcagtctccaacaaccatagctgcatctccaggggagaaggtcaccatcacctgccgtgccagctccagtgt aagttacatgtactggtaccagcagaagtcaggcgcctcccctaaactctggatttatgacacatccaagctggcttctggagttcca aatgcttcagtggcagtgggtctgggacctcttattctctcgcaatcaacaccatggaggctgaagatgctgccacttattactgtca gcagtggagtagtactccgctcacgttcgggtctgggaccaagctggagatcaaacgg

1D8 VL (amino acid sequence) (SEQ ID NO ___)

DIVLTQSPTTIAASPGEKVTITCRASSSVSYMYWYQQKSGASPKLWIYDTSKLASG VPNRFSGSGSGTSYSLAINTMETEDAATYYCQQWSSTPLTFGSGTKLEIKR

1D8 scFv (nucleotide sequence) (SEQ ID NO ___)

aagcttatggatttcaagtgcagatttcagcttcctgctaatcagtgcttcagtcataatgtccagaggagtcgacattgtgctcactc agtctccaacaaccatagctgcatctccaggggagaaggtcaccatcacctgccgtgccagctccagtgtaagttacatgtactggt accagcagaagtcaggcgcctcccctaaactctggatttatgacacatccaagctggcttctggagttccaaatcgcttcagtggca

gtgggtctgggacctcttattctctcgcaatcaacaccatggagactgaagatgctgccacttattactgtcagcagtggagtagtact
ccgctcacgttcgggtctgggaccaagctggagatcaaacggggtggcggtggctcgggcggtggtgggtcgggtggcggcg
gatctcaggtgcagctgaaggaggcaggacctggcctggtgcaaccgacacagaccctgtccctcacatgcactgtctctgggtt
ctcattaaccagcgatggtgtacactggattcgacagcctccaggaaagggtctggaatggatgggaataatatattatgatggagg
cacagattataattcagcaattaaatccagactgagcatcagcagggacacctccaagagccaagtttttcttaaaaatcaacagtctg
caaactgatgacacagccatgtattactgtgccagaatccactttgattactggggccaaggagtcatggtcacagtctcctctgatc
a

1D8 scFv (amino acid sequence) (SEQ ID NO ___)

MDFQVQIFSFLLISASVIMSRGVDIVLTQSPTTIAASPGEKVTITCRASSSVSYMYWY
QQKSGASPKLWIYDTSKLASGVPNRFSGSGSGTSYSLAINTMETEDAATYYCQQW
SSTPLTFGSGTKLEIKRGGGGSGGGGSGGGGSQVQLKEAGPGLVQPTQTLSLTCTV
SGFSLTSDGVHWIRQPPGKGLEWMGIIYYDGGTDYNSAIKSRLSISRDTSKSQVFLK
INSLQTDDTAMYYCARIHFDYWGQGVMVTVSS

1D8 scFv IgG WTH WTCH2CH3 (nucleotide sequence) (SEQ ID NO ___)

aagcttatggattttcaagtgcagattttcagcttcctgctaatcagtgcttcagtcataatgtccagaggagtcgacattgtgctcactc
agtctccaacaaccatagctgcatctccaggggagaaggtcaccatcacctgccgtgccagctccagtgtaagttacatgtactggt
accagcagaagtcaggcgcctcccctaaactctggatttatgacacatccaagctggcttctggagttccaaatcgcttcagtggca
gtgggtctgggacctcttattctctcgcaatcaacaccatggagactgaagatgctgccacttattactgtcagcagtggagtagtact
ccgctcacgttcgggtctgggaccaagctggagatcaaacggggtggcggtggctcgggcggtggtgggtcgggtggcggcg
gatctcaggtgcagctgaaggaggcaggacctggcctggtgcaaccgacacagaccctgtccctcacatgcactgtctctgggtt
ctcattaaccagcgatggtgtacactggattcgacagcctccaggaaagggtctggaatggatgggaataatatattatgatggagg
cacagattataattcagcaattaaatccagactgagcatcagcagggacacctccaagagccaagtttttcttaaaaatcaacagtctg
caaactgatgacacagccatgtattactgtgccagaatccactttgattactggggccaaggagtcatggtcacagtctcctctgatc
aggagcccaaatcttgtgacaaaactcacacatgcccaccgtgcccagcacctgaactcctgggggggaccgtcagtcttcctcttc
ccccaaaacccaaggacaccctcatgatctcccggacccctgaggtcacatgcgtggtggtggacgtgagccacgaagaccct
gaggtcaagttcaactggtacgtggacggcgtggaggtgcataatgccaagacaaagccgcgggaggagcagtacaacagcac
gtaccgtgtggtcagcgtcctcaccgtcctgcaccaggactggctgaatggcaaggagtacaagtgcaaggtctccaacaaagcc
ctcccagccccatcgagaaaacaatctccaaagccaaagggcagccccgagaaccacaggtgtacaccctgcccccatcccg
ggatgagctgaccaagaaccaggtcagcctgacctgcctggtcaaaggcttctatcccagcgacatcgccgtggagtgggagag

caatgggcagccggagaacaactacaagaccacgcctcccgtgctggactccgacggctccttctttcctctacagcaagctcacc
gtggacaagagcaggtggcagcaggggaacgtcttctcatgctccgtgatgcatgaggctctgcacaaccactacacgcagaag
agcctctccctgtctccgggtaaatgatctaga

1D8 scFv IgG WTH WTCH2CH3 (amino acid sequence) (SEQ ID NO ___)

MDFQVQIFSFLLISASVIMSRGVDIVLTQSPTTIAASPGEKVTITCRASSSVSYMYWY
QQKSGASPKLWIYDTSKLASGVPNRFSGSGSGTSYSLAINTMETEDAATYYCQQW
SSTPLTFGSGTKLEIKRGGGGSGGGGSGGGGSQVQLKEAGPGLVQPTQTLSLTCTV
SGFSLTSDGVHWIRQPPGKGLEWMGIIYYDGGTDYNSAIKSRLSISRDTSKSQVFLK
INSLQTDDTAMYYCARIHFDYWGQGVMVTVSSDQEPKSCDKTHTCPPCPAPELLG
GPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKP
REEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREP
QVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDG
SFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

1D8 scFv IgG MTH MTCH2CH3-CD80 (nucleotide sequence) (SEQ ID NO ___)

aagcttatggattttcaagtgcagattttcagcttcctgctaatcagtgcttcagtcataatgtccagaggagatcgacattgtgctcactc
agtctccaacaaccatagctgcatctccaggggagaaggtcaccatcacctgccgtgccagctccagtgtaagttacatgtactggt
accagcagaagtcaggcgcctcccctaaactctggatttatgacacatccaagctggcttctggagttccaaatcgcttcagtggca
gtgggtctgggacctcttattctctcgcaatcaacaccatggagctgaagatgctgccactattactgtcagcagtggagtagtact
ccgctcacgttcgggtctgggaccaagctggagatcaaacggggtggcggtggctcgggcggtggtgggtcgggtggcggcgg
atctcaggtgcagctgaaggaggcaggacctggcctggtgcaaccgacacagaccctgtccctcacatgcactgtctctgggtt
ctcattaaccagcgatggtgtacactggattcgacagcctccaggaaagggtctggaatggatgggaataatatattatgatggagg
cacagattataattcagcaattaaatccagactgagcatcagcagggacacctccaagagccaagttttcttaaaaatcaacagtctg
caaactgatgacacagccatgtattactgtgccagaatccacttgattactggggccaaggagtcatggtcacagtctcctctgatct
ggagcccaaatcttctgacaaaactcacacaagcccaccgagcccagcacctgaactcctgggggggatcgtcagtcttcctcttcc
ccccaaaacccaaggacaccctcatgatctcccggaccccctgaggtcacatgcgtggtggtggacgtgagccacgaagaccctg
aggtcaagttcaactggtacgtggacggcgtggaggtgcataatgccaagacaaagccgcgggaggagcagtacaacagcacg
taccgtgtggtcagcgtcctcaccgtcctgcaccaggactggctgaatggcaaggagtacaagtgcaaggtctccaacaaagccc
tcccagcccccatcgagaaaaccatctccaaagccaaagggcagccccgagaaccacaggtgtacaccctgcccccatcccgg
gatgagctgaccaagaaccaggtcagcctgacctgcctggtcaaaggcttctatcccagcgacatcgccgtggagtgggagagc

aatgggcagccggagaacaactacaagaccacgcctcccgtgctggactccgacggctccttcttcctctacagcaagctcaccg
tggacaagagcaggtggcagcaggggaacgtcttctcatgctccgtgatgcatgaggctctgcacaaccactacacgcagaaga
gcctctccctgtctccgggtaaagcggatccttcgaacctgctcccatcctgggccattaccttaatctcagtaaatggaatttttgtga
tatgctgcctgacctactgctttgccccaagatgcagagagagaaggaggaatgagagattgagaagggaaagtgtacgccctgt
5 ataaatcgata

1D8 scFv IgG MTH MTCH2CH3-CD80 (amino acid sequence) (SEQ ID NO ___)

MDFQVQIFSFLLISASVIMSRGVDIVLTQSPTTIAASPGEKVTITCRASSSVSYMYWY
QQKSGASPKLWIYDTSKLASGVPNRFSGSGSGTSYSLAINTMETEDAATYYCQQW
10 SSTPLTFGSGTKLEIKRGGGGSGGGGSGGGGSQVQLKEAGPGLVQPTQTLSLTCTV
SGFSLTSDGVHWIRQPPGKGLEWMGIIYYDGGTDYNSAIKSRLSISRDTSKSQVFLK
INSLQTDDTAMYYCARIHFDYWGQGVMVTVSSDLEPKSSDKTHTSPPSPAPELLG
GSSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKP
REEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREP
15 QVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDG
SFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGKADPSNLLPSW
AITLISVNGIFVICCLTYCFAPRCRERRRNERLRRESVRPV

1D8 scFv IgG WTH WTCH2CH3-CD80 (nucleotide sequence) (SEQ ID NO ___)

20 aagcttatggattttcaagtgcagattttcagcttcctgctaatcagtgcttcagtcataatgtccagaggagtcgacattgtgctcactc
agtctccaacaaccatagctgcatctccaggggagaaggtcaccatcacctgccgtgccagctccagtgtaagttacatgtactggt
accagcagaagtcaggcgcctcccctaaactctggatttatgacacatccaagctggcttctggagttccaaatcgcttcagtggca
gtgggtctgggacctcttattctctcgcaatcaacaccatggagactgaagatgctgccacttattactgtcagcagtggagtagtact
ccgctcacgttcgggtctgggaccaagctggagatcaaacggggtggcggtggctcgggcggtggtgggtcgggtggcggcg
25 gatctcaggtgcagctgaaggaggcaggacctggcctggtgcaaccgacacagaccctgtccctcacatgcactgtctctgggtt
ctcattaaccagcgatggtgtacactggattcgacagcctccaggaaagggtctggaatggatgggaataatatattatgatggagg
cacagattataattcagcaattaaatccagactgagcatcagcagggacacctccaagagccaagttttcttaaaaatcaacagtctg
caaactgatgacacagccatgtattactgtgccagaatccacttgattactggggccaaggagtcatggtcacagtctcctctgatct
ggagcccaaatcttgtgacaaaactcacacatgcccaccgtgcccagcacctgaactcctgggggggaccgtcagtcttcctcttcc
30 ccccaaaacccaaggacaccctcatgatctcccggacccctgaggtcacatgcgtggtggtggacgtgagccacgaagaccctg
aggtcaagttcaactggtacgtggacggcgtggaggtgcataatgccaagacaaagccgcgggaggagcagtacaacagcacg

330

taccgtgtggtcagcgtcctcaccgtcctgcaccaggactggctgaatggcaaggagtacaagtgcaaggtctccaacaaagccc
tcccagccccccatcgagaaaaccatctccaaagccaaagggcagccccgagaaccacaggtgtacaccctgccccccatcccgg
gatgagctgaccaagaaccaggtcagcctgacctgcctggtcaaaggcttctatcccagcgacatcgccgtggagtgggagagc
aatgggcagccggagaacaactacaagaccacgcctcccgtgctggactccgacggctccttcttcctctacagcaagctcaccg
tggacaagagcaggtggcagcaggggaacgtcttctcatgctccgtgatgcatgaggctctgcacaaccactacacgcagaaga
gcctctccctgtctccgggtaaagcggatccttcgaacctgctcccatcctgggccattacctaatctcagtaaatggaatttttgtga
tatgctgcctgacctactgcttttgccccaagatgcagagagagaaggaggaatgagagattgagaagggaaagtgtacgccctgt
ataaatcgata

1D8 scFv IgG WTH WTCH2CH3-CD80 (amino acid sequence) (SEQ ID NO ___)

MDFQVQIFSFLLISASVIMSRGVDIVLTQSPTTIAASPGEKVTITCRASSSVSYMYWY
QQKSGASPKLWIYDTSKLASGVPNRFSGSGSGTSYSLAINTMETEDAATYYCQQW
SSTPLTFGSGTKLEIKRGGGGSGGGGSGGGGSQVQLKESGPGLVQPTQTLSLTCTV
SGFSLTSDGVHWIRQPPGKGLEWMGIIYYDGGTDYNSAIKSRLSISRDTSKSQVFLK
INSLQTDDTAMYYCARIHFDYWGQGVMVTVSSDLEPKSCDKTHTCPPCPAPELLG
GPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKP
REEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREP
QVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDG
SFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGKADPSNLLPSW
AITLISVNGIFVICCLTYCFAPRCRERRRNERLRRESVRPV

Anti-human CD3 scFv WTH WTCH2CH3 (nucleotide sequence) (SEQ ID NO ___)

aagcttatggattttcaagtgcagattttcagcttcctgctaatcagtgtttcagtcatcatgtccagaggagtcgacatccagatgaca
cagactacatcctccctgtctgcctctctgggagacagagtcaccatcagttgcagggcaagtcaggacattcgcaatttttaaact
ggtatcagcagaaaccagatggaactgttaaactcctgatctactacacatcaagattacactcaggagtcccatcaaggttcagtg
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gcttccgtggacgttcggtggaggcaccaaactggtaaccaaacgggagctcggtggcggtggctcgggcggtggtgggtcgg
gtggcggcggatctatcgatgaggtccagctgcaacagtctggacctgaactggtgaagcctggagcttcaatgtcctgcaaggc
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acaaaggtcttactacctacaaccagaaattcaagggcaaggccacattaactgtagacaagtcatccagcacagcctacatggag
ctcctcagtctgacatctgaagactctgcagtctattactgtgcaagatctgggtactatggtgactcggactggtacttcgatgtctgg

ggcgcagggaccacggtcaccgtctcctctgatcaggagcccaaatcttgtgacaaaactcacacatgcccaccgtgcccagcac
ctgaactcctgggggggaccgtcagtcttcctcttccccccaaaacccaaggacaccctcatgatctcccggacccctgaggtcaca
tgcgtggtggtggacgtgagccacgaagaccctgaggtcaagttcaactggtacgtggacggcgtggaggtgcataatgccaag
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agaaccacaggtgtacaccctgcccccatcccgggatgagctgaccaagaaccaggtcagcctgacctgcctggtcaaaggct
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tccgacggctccttcttcctctacagcaagctcaccgtggacaagagcaggtggcagcaggggaacgtcttctcatgctccgtgat
gcatgaggctctgcacaaccactacacgcagaagagcctctccctgtctccgggtaaatgatctaga

Anti-human CD3 scFv WTH WTCH2CH3 (amino acid sequence) (SEQ ID NO ___)

MDFQVQIFSFLLISASVIMSRGVDIQMTQTTSSLSASLGDRVTISCRASQDIRNYLN
WYQQKPDGTVKLLIYYTSRLHSGVPSRFSGSGSGTDYSLTIANLQPEDIATYFCQQ
GNTLPWTFGGGTKLVTKRELGGGGSGGGGSGGGGSIDEVQLQQSGPELVKPGAS
MSCKASGYSFTGYIVNWLKQSHGKNLEWIGLINPYKGLTTYNQKFKGKATLTVD
KSSSTAYMELLSLTSEDSAVYYCARSGYYGDSDWYFDVWGAGTTVTVSSDQEPK
SCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKF
NWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKA
LPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESN
GQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQ
KSLSLSPGK

2H7-antiCD40 scFv MTH (SSS) MTCH2WTCH3 (2h7-40.2 220Ig + restriction sites) (nucleotide sequence) (SEQ ID NO ___)

aagcttgccgccatggattttcaagtgcagattttcagcttcctgctaatcagtgcttcagtcataattgccagaggacaaattgttctct
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ggtaccagcagaagccaggatcctcccccaaaccctggatttatgccccatccaacctggcttctggagtccctgcttcgcttcagtg
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ggagctctcaggcttatctacagcagtctggggctgagctggtgaggcctggggcttcagtgaagatgtcctgcaaggcttctggc
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caacactgcatatttacagataagcaacctcaaagatgaggacacggctacgtatttctgtgtgagatccgggaatggtaactatga
cctggcctactttgcttactggggccaagggacactggtcactgtctctgatcaggagcccaaatcttctgacaaaactcacacatcc
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2H7-antiCD40 scFv MTH (SSS) MTCH2WTCH3 (2H7-40 2.220Ig) (amino acid sequence) (SEQ ID NO ___)

MDFQVQIFSFLLISASVIIARGQIVLSQSPAILSASPGEKVTMTCRASSSVSYMHWY
QQKPGSSPKPWIYAPSNLASGVPARFSGSGSGTSYSLTISRVEAEDAATYYCQQWS
FNPPTFGAGTKLELKGGGGSGGGGSGGGGSSQAYLQQSGAELVRPGASVKMSCK
ASGYTFTSYNMHWVKQTPRQGLEWIGAIYPGNGDTSYNQKFKGKATLTVDKSSS
TAYMQLSSLTSEDSAVYFCARVVYYSNSYWYFDVWGTGTTVTVSSDQSNSEEAK
KEEAKKEEAKKSNSVDIVLTQSPATLSVTPGDRVSLSCRASQSISDYLHWYQQKSH
ESPRLLIKYASHSISGIPSRFSGSGSGSDFTLSINSVEPEDVGIYYCQHGHSFPWTFGG
GTKLEIKRGGGGSGGGGSGGGGSQIQLVQSGPELKKPGETVRISCKASGYAFTTTG
MQWVQEMPGKGLKWIGWINTPLWSAKICRRLQGRFAFSLETSANTAYLQISNLKD

EDTATYFCVRSGNGNYDLAYFAYWGQGTLVTVSDQEPKSSDKTHTSPPSPAPELL
GGSSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTK
PREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPRE
PQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSD
GSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

5B9 VH (includes the VH leader peptide) (nucleotide sequence) (SEQ ID NO ___)

atggctgtcttggggctgctcttctgcctggtgacatttccaagctgtgtcctatcccaggtgcagctgaagcagtcaggacctggcc
tagtgcagtcctcacagagcctgtccatcacctgcacagtctctggtttctcattaactacctatgctgtacactgggttcgccagtctc
caggaaagggtctggagtggctgggagtgatatggagtggtggaatcacagactataatgcagctttcatatccagactgagcatc
accaaggacgattccaagagccaagttttctttaaaatgaacagtctgcaacctaatgacacagccatttattactgtgccagaaatg
ggggtgataactaccctattactatgctatggactactggggtcaaggaacctcagtcaccgtctcctca

5B9 VH (minus the leader) (nucleotide sequence) (SEQ ID NO ___)

caggtgcagctgaagcagtcaggacctggcctagtgcagtcctcacagagcctgtccatcacctgcacagtctctggtttctcatta
actacctatgctgtacactgggttcgccagtctccaggaaagggtctggagtggctgggagtgatatggagtggtggaatcacaga
ctataatgcagctttcatatccagactgagcatcaccaaggacgattccaagagccaagttttctttaaaatgaacagtctgcaacctaa
atgacacagccatttattactgtgccagaaatgggggtgataactaccctattactatgctatggactactggggtcaaggaacctca
gtcaccgtctcctca

5B9 VH (includes leader peptide) (amino acid sequence) (SEQ ID NO ___)

MAVLGLLFCLVTFPSCVLSQVQLKQSGPGLVQSSQSLSITCTVSGFSLTTYAVHWV
RQSPGKGLEWLGVIWSGGITDYNAAFISRLSITKDDSKSQVFFKMNSLQPNDTAIY
YCARNGGDNYPYYYAMDYWGQGTSVTVSS

5B9 VH (no leader peptide) (amino acid sequence) (SEQ ID NO ___)

QVQLKQSGPGLVQSSQSLSITCTVSGFSLTTYAVHWVRQSPGKGLEWLGVIWSGGI
TDYNAAFISRLSITKDDSKSQVFFKMNSLQPNDTAIYYCARNGGDNYPYYYAMDY
WGQGTSVTVSS

5B9 VL (nucleotide sequence) (SEQ ID NO ___)

atgaggttctctgctcagcttctggggctgcttgtgctctggatccctggatccactgcagatattgtgatgacgcaggctgcattctc
caatccagtcactcttggaacatcagcttccatctcctgcaggtctagtaagagtctcctacatagtaatggcatcacttatttgtattgg
tatctgcagaagccaggccagtctcctcagctcctgatttatcagatgtccaacctigcctcaggagtcccagacaggttcagtagca
gtgggtcaggaactgatttcacactgagaatcagcagagtggaggctgaggatgtgggtgtttattactgtgctcaaaatctagaact
tccgctcacgttcggtgctgggaccaagctggagctgaaacgg

5B9 VL (amino acid sequence) (SEQ ID NO ___)

MRFSAQLLGLLVLWIPGSTADIVMTQAAFSNPVTLGTSASISCRSSKSLLHSNGITY
LYWYLQKPGQSPQLLIYQMSNLASGVPDRFSSSGSGTDFTLRISRVEAEDVGVYYC
AQNLELPLTFGAGTKLELKR

5B9 scFv (nucleotide sequence) (SEQ ID NO ___)

aagcttgccgccatgaggttctctgctcagcttctggggctgcttgtgctctggatccctggatccactgcagatattgtgatgacgca
ggctgcattctccaatccagtcactcttggaacatcagcttccatctcctgcaggtctagtaagagtctcctacatagtaatggcatca
cttatttgtattggtatctgcagaagccaggccagtctcctcagctcctgatttatcagatgtccaacctigcctcaggagtcccagaca
ggttcagtagcagtgggtcaggaactgatttcacactgagaatcagcagagtggaggctgaggatgtgggtgtttattactgtgctc
aaaatctagaacttccgctcacgttcggtgctgggaccaagctggagctgaaacggggtggcggtggctcgggcggtggtgggt
cgggtggcggcggatcgtcacaggtgcagctgaagcagtcaggacctggcctagtgcagtcctcacagagcctgtccatcacct
gcacagtctctggtttctcattaactacctatgctgtacactgggttcgccagtctccaggaaagggtctggagtggctgggagtgat
atggagtggtggaatcacagactataatgcagctttcatatccagactgagcatcaccaaggacgattccaagagccaagttttctit
aaaatgaacagtctgcaacctaatgacacagccatttattactgtgccagaaatgggggtgataactacccttattactatgctatgga
ctactggggtcaaggaacctcagtcaccgtctcctct

5B9 scFv (amino acid sequence) (SEQ ID NO ___)

MRFSAQLLGLLVLWIPGSTADIVMTQAAFSNPVTLGTSASISCRSSKSLLHSNGITY
LYWYLQKPGQSPQLLIYQMSNLASGVPDRFSSSGSGTDFTLRISRVEAEDVGVYYC
AQNLELPLTFGAGTKLELKRGGGGSGGGGSGGGGSSQVQLKQSGPGLVQSSQSLS
ITCTVSGFSLTTYAVHWVRQSPGKGLEWLGVIWSGGITDYNAAFISRLSITKDDSK
SQVFFKMNSLQPNDTAIYYCARNGGDNYPYYYAMDYWGQGTSVTVSS

5B9 scFv-hmIgG1-hCD80 (nucleotide sequence) (SEQ ID NO ___)

aagcttgccgccatgaggttctctgctcagcttctggggctgcttgtgctctggatccctggatccactgcagatattgtgatgacgca
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cttatttgtattggtatctgcagaagccaggccagtctcctcagctcctgatttatcagatgtccaaccttgcctcaggagtcccagaca
ggttcagtagcagtgggtcaggaactgatttcacactgagaatcagcagagtggaggctgaggatgtgggtgtttattactgtgctc
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cgggtggcggcggatcgtcacaggtgcagctgaagcagtcaggacctggcctagtgcagtcctcacagagcctgtccatcacct
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gccaagacaaagccgcgggaggagcagtacaacagcacgtaccgtgtggtcagcgtcctcaccgtcctgcaccaggactggct
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agccccgagaaccacaggtgtacaccctgcccccatcccgggatgagctgaccaagaaccaggtcagcctgacctgcctggtca
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atcctgggccattacctaatctcagtaaatggaatttttgtgatatgctgcctgacctactgctttgccccaagatgcagagagaga
aggaggaatgagagattgagaagggaaagtgtacgccctgtataaatcgatactcgag

5B9 scFv-hmIgG1-hCD80 (amino acid sequence) (SEQ ID NO ___)

MRFSAQLLGLLVLWIPGSTADIVMTQAAFSNPVTLGTSASISCRSSKSLLHSNGITY
LYWYLQKPGQSPQLLIYQMSNLASGVPDRFSSSGSGTDFTLRISRVEAEDVGVYYC
AQNLELPLTFGAGTKLELKRGGGGSGGGGSGGGGSSQVQLKQSGPGLVQSSQSLS
ITCTVSGFSLTTYAVHWVRQSPGKGLEWLGVIWSGGITDYNAAFISRLSITKDDSK
SQVFFKMNSLQPNDTAIYYCARNGGDNYPYYYAMDYWGQGTSVTVSSDLEPKSS
DKTHTSPPSPAPELLGGSSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNW
YVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPA
PIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQP

ENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLS
LSPGKADPSNLLPSWAITLISVNGIFVICCLTYCFAPRCRERRRNERLRRESVRPV

2e12 scFv WTH CH2 CH3 (2e12 scFv-WthIgG-CD80) (nucleotide sequence) (SEQ ID NO ___)

aagcttatggattttcaagtgcagattttcagcttcctgctaatcagtgcttcagtcataatgtccagaggagtcgacattgtgctcaccc
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taatgcagtggtaccaacagaaaccaggacagccacccaaactcctcatctctgctgcatccaacgtagaatctggggtccctgcc
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ggaggaatgagagattgagaagggaaagtgtacgccctgtataaatcgat

2e12 scFv WTH CH2 CH3 (2e12 scFv-WthIgG-CD80) (amino acid sequence) (SEQ ID NO ___)

MDFQVQIFSFLLISASVIMSRGVDIVLTQSPASLAVSLGQRATISCRASESVEYYVTS
LMQWYQQKPGQPPKLLISAASNVESGVPARFSGSGSGTDFSLNIHPVEEDDIAMYF
CQQSRKVPWTFGGGTKLEIKRGGGGSGGGGSGGGGSQVQLKESGPGLVAPSQSLS
ITCTVSGFSLTGYGVNWVRQPPGKGLEWLGMIWGDGSTDYNSALKSRLSITKDNS

KSQVFLKMNSLQTDDTARYYCARDGYSNFHYYVMDYWGQGTSVTVSSDLEPKS
CDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFN
WYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALP
APIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQ
PENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSL
SLSPGKADPSNLLPSWAITLISVNGIFVICCLTYCFAPRCRERRRNERLRRESVRPV

2H7-human IgE Fc (CH2-CH3-CH4) (nucleotide sequence) (SEQ ID NO ___)

aagcttgccgccatggattttcaagtgcagattttcagcttcctgctaatcagtgcttcagtcataattgccagaggacaaattgttctct
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ggatcgaggggagacctaccagtgcagggtgacccaccccccacctgcccagggccctcatgcggtccacgaccaagaccag
cggcccgcgtgctgcccccggaagtctatgcgtttgcgacgccggagtggccggggagccgggacaagcgcacccctcgcctgc
ctgatccagaacttcatgcctgaggacatctcggtgcagtggctgcacaacgaggtgcagctcccggacgcccggcacagcacg
acgcagccccgcaagaccaagggctccggcttcttcgtcttcagccgcctggaggtgaccagggccgaatgggagcagaaaga
tgagttcatctgccgtgcagtccatgaggcagcgagccccttacagaccgtccagcgagcggtgtctgtaaatcccggtaaatgat
aatctaga

2H7 scFv IgE (CH2-CH3-CH4) (amino acid sequence) (SEQ ID NO ___)

MDFQVQIFSFLLISASVIIARGQIVLSQSPAILSASPGEKVTMTCRASSSVSYMHWY
QQKPGSSPKPWIYAPSNLASGVPARFSGSGSGTSYSLTISRVEAEDAATYYCQQWS
FNPPTFGAGTKLELKGGGGSGGGGSGGGGSSQAYLQQSGAELVRPGASVKMSCK
ASGYTFTSYNMHWVKQTPRQGLEWIGAIYPGNGDTSYNQKFKGKATLTVDKSSS
TAYMQLSSLTSEDSAVYFCARVVYYSNSYWYFDVWGTGTTVTVSDHVCSRDFTP
PTVKILQSSCDGGGHFPPTIQLLCLVSGYTPGTINITWLEDGQVMDVDLSTASTTQE
GELASTQSELTLSQKHWLSDRTYTCQVTYQGHTFEDSTKKCADSNPRGVSAYLSR
PSPFDLFIRKSPTITCLVVDLAPSKGTVNLTWSRASGKPVNHSTRKEEKQRNGTLTV
TSTLPVGTRDWIEGETYQCRVTHPHLPRALMRSTTKTSGPRAAPEVYAFATPEWP
GSRDKRTLACLIQNFMPEDISVQWLHNEVQLPDARHSTTQPRKTKGSGFFVFSRLE
VTRAEWEQKDEFICRAVHEAASPSQTVQRAVSVNPGK

2H7 scFv MH (SSS) MCH2WTCH3 (nucleotide sequence) (SEQ ID NO ___)

aagcttgccgccatggattttcaagtgcagattttcagcttcctgctaatcagtgcttcagtcataattgccagaggacaaattgttctct
cccagtctccagcaatcctgtctgcatctccaggggagaaggtcacaatgacttgcagggccagctcaagtgtaagttacatgcact
ggtaccagcagaagccaggatcctccccaaaccctggatttatgcccatccaacctggcttctggagtccctgctcgcttcagtg
gcagtgggtctgggacctcttactctctcacaatcagcagagtggaggctgaagatgctgccactttttactgccagcagtggagttt
tnaccoacccacgttcggtgctgggaccaagctggagctgaaagatggcggtggctcgggcggtggtggatctggaggaggtg
ggagctctcaggcttatctacagcagtctggggctgagctggtgaggcctggggcctcagtgaagatgtcctgcaaggcttctggc
tacacatttaccagttacaatatgcactgggtaaagcagacacctagacagggcctggaatggattggagctatttatccaggaaat
ggtgatacttcctacaatcagaagttcaagggcaaggccacactgactgtagacaaatcctccagcacagcctacatgcagctcag
cagcctgacatctgaagactctgcggtctatttctgtgcaagagtggtgtactatagtaactcttactggtacttcgatgtctggggcac
agggaccacggtcaccgtctcttctgatcaggagcccaaatcttctgacaaaactcacacatcccccaccgtccccagcacctgaac
tcctgggggggatcgtcagtcttcctcttccccccaaaacccaaggacaccctcatgatctcccggacccctgaggtcacatgcgtg
gtggtggacgtgagccacgaagaccctgaggtcaagttcaactggtacgtggacggcgtggaggtgcataatgccaagacaaag
ccgcgggaggagcagtacaacagcacgtaccgtgtggtcagcgtcctcaccgtcctgcaccaggactggctgaatggcaagga
gtacaagtgcaaggtctccaacaaagccctcccagcccccatcgagaaaacaatctccaaagccaaagggcagccccgagaac
cacaggtgtacaccctgcccccatcccgggatgagctgaccaagaaccaggtcagcctgacctgcctggtcaaaggcttctatcc
cagcgacatcgccgtggagtgggagagcaatgggcagccggagaacaactacaagaccacgcctcccgtgctggactccgac
ggctccttcttcctctacagcaagctcaccgtggacaagagcaggtggcagcaggggaacgtcttctcatgctccgtgatgcatga
ggctctgcacaaccactacacgcagaagagcctctccctgtctccgggtaaatgatctaga

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2H7 scFv MH (SSS) MCH2WTCH3 (amino acid sequence) (SEQ ID NO ___)

MDFQVQIFSFLLISASVIIARGQIVLSQSPAILSASPGEKVTMTCRASSSVSYMHWY
QQKPGSSPKPWIYAPSNLASGVPARFSGSGSGTSYSLTISRVEAEDAATYYCQQWS
FNPPTFGAGTKLELKDGGGSGGGGSGGGGSSQAYLQQSGAELVRPGASVKMSCK
ASGYTFTSYNMHWVKQTPRQGLEWIGAIYPGNGDTSYNQKFKGKATLTVDKSSS
TAYMQLSSLTSEDSAVYFCARVVYYSNSYWYFDVWGTGTTVTVSSDQEPKSSDK
THTSPPSPAPELLGGSSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYV
DGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIE
KTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPEN
NYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLS
PGK

5B9 scFv MTHWTCH2CH3 (nucleotide sequence) (SEQ ID NO ___)

aagcttgccgccatgaggttctctgctcagcttctggggctgcttgtgctctggatccctggatccactgcagatattgtgatgacgca
ggctgcattctccaatccagtcactcttggaacatcagcttccatctcctgcaggtctagtaagagtctcctacatagtaatggcatca
cttatttgtattggtatctgcagaagccaggccagtctcctcagctcctgatttatcagatgtccaacttgcctcaggagtcccagaca
ggttcagtagcagtgggtcaggaactgatttcacactgagaatcagcagagtggaggctgaggatgtgggtgttattactgtgctc
aaaatctagaacttccgctcacgttcggtgctgggaccaagctggagctgaaacggggtggcggtggctcgggcggtggtgggt
cgggtggcggcggatcgtcacaggtgcagctgaagcagtcaggacctggcctagtgcagtcctcacagagcctgtccatcacct
gcacagtctctggtttctcattaactacctatgctgtacactgggttcgccagtctccaggaaagggtctggagtggctgggagtgat
atggaggtggtggaatcacagactataatgcagctttcatatccagactgagcatcaccaaggacgattccaagagccaagttttcttt
aaaatgaacagtctgcaacctaatgacacagccatttattactgtgccagaaatgggggtgataactaccctattactatgctatgga
ctactggggtcaaggaacctcagtcaccgtctcctctgatcaggagcccaaatcttctgacaaaactcacacatcccccaccgtcccc
agcacctgaactcctgggggggaccgtcagtcttcctcttccccccaaaacccaaggacaccctcatgatctcccggacccctgag
gtcacatgcgtggtggtggacgtgagccacgaagaccctgaggtcaagttcaactggtacgtggacggcgtggaggtgcataat
gccaagacaaagccgcgggaggagcagtacaacagcacgtaccgtgtggtcagcgtcctcaccgtcctgcaccaggactggct
gaatggcaaggagtacaagtgcaaggtctccaacaaagccctcccagccccatcgagaaaacaatctccaaagccaaagggc
agccccgagaaccacaggtgtacaccctgccccatcccgggatgagctgaccaagaaccaggtcagcctgacctgcctggtca
aaggcttctatcccagcgacatcgccgtggagtgggagagcaatgggcagccggagaacaactacaagaccacgcctcccgtg
ctggactccgacggctccttcttcctctacagcaagctcaccgtggacaagagcaggtggcagcaggggaacgtcttctcatgctc
cgtgatgcatgaggctctgcacaaccactacacgcagaagagcctctccctgtctccgggtaaatgatctaga

340 gpt

5B9 scFv MTHWTCH2CH3 (amino acid sequence) (SEQ ID NO ___)

MRFSAQLLGLLVLWIPGSTADIVMTQAAFSNPVTLGTSASISCRSSKSLLHSNGITY
LYWYLQKPGQSPQLLIYQMSNLASGVPDRFSSSGSGTDFTLRISRVEAEDVGVYYC
AQNLELPLTFGAGTKLELKRGGGGSGGGGSGGGGSSQVQLKQSGPGLVQSSQSLS
ITCTVSGFSLTTYAVHWVRQSPGKGLEWLGVIWSGGITDYNAAFISRLSITKDDSK
SQVFFKMNSLQPNDTAIYYCARNGGDNYPYYYAMDYWGQGTSVTVSSDQEPKSS
DKTHTSPPSPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNW
YVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPA
PIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQP
ENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLS
LSPGK

Human IgG1 hinge mutations

2B7 scFv- MTH (CSS) WTCH2CH3 (nucleotide sequence) (SEQ ID NO ___)

aagcttgccgccatggatttttcaagtgcagattttcagcttcctgctaatcagtgcttcagtcataattgccagaggacaaattgttctct
cccagtctccagcaatcctgtctgcatctccaggggagaaggtcacaatgacttgcagggccagctcaagtgtaagttacatgcact
ggtaccagcagaagccaggatcctcccccaaaccctggatttatgccccatccaacctggcttctggagtccctgctcgcttcagtg
gcagtgggtctgggacctcttactctctcacaatcagcagagtggaggctgaagatgctgccacttattactgccagcagtggagttt
taacccacccacgttcggtgctgggaccaagctggagctgaaagatggcggtggctcgggcggtggtgggatctggaggaggtg
ggagctctcaggcttatctacagcagtctggggctgagctggtgaggcctggggcctcagtgaagatgtcctgcaaggcttctggc
tacacatttaccagttacaatatgcactgggtaaagcagacacctagacagggcctggaatggattggagctatttatccaggaaat
ggtgatacttcctacaatcagaagttcaagggcaaggccacactgactgtagacaaatcctccagcacagcctacatgcagctcag
cagcctgacatctgaagactctgcggtctatttctgtgcaagagtggtgtactatagtaactcttactggtacttcgatgtctgggcac
agggaccacggtcaccgtctcttctgatcaggagcccaaatcttgtgacaaaactcacacatcccccaccgtcccagcacctgaac
tcctgggggggaccgtcagtcttcctcttccccccaaaacccaaggacaccctcatgatctcccggaccccctgaggtcacatgcgtg
gtggtggacgtgagccacgaagaccctgaggtcaagttcaactggtacgtggacggcgtggaggtgcataatgccaagacaaag
ccgcgggaggagcagtacaacagcacgtaccgtgtggtcagcgtcctcaccgtcctgcaccaggactggctgaatggcaagga
gtacaagtgcaaggtctccaacaaagccctcccagcccccatcgagaaaacaatctccaaagccaaagggcagccccgagaac
cacaggtgtacaccctgcccccatcccgggatgagctgaccaagaaccaggtcagcctgacctgcctggtcaaaggcttctatcc
cagcgacatcgccgtggagtgggagagcaatgggcagccggagaacaactacaagaccacgcctcccgtgctggactccgac

ggctcctctttcctctacagcaagctcaccgtggacaagagcaggtggcagcaggggaacgtcttctcatgctccgtgatgcatga
ggctctgcacaaccactacacgcagaagagcctctccctgtctccgggtaaatgatctaga

2H7 scFv- MTH (CSS) WTCH2CH3 (amino acid sequence) (SEQ ID NO ___)

MDFQVQIFSFLLISASVIIARGQIVLSQSPAILSASPGEKVTMTCRASSSVSYMHWY
QQKPGSSPKPWIYAPSNLASGVPARFSGSGSGTSYSLTISRVEAEDAATYYCQQWS
FNPPTFGAGTKLELKDGGGSGGGGSGGGGSSQAYLQQSGAELVRPGASVKMSCK
ASGYTFTSYNMHWVKQTPRQGLEWIGAIYPGNGDTSYNQKFKGKATLTVDKSSS
TAYMQLSSLTSEDSAVYFCARVVYYSNSYWYFDVWGTGTTVTVSSDQEPKSCDK
THTSPPSPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYV
DGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIE
KTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPEN
NYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLS
PGK

2H7 scFv- MTH (SCS) WTCH2CH3 (nucleotide sequence) (SEQ ID NO ___)

atgctgccgccatggattttcaagtgcagattttcagcttcctgctaatcagtgcttcagtcataattgccagaggacaaattgttctct
cccagtctccagcaatcctgtctgcatctccaggggagaaggtcacaatgacttgcagggccagctcaagtgtaagttacatgcact
ggtaccagcagaagccaggatcctcccccaaaccctggatttatgccccatccaacctggcttctggagtccctgctcgcttcagtg
gcagtgggtctgggacctcttactctctcacaatcagcagagtggaggctgaagatgctgccacttattactgccagcagtggagttt
tacccacccacgttcggtgctgggaccaagctggagctgaaagatggcggtggctcgggcggtggtggatctggaggaggtg
ggagcctcaggcttatctacagcagtctggggctgagctggtgaggcctggggcctcagtgaagatgtcctgcaaggcttctggc
tacacatttaccagttacaatatgcactgggtaaagcagacacctagacagggcctggaatggattggagctatttatcccaggaaat
ggtgatacttcctacaatcagaagttcaagggcaaggccacactgactgtagacaaatcctccagcacagcctacatgcagctcag
cagcctgacatctgaagactctgcggtctatttctgtgcaagagtggtgtactatagtaactcttactggtacttcgatgtctggggcac
agggaccacggtcaccgtctcttctgatcaggagcccaaatcttctgacaaaactcacacatgcccaccgtcccagcacctgaac
tcctgggggggaccgtcagtcttcctcttccccccaaaacccaaggacaccctcatgatctcccggacccctgaggtcacatgcgtg
gtggtggacgtgagccacgaagaccctgaggtcaagttcaactggtacgtggacggcgtggaggtgcataatgccaagacaaag
ccgcgggaggagcagtacaacagcacgtaccgtgtggtcagcgtcctcaccgtcctgcaccaggactggctgaatggcaagga
gtacaagtgcaaggtctccaacaaagccctcccagccccccatcgagaaaacaatctccaaagccaaagggcagccccgagaac
cacaggtgtacaccctgcccccatcccgggatgagctgaccaagaaccaggtcagcctgacctgcctggtcaaaggcttctatcc

cagcgacatcgccgtggagtgggagagcaatgggcagccggagaacaactacaagaccacgcctcccgtgctggactccgac
ggctccttcttcctctacagcaagctcaccgtggacaagagcaggtggcagcaggggaacgtcttctcatgctccgtgatgcatga
ggctctgcacaaccactacacgcagaagagcctctccctgtctccgggtaaatgatctaga

5 2H7 scFv- MTH (SCS) WTCH2CH3 (amino acid sequence) (SEQ ID NO ___)

MDFQVQIFSFLLISASVIIARGQIVLSQSPAILSASPGEKVTMTCRASSSVSYMHWY
QQKPGSSPKPWIYAPSNLASGVPARFSGSGSGTSYSLTISRVEAEDAATYYCQQWS
FNPPTFGAGTKLELKDGGGSGGGGSGGGGSSQAYLQQSGAELVRPGASVKMSCK
ASGYTFTSYNMHWVKQTPRQGLEWIGAIYPGNGDTSYNQKFKGKATLTVDKSSS
10 TAYMQLSSLTSEDSAVYFCARVVYYSNSYWYFDVWGTGTTVTVSSDQEPKSSDK
THTCPPSPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWY
VDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPI
EKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPE
NNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSL
15 SPGK

2H7 scFv- MTH (SSC) WTCH2CH3 (nucleotide sequence) (SEQ ID NO ___)

aagcttgccgccatggattttcaagtgcagattttcagcttcctgctaatcagtgcttcagtcataattgccagaggacaaattgttctct
cccagtctccagcaatcctgtctgcatctccaggggagaaggtcacaatgacttgcagggccagctcaagtgtaagttacatgcact
20 ggtaccagcagaagccaggatcctccccaaaccctggatttatgccccatccaacctggcttctggagtccctgctcgcttcagtg
gcagtgggtctgggacctcttactctctcacaatcagcagagtggaggctgaagatgctgccactattactgccagcagtggagtt
taacccacccacgttcggtgctgggaccaagctggagctgaaagatggcggtggctcgggcggtggtggatctggaggaggtg
ggagctctcaggcttatctacagcagtctggggctgagctggtgaggcctggggcctcagtgaagatgtcctgcaaggcttctggc
tacacatttaccagttacaatatgcactgggtaaagcagacacctagacagggcctggaatggattggagctatttatccaggaaat
25 ggtgatacttcctacaatcagaagttcaagggcaaggccacactgactgtagacaaatcctccagcacagcctacatgcagctcag
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agggaccacggtcaccgtctcttctgatcaggagcccaaatcttctgacaaaactcacacatcccccaccgtgcccagcacctgaac
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gtggtggacgtgagccacgaagaccctgaggtcaagttcaactggtacgtggacggcgtggaggtgcataatgccaagacaaag
30 ccgcgggaggagcagtacaacagcacgtaccgtgtggtcagcgtcctcaccgtcctgcaccaggactggctgaatggcaagga
gtacaagtgcaaggtctccaacaaagccctcccagccccatcgagaaaacaatctccaaagccaaagggcagccccgagaac

cacaggtgtacaccctgccccatcccgggatgagctgaccaagaaccaggtcagcctgacctgcctggtcaaaggcttctatcc
cagcgacatcgccgtggagtgggagagcaatgggcagccggagaacaactacaagaccacgcctcccgtgctggactccgac
ggctccttcttcctctacagcaagctcaccgtggacaagagcaggtggcagcaggggaacgtcttctcatgctccgtgatgcatga
ggctctgcacaaccactacacgcagaagagcctctccctgtctccgggtaaatgatctaga

5

2H7 scFv- MTH (SSC) WTCH2CH3 (amino acid sequence) (SEQ ID NO ___)
MDFQVQIFSFLLISASVIIARGQIVLSQSPAILSASPGEKVTMTCRASSSVSYMHWY
QQKPGSSPKPWIYAPSNLASGVPARFSGSGSGTSYSLTISRVEAEDAATYYCQQWS
FNPPTFGAGTKLELKDGGGSGGGGSGGGGSSQAYLQQSGAELVRPGASVKMSCK
10 ASGYTFTSYNMHWVKQTPRQGLEWIGAIYPGNGDTSYNQKFKGKATLTVDKSSS
TAYMQLSSLTSEDSAVYFCARVVYYSNSYWYFDVWGTGTTVTVSSDQEPKSSDK
THTSPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWY
VDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPI
EKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPE
15 NNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSL
SPGK

HIgGMHcys1 (nucleotide sequence) (SEQ ID NO ___)
gtt gtt gat cag gag ccc aaa tct tct gac aaa act cac aca tg

20

HIgGMHcys2 (nucleotide sequence) (SEQ ID NO ___)
gtt gtt gat cag gag ccc aaa tct tgt gac aaa act cac aca tct cca ccg tgc

HIgGMHcys3 (nucleotide sequence) (SEQ ID NO ___)
25 gtt gtt gat cag gag ccc aaa tct tgt gac aaa act cac aca tgt cca ccg tcc cca gca cct

HuIgG1 MTCH3Y405 (nucleotide sequence) (SEQ ID NO ___)
gggcagccccgagaaccacaggtgtacaccctgcccccatcccgggaggagatgaccaagaaccaggtcagcctgacctgcct
ggtcaaaggcttctatcccagcgacatcgccgtggagtgggagagcaatgggcagccggagaacaactacaagaccacgcctc

ccgtgctggactccgacggctccttctacctctatagcaagctcaccgtggacaagagcaggtggcagcaggggaacgtcttctc
atgctccgtgatgcatgaggctctgcacaaccactacacgcagaagagcctctccctgtcccgggtaaatga

HuIgG1_MTCH3Y405 (amino acid sequence) (SEQ ID NO ___)

GQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPP
VLDSDGSFYLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

HuIgG1 MTCH3A405 (nucleotide sequence) (SEQ ID NO ___)

gggcagccccgagaaccacaggtgtacaccctgccccatcccgggaggagatgaccaagaaccaggtcagcctgacctgcct
ggtcaaaggcttctatcccagcgacatcgccgtggagtgggagagcaatgggcagccggagaacaactacaagaccacgcctc
ccgtgctggactccgacggctccttcgccctctatagcaagctcaccgtggacaagagcaggtggcagcaggggaacgtcttctc
atgctccgtgatgcatgaggctctgcacaaccactacacgcagaagagcctctccctgtcccgggtaaatga

HuIgG1 MTCH3A405 (amino acid sequence) (SEQ ID NO ___)

GQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPP
VLDSDGSFALYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

HuIgG1 MTCH3A407 (nucleotide sequence) (SEQ ID NO ___)

Ggcagccccgagaaccacaggtgtacaccctgccccatcccgggaggagatgaccaagaaccaggtcagcctgacctgcc
tggtcaaaggcttctatcccagcgacatcgccgtggagtgggagagcaatgggcagccggagaacaactacaagaccacgcct
cccgtgctggactccgacggctccttcttcctcgccagcaagctcaccgtggacaagagcaggtggcagcaggggaacgtcttct
catgctccgtgatgcatgaggctctgcacaaccactacacgcagaagagcctctccctgtcccgggtaaatga

HuIgG1 MTCH3A407 (amino acid sequence) (SEQ ID NO ___)

GQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPP
VLDSDGSFFLASKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

HuIgG1 MTCH3Y405A407 (nucleotide sequence) (SEQ ID NO ___)

345 bis

gggcagccccgagaaccacaggtgtacaccctgccccatcccgggaggagatgaccaagaaccaggtcagcctgacctgcct
ggtcaaaggcttctatcccagcgacatcgccgtggagtgggagagcaatgggcagccggagaacaactacaagaccacgcctc
ccgtgctggactccgacggctccttctacctcgccagcaagctcaccgtggacaagagcaggtggcagcaggggaacgtcttctc
atgctccgtgatgcatgaggctctgcacaaccactacacgcagaagagcctctccctgtccccgggtaaatga

5

HuIgG1 MTCH3Y405A407 (amino acid sequence) (SEQ ID NO ___)

GQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPP
VLDSDGSFYLASKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

10 **HuIgG1 MTCH3A405A407 (nucleotide sequence) (SEQ ID NO ___)**

gggcagccccgagaaccacaggtgtacaccctgccccatcccgggaggagatgaccaagaaccaggtcagcctgacctgcct
ggtcaaaggcttctatcccagcgacatcgccgtggagtgggagagcaatgggcagccggagaacaactacaagaccacgcctc
ccgtgctggactccgacggctccttcgccctcgccagcaagctcaccgtggacaagagcaggtggcagcaggggaacgtcttct
catgctccgtgatgcatgaggctctgcacaaccactacacgcagaagagcctctccctgtccccgggtaaatga

15

HuIgG1 MTCH3A405A407 (amino acid sequence) (SEQ ID NO ___)

GQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPP
VLDSDGSFALASKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

20 **2H7 scFv MTH (SSS) WTCH2MTCH3Y405 (nucleotide sequence) (SEQ ID NO ___)**

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346

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2H7 scFv MTH (SSS) WTCH2MTCH3Y405 (amino acid sequence) (SEQ ID NO ___)

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PGK

2H7 scFv MTH (SSS) WTCH2MTCH3A405 (nucleotide sequence) (SEQ ID NO ___)

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2H7 scFv MTH (SSS) WTCH2MTCH3A405 (nucleotide sequence) (SEQ ID NO ___)

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2H7 scFv MTH (SSS) WTCH2MTCH3A407 (nucleotide sequence) (SEQ ID NO ___)

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2H7 scFv MTH (SSS) WTCH2MTCH3A407 (amino acid sequence) (SEQ ID NO ___)

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2H7 scFv MTH (SSS) WTCH2MTCH3Y405A407 (nucleotide sequence) (SEQ ID NO ___)

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2H7 scFv MTH (SSS) WTCH2MTCH3Y405A407 (amino acid sequence) (SEQ ID NO ___)

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TAYMQLSSLTSEDSAVYFCARVVYYSNSYWYFDVWGTGTTVTVSSDQEPKSSDK
THTSPPSPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYV
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KTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPEN
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PGK

2H7 scFv MTH (SSS) WTCH2MTCH3A405A407 (nucleotide sequence) (SEQ ID NO ___)

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2H7 scFv MTH (SSS) WTCH2MTCH3A405A407 (amino acid sequence) (SEQ ID NO ___)

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PGK

2H7 scFv MTH (SCC) WTCH2CH3 (nucleotide sequence)

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2H7 scFv MTH (SCC) WTCH2CH3 (amino acid sequence)

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2H7 scFv MTH (CSC) WTCH2CH3 (nucleotide sequence)

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2H7 scFv MTH (CSC) WTCH2CH3 (amino acid sequence)

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NNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSL
SPGK

2H7 scFv MTH (CCS) WTCH2CH3 (nucleotide sequence)

aagcttgccgccatggattttcaagtgcagattttcagcttcctgctaatcagtgcttcagtcataattgccagaggacaaattgttctct
cccagtctccagcaatcctgtctgcatctccaggggagaaggtcacaatgacttgcagggccagctcaagtgtaagttacatgcact

ggtaccagcagaagccaggatcctccccaaaccctggatttatgcccatccaacctggcttctggagtcctgctcgcttcagtg
gcagtgggtctgggacctcttactctctcacaatcagcagagtggaggctgaagatgctgccacttattactgccagcagtggagttt
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ggctccttcttcctctacagcaagctcaccgtggacaagagcaggtggcagcaggggaacgtcttctcatgctccgtgatgcatga
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2H7 scFv MTH (CCS) WTCH2CH3 (amino acid sequence)

MDFQVQIFSFLLISASVIIARGQIVLSQSPAILSASPGEKVTMTCRASSSVSYMHWY
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FNPPTFGAGTKLELKDGGGSGGGGSGGGGSSQAYLQQSGAELVRPGASVKMSCK
ASGYTFTSYNMHWVKQTPRQGLEWIGAIYPGNGDTSYNQKFKGKATLTVDKSSS
TAYMQLSSLTSEDSAVYFCARVVYYSNSYWYFDVWGTGTTVTVSSDQEPKSCDK
THTCPPSPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWY
VDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPI
EKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPE
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SPGK

HpIgAHIgA-T4-ORF (nucleotide sequence)

tgatcagccagttccctcaactccacctaccccatctccctcaactccacctaccccatctccctcatgctgccaccccccgactgtca
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ac

HpIgAHIgA-T4-ORF (amino acid sequence)

DQPVPSTPPTPSPSTPPTPSPSCCHPRLSLHRPALEDLLLGSEAILTCTLTGLRDASGV
TFTWTPSSGKSAVQGPPDRDLCGCYSVSSVLPGCAEPWNHGKTFTCTAAYPESKT
PLTATLSKSGNTFRPEVHLLPPPSEELALNELVTLTCLARGFSPKDVLVRWLQGSQ
ELPREKYLTWASRQEPSQGTTTFAVTSILRVAAEDWKKGDTFSCMVGHEALPLAF
TQKTIDRLAGKPTHVNVSVVMAEVDADPSN

1D8-IgAH IgA-T4-CD80 (nucleotide sequence)

aagcttatggattttcaagtgcagattttcagcttcctgctaatcagtgcttcagtcataatgtccagaggagtcgacattgtgctcactc
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accagcagaagtcaggcgcctcccctaaactctggatttatgacacatccaagctggcttctggagttccaaatcgcttcagtggca
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aaccgccaccctctcaaaatccggaaacacattccggcccgaggtccacctgctgccgccgccgtcggaggagctggccctgaa
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AA

1D8 scFv IgAH IgA-T4-CD80 (amino acid sequence)

MDFQVQIFSFLLISASVIMSRGVDIVLTQSPTTIAASPGEKVTITCRASSSVSYMYWY
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SSTPLTFGSGTKLEIKRGGGGSGGGGSGGGGSQVQLKEAGPGLVQPTQTLSLTCTV
SGFSLTSDGVHWIRQPPGKGLEWMGIIYYDGGTDYNSAIKSRLSISRDTSKSQVFLK
INSLQTDDTAMYYCARIHFDYWGQGVMVTVSSDQPVPSTPPTPSPSTPPTPSPSCC
HPRLSLHRPALEDLLLGSEAILTCTLTGLRDASGVTFTWTPSSGKSAVQGPPDRDL
CGCYSVSSVLPGCAEPWNHGKTFTCTAAYPESKTPLTATLSKSGNTFRPEVHLLPP
PSEELALNELVTLTCLARGFSPKDVLVRWLQGSQELPREKYLTWASRQEPSQGTTT
FAVTSILRVAAEDWKKGDTFSCMVGHEALPLAFTQKTIDRLAGKPTHVNVSVVM
AEVDADPSNNLLPSWAITLISVNGIFVICCLTYCFAPRCRERRRNERLRRESVRPV

human IgE Fc (CH2-CH3-CH4) ORF (nucleotide sequence)

tgatcacgtctgctccagggacttcaccccgcccaccgtgaagatcttacagtcgtcctgcgacggcggcgggcacttccccccg
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tggacttgtccaccgcctctaccacgcaggagggtgagctggcctccacacaaagcgagctcaccctcagccagaagcactggc
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tccatgaggcagcgagccccctcacagaccgtccagcgagcggtgtctgtaaatcccggtaaagcggatccttcgaa

AA

human IgE Fc (CH2-CH3-CH4) ORF (amino acid sequence)

DHVCSRDFTPPTVKILQSSCDGGGHFPPTIQLLCLVSGYTPGTINITWLEDGQVMDV
DLSTASTTQEGELASTQSELTLSQKHWLSDRTYTCQVTYQGHTFEDSTKKCADSN
PRGVSAYLSRPSPFDLFIRKSPTITCLVVDLAPSKGTVNLTWSRASGKPVNHSTRKE
EKQRNGTLTVTSTLPVGTRDWIEGETYQCRVTHPHLPRALMRSTTKTSGPRAAPE
VYAFATPEWPGSRDKRTLACLIQNFMPEDISVQWLHNEVQLPDARHSTTQPRKTK
GSGFFVFSRLEVTRAEWEQKDEFICRAVHEAASPSQTVQRAVSVNPGKADPS

1D8 scFv-human IgE Fc (CH2-CH3-CH4)-CD80 (nucleotide sequence)

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1D8-scFv-human IgE Fc (CH2-CH3-CH4)-CD80 (amino acid sequence)

MDFQVQIFSFLLISASVIMSRGVDIVLTQSPTTIAASPGEKVTITCRASSSVSYMYWY
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SSTPLTFGSGTKLEIKRGGGGSGGGGSGGGGSQVQLKEAGPGLVQPTQTLSLTCTV
SGFSLTSDGVHWIRQPPGKGLEWMGIIYYDGGTDYNSAIKSRLSISRDTSKSQVFLK
INSLQTDDTAMYYCARIHFDYWGQGVMVTVSSDHVCSRDFTPPTVKILQSSCDGG
GHFPPTIQLLCLVSGYTPGTINITWLEDGQVMDVDLSTASTTQEGELASTQSELTLS
QKHWLSDRTYTCQVTYQGHTFEDSTKKCADSNPRGVSAYLSRPSPFDLFIRKSPTI
TCLVVDLAPSKGTVNLTWSRASGKPVNHSTRKEEKQRNGTLTVTSTLPVGTRDWI
EGETYQCRVTHPHLPRALMRSTTKTSGPRAAPEVYAFATPEWPGSRDKRTLACLI
QNFMPEDISVQWLHNEVQLPDARHSTTQPRKTKGSGFFVFSRLEVTRAEWEQKDE
FICRAVHEAASPSQTVQRAVSVNPGKADPSKLPSWAITLISVNGIFVICCLTYCFAP
RCRERRRNERLRRESVRPV

5B9-IgAH IgA-T4-CD80 (nucleotide sequence)

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358

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5B9-IgAH IgA-T4-CD80 (amino acid sequence)

MRFSAQLLGLLVLWIPGSTADIVMTQAAFSNPVTLGTSASISCRSSKSLLHSNGITY
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AQNLELPLTFGAGTKLELKRGGGGSGGGGSGGGGSSQVQLKQSGPGLVQSSQSLS
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SQVFFKMNSLQPNDTAIYYCARNGGDNYPYYYAMDYWGQGTSVTVSSDQPVPST
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KSGNTFRPEVHLLPPPSEELALNELVTLTCLARGFSPKDVLVRWLQGSQELPREKY
LTWASRQEPSQGTTTFAVTSILRVAAEDWKKGDTFSCMVGHEALPLAFTQKTIDR
LAGKPTHVNVSVVMAEVDADPSNNLLPSWAITLISVNGIFVICCLTYCFAPRCRER
RRNERLRRESVRPV

5B9-scFv-human IgE Fc (CH2-CH3-CH4)-CD80 (nucleotide sequence)

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359

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5B9-scFv-human IgE Fc (CH2-CH3-CH4)-CD80 (amino acid sequence)

MRFSAQLLGLLVLWIPGSTADIVMTQAAFSNPVTLGTSASISCRSSKSLLHSNGITY
LYWYLQKPGQSPQLLIYQMSNLASGVPDRFSSSGSGTDFTLRISRVEAEDVGVYYC
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RLEVTRAEWEQKDEFICRAVHEAASPSQTVQRAVSVNPGKADPSKLPSWAITLISV
NGIFVICCLTYCFAPRCRERRRNERLRRESVRPV

360

2e12-scFv-IgAH IgA-T4-CD80 (nucleotide sequence)

aagcttatggattttcaagtgcagattttcagcttcctgctaatcagtgcttcagtcataatgtccagaggagtcgacattgtgctcaccc
aatctccagcttctttggctgtgtctctaggtcagagagccaccatctcctgcagagccagtgaaagttgttgatattttatgtcacaagtt
tattgcagtggtaccaacagaaaccaggacagccacccaaactcctcatctctgctgcatccaacgtagaatctggggtccctgcc
aggtttagtggcagtgggtctgggacagacttcagcctcaacatccatcctgtggaggaggatgatattgcaatgtatttctgtcagc
aaagtaggaaggttccttggacgttcggtggaggcaccaagctggaaatcaaacggggtggcggtggctcgggcggaggtggg
tcgggtggcggcggatctcaggtgcagctgaaggagtcaggacctggcctggtggcgccctcacagagcctgtccatcacatgc
accgtctcagggttctcattaaccggctatggtgtaaactgggttcgccagcctccaggaaagggtctggagtggctgggaatgat
atggggtgatggaagcacagactataattcagctctcaaatccagactgagcatcaccaaggacaactccaagagccaagttttctt
aaaaatgaacagtctgcaaactgatgacacagccagatactactgtgccagagatggttatagtaacttttcattactatgttatggact
actggggtcaaggaacctcagtcaccgtctcctcagatcagccagttccctcaactccacctaccccatctccctcaactccaccta
ccccatctcctcatgctgccaccccccgactgtcactgcaccgaccggccctcgaggacctgctcttaggttcagaagcgatcctc
acgtgcacactgaccggcctgagagatgcctcaggtgtcaccttcacctggacgccctcaagtgggaagagcgctgttcaaggac
cacctgaccgtgacctctgtggctgctacagcgtgtccagtgtcctgccgggctgtgccgagccatggaaccatgggaagacctt
acttgcactgctgcctaccccgagtccaagaccccgctaaccgccaccctctcaaaatccggaaacacattccggcccgaggtcc
acctgctgccgccgccgtcggaggagctggccctgaacgagctggtgacgctgacgtgcctggcacgtggcttcagccccaag
gatgtgctggttcgctggctgcaggggtcacaggagctgccccgcgagaagtacctgacttgggcatcccggcaggagcccag
ccagggcaccaccaccttcgctgtgaccagcatactgcgcgtggcagccgaggactggaagaggggacacctttctcctgcat
ggtgggccacgaggccctgccgctggccttcacacagaagaccatcgaccgcttggcgggtaaacccacccatgtcaatgtgtct
gttgtcatggcggaggtggacgcggatccttcgaacaacctgctcccatcctgggccattaccttaatctcagtaaatggaatttttgt
gatatgctgcctgacctactgctttgccccaagatgcagagagagaaggaggaatgagagattgagaagggaaagtgtacgccct
gtataaatcgatac

2e12-scFv-IgAH IgA-T4-CD80 (amino acid sequence)

MDFQVQIFSFLLISASVIMSRGVDIVLTQSPASLAVSLGQRATISCRASESVEYYVTS
LMQWYQQKPGQPPKLLISAASNVESGVPARFSGSGSGTDFSLNIHPVEEDDIAMYF
CQQSRKVPWTFGGGTKLEIKRGGGGSGGGGSGGGGSQVQLKESGPGLVAPSQSLS
ITCTVSGFSLTGYGVNWVRQPPGKGLEWLGMIWGDGSTDYNSALKSRLSITKDNS
KSQVFLKMNSLQTDDTARYYCARDGYSNFHYYVMDYWGQGTSVTVSSDQPVPS
TPPTPSPSTPPTPSPSCCHPRLSLHRPALEDLLLGSEAILTCTLTGLRDASGVTFTWTP
SSGKSAVQGPPDRDLCGCYSVSSVLPGCAEPWNHGKTFTCTAAYPESKTPLTATLS

KSGNTFRPEVHLLPPPSEELALNELVTLTCLARGFSPKDVLVRWLQGSQELPREKY
LTWASRQEPSQGTTTFAVTSILRVAAEDWKKGDTFSCMVGHEALPLAFTQKTIDR
LAGKPTHVNVSVVMAEVDADPSNNLLPSWAITLISVNGIFVICCLTYCFAPRCRER
RRNERLRRESVRPV

5

2e12-scFv-human IgE Fc (CH2-CH3-CH4)-CD80 (nucleotide sequence)

aagcttatggattttcaagtgcagattttcagcttcctgctaatcagtgcttcagtcataatgtccagaggagtcgacattgtgctcaccc
aatctccagcttctttggctgtgtctctaggtcagagagccaccatctcctgcagagccagtgaaagtgttgaatattatgtcacaagtt
taatgcagtggtaccaacagaaaccaggacagccacccaaactcctcatctctgctgcatccaacgtagaatctggggtccctgcc
aggttttagtggcagtgggtctgggacagacttcagcctcaacatccatcctgtggaggaggatgatattgcaatgtatttctgtcagc
aaagtaggaaggttccttggacgttcggtggaggcaccaagctggaaatcaaacgggtggcggtggctcgggcggaggtggg
tcgggtggcggcggatctcaggtgcagctgaaggagtcaggacctggcctggtggcgccctcacagagcctgtccatcacatgc
accgtctcagggttctcattaccggctatggtgtaaactgggttcgccagcctccaggaaagggtctggagtggctgggaatgat
atggggtgatggaagcacagactataattcagctctcaaatccagactgagcatcaccaaggacaactccaagagccaagttttctt
aaaaatgaacagtctgcaaactgatgacacagccagatactactgtgccagagatggttatagtaactttcattacfatgttatggact
actggggtcaaggaacctcagtcaccgtctcctcagatcacgtctgctccagggacttcaccccgccccaccgtgaagatcttacag
tcgtcctgcgacggcggcgggcacttcccccgaccatccagctcctgtgcctcgtctctgggtacaccccagggactatcaacat
cacctggctggaggacgggcaggtcatggacgtggacttgtccaccgcctctaccacgcaggaggggtgagctggcctccacac
aaagcgagctcaccctcagccagaagcactggctgtcagaccgcacctacacctgccaggtcacctatcaaggtcacaccttga
ggacagcaccaagaagtgtgcagattccaacccgagaggggtgagcgcctacctaagccggcccagccccgttcgacctgttcat
ccgcaagtcgcccacgatcacctgtctggtggtggacctggcacccagcaaggggaccgtgaacctgacctggtcccgggcca
gtgggaagcctgtgaaccactccaccagaaaggaggagaagcagcgcaatggcacgttaaccgtcacgtccacctgccggtg
ggcacccgagactggatcgagggggagacctaccagtgcagggtgacccaccccacctgcccagggccctcatgcggtcca
cgaccaagaccagcggcccgcgtgctgcccccggaagtctatgcgttgcgacgccggagtggccgggggagccgggacaagc
gcaccctcgcctgcctgatccagaacttcatgcctgaggacatctcggtgcagtggctgcacaacgaggtgcagctcccggacgc
ccggcacagcacgacgcagcccccgcaagaccaagggctccggcttcttcgtcttcagccgcctggaggtgaccagggccgaat
gggagcagaaagatgagttcatctgccgtgcagtccatgaggcagcgagcccctcacagaccgtccagcgagcggtgtctgtaa
atcccggtaaagcggatccttcgaagctcccatcctgggccattaccttaatctcagtaaatggaatttttgtgatatgctgcctgacct
actgctttgccccaagatgcagagagagaaggaggaatgagagattgagaagggaaagtgtacgccctgtataaatcgata

30

2e12-scFv-human IgE Fc (CH2-CH3-CH4)-CD80 (amino acid sequence)

362

MDFQVQIFSFLLISASVIMSRGVDIVLTQSPASLAVSLGQRATISCRASESVEYYVTS
LMQWYQQKPGQPPKLLISAASNVESGVPARFSGSGSGTDFSLNIHPVEEDDIAMYF
CQQSRKVPWTFGGGTKLEIKRGGGGSGGGGSGGGGSQVQLKESGPGLVAPSQSLS
ITCTVSGFSLTGYGVNWVRQPPGKGLEWLGMIWGDGSTDYNSALKSRLSITKDNS
KSQVFLKMNSLQTDDTARYYCARDGYSNFHYYVMDYWGQGTSVTVSSDHVCSR
DFTPPTVKILQSSCDGGGHFPPTIQLLCLVSGYTPGTINITWLEDGQVMDVDLSTAS
TTQEGELASTQSELTLSQKHWLSDRTYTCQVTYQGHTFEDSTKKCADSNPRGVSA
YLSRPSPFDLFIRKSPTITCLVVDLAPSKGTVNLTWSRASGKPVNHSTRKEEKQRNG
TLTVTSTLPVGTRDWIEGETYQCRVTHPHLPRALMRSTTKTSGPRAAPEVYAFATP
EWPGSRDKRTLACLIQNFMPEDISVQWLHNEVQLPDARHSTTQPRKTKGSGFFVFS
RLEVTRAEWEQKDEFICRAVHEAASPSQTVQRAVSVNPGKADPSKLPSWAITLISV
NGIFVICCLTYCFAPRCRERRRNERLRRESVRPV

500A2 scFv (nucleotide sequence)

atgttgtatacatctcagctccttgggctttttactcttctggatttcagcctccagaagtgacatagtgctgactcagactccagccactct
tgtctctaatttcctggagaaagagtcacaatgacctgtaagaccagtcagaatattggcacaatcttacactggtatcaccaaaaacc
aaaggaggctccaagggctctcatcaagtatgcttcgcagtccatttcctgggatcccctccagattcagtggcagtggttcggaaac
agatttcactctcagcatcaataacctggagcctgatgatatcgcaatttattactgtcaacaaagtagaagctggcctgtcacgttcg
gtcctggcaccaagctggagataaaacggggtggcggtggctcgggcggaggtgggtcgggtggcggcggatctcaggtcaa
gctgcagcagtccggttctgaactagggaaacctggggcctcagtgaaactgtcctgcaagacttcaggctacatatttcacagatc
actatatttcttgggtgaaacagaagcctggaggaagcctgcagtggataggaaatgtttatggtggaaatggtggtacaagctaca
atcaaaaatttccagggcaaggccacactgactgtagataaaatctctagcacagcctacatggaactcagcagcctgacatctgag
gattctgccatctattactgtgcaagaggccggtagcgacgggccatgctatggactactggggtcaggggatccaagttaccgt
ctcctctgatc

500A2 scFv (amino acid sequence)

MLYTSQLLGLLLFWISASRSDIVLTQTPATLSLIPGERVTMTCKTSQNIGTILHWYH
QKPKEAPRALIKYASQSIPGIPSRFSGSGSETDFTLSINNLEPDDIGIYYCQQSRSWPV
TFGPGTKLEIKRGGGGSGGGGSGGGGSQVKLQQSGSELGKPGASVKLSCKTSGYIF
TDHYISWVKQKPGESLQWIGNVYGGNGGTSYNQKFQGKATLTVDKISSTAYMEL
SSLTSEDSAIYYCARRPVATGHAMDYWGQGIQVTVSSD

363

NT

5' oligo

Name IgGWT3

GTTGTTTTCGAAGGATCCGCTTTACCCGGAGACAGGGAGAGGCTCTT

NT

3' oligo

Name hIgGWT5

GTTGTTAGATCTGGAGCCCAAATCTTGTGACAAAACTCACACATG

NT

5' oligo

Name FADD5

Sequence

GTTGTGGATCCTTCGAACCCGTTCCTGGTGCTGCTGCACTCGGTGTCG

NT

3' oligo

Name FADD3

Sequence

GTTGTTATCGATCTCGAGTTATCAGGACGCTTCGGAGGTAGATGCGTC

NT

FADD-CSSCFV (nucleotide sequence)

gtggatccttcgaacccgttcctggtgctgctgcactcggtgtcgtccagcctgtcgagcagcgagctgaccgagctcaagttccta
tgcctcgggcgcgtgggcaagcgcaagctggagcgcgtgcagagcggcctagacctcttctccatgctgctggagcagaacga
cctggagcccgggcacaccgagctcctgcgcgagctgctcgcctccctgcggcgccacgacctgctgcggcgcgtcgacgact
tcgaggcgggggcggcggccggggccgcgcctggggaagaagacctgtgtgcagcatttaacgtcatatgtgataatgtgggg
aaagattggagaaggctggctcgtcagctcaaagtctcagacaccaagatcgacagcatcgaggacagataccccgcaacctg
acagagcgtgtgcgggagtcactgagaatctggaagaacacagagaaggagaacgcaacagtggcccacctggtgggggctc
tcaggtcctgccagatgaacctggtggctgacctggtacaagaggttcagcaggcccgtgacctccagaacaggagtggggcca
tgtccccgatgtcatggaactcagacgcatctacctccgaagcgtcctgataactcgagatcgataacaac

FADD-CSSCFV (amino acid sequence)

VDPSNPFLVLLHSVSSSLSSSELTELKFLCLGRVGKRKLERVQSGLDLFSMLLEQND
LEPGHTELLRELLASLRRHDLLRRVDDFEAGAAAGAAPGEEDLCAAFNVICDNVG
KDWRRLARQLKVSDTKIDSIEDRYPRNLTERVRESLRIWKNTEKENATVAHLVGA
LRSCQMNLVADLVQEVQQARDLQNRSGAMSPMSWNSDASTSEAS

5

HCD28tm5B (nucleotide sequence)
GTTGTGGATCCTCCCTTTTGGGTGCTGGTGGTGGTTGGTGTCCTGGCTTGCTAT
AGCTTG

10 **HCD28tm3S (nucleotide sequence)**
GTTGTTTCGAACCCAGAAAATAATAAAGGCCACTGTTACTAGCAAGCTATAGC
AAGCCAG

HCD28tm5' (nucleotide sequence)
15 GTTGTGGATCCTCCCTTTTGGGTGCTGGTGGT

HCD28tm3' (nucleotide sequence)
GTTGTTTCGAACCCAGAAAATAATAAAGGCCAC

20 **HCD80tm5' (nucleotide sequence)**

GTTGTGGATCCTCCTGCTCCCATCCTGG

HCD80tm3' (nucleotide sequence)
25 GTTGTTTCGAACGGCAAAGCAGTAGGTCAGGC

MFADD5BB (nucleotide sequence)
GTTGTGGATCCTTCGAACCCATTCCTGGTGCTGCTGCACTCGCTG

MFADD3XC (nucleotide sequence)

GTTGTTATCGATCTCGAGTCAGGGTGTTTCTGAGGAAGACAC

Murine FADD nucleotide sequence (full length, but without flanking –Ig or transmembrane sequences) (nucleotide sequence)

gtggatccttcgaacatggacccattcctggtgctgctgcactcgctgtccggcagcctgtcgggcaacgatctgatggagctcaa
gttcttgtgccgcgagcgcgtgagcaaacgaaagctggagcgcgtgcagagtggcctggacctgttcacggtgctgctggagca
gaacgacctggagcgcgggcacaccgggctgctgcgcgagttgctggcctcgctgcgccgacacgatctactgcagcgcctgg
acgacttcgaggcggggacggcgaccgctgcgccccccgggggaggcagatctgcaggtggcatttgacattgtgtgtgacaatg
tggggagagactggaaaagactggcccgcgagctgaaggtgtctgaggccaagatggatgggattgaggagaagtacccccg
aagtctgagtgagcgggtaagggagagtctgaaagtctggaagaatgctgagaagaagaacgcctcggtggccggactggtca
aggcgctgcggacctgcaggctgaatctggtggctgacctggtggaagaagcccaggaatctgtgagcaagagtgagaatatgt
ccccagtactaagggattcaactgtgtcttcctcagaaacaccctgactcgagatcgat

Murine FADD (amino acid sequence)

VDPSNMDPFLVLLHSLSGSLSGNDLMELKFLCRERVSKRKLERVQSGLDLFTVLLE
QNDLERGHTGLLRELLASLRRHDLLQRLDDFEAGTATAAPPGEADLQVAFDIVCD
NVGRDWKRLARELKVSEAKMDGIEEKYPRSLSERVRESLKVVWKNAEKKNASVA
GLVKALRTCRLNLVADLVEEAQESVSKSENMSPVLRDSTVSSSETP

MCASP3-5 (nucleotide sequence)

GTTGTGGATCCTTCGAACATGGAGAACAACAAAACCTCAGTGGATTCA

MCASP3-3 (nucleotide sequence)

GTTGTTATCGATCTCGAGCTAGTGATAAAAGTACAGTTCTTTCGT

MCASP8-5 (nucleotide sequence)

GTTGTTTCGAACATGGATTTCCAGAGTTGTCTTTATGCTATTGCTG

MCASP8-3 (nucleotide sequence)

GTTGTTATCGATCTCGAGTCATTAGGGAGGGAAGAAGAGCTTCTTCCG

hcasp3-5(nucleotide sequence)

GTTGTGGATCCTTCGAACATGGAGAACACTGAAAACTCAGTGGAT

hcasp3-3 (nucleotide sequence)

GTTGTTATCGATCTCGAGTTAGTGATAAAAATAGAGTTCTTTTGTGAG

hcasp8-5 (nucleotide sequence)

GTTGTGGATCCTTCGAACATGGACTTCAGCAGAAATCTTTATGAT

hcasp8-3 (nucleotide sequence)

GTTGTTATCGATGCATGCTCAATCAGAAGGGAAGACAAGTTTTTTTCT

1 2H7 scFv with alternative VHL11 mutations

Nucleotide sequence

Aagcttgccgccatggattttcaagtgcagattttcagcttcctgctaatcagtgcttcagtcataattgccagaggacaaattgttctc
tcccagtctccagcaatcctgtctgcatctccaggggagaaggtcacaatgacttgcagggccagctcaagtgtaagttacatgcac
tggtaccagcagaagccaggatcctcccccaaaccctggatttatgcccatccaacctggcttctggagtccctgctcgcttcagt
ggcagtgggtctgggacctcttactctctcacaatcagcagagtggaggctgaagatgctgccacttattactgccagcagtggagt
ttaacccacccacgttcggtgctgggaccaagctggagctgaaagatggcggtggctcgggcggtggtgggtctggaggaggt
gggagctctcaggcttatctacagcagtctggggctgag (one of the following tcn, acn, gan, can, aan,
cgn, agn)
gtgaggcctgggcctcagtgaagatgtcctgcaaggcttctggctacacatttaccagttacaatatgcactgggtaaagcagaca
cctagacagggcctggaatggattggagctatttatccaggaaatggtgatacttcctacaatcagaagttcaagggcaaggccac
actgactgtagacaaatcctccagcacagcctacatgcagctcagcagcctgacatctgaagactctgcggtctatttctgtgcaag
agtggtgtactatagtaactcttactggtacttcgatgtctggggcacagggaccacggtcaccgtctcttctgatcag

Amino acid sequence

MDFQVQIFSFLLISASVIIARGQIVLSQSPAILSASPGEKVTMTCRASSSVSYMHWY
QQKPGSSPKPWIYAPSNLASGVPARFSGSGSGTSYSLTISRVEAEDAATYYCQQWS
FNPPTFGAGTKLELKDGGGSGGGGSGGGGSSQAYLQQSGAE (one of the following
S, T, D, E, Q, N, R, K, H)
VRPGASVKMSCKASGYTFTSYNMHWVKQTPRQGLEWIGAIYPGNGDTSYNQKFK

GKATLTVDKSSSTAYMQLSSLTSEDSAVYFCARVVYYSNSYWYFDVWGTGTTVT
VSSDQ

2. VHL11 deletion

Nucleotide sequence:

Aagcttgccgccatggattttcaagtgcagattttcagcttcctgctaatcagtgcttcagtcataattgccagaggacaaattgttctc
tcccagtctccagcaatcctgtctgcatctccaggggagaaggtcacaatgacttgcagggccagctcaagtgtaagttacatgcac
tggtaccagcagaagccaggatcctccccaaaccctggatttatgccccatccaacctggcttctggagtcctgctcgcttcagt
ggcagtgggtctgggacctcttactctctcacaatcagcagagtggaggctgaagatgctgccacttattactgccagcagtggagt
tttaacccacccacgttcggtgctgggaccaagctggagctgaaagatggcggtggctcgggcggtggtgggatctggaggaggt
gggagctctcaggcttatctacagcagtctggggctgaggtgaggcctggggcctcagtgaagatgtcctgcaaggcttctggct
acacatttaccagttacaatatgcactgggtaaagcagacacctagacagggcctggaatggattggagctatttatccaggaaatg
gtgatacttcctacaatcagaagttcaaggccaaggccacactgactgtagacaaaatcctccagcacagcctacatgcagctcagc
agcctgacatctgaagactctgcggtctatttctgtgcaagagtggtgtactatagtaactcttactggtacttcgatgtctggggcaca
gggaccacggtcaccgtctcttctgatcag

Amino acid sequence

MDFQVQIFSFLLISASVIIARGQIVLSQSPAILSASPGEKVTMTCRASSSVSYMHWY
QQKPGSSPKPWIYAPSNLASGVPARFSGSGSGTSYSLTISRVEAEDAATYYCQQWS
FNPPTFGAGTKLELKDGGGSGGGGSGGGGSSQAYLQQSGAEVRPGASVKMSCKA
SGYTFTSYNMHWVKQTPRQGLEWIGAIYPGNGDTSYNQKFKGKATLTVDKSSST
AYMQLSSLTSEDSAVYFCARVVYYSNSYWYFDVWGTGTTVTVSSDQ

3 2H7 VL L106 with alternative mutations

Nucleotide sequence

aagcttgccgccatggattttcaagtgcagattttcagcttcctgctaatcagtgcttcagtcataattgccagaggacaaattgttctct
cccagtctccagcaatcctgtctgcatctccaggggagaaggtcacaatgacttgcagggccagctcaagtgtaagttacatgcact
ggtaccagcagaagccaggatcctccccaaaccctggatttatgccccatccaacctggcttctggagtcctgctcgcttcagtg
gcagtgggtctgggacctcttactctctcacaatcagcagagtggaggctgaagatgctgccacttattactgccagcagtggagtt
taacccacccacgttcggtgctgggaccaagctggag (tcn, agn, aan, cgn, can, gan, and non-natural
derivatives of these codons) aaagatggcggtggctcgggcggtggtgggatctggaggaggtgggagctc

Amino acid sequence

MDFQVQIFSFLLISASVIIARGQIVLSQSPAILSASPGEKVTMTCRASSSVSYMHWY
QQKPGSSPKPWIYAPSNLASGVPARFSGSGSGTSYSLTISRVEAEDAATYYCQQWS
FNPPTFGAGTKLE (S, T, R, K, H, Q, N, D, E, and non-natural derivatives of these
amino acids at position 106)KDGGGSGGGGSGGGGSS

4 VL L106 deletion

Nucleotide sequence

Aagcttgccgccatggattttcaagtgcagattttcagcttcctgctaatcagtgcttcagtcataattgccagaggacaaattgttctc
tcccagtctccagcaatcctgtctgcatctccaggggagaaggtcacaatgacttgcagggccagctcaagtgtaagttacatgcac
tggtaccagcagaagccaggatcctccccaaaccctggatttatgccccatccaacctggcttctggagtcctgctcgcttcagt
ggcagtgggtctgggacctcttactctctcacaatcagcagagtggaggctgaagatgctgccacttattactgccagcagtggagt
tttaacccacccacgttcggtgctgggaccaagctggagaaagatggcggtggctcgggcggtggtgggatctggaggaggtgg
gagctc

Amino acid sequence

MDFQVQIFSFLLISASVIIARGQIVLSQSPAILSASPGEKVTMTCRASSSVSYMHWY
QQKPGSSPKPWIYAPSNLASGVPARFSGSGSGTSYSLTISRVEAEDAATYYCQQWS
FNPPTFGAGTKLEKDGGGSGGGGSGGGGSS

5 IgE CH3 CH4

Nucleotide sequence

tccaacccgagaggggtgagcgcctacctaagccggcccagcccgttcgacctgttcatccgcaagtcgcccacgatcacctgtc
tggtggtggacctggcacccagcaaggggaccgtgaacctgacctggtcccgggccagtgggaagcctgtgaaccactccacc
agaaaggaggagaagcagcgcaatggcacgttaaccgtcacgtccacccctgccggtgggcacccgagactggatcgagggag
agacctaccagtgcagggtgacccacccccacctgcccagggccctcatgcggtccacgaccaagaccagcggcccgcgtgct
gccccggaagtctatgcgtttgcgacgccggagtggccggggagccgggacaagcgcaccctcgcctgcctgatccagaactt
catgcctgaggacatctcggtgcagtggctgcacaacgaggtgcagctcccggacgcccggcacagcacgacgcagcccgc
aagaccaagggctccggcttcttcgtcttcagccgcctggaggtgaccagggccgaatgggagcagaaagatgagttcatctgcc
gtgcagtccatgaggcagcgagccctcacagaccgtccagcgagcggtgtctgtaaatccggtaaatgataatctagaa

Amino acid sequence.

SNPRGVSAYLSRPSPFDLFIRKSPTITCLVVDLAPSKGTVNLTWSRASGKPVNHSTR
KEEKQRNGTLTVTSTLPVGTRDWIEGETYQCRVTHPHLPRALMRSTTKTSGPRAA
PEVYAFATPEWPGSRDKRTLACLIQNFMPEDISVQWLHNEVQLPDARHSTTQPRK
TKGSGFFVFSRLEVTRAEWEQKDEFICRAVHEAASPSQTVQRAVSVNPGK

6. hIgG1H/IgE WCH3 WCH4

Nucleotide sequence

tgatcaggagcccaaatcttctgacaaaactcacacatcccccaccgtccccagcatccaacccgagaggggtgagcgcctaccta
agccggcccagccc gttcgacctgttcatccgcaagtcgcccacgatcacctgtctggtggtggacctggcacccagcaagggg
accgtgaacctgacctggtcccgggccagtgggaagcctgtgaaccactccaccagaaaggaggagaagcagcgcaatggca
cgttaaccgtcacgtccaccctgccggtgggcacccgagactggatcgaggggagaccctaccagtgcagggtgacccacccc
cacctgcccagggccctcatgcggtccacgaccaagaccagcggcccgcgtgctgccccggaagtctatgcgtttgcgacgcc
ggagtggccggggagccgggacaagcgcaccctcgcctgcctgatccagaacttcatgcctgaggacatctcggtgcagtggct
gcacaacgaggtgcagctcccggacgcccggcacagcacgacgcagcccgcaagaccaagggctccggcttcttcgtcttca
gccgcctggaggtgaccagggccgaatgggagcagaaagatgagttcatctgccgtgcagtccatgaggcagcgagcctcctca
cagaccgtccagcgagcggtgtctgtaaatccggtaaatgataatctagaa

Amino acid sequence

DQEPKSSDKTHTSPPSPASNPRGVSAYLSRPSPFDLFIRKSPTITCLVVDLAPSKGTV
NLTWSRASGKPVNHSTRKEEKQRNGTLTVTSTLPVGTRDWIEGETYQCRVTHPHL
PRALMRSTTKTSGPRAAPEVYAFATPEWPGSRDKRTLACLIQNFMPEDISVQWLH
NEVQLPDARHSTTQPRKTKGSGFFVFSRLEVTRAEWEQKDEFICRAVHEAASPSQT
VQRAVSVNPGK

7 IgE WCH2 WCH3 WCH4

Nucleotide sequence

Tgatcacgtctgctccagggacttcacccccgcccaccgtgaagatcttacagtcgtcctgcgacggcggcgggcacttcccccccg
accatccagctcctgtgcctcgtctctgggtacaccccagggactatcaacatcacctggctggaggacgggcaggtcatggacg
tggacttgtccaccgcctctaccacgcaggagggtgagctggctccacacaaagcgagctcaccctcagccagaagcactggc
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369

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tccatgaggcagcgagcccctcacagaccgtccagcgagcggtgtctgtaaatcccggtaaatgataatctaga

Amino acid sequence
DHVCSRDFTPPTVKILQSSCDGGGHFPPTIQLLCLVSGYTPGTINITWLEDGQVMDV
DLSTASTTQEGELASTQSELTLSQKHWLSDRTYTCQVTYQGHTFEDSTKKCADSN
PRGVSAYLSRPSPFDLFIRKSPTITCLVVDLAPSKGTVNLTWSRASGKPVNHSTRKE
EKQRNGTLTVTSTLPVGTRDWIEGETYQCRVTHPHLPRALMRSTTKTSGPRAAPE
VYAFATPEWPGSRDKRTLACLIQNFMPEDISVQWLHNEVQLPDARHSTTQPRKTK
GSGFFVFSRLEVTRAEWEQKDEFICRAVHEAASPSQTVQRAVSVNPGK

8 hIgG1H/IgE CH3 CH4 (ORF)

Nucleotide sequence

tgatcaggagcccaaatcttctgacaaaactcacacatcccccaccgtcccccagcatccaaccccgagaggggtgagcgcctacctg
agccggcccagccccgttcgacctgttcatccgcaagtcgcccacgatcacctgtctggtggtggacctggcacccagcaagggg
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cagaccgtccagcgagcggtgtctgtaaatcccggtaaagcggatccttcgaa

Amino acid sequence.
DQEPKSSDKTHTSPPSPASNPRGVSAYLSRPSPFDLFIRKSPTITCLVVDLAPSKGTV
NLTWSRASGKPVNHSTRKEEKQRNGTLTVTSTLPVGTRDWIEGETYQCRVTHPHL
PRALMRSTTKTSGPRAAPEVYAFATPEWPGSRDKRTLACLIQNFMPEDISVQWLH
NEVQLPDARHSTTQPRKTKGSGFFVFSRLEVTRAEWEQKDEFICRAVHEAASPSQT
VQRAVSVNPGKSGSFE

9 2H7 VHL11S scFv hIgG1(SSS-S)H hIgE WCH3 WCH4

Nucleotide sequence

aagcttgccgccatggatttttcaagtgcagattttcagcttcctgctaatcagtgcttcagtcataattgccagaggacaaattgttctct
cccagtctccagcaatcctgtctgcatctccaggggagaaggtcacaatgacttgcagggccagctcaagtgtaagttacatgcact
ggtaccagcagaagccaggatcctcccccaaaccctggatttatgccccatccaacctggcttctggagtccctgctcgcttcagtg
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370

Amino acid sequence
MDFQVQIFSFLLISASVIIARGQIVLSQSPAILSASPGEKVTMTCRASSSVSYMHWY
QQKPGSSPKPWIYAPSNLASGVPARFSGSGSGTSYSLTISRVEAEDAATYYCQQWS
FNPPTFGAGTKLELKDGGGSGGGGSGGGGSSQAYLQQSGAESVRPGASVKMSCK
ASGYTFTSYNMHWVKQTPRQGLEWIGAIYPGNGDTSYNQKFKGKATLTVDKSSS
TAYMQLSSLTSEDSAVYFCARVVYYSNSYWYFDVWGTGTTVTVSSDQEPKSSDK
THTSPPSSASNPRGVSAYLSRPSPFDLFIRKSPTITCLVVDLAPSKGTVNLTWSRASG
KPVNHSTRKEEKQRNGTLTVTSTLPVGTRDWIEGETYQCRVTHPHLPRALMRSTT
KTSGPRAAPEVYAFATPEWPGSRDKRTLACLIQNFMPEDISVQWLHNEVQLPDAR
HSTTQPRKTKGSGFFVFSRLEVTRAEWEQKDEFICRAVHEAASPSQTVQRAVSVNP
GK

10 2H7 VHL11S scFv hIgG1(SSS-P)H hIgE WCH3 WCH4

Nucleotide sequence
aagcttgccgccatggattttcaagtgcagattttcagcttcctgctaatcagtgcttcagtcataattgccagaggacaaattgttctct
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Amino acid sequence
MDFQVQIFSFLLISASVIIARGQIVLSQSPAILSASPGEKVTMTCRASSSVSYMHWY
QQKPGSSPKPWIYAPSNLASGVPARFSGSGSGTSYSLTISRVEAEDAATYYCQQWS
FNPPTFGAGTKLELKDGGGSGGGGSGGGGSSQAYLQQSGAESVRPGASVKMSCK
ASGYTFTSYNMHWVKQTPRQGLEWIGAIYPGNGDTSYNQKFKGKATLTVDKSSS
TAYMQLSSLTSEDSAVYFCARVVYYSNSYWYFDVWGTGTTVTVSSDQEPKSSDK
THTSPPSPASNPRGVSAYLSRPSPFDLFIRKSPTITCLVVDLAPSKGTVNLTWSRASG
KPVNHSTRKEEKQRNGTLTVTSTLPVGTRDWIEGETYQCRVTHPHLPRALMRSTT
KTSGPRAAPEVYAFATPEWPGSRDKRTLACLIQNFMPEDISVQWLHNEVQLPDAR
HSTTQPRKTKGSGFFVFSRLEVTRAEWEQKDEFICRAVHEAASPSQTVQRAVSVNP
GK

10 2H7 VL L106S
aagcttgccgccatggattttcaagtgcagattttcagcttcctgctaatcagtgcttcagtcataattgccagaggacaaattgttctct
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taacccacccacgttcggtgctgggaccaagctggagctgaaagatggcggtggctcgggcggtggtggatctggaggaggtg
ggagctc

Amino acid sequence
MDFQVQIFSFLLISASVIIARGQIVLSQSPAILSASPGEKVTMTCRASSSVSYMHWY
QQKPGSSPKPWIYAPSNLASGVPARFSGSGSGTSYSLTISRVEAEDAATYYCQQWS
FNPPTFGAGTKLESKDGGGSGGGGSGGGGSS

11 2H7 VL L106S scFv
Nucleotide sequence
aagcttgccgccatggattttcaagtgcagattttcagcttcctgctaatcagtgcttcagtcataattgccagaggacaaattgttctct
cccagtctccagcaatcctgtctgcatctccaggggagaaggtcacaatgacttgcagggccagctcaagtgtaagttacatgcact
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Amino acid sequence
MDFQVQIFSFLLISASVIIARGQIVLSQSPAILSASPGEKVTMTCRASSSVSYMHWY
QQKPGSSPKPWIYAPSNLASGVPARFSGSGSGTSYSLTISRVEAEDAATYYCQQWS
FNPPTFGAGTKLESKDGGGSGGGGSGGGGSSQAYLQQSGAELVRPGASVKMSCK
ASGYTFTSYNMHWVKQTPRQGLEWIGAIYPGNGDTSYNQKFKGKATLTVDKSSS
TAYMQLSSLTSEDSAVYFCARVVYYSNSYWYFDVWGTGTTVTVSSDQ

12 2H7 scFv VL L106S VHL11S scFv
Nucleotide sequence
Aagcttgccgccatggattttcaagtgcagattttcagcttcctgctaatcagtgcttcagtcataattgccagaggacaaattgttctc
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ggcagtgggtctgggacctcttactctctcacaatcagcagagtggaggctgaagatgctgccacttattactgccagcagtggagt
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acagggaccacggtcaccgtctcttctgatcag

Amino acid sequence
MDFQVQIFSFLLISASVIIARGQIVLSQSPAILSASPGEKVTMTCRASSSVSYMHWY
QQKPGSSPKPWIYAPSNLASGVPARFSGSGSGTSYSLTISRVEAEDAATYYCQQWS
FNPPTFGAGTKLESKDGGGSGGGGSGGGGSSQAYLQQSGAESVRPGASVKMSCK
ASGYTFTSYNMHWVKQTPRQGLEWIGAIYPGNGDTSYNQKFKGKATLTVDKSSS
TAYMQLSSLTSEDSAVYFCARVVYYSNSYWYFDVWGTGTTVTVSSDQ

10. Human IgD hinge linker with attached restriction sites
Nucleotide.

gtggatccaggttcgaagtctccaaaggcacaggcctcctccgtgcccactgcacaaccccaagcagagggcagcctcgccaa
ggcaaccacagccccagccaccaccctgtaacacaggaagaggaggagaagagaagaaggaggaaggaggaaagaggaa
caagaagagagagagacaaagaccggtgcagtcgacg

Amino acid
VDPGSKSPKAQASSVPTAQPQAEGSLAKATTAPATTRNTGRGGEEKKKEKEKEEQ
EERETKTGAVD

Sequence of Native IgD hinge domain
(includes a cysteine residue—we truncated the hinge prior to that residue for these constructs.)
Nucleotide
gagtctccaaaggcacaggcctcctccgtgcccactgcacaaccccaagcagagggcagcctcgccaaggcaaccacagccc
cagccaccaccctgtaacacaggaagaggaggagaagagaagaaggaggaaggaggaaagaggaacaagaagagagaga
gacaaagacaccagagtgtccgagccacacccagcctcttggcgtctacctgctaaccct

Amino acid sequence
ESPKAQASSVPTAQPQAEGSLAKATTAPATTRNTGRGGEEKKKEKEKEEQEERET
KTPECPSHTQPLGVYLLTP

12 2H7 VH L11S
Nucleotide sequence
caggcttatctacagcagtctggggctgagtcggtgaggcctggggcctcagtgaagatgtcctgcaaggcttctggctacacattt
accagttacaatatgcactgggtaaagcagacacctagacagggcctggaatggattggagctatttatcccggaaatggtgatact
tcctacaatcagaagttcaagggcaaggccacactgactgtagacaaatcctccagcacagcctacatgcagctcagcagcctga
catctgaagactctgcggtctatttctgtgcaagagtggtgtactatagtaactcttactggtacttcgatgtctggggcacagggacc
acggtcaccgtctcttct

Amino acid sequence
QAYLQQSGAESVRPGASVKMSCKASGYTFTSYNMHWVKQTPRQGLEWIGAIYPG
NGDTSYNQKFKGKATLTVDKSSSTAYMQLSSLTSEDSAVYFCARVVYYSNSYWY
FDVWGTGTTVTVSS

13 2H7 VH L11S scFv
Nucleotide sequence
aagcttgccgccatggattttcaagtgcagattttcagcttcctgctaatcagtgcttcagtcataattgtccagaggacaaattgttctct
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Amino acid sequence
MDFQVQIFSFLLISASVIIARGQIVLSQSPAILSASPGEKVTMTCRASSSVSYMHWY
QQKPGSSPKPWIYAPSNLASGVPARFSGSGSGTSYSLTISRVEAEDAATYYCQQWS
FNPPTFGAGTKLELKDGGGSGGGGSGGGGSSQAYLQQSGAESVRPGASVKMSCK

ASGYTFTSYNMHWVKQTPRQGLEWIGAIYPGNGDTSYNQKFKGKATLTVDKSSS
TAYMQLSSLTSEDSAVYFCARVVYYSNSYWYFDVWGTGTTVTVSSDQ

14. 2H7 scFv VH L11S hIgG1 (CSC-S)H WCH2 WCH3

Nucleotide sequence.

aagcttgccgccatggattttcaagtgcagattttcagcttcctgctaatcagtgcttcagtcataattgccagaggacaaattgttctct
cccagtctccagcaatcctgtctgcatctccaggggagaaggtcacaatgacttgcagggccagctcaagtgtaagttacatgcact
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gctctgcacaaccactacacgcagaagagcctctccctgtctccgggtaaatgatctaga

Amino acid sequence

MDFQVQIFSFLLISASVIIARGQIVLSQSPAILSASPGEKVTMTCRASSSVSYMHWY
QQKPGSSPKPWIYAPSNLASGVPARFSGSGSGTSYSLTISRVEAEDAATYYCQQWS
FNPPTFGAGTKLELKDGGGSGGGGSGGGGSSQAYLQQSGAELVRPGASVKMSCK
ASGYTFTSYNMHWVKQTPRQGLEWIGAIYPGNGDTSYNQKFKGKATLTVDKSSS
TAYMQLSSLTSEDSAVYFCARVVYYSNSYWYFDVWGTGTTVTVSSDQEPKSCDK
THTSPPCSAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWY
VDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPI
EKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPE
NNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSL
SPGK

15. 2H7 scFv VH L11S IgE WCH2 WCH3 WCH4

Nucleotide sequence

aagcttgccgccatggattttcaagtgcagattttcagcttcctgctaatcagtgcttcagtcataattgccagaggacaaattgttctct
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tgataatctaga

Amino acid sequence
MDFQVQIFSFLLISASVIIARGQIVLSQSPAILSASPGEKVTMTCRASSSVSYMHWY
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FNPPTFGAGTKLELKDGGGSGGGGSGGGGSSQAYLQQSGAESVRPGASVKMSCK
ASGYTFTSYNMHWVKQTPRQGLEWIGAIYPGNGDTSYNQKFKGKATLTVDKSSS
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16. 2H7 scFv VH L11S mIgk WCH2 WCH3 WCH4

Nucleotide sequence
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Amino acid sequence
MDFQVQIFSFLLISASVIIA t tQIVLSQSPAILSASPGEKVTMTCRASSSVSYMHWY
QQKPGSSPKPWIYAPSN L VPARFS SGSGTSY SLTISRVEAEDAATYYCQQWS
FNPPTFGAGTKLELKDG t t GGSGGGGSGGGGSSQAYLQQSGAESVRPGASVKMSCK

agaagtgtgcagattccaacccgagaggggtgagcgcctacctaagccggcccagcccgttcgacctgttcatccgcaagtcgc
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tgataatctaga

Amino acid sequence
MDFQVQIFSFLLISASVIIARGQIVLSQSPAILSASPGEKVTMTCRASSSVSYMHWY
QQKPGSSPKPWIYAPSNLASGVPARFSGSGSGTSYSLTISRVEAEDAATYYCQQWS
FNPPTFGAGTKLELKDGGGSGGGGSGGGGSSQAYLQQSGAESVRPGASVKMSCK
ASGYTFTSYNMHWVKQTPRQGLEWIGAIYPGNGDTSYNQKFKGKATLTVDKSSS
TAYMQLSSLTSEDSAVYFCARVVYYSNSYWYFDVWGTGTTVTVSSDHVCSRDFT
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RPSPFDLFIRKSPTITCLVVDLAPSKGTVNLTWSRASGKPVNHSTRKEEKQRNGTLT
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PGSRDKRTLACLIQNFMPEDISVQWLHNEVQLPDARHSTTQPRKTKGSGFFVFSRL
EVTRAEWEQKDEFICRAVHEAASPSQTVQRAVSVNPGK

16 2H7 scFv VH L11S mIgE WCH2 WCH3 WCH4

Nucleotide sequence

aagcttgccgccatggatttcaagtgcagattttcagcttcctgctaatcagtgcttcagtcataattgccagaggacaaaattgttctct
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Amino acid sequence
MDFQVQIFSFLLISASVIIARGQIVLSQSPAILSASPGEKVTMTCRASSSVSYMHWY
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FNPPTFGAGTKLELKDGGGSGGGGSGGGGSSQAYLQQSGAESVRPGASVKMSCK

ASGYTFTSYNMHWVKQTPRQGLEWIGAIYPGNGDTSYNQKFKGKATLTVDKSSS
TAYMQLSSLTSEDSAVYFCARVVYYSNSYWYFDVWGTGTTVTVSSDHVRPVNIT
EPTLELLHSSCDPNAFHSTIQLYCFIYGHILNDVSVSWLMDDREITDTLAQTVLIKE
EGKLASTCSKLNITEQQWMSESTFTCKVTSQGVDYLAHTRRCPDHEPRGVITYLIP
PSPLDLYQNGAPKLTCLVVDLESEKNVNVTWNQEKKTSVSASQWYTKHHNNATT
SITSILPVVAKDWIEGYGYQCIVDHPDFPKPIVRSITKTPGQRSAPEVYVFPPPEEESE
DKRTLTCLIQNFFPEDISVQWLGDGKLISNSQHSTTTPLKSNGSNQGFFIFSRLEVAK
TLWTQRKQFTCQVIHEALQKPRKLEKTISTSLGNTSLRPS

17 2H7 scFv VH L11S hIgA WH WCH2 T4CH3

Nucleotide sequence

aagcttgccgccatggattttcaagtgcagattttcagcttcctgctaatcagtgcttcagtcataattgccagaggacaaattgttctct
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Amino acid sequence

MDFQVQIFSFLLISASVIIARGQIVLSQSPAILSASPGEKVTMTCRASSSVSYMHWY
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TAYMQLSSLTSEDSAVYFCARVVYYSNSYWYFDVWGTGTTVTVSSDQPVPSTPPT
PSPSTPPTPSPSCCHPRLSLHRPALEDLLLGSEAILTCTLTGLRDASGVTFTWTPSSG
KSAVQGPPDRDLCGCYSVSSVLPGCAEPWNHGKTFTCTAAYPESKTPLTATLSKS
GNTFRPEVHLLPPPSEELALNELVTLTCLARGFSPKDVLVRWLQGSQELPREKYLT
WASRQEPSQGTTTFAVTSILRVAAEDWKKGDTFSCMVGHEALPLAFTQKTIDRLA
GKPTHVNVSVVMAEVD

18 2H7 scFv VH L11S mIgA WH WCH2 T4 CH3

Nucleotide sequence

aagcttgccgccatggattttcaagtgcagattttcagcttcctgctaatcagtgcttcagtcataattgccagaggacaaattgttctct
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Amino acid sequence:

MDFQVQIFSFLLISASVIIARGQIVLSQSPAILSASPGEKVTMTCRASSSVSYMHWY
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ASGYTFTSYNMHWVKQTPRQGLEWIGAIYPGNGDTSYNQKFKGKATLTVDKSSS
TAYMQLSSLTSEDSAVYFCARVVYYSNSYWYFDVWGTGTTVTVSSDHICSPPTTP
PPPSCQPSLSLQRPALEDLLLGSDASITCTLNGLRDPEGAVFTWEPSTGKDAVQKK
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VIMSEGD

A. mIgA WCH2 T4CH3

Nucleotide sequence

Gttgttgatcacatctgttctcctcctactactcctcttccaccttcctgccagcccagcctgtcactgcagcggccagctcttgagga
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caccaatgtcagcgtgtctgtgatcatgtcagagggagattgataatctagat

Amino acid sequence:

DHICSPPTTPPPPSCQPSLSLQRPALEDLLLGSDASITCTLNGLRDPEGAVFTWEPST
GKDAVQKKAVQNSCGCYSVSSVLPGCAERWNSGASFKCTVTHPESDTLTGTIAKV
TVNTFPPQVHLLPPPSEELALNELVSLTCLVRAFNPKEVLVRWLHGNEELSPESYL
VFEPLKEPGEGATTYLVTSVLRVSAEIWKQGDQYSCMVGHEALPMNFTQKTIDRL
SGKPTNVSVSVIMSEGD

20 K322S CH2 region

Nucleotide sequence

cctgaactcctgggggggaccgtcagtcttcctcttccccccaaaacccaaggacaccctcatgatctcccggacccctgaggtcac
atgcgtggtggtggacgtgagccacgaagaccctgaggtcaagttcaactggtacgtggacggcgtggaggtgcataatgccaa
gacaaagccgcgggaggagcagtacaacagcacgtaccgtgtggtcagcgtcctcaccgtcctgcaccaggactggctgaatg
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Amino acid sequence
PELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHN
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21 K322S CH2 WCH3
Nucleotide sequence
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Amino acid sequence
PELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHN
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LDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

1 K322L CH2 WCH3
Nucleotide sequence
tgatcaggagcccaaatcttctgacaaaactcacacatcccccaccgtcctcagcacctgaactcctgggggggaccgtcagtcttcct
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gcacgtaccgtgtggtcagcgtcctcaccgtcctgcaccaggactggctgaatggcaaggagtacaagtgctcggtctccaacaa
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caccgtggacaagagcaggtggcagcaggggaacgtcttctcatgctccgtgatgcatgaggctctgcacaaccactacacgca
gaagagcctctccctgtctccgggtaaatgatctaga

Amino acid sequence
DQEPKSSDKTHTSPPSSAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHED
PEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCLV
SNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVE
WESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHN
HYTQKSLSLSPGK

22 2H7 scFv VHL11S hIgG1 (SSS-S)H K322SCH2 WCH3
Nucleotide sequence.
aagcttgccgccatggattttcaagtgcagattttcagcttcctgctaatcagtgcttcagtcataattgccagaggacaaattgttctct
cccagtctccagcaatcctgtctgcatctccaggggagaaggtcacaatgacttgcagggccagctcaagtgtaagttacatgcact
ggtaccagcagaagccaggatcctccccaaacccatggatttatgccccatccaacctggcttctggagtccctgctcgcttcagtg
gcagtgggtctgggacctcttactctctcacaatcagcagagtggaggctgaagatgctgccacttattactgccagcagtggagtit
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caggtgtacaccctgcccccatcccgggatgagctgaccaagaaccaggtcagcctgacctgcctggtcaaaggcttctatccca
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ctccttcttcctctacagcaagctcaccgtggacaagagcaggtggcagcaggggaacgtcttctcatgctccgtgatgcatgagg
ctctgcacaaccactacacgcagaagagcctctccctgtctccgggtaaatgatctaga

Amino acid sequence

MDFQVQIFSFLLISASVIIARGQIVLSQSPAILSASPGEKVTMTCRASSSVSYMHWY
QQKPGSSPKPWIYAPSNLASGVPARFSGSGSGTSYSLTISRVEAEDAATYYCQQWS
FNPPTFGAGTKLELKDGGGSGGGGSGGGGSSQAYLQQSGAESVRPGASVKMSCK
ASGYTFTSYNMHWVKQTPRQGLEWIGAIYPGNGDTSYNQKFKGKATLTVDKSSS
TAYMQLSSLTSEDSAVYFCARVVYYSNSYWYFDVWGTGTTVTVSSDQEPKSSDK
THTSPPSSAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYV
DGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCSVSNKALPAPIE
KTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPEN
NYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLS
PGK

23 2H7 scFv VHL11S hIgG1 (SSS-S)H K322LCH2 WCH3

Nucleotide sequence

aagcttgccgccatggattttcaagtgcagatttcagcttcctgctaatcagtgcttcagtcataattgccagaggacaaattgttctct
cccagtctctcagcaatcctgtctgcatctccaggggagaggtcacaatgacttgcagggccagctcaagtgtaagttacatgcact
ggtaccagcagaagccaggatcctccccaaacctggatttatgccccatccaacctggcttctggagtccctgctcgcttcagtg
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cctgggggggaccgtcagtcttcctcttccccccaaaacccaaggacaccctcatgatctcccggacccctgaggtcacatgcgtgg
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cgcgggaggagcagtacaacagcacgtaccgtgtggtcagcgtcctcaccgtcctgcaccaggactggctgaatggcaaggag
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ctccttcttcctctacagcaagctcaccgtggacaagagcaggtggcagcaggggaacgtcttctcatgctccgtgatgcatgagg
ctctgcacaaccactacacgcagaagagcctctccctgtctccgggtaaatgatctaga

Amino acid sequence

MDFQVQIFSFLLISASVIIARGQIVLSQSPAILSASPGEKVTMTCRASSSVSYMHWY
QQKPGSSPKPWIYAPSNLASGVPARFSGSGSGTSYSLTISRVEAEDAATYYCQQWS
FNPPTFGAGTKLELKDGGGSGGGGSGGGGSSQAYLQQSGAESVRPGASVKMSCK
ASGYTFTSYNMHWVKQTPRQGLEWIGAIYPGNGDTSYNQKFKGKATLTVDKSSS
TAYMQLSSLTSEDSAVYFCARVVYYSNSYWYFDVWGTGTTVTVSSDQEPKSSDK

THTSPPSSAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYV
DGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCLVSNKALPAPIE
KTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPEN
NYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLS
PGK

24. 2H7 scFv VHL11S hIgG1 (CSS-S)H K322SCH2 WCH3

Nucleotide sequence.

aagcttgccgccatggattttcaagtgcagattttcagcttcctgctaatcagtgcttcagtcataattgccagaggacaaattgttctct
cccagtctccagcaatcctgtctgcatctccaggggtgaaggtcacaatgacttgcagggccagctcaagtgtaagttacatgcact
ggtaccagcagaagccaggatcctcccccaaaccctggatttatgccccatccaacctggcttctggagtcctgctcgcttcagtg
gcagtgggtctgggacctcttactctctcacaatcagccgagtggaggctgaagatgctgccacttattactgccagcagtggagttt
taaccccacccacgttcggtgctgggaccaagctggagctgaaagatggcggtggctcgggcggtggtggatctggaggaggtg
ggagctctcaggcttatctacagcagtctggggctgagtcggtgaggcctggggcctcagtgaagatgtcctgcaaggcttctggc
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cgcgggaggagcagtacaacagcacgtaccgtgtggtcagcgtcctcaccgtcctgcaccaggactggctgaatggcaaggag
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caggtgtacaccctgcccccatcccgggatgagctgaccaagaaccaggtcagcctgacctgcctggtcaaaggcttctatcccca
gcgacatcgccgtggagtgggagagcaatgggcagccggagacaactacaagaccacgcctcccgtgctggactccgacgg
ctccttcttcctctacagcaagctcaccgtggacaagagcaggtggcagcaggggaacgtcttctcatgctccgtgatgcatgagg
ctctgcacaaccactacacgcagaagagcctctccctgtctccgggtaaatgatctaga

Amino acid sequence

MDFQVQIFSFLLISASVIIARGQIVLSQSPAILSASPGEKVTMTCRASSSVSYMHWY
QQKPGSSPKPWIYAPSNLASGVPARFSGSGSGTSYSLTISRVEAEDAATYYCQQWS
FNPPTFGAGTKLELKDGGGSGGGGSGGGGSSQAYLQQSGAESVRPGASVKMSCK
ASGYTFTSYNMHWVKQTPRQGLEWIGAIYPGNGDTSYNQKFKGKATLTVDKSSS
TAYMQLSSLTSEDSAVYFCARVVYYSNSYWYFDVWGTGTTVTVSSDQEPKSCDK
THTSPPSSAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYV
DGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCSVSNKALPAPIE
KTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPEN
NYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLS
PGK

25. P331S CH2

Nucleotide sequence

cctgaacfcctgggggggaccgtcagtcttcctcttcccccaaaacccaaggacaccctcatgatctcccggaccccctgaggtcac
atgcgtggtggtggacgtgagccacgaagaccctgaggtcaagttcaactggtacgtggacggcgtggaggtgcataatgccaa
gacaaagccgcgcgggaggagcagtacaatcagcacgtaccgtgtggtcagcgtcctcaccgtcctgcaccaggactggctgaatg
gcaaggagtacaagtgcaaggtctccaacaaagccctcccagcctccatcgagaaaacaatctccaaagccaaa

Amino acid sequence

PELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHN
AKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPASIEKTISKAK

26. P331S CH2 WCH3

Nucleotide sequence

cctgaactcctgggggggaccgtcagtcttcctcttccccccaaaacccaaggacaccctcatgatctcccggacccctgaggtcac
atgcgtggtggtggacgtgagccacgaagaccctgaggtcaagttcaactggtacgtggacggcgtggaggtgcataatgccaa
gacaaagccgcgggaggagcagtacaacagcacgtaccgtgtggtcagcgtcctcaccgtcctgcaccaggactggctgaatg
gcaaggagtacaagtgcaaggtctccaacaaagccctcccagcctccatcgagaaaacaatctccaaagccaaagggcagccc
cgagaaccacaggtgtacaccctgcccccatcccgggatgagctgaccaagaaccaggtcagcctgacctgcctggtcaaagg
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actccgacggctccttcttcctctacagcaagctcaccgtggacaagagcaggtggcagcaggggaacgtcttctcatgctccgtg
atgcatgaggctctgcacaaccactacacgcagaagagcctctccctgtctccgggtaaatgatctaga

Amino acid sequence

PELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHN
AKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPASIEKTISKAKG
QPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPV
LDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

27 2H7 scFv VH L11S (SSS-S)H P331S CH2 WCH3

Nucleotide sequence

aagcttgccgccatggatttttcaagtgcagattttcagcttcctgctaatcagtgcttcagtcataattgccagaggacaaattgttctct
cccagtctctcagcaatcctgtctgcatctccagggggagaggtcacaatgacttgcagggccagctcaagtgtaagttacatgcact
ggtaccagcagaagccaggatcctcccccaaaccctggatttatgccccatccaacctggcttctggagtccctgctcgcttcagtg
gcagtgggtctgggacctcttactctcacaatcagcagagtggaggctgaagatgctgccactattactgccagcagtggagttt
tacccacccacgttcggtgctgggaccaagctggagctgaaagatggcggtggctcgggcggtggtggatcgggaggaggtg
ggaggctctcaggcttatctacagcagtctggggctgagtcggtgaggcctggggcctcagtgaagatgtcctgcaaggcttctggc
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ctccttcttcctctacagcaagctcaccgtggacaagagcaggtggcagcaggggaacgtcttctcatgctccgtgatgcatgagg
ctctgcacaaccactacacgcagaagagcctctccctgtctccgggtaaatgatctaga

Amino acid sequence

MDFQVQIFSFLLISASVIIARGQIVLSQSPAILSASPGEKVTMTCRASSSVSYMHWY
QQKPGSSPKPWIYAPSNLASGVPARFSGSGSGTSYSLTISRVEAEDAATYYCQQWS
FNPPTFGAGTKLELKDGGGSGGGGSGGGGSSQAYLQQSGAESVRPGASVKMSCK
ASGYTFTSYNMHWVKQTPRQGLEWIGAIYPGNGDTSYNQKFKGKATLTVDKSSS
TAYMQLSSLTSEDSAVYFCARVVYYSNSYWYFDVWGTGTTVTVSSDQEPKSSDK
THTSPPSSAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYV
DGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPASIE
KTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPEN
NYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLS
PGK

28. 2H7 scFv VH L11S (CSS-S)H P331S CH2 WCH3

376

Nucleotide sequence

aagcttgccgccatggatttfcaagtgcagatttfcagcttcctgctaatcagtgcttcagtcataattgccagaggacaaattgttctct
cccagtctccagcaatcctgtctgcatctccagggggagaggtcacaatgacttgcagggccagctcaagtgtaagttacatgcact
ggtaccagcagaagccaggatcctcccctcaaacctggatttatgcccatccaacctggcttctggagtccctgctcgcttcagtg
gcagtgggtctgggacctcttactctcacaatcagcagagtggaggctgaagatgctgccacttattactgccagcagtggagtt
tacccacccacgttcggtgctgggaccaagctggagctgaaagatggcggtggctcgggcggtggtggatctggaggaggtg
ggagctctcaggcttatctacagcagtctggggctgagtcggtgaggcctggggcctcagtgaagatgtcctgcaaggcttctggc
tacacatttaccagttacaatatgcactgggtaaagcagacacctagacagggcctggaatggattggagctatttatccaggaaat
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cagcctgacatctgaagactctgcggtctatttctgtgcaagagtggtgtactatagtaactcttactggtacttcgatgtctggggcac
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cgcgggaggagcagtacaacagcacgtaccgtgtggtcagcgtcctcaccgtcctgcaccaggactggctgaatggcaaggag
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ctccttcttcctctacagcaagctcaccgtggacaagagcaggtggcagcaggggaacgtcttctcatgctccgtgatgcatgagg
ctctgcacaaccactacacgcagaagagcctctccctgtctccgggtaaatgatctaga

Amino acid sequence

MDFQVQIFSFLLISASVIIARGQIVLSQSPAILSASPGEKVTMTCRASSSVSYMHWY
QQKPGSSPKPWIYAPSNLASGVPARFSGSGSGTSYSLTISRVEAEDAATYYCQQWS
FNPPTFGAGTKLELKDGGGSGGGGSGGGGSSQAYLQQSGAESVRPGASVKMSCK
ASGYTFTSYNMHWVKQTPRQGLEWIGAIYPGNGDTSYNQKFKGKATLTVDKSSS
TAYMQLSSLTSEDSAVYFCARVVYYSNSYWYFDVWGTGTTVTVSSDQEPKSCDK
THTSPPSSAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYV
DGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIE
KTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPEN
NYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLS
PGK

29 T256N CH2 region

Nucleotide sequence

Cctgaactcctgggggggaccgtcagtcttcctcttccccccaaaacccaaggacaccctcatgatctcccggaccctgaggtca
catgcgtggtggtggacgtgagccacgaagaccctgaggtcaagttcaactggtacgtggacggcgtggaggtgcataatgcca
agacaaagccgcgggaggagcagtacaacagcacgtaccgtgtggtcagcgtcctcaccgtcctgcaccaggactggctgaat
ggcaaggagtacaagtgcaaggtctccaacaaagccctcccagccccatcgagaaaacaatctccaaagccaaa

Amino acid sequence

PELLGGPSVFLFPPKPKDTLMISRNPEVTCVVVDVSHEDPEVKFNWYVDGVEVHN
AKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAK

30 T256N CH2 WCH3

Nucleotide sequence

cctgaactcctgggggggaccgtcagtcttcctcttccccccaaaacccaaggacaccctcatgatctcccggaccctgaggtcac
atgcgtggtggtggacgtgagccacgaagaccctgaggtcaagttcaactggtacgtggacggcgtggaggtgcataatgccaa
gacaaagccgcgggaggagcagtacaacagcacgtaccgtgtggtcagcgtcctcaccgtcctgcaccaggactggctgaatg
gcaaggagtacaagtgcaaggtctccaacaaagccctcccagccccatcgagaaaacaatctccaaagccaaagggcagccc
cgagaaccacaggtgtacaccctgcccccatcccgggatgagctgaccaagaaccaggtcagcctgacctgcctggtcaaagg
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agttccgacggctccttcttcctctacagcaagctcaccgtggacaagagcaggtggcagcaggggaacgtcttctcatgctccgtg
atgcatgaggctctgcacaaccactacacgcagaagagcctctccctgtctccgggtaaatgatctaga

Amino acid sequence

PELLGGPSVFLFPPKPKDTLMISRNPEVTCVVVDVSHEDPEVKFNWYVDGVEVHN
AKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKG
QPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPV
LDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

31 2H7 scFv VH L11S (SSS-S)H T256N CH2 WCH3

Nucleotide sequence

aagcttgccgccatggattttcaagtgcagattttcagcttcctgctaatcagtgcttcagtcataattgccagaggacaaattgttctct
cccagtctccagcaatcctgtctgcatctccaggggagaaggtcacaatgacttgcagggccagctcaagtgtaagttacatgcact
ggtaccagcagaagccaggatcctccccaaaccctggatttatgccccatccaacctggcttctggagtccctgctcgcttcagtg
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ggagctctcaggcttatctacagcagtctggggctgagtcggtgaggcctggggcctcagtgaagatgtcctgcaaggcttctggc
tacacatttaccagttacaatatgcactgggtaaagcagacacctagacagggcctggaatggattggagctatttatccaggaaat
ggtgatacttcctacaatcagaagttcaagggcaaggccacactgactgtagacaaatcctccagcacagcctacatgcagctcag
cagcctgacatctgaagactctgcggtctatttctgtgcaagagtggtgtactatagtaactcttactggtacttcgatgtctggggcac
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cgcgggaggagcagtacaacagcacgtaccgtgtggtcagcgtcctcaccgtcctgcaccaggactggctgaatggcaaggag
tacaagtgcaaggtctccaacaaagccctcccagcccccatcgagaaaacaatctccaaagccaaagggcagccccgagaacc
acaggtgtacaccctgcccccatcccgggatgagctgaccaagaaccaggtcagcctgacctgcctggtcaaaggcttctatccc
agcgacatcgccgtggagtgggagagcaatgggcagccggagaacaactacaagaccacgcctcccgtgctggactccgacg
gctccttcttcctctacagcaagctcaccgtggacaagagcaggtggcagcaggggaacgtcttctcatgctccgtgatgcatgag
gctctgcacaaccactacacgcagaagagcctctccctgtctccgggtaaatgatctaga

Amino acid sequence

MDFQVQIFSFLLISASVIIARGQIVLSQSPAILSASPGEKVTMTCRASSSVSYMHWY
QQKPGSSPKPWIYAPSNLASGVPARFSGSGSGTSYSLTISRVEAEDAATYYCQQWS
FNPPTFGAGTKLELKDGGGSGGGGSGGGGSSQAYLQQSGAESVRPGASVKMSCK
ASGYTFTSYNMHWVKQTPRQGLEWIGAIYPGNGDTSYNQKFKGKATLTVDKSSS
TAYMQLSSLTSEDSAVYFCARVVYYSNSYWYFDVWGTGTTVTVSSDQEPKSSDK
THTSPPSSAPELLGGPSVFLFPPKPKDTLMISRNPEVTCVVVDVSHEDPEVKFNWY
VDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPI
EKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPE
NNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSL
SPGK

32. 2H7 scFv VH L11S (CSS-S)H T256N CH2 WCH3

Nucleotide sequence

aagcttgccgccatggattttcaagtgcagattttcagcttcctgctaatcagtgcttcagtcataattgccagaggacaaattgttctct
cccagtctccagcaatcctgtctgcatctccaggggagaaggtcacaatgacttgcagggccagctcaagtgtaagttacatgcact
ggtaccagcagaagccaggatcctccccaaacccctggatttatgccccatccaacctggcttctggagtccctgctcgcttcagtg
gcagtgggtctgggacctcttactctctcacaatcagcagagtggaggctgaagatgctgccacttattactgccagcagtggagttt
taacccacccacgttcggtgctgggaccaagctggagctgaaagatggcggtggctcgggcggtggtggatctggaggaggtg

ggagctctcaggcttatctacagcagtctggggctgagtcggtgaggcctggggcctcagtgaagatgtcctgcaaggcttctggc
tacacatttaccagttacaatatgcactgggtaaagcaggacacctagacagggcctggaatggattggaggctatttatccaggaaat
ggtgatacttcctacaatcagaagttcaagggcaaggccacactgactgtagacaaatcctccagcacagcctacatgcagctcag
cagcctgacatctgaagactctgcggtctatttctgtgcaagagtggtgtactatagtaactcttactggtacttcgatgtctggggcac
agggaccacggtcaccgtctcttcttcatcaggagcccaaatcttgtgacaaaactcacacatcccccaccgtcctcagcacctgaact
cctgggggggaccgtcagtcttcctcttccccccaaaacccaaggacaccctcatgatctcccggaacccctgaggtcacatgcgtgg
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cgcgggaggagcagtacaacagcacgtaccgtgtggtcagcgtcctcaccgtcctgcaccaggactggctgaatggcaaggag
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acaggtgtacaccctgcccccatcccgggatgagctgaccaagaaccaggtcagcctgacctgcctggtcaaaggcttctatccc
agcgacatcgccgtggagtgggagagcaatgggcagccggagaacaactacaagaccacgcctcccgtgctggactccgacg
gctccttcttcctctacagcaagctcaccgtggacaagagcaggtggcagcaggggaacgtcttctcatgctccgtgatgcatgag
gctctgcacaaccactacacgcagaagagcctctccctgtctccgggtaaatgatctaga

Amino acid sequence
MDFQVQIFSFLLISASVIIARGQIVLSQSPAILSASPGEKVTMTCRASSSVSYMHWY
QQKPGSSPKPWIYAPSNLASGVPARFSGSGSGTSYSLTISRVEAEDAATYYCQQWS
FNPPTFGAGTKLELKDGGGSGGGGSGGGGSSQAYLQQSGAESVRPGASVKMSCK
ASGYTFTSYNMHWVKQTPRQGLEWIGAIYPGNGDTSYNQKFKGKATLTVDKSSS
TAYMQLSSLTSEDSAVYFCARVVYYSNSYWYFDVWGTGTTVTVSSDQEPKSCDK
THTSPPSSAPELLGGPSVFLFPPKPKDTLMISRNPEVTCVVVDVSHEDPEVKFNWY
VDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPI
EKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPE
NNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSL
SPGK

33 RTPE/QNAK (255-258) CH2
Nucleotide sequence
cctgaactcctgggggggaccgtcagtcttcctcttccccccaaaacccaaggacaccctcatgatctcccggaacgctaaggtcac
atgcgtggtggtggacgtgagccacgaagaccctgaggtcaagttcaactggtacgtggacggcgtggaggtgcataatgccaa
gacaaagccgcgggaggagcagtacaacagcacgtaccgtgtggtcagcgtcctcaccgtcctgcaccaggactggctgaatg
gcaaggagtacaagtgcaaggtctccaacaaagccctcccagcccccatcgagaaaacaatctccaaagccaaa

Amino acid sequence
PELLGGPSVFLFPPKPKDTLMISQNAKVTCVVVDVSHEDPEVKFNWYVDGVEVHN
AKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAK

34. RTPE/QNAK (255-258)CH2 WCH3
Nucleotide sequence
cctgaactcctgggggggaccgtcagtcttcctcttccccccaaaacccaaggacaccctcatgatctcccggaacgctaaggtcac
atgcgtggtggtggacgtgagccacgaagaccctgaggtcaagttcaactggtacgtggacggcgtggaggtgcataatgccaa
gacaaagccgcgggaggagcagtacaacagcacgtaccgtgtggtcagcgtcctcaccgtcctgcaccaggactggctgaatg
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cgagaaccacaggtgtacaccctgcccccatcccgggatgagctgaccaagaaccaggtcagcctgacctgcctggtcaaagg
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actccgacggctccttcttcctctacagcaagctcaccgtggacaagagcaggtggcagcaggggaacgtcttctcatgctccgtg
atgcatgaggctctgcacaaccactacacgcagaagagcctctccctgtctccgggtaaatgatctaga

Amino acid sequence
PELLGGPSVFLFPPKPKDTLMISQNAKVTCVVVDVSHEDPEVKFNWYVDGVEVHN
AKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKG

QPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPV
LDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

35. 2H7 scFv VH L11S (SSS-S)H RTPE/QNAK (255-258)CH2 WCH3

Nucleotide sequence

aagcttgccgccatggattttcaagtgcagattttcagcttcctgctaatcagtgcttcagtcataattgccagaggacaaattgttctct
cccagtctccagcaatcctgtctgcatctccaggggagaaggtcacaatgacttgcagggccagctcaagtgtaagttacatgcact
ggtaccagcagaagccaggatcctcccccaaaccctggatttatgccccatccaacctggcttctggagtccctgctcgcttcagtg
gcagtgggtctgggacctcttactctctcacaatcagcagagtggaggctgaagatgctgccacttattactgccagcagtggagttt
taacccacccacgttcggtgctgggaccaagctggagctgaaagatggcggtggctcgggcggtggtgggatctggaggaggtg
ggagctctcaggcttatctacagcagtctggggctgagtcggtgaggcctgggacctcagtgaagatgtcctgcaaggcttctggc
tacacatttaccagttacaatatgcactgggtaaagcagacacctagacagggcctggaatggattggagctatttatccaggaaat
ggtgatacttcctacaatcagaagttcaagggcaaggccacactgactgtagacaaatcctccagcacagcctacatgcagctcag
cagcctgacatctgaagactctgcggtctatttctgtgcaagagtggtgtactatagtaactcttactggtacttcgatgtctggggcac
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gctccttcttcctctacagcaagctcaccgtggacaagagcaggtggcaggcaggggaacgtcttctcatgctccgtgatgcatgag
gctctgcacaaccactacacgcagaagagcctctccctgtctccgggtaaatgatctaga

Amino acid sequence

MDFQVQIFSFLLISASVIIARGQIVLSQSPAILSASPGEKVTMTCRASSSVSYMHWY
QQKPGSSPKPWIYAPSNLASGVPARFSGSGSGTSYSLTISRVEAEDAATYYCQQWS
FNPPTFGAGTKLELKDGGGSGGGGSGGGGSSQAYLQQSGAESVRPGASVKMSCK
ASGYTFTSYNMHWVKQTPRQGLEWIGAIYPGNGDTSYNQKFKGKATLTVDKSSS
TAYMQLSSLTSEDSAVYFCARVVYYSNSYWYFDVWGTGTTVTVSSDQEPKSSDK
THTSPPSSAPELLGGPSVFLFPPKPKDTLMISQNAKVTCVVVDVSHEDPEVKFNWY
VDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPI
EKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPE
NNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSL
SPGK

36. 2H7 scFv VH L11S (CSS-S)H RTPE/QNAK (255-258)CH2 WCH3

Nucleotide sequence

aagcttgccgccatggattttcaagtgcagattttcagcttcctgctaatcagtgcttcagtcataattgccagaggacaaattgttctct
cccagtctccagcaatcctgtctgcatctccaggggagaaggtcacaatgacttgcagggccagctcaagtgtaagttacatgcact
ggtaccagcagaagccaggatcctcccccaaaccctggatttatgccccatccaacctggcttctggagtccctgctcgcttcagtg
gcagtgggtctgggacctcttactctctcacaatcagcagagtggaggctgaagatgctgccacttattactgccagcagtggagttt
taacccacccacgttcggtgctgggaccaagctggagctgaaagatggcggtggctcgggcggtggtgggatctggaggaggtg
ggagctctcaggcttatctacagcagtctggggctgagtcggtgaggcctgggacctcagtgaagatgtcctgcaaggcttctggc
tacacatttaccagttacaatatgcactgggtaaagcagacacctagacagggcctggaatggattggagctatttatccaggaaat
ggtgatacttcctacaatcagaagttcaagggcaaggccacactgactgtagacaaatcctccagcacagcctacatgcagctcag
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cctgggggggaccgtcagtcttcctcttccccccaaaacccaaggacaccctcatgatctcccggaacgctaaggtcacatgcgtgg
tggtggacgtgagccacgaagaccctgaggtcaagttcaactggtacgtggacggcgtggaggtgcataatgccaagacaaagc

cgcgggaggagcagtacaacagcacgtaccgtgtggtcagcgtcctcaccgtcctgcaccaggactggctgaatggcaaggag
tacaagtgcaaggtctccaacaaagccctcccagcccccatcgagaaaacaatctccaaagccaaagggcagccccgagaacc
acaggtgtacaccctgcccccatcccgggatgagctgaccaagaaccaggtcagcctgacctgcctggtcaaaggcttctatccc
agcgacatcgccgtggagtgggagagcaatgggcagccggagaacaactacaagaccacgcctcccgtgctggactccgacg
gctccttcttcctctacagcaagctcaccgtggacaagagcaggtggcagcaggggaacgtcttctcatgctccgtgatgcatgag
gctctgcacaaccactacacgcagaagagcctctccctgtctccgggtaaatgatctaga

Amino acid sequence
MDFQVQIFSFLLISASVIIARGQIVLSQSPAILSASPGEKVTMTCRASSSVSYMHWY
QQKPGSSPKPWIYAPSNLASGVPARFSGSGSGTSYSLTISRVEAEDAATYYCQQWS
FNPPTFGAGTKLELKDGGGSGGGGSGGGGSSQAYLQQSGAESVRPGASVKMSCK
ASGYTFTSYNMHWVKQTPRQGLEWIGAIYPGNGDTSYNQKFKGKATLTVDKSSS
TAYMQLSSLTSEDSAVYFCARVVYYSNSYWYFDVWGTGTTVTVSSDQEPKSCDK
THTSPPSSAPELLGGPSVFLFPPKPKDTLMISQNAKVTCVVVDVSHEDPEVKFNWY
VDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPI
EKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPE
NNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSL
SPGK

36. K290Q CH2 region

Nucleotide sequence
cctgaactcctgggggggaccgtcagtcttcctcttccccccaaaacccaaggacaccctcatgatctcccggacccctgaggtcac
atgcgtggtggtggacgtgagccacgaagaccctgaggtcaagttcaactggtacgtggacggcgtggaggtgcataatgccaa
gacacagccgcgcgggaggagcagtacaacagcacgtaccgtgtggtcagcgtcctcaccgtcctgcaccaggactggctgaatg
gcaaggagtacaagtgcaaggtctccaacaaagccctcccagcccccatcgagaaaacaatctccaaagccaaa

Amino acid sequence
PELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHN
AKTQPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAK

37 K290Q CH2 WCH3

Nucleotide sequence
Cctgaactcctgggggggaccgtcagtcttcctcttccccccaaaacccaaggacaccctcatgatctcccggacccctgaggtca
catgcgtggtggtggacgtgagccacgaagaccctgaggtcaagttcaactggtacgtggacggcgtggaggtgcataatgcca
agacacagccgcgcgggaggagcagtacaacagcacgtaccgtgtggtcagcgtcctcaccgtcctgcaccaggactggctgaat
ggcaaggagtacaagtgcaaggtctccaacaaagccctcccagcccccatcgagaaaacaatctccaaagccaaagggcagcc
ccgagaaccacaggtgtacaccctgcccccatcccgggatgagctgaccaagaaccaggtcagcctgacctgcctggtcaaag
gcttctatcccagcgacatcgccgtggagtgggagagcaatgggcagccggagaacaactacaagaccacgcctcccgtgctg
gactccgacggctccttcttcctctacagcaagctcaccgtggacaagagcaggtggcagcaggggaacgtcttctcatgctccgt
gatgcatgaggctctgcacaaccactacacgcagaagagcctctccctgtctccgggtaaatgatctaga

Amino acid sequence
PELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHN
AKTQPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKG
QPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPV
LDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

38 2H7 scFv VH L11S (SSS-S)H K290Q CH2 WCH3

Nucleotide sequence
aagcttgccgccatggattttcaagtgcagattttcagcttcctgctaatcagtgtcttcagtcataattgccagaggacaaattgttctct
cccagtctccagcaatccttgtctgcatctccaggggagaaggtcacaatgacttgcagggccagctcaagtgtaagttacatgcact

ggtaccagcagaagccaggatcctccccaaaccctggatttgtgcccatccaacctggcttctggagtccctgctcgcttcagtg
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taacccacccacgttcggtgctgggaccaagctggagctgaaagatggcggtggctcgggcggtggtggatctggaggaggtg
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gctccttcttcctctacagcaagctcaccgtggacaagagcaggtggcagcaggggaacgtcttctcatgctccgtgatgcatgag
gctctgcacaaccactacacgcagaagagcctctccctgtctccgggtaaatgatctaga

Amino acid sequence
MDFQVQIFSFLLISASVIIARGQIVLSQSPAILSASPGEKVTMTCRASSSVSYMHWY
QQKPGSSPKPWIYAPSNLASGVPARFSGSGSGTSYSLTISRVEAEDAATYYCQQWS
FNPPTFGAGTKLELKDGGGSGGGGSGGGGSSQAYLQQSGAESVRPGASVKMSCK
ASGYTFTSYNMHWVKQTPRQGLEWIGAIYPGNGDTSYNQKFKGKATLTVDKSSS
TAYMQLSSLTSEDSAVYFCARVVYYSNSYWYFDVWGTGTTVTVSSDQEPKSSDK
THTSPPSSAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYV
DGVEVHNAKTQPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIE
KTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPEN
NYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLS
PGK

39 2H7 scfv VH L11S (CSS-S)H K290Q CH2 WCH3
Nucleotide sequence
aagcttgccgccatggatttccaagtgcagattttcagcttcctgctaatcagtgcttcagtcataattgccagaggacaaattgttctct
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gctccttcttcctctacagcaagctcaccgtggacaagagcaggtggcagcaggggaacgtcttctcatgctccgtgatgcatgag
gctctgcacaaccactacacgcagaagagcctctccctgtctccgggtaaatgatctaga

Amino acid sequence

MDFQVQIFSFLLISASVIIARGQIVLSQSPAILSASPGEKVTMTCRASSSVSYMHWY
QQKPGSSPKPWIYAPSNLASGVPARFSGSGSGTSYSLTISRVEAEDAATYYCQQWS
FNPPTFGAGTKLELKDGGGSGGGGSGGGGSSQAYLQQSGAESVRPGASVKMSCK
ASGYTFTSYNMHWVKQTPRQGLEWIGAIYPGNGDTSYNQKFKGKATLTVDKSSS
TAYMQLSSLTSEDSAVYFCARVVYYSNSYWYFDVWGTGTTVTVSSDQEPKSCDK
THTSPPSSAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYV
DGVEVHNAKTQPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIE
KTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPEN
NYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLS
PGK

40. A339PCH2

Nucleotide sequence

cctgaactcctgggggaccgtcagtcttcctcttccccccaaaacccaaggacaccctcatgatctcccggacccctgaggtcac
atgcgtggtggtggacgtgagccacgaagaccctgaggtcaagttcaactggtacgtggacggcgtggaggtgcataatgccaa
gacaaagccgcgggaggagcagtacaacagcacgtaccgtgtggtcagcgtcctcaccgtcctgcaccaggactggctgaatg
gcaaggagtacaagtgcaaggtctccaacaaagccctcccagcccccatcgagaaaacaatctccaaacccaaa

Amino acid sequence

PELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHN
AKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKPK

41 A339P-CH2 WCH3

Nucleotide sequence

cctgaactcctgggggaccgtcagtcttcctcttccccccaaaacccaaggacaccctcatgatctcccggacccctgaggtcac
atgcgtggtggtggacgtgagccacgaagaccctgaggtcaagttcaactggtacgtggacggcgtggaggtgcataatgccaa
gacaaagccgcgggaggagcagtacaacagcacgtaccgtgtggtcagcgtcctcaccgtcctgcaccaggactggctgaatg
gcaaggagtacaagtgcaaggtctccaacaaagccctcccagcccccatcgagaaaacaatctccaaacccaaagggcagccc
cgagaaccacaggtgtacaccctgcccccatcccgggatgagctgaccaagaaccaggtcagcctgacctgcctggtcaaagg
cttctatcccagcgacatcgccgtggagtgggagagcaatgggcagccggagaacaactacaagaccacgcctcccgtgctgg
actccgacggctccttcttcctctacagcaagctcaccgtggacaagagcaggtggcagcaggggaacgtcttctcatgctccgtg
atgcatgaggctctgcacaaccactacacgcagaagagcctctccctgtctccgggtaaatgatctaga

Amino acid sequence

PELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHN
AKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKPKG
QPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPV
LDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

42 2H7 scFv VHL11S (SS8-S)H A339P CH2 WCH3

Nucleotide sequence

aagcttgccgccatggatttttcaagtgcagattttcagcttcctgctaatcagtgcttcagtcataattgccagaggacaaaattgttctct
cccagtctccagcaatcctgtctgcatctccagggggagaaggtcacaatgacttgcagggccagctcaagtgtaagttacatgcact
ggtaccagcagaagccaggatcctcccccaaaccctggatttatgccccatccaacctggcttctggagtccctgctcgcttcagtg
gcagtgggtctgggacctcttactctctcacaatcagcagagtggaggctgaagatgctgccacttattactgccagcagtggagttt
tacccacccacgttcggtgctgggaccaagctggagctgaaagatggcggtggctcgggcggtggtggatctggaggaggtg
ggagctctcaggcttatctacagcagtctggggctgagtcggtgaggcctggggcctcagtgaagatgtcctgcaaggcttctggc
tacacatttaccagttacaatatgcactgggtaaagcagacacctagacagggcctggaatggattggagctatttatccaggaaat
ggtgatacttcctacaatcagaagttcaagggcaaggccacactgactgtagacaaatcctccagcacagcctacatgcagctcag
cagcctgacatctgaagactctgcggtctatttctgtgcaagagtggtgtactatagtaactcttactggtacttcgatgtctggggcac

agggaccacggtcaccgtctcttctgatcaggagcccaaatcttctgacaaaactcacacatcccccaccgtcctcagcacctgaact
cctgggggggatcgtcagtcttcctcttcccccaaaacccaaggacaccctcatgatctcccggacccctgaggtcacatgcgtgg
tggtggacgtgagccacgaagaccctgaggtcaagttcaactggtacgtggacggcgtggaggtgcataatgccaagacaaagc
cgcgggaggagcagtacaacagcacgtaccgtgtggtcagcgtcctcaccgtcctgcaccaggactggctgaatggcaaggag
tacaagtgcaaggtctccaacaaagccctcccagcccccatcgagaaaacaatctccaaacccaaagggcagccccgagaacc
acaggtgtacaccctgcccccatcccgggatgagctgaccaagaaccaggtcagcctgacctgcctggtcaaaggcttctatccc
agcgacatcgccgtggagtgggagagcaatgggcagccggagaacaactacaagaccacgcctcccgtgctggactccgacg
gctccttcttcctctacagcaagctcaccgtggacaagagcaggtggcagcaggggaacgtcttctcatgctccgtgatgcatgag
gctctgcacaaccactacacgcagaagagcctctccctgtctccgggtaaatgatctaga

Amino acid sequence
MDFQVQIFSFLLISASVIIARGQIVLSQSPAILSASPGEKVTMTCRASSSVSYMHWY
QQKPGSSPKPWIYAPSNLASGVPARFSGSGSGTSYSLTISRVEAEDAATYYCQQWS
FNPPTFGAGTKLELKDGGGSGGGGSGGGGSSQAYLQQSGAESVRPGASVKMSCK
ASGYTFTSYNMHWVKQTPRQGLEWIGAIYPGNGDTSYNQKFKGKATLTVDKSSS
TAYMQLSSLTSEDSAVYFCARVVYYSNSYWYFDVWGTGTTVTVSSDQEPKSSDK
THTSPPSSAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYV
DGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIE
KTISKPKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPEN
NYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLS
PGK

43. 2H7 scFv VHL11S (CSS-S)H A339P CH2 WCH3

Nucleotide sequence:
aagcttgccgccatggatttttcaagtgcagatttttcagcttcctgctaatcagtgcttcagtcatcattgccagaggacaaatttgttctct
cccagtctccagcaatcctgtctgcatctccagggggagaaggtcacaatgacttgcagggccagctcaagtgtaagttacatgcact
ggtaccagcagaagccaggatcctcccccaaacctggatttatgccccatccaacctggcttctggagtccctgctcgcttcagtg
gcagtgggtctgggacctcttactctctcacaatcagcagagtggaggctgaagatgctgccacttattactgccagcagtggagttt
taacccacccacgttcggtgctgggaccaagctggagctgaaagatggcggtggctcgggcggtggtgggtcgggaggaggtg
ggagctctcaggcttatctacagcagtctggggctgagtcggtgaggcctggggcctcagtgaagatgtcctgcaaggcttctggc
tacacatttaccagttacaatatgcactgggtaaagcagacacctagacagggcctggaatggattggagctatttatccaggaaat
ggtgatacttcctacaatcagaagttcaaggacaaggccacactgactgtagacaaatcctccagcacagcctacatgcagctcag
cagcctgacatctgaagactctgcggtctatttctgtgcaagagtggtgtactatagtaactcttactggtacttcgatgtctggggcac
agggaccacggtcaccgtctcttctgatcaggagcccaaatcttgtgacaaaactcacacatcccccaccgtcctcagcacctgaact
cctgggggggaccgtcagtcttcctcttcccccaaaacccaaggacaccctcatgatctcccggaccccctgaggtcacatgcgtgg
tggtggacgtgagccacgaagaccctgaggtcaagttcaactggtacgtggacggcgtggaggtgcataatgccaagacaaagc
cgcggaggaggagcagtacaacagcacgtaccgtgtggtcagcgtcctcaccgtcctgcaccaggactggctgaatggcaaggag
tacaagtgcaaggtctccaacaaagccctcccagcccccatcgagaaaacaatctccaaacccaaagggcagccccgagaacc
acaggtgtacaccctgcccccatcccgggatgagctgaccaagaaccaggtcagcctgacctgcctggtcaaaggcttctatccc
agcgacatcgccgtggagtgggagagcaatgggcagccggagaacaactacaagaccacgcctcccgtgctggactccgacg
gctccttcttcctctacagcaagctcaccgtggacaagagcaggtggcagcaggggaacgtcttctcatgctccgtgatgcatgag
gctctgcacaaccactacacgcagaagagcctctccctgtctccgggtaaatgatctaga

Amino acid sequence
MDFQVQIFSFLLISASVIIARGQIVLSQSPAILSASPGEKVTMTCRASSSVSYMHWY
QQKPGSSPKPWIYAPSNLASGVPARFSGSGSGTSYSLTISRVEAEDAATYYCQQWS
FNPPTFGAGTKLELKDGGGSGGGGSGGGGSSQAYLQQSGAESVRPGASVKMSCK
ASGYTFTSYNMHWVKQTPRQGLEWIGAIYPGNGDTSYNQKFKGKATLTVDKSSS
TAYMQLSSLTSEDSAVYFCARVVYYSNSYWYFDVWGTGTTVTVSSDQEPKSCDK
THTSPPSSAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYV

DGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIE
KTISKPKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPEN
NYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLS
PGK

44. G28-1VH

Nucleotide sequence.

gcggtccagctgcagcagtctggacctgagctggaaaagcctggcgcttcagtgaagatttcctgcaaggcttctggttactcattc
actggctacaatatgaactgggtgaagcagaatagtggaaagagccttgagtggattggagatattgatccttattatggtggtacta
cctacaaccggaagttcaagggcaaggccacattgactgtagacaaatcctccagcacagcctacatgcagctcaagagtctgac
atctgaggactctgcagtctattactgtgcaagatcggtcggccctatggactactggggtcaaggaacctcagtcaccgtctcttct
gatcag

Amino acid sequence

AVQLQQSGPELEKPGASVKISCKASGYSFTGYNMNWVKQNNGKSLEWIGNIDPY
YGGITYNRKFKGKATLTVDKSSSTAYMQLKSLTSEDSAVYYCARSVGPMDYWG
QGTSVTVSSDQ

45. G28-1VL

Nucleotide sequence

aagcttgccgccatggtatccacagctcagttccttgggttgctgctgctgtggcttacaggtggcagatgtgacatccagatgactc
agtctccagcctccctatctgcatctgtgggagagactgtcaccatcacatgtcgaacaagtgaaaatgtttacagttatttggcttggt
atcagcagaaacagggaaatctcctcagctcctggtctctttgcaaaaaccttagcagaaggtgtgccatcaaggttcagtggca
gtggatcaggcacacagttttctctgaagatcagcagcctgcagcctgaagattctggaagttattactgtcaacatcattccgatgat
ccgtggacgttcggtggaggcaccgaactggagatcaaaggtggcggtggctcgggcggtggtgggtcgggtggcggcggat
cgtca

Amino acid sequence

MVSTAQFLGLLLLWLTGGRCDIQMTQSPASLSASVGETVTITCRTSENVYSYLAW
YQQKQGKSPQLLVSFAKTLAEGVPSRFSGSGSGTQFSLKISSLQPEDSGSYFCQHHS
DNPWTFGGGTELEIKGGGGSGGGGSGGGGS

46. G28-1 scFv

Nucleotide sequence

aagcttgccgccatggtatccacagctcagttccttgggttgctgctgctgtggcttacaggtggcagatgtgacatccagatgactc
agtctccagcctccctatctgcatctgtgggagagactgtcaccatcacatgtcgaacaagtgaaaatgtttacagttatttggcttggt
atcagcagaaacagggaaatctcctcagctcctggtctctttgcaaaaaccttagcagaaggtgtgccatcaaggttcagtggca
gtggatcaggcacacagttttctctgaagatcagcagcctgcagcctgaagattctggaagttattactgtcaacatcattccgatgat
ccgtggacgttcggtggaggcaccgaactggagatcaaaggtggcggtggctcgggcggtggtgggtcgggtggcggcggat
cgtcagcggtccagctgcagcagtctggacctgagctggaaaagcctggcgcttcagtgaagatttcctgcaaggcttctggttact
cattcactggctacaatatgaactgggtgaagcagaatagtggaaagagccttgagtggattggagatattgatccttattatggtggt
actacctacaaccggaagttcaagggcaaggccacattgactgtagacaaatcctccagcacagcctacatgcagctcaagagtct
gacatctgaggactctgcagtctattactgtgcaagatcggtcggccctatggactactggggtcaaggaacctcagtcaccgtctc
ttctgatcag

Amino acid sequence

MVSTAQFLGLLLLWLTGGRCDIQMTQSPASLSASVGETVTITCRTSENVYSYLAW
YQQKQGKSPQLLVSFAKTLAEGVPSRFSGSGSGTQFSLKISSLQPEDSGSYFCQHHS
DNPWTFGGGTELEIKGGGGSGGGGSGGGGSSAVQLQQSGPELEKPGASVKISCKA

agtcttcctcttcccccaaaacccaaggacaccctcatgatctcccggacccctgaggtcacatgcgtggtggtggacgtgagcc
acgaagaccctgaggtcaagttcaactggtacgtggacggcgtggaggtgcataatgccaagacaaagccgcgggaggagca
gtacaacagcacgtaccgtgtggtcagcgtcctcaccgtcctgcaccaggactggctgaatggcaaggagtacaagtgcaaggtc
tccaacaaagccctcccagcccccatcgagaaaacaatctccaaagccaaagggcagccccgagaaccacaggtgtacaccct
gcccccatcccgggatgagctgaccaagaaccaggtcagcctgacctgcctggtcaaaggcttctatcccagcgacatcgccgtg
gagtgggagagcaatgggcagccggagaacaactacaagaccacgcctcccgtgctggactccgacggctccttcttcctctac
agcaagctcaccgtggacaagagcaggtggcagcaggggaacgtcttctcatgctccgtgatgcatgaggctctgcacaaccact
acacgcagaagagcctctccctgtctccgggtaaatgatctaga

Amino acid sequence·
MVSTAQFLGLLLLWLTGGRCDIQMTQSPASLSASVGETVTITCRTSENVYSYLAW
YQQKQGKSPQLLVSFAKTLAEGVPSRFSGSGSGTQFSLKISSLQPEDSGSYFCQHHS
DNPWTFGGGTELEIKGGGGSGGGGSGGGGSSAVQLQQSGPELEKPGASVKISCKA
SGYSFTGYNMNWVKQNNGKSLEWIGNIDPYYGGTTYNRKFKGKATLTVDKSSST
AYMQLKSLTSEDSAVYYCARSVGPMDYWGQGTSVTVSSDHDQEPKSSDKTHTSP
PSSAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVE
VHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISK
AKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKT
TPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

50. G28-1 scFv IgAW H IgG1WCH2 WCH3

Nucleotide sequence

aagcttgccgccatggatccacagctcagttccttgggttgctgctgctgtggcttacaggtggcagatgtgacatccagatgactc
agtctccagcctccctatctgcatctgtgggagagactgtcaccatcacatgtcgaacaagtgaaaatgtttacagttatttggcttggt
atcagcagaaacagggaaaatctcctcagctcctggtctctttgcaaaaaccttagcagaaggtgtgccatcaaggttcagtggca
gtggatcaggcacacagttttctctgaagatcagcagcctgcagcctgaagatictggaagttatttctgtcaacatcattccgatgat
ccgtggacgttcggtggaggcaccgaactggagatcaaagcggccggtggctcgggcggtggtgggtcgggtggcggcggat
cgtcagcggtccagctgcagcagtctggacctgagctggaaaagcctggcgcttcagtgaagatttcctgcaaggcttctggttact
cattcactggctacaatatgaactgggtgaagcagaatagtggaaagagccttgagtggattggaaatattgatccttattatggtggt
actacctacaaccggaagttcaaggcaaggccacattgactgtagacaaatcctccagcacagcctacatgcagctcaagagtct
gacatctgaggactctgcagtctattactgtgcaagatcggtcggcctatggactactggggtcaaggaacctcagtcaccgtctc
ttctgatcagccagttcctcaactccacctaccccatctcctcaactccacctaccccatctcctcatgcgcacctgaactcctgg
ggggaccgtcagtcttcctcttccccccaaaacccaaggacaccctcatgatctcccggacccctgaggtcacatgcgtggtggtg
gacgtgagccacgaagaccctgaggtcaagttcaactggtacgtggacggcgtggaggtgcataatgccaagacaaagccgcg
ggaggagcagtacaacagcacgtaccgtgtggtcagcgtcctcaccgtcctgcaccaggactggctgaatggcaaggagtacaa
gtgcaaggtctccaacaaagccctcccagcccccatcgagaaaacaatctccaaagccaaagggcagccccgagaaccacagg
tgtacaccctgcccccatcccgggatgagctgaccaagaaccaggtcagcctgacctgcctggtcaaaggcttctatcccagcga
catcgccgtggagtgggagagcaatgggcagccggagaacaactacaagaccacgcctcccgtgctggactccgacggctcct
tcttcctctacagcaagctcaccgtggacaagagcaggtggcagcaggggaacgtcttctcatgctccgtgatgcatgaggctctg
cacaaccactacacgcagaagagcctctccctgtctccgggtaaatgatctaga

Amino acid sequence.
MVSTAQFLGLLLLWLTGGRCDIQMTQSPASLSASVGETVTITCRTSENVYSYLAW
YQQKQGKSPQLLVSFAKTLAEGVPSRFSGSGSGTQFSLKISSLQPEDSGSYFCQHHS
DNPWTFGGGTELEIKGGGGSGGGGSGGGGSSAVQLQQSGPELEKPGASVKISCKA
SGYSFTGYNMNWVKQNNGKSLEWIGNIDPYYGGTTYNRKFKGKATLTVDKSSST
AYMQLKSLTSEDSAVYYCARSVGPMDYWGQGTSVTVSSDQPVPSTPPTPSPSTPPT
PSPSCAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDG
VEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTI
SKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNY

KTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG
K

51 G28-1 scFv VHL11S (SSS-S)H WCH2 WCH3

Nucleotide sequence

aagcttgccgccatggtatccacagctcagttccttgggttgctgctgctgtggcttacaggtggcagatgtgacatccagatgactc
agtctccagccteccttatctgcatctgtgggagagactgtcaccatcacatgtcgaacaagtgaaaatgtttacagttatttggcttggt
atcagcagaaacagggaaaatctcctcagctcctggtctctttgcaaaaaccttagcagaaggtgtgccatcaaggttcagtggca
gtggatcaggcacacagttttctctgaagatcagcagcctgcagcctgaagattctggaagttatttctgtcaacatcattccgatgat
ccgtggacgttcggtggaggcaccgaactggagatcaaaggtggcggtggctcgggcggtggtgggtcgggtggcggcggat
cgtcagcggtccagctgcagcagtctggacctgagtcggaaaagcctggcgcttcagtgaagatttcctgcaaggcttctggttact
cattcactggctacaatatgaactgggtgaagcagaatagtggaaagagccttgagtggattggaaatattgatccttattatggtggt
actacctacaaccggaagttcaagggcaaggccacattgactgtagacaaatcctccagcacagcctacatgcagctcagagtct
gacatctgaggactctgcagtctattactgtgcaagatcggtcggccctatggactactggggtcaaggaacctcagtcaccgtctc
ttctgatcaggagcccaaatcttctgacaaaactcacacatcccccaccgtcctcagcacctgaactcctgggggaccgtcagtctt
cctcttccccccaaaacccaaggacaccctcatgatctcccggaccccctgaggtcacatgcgtggtggtggacgtgagccacgaa
gaccctgaggtcaagttcaactggtacgtggacggcgtggaggtgcataatgccaagacaaagccgcgcgggaggagcagtacaa
cagcacgtaccgtgtggtcagcgtcctcaccgtcctgcaccaggactggctgaatggcaaggagtacaagtgcaaggtctccaac
aaagccctcccagcccccatcgagaaaacaatctccaaagccaaagggcagccccgagaaccacaggtgtacaccctgcccccc
atcccgggatgagctgaccaagaaccaggtcagcctgacctgcctggtcaaaggcttctatcccagcgacatcgccgtggagtgg
gagagcaatgggcagccggagaacaactacaagaccacgcctcccgtgctggactccgacggctccttcttcctctacagcaag
ctcaccgtggacaagagcaggtggcagcaggggaacgtcttctcatgctccgtgatgcatgaggctctgcacaaccactacacgc
agaagagcctctccctgtctccgggtaaatgatctaga

Amino acid sequence

MVSTAQFLGLLLLWLTGGRCDIQMTQSPASLSASVGETVTITCRTSENVYSYLAW
YQQKQGKSPQLLVSFAKTLAEGVPSRFSGSGSGTQFSLKISSLQPEDSGSYFCQHHS
DNPWTFGGGTELEIKGGGGSGGGGSGGGGSSAVQLQQSGPESEKPGASVKISCKA
SGYSFTGYNMNWVKQNNGKSLEWIGNIDPYYGGTTYNRKFKGKATLTVDKSSST
AYMQLKSLTSEDSAVYYCARSVGPMDYWGQGTSVTVSSDHDQEPKSSDKTHTSP
PSSAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVE
VHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISK
AKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKT
TPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

52. G28-1 scFv VHL11S (CSS-S)H WCH2 WCH3

Nucleotide sequence.

aagcttgccgccatggtatccacagctcagttccttgggttgctgctgctgtggcttacaggtggcagatgtgacatccagatgactc
agtctccagccteccttatctgcatctgtgggagagactgtcaccatcacatgtcgaacaagtgaaaatgtttacagttatttggcttggt
atcagcagaaacagggaaaatctcctcagctcctggtctctttgcaaaaaccttagcagaaggtgtgccatcaaggttcagtggca
gtggatcaggcacacagttttctctgaagatcagcagcctgcagcctgaagattctggaagttatttctgtcaacatcattccgatgat
ccgtggacgttcggtggaggcaccgaactggagatcaaaggtggcggtggctcgggcggtggtgggtcgggtggcggcggat
cgtcagcggtccagctgcagcagtctggacctgagtcggaaaagcctggcgcttcagtgaagatttcctgcaaggcttctggttact
cattcactggctacaatatgaactgggtgaagcagaatagtggaaagagccttgagtggattggaaatattgatccttattatggtggt
actacctacaaccggaagttcaagggcaaggccacattgactgtagacaaatcctccagcacagcctacatgcagctcagagtct
gacatctgaggactctgcagtctattactgtgcaagatcggtcggccctatggactactggggtcaaggaacctcagtcaccgtctc
ttctgatcaggagcccaaatcttgtgacaaaactcacacatcccccaccgtcctcagcacctgaactcctgggggaccgtcagtctt
cctcttccccccaaaacccaaggacaccctcatgatctcccggaccccctgaggtcacatgcgtggtggtggacgtgagccacgaa
gaccctgaggtcaagttcaactggtacgtggacggcgtggaggtgcataatgccaagacaaagccgcgcgggaggagcagtacaa
cagcacgtaccgtgtggtcagcgtcctcaccgtcctgcaccaggactggctgaatggcaaggagtacaagtgcaaggtctccaac
aaagccctcccagcccccatcgagaaaacaatctccaaagccaaagggcagccccgagaaccacaggtgtacaccctgcccccc

atcccgggatgagctgaccaagaaccaggtcagcctgacctgcctggtcaaaggcttctatcccagcgacatcgccgtggagtgg
gagagcaatgggcagccggagaacaactacaagaccacgcctcccgtgctggactccgacggctccttcttcctctacagcaag
ctcaccgtggacaagagcaggtggcagcaggggaacgtcttctcatgctccgtgatgcatgaggctctgcacaaccactacacgc
agaagagcctctccctgtctccgggtaaatgatctaga

Amino acid sequence
MVSTAQFLGLLLLWLTGGRCDIQMTQSPASLSASVGETVTITCRTSENVYSYLAW
YQQKQGKSPQLLVSFAKTLAEGVPSRFSGSGSGTQFSLKISSLQPEDSGSYFCQHHS
DNPWTFGGGTELEIKGGGGSGGGGSGGGGSSAVQLQQSGPESEKPGASVKISCKA
SGYSFTGYNMNWVKQNNGKSLEWIGNIDPYYGGTTYNRKFKGKATLTVDKSSST
AYMQLKSLTSEDSAVYYCARSVGPMDYWGQGTSVTVSSDQEPKSCDKTHTSPPSS
APELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVH
NAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAK
GQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPP
VLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

53 G28-1 scFv VH L11S (CSC-S)H WCH2 WCH3
Nucleotide sequence:
aagcttgccgccatggtatccacagctcagttccttgggttgctgctgctgtggcttacaggtgccagatgtgacatccagatgactc
agtctccagcctccctatctgcatctgtgggagagactgtcaccatcacatgtcgaacaagtgaaaatgtttacagttatttggcttggt
atcagcagaaacagggaaaatctcctcagctcctggtctttttgcaaaaaccttagcagaaggtgtgccatcaaggttcagtggca
gtggatcaggcacacagttttctctgaagatcagcagcctgcagcctgaagattctgggagttattctgtcaacatcattccgataat
ccgtggacgttcggtggaggcaccgaactggagatcaaaggtggcggtggctcgggcggtggtgggtcgggtggcggcggat
cgtcagcggtccagctgcagcagtctggacctgagtcggaaaagcctggcgttcagtgaagatttcctgcaaggcttctggttact
cattcactggctacaatatgaactgggtgaagcagaataatggaaagagccttgagtggattggaaatattgatccttattatggtggt
actacctacaaccggaagttcaagggcaaggccacattgactgtagacaaatcctccagcacagcctacatgcagctcaagagtct
gacatctgaggactctgcagtctattactgtgcaagtcgggtcggccctatggactactggggtcaaggaacctcagtcaccgtctc
ttctgatcaggagcccaaatcttgtgacaaaactcacacatctccaccgtgtcagcacctgaactcctgggtggaccgtcagtcttc
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agcacgtaccgtgtggtcagcgtcctcaccgtcctgcaccaggactggctgaatggcaaggagtacaagtgcaaggtctccaaca
aagccctcccagcccccatcgagaaaacaatctccaaagccaaagggcagccccgagaaccacaggtgtacaccctgcccccca
tcccgggatgagctgaccaagaaccaggtcagcctgacctgcctggtcaaaggcttctatccaagcgacatcgccgtggagtgg
gagagcaatgggcagccggagaacaactacaagaccacgcctcccgtgctggactccgacggctccttcttcctctacagcaag
ctcaccgtggacaagagcaggtggcagcaggggaacgtcttctcatgctccgtgatgcatgaggctctgcacaaccactacacgc
agaagagcctctccctgtctccgggtaaatgatctaga

Amino acid sequence
MVSTAQFLGLLLLWLTGGRCDIQMTQSPASLSASVGETVTITCRTSENVYSYLAW
YQQKQGKSPQLLVSFAKTLAEGVPSRFSGSGSGTQFSLKISSLQPEDSGSYFCQHHS
DNPWTFGGGTELEIKGGGGSGGGGSGGGGSSAVQLQQSGPESEKPGASVKISCKA
SGYSFTGYNMNWVKQNNGKSLEWIGNIDPYYGGTTYNRKFKGKATLTVDKSSST
AYMQLKSLTSEDSAVYYCARSVGPMDYWGQGTSVTVSSDQEPKSCDKTHTSPPC
SAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEV
HNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKA
KGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTP
PVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

54 G28-1 scFv VH L11S (SSC-F)H WCH2 WCH3
Nucleotide sequence.

aagcttgccgccatggatccacagctcagttccttgggttgctgctgctgtggcttacaggtggcagatgtgacatccagatgactc
agtctccagccteeetatctgcatctgtgggagagactgtcaccatcacatgtcgaacaagtgaaaatgtttacagttatttggcttggt
atcagcagaaacagggaaatctcctcagctcctggtctctttgcaaaaaccttagcagaaggtgtgccatcaaggttcagtggca
gtggatcaggcacacagttttctctgaagatcagcagcctgcagcctgaagattctggaagttatttctgtcaacatcattccgatnat
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cgtcagcggtccagctgcagcagtctggacctgagtcggaaaagcctggcgcttcagtgaagatttcctgcaaggcttctggttact
cattcactggctacaatatgaactgggtgaagcagaatnatggaaagagccttgagtggattggaaatattgatccttattatggtggt
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cagcacgtaccgtgtggtcagcgtcctcaccgtcctgcaccaggactggctgaatggcaaggagtacaagtgcaaggtctccaac
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gagagcaatgggcagccggagaacaactacaagaccacgcctcccgtgctggactccgacggctccttcttcctctacagcaag
ctcaccgtggacaagagcaggtggcagcaggggaacgtcttctcatgctccgtgatgcatgaggctctgcacaaccactacacgc
agaagagcctctccctgtctccgggtaaatgatctaga

Amino acid sequence:
MVSTAQFLGLLLLWLTGGRCDIQMTQSPASLSASVGETVTITCRTSENVYSYLAW
YQQKQGKSPQLLVSFAKTLAEGVPSRFSGSGSGTQFSLKISSLQPEDSGSYFCQHHS
DNPWTFGGGTELEIKGGGGSGGGGSGGGGSSAVQLQQSGPELVKPGASVKISCKA
SGYSFTGYNMNWVKQNNGKSLEWIGNIDPYYGGTTYNRKFKGKATLTVDKSSST
AYMQLKSLTSEDSAVYYCARSVGPMDYWGQGTSVTVSSDQEPKSSDKTHTSPPCP
APELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVH
NAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAK
GQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPP
VLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

II. 54. HCTLA4 HIGG1 (SSS-S)H P238SCH2 WCH3

Nucleotide sequence:
atggcttgcctggatttcagcggcacaaggctcagctgaacctggctgccaggacctggccctgcactctcctgttttttcttctcttc
atccctgtcttctgcaaagcaatgcacgtggcccagcctgtgtggtactggccagcagccgaggcatcgccagcttttgtgtgtga
gtatgcatctccaggcaaagccactgaggtccgggtgacagtgcttcggcaggctgacagccaggtgactgaagtctgtgcggc
aacctacatgacggggaatgagttgaccttcctagatgattccatctgcacgggcacctccagtggaaatcaagtgaacctcactat
ccaaggactgaggggccatggacacgggactctacatctgcaaggtggagctcatgtacccaccgccatactacctgggcatagg
caacggaacccagatttatgtaattgatccagaccgtgcccagattctgatcaacccaaatcttctgacaaaactcacacatcccca
ccgtcctcagcacctgaactcctgggggggatcgtcagtcttcctcttccccccaaaacccaaggacaccctcatgatctcccggac
ccctgaggtcacatgcgtggtggtggacgtgagccacgaagaccctgaggtcaagttcaactggtacgtggacggcgtggaggt
gcataatgccaagacaaagccgcgggaggagcagtacaacagcacgtaccgtgtggtcagcgtcctcaccgtcctgcaccagg
actggctgaatggcaaggagtacaagtgcaaggtctccaacaaagccctcccagcccccatcgagaaaacaatctccaaagcca
aagggcagccccgagaaccacaggtgtacaccctgcccccatcccgggatgagctgaccaagaaccaggtcagcctgacctgc
ctggtcaaaggcttctatcccagcgacatcgccgtggagtgggagagcaatgggcagccggagaacaactacaagaccacgcc
tcccgtgctggactccgacggctccttcttcctctacagcaagctcaccgtggacaagagcaggtggcagcaggggaacgtcttct
catgctccgtgatgcatgaggctctgcacaaccactacacgcagaagagcctctccctgtctccgggtaaatga

Amino acid sequence:
MACLGFQRHKAQLNLAARTWPCTLLFFLLFIPVFCKAMHVAQPAVVLASSRGIAS
FVCEYASPGKATEVRVTVLRQADSQVTEVCAATYMTGNELTFLDDSICTGTSSGN

QVNLTIQGLRAMDTGLYICKVELMYPPPYYLGIGNGTQIYVIDPEPCPDSDQPKSSD
KTHTSPPSSAPELLGGSSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWY
VDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPI
EKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPE
NNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSL
SPGK

55. Fc2-2 VL

Nucleotide sequence

gttgttaagcttgccgccatggatttcacaggcccaggttcttatgttactgctgctatgggtatctggtacctgtggggacattgtgatg
tcacagtctccatcctccctagctgtgtcagttggagagaaggttctatgagctgcaagtccagtcagagccttttatataatcacaat
caaaagaactacttggcctggtaccagcagataccagggcagtctcctaaactgctgatttactgggcatccactagggaatctgg
ggtccctgatcgcttcacaggcagtggatctgggacagatttcactctcaccatcagcagagtgaaggctgaagacctggcagttta
ttactgtcagcaatattatacctatcctccacgttcggaggtggcaccaagctggaaataaaaggtggcggtggctcgggcggtg
gtgggtcgggtggcggcggatctg

Amino acid sequence

MDSQAQVLMLLLLWVSGTCGDIVMSQSPSSLAVSVGEKVSMSCKSSQSLLYNHN
QKNYLAWYQQIPGQSPKLLIYWASTRESGVPDRFTGSGSGTDFTLTISRVKAEDLA
VYYCQQYYTYPPTFGGGTKLEIKGGGGSGGGGSGGGGSS

56. FC2-2VH

Nucleotide sequence.

Gggagctcgcaggtgcagttgaaggagtcaggacctggcctggtggcgccctcacagagcctgtccatcacatgcaccgtctca
gggttctcattaccgtctatggtgttaactgggttccgccagcctccaggaaagggtctggagtggctgggagtgatatggggtgat
ggaagcacagactataattcagctctcaaatccagactgagcatcagtaaggacaactccaagagccaagttttcttaaaaatggac
agtctacaaactgatgacacagccaggtactactgtgccagagatcactatggtacccactatgctatggactactggggtcaagga
acctcagtcaccgtctcctctgatcag

Amino acid sequence.

GSSQVQLKESGPGLVAPSQSLSITCTVSGFSLTVYGVNWVRQPPGKGLDWLGMIW
GDGSTDYNSALKSRLSISKDNSKSQVFLKMDSLQTDDTARYYCARDHYGTHYAM
DYWGQGTSVTVSSDQ

57 FC2-2scFv

Nucleotide sequence.

gttgttaagcttgccgccatggatttcacaggcccaggttcttatgttactgctgctatgggtatctggtacctgtggggacattgtgatg
tcacagtctccatcctccctagctgtgtcagttggagagaaggttctatgagctgcaagtccagtcagagccttttatataatcacaat
caaaagaactacttggcctggtaccagcagataccagggcagtctcctaaactgctgatttactgggcatccactagggaatctgg
ggtccctgatcgcttcacaggcagtggatctgggacagatttcactctcaccatcagcagagtgaaggctgaagacctggcagttta
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gtgggtcgggtggcggcggatctgaggtgcagttgaaggagtcaggacctggcctggtggcgccctcacagagcctgtcc
atcacatgcaccgtctcagggttctcattaccgtctatggtgttaactgggttccgccagcctccaggaaagggtctggagtggctgg
gagtgatatggggtgatggaagcacagactataattcagctctcaaatccagactgagcatcagtaaggacaactccaagagccaa
gttttcttaaaaatggacagtctacaaactgatgacacagccaggtactactgtgccagagatcactatggtacccactatgctatgg
actactggggtcaaggaacctcagtcaccgtctcctctgatcag

Amino acid sequence

MDSQAQVLMLLLLWVSGTCGDIVMSQSPSSLAVSVGEKVSMSCKSSQSLLYNHN
QKNYLAWYQQIPGQSPKLLIYWASTRESGVPDRFTGSGSGTDFTLTISRVKAEDLA
VYYCQQYYTYPPTFGGGTKLEIKGGGGSGGGGSGGGGSSQVQLKESGPGLVAPSQ

SLSITCTVSGFSLTVYGVNWVRQPPGKGLDWLGMIWGDGSTDYNSALKSRLSISK
DNSKSQVFLKMDSLQTDDTARYYCARDHYGTHYAMDYWGQGTSVTVSSDQ

58. FC2-2 VHL11S

Nucleotide sequence

gggagctctcaggtgcagttgaaggagtcaggacctggctcggtggcgccctcacagagcctgtccatcacatgcaccgtctcag
ggttctcattaaccgtctatggtgttaactgggttcgccagcctccaggaaagggtctggactggctgggaatgatatggggtgatg
gaagcacagactataattcagctctcaaatccagactgagcatcagtaaggacaactccaagagccaagttttcttaaaaatggaca
gtctacaaactgatgacacagccaggtactactgtgccagagatcactatggtacccactatgctatggactactggggtcaaggaa
cctcagtcaccgtctcctctgatcag

Amino acid sequence

(GSS)QVQLKESGPGSVAPSQSLSITCTVSGFSLTVYGVNWVRQPPGKGLDWLGMI
WGDGSTDYNSALKSRLSISKDNSKSQVFLKMDSLQTDDTARYYCARDHYGTHYA
MDYWGQGTSVTVSSDQ

59 FC2-2 VH L11S scFv

Nucleotide sequence

gttgttaagcttgccgccatggatttcacaggcccaggttcttatgttactgctgctatgggtatctggtacctgtggggacattgtgatg
tcacagtctccatcctccctagctgtgtcagttggagagaaggtttctatgagctgcaagtccagtcagagccttttatataatcacaat
caaaagaactacttggcctggtaccagcagaaaccagggcagtctcctaaactgctgatttactgggcatccactagggaatctgg
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gttttcttaaaaatggacagtctacaaactgatgacacagccaggtactactgtgccagagatcactatggtacccactatgctatgg
actactggggtcaaggaacctcagtcaccgtctcctctgatcag

Amino acid sequence

MDSQAQVLMLLLLWVSGTCGDIVMSQSPSSLAVSVGEKVSMSCKSSQSLLYNHN
QKNYLAWYQQIPGQSPKLLIYWASTRESGVPDRFTGSGSGTDFTLTISRVKAEDLA
VYYCQQYYTYPPTFGGGTKLEIKGGGGSGGGGSGGGGSSQVQLKESGPGSVAPSQ
SLSITCTVSGFSLTVYGVNWVRQPPGKGLDWLGMIWGDGSTDYNSALKSRLSISK
DNSKSQVFLKMDSLQTDDTARYYCARDHYGTHYAMDYWGQGTSVTVSSDQ

60. FC2-2 (SSS-S)H WCH2 WCH3

Nucleotide sequence

gttgttaagcttgccgccatggatttcacaggcccaggttcttatgttactgctgctatgggtatctggtacctgtggggacattgtgatg
tcacagtctccatcctccctagctgtgtcagttggagagaaggtttctatgagctgcaagtccagtcagagccttttatataatcacaat
caaaagaactacttggcctggtaccagcagaaaccagggcagtctcctaaactgctgatttactgggcatccactagggaatctgg
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gttttcttaaaaatggacagtctacaaactgatgacacagccaggtactactgtgccagagatcactatggtacccactatgctatgg
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cagcacctgaactcctgggtggaccgtcagtcttcctcttccccccaaaacccaaggacaccctcatgatctcccggacccctgag
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gaatggcaaggagtacaagtgcaaggtctccaacaaagccctcccagccccatcgagaaaaccatctccaaagccaaagggc
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aaggcttctatccaagcgacatcgccgtggagtgggagagcaatgggcagccggagaacaactacaagaccacgcctcccgtg
ctggactccgacggctccttcttcctctacagcaagctcaccgtggacaagagcaggtggcagcaggggaacgtcttctcatgctc
cgtgatgcatgaggctctgcacaaccactacacgcagaagagcctctccctgtctccgggtaaatgatctaga

Amino acid sequence
MDSQAQVLMLLLLWVSGTCGDIVMSQSPSSLAVSVGEKVSMSCKSSQSLLYNHN
QKNYLAWYQQIPGQSPKLLIYWASTRESGVPDRFTGSGSGTDFTLTISRVKAEDLA
VYYCQQYYTYPPTFGGGTKLEIKGGGGSGGGGSGGGGSSQVQLKESGPGLVAPSQ
SLSITCTVSGFSLTVYGVNWVRQPPGKGLDWLGMIWGDGSTDYNSALKSRLSISK
DNSKSQVFLKMDSLQTDDTARYYCARDHYGTHYAMDYWGQGTSVTVSSDQEPK
SSDKTHTSPPSSAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKF
NWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKA
LPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESN
GQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQ
KSLSLSPGK

61 FC2-2 VHL11S (SSS-S)H WCH2 WCH3
Nucleotide sequence
gttgttaagcttgccgccatggattcacaggcccaggttcttatgttactgctgctatgggtatctggtacctgtggggacattgtgatg
tcacagtctccatcctccctagctgtgtcagttggagagaaggtttctatgagctgcaagtccagtcagagccttttatatnatcacaat
caaaagaactacttggcctggtaccagcagaaaccagggcagtctcctaaactgctgatttactgggcatccactagggaatctgg
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cagcacctgaactcctgggtggaccgtcagtcttcctcttccccccaaaacccaaggacacctcatgatctcccggacccctgag
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gccaagacaaagccgcgggaggagcagtacaacagcacgtaccgtgtggtcagcgtcctcaccgtcctgcaccaggactggct
gaatggcaaggagtacaagtgcaaggtctccaacaaagccctcccagccccatcgagaaaaccatctccaaagccaaagggc
agccccgagaaccacaggtgtacaccctgccccatcccgggatgagctgaccaagaaccaggtcagcctgacctgcctggtca
aaggcttctatccaagcgacatcgccgtggagtgggagagcaatgggcagccggagaacaactacaagaccacgcctcccgtg
ctggactccgacggctccttcttcctctacagcaagctcaccgtggacaagagcaggtggcagcaggggaacgtcttctcatgctc
cgtgatgcatgaggctctgcacaaccactacacgcagaagagcctctccctgtctccgggtaaatgatctaga

Amino acid sequence
MDSQAQVLMLLLLWVSGTCGDIVMSQSPSSLAVSVGEKVSMSCKSSQSLLYNHN
QKNYLAWYQQIPGQSPKLLIYWASTRESGVPDRFTGSGSGTDFTLTISRVKAEDLA
VYYCQQYYTYPPTFGGGTKLEIKGGGGSGGGGSGGGGSSQVQLKESGPGSVAPSQ
SLSITCTVSGFSLTVYGVNWVRQPPGKGLDWLGMIWGDGSTDYNSALKSRLSISK
DNSKSQVFLKMDSLQTDDTARYYCARDHYGTHYAMDYWGQGTSVTVSSDQEPK
SSDKTHTSPPSSAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKF
NWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKA
LPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESN
GQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQ
KSLSLSPGK

62 UCHL-1 VH

Nucleotide sequence:

atgggcaggcttacttcttcattcctgctactgattgttcctgcatatgtcctctcccagattactctgaaagagtctggccctgggatctt
gcagcctcccagaccctcagtctgacttgttcttctctgggttttcactgaccacttatggtataggagtaggttggattcgtcagcct
ccagggaagggtctggagtggctgacacacattggtgggatgataataagtactataacacagccctgaggagccggctcacaa
tctccaaggattcctccaacaaccaagtactcctcaagatcgccaatgtggacactgcagataccgccacatactactgtctctacg
gctacacttactggggccaagggactctggtcactgtctctgca

Amino acid sequence

MGRLTSSFLLLIVPAYVLSQITLKESGPGILQPSQTLSLTCSFSGFSLTTYGIGVGWIR
QPPGKGLEWLTHIWWNDNKYYNTALRSRLTISKDSSNNQVLLKIANVDTADTAT
YYCLYGYTYWGQGTLVTVSA

63. UCHL-1 VL

Nucleotide sequence

atgaagttgcctgttaggctgttggtgctgatgttctggattcctgcttccatcagtgatgttgtgatgacccaaactccactctccctgc
ctgtcagtcttggagatcaggcctccatctcttgcagatctagtcagagccttcttacagtaatggaaacacctatttacattggtacct
gcagaagccaggccagtctccaaaactcctgatctacaaactttccaaccgattttctggggtcccagacaggttcagtggcagtgg
atcagggacagatttcacactcaagatcagcagagtggaggctgaggatctgggagtttatttctgctctcaaagtacacatgttccg
tggacgttcggtggaggcaccaagctggaaatcaaa

Amino acid sequence

MKLPVRLLVLMFWIPASISDVVMTQTPLSLPVSLGDQASISCRSSQSLLYSNGNTYL
HWYLQKPGQSPKLLIYKLSNRFSGVPDRFSGSGSGTDFTLKISRVEAEDLGVYFCS
QSTHVPWTFGGGTKLEIK

64. UCHL-1 scFv

Nucleotide sequence

gttgttaagcttgccgccatgaagttgcctgttaggctgttggtgctgatgttctggattcctgcttccatcagtgatgttgtgatgaccc
aaactccactctccctgcctgtcagtcttggagatcaggcctccatctcttgcagatctagtcagagccttcttacagtaatggaaac
acctatttacattggtacctgcagaagccaggccagtctccaaaactcctgatctacaaactttccaaccgattttctggggtcccaga
caggttcagtggcagtggatcagggacagatttcacactcaagatcagcagagtggaggctgaggatctgggagtttatttctgctc
tcaaagtacacatgttccgtggacgttcggtggaggcaccaagctggaaatcaaagatggcggtggctcgggcggtggtggatct
ggaggaggtgggagctctcagattactctgaaagagtctggccctgggatcttgcagccctccagaccctcagtctgacttgttctt
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ttggtggaatgataataagtactataacacagccctgaggagccggctcacaatctccaaggattcctccaacaaccaagtactcct
caagatcgccaatgtggacactgcagataccgccacatactactgtctctacggctacacttactggggccaagggactctggtca
ctgtctctgctgatca

Amino acid sequence

MKLPVRLLVLMFWIPASISDVVMTQTPLSLPVSLGDQASISCRSSQSLLYSNGNTYL
HWYLQKPGQSPKLLIYKLSNRFSGVPDRFSGSGSGTDFTLKISRVEAEDLGVYFCS
QSTHVPWTFGGGTKLEIKDGGGSGGGGSGGGGSSQITLKESGPGILQPSQTLSLTCS
FSGFSLTTYGIGVGWIRQPPGKGLEWLTHIWWNDNKYYNTALRSRLTISKDSSNN
QVLLKIANVDTADTATYYCLYGYTYWGQGTLVTVSAD

65 UCHL-1 VH I118L128

Nucleotide sequence

gggagctctcagattactctgaaagagtctggccctgggatcttgcagccctccagaccctcagtctgacttgttctttctctgggttt
tcactgaccacttatggtataggagtaggttggattcgtcagcctccagggaagggtctggagtggctgacacacatttggtggaat

gatcatcagtactatcacacagccctgaggagccggctcacaatctccaaggattcctccaacaaccaagtactcctcaagatcgc
caatgtggacactgcagatcccgccacatactactgtctctacggctacacttactggggccaagggactctggtcactgtctctgct
gatca

Amino acid sequence:
(GSS)QITLKESGPGSSQPSQTLSLTCSFSGFSLTTYGIGVGWIRQPPGKGLEWLTHIW
WNDNKYYNTALRSRLTISKDSSNNQVLLKIANVDTADTATYYCLYGYTYWGQGT
LVTVSAD

66. UCHL-1 scFv VH L11S
Nucleotide sequence
gttgttaagcttgccgccatgaagttgcctgttaggctgttggtgctgatgttctggattcctgcttccatcagtgatgttgtgatgaccc
aaactccactctccctgcctgtcagtcttggagatcaggcctccatctcttgcagatctagtcagagccttctttacagtaatggaaac
acctatttacattggtacctgcagaagccaggccagtctccaaaactcctgatctacaaacttccaaccgatttttctggggtcccaga
caggttcagtggcagtggatcagggacagatttcacactcaagatcagcagagtggaggctgaggatctgggagtttatttctgctc
tcaaagtacacatgttccgtggacgttcggtggaggcaccaagctggaaatcaaagatggcggtggctcgggcggtggtgggatct
ggaggaggtgggagctctcagattactctgaaagagtctggccctgggagctccagccctccagaccctcagtctgacttgttc
tttctctgggtttcactgaccacttatggtataggagtaggttggattcgtcagcctccagggaagggtctggagtggctgacacac
attggtggaatgataataagtactataacacagccctgaggagccggctcacaatctccaaggattcctccaacaaccaagtactc
ctcaagatcgccaatgtggacactgcagataccgccacatactactgtctctacggctacacttactggggccaagggactctggtc
actgtctctgctgatca

Amino acid sequence:
MKLPVRLLVLMFWIPASISDVVMTQTPLSLPVSLGDQASISCRSSQSLLYSNGNTYL
HWYLQKPGQSPKLLIYKLSNRFSGVPDRFSGSGSGTDFTLKISRVEAEDLGVYFCS
QSTHVPWTFGGGTKLEIKDGGGSGGGGSGGGGSSQITLKESGPGSSQPSQTLSLTC
SFSGFSLTTYGIGVGWIRQPPGKGLEWLTHIWWNDNKYYNTALRSRLTISKDSSNN
QVLLKIANVDTADTATYYCLYGYTYWGQGTLVTVSAD

67 UCHL-1 scFv (SSS-S)H WCH2 WCH3
Nucleotide sequence:
gttgttaagcttgccgccatgaagttgcctgttaggctgttggtgctgatgttctggattcctgcttccatcagtgatgttgtgatgaccc
aaactccactctccctgcctgtcagtcttggagatcaggcctccatctcttgcagatctagtcagagccttctttacagtaatggaaac
acctatttacattggtacctgcagaagccaggccagtctccaaaactcctgatctacaaacttccaaccgatttttctggggtcccaga
caggttcagtggcagtggatcagggacagatttcacactcaagatcagcagagtggaggctgaggatctgggagtttatttctgctc
tcaaagtacacatgttccgtggacgttcggtggaggcaccaagctggaaatcaaagatggcggtggctcgggcggtggtgggatct
ggaggaggtgggagctctcagattactctgaaagagtctggccctgggagcttgcagccctccagaccctcagtctgacttgttctt
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cagtcttcctcttccccccaaaacccaaggacaccctcatgatctcccggacccctgaggtcacatgcgtggtggtggacgtgagc
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acacgcagaagagcctctccctgtctccgggtaaatgatctaga

Amino acid sequence
MKLPVRLLVLMFWIPASISDVVMTQTPLSLPVSLGDQASISCRSSQSLLYSNGNTYL
HWYLQKPGQSPKLLIYKLSNRFSGVPDRFSGSGSGTDFTLKISRVEAEDLGVYFCS
QSTHVPWTFGGGTKLEIKDGGGSGGGGSGGGGSSQITLKESGPGILQPSQTLSLTCS
FSGFSLTTYGIGVGWIRQPPGKGLEWLTHIWWNDNKYYNTALRSRLTISKDSSNN
QVLLKIANVDTADTATYYCLYGYTYWGQGTLVTVSADQEPKSSDKTHTSPPSSAP
ELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNA
KTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQ
PREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVL
DSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

68. UCHL-1 scFv VHL11S (SSS-S)H WCH2 WCH3

Nucleotide sequence.
gtgttaagcttgccgccatgaagttgcctgttaggctgttggtgctgatgttctggattcctgcttccatcagtgatgttgtgatgaccc
aaactccactctccctgcctgtcagtcttggagatcaggcctccatctcttgcagatctagtcagagccttcttacagtaatggaaac
acctatttacattggtacctgcagaagccaggccagtctccaaaactcctgatctacaaacttccaaccgattttctggggtcccaga
caggttcagtggcagtggatcagggacagatttcacactcaagatcagcagagtggaggctgaggatctgggagtttatttctgctc
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tgccccatcccgggatgagctgaccaagaaccaggtcagcctgacctgcctggtcaaaggcttctatccaagcgacatcgccgt
ggagtgggagagcaatgggcagccggagaacaactacaagaccacgcctcccgtgctggactccgacggctccttcttcctcta
cagcaagctcaccgtggacaagagcaggtggcagcaggggaacgtcttctcatgctccgtgatgcatgaggctctgcacaacca
ctacacgcagaagagcctctccctgtctccgggtaaatgatctagaa

Amino acid sequence
MKLPVRLLVLMFWIPASISDVVMTQTPLSLPVSLGDQASISCRSSQSLLYSNGNTYL
HWYLQKPGQSPKLLIYKLSNRFSGVPDRFSGSGSGTDFTLKISRVEAEDLGVYFCS
QSTHVPWTFGGGTKLEIKDGGGSGGGGSGGGGSSQITLKESGPGSSQPSQTLSLTC
SFSGFSLTTYGIGVGWIRQPPGKGLEWLTHIWWNDNKYYNTALRSRLTISKDSSNN
QVLLKIANVDTADTATYYCLYGYTYWGQGTLVTVSADQEPKSSDKTHTSPPSSAP
ELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNA
KTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQ
PREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVL
DSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

69 5B9 VH L11S

Nucleotide sequence
gggagctctcaggtgcagctgaagcagtcaggacctggctcagtgcagtcctcacagagcctgtccatcacctgcacagtctctg
gtttctcattaactacctatgctgtacactgggttcgccagtctccaggaaagggtctggagtggctgggagtgatatggagtggtgg
aatcacagactataatgcagcttttatatccagactgagcatcaccaaggacgattccaagagccaagttttcttaaaatgaacagtc

tgcaacctaatgacacagccatttattactgtgccagaaatgggggtgataactacccttattactatgctatggactactggggtcaa
ggaacctcagtcaccgtctcctcag

Amino acid sequence:

(GSS)QVQLKQSGPGSVQSSQSLSITCTVSGFSLTTYAVHWVRQSPGKGLEWLGVI
WSGGITDYNAAFISRLSITKDDSKSQVFFKMNSLQPNDTAIYYCARNGGDNYPYY
YAMDYWGQGTSVTVSS

73. 5B9 VH L11S scFv

Nucleotide sequence

aagcttgccgccatgaggttctctgctcagcttctggggctgcttgtgctctggatccctggatccactgcagatattgtgatgacgca
ggctgcattctccaatccagtcactcttggaacatcagcttccatctcctgcaggtctagtaagagtctcctacatagtaatggcatca
cttatttgtattggtatctgcagaagccaggccagtctcctcagctcctgatttatcagatgtccaaccttgcctcaggagtcccagaca
ggttcagtagcagtgggtcaggaactgatttcacactgagaatcagcagagtggaggctgaggatgtgggtgtttattactgtgctc
aaaatctagaacttccgctcacgttcggtgctgggaccaagctggagctgaaacgggtggcggtggctcgggcggtggtgggt
cgggtggcggcgggagctctcaggtgcagctgaagcagtcaggacctggctcagtgcagtcctcacagagcctgtccatcacct
gcacagtctctggtttctcattaactacctatgctgtacactgggttccgccagtctccaggaaagggtctggagtggctgggagtgat
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aaaatgaacagtctgcaacctaatgacacagccatttattactgtgccagaaatgggggtgataactacccttattactatgctatgga
ctactggggtcaaggaacctcagtcaccgtctcctcag

Amino acid sequence

MRFSAQLLGLLVLWIPGSTADIVMTQAAFSNPVTLGTSASISCRSSKSLLHSNGITY
LYWYLQKPGQSPQLLIYQMSNLASGVPDRFSSSGSGTDFTLRISRVEAEDVGVYYC
AQNLELPLTFGAGTKLELKRGGGGSGGGGSGGGGSSQVQLKQSGPGSVQSSQSLSI
TCTVSGFSLTTYAVHWVRQSPGKGLEWLGVIWSGGITDYNAAFISRLSITKDDSKS
QVFFKMNSLQPNDTAIYYCARNGGDNYPYYYAMDYWGQGTSVTVSS

70. 5B9 scFv VHL11S (SSS-S)H WCH2 WCH3

Nucleotide sequence

aagcttgccgccatgaggttctctgctcagcttctggggctgcttgtgctctggatccctggatccactgcagatattgtgatgacgca
ggctgcattctccaatccagtcactcttggaacatcagcttccatctcctgcaggtctagtaagagtctcctacatagtaatggcatca
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ggttcagtagcagtgggtcaggaactgatttcacactgagaatcagcagagtggaggctgaggatgtgggtgtttattactgtgctc
aaaatctagaacttccgctcacgttcggtgctgggaccaagctggagctgaaacgggtggcggtggctcgggcggtggtgggt
cgggtggcggcgggagctctcaggtgcagctgaagcagtcaggacctggctcagtgcagtcctcacagagcctgtccatcacct
gcacagtctctggtttctcattaactacctatgctgtacactgggttccgccagtctccaggaaagggtctggagtggctgggagtgat
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ctactggggtcaaggaacctcagtcaccgtctcctctgatcaggagcccaaatcttctgacaaaactcacacatcccccaccgtcctc
agcacctgaactcctgggtggaccgtcagtcttcctcttccccccaaaacccaaggacaccctcatgatctcccggacccctgagg
tcacatgcgtggtggtggacgtgagccacgaagaccctgaggtcaagttcaactggtacgtggacggcgtggaggtgcataatgc
caagacaaagccgcgggaggagcagtacaacagcacgtaccgtgtggtcagcgtcctcaccgtcctgcaccaggactggctga
atggcaaggagtacaagtgcaaggtctccaacaaagccctcccagcccccatcgagaaaaccatctccaaagccaaagggcag
cccgagaaccacaggtgtacaccctgcccccatcccgggatgagctgaccaagaaccaggtcagcctgacctgcctggtcaaa
ggcttctatccaagcgacatcgccgtggagtgggagagcaatgggcagccggagaacaactacaagaccacgcctcccgtgct
ggactccgacggctccttcttcctctacagcaagctcaccgtggacaagagcaggtggcagcaggggaacgtcttctcatgctcc
gtgatgcatgaggctctgcacaaccactacacgcagaagagcctctccctgtctccgggtaaatgatctagag

Amino acid sequence

MRFSAQLLGLLVLWIPGSTADIVMTQAAFSNPVTLGTSASISCRSSKSLLHSNGITY
LYWYLQKPGQSPQLLIYQMSNLASGVPDRFSSSGSGTDFTLRISRVEAEDVGVYYC
AQNLELPLTFGAGTKLELKRGGGGSGGGGSGGGGSSQVQLKQSGPGSVQSSQSLSI
TCTVSGFSLTTYAVHWVRQSPGKGLEWLGVIWSGGITDYNAAFISRLSITKDDSKS
QVFFKMNSLQPNDTAIYYCARNGGDNYPYYYAMDYWGQGTSVTVSSDQEPKSS
DKTHTSPPSSAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNW
YVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPA
PIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQP
ENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLS
LSPGK

76. 2H7 scFv VH L11S (SSS-S)H P238SCH2 WCH3

Nucleotide sequence.

aagcttgccgccatggattttcaagtgcagattttcagcttcctgctaatcagtgcttcagtcataattgccagaggacaaattgttctct
cccagtctccagcaatcctgtctgcatctccaggggagaaggtcacaatgacttgcagggccagctcaagtgtaagttacatgcact
ggtaccagcagaagccaggatcctcccccaaaccctggatttatgccccatccaacctggcttctggagtcccctgctcgcttcagtg
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agcgacatcgccgtggagtgggagagcaatgggcagccggagaacaactacaagaccacgcctcccgtgctggactccgacg
gctccttcttcctctacagcaagctcaccgtggacaagagcaggtggcagcaggggaacgtcttctcatgctccgtgatgcatgag
gctctgcacaaccactacacgcagaagagcctctccctgtctccgggtaaatgatctaga

Amino acid sequence

MDFQVQIFSFLLISASVIIARGQIVLSQSPAILSASPGEKVTMTCRASSSVSYMHWY
QQKPGSSPKPWIYAPSNLASGVPARFSGSGSGTSYSLTISRVEAEDAATYYCQQWS
FNPPTFGAGTKLELKDGGGSGGGGSGGGGSSQAYLQQSGAESVRPGASVKMSCK
ASGYTFTSYNMHWVKQTPRQGLEWIGAIYPGNGDTSYNQKFKGKATLTVDKSSS
TAYMQLSSLTSEDSAVYFCARVVYYSNSYWYFDVWGTGTTVTVSSDQEPKSSDK
THTSPPSSAPELLGGSSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYV
DGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIE
KTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPEN
NYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLS
PGK

78. 2H7 scFv VH L11S (SSS-S)H WCH2 WCH3

Nucleotide sequence

aagcttgccgccatggattttcaagtgcagattttcagcttcctgctaatcagtgcttcagtcataattgccagaggacaaattgttctct
cccagtctccagcaatcctgtctgcatctccaggggagaaggtcacaatgacttgcagggccagctcaagtgtaagttacatgcact

ggtaccagcagaagccaggatcctctccccaaaccctggatttatgccccatccaacctggcttctggagtccctgctcgcttcagtg
gcagtgggtctgggacctcttactctctcacaatcagcagagtggaggctgaagatgctgccactiattactgccagcagtggagtit
taacccacccacgttcggtgctgggaccaagctggagctgaaagatggcggtggctcgggcggtggtggatctggaggaggtg
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Amino acid sequence
MDFQVQIFSFLLISASVIIARGQIVLSQSPAILSASPGEKVTMTCRASSSVSYMHWY
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FNPPTFGAGTKLELKDGGGSGGGGSGGGGSSQAYLQQSGAESVRPGASVKMSCK
ASGYTFTSYNMHWVKQTPRQGLEWIGAIYPGNGDTSYNQKFKGKATLTVDKSSS
TAYMQLSSLTSEDSAVYFCARVVYYSNSYWYFDVWGTGTTVTVSSDQEPKSSDK
THTSPPSSAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYV
DGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIE
KTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPEN
NYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLS
PGK

79 2H7 scFv VH L11S (CSS-S)H WCH2 WCH3
Nucleotide sequence:
aagcttgccgccatggattttcaagtgcagattttcagcttcctgctaatcagtgtttcagtcataattgtccagaggacaaattgttctct
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Amino acid sequence.
MDFQVQIFSFLLISASVIIARGQIVLSQSPAILSASPGEKVTMTCRASSSVSYMHWY
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DGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIE
KTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPEN
NYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLS
PGK

77 G8-1 scFv VHL11S (SCS-S)H WCH2 WCH3
Nucleotide sequence:
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Amino acid sequence:
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DNPWTFGGGTELEIKGGGGSGGGGSGGGGSSAVQLQQSGPESEKPGASVKISCKA
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APELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVH
NAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAK
GQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPP
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79.. G28-1 scFv VHL11S (CCS-P)H WCH2 WCH3
Nucleotide sequence
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406

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Amino acid sequence
MVSTAQFLGLLLLWLTGGRCDIQMTQSPASLSASVGETVTITCRTSENVYSYLAW
YQQKQGKSPQLLVSFAKTLAEGVPSRFSGSGSGTQFSLKISSLQPEDSGSYFCQHHS
DNPWTFGGGTELEIKGGGGSGGGGSGGGGSSAVQLQQSGPESEKPGASVKISCKA
SGYSFTGYNMNWVKQNNGKSLEWIGNIDPYYGGTTYNRKFKGKATLTVDKSSST
AYMQLKSLTSEDSAVYYCARSVGPMDYWGQGTSVTVSSDQEPKSCDKTHTCPPS
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KGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTP
PVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

80 G28-1 scFv VH L11S (SCC-P)H WCH2 WCH3

Nucleotide sequence

gttgttaagcttgccgccatggtatccacagtcagttcctgggtgctgctgctgtggcttacaggtggcagatgtgacatccagat
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Amino acid sequence.
MVSTAQFLGLLLLWLTGGRCDIQMTQSPASLSASVGETVTITCRTSENVYSYLAW
YQQKQGKSPQLLVSFAKTLAEGVPSRFSGSGSGTQFSLKISSLQPEDSGSYFCQHHS
DNPWTFGGGTELEIKGGGGSGGGGSGGGGSSAVQLQQSGPESEKPGASVKISCKA
SGYSFTGYNMNWVKQNNGKSLEWIGNIDPYYGGTTYNRKFKGKATLTVDKSSST

AYMQLKSLTSEDSAVYYCARSVGPMDYWGQGTSVTVSSDQEPKSSDKTHTCPPC
PAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEV
HNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKA
KGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTP
PVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

81 G28-1 scFv VH L11S mIgE CH2 CH3 CH4

Nucleotide sequence.

aagcttgccgccatggtatccacagctcagttccttgggttgctgctgctgtggcttacaggtggcagatgtgacatccagatgactc
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Amino acid sequence

MVSTAQFLGLLLLWLTGGRCDIQMTQSPASLSASVGETVTITCRTSENVYSYLAW
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DNPWTFGGGTELEIKGGGGSGGGGSGGGGSSAVQLQQSGPESEKPGASVKISCKA
SGYSFTGYNMNWVKQNNGKSLEWIGNIDPYYGGTTYNRKFKGKATLTVDKSSST
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AKDWIEGYGYQCIVDHPDFPKPIVRSITKTPGQRSAPEVYVFPPPEEEKSEDKRTLTC
LIQNFFPEDISVQWLGDGKLISNSQHSTTTPLKSNGSNQGFFIFSRLEVAKTLWTQR
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82 G28-1 scFv VH L11S mIgA WH WCH2 T4CH3

Nucleotide sequence

aagcttgccgccatggtatccacagctcagttccttgggttgctgctgctgtggcttacaggtggcagatgtgacatccagatgactc
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Amino acid sequence

MVSTAQFLGLLLLWLTGGRCDIQMTQSPASLSASVGETVTITCRTSENVYSYLAW
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DNPWTFGGGTELEIKGGGGSGGGGSGGGGSSAVQLQQSGPESEKPGASVKISCKA
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83. G28-1 scFv Vh L11S hIgE CH2 CH3 CH4

Nucleotide sequence

aagcttgccgccatggtatccacagctcagttccttgggttgctgctgtggcttacaggtggcagatgtgacatccagatgactc
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cgtcagcggtccagctgcagcagtctggacctgagtcggaaaagcctggcgttcagtgaagatttcctgcaaggcttctggttact
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tccatgaggcagcgagccctcacagaccgtccagcgagcggtgtctgtaaatcccggtaaatgataatctaga

Amino acid sequence.
MVSTAQFLGLLLLWLTGGRCDIQMTQSPASLSASVGETVTITCRTSENVYSYLAW
YQQKQGKSPQLLVSFAKTLAEGVPSRFSGSGSGTQFSLKISSLQPEDSGSYFCQHHS
DNPWTFGGGTELEIKGGGGSGGGGSGGGGSSAVQLQQSGPESEKPGASVKISCKA
SGYSFTGYNMNWVKQNNGKSLEWIGNIDPYYGGTTYNRKFKGKATLTVDKSSST
AYMQLKSLTSEDSAVYYCARSVGPMDYWGQGTSVTVSSDHVCSRDFTPPTVKIL
QSSCDGGGHFPPTIQLLCLVSGYTPGTINITWLEDGQVMDVDLSTASTTQEGELAS
TQSELTLSQKEWLSDRTYTCQVTYQGHTFEDSTKKCADSNPRGVSAYLSRPSPFD
LFIRKSPTITCLVVDLAPSKGTVNLTWSRASGKPVNHSTRKEEKQRNGTLTVTSTLP
VGTRDWIEGETYQCRVTHPHLPRALMRSTTKTSGPRAAPEVYAFATPEWPGSRDK
RTLACLIQNFMPEDISVQWLHNEVQLPDARHSTTQPRKTKGSGFFVFSRLEVTRAE
WEQKDEFICRAVHEAASPSQTVQRAVSVNPGK

84. G28-1 scFv VH L11S hIgA WH WCH2 T4CH3
Nucleotide sequence
atggtatccacagctcagttccttgggttgctgctgtggcttacaggtggcagatgtgacatccagatgactcagtctccagcct
ccctatctgcatctgtgggagagactgtcaccatcacatgtcgaacaagtgaaaatgtttacagttatttggcttggtatcagcagaaa
cagggaaaatctcctcagctcctggtctctttgcaaaaaccttagcagaggtgtgccatcaaggttcagtggcagtggatcaggc
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gcagccgaggactggaagaggggagcacctttctcctgcatggtgggccacgaggccctgccgctggccttcacacagaagac
catcgaccgcttggcgggtaaacccacccatgtcaatgtgtctgttgtcatggcggaggtggactgataatctaga

Amino acid sequence
MVSTAQFLGLLLLWLTGGRCDIQMTQSPASLSASVGETVTITCRTSENVYSYLAW
YQQKQGKSPQLLVSFAKTLAEGVPSRFSGSGSGTQFSLKISSLQPEDSGSYFCQHHS
DNPWTFGGGTELEIKGGGGSGGGGSGGGGSSAVQLQQSGPESEKPGASVKISCKA
SGYSFTGYNMNWVKQNNGKSLEWIGNIDPYYGGTTYNRKFKGKATLTVDKSSST
AYMQLKSLTSEDSAVYYCARSVGPMDYWGQGTSVTVSSDQPVPSTPPTPSPSTPPT
PSPSCCHPRLSLHRPALED

85. HD37 VL
Nucleotide sequence:
aagcttgccgccatggagacagacacactcctgctatgggtgctgctgctctgggttccaggctccactggtgacattgtgctgacc
caatctccagcttctttggctgtgtctctagggcagagggccaccatctcctgcaaggccagccaaagtgttgatttatgatggtgata
gttatttgaactggtaccaacagattccaggacagccacccaaactcctcatctatgatgcatccaatctagtttctgggatccccacc
aggtttagtggcagtgggtctgggacagacttcaccctcaacatccatcctgtggaggaggtggatgctgcaacctatcactgtcag
caaagtactgaggatccgtggacgttcggtggaggcaccaagctggaaatcaaaggtggcggtggctcgggcggtggtgggtc
gggtggcggcggatctcg

Amino acid sequence
METDTLLLWVLLLWVPGSTGDIVLTQSPASLAVSLGQRATISCKASQSVDYDGDS
YLNWYQQIPGQPPKLLIYDASNLVSGIPPRFSGSGSGTDFTLNIHPVEKVDAATYHC
QQSTEDPWTFGGGTKLEIKGGGGSGGGGSGGGGSS

86. HD37 VH
Nucleotide sequence:
gggagctcgcaggttcagctgcagcagtctggggctgagctggtgaggcctgggtcctcagtgaagatttcctgcaaggcttctg
gctatgcattcagtagctactggatgaactgggtgaagcagaggcctggacagggtcttgagtggattggacagatttggcctgga
gatggtgatactaactacaatggaaagttcaagggtaaagccactctgactgcagacgaatcctccagcacagcctacatgcaact
cagcagcctagcatctgaggactctgcggtctatttctgtgcaagacgggagactacgacggtaggccgttattactatgctatgga
ctactggggtcaaggaacctcagtcaccgtctcctctgatcag

Amino acid sequence
(GSS)QVQLQQSGAELVRPGSSVKISCKASGYAFSSYWMNWVKQRPGQGLEWIGQI
WPGDGDTNYNGKFKGKATLTADESSSTAYMQLSSLASEDSAVYFCARRETTTVG
RYYYAMDYWGQGTSVTVSSDQ

87 HD37 scFv
Nucleotide sequence:
aagcttgccgccatggaggacagacacactcctgctatgggtgctgctgctctgggttccaggctccactggtgacattgtgctgacc
caatctccagcttctttggctgtgtctctagggcagagggccaccatctcctgcaaggccagccaaagtgttgatttatgatggtgata
gttatttgaactggtaccaacagattccaggacagccacccaaactcctcatctatgatgcatccaatctagtttctgggatcccaccc
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caaagtactgaggatccgtggacgttcggtggaggcaccaagctggaaatcaaaggtggcggtggctcgggcggtggtgggtc
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aaggcttctggctatgcattcagtagctactggatgaactgggtgaagcagaggcctggacagggtcttgagtggattggacagatt
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catgcaactcagcagcctagcatctgaggactctgcggtctatttctgtgcaagacgggagactacgacggtaggccgttattacta
tgctatggactactggggtcaaggaacctcagtcaccgtctcctctgatcag

Amino acid sequence
METDTLLLWVLLLWVPGSTGDIVLTQSPASLAVSLGQRATISCKASQSVDYDGDS
YLNWYQQIPGQPPKLLIYDASNLVSGIPPRFSGSGSGTDFTLNIHPVEKVDAATYHC
QQSTEDPWTFGGGTKLEIKGGGGSGGGGSGGGGSSQVQLQQSGAELVRPGSSVKI
SCKASGYAFSSYWMNWVKQRPGQGLEWIGQIWPGDGDTNYNGKFKGKATLTAD
ESSSTAYMQLSSLASEDSAVYFCARRETTTVGRYYYAMDYWGQGTSVTVSSDQ

88. HD37 VHL11S
Nucleotide sequence
gggagctcgcaggttcagctgcagcagtctggggctgagtcggtgaggcctgggtcctcagtgaagatttcctgcaaggcttctg
gctatgcattcagtagctactggatgaactgggtgaagcagaggcctggacagggtcttgagtggattggacagatttggcctgga
gatggtgatactaactacaatggaaagttcaagggtaaagccactctgactgcagacgaatcctccagcacagcctacatgcaact
cagcagcctagcatctgaggactctgcggtctatttctgtgcaagacgggagactacgacggtaggccgttattactatgctatgga
ctactggggtcaaggaacctcagtcaccgtctcctctgatcag

Amino acid sequence:
(GSS)QVQLQQSGAESVRPGSSVKISCKASGYAFSSYWMNWVKQRPGQGLEWIGQI
WPGDGDTNYNGKFKGKATLTADESSSTAYMQLSSLASEDSAVYFCARRETTTVG
RYYYAMDYWGQGTSVTVSSDQ

89 HD37 scFv VHL11S

Nucleotide sequence

Aagcttgccgccatggagacagacacactcctgctatgggtgctgctgctctgggttccaggctccactggtgacattgtgctgac
ccaatctccagcttctttggctgtgtctctagggcagagggccaccatctcctgcaaggccagccaaagtgttgattatgatggtgat
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cgggtggcggcgggagctcgcaggttcagctgcagcagtctggggctgagtcggtgaggcctgggtcctcagtgaagatttcct
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ctacatgcaactcagcagcctagcatctgaggactctgcggtctatttctgtgcaagacgggagactacgacggtaggccgttatta
ctatgctatggactactggggtcaaggaacctcagtcaccgtctcctctgatcag

Amino acid sequence.

METDTLLLWVLLLWVPGSTGDIVLTQSPASLAVSLGQRATISCKASQSVDYDGDS
YLNWYQQIPGQPPKLLIYDASNLVSGIPPRFSGSGSGTDFTLNIHPVEKVDAATYHC
QQSTEDPWTFGGGTKLEIKGGGGSGGGGSGGGGSSQVQLQQSGAESVRPGSSVKI
SCKASGYAFSSYWMNWVKQRPGQGLEWIGQIWPGDGDTNYNGKFKGKATLTAD
ESSSTAYMQLSSLASEDSAVYFCARRETTTVGRYYYAMDYWGQGTSVTVSSDQ

90. HD37 scFv IgAH hIgG1 WCH2 T4CH3

Nucleotide sequence

agcttgccgccatggagacagacacactcctgctatgggtgctgctgctctgggttccaggctccactggtgacattgtgctgacc
caatctccagcttctttggctgtgtctctagggcagagggccaccatctcctgcaaggccagccaaagtgttgattatgatggtgata
gtttttgaactggtaccaacagattccaggacagccacccaaactcctcatctatgatgcatccaatctagtttctgggatcccacc
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gtcaatgtgtctgttgtcatggcggtggagtggactgataatctaga

Amino acid sequence

METDTLLLWVLLLWVPGSTGDIVLTQSPASLAVSLGQRATISCKASQSVDYDGDS
YLNWYQQIPGQPPKLLIYDASNLVSGIPPRFSGSGSGTDFTLNIHPVEKVDAATYHC
QQSTEDPWTFGGGTKLEIKGGGGSGGGGSGGGGSSQVQLQQSGAESVRPGSSVKI
SCKASGYAFSSYWMNWVKQRPGQGLEWIGQIWPGDGDTNYNGKFKGKATLTAD
ESSSTAYMQLSSLASEDSAVYFCARRETTTVGRYYYAMDYWGQGTSVTVSSDQP
VPSTPPTPSPSTPPTPSPSCCHPRLSLHRPALEDLLLGSEAILTCTLTGLRDASGVTFT
WTPSSGKSAVQGPPDRDLCGCYSVSSVLPGCAEPWNHGKTFTCTAAYPESKTPLT

ATLSKSGNTFRPEVHLLPPPSEELALNELVTLTCLARGFSPKDVLVRWLQGSQELP
REKYLTWASRQEPSQGTTTFAVTSILRVAAEDWKKGDTFSCMVGHEALPLAFTQK
TIDRLAGKPTHVNVSVVMAEVD

91 HD37 scFv (SSS-S)H WCH2 WCH3

Nucleotide sequence

aagcttgccgccatggaggacagacacactcctgctatgggtgctgctgctctgggttccaggctccactggtgacattgtgctgacc
caatctccagcttctttggctgtgtctctagggcagagggccaccatctcctgcaaggccagccaaagtgttgattatgatggtgata
gttatttgaactggtaccaacagattccaggacagccacccaaactcctcatctatgatgcatccaatctagtttctgggatcccaccc
aggtttagtggcagtgggtctgggacagacttcaccctcaacatccatcctgtggaggaggtggatgctgcaacctatcactgtcag
caaagtactgaggatccgtggacgttcggtggaggcaccaagctggaaatcaaaggtggcggtggctcgggcggtggtgggtc
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ctggtcaaaggcttctatcccagcgacatcgccgtggagtgggagagcaatgggcagccggagaacaactacaagaccacgcc
tcccgtgctggactccgacggctccttcttcctctacagcaagctcaccgtggacaagagcaggtggcagcaggggaacgtcttct
catgctccgtgatgcatgaggctctgcacaaccactacacgcagaagagcctctccctgtctccgggtaaatgatctaga

Amino acid sequence

METDTLLLWVLLLWVPGSTGDIVLTQSPASLAVSLGQRATISCKASQSVDYDGDS
YLNWYQQIPGQPPKLLIYDASNLVSGIPPRFSGSGSGTDFTLNIHPVEKVDAATYHC
QQSTEDPWTFGGGTKLEIKGGGGSGGGGSGGGGSSQVQLQQSGAELVRPGSSVKI
SCKASGYAFSSYWMNWVKQRPGQGLEWIGQIWPGDGDTNYNGKFKGKATLTAD
ESSSTAYMQLSSLASEDSAVYFCARRETTTVGRYYYAMDYWGQGTSVTVSSDQE
PKSSDKTHTSPPSSAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEV
KFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNK
ALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWES
NGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYT
QKSLSLSPGK

92. HD37 scFv VH L11S (SSS-S)H WCH2 WCH3

Nucleotide sequence.

aagcttgccgccatggaggacagacacactcctgctatgggtgctgctgctctgggttccaggctccactggtgacattgtgctgacc
caatctccagcttctttggctgtgtctctagggcagagggccaccatctcctgcaaggccagccaaagtgttgattatgatggtgata
gttatttgaactggtaccaacagattccaggacagccacccaaactcctcatctatgatgcatccaatctagtttctgggatcccaccc
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caaagtactgaggatccgtggacgttcggtggaggcaccaagctggaaatcaaaggtggcggtggctcgggcggtggtgggtc
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gactggctgaatggcaaggagtacaagtgcaaggtctccaacaaagccctcccagccccccatcgagaaaacaatctccaaagcc
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ctcccgtgctggactccgacggctccttcttcctctacagcaagctcaccgtggacaagagcaggtggcagcaggggaacgtctt
ctcatgctccgtgatgcatgaggctctgcacaaccactacacgcagaagagcctctccctgtctccgggtaaatgatctaga

Amino acid sequence:
METDTLLLWVLLLWVPGSTGDIVLTQSPASLAVSLGQRATISCKASQSVDYDGDS
YLNWYQQIPGQPPKLLIYDASNLVSGIPPRFSGSGSGTDFTLNIHPVEKVDAATYHC
QQSTEDPWTFGGGTKLEIKGGGGSGGGGSGGGGSSQVQLQQSGAESVRPGSSVKI
SCKASGYAFSSYWMNWVKQRPGQGLEWIGQIWPGDGDTNYNGKFKGKATLTAD
ESSSTAYMQLSSLASEDSAVYFCARRETTTVGRYYYAMDYWGQGTSVTVSSDQE
PKSSDKTHTSPPSSAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEV
KFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNK
ALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWES
NGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYT
QKSLSLSPGK

91 L6 VL
Nucleotide sequence
atggattttcaagtgcagattttcagcttcctgctaatcagtgcttcagtcataatgtccagaggacaaattgttctctcccagtctccag
caatcctgtctgcatctccaggggagaaggtcacattgacttgcagggccagctcaagtgtaagtttcatgaactggtaccagcaga
agccaggatcctcccccaaaccctggatttatgccacatccaatttggcttctgagttccctggtcgcttcagtggcgagtggtctgg
gacctcttactctctcgcaatcagcagagtggaggctgaagatgctgccactattactgccagcagtggaatagtaacccactcac
gttcggtgctgggaccaagctggagctgaaagagctctctggtggcggtggctcgggcggtggtgggtcgggtggcggcggat
ct

Amino acid sequence.
MDFQVQIFSFLLISASVIMSRGQIVLSQSPAILSASPGEKVTLTCRASSSVSFMNWYQ
QKPGSSPKPWIYATSNLASHFPGRFSGEWSGTSYSLAISRVEAEDAATYYCQQWNS
NPLTFGAGTKLELKELSGGGGSGGGGSGGGGS

92. L6 VH
Nucleotide sequence
cagatccagttggtgcagtctggacctgagctgaagaagcctggagagacagtcaagatctcctgcaaggcttctgggtataccтt
cacaaactatggaatgaactgggtgaagcaggctccaggaaagggtttaagtggatgggctggataaacacctacactggaca
gccaacatatgctgatgacttcaagggacggtttgccttctctttggaaacctctgcctacactgcctatttgcagatcaacaacctca
aaaatgaggacatggctacatatttctgtgcaagatttagctatggtaactcacgtacgctgactactggggccaaggcaccactct
cacagtctcctctgatca

Amino acid sequence
QIQLVQSGPELKKPGETVKISCKASGYTFTNYGMNWVKQAPGKGLKWMGWINTY
TGQPTYADDFKGRFAFSLETSAYTAYLQINNLKNEDMATYFCARFSYGNSRYADY
WGQGTTLTVSSD

93 L6 scFv
Nucleotide sequence.
atggattttcaagtgcagattttcagcttcctgctaatcagtgcttcagtcataatgtccagaggacaaattgttctctcccagtctccag
caatcctgtctgcatctccaggggagaaggtcacattgacttgcagggccagctcaagtgtaagtttcatgaactggtaccagcaga
agccaggatcctcccccaaaccctggatttatgccacatccaatttggcttctgagttccctggtcgcttcagtggcgagtggtctgg

gacctcttactctctgcaatcagcagagtggaggctgaagatgctgccacttattactgccagcagtggaatagtaacccactcac
gttcggtgctgggaccaagctggagctgaaagagctctctggtggcggtggctcgggcggtggtgggtcgggtggcggcggat
ctctgcagatccagttggtgcagtctggacctgagctgaagaagcctggagagacagtcaagatctcctgcaaggcttctgggtat
accttcacaaactatggaatgaactgggtgaagcaggctccaggaaagggtttaagtggatgggctggataaacacctacactg
gacagccaacatatgctgatgacttcaagggacggtttgccttctctttggaaacctctgcctacactgcctatttgcagatcaacaac
ctcaaaaatgaggacatggctacatatttctgtgcaagatttagctatggtaactcacgttacgctgactactggggccaaggcacca
ctctcacagtctcctctgatca

Amino acid sequence
MDFQVQIFSFLLISASVIMSRGQIVLSQSPAILSASPGEKVTLTCRASSSVSFMNWYQ
QKPGSSPKPWIYATSNLASEFPGRFSGEWSGTSYSLAISRVEAEDAATYYCQQWNS
NPLTFGAGTKLELKELSGGGGSGGGGSGGGGSLQIQLVQSGPELKKPGETVKISCK
ASGYTFTNYGMNWVKQAPGKGLKWMGWINTYTGQPTYADDFKGRFAFSLETSA
YTAYLQINNLKNEDMATYFCARFSYGNSRYADYWGQGTTLTVSSD

94. L6 VHL11S

Nucleotide sequence
ctgcagatccagttggtgcagtctggacctgagtcgaagaagcctggagagacagtcaagatctcctgcaaggcttctgggtatac
cttcacaaactatggaatgaactgggtgaagcaggctccaggaaagggtttaagtggatgggctggataaacacctacactgga
gccaacatatgctgatgacttcaagggacggtttgccttctctttggaaacctctgcctacactgcctatttgcagatcaacaacct
caaaaatgaggacatggctacatatttctgtgcaagatttagctatggtaactcacgttacgctgactactggggccaaggcaccact
ctcacagtctcctctgatca

Amino acid sequence
QIQLVQSGPESKKPGETVKISCKASGYTFTNYGMNWVKQAPGKGLKWMGWINTY
TGQPTYADDFKGRFAFSLETSAYTAYLQINNLKNEDMATYFCARFSYGNSRYADY
WGQGTTLTVSSD

95 L6 VH L11S scFv

Nucleotide sequence,
Aagcttgttgtiatggattttcaagtgcagattttcagcttcctgctaatcagtgcttcagtcataatgtccagaggacaaattgttctctc
ccagtctccagcaatcctgtctgcatctccaggggagaaggtcacattgacttgcagggccagctcaagtgtaagtttcatgaactg
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ggcggcggatctctgcagatccagttggtgcagtctggacctgagtcgaagaagcctggagagacagtcaagatctcctgcaag
gcttctgggtataccttcacaaactatggaatgaactgggtgaagcaggctccaggaaagggtttaagtggatgggctggataaa
cacctacactggacagccaacatatgctgatgacttcaagggacggtttgccttctctttggaaacctctgcctacactgcctatttgc
agatcaacaacctcaaaaatgaggacatggctacatatttctgtgcaagatttagctatggtaactcacgttacgctgactactgggg
ccaaggcaccactctcacagtctcctctgatca

Amino acid sequence
MDFQVQIFSFLLISASVIMSRGQIVLSQSPAILSASPGEKVTLTCRASSSVSFMNWYQ
QKPGSSPKPWIYATSNLASEFPGRFSGEWSGTSYSLAISRVEAEDAATYYCQQWNS
NPLTFGAGTKLELKELSGGGGSGGGGSGGGGSLQIQLVQSGPESKKPGETVKISCK
ASGYTFTNYGMNWVKQAPGKGLKWMGWINTYTGQPTYADDFKGRFAFSLETSA
YTAYLQINNLKNEDMATYFCARFSYGNSRYADYWGQGTTLTVSSD

96 L6 Or L6 VHL11S scFv IgAH hIgG1 WCH2 WCH3

Nucleotide sequence (L6 VHL11S is shown)

aagcttgttgttatggattttcaagtgcagattttcagcttcctgctaatcagtgcttcagtcataatgtccagaggacaaattgttctctcc
cagtctccagcaatcctgtctgcatctccaggggagaaggtcacattgacttgcagggccagctcaagtgtaagtttcatgaactgg
taccagcagaagccaggatcctccccaaaccctggatttatgccacatccaatttggcttctgagttcctggtcgcttcagtggcg
agtggtctgggacctcttactctctcgcaatcagcagagtggaggctgaagatgctgccacttattactgccagcagtggaatagta
acccactcacgttcggtgctgggaccaagctggagctgaaagagctctctggtggcggtggctcgggcggtggtgggtcgggtg
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ataatgccaagacaaagccgcgggaggagcagtacaacagcacgtaccgtgtggtcagcgtcctcaccgtcctgcaccaggact
ggctgaatggcaaggagtacaagtgcaaggtctccaacaaagccctcccagccccccatcgagaaaacaatctccaaagccaaag
ggcagccccgagaaccacaggtgtacaccctgcccccatcccgggatgagctgaccaagaaccaggtcagcctgacctgcctg
gtcaaaggcttctatcccagcgacatcgccgtggagtgggagagcaatgggcagccggagaacaactacaagaccacgcctcc
cgtgctggactccgacggctccttcttcctctacagcaagctcaccgtggacaagagcaggtggcagcaggggaacgtcttctcat
gctccgtgatgcatgaggctctgcacaaccactacacgcagaagagcctctccctgtctccgggtaaatgatctaga

Amino acid sequence.
MDFQVQIFSFLLISASVIMSRGQIVLSQSPAILSASPGEKVTLTCRASSSVSFMNWYQ
QKPGSSPKPWIYATSNLASEFPGRFSGEWSGTSYSLAISRVEAEDAATYYCQQWNS
NPLTFGAGTKLELKELSGGGGSGGGGSGGGGSLQIQLVQSGPESKKPGETVKISCK
ASGYTFTNYGMNWVKQAPGKGLKWMGWINTYTGQPTYADDFKGRFAFSLETSA
YTAYLQINNLKNEDMATYFCARFSYGNSRYADYWGQGTTLTVSSDQPVPSTPPTP
SPSTPPTPSPSCAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFN
WYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALP
APIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQ
PENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSL
SLSPGK

97 L6 scFv VHL11S (SSS-S)H WCH2 WCH3
Nucleotide sequence
aagcttgttgttatggattttcaagtgcagattttcagcttcctgctaatcagtgcttcagtcataatgtccagaggacaaattgttctctcc
cagtctccagcaatcctgtctgcatctccaggggagaaggtcacattgacttgcagggccagctcaagtgtaagtttcatgaactgg
taccagcagaagccaggatcctccccaaaccctggatttatgccacatccaatttggcttctgagttcctggtcgcttcagtggcg
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acccactcacgttcggtgctgggaccaagctggagctgaaagagctctctggtggcggtggctcgggcggtggtgggtcgggtg
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cctacactggacagccaacatatgctgatgacttcaagggacggtttgccttctctttggaaacctctgcctacactgcctatttgcag
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ccgcgggaggagcagtacaacagcacgtaccgtgtggtcagcgtcctcaccgtcctgcaccaggactggctgaatggcaagga
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cacaggtgtacaccctgcccccatcccgggatgagctgaccaagaaccaggtcagcctgacctgcctggtcaaaggcttctatcc
cagcgacatcgccgtggagtgggagagcaatgggcagccggagaacaactacaagaccacgcctcccgtgctggactccgac

ggctccttcttcctctacagcaagctcaccgtggacaagagcaggtggcagcaggggaacgtcttctcatgctccgtgatgcatga
ggctctgcacaaccactacacgcagaagagcctctccctgtctccgggtaaatgatctaga

Amino acid sequence

MDFQVQIFSFLLISASVIMSRGQIVLSQSPAILSASPGEKVTLTCRASSSVSFMNWYQ
QKPGSSPKPWIYATSNLASGFPGRFSGSGWSGTSYSLAISRVEAEDAATYYCQQWNS
NPLTFGAGTKLELKRLSGGGGSGGGGSGGGGSLQIQLVQSGPESKKPGETVKISCK
ASGYTFTNYGMNWVKQAPGKGLKWMGWINTYTGQPTYADDFKGRFAFSLETSA
YTAYLQINNLKNEDMATYFCARFSYGNSRYADYWGQGTTLTVSSDQEPKSSDKT
HTSPPSSAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYV
DGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIE
KTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPEN
NYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLS
PGK

98. P238 CH2 WCH3

a. P238S CH2 WCH3

Nucleotide sequence

cctgaactcctgggggatcgtcagtcttcctcttccccccaaaacccaaggacaccctcatgatctcccggaccctgaggtcac
atgcgtggtggtggacgtgagccacgaagaccctgaggtcaagttcaactggtacgtggacggcgtggaggtgcataatgccaa
gacaaagccgcgggaggagcagtacaacagcacgtaccgtgtggtcagcgtcctcaccgtcctgcaccaggactggctgaatg
gcaaggagtacaagtgcaaggtctccaacaaagccctcccagccccatcgagaaaacaatctccaaagccaaagggcagccc
cgagaaccacaggtgtacaccctgcccccatcccgggatgagctgaccaagaaccaggtcagcctgacctgcctggtcaaagg
cttctatcccagcgacatcgccgtggagtgggagagcaatgggcagccggagaacaactacaagaccacgcctcccgtgctgg
actccgacggctccttcttcctctacagcaagctcaccgtggacaagagcaggtggcagcaggggaacgtcttctcatgctccgtg
atgcatgaggctctgcacaaccactacacgcagaagagcctctccctgtctccgggtaaatgatctaga

Amino acid sequence

PELLGGSSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHN
AKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKG
QPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPV
LDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

b (SSS-S)H P238S CH2 WCH3

Nucleotide sequence

tgatcaggagcccaaatcttctgacaaaactcacacatcccccaccgtcctcagcacctgaactcctgggggatcgtcagtcttcct
cttccccccaaaacccaaggacaccctcatgatctcccggaccctgaggtcacatgcgtggtggtggacgtgagccacgaaga
ccctgaggtcaagttcaactggtacgtggacggcgtggaggtgcataatgccaagacaaagccgcgggaggagcagtacaaca
gcacgtaccgtgtggtcagcgtcctcaccgtcctgcaccaggactggctgaatggcaaggagtacaagtgcaaggtctccaacaa
agccctcccagccccatcgagaaaacaatctccaaagccaaagggcagccccgagaaccacaggtgtacaccctgcccccat
cccgggatgagctgaccaagaaccaggtcagcctgacctgcctggtcaaaggcttctatcccagcgacatcgccgtggagtggg
agagcaatgggcagccggagaacaactacaagaccacgcctcccgtgctggactccgacggctccttcttcctctacagcaagct
caccgtggacaagagcaggtggcagcaggggaacgtcttctcatgctccgtgatgcatgaggctctgcacaaccactacacgca
gaagagcctctccctgtctccgggtaaatgatctaga

Amino acid sequence:

DQEPKSSDKTHTSPPSSAPELLGGSSVFLFPPKPKDTLMISRTPEVTCVVVDVSHED
PEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKV
SNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVE

WESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHN
HYTQKSLSLSPGK*

99 a.CD16-6 low (ED) +NL+ (SSS-S)H P238S CH2 WCH3

Nucleotide sequence

ggagcccaaatcttctgacaaaactcacacatcccccaccgtcctcagcacctgaactcctgggggggatcgtcagtcttcctcttccc
ccccaaaacccaaggacaccctcatgatctcccggacccctgaggtcacatgcgtggtggtggacgtgagccacgaagaccctga
ggtcaagttcaactggtacgtggacggcgtggaggtgcataatgccaagacaaagccgcgggaggagcagtacaacagcacgt
accgtgtggtcagcgtcctcaccgtcctgcaccaggactggctgaatggcaaggagtacaagtgcaaggtctccaacaaagccct
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ggacaagagcaggtggcagcaggggaacgtcttctcatgctccgtgatgcatgaggctctgcacaaccactacacgcagaagag
cctctccctgtctccgggtaaatgatctaga

Amino acid sequence

MWQLLLPTALLLLVSAGMRTEDLPKAVVFLEPQWYRVLEKDSVTLKCQGAYSPE
DNSTQWFHNESLISSQASSYFIDAATVDDSGEYRCQTNLSTLSDPVQLEVHIGWLL
LQAPRWVFKEEDPIHLRCHSWKNTALHKVTYLQNGKGRKYFHHNSDFYIPKATL
KDSGSYFCRGLVGSKNVSSETVNITITQGLADQEPKSSDKTHTSPPSSAPELLGGSS
VFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREE
QYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVY
TLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFF
LYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

100. b CD16-6 low (ED)+HE4LP+(SSS-S)H P238S CH2 WCH3

Nucleotide sequence

aagcttgccgccatgcctgcttgtcgcctaggcccgctagccgccgccctcctcctcagcctgctgctgttcggcttcaccctagtct
caggcaccggtgcaatgcggactgaagatctcccaaaggctgtggtgttcctggagcctcaatggtacagggtgctcgagaagg
acagtgtgactctgaagtgccagggagcctactcccctgaggacaattccacacagtggtttcacaatgagagcctcatctcaagc
caggcctcgagctacttcattgacgctgccacagtcgacgacagtggagagtacaggtgccagacaaacctctccacccctcagtg
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agaaccacaggtgtacaccctgcccccatcccgggatgagctgaccaagaaccaggtcagcctgacctgcctggtcaaaggctt
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ccgacggctccttcttcctctacagcaagctcaccgtggacaagagcaggtggcagcaggggaacgtcttctcatgctccgtgatg
catgaggctctgcacaaccactacacgcagaagagcctctccctgtctccgggtaaatgatctagaaa

Amino acid sequence

MPACRLGPLAAALLLSLLLFGFTLVSGTGAMRTEDLPKAVVFLEPQWYRVLEKDS
VTLKCQGAYSPEDNSTQWFHNESLISSQASSYFIDAATVDDSGEYRCQTNLSTLSD
PVQLEVHIGWLLLQAPRWVFKEEDPIHLRCHSWKNTALHKVTYLQNGKGRKYFH
HNSDFYIPKATLKDSGSYFCRGLVGSKNVSSETVNITITQGLADQEPKSSDKTHTSP
PSSAPELLGGSSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVE
VHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISK

AKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKT
TPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

101 CD16-9 high (ED) (SSS-S)H P238S CH2 CH3

a. CD16-9 high (ED)NL+(SSS-S)H P238S CH2 CH3

Nucleotide sequence

gttgttaagcttgccgccatgtggcagctgctcctcccaactgctctgctacttctagtttcagctggcatgcggactgaagatctccc
aaaggctgtggtgttcctggaggcctcaatggtacagggtgctcgagaaggacagtgtgactctgaagtgccagggagcctactcc
cctgaggacaattccacacagtggtttcacaatgagagcctcatctcaagccaggcctcgagctacttcattgacgctgccacagtc
gacgacagtggagagtacaggtgccagacaaacctctccacccctcagtgacccggtgcagctagaagtccatatcggctggctgt
tgctccaggccctcggtgggtgttcaaggaggaagaccctattcacctgaggtgtcacagctggaagaacactgctctgcataag
gtcatatatttacagaatggcaaggcaggagtattttcatcataattctgacttctacattccaaaagccacactcaaagacagcg
gctcctacttctgcaggggggcttttgggagtaaaaatgtgtcttcagagactgtgaacatcaccatcactcaaggtttggctgatcag
gagcccaaatcttctgacaaaactcacacatcccccaccgtcctcagcacctgaactcctgggggggatcgtcagtcttcctcttcccc
ccaaaacccaaggacaccctcatgatctcccggacccctgaggtcacatgcgtggtggtggacgtgagccacgaagaccctgag
gtcaagttcaactggtacgtggacggcgtggaggtgcataatgccaagacaaagccgcgggaggagcagtacaacagcacgta
ccgtgtggtcagcgtcctcaccgtcctgcaccaggactggctgaatggcaaggagtacaagtgcaaggtctccaacaaagccctc
tccagcccccatcgagaaaacaatctccaaagccaaagggcagccccgagaaccacaggtgtacaccctgcccccatcccgggga
tgagctgaccaagaaccaggtcagcctgacctgcctggtcaaaggcttctatcccagcgacatcgccgtggagtgggagagcaat
gggcagccggagaacaactacaagaccacgcctcccgtgctggactccgacggctccttcttcctctacagcaagctcaccgtgg
acaagagcaggtggcagcaggggaacgtcttctcatgctccgtgatgcatgaggctctgcacaaccactacacgcagaagagcc
tctccctgtctccgggtaaatgatctagaaa

Amino acid sequence

MWQLLLPTALLLLVSAGMRTEDLPKAVVFLEPQWYRVLEKDSVTLKCQGAYSPE
DNSTQWFHNESLISSQASSYFIDAATVDDSGEYRCQTNLSTLSDPVQLEVHIGWLL
LQAPRWVFKEEDPIHLRCHSWKNTALHKVTYLQNGKGRKYFHHNSDFYIPKATL
KDSGSYFCRGLFGSKNVSSETVNITITQGLADQEPKSSDKTHTSPPSSAPELLGGSS
VFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREE
QYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVY
TLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFF
LYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

CD16-9 high (ED)+HE4LP+hIgG1 (SSS-S)H P238S CH2 CH3

Nucleotide sequence

aagcttgccgccatgcctgcttgtcgcctaggccgctagccgccgccctcctcctcagcctgctgctgttcggcttcaccctagtct
caggcaccggtgcaatgcggactgaagatctcccaaaggctgtggtgttcctggaggcctcaatggtacagggtgctcgagaagg
acagtgtgactctgaagtgccagggagcctactcccctgaggacaattccacacagtggtttcacaatgagagcctcatctcaagc
caggcctcgagctacttcattgacgctgccacagtcgacgacagtggagagtacaggtgccagacaaacctctccacccctcagtg
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Amino acid sequence.
MPACRLGPLAAALLLSLLLFGFTLVSGTGAMRTEDLPKAVVFLEPQWYRVLEKDS
VTLKCQGAYSPEDNSTQWFHNESLISSQASSYFIDAATVDDSGEYRCQTNLSTLSD
PVQLEVHIGWLLLQAPRWVFKEEDPIHLRCHSWKNTALHKVTYLQNGKGRKYFH
HNSDFYIPKATLKDSGSYFCRGLFGSKNVSSETVNITITQGLADQEPKSSDKTHTSP
PSSAPELLGGSSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVE
VHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISK
AKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKT
TPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

102. a. CD16 ED low (native leader)
Nucleotide sequence
aagcttgccgccatgtggcagctgctcctcccactgctctgctacttctagtttcagctggcatgcggactgaagatctcccaaagg
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Amino acid sequence.
mwqllipfallivsagmrtedlpkavvflepqwyrvlekdsvtlkcqgayspednstqwfhneslissqassyfidaatvdds
geyrcqtnlstlsdpvqlevhigwlllqaprwvfkeedpihlrchswkntalhkvtylqngkgrkyfhhnsdfyipkatlkdsg
syfcrglvgsknvssetvnititqgladq

b CD16 ED low (HE4 leader)
Nucleotide sequence
aagcttgccgccatgcctgcttgtcgcctaggcccgctagccgccgccctcctcctcagcctgctgctgttcggcttcaccctagtct
cagccaccggtgcaatgcggactgaagatctcccaaaggctgtggtgttcctggagcctcaatggtacagggtgctcgagaagg
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Amino acid sequence
mpacrlgplaaalllsllfgftlvsgtgamrtedlpkavvflepqwyrvlekdsvtlkcqgayspednstqwfhneslissqass
yfidaatvddsgeyrcqtnlstlsdpvqlevhigwlllqaprwvfkeedpihlrchswkntalhkvtylqngkgrkyfhhnsdf
yipkatlkdsgsyfcrglvgsknvssetvnititqgladq

103 a. CD16 ED high (native leader)
Nucleotide sequence
gttgttaagcttgccgccatgtggcagctgctcctcccaactgctctgctacttctagtttcagctggcatgcggactgaagatctccc
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420

gtcacatatttacagaatggcaaaggcaggaagtatttcatcataattctgacttctacattccaaaagccacactcaaagacagcg
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a

Amino acid sequence
mwqlllptalllvsagmrtedlpkavvflepqwyrvlekdsvtlkcqgayspednstqwfhneslissqassyfidaatvddsgeyrcqtnlstlsdpvqlevhigwlllqaprwvfkeedpihlrchswknialhkvtylqngkgrkyfhhnsdfyipkatlkdsgsyfcrglfgsknvsseivnititqgladq

b CD16 ED high (HE4 leader)

Nucleotide sequence
aagcttgccgccatgcctgcttgtcgcctaggcccgctagccgccgccctcctcctcagcctgctgctgttcggcttcaccctagtct
caggcaccggtgcaatgcggactgaagatctcccaaaggctgtggtgttcctggagcctcaatggtacagggtgctcgagaagg
acagtgtgactctgaagtgccagggagcctactccctgaggacaattccacacagtggtttcacaatgagagcctcatctcaagc
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Amino acid sequence
MPACRLGPLAAALLLSLLLFGFTLVSGTGAMRTEDLPKAVVFLEPQWYRVLEKDS
VTLKCQGAYSPEDNSTQWFHNESLISSQASSYFIDAATVDDSGEYRCQTNLSTLSD
PVQLEVHIGWLLLQAPRWVFKEEDPIHLRCHSWKNTALHKVTYLQNGKGRKYFH
HNSDFYIPKATLKDSGSYFCRGLFGSKNVSSETVNITITQGLADQ

104. 2e12 scFv (SSS-S)H P238S CH2 WCH3-hCD80TM/CT

Nucleotide sequence
aagcttatggattttcaagtgcagattttcagcttcctgctaatcagtgcttcagtcataatgtccagaggagtcgacattgtgctcaccc
aatctccagcttctttggctgtgtctctaggtcagagagccaccatcttctgcagagccagtgaaagttgttgatattatgtcacaagtt
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Amino acid sequence

421

MDFQVQIFSFLLISASVIMSRGVDIVLTQSPASLAVSLGQRATISCRASESVEYYVTS
LMQWYQQKPGQPPKLLISAASNVESGVPARFSGSGSGTDFSLNIHPVEEDDIAMYF
CQQSRKVPWTFGGGTKLEIKRGGGGSGGGGSGGGGSQVQLKESGPGLVAPSQSLS
ITCTVSGFSLTGYGVNWVRQPPGKGLEWLGMIWGDGSTDYNSALKSRLSITKDNS
KSQVFLKMNSLQTDDTARYYCARDGYSNFHYYVMDYWGQGTSVTVSSDLEPKS
SDKTHTSPPSPAPELLGGSSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFN
WYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALP
APIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQ
PENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSL
SLSPGKADPSNLLPSWAITLISVNGIFVICCLTYCFAPRCRERRRNERLRRESVRPV

105 10A8 scFv (SSS-S)H P238SCH2 WCH3-hCD80TM/CT

Nucleotide sequence

aagcttatggattttcaagtgcagattttcagcttcctgctaatcagtgcttcagtcataatgtccagaggagtcgacatccagatgaca
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gaaagtgtacgccctgtataaatcgat

Amino acid sequence

MDFQVQIFSFLLISASVIMSRGVDIQMTQSPSSLSASLGGKVTITCKASQDIKKYIG
WYQHKPGKGPRLLIYYTSTLQPGIPSRFSGSGSGRDYSLSIRNLEPEDIATYYCQQY
DNLPLTFGSGTKLEIKRGGGGSGGGGSGGGGSDVQLQESGPGLVKPSQSLSLTCSV
TGYSITSGFYWNWIRQFPGNKLEWMGHISHDGRNNYNPSLINRISITRDTSKNQFFL
KLSSVTTEDTATYFCARHYGSSGAMDYWGQGTSVTVSSDLEPKSSDKTHTSPPSP
APELLGGSSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVH
NAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAK
GQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPP
VLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGKADPS
NLLPSWAITLISVNGIFVICCLTYCFAPRCRERRRNERLRRESVRPV

106. 2H7 scFv VHL11S (SSS-P)H P238SCH2CH3-hCD80TM/CT

Nucleotide sequence

aagcttgccgccatggattttcaagtgcagattttcagcttcctgctaatcagtgcttcagtcataattgccagaggacaaattgttctct
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Amino acid sequence
MDFQVQIFSFLLISASVIIARGQIVLSQSPAILSASPGEKVTMTCRASSSVSYMHWY
QQKPGSSPKPWIYAPSNLASGVPARFSGSGSGTSYSLTISRVEAEDAATYYCQQWS
FNPPTFGAGTKLELKDGGGSGGGGSGGGGSSQAYLQQSGAESVRPGASVKMSCK
ASGYTFTSYNMHWVKQTPRQGLEWIGAIYPGNGDTSYNQKFKGKATLTVDKSSS
TAYMQLSSLTSEDSAVYFCARVVYYSNSYWYFDVWGTGTTVTVSSDLEPKSSDK
THTSPPSPAPELLGGSSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYV
DGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIE
KTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPEN
NYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLS
PGKADPSNLLPSWAITLISVNGIFVICCLTYCFAPRCRERRRNERLRRESVRPV

107 G19-4 scFv (SSS-P)H P238SCH2 WCH3-hCD80TM/CT
Nucleotide sequence
aagcttatggattttcaagtgcagattttcagcttcctgctaatcagtgcttcagtcataatgtccagaggagtcgacatccagatgaca
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Amino acid sequence:

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GNTLPWTFGGGTKLVTKRELGGGGSGGGGSGGGGSIDEVQLQQSGPELVKPGAS
MSCKASGYSFTGYIVNWLKQSHGKNLEWIGLINPYKGLTTYNQKFKGKATLTVD
KSSSTAYMELLSLTSEDSAVYYCARSGYYGDSDWYFDVWGAGTTVTVSSDLEPK
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PV

108 2e12 scFv IgA WH WCH2 T4 CH3-hCD80TM/CT

Nucleotide sequence

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Amino acid sequence

MDFQVQIFSFLLISASVIMSRGVDIVLTQSPASLAVSLGQRATISCRASESVEYYVTS
LMQWYQQKPGQPPKLLISAASNVESGVPARFSGSGSGTDFSLNIHPVEEDDIAMYF
CQQSRKVPWTFGGGTKLEIKRGGGGSGGGGSGGGGSQVQLKESGPGLVAPSQSLS
ITCTVSGFSLTGYGVNWVRQPPGKGLEWLGMIWGDGSTDYNSALKSRLSITKDNS
KSQVFLKMNSLQTDDTARYYCARDGYSNFHYYVMDYWGQGTSVTVSSDQPVPS
TPPTPSPSTPPTPSPSCCHPRLSLHRPALEDLLLGSEAILTCTLTGLRDASGVTFTWTP
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KSGNTFRPEVHLLPPPSEELALNELVTLTCLARGFSPKDVLVRWLQGSQELPREKY
LTWASRQEPSQGTTTFAVTSILRVAAEDWKKGDTFSCMVGHEALPLAFTQKTIDR

424

LAGKPTHVNVSVVMAEVDADPSNNLLPSWAITLISVNGIFVICCLTYCFAPRCRER
RRNERLRRESVRPV

109 2e12 scFV (SSS-P)H P238S CH2 WCH3-mFADD-TM/CT

Nucleotide sequence,

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Amino acid sequence

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SDKTHTSPPSPAPELLGGSSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFN
WYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALP
APIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQ
PENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSL
SLSPGKADPSNMDPFLVLLHSLSGSLSGNDLMELKFLCRERVSKRKLERVQSGLDL
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FDIVCDNVGRDWKRLARELKVSEAKMDGIEEKYPRSLSERVRESLKVWKNAEKK
NASVAGLVKALRTCRLNLVADLVEEAQESVSKSENMSPVLRDSTVSSSETP

110 2e12 scFv (SSS-P)H WCH2WCH3-mFADD-TM/CT

Nucleotide sequence

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425 of 464

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Amino acid sequence
MDFQVQIFSFLLISASVIMSRGVDIVLTQSPASLAVSLGQRATISCRASESVEYYVTS
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PENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSL
SLSPGKADPSNMDPFLVLLHSLSGSLSGNDLMELKFLCRERVSKRKLERVQSGLDL
FTVLLEQNDLERGHTGLLRELLASLRRHDLLQRLDDFEAGTATAAPPGEADLQVA
FDIVCDNVGRDWKRLARELKVSEAKMDGIEEKYPRSLSERVRESLKVVWKNAEKK
NASVAGLVKALRTCRLNLVADLVEEAQESVSKSENMSPVLRDSTVSSSETP

111 **mcasp3-TM/CT**
Nucleotide sequence
Ggatccttcgaacatggagaacacaaaacctcagtggattcaaaatccattaataatttgaagtaaagaccatacatgggagcaa
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Amino acid sequence

DPSNMENNKTSVDSKSINNFEVKTIHGSKSVDSGIYLDSSYKMDYPEMGICIIINNK
NFHKSTGMSSRSGTDVDAANLRETFMGLKYQVRNKNDLTREDILELMDSVSKED
HSKRSSFVCVILSHGDEGVIYGTNGPVELKKLTSFFRGDYCRSLTGKPKLFIIQACR
GTELDCGIETDSGTDEEMACQKIPVEADFLYAYSTAPGYYSWRNSKDGSWFIQSL
CSMLKLYAHKLEFMHILTRVNRKVATEFESFSLDSTFHAKKQIPCIVSMLTKELYF
YH

112. 2e12 scFv (SSS-P)H WCH2WCH3-mesp3-TM/CT

Nucleotide sequence

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Amino acid sequence

MDFQVQIFSFLLISASVIMSRGVDIVLTQSPASLAVSLGQRATISCRASESVEYYVTS
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CQQSRKVPWTFGGGTKLEIKRGGGGSGGGGSGGGGSQVQLKESGPGLVAPSQSLS
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APIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQ

PENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSL
SLSPGKADPSNMENNKTSVDSKSINNFEVKTIHGSKSVDSGIYLDSSYKMDYPEMG
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IIQACRGTELDCGIETDSGTDEEMACQKIPVEADFLYAYSTAPGYYSWRNSKDGS
WFIQSLCSMLKLYAHKLEFMHILTRVNRKVATEFESFSLDSTFHAKKQIPCIVSMLT
KELYFYH

113 **2e12 scFv (SSS-P)H P238SCH2WCH3-mcasp3-TM/CT**

Nucleotide sequence

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Amino acid sequence

MDFQVQIFSFLLISASVIMSRGVDIVLTQSPASLAVSLGQRATISCRASESVEYYVTS
LMQWYQQKPGQPPKLLISAASNVESGVPARFSGSGSGTDFSLNIHPVEEDDIAMYF
CQQSRKVPWTFGGGTKLEIKRGGGGSGGGGSGGGGSQVQLKESGPGLVAPSQSLS
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KSQVFLKMNSLQTDDTARYYCARDGYSNFHYYVMDYWGQGTSVTVSSDLEPKS
SDKTHTSPPSPAPELLGGSSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFN
WYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALP
APIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQ
PENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSL
SLSPGKADPSNSNMENNKTSVDSKSINNFEVKTIHGSKSVDSGIYLDSSYKMDYPE

MGICIIINNKNFHKSTGMSSRSGTDVDAANLRETFMGLKYQVRNKNDLTREDILEL
MDSVSKEDHSKRSSFVCVILSHGDEGVIYGTNGPVELKKLTSFFRGDYCRSLTGKP
KLFIIQACRGTELDCGIETDSGTDEEMACQKIPVEADFLYAYSTAPGYYSWRNSKD
GSWFIQSLCSMLKLYAHKLEFMHILTRVNRKVATEFESFSLDSTFHAKKQIPCIVSM
LTKELYFYH

114 mcasp8-TM/CT

Nucleotide sequence

ttcgaacatggatttccagagttgtctttatgctattgctgaagaactgggcagtgaagacctggctgccctcaagttcctgtgcttgga
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Amino acid sequence

SNMDFQSCLYAIAEELGSEDLAALKFLCLDYIPHKKQETIEDAQKLFLRLREKGML
EEGNLSFLKELLFHISRWDLLVNFLDCNREEMVRELRDPDNAQISPYRVMLFKLSE
EVSELELRSFKFLLNNEIPKCKLEDDLSLLEIFVEMEKRTMLAENNLETLKSICDQV
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115 2e12 scFv hIgG1 (SSS-P)H WCH2 WCH3-mcasp8-TM/CT

Nucleotide sequence

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Amino acid sequence.
MDFQVQIFSFLLISASVIMSRGVDIVLTQSPASLAVSLGQRATISCRASESVEYYVTS
LMQWYQQKPGQPPKLLISAASNVESGVPARFSGSGSGTDFSLNIHPVEEDDIAMYF
CQQSRKVPWTFGGGTKLEIKRGGGGSGGGGSGGGGSQVQLKESGPGLVAPSQSLS
ITCTVSGFSLTGYGVNWVRQPPGKGLEWLGMIWGDGSTDYNSALKSRLSITKDNS
KSQVFLKMNSLQTDDTARYYCARDGYSNFHYYVMDYWGQGTSVTVSSDLEPKS
SDKTHTSPPSPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFN
WYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALP
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PENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSL
SLSPGKADPSNMDFQSCLYAIAEELGSEDLAALKFLCLDYIPHKKQETIEDAQKLFL
RLREKGMLEEGNLSFLKELLFHISRWDLLVNFLDCNREEMVRELRDPDNAQISPYR
VMLFKLSEEVSELELRSFKFLLNNEIPKCKLEDDLSLLEIFVEMEKRTMLAENNLET
LKSICDQVNKSLLGKIEDYERSSTERRMSLEGREELPPSVLDEMSLKMAELCDSPR
EQDSESRTSDKVYQMKNKPRGYCLIINNHDFSKAREDITQLRKMKDRKGTDCDKE
ALSKTFKELHFEIVSYDDCTANEIHEILEGYQSADHKNKDCFICCILSHGDKGVVYG
TDGKEASIYDLTSYFTGSKCPSLSGKPKIFFIQACQGSNFQKGVPDEAGFEQQNHTL
EVDSSSHKNYIPDEADFLLGMATVKNCVSYRDPVNGTWYIQSLCQSLRERCPQGD
DILSILTGVNYDVSNKDDRRNKGKQMPQPTFTLRKKLFFPP

116. 2e12 scFv hIgG1 (SSS-P)H P238SCH2 WCH3-mcasp8-TM/CT

Nucleotide sequence

aagcttatggattttcaagtgcagattttcagcttcctgctaatcagtgcttcagtcataatgtccagaggagtcgacattgtgctcaccc
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Amino acid sequence

MDFQVQIFSFLLISASVIMSRGVDIVLTQSPASLAVSLGQRATISCRASESVEYYVTS
LMQWYQQKPGQPPKLLISAASNVESGVPARFSGSGSGTDFSLNIHPVEEDDIAMYF
CQQSRKVPWTFGGGTKLEIKRGGGGSGGGGSGGGGSQVQLKESGPGLVAPSQSLS
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KSQVFLKMNSLQTDDTARYYCARDGYSNFHYYVMDYWGQGTSVTVSSDLEPKS
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SLSPGKADPSNMDFQSCLYAIAEELGSEDLAALKFLCLDYIPHKKQETIEDAQKLFL
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EVDSSSHKNYIPDEADFLLGMATVKNCVSYRDPVNGTWYIQSLCQSLRERCPQGD
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117 hcasp3-TM/CT

Nucleotide sequence

Ggatccttcgaacttggaghacactgaaaactcagtggattcaaaatccattaaaaatttggaaccaaagatcatacatggaagcg
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Amino acid sequence

DPSNMENTENSVDSKSIKNLEPKIIHGSESMDSGISLDNSYKMDYPEMGLCIIINNK
NFHKSTGMTSRSGTDVDAANLRETFRNLKYEVRNKNDLTREEIVELMRDVSKED
HSKRSSFVCVLLSHGEEGIIFGTNGPVDLKKITNFFRGDRCRSLTGKPKLFIIQACRG
TELDCGIETDSGVDDDMACHKIPVEADFLYAYSTAPGYYSWRNSKDGSWFIQSLC
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118. 2e12 scFv hIgG1 (SSS-P)H WCH2 WCH3-hcasp3-TM/CT

Nucleotide sequence

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Amino acid sequence
MDFQVQIFSFLLISASVIMSRGVDIVLTQSPASLAVSLGQRATISCRASESVEYYVTS
LMQWYQQKPGQPPKLLISAASNVESGVPARFSGSGSGTDFSLNIHPVEEDDIAMYF
CQQSRKVPWTFGGGTKLEIKRGGGGSGGGGSGGGGSQVQLKESGPGLVAPSQSLS
ITCTVSGFSLTGYGVNWVRQPPGKGLEWLGMIWGDGSTDYNSALKSRLSITKDNS
KSQVFLKMNSLQTDDTARYYCARDGYSNFHYYVMDYWGQGTSVTVSSDLEPKS
SDKTHTSPPSPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFN
WYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALP
APIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQ
PENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSL
SLSPGKADPSNMENTENSVDSKSIKNLEPKIIHGSESMDSGISLDNSYKMDYPEMG
LCIIINNKNFHKSTGMTSRSGTDVDAANLRETFRNLKYEVRNKNDLTREEIVELMR
DVSKEDHSKRSSFVCVLLSHGEEGIIFGTNGPVDLKKITNFFRGDRCRSLTGKPKLFI
IQACRGTELDCGIETDSGVDDDMACHKIPVEADFLYAYSTAPGYYSWRNSKDGS
WFIQSLCAMLKQYADKLEFMHILTRVNRKVATEFESFSFDATFHAKKQIPCIVSML
TKELYFYH

123 2e12 scFv hIgG1 (SSS-P)H P238SCH2 WCH3-heasp3-TM/CT
Nucleotide sequence
aagcttatggattttcaagtgcagattttcagcttcctgctaatcagtgcttcagtcataatgtccagaggagtcgacattgtgctcaccc
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Amino acid sequence

MDFQVQIFSFLLISASVIMSRGVDIVLTQSPASLAVSLGQRATISCRASESVEYYVTS
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KSQVFLKMNSLQTDDTARYYCARDGYSNFHYYVMDYWGQGTSVTVSSDLEPKS
SDKTHTSPPSPAPELLGGSSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFN
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PENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSL
SLSPGKADPSNMENTENSVDSKSIKNLEPKIIHGSESMDSGISLDNSYKMDYPEMG
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DVSKEDHSKRSSFVCVLLSHGEEGIIFGTNGPVDLKKITNFFRGDRCRSLTGKPKLFI
IQACRGTELDCGIETDSGVDDDMACHKIPVEADFLYAYSTAPGYYSWRNSKDGS
WFIQSLCAMLKQYADKLEFMHILTRVNRKVATEFESFSFDATFHAKKQIPCIVSML
TKELYFYH

124. hcasp8-TM/CT

hCaspase8B

Nucleotide sequence

ggatccttcgaacatggacttcagcagaaatcttttatgatattgggggaacaactggacagtgaagatctggcctccctcaagttcctg
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Amino acid sequence

DPSNMDFSRNLYDIGEQLDSEDLASLKFLSLDYIPQRKQEPIKDALMLFQRLQEKR
MLEESNLSFLKELLFRINRLDLLITYLNTRKEEMERELQTPGRAQISAYRVMLYQIS
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NKSLLKIINDYEEFSKERSSSLEGSPDEFSNGEELCGVMTISDSPREQDSESQTLDKV
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KPHDDCTVEQIYEILKIYQLMDHSNMDCFICCILSHGDKGIIYGTDGQEAPIYELTS
QFTGLKCPSLAGKPKVFFIQACQGDNYQKGIPVETDSEEQPYLEMDLSSPQTRYIPD
EADFLLGMATVNNCVSYRNPAEGTWYIQSLCQSLRERCPRGDDILTILTEVNYEVS
NKDDKKNMGKQMPQPTFTLRKKLVFPSD

hCaspase8C

Nucleotide sequence

ggatccttcgaatcatggacttcagcagaaatctttatgatattggggaacaactggacagtgaagatctggcctccctcaagttcctg
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Amino acid sequence

DPSNMDFSRNLYDIGEQLDSEDLASLKFLSLDYIPQRKQEPIKDALMLFQRLQEKR
MLEESNLSFLKELLFRINRLDLLITYLNTRKEEMERELQTPGRAQISAYRVMLYQIS
EEVSRSELRSFKFLLQEEISKCKLDDDMNLLDIFIEMEKRVILGEGKLDILKRVCAQI
NKSLLKIINDYEEFSKGEELCGVMTISDSPREQDSESQTLDKVYQMKSKPRGYCLII
NNHNFAKAREKVPKLHSIRDRNGTHLDAGALTTTFEELHFEIKPHDDCTVEQIYEIL
KIYQLMDHSNMDCFICCILSHGDKGIIYGTDGQEAPIYELTSQFTGLKCPSLAGKPK
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VSYRNPAEGTWYIQSLCQSLRERCPRGDDILTILTEVNYEVSNKDDKKNMGKQMP
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125 2e12 scFv hIgG1-(SSS-P)H WCH2 WCH3-hcasp8-TM/CT

Nucleotide sequence

aagcttatggattttcaagtgcagattttcagcttcctgctaatcagtgcttcagtcataatgtccagaggagtcgacattgtgctcaccc
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Amino acid sequence
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LMQWYQQKPGQPPKLLISAASNVESGVPARFSGSGSGTDFSLNIHPVEEDDIAMYF
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KSQVFLKMNSLQTDDTARYYCARDGYSNFHYYVMDYWGQGTSVTVSSDLEPKS
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VNYEVSNKDDKKNMGKQMPQPTFTLRKKLVFPSD

126 2e12 scFv hIgG1 (SSS-P)H P238SCH2 WCH3-hcasp8B-TM/CT
(other caspase 8 isoforms are similar)
Nucleotide sequence
aagcttatggattttcaagtgcagattttcagcttcctgctaatcagtgcttcagtcataatgtccagaggagtcgacattgtgctcaccc
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436

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Amino acid sequence

MDFQVQIFSFLLISASVIMSRGVDIVLTQSPASLAVSLGQRATISCRASESVEYYVTS
LMQWYQQKPGQPPKLLISAASNVESGVPARFSGSGSGTDFSLNIHPVEEDDIAMYF
CQQSRKVPWTFGGGTKLEIKRGGGGSGGGGSGGGGSQVQLKESGPGLVAPSQSLS
ITCTVSGFSLTGYGVNWVRQPPGKGLEWLGMIWGDGSTDYNSALKSRLSITKDNS
KSQVFLKMNSLQTDDTARYYCARDGYSNFHYYVMDYWGQGTSVTVSSDLEPKS
SDKTHTSPPSPAPELLGGSSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFN
WYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALP
APIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQ
PENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSL
SLSPGKADPSNMDFSRNLYDIGEQLDSEDLASLKFLSLDYIPQRKQEPIKDALMLFQ
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MLYQISEEVSRSELRSFKFLLQEEISKCKLDDDMNLLDIFIEMEKRVILGEGKLDILK
RVCAQINKSLLKIINDYEEFSKERSSSLEGSPDEFSNGEELCGVMTISDSPREQDSES
QTLDKVYQMKSKPRGYCLIINNHNFAKAREKVPKLHSIRDRNGTHLDAGALTTTT
EELHFEIKPHDDCTVEQIYEILKIYQLMDHSNMDCFICCILSHGDKGIIYGTDGQEAP
IYELTSQFTGLKCPSLAGKPKVFFIQACQGDNYQKGIPVETDSEEQPYLEMDLSSPQ
TRYIPDEADFLLGMATVNNCVSYRNPAEGTWYIQSLCQSLRERCPRGDDILTILTE
VNYEVSNKDDKKNMGKQMPQPTFTLRKKLVFPSD

431

128. 2e12 scFv-hIgG1 (SSS-P)H WCH2 WCH3-hFADD-TM/CT

Nucleotide sequence

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Amino acid sequence

MDFQVQIFSFLLISASVIMSRGVDIVLTQSPASLAVSLGQRATISCRASESVEYYVTS
LMQWYQQKPGQPPKLLISAASNVESGVPARFSGSGSGTDFSLNIHPVEEDDIAMYF
CQQSRKVPWTFGGGTKLEIKRGGGGSGGGGSGGGGSQVQLKESGPGLVAPSQSLS
ITCTVSGFSLTGYGVNWVRQPPGKGLEWLGMIWGDGSTDYNSALKSRLSITKDNS
KSQVFLKMNSLQTDDTARYYCARDGYSNFHYYVMDYWGQGTSVTVSSDLEPKS
SDKTHTSPPSPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFN
WYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALP
APIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQ
PENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSL
SLSPGKADPSNPFLVLLHSVSSSLSSSELTELKFLCLGRVGKRKLERVQSGLDLFSM
LLEQNDLEPGHTELLRELLASLRRHDLLRRVDDFEAGAAAGAAPGEEDLCAAFNV
ICDNVGKDWRRLARQLKVSDTKIDSIEDRYPRNLTERVRESLRIWKNTEKENATV
AHLVGALRSCQMNLVADLVQEVQQARDLQNRSGAMSPMSWNSDASTSEAS

129 2e12 scFv-hIgG1 (SSS-P)H P238SCH2 WCH3-hFADD-TM/CT

Nucleotide sequence

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Amino acid sequence

MDFQVQIFSFLLISASVIMSRGVDIVLTQSPASLAVSLGQRATISCRASESVEYYVTS
LMQWYQQKPGQPPKLLISAASNVESGVPARFSGSGSGTDFSLNIHPVEEDDIAMYF
CQQSRKVPWTFGGGTKLEIKRGGGGSGGGGSGGGGSQVQLKESGPGLVAPSQSLS
ITCTVSGFSLTGYGVNWVRQPPGKGLEWLGMIWGDGSTDYNSALKSRLSITKDNS
KSQVFLKMNSLQTDDTARYYCARDGYSNFHYYVMDYWGQGTSVTVSSDLEPKS
SDKTHTSPPSPAPELLGGSSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFN
WYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALP
APIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQ
PENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSL
SLSPGKADPSNPFLVLLHSVSSSLSSSELTELKFLCLGRVGKRKLERVQSGLDLFSM
LLEQNDLEPGHTELLRELLASLRRHDLLRRVDDFEAGAAAGAAPGEEDLCAAFNV
ICDNVGKDWRRLARQLKVSDTKIDSIEDRYPRNLTERVRESLRIWKNTEKENATV
AHLVGALRSCQMNLVADLVQEVQQARDLQNRSGAMSPMSWNSDASTSEAS

130 1D8 scFv hIgG1 (SSS-P)H WCH2 WCH3-hCD80TM/CT

Nucleotide sequence

aagcttatggatttcaagtgcagatttcagcttcctgctaatcagtgcttcagtcataatgtccagaggagtcgacattgtgctcaccc
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Amino acid sequence
MDFQVQIFSFLLISASVIMSRGVDIVLTQSPASLAVSLGQRATISCRASESVEYYVTS
LMQWYQQKPGQPPKLLISAASNVESGVPARFSGSGSGTDFSLNIHPVEEDDIAMYF
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YMYWYQQKSGASPKLWIYDTSKLASGVPNRFSGSGSGTSYSLAINTMETEDAATY
YCQQWSSTPLTFGSGTKLEIKRGGGGSGGGGSGGGGSQVQLKEAGPGLVQPTQTL
SLTCTVSGFSLTSDGVHWIRQPPGKGLEWMGIIYYDGGTDYNSAIKSRLSISRDTSK
SQVFLKINSLQTDDTAMYYCARIHFDYWGQGVMVTVSSDLEPKSSDKTHTSPPSP
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NAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAK
GQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPP
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131 1D8 scFv mIgAT4-hCD80TM/CT
Nucleotide sequence
aagcttatggattttcaagtgcagattttcagcttcctgctaatcagtgtcttcagtcataatgtccagaggagtcgacattgtgctcactc
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Amino acid sequence
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SSTPLTFGSGTKLEIKRGGGGSGGGGSGGGGSQVQLKEAGPGLVQPTQTLSLTCTV
SGFSLTSDGVHWIRQPPGKGLEWMGIIYYDGGTDYNSAIKSRLSISRDTSKSQVFLK
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ELVSLTCLVRAFNPKEVLVRWLHGNEELSPESYLVFEPLKEPGEGATTYLVTSVLR
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132.1D8 scFv hIgG1 (SSS-P)H WCH2 WCH3-mFADD-TM/CT
Nucleotide sequence
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Amino acid sequence
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SSTPLTFGSGTKLEIKRGGGGSGGGGSGGGGSQVQLKEAGPGLVQPTQTLSLTCTV
SGFSLTSDGVHWIRQPPGKGLEWMGIIYYDGGTDYNSAIKSRLSISRDTSKSQVFLK
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SFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGKADPSNMDPFL
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133 1D8 scFv hIgG1 (SSS-P)H PZ38S CH2 WCH3-mFADD-TM/CT

Nucleotide sequence

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Amino acid sequence

MDFQVQIFSFLLISASVIMSRGVDIVLTQSPTTIAASPGEKVTITCRASSSVSYMYWY
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QVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDG
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VLLHSLSGSLSGNDLMELKFLCRERVSKRKLERVQSGLDLFTVLLEQNDLERGHT
GLLRELLASLRRHDLLQRLDDFEAGTATAAPPGEADLQVAFDIVCDNVGRDWKR
LARELKVSEAKMDGIEEKYPRSLSERVRESLKVVWKNAEKKNASVAGLVKALRTC
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134 1D8 scFv hIgG1 (SSS-P)H WCH2 WCH3-mcasp3-TM/CT
Nucleotide sequence

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Amino acid sequence:
MDFQVQIFSFLLISASVIMSRGVDIVLTQSPTTIAASPGEKVTITCRASSSVSYMYWY
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135 1D8 scFv hIgG1 (SSS-P)H P238S CH2 WCH3-mcasp3-TM/CT
Nucleotide sequence

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a

Amino acid sequence
MDFQVQIFSFLLISASVIMSRGVDIVLTQSPTTIAASPGEKVTITCRASSSVSYMYWY
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136 1D8 scFv hIgG1 (SSS-P)H WCH2 WCH3-mcasp8-TM/CT
Nucleotide sequence
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444

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Amino acid sequence
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137 1D8 scFv hIgG1 (SSS-P)H P238SCH2 WCH3-mcasp8-TM/CT
Nucleotide sequence
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Amino acid sequence.

MDFQVQIFSFLLISASVIMSRGVDIVLTQSPTTIAASPGEKVTITCRASSSVSYMYWY
QQKSGASPKLWIYDTSKLASGVPNRFSGSGSGTSYSLAINTMETEDAATYYCQQW
SSTPLTFGSGTKLEIKRGGGGSGGGGSGGGGSQVQLKEAGPGLVQPTQTLSLTCTV
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SFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGKADPSNMDFQS
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KNKPRGYCLIINNHDFSKAREDITQLRKMKDRKGTDCDKEALSKTFKELHFEIVSY
DDCTANEIHEILEGYQSADHKNKDCFICCILSHGDKGVVYGTDGKEASIYDLTSYF
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138.1D8 scFv hIgG1 (SSS-P)H WCH2 WCH3-hcasp3-TM/CT

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Amino acid sequence
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LLSHGEEGIIFGTNGPVDLKKITNFFRGDRCRSLTGKPKLFIIQACRGTELDCGIETD
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139 1D8 scFv hIgG1 (SSS-P)H P238SCH2 WCH3-hcasp3-TM/CT
Nucleotide sequence
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Amino acid sequence.
MDFQVQIFSFLLISASVIMSRGVDIVLTQSPTTIAASPGEKVTITCRASSSVSYMYWY
QQKSGASPKLWIYDTSKLASGVPNRFSGSGSGTSYSLAINTMETEDAATYYCQQW
SSTFLTFGSGTKLEIKRGGGGSGGGGSGGGGSQVQLKEAGPGLVQPTQTLSLTCTV
SGFSLTSDGVHWIRQPPGKGLEWMGIIYYDGGTDYNSAIKSRLSISRDTSKSQVFLK
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QVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDG
SFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGKADPSNMENTE
NSVDSKSIKNLEPKIIHGSESMDSGISLDNSYKMDYPEMGLCIIINNKNFHKSTGMT
SRSGTDVDAANLRETFRNLKYEVRNKNDLTREEIVELMRDVSKEDHSKRSSFVCV
LLSHGEEGIIFGTNGPVDLKKITNFFRGDRCRSLTGKPKLFIIQACRGTELDCGIETD
SGVDDDMACHKIPVEADFLYAYSTAPGYYSWRNSKDGSWFIQSLCAMLKQYADK
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140 1D8 scFv hIgG1 (SSS-S)H WCH2 WCH3-hcasp8-TM/CT
Nucleotide sequence
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catcgata

Amino acid sequence

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141 1D8 scFy hIgG1 (SSS-S)H P238SCH2 WCH3-hcasp8-TM/CT

Nucleotide sequence

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catcgataa

Amino acid sequence
MDFQVQIFSFLLISASVIMSRGVDIVLTQSPTTIAASPGEKVTITCRASSSVSYMYWY
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145 hCTLA4 IgAH IgACH2CH3
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450

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Amino acid sequence
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146 hCTLA4 IgA WH WCH2 T4CH3 (hCTLA4 IgAH IgACH2CH3)
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Amino acid sequence
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147 hIGA WH WCH2 T18CH3
Nucleotide sequence

451 of

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Amino acid sequence
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150 G19-4 scFv (SSS-P)WH WCH2 WCH3-hCD80TMCT

Nucleotide sequence
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Amino acid sequence
MDFQVQIFSFLLISASVIMSRGVDIQMTQTTSSLSASLGDRVTISCRASQDIRNYLN
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452

KSLSLSPGKADPSNLLPSWAITLISVNGIFVICCLTYCFAPRCRERRRRNERLRRESVR
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HCD16lowFL+NL

Nucleotide sequence

aagcttgccgccatgtggcagctgctcctcccaactgctctgctacttctagtttcagctggcatgcggactgaagatctcccaaagg
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Amino acid sequence

MWQLLLPTALLLLVSAGMRTEDLPKAVVFLEPQWYRVLEKDSVTLKCQGAYSPE
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DTGLYFSVKTNIRSSTRDWKDHKFKWRKDPQDK

From the foregoing, it will be appreciated that, although specific embodiments of the invention have been described herein for the purpose of illustration, various modifications may be made without deviating from the spirit and scope of the invention Accordingly, the present invention is not limited except as by the appended claims.

All patents, patent applications, publications, scientific articles, web sites, and other documents and materials referenced or mentioned herein are indicative of the levels of skill of those skilled in the art to which the invention pertains, and each such referenced document and material is hereby incorporated by reference to the same extent as if it had been incorporated by reference in its entirety individually or set forth herein in its entirety Additionally, all claims in this application, and all priority applications, including but not limited to original claims, are hereby incorporated in their entirety into, and form a part of, the written description of the invention Applicants reserve the right to physically incorporate into this specification any and all materials and information from any such patents, applications, publications, scientific articles, web sites, electronically available information, and other referenced materials or documents Applicants reserve the right to physically incorporate into any part of this document, including any part of the written description, the claims referred to above including but not limited to any original claims

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The specific methods and compositions described herein are representative of preferred embodiments and are exemplary and not intended as limitations on the scope of the invention Other objects, aspects, and embodiments will occur to those skilled in the art upon consideration of this specification, and are encompassed within the spirit of the invention as defined by the scope of the claims It will be readily apparent to one skilled in the art that varying substitutions and modifications may be made to the invention disclosed herein without departing from the scope and spirit of the invention. The invention illustratively described herein suitably may be practiced in the absence of any element or elements, or limitation or limitations, which is not specifically disclosed herein as essential Thus, for example, in each instance herein, in embodiments or examples of the present invention, any of the terms "comprising", "consisting essentially of", and "consisting of" may be replaced with either of the other two terms in the specification Also, the terms 'comprising", "including", containing", *etc.* are to be read expansively and without limitation The methods and processes illustratively described herein suitably may be practiced in differing orders of steps, and that they are not necessarily restricted to the orders of steps indicated herein or in the claims It is also that as used herein and in the appended claims, the singular forms 'a," "an," and "the" include plural reference unless the context clearly dictates otherwise. Thus, for example, a reference to "a host cell" includes a plurality (for example, a culture or population) of such host cells, and so forth. Under no circumstances may the patent be interpreted to be limited to the specific examples or embodiments or methods specifically disclosed herein Under no circumstances may the patent be interpreted to be limited by any statement made by any Examiner or any other official or employee of the Patent and Trademark Office unless such statement is specifically and without qualification or reservation expressly adopted in a responsive writing by Applicants

The terms and expressions that have been employed are used as terms of description and not of limitation, and there is no intent in the use of such terms and expressions to exclude any equivalent of the features shown and described or portions thereof, but it is recognized that various modifications are possible within the scope of the invention as claimed Thus, it will be understood that although the present invention has been specifically disclosed by preferred embodiments and optional features, modification and variation of the concepts herein disclosed may be resorted to by those skilled in the art,

and that such modifications and variations are considered to be within the scope of this invention as defined by the appended claims

The invention has been described broadly and generically herein. Each of the narrower species and subgeneric groupings falling within the generic disclosure also form part of the invention. This includes the generic description of the invention with a proviso or negative limitation removing any subject matter from the genus, regardless of whether or not the excised material is specifically recited herein For example, the subject matter of the instant invention may optionally exclude any of the subject matter or sequences described or included in related application published as U S S N 2003/0118592 A1 on June 26, 2003 (and the sequence listings of SEQ ID NOS 1-427 published by USPTO), by Ledbetter et al entitled "Binding Domain-Immunoglobulin Fusion Proteins"

Other embodiments are within the following claims In addition, where features or aspects of the invention are described in terms of Markush groups, those skilled in the art will recognize that the invention is also thereby described in terms of any individual member or subgroup of members of the Markush group

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In the claims

1 A non-naturally occurring single chain protein comprising i) a first polypeptide having a binding domain polypeptide capable of binding to a target molecule, said binding domain polypeptide comprising a heavy chain variable region, said heavy chain variable region comprising an amino acid substitution or deletion at one or more amino acid residues, ii) a second polypeptide comprising a connecting region attached to said first polypeptide, and iii) a third polypeptide comprising an N-terminally truncated immunoglobulin heavy chain constant region polypeptide attached to the second polypeptide, wherein said non-naturally occurring single-chain protein is capable of at least one immunological activity

2 A protein of claim 1 wherein said binding domain polypeptide is a single chain Fv

3 A protein of claim 1 wherein the one or more amino acid substitution or deletion in said heavy chain variable region is effective to increase expression or stability of said protein relative to a protein without said deletion or substitution

4 A protein of claim 1 wherein said binding domain polypeptide comprises an immunoglobulin light chain variable region polypeptide and an immunoglobulin heavy chain variable region polypeptide

5 A protein of claim 4 further comprising a second binding domain polypeptide capable of binding a second target molecule, said second binding domain polypeptide comprising an immunoglobulin light chain variable region polypeptide and an immunoglobulin heavy chain variable region polypeptide.

6 A protein of claim 5 wherein the first target molecule and the second target molecule are different

7 A protein of claim 5 wherein the first target molecule and the second target molecule are the same.

8 A protein of claim 1 wherein said binding domain polypeptide is a single chain Fv comprising one or more amino acid substitution in positions 9, 10, 11, 12, 108, 110, 112 in said heavy chain variable region

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9 A protein of claim 1 wherein said binding domain polypeptide is a single chain Fv comprising a an amino acid substitution at position 11 in said heavy chain variable region

10 A protein of claim 9 wherein the amino acid substituted for the amino acid at position of 11 of the single chain Fv heavy chain variable region is selected from serine, threonine, cysteine, tyrosine, asparagine, glutamine, aspartic acid, glutamic acid, lysine, arginine, and histidine.

11 A protein of claim 9 wherein a leucine at position of 11 of the single chain Fv heavy chain variable region is replaced with an amino acid other than serine

12 A protein of claim 9 where leucine is replaced by serine at position 11, and wherein said protein is capable of antibody dependent cell-mediated cytotoxicity and complement fixation, and is capable of binding to said target molecule decreasing a number of target cells

13 A protein of claim 9 where leucine is replaced by des-leucine at position 11

14 A protein of claim 12 having an increased recombinant expression or stability relative to said protein not having an amino acid substitution at position 11

15 A protein of claim 14 wherein the expression of said protein having an amino acid substitution at position 11 is 10-100 fold greater than said protein without a substitution at position 11

16 A protein of claim 14 wherein said expression is in mammalian cells

17 A protein of claim 1 wherein said binding domain polypeptide is a single chain Fv and the amino acid at position 11 of the heavy chain variable region of said single chain Fv has been deleted.

18 A protein of claim 1 wherein said binding domain polypeptide is a single chain Fv and said binding domain polypeptide comprises a light chain variable region, wherein said light chain variable region has an amino acid deletion or substitution at one or more of amino acid positions 12, 80, 81, 83, 105, 106, and 107

19 A protein of claim 18 wherein the amino acid at position 106 has been substituted or deleted

20 A protein of claim 2 wherein said binding domain polypeptide binds to a tumor antigen.

21 A protein of claim 2 wherein said binding domain polypeptide binds to an antigen on an immune effector cell.

22 A protein of claim 2 wherein said binding domain polypeptide binds to a cancer cell antigen.

23 A protein of claim 22 wherein said cancer cell antigen is a surface antigen

24 A protein of claim 22 wherein said cancer cell antigen is an intracellular antigen.

25 A protein of claim 1 wherein said binding domain polypeptide binds to a B cell antigen

26 A protein of claim 25 wherein said B cell antigen is selected from CD19, CD20, CD22, CD37, CD40, CD80, and CD86

27 A protein of claim 2 wherein said single chain Fv binds to a B cell antigen

28 A protein of claim 27 wherein said B cell antigen is selected from CD19, CD20, CD22, CD37, CD40, CD80, and CD86

29 A protein of claim 28 wherein said single chain Fv is selected from HD37 single chain Fv, 2H7 single chain Fv, G28-1 single chain Fv, and 4 4.220 single chain Fv

30 A protein of claim 2 wherein said single chain Fv is selected from HD37 single chain Fv, 2H7 single chain Fv, G28-1 single chain Fv, FC2-2, UCHL-1, 5B9, L6, 10A8, 2e12, 40.2 36, G19-4, 1D8, and 4 4 220 single chain Fv

31 A protein of claim 1 wherein said binding domain polypeptide is an scFv that binds to a B cell differentiation antigen

32 A protein of claim 31 wherein said B cell antigen is selected from CD19, CD20, CD22, CD37, and CD40

33 A protein of claim 1 wherein said binding domain polypeptide binds to a target selected from CD2, CD3, CD4, CD5, CD6, CD8, CD10, CD11b, CD14, CD19, CD20, CD21, CD22, CD23, CD24, CD25, CD28, CD30, CD37, CD40, CD43, CD50 (ICAM3), CD54 (ICAM1), CD56, CD69, CD80, CD86, CD134 (OX40), CD137 (41BB),

CD152 (CTLA-4), CD153 (CD30 ligand), CD154 (CD40 ligand), ICOS, L6, B7-H1, and HLA class II

34 A protein of claim 1 wherein said protein is capable of forming a complex comprising two or more of said proteins

35 A protein of claim 34 wherein said complex is a dimer

36 A protein of claim 1 wherein said protein is a monomer

37 A protein of claim 1 coupled to a drug, toxin, immunomodulator, polypeptide effector, isotope, label, or effector moiety

38 A protein of claim 1 wherein said at least immunological activity is selected from antibody dependent cell-mediated cytotoxicity, complement fixation, induction of apoptosis, induction of one or more biologically active signals, induction of one or more immune effector cells, activation of cellular differentiation, cellular activation, release of one or more biologically active molecules, and neutralization of an infectious agent or toxin.

39 A protein of claim 38 wherein said immunological activity comprises two immunological activities selected from antibody dependent cell-mediated cytotoxicity, complement fixation, induction of apoptosis, induction of one or more biologically active signals, induction of one or more immune effector cells, activation of cellular differentiation, cellular activation, release of one or more biologically active molecules, and neutralization of an infectious agent or toxin

40 A protein of claim 38 which is capable of induction of biologically active signals by activation or inhibition of one or more molecules selected from protein kinases protein phosphatases, G-proteins, cyclic nucleotides or other second messengers, ion channels, and secretory pathway components, or which is capable of induction of one or more immune effector cells selected from NK cells, monocytes, macrophages, B cells, T cells, mast cells, neutrophils, eosinophils, and basophils

41 A protein of claim 40 wherein said induction of one or more immune effector cells leads to antibody dependent cell-mediated cytotoxicity or the release of one or more biologically active molecules

42 A protein of claim 38 which is capable of cellular activation, wherein said activation leads to changes in cellular transcriptional activity

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43 A protein of claim 42 wherein said cellular transcriptional activity is increased.

44 A protein of claim 42 wherein said cellular transcriptional activity is decreased.

45 A protein of claim 38 wherein said one or more biologically active molecules is a protease

46 A protein of claim 38 wherein said one or more biologically active molecules is a cytokine.

47 A protein of claim 46 wherein said cytokine is selected from monokines, lymphokines, chemokines, growth factors, colony stimulating factors, interferons, and interleukins

48 A protein of claim 38 which is capable of neutralization of an infectious agent, wherein said infectious agent is a bacterium, a virus, a parasite, or a fungus

49 A protein of claim 38 which is capable of neutralization of a toxin, wherein said toxin is selected from endotoxins and exotoxins

50 A protein of claim 38 which is capable of neutralization of a toxin, wherein said toxin is an exotoxin selected from anthrax toxin, cholera toxin, diphtheria toxin, pertussis toxin, *E coli* heat-labile toxin LT, *E. coli* heat stable toxin ST, shiga toxin *Pseudomonas* Exotoxin A, botulinum toxin, tetanus toxin, *Bordetella pertussis* AC toxin, and *Bacillus anthracus* EF

51 A protein of claim 38 which is capable of neutralization of a toxin, wherein said toxin is an endotoxin selected from saxitoxins, tetrodotoxin, mushroom toxins, aflatoxins, pyrrolizidine alkaloids, phytohemagglutinins, and grayanotoxins

52. A protein of claim 1 wherein said protein is capable of binding to an intracellular target to effect a cellular function.

53 A protein of claim 1 wherein said binding domain polypeptide comprises a light chain variable region attached to said heavy chain variable region by a binding domain linker, wherein said binding domain linker comprises one or more peptide having a sequence Gly-Gly-Gly-Gly-Ser

54 A protein of claim 53 comprising three Gly-Gly-Gly-Gly-Ser peptides

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55 A protein of claim 1 wherein said binding domain polypeptide comprises wild type or engineered immunoglobulin variable region obtained from species selected from human, murine, rat, pig, and monkey

56 A protein of claim 1 wherein said binding domain polypeptide comprises a humanized immunoglobulin variable region.

57 A protein of claim 2 wherein said N-terminally truncated immunoglobulin heavy chain constant region polypeptide comprises an IgG CH2 constant region polypeptide attached to an immunoglobulin heavy chain IgG CH3 constant region polypeptide.

58 A protein of claim 2 wherein said N terminally truncated immunoglobulin heavy chain constant region polypeptide consist essentially of an IgG CH2 constant region polypeptide attached to an immunoglobulin heavy chain IgG CH3 constant region polypeptide.

59 A protein of claim 2 wherein said N-terminally truncated immunoglobulin heavy chain constant region polypeptide comprises an IgG CH2 constant region polypeptide attached to an immunoglobulin heavy chain IgG CH3 constant region polypeptide

60 A protein of claim 2 wherein said N-terminally truncated immunoglobulin heavy chain constant region polypeptide consist essentially of an IgG CH2 constant region polypeptide attached to an immunoglobulin heavy chain IgG CH3 constant region polypeptide

61 A protein of claim 1 wherein said binding domain polypeptide is a single chain Fv that comprises at least a portion of a human constant region.

62 A protein of claim 2 wherein said binding domain polypeptide is a single chain Fv that comprises at least a portion of a human constant region

63 A protein of claim 1 wherein said connecting region comprises a naturally occurring hinge region selected from a human hinge or portion thereof, human IgG hinge or a portion thereof, human IgA hinge or a portion thereof, human IgE hinge or a portion thereof, camelid hinge region or a portion thereof, IgG1 llama hinge region or portion thereof, nurse shark hinge region or portion thereof, and spotted ratfish hinge region or a portion thereof

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64 A protein of claim 1 wherein said connecting region comprises a human IgE hinge or a portion thereof

65 A protein of claim 1 wherein said connecting region comprises a human IgG1, IgG2, IgG3 or IgG4 hinge region having either zero or one cysteine residue.

66 A protein of claim 1 wherein said connecting region comprises a human IgGA hinge region having between zero and two cysteine residues

67 A protein of claim 1 wherein said connecting region comprises a wild type human IgG1 immunoglobulin hinge region.

68 A protein of claim 1 wherein said connecting region comprises a glycosylation site.

69 A protein of claim 1 wherein said connecting region has no cysteine residues capable of forming disulfide bonds

70 A protein of claim 1 wherein said connecting region has one cysteine residue.

71 A protein of claim 1 wherein said connecting region comprises a mutated wild-type immunoglobulin hinge region polypeptide comprising no more than one cysteine residue.

72 A protein of claim 1 wherein said connecting region is altered so that said protein has a reduced ability to dimerize

73 A protein of claim 1 where said connecting region comprises three cysteine residues and one proline residue, wherein one or more of said cysteine residues is deleted or substituted and said proline reside is substituted or deleted

74 A protein of claim 1 wherein said connecting region comprises a mutated wild-type immunoglobulin hinge region polypeptide comprising first, second, and third cysteine residues, where said first cysteine reside is N-terminal to said second cysteine and said second cysteine is N-terminal to said third cysteine, wherein said first cysteine residue is substituted or deleted

75 A protein of claim 74 wherein said wild-type hinge region polypeptide is from human IgG1

76 A non-naturally occurring single chain protein comprising 1) a first polypeptide having a binding domain polypeptide capable of binding to a target molecule, said binding domain polypeptide comprising a heavy chain variable region comprising one

or more amino acid deletion or substitution in positions 9, 10, 11, 12, 108, 110, 112, ii) a second polypeptide comprising a connecting region attached to said first polypeptide, and iii) a third polypeptide comprising an N-terminally truncated immunoglobulin heavy chain constant region polypeptide attached to the second polypeptide, wherein said non-naturally occurring single-chain protein is capable of at least one immunological activity, and wherein said protein has an increased recombinant expression or stability relative to said protein not having an amino acid deletion or substitution.

77 A non-naturally occurring single chain Fv protein comprising i) a first polypeptide having a binding domain polypeptide capable of binding to a target molecule on a target cell, said binding domain polypeptide comprising a heavy chain variable region wherein leucine at position 11 in the first framework region of the heavy chain variable region is deleted or substituted with another amino acid, ii) a second polypeptide comprising a connecting region attached to said first polypeptide, and iii) a third polypeptide comprising an N-terminally truncated immunoglobulin heavy chain constant region polypeptide attached to the second polypeptide, wherein said non-naturally occurring single-chain protein (1) is capable of binding to said target molecule, and (2) is capable of antibody dependent cell-mediated cytotoxicity and complement fixation, and (3) is capable of decreasing the number of target cells

78 A protein of claim 77 further comprising a substitution or deletion of the amino acid at position 10 in the first framework region of the heavy chain variable region.

79 A protein of claim 77 wherein the amino acid substitution at position 11 is effective to increase expression or stability of said single chain Fv protein relative to a single chain Fv protein without said deletion or substitution.

80 A protein of claim 77 wherein the numbering of the residues is the EU index according to Kabat.

81 A protein of claim 77 wherein said connecting region comprises a proline and first, second, and third cysteine residues, where said first cysteine reside is N-terminal to said second cysteine and said second cysteine is N-terminal to said third cysteine, said third cysteine residue is N-terminal to said proline residue

82 A protein of claim 77 wherein said connecting region comprises an IgA hinge region or portion thereof

83. A protein of claim 81 where said single chain protein comprises a single chain Fv binding domain from a 2H7 hybridoma, wherein said second cysteine residue is replaced by serine and said proline residue is replaced by serine in the connecting region, and said heavy chain constant region comprises CH2 and CH3 domains from IgG_1

84 A protein of claim 82 wherein said single chain protein comprises a single chain Fv binding domain from a 2H7 hybridoma, said connecting region comprises a murine IgA hinge or portion thereof, and said heavy chain constant region comprises CH2 and CH3 domains from murine IgA, wherein said CH3 comprises a deletion or substitution in four amino acids that render the IgA heavy chain constant region incapable of associating with a J chain polypeptide

85 A protein of claim 81 where said single chain protein comprises a 2H7 single chain Fv binding domain, wherein said first, second, and third cysteine residues in the connecting region are replaced by serine and said proline residue in the connecting region is replaced by serine, said heavy chain constant region comprising CH2 and CH3 domains from IgG_1 wherein lysine is replaced by serine at position 322 in said CH2 region.

86 A protein of claim 81 where said single chain protein comprises a single chain Fv binding domain from a 2H7 hybridoma, wherein in the connecting region said second and third cysteine residues are replaced by serine and said proline residue is replaced by serine, said heavy chain constant region comprising CH2 and CH3 domains from IgG_1 wherein lysine is replaced by serine at position 322 in said CH2 region

87 A protein of claim 81 where said single chain protein comprises a single chain Fv binding domain from a 2H7 hybridoma, wherein in the connecting region said first, second, and third cysteine residues are replaced by serine and said proline residue is replaced by serine, said heavy chain constant region comprising CH2 and CH3 domains from IgG_1 wherein proline is replaced by serine at position 331 in said CH2 region.

88 A protein of claim 81 where said single chain protein comprises a single chain Fv binding domain from a 2H7 hybridoma, wherein in the connecting region said second and third cysteine residues are replaced by serine and said proline residue is replaced by serine, said heavy chain constant region comprising CH2 and CH3 domains from IgG_1 wherein proline is replaced by serine at position 331 in said CH2 region

89 A protein of claim 81 where said single chain protein comprises a 2H7 single chain Fv binding domain, wherein in the connecting region said first, second, and third cysteine residues are replaced by serine and said proline residue is replaced by serine, said heavy chain constant region comprising CH2 and CH3 domains from IgG_1 wherein threonine is replaced by asparagine at position 256 in said CH2 region.

90 A protein of claim 81 where said single chain protein comprises a 2H7 single chain Fv binding domain, where in the connecting region said first, second, and third cysteine residues are replaced by serine and said proline residue is replaced by serine, said heavy chain constant region comprises 2H7 single chain Fv CH2 and CH3 domains from IgG_1 where in the CH2 domain arginine is replaced by glutamine at position 255 threonine is replaced by asparagine at position 256, proline is replaced by alanine at position 257, and glutamic acid is replaced by lysine at position 258

91 A protein of claim 81 where said single chain protein comprises a single chain Fv binding domain from a 2H7 hybridoma, where in the connecting region said first, second and third cysteine residues are replaced by serine and said proline residue is replaced by serine, said heavy chain constant region comprising CH2 and CH3 domains from IgG_1 wherein lysine is replaced by glutamine at position 290 in said CH2 region

92 A protein of claim 81 where said single chain protein comprises a single chain Fv binding domain from a 2H7 hybridoma, where in the connecting region said first, second and third cysteine residues are replaced by serine and said proline residue is replaced by serine, said heavy chain constant region comprising CH2 and CH3 domains from IgG_1 wherein alanine is replaced by proline at position 339 in said CH2 region.

93 A protein of claim 81 where said single chain protein comprises a single chain Fv binding domain from a G28-1 hybridoma, where in the connecting region said first, second, and third cysteine residues are replaced by serine and said proline residue is replaced by serine, said heavy chain constant region comprising CH2 and CH3 domains from IgG1

94 A protein of claim 81 where said single chain protein comprises a single chain Fv binding domain from a G28-1 hybridoma, where in the connecting region said second and third cysteine residues are replaced by serine and said proline residue is replaced by serine, said heavy chain constant region comprising CH2 and CH3 domains from IgG_1

95 A protein of claim 81 where said single chain protein comprises a single chain Fv binding domain from a G28-1 hybridoma, where in the connecting region said second cysteine residue is replaced by serine and said proline residue is replaced by serine, said heavy chain constant region comprising CH2 and CH3 domains from IgG_1

96 A protein of claim 81 said single chain protein comprises a single chain Fv binding domain from a G28-1 hybridoma, where in the connecting region said first and second cysteine residues are replaced by serine, said heavy chain constant region comprising CH2 and CH3 domains from IgG_1

97 A protein of claim 81 said single chain protein comprises a single chain Fv binding domain from a FC2-2 hybridoma, where in the connecting region said first, second, and third cysteine residues are replaced by serine and said proline residue is replaced by serine, said heavy chain constant region comprising CH2 and CH3 domains from IgG_1

98 A protein of claim 81 where said single chain protein comprises a single chain Fv binding domain from a UCHL-1 hybridoma, where in the connecting region said first, second, and third cysteine residues are replaced by serine and said proline residue is replaced by serine, said heavy chain constant region comprising CH2 and CH3 domains from IgG_1

99 A protein of claim 81 where said single chain protein comprises a single chain Fv binding domain from a 5B9 hybridoma, where in the connecting region said first, second, and third cysteine residues are replaced by serine and said proline residue is replaced by serine, where said heavy chain constant region comprises CH2 and CH3 domains from IgG_1

100 A protein of claim 81 where said single chain protein comprises a single chain Fv binding domain from a 2H7 hybridoma, where in the connecting region said first, second and third cysteine residues are replaced by serine and said proline residue is replaced by serine, said heavy chain constant region comprising CH2 and CH3 domains from IgG_1

101 A protein of claim 81 where said single chain protein comprises a single chain Fv binding domain from a 2H7 hybridoma, where in the connecting region said second and third cysteine residues are replaced by serine and said proline residue is

replaced by serine, where said heavy chain constant region comprises CH2 and CH3 domains from IgG_1

102 A protein of claim 81 where said single chain protein comprises a single chain Fv binding domain from a G28-1 hybridoma, where in the connecting region said first and third cysteine residues are replaced by serine and said proline residue is replaced by serine, said heavy chain constant region comprising CH2 and CH3 domains from IgG_1

103 A protein of claim 81 where said single chain protein comprises a single chain Fv binding domain from a G28-1 hybridoma comprising a connecting region wherein said third cysteine residue is replaced by serine and said proline residue is replaced by serine, said heavy chain constant region comprising CH2 and CH3 domains from IgG_1

104 A protein of claim 81 where said single chain protein comprises a single chain Fv binding domain from a G28-1 hybridoma, where in the connecting region said first cysteine residue is replaced by serine, said heavy chain constant region comprising CH2 and CH3 domains from IgG_1

105 A protein of claim 82 wherein said single chain protein comprises a single chain Fv binding domain from a G28-1 hybridoma, wherein said connecting region comprising a murine IgA hinge region, said heavy chain constant region comprises CH2 and CH3 domains from murine IgA and said CH3 comprises a deletion or substitution in four amino acids that render the IgA heavy chain constant region incapable of associating with a J chain polypeptide

106 A protein of claim 82 wherein said single chain protein comprises a single chain Fv binding domain from a G28-1 hybridoma, wherein said connecting region comprising a human IgA hinge region and said heavy chain constant region comprises CH2 and CH3 domains from human IgA, where said CH3 comprises a deletion or substitution in four amino acids that render the IgA heavy chain constant region incapable of associating with a J chain polypeptide.

107 A protein of claim 81 where said single chain protein comprises a single chain Fv binding domain from a HD37 hybridoma, where in the connecting region said first, second, and third cysteine residues are replaced by serine and said proline reside is replaced by serine, and said heavy chain constant region comprising CH2 and CH3 domains from IgG_1

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108 A protein of claim 81 where said single chain protein comprises a L6 single-chain Fv binding domain, where in the connecting region said first, second, and third cysteine residues are replaced by serine and said proline reside is replaced by serine, and said heavy chain constant region comprises CH2 and CH3 domains from IgG_1

109 A non-naturally-occurring single chain Fv protein comprising i) a first polypeptide having a binding domain polypeptide capable of binding to a target molecule, said binding domain polypeptide comprising a heavy chain variable region where leucine is replaced by serine at position 11 in the first framework region of the heavy chain variable region, wherein said protein has an increased expression or stability in mammalian cells relative to a protein not having said amino acid substitution, ii) a second polypeptide comprising a connecting region attached to said first polypeptide, and iii) a third polypeptide comprising an N-terminally truncated immunoglobulin heavy chain constant region polypeptide attached to the second polypeptide, wherein said non-naturally occurring single-chain Fv protein is capable of at least one immunological activity

110 A non-naturally occurring single chain Fv protein comprising i) a first polypeptide having a binding domain polypeptide capable of binding to a target molecule, said binding domain polypeptide comprising a heavy chain variable region wherein leucine at is replaced by serine at position 11 in the first framework region of the heavy chain variable region; and ii) a second polypeptide comprising an N-terminally truncated IgE immunoglobulin heavy chain constant region polypeptide attached to the second polypeptide, wherein said non-naturally occurring single-chain protein is capable of at least one immunological activity

111 A protein of claim 110 wherein the amino acid substitution at position 11 is effective to increase expression or stability of said single chain Fv protein relative to a single chain Fv protein without said deletion or substitution

112 A protein of claim 110 where said single chain protein comprises a single chain Fv binding domain from a 2H7 hybridoma and said heavy chain constant region comprises CH2, CH3, and CH4 domains from IgE

113 A protein of claim 110 wherein said single chain protein comprises a single chain Fv binding domain from a 2H7 hybridoma and said heavy chain constant region comprises CH2, CH3, and CH4 domains from murine IgE

114 A protein of claim 110 wherein said single chain protein comprises a single chain Fv binding domain from a G28-1 hybridoma, wherein said heavy chain constant region comprises CH2, CH3, and CH4 domains from murine IgE.

115 A protein of claim 110 wherein said single chain protein comprises a single chain Fv binding domain from a G28-1 hybridoma, wherein said heavy chain constant region comprises CH2, CH3, and CH4 domains from human IgE

116 A non-naturally occurring single chain protein, including a first polypeptide comprising a binding domain polypeptide capable of binding to a target molecule, a second polypeptide comprising a connecting region attached to said first polypeptide, a third polypeptide comprising an N-terminally truncated immunoglobulin heavy chain constant region polypeptide attached to the second polypeptide, wherein said connecting region comprises first, second, and third cysteine residues, where said first cysteine reside is N-terminal to said second cysteine and said second cysteine is N-terminal to said third cysteine, wherein one or both of said second and third cysteine residues is substituted or deleted, and wherein said non-naturally occurring single-chain protein is capable of at least one immunological activity

117 A protein of claim 116 wherein said binding domain polypeptide is a single chain Fv

118 A protein of claim 116 wherein said binding domain polypeptide comprises an immunoglobulin light chain variable region polypeptide and an immunoglobulin heavy chain variable region polypeptide

119 A protein of claim 118 further comprising a second binding domain polypeptide capable of binding a second target molecule, said second binding domain polypeptide comprising an immunoglobulin light chain variable region polypeptide and an immunoglobulin heavy chain variable region polypeptide

120 A protein of claim 116 wherein said binding domain polypeptide is a single chain Fv comprising a heavy chain variable region, wherein said heavy chain variable region has an amino acid deletion or substitution at one or more of amino acid positions 9, 10, 11, 12, 108, 110, 112

121 A protein of claim 117 wherein said binding domain polypeptide is a single chain Fv comprising a light chain variable region, wherein said light chain variable

region has an amino acid deletion or substitution at one or more of amino acid positions 12, 80, 81, 83, 105, 106, and 107

122 A protein of claim 116 wherein said binding domain polypeptide is a single chain Fv comprising a heavy chain variable region, wherein said heavy chain variable region has an amino acid substitution at amino acid position 11

123 A protein of claim 122 wherein acid at position of 11 of the single chain Fv heavy chain variable region is selected from serine, threonine, cysteine, tyrosine, asparagine, glutamine, aspartic acid, glutamic acid, lysine, arginine, and histidine

124 A protein of claim 122 wherein the amino acid substituted for the amino acid at position of 11 of the single chain Fv heavy chain variable region is selected from serine, threonine, cysteine, tyrosine, asparagine, and glutamine

125 A protein of claim 122 wherein leucine is replaced by serine at position 11

126 A protein of claim 122 wherein leucine is replaced by des-leucine at position 11

127 A protein of claim 116 wherein said binding domain polypeptide is a single chain Fv and the amino acid at position 11 of the heavy chain variable region of said single chain Fv has been deleted

128 A protein of claim 121 where leucine is replaced by serine at position 105

129 A protein of claim 117 wherein said binding domain polypeptide binds to a tumor antigen.

130 A protein of claim 117 wherein said binding domain polypeptide binds to an antigen on an immune effector cell

131 A protein of claim 117 wherein said binding domain polypeptide binds to a cancer cell antigen

132 A protein of claim 131 wherein said cancer cell antigen is a surface antigen

133 A protein of claim 131 wherein said cancer cell antigen is an intracellular antigen

134 A protein of claim 116 wherein said binding domain polypeptide binds to a B cell antigen

135 A protein of claim 135 wherein said B cell antigen is selected from CD19, CD20, CD22, CD37, CD40, CD80, and CD86

136 A protein of claim 117 wherein said single chain Fv binds to a B cell antigen.

137 A protein of claim 136 wherein said B cell antigen is selected from CD19, CD20, CD22, CD37, CD40, CD80, and CD86

138 A protein of claim 137 wherein said single chain Fv is selected from HD37 single chain Fv, 2H7 single chain Fv, G28-1 single chain Fv, and 4.4 220 single chain Fv

139 A protein of claim 117 wherein said single chain Fv is selected from HD37 single chain Fv, 2H7 single chain Fv, G28-1 single chain Fv, FC2-2, UCHL-1, 5B9, L6, 10A8, 2e12, 40 2 36, G19-4, 1D8, and 4 4 220 single chain Fv

140 A protein of claim 116 wherein said binding domain polypeptide is an scFv that binds to a B cell differentiation antigen.

141 A protein of claim 136 wherein said B cell antigen is selected from CD19, CD20, CD22, CD37, and CD40

142 A protein of claim 116 wherein said binding domain polypeptide binds to a target selected from CD2, CD3, CD4, CD5, CD6, CD8, CD10, CD11b, CD14, CD19, CD20, CD21, CD22, CD23, CD24, CD25, CD28, CD30, CD37, CD40, CD43, CD50 (ICAM3), CD54 (ICAM1), CD56, CD69, CD80, CD86, CD134 (OX40), CD137 (41BB), CD152 (CTLA-4), CD153 (CD30 ligand), CD154 (CD40 ligand), ICOS, L6, B7-H1, and HLA class II

143 A protein of claim 116 wherein said protein is capable of forming a complex comprising two or more of said proteins

144 A protein of claim 143 wherein said complex is a dimer

145 A protein of claim 116 wherein said protein is a monomer

146 A protein of claim 116 coupled to a drug, toxin, immunomodulator, polypeptide effector, isotope, label, or effector moiety

147 A protein of claim 116 wherein said immunological activity is selected from antibody dependent cell-mediated cytotoxicity, complement fixation, induction of apoptosis, induction of one or more biologically active signals, induction of one or more immune effector cells, activation of cellular differentiation, cellular activation,

release of one or more biologically active molecules, and neutralization of an infectious agent or toxin.

148 A protein of claim 147 which is capable of induction of biologically active signals by activation or inhibition of one or more molecules selected from protein kinases, protein phosphatases, G-proteins, cyclic nucleotides or other second messengers, ion channels, and secretory pathway components

149 A protein of claim 147 which is capable of induction of one or more immune effector cells selected from NK cells, monocytes, macrophages, B cells, T cells, mast cells, neutrophils, eosinophils, and basophils

150 A protein of claim 149 wherein said induction of one or more immune effector cells leads to antibody dependent cell-mediated cytotoxicity or the release of one or more biologically active molecules

151 A protein of claim 147 which is capable of cellular activation, wherein said activation leads to changes in cellular transcriptional activity

152 A protein of claim 151 wherein said cellular transcriptional activity is increased.

153 A protein of claim 151 wherein said cellular transcriptional activity is decreased.

154 A protein of claim 147 wherein said one or more biologically active molecules is a protease.

155 A protein of claim 147 wherein said one or more biologically active molecules is a cytokine.

156 A protein of claim 155 wherein said cytokine is selected from monokines, lymphokines, chemokines, growth factors, colony stimulating factors, interferons, and interleukins

157 A protein of claim 147 which is capable of neutralization of an infectious agent, wherein said infectious agent is a bacterium, a virus, a parasite, or a fungus

158 A protein of claim 147 A protein which is capable of neutralization of a toxin, wherein said toxin is selected from endotoxins and exotoxins

159 A protein of claim 147 which is capable of neutralization of a toxin, wherein said toxin is an exotoxin selected from anthrax toxin, cholera toxin, diphtheria

toxin, pertussis toxin, *E coli* heat-labile toxin LT, *E. coli* heat stable toxin ST, shiga toxin *Pseudomonas* Exotoxin A, botulinitm toxin, tetanus toxin, *Bordetella pertussis* AC toxin, and *Bacillus anthracis* EF

160 A protein of claim 147 which is capable of neutralization of a toxin, wherein said toxin is an endotoxin selected from saxitoxins, tetrodotoxin, mushroom toxins, aflatoxins, pyrrolizidine alkaloids, phytohemagglutinins, and grayanotoxins

161 A protein of claim 116 wherein said binding domain polypeptide comprises a light chain variable region attached to said heavy chain variable region by a binding domain linker, wherein said binding domain linker comprises one or more peptide having a sequence Gly-Gly-Gly-Gly-Ser

162 A protein of claim 116 comprising three Gly-Gly-Gly-Gly-Ser peptides

163 A protein of claim 116 wherein said binding domain polypeptide comprises wild type or engineered immunoglobulin variable region obtained from species selected from human, murine, rat, pig, and monkey

164 A protein of claim 116 wherein said protein is capable of binding to an intracellular target to effect a cellular function.

165 A protein of claim 116 wherein said N-terminally truncated immunoglobulin heavy chain constant region polypeptide comprises an IgG CH2 constant region polypeptide attached to an immunoglobulin heavy chain IgG CH3 constant region polypeptide

166 A protein of claim 116 wherein said immunoglobulin heavy chain constant region polypeptide is without a functionally active CH1 domain.

167 A protein of claim 116 said N-terminally truncated immunoglobulin heavy chain constant region polypeptide consist essentially of an IgG CH2 constant region polypeptide attached to an immunoglobulin heavy chain IgG CH3 constant region polypeptide

168 A protein of claim 116 comprising at least a portion of a human constant region.

169 A protein of claim 116 wherein said connecting region comprises a naturally occurring hinge region selected from a human hinge or portion thereof, human IgG hinge or a portion thereof, human IgA hinge or a portion thereof, human IgE hinge or

a portion thereof, camelid hinge region or a portion thereof, IgG1 llama hinge region or portion thereof, nurse shark hinge region or portion thereof, and spotted ratfish hinge region or a portion thereof

170 A protein of claim 116 wherein said connecting region comprises a human IgE hinge or a portion thereof.

171 A protein of claim 116 wherein said connecting region comprises a human IgG1, IgG2, IgG3 or IgG4 hinge region having either zero or one cysteine residue.

172 A protein of claim 116 wherein said connecting region comprises a human IgGA hinge region having between zero and two cysteine residues

173 A protein of claim 116 wherein said connecting region comprises a wild type human IgG1 immunoglobulin hinge region.

174 A protein of claim 116 wherein said connecting region comprises a glycosylation site

175 A protein of claim 116 wherein said connecting region has no cysteine residues capable of forming disulfide bonds

176 A protein of claim 116 wherein said connecting region has one cysteine residue

177 A protein of claim 116 wherein said connecting region comprises a mutated wild-type immunoglobulin hinge region polypeptide comprising no more than one cysteine residue

178 A protein of claim 116 wherein said connecting region is altered so that said protein has a reduced ability to dimerize.

179 A protein of claim 116 wherein said connecting region comprises a proline and first, second, and third cysteine residues, where said first cysteine reside is N-terminal to said second cysteine and said second cysteine is N-terminal to said third cysteine, said third cysteine residue is N-terminal to said proline residue.

180 A protein of claim 179 where said single chain protein comprises a CTLA-4 single chain Fv binding domain, wherein in the connecting region said first, second, and third cysteine residues are replaced by serine and said proline residue is replaced by serine, said heavy chain constant region comprising CH2 and CH3 domains from IgG_1 wherein proline is replaced by serine at position 238 in said CH2 domain

181 A protein of claim 179 where said single chain protein comprises a CTLA-4 single chain Fv binding domain and said heavy chain constant region comprises CH2 and CH3 domains from IgG_1

182 A protein of claim 179 where said single chain protein comprises a FC2-2 single chain Fv binding domain, wherein in the connecting region said first, second, and third cysteine residues are replaced by serine and said proline residue is replaced by serine, said heavy chain constant region comprising CH2 and CH3 domains from IgG_1

183 A protein of claim 179 where said single chain protein comprises a UCHL-1 single chain Fv binding domain, wherein in the connecting region said first, second, and third cysteine residues are replaced by serine and said proline residue is replaced by serine, said heavy chain constant region comprising CH2 and CH3 domains from IgG_1

184 A protein of claim 179 where said single chain protein comprises a 5B9 single chain Fv binding domain, wherein in the connecting region said first, second, and third cysteine residues are replaced by serine and said proline residue is replaced by serine, said heavy chain constant region comprising CH2 and CH3 domains from IgG_1

185 A protein of claim 179 where said single chain protein comprises a 2H7 scFv single chain Fv binding domain, wherein in the connecting region said first, second, and third cysteine residues are replaced by serine and said proline residue is replaced by serine, said heavy chain constant region comprising CH2 and CH3 domains from IgG_1

186. A protein of claim 179 where said single chain protein comprises a 2H7 single chain Fv binding domain, wherein in the connecting region said proline residue is replaced by serine, said heavy chain constant region comprising CH2 and CH3 domains from IgG_1

187 A protein of claim 179 where said single chain protein comprises a 2H7 single chain Fv comprising a connecting region wherein said first, second, and third cysteine residues are replaced by serine and said proline residue is replaced by serine, said heavy chain constant region comprising CH2 and CH3 domains from IgG_1 wherein phenylalanine is replaced by tyrosine at position 405 in said CH3 region

188 A protein of claim 179 where said single chain protein comprises a 2H7 single chain Fv binding domain, wherein in the connecting region said first, second,

and third cysteine residues are replaced by serine and said proline residue is replaced by serine, said heavy chain constant region comprises CH2 and CH3 domains from IgG_1 wherein phenylalanine is replaced by alanine at position 405 in said CH3 region.

189. A protein of claim 179 where said single chain protein comprises a 2H7 single chain Fv binding domain, wherein in the connecting region said first, second, and third cysteine residues are replaced by serine and said proline residue is replaced by serine, said heavy chain constant region comprising CH2 and CH3 domains from IgG_1 wherein tyrosine is replaced by alanine at position 407 in said CH3 region.

190. A protein of claim 189 having an apparent molecular weight of about 75 kDa.

191 A protein of claim 179 where said single chain protein comprises a 2H7 single chain Fv binding domain, wherein in the connecting region said first, second, and third cysteine residues are replaced by serine and said proline residue is replaced by serine, said heavy chain constant region comprising CH2 and CH3 domains from IgG_1 wherein phenylalanine is replaced by alanine at position 405 and tyrosine is replaced by alanine at position 407 in said CH3 region

192 A protein of claim 179 where said single chain protein comprises a 2H7 single chain Fv binding domain, wherein in the connecting region said second and third cysteine residues are replaced by serine and said proline residue is replaced by serine, said heavy chain constant region comprising CH2 and CH3 domains from IgG_1

193 A protein of claim 179 where said single chain protein comprises a 2H7 single chain Fv binding domain, wherein in the connecting region said first and third cysteine residues are replaced by serine and said proline residue is replaced by serine, said heavy chain constant region comprising CH2 and CH3 domains from IgG_1

194 A protein of claim 179 where said single chain protein comprises a 2H7 single chain Fv binding domain, wherein in the connecting region said first and second cysteine residues are replaced by serine, said heavy chain constant region comprising CH2 and CH3 domains from IgG_1

195 A protein of claim 179 where said single chain protein comprises a 2H7 single chain Fv comprising a connecting region wherein said second cysteine residue is replaced by serine and said proline residue is replaced by serine, said heavy chain constant region comprising CH2 and CH3 domains from IgG_1

196 A protein of claim 179 where said single chain protein comprises a 2H7 single chain Fv comprising a connecting region wherein said third cysteine residue is replaced by serine, said heavy chain constant region comprising CH2 and CH3 domains from IgG_1

197 A protein of claim 179 where said single chain protein comprises a 2H7 single chain Fv binding domain, wherein in the connecting region said first cysteine residue is replaced by serine, and where said heavy chain constant region comprises 2H7 single chain Fv CH2 and CH3 domains

198. A protein of claim 179 where said single chain protein comprises a HD37 single chain Fv binding domain, wherein in the connecting region said first, second and third cysteine residues are replaced by serine and said proline residue is replaced by serine, said heavy chain constant region comprising CH2 and CH3 domains from IgG_1

199 A protein of claim 179 where said single chain protein comprises a 2H7 single chain Fv binding domain and said heavy chain constant region comprises CH2 and CH3 constant region polypeptides from llama IgG_1

200 A protein of claim 179 where said single chain protein comprises a 2H7 single chain Fv binding domain and said heavy chain constant region comprises CH2 and CH3 constant region polypeptides from llama IgG_2

201 A protein of claim 179 where said single chain protein comprises a 2H7 single chain Fv binding domain and said heavy chain constant region comprises CH2 and CH3 constant region polypeptides from llama IgG_3

202 A protein of claim 179 where said single chain protein comprises a CD-16-6 single chain Fv binding domain, wherein in the connecting region said first, second, and third cysteine residues are replaced by serine and said proline residue is replaced by serine, said heavy chain constant region comprising CH2 and CH3 domains from IgG_1 wherein proline is replaced by serine at position 238 in said CH2 region

203 A protein of claim 179 where said single chain protein comprises a CD-16 receptor binding domain, wherein in the connecting region said first, second, and third cysteine residues are replaced by serine and said proline residue is replaced by serine, said heavy chain constant region comprising CH2 and CH3 domains from IgG_1 wherein proline is replaced by serine at position 238 in said CH2 region

204 A protein of claim 179 where said single chain protein comprises a 2e12 single chain Fv binding domain, wherein in the connecting region said first, second, and third cysteine residues are replaced by serine and said proline residue is replaced by serine, said heavy chain constant region comprising CH2 and CH3 domains from IgG_1 wherein proline is replaced by serine at position 238 in said CH2 region, and wherein said CH3 domain is attached to a polypeptide comprising CD80 transmembrane and cytoplasmic tail domains

205 A protein of claim 179 where said single chain protein comprises a 10A8 scFv single chain Fv binding domain, wherein in the connecting region said first, second, and third cysteine residues are replaced by serine and said proline residue is replaced by serine, said heavy chain constant region comprising CH2 and CH3 domains from IgG_1 wherein proline is replaced by serine at position 238 in said CH2 region, and wherein said CH3 domain is attached to a polypeptide comprising CD80 transmembrane and cytoplasmic tail domains

206 A protein of claim 179 where said single chain protein comprises a single chain Fv binding domain from a 40 2 36 hybridoma, where in the connecting region said first second and third cysteine residues are replaced by serine and said proline residue is replaced by serine, said heavy chain constant region comprising CH2 and CH3 domains from IgG_1 wherein proline is replaced by serine at position 238 in said CH2 region, and wherein said CH3 domain is attached to a polypeptide comprising CD80 transmembrane and cytoplasmic tail domains

207 A protein of claim 179 where said single chain protein comprises a single chain Fv binding domain from a 2H7 hybridoma, where in the connecting region said first, second and third cysteine residues are replaced by serine and said proline residue is replaced by serine, said heavy chain constant region comprising CH2 and CH3 domains from IgG_1 wherein proline is replaced by serine at position 238 in said CH2 region, and wherein said CH3 domain is attached to a polypeptide comprising CD80 transmembrane and cytoplasmic tail domains

208 A protein of claim 179 where said single chain protein comprises a single chain Fv binding domain from a G19-4 hybridoma, where in the connecting region said first, second and third cysteine residues are replaced by serine and said proline residue is replaced by serine, said heavy chain constant region comprising CH2 and CH3 domains

from IgG_1 wherein proline is replaced by serine at position 238 in said CH2 region, and wherein said CH3 domain is attached to a polypeptide comprising CD80 transmembrane and cytoplasmic tail domains

209 A protein of claim 179 where said single chain protein comprises a single chain Fv binding domain from a G19-4 hybridoma, said heavy chain constant region comprising CH2 and CH3 domains from IgG_1 wherein said CH3 domain is attached to a polypeptide comprising CD80 transmembrane and cytoplasmic tail domains

210 A protein of claim 179 where said single chain protein comprises a single chain Fv binding domain from a 2e12 hybridoma, where in the connecting region said first, second and third cysteine residues are replaced by serine and said proline residue is replaced by serine, said heavy chain constant region comprising CH2 and CH3 domains from IgG_1, wherein said CH3 domain is attached to a polypeptide comprising CD80 transmembrane and cytoplasmic tail domains

211 A protein of claim 179 where said single chain protein comprises a single chain Fv binding domain from a 2e12 hybridoma, where in the connecting region said first, second and third cysteine residues are replaced by serine and said proline residue is replaced by serine, said heavy chain constant region comprising CH2 and CH3 domains from IgG_1 wherein proline is replaced by serine at position 238 in said CH2 region and said CH3 domain is attached to a polypeptide comprising mFADD transmembrane and cytoplasmic tail domains

212 A protein of claim 179 where said single chain protein comprises a single chain Fv binding domain from a 2e12 hybridoma, where in the connecting region said first, second and third cysteine residues are replaced by serine and said proline residue is replaced by serine, said heavy chain constant region comprising CH2 and CH3 domains from IgG_1, wherein said CH3 domain is attached to a polypeptide comprising mFADD transmembrane and cytoplasmic tail domains

213 A protein of claim 179 where said single chain protein comprises a single chain Fv binding domain from a 2e12 hybridoma, where in the connecting region said first, second and third cysteine residues are replaced by serine and said proline residue is replaced by serine, said heavy chain constant region comprising CH2 and CH3 domains from IgG_1, wherein said CH3 domain is attached to a polypeptide comprising mcasp3 transmembrane and cytoplasmic tail domains

214 A protein of claim 179 where said single chain protein comprises a single chain Fv binding domain from a 2e12 hybridoma, where in the connecting region said first, second and third cysteine residues are replaced by serine and said proline residue is replaced by serine, said heavy chain constant region comprising CH2 and CH3 domains from IgG_1 wherein proline is replaced by serine at position 238 in said CH2 region and said CH3 domain is attached to a polypeptide comprising mcasp3 transmembrane and cytoplasmic tail domains

215 A protein of claim 179 where said single chain protein comprises a single chain Fv binding domain from a 2e12 hybridoma, where in the connecting region said first, second and third cysteine residues are replaced by serine and said proline residue is replaced by serine, said heavy chain constant region comprising CH2 and CH3 domains from IgG_1, wherein said CH3 domain is attached to a polypeptide comprising mcasp8 transmembrane and cytoplasmic tail domains

216 A protein of claim 179 where said single chain protein comprises a single chain Fv binding domain from a 2e12 hybridoma, where in the connecting region said first, second and third cysteine residues are replaced by serine and said proline residue is replaced by serine, said heavy chain constant region comprising CH2 and CH3 domains from IgG_1 wherein proline is replaced by serine at position 238 in said CH2 region and said CH3 domain is attached to a polypeptide comprising mcasp8 transmembrane and cytoplasmic tail domains

217 A protein of claim 179 where said single chain protein comprises a single chain Fv binding domain from a 2e12 hybridoma, where in the connecting region said first, second and third cysteine residues are replaced by serine and said proline residue is replaced by serine, said heavy chain constant region comprising CH2 and CH3 domains from IgG_1, wherein said CH3 domain is attached to a polypeptide comprising hcasp3 transmembrane and cytoplasmic tail domains

218 A protein of claim 179 where said single chain protein comprises a single chain Fv binding domain from a 2e12 hybridoma, where in the connecting region said first, second and third cysteine residues are replaced by serine and said proline residue is replaced by serine, said heavy chain constant region comprising CH2 and CH3 domains from IgG_1 wherein proline is replaced by serine at position 238 in said CH2 region and said

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CH3 domain is attached to a polypeptide comprising hcasp3 transmembrane and cytoplasmic tail domains

219 A protein of claim 179 where said single chain protein comprises a single chain Fv binding domain from a 2e12 hybridoma, where in the connecting region said first, second and third cysteine residues are replaced by serine and said proline residue is replaced by serine, said heavy chain constant region comprising CH2 and CH3 domains from IgG_1, wherein said CH3 domain is attached to a polypeptide comprising hcasp8 transmembrane and cytoplasmic tail domains.

220 A protein of claim 179 where said single chain protein comprises a single chain Fv binding domain from a 2e12 hybridoma, where in the connecting region said first, second and third cysteine residues are replaced by serine and said proline residue is replaced by serine, said heavy chain constant region comprising CH2 and CH3 domains from IgG_1 wherein proline is replaced by serine at position 238 in said CH2 region and said CH3 domain is attached to a polypeptide comprising hcasp8 transmembrane and cytoplasmic tail domains

221 A protein of claim 179 where said single chain protein comprises a single chain Fv binding domain from a 2e12 hybridoma, said heavy chain constant region comprising CH2 and CH3 domains from IgG_1 wherein said CH3 domain is attached to a polypeptide comprising CD80 transmembrane and cytoplasmic tail domains

222 A protein of claim 179 where said single chain protein comprises a single chain Fv binding domain from a 1D8 hybridoma, where in the connecting region said first, second and third cysteine residues are replaced by serine and said proline residue is replaced by serine, said heavy chain constant region comprising CH2 and CH3 domains from IgG_1 wherein proline is replaced by serine at position 238 in said CH2 region and said CH3 domain is attached to a polypeptide comprising CD80 transmembrane and cytoplasmic tail domains

223 A protein of claim 179 where said single chain protein comprises a single chain Fv binding domain from a 1D8 scFv hybridoma, where in the connecting region said first, second and third cysteine residues are replaced by serine and said proline residue is replaced by serine, said heavy chain constant region comprising CH2 and CH3 domains from IgG_1, wherein said CH3 domain is attached to a polypeptide comprising CD80 transmembrane and cytoplasmic tail domains

224 A protein of claim 179 where said single chain protein comprises a single chain Fv binding domain from a 1D8 hybridoma, where in the connecting region said first, second and third cysteine residues are replaced by serine and said proline residue is replaced by serine, said heavy chain constant region comprising CH2 and CH3 domains from IgG_1 wherein proline is replaced by serine at position 238 in said CH2 region and said CH3 domain is attached to a polypeptide comprising mFADD transmembrane and cytoplasmic tail domains

225 A protein of claim 179 where said single chain protein comprises a single chain Fv binding domain from a 1D8 hybridoma, where in the connecting region said first, second and third cysteine residues are replaced by serine and said proline residue is replaced by serine, said heavy chain constant region comprising CH2 and CH3 domains from IgG_1, wherein said CH3 domain is attached to a polypeptide comprising mFADD transmembrane and cytoplasmic tail domains.

226 A protein of claim 179 where said single chain protein comprises a single chain Fv binding domain from a 1D8 hybridoma, where in the connecting region said first, second and third cysteine residues are replaced by serine and said proline residue is replaced by serine, said heavy chain constant region comprising CH2 and CH3 domains from IgG_1, wherein said CH3 domain is attached to a polypeptide comprising mcasp3 transmembrane and cytoplasmic tail domains

227 A protein of claim 179 where said single chain protein comprises a single chain Fv binding domain from a 1D8 hybridoma, where in the connecting region said first, second and third cysteine residues are replaced by serine and said proline residue is replaced by serine, said heavy chain constant region comprising CH2 and CH3 domains from IgG_1 wherein proline is replaced by serine at position 238 in said CH2 region and said CH3 domain is attached to a polypeptide comprising mcasp3 transmembrane and cytoplasmic tail domains

228 A protein of claim 179 where said single chain protein comprises a single chain Fv binding domain from a 1D8 hybridoma, where in the connecting region said first, second and third cysteine residues are replaced by serine and said proline residue is replaced by serine, said heavy chain constant region comprising CH2 and CH3 domains from IgG_1, wherein said CH3 domain is attached to a polypeptide comprising mcasp8 transmembrane and cytoplasmic tail domains.

229 A protein of claim 179 where said single chain protein comprises a single chain Fv binding domain from a 1D8 hybridoma, where in the connecting region said first, second and third cysteine residues are replaced by serine and said proline residue is replaced by serine, said heavy chain constant region comprising CH2 and CH3 domains from IgG_1 wherein proline is replaced by serine at position 238 in said CH2 region and said CH3 domain is attached to a polypeptide comprising mcasp8 transmembrane and cytoplasmic tail domains

230 A protein of claim 179 where said single chain protein comprises a single chain Fv binding domain from a 1D8 hybridoma, where in the connecting region said first, second and third cysteine residues are replaced by serine and said proline residue is replaced by serine, said heavy chain constant region comprising CH2 and CH3 domains from IgG_1, wherein said CH3 domain is attached to a polypeptide comprising hcasp3 transmembrane and cytoplasmic tail domains

231 A protein of claim 179 where said single chain protein comprises a single chain Fv binding domain from a 1D8 hybridoma, where in the connecting region said first, second and third cysteine residues are replaced by serine and said proline residue is replaced by serine, said heavy chain constant region comprising CH2 and CH3 domains from IgG_1 wherein proline is replaced by serine at position 238 in said CH2 region and said CH3 domain is attached to a polypeptide comprising hcasp3 transmembrane and cytoplasmic tail domains

232 A protein of claim 179 where said single chain protein comprises a single chain Fv binding domain from a 1D8 hybridoma, where in the connecting region said first, second and third cysteine residues are replaced by serine and said proline residue is replaced by serine, said heavy chain constant region comprising CH2 and CH3 domains from IgG_1, wherein said CH3 domain is attached to a polypeptide comprising hcasp8 transmembrane and cytoplasmic tail domains

233 A protein of claim 179 where said single chain protein comprises a single chain Fv binding domain from a 1D8 hybridoma, where in the connecting region said first, second and third cysteine residues are replaced by serine and said proline residue is replaced by serine, said heavy chain constant region comprising CH2 and CH3 domains from IgG_1 wherein proline is replaced by serine at position 238 in said CH2 region and said

CH3 domain is attached to a polypeptide comprising hcasp8 transmembrane and cytoplasmic tail domains

234 A protein of claim 179 where said single chain protein comprises a L6 single chain Fv binding domain, where in the connecting region said first, second and third cysteine residues are replaced by serine and said proline residue is replaced by serine, said heavy chain constant region comprising CH2 and CH3 domains from IgG_1

235 A protein of claim 179 where said single chain protein comprises a G28-1 single chain Fv comprising a connecting region wherein said first, second, and third cysteine residues are replaced by serine and said proline residue is replaced by serine, where said heavy chain constant region comprises G28-1 single chain Fv CH2 and CH3 domains.

236 A method of reducing a target cell population in a subject comprising administering to said subject a therapeutically effective amount of a protein that is less than about 150kD comprising the steps of a) treating the target cell population with a first protein or peptide that binds to cells within said target cell population, and b) treating the target cell population with a second protein or peptide that capable of at least one of i) binding an Fc receptor, ii) inducing target cell apoptosis, or iii) fix complements, wherein said first protein or peptide molecule is directly connected to said second protein or peptide molecule or, optionally, said first protein or peptide molecule and said second protein or peptide molecule are linked by a third protein or peptide molecule, and wherein said protein molecule is not an antibody, a member of the TNF family or the TNF receptor family and is not conjugated with a bacterial toxin, a cytotoxic drug, or a radioisotope.

237 A method of reducing a target cell population in a subject comprising administering to said subject a therapeutically effective amount of a protein that is less than about 150kD consisting essentially of the steps of a) treating the target cell population with a first protein or peptide that binds to cells within said target cell population, and b) treating the target cell population with a second protein or peptide that capable of at least one of i) binding an Fc receptor, ii) inducing target cell apoptosis, or iii) fix complements, wherein said first protein or peptide molecule is directly connected to said second protein or peptide molecule or, optionally, said first protein or peptide molecule and said second protein or peptide molecule are linked by a third protein or peptide molecule, and wherein said protein molecule is not an antibody, a member of the

TNF family or the TNF receptor family, and is not conjugated with a bacterial toxin, a cytotoxic drug, or a radioisotope

238 A non-naturally occurring single chain protein comprising i) a first polypeptide having a binding domain polypeptide capable of binding to a target molecule, said binding domain polypeptide comprising a light chain variable region comprising one or more amino acid deletion or substitution, ii) a second polypeptide comprising a connecting region attached to said first polypeptide, and iii) a third polypeptide comprising an N-terminally truncated immunoglobulin heavy chain constant region polypeptide attached to the second polypeptide, wherein said non-naturally occurring single-chain protein is capable of at least one immunological activity

239 A non-naturally occurring single chain protein comprising i) a first polypeptide having a binding domain polypeptide capable of binding to a target molecule, said binding domain polypeptide comprising a light chain variable region comprising one or more amino acid deletion or substitution in positions 12, 80, 81, 83, 105, 106, and 107, ii) a second polypeptide comprising a connecting region attached to said first polypeptide, and iii) a third polypeptide comprising an N-terminally truncated immunoglobulin heavy chain constant region polypeptide attached to the second polypeptide, wherein said non-naturally occurring single-chain protein is capable of at least one immunological activity

240 A protein of claim 238 wherein the one or more amino acid substitution or deletion in said light chain variable region is effective to increase expression or stability of said protein relative to a protein without said deletion or substitution.

241 A protein of claim 238 wherein said binding domain polypeptide is a single chain Fv

242 A protein of claim 238 wherein said binding domain polypeptide comprises an immunoglobulin light chain variable region polypeptide and an immunoglobulin heavy chain variable region polypeptide.

243 A protein of claim 242 further comprising a second binding domain polypeptide capable of binding a second target molecule, said second binding domain polypeptide comprising an immunoglobulin light chain variable region polypeptide and an immunoglobulin heavy chain variable region polypeptide.

244 A protein of claim 241 wherein said binding domain polypeptide is a single chain Fv and said light chain variable region of said single chain Fv has an amino acid deletion or substitution at position 106

245 A protein of claim 241 wherein said binding domain polypeptide is a single chain Fv and the amino acid at position 106 of the heavy chain variable region of said single chain Fv has been deleted.

246 A protein of claim 238 wherein said binding domain polypeptide is a single chain Fv and said light chain variable region of said single chain Fv has an amino acid substitution at position 106

247 A protein of claim 244 wherein leucine is replaced by serine at position of 106

248 A protein of claim 244 wherein leucine is replaced by des-leucine at position of 106

249 A protein of claim 244 wherein the amino acid substituted for the amino acid at position 106 of the single chain Fv light chain variable region is selected from serine, threonine, cysteine, tyrosine, asparagine, glutamine, aspartic acid, glutamic acid, lysine, arginine, and histidine

250 A protein of claim 244 wherein said binding domain polypeptide is a single chain Fv comprising a heavy chain variable region, wherein said heavy chain variable region has an amino acid substitution at amino acid position 11

251 A protein of claim 241 wherein said binding domain polypeptide binds to a tumor antigen.

252 A protein of claim 241 wherein said binding domain polypeptide binds to an antigen on an immune effector cell

253 A protein of claim 241 wherein said binding domain polypeptide binds to a cancer cell antigen

254 A protein of claim 253 wherein said cancer cell antigen is a surface antigen

255 A protein of claim 253 wherein said cancer cell antigen is an intracellular antigen

256 A protein of claim 238 wherein said binding domain polypeptide binds to a B cell antigen.

257 A protein of claim 256 wherein said B cell antigen is selected from CD19, CD20, CD22, CD37, CD40, CD80, and CD86

258 A protein of claim 241 wherein said single chain Fv binds to a B cell antigen

259 A protein of claim 258 wherein said B cell antigen is selected from CD19, CD20, CD22, CD37, CD40, CD80, and CD86

260 A protein of claim 259 wherein said single chain Fv is selected from HD37 single chain Fv, 2H7 single chain Fv, G28-1 single chain Fv, and 4 4 220 single chain Fv

261 A protein of claim 238 wherein said binding domain polypeptide is an scFv that binds to a B cell differentiation antigen.

262 A protein of claim 261 wherein said B cell antigen is selected from CD19, CD20, CD22, CD37, and CD40.

263. A protein of claim 238 wherein said binding domain polypeptide binds to a target selected from CD2, CD3, CD4, CD5, CD6, CD8, CD10, CD11b, CD14, CD19, CD20 CD21, CD22, CD23, CD24, CD25, CD28, CD30, CD37, CD40, CD43, CD50 (ICAM3), CD54 (ICAM1), CD56, CD69, CD80, CD86, CD134 (OX40), CD137 (41BB), CD152 (CTLA-4), CD153 (CD30 ligand), CD154 (CD40 ligand), ICOS, L6, B7-H1, and HLA class II.

264 A protein of claim 238 wherein said protein is capable of forming a complex comprising two or more of said proteins

265 A protein of claim 264 wherein said complex is a dimer

266 A protein of claim 238 wherein said protein is a monomer

267 A protein of claim 238 wherein said immunological activity is selected from antibody dependent cell-mediated cytotoxicity, complement fixation, induction of apoptosis, induction of one or more biologically active signals, induction of one or more immune effector cells, activation of cellular differentiation, cellular activation, release of one or more biologically active molecules, and neutralization of an infectious agent or toxin.

268 A protein of claim 267 which is capable of induction of biologically active signals by activation or inhibition of one or more molecules selected from protein

kinases, protein phosphatases, G-proteins, cyclic nucleotides or other second messengers, ion channels, and secretory pathway components

269 A protein of claim 267 which is capable of induction of one or more immune effector cells selected from NK cells, monocytes, macrophages, B cells, T cells, mast cells, neutrophils, eosinophils, and basophils

270 A protein of claim 267 wherein said induction of one or more immune effector cells leads to antibody dependent cell-mediated cytotoxicity or the release of one or more biologically active molecules

271 A protein of claim 267 which is capable of cellular activation, wherein said activation leads to changes in cellular transcriptional activity

272 A protein of claim 271 wherein said cellular transcriptional activity is increased

273 A protein of claim 271 wherein said cellular transcriptional activity is decreased

274. A protein of claim 267 wherein said one or more biologically active molecules is a protease

275 A protein of claim 267 wherein said one or more biologically active molecules is a cytokine

276 A protein of claim 275 wherein said cytokine is selected from monokines, lymphokines, chemokines, growth factors, colony stimulating factors, interferons, and interleukins

277 A protein of claim 267 which is capable of neutralization of an infectious agent, wherein said infectious agent is a bacterium, a virus, a parasite, or a fungus

278 A protein of claim 267 which is capible of neutralization of a toxin, wherein said toxin is selected from endotoxins and exotoxins

279 A protein of claim 267 which is capable of neutralization of a toxin, wherein said toxin is an exotoxin selected from anthrax toxin, cholera toxin, diphtheria toxin, pertussis toxin, *E. coli* heat-labile toxin LT, *E coli* heat stable toxin ST, shiga toxin *Pseudomonas* Exotoxin A, botulinum toxin, tetanus toxin, *Bordetella pertussis* AC toxin, and *Bacillus anthracis* EF

280 A protein of claim 267 which is capable of neutralization of a toxin, wherein said toxin is an endotoxin selected from saxitoxins, tetrodotoxin, mushroom toxins, aflatoxins, pyrrolizidine alkaloids, phytohemagglutinins, and grayanotoxins

281 A protein of claim 238 wherein said protein is capable of binding to an intracellular target to effect a cellular function.

282 A protein of claim 238 wherein said N-terminally truncated immunoglobulin heavy chain constant region polypeptide comprises an IgG CH2 constant region polypeptide attached to an immunoglobulin heavy chain IgG CH3 constant region polypeptide

283 A protein of claim 238 wherein said N-terminally truncated immunoglobulin heavy chain constant region polypeptide consist essentially of an IgG CH2 constant region polypeptide attached to an immunoglobulin heavy chain IgG CH3 constant region polypeptide.

284 A protein of claim 238 comprising at least a portion of a human constant region.

285 A protein of claim 238 wherein said connecting region comprises a naturally occurring hinge region selected from a human hinge or portion thereof, human IgG hinge or a portion thereof, human IgA hinge or a portion thereof, human IgE hinge or a portion thereof, camelid hinge region or a portion thereof, IgG1 llama hinge region or portion thereof, nurse shark hinge region or portion thereof, and spotted ratfish hinge region or a portion thereof

286 A protein of claim 238 wherein said connecting region comprises a human IgE hinge or a portion thereof

287 A protein of claim 238 wherein said connecting region comprises a human IgG1, IgG2, IgG3 or IgG4 hinge region having either zero or one cysteine residue.

288 A protein of claim 238 wherein said connecting region comprises a human IgGA hinge region having between zero and two cysteine residues

289 A protein of claim 238 wherein said connecting region comprises a wild type human IgG1 immunoglobulin hinge region

290 A protein of claim 238 wherein said connecting region comprises a glycosylation site

291 A protein of claim 238 wherein said connecting region has no cysteine residues capable of forming disulfide bonds

292 A protein of claim 238 wherein said connecting region has one cysteine residue

293 A protein of claim 238 wherein said connecting region comprises a mutated wild-type immunoglobulin hinge region polypeptide comprising no more than one cysteine residue

294 A protein of claim 238 wherein said connecting region is altered so that said protein has a reduced ability to dimerize.

295 A protein of claim 238 wherein said connecting region comprises a proline and first, second, and third cysteine residues, where said first cysteine reside is N-terminal to said second cysteine and said second cysteine is N-terminal to said third cysteine, said third cysteine residue is N-terminal to said proline residue.

296 A non-naturally occurring single chain protein, including a first polypeptide comprising a binding domain polypeptide capable of binding to a target molecule, a second polypeptide comprising a connecting region attached to said first polypeptide, said connecting region comprises at least a portion of an IgA hinge region, a third polypeptide comprising an N-terminally truncated immunoglobulin heavy chain constant region polypeptide attached to the second polypeptide, and wherein said non-naturally occurring single-chain protein is capable of at least one immunological activity

297 A protein of claim 296 wherein said binding domain polypeptide comprises an immunoglobulin light chain variable region polypeptide and an immunoglobulin heavy chain variable region polypeptide

298 A protein of claim 297 further comprising a second binding domain polypeptide capable of binding a second target molecule, said second binding domain polypeptide comprising an immunoglobulin light chain variable region polypeptide and an immunoglobulin heavy chain variable region polypeptide

299 A protein of claim 296 wherein said connecting region consist essentially of at least a portion of an IgA hinge region

300. A protein of claim 296 wherein said connecting region consist essentially of an IgA hinge region

301 A protein of claim 300 that is expressed as a protein having an apparent molecular of at least about 70 kDa.

302 A protein of claim 300 that is expressed as a protein having an apparent molecular of between about 70 kDa and about 700 kDa.

303 A protein of claim 296 wherein said connecting region comprises between 10 to 50 amino acids.

304 A protein of claim 296 wherein said connecting region comprises a modified human IgA immunoglobulin hinge region polypeptide

305 A protein of claim 296 wherein said connecting region has only one cysteine residue.

306 A protein of claim 296 wherein said connecting region two cysteine residues

307 A protein of claim 296 wherein said N-terminally truncated immunoglobulin heavy chain constant region polypeptide in the third polypeptide comprises IgA immunoglobulin heavy chain constant regions

308 A protein of claim 307 comprising CH2 and CH3 domains from IgA immunoglobulin heavy chain constant regions

309 A protein of claim 308 wherein said IgA CH3 constant region domain comprises a substitution or deletion in one or more amino acids that render the IgA heavy chain constant region incapable of associating with a J chain polypeptide.

310 A protein of claim 308 wherein said IgA heavy chain constant region is capable of associating with a J chain polypeptide

311 A protein of claim 309 wherein said IgA CH3 region domain comprises a deletion in between four and twenty amino acids.

312 A protein of claim 309 wherein said IgA region domain comprises a deletion of four amino acids

313 A protein of claim 309 wherein said IgA region domain comprises a deletion of twenty amino acids

314 A protein of claim 296 wherein said single chain protein comprises a L6 single chain Fv binding domain, wherein the connecting region comprises an IgA hinge region or portion thereof, said heavy chain constant region comprising CH2 and CH3 domains from IgG_1

315 A protein of claim 296 wherein said single chain protein comprises a single chain Fv binding domain from a L6 hybridoma, wherein the connecting region comprises an IgA hinge region or portion thereof, said heavy chain constant region comprising CH2 and CH3 domains from IgG_1

316 A protein of claim 296 wherein said single chain protein comprises a single chain Fv binding domain from a CTLA-4 hybridoma, wherein the connecting region comprises an IgA hinge region or portion thereof, said heavy chain constant region comprises CH2 and CH3 domains from murine IgA.

317 A protein of claim 296 wherein said single chain protein comprises a single chain Fv binding domain from a CTLA-4 hybridoma, wherein the connecting region comprises an IgA hinge region or portion thereof, said heavy chain constant region comprises CH2 and CH3 domains from murine IgA and said CH3 comprises a deletion or substitution in four amino acids that render the IgA heavy chain constant region incapable of associating with a J chain polypeptide

318 A protein of claim 296 wherein said single chain protein comprises a single chain Fv binding domain from a 2H7 hybridoma, wherein the connecting region comprises an IgA hinge region or portion thereof, said heavy chain constant region comprises CH2 and CH3 domains from murine IgA.

319 A protein of claim 296 wherein said single chain protein comprises a single chain Fv binding domain from a 2H7 hybridoma, said connecting region comprises a IgA hinge or portion thereof, and said heavy chain constant region comprises CH2 and CH3 domains from IgA, wherein said CH3 comprises a deletion or substitution in eighteen amino acids that render the IgA heavy chain constant region incapable of associating with a J chain polypeptide.

320 A protein of claim 296 wherein said single chain protein comprises a single chain Fv binding domain from a 2e12 hybridoma, said connecting region comprises a IgA hinge or portion thereof, and said heavy chain constant region comprises CH2 and CH3 domains from IgA, wherein said CH3 comprises a deletion or substitution in four amino acids that render the IgA heavy chain constant region incapable of associating with a J chain polypeptide and said CH3 domain is attached to a polypeptide comprising CD80 transmembrane and cytoplasmic tail domains

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321 A protein of claim 296 wherein said single chain protein comprises a single chain Fv binding domain from a 2e12 hybridoma, said connecting region comprises a IgA hinge or portion thereof, said heavy chain constant region comprises CH2 and CH3 domains from IgA, wherein said CH3 comprises a deletion or substitution in four amino acids that render the IgA heavy chain constant region incapable of associating with a J chain polypeptide and said CH3 domain is attached to a polypeptide comprising CD80 transmembrane and cytoplasmic tail domains

322. A protein of claim 296 wherein said single chain protein comprises a single chain Fv binding domain from a 1D8 hybridoma, said connecting region comprises a IgA hinge or portion thereof, said heavy chain constant region comprises CH2 and CH3 domains from IgA, wherein said CH3 comprises a deletion or substitution in four amino acids that render the IgA heavy chain constant region incapable of associating with a J chain polypeptide and said CH3 domain is attached to a polypeptide comprising CD80 transmembrane and cytoplasmic tail domains

323 A protein of claim 296 wherein said single chain protein comprises a single chain Fv binding domain from a G28-1 hybridoma, wherein the connecting region comprises an IgA hinge region or portion thereof, said heavy chain constant region comprising CH2 and CH3 domains from IgG_1

324 A protein of claim 296 wherein said single chain protein comprises a single chain Fv binding domain from a 2H7 hybridoma, wherein the connecting region comprises an IgA hinge region or portion thereof, said heavy chain constant region comprising CH2 and CH3 domains from IgG_1

325 A protein of claim 296 wherein said single chain protein comprises a single chain Fv binding domain from a 2H7 hybridoma, wherein the connecting region comprises an IgA hinge region or portion thereof, said said heavy chain constant region comprises CH2 and CH3 domains from IgA, wherein said CH3 comprises a deletion or substitution in four amino acids that render the IgA heavy chain constant region incapable of associating with a J chain polypeptide and said CH3 domain is attached to a polypeptide comprising CD80 transmembrane and cytoplasmic tail domains

326 A protein of claim 296 wherein said single chain protein comprises a single chain Fv binding domain from a 2H7 hybridoma, wherein the connecting region comprises an IgA hinge region or portion thereof, said heavy chain constant region

comprises CH2 and CH3 domains from IgA, wherein the IgA heavy chain constant region is capable of associating with a J chain polypeptide

327 A protein of claim 296 wherein said single chain protein comprises a single chain Fv binding domain from a HD37 hybridoma, wherein the connecting region comprises an IgA hinge region or portion thereof, said heavy chain constant region comprises CH2 and CH3 domains from IgA

328 A non-naturally occurring single chain protein comprising 1) a first polypeptide comprising a binding domain polypeptide capable of binding to a target molecule, and ii) a second polypeptide comprising at least a portion of an IgE immunoglobulin heavy chain constant region polypeptide attached to the second polypeptide, wherein said non-naturally occurring single-chain protein is capable of at least one immunological activity

329 A protein of claim 328 wherein said binding domain polypeptide comprises a non-IgE variable region

330 A protein of claim 328 wherein said binding domain polypeptide is a single chain Fv selected from HD37 single chain Fv, 2H7 single chain Fv G28-1 single chain Fv, FC2-2, UCHL-1, 5B9, L6, 10A8 2e12, 40.2 36, G19-4, 1D8, and 4 4 220 single chain Fv

331 A protein of claim 328 wherein said binding domain polypeptide comprises an immunoglobulin light chain variable region polypeptide and an immunoglobulin heavy chain variable region polypeptide

332 A protein of claim 331 further comprising a second binding domain polypeptide capable of binding a second target molecule, said second binding domain polypeptide comprising an immunoglobulin light chain variable region polypeptide and an immunoglobulin heavy chain variable region polypeptide

333 A protein of claim 332 wherein said immunoglobulin light and heavy chains comprise IgG immunoglobulin light and heavy chains or a portions thereof

334 A protein of claim 328 wherein said binding domain polypeptide comprises at least a portion of an IgG immunoglobulin variable region.

335 A protein of claim 334 wherein said IgG variable region comprises a heavy chain variable region and a light chain variable region.

336 A protein of claim 328 that is capable of binding to a Fc receptor specific for IgE.

337 A protein of claim 328 that does not have IgE variable chain region.

338. A protein of claim 328 wherein the binding domain consist essentially of an IgG immunoglobulin light chain region and a IgG immunoglobulin light and heavy chain region

339 A protein of claim 328 wherein said IgE immunoglobulin heavy chain constant region polypeptide comprises CH2 and CH3 domains

340 A protein of claim 328 wherein said IgE immunoglobulin heavy chain constant region polypeptide comprises CH2, CH3 and CH4 domains

341 A protein of claim 328 wherein said binding domain polypeptide is a single chain Fv comprising a heavy chain variable region, wherein said heavy chain variable region has an amino acid substitution at amino acid position 11

342 A protein of claim 328 wherein said single chain protein is a single chain Fv selected from HD37 single chain Fv, 2H7 single chain Fv, G28-1 single chain Fv, and 4 4 220 single chain Fv

343 A protein of claim 328 wherein said single chain protein is a single chain Fv comprising a 2H7 single chain Fv or a G28-1 single chain Fv

344 A method of reducing a target cell population in a subject comprising administering to said subject a therapeutically effective amount of a non-naturally occurring single chain protein, said protein comprising a first polypeptide comprising a binding domain polypeptide, said binding domain polypeptide comprising at least a portion of an IgG variable region and being capable of binding to a target molecule a second polypeptide comprising a connecting region attached to said first polypeptide, a third polypeptide comprising an IgE immunoglobulin heavy chain constant region polypeptide attached to the second polypeptide, wherein said non-naturally occurring single-chain protein is capable of at least one immunological activity

345 The method of claim 344 wherein said protein is administered into the blood stream of a patient

346 The method of claim 344 wherein monocytes are activated but mast cells are not activated as a result of administration of the protein

347 The method of claim 344 wherein the protein is administered or coadministered with a histamine release blocker

348 A method of depleting cells in an animal comprising administering a modified IgE protein into the blood stream of an animal.

349 The method of claim 348 wherein the modified IgE protein is administered or coadministered with a histamine release blocker

350 A protein of claim 328 wherein said connecting region comprises a IgG hinge or portion thereof, and said heavy chain constant region is from IgE and comprises CH3 and CH4 domains without a CH2 domain

351 A protein of claim 328 wherein said single chain protein comprises a single chain Fv binding domain from a 2e12, and said heavy chain constant region comprises IgE CH2, CH3, and CH4 domains, and wherein said heavy chain constant region is attached to a polypeptide comprising CD80 transmembrane and cytoplasmic tail domains

352 A non-naturally occurring single chain protein comprising i) a first polypeptide having a binding domain polypeptide capable of binding to a target molecule, ii) a second polypeptide comprising a connecting region attached to said first polypeptide, said connecting region comprising three cysteine residues and one proline residue, wherein one or more of said cysteine residues is deleted or substituted, and iii) a third polypeptide comprising an N-terminally truncated immunoglobulin heavy chain constant region polypeptide attached to the second polypeptide, wherein said non-naturally occurring single-chain protein is capable of at least one immunological activity

353 A protein of claim 352 wherein said connecting region comprises first, second, and third cysteine residues, where said first cysteine reside is N-terminal to said second cysteine and said second cysteine is N-terminal to said third cysteine, wherein said first cysteine residue is not substituted or deleted, and where said second and third cysteine are substituted or deleted

354 A protein of claim 353 wherein said second and third cysteine residues are substituted by another amino acid

355 A protein of claim 354 wherein said second and third cysteine residues are substituted by serine residues

356 A protein of claim 352 that is expressed as a homogeneous protein having an apparent molecular of about 75 kDa.

357 A protein of claim 352 wherein said connecting region comprises first, second, and third cysteine residues, where said first cysteine reside is N-terminal to said second cysteine and said second cysteine is N-terminal to said third cysteine, wherein said first cysteine residue is substituted or deleted

358 A protein of claim 352 where said second and third cysteine residues in said connecting region are not substituted or deleted.

359 A protein of claim 352 wherein said connecting region comprises first, second, and third cysteine residues, where said first cysteine reside is N-terminal to said second cysteine and said second cysteine is N-terminal to said third cysteine, wherein said second cysteine residue is substituted or deleted.

360 A protein of claim 352 wherein said first and third cysteine residues in said connecting region are not substituted or deleted.

361 A protein of claim 352 wherein said connecting region comprises first, second, and third cysteine residues, where said first cysteine reside is N-terminal to said second cysteine and said second cysteine is N-terminal to said third cysteine, wherein said third cysteine residue is substituted or deleted

362 The protein of claim 10 where said first and second cysteine residues in said connecting region are not substituted or deleted.

363 A protein of claim 352 wherein said connecting region comprises between 5 and 65 amino acids

364 A protein of claim 352 wherein said connecting region comprises between 10 and 50 amino acids

365 A protein of claim 352 wherein said connecting region comprises between 15 and 35 amino acids

366 A protein of claim 352 wherein said connecting region comprises at least five consecutive hinge region amino acids

367 A protein of claim 352 wherein said connecting region comprises at least ten consecutive hinge region amino acids

368 A protein of claim 352 wherein said connecting region comprises at least fifteen consecutive hinge region amino acids

369 A protein of claim 353 wherein said second and third cysteines are replaced by serine residues and said connecting region comprises at least a portion of a IgG_1 hinge region

370 A protein of claim 369 wherein said connecting region comprises at least 5 consecutive amino acids of an IgG_1 hinge region

371 A protein of claim 353 wherein said second and third cysteines are replaced by serine residues and said connecting region comprises at least a portion of a IgG_2 hinge region

372 A protein of claim 371 wherein said connecting region comprises at least 5 consecutive amino acids of an IgG_2 hinge region

373 A protein of claim 353 wherein said second and third cysteine residues are replaced by serine residues and said connecting region comprises at least a portion of a IgG_3 hinge region

374 A protein of claim 373 wherein said connecting region comprises at least 5 consecutive amino acids of an IgG_3 hinge region

375 A protein of claim 353 wherein said second and third cysteine residues are replaced by serine residues and said connecting region comprises at least a portion of a IgG_4 hinge region

376 A protein of claim 375 wherein said connecting region comprises at least 5 consecutive amino acids of an IgG_4 hinge region

377 A protein of claim 352 wherein said second and third cysteine residues are substituted or deleted and said proline residue is substituted or deleted, wherein said heavy chain constant region polypeptide comprises a CH3 domain wherein tyrosine is replaced by alanine at position 407, and wherein said protein has an apparent molecular weight of about 75kDa.

378 A protein of claim 352 wherein said first and third cysteine residues are substituted or deleted and said proline residue is substituted or deleted, wherein said heavy chain constant region polypeptide comprises a CH3 domain wherein tyrosine is replaced by alanine at position 407, and wherein said protein has an apparent molecular weight of about 75kDa.

379 A non-naturally occurring single chain protein, including a first polypeptide comprising a binding domain polypeptide capable of binding to a target

molecule, a second polypeptide comprising a connecting region attached to said first polypeptide, a third polypeptide comprising an N-terminally truncated immunoglobulin heavy chain constant region polypeptide attached to the second polypeptide, wherein said connecting region comprises first, second, and third cysteine residues, where said first cysteine reside is N-terminal to said second cysteine and said second cysteine is N-terminal to said third cysteine, wherein one or both of said second and third cysteine residues is substituted or deleted, and wherein said non-naturally occurring single-chain protein is capable of at least one immunological activity

380 A protein of claim 352 wherein said connecting region comprises a modified human IgG1 immunoglobulin hinge region polypeptide

381 A protein of claim 352 wherein said connecting region has only one cysteine residue.

382 A protein of claim 381 wherein said connecting region comprises a modified human IgG1 immunoglobulin hinge region polypeptide

383 A protein of claim 352 wherein said N-terminally truncated immunoglobulin heavy chain constant region polypeptide comprises an IgG CH2 constant region polypeptide attached to an immunoglobulin heavy chain IgG CH3 constant region polypeptide

384 A protein of claim 352 wherein said N-terminally truncated immunoglobulin heavy chain constant region polypeptide consist essentially of an IgG CH2 constant region polypeptide attached to an immunoglobulin heavy chain IgG CH3 constant region polypeptide

385 A non-naturally occurring single chain protein, including a first polypeptide comprising a binding domain polypeptide capable of binding to a target molecule, a second polypeptide comprising a connecting region attached to said first polypeptide, a third polypeptide comprising an immunoglobulin heavy chain CH2 constant region polypeptide and an immunoglobulin heavy chain CH3 constant region polypeptide, said CH3 constant region polypeptide comprising one or more amino acid substitution or deletion that inhibits two of said proteins from associating together to form a non covalent dimer, wherein said non-naturally occurring single-chain protein is capable of at least one immunological activity

386 A non-naturally occurring single chain protein, including a first polypeptide comprising a binding domain polypeptide capable of binding to a target molecule, a second polypeptide comprising a connecting region attached to said first polypeptide, a third polypeptide comprising an immunoglobulin heavy chain CH2 constant region polypeptide and an immunoglobulin heavy chain CH3 constant region polypeptide, said CH3 constant region polypeptide comprising one or more amino acid substitution or deletion at positions 405 and 407, wherein said non-naturally occurring single-chain protein is capable of at least one immunological activity

387 A protein of claim 252 or 253 comprising amino acid substitutions at position 405 and position 407

388 A protein of claim 387 wherein phenylalanine is replaced with alanine at position 405 and tyrosine is substituted with alanine at position 407

389 A protein of claim 387 having an apparent molecular weight of between about 40 kDa and about 50 kDa.

390 A protein of claim 387 that has a biological activity of an agonist or antagonist.

391 A protein of claim 390 that has a biological activity of an antagonist

392 A protein of claim 387 wherein only one amino acid at positions 405 and 407 is substituted.

393 A protein of claim 392 having an apparent molecular weight of between about 70 kDa and about 80 kDa.

394 A protein of claim 387 where phenylalanine is replaced by alanine at position 405

395 A protein of claim 387 where phenylalanine is replaced by tyrosine at position 405

396 A protein of claim 386 where tyrosine is replaced by alanine at position 407

397 A protein of claim 386 comprising amino acid substitutions at position 405 and position 407

398 A protein of claim 393 wherein phenylalanine is replaced by alanine at position 405 and tyrosine is replaced by alanine at position 407

399 A polynucleotide that encodes a protein according to any one of claims 1, 8, 9, 12, 18, 30, and 112-109

400 A polynucleotide that encodes a protein according to any one of claims 116, 120, 121, and 180-235

401 A polynucleotide that encodes a protein according to any one of claims 238, 239, 296, 328, 352, 353, 385, and 386

402 A polynucleotide of any one of claims 399-401 that is operably linked to a promoter or other sequence that enhances expression of the polynucleotide in a cell

403 A cell containing a polynucleotide of any one of claims 399-401

404 A recombinant vector capable of expressing a protein according to any one of claims 1, 8, 9, 12, 18, 30, and 112-109

405 A recombinant vector capable of expressing a protein according to any one of claims 116, 120, 121, and 180-235

406 A method of expressing a protein according to any one of claims 1, 8, 9, 12 18, 30, and 112-109 under conditions in which the protein is expressed

407 A method of expressing a protein according to any one of claims 116, 120, 121, and 180-235 under conditions in which the protein is expressed

408 A method of expressing a protein according to any one of claims 238, 239, 296, 328, 352, 353, 385, and 386 under conditions in which the protein is expressed

409 A composition comprising a protein according to any one of claims 238, 239, 296, 328, 352, 353, 385, and 386 in combination with one or more additional therapeutic compounds

410 A method of treating a subject comprising the steps of administering to said subject a one or more first compound according to any one of claims 238, 239, 296, 328, 352, 353, 385, and 386, administering or coadministering a second compound to the subject in combination said first compound, wherein the treatment is effective to treat or prevent a disease, ailment, or undesired condition.

411 The method according to claim 410 wherein the second compound is selected from a chemotherapeutic compound, therapeutic drug, angiogenesis inhibitors, steroids, B-cell activators, T-cell activators, colony stimulating factors, tumor necrosis

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factors, interferons, antibody, binding construct of the invention, gene therapy, retinoids, surgical procedures, alkylating agents, nucleoside analogues, topoisomerase II, VEGF, IFN-alpha, DMAR agents, interleukin-1, glucocorticoids, p38 inhibitors, interleukin-4, interleukin-6, interleukin-2, interleukin-12, IFN-γ, GM-CSF, G-CSF, M-CSF, anti- CTLA-4, Bcl-2 antisense, vitamin A, anti-CD19, anti-CD20, anti-CD22, anti-CD28 or anti-CD3

412. A method for displaying recombinant molecules, which molecules include a native or engineered immunoglobulin heavy chain variable region, the improvement comprising a immunoglobulin heavy chain region that includes one or more mutation, substitution, alteration, and/or deletion at one or more amino acid residue corresponding to positions 9, 10, 11, 12, 108, 110, and 112 in said heavy chain variable region

413 A non-naturally occurring single chain antigen-binding protein comprising protein having a formula selected from 2H7 scFv VH L11S (CSC-S) H WCH2 WCH3, 2H7 scFv VH L11S IgE CH2 CH3 CH4, 2H7 scFv VH L11S mIgE CH2 CH3 CH4, 2H7 scFv VH L11S mIgAH WIgACH2 T4CH3, 2H7 scFv VH L11S (SSS-S) H K322S CH2 WCH3, 2H7 scFv VH L11S (CSS-S) H K322S CH2 WCH3, 2H7 scFv VH L11S (SSS-S) H P331S CH2 WCH3, 2HU scFv VH L11S (CSS-S) H P331S CH2 WCH3, 2H7 scFv VH L11S (SSS-S) H T256N CH2 WCH3, 2H7 scFv VH L11S (SSS-S) H RTPE/QNAK (255-258) CH2 WCH3, 2H7 scFv VH L11S (SSS-S) H K290Q CH2 WCH3, 2H7 scFv VH L11S (SSS-S) H A339P CH2 WCH3, G28-1 scFv (SSS-S) H WCH2 WCH3, G28-1 scFv IgAH WCH2 WCH3, G28-1 scFv VH L11S (SSS-S) H WCH2 WCH3, G28-1 scFv VH L11S (CSS-S) H WCH2 WCH3, G28-1 scFv VH L11S (CSC-S) H WCH2 WCH3, G28-1 scFv VH L11S (SSC-P) H WCH2 WCH3, CTLA4 (SSS-S) H P238SCH2 WCH32, CTLA4 (CCC-P) WH WCH2 WCH3, FC2-2 scFv (SSS-S) H WCH2 WCH3, FC2-2 scFv VHL11S (SSS-S) H WCH2 WCH3, UCHL-1 scFv (SSS-S) H WCH2 WCH3, UCHL-1 scFv VHL11S (SSS-S) H WCH2 WCH3, 5B9 scFv (SSS-S) H WCH2 WCH3, 5B9 scFv VHL11S (SSS-S) H WCH2 WCH3, 2H7 scFv (SSS-S) H WCH2 WCH3, 2H7 scFv (SSS-S) H P238SCH2 WCH3, 2H7 scFv IgAH WCH2 WCH3, 2H7 scFv IgAH WIgACH2 T4CH3, 2H7 scFv IgAH WIgACH2 WCH3 + JChain, 2H7 scFv (CCC-P) WH WCH2 WCH3, 2H7 scFv (SSS-S) H WCH2 F405YCH3, 2H7 scFv (SSS-S) H WCH2 F405ACH3, 2H7 scFv (SSS-S) H WCH2 Y407ACH3, 2H4 scFv (SSS-S) HWCH2 F405A, Y407ACH3, 2H7 scFv (CSS-S) H WCH2 WCH3, 2H7 scFv (SCS-S) H

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WCH2 WCH3, 2H7 scFv (SSC-P) H WCH2 WCH3, 2H7 scFv (CSC-S) H WCH2 WCH3, 2H7 scFv (CCS-P) H WCH2 WCH3, 2H7 scFv (SCC-P) H WCH2 WCH3, 2H7 scFv VH L11S (SSS-S) H WCH2 WCH3, 2H7 scFv VH L11S (CSS-S) H WCH2 WCH3, G28-1 scFv VH L11S (SCS-S) H WCH2 WCH3, G28-1 scFv VH L11S (CCS-P) H WCH2 WCH3, G28-1 scFv VH L11S (SCC-P) H WCH2 WCH3, G28-1 scFv VH L11S mIgE CH2 CH3 CH4, G28-1 scFv VH L11S mIgAH WIgACH2 T4CH3, G28-1 scFv VH L11S hIgE CH2 CH3 CH4, G28-1 scFv VH L11S hIgAH WIgACH2 T4CH3, HD37 scFv IgAH WCH2 WCH3, HD37 scFv (SSS-S) H WCH2 WCH3, HD37 scFv VH L11S (SSS-S) H WCH2 WCH3, L6 scFv IgAH WCH2 WCH3, L6 scFv VHL11S (SSS-S) H WCH2 WCH3, 2H7 scFv-llama IgG1, 2H7 scFv-llama IgG2, 2H7 scFv-llama IgG3, CD16-6 low (ED)(SSS-S) H P238SCH2 WCH3, CD16-9 high (ED)(SSS-S) H P238SCH2 WCH3, 2e12 scFv (SSS-s)H P238SCH2 WCH3—hCD80TM/CT, 10A8 scFv (SSS-s)H P238SCH2 WCH3—hCD80TM/CT, 40 2 36 scFv (SSS-s)H P238SCH2 WCH3—hCD80TM/CT, 2H7 scFv (SSS-s)H P238SCH2 WCH3—hCD80TM/CT, G19-4 scFv (SSS-s)H P238SCH2 WCH3—hCD80TM/CT, 2e12 scFv (SSS-s)H WCH2 WCH3—hCD80TM/CT, 2e12 scFv IgAH IgACH2 T4CH3—hCD80TM/CT 2e12 scFv IgE CH2CH3CH4—hCD80TM/CT, 2e12 scFv (SSS-s)H P238SCH2 WCH3—mFADD-TM/CT, 2e12 scFv (SSS-s)H WCH2 WCH3—mFADD-TM/CT, 2e12 scFv (SSS-s)H WCH2 WCH3—mcasp3-TM/CT, 2e12 scFv (SSS-s)H P238SCH2 WCH3—mcasp3-TM/CT, 2e12 scFv (SSS-s)H WCH2 WCH3—mcasp8-TM/CT, 2e12 scFv (SSS-s)H P238SCH2 WCH3—mcasp8-TM/CT, 2e12 scFv (SSS-s)H WCH2 WCH3—hcasp3-TM/CT, 2e12 scFv (SSS-s)H P238SCH2 WCH3—hcasp3-TM/CT, 2e12 scFv (SSS-s)H WCH2 WCH3—hcasp8-TM/CT, 2e12 scFv (SSS-s)H P238SCH2 WCH3—hcasp8-TM/CT, 1D8 scFv—hIgG1 (SSS-s)H P238SCH2 WCH3—hCD80TM/CT, 1D8 scFv—hIgG1 (SSS-s)H WCH2 WCH3—hCD80TM/CT, 1D8 scFv—mIgAT4—hCD80TM/CT, 1D8 scFv—hIgE—hCD80TM/CT, 1D8 scFv—hIgG1 (SSS-s)H P238SCH2 WCH3—mFADD-TM/CT, 1D8 scFv—hIgG1 (SSS-s)H WCH2 WCH3—mFADD-TM/CT, 1D8 scFv—hIgG1 (SSS-s)H WCH2 WCH3—mcasp3-TM/CT, 1D8 scFv—hIgG1 (SSS-s)H P238SCH2 WCH3—mcasp3-TM/CT, 1D8 scFv—hIgG1 (SSS-s)H WCH2 WCH3—mcasp8-TM/CT, 1D8 scFv—hIgG1 (SSS-s)H P238SCH2 WCH3—mcasp8-TM/CT, 1D8 scFv—hIgG1 (SSS-s)H WCH2 WCH3—hcasp3-TM/CT, 1D8 scFv—hIgG1 (SSS-s)H P238SCH2 WCH3—hcasp3-TM/CT, 1D8 scFv—hIgG1 (SSS-s)H WCH2 WCH3—hcasp8-TM/CT, 1D8 scFv—hIgG1 (SSS-s)H

P238SCH2 WCH3—hcasp8-TM/CT, L6 scFv (SSS-S) H WCH2 WCH3, 2H7 scFv CD154 (L2), 2H7 scFv CD154 (S4), CTLA4 IgAH IGACH2CH3, CTLA4 IgAH IgACH2 T4CH3, 2H7 scFv IgAH IgACH2CH3, 2H7 scFv IgAH IgAHCH2 T18CH3, 2H&-40 2 220 scFv (SSS-S) H WCH2 WCH3 (bispecific anti-ccd20-anti-od40), 2H7 scFv IgAH IgACH2 T4CH3-hCD89 TM/CT, G19-4 scFv (CCC-P) WH WCH2 WCH3-hCD89 TM/CT, and 2e12 scFv (CCC-P) WH WCH2 WCH3-hCD89 TM/CT

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Fig. 1

2H7scFv-Ig cDNA and predicted amino acid sequence

HindIII NcoI 2H7 V_L Leader Peptide→

```
                  M  D  F  Q  V  Q  I  F  S  F  L  L  I  S  A  S
1     AAGCTTGCCG CCATGGATTT TCAAGTGCAG ATTTTCAGCT TCCTGCTAAT CAGTGCTTCA

                                    2H7 V_L→
      V  I  I  A  R  G  Q  I  V  L  S  Q  S  P  A  I  L  S  A  S
61    GTCATAATTG CCAGAGGACA AATTGTTCTC TCCCAGTCTC CAGCAATCCT GTCTGCATCT

      P  G  E  K  V  T  M  T  C  R  A  S  S  S  V  S  Y  M  H  W
121   CCAGGGGAGA AGGTCACAAT GACTTGCAGG GCCAGCTCAA GTGTAAGTTA CATGCACTGG

                  BamHI
                  --------
      Y  Q  Q  K  P  G  S  S  P  K  P  W  I  Y  A  P  S  N  L  A
181   TACCAGCAGA AGCCAGGATC CTCCCCCAAA CCCTGGATTT ATGCCCCATC CAACCTGGCT

      S  G  V  P  A  R  F  S  G  S  G  S  G  T  S  Y  S  L  T  I
241   TCTGGAGTCC CTGCTCGCTT CAGTGGCAGT GGGTCTGGGA CCTCTTACTC TCTCACAATC

      S  R  V  E  A  E  D  A  A  T  Y  Y  C  Q  Q  W  S  F  N  P
301   AGCAGAGTGG AGGCTGAAGA TGCTGGCACT TATTACTGCC AGCAGTGGAG TTTTAACCCA

(Gly4Ser)3 Linker
      P  T  F  G  A  G  T  K  L  E  L  K  G  G  G  G  S  G  G  G
361   CCCACGTTCG GTGCTGGGAC CAAGCTGGAG CTGAAAGGTG GCGGTGGCTC GGGCGGTGGT

                                                    2H7 V_H→
      G  S  G  G  G  G  S  S  Q  A  Y  L  Q  Q  S  G  A  E  L  V
421   GGATCTGGAG GAGGTGGGAG CTCTCAGGCT TATCTACAGC AGTCTGGGGC TGAGCTGGTG

      R  P  G  A  S  V  K  M  S  C  K  A  S  G  Y  T  F  T  S  Y
481   AGGCCTGGGG CCTCAGTGAA GATGTCCTGC AAGGCTTCTG GCTACACATT TACCAGTTAC

      N  M  H  W  V  K  Q  T  P  K  Q  G  L  E  W  I  G  A  I  Y
541   AATATGCACT GGGTAAAGCA GACACCTAGA CAGGGCCTGG AATGGATTGG AGCTATTTAT

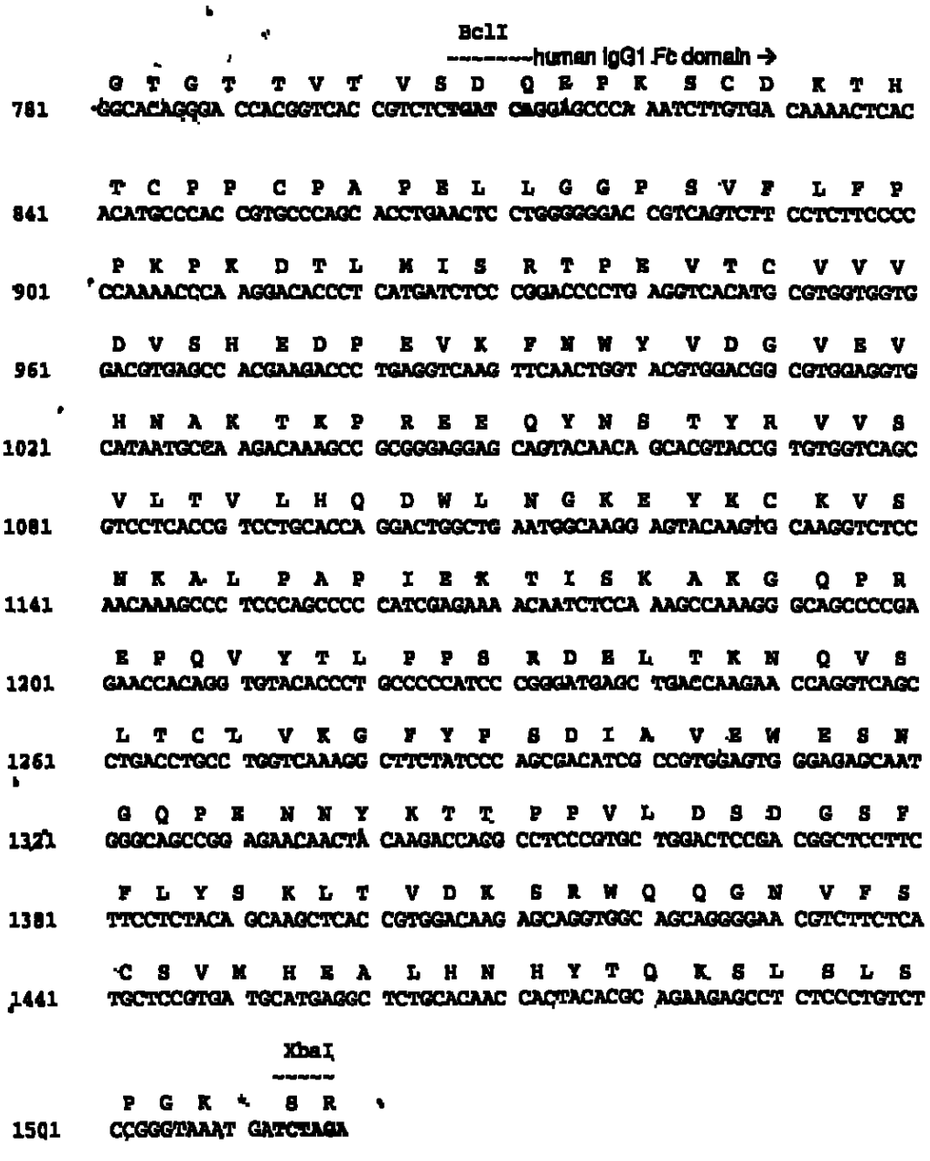
      P  G  N  G  D  T  S  Y  N  Q  K  F  K  G  K  A  T  L  T  V
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      D  K  S  S  S  T  A  Y  M  Q  L  S  S  L  T  S  E  D  S  A
661   GACAAATCCT CCAGCACAGC CTACATGCAG CTCAGCAGCC TGACATCTGA AGACTCTGCG

      V  Y  F  C  A  R  V  V  Y  Y  S  N  S  Y  W  Y  F  D  V  W
721   GTCTATTTCT GTGCAAGAGT GGTGTACTAT AGTAACTCTT ACTGGTACTT CGATGTCTGG
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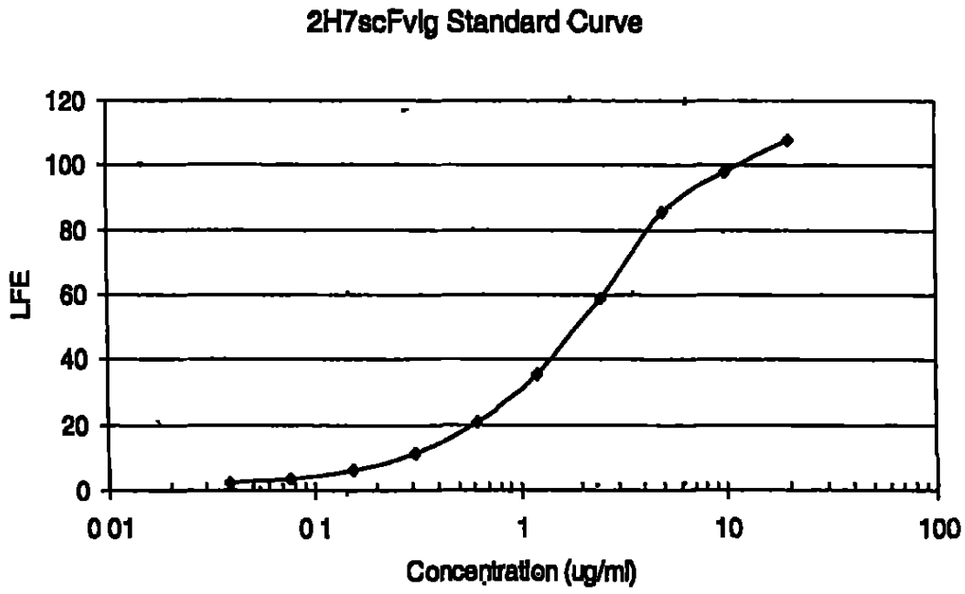
Fig 1 (continued)



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Fig 2

Production Levels of 2H7 scFvIgG1 (SSS-S)H WCH2 WCH3 by Stable CHO Lines



Clone	LFE @ 1 50 Estimated Concentration (µg/ml)
D2	26 156
IIIC6	25 755
IVA3	28 661
Spent bulk	29 664

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Fig 3

SDS-PAGE Analysis of
2H7 scFvIgG1 (SSS-S)H WCH2 WCH3 Protein

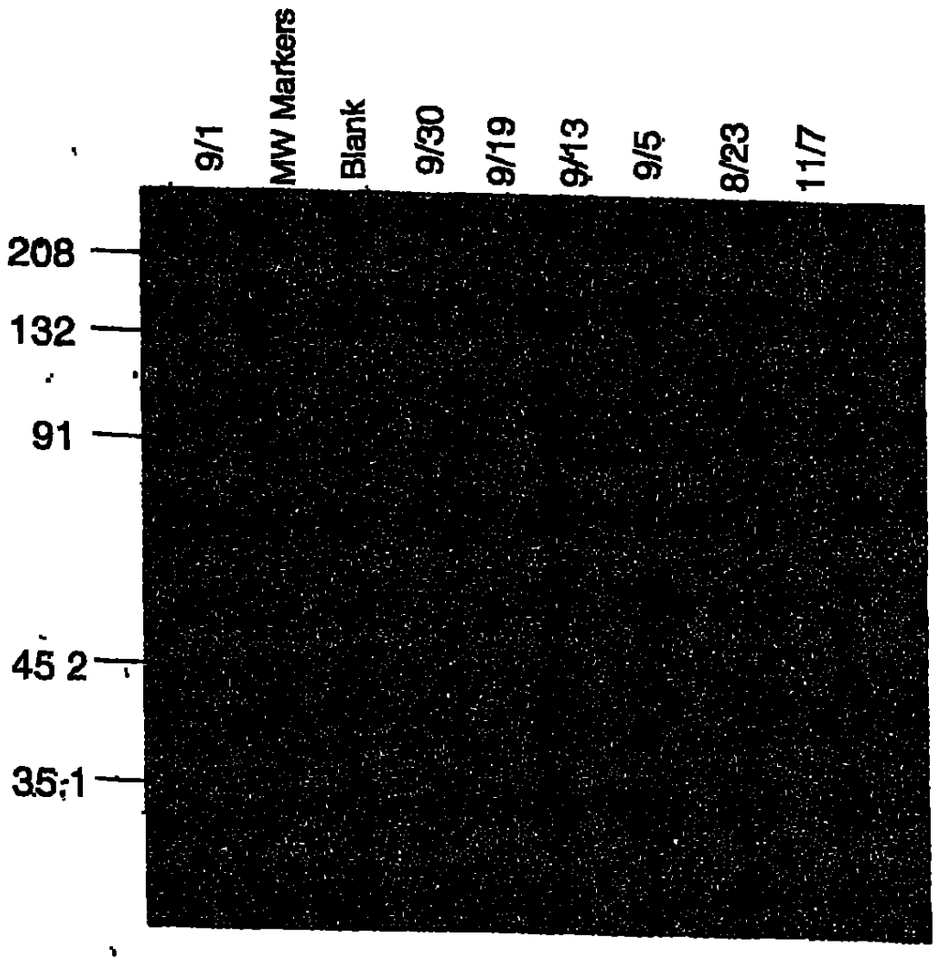




Fig 4A

Complement Mediated B Cell Killing After Binding of CD20-targeted 2H7 scFvIgG1 (SSS-S)H WCH2 WCH3

2H7scFv-Ig Concentration	RAMOS # live cells/total cells	BJAB #-live cells/total cells
20 µg/ml + complement	0 16	0 07
5 µg/ml + complement	0 2	N D
1 25 µg/ml + complement	0 32	0 1
Complement alone	0.98	0 94

*Viability was determined by trypan blue exclusion and is tabulated as the fraction of viable cells out of the total number of cells counted.

**N D (not determined)

Fig 4B

Antibody-dependent cellular cytotoxicity (ADCC) mediated by 2H7scFv-IgG1 (SSS-S)H WCH2 WCH3

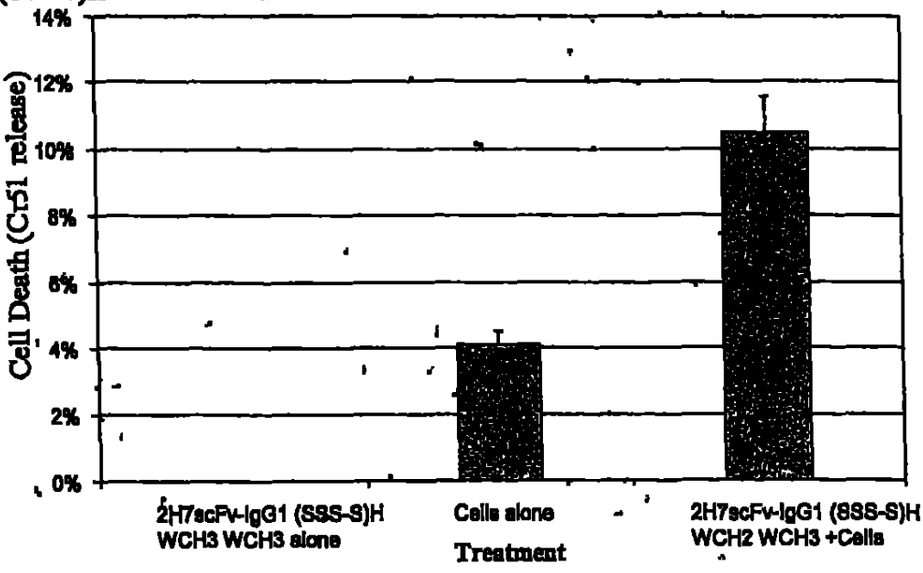
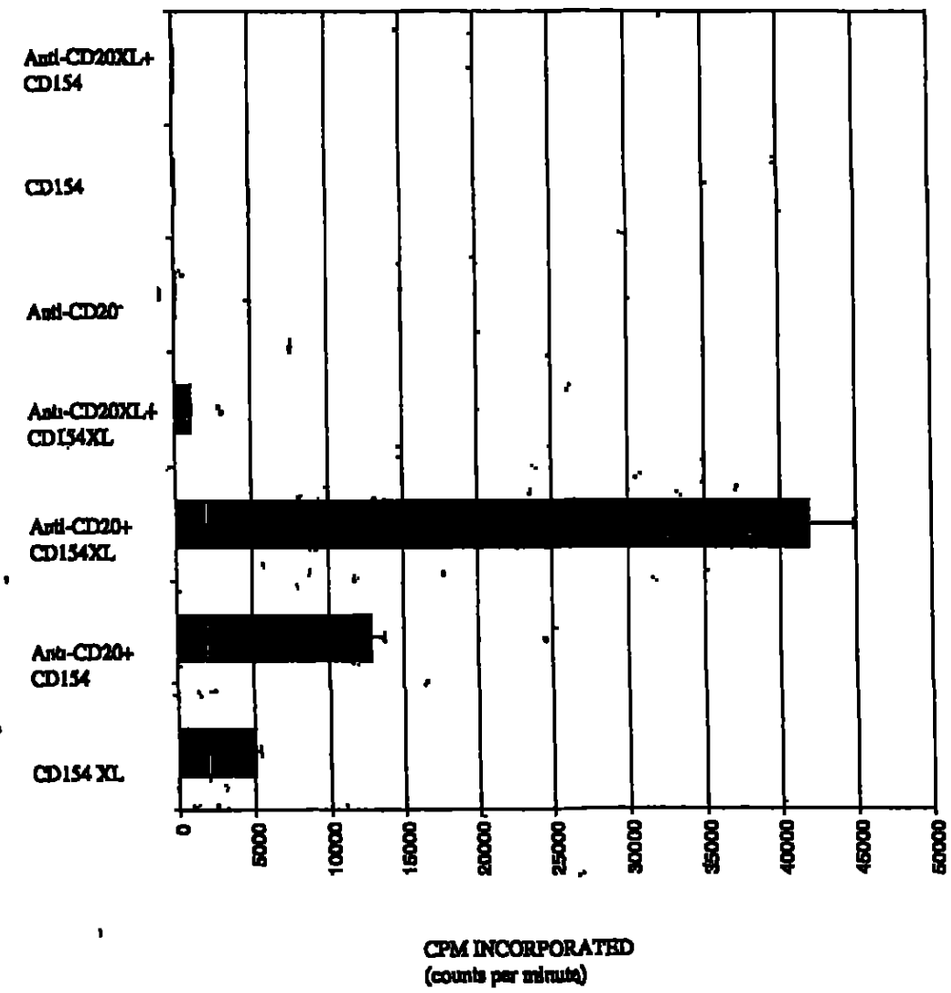


Fig 5

Effects of Crosslinking of CD20 and CD40 Cell Surface Receptors on B Cell Proliferation



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Fig. 6

Effect of Simultaneous ligation of CD20 and CD40 on CD95 and apoptosis.

Fig 6A

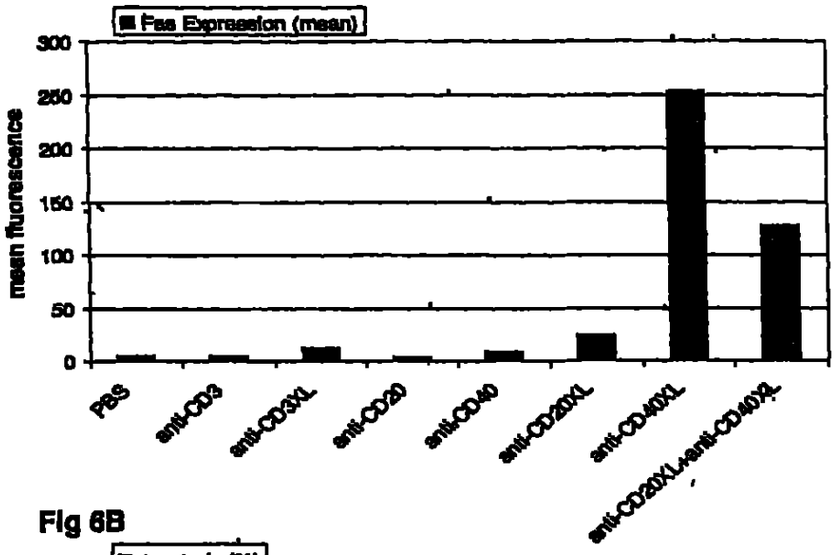


Fig 6B

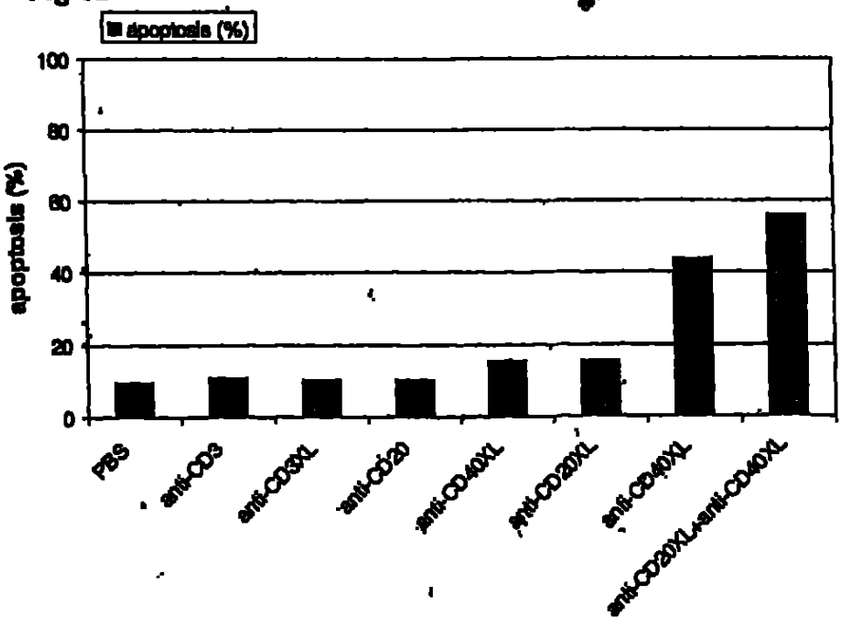


Fig. 7A

2H7-CD154 L2 cDNA and predicted amino acid sequence

HindIII NcoI 2H7 V_L Leader Peptide →

```
                    M  D  F  Q  V  Q  I  F  S  F  L  L  I  S  A  S
1   AAGCTTGCCG CC   ATGGATTT TCAAGTGCAG ATTTTCAGCT TCCTGCTAAT CAGTGCTTCA

                                    2H7 V_L →
    V  I  I  A  R  G  Q  I  V  L  S  Q  S  P  A  I  L  S  A  S
61  GTCATAATTG CCAGAGGACA AATTGTTCTC TCCCAGTCTC CAGCAATCCT GTCTGCATCT

    P  G  E  K  V  T  M  T  C  R  A  S  S  S  V  S  Y  M  H  W
121 CCAGGGGAGA AGGTCACAAT GACTTGCAGG GCCAGCTCAA GTGTAAGTTA CATGCACTGG

                BamHI
                ~~~~~~
    Y  Q  Q  K  P  G  S  S  P  K  P  W  I  Y  A  P  S  N  L  A
181 TACCAGCAGA AGCCAGGATC CTCCCCCAAA CCCTGGATTT ATGCCCCATC CAACCTGGCT

    S  G  V  P  A  R  F  S  G  S  G  S  G  T  S  Y  S  L  T  I
241 TCTGGAGTCC CTGCTCGCTT CAGTGGCAGT GGGTCTGGGA CCTCTTACTC TCTCACAATC

    S  R  V  E  A  E  D  A  A  T  Y  Y  C  Q  Q  W  S  F  N  P
301 AGCAGAGTGG AGGCTGAAGA TGCTGCCACT TATTACTGCC AGCAGTGGAG TTTTAACCCA

(Gly4Ser)3 Linker →
    P  T  F  G  A  G  T  K  L  E  L  K  G  G  G  G  S  G  G  G
361 CCCACGTTCG GTGCTGGGAC CAAGCTGGAG CTGAAAGGTG GCGGTGGCTC GGGCGGTGGT

                                                2H7 V_H →
    G  S  G  G  G  G  S  S  Q  A  Y  L  Q  Q  S  G  A  E  L  V
421 GGATCTGGAG GAGGTGGGAG CTCTCAGGCT TATCTACAGC AGTCTGGGGC TGAGCTGGTG

    R  P  G  A  S  V  K  M  S  C  K  A  S  G  Y  T  F  T  S  Y
481 AGGCCTGGGG CCTCAGTGAA GATGTCCTGC AAGGCTTCTG GCTACACATT TACCAGTTAC

    N  M  H  W  V  K  Q  T  P  R  Q  G  L  E  W  I  G  A  I  Y
541 AATATGCACT GGGTAAAGCA GACACCTAGA CAGGGCCTGG AATGGATTGG AGCTATTTAT

    P  G  N  G  D  T  S  Y  N  Q  K  F  K  G  K  A  T  L  T  V
601 CCAGGAAATG GTGATACTTC CTACAATCAG AAGTTCAAGG GCAAGGCCAC ACTGACTGTA

    D  K  S  S  S  T  A  Y  M  Q  L  S  S  L  T  S  E  D  S  A
661 GACAAATCCT CCAGCACAGC CTACATGCAG CTCAGCAGCC TGACATCTGA AGACTCTGCG

    V  Y  F  C  A  R  V  Y  Y  Y  S  N  S  Y  W  Y  F  D  V  W
721 GTCTATTTCT GTGCAAGAGT GGTGTACTAT AGTAACTCTT ACTGGTACTT CGATGTCTGG
```

512 pc

Fig 7A (continued)

human CD154/amino acid 48→

Bcl/Bam hybrid site

```
      G  T  G  T  T  V  T  V  S  D  P  R  R  L  D  K  I  E  D  E
781   GGCACAGGGA CCACGGTCAC CGTCTCTGAT CCAAGAAGGT TGGACAAGAT AGAAGATGAA

      R  N  L  H  E  D  F  V  F  M  K  T  I  Q  R  C  N  T  G  E
841   AGGAATCTTC ATGAAGATTT TGTATTCATG AAAACGATAC AGAGATGCAA CACAGGAGAA

      R  S  L  S  L  L  N  C  E  E  I  K  S  Q  F  E  G  F  V  K
901   AGATCCTTAT CCTTACTGAA CTGTGAGGAG ATTAAAAGCC AGTTTGAAGG CTTTGTGAAG

                                                              BclI
      D  I  M  L  N  K  E  E  T  K  K  E  N  S  F  E  M  Q  K  G
961   GATATAATGT TAAACAAAGA GGAGACGAAG AAAGAAAACA GCTTTGAAAT GCAAAAAGGT

      BclI
      ~~~~~
      D  Q  M  P  Q  I  A  A  H  V  I  S  E  A  S  S  K  T  T  S
1021  GATCAGAATC CTCAAATTGC GGCACATGTC ATAAGTGAGG CCAGCAGTAA AACAACATCT

      V  L  Q  W  A  E  K  G  Y  Y  T  M  S  N  N  L  V  T  L  E
1081  GTGTTACAGT GGGCTGAAAA AGGATACTAC ACCATGAGCA ACAACTTGGT AACCCTGGAA

      N  G  K  Q  L  T  V  K  R  Q  G  L  Y  Y  I  Y  A  Q  V  T
1141  AATGGGAAAC AGCTGACCGT TAAAAGACAA GGACTCTATT ATATCTATGC CCAAGTCACC

                  HindIII
                  ~~~~~~~
      F  C  S  N  R  E  A  S  S  Q  A  P  F  I  A  S  L  C  L  K
1201  TTCTGTTCCA ATCGGGAAGC TTCGAGTCAA GCTCCATTTA TAGCGAGCCT CTGCCTAAAG

      S  P  G  R  F  E  R  I  L  L  R  A  A  N  T  H  S  S  A  K
1261  TCCCCCGGTA GATTCGAGAG AATCTTACTC AGAGCTGCAA ATACCCACAG TTCCGCCAAA

      P  C  G  Q  Q  S  I  H  L  G  G  V  F  E  L  Q  P  G  A  S
1321  CCTTGCGGGC AACAATCCAT TCACTTGGGA GGAGTATTTG AATTGCAACC AGGTGCTTCG

                                        NcoI
                                        ~~~~~~
      V  F  V  N  V  T  D  P  S  Q  V  S  H  G  T  G  F  T  S  F
1381  GTGTTTGTCA ATGTGACTGA TCCAAGCCAA GTGAGCCATG GCACTGGCTT CACGTCCTTT

                  XhoI          XbaI
                  ~~~~~~        ~~~~
      G  L  L  K  L  E  *  *  S  R
1441  GGCTTACTCA AACTCGAGTG ATAATCTAGA
```

S13 part

Fig 7B.

2H7scFv-CD154 S4 cDNA and predicted amino acid sequence.

HindIII NcoI
2H7 V_L Leader Peptide→
M D F Q V Q I F S F L L I S A S
1 AAGCTTGCCG CC ATGGATTT TCAAGTGCAG ATTTTCAGCT TCCTGCTAAT CAGTGCTTCA

2H7 V_L →
V I I A R G Q I V L S Q S P A I L S A S
61 GTCATAATTG CCAGAGGACA AATTGTTCTC TCCCAGTCTC CAGCAATCCT GTCTGCATCT

P G E K V T M T C R A S S S V S Y M H W
121 CCAGGGGAGA AGGTCACAAT GACTTGCAGG GCCAGCTCAA GTGTAAGTTA CATGCACTGG

BamHI
Y Q Q K P G S S P K P W I Y A P S N L A
181 TACCAGCAGA AGCCAGGATC CTCCCCCAAA CCCTGGATTT ATGCCCCATC CAACCTGGCT

S G V P A R F S G S G S G T S Y S L T I
241 TCTGGAGTCC CTGCTCGCTT CAGTGGCAGT GGGTCTGGGA CCTCTTACTC TCTCACAATC

S R V E A E D A A T Y Y C Q Q W S F N P
301 AGCAGAGTGG AGGCTGAAGA TGCTGCCACT TATTACTGCC AGCAGTGGAG TTTTAACCCA

(Gly$_4$Ser)$_3$ Linker →
P T F G A G T K L E L K G G G G S G G G
361 CCCACGTTCG GTGCTGGGAC CAAGCTGGAG CTGAAAGGTG GCGGTGGCTC GGGCGGTGGT

2H7 V_H →
G S G G G G S S Q A Y L Q Q S G A E L V
421 GGATCTGGAG GAGGTGGGAG CTCTCAGGCT TATCTACAGC AGTCTGGGGC TGAGCTGGTG

R P G A S V K M S C K A S G Y T F T S Y
481 AGGCCTGGGG CCTCAGTGAA GATGTCCTGC AAGGCTTCTG GCTACACATT TACCAGTTAC

N M H W V K Q T P R Q G L E W I G A I Y
541 AATATGCACT GGGTAAAGCA GACACCTAGA CAGGGCCTGG AATGGATTGG AGCTATTTAT

P G N G D T S Y N Q K F K G K A T L T V
601 CCAGGAAATG GTGATACTTC CTACAATCAG AAGTTCAAGG GCAAGGCCAC ACTGACTGTA

D K S S S T A Y M Q L S S L T S E D S A
661 GACAAATCCT CCAGCACAGC CTACATGCAG CTCAGCAGCC TGACATCTGA AGACTCTGCG

V Y F C A R V V Y Y S N S Y W Y F D V W
721 GTCTATTTCT GTGCAAGAGT GGTGTACTAT AGTAACTCTT ACTGGTACTT CGATGTCTGG

Fig 7B

human CD154/amino acid 108 →

Bcl/Bam hybrid site

BclI

G T G T T V T V S D P E N S F E M Q K G
781 GGCACAGGGA CCACGGTCAC CGTCTCTGAT CCAGAAAACA GCTTTGAAAT GCAAAAAGGT

BclI

D Q N P Q I A A H V I S E A S S K T T S
841 GATCAGAATC CTCAAATTGC GGCACATGTC ATAAGTGAGG CCAGCAGTAA AACAACATCT

V L Q W A E K G Y Y T M S N N L V T L E
901 GTGTTACAGT GGGCTGAAAA AGGATACTAC ACCATGAGCA ACAACTTGGT AACCCTGGAA

N G K Q L T V K R Q G L Y Y I Y A Q V T
961 AATGGGAAAC AGCTGACCGT TAAAAGACAA GGACTCTATT ATATCTATGC CCAAGTCACC

HindIII

F C S N R E A S S Q A P F I A S L C L K
1021 TTCTGTTCCA ATCGGGAAGC TTCGAGTCAA GCTCCATTTA TAGCCAGCCT CTGCCTAAAG

S P G R F E R I L L R A A N T K S S A K
1081 TCCCCCGGTA GATTCGAGAG AATCTTACTC AGAGCTGCAA ATACCCACAG TTCCGCCAAA

P C G Q Q S I H L G G V F E L Q P G A S
1141 CCTTGCGGGC AACAATCCAT TCACTTGGGA GGAGTATTTG AATTGCAACC AGGTGCTTCG

NcoI

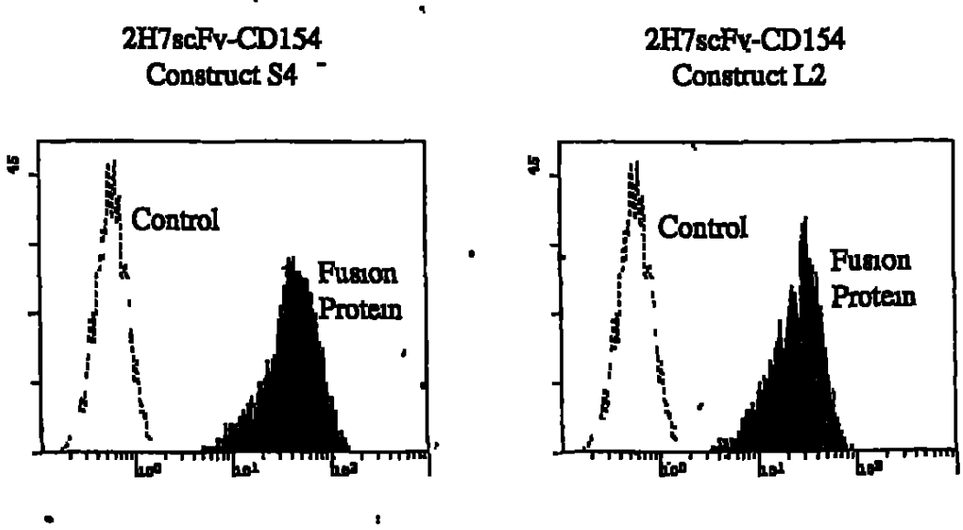
V F V N V T D P S Q V S H G T G F T S F
1201 GTGTTTGTCA ATGTGACTGA TCCAAGCCAA GTGAGCCATG GCACTGGCTT CACGTCCTTT

XhoI XbaI

G L L K L E * * S R
1261 GGCTTACTCA AACTCGAGTG ATAATCTAGA

Fig 8

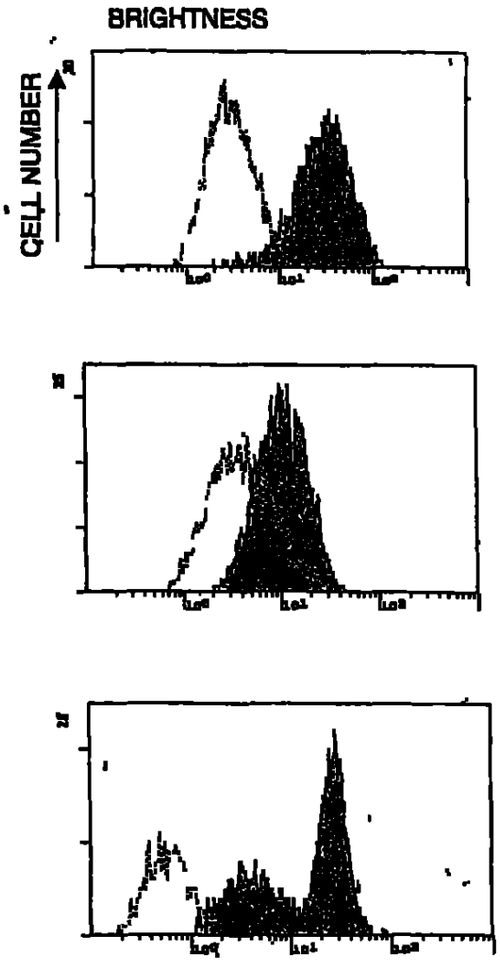
Simultaneous Binding of 2H7scFv-CD154 Fusion Proteins to CD20 and CD40



CD20 CHO cell targets + (control of fusion protein)
+ Biotin-CD40Ig + PE-SA

Fig 9

Induction of Apoptosis Measured by Binding of Annexin V after incubation with 2H7scFv-CD154



control supernatant ___2H7scFv-CD154 supernatant

Fig 10

Proliferation of T51 B Cell Line After Incubation with 2H7 scFv-CD154 S4 or 2H7 scFv-CD154 L2 Constructs

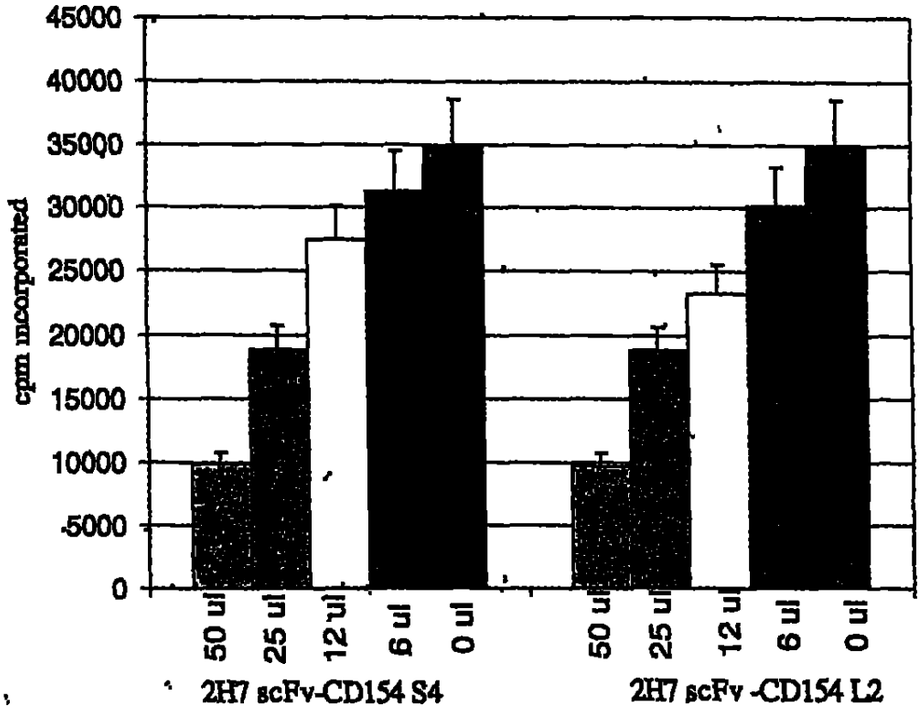
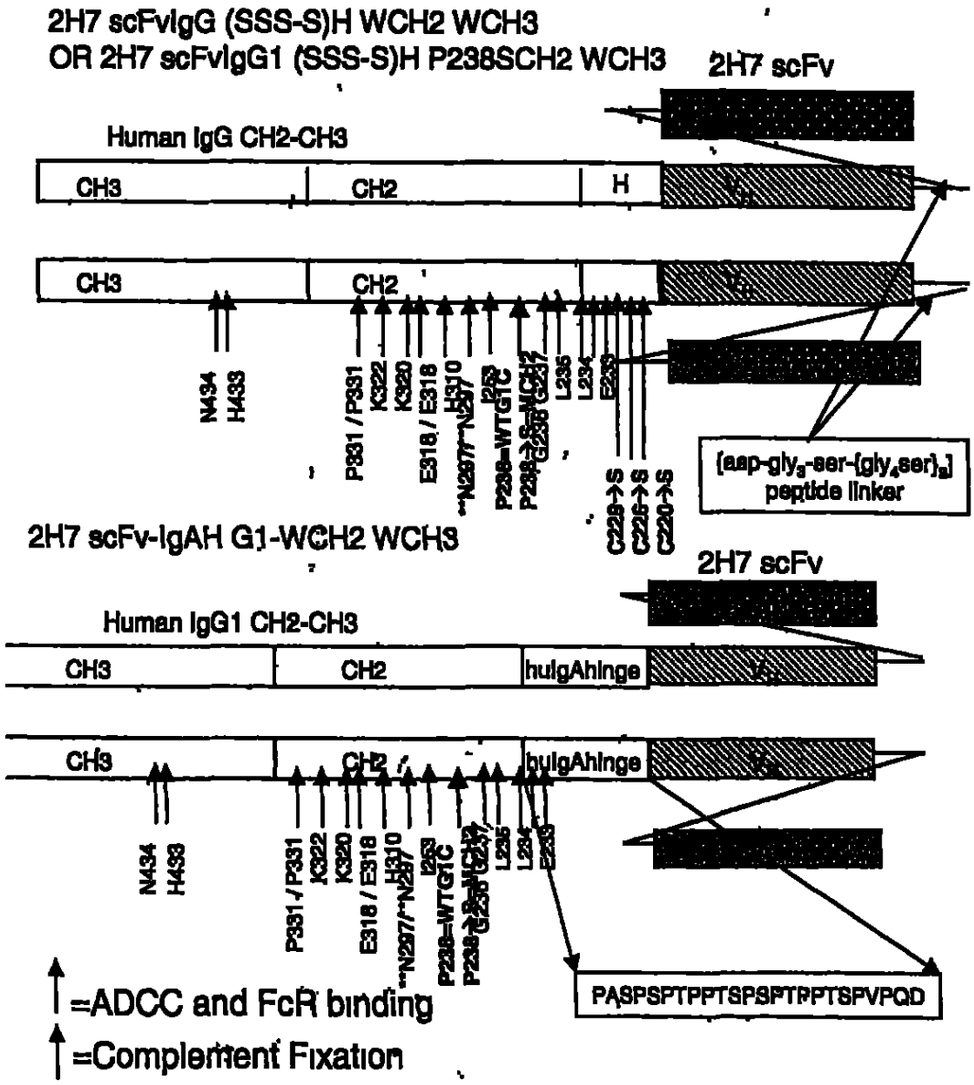


Fig 11

Schematic Representation of 2H7 scFvIg Constructs



519

Fig 12

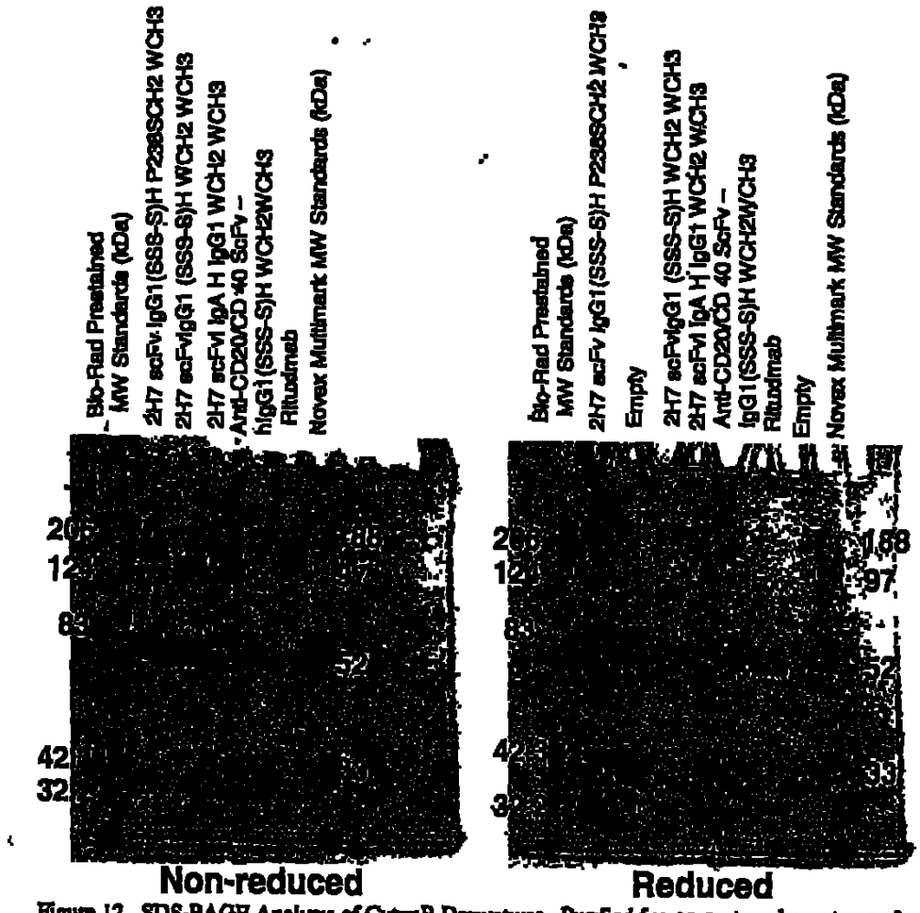


Figure 12. SDS-PAGE Analysis of CytoxB Derivatives Purified fusion protein derivatives of CytoxB-scFvIg molecules and Rituximab were resuspended SDS sample buffer, boiled, loaded onto 10% Novex Tris-Bis gels (Invitrogen, San Diego, CA) and subjected to nonreducing (left panel) or reducing (right panel) SDS-PAGE electrophoresis at 175 volts Two different molecular weight markers, BioRad prestained markers, and Novex Multimark molecular weight markers were also loaded onto each gel and the approximate size in kDa of each marker band is indicated along each side of the photographed gels Gels were stained in Coomassie Blue stain and photographed with a SONY Mavica Digital camera. The mutant hinge forms of 2H7 scFvIgG1 migrate at approximately 70 kDa under both nonreducing and reducing conditions, indicating that these molecules are monomeric rather than dimeric in structure. The IgA hinge form of 2H7scFvIg migrates at approximately 75 kDa under reducing conditions, but migrates predominately as a dimer of 140 kDa with a fraction of the protein migrating at 75 kDa under nonreducing conditions. Under nonreducing conditions, rituximab migrates as a diffuse band of between 150 and 200 kDa. The heavy and light chains resolve into separate bands of approximately 32 and 50 kDa when rituximab is reduced and subjected to SDS-PAGE

Fig 13

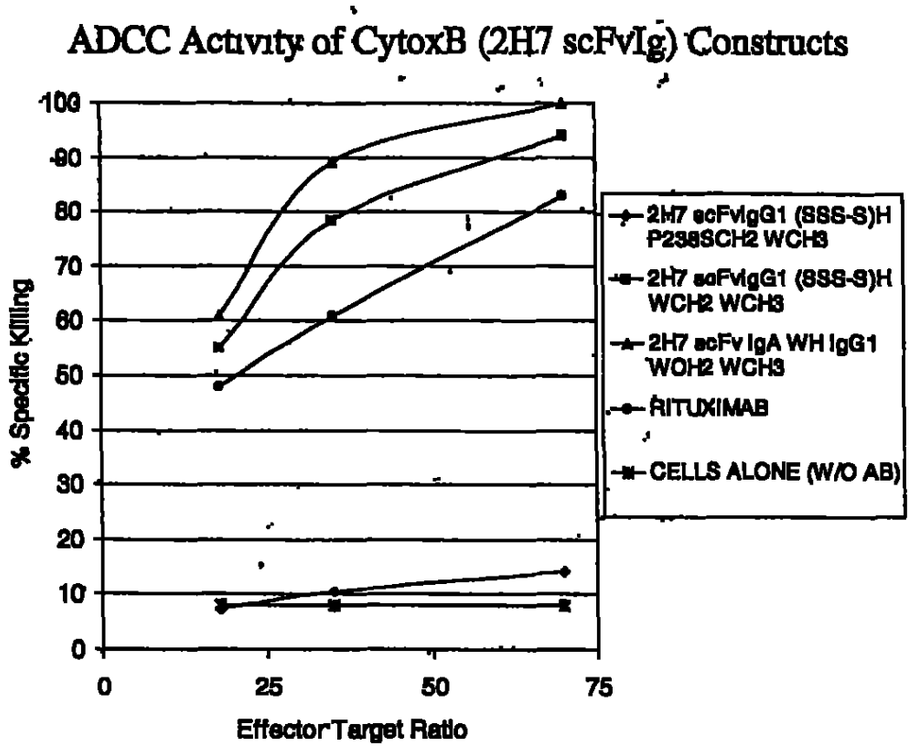


Figure 13 ADCC Activity of CytoxB Derivatives Compared to Rituximab ADCC activity of CytoxB Derivatives or Rituximab was measured *in vitro* against BJAB B lymphoma cell line as target and using fresh human PBMC as effector cells Effector to target ratios were varied as follows 70 1, 35 1, and 18 1, with the number of BJAB cells per well remaining constant but varying the number of PBMC Bjab cells were labeled for 2 hours with ^{51}Cr and aliquoted at a cell density of $5x10^4$ cells/well to each well of flat-bottom 96 well plates Purified fusion proteins or rituximab were added at a concentration of 10 mg/ml, and PBMC were added at $9x10^5$ cells /well (18 1), $1.8x10^6$ cells/well (35 1), or $3.6x10^6$ cells/well (70 1), in a final volume of 200 µl Spontaneous release was measured without addition of PBMC or fusion protein, and maximal release was measured by the addition of detergent (1% NP-40) to the appropriate wells Reactions were incubated for 4 hours, and 100 ml culture supernatant harvested to a Lumaplate (Packard Instruments) and allowed to dry overnight prior to counting cpm released on a Packard Top Count NXT Microplate Scintillation Counter

Fig 14.

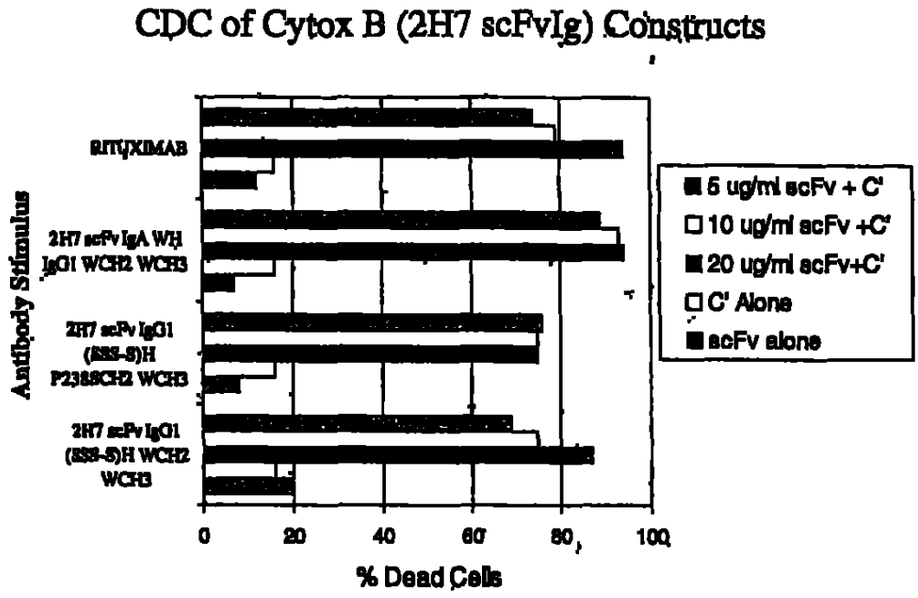
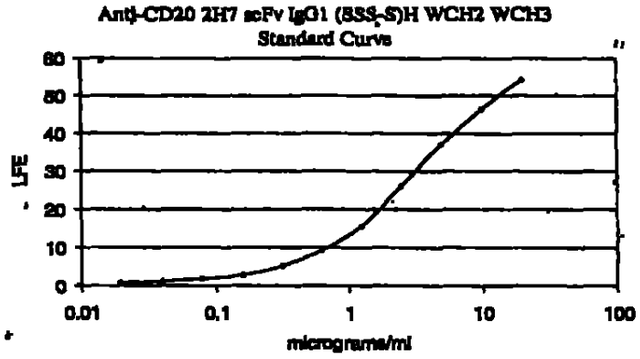


Figure 14 Complement Dependent Cytoxicity (CDC) Activity of CytoxB Derivatives Compared to Rituximab 2H7 scFvIgG1 (SSS-S)H WCH2 WCH3, 2H7 scFvIgG1 (SSS-S)H WCH2 WCH3, and 2H7scFv IgA WH IgG1 WCH2 WCH3 derivatives and Rituximab were compared for their ability to mediate complement dependent cytoxicity Rabbit complement (Pel-Freez) was diluted 1 10 and added to BJAB cells along with dilutions of each antibody derivative (20 µg/ml, 10 µg/ml, and 5 µg/ml) Controls were also included without addition of complement (C') or scFv derivative Reactions were allowed to continue for 1 hour, and cells from each well were then stained with trypan blue and the cell viability counted using a hemacytometer Data is graphed as % of dead cells/total cells counted for each condition assayed.

52

Fig 15

2H7 (anti-CD20) scFv IgG1 (SSS-S)H WCH2 WCH3
In Vivo Half Life



Macaque A99314

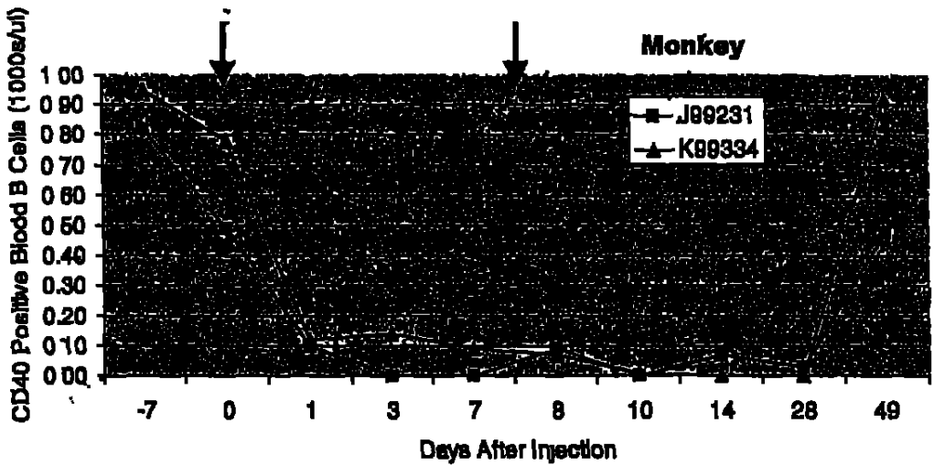
Day	Binding Intensity (LFE) @ 1:50 dilution of serum	estimated concentration (µg/ml)
–7	0 213	<0 1
0 (Injection #1)	0 227	<0 1
1	7 79	25 1
3	5 51	15 6
7 (Injection #2)	3 37	9 4
8	11 33	41 7
10	5 45	15 4
14	0.27	<0 1

Macaque F98081

Day	Binding Intensity (LFE) @ 1:50 dilution of serum	estimated concentration (µg/ml)
–7	0 208	<0 1
0 (Injection #1)	0 219	<0 1
1	6 73	21 9
3	6 14	19 3
7 (Injection #2)	3 04	8 7
8	9 83	33 8
10	4 77	14 4
14	0 231	<0 1

Fig. 16

B Cell Depletion in macaques mediated by Cytox B20 (2H7 scFv IgG1 (SSS-S)H WCH2 WCH3) Construct

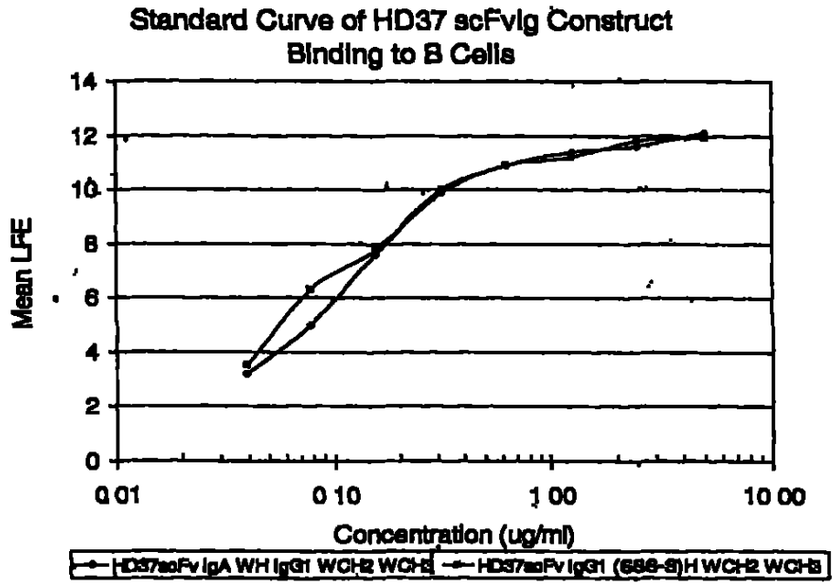


- **CytoxB20 injections of 6mg/kg yields 3 week B-cell depletion**
- **3-4 day half-life *in vivo***
- **CD20 saturation in lymph node B-cells at d14**
- **No first dose effects**
- **No anti-chimeric antibody development**

524

Fig. 17

Production Levels of HD37 scFvIg Constructs by CHO Cell Lines

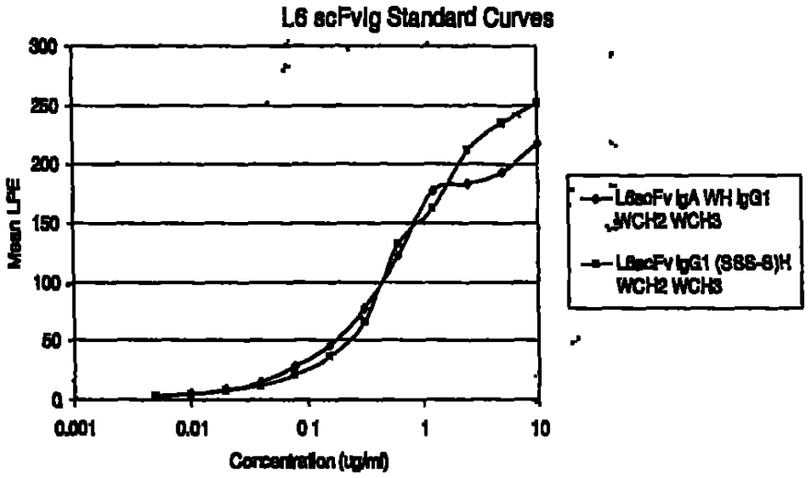


Clone/Isolate	Mean LFE at 1 100	Estimated Concentration
Bulk HD37 scFv		
IgA WH IgG1 WCH2 WCH3	11 2	> 60 ug/ml
1B2	10 4	>50 ug/ml
6C5	10 5	>50 ug/ml
4B1	8 6	>40 ug/ml
Bulk HD37 scFv		
IgG1 (SSS-S)H WCH2 WCH3	10 9	> 50 ug/ml
2G8	10 6	> 50 ug/ml
3F3	8 3	>40 ug/ml
3D9	11 1	> 60 ug/ml

525

Fig 18

Production of L6 scFvIg constructs by CHO Cells



Construct	Mean LFE 1 20	Estimated Concentration
L6scFv IgA WH IgG1 WCH2 WCH3 unamplified CHO sup	51 1	6 25 ug/ml
L6scFv IgG1(SSS-S)H WCH2 WCH3 unamplified CHO sup	28 0	3 2 ug/ml

Fig. 19

ADCC Activity of 2H7 scFvIg, G28-1 scFvIg, and HD37 scFvIg Constructs

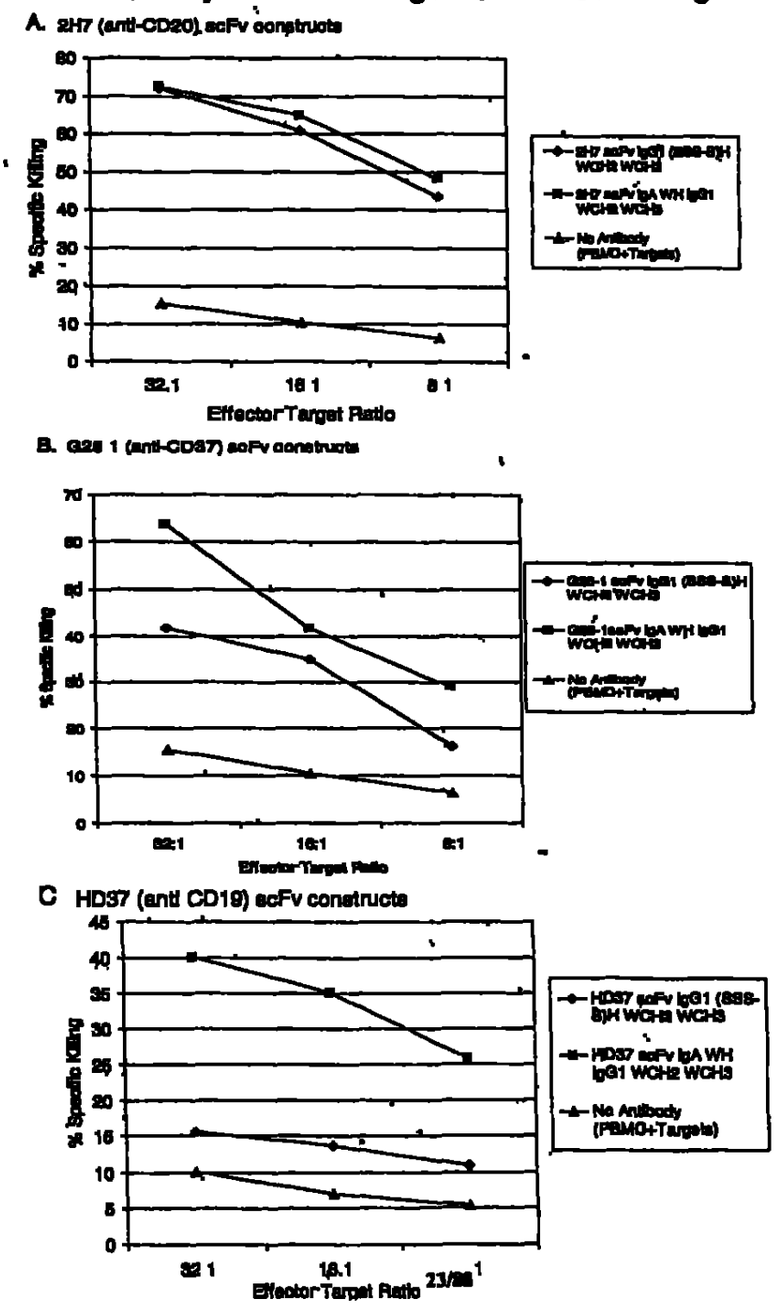
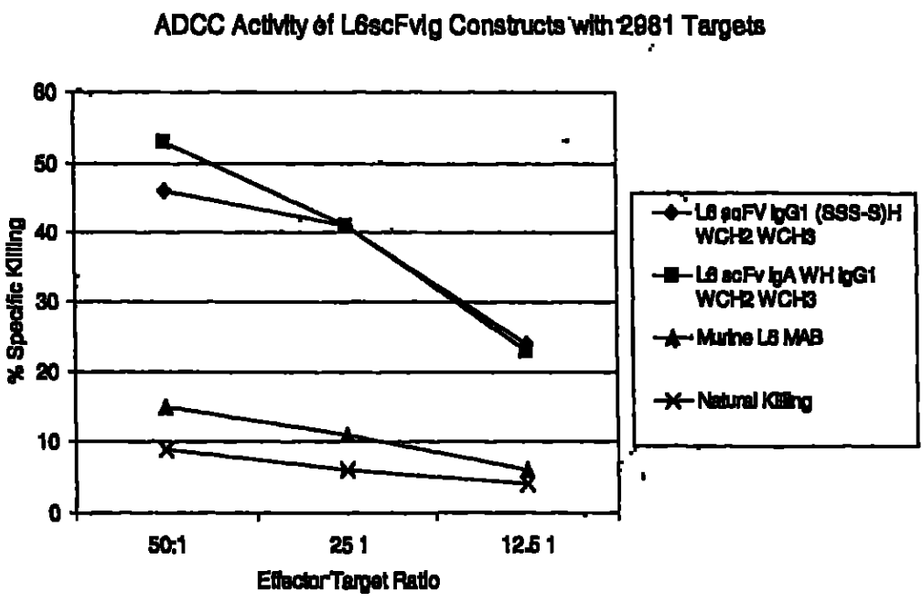


Fig 20

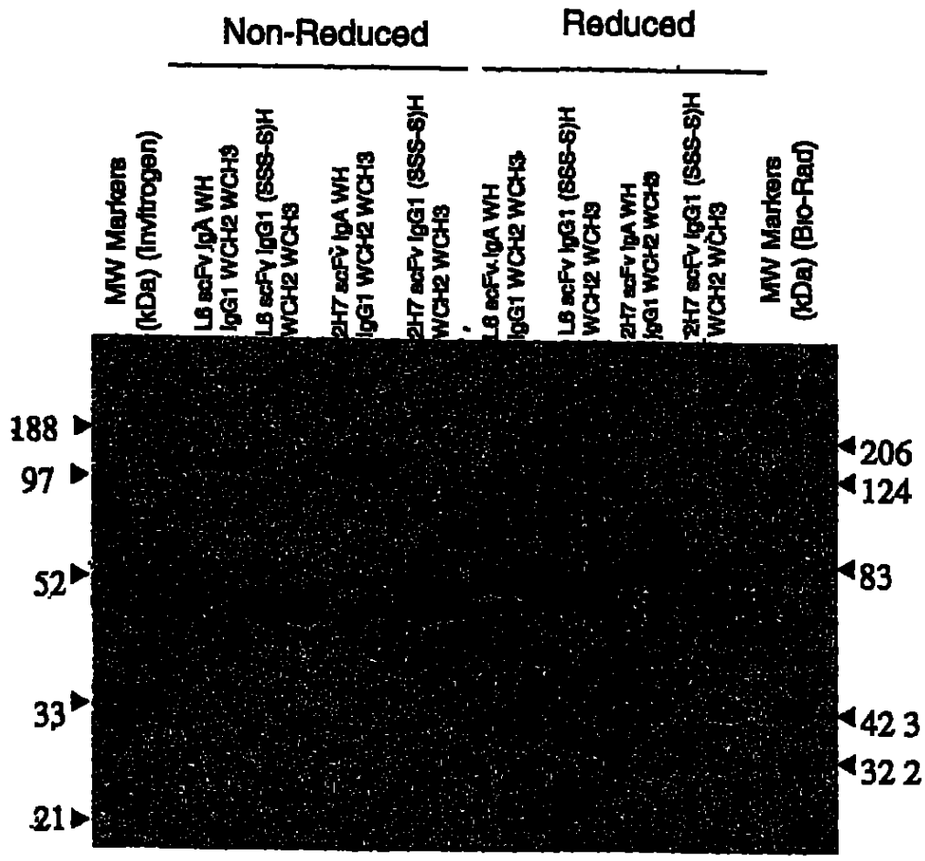
ADCC Activity of L6 scFvIg Constructs



528

Fig 21

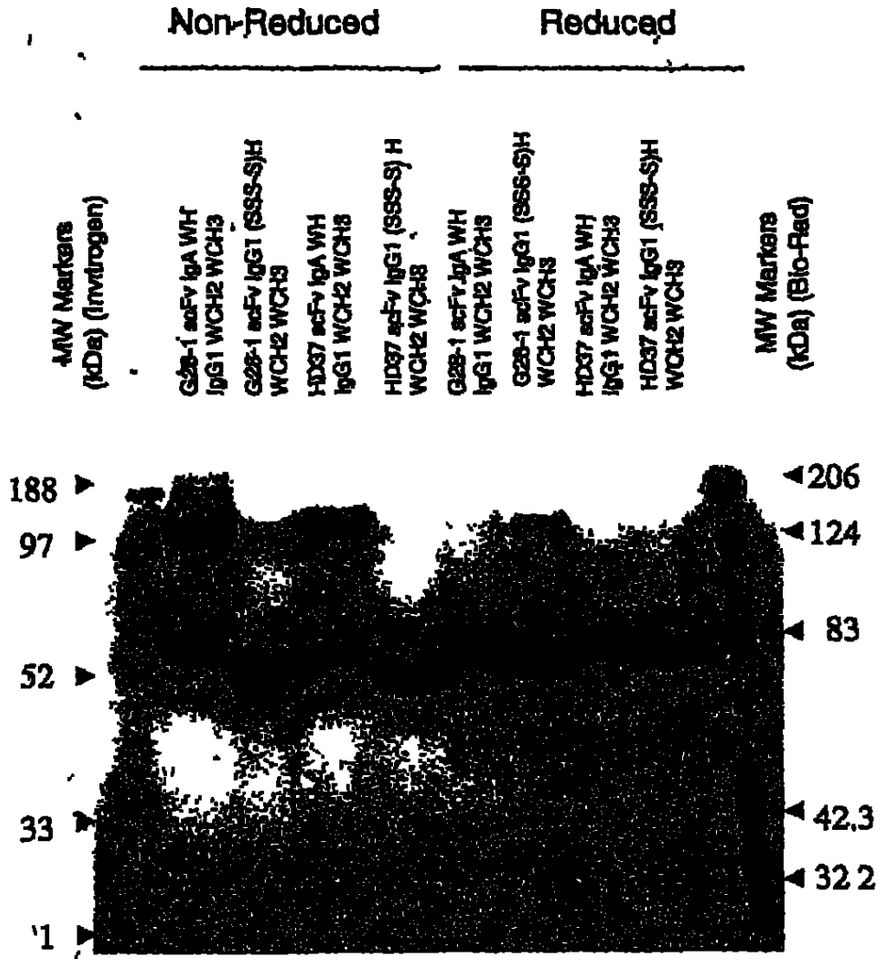
SDS-PAGE Analysis of L6 and 2H7
scFvIg Fusion Proteins



529

Fig 22

SDS-PAGE Analysis of G28-1 and HD37 scFvIg Constructs.



530 f34

Fig 23

Sequence alignment of human and llama Fc regions.

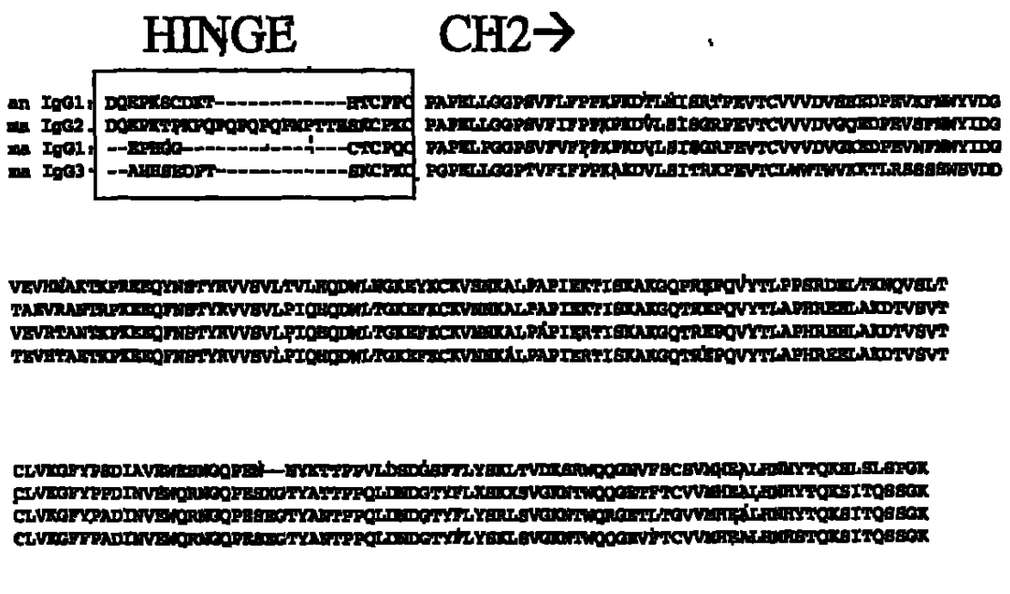
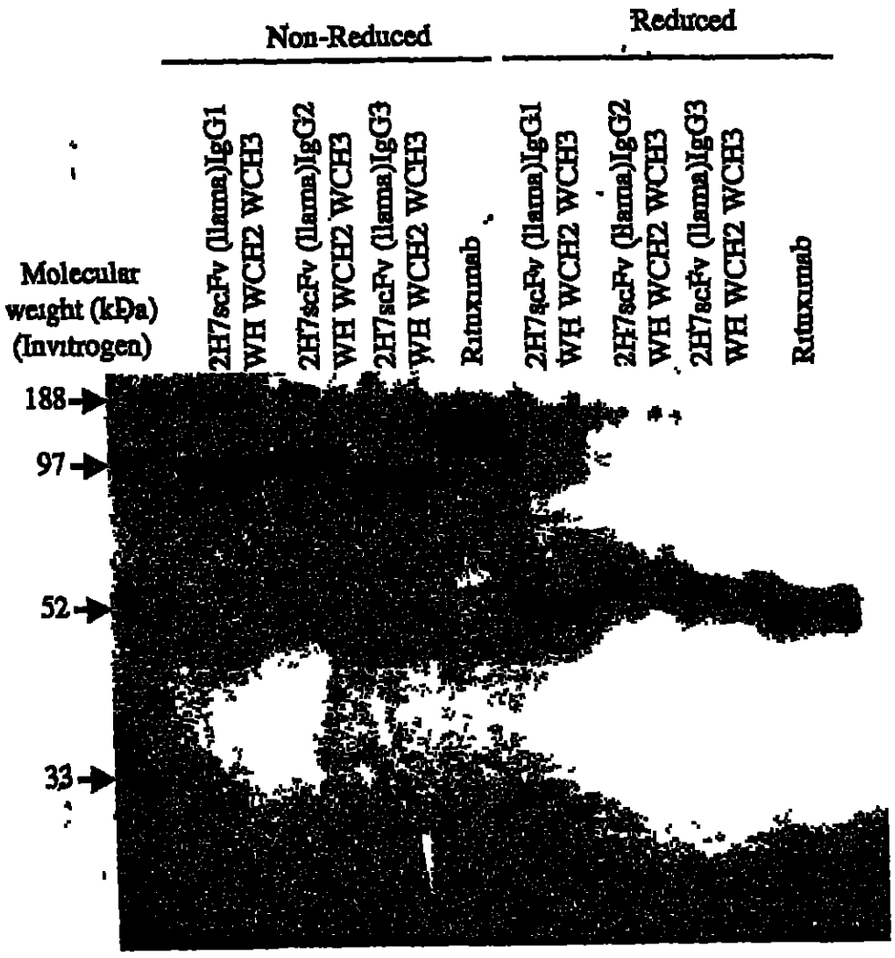
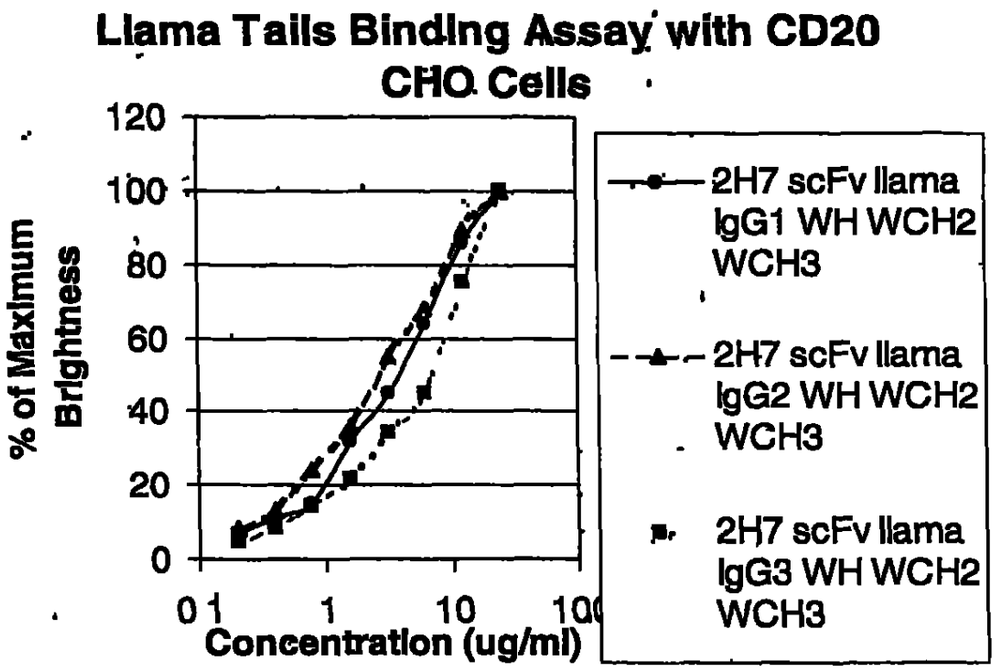


Fig 24


Non-Reduced
Reduced
2H7scFv (llama)IgG1 WH WCH2 WCH3
2H7scFv (llama)IgG2 WH WCH2 WCH3
2H7scFv (llama)IgG3 WH WCH2 WCH3
Rituximab
2H7scFv (llama)IgG1 WH WCH2 WCH3
2H7scFv (llama)IgG2 WH WCH2 WCH3
2H7scFv (llama)IgG3 WH WCH2 WCH3
Rituximab
Molecular weight (kDa) (Invitrogen)
188
97
52
33

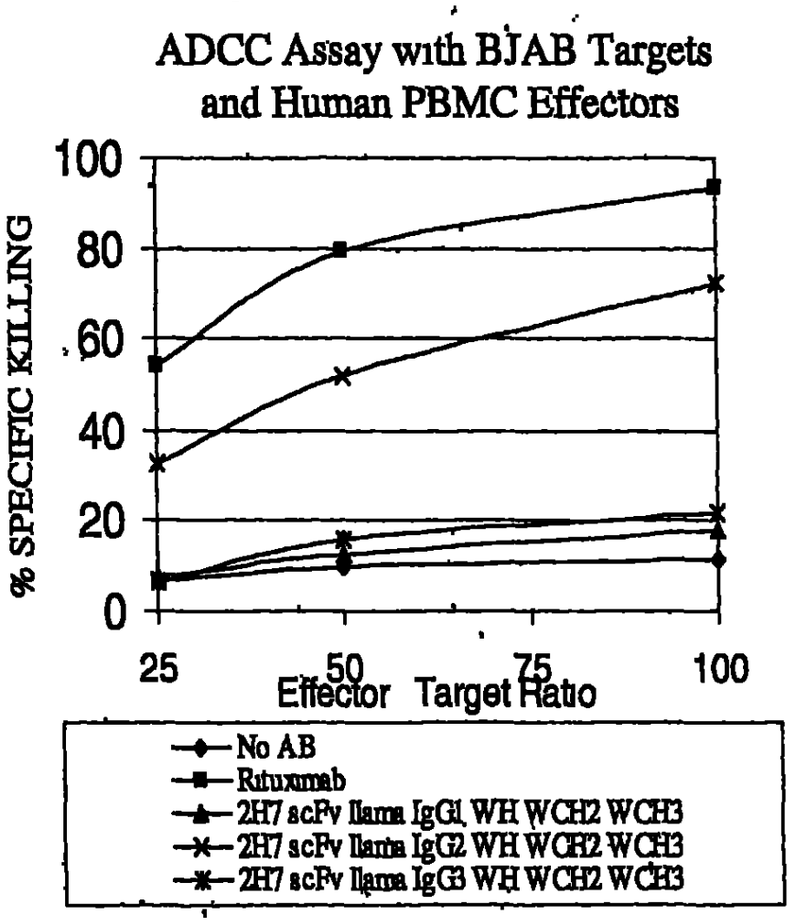
532

Fig 25


Llama Tails Binding Assay with CD20
CHO Cells
% of Maximum Brightness
120
100
80
60
40
20
0
0.1
1
10
100
Concentration (ug/ml)
2H7 scFv llama IgG1 WH WCH2 WCH3
2H7 scFv llama IgG2 WH WCH2 WCH3
2H7 scFv llama IgG3 WH WCH2 WCH3

534 66

Fig 27


ADCC Assay with BJAB Targets
and Human PBMC Effectors
% SPECIFIC KILLING
100
80
60
40
20
0
25
50
75
100
Effector Target Ratio
No AB
Rituximab
2H7 scFv llama IgG1 WH WCH2 WCH3
2H7 scFv llama IgG2 WH WCH2 WCH3
2H7 scFv llama IgG3 WH WCH2 WCH3

535

Fig 28

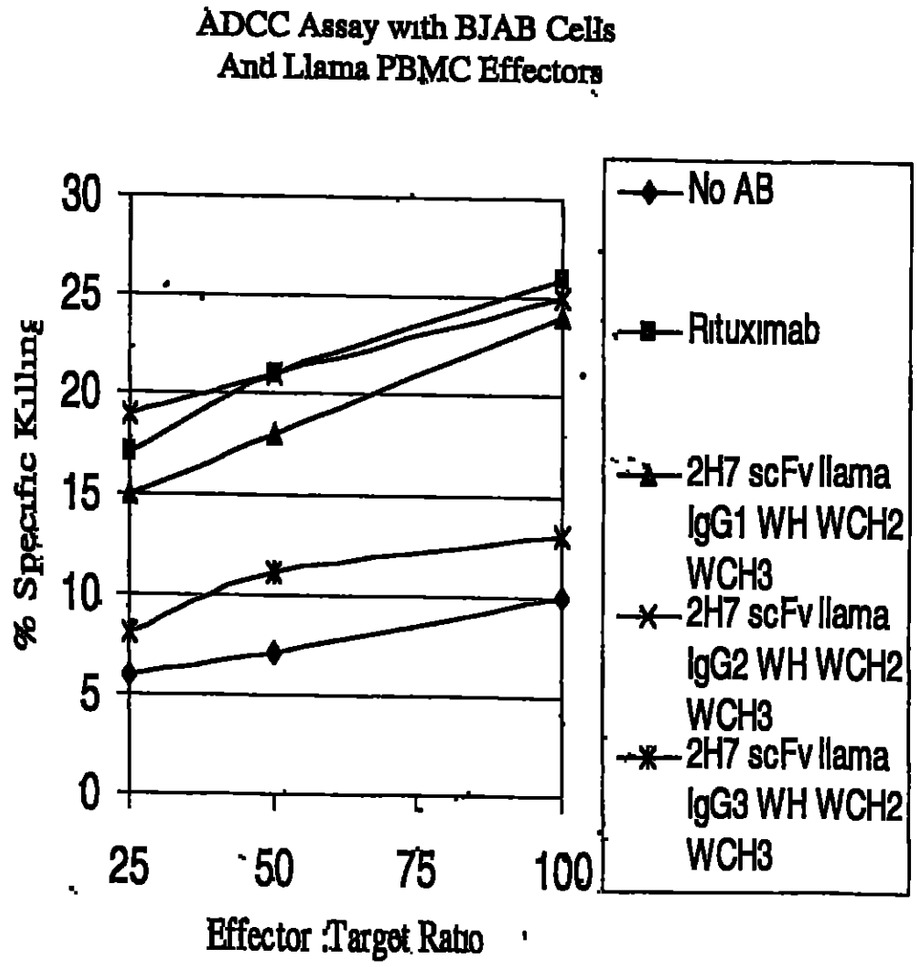
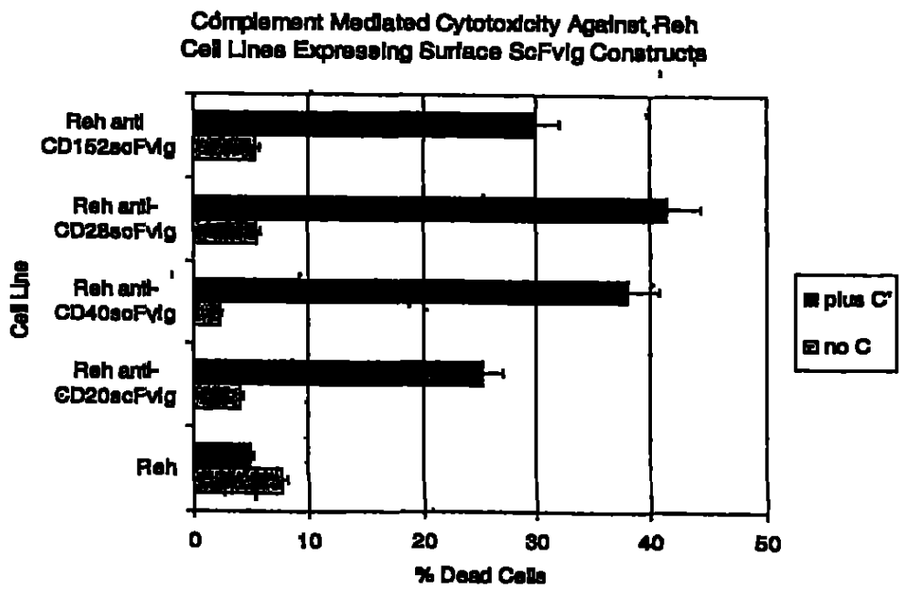
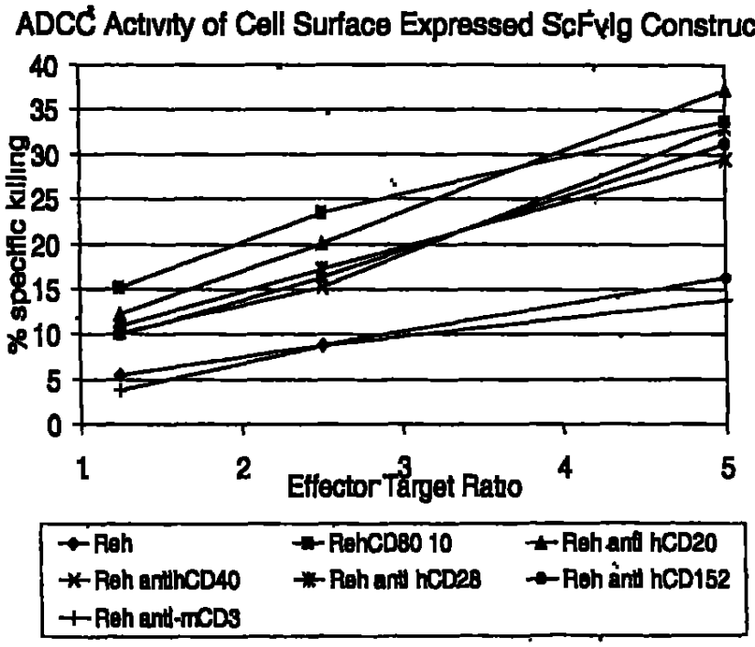

ADCC Assay with BJAB Cells
And Llama PBMC Effectors
% Specific Killing
30
25
20
15
10
5
0
25
50
75
100
Effector :Target Ratio
No AB
Rituximab
2H7 scFv llama IgG1 WH WCH2 WCH3
2H7 scFv llama IgG2 WH WCH2 WCH3
2H7 scFv llama IgG3 WH WCH2 WCH3

Fig 29



Surface scFvIg and Effector Functions

Fig 30



Surface scFvIg and Effector Functions

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Fig 31

Ig Constructs and Nomenclature

Name Identifier	Hinge Sequence	CH2 Sequence	CH3 Sequence
hIgG1 (CCC-P)H WCH2 WCH3	IgG1 WT Hinge (CCC-P)	Wild Type CH2	Wild Type CH3
hIgG1 (SSS-S)H WCH2 WCH3	IgG1 Mutant Hinge (SSS-S)	Wild type CH2 (IgG1)	Wild type CH3 (IgG1)
VH L11S hIgG1 (SSS-S)H WCH2 WCH3	IgG1 Mutant Hinge (SSS-S)	Wild type CH2 (IgG1)	Wild type CH3 (IgG1)
IgG1 (SSC-S)H WCH2 WCH3	IgG1 Mutant Hinge (SSC-S)	Wild type CH2 (IgG1)	Wild type CH3 (IgG1)
IgG1 (SCS-S)H WCH2 WCH3	IgG1 Mutant Hinge (SCS-S)	Wild type CH2 (IgG1)	Wild type CH3 (IgG1)
IgG1 (CSS-S)H WCH2 WCH3	IgG1 Mutant Hinge (CSS-S)	Wild type CH2 (IgG1)	Wild type CH3 (IgG1)
IgG1 (SSS-S)H P238S CH2 WCH3	IgG1 Mutant Hinge (SSS-S)	Mutant CH2 (IgG1) Pro→Ser 238	Wild type CH3 (IgG1)
IgA WH hIgG1 WCH2 WCH3	IgA Hinge	Wild type CH2 (IgG1)	Wild type CH3 (IgG1)
IgA WH IgA WCH2 WCH3	IgA Hinge	Wild type CH2 (IgA)	Wild type CH3 (IgA)
IgA WH IgA WCH2 T4CH3	IgA Hinge	Wild type CH2 (IgA)	Truncated CH3 (IgA) Missing 4 aa at COOH

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Fig. 32

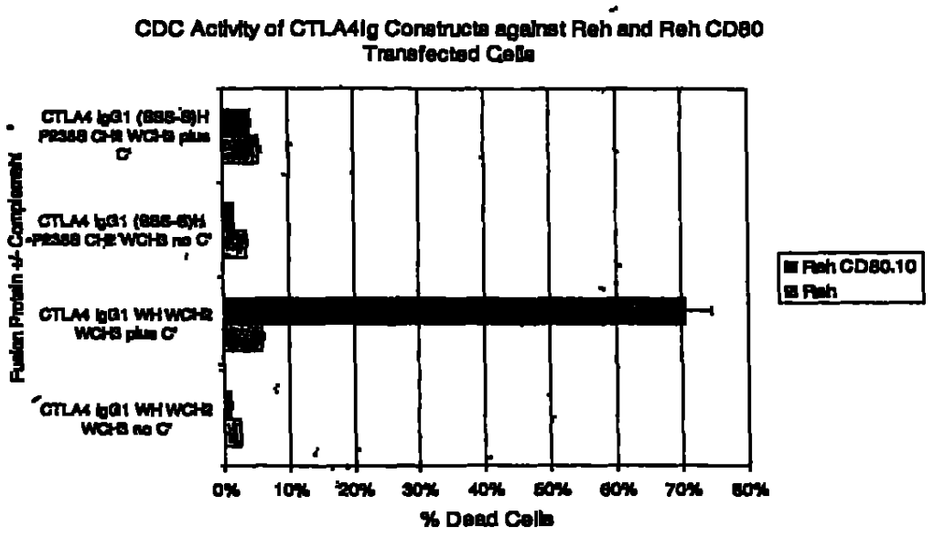
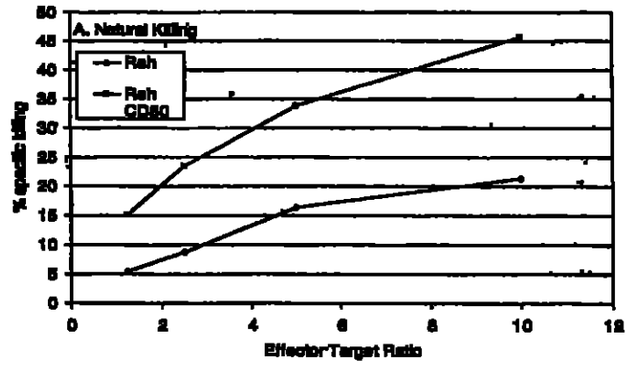
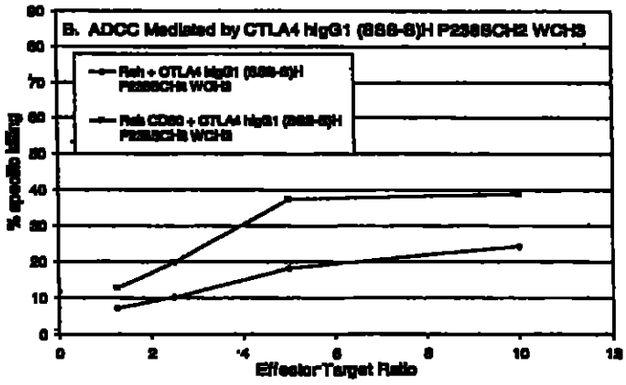

CDC Activity of CTLA4Ig Constructs against Reh and Reh CD80 Transfected Cells
CTLA4 IgG1 (SSS-S)H P93S CH2 WCH-S plus C'
CTLA4 IgG1 (SSS-S)H P93S CH2 WCH-S no C'
CTLA4 IgG1 WH WCH-2 WCH-S plus C'
CTLA4 IgG1 WH WCH-2 WCH-S no C'
Fusion Protein +/- Complement
Reh CD80.10
Reh
0%
10%
20%
30%
40%
50%
60%
70%
80%
% Dead Cells

Fig. 33


A. Natural Killing
Raji
Raji CD80
% specific killing
Effector:Target Ratio


B. ADCC Mediated by CTLA4 hIgG1 (SSS-S)H P238SCH2 WCH3
% specific killing
Effector:Target Ratio

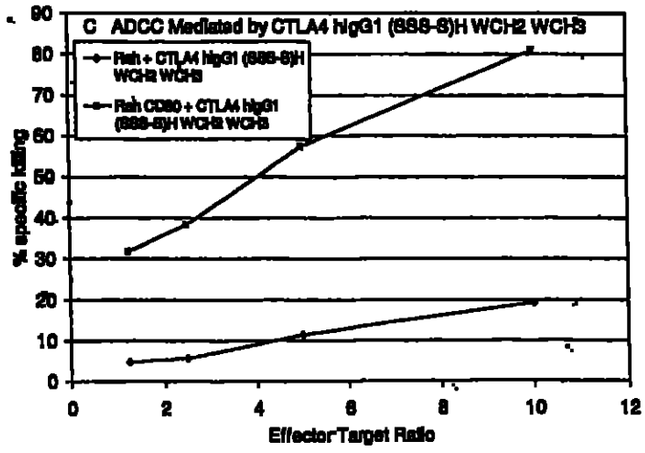
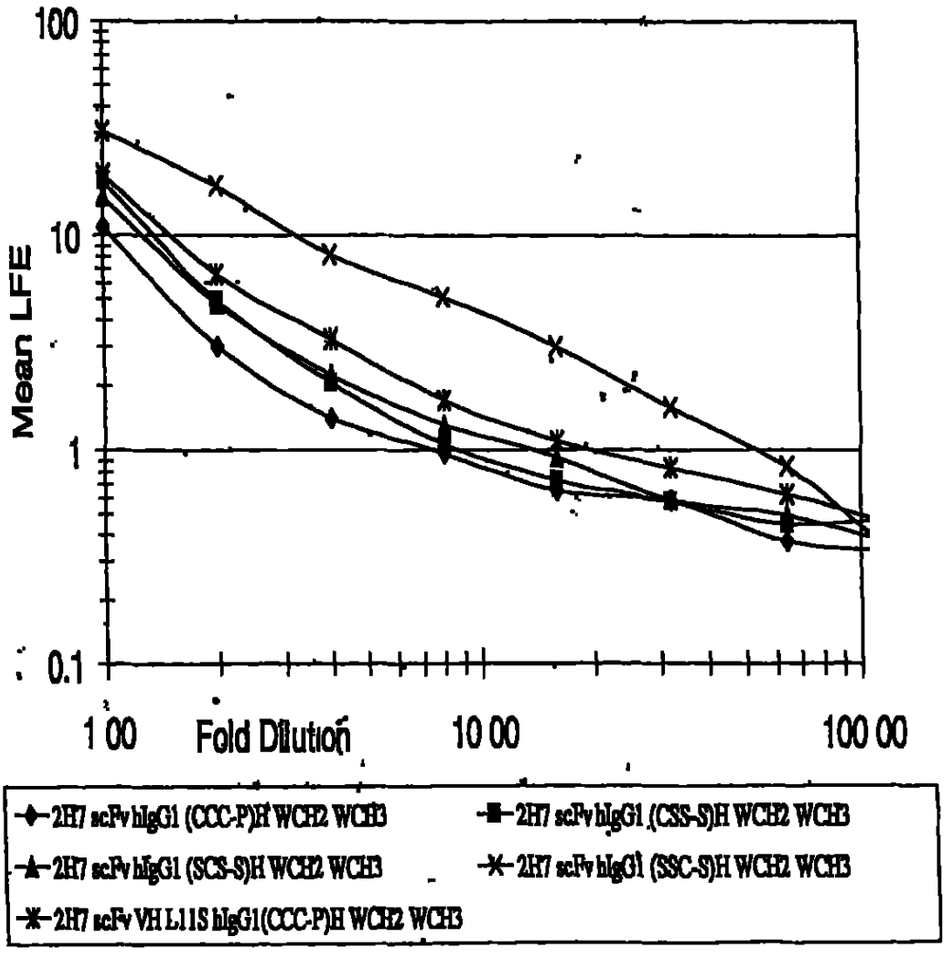

C ADCC Mediated by CTLA4 hIgG1 (SSS-S)H WCH2 WCH3
Raji + CTLA4 hIgG1 (SSS-S)H WCH2 WCH3
Raji CD80 + CTLA4 hIgG1 (SSS-S)H WCH2 WCH3
% specific killing
Effector:Target Ratio

Fig. 34

Binding of 2H7 scFvIg Constructs
with Alternative Tails to CD20 CHO Cells



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Fig 35

Immunoblot Analysis of protein immunoprecipitates from COS transfections of 2H7 scFvIg Constructs

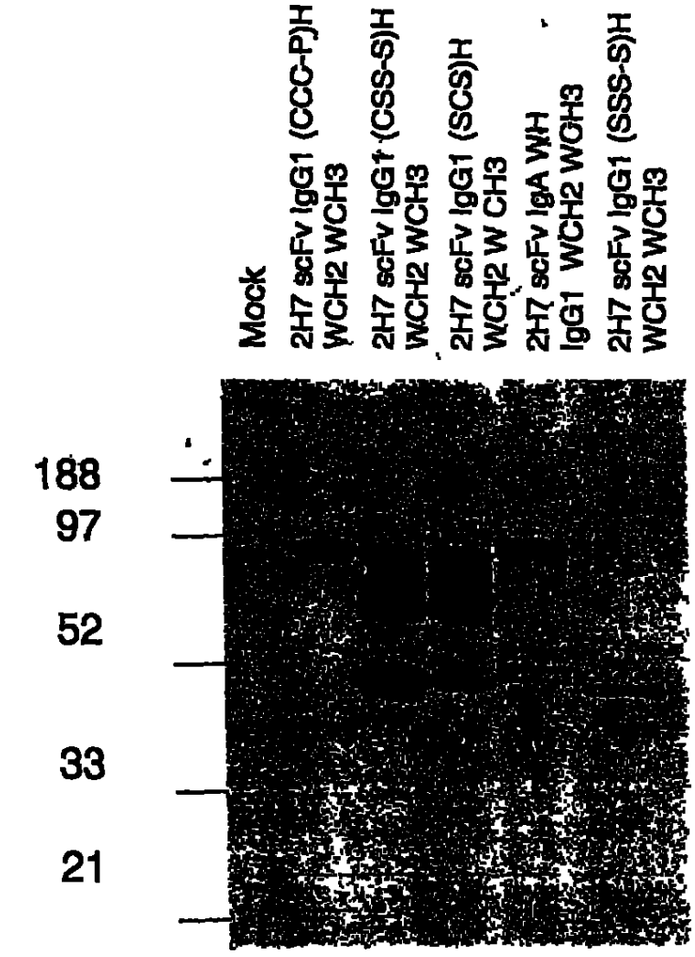


Fig 36

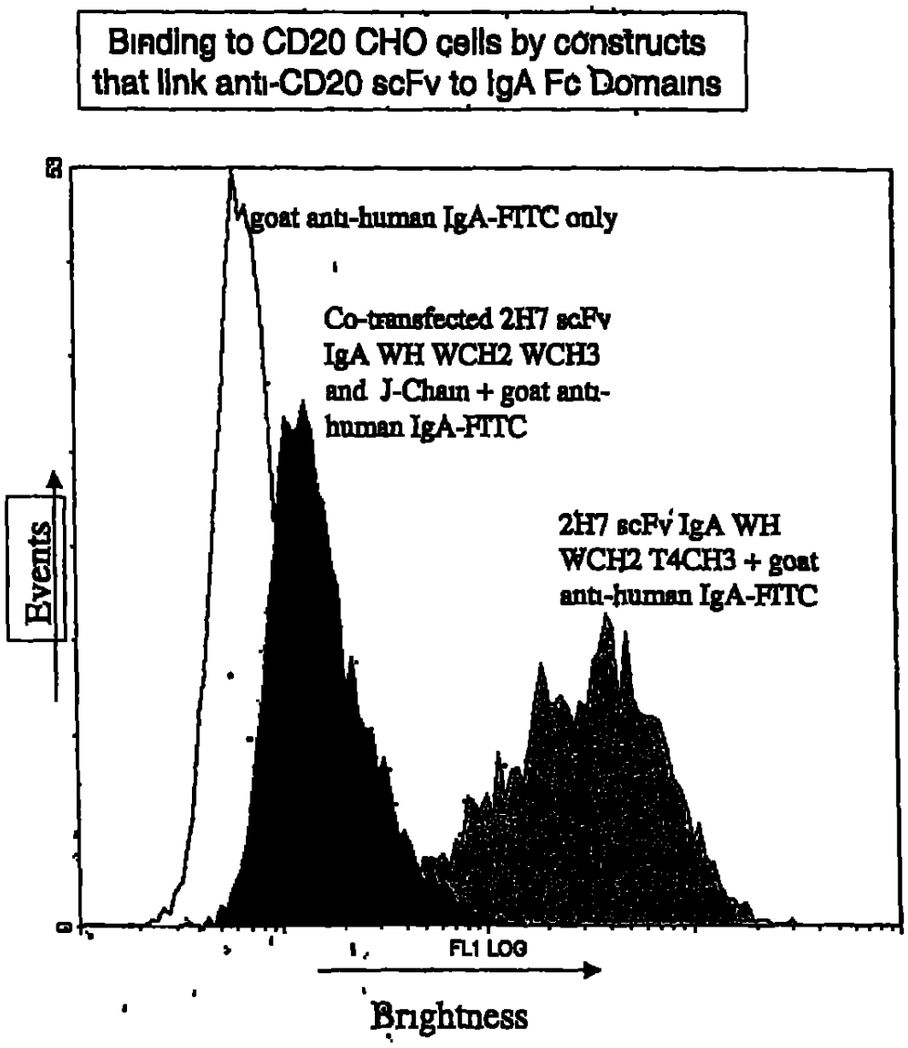
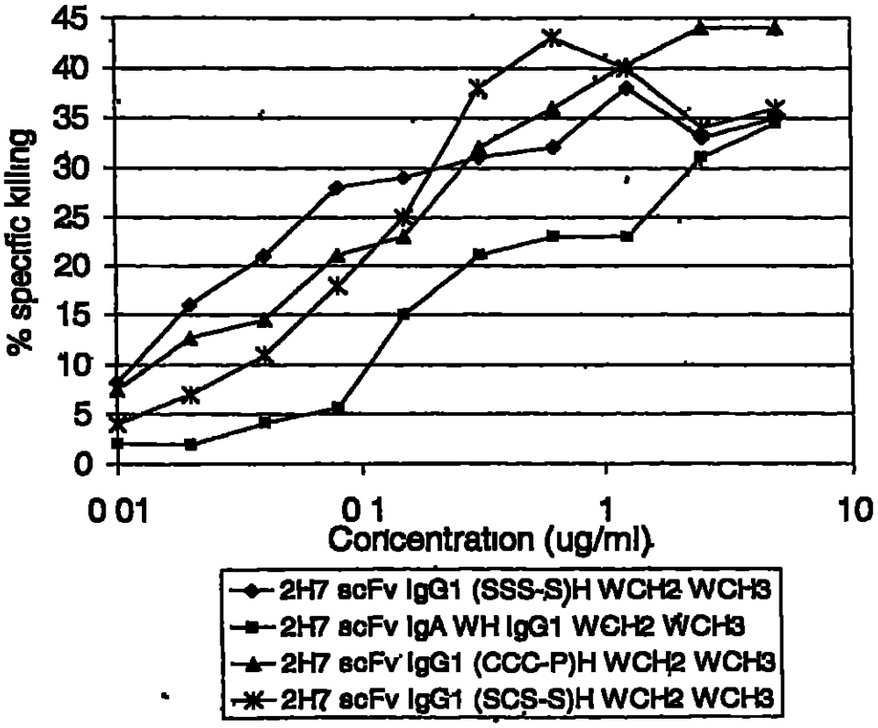

Binding to CD20 CHO cells by constructs that link anti-CD20 scFv to IgA Fc Domains
goat anti-human IgA-FITC only
Co-transfected 2H7 scFv IgA WH WCH2 WCH3 and J-Chain + goat anti-human IgA-FITC
2H7 scFv IgA WH WCH2 T4CH3 + goat anti-human IgA-FITC
Events
FL1 LOG
Brightness

Fig 37

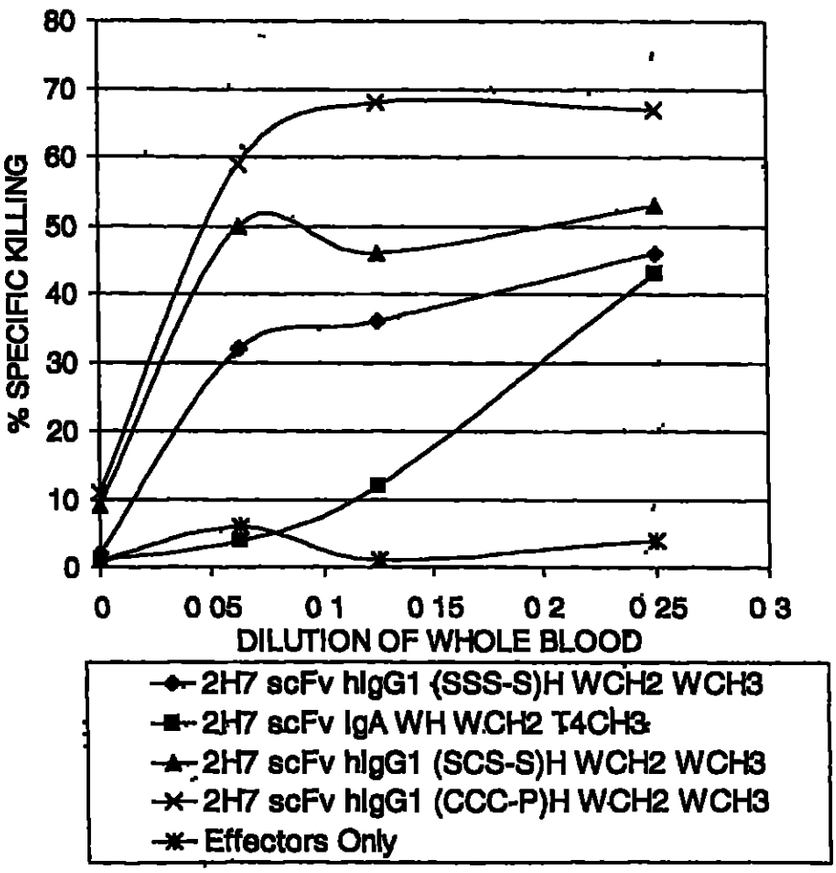
Titration of CD20 specific scFvIg Constructs
for ADCC Activity Using Whole Blood Effectors



545

Fig .38

ADCC Assay of anti-CD20 constructs with alternative tails
(Whole Blood Effectors / BJAB Targets

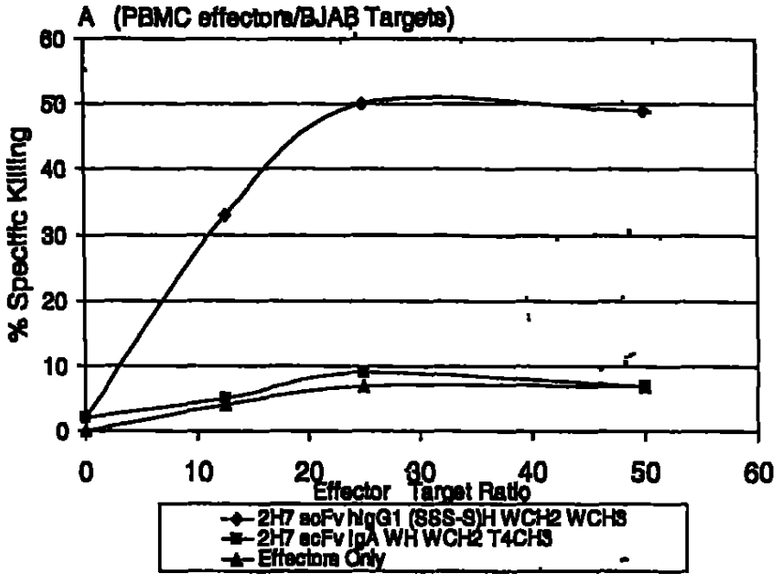


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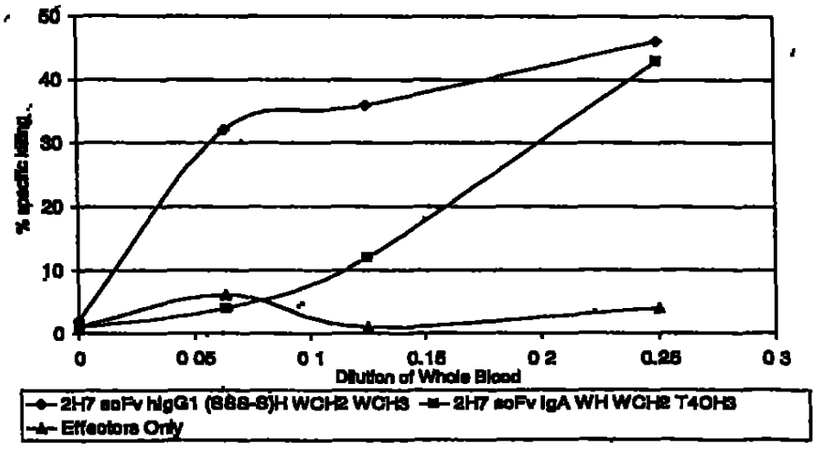
Fig. 39

ADCC Assay of Anti-CD20 scFvIg Constructs Using Different Effector Populations Against BJAB Targets

A (PBMC effectors/BJAB Targets)



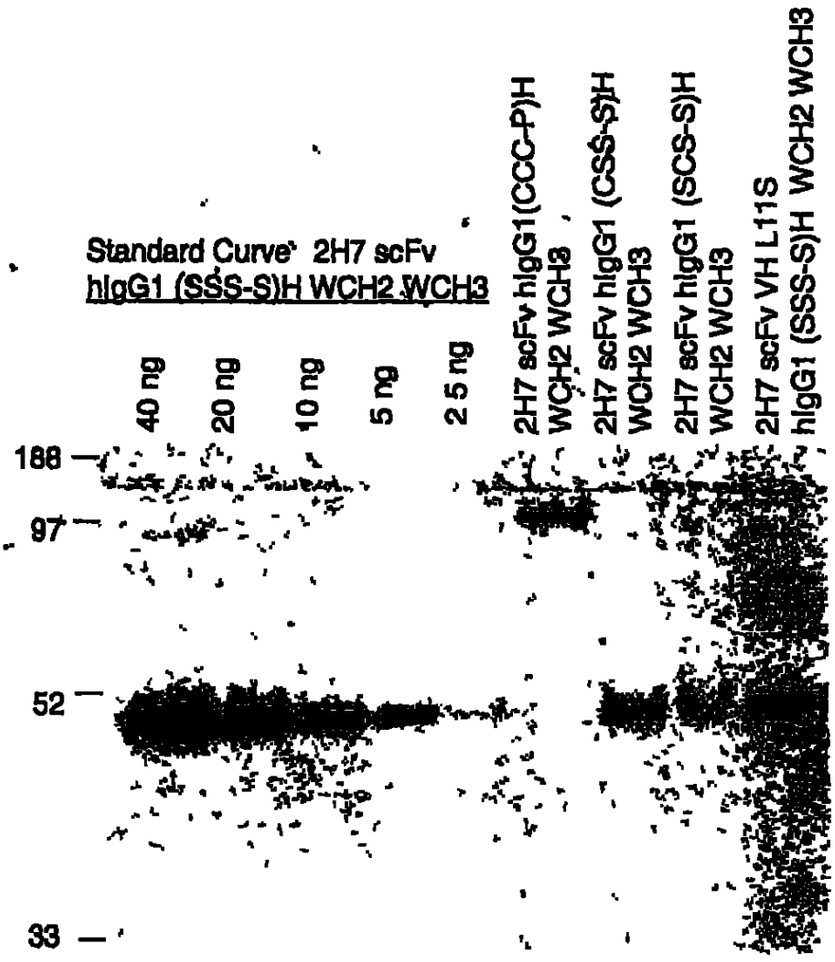
B. WHOLE BLOOD EFFECTORS/BJAB TARGETS



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Fig 40

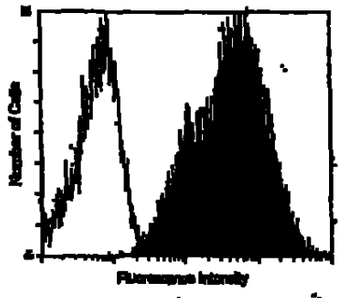
Immunoblot of 2H7 scFv Ig constructs from COS Transfections (1 µl/well) compared to a Concentration Standard

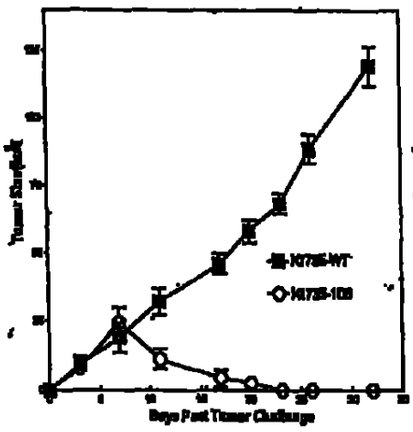


548

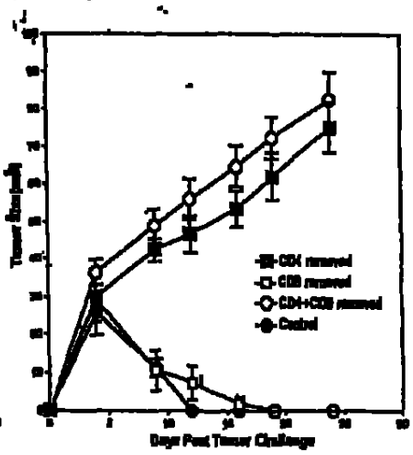
Figures 41A, 41B and 41C

A.





B.



C.

549

Fig. 42

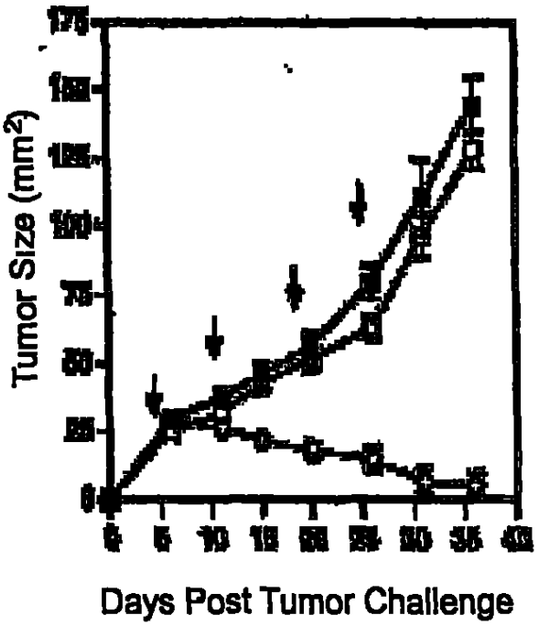


Fig. 43

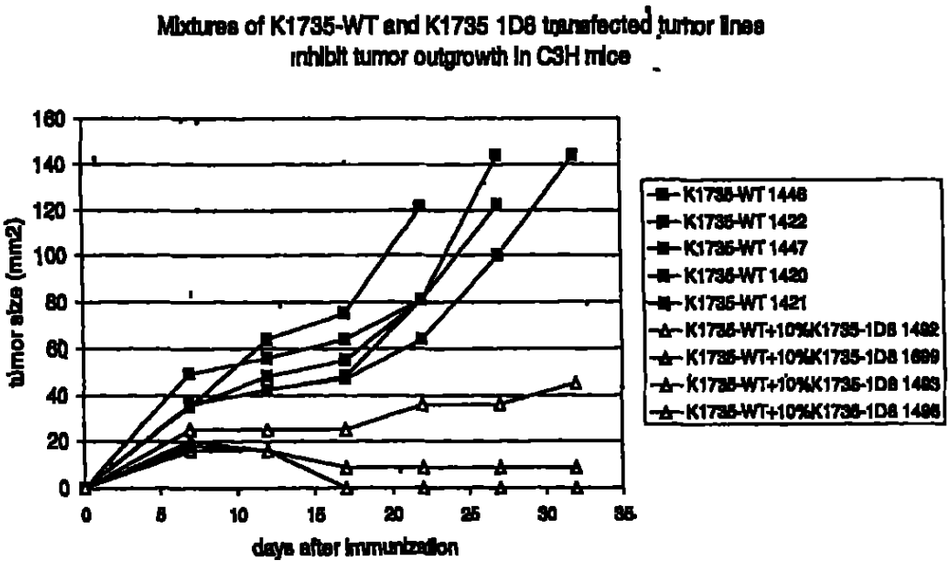
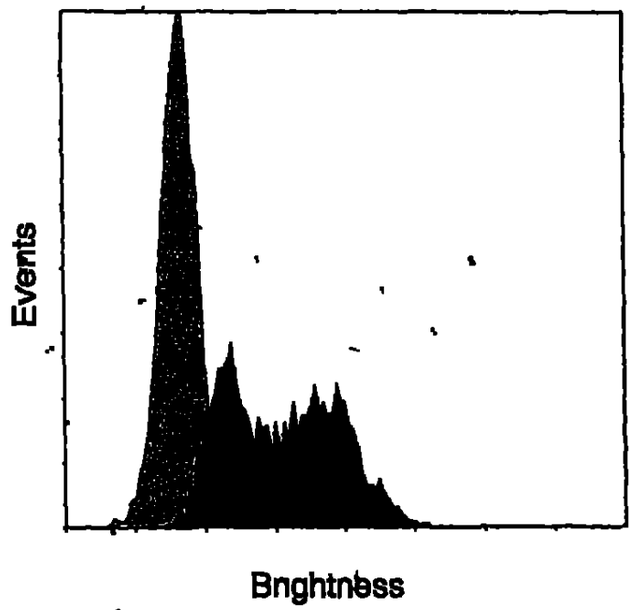

Mixtures of K1735-WT and K1735 1D8 transfected tumor lines inhibit tumor outgrowth in C3H mice
tumor size (mm2)
days after immunization
K1735-WT 1446
K1735-WT 1422
K1735-WT 1447
K1735-WT 1420
K1735-WT 1421
K1735-WT+10%K1735-1D8 1492
K1735-WT+10%K1735-1D8 1499
K1735-WT+10%K1735-1D8 1493
K1735-WT+10%K1735-1D8 1495

Fig. 44

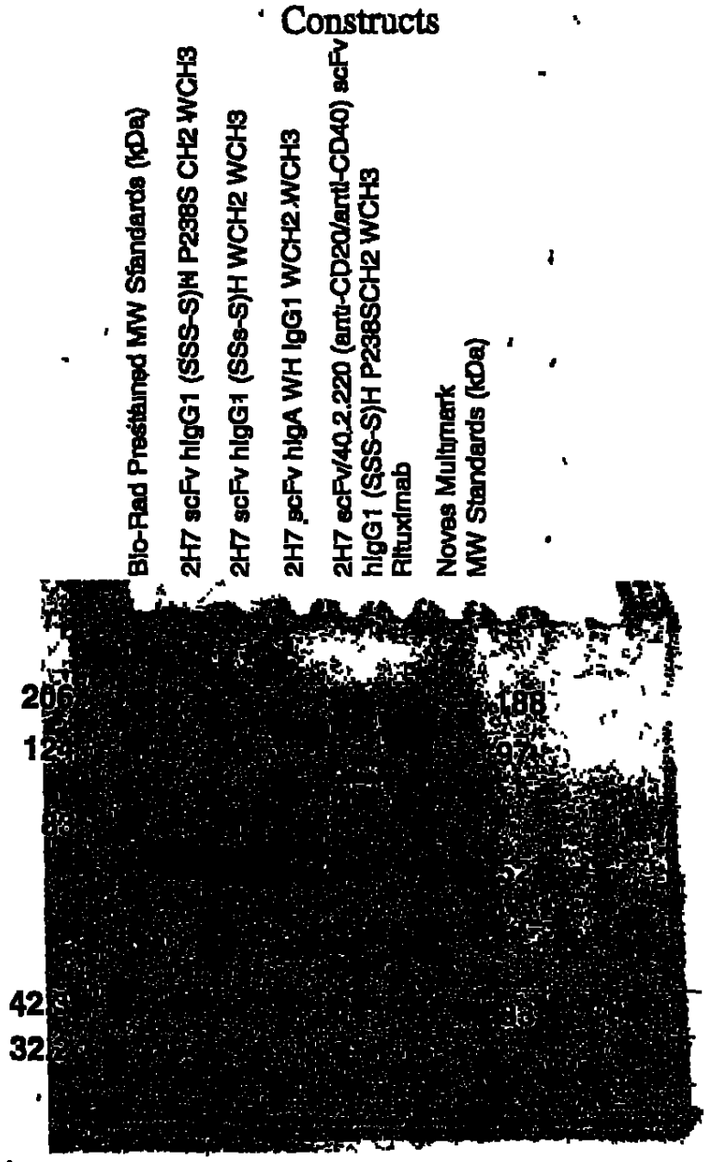
Expression of anti-mouse CD137 (1D8) scFv-hIgG1 (SSS-S)H P238SCH2 WCH3
On the surface of panned Ag104-1D8 Transfected Tumor Cells



552

Fig. 45

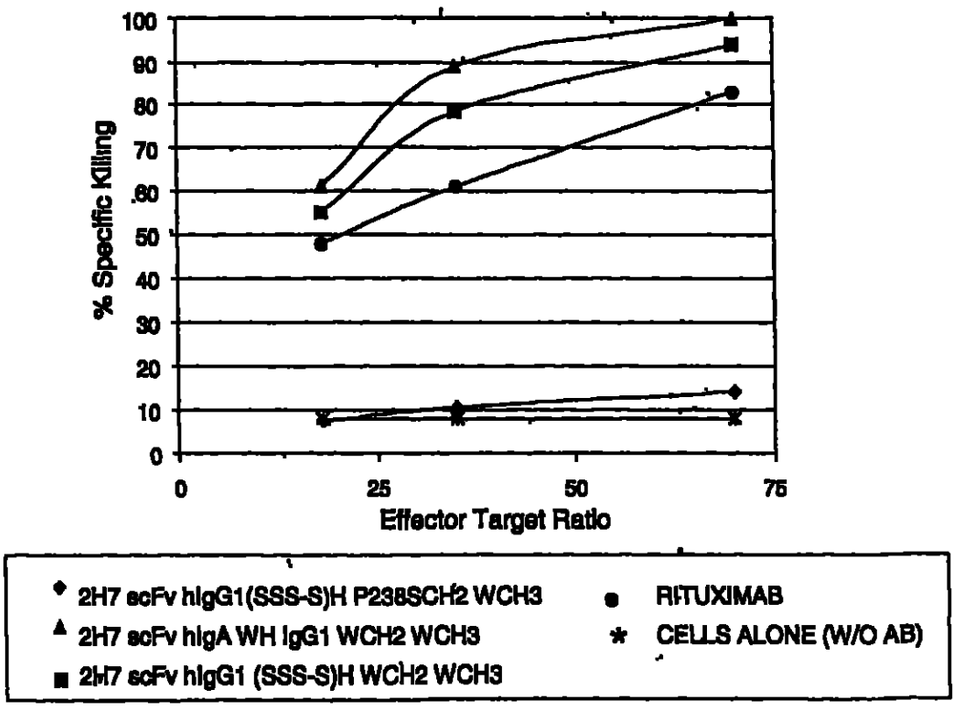
Coomassie Stained SDS-PAGE Gel of 2H7 scFv Ig Constructs



553

Fig. 46

ADCC mediated by 2H7 scFvIg Constructs by human PBMC effector cells against Bjab targets

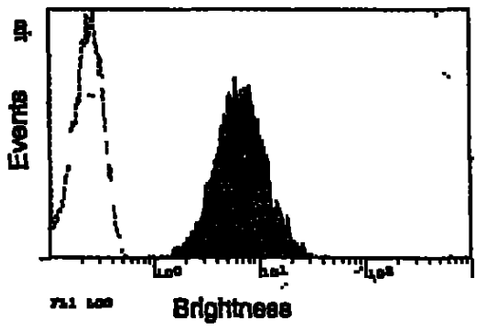


554/560

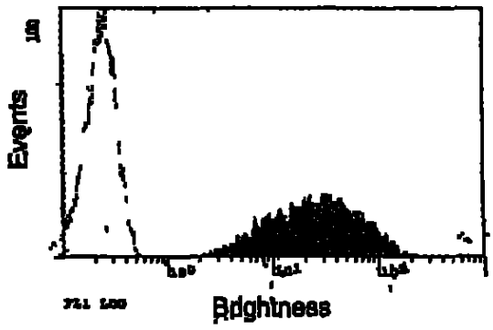
Fig 47

Cell surface expression of anti-human CD3 G19-4 scFv hIgG1 (SSS-S)H P238SCH2 WCH3-hCD80TM/CT on Reh and T51 Cells.

Reh anti-CD3 (G19-4) scFv hIgG1 (SSS-S)H P238SCH2 WCH3-hCD80TM/CT



T51 G19-4 scFv hIgG1 (SSS-S)H P238SCH2 WCH3-hCD80TM/CT



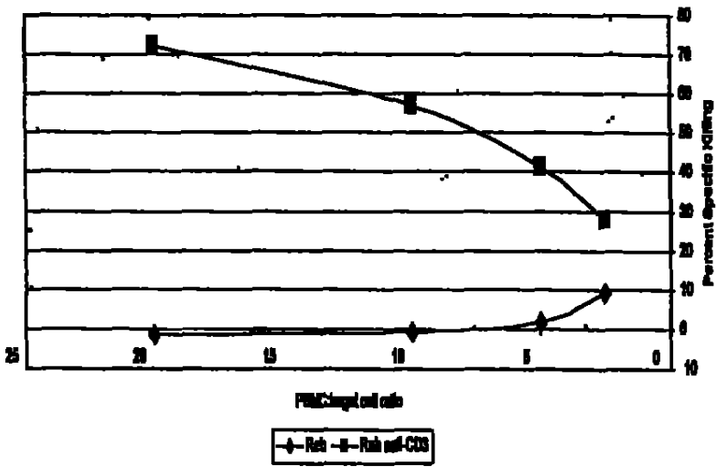
555/858

Figure 48

Targeting of Cytotoxicity to Transfected Cell Lines by Surface expression of CD3 scFvIg

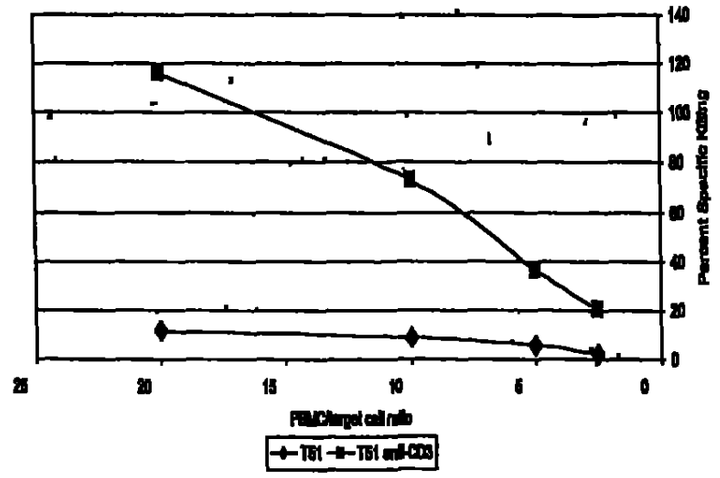
Cytotoxic activity of resting PBMC towards transfected Reh cells

A



Cytotoxic activity of resting PBMC towards transfected T51 lymphoblastoid cells

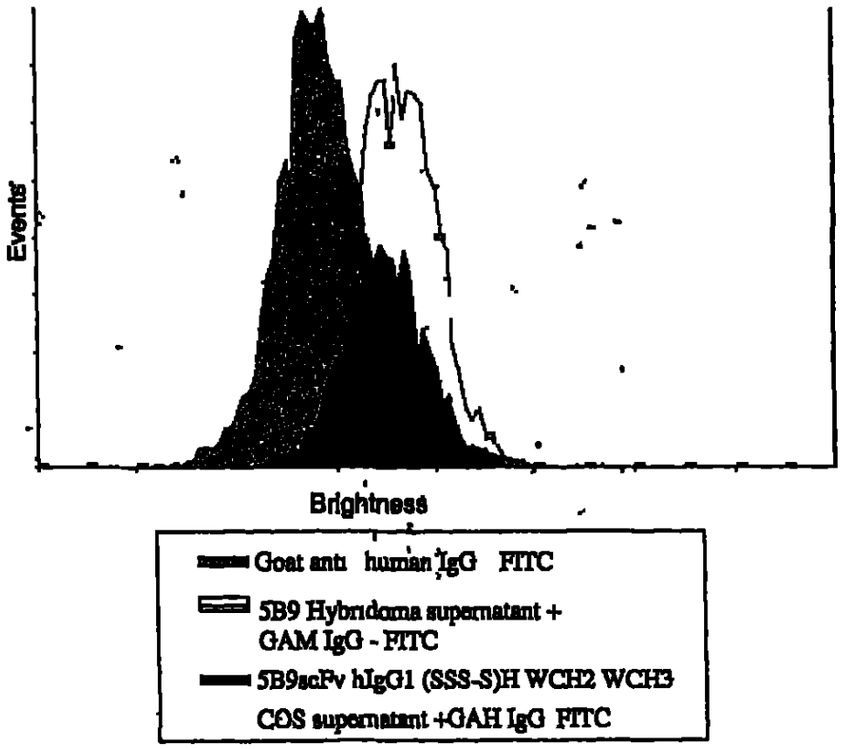
B



556 #B

Fig 49

Binding of 5B9, a mouse anti-human CD137 scFv hIgG1 (SSS-S)H WCH2WCH3 to stimulated human PBMC

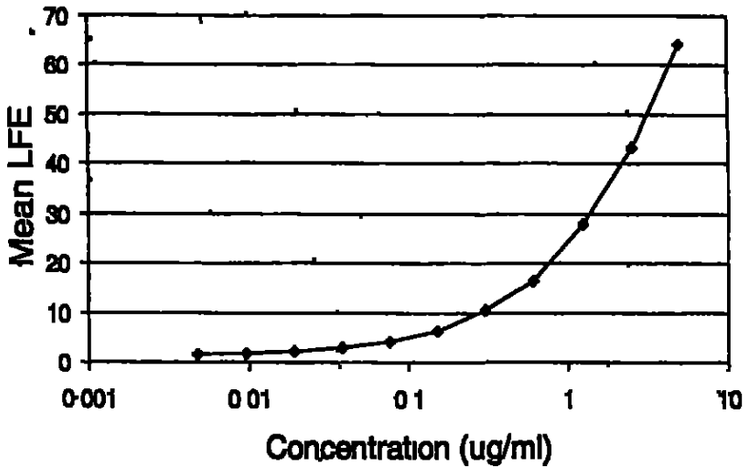


557

Fig 50

Effect of V_HL11S Mutation on CytoxB20
2H7 scFv hIgG1 (SSS-S)H WCH2 WCH3 Protein Expression

50A Standard Curve 2H7VH-L11S-IgG1 (SSS-S)H WCH2 WCH3



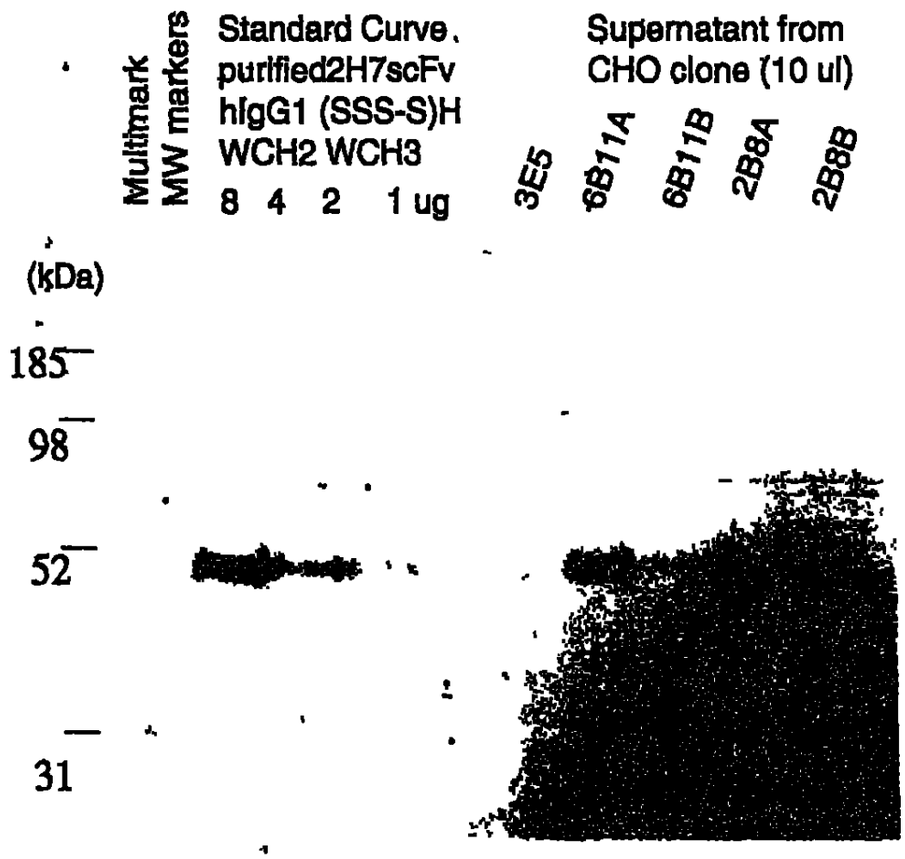
50B CHO supernatant Brightness and Estimation of Protein concentrations from Standard Curve

	CHO clone name				
	4F2	4F5	3E5	6B11A	2B8A
Mean LFE					
1/100	71 7	40 6	31 5	99 7	101 5
1/500	27 1	12 4	11 2	40 8	43
approx conc µg/ml	600	225	125	1000	1250

558

Fig. 5I

Production Levels of 2H7scFv VH L11S hIgG1 (SSS-S)H WCH2 WCH3 From CHO Clone Culture Supernatants



559

Fig 52

Effect of VHL11S Mutation on G28-1 scFvIg Construct Protein Production from COS cells

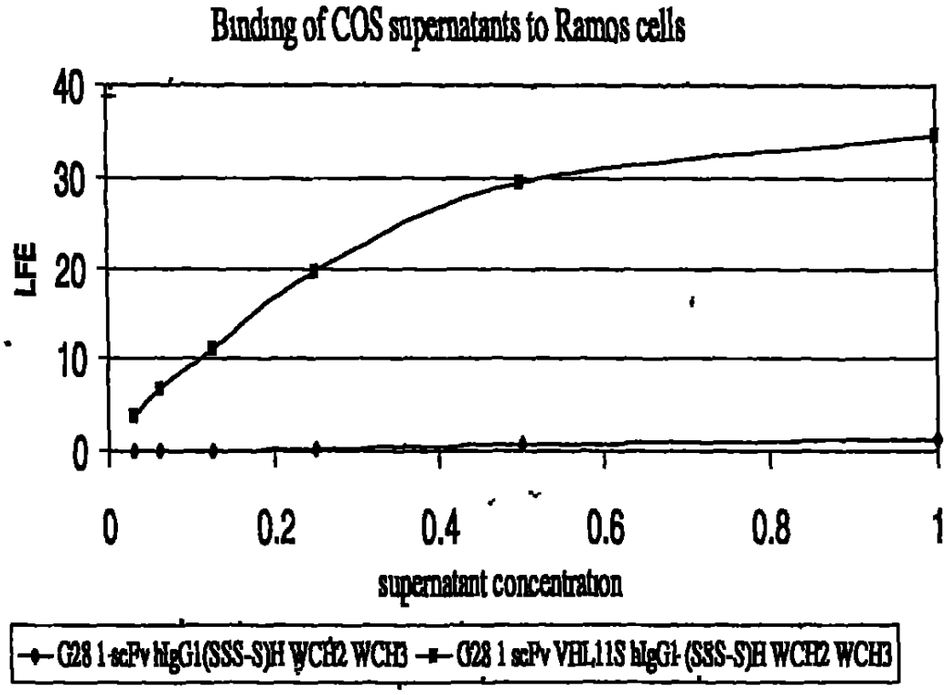


Fig. 53

Immunoblot of G28-1 scFvIg Constructs

Increased Protein Levels in COS supernatants
transfected with G28-1scFv hIgG1 (SSS-S)H WCH2 WCH3
After Substitution of Leucine with Serine at position 11 of VH (VHL11S)

Fig 53A

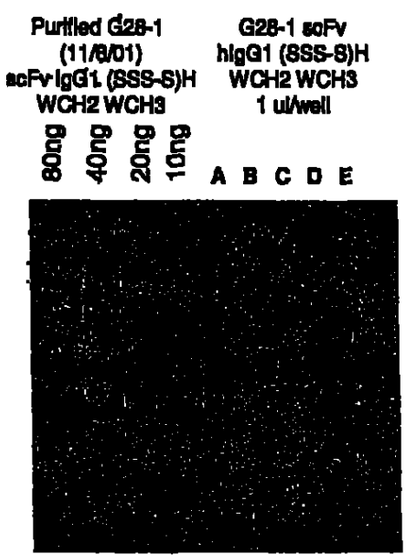


Fig 53B

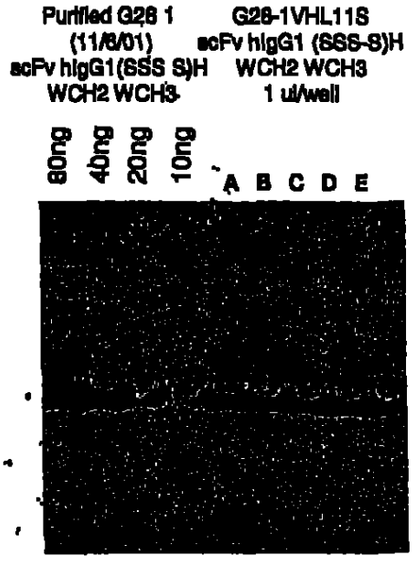
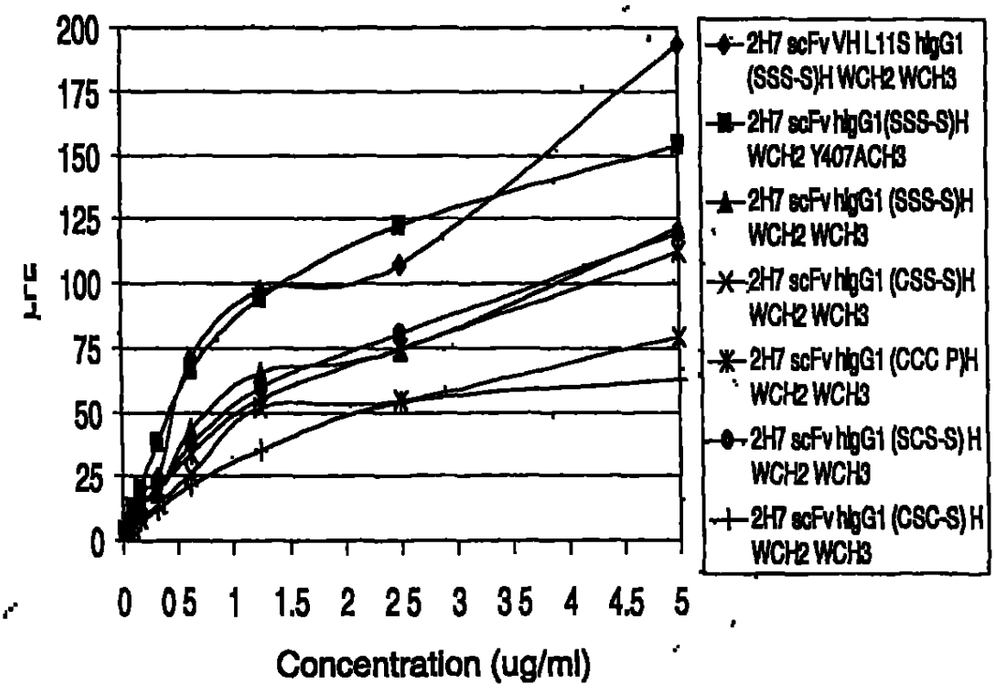


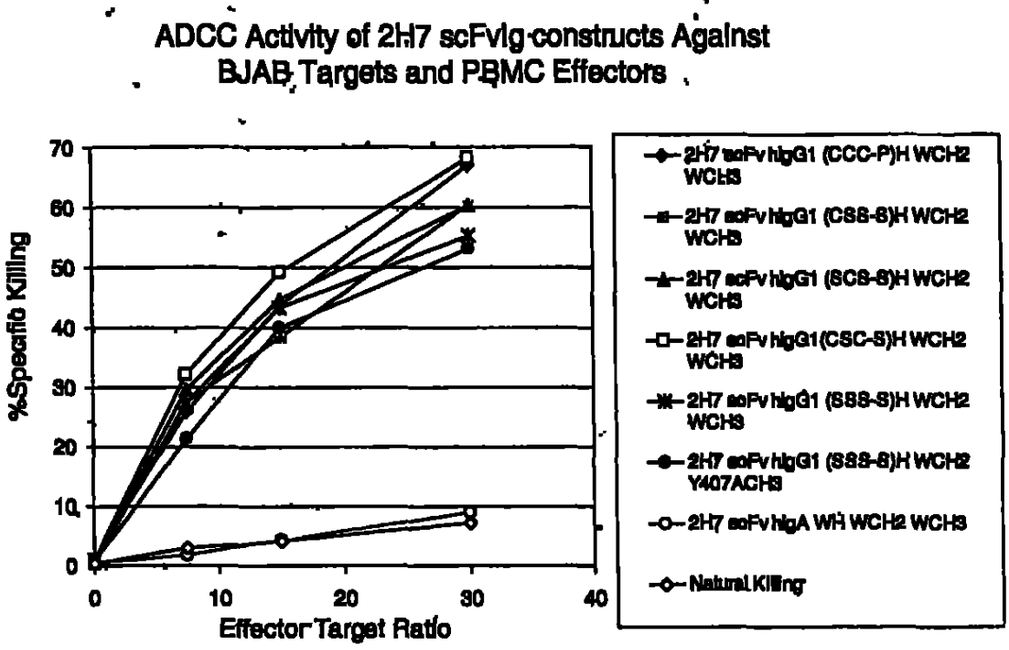
Fig. 54

Binding of 2H7 scFvIg Constructs with Altered Hinges and CH3 domains to CD20 CHO Cells



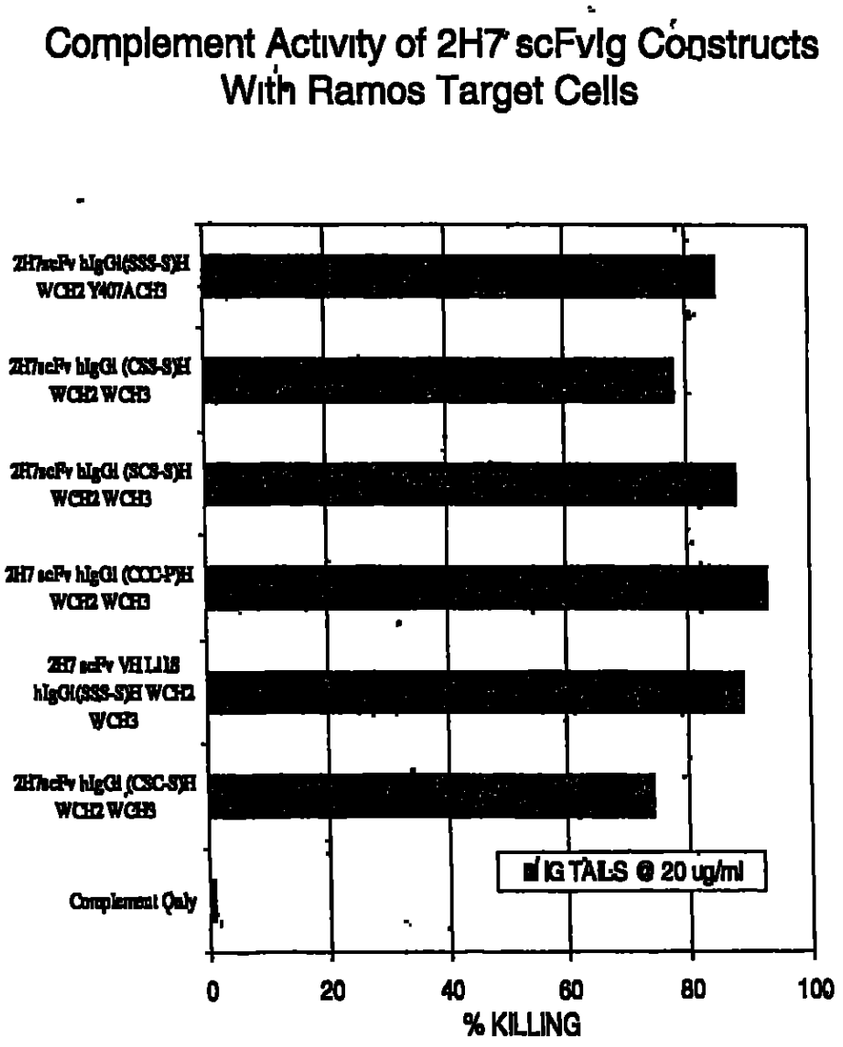
362

Fig. 55


ADCC Activity of 2H7 scFvIg constructs Against BJAB Targets and PBMC Effectors
%Specific Killing
Effector Target Ratio
0
10
20
30
40
50
60
70
2H7 scFv hIgG1 (CCC-P)H WCH2 WCH3
2H7 scFv hIgG1 (CSS-S)H WCH2 WCH3
2H7 scFv hIgG1 (SCS-S)H WCH2 WCH3
2H7 scFv hIgG1(CSC-S)H WCH2 WCH3
2H7 scFv hIgG1 (SSS-S)H WCH2 WCH3
2H7 scFv hIgG1 (SSS-S)H WCH2 Y407ACH3
2H7 scFv hIgA WH WCH2 WCH3
Natural Killing

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Fig. 56


Complement Activity of 2H7 scFvIg Constructs
With Ramos Target Cells
2H7scFv hIgG1(SSS-S)H WCH2 Y407ACH3
2H7scFv hIgG1 (CSS-S)H WCH2 WCH3
2H7scFv hIgG1 (SCS-S)H WCH2 WCH3
2H7 scFv hIgG1 (CCC-P)H WCH2 WCH3
2H7 scFv VH LH5 hIgG1(SSS-S)H WCH2 WCH3
2H7scFv hIgG1 (CSC-S)H WCH2 WCH3
Complement Only
IG TALS @ 20 ug/ml
0
20
40
60
80
100
% KILLING

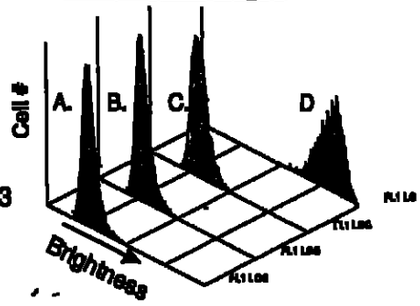
564

Fig 57

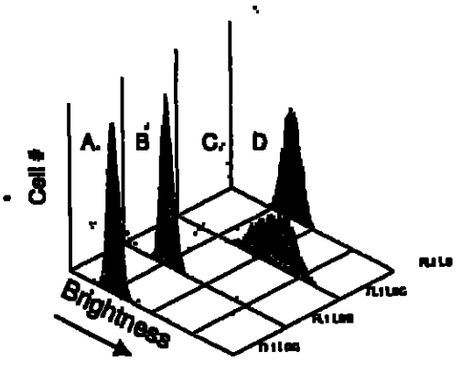
Binding of 2H7 scFvIg Derivatives to CD20CHO Cells

A ■ No fusion protein
B ■ 2H7 scFv hIgE CH2CH3CH4
C ■ 2H7 scFv hIgA WH WCH2 WCH3
D ■ 2H7 scFv hIgG1 (SSS-S)H WCH2 WCH3

FITC anti-hIgG



FITC anti-hIgA



FITC anti-hIgE

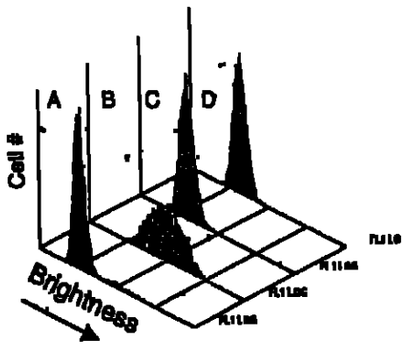


Fig. 58

Fig 58A 2H7 scFv VH L11S human IgE (WCH2 WCH3 WCH4) Binding to CD20 CHO at 30 ug/ml

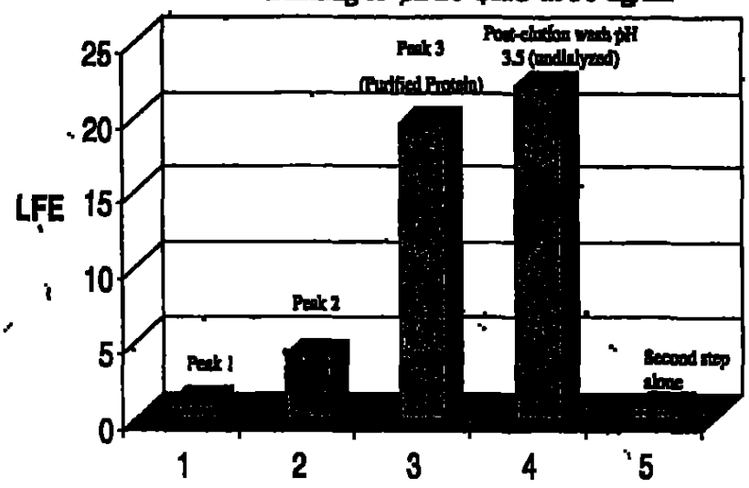
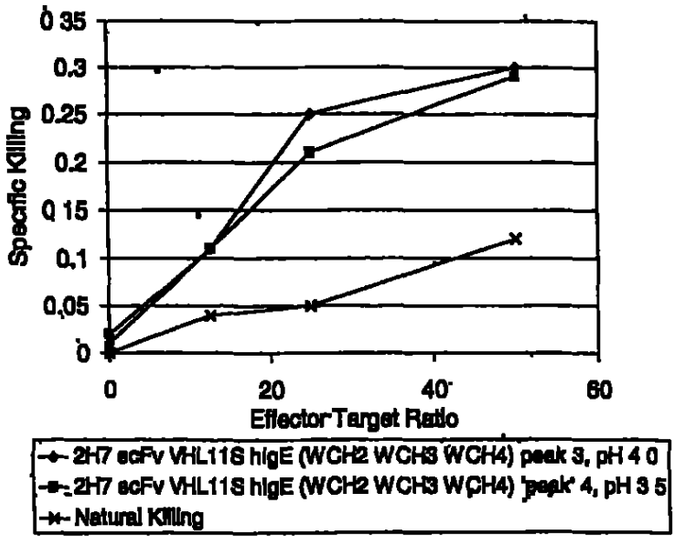


Fig 58B ADCC Activity of 2H7 VHL11S IgE (WCH2 WCH3 WCH4) Protein Fractions with PBMC Effectors and Bjab Targets



566

Fig. 59

Binding Data for COS derived α-CD20 (2H7) scFv VHL11S mIg E (WCH2 WCH3 WCH4) and mIgA (WH WCH2 WCH3)Tailed Molecules

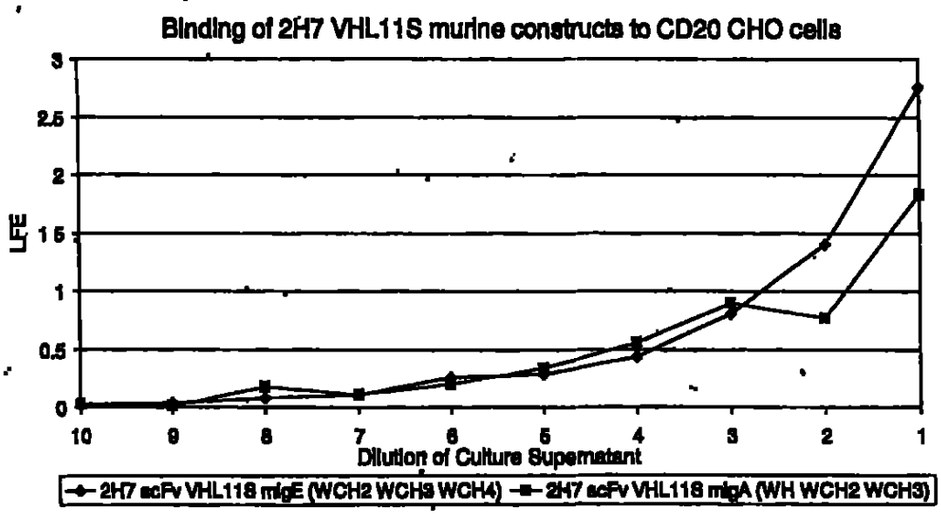
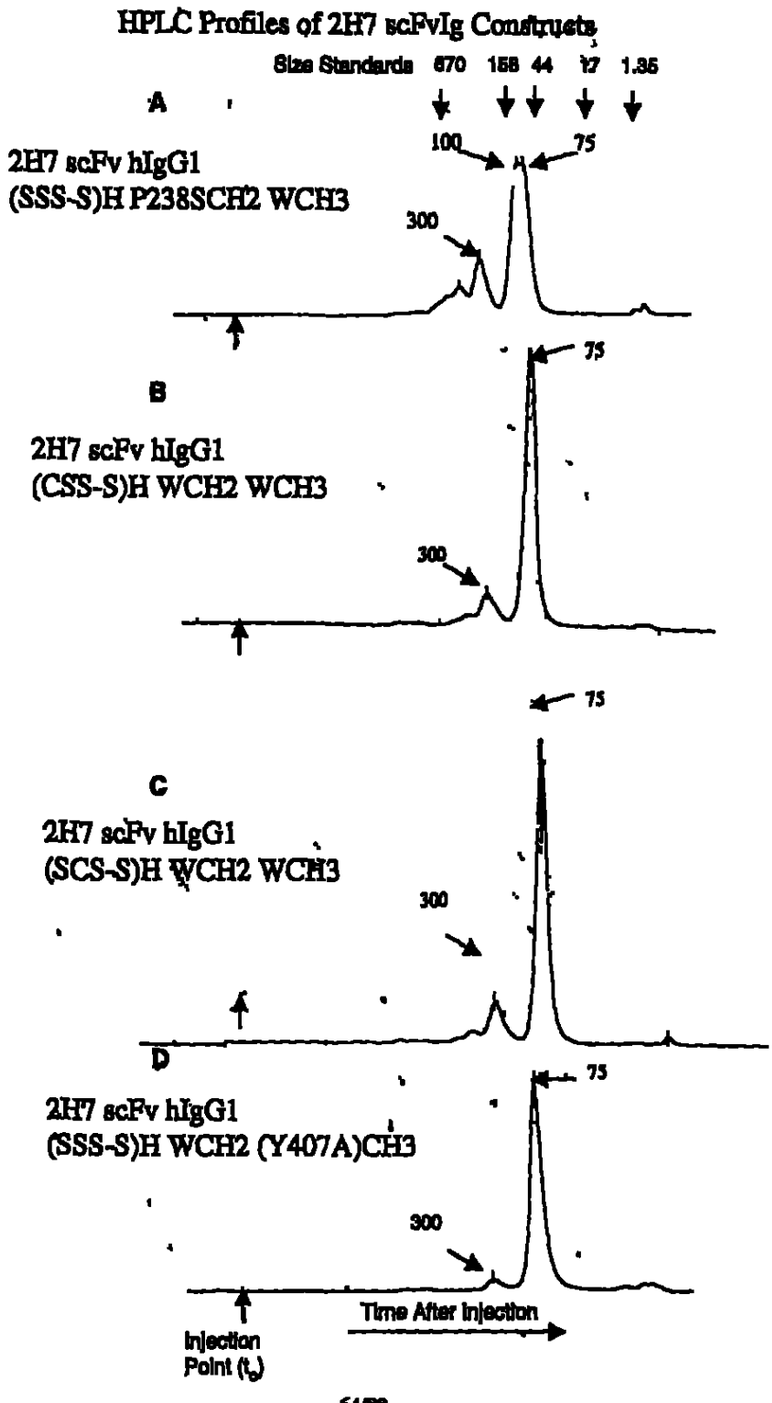


Fig 60


HPLC Profiles of 2H7 scFvIg Constructs
Size Standards 670 158 44 17 1.35
A
2H7 scFv hIgG1
(SSS-S)H P238SCH2 WCH3
100
75
300
B
2H7 scFv hIgG1
(CSS-S)H WCH2 WCH3
75
300
C
2H7 scFv hIgG1
(SCS-S)H WCH2 WCH3
75
300
D
2H7 scFv hIgG1
(SSS-S)H WCH2 (Y407A)CH3
75
300
Injection Point (t_o)
Time After Injection

568

Fig 61

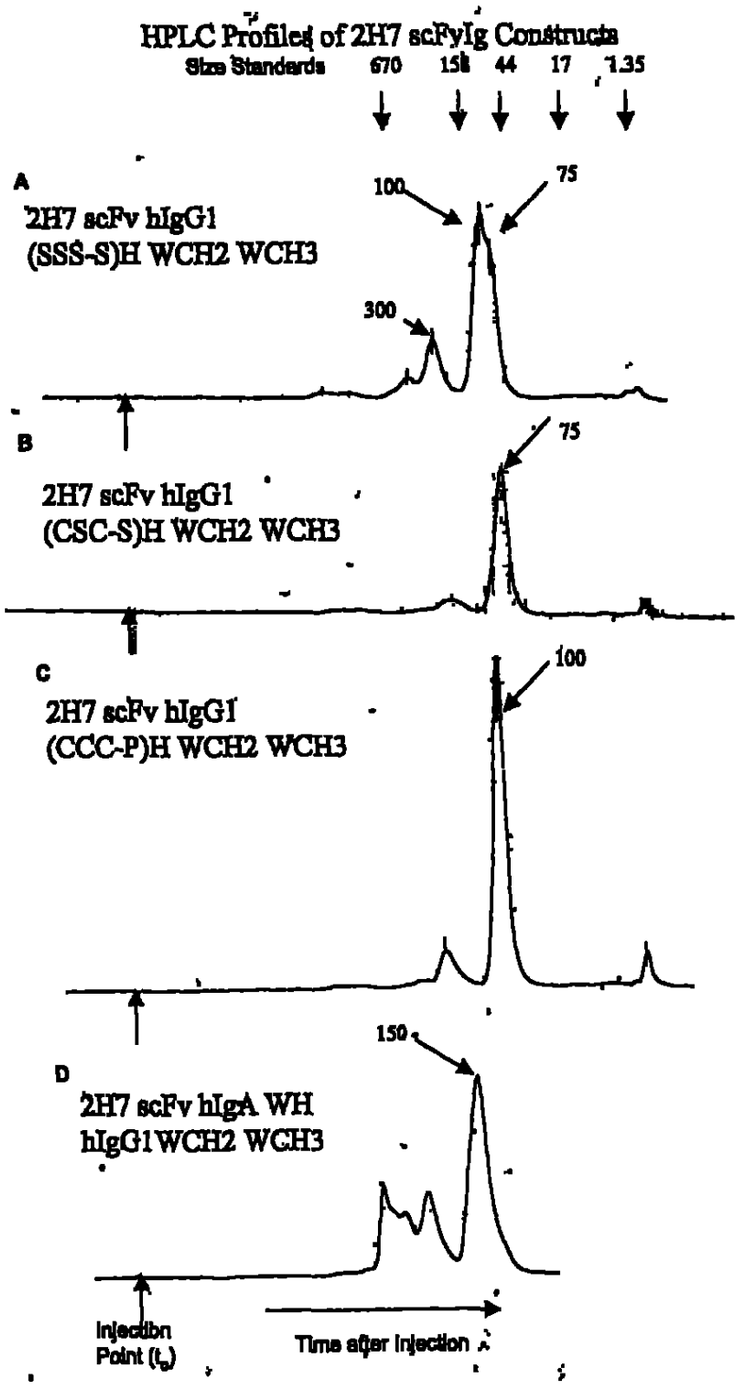

HPLC Profiles of 2H7 scFvIg Constructs
Size Standards
670
158
44
17
1.35
A
2H7 scFv hIgG1
(SSS-S)H WCH2 WCH3
100
75
300
B
2H7 scFv hIgG1
(CSC-S)H WCH2 WCH3
75
C
2H7 scFv hIgG1
(CCC-P)H WCH2 WCH3
100
D
2H7 scFv hIgA WH
hIgG1WCH2 WCH3
150
Injection
Point (t_0)
Time after Injection

Fig 62

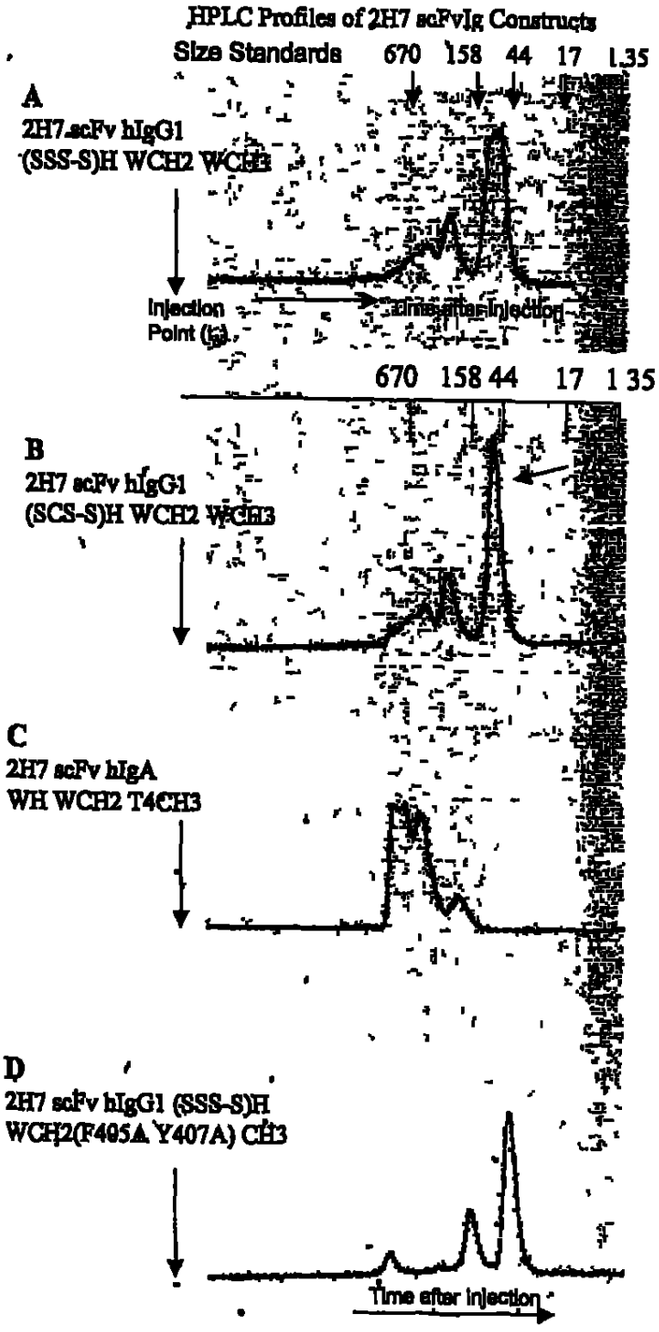

HPLC Profiles of 2H7 scFvIg Constructs
Size Standards 670 158 44 17 1.35
A
2H7 scFv hIgG1
(SSS-S)H WCH2 WCH3
Injection Point (t0)
Time after Injection
670 158 44 17 1 35
B
2H7 scFv hIgG1
(SCS-S)H WCH2 WCH3
C
2H7 scFv hIgA
WH WCH2 T4CH3
D
2H7 scFv hIgG1 (SSS-S)H
WCH2(F405A Y407A) CH3
Time after Injection

Fig. 63

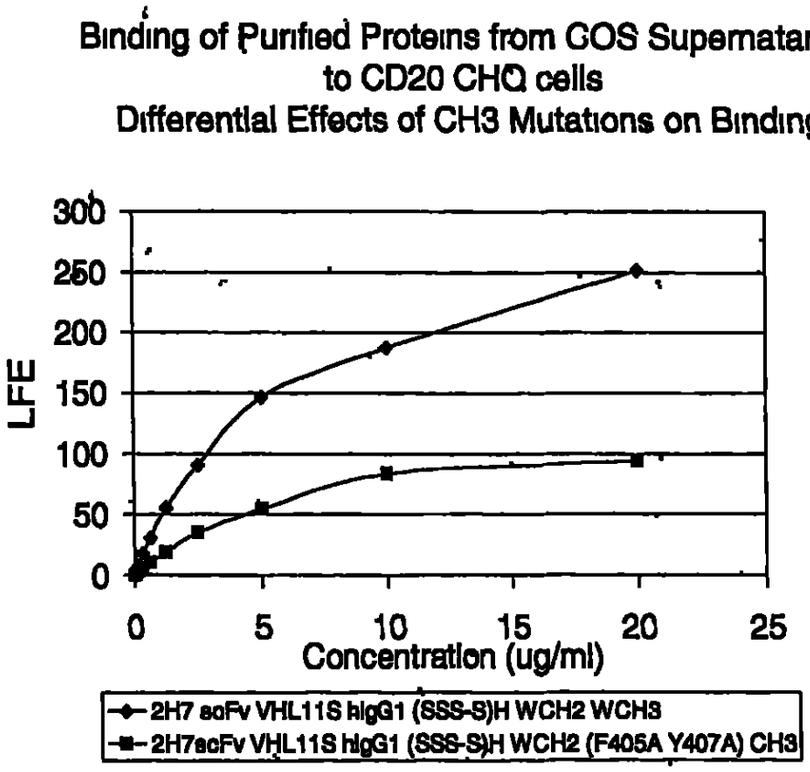
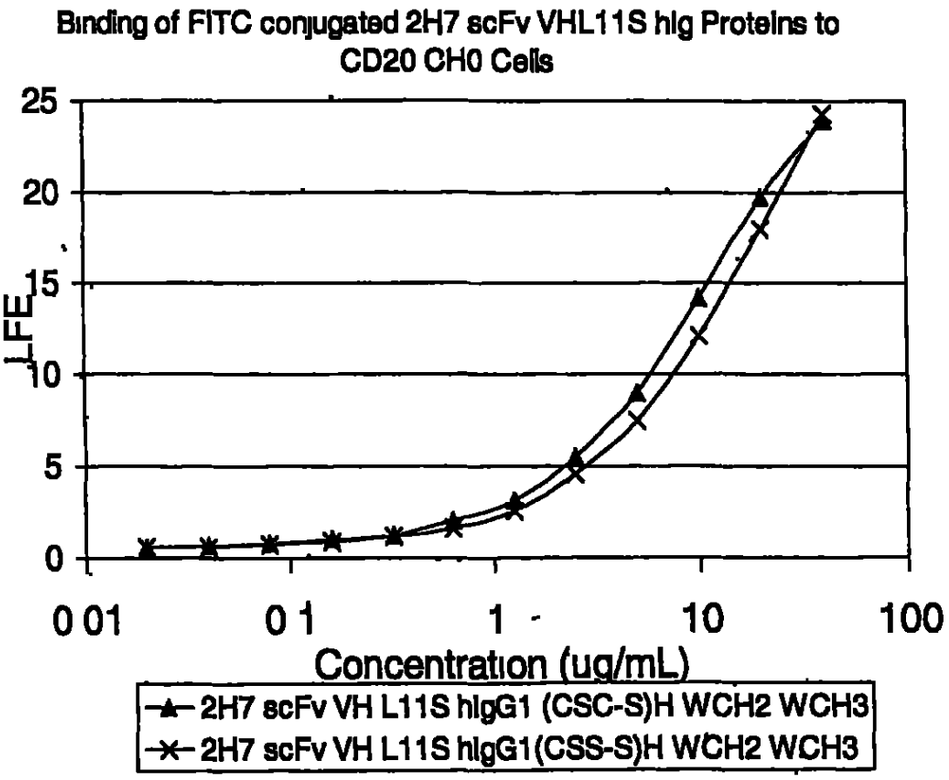

Binding of Purified Proteins from COS Supernatants
to CD20 CHO cells
Differential Effects of CH3 Mutations on Binding
LFE
300
250
200
150
100
50
0
0
5
10
15
20
25
Concentration (ug/ml)
2H7 scFv VHL11S hIgG1 (SSS-S)H WCH2 WCH3
2H7scFv VHL11S hIgG1 (SSS-S)H WCH2 (F405A Y407A) CH3

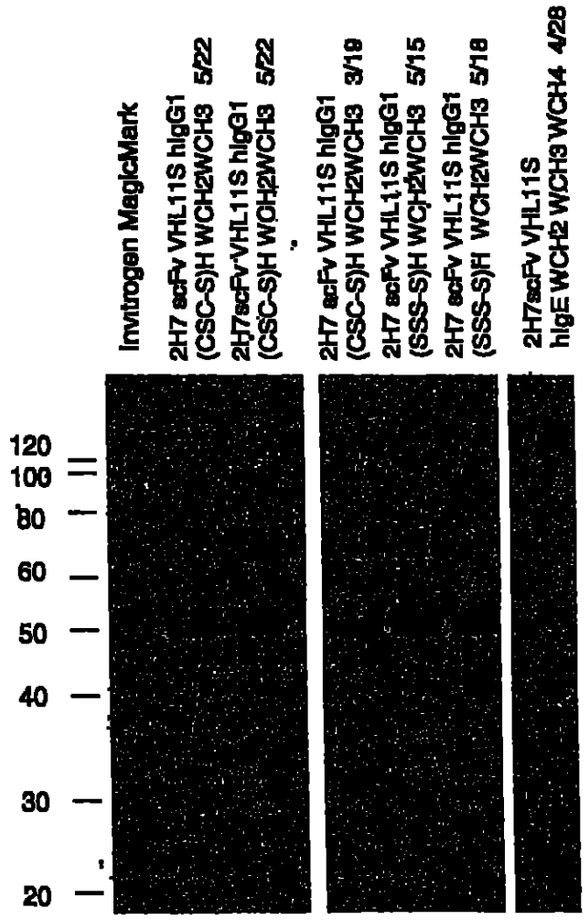
Fig. 64


Binding of FITC conjugated 2H7 scFv VHL11S hIg Proteins to CD20 CH0 Cells
25
20
15
10
5
0
LFE
0 01
0 1
1
10
100
Concentration (ug/mL)
2H7 scFv VH L11S hIgG1 (CSC-S)H WCH2 WCH3
2H7 scFv VH L11S hIgG1(CSS-S)H WCH2 WCH3

572

Fig. 65

Nonreducing SDS-PAGE on Protein A-Purified Lots of 2H7 scFv VHL11S hIg Constructs (1.0 ug/lane)



573

Fig 66

Alterations in Human IgG Fc sequence
that differentially change effector function efficiency

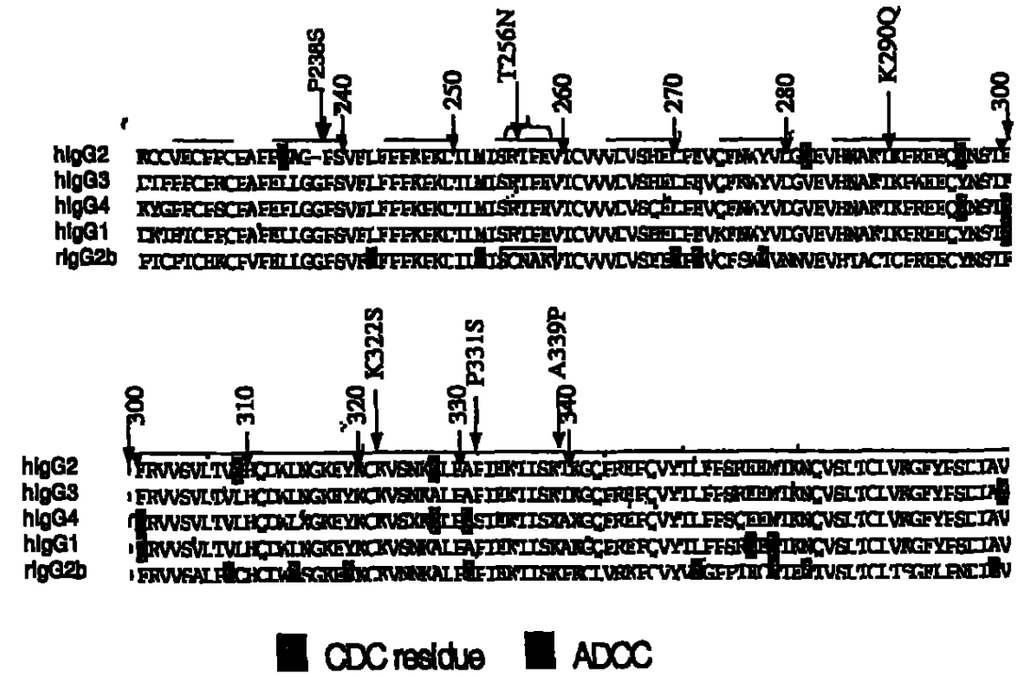
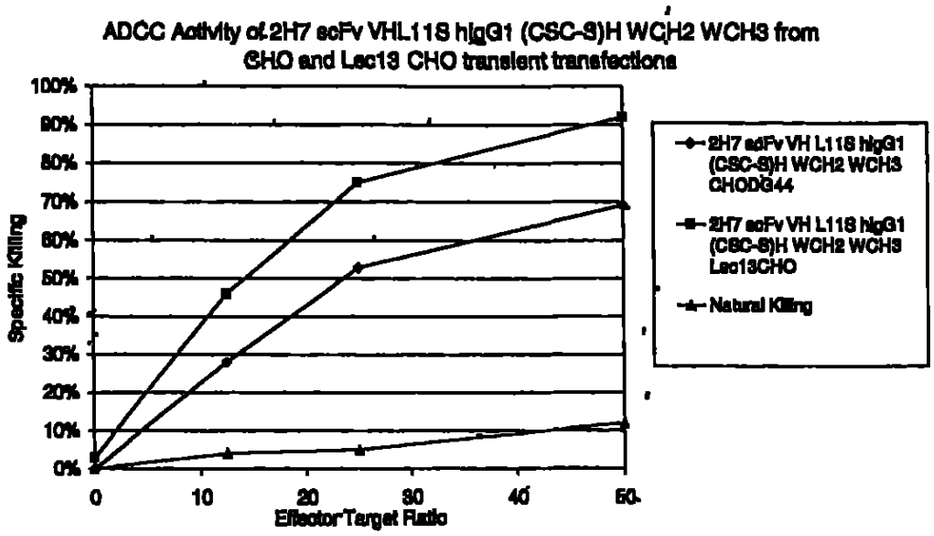


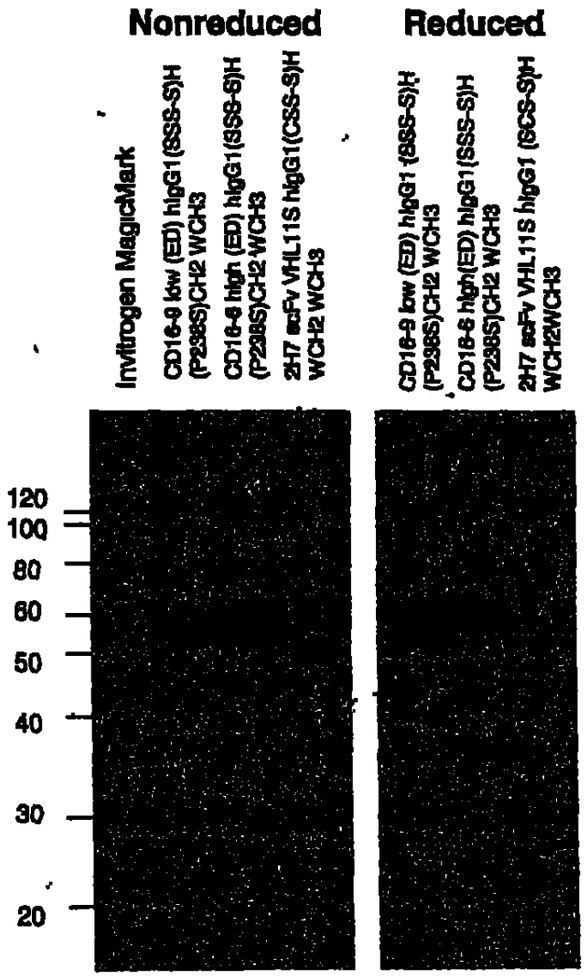
Figure 67.


ADCC Activity of 2H7 scFv VHL11S hIgG1 (CSC-S)H WCH2 WCH3 from CHO and Lec13 CHO transient transfections
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
Specific Killing
0
10
20
30
40
50
Effector:Target Ratio
2H7 scFv VH L11S hIgG1 (CSC-S)H WCH2 WCH3 CHODG44
2H7 scFv VH L11S hIgG1 (CSC-S)H WCH2 WCH3 Lec13CHO
Natural Killing

575

Fig. 68

CD16(ED) hIgG1(SSS-S)H P238S CH2 WCH3 high and low affinity alleles expressed as soluble molecules



576

Fig. 69

Binding of soluble CD16-FITC high and low affinity fusion proteins to 2H7 scFv VHL11S hIgG1 (CSC-S)H WCH2WCH3 or (SSS-S)H (P238S)CH2WCH3 on CD20CHO Targets

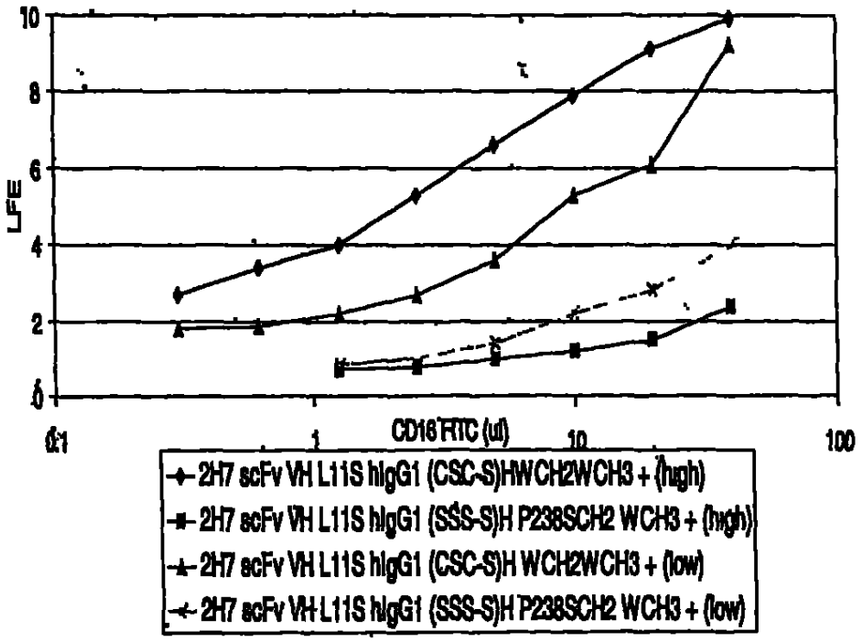
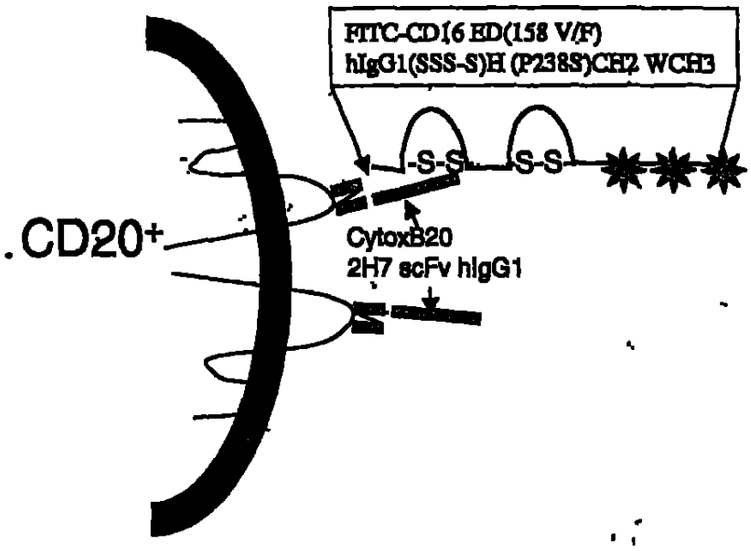


Fig 70

Binding of FITC Labeled, Recombinant Human CD16(ED) extracellular domain -Ig Fusion Protein to CytoxB Derivatives on CD20 CHO Cells



Expression of surface displayed SMIPs links modified cDNAs with the altered fusion proteins

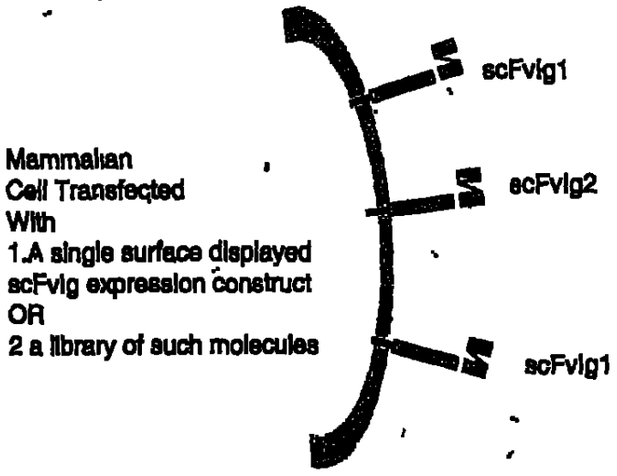
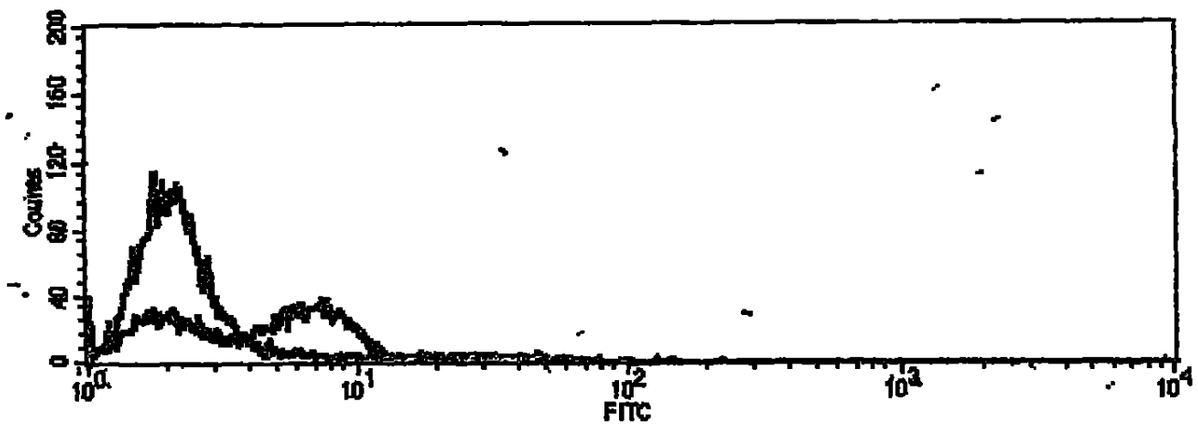


Fig. 71

CD37 mAbs and scFvIg Induce Apoptosis

	Bjab Staining	Annexin V Positive	
	No scFvIg	17 5	
	2H7 MH	27	
scFvIg	G28-1 MH	30 6	
	G28-1 IgAH	28.9	
	HD37 MH	29 1	
	(2H7+G28-1)MH	41	
	(2H7+HD37) MH	37 1	
	(G28-1+HD37) MH	35.3	
			plus GAM
	Ramos	AnnexinV Positive	AnnexinV positive
	cells alone	3	3.3
	2H7 Mab	1 4	3.1
	G28-1 Mab	18.3	8.7
mAbs	HD37 Mab	3.7	3.1
	G28-5	3.9	8.3
	2H7+G28-1	32.3	35 7
	2H7+HD37	5	10 5
	2H7+G28-5	5.7	19 4
	HD37+G28-1	26.9	50
	HD37+G28-5	8.2	18.4
	G28-1+G28-5	39 5	68.3

Caspase 3 Activity in Ramos Cells after 4 Hour Incubation With CytoxB20G SMIP



media
CD20 SMIP

Fig. 72

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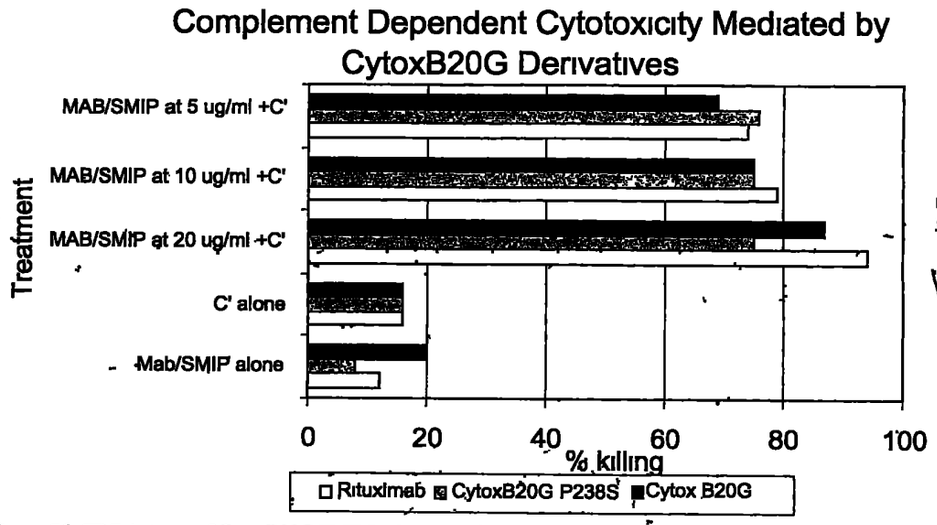


Fig 73

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Figure 76 CDC Activity of CytoxB20G SMIPS CytoxB20G, CytoxB20GP238, or Rituximab were incubated at increasing concentrations with 10^4 Bjab Target Cells and a 1 10 dilution of rabbit complement (PelFreez) in a volume of 100 microliters for sixty minutes Aliquots were stained with trypan blue (Invitrogen), and counted using a hemacytometer to determine the percentage of the cell population killed during treatment. Negative controls with cells and only one reagent were also included.

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ADCC Activity of CytoxB20G SMIPS

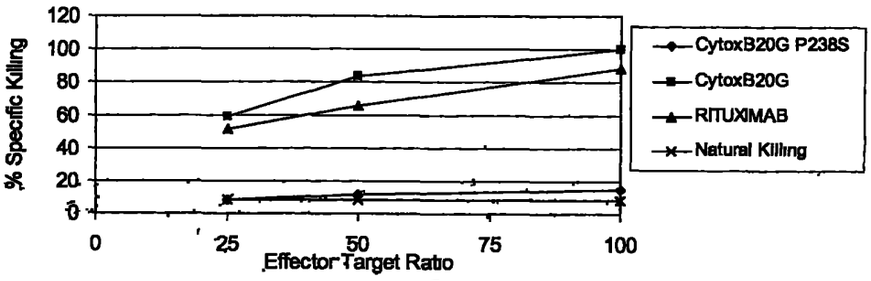


Fig 74

Figure 77 ADCC Activity of CytoxB20G SMIPS ADCC activity of CytoxB20G or Rituximab was measured *in vitro* against BJAB B lymphoma cell line as target and using fresh human PBMC as effector cells Effector to target ratios were varied as follows 100 1, 50 1, and 25 1, with the number of BJAB cells per well remaining constant but varying the number of PBMC Bjab cells were labeled for 2 hours with ^{51}Cr and aliquoted at a cell density of $5x10^4$ cells/well to each well of flat-bottom 96 well plates Purified fusion proteins or rituximab were added at a concentration of 10 µg/ml, and PBMC were added at 1 25 x 10^6 cells /well (25 1), 2 5 x 10^6 cells/well (50 1), or 5 x 10^6 cells/well (100 1), in a final volume of 200 µl Natural Killing was measured at each effector target ratio by omission of SMIP or MAb Spontaneous release was measured without addition of PBMC or fusion protein, and maximal release was measured by the addition of detergent (1% NP-40) to the appropriate wells Reactions were incubated for 5 hours, and 100 µl culture supernatant harvested to a Lumaplate (Packard Instruments) and allowed to dry overnight prior to counting cpm released on a Packard Top Count NXT Microplate Scintillation Counter

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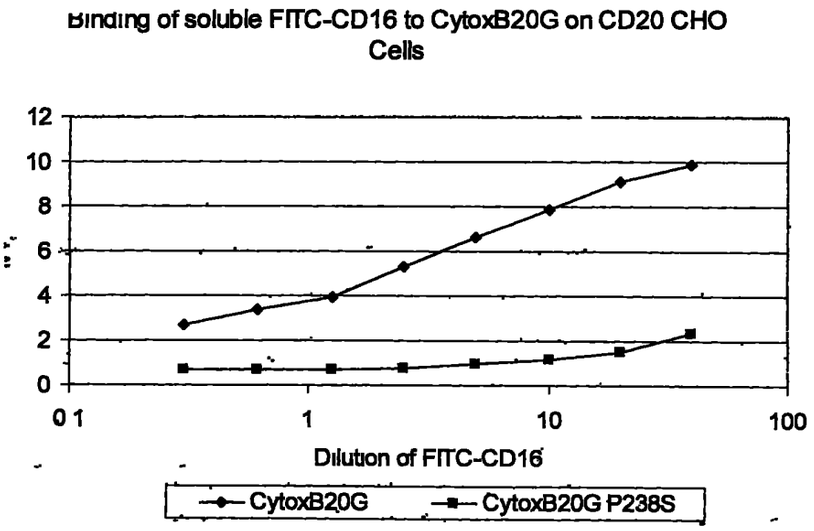


Fig 75

re 78 Binding of soluble FITC-CD16 to CytoxB20G on CD20 CHO cells CD20 CHO cells (10⁶) were incubated with saturating amounts of CytoxB20G or CytoxB20G P238S (10 ug/ml) for one hour on ice in PBS/2% FBS Cells washed in PBS/2% FBS and incubated with serial dilutions of 0 5 mg/ml FITC-CD16 for one hour on ice. Cells washed and specific binding measured by flow cytometry using a Beckman-Coulter Epics C machine Results analyzed using Expo analysis software and normalized fluorescence units graphed as a function of concentration

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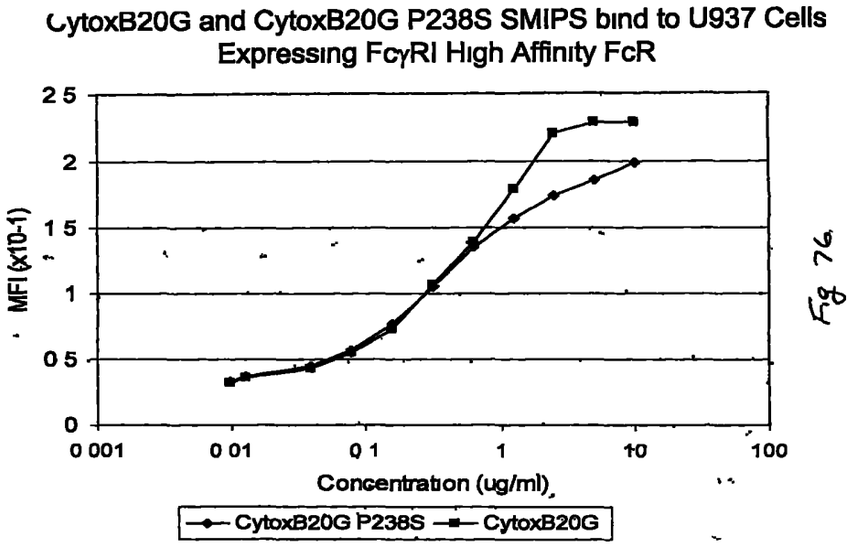


Fig 76.

re 79 CytoxB20G SMIPs bind similarly to U937 cells expressing the high affinity FcR (FcγRI, CD64) U937 cells
expressing CD64 were incubated in PBS/2%FBS for one hour on ice with CytoxB20G orCytoxB20G P238S Cells
washed and incubated for one hour on ice with FITC-goat anti-human IgG1 (Fc specific) (Caltag) at a final dilution
100. Cells were washed and fluorescence analysed on a Beckman-Coulter EpicsC flow cytometer Data was
yzed using Expo analysis software and fluorescence intensity graphed as a function of SMIP concentration

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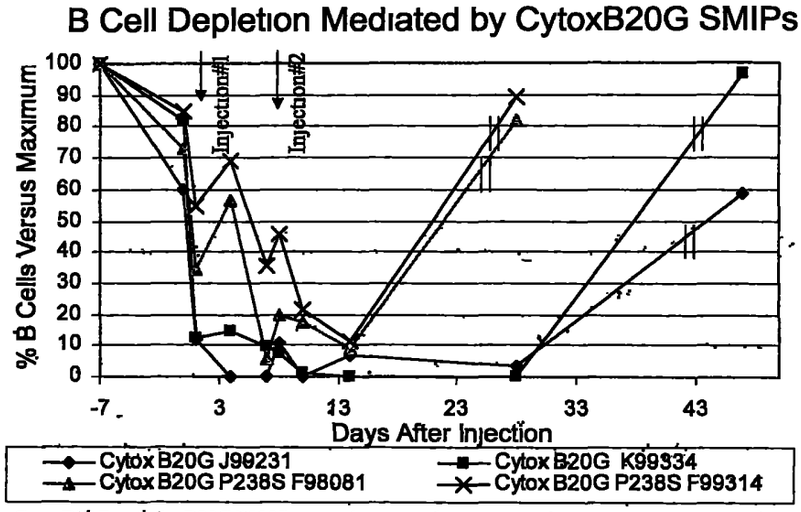


Fig 77

10 CytoxB20G or CytoxB20G P238S were administered to macaques by intravenous injection at 6 mg/kg, with
sions given one week apart. The effect on circulating B cells was measured by detection of CD40 positive B cells
heral blood Blood samples were drawn from injected animals at days -7,0,1,3,7,8,10,14, 28, and 43 B cell
n was estimated by performing CBC (complete blood counts) and two color flow cytometry analysis on monkey
FITC or PE conjugates of antibodies against CD40, CD19,CD20, IgG, CD3, CD8 were used in various
itions Data are plotted as the number of CD40 positive blood B cells tabulated in thousands of cells per
ar over time relative to the initial pre-injection time point level of B cells (maximum)

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Figure 81 SEC on CytoxB37G SMIPs containing SSS and SSC hinge Domains from Human IgG1

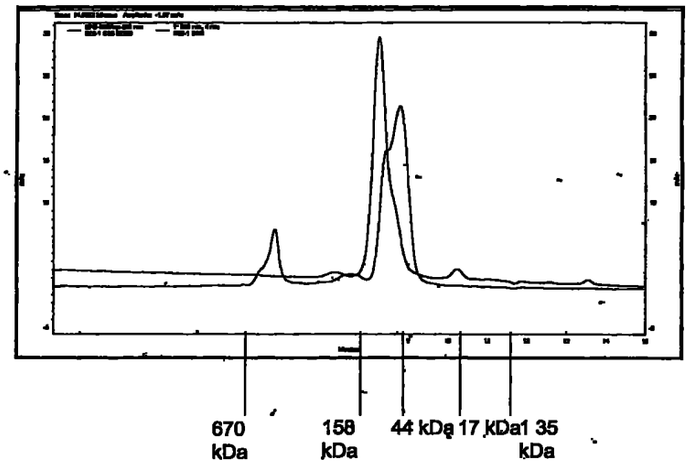


Fig 78

Figure 81; SEC (Size Exclusion Chromatography) CytoxB37G SMIP's were purified from CHO culture supernatants by Protein A affinity chromatography Purified aliquots of 10-25 µg were subjected to HPLC over a Tosoh Biosep, Inc TSK 3000 SWXL HPLC column, pore size 5 µm. The flow rate was 1ml/min, in PBS, pH 7 2 running buffer Migration rates of molecular weight standards are indicated below the tracing The CytoxB37G (SSS)H SMIP indicated in blue, while the CytoxB37G (CSS)H is indicated in red.

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gure 82 Binding of CytoxB37G SMIPs to B Cell Lymphoma Cell Lines

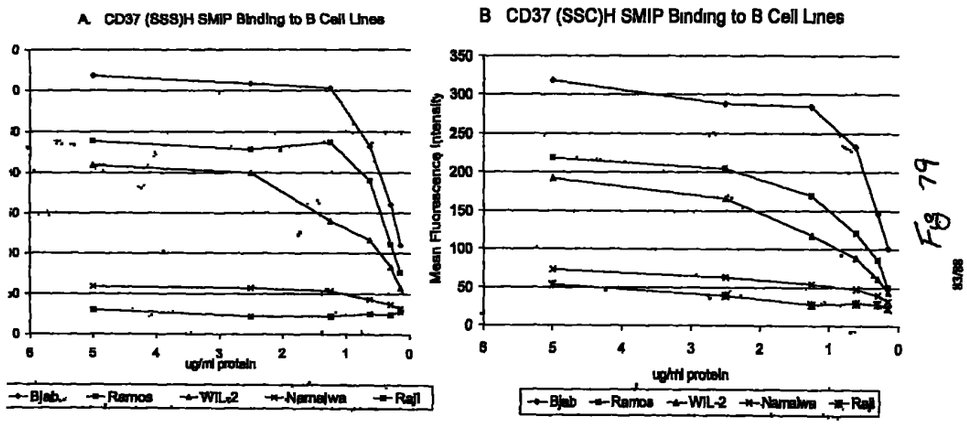


Fig 79

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ure 82 Binding of CytoxB37G SMIPS to B cell lymphoma cell lines Serial dilutions of ified CytoxB37 (SSS)H G or CytoxB37 (SSC)H G SMIPS were incubated with 10^6 cells of each cell type for 60 utes on ice in PBS/2%FBS Samples were washed twice, and incubated with a mixture of FITC goat anti-human, r and FITC goat anti-human IgG F(ab')2 (CalTag) at 1 100 each, on ice for 45 minutes Samples were washed and lyzed by flow cytometry using a FACsCalibur (Becton-Dickinson)

Fig 80: AnnexinV-PI Staining of Ramos Cells Incubated 24 hours with CD37 SMIPS

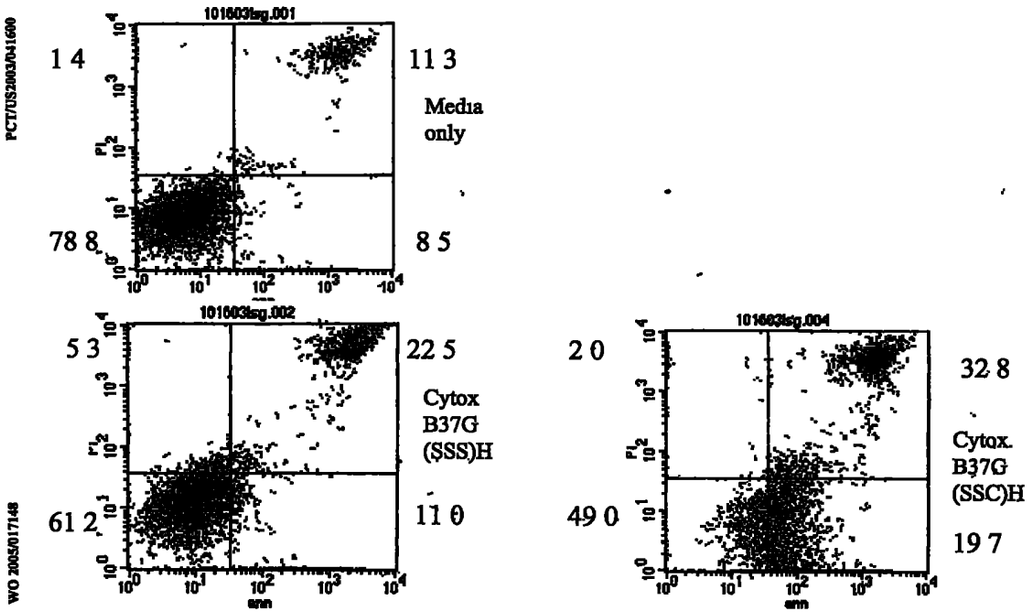


Figure 84 Thymidine Incorporation (Growth Inhibition) in Ramos B-cells after a 48 Hour Incubation with anti-CD37 SMIPS

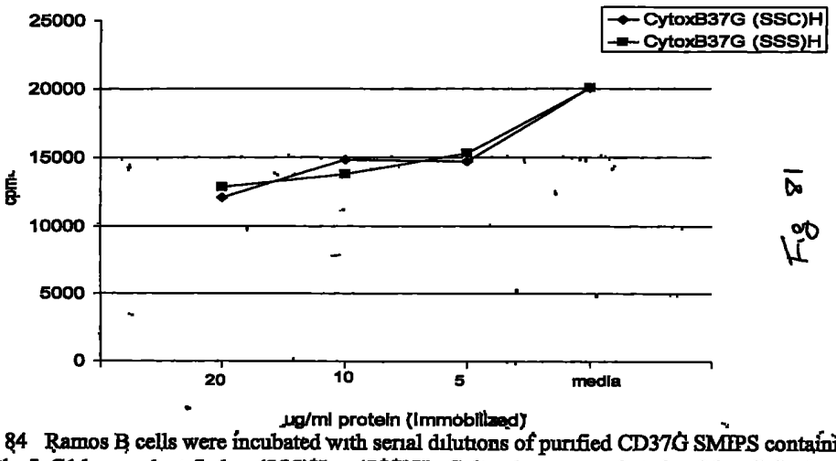


Fig 81

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ıre 84 Ramos B cells were incubated with serial dilutions of purified CD37G SMIPS containing .
ər the IgG1 hinge identified as (SSS)H or (SSC)H Cultures were incubated in 96 well flat bottom
ıe culture dishes (Costar) at 37°C, 5%CO_2 for 36 hours prior to pulsing with ^{3}H-thymidine for the
12 hours of a 48 hour incubation (0 75 µCı/well) Cells were harvested onto 96-well GFC plates
g a Packard harvester, dried, and 25 µl Microscint scintillation fluid added to each well prior to
ıting on a TopCount NXT microplate (Packard) scintillation counter Data are plotted as cpm
rporated versus protein concentratı n Each SMIP sh w increasing inhibitı n f prolıferatı n with
.asing protein concentration

ure 85 The Induction of Apoptosis in Ramos B-cells after a 20 hour incubation with different combinations of CD20 and CD37 targeted SMIPS

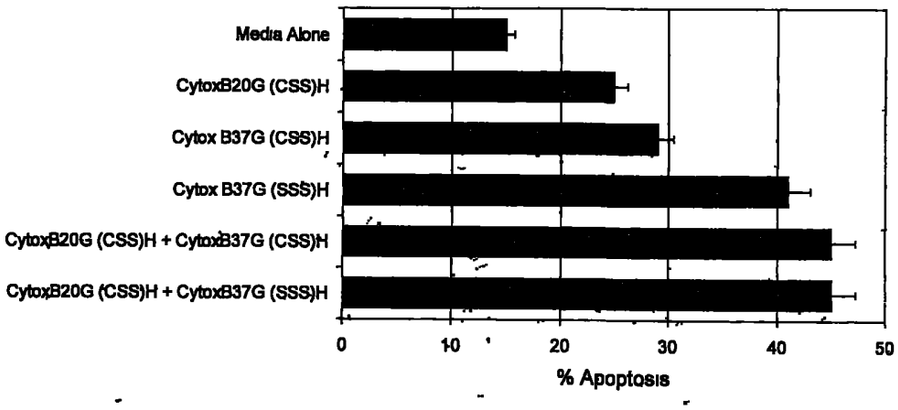


Fig 82

gure 85 Ramos B cells were incubated with CD20 and/or CD37 targeted SMIPs (10 /ml) in solution for 20 hours Cells were then harvested, washed, and incubated in nexinV and propidium iodide using a staining kit from Immunotech prior to two color w cytometry using a FACsCalibur flow cytometer (Becton-Dickinson) The graph show percentage of annexin V positive cells identified by their staining in the right quadrants the dot plots

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Figure 86 Complement Mediated Killing of Ramos Cells by CD37 SMIPs

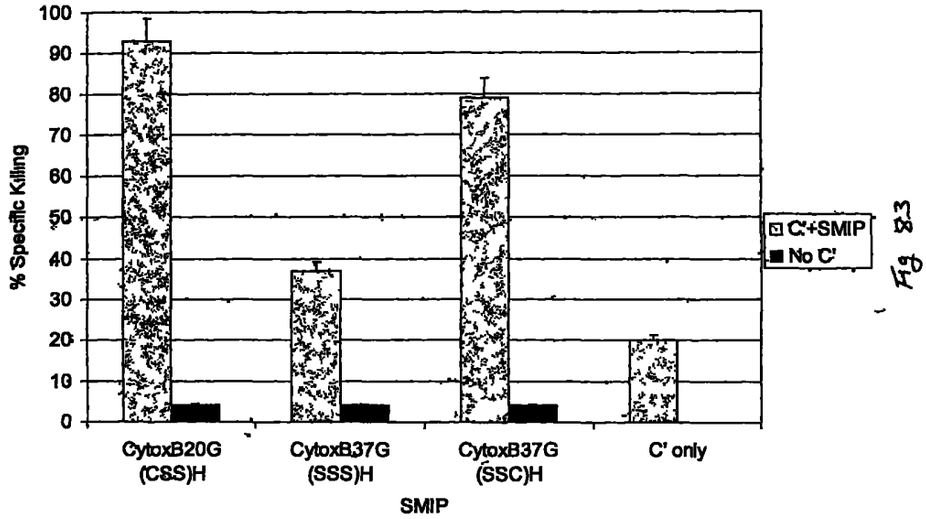


Fig 83

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Figure 86 CDC Activity of CytoxB37G SMIPS CytoxB20G, CytoxB37 (SSS)H G, CytoxB37 (SCS)H, CytoxB37 (CSS)H, or CytoxB37 (SSC)H were incubated at 10 μg/ml with 10^4 Ramos Target Cells and a 1 10 dilution of rabbit complement (PelFreez) in a volume of 150 μl for 90 minutes Aliquots were stained with trypan blue (Invitrogen), and counted using a hemacytometer to determine the percentage of the cell population killed during treatment. Negative controls with cells and only one reagent were also included

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Figure 87 ADCC Activity of CD37 SMIPs Against Ramos Targets

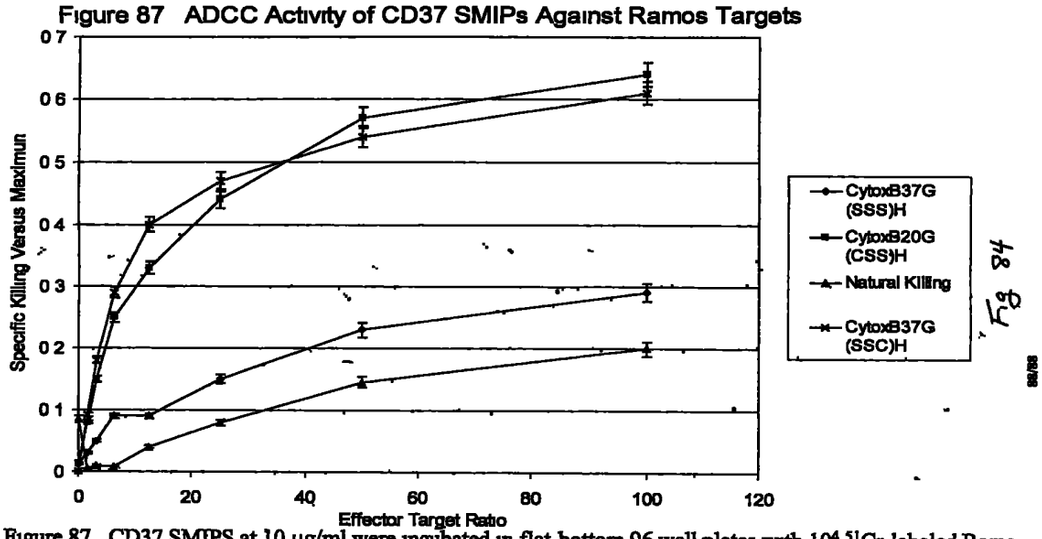
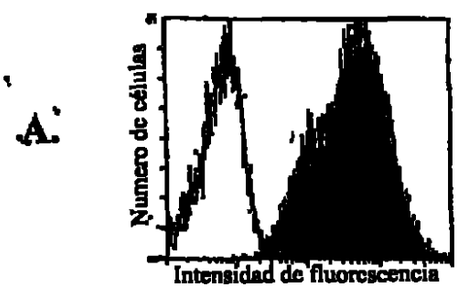


Fig 84

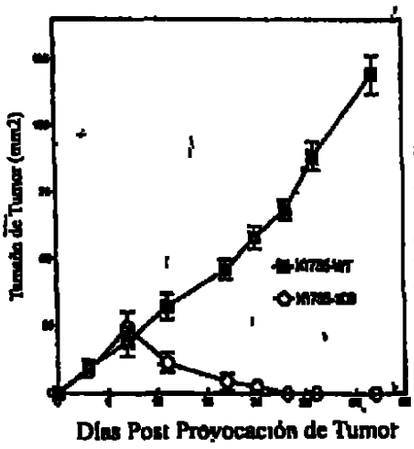
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Figure 87 CD37 SMIPS at 10 µg/ml were incubated in flat-bottom 96 well plates with 10^4 ^{51}Cr-labeled Ramo cells and resting human PBMCs at different effector:target ratios ranging from 0 to 100 · All incubations were performed in triplicate at each effector target ratio Natural Killing was measured at each effector target ratio by omission of SMIP Spontaneous release was measured without addition of PBMC or fusion protein, and maximal release was measured by the addition of detergent (1% NP-40) to the appropriate wells Reactions were incubated for 6 hours, and 100 ml culture supernatant harvested to a Lumaplate (Packard Instruments) and allowed to dry overnight prior to counting cpm released in a Packard Top Count NXT Microplate Scintillation Counter,

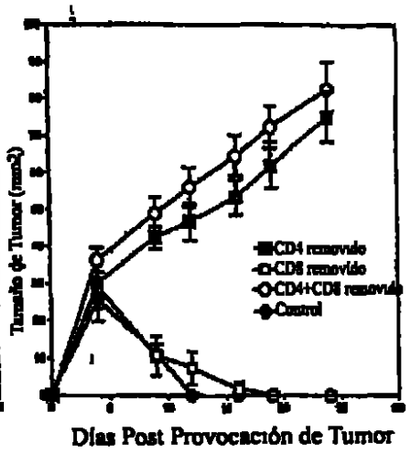
Figuras 41A, 41B y 41C



A



B



C

593

Fig 42

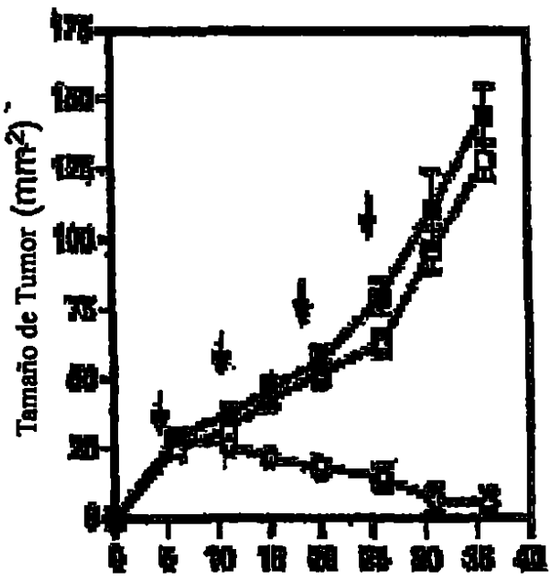
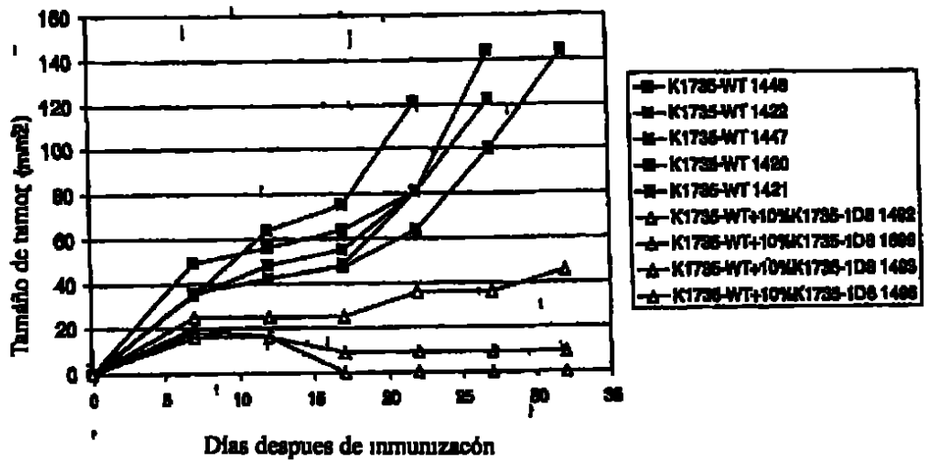


Fig. 43

Mezclas de líneas de tumor transfectadas K1735-WT y K1735-1D8 inhiben el sobrecrecimiento de tumor en ratones C3H



595

Fig 44

Expresión de CD137 (1D8) 2H7 scFv-IgG1 (SSS-S)H P238SCH2 WCH3 anti-ratón Sobre la superficie de Celulas de Tumor Transfectadas Ag104-1D8 filtradas

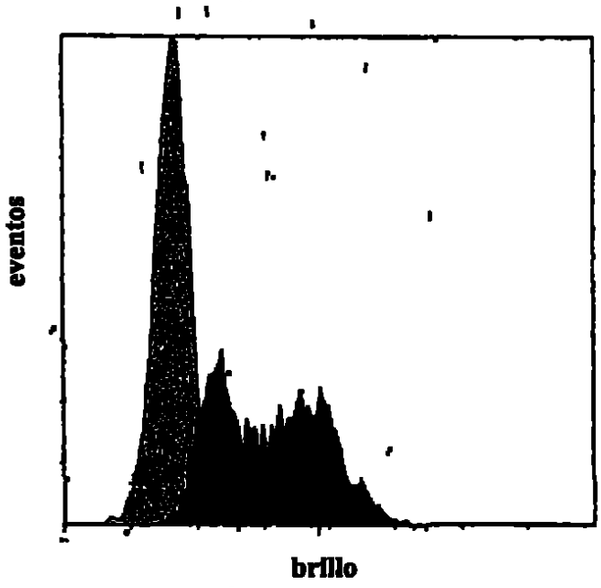


Fig. 45.

Gel SDS-PAGE Teñido Coomassie de Construcciones
2H7 scFv Ig

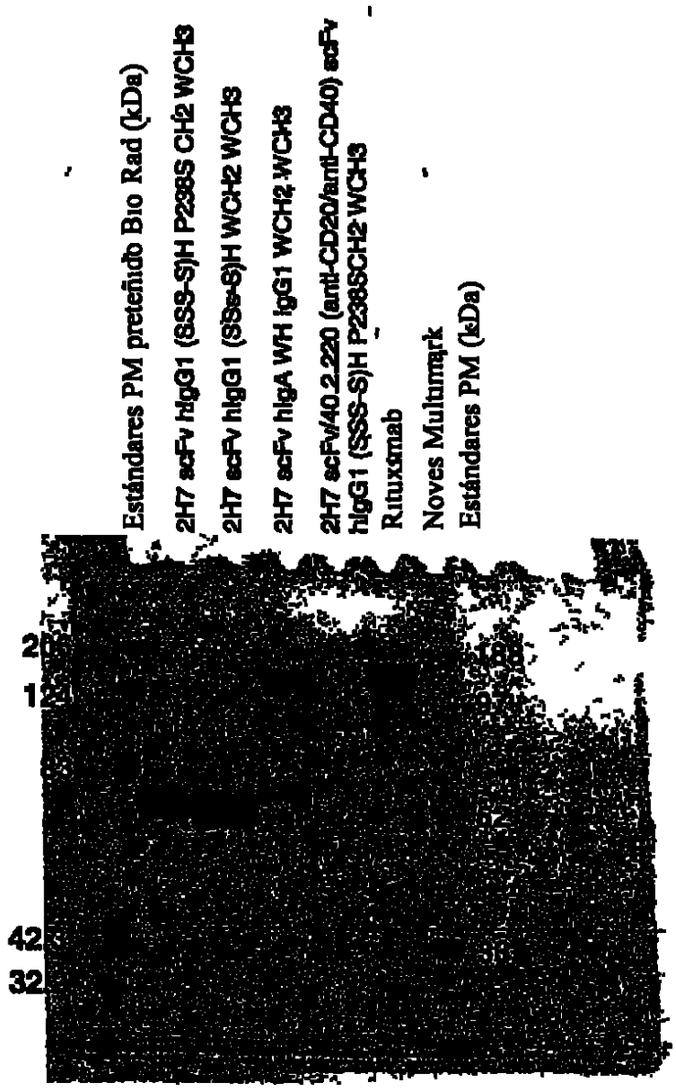


Fig 46

ADCC mediado por Construcciones 2H7 scFvIg por células efectoras PBMC humanas contra objetivos Bjab

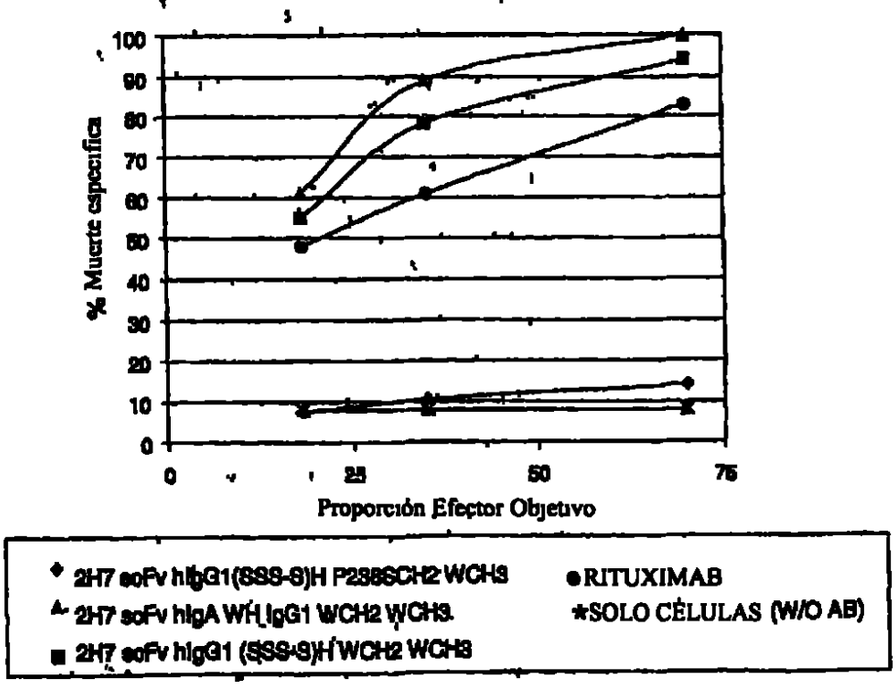
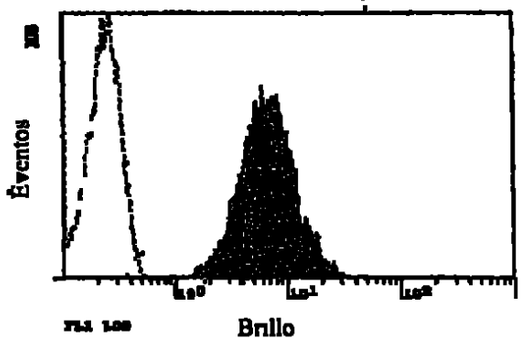


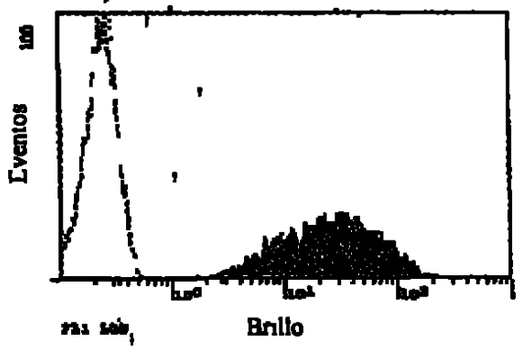
Fig 47

Expresion de superficie celular de CD3 G19-4 scFv hIgG1 (SSS-S)H P238SCH2 WCH3-HCD80TM/CT anti-humano en Células Reh y T51

Reh anti-CD3 (G19-4) scFv hIgG1 (SSS-S)H P238SCH2 WCH3-hCD80TM/CT

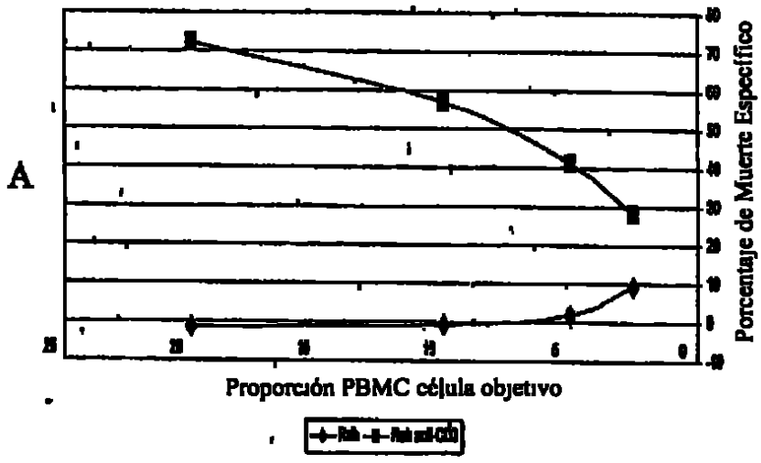


T51 G19-4 scFv hIgG1 (SSS-S)H P238SCH2 WCH3-hCD80TM/CT



Objetivo de Citotoxicidad para Líneas de Células Transfectadas por expresion de superficie de Cd3 scFvIg

Actividad citotóxica de PBMC que se inclina hacia células Ren transfectadas



Actividad citotóxica de PBMC que se inclina hacia células linfoblastoides T51

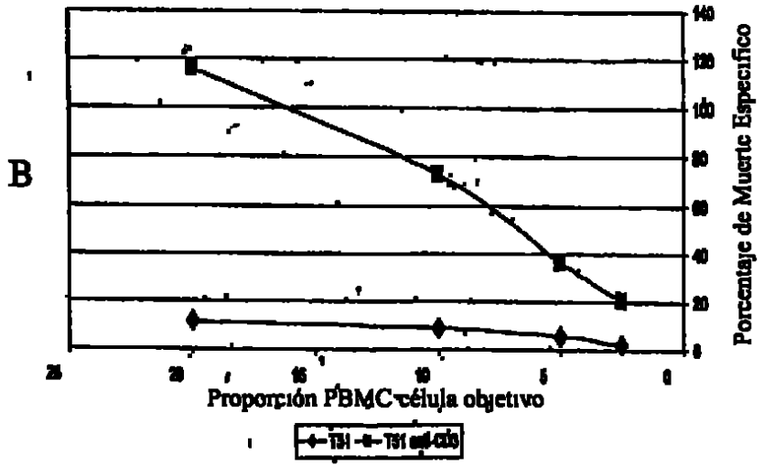


Fig 49

Unión de 5B9, un CD137 scFv hIgG1 (SSS-S)H WCH2 WCH3 anti-humano de ratón para PBMC humano estimulado

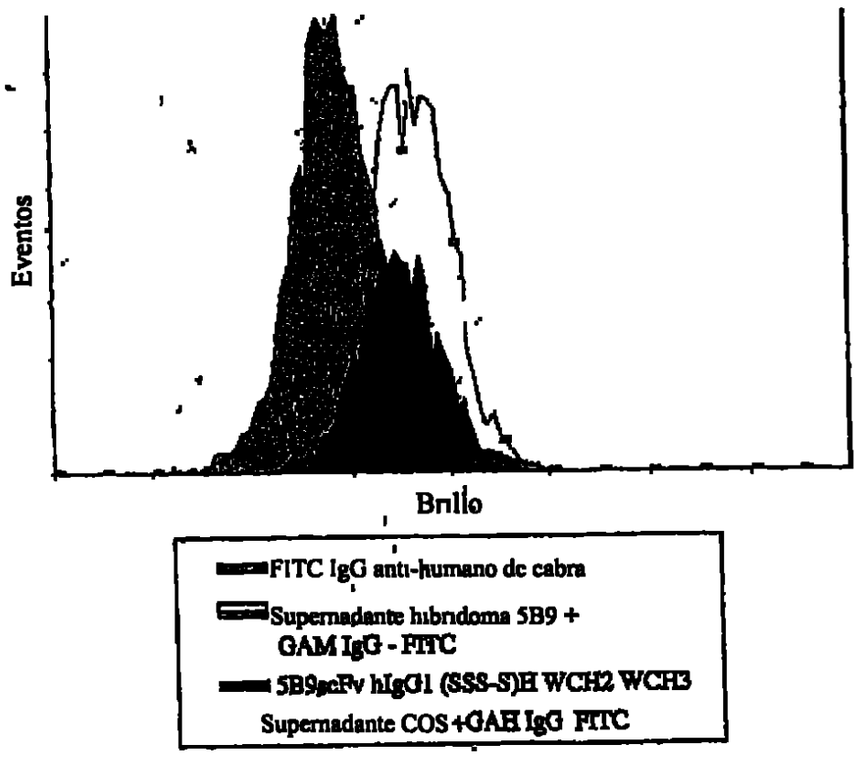
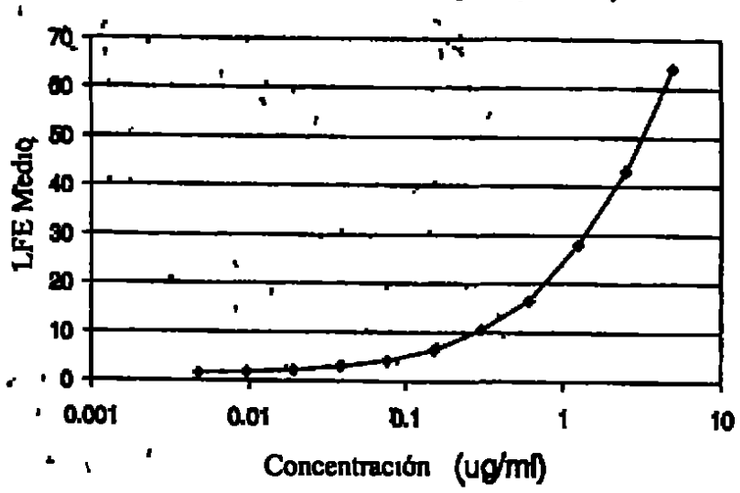


Fig 50

Efectos de Mutación VHL11 en CitoxB20 Proteina de Expresión
2H7 scFv hIgG1 (SSS-S)H WCH2 WCH3

50A, Curva Estándar, 2H7VH-L11S-IgG1 (SSS-S)H WCH2 WCH3

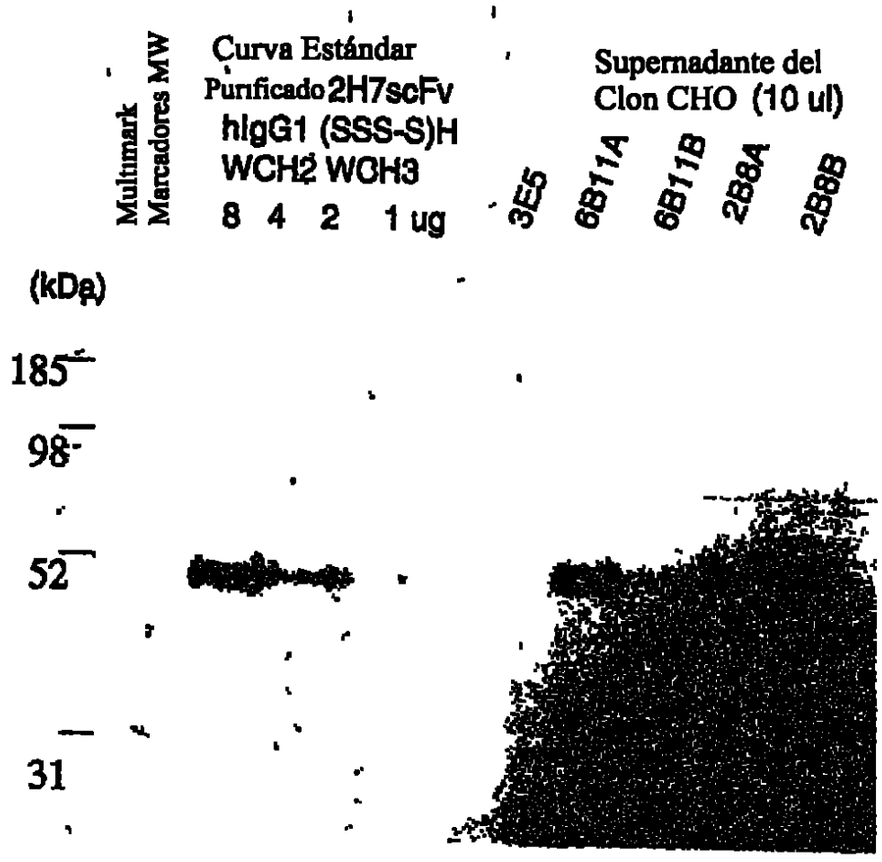


50B Brillo y Estimacion de supernadantes CHO de concentracion de Proteina de la Curva Estándar

Nombre de Clon CHO	4F2	4F5	3E5	6B11A	2B8A
LFE Medio					
1/100	71 7	40 6	31 5	99 7	101 5
1/500	27 1	12.4	11 2	40 8	43
Concent Aprox µg/ml	600	225	125	1000	1250

Fig. 51

Niveles de Producción de 2H7 scFv VHL11S-hIgG1 (SSS-S)H WCH2 WCH3 de Supernadantes de Cultivo de Clon CHO



603

Fig 52

Efecto de Mutación VHL11S en la Producción de Proteína de Construcción G28-1 scFvIg de celulas COS

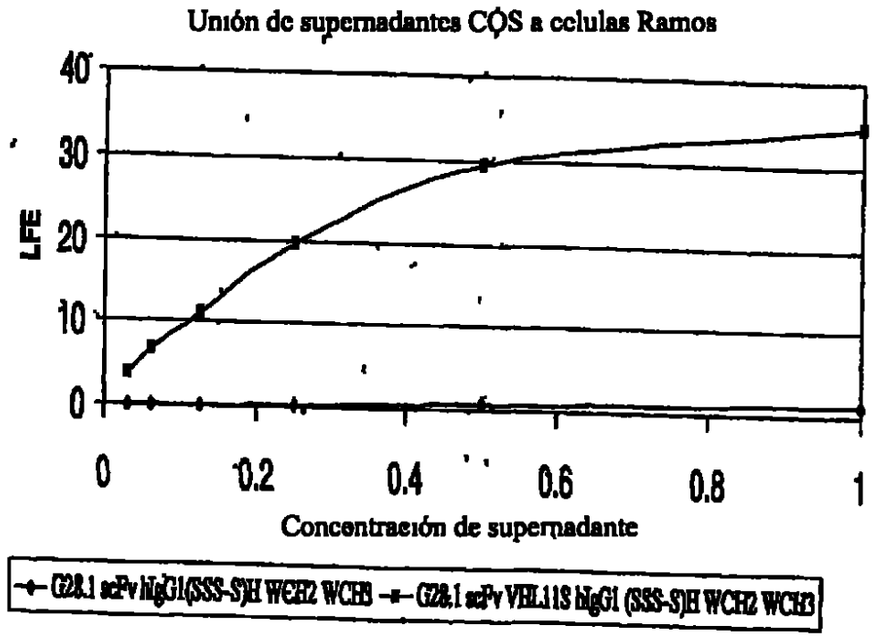


Fig. 53

Inmunoblot de Construcciones G28-1 scfvIg

Niveles de Proteina incrementados en los supernadantes COS transfectados con G28-1 scFv hIgG1 (SSS-S)H WCH2 WCH3 después de sustitución de leucina con serina en la posición 11 de VH(VHL11S)

Fig 53A

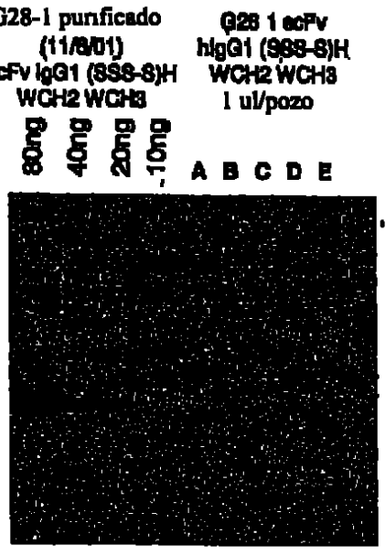


Fig 53B

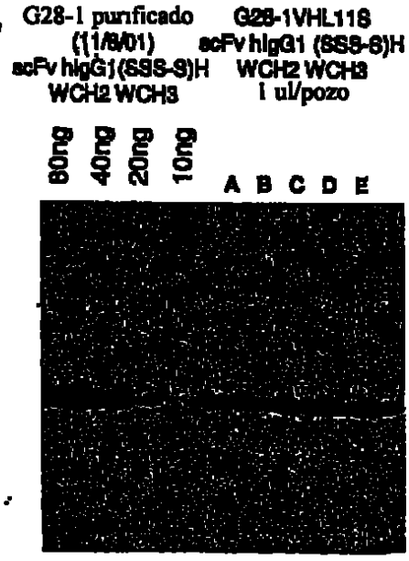


Fig 54

Unión de Construcciones 2H7 scFvIg con Pivotes Alterados y dominios CH3 a Células CD20 CHO

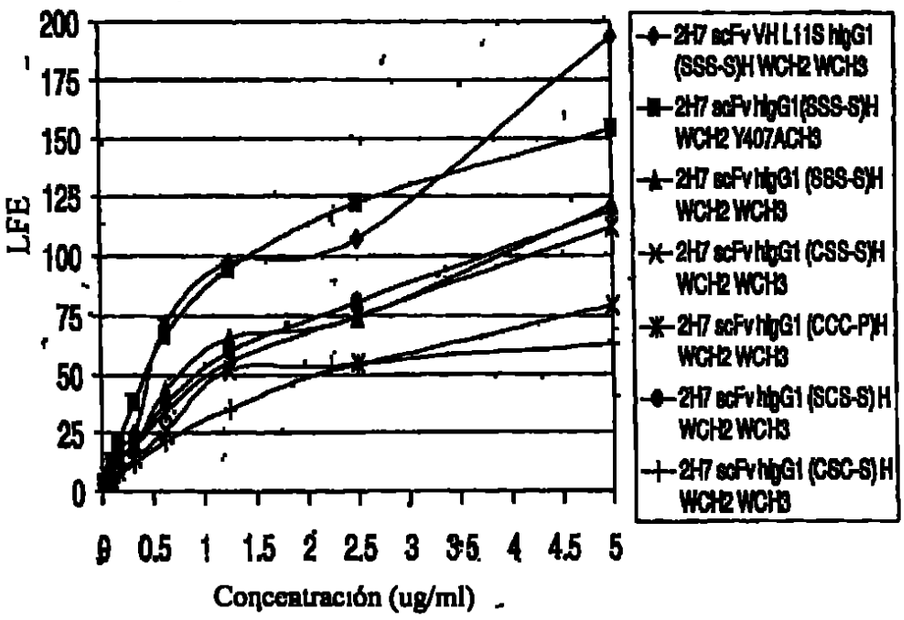
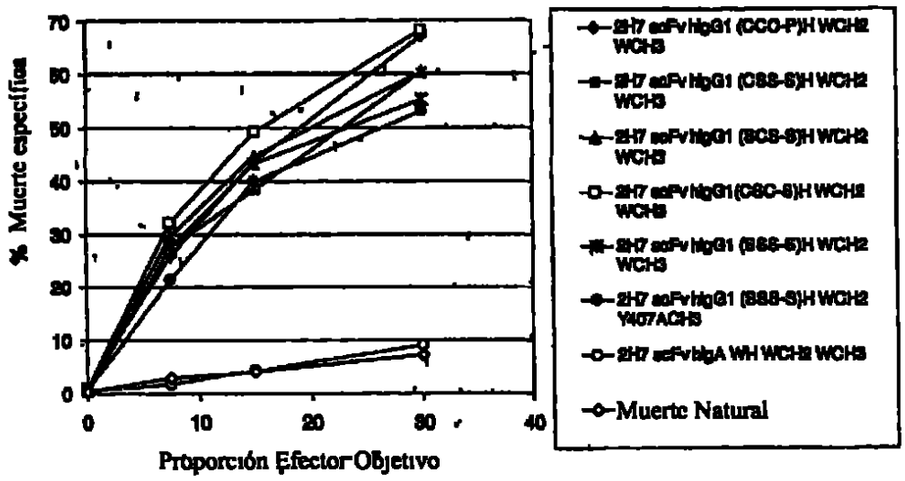


Fig 55

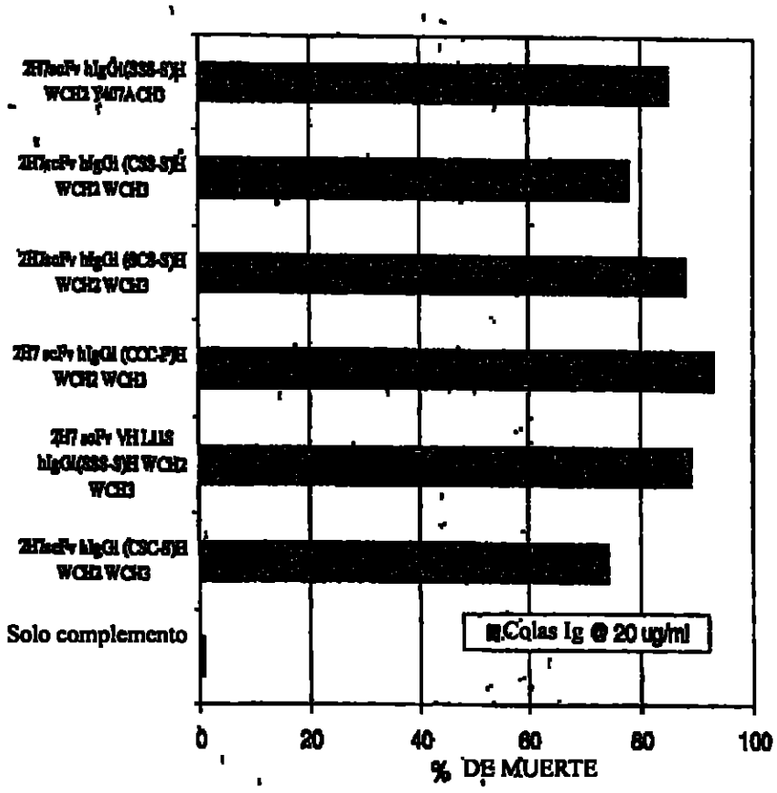
Actividad ADCC de construcciones 2H7 scFvIg contra Objetivos BJAB y Efectores PBMC



607 pgs

Fig 56

Actividad de Complemento de Construcciones
2H7 scFvIg con Células Objetivo Ramos



608 Sept

Fig 57

Unión de Derivados 2H7 scFvIg a Celulas CD20CHO

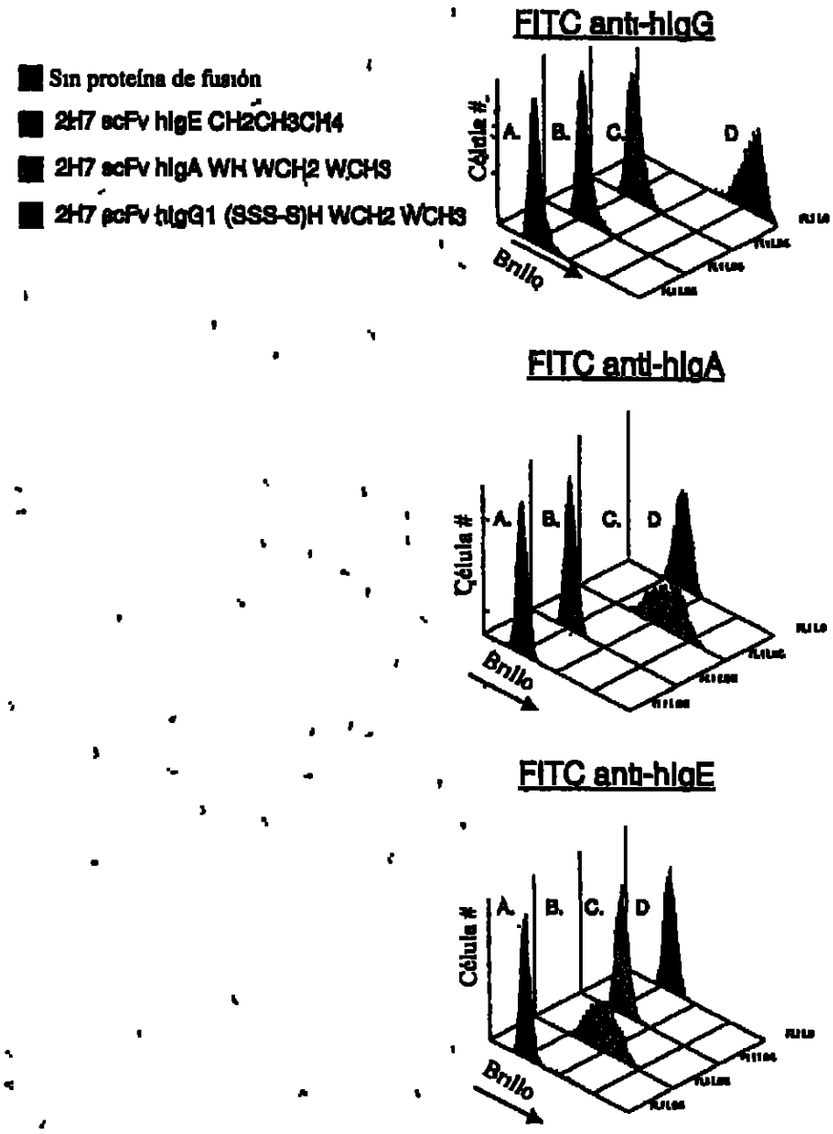
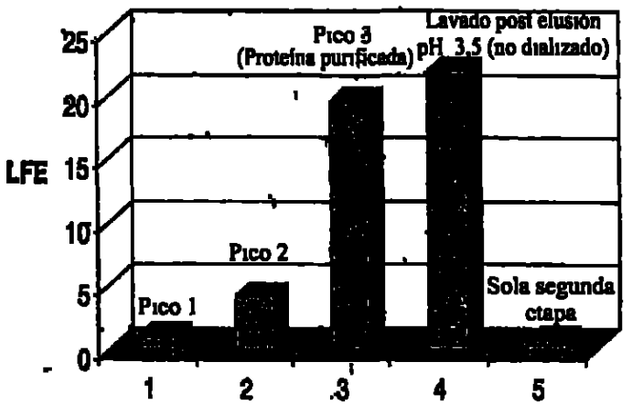


Fig. 58

Figura 58A 2H7 scFv VHL11S humano IgE (WCH2 WCH3 WCH4) que se une al CD20 CHO en 30 ug/ml



58B Actividad ADCC de fracciones de proteína 2H7 scFv VHL11S IgE (WCH2 WCH3 WCH4) con Efectores PBMC y Objetivos Bjab

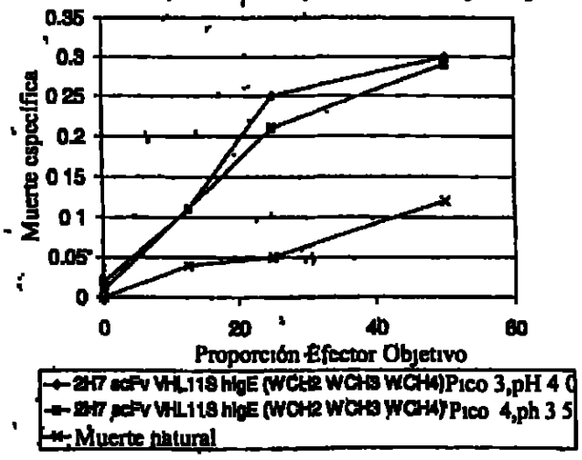


Fig 59

Datos de Unión para COS derivados de α-Cd20 (2H7) scFv VHL11S mIg E (WCH2 WCH3 WCH4) y Moleculas de Cola mIgA (WH WCH2 WCH3 WCH4)

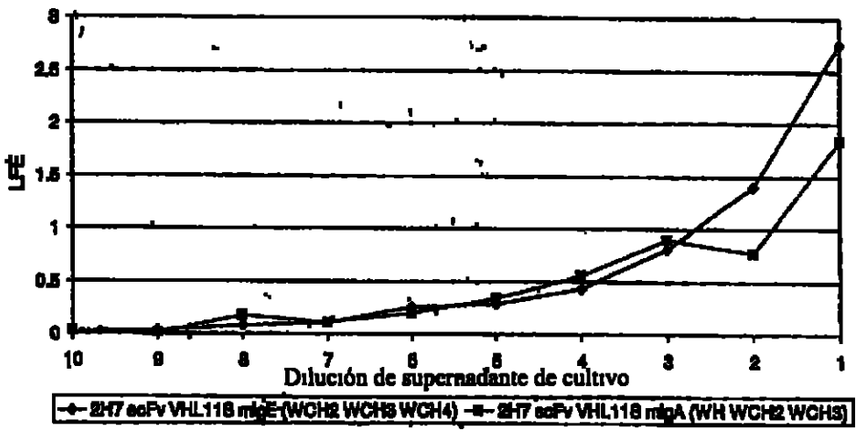


Fig 60

Perfiles HPLC de Construcciones 2H7 scFvIg

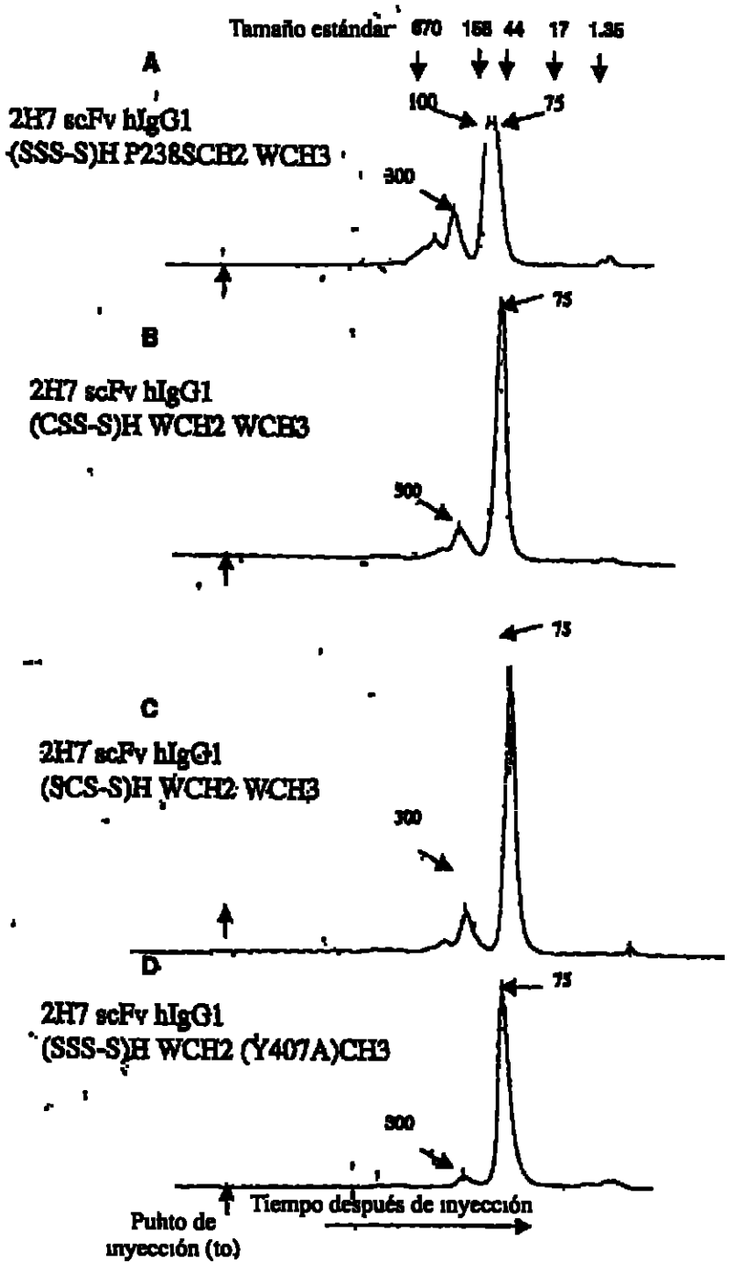


Fig 61

Perfiles HPLC de Construcciones 2H7 scFvIg

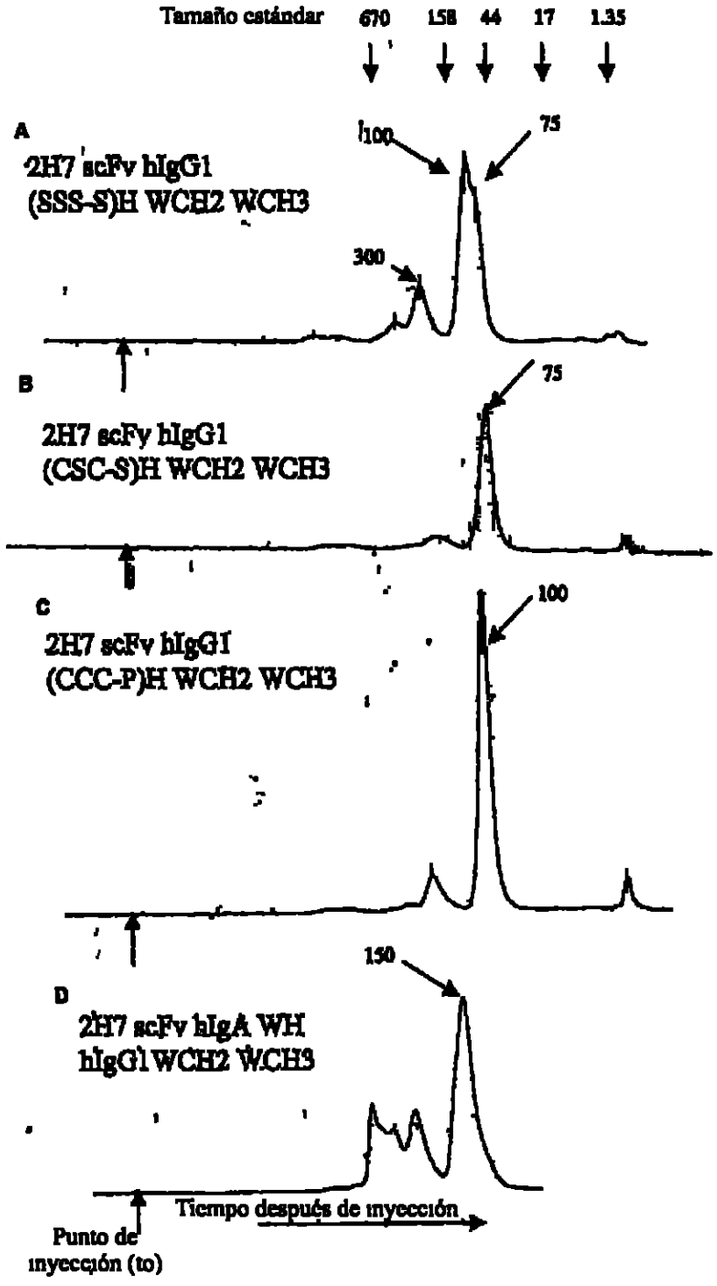


Fig 62

Perfiles HPLC de Construcciones 2H7 scFvIg

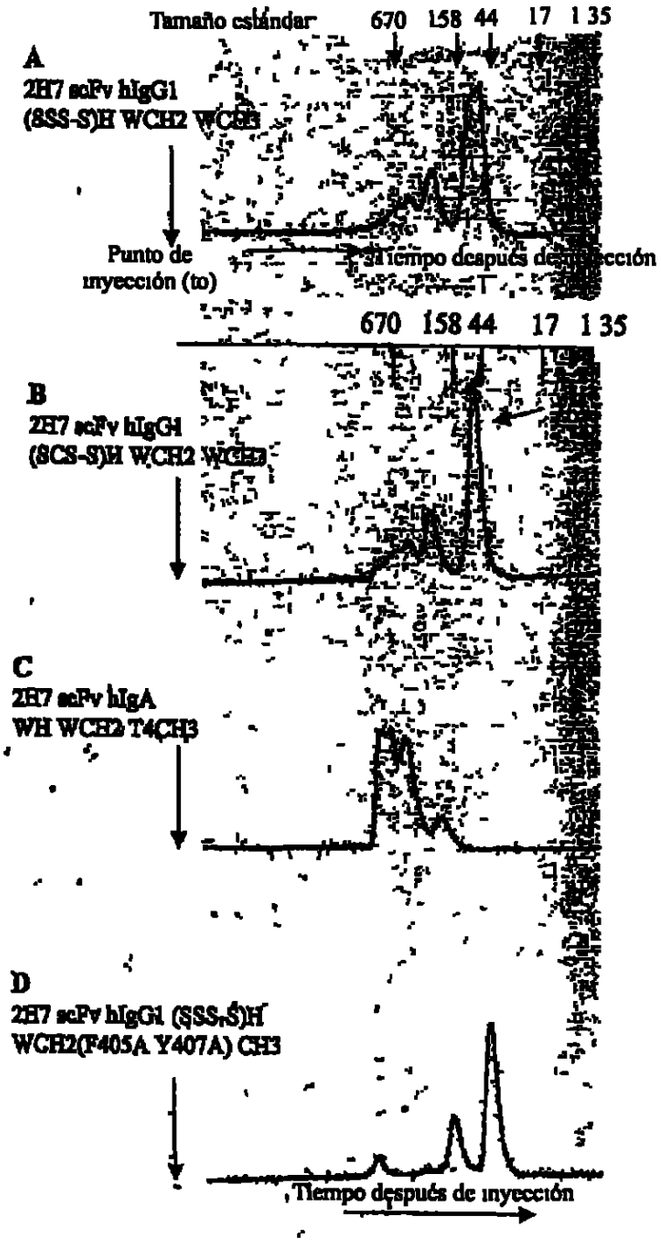
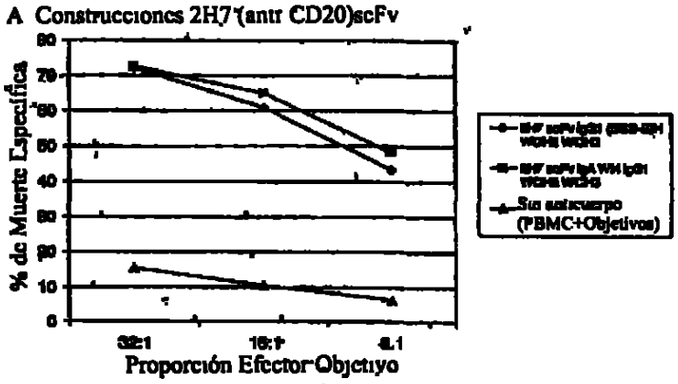


Fig. 19

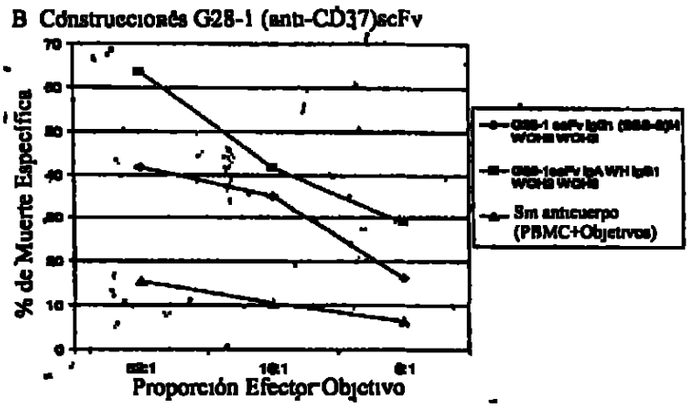
Actividad ADCC de Construcciones 2H7 scFvIg, G28-1 scFvIg, y HD37 scFvIg

Actividad ADCC de scFvs objetivo a Antígenos de Célula B

A Construcciones 2H7 (anti-CD20)scFv



B Construcciones G28-1 (anti-CD37)scFv



C Construcciones HD37 (anti-CD19)scFv

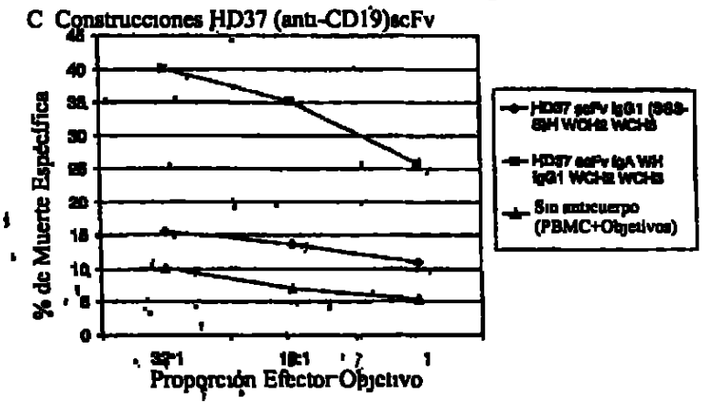


Fig. 20

Actividad ADCC de Construcciones L6 scFvIg

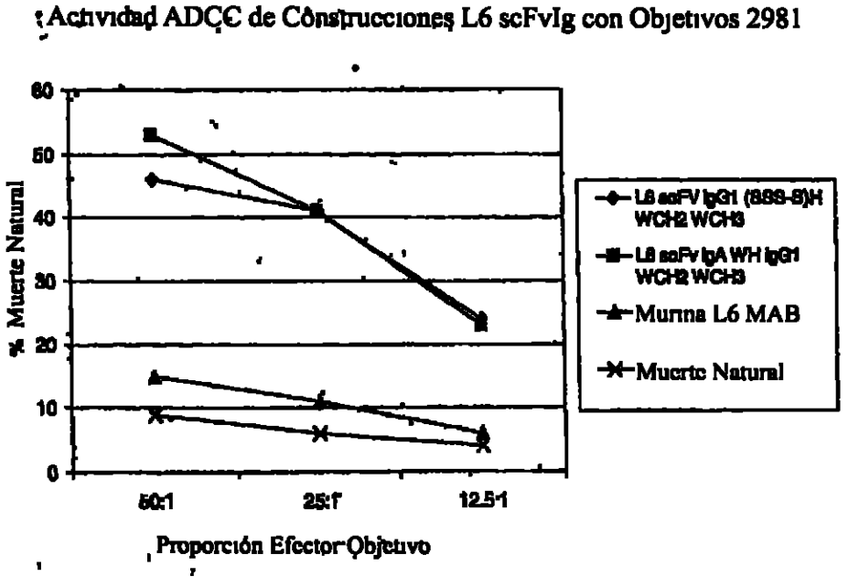


Fig 21

Analisis SDS-PAGE de Proteinas de Fusión L6 y 2H7 scFvIg

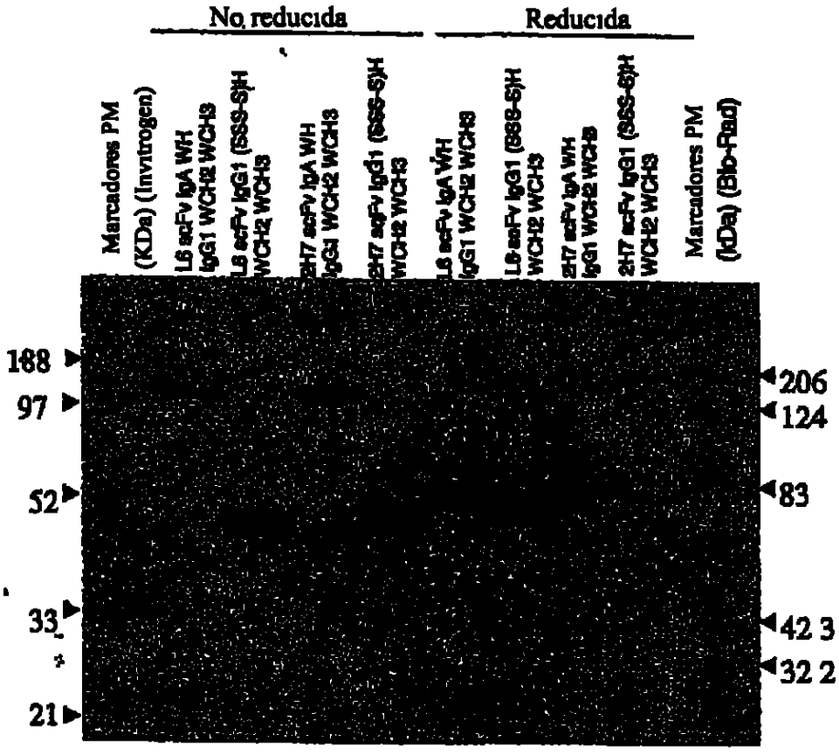


Fig 22

Analisis SDS-PAGE de Construcciones G28-1 y HD37 scFvIg

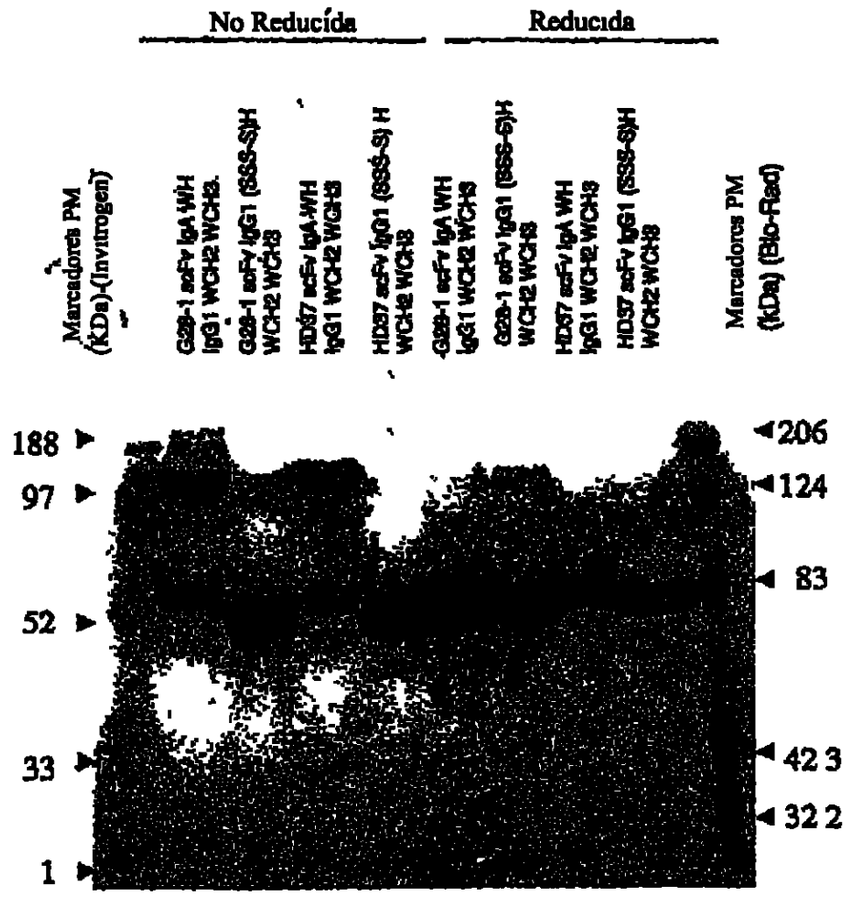


Fig 23

Alineación de secuencia de regiones Fc de llama y humano

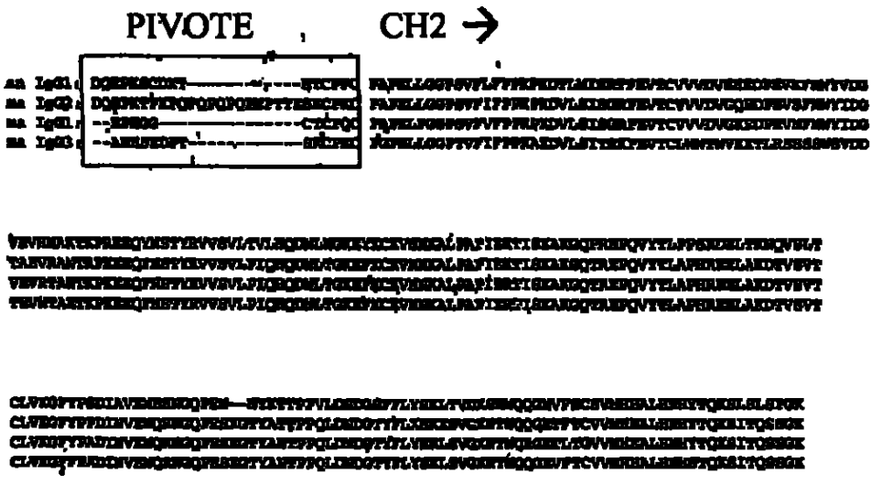


Fig. 24

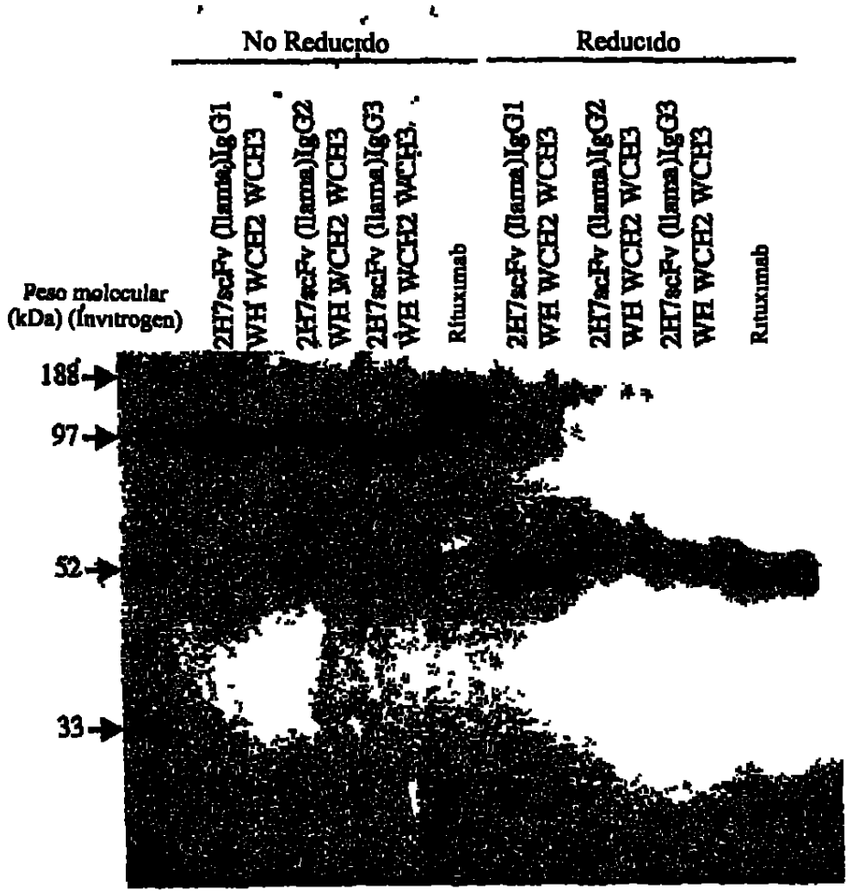

No Reducido
Reducido
2H7scFv (llama)IgG1 WH WCH2 WCH3
2H7scFv (llama)IgG2 WH WCH2 WCH3
2H7scFv (llama)IgG3 WH WCH2 WCH3
Rituximab
Peso molecular (kDa) (Invitrogen)
188
97
52
33

Fig. 25

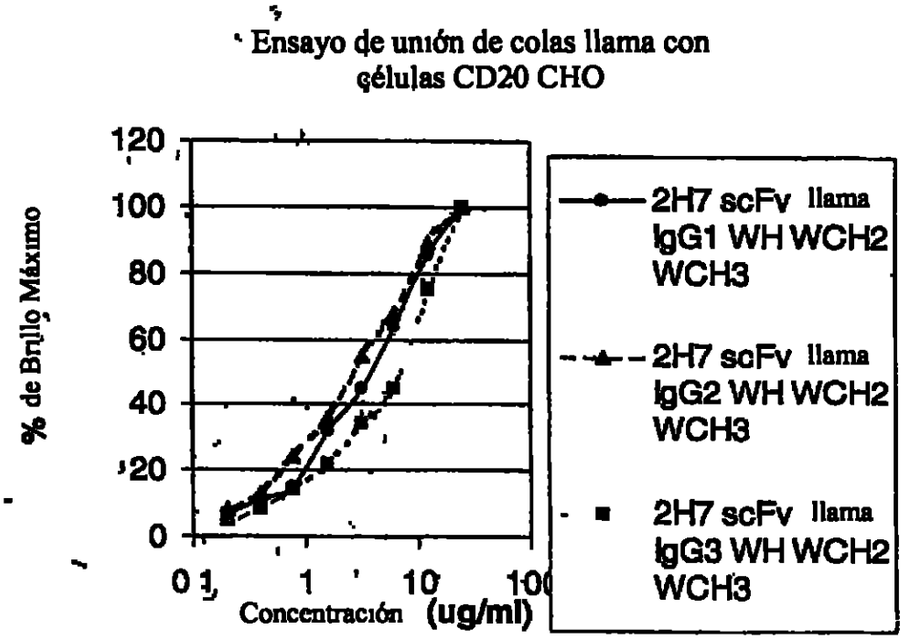

Ensayo de unión de colas llama con células CD20 CHO
% de Brillo Máximo
120
100
80
60
40
20
0
0.1
1
10
100
Concentración (ug/ml)
2H7 scFv llama IgG1 WH WCH2 WCH3
2H7 scFv llama IgG2 WH WCH2 WCH3
2H7 scFv llama IgG3 WH WCH2 WCH3

Fig 26

Ensayos de unión de colas 2H7 scFv Ig de Llama con celulas CD20 CHO

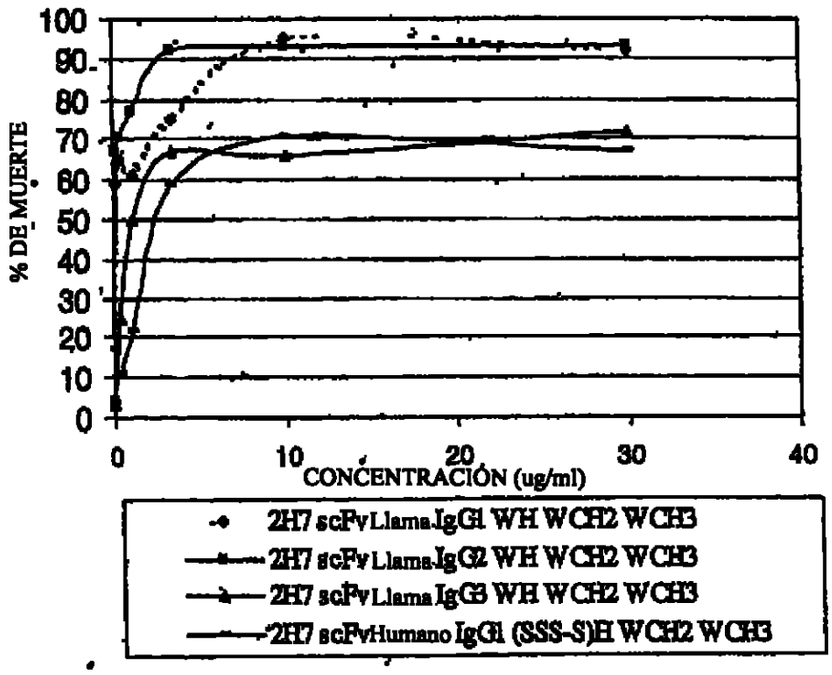


Fig. 27

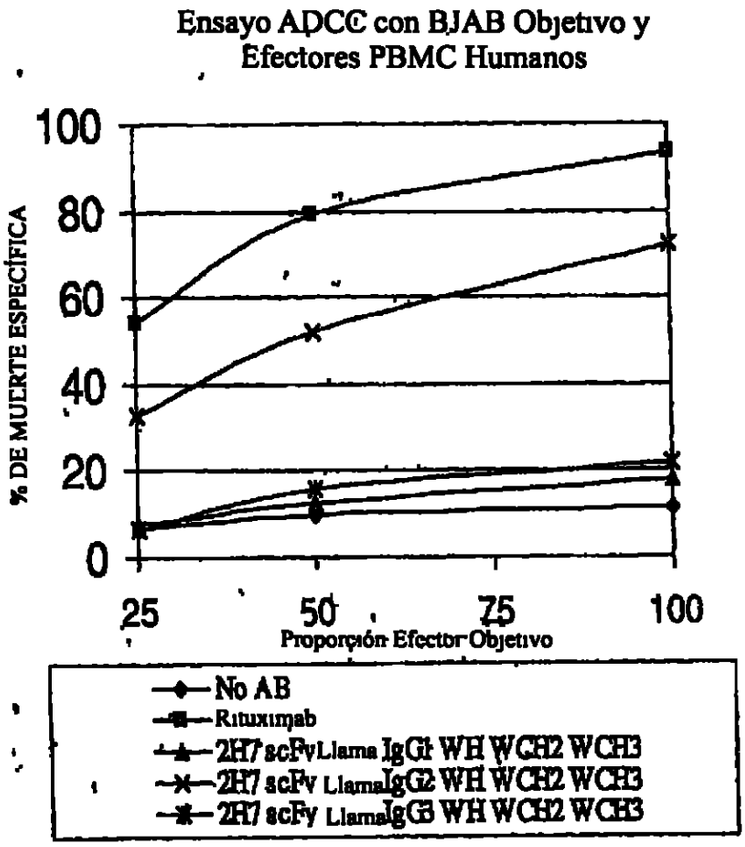
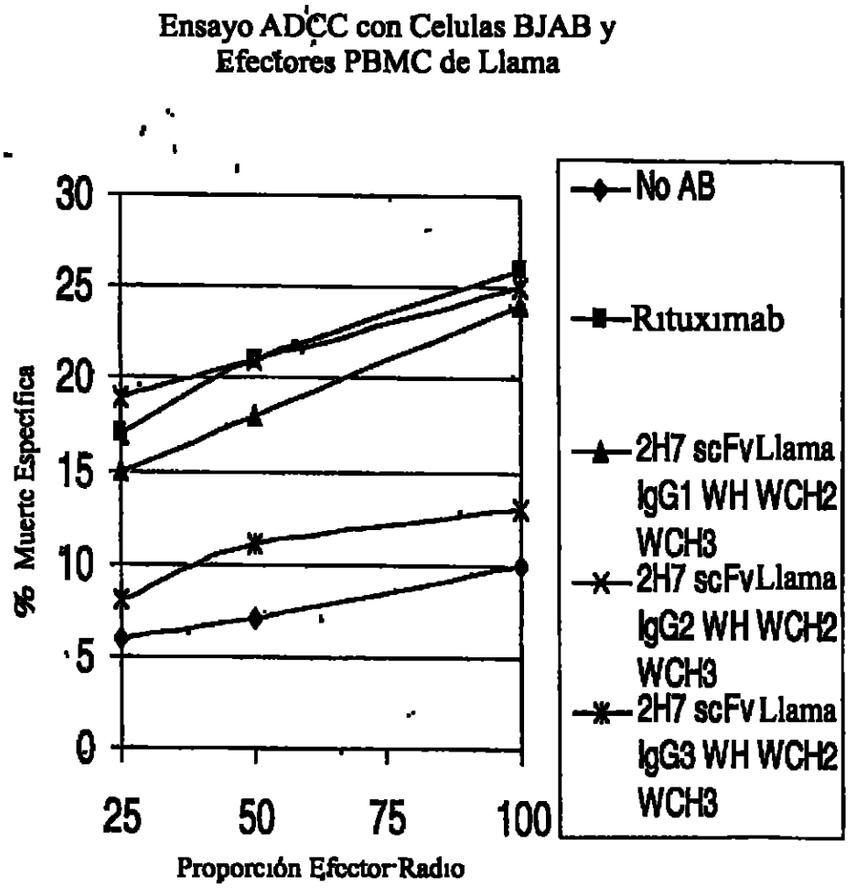

Ensayo ADCC con BJAB Objetivo y Efectores PBMC Humanos
% DE MUERTE ESPECÍFICA
100
80
60
40
20
0
25
50
75
100
Proporción Efector-Objetivo
No AB
Rituximab
2H7 scFv Llama IgG1 WH WCH2 WCH3
2H7 scFv Llama IgG2 WH WCH2 WCH3
2H7 scFv Llama IgG3 WH WCH2 WCH3

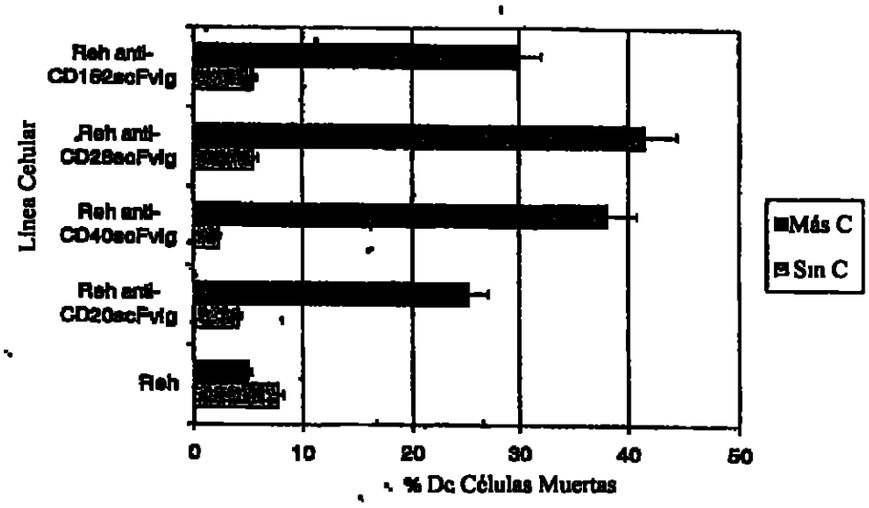
Fig 28


Ensayo ADCC con Celulas BJAB y Efectores PBMC de Llama
% Muerte Específica
30
25
20
15
10
5
0
25
50
75
100
Proporción Efector-Radio
No AB
Rituximab
2H7 scFvLlama IgG1 WH WCH2 WCH3
2H7 scFvLlama IgG2 WH WCH2 WCH3
2H7 scFvLlama IgG3 WH WCH2 WCH3

624

Fig 29

Citotoxicidad Mediada por Complemento contra Líneas de Células Reh que Expresan Construcciones scFvIg de Superficie

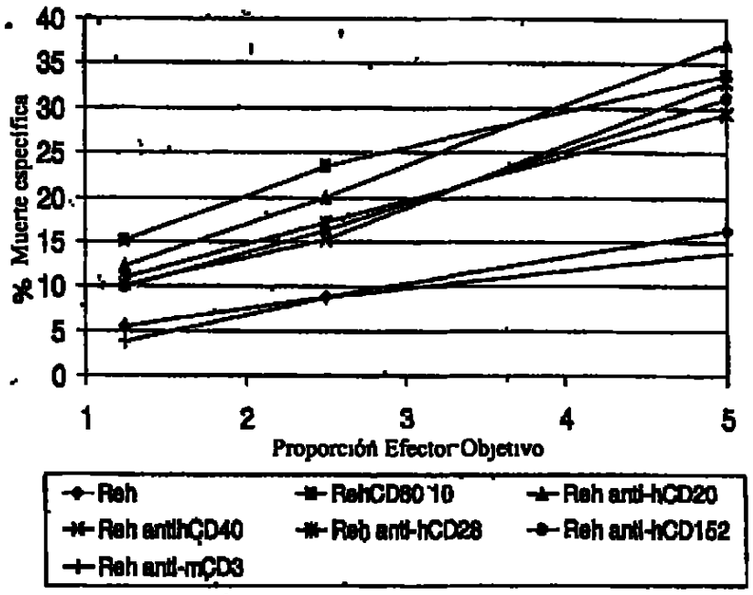


Funciones Efectoras y de Superficie scFvIg

625

Fig. 30

Actividad ADCC de Construcciones ScFvIg
que Expresan Superficie Celular



Funciones Efectoras y de Superficie scFvIg

Fig 31

Construcciones Ig y Nomenclatura

Identificador del Nombre	Secuencia de Pivote	Secuencia CH2	Secuencia CH3
hIgG1 (CCC-P)H WCH2 WCH3	Pivote IgG1, WT (CCC-P)	CH2 Tipo silvestre	CH3 Tipo silvestre
hIgG1 (SSS-S)H WCH2, WCH3	Pivote IgG1 Mutante (SSS-S)	CH2 Tipo silvestre (IgG1)	CH3 Tipo silvestre (IgG1)
VH L11S hIgG1 (SSS-S)H WCH2 WCH3	Pivote IgG1 Mutante (SSS-S)	Ch2 Tipo silvestre (IgG1)	CH3 Tipo silvestre (IgG1)
IgG1 (SSC-S)H WCH2 WCH3	Pivote IgG1 Mutante (SSC-S)	CH2 Tipo silvestre (IgG1)	CH3 Tipo silvestre (IgG1)
IgG1 (SCS-S)H WCH2 WCH3	Pivote IgG1 Mutante (SCS-S)	CH2 Tipo silvestre (IgG1)	CH3 Tipo silvestre (IgG1)
IgG1 (CSS-S)H WCH2 WCH3	Pivote IgG1 Mutante (CSS-S)	CH2 Tipo silvestre (IgG1)	CH3 Tipo silvestre (IgG1)
IgG1 (SSS-S)H P238S CH2 WCH3	Pivote IgG1 Mutante (SSS-S)	CH2 Mutante (IgG1) Pro→Ser 238	Ch3 Tipo silvestre (IgG1)
IgA WH hIgG1 WCH2 WCH3	Pivote IgA	CH2 Tipo silvestre (IgG1)	CH3 Tipo silvestre (IgG1)
IgA WH IgA WCH2 WCH3	Pivote IgA	CH2 Tipo silvestre (IgA)	CH3 Tipo silvestre (IgA)
IgA WH IgA WCH2 T4CH3	Pivote IgA	CH2 Tipo silvestre (IgA)	CH3 Truncado (IgA) Desaparece 4 aa en COOH

Fig. 32

Actividad CDC de Construcciones CTLA4Ig contra Células Transfectadas Reh y Reh CD80

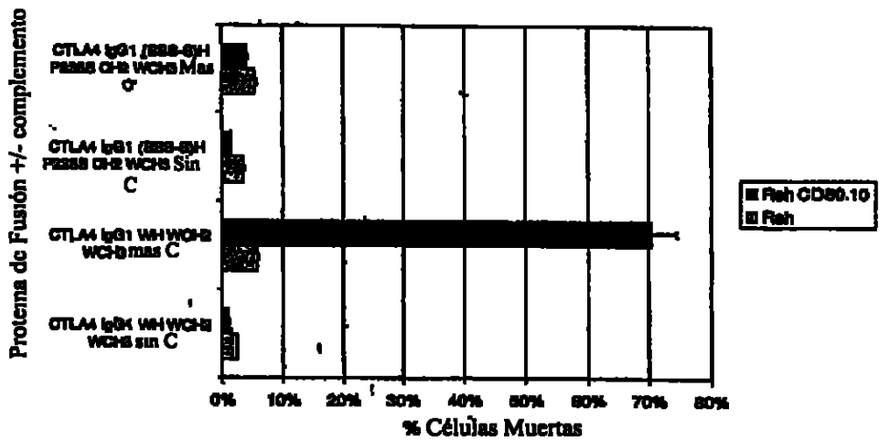
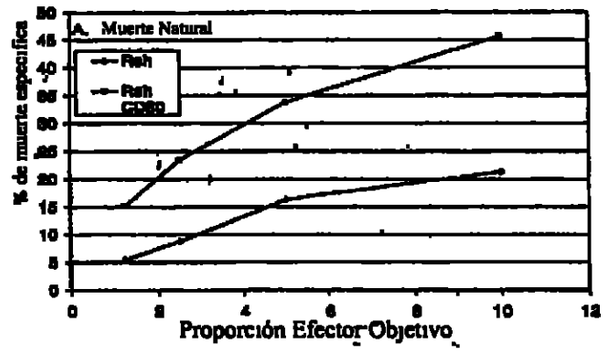
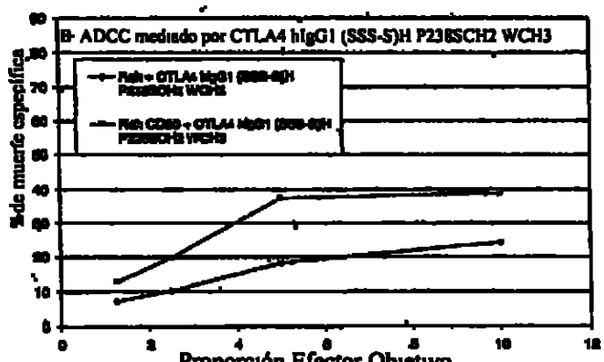
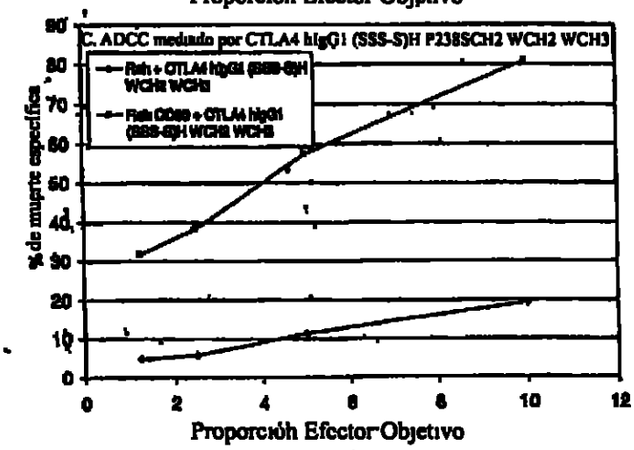


Fig. 33


A. Muerte Natural
Raji
Raji CD80
% de muerte específica
Proporción Efector Objetivo


B- ADCC mediado por CTLA4 hIgG1 (SSS-S)H P238SCH2 WCH3
% de muerte específica
Proporción Efector Objetivo


C. ADCC mediado por CTLA4 hIgG1 (SSS-S)H P238SCH2 WCH2 WCH3
% de muerte específica
Proporción Efector Objetivo

629

Fig. 34

Unión de Construcciones 2H7 scFvIg con Colas Alternativas para Células CD20 CHO

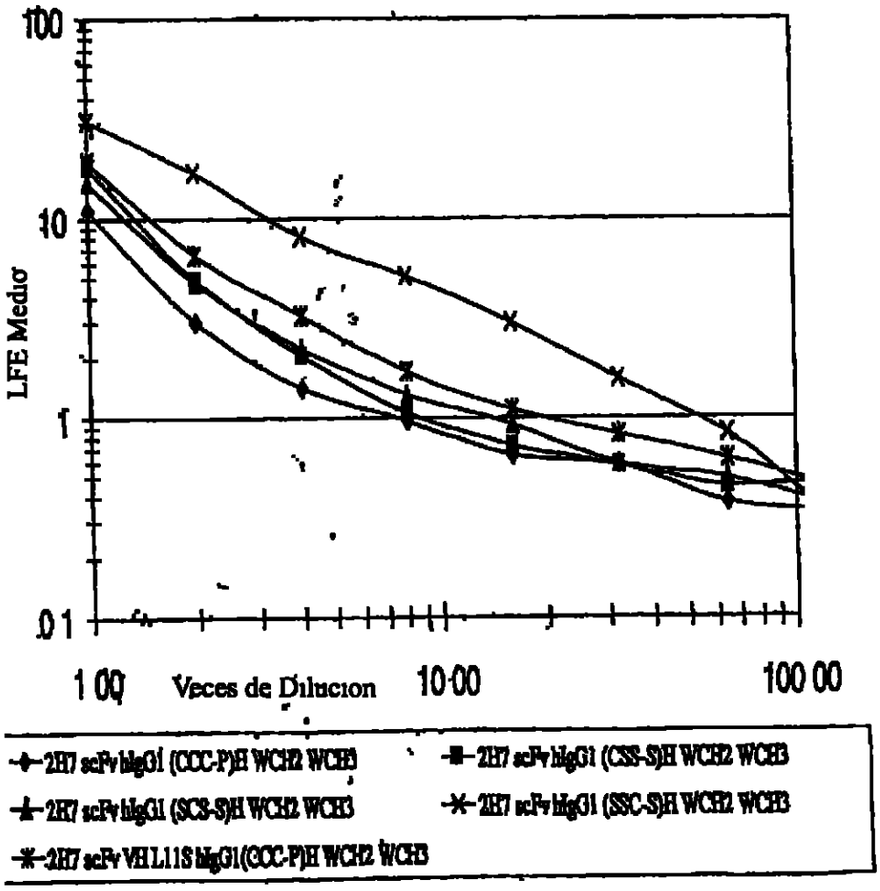


Fig 35

Analisis Inmunoblot de inmunoprecipitados de proteina de transfecciones COS de Construcciones 2H7 scFvIg

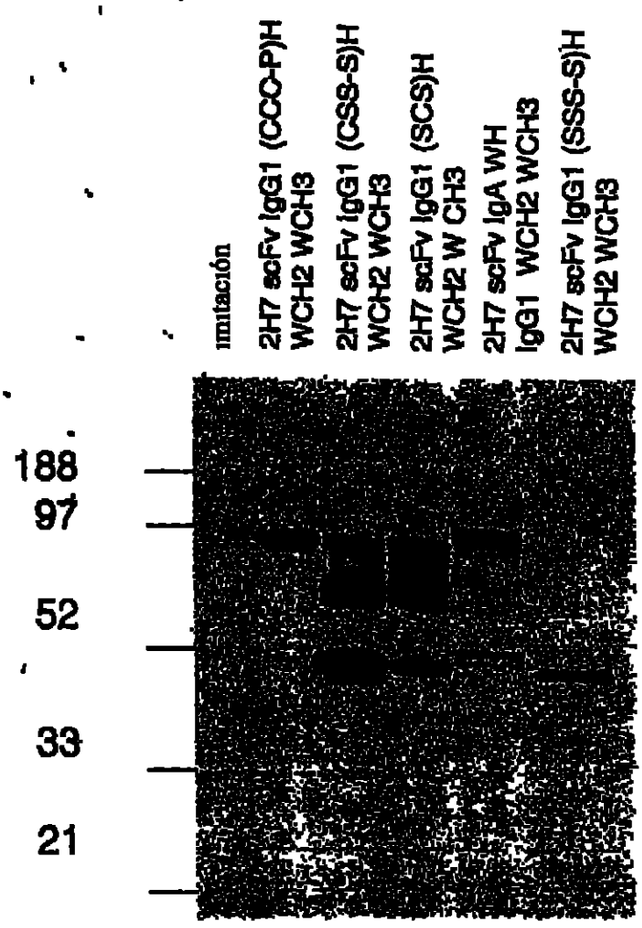


Fig. 36

Union a células CD20 CHO mediante construcciones que ligan anti-CD20 scFv a dominios IgA Fc

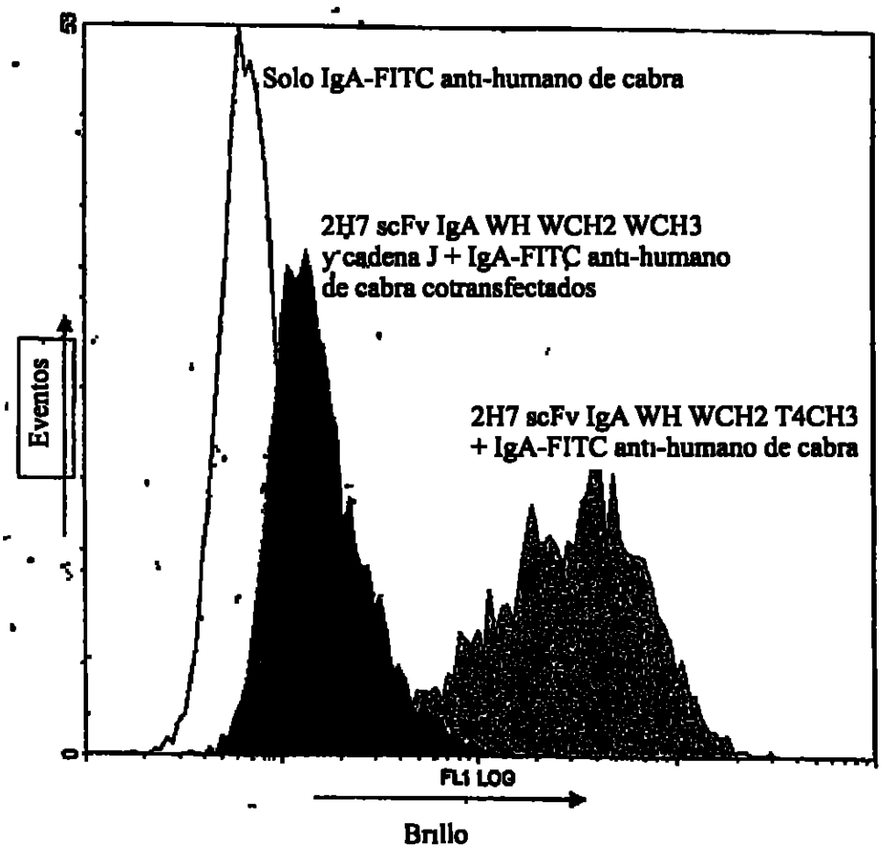


Fig 37

Titulación de Construcciones scFvIg específico CD20 para Actividad ADCC que Utiliza Efectores Sanguíneos Completos

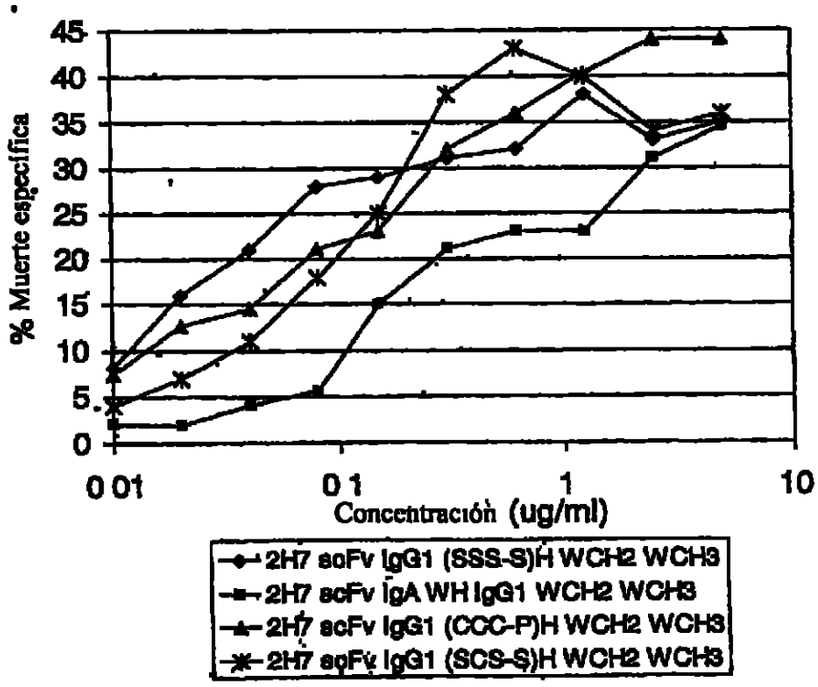


Fig. 38

Ensayo ADCC de construcciones anti-CD20 con colas alternativas
(Efectores Sanguíneos Completos/Objetivos BJAB

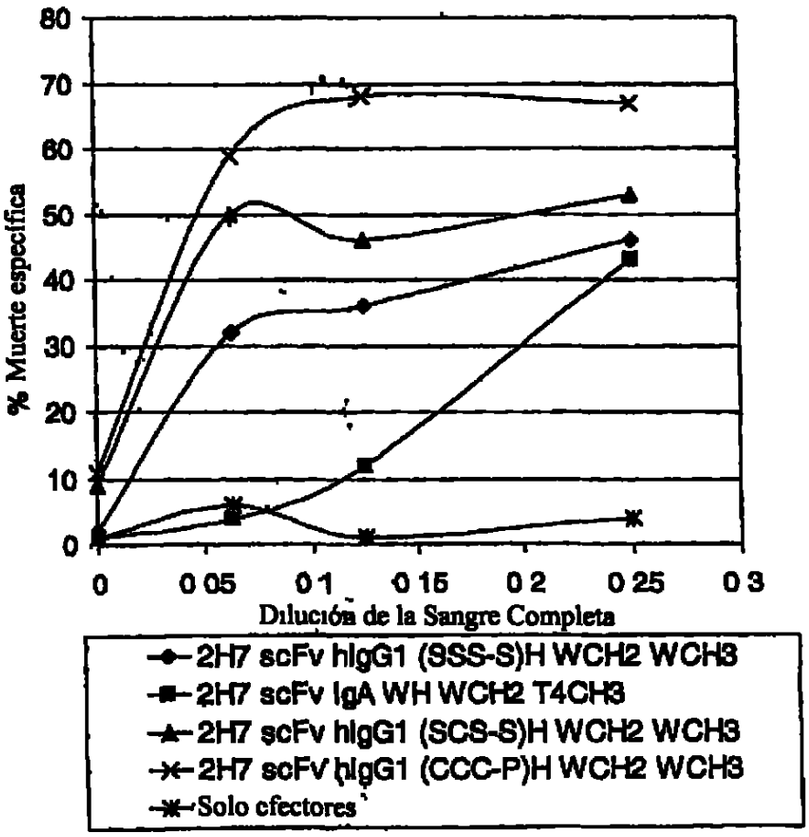
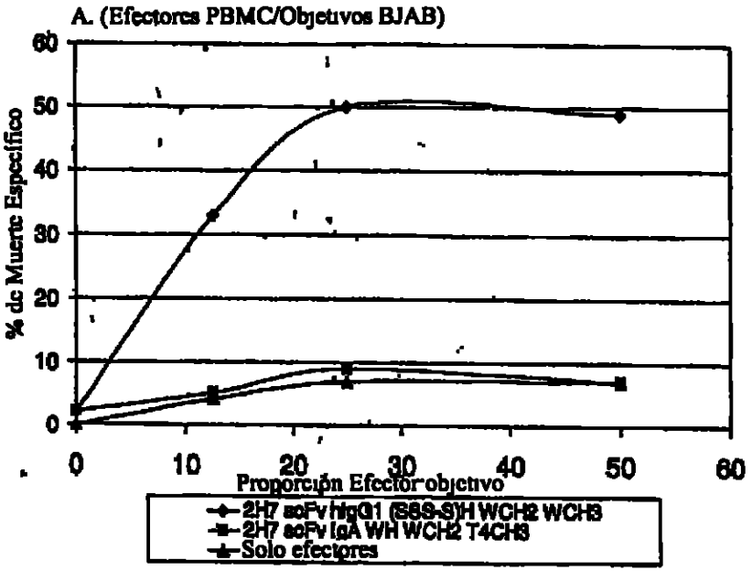


Fig. 39

Ensayo ADCC de Construcciones scFvIg Anti-CD20 que Utilizan Diferentes Poblaciones Efectoras Contra Objetivos BJAB



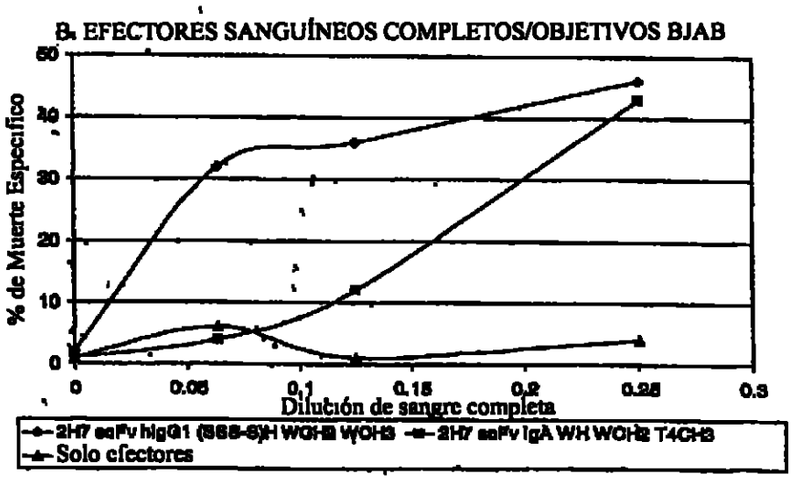


Fig 40

Inmunoblot de construcciones 2H7 scFv Ig de transfecciones COS (1 µl/pozo) comparado con una Concentracion Estandar

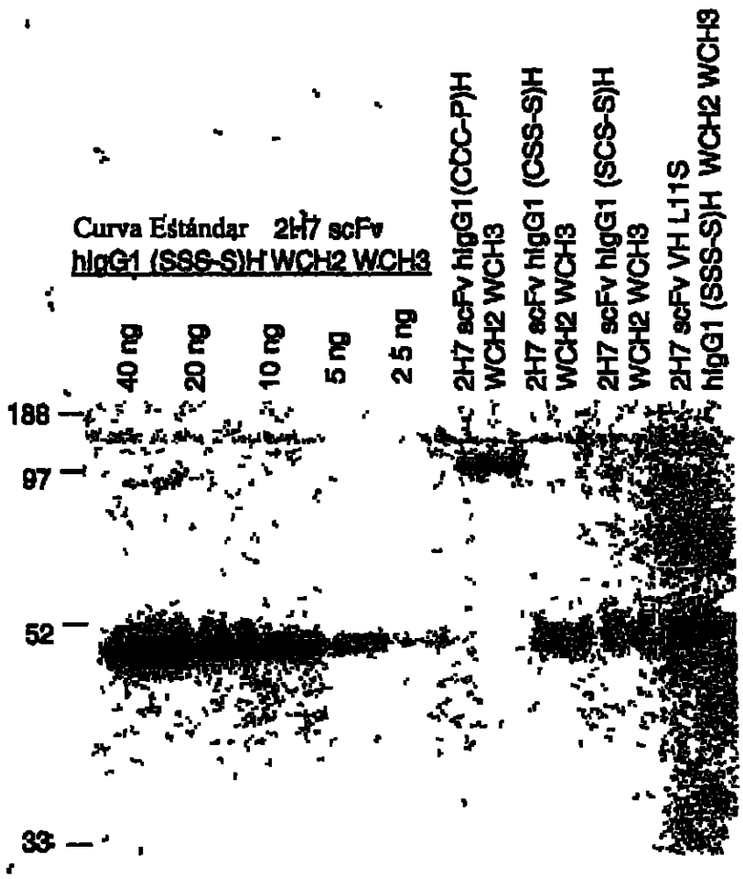


Fig 1

Secuência de aminoácido predicho y cADN 2H7 scFv-Ig

HindIII NcoI Péptido líder 2H7 V_L →

```
                  M  D  F  Q  V  Q  I  F  S  F  L  L  I  S  A  S
    1 AAGCTTGCCG CCATGGATTT TCAGGTGCAG ATTTTCAGCT TCCTGCTAAT CAGTGCTTCA

                                        2H7 V_L→
      V  I  I  A  R  G  Q  I  V  L  S  Q  S  P  A  I  L  S  A  S
   61 GTCATAATTG CCAGAGGACA AATTGTTCTC TCCCAGTCTC CAGCAATCCT GTCTGCATCT

      P  G  E  K  V  T  M  T  C  R  A  S  S  S  V  S  Y  M  H  W
  121 CCAGGGGAGA AGGTCACAAT GACTTGCAGG GCCAGCTCAA GTGTAAGTTA CATGCACTGG

                  BamHI
      Y  Q  Q  K  P  G  S  S  P  K  P  W  I  Y  A  P  S  N  L  A
  181 TACCAGCAGA AGCCAGGATC CTCCCCCAAA CCCTGGATTT ATGCCCCATC CAACCTGGCT

      S  G  V  P  A  R  F  S  G  S  G  S  G  T  S  Y  S  L  T  I
  241 TCTGGAGTCC CTGCTCGCTT CAGTGGCAGT GGGTCTGGGA CCTCTTACTC TCTCACAATC

      S  R  V  E  A  E  D  A  A  T  Y  Y  C  Q  Q  W  S  F  N  P
  301 AGCAGAGTGG AGGCTGAAGA TGCTGCCACT TATTACTGCC AGCAGTGGAG TTTTAACCCA

Ligador $(Gly_4Ser)_3$

      P  T  F  G  A  G  T  K  L  E  L  K  G  G  G  G  S  G  G  G
  361 CCCACGTTCG GTGCTGGGAC CAAGCTGGAG CTGAAAGGTG GCGGTGGCTC GGGCGGTGGT

                                                        2H7 V_H→
      G  S  G  G  G  G  S  S  Q  A  Y  L  Q  Q  S  G  A  E  L  V
  421 GGATCTCAAG GAGGTGGCAG CTCTCAGGCT TATCTACAGC AGTCTGGGGC TGAGCTGGTG

      R  P  G  A  S  V  K  M  S  C  K  A  S  G  Y  T  F  T  S  Y
  481 AGGCCTGGGG CCTCAGTGAA GATGTCCTGC AAGGCTTCTG GCTACACATT TACCAGTTAC

      N  M  H  W  V  K  Q  T  P  R  Q  G  L  E  W  I  G  A  I  Y
  541 AATATGCACT GGGTAAAGCA GACACCTAGA CAGGGCCTGG AATGGATTGG AGCTATTTAT

      P  G  N  G  D  T  S  Y  N  Q  K  F  K  G  K  A  T  L  T  V
  601 CCAGGAAATG GTGATACTTC CTACAATCAG AAGTTCAAGG GCAAGGCCAC ACTGACTGTA

      D  K  S  S  S  T  A  Y  M  Q  L  S  S  L  T  S  E  D  S  A
  661 GACAAATCCT CCAGCACAGC CTACATGCAG CTCAGCAGCC TGACATCTGA AGACTCTGCG

      V  Y  F  C  A  R  V  Y  Y  S  N  S  Y  W  Y  F  D  V  W
  721 GTCTATTTCT GTGCAAGAGT GGTGTACTAT AGTAACTCTT ACTGGTACTT CGATGTCTGG
```

637

Fig 1 (Continuación)

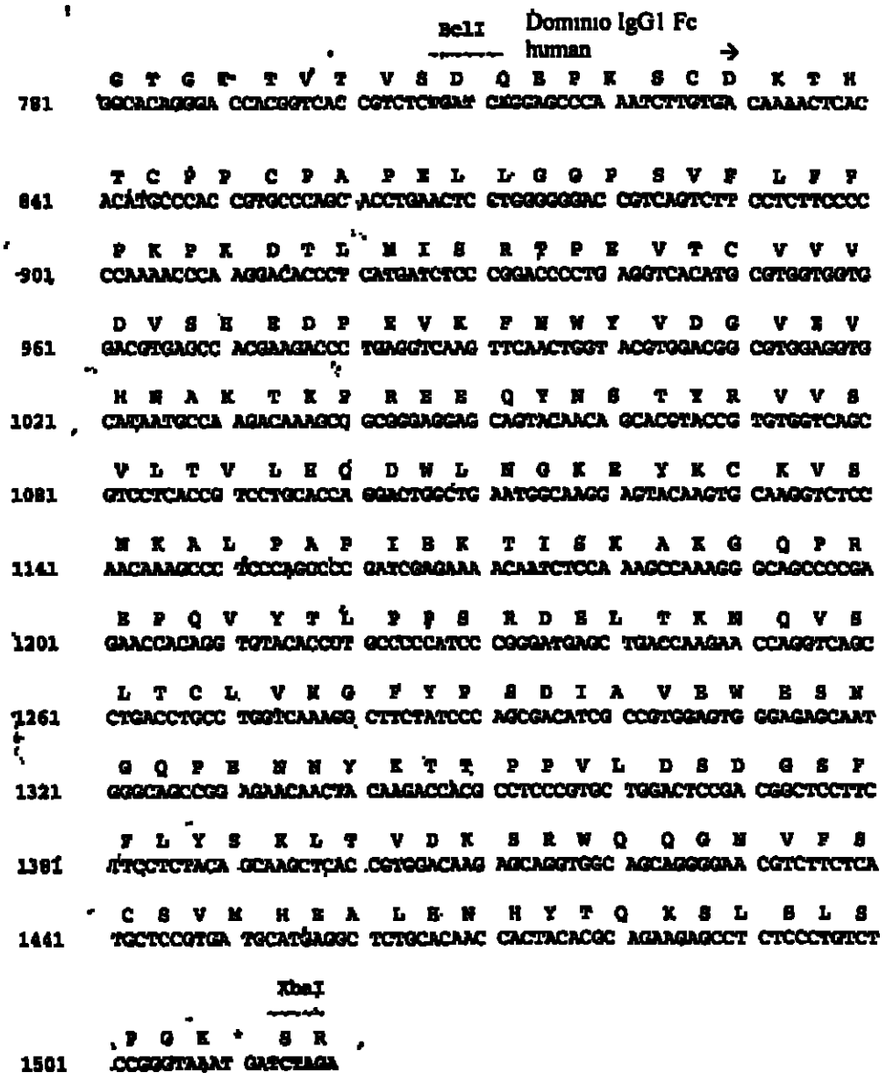
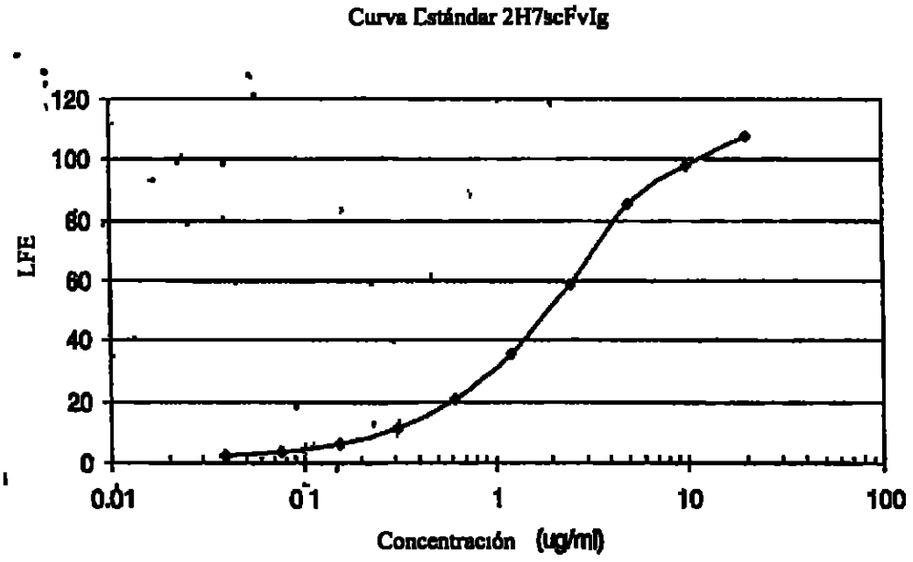


Fig 2

Niveles de Producción de 2nH7scFvIgG1 (SSS-S)H WCH2 WCH3 mediante Líneas CHO Estables



Clon	Concentración Estimada LFE @ 1 50 (µg/ml)
D2	26.166
IIIC8	25 755
IVA3	28 661
Masa Agotada	29.664

639

Fig 3

Análisis SDS-PAGE de Proteína 2H7 scFv IgG1 (SSS-S)H WCH2 WCH3

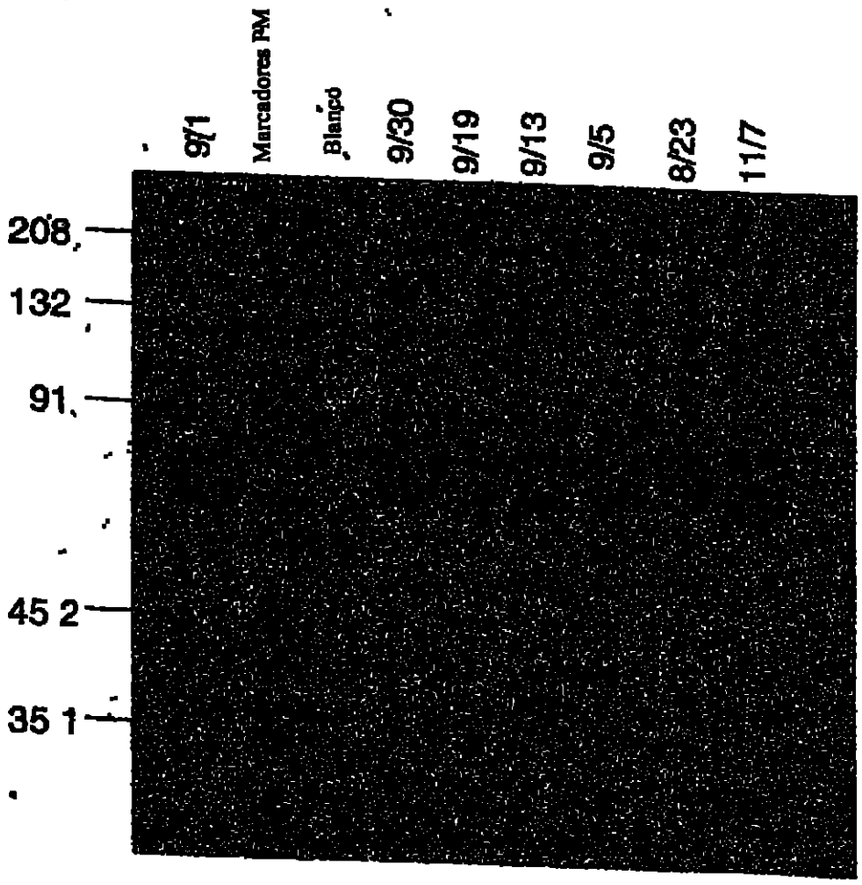


Fig 4A

Muerte de Células B Mediada por Complemento Después de Unión de CD20-objetivo 2H7 scFvIgG1 (SSS-S)H WCH2 WCH3

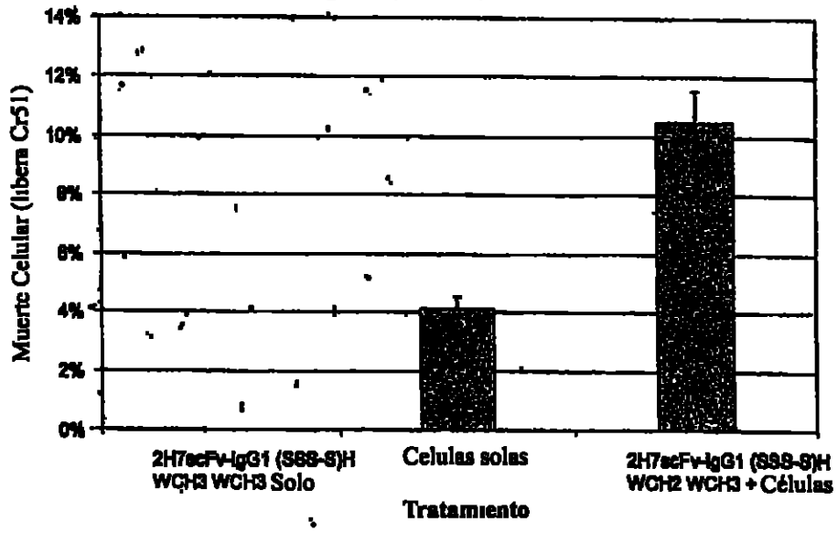
Concentración 2H7 scFv-Ig	RAMOS # células vivas/células totales	BJAB # células vivas/células totales
20 µg/ml + Complemento	0.16	0.07
5 µg/ml + Complemento	0.2	N.D
1.25 µg/ml + Complemento	0.32	0.1
Complemento solo	0.98	0.94

*La viabilidad se determinó mediante exclusión de azul trypan y se tabuló como la fracción de células viables del número total de células contadas

**N D (no determinado)

Fig 4B

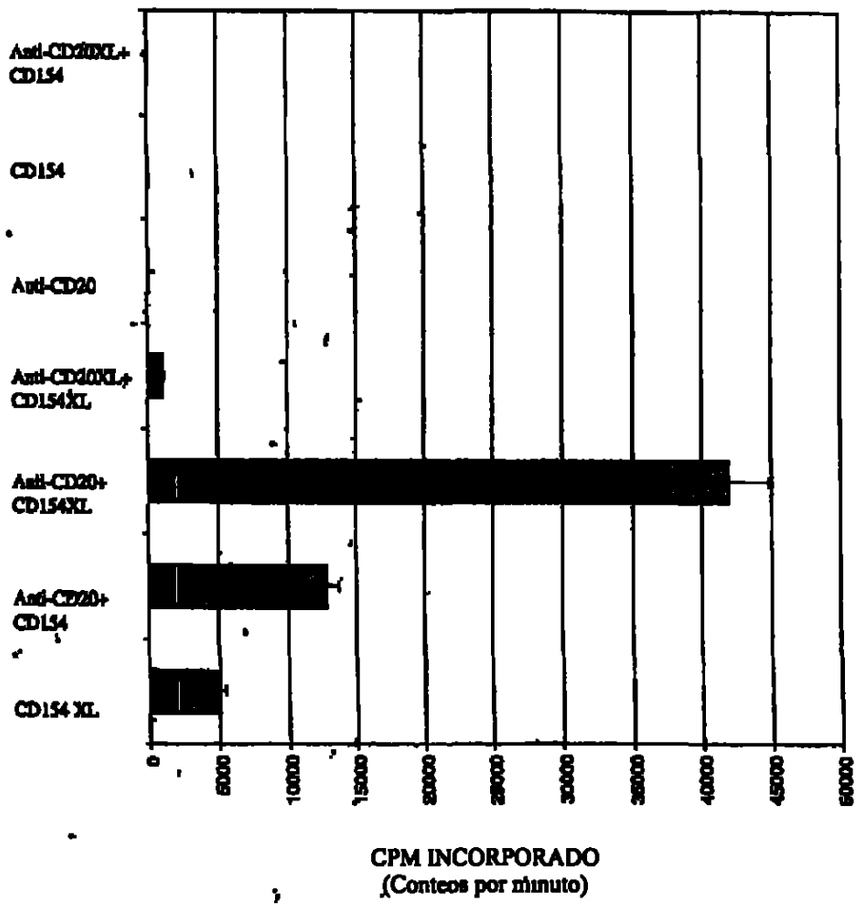
Citotoxicidad celular dependiente de anticuerpo (ADCC) mediada por 2H7 scFv-IgG1 (SSS-S)H WCH2 WCH3



641 69

Fig. 5

Efectos de Reticulación de Receptores de Superficie Celular CD20 y CD40 en la Proliferacion de Células B



CPM INCORPORADO
(Conteos por minuto)

Fig 6

Efecto de ligacion simultánea de CD20 y Cd40 en CD95 y apoptosis

Fig 6A.

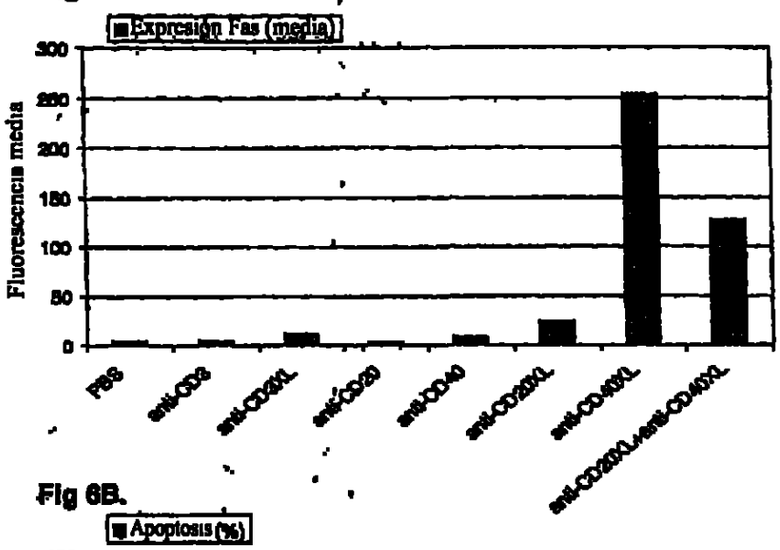


Fig 6B.

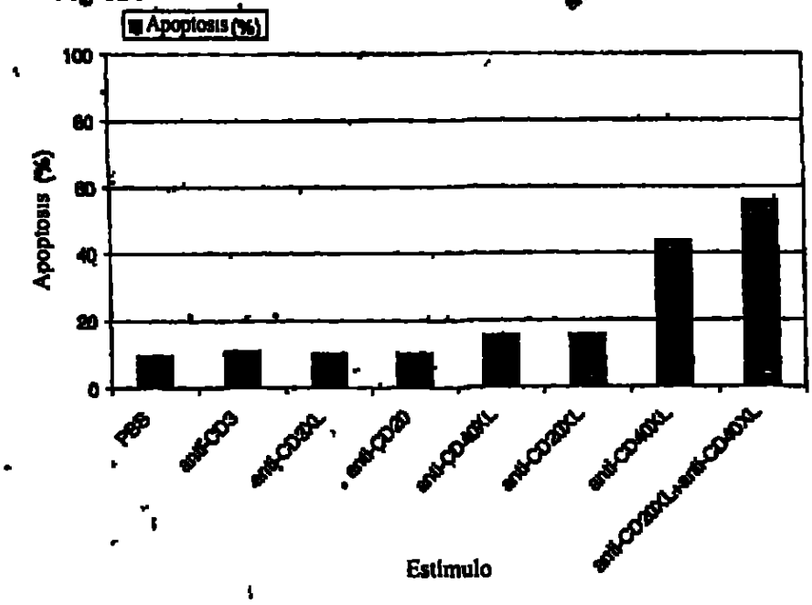


Fig. 7A

Secuencia de aminoácido predicha y cADN 2H7-CD154 L2

HindIII NcoI Péptido Líder 2H7 VL→

```
                                M  D  F  Q  V  Q  I  F  S  F  L  L  I  S  A  S
1   AAGCTTGCCG CC  ATGGATTT TCAAGTGCAG ATTTTCAGCT TCCTGCTAAT CAGTGCTTCA

                                        2H7 VL →
    V  L  T  Q  R  G  Q  I  V  L  S  Q  S  P  A  I  L  S  A  S
61  GTCTTAATTG CCAGAGGACA AATTGTTCTC TCCCAGTCTC CAGCAATCCT GTCTGCATCT

    P  G  E  K  V  T  M  T  C  R  A  S  S  S  V  S  Y  M  H  W
121 CCAGGGGAGA AGGTCACAAT GACTTGCAGG GCCAGCTCAA GTGTAAGTTA CATGCACTGG

                    BamHI
    Y  Q  Q  K  P  G  S  S  P  K  P  W  I  Y  A  P  S  N  L  A
181 TACCAGCAGA AGCCAGGATC CTCCCCCAAA CCCTGGATTT ATGCCCCAAC CAACCTGGCT

    S  G  V  P  A  R  F  S  G  S  G  S  G  T  S  Y  S  L  T  I
241 TCTGGAGTCC CTGCTCGCTT CAGTGGCAGT GGGTCTGGGA CCTCTTACTC TCTCACAATC

    S  R  V  E  A  E  D  A  A  T  Y  Y  C  Q  Q  W  S  F  N  P
301 AGCAGAGTGG AGGCTGAAGA TGCTGCCACT TATTACTGCC AGCAGTGGAG TTTTAACCCA

Ligador (Gly4Ser)3→
    P  T  F  G  A  G  T  K  L  E  L  K  G  G  G  G  S  G  G  G
361 CCCACGTTCG GTGCTGGGAC CAAGCTGGAG CTGAAAGGTG GCGGTGGCTC GGGCGGTGGT

                                                    2H7 VH →
    G  S  G  G  G  G  S  S  Q  A  Y  L  Q  Q  S  G  A  E  L  V
421 GGATCTGGAG GAGGTGGGAG CTCTCAGGCT TATCTACAGC AGTCTGGGGC TGAGCTGGTG

    R  P  G  A  S  V  K  M  S  C  K  A  S  G  Y  T  F  T  S  Y
481 AGGCCTGGGG CCTCAGTGAA GATGTCCTGC AAGGCTTCTG GCTACACATT TACCAGTTAC

    N  M  H  W  V  K  Q  T  P  R  Q  G  L  E  W  I  G  A  I  Y
541 AATATGCACT GGGTAAAGCA GACACCTAGA CAGGGCCTGG AATGGATTGG AGCTATTTAT

    P  G  N  G  D  T  S  Y  N  Q  K  F  K  G  K  A  T  L  T  V
601 CCAGGAAATG GTGATACTTC CTACAATCAG AAGTTCAAGG GCAAGGCCAC ACTGACTGTA

    D  K  S  S  S  T  A  Y  M  Q  L  S  S  L  T  S  E  D  S  A
661 GACAAATCCT CCAGCACAGC CTACATGCAG CTCAGCAGCC TGACATCTGA GGACTCTGCG

    V  Y  F  C  A  R  V  V  Y  Y  S  N  S  Y  W  Y  F  D  V  W
721 GTCTATTTCT GTGCAAGAGT GGTGTACTAT AGTAACTCTT ACTGGTACTT CGATGTCTGG
```

Fig 7A (Continuación)

CD154 humano/aminoácido 48

Sitio híbrido Bcl/Bam

```
      G  T  G  T  T  V  T  V  S  D  P  R  R  L  D  K  I  E  D  E
781   GGCACAGGGA CCACGGTCAC CGTCTCTGAT CCAAGAAGGT TGGACAAGAT AGAAGATGAA

      K  W  L  H  E  D  F  V  F  M  K  T  I  Q  R  C  N  T  G  E
841   AGGAATGTTC ATGAAGATTT TGTATTCATG AAAACGATAC AGAGATGCAA CACAGGAGAA

      R  S  L  S  L  L  M  C  E  E  I  K  S  Q  F  E  G  F  V  K
901   AGATCCTTAT CCTTACTGAA CTGTGAGGAG ATTAAAAGCC AGTTTGAAGG CTTTGTGAAG

                                                              BclI
      D  I  M  L  N  K  E  E  T  K  K  E  N  S  F  E  M  Q  K  G
961   GATATAATGT TAAACAAAGA GGAGACGAAG AAAGAAAACA GCTTTGAAAT GCAAAAAGGT

      BclI
      ----
      D  Q  N  P  Q  I  A  A  H  V  I  S  E  A  S  S  K  T  T  S
1021  GATCAGAATC CTCAAATTGC GGCACATGTC ATAAGTGAGG CCAGCAGTAA AACAACATCT

      V  L  Q  W  A  E  K  G  Y  Y  T  M  S  N  N  L  V  T  L  E
1081  GTGTTACAGT GGGCTGAAAA AGGATACTAC ACCATGAGCA ACAACTTGGT AACCCTGGAA

      N  G  K  Q  L  T  V  K  R  Q  G  L  Y  Y  I  Y  A  Q  V  T
1141  AATGGGAAAC AGCTGACCGT TAAAAGACAA GGACTCTATT ATATCTATGC CCAAGTCACC

                  HindIII
                  -------
      F  C  S  N  R  E  A  S  S  Q  A  P  F  I  A  S  L  C  L  K
1201  TTCTGTTCCA ATCGGGAAGC TCCGAGTCAA GCTCCATTTA TAGCCAGCCT CTGCCTAAAG

      S  P  Q  R  F  E  R  I  L  L  R  A  A  N  T  H  S  S  A  K
1261  TCCCCCAGTA GATTCGAGAG AATCTTACTC AGAGCTGCAA ATACCCACAG TTCCGCCAAA

      P  C  G  Q  Q  S  I  H  L  G  G  V  F  E  L  Q  P  G  A  S
1321  CCTTGCGGGC AACAATCCAT TCACTTGGGA GGAGTATTTG AATTGCAACC AGGTGCTTCG

                                                  NcoI
                                                  ------
      V  F  V  N  V  T  D  P  S  Q  V  S  H  G  T  G  F  T  S  F
1381  GTGTTTGTCA ATGTGACTGA TCCAAGCCAA GTGAGCCATG GCACTGGCTT CACGTCCTTT

                  XhoI          XbaI
                  ------        ----
      G  L  L  K  L  E  *  *  S  R
1441  GGCTTACTCA AACTCGAGTG ATAATCTAGA
```

645

Fig 7B.

Secuencia de aminoacido predicha y cADN 2H7-scFv-CD154 S4

HindIII NcoI Péptido Líder
2H7 VL →

```
                  M  D  V  Q  V  Q  I  F  S  F  L  L  I  S  A  S
1   AAGCTTGCCG CC  ATGGATTT TCAAGTGCAG ATTTTCAGCT TCCTGCTAAT CAGTGCTTCA

                                        2H7 VL →
    V  I  L  A  R  G  Q  I  V  L  S  Q  S  P  A  I  L  S  A  S
61  GTCATAATTG CCAGAGGACA AATTGTTCTC TCCCAGTCTC CAGCAATCCT GTCTGCATCT

    P  G  E  K  V  T  M  T  C  R  A  S  S  S  V  S  Y  M  H  W
121 CCAGGGGAGA AGGTCACAAT GACTTGCAGG GCCAGCTCAA GTGTAAGTTA CATGCACTGG

                    BamHI
                    ------
    Y  Q  Q  K  P  G  S  S  P  K  P  W  I  Y  A  P  S  N  L  A
181 TACCAGCAGA AGCCAGGATC CTCCCCCAAA CCCTGGATTT ATGCCCCATC CAACCTGGCT

    S  G  V  P  A  R  F  S  G  S  G  S  G  T  S  Y  S  L  T  I
241 TCTGGAGTCC CTGCTCGCTT CAGTGGCAGT GGGTCTGGGA CCTCTTACTC TCTCACAATC

    S  R  V  E  A  E  D  A  A  T  Y  Y  C  Q  Q  W  S  F  N  P
301 AGCAGAGTGG AGGCTGAAGA TGCTGCCACT TATTACTGCC AGCAGTGGAG TTTTAACCCA

Ligador (Gly4Ser)3 →
    P  T  F  G  A  G  T  K  L  E  L  K  G  G  G  G  S  G  G  G
361 CCCACGTTCG GTGCTGGGAC CAAGCTGGAG CTGAAAGGTG GCGGTGGCTC GGGCGGTGGT

                                                    2H7 VH →
    G  S  Q  G  G  G  S  E  Q  A  Y  L  Q  Q  S  G  A  E  L  V
421 GGATCTGGAG GAGGTGGGAG CTCTCAGGCT TATCTACAGC AGTCTGGGGC TGAGCTGGTG

    R  P  G  A  S  V  K  M  S  C  K  A  S  G  Y  T  F  T  S  Y
481 AGGCCTGGGG CCTCAGTGAA GATGTCCTGC AAGGCTTCTG GCTACACATT TACCAGTTAC

    N  M  H  W  V  K  Q  T  P  R  Q  G  L  E  W  I  G  A  I  Y
541 AATATGCACT GGGTAAAGCA GACACCTAGA CAGGGCCTGG AATGGATTGG AGCTATTTAT

    P  G  N  G  D  T  S  Y  N  Q  K  F  K  G  K  A  T  L  T  V
601 CCAGGAAATG GTGATACTTC CTACAATCAG AAGTTCAAGG GCAAGGCCAC ACTGACTGTA

    D  K  S  S  S  T  A  Y  M  Q  L  S  S  L  T  S  E  D  S  A
661 GACAAATCCT CCAGCACAGC CTACATGCAG CTCAGCAGCC TGACATCTGA AGACTCTGCG

    V  Y  F  C  A  R  V  V  Y  Y  S  N  S  Y  W  Y  F  D  V  W
721 GTCTATTTCT GTGCAAGAGT GGTTTACTAT AGTAACTCTT ACTGGTACTT CGATGTCTGG
```

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Fig 7B

CD154 humano/aminoácido 108

Sitio híbrido BclI/BamHI

BclI
G T G T T V T V S D P E N S F E M Q K G
781 GGCACAGGGA CCACGGTCAC CGTCTCTGAA GAAGATAACA GCTTTGAAAT GCAAAAAGGT

BclI
D Q N P Q I A A H V I S E A S S K T T S
841 GATCAGAATC CTCAAATTGC GGCACATGTC ATAAGTGAGG CCAGCAGTAA AACAACATCT

V L Q W A E K G Y Y T M S N N L V T L E
901 GTGTTACAGT GGGCTGAAAA AGGATACTAC ACCATGAGCA ACAACTTGGT AACCCTGGAA

N G K Q L T V K R Q G L Y Y I Y A Q V T
961 AATGGGAAAC AGCTGACCGT TAAAAGACAA GGACTCTATT ATATCTATGC CCAAGTCACC

HindIII
F C S N R E A S S Q A P F I A S L C L K
1021 TTCTGTTCCA ATCGGGAAGC TTCGAGTCAA GCTCCATTTA TAGCCAGCCT CTGCCTAAAG

S P G R F E R I L L R A A N T H S S A K
1081 TCCCCCGGTA GATTCGAGAG AATCTTACTC AGAGCTGCAA ATACCCACAG TTCCGCCAAA

P C G Q Q S I H L G G V F E L Q P G A S
1141 GCTTGCGGGC AACAATCCAT TCACTTGGGA GGAGTATTTG AATTGCAACC AGGTGCTTCG

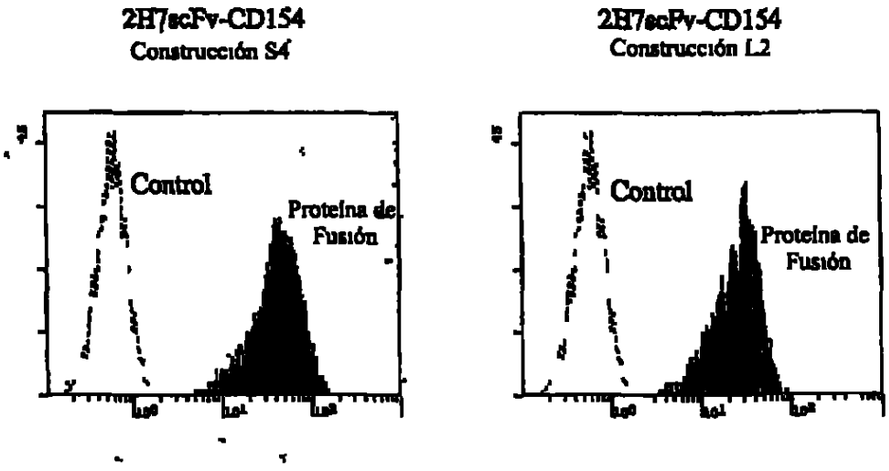
EcoI
V F V N V T D P S Q V S H G T G F T S F
1201 GTGTTTGTCA ATGTGACTGA TCCAAGCCAA GTGAGCCATG GCACTGGCTT CACGTCCTTT

XhoI XbaI
G L L K L E * * S R
1261 GGCTTACTCA AACTCGAGTG ATAATCTAGA

647

Fig 8

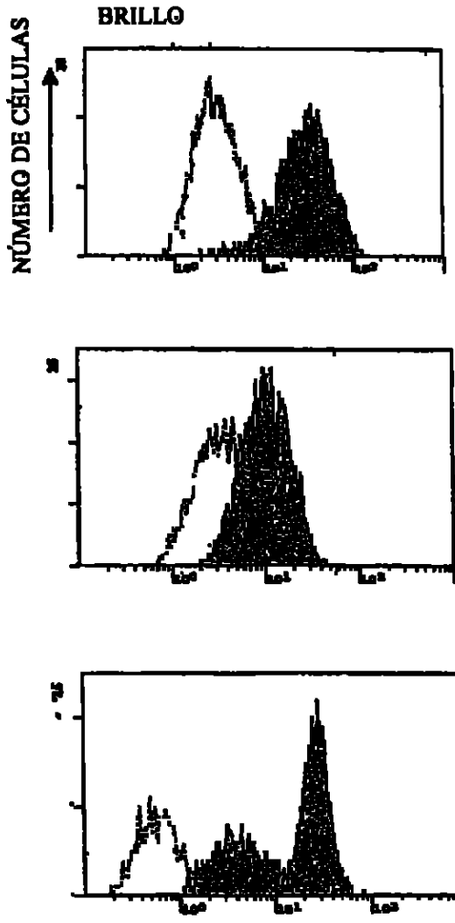
Unión Simultanea de Proteínas de Fusión 2H7-scFv-CD154 a CD20 y CD40



Objetivos de célula CD20 CHO + (proteína de fusión o control) + Biotin-CD40Ig + PE-SA

Fig. 9

Inducción de Apoptosis Medida por la Unión de Anexina V después de Incubación con 2H7-scFv-CD154

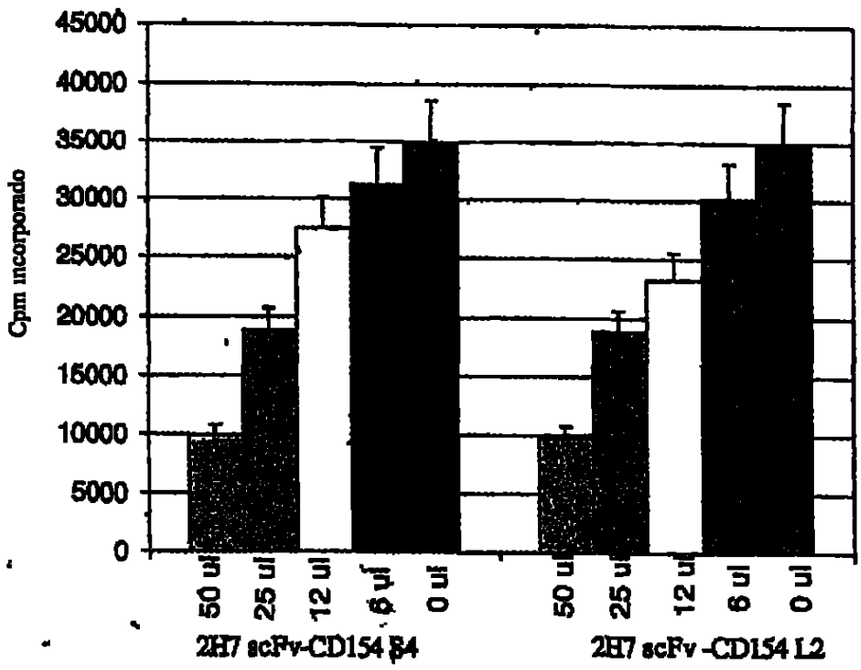


Supernadante de control ____ Supernadante 2H7-scFv-CD154

649

Fig. 10

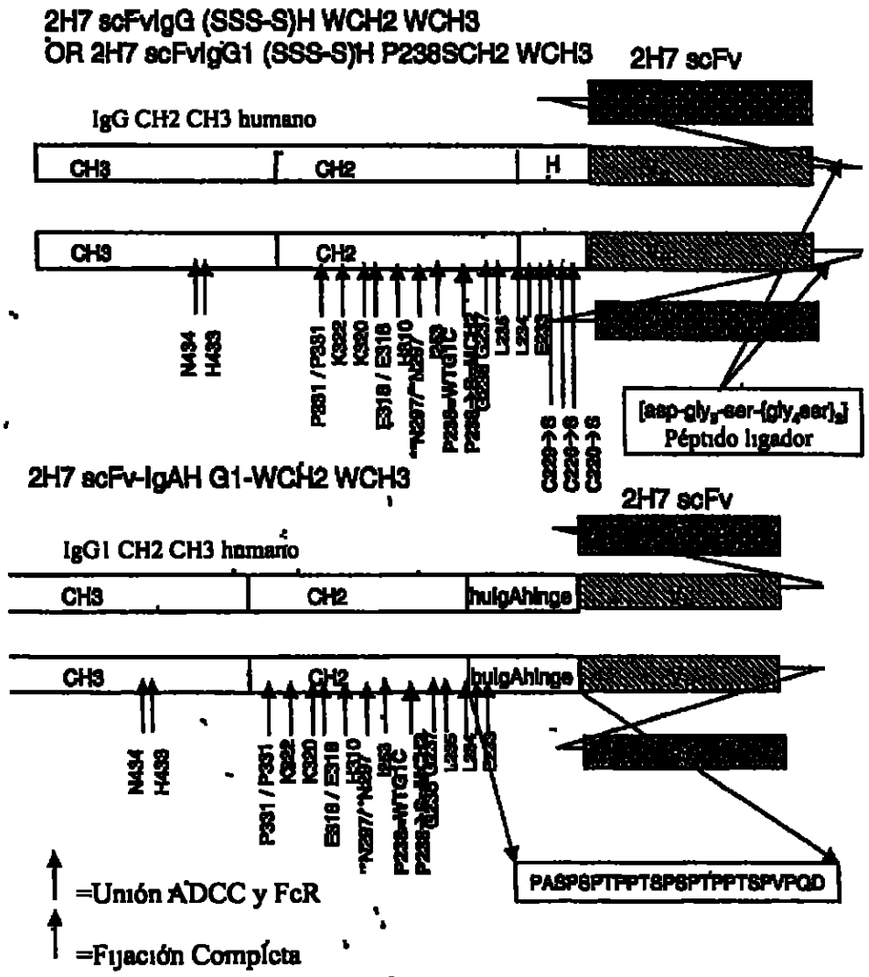
Proliferación de Línea de Celulas B T51 después de Incubación con las Construcciones 2H7-scFv-CD154 S4 o 2H7-scFv-CD154 L2



650

Fig 11

Representación Esquemática de Construcciones 2H7 scFvIg



651

Fig. 12

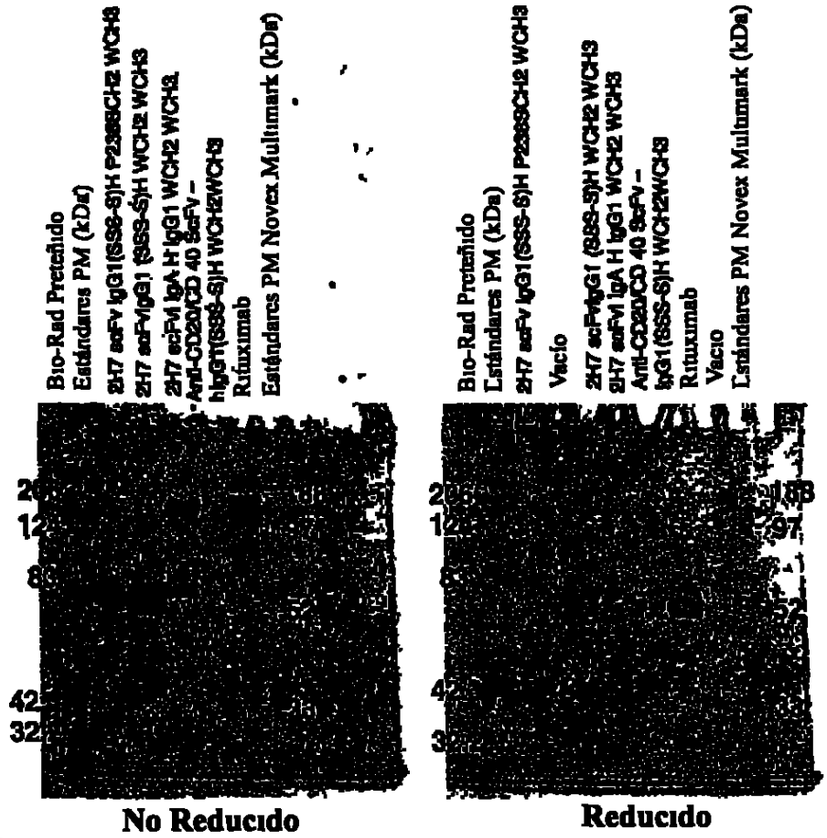


Figura 12 Análisis SDS-PAGE de Derivados CytoxB Los derivados de proteína de fusión purificada de las moléculas CytoxB-scFvIg y Rituximab se resuspendieron en amortiguador de muestra SDS, hervidos, cargados en 10% de gel Novex Tris-Bis (Invitrogen, San Diego, CA) y se sometieron a electroforesis SDS-PAGE de reducción (panel derecho) o sin reducción (panel izquierdo) a 175 voltios Dos marcadores de diferente peso molecular, marcadores pretefiidos BioRad, y marcadores de peso molecular Novex Multimark también se cargaron en cada gel y el tamaño aproximado en kDa de cada banda de marcador se indica junto con cada lado de los geles fotografiados Los geles se tiñeron con tinte Azul Coomassie y se fotografiaron con cámara,Digital SONY Mavica Las formas de pivote mutante de 2H7 scFv IgG1 migran en aproximadamente 70 kDa bajo ambas condiciones de reducción y no reducción, que indican que estas moléculas son monoméricas a diferencia de las diméricas en estructura La forma de pivote IgA del 2H7 scFv Ig migra en aproximadamente 75 kDa bajo condiciones de reducción, pero migra predominantemente como un dímero de 140 kDa con una fracción de la proteína que migra en 75 kDa bajo condiciones de no reducción Bajo condiciones de no reducción, el Rituximab migra como una banda difusa de entre 150 y 200 kDa Las cadenas liviana y pesada se resuelven en bandas separadas de aproximadamente 32 y 50 kDa cuando el Rituximab se reduce y se somete a SDS-PAGE

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Fig. 13

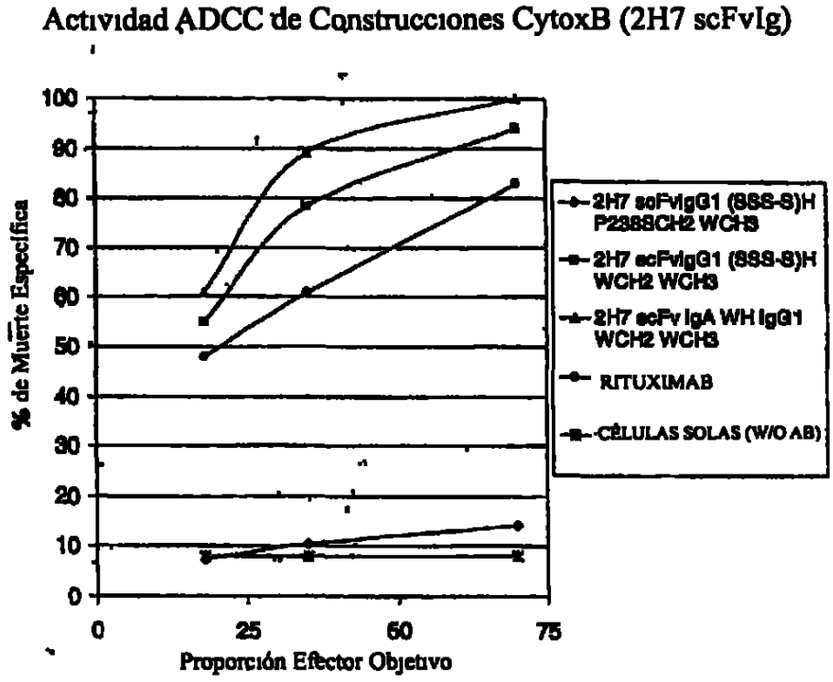


Figura 13 Actividad ADCC de los Derivados CytoxB Comparados con Rituximab La actividad ADCC de los Derivados CytoxB o Rituximab se miden in vitro contra la línea de célula de linfoma B BJAB como objetivo y utiliza PBMC humano fresco como células efectoras El efector para los índices objetivo se varían como sigue 70 1, 35 1 y 18 1, con el número de células BJAB por pozo remanente constante pero que varía el número de PBMC Las células BJAB se etiquetan durante 2 horas con 51Cr y se alicuotan en una densidad celular de 5x104 celulas/pozo para cada pozo de placas de 96 pozos de fondo plano Las proteínas de fusión purificas o Rituximab se agregan en una concentración de 10 mg/ml y el PBMC se agrega en 9x105 células/pozo (18 1), 1 8x106 células/pozo (35 1), o 3 6x106 células/pozo (70 1), en un volumen final de 200 1 La liberación espontánea se midió sin adición de PBMC o proteína de fusión, y la liberación máxima se midió mediante la adición de detergente (1% NP-40) a los pozos apropiados Las reacciones se incubaron durante 4 horas, y 100 ml del supernadante de cultivo cosechado a un Lumaplate (Packard Instruments) y se le permitió secar durante la noche antes de continuar la liberación cpm en un Contador de Centelleo de Microplaca Packard Top Count NXT

653

Fig 14

CDC de Construcciones CytoxB (2H7 scFvIg)

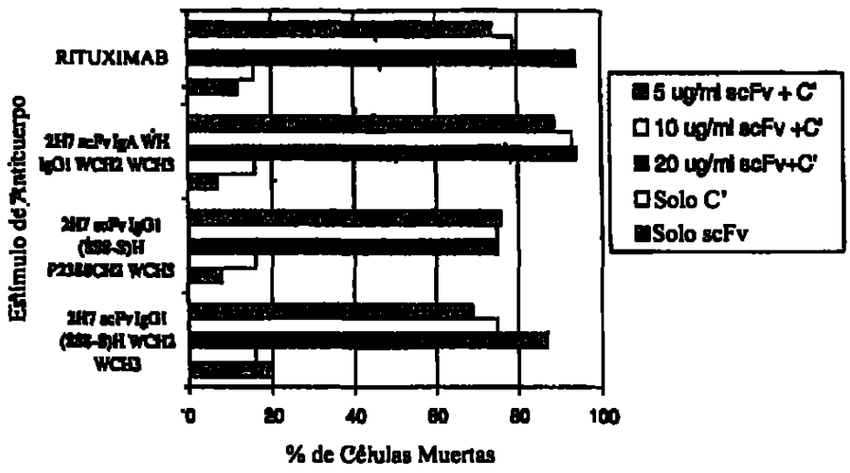
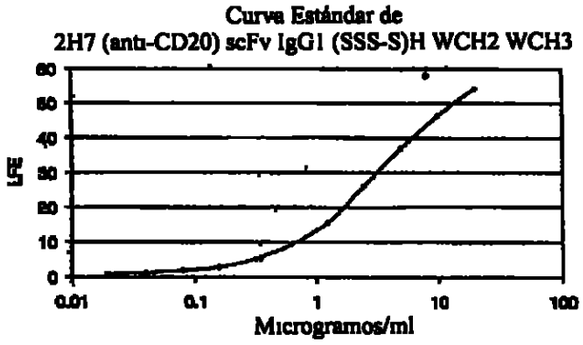


Figura 14 Actividad de Citotoxicidad Dependiente de Complemento (CDC) de los Derivados CytoxB Comparados con Rituximab Los derivados de 2H7 scFvIgG1 (SSS-S)H WCH2 WCH3 2H7 scFvIgG1 (SSS-S)H WCH2 WCH3, y 2H7 scFv IgA WH IgG1 WCH2 WCH3 y Rituximab se compararon con su capacidad para mediar la citotoxicidad dependiente de complemento El complemento de conejo (Pel-Freez) se diluyó 1 10 y se agregó a células BJAB junto con las diluciones de cada derivado de anticuerpo (20 g/ml, 10 g/ml, y 5 g/ml) Los controles también se incluyen sin la adición del complemento (C') o derivado scFv A las reacciones se les permitió continuar durante 1 hora, y las células de cada pozo se tiñeron luego con trypan azul y la viabilidad de las células utilizando un hemacitómetro Los datos se grafican como % de celulas muertas/células totales contadas para cada condición ensayada

654

Fig 15

Vida Media In Vivo de 2H7 (anti-CD20) scFv IgG1 (SSS-S)H WCH2 WCH3



Macaco A99314

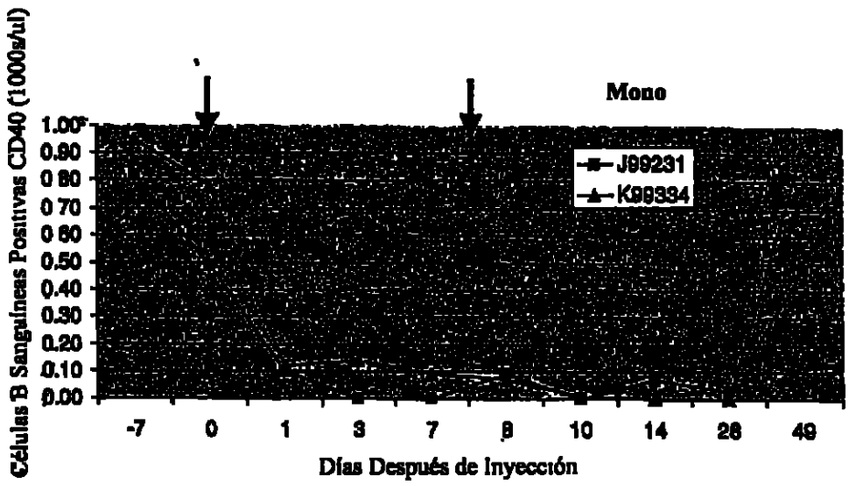
	Día	Intensidad de Unión (LFE) @ 1 50 dilución de suero	Concentración Estimada (g/ml)
Inyección #1 →	-7	0 219	<0 1
	0	0.227	<0 1
	1	7 79	25 1
	3	5 51	15 6
Inyección #2 →	7	3 37	9.4
	8	11 33	41 7
	10	5.45	15.4
	14	0 27	<0 1

Macaco F98081

	Día	Intensidad de Unión (LFE) @ 1 50 dilución de suero	Concentración Estimada (g/ml)
	-7	0 208	<0 1
Inyección #1 →	0	0 219	<0 1
	1	6 73	21 9
	3	6 14	19 3
Inyección #2 →	7	3.04	8 7
	8	9.83	33 8
	10	4 77	14.4
	14	0.231	<0 1

Fig. 16

Agotamiento de Célula B en Macacos Mediada por Construccion Cytox B20 (2H7 scFv IgG1 (SSS-S)H WCH2 WCH3)

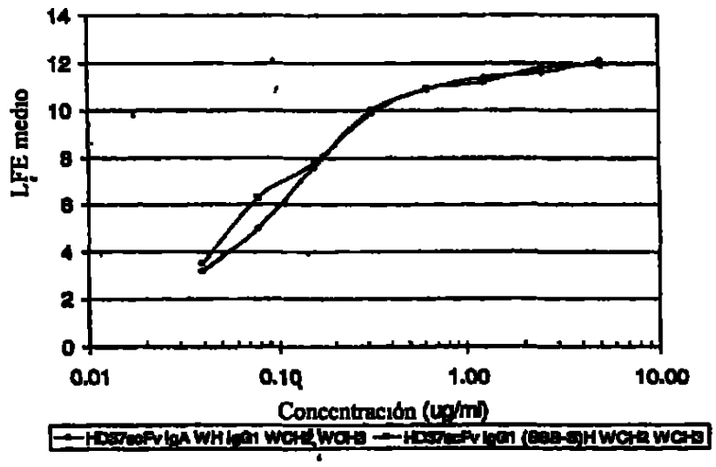


- Inyecciones de CytoxB20 de 6 mg/kg produce 3 semanas de agotamiento de célula B
- 3-4 días de vida media in vivo
- Saturación CD20 en células B de nodo de plasma en d14
- Sin efectos de primera dosis
- Sin desarrollo de anticuerpo anti-quimérico

Fig 17

Niveles de Producción de Construcciones HD37 scFvIg mediante Líneas de Células CHO

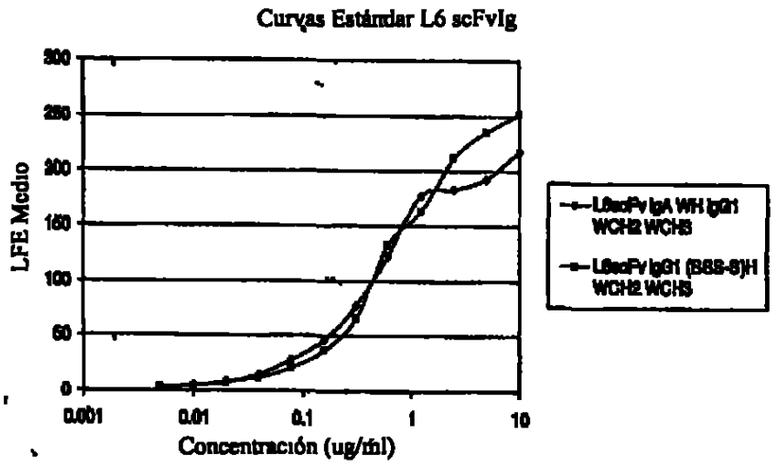
Curva Estándar de la Unión de Construcción HD37 scFvIg a Células B



Clon/Aislado	LFE Medio en 1 100	Concentración Estimada
HD37 scFv Masa		
IgA WH IgG1 WCH2 WCH3	11 2	> 60 ug/ml
1B2	10 4	>50 ug/ml
6C5	10 5	>50 ug/ml
4B1	8 6	>40 ug/ml
Hd37 scFv Masa		
IgG1 (SSS-S)H WCH2 WCH3	10 9	> 50 ug/ml
2G8	10 6	> 50 ug/ml
3F3	8 3	>40 ug/ml
3D9	11 1	> 60 ug/ml

Fig 18

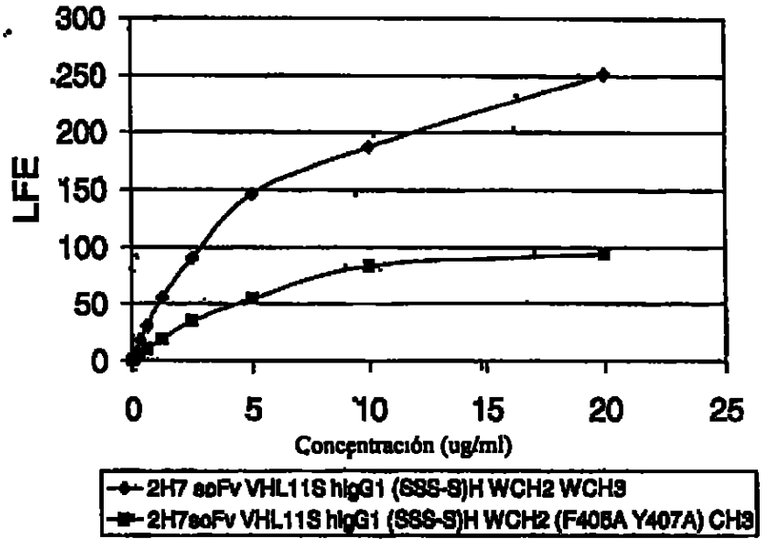
Producción de Construcciones L6 scFvIg mediante Celulas CHO



Construcción	LFE Medio 1 20	Concentración Estimada
L6scFv IgA WH IgG1 WCH2 WCH3 CHO no amplificado sup	51 1	6.25 ug/ml
L6scFv IgG1(SSS-S)H WCH2 WCH3 CHO no amplificado sup	23 0	3.2ug/ml

Fig. 63

Unión de Proteínas Purificadas de Supernadantes COS
a células CD20 CHO
Efecto Diferencial de Mutaciones CH3 en la Unión



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Fig. 64

Union de Proteinas 2H7 scFv VHL11S hIg FITC conjugadas a Células CD20 CHO

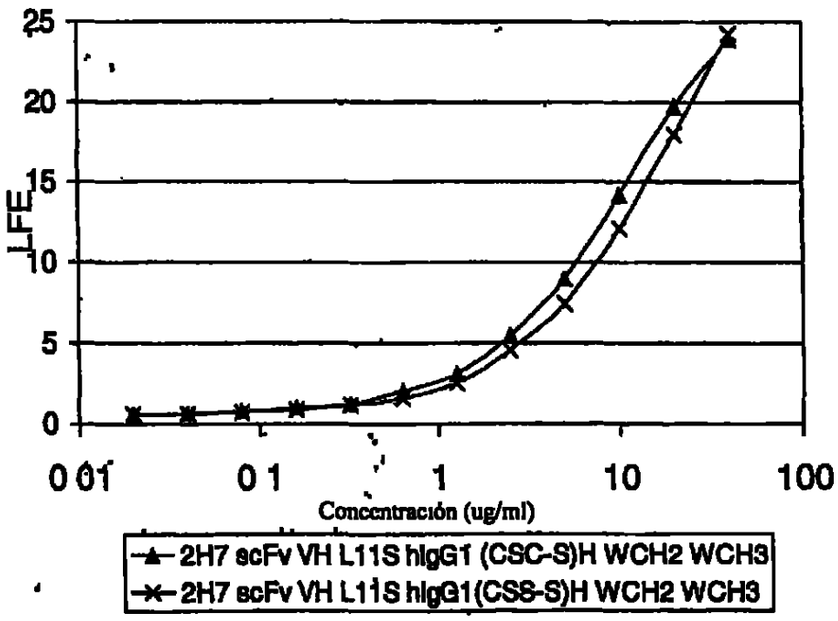
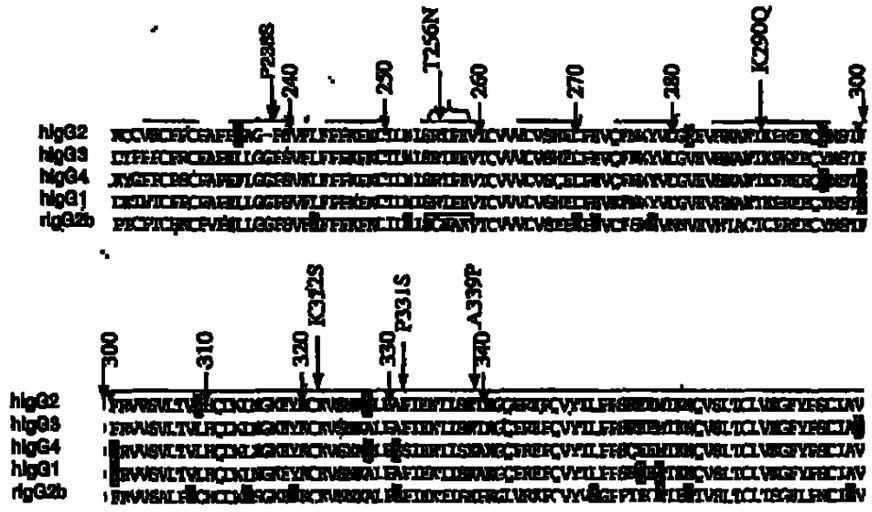


Fig. 66

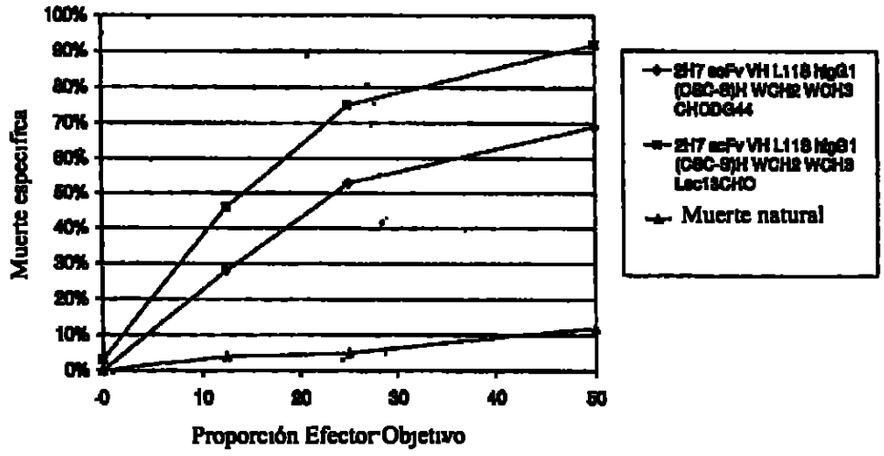
Alteraciones en la secuencia IgG Fc humana que cambia diferencialmente la eficiencia de la función efectora



■Residuo CDC ■ ADCC

Figure 67.

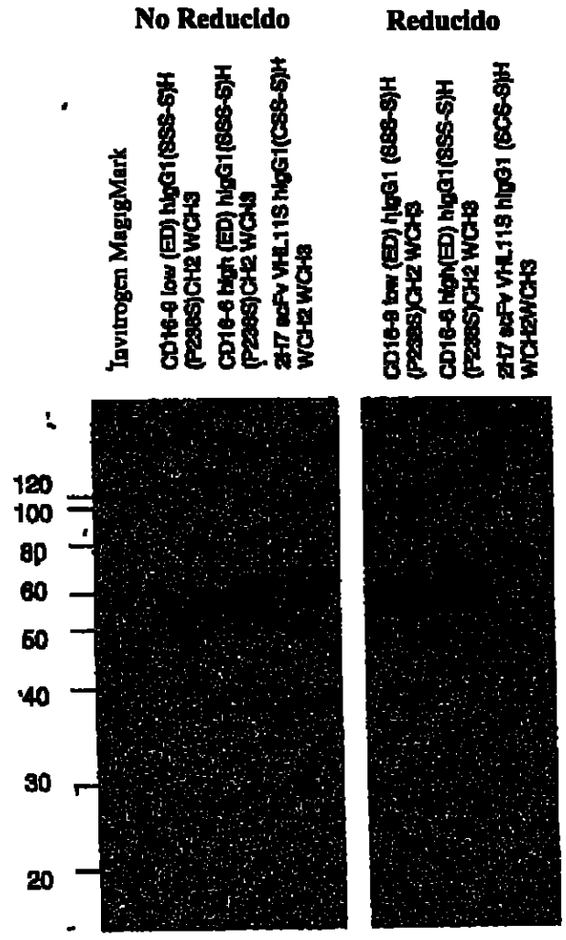
Actividad ADCC de 2H7 scFv VHL11S hIgG1 (CSC-S)H WCH2 WCH3 de transfecciones transitorias CHO y Lec13-CHO



663

Fig. 68

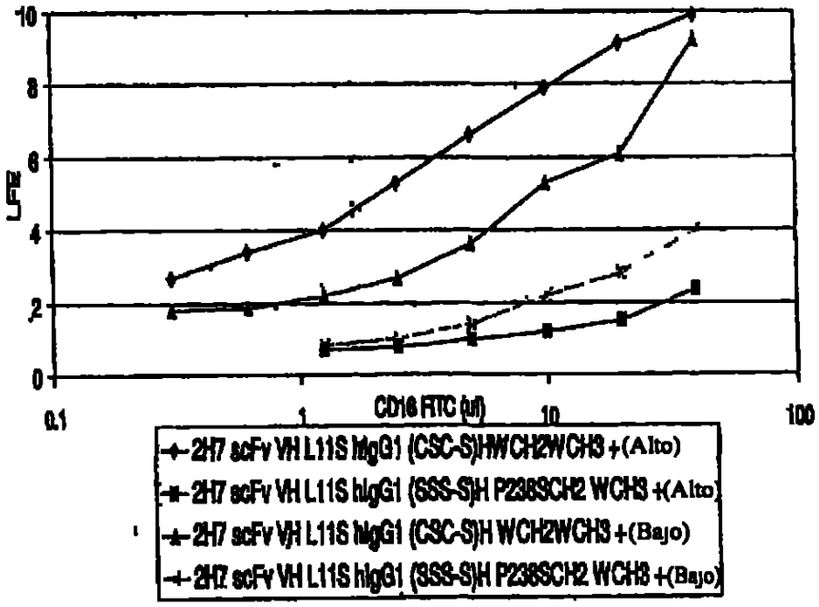
Alelos de alta y baja afinidad CD16 (ED) hIgG1 (SSS-S) H P238S CH2 WCH3 expresado como moléculas solubles



69

Fig. 69

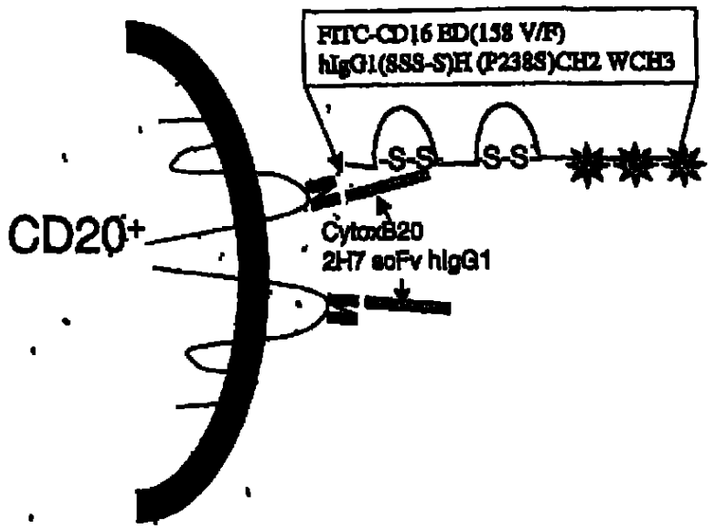
Unión de proteínas de fusion de alta y baja afinidad CD16 FITC a 2H7 scFv VHL11S hIGg1 (CSC-S)H WCH2WCH3 o (SSS-S)H(P238S)CH2WCH3 en Objetivos CD20CHO



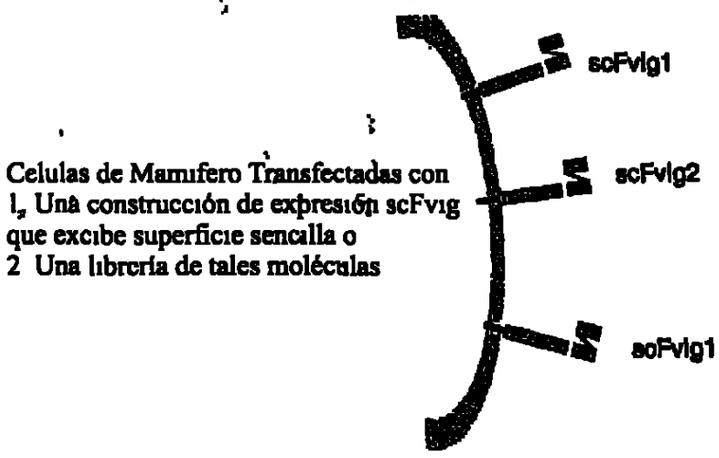
665

Fig. 70

Unión de FITC Etiquetado, dominio extracelular CD16(ED) Recombinante Humano-Proteina Ig de Fusion a Derivados CitoxB sobre Celulas CD20 CHO



Expresión de SMIP que exhibe superficie que enlaza el cADN modificado con proteinas de fusión alteradas



Celulas de Mamifero Transfectadas con
1, Una construcción de expresión scFvIg que excibe superficie sencilla o
2 Una libreria de tales moléculas

Fig. 71

CD37 mAbs y scFvIg que Inducen a Apoptosis

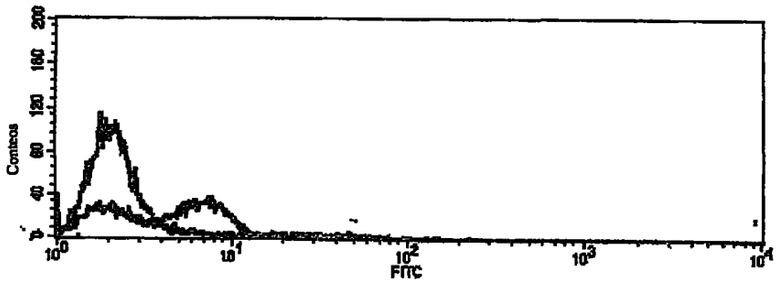
	Tejido Bjab	Anexina V positiva	
	No scFvIg	17 5	
	2H7 MH	27	
scFvIg	G28-1 MH	30.6	
	G28-1 IgAH	28.9	
	HD37 MH	29.1	
	(2H7+G28-1)MH	41	
	(2H7+HD37) MH	37 1	
	(G28-1+HD37) MH	35.3	
			Más GAM
	Ramos	Anexina V positiva	Anexina V positiva
	Células solas	3	3.3
	2H7 Mab	1 4	3.1
	G28-1 Mab	18.3	8.7
mAbs	HD37 Mab	3.7	3.1
	G28-5	3.9	8.3
	2H7+G28-1	32.3	35 7
	2H7+HD37	5	10.5
	2H7+G28-5	5.7	19.4
	HD37+G28-1	26.9	50
	HD37+G28-5	8.2	18.4
	G28-1+G28-5	39.5	68.3

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Actividad de la Caspasa 3 en Células Ramos después de Incubación de 4 horas con CitoxB20G SMIP



Medio CD20 SMIP

Fig 72

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Citoxicidad Dependiente de Complemento Mediada por Derivados CitoxB20G

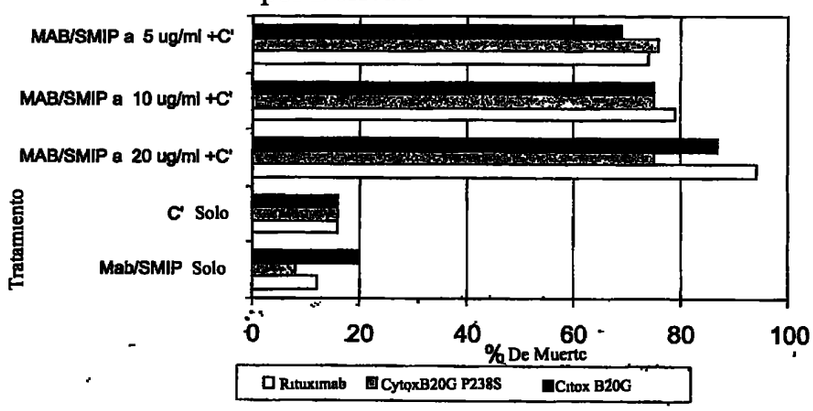


Fig 73

Figura 73 Actividad de CDC de CitoxB20G SMIPS El CitoxB20G, CitoxB20GP238, o Rituximab se incuban en concentraciones que se incrementan con 104 Células Objetivo Bjab y una dilución 1 10 de complemento de conejo (PelFreez) en un volumen de 100 microlitros durante sesenta minutos Las alicuotas se tiñen con trypan azul (Invitrogen), y se cuenta utilizando un hemacitometro para determinar el porcentaje de población celular muerta durante el tratamiento Los controles negativos con las células y solo un reactivo también se incluyen

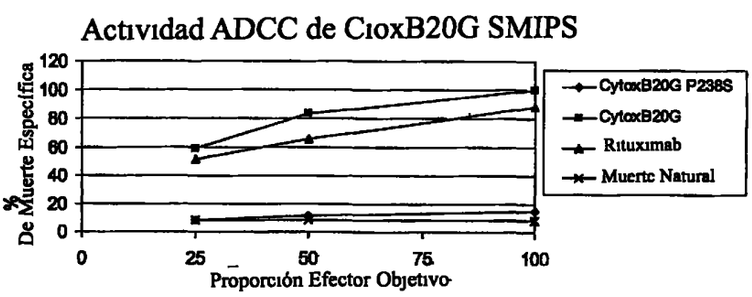


Fig 74

Figura 74 Actividad de ADCC de CitoxB20G SMIPS La actividad de ADCC de CitoxB20G SMIPS o Rituximab se mide in vitro contra líneas celulares de linfoma BJAB como objetivo y utiliza PBMC humano fresco como células efectoras El efector para los índices objetivo se varían como sigue 100 1, 50 1, y 25 1, con el numero de células BJAB por pozo que permanece constante pero varia el número de PBMC Las células Bjab se etiquetan durante 2 horas con 51Cr y se alicuotan en una densidad de células de 5x104 células/pozo para cada pozo de placas de 96 pozos de fondo plano Las proteínas de fusión purificadas o Rituximab se agregan en una concentración de 10$g/ml, y se agrega PBMC en 1,25 x106 células/pozo (25 1), 2,5 x106 células/pozo (50 1), o 5x106 células/pozo (100'1), en un volumen final de 200 il La muerte natural se mide en cada proporción efector objetivo mediante la omisión del SMIP o MAB La liberación espontánea se mide sin la adición del PBMC o proteínas de fusion, y la liberación máxima se mide por la adición de detergente (1% NP-40) para pozos apropiados Las reacciones se incuban durante 5 horas, y 100 il de supernadante de cultivo se cosecha a un Lumaplate (Packard Instruments) y se le permite secar durante la noche antes de contar el cpm liberado en un Contador de Centellco de Microplaca Packard Top Count NXT

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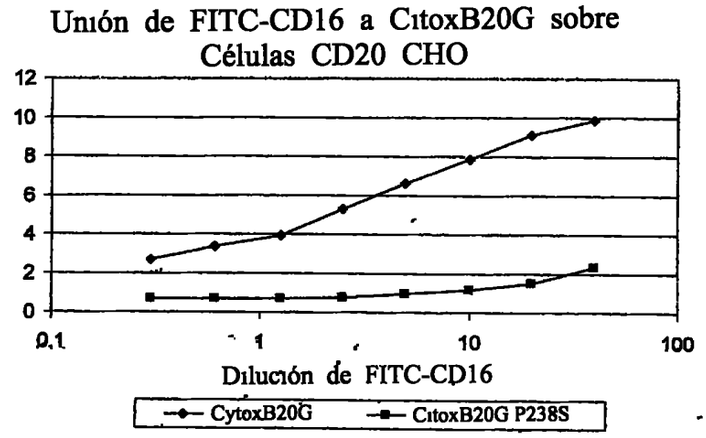


Fig 75

Figura 75 Unión de FITC-CD16 a de CitoxB20G sobre células CD20 CHO Las células CD20 CHO (106) se incubaron en cantidades de saturación de actividad de CitoxB20G o CitoxB20G P238S (10 ug/ml) durante una hora en hielo en PBS/2% de FBS Las células se lavaron en PBS/2% FBS e incubaron con diluciones seriales de 0,5 mg/mlFITC-CD16 durante una hora en hielo Las celulas se lavaron, y se mide la unión específica mediante citometría de flujo que utiliza una máquina Beckman Coulter Epics C Los resultados se analizaron utilizando software de análisis Expo y las unidades de fluorescencia normalizada se graficaron como una función de la concentración

CitoxB20G y de CitoxB20G P238S SMIPS se unen a Células U937 que expresan alta afinidad FcR FcγRI

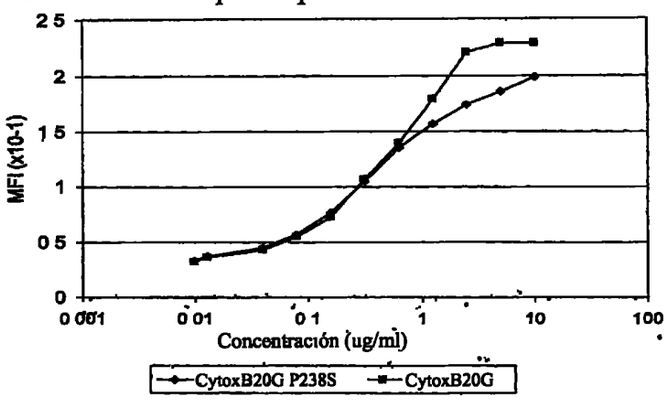


Fig 76

Figura 76 CitoxB20G SMIPs se unen en forma a las células U937 que expresan alta afinidad-FcR (FcãRI, Cd64) Las células U937 que expresan CD64 se incuban en PBS/2% FBS durante una hora en hielo con CitoxB20G o CitoxB20G P238S Las células se lavan e incuban durante una hora en hielo con IgG1 anti-humano FITC-cabra (Fc específico) (Caltag) en una dilución final de 100 las células se lavan y se analiza la fluorescencia en un citometro de flujo Beckman-Coulter EpicsC Los datos se analizan utilizando software de análisis Expo y la intensidad de fluorescencia se grafica como una función de la concentración SMIP

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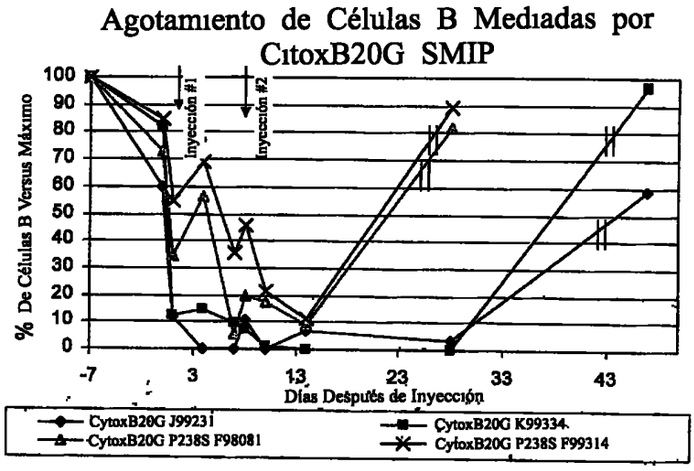


Fig 77

Figura 77 CitoxB20G oCitoxB20G P238S se administran a macacos mediante inyección intravenosa en 6 mg/kg, con dos infusiones dadas una semana aparte El efecto de las células B circulantes se midió mediante la detección sobre las células B circulantes se mide mediante las células sanguíneas periféricas B positivas CD40 Las muestras de sangre se retiran de animales inyectados en los días -7, 0, 1, 3, 7, 8, 10, 14, 28 y 43 El agotamiento de las células B se estiman al desarrollar CBC (conteo sanguíneos completos) y análisis de citometría de flujo de dos colores en sangre de mono Los conjugados FITC o PE de los anticuerpos contra CD40, CD19 CD20, IgG, Cd3, CD8 se utilizan en varias combinaciones Los dátos se grafican como el número de células B sanguíneas positivas CD40 tabuladas en miles de células por microlitro durante el tiempo relacionado con el nivel de punto de tiempo de preinyección inicial de células B (máximo)

Figura 78 SEC sobre CytoxB37G SMIP que contiene Dominios de Pivote SSS y SSC del IgG1 Humano

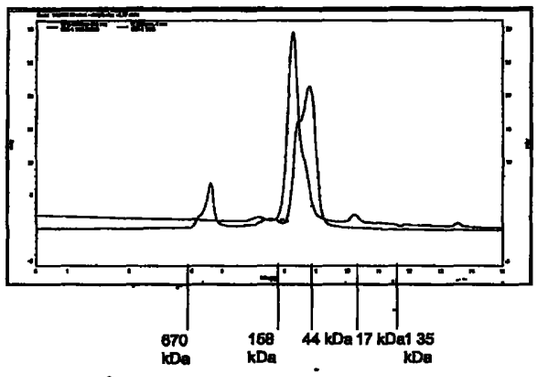


Fig 78

Figura 78 Secuencia (Cromatografía de Exclusión de Tamaño) CitoxB37G SIMP se purificó de los sobrenadantes de cultivó CHO mediante cromatografía de afinidad de proteína A.-Alícuotas purificadas de 10-25 lg se sometieron a HPLC sobre una columna HPLC Tosoh Biogep, Inc TSK 3000 SWXL, tamaño de poro 5 lm La proporción de flujo era de 1 ml/min, en PBS, amortiguador pH 7,2 Las proporciones de migración de los estándares de pesó molecular se indican adelante del trazado El CitoxB37G (SSS) SMIP se indica en azul mientras que el CitoxB37G (CSS)H se indica en rojo

Figura 79 Unión de CytoxB37G SIMP a Líneas Celulares de Linfoma de Célula B

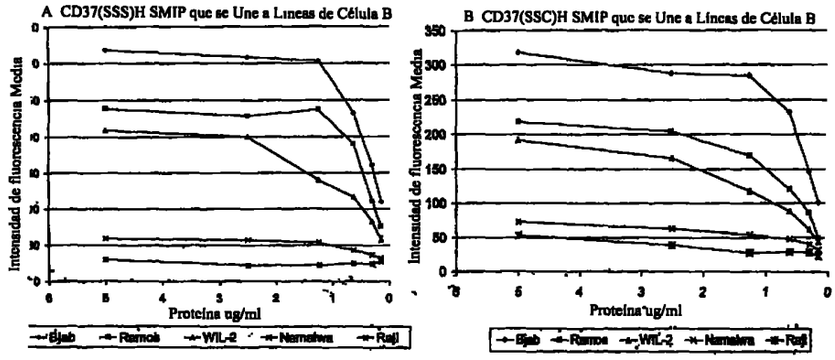


Fig 79

Figura 79 Unión de CytoxB37G SMIP a lineas de celula de linfoma de celula B Diluciones seriales de CytoxB37 (SSS)H G o CytoxB37 (SSC)H G SMIPS modificado se incuban con 106 células de cada tipo de célula durante 60 minutos en PBS/2%FBS en hielo Las mezclas se lavan dos veces e incuban con una mezcla de IgG anti humano de cabra FITC y IgG F(ab')2(Caltag) anti humano de cabra FITC,én 1 100 cada uno, en hielo durante 45 minutos Las muestras se lavaron y analizaron mediante citometría de flujo que utiliza un FacsCalibur (Becton-Dickinson)

Figura 80 Teñido de Anexina V-PI de Células Ramos Incubadas 24 horas con Cd37 SIMP

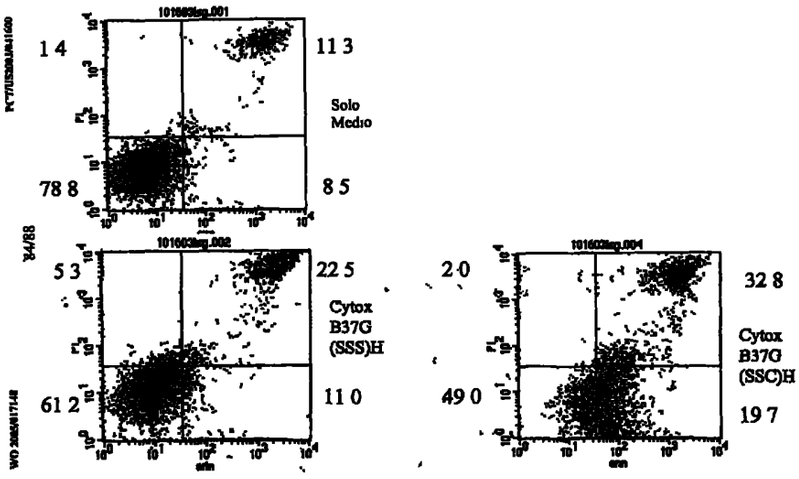


Fig 80

Figura 81 Incorporación de Timidina (Inhibición de crecimiento) en Células B Ramos después de Incubación de 48 horas con anti-CD37 SIMPS

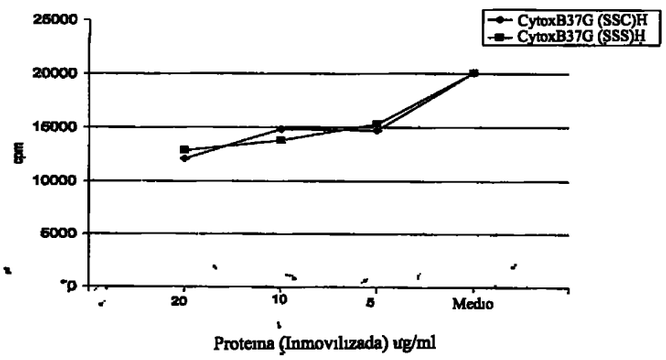


Fig 81

Figura 81 Las células B Ramos se incuban con diluciones en serie de CD37G SMIPS purificado que contiene el pivote IgG1 identificado como (SSS)H o (SSC)H Los cultivos se incuban en platos de cultivo de tejido de fondo plano de 96 pozos (Costar) a 37° C, 5% CO2 durante 36 horas antes de pulsar con 3H-timidina durante al menos 12 horas de una incubación de 48 horas (0,75 lCi/pozo) Las células se cosechan en placas GFC de 96 pozos que utilizan un cosechador Packard, se secan, y se agrega 25 il de fluido de centelleo Microscint a cada pozo antes del conteo en un contador de centelleo de microplaca TopCount NJXT (Packard) Los datos se grafican como concentración de proteína versus cpm incorporado Cada SMIP muestra inhibición de incremento de proliferación con concentración de proteína incrementada

Figura 82 La Induccion de Apoptosis en Células B Ramos después de Incubación de 20 horas con diferentes combinaciones Cd20 y Cd37 objetivado SIMPS

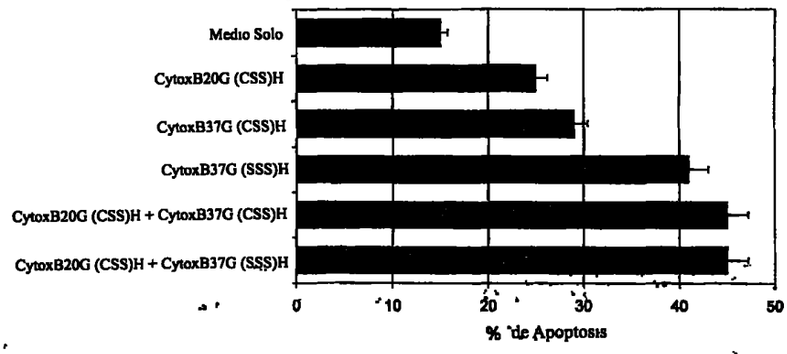


Fig 82

Figura 82 Las células B Ramos se incuban con CD20 y/o CD37 objetivado SMIP (10 mg/ml) en solución durante 20 horas Las células se cosechan luego, se lavan, e incuban en Annexina V y yoduro de propidio que utiliza un kit de teñido de Immunotech antes de citometria de flujó de dos colores que utiliza un citómetro de flujo FACsCalibur (Becton-Dickinson) La gráfica muestra el porcentaje de células positivas de Anexina V identificadas por su teñido en los cuadrantes derecho de los graficos de puntos

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Figura 83 Muerte Mediada por Complemento de Células Ramos CD37 SMIP

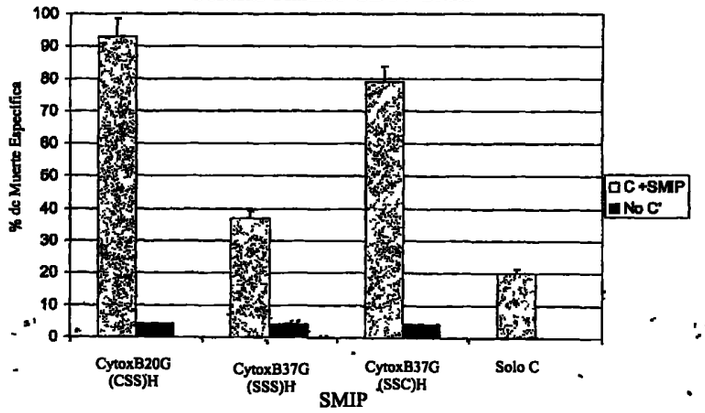


Fig 83

Figura 83 Actividad CDC del CytoxB37G SMIPS CytoxB20G, CytoxB37(SSS)h g, CytoxB37 (SCS)H, CytoxB37(CSS)H, o CytoxB37 (SSC)H se incuban en 10 µg/ml con 104 Células Objetivo Ramos y una dilución de 1 10 de complemento de conejo (PelFreez) en un volumen de 150 µl durante 90 minutos Las alícuotas se tiñen con trypan azul (Invitrogen), y se cuentan utilizando un hemacitómetro para determinar el porcentaje de la población muerta durante tratamiento Los controles negativos con células y solo un reactivo también se incluyen

Figura 84 Actividad ADCC de CD37 SMIP contra Objetivos Ramos

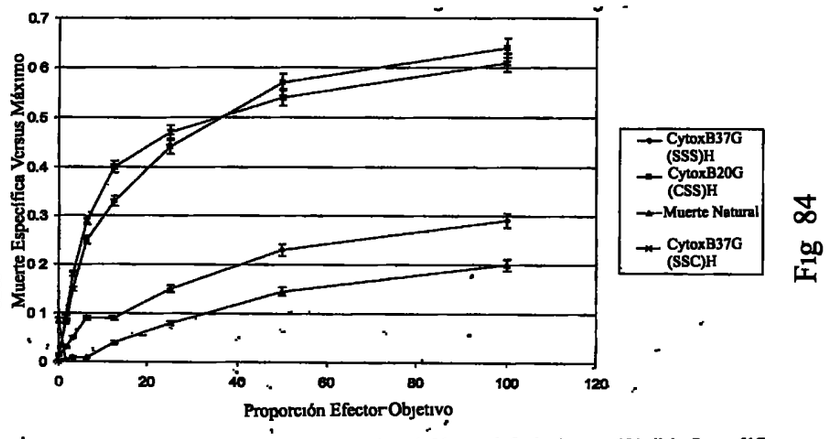


Fig 84

Figura 84 CD37 SMIPS en 10 mg/ml se incuba en placas de 96 pozos de fondo plano con 104 células Ramos 51Cr etiquetado y PBMC humano en diferentes proporciones efector objetivo que varía de 0 a 100 Todas las incubaciones se desarrollan por triplicado en cada proporción efector objetivo La muerte natural se mide en cada proporción efector objetivo por la omisión del SMIP La liberación espontánea se mide sin la adición de PBMC o proteína de fusión, y la liberación máxima se mide por la adición de detergente (1% NP-40) a los pozos apropiados Las reacciones se incuban durante 6 horas; y 100 ml de sobrenadante de cultivo cosechado a un Lumáplate (Packard Instruments) y se le permite secarse durante la noche antes de conteo de liberación de cpm en un Contador de Centelleo de Microplaca Packard Top Count NXT

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CONSTRUCCIONES DE UNIÓN Y MÉTODOS PARA USO DE ELLAS

Todas las solicitudes de las cuales tiene prioridad esta solicitud se incorporan aquí como referencia en su totalidad

Campo de la Invención

La presente invención se relaciona de manera general con compuestos que tienen varias utilidades incluyendo usos para investigación, diagnóstico, y terapia, por ejemplo, inmunoterapia Los compuestos de la invención incluyen proteínas inmunológicamente activas y conjugados de proteína Tales proteínas incluyen proteínas de unión recombinantes o trabajadas con ingeniería tales como, por ejemplo, proteínas de fusión de inmunoglobulina de dominio de unión, que pueden incluir proteínas de fusión de inmunoglobulina Fv de cadena simple y compuestos que contienen inmunoglobulinas Fv de cadena simple La presente invención también se relaciona con composiciones y métodos para tratar condiciones, enfermedades, y desórdenes que se mejorarían, terminarían, o disminuirán con la administración de, por ejemplo, polipéptido y/o construcciones de ácido nucleico de la invención, incluyendo, por ejemplo, condiciones malignas y desórdenes de la célula B, incluyendo enfermedades caracterizadas por la producción de auto anticuerpos y/o inflamación

Antecedentes de la Invención

El sistema inmune es uno de los más complejos de los muchos sistemas intrincados del cuerpo Una basta y complicada disposición hecha de muchos diferentes tipos de de células que involucra muchas diferentes clases de moléculas, el sistema inmune humano le permite al cuerpo responder a invasores extraños tales como bacterias, virus, y otros agentes infecciosos, así como también material extraño tal como polen En general, el sistema inmune humano se divide en dos partes principales, inmunidad mediada por anticuerpo (también denominada inmunidad "humoral" o "circulante") e inmunidad mediada por célula, las cuales son manejadas por linfocitos Los linfocitos son una de las cinco clases de células sanguíneas blancas (leucocitos) que circulan en la sangre Existen varias clases de linfocitos, cada una con diferentes funciones a desarrollar Los tipos más comunes de linfocitos son los linfocitos B (células B), que son responsables para la elaboración de anticuerpos y linfocitos T (células T) Las células del sistema inmune no solamente incluyen células T y células B sino también células Asesinas Naturales, granulocitos (o leucocitos polimorfonucleares (PMN)), macrófagos, y células dendríticas El sistema humoral se maneja por las células B con la ayuda de las células T y trata con los agentes infecciosos en la sangre y los tejidos

del cuerpo El sistema mediado por células se maneja por células T y trata con las células del cuerpo que se han infectado

Un antígeno es una sustancia, usualmente macromolecular, que estimula o induce una respuesta inmune En razón a su estructura macromolecular compleja, un macroorganismo simple consiste de multiples antígenos (por ejemplo, estructuras de superficie tal como componentes de pared celular, fimbria, flagelos, etc , o proteínas extracelulares, tales como toxinas o enzimas producidas por el microorganismo) Las proteínas de recubrimiento y algunas de las proteínas de cubierta de los virus animales también son usualmente antigénicas Un huésped es generalmente capaz de responder específicamente a antígenos que entran en contacto con componentes de su sistema inmune Tanto los sistemas de inmunidad mediada por anticuerpo como de inmunidad mediada por célula involucran inter relaciones complejas que les permiten montar reacciones inmunes y a casi cualquier antígeno En otras palabras, el sistema inmune es capaz de reconocer sustancias extrañas (antígenos) que estimulan el sistema para producir inmunidad mediada por anticuerpo, inmunidad mediada por célula, o ambos

Los complejos del sistema inmune están constituidos por una variedad de diferentes tipos de célula y órganos diseminados

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a lo largo del cuerpo Estos incluyen los órganos linfoides primarios y secundarios Los órganos linfoides primarios son la médula espinal y el timo Todas las células del sistema inmune son inicialmente derivadas de la médula ósea en un proceso denominado hematopoiesis Durante la hematopoyesis las células madre derivadas de la médula ósea se diferencian en células maduras del sistema inmune (células "B") o en precursores de células que emigran hacia afuera de la médula ósea para madurar en el timo (células "T") Además de las células sanguíneas rojas, plaquetas, y células B, la médula ósea también produce células Asesinas Naturales granulocitos y timocitos inmaduros La función del timo es producir células T maduras Los timocitos inmaduros, también conocidos como protimocitos, dejan la médula espinal y migran hacia el timo donde ellos maduran y entonces se liberan hacia el torrente sanguíneo El complejo del sistema inmune también incluye órganos linfoides secundarios, por ejemplo, el bazo, los nodos linfáticos, etc , así como también un sistema circulatorio que se separa de los vasos sanguíneos

El bazo, hecho de células B, células T, macrófagos, células dendríticas, células Asesinas Naturales, y células sanguíneas rojas, son un filtro inmunológico de la sangre Los macrófagos migratorios y las células dendríticas capturan antígenos de la sangre que pasan a través del bazo Los macrófagos migratorios y las células dendríticas también

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traen antígenos del bazo por vía del torrente sanguíneo Una respuesta inmune se inicia en el bazo cuando los macrófagos o las células dendríticas presentan el antígeno a las células B o T apropiadas, y las células B se activan y producen grandes cantidades de anticuerpo

Los vasos linfáticos y los nodos linfáticos son partes del sistema circulatorio especial que lleva linfa La linfa es un fluido transparente que contiene células sanguíneas blancas, principalmente linfocitos La linfa baña el tejido del cuerpo, y es luego recolectada en los vasos linfáticos Los nodos linfáticos puntean una red de vasos linfáticos y, cuando diferentes ductos de linfa llevan antígeno que contiene linfa hacia el nodo, funcionan como un filtro inmunológico para la linfa Compuestos principalmente de células T, células B, células dendríticas, y macrófagas, los nodos de la linfa drenan fluido de la mayoría de los tejidos Los antígenos son filtrados hacia fuera de la linfa en el nodo linfático antes de que la linfa sea regresada a la circulación Los macrófagos y las células dendríticas que capturan antígenos también presentan estos materiales extraños a las células T y B en los nodos linfáticos, dando como resultado la estimulación de las células B para que se desarrollen allí en las células de plasma que secretan anticuerpos Los anticuerpos dejan el nodo linfático por medio de los ductos eferentes que se emplean en el torrente

sanguíneo Los linfocitos también pueden dejar el nodo mediante un ducto eferente y viajar a otros sitios en el sistema linfático o ingresar a la circulación sanguínea Un linfocito simple completa un circuito a través del torrente sanguíneo y los sistemas linfáticos una vez cada 24 horas

Las tonsilas, adenoides, partículas de Peyer, y el apéndice también son tejidos linfáticos Los parches de Peyer (masas de linfocitos) son similares a los tonsilos y se encuentran a través del cuerpo, especialmente en los recubrimientos interiores de la mucosa de los tractos digestivo y respiratorio Es la función de las células fagocíticas encontradas en los parches de Peyer y otros folículos agregados linfáticos defender el cuerpo contra, por ejemplo, partículas alimenticias inadecuadamente digeridas que cruzan la pared de la tripa e ingresan a la sangre, a atacar invasores extraños no deseados mientras ellos están aún en el intestino

La función principal de las células B es la producción de anticuerpos en respuesta a proteínas extrañas de bacterias, virus, y células tumorales Las células T están usualmente divididas en dos grupos principales, a saber, los linfocitos T citotóxicos (células "Tc" o los CTL) y las células T ayudantes (células "Th" o células T ayudantes) Las células Th, también denominadas células CD4+ T funcionan para

aumentar o potenciar las respuestas inmunes de ante la secreción de factores especializados que actúan otras células sanguíneas blancas para combatir la infección Ellos mejoran la producción de los anticuerpos mediante las células B Las células Tc, también denominadas células CD8+ T, pueden matar directamente ciertas células tumorales, células infectadas con virus, y algunas veces parásitos Las células Tc también son importantes en la subregulación de las respuestas inmunes Ambos tipos de células T dependen a menudo de órganos linfoides secundarios (los nodos linfoides y el bazo) como sitios donde ocurre la activación, pero ellos también se encuentran en otros tejidos del cuerpo, incluyendo el hígado, pulmón, sangre, y los tractos intestinales y reproductivos

Las células Asesinas Naturales, denominadas a menudo como células NK, representan otro tipo de linfocito y son similares al subconjunto de células Tc Ellas funcionan como células efectuadoras que matan directamente ciertos tumores tales como melanomas y linfomas y células infectadas con virus Ellas son denominadas asesinas "naturales" porque, a diferencia de las células T citotóxicas, ellas no necesitan reconocer un antígeno específico antes de llevar a cabo su función Mientras las células NK, a diferencia de las células Tc, matan sus objetivos sin activación anterior en los órganos linfoides, las células NK activadas por las secreciones de las células Th matarán tumores u objetivos

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infectados con virus más efectivamente Las células NK apuntan a las células tumorales y protegen contra una amplia variedad de infecciones microbianas En varias enfermedades de inmunodeficiencia, que incluyen SIDA, la función de la célula Asesina Natural es anormal Las células Asesinas Naturales también pueden contribuir en la inmunoregulación al secretar altos niveles de linfocinas influenciales

Algunas células MK tienen receptores de superficie (FcγRIII, también denominado C16) para la porción Fc del anticuerpo IgG Ellos se unen a las células objetivo a través de receptores para la porción Fc de un anticuerpo que ha reaccionado con antígeno sobre una célula objetivo Este tipo de inmunidad mediada por célula es denominado citotoxicidad mediada por célula dependiente de anticuerpo (ADCC) Las células MK también pueden tener receptores para el componente C3 del complemento, otro sistema de defensa inmune, y de esta manera reconocer células que son recubiertas con C3 como objetivos El ADCC se cree que es una defensa importante contra una variedad de infecciones parasíticas originadas, por ejemplo, por protozoos y helmintos

Aunque los linfocitos pequeños parecen idénticos, ellos se pueden distinguir mediante moléculas llevadas sobre su superficie celular No solamente tales marcadores distinguen entre células B y células T, ellos distinguen entre varios

subconjuntos de células que se comportan de manera diferente Cada célula T madura, por ejemplo, lleva un marcador conocido como T3 (o CD3) Además, la mayoría de las células T ayudantes llevan un marcador T4 (CD4), una molécula que reconoce los antígenos del complejo de histocompatibilidad principal clase II ("MHC") Una molécula conocida como T8 (CD8), que reconoce los antígenos MHC clase I se encuentra sobre muchas células T supresoras/citotóxicas

Otro grupo de células sanguíneas blancas colectivamente denominadas como granulocito, o leucocitos polimorfonucleares (PMN), se componen de tres tipos de células Estas células, neutrofilos, eosinófilos, y basófilos son importantes en la remoción de bacterias y parásitos provenientes del cuerpo Los neutrófilos migran a través de las paredes capilares hacia los tejidos infectados donde ellos matan los invasores (por ejemplo bacterias) y luego engullen los remanentes mediante fagocitosis Los eosinófilos son citotóxicos, liberando los contenidos de sus gránulos sobre un invasor Los basófilos dejan la sangre y se acumulan en el sitio de una infección u otra inflamación y descargan los contenidos de sus gránulos, liberando una variedad de mediadores tales como histamina, serotonina, prostaglandinas, y leucotrienos que, por ejemplo, incrementan el flujo sanguíneo al área Los mediadores liberados por basófilos también juegan una parte importante en algunas respuestas alérgicas tales como la

fiebre de heno y las respuestas anafilácticas a picaduras de insectos

Los monocitos son células sanguíneas blancas fagocíticas liberadas de la médula ósea en la circulación sanguínea Cuando un monocito ingresa al tejido, se convierte en un macrófago Los macrófagos también son células fagocíticas grandes que engullen material extraño (antígeno) que ingresan al cuerpo, así como también células muertas y que están muriendo en el cuerpo Los macrófagos son importantes en la regulación de respuestas inmunes, y son a menudo denominados como carroñeros, o células que presentan antígeno (APC) por que ellos recogen e ingieren materiales extraños y presentan estos antígenos a otras células del sistema inmune tales como las células T y las células B Esta es una de las primeras etapas importantes en la iniciación de una respuesta inmune Los macrófagos estimulados exhiben niveles crecientes de fagocitosis y también secretan interleucina -1 (IL-1), un producto que ayuda a activar las células B y las células T

Las células dendríticas también se originan en la médula ósea y funcionan como los APC Ellas son usualmente encontradas en el compartimiento estructural de los órganos linfoides tales como el timo, los nodos linfáticos y el bazo, pero también son encontradas en el torrente sanguíneo y otros tejidos Se cree que las células dendríticas capturan antigenos o lo

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llevan a los órganos linfoides donde se inicia una respuesta inmune

Características importantes del sistema inmunológico relevantes para la defensa del huésped y/o la inmunidad de los microorganismos patógenos incluyen la especificidad, la memoria, y la tolerancia Se entiende, por ejemplo, que un anticuerpo o célula T reactiva reaccionará específicamente con el antígeno que indujo su formación, éste no reaccionará con otros antígenos De manera general, ésta especificidad es del mismo orden que aquella de la especificidad del sustrato enzima o la especificidad del ligando receptor, aunque una reactividad cruzada es posible La especificidad de la respuesta inmune está explicada por selección clonal

Durante la respuesta inmune primaria, un antígeno específico selecciona un clon pre-existente de leucocitos específicos y estimula su activación, proliferación y diferenciación También se entiende que una vez que el sistema inmune ha respondido para producir un tipo específico de anticuerpo o célula T reactiva, es capaz de producir más del anticuerpo o la célula T activada más rápidamente en mayores cantidades, esto es denominado respuesta secundaria (o memoria) También se reconoce que un animal generalmente no sufre una respuesta inmunológica a sus propios componentes (potencialmente antigénico) Se dice que el animal es tolerante, o incapaz de

reaccionar a sus propios componentes potencialmente antigénicos Esto asegura que bajo condiciones normales, una respuesta inmune a "auto" antígenos (denominado una respuesta auto inmune) no ocurra La tolerancia se efectúa de números maneras, pero en esencia el sistema inmune es capaz de distinguir "auto" componentes de "no auto" (extraño) antígenos, este responderá a "no auto" pero no a "auto" Algunas veces en un animal, la tolerancia puede ser "rota", lo cual puede dar como resultado una enfermedad auto inmune

Las actividades biológicas de las respuestas inmunes mediada por anticuerpo y mediada por célula son diferentes y varían de un tipo de infección a otro Existen varias clases de tipos de anticuerpos (y subclases de varios tipos) involucradas en inmunidad mediada por anticuerpo Todas las clases de anticuerpo se producen en respuesta a un antígeno específico que reacciona estereoquímicamente con ese antígeno y no con otros (diferentes) antígenos El huésped tiene la capacidad genética de producir anticuerpos específicos a miles de diferentes antígenos, pero no lo hace así hasta que existe un estimula antigénico apropiado (específico) Debido a la selección clonal, el huésped produce solamente los anticuerpos homólogos que reaccionaran con ese antígeno el cual, como se anotó anteriormente se encuentra en la sangre (plasma) linfa, y muchos tejidos extravasculares Una vez que ocurra la respuesta inmune mediada por anticuerpo luego de la

interacción con los linfocitos B con antígeno y su diferenciación en células de plasma que secretan anticuerpos, el anticuerpo secretado se une al antígeno el cual, a su vez, da como resultado la neutralización o eliminación del cuerpo

La inmunidad mediada por célula, de otra parte es mediada por subpoblaciones específicas de linfocitos T denominadas células T efectuadoras que existen en las formas precursoras como "células T de descanso" (célula pT) Estas células llevaban receptores para antígenos específicos y reconocen aquellos antígenos sobre la superficie de otras células La estimulación con ese antígeno da como resultado la activación de células T Las células T se agrandan ingresan al ciclo mitótico, se reproducen y se desarrollan en células T efectuadoras cuyas actividades son responsables de este tipo de inmunidad Ellas también se desarrollan en clones de células T reactivas idénticas denominadas células T memoria Como se notó anteriormente, la mayoría de las células T en el cuerpo pertenecen a uno de dos sub conjuntos y se distingue por la presencia sobre su superficie de uno o del otro de dios glicoproteínas designadas CD4 y CD8 Cuales de estas moléculas están presentes determina los tipos de células a los cuales las células T pueden unirse Las células T que llevan CD8 (células T $CD8^+$) siempre reconocen antígeno en asocio con las proteínas MHC clase 1 y típicamente funcionan como células T citotóxicas Casi todas las células del cuerpo

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expresan moléculas MHC clase I Las células T que llevan CD4 (células T $CD4^+$) siempre reconocen antígenos en asocıo con las proteínas MHC clase II sobre la superfıcıe de otras células Solamente las células que presentan antígeno especializado expresan moléculas MHC clase II, que ıncluyen células dendrítıcas, células fagocíticas tales como macrófagos y células B Los lınfocıtos T $CD4^+$ generalmente funcıonan como células ayudantes T

Las células ayudantes T que ıncluyen células Th1 y células Th2 responden al antígeno con la producción de lınfocınas Las células Th1 y Th2 se pueden distınguır con base en sus perfıles de lınfocina Como todas las células T, las células Th surgen en el tımo Cuando ellas se presentan con un antígeno por las células dendrítıcas que presentan antígeno ellas ınıcıan su prolıferación y se actıvan, Exısten dos clases de células dendrítıcas, las células DC1 (descendıentes de monocitos) y las células DC2 (que parecen derıvarse de los lınfocıtos)

Las células Th1 (células Th1 ınflamatorias ınvolucradas en la elımınación de patógenos que reciden ıntracelularmente en los compartımentos vesiculares) se producen cuando las células dendrítıcas tıpo DC1 presentan antígeno en el receptor de la célula T para el antígeno (TCR) y secretan ınterleucına 12 (IL-12) Esta estımulación de paracrına actıva las células

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Th1 para secretar sus propias linfocimas, en particular, el Factor de Necrosis Tumoral beta (TNF-β) (también denominado como linfotoxina) y el interferón-gama (INF-γ) Estas linfocinas estimulan los macrófagos para matar bacteria que ellas han engullido mediante fagocitosis y ellas reclutaran otros leucocitos al sitio que produce la inflamación Las células Th1 son esenciales para inmunidad mediada por célula y para controlar los patógenos intracelulares tales como, por ejemplo, *Listeria y Mycobacterium tuberculosis*

Las células Th2 (células Th2 ayudantes "reales", que se requieren para producción de anticuerpo por las células B) se producen cuando las células dendríticas tipo DC2 presentan antígeno al receptor de la célula T para antígeno y, presumiblemente, uno o más estimulantes de paracrina Las linfocinas principales secretadas por las células Th2 son interleucina (IL-4) que estimulan el cambio de clase en las células B y promueven su síntesis de anticuerpos IgE, actúan como un dispositivo de retroalimentación positiva que promueven más células pre-Th para ingresar a la senda Th2, y bloquean la expresión del receptor IL-12 inhibiendo de esta manera las células pre-Th en el timo de ingresar a la senda Th1 El IL-4 también origina que las células B proliferen y se diferencien en células de plasma que secretan anticuerpos y en células B de memoria El IL-4 activa solamente células B en la vecindad cuando ellas mismas han encontrado el

antígeno, y no otros, con el fin de sostener la especificidad de la respuesta inmune Las células Th2 también producen interleucina 5 (IL-5), que atrae y activa los eosinófilos), la interleucina 10 (IL-10, que inhibe la producción de IL-12 por los DC y evita la maduración de las células pre-Th hacia los Th1), y la interleucina 13 (IL-13, que también promueve las síntesis de anticuerpos IgE)

La activación de la célula Th2 también la origina el inicio de la producción de interleucina 2 (IL-2), y expresa un receptor de membrana para IL-2 La IL-2 secretada autoestimula la proliferación de células Th2 Por ejemplo, en IL-2 se une a los receptores IL-2 sobre otras células T (que se han unido al antígeno) y estimulan su proliferación Además IL-2, las células Th2 estimuladas también producen IFN-γ e interleucina 6 (IL-6), que median varios aspectos de la respuesta inmune El IFN-γ activa las células Asesinas Naturales a su potencial citolítico completo, y es un activador de macrófagos e incrementan así sus actividades antitumorales Si los macrófagos son infectados por parásitos intracelulares, este activa los macrófagos, que a su vez destruyen los parásitos El IFN-γ también refuerza la actividad antitumoral de los linfocitos citotóxico, incrementa las actividades no específicas de las células NK, y es uno de los factores que controla la diferenciación de las células B e incrementa la secreción de las

inmunoglobulinas El IL-6 estimula varios tipos de leucocitos, así como también la producción de Proteínas de Fase Aguda en el hígado Es particularmente importante para inducir las células B diferenciar entre células formadoras de anticuerpos (plasma) De esta manera, las células Th2 suministran ayuda a las células B y son esenciales para la inmunidad mediada por anticuerpo

Los linfocitos T citotóxicos son capaces de matar las células que muestran un antígeno nuevo o extraño sobre su superficie (por ejemplo, células infectadas por virus, o células tumorales, o células de tejido trasplantada) Los CTL $CD8^+$ también vienen en dos sub conjuntos Tcl que, como las células Th1, secretan IFN-γ y Tc2 que, como las células Th2, secretan IL-4

La respuesta a la inmunidad mediada por célula también juega un papel en la destrucción de las células tumorales y en el rechazo de los trasplantes de tejido de animales Un problema principal en los trasplantes de tejido es el rechazo, que está a menudo basado en la respuesta a la inmunidad mediada por célula a células "extrañas" (por que ellas no son una coincidencia antigénica perfecta) En razón a que las células tumorales contienen antígenos específicos no vistos sobre células normales ellas también se pueden reconocer como extrañas y distribuidas por las fuerzas de inmunidad mediada

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por célula Si las células tumorales se desarrollan sobre una base regular en los animales esta puede ser inmunidad mediada por célula que las elimina o las mantiene en revisión El incremento de la incidencia en muchos tipos de cáncer (tumores) en humanos con el avance de la edad puede estar correlacionado con una declinación en la eficiencia pico del sistema inmune que ocurre aproximadamente a los 25 años de edad

Un resumen de los tipos de célula involucrado en la expresión de inmunidad mediada por célula sigue Los linfocitos Tc matan células que llevan antígenos extraños sobre la superficie en asocio con MCH Clase 1 y pueden matar células que posean parásitos intracelulares (o arterias o virus) en tanto que la célula infectada esté desplegando un antígeno microbiano sobre su superficie Las células Tc matan las células tumorales y cuentan para el rechazo de células trasplantadas Las células Tc se reconocen los complejos MHC Clase 1 de antígeno sobre células objetivo, los contactan, y liberan los contenidos de los gránulos directamente hacia la membrana de la célula objetivo que rompe la célula Los linfocitos Th producen linfocinas que son factores de "ayuda" para el desarrollo de las células B en células de plasma que secretan anticuerpo Ellas también producen ciertas linfosinas que estimulan la diferenciación de las células T efectuadoras y la actividad de los macrófagos Las células

Th1 reconocen antígenos sobre macrófagos en asocio con el MHC Clase II y se activan (por IL-1) para producir linfosimas que incluyen IFN-γ que activa macrófagos y células NK Estas células median en varios aspectos de las respuestas a la inmunidad mediada por célula que incluye reacciones de hipersensibilidad tipo retrazado Las células Th2 reconocen antígeno en asocio con el MHC Clase II sobre un APC y luego producen interleucinas y otras sustancias que estimulan la proliferación y la actividad de las células B y células T específicas Los macrófagos son importantes como células que presentan antígeno que inician las interacciones de la célula T, el desarrollo, y la proliferación Los macrófagos están también involucrados en la expresión de la inmunidad mediada por célula Los macrófagos activados han incrementado su potencial fagocítico y liberan sustancias solubles que originan la inflamación y destruyen muchas bacterias y otras células Las células Asesinas Naturales son células citotóxicas que rompen las células que llevan nuevos antígenos sin importar su tipo MHC y aun rompen algunas células que no llevan proteínas MHC Las células NK se definen por su capacidad para matar células que despliegan un antígeno extraño (por ejemplo células tumorales) sin importar el tipo MHC y sin importar la sensibilización previa (exposición) al antígeno Las células NK se pueden activar por el IL-2 y el IFN-γ y rompen las células de la misma manera que los linfocitos T citotóxicos Algunas células NK

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tienen receptores para el dominio Fc y el anticuerpo IgG y son así capaces de unirse a la porción Fc del IgG sobre la superficie de una célula objetivo y liberar componentes citolíticos que matan la célula objetivo por vía de citotoxicidad mediada por célula dependiente de anticuerpo

Los factores extracelulares que afectan la proliferación y la diferenciación de la célula se han definido como citoquinas Estas incluyen las linfocinas, que son proteínas producidas por linfocitos T que tienen efecto sobre la diferenciación, proliferación, y actividad de varias células involucradas en la expresión de la inmunidad mediada por célula En general, la función de las linfocinas al (1) enfocar los leucocitos circulantes y los linfocitos en el sitio de interacción inmunológica, (2) estimular el desarrollo y proliferación de la células B y las células T, (3) estimular y preparar los macrófagos para sus tareas fagocíticas, (4) estimular las células Asesinas Naturales, y (5) suministrar cubierta y actividad antiviral Un resumen de varias linfocinas importantes sigue Inicialmente denominada como un factor de activación de linfocito, el IL-1 es principalmente un producto de macrófagos, y tiene una variedad de efectos sobre varios tipos de célula Este actúa como un regulador de crecimiento de las células T y las células B, y e induce otras células tales como los hepatocitos para producir proteína relevante a la defensa del huésped El IL-1 forma un

gradiente quimiotático para los neutrófilos y sirve como un pirógeno endógeno que produce fiebre Así, el IL-2 juega un papel importante tanto en las respuestas inmunes como en las respuestas inflamatorias EL IL-2 estimula la proliferación de las células T y activa las células NK El IL-13 regula la proliferación de las células madre y la diferenciación de las células mástil El IL-4 origina la penetración de las células B y la síntesis mejorada de anticuerpo El IL-6 (también denominado como interferón beta 2, factor de diferenciación de célula B y factor estimulatorio hepatocito) tiene efecto sobre la diferenciación de la célula B y sobre la producción de anticuerpo y sobre la activación de célula T, crecimiento, y diferenciación, y probablemente tiene un papel importante en la mediación de la respuesta inflamatoria e inmune iniciada por la infección o el daño El IL-8 es un atrayente quimiotáctico para los neutrófilos El IL-13 comparte muchas de las propiedades del IL-4 y es un regulador potente de las respuestas inflamatoria e inmune El IFN-γ es producido por las células T y puede ser considerado como una linfocina Este es algunas veces denominado "interferón inmune" (Interferón alfa que es denominado como "interferón leucocito" e interferón-beta que es denominado como "interferón fibroblasto") El IFN-γ tiene varios efectos antivirales que incluyen la inhibición de la síntesis de proteína viral en células infectadas Este también activa los macrófagos y las células NK, y estimula el IL-1, IL-2, y la

producción de anticuerpo Las linfotoxinas incluyen los factores de necrosis tumoral Las células T producen TNF-beta, mientras que los TNF-alfa son producidos por células T así como también otros tipos de célula

Los TNF funcionan para matar células, incluyendo células tumorales (a distancia) Existen varios Factores Estimulantes de Colonia (CSF), que incluyen factor estimulante de colonia de macrófago de granulocito (GMCSS), que origina células blancas fagocíticas de todos los tipos para diferenciarse y dividirse

La naturaleza de los receptores de membrana para el antígeno sobre las células B y las células T es claramente bien entendido Cada célula B tiene aproximadamente 10^5 moléculas de anticuerpo unidas a membrana (IgD o IgM) que corresponden en especificidad al anticuerpo la célula se programa para producir (siendo estos receptores denominados como BCR) El CD32 ($F_{c(}$RII) sobre las células B son receptores para la región Fc de IgG El CD21 y CD35 sobre las células B son receptores para los componentes complemento Cada célula T tiene aproximadamente 10^5 moléculas de un receptor de célula T de unión de antígeno específico (un TCR) expuesto sobre su superficie El TCR es similar, pero no idéntico, a un anticuerpo Existen dos tipos de célula T que difieren en sus TCR, células T alfa/beta (αβ) y células T gama/delta ((δ) El

TCR de las células T alfa/beta se unen a un complejo molecular desplegado por una molécula MHC Clase I en la superficie de la célula que presenta antígeno Como se anotó anteriormente, la mayoría de las células Th expresan CD4, mientras que las mayorías de las células Tc expresan CD8

Tanto los DCR como los TCR son similares por que ellos son proteínas de membrana integrales, ellos están presentes en miles de copias idénticas expuestas en la superficie celular, ellos son hechos antes de que la célula encuentre un antígeno, ellos son codificados mediante genes ensamblados mediante la recombinación de segmentos de ADN, ellos tienen un sitio de unión único que se une a través de fuerzas no covalentes a una porción del antígeno denominado un epítopo (o antígeno determinante) que dependen de la complementariedad de la superficie del receptor y la superficie del epítopo, y la unión exitosa del receptor de antígeno al epítopo, si se acompaña por señales adicionales, da como resultado la estimulación de la célula para dejar G_o y ingresar al ciclo celular y repetir la mitosis que conduce a un desarrollo de un clon de células que lleva el mismo receptor antígeno, es decir, un clon de células de especificidad idéntica Los BCR y los TCR difieren en su estructura, los genes que los codifican, y el tipo de epítopo al cual ellos se unen

La inducción de la respuesta inmune primaria inicia cuando un antigeno penetra en las superficies epiteliales Este eventualmente entrará en contacto con los macrófagos de ciertas otras clases de células que presentan antígeno, incluyendo células B, monocitos, células dendríticas, células Langerhans, y células endoteliales Los antígenos, tales como las células bacteriales, son internacionalizados mediante endocitosis y "procesados" mediante los APC, luego "presentado" a los linfocitos inmunocompetentes para iniciar las etapas tempranas de la respuesta inmunológica El procesamiento por un macrófago, (por ejemplo) da como resultado la unión de materiales antigénicos a la superficie de la membrana en asocio con las moléculas MHC Clase II sobre la superficie de la célula El complejo MHC Clase II de antígeno se presenta a una célula ayudante T (Th2), que es capaz de reconocer antígeno procesado asociado con una molécula MCH Clase II sobre la membrana del macrófago La interacción, junto con la estimulación por el IL-1 secretada por el macrófago, activará la célula Th2

Como se indicó anteriormente, las células B mismas se comportan como APC Los antígenos reticulados unidos a los receptores de anticuerpo sobre la superficie de la célula de una célula B originan la internacionalización de algunos de los antígenos y la expresión sobre la membrana de la célula B junto con moléculas MHC Clase II La célula Th2 reconoce el

antígeno junto con las moléculas MHC Clase II, y secreta las varias linfocinas que activan las células B para volverse células de plasma que secretan anticuerpos y células B de memoria Aún si el antígeno no puede reticular el receptor, este puede ser endocitosado por la célula B, procesado y regresado a la superficie del asocio con el MHC Clase II donde este puede ser reconocido por células Th2 específicas que se activarán para iniciar la diferenciación y proliferación de la célula B En cualquier caso, la respuesta de la célula B total conduce a inmunidad mediada por anticuerpo

Los receptores de antígeno sobre la superficie de la célula B se consideran como los tipos específicos de anticuerpos que ellos son genéticamente programados para producir De esta manera, existen miles de subpoblaciones de células B distinguidas por su capacidad para producir una molécula de anticuerpo única, Las células B también pueden reaccionar con un antígeno homólogo sobre la superficie del macrófago, o con antigenos solubles Cuando una célula B se une al antigeno y simultáneamente se estimula por el IL-4 producido por una célula Th2 vecina, la célula B se estimula para crecer y dividirse para formar un clon de células B idénticas, cada una capaz de producir moléculas de anticuerpo idénticas Las células B activadas se diferencian adicionalmente en células de plasma que sintetizan y secretan grandes cantidades de

anticuerpo, y en células B de memoria Los anticuerpos producidos y secretados por las células de plasma reaccionarán específicamente con el antígeno homólogo que indujo su formación Muchas de estas reacciones conducen a la defensa del huésped y evitar la reinfección por patógenos Las células memoria juegan un papel en las respuestas inmunes secundarias Las células de plasma son relativamente de corta vida (aproximadamente una semana) pero producen grandes cantidades de anticuerpo durante este periodo Las células memoria, de otra parte, son relativamente de larga vida y luego de subsecuente exposición al antígeno se transforman rápidamente en células de plasma que producen anticuerpo

La generación de inmunidad mediada por célula inicia cuando por ejemplo, una célula T citotóxica reconoce un antígeno procesado asociado con el MHC Clase I sobre la membrana de una célula (usualmente una célula autoalterada, pero posiblemente una célula de tejido trasplantada, o un parásito eucariótico) Bajo la estimulación del IL-2 producido por las células Th2, la célula Tc se activa para volverse un linfocito T citotóxico capaz de lisar la célula que está mostrando el nuevo antígeno extraño sobre su superficie, una manifestación primaria de la inmunidad mediada por célula La interacción entre un macrófago que presenta antígeno y una célula Th estimula el macrófago para producir y secretar una interleucina-1 que actúa localmente sobre la célula Th,

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estimulando la célula Th para diferenciarse y producir sus propias citoquinas (que pueden ser llanadas aquí linfocinas por que ellas surgen de un linfocito) Estas linfocinas tienen varias funciones La IL-4 tiene un efecto inmediato sobre las células B vecinas El IL-2 tiene un efecto inmediato sobre las células T como se describieron anteriormente

Los leucocitos también expresan los sectores que promueven la adhesión que median interacciones célula-célula y célula-matriz Estas interacciones adhesivas son cruciales para la regulación de la hemopoiesis y la maduración de timocito, la dirección y el control del tráfico de leucocito y la migración a través de tejidos, y el desarrollo de respuestas inflamatorias inmunes y no inmunes Varias familias de receptores de adhesión han sido identificadas La familia integrina de leucocito comprende tres glicoproteinas de membrana heterodimérica alfa-beta que comparten una sub unidad beta común, designada CD18 Las sub unidades alfa de cada uno de los tres miembros, antígeno-1 asociado con función de linfocito (LFA-1), antigeno-1 de macrófago (Mac-1) y p150, son designados CD11a, b y c respectivamente Estas moléculas de adhesión juegan una parte crítica en las respuestas inmunes e inflamatorias de los leucocitos La familia integrina de leucocito es, a su vez, parte de la superfamilia integrina, cuyos miembros están

evolucionalmente, estructuralmente y funcionalmente relacionados Otra subfamilia integrina encontrada en los leucocitos es el grupo VLA, así denominado por que los "antígenos de activación muy tardía" VLA-1 y VLA-2 fueron originalmente encontrados para aparecer tarde en la activación de la célula T Los miembros de esta familia funcionan principalmente como receptores de adhesión de matriz extracelular y se encuentran tanto en células hematopoieticas como no hematopoyéticas Ellos juegan una parte en diversas funciones celulares que incluyen la organización de tejido, la recirculación de linfocito y las respuestas inmunes de células T Otra subfamilia integrina, las citoadesinas, son receptores sobre plaquetas y células endoteliales que se unen a proteínas de matriz extracelulares Una segunda familia de receptores de adhesión es la superfamilia de inmunoglobulina, cuyos miembros incluyen CD2, LFA-3, y ICAM-1, que participan en las interacciones adhesivas de la célula T, y los receptores específicos de antígeno de las células T y B, CD4, CD8, y las moléculas Clase I y Clase II de MHC Otra familia reconocida de receptores de adhesión es la selectina, caracterizadas por un dominio de lectina común La molécula-1 de adhesión de leucocito (LAM-1), que es el homólogo humano del receptor guía de murino, MEL-14, se expresa sobre leucocitos, aunque la molécula-1 de adhesión de leucocito endotelial (ELAM-1) y

la proteína de membrana de gránulo (GMP-140) se expresan sobre células endoteliales estimuladas y plaquetas activadas

La activación de una respuesta inmune requiere contacto físico célula-célula además de las citoquinas Así, por ejemplo, el desarrollo de los precursores de célula B y T requieren contacto íntimo con células estromales Al menos tres eventos de contacto críticos célula-célula se requieren para la generación de respuestas inmunes El primero es el contacto inicial de un antígeno específico con una célula T sencilla En razón del requisito para la presentación MHC, este es un evento de contacto de célula obligado En situaciones normales la célula que presenta antígeno crítico es la célula dendrítica Además de la interacción MHC/péptido-TCR existen otros pares ligando receptor unidos a membrana específicos de no antígeno que son importantes para la interacción de células dendríticas-células T La principal es el asocio de la molécula CD28 sobre la célula T con cualquiera de dos ligandos, B7 1 (CD80) y B7 2 (CD86), sobre la célula dendrítica Estas moléculas son denominadas moléculas accesorias y se entenderá que la molécula CD28 suministra una segunda señal esencial a la célula T sin la cual la célula T no se activa

Un segundo contacto esencial célula-célula es entre la célula T activada y la célula B específica de antígeno La mayoría

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de los antígenos son dependientes de las células T, esto es, una respuesta de anticuerpo al antígeno requiere absolutamente la ayuda de la célula T Esta ayuda se suministra tanto por la citoquina como por el contacto célula-célula Las células unen antígeno específico vía el Ig de superficie, luego internalizan, procesan, y lo presentan sobre las moléculas MHC Clase II Esto les posibilita ser reconocidas por las células T específicas para los epítopos ayudantes del antígeno específico Esta interacción célula-célula también requiere el CD28 que se une al B7 sobre la célula B Además, la molécula denominada ligando CD40 o CD154, cuya expresión se induce luego de la activación de la célula T, se une a la CD40 sobre las células B La reticulación de la CD40 promueve la proliferación de la célula B, evita la apoptosis de la célula B centro germinal, y promueve la conmutación y cambio del isótopo de inmunoglobulina Las interacciones de ligando del receptor CD28-B7 y CD40-CD40L son ambas esenciales para el dialogo entre las célula B y T que originan su activación mutua

Una tercera interacción célula- célula que es esencial en las respuestas inmunes es la unión de las célula B activadas (que han migrado a una estructura especializada en órganos linfoides denominados centros germinales) a células dendríticas foliculares (FDC) Las FDC son células estromales especializadas que mantienen intacto, es decir, sin procesar

el antígeno sobre su superficie en la forma de complejos inmunes de larga vida Entre otras moléculas, las FDC expresan CD23, que se unen a las células B centro germinales por vía de un receptor CR2 y estimulan la diferenciación a célula de plasma

Se requiere tiempo antes que la respuesta inmune primaria sea efectiva como una defensa de huésped Los antígenos tienen que ser reconocidos, tomados, digeridos, procesados y presentados por los APC Unas pocas células Th seleccionadas deben reaccionar con antígeno y responder, los linfocitos B y T preexistentes deben encontrar el antígeno y proliferar y diferenciarse en células efectuadoras (células de plasma o células Tc) En el caso de inmunidad mediada por anticuerpo, el nivel del anticuerpo tiene que construir a una concentración fisiológica efectiva para hacer su huésped resistente Esto puede tomar varios días o semanas alcanzar un nivel de inmunidad efectivo, aunque esta inmunidad puede persistir para muchos durante muchos meses o años, o aún el tiempo de vida, debido la presencia de los anticuerpos En infecciones naturales, el inóculo es pequeño, y aunque el estímulo antigénico se incrementa durante la replicación microbiana, solamente pequeñas cantidades de anticuerpo se forman en los primeros pocos días, y el anticuerpo circulante no es detectable hasta aproximadamente una semana después de la infección

Con relación a la inducción de una respuesta inmune secundaria, se entiende que a la re-exposición a los antígenos microbianos (exposición secundaria al antígeno), existe una respuesta inmunológica acelerada, es decir, la respuesta secundaria o de memoria Cantidades más grandes de anticuerpos se forman en solamente 1-2 días Esto se debe a las actividades de las células B de memoria específica o a las células T de memoria que fueron formadas durante la respuesta inmune primaria Estas células de memoria, cuando se estimulan por el antígeno, "recuerdan" haber visto previamente el antígeno, y son capaces de dividirse rápidamente y diferenciarse en células efectuadoras Estimular las células de memoria para producir rápidamente muy altos niveles (efectivos) de anticuerpos circulantes persistentes es la base para dar vacunas tipo "potenciadas" a humanos y animales domésticos Así, luego de la primer exposición al antígeno la respuesta inmune (como se evidencia en lo que sigue la concentración del anticuerpo específico en el suero) desarrolla globalmente durante un periodo de días, y alcanza una meseta baja entre 2 a 3 semanas y usualmente inicia su declinación en un relativamente corto periodo de tiempo Cuando el antígeno se encuentra una segunda vez, una respuesta secundaria (memoria) origina una rápida elevación en la concentración del anticuerpo, alcanzando un nivel mucho más alto en el suero, que puede persistir durante un periodo

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relativamente largo de tiempo Un nivel de protección del anticuerpo se puede alcanzar mediante una exposición primaria sola, pero usualmente para asegurar un alto nivel del anticuerpo protector que persiste durante un largo periodo de tiempo, es necesario tener estimulación antigénica repetida del sistema inmune

Una molécula de inmunoglobulina (abreviada Ig), es una proteína multimérica compuesta de dos polipéptidos de cadena ligera idénticos y dos polipéptidos de cadena pesada idénticos (H_2L_2)que están unidos en un complejo macromolecular mediante enlaces disulfuro intercadena, es decir enlaces covalentes entre los grupos sulfidrilo y los residuos de cisteína adhesivos Existen varias clases de proteinas de anticuerpo humano, cada una de las cuales se produce por un clon específico de células de plasma Cinco clases de inmunoglobulina humana se definen sobre la base de su composición de cadena pesada, y son denominadas IgG, IgM, IgA, IgE, e IgD Los anticuerpos de clase IgG y de clase IgA están además divididos en subclases a saber, IgG1, IfG2, IgG3, y IgG4 e IgA1 y IgA2 Las uniones disulfuro intracadena une diferentes áreas de la misma cadena de polipéptido, lo que da como resultado la formación de aros que, junto con los aminoácidos adyacentes, constituyen los dominios de la inmunoglobulina En la porción amino terminal (también denominada el "NH_2-terminal" o el "N-terminal"), cada cadena

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ligera y cada cadena pesada tienen una región variable simple que muestra variación considerable en la composición de aminoácido de un anticuerpo a otro La región variable de cadena ligera, V_L, se asocia con la región variable de la cadena pesada, V_H, para formar el sitio de unión de antigeno de la inmunoglobulina, denominado el Fv

Además de las regiones variables, cada una de las cadenas de anticuerpo tiene una o más regiones constantes Las cadenas ligeras tienen un dominio de región constante simple Así, las cadenas ligeras tienen una región variable y una región constante Las cadenas pesadas tienen varios dominios de región constante Las cadenas pesadas de los anticuerpos IgG, IgA, e IgD tienen tres dominios de región constante, que son designados CH1, CH2, y CH3, y las cadenas pesadas en los anticuerpos IgM e IgE tienen cuatro dominios de región constante, CH1, CH2, CH3, y CH4 Así, las cadenas pesadas tienen una región variable y tres o cuatro regiones constantes La estructura de la inmunoglobulina y la función son revisadas, por ejemplo, en Harlow *et al* , Eds , *Antibodies A Laboratory Manual,* Chapter 14, Cold Spring Habor Laboratory, Cold Spring Harbor (1988)

Las cadenas pesadas de las inmunoglobulinas también se pueden dividir en tres regiones funcionales la región Fd (un fragmento que comprende V_H y CH1, es decir, los dos dominios

N-terminales de la cadena pesada), la región de pivote, y la región Fc (la región del "fragmento cristalizable", derivado de las regiones constantes y formadas después de la digestión de pepsina) La región Fd en combinación con la cadena ligera forma un Fab (en "fragmento de unión de antígeno") En razón a que el antígeno reaccionará esteroquímicamente con la región de unión de antígeno en cada amino terminal de cada Fab la molécula IgG es divalente, es decir, se puede unir a dos moléculas de antigeno El Fc contiene los dominios que interactúan con los receptores de inmunoglobulina sobre las células y con los elementos iniciales de la cascada complemento Así, el fragmento Fc es generalmente considerado responsable de cada función efectuadora de una inmunoglobulina, tal como la fijación complemento de la unión a los receptores Fc La pepsina algunas veces también se cliva antes del tercer dominio constante (CH3) de la cadena pesada para dar un fragmento largo F(abc) y un fragmento pequeño pFcb Estos términos son también utilizados para regiones análogas de otras inmunoglobulinas La región de pivote, encontrada en anticuerpos clase IgG IgA e IgD, actua como un espaciador flexible, que le permite a la porción Fab moverse libremente en el espacio Como contraste a las regiones constantes, los dominios de pivote son estructuralmente diversos, variando tanto en secuencia como en longitud entre las clases y subclases de inmunoglobulina

Por ejemplo, la longitud y flexibilidad en la región de pivote varía entre las subclases IgG La región de pivote del IgG1 comprende según lo reportado los aminoácidos 216-231 y por que esta es libremente flexible, los fragmentos Fab pueden rotar alrededor de sus ejes de simetría y moverse dentro de una esfera centrada en el primero de dos puentes disulfuro de cadena interpesada El IgG2 tiene un pivote más corto que el IgG1, segun lo reportado 12 residuos de aminoácido y cuatro puentes disulfuro A la región del pivote del IgG2 le falta un residuo glicina, es este relativamente corto y contiene una doble hélice de poli-prolina rígida, estabilizada con puentes disulfuro de cadena interpesada extra Estas propiedades restringen la flexibilidad de la molécula IgG2 El IgG3 difiere de las cuatro subclases de su única región de pivote extendida (aproximadamente cuatro veces tan larga como el pivote IgG1), y se reporta para contener 62 aminoácidos (incluyendo 21 prolinas y 11 cisteínas), que forman una hélice doble de poli-prolina inflexible En el IgG3 los fragmentos Fab están relativamente alejadas del fragmento Fc, dándole a la molécula una mayor flexibilidad El pivote alongado en el IgG3 también es responsable por su mayor peso molecular comparado con las otras subclases La región de pivote del IgG4 es más corta que aquella del IgG1 y su flexibilidad es intermedia entre el IgG1 y el IgG2 La flexibilidad de la región de pivote según lo reportado disminuye el orden IgG3 > IgG1 > IgG4 > IgG2

Las cuatro subclases IgG también difieren una de la otra con respecto a sus funciones efectuadoras Esta diferencia se relaciona con las diferencias en la estructura, que incluyen con respecto a la interacción entre la región variable, los fragmentos Fab, y el fragmento Fc constante Sin embargo, además de la glicolización dentro de la región CH2, por ejemplo, y a pesar del conocimiento no existen reglas establecidas o convenciones con relación a los medios o métodos para cambiar las características, incluyendo secuencias, de estas subclases de molécula para cambiar, controlar, agregar, o remover diferentes funciones, por ejemplo, ADCC, CDC, y otras funciones

De acuerdo con los estudios cristalográficos, la región de pivote de la inmunoglobulina puede ser adicionalmente subdividida funcionalmente en tres regiones la región de pivote superior, la región núcleo, y la región de pivote inferior Shin et al , 1992 *Innunological Reviews* 130 87 La región de pivote superior incluye los aminoácidos desde el extremo carboxilo del CH1 al primer residuo en el pivote que restringe el movimiento, generalmente el primer residuo de cisteína que forma una unión disulfuro intercadena entre las dos cadenas pesadas La longitud de la región de pivote superior se correlaciona con la flexibilidad del segmento del anticuerpo La región de pivote núcleo contiene los puentes disulfuro de cadena interpesada, y la región de pivote

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inferior une los extremos aminoterminales del dominio CH2 e incluye los residuos en el CH2 La región de pivote núcleo del IgG1 humano contiene una secuencia Cys-Pro-Pro-Cys la cual, cuando se dimeriza por la formación de iones disulfuro, da como resultado un octapéptido cíclico que se considera que actúa como un pivote, confiriendo así flexibilidad La región de pivote también puede contener uno o más sitios de glicolización, que incluye un numero de tipos estructuralmente distintos de sitios de uniones carbohidrato Por ejemplo, el IgA1 contiene cinco sitios de glicolización dentro de un segmento de 17 aminoácidos de la región de pivote, que reconfiere resistencia del polipéptido de región de pivote a las proteasas intestinales, considerada una propiedad ventajosa para una inmunoglobulina secretoria

Los cambios conformacionales permitidos por la estructura de la flexibilidad de la secuencia de polipéptido de la región de pivote de la inmunoglobulina también puede aceptar las funciones efectuadoras de la porción Fc del anticuerpo Tres categorías generales de la función efectuadora asociada con la región Fc incluyen (1) la activación de la cascada complemento clásica, (2) la interacción con las células efectuadoras, y (3) la compartimentación de las inmunoglobulinas Las diferentes subclases IgG humanas varían en relación a la eficacia con la cual ellas fijan el complemento, o activan y amplifican la etapa de la cascada

complemento Ver, por ejemplo, Kirschfink 2001 *Immunol Rev* 180 177, Chakraborti *et al* , 2000 *cell Signal* 12 607, Kohl *et al* , 1999 *Mol Immunol* 36 893, Marsh *et al* , 1999 *Curr Opin Nephrol Hypertens* 8 557, Speth *et al* , 1999 *Wien Klin, Wochenschr* 111 378

La citotoxicidad dependiente de complemento (CDC) se cree que es un mecanismo significativo para despejar las células objetivo específicas tales como las células tumorales El CDC es una corriente de eventos que consiste de una serie de enzimas que se activa una por la otra en forma de cascada El complemento tiene un papel importante en la limpieza de antígeno, lograda por sus cuatro funciones principales (1) vasodilatación local, (2) atracción de las células inmunes, específicamente fagocitos (quimiotaxis), (3) etiquetado de los organismos extraños para fagocitosis (opsonización), y (4) destrucción de los organismos invasores por medio del complejo de ataque de membrana (ataque MAC) La molécula central en la proteína C3 Esta es una enzima que se divide en dos fragmentos por los componentes de cualquiera de las sendas clásicas o de la senda alternativa Los anticuerpos, específicamente IgG e IgM, inducen la senda clásica aunque la senda alternativa no es específicamente estimulada por los productos bacterianos como el lipopilisacárido (LPS) En resumen, los productos de del C3 se dividen incluyendo un péptido pequeño C3a que es quimiotáctico para las células

inmunes fagocíticas y da como resultado una vasodilatación local al originar la liberación del fragmento C5a del C5 La otra parte del C3, C3b recubre los antígenos sobre la superficie del organismo extraño y actúa para opsonizar el organismo para destrucción El C3b también reacciona con otros componentes del sistema complemento para formar un MAC que consiste de C5b, C6, C7, C8 y C9

En general, el IgG1 y el IgG3 fijan el complemento más efectivamente, el IgG2 es menos efectivo, y el IgG4 no activa el complemento La activación del complemento se inicia por la unión C1q, una subunidad del primer componente C1 en la cascada, a los complejos antígeno-anticuerpo Aunque el sitio de unión para el C1q está cargado en el dominio CH2 del anticuerpo, la región de pivote influencia la capacidad del anticuerpo para activar la cascada Por ejemplo, las inmunoglobulinas recombinantes a las que les falta la región de pivote son de acuerdo con lo reportado y capaces de activar complemento Shin et al , 1992 Sin la flexibilidad conferida la región de pivote la porción Fab del anticuerpo unida al antígeno puede no ser capaz de adoptar la conformación requerida para permitir al C1q unirse al CH2 *ver id* La longitud de pivote y la flexibilidad segmental se han reportado por correlacionarse con la activación complemento, sin embargo, la correlación no es absoluta Las moléculas IGg3 humanas con regiones de pivote alteradas que

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son tan rígidas como el IgG4, por ejemplo, pueden aún activa efectivamente la cascada

La ausencia de una región de pivote, o la falta de una región de pivote funcional, también puede afectar la capacidad de ciertas inmunoglobulinas IgG humanas para unirse a los receptores Fc sobre las células efectuadoras La unión de una inmunoglobulina a un receptor Fc facilita la citotoxicidad mediada por célula dependiente del anticuerpo, la cual como se anotó anteriormente se presume que es un mecanismo importante para la eliminación de las células tumorales La familia del receptor IgG (FcR) se divide en tres grupos, Fcγ(RI(CD64), que es capaz de unirse al IgG con una alta afinidad, y el Fcγ(RII (CD32) y el Fcγ(RIII (CD16), ambos los cuales tienen receptores de afinidad inferior La interacción molecular entre cada uno de los tres receptores y una inmunoglobulina no se ha definido precisamente, pero la evidencia experimental indica que los residuos en la región próxima de pivote del dominio CH2 puede ser importante a la especificidad de la interacción entre el anticuerpo y el receptor Fc Las proteínas de mieloma IgG1 y los anticuerpos quiméricos IgG3 recombinantes a los que les falta una región de pivote son incapaces de sacuelo con lo reportado a unirse al FcγRI quizás por que la accesibilidad al CH2 se disminuye Shin et al , 1993 *Inter, Rev Immunol* 10 17, 178-79

Las excepciones inusuales y aparentemente evolucionalmente no relacionadas con la estructura H_2L_2 de los anticuerpos convencionales ocurren algunos tipos de inmunoglobulinas encontrados en camélidos (camellos, dromedarios y llamas, Hamers-Casterman et al , 1993 *Nature* 3363 446, Nguyen *et al* , 1998 *J Mol Biol* 275 413), tiburones nodriza (Roux *et al* , 1998 *Proc Nat Acad Sci* USA 95 11804), y peces ratas, manchados (Nguyen et al , "Heavy-Chain antibodyes in Camelidae, a case of evolutionary innovation", 2002 *Immunogenetics*)54(1) 39-47) Estos anticuerpos pueden formar aparentemente regiones de unión a antígeno que utiliza solamente la región variable de cadena pesada, es decir, estos anticuerpos funcionales son homodímeros de cadenas pesadas solamente (denominados como anticuerpos de cadena pesada"o "HCAbs") En ambas especies, estas regiones variables a menudo contienen una tercera región de complementariedad determinante extendida (CD3) que puede ayudar a compensar la falta de regiones variables de cadena ligera, y existen frecuentes uniones disulfuro entre las regiones CDR que presumiblemente ayudan estabilizar el sitio de unión Muyldermans et al , 1994 *Prot Engineer*, 7 1129, Roux et al Sin embargo, la función precisa de la sola cadena pesada "anticuerpos" es desconocida, y la presión evolucionaria que conduce a su formación no se ha identificado Ver, por ejemplo, Nguyen, et al , 2002 *supra* Los camélidos, incluyendo los camellos, llamas, y alpacas,

también expresan anticuerpos H_2L_2 convencionales, y los anticuerpos de solo cadena pesada no parecen así estar presentes en estos animales simplemente como una estructura de anticuerpo alternativa

Las regiones variables (V_HH) de las inmunoglobulinas de solo cadena pesada de camélido y las regiones variables de cadena pesada convencionales (H_2L_2) contienen diferencia de aminoácido, que incluyen diferencias en varias posiciones que se pueden involucrar en la interferencia entre los dominios V_H y V_L convencionales Nguyen *et al* , 1998 J *Mol* Biol 275 413, Muyldermans et al , 1994 Prot *Engineer* 7 1129 Los V_HH de camélido según los reportes se recombina con las regiones constantes IgG2 e IgG3 que contienen los dominios pivote, CH2 y CH3, y les falta el dominio CH1 Hamers-Casterman et al , 1993 Nature 363 446 De manera interesante, los V_HH son codificados por un locus cromosomal distinto del locus V_H (Nguyen et al , 1998, *supra*)que indica que las células B de camélido han evolucionado mecanismos complejos de reconocimiento de antígeno y diferenciación Así, por ejemplo, el IgG1 en llama es un isótopo de anticuerpo convencional (H_2L_2) en el cual el V_H se recombina con una región constante que contiene los dominios pivote CH1, CH2 y CH3 mientras que el IgG2 y el IgG3 de llama son isótopos de solo cadena pesada a los que les falta los dominios CH1 y que no contienen cadenas ligeras

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Las clases de inmunoglobulina tienen diferentes características físicas y químicas y ellas exhiben propiedades biológicas únicas Su síntesis ocurre en diferentes etapas y proporciones durante una respuesta inmune y/o durante el curso de una infección Su importancia y funciones en resistencia del huésped (inmunidad) son diferentes

La inmunoglobulina G(IgG), una proteína con peso molecular de aproximadamente 150,000 daltons (150kD), es la Ig predominante en el suero Esta corresponde aproximadamente 80% del anticuerpo total encontrado en un animal en cualquier momento dado, que es 75% del anticuerpo de suero total Esta puede difundirse hacia fuera del torrente sanguíneo en espacios extravasculares y es el Ig más común encontrado allí Su concentración de fluidos de tejido se incrementa durante la inflamación, y es particularmente efectiva en la neutralización de las toxinas y virus extracelulares bacteriales Esta también tiene la capacidad opsonizante y la capacidad de fijar complemento La composición de polipéptido de la región Fc y todas las moléculas de anticuerpo IgG1 son relativamente constantes sin importar la especificidad del anticuerpo, sin embargo, como se anotó anteriormente, la región Fab siempre difiere en su secuencia de aminoácido exacta dependiendo de su especificidad antigénica Las

regiones de aminoácido específico de la porción Fc de la molécula son reconocidas por los receptores sobre fagocitos y ciertas otras células, y el dominio Fc contiene una región de péptido que se unirá y complemento activo que es a menudo requerido para la manifestación de la inmunidad mediada por anticuerpo En razón a que la molécula IgG es divalente, ésta puede reticular moléculas de antígeno que puede conducir a la precipitación o aglutinación de los antígenos, si el IgG está unido al antígeno sobre una célula microbiana o de superficie, su región Fc puede suministrar un ligando extrínseco que será reconocido por los receptores específicos sobre fagocitos Las células microbianas o los virus recubiertos con moléculas IgG son opsonizados para fagocitosis, y los patógenos opsonizados son tomados e instruidos mucho más fácilmente por los fagocitos que sus contrapartes no opsonizadas El IgG, así como también el IgM y el IgA, neutralizarán la actividad de las toxinas, incluyendo las exotocinas bacterianas Adicionalmente, las moléculas IgG reticulazas sobre la superficie de una célula se pueden unir a un complemento activo del suero y establecer una cascada de reacciones que puedan conducir a la destrucción de la célula

El IgM es la primera inmunoglobulina que aparece en el torrente sanguíneo durante el curso de una inyección Esta está principalmente confinada al torrente sanguíneo y

suministra protección contra patógenos de origen sanguíneo El IgM elabora hasta aproximadamente el 10% de las inmunoglobulinas del suero, y está dispuesta para semejar un pentámero de 5 moléculas de inmunoglobulina (que tienen un peso molecular de aproximadamente 900kD) atadas juntas en los dominios Fc Además de los enlaces covalentes entre las subunidades monoméricas, el pentámero se estabiliza por un polipéptido 15kD denominado cadena J El IgM, por lo tanto, tiene una "valencia" teórica de 10 (es decir tiene 10 dominios Fab expuestos) Probablemente, el papel más importante del IgM es la capacidad para funcionar tempranamente en las respuestas inmunes contra los patógenos de origen sanguíneo dando su eficacia al aglutinar antígenos en partículas El IgM también une el complemento fuertemente y los anticuerpos IgM unidos a una superficie microbiana actúan como opsoninos, haciendo el microbio más susceptible a fagocitosis En la presencia del complemento y las células microbianas completas IgM pueden ser muertas y lisadas Como se anotó anteriormente, el IgM también aparece en las superficies de las células B maduras como un monómero transmembranoso donde este funciona como un receptor antígeno, capaz de activar células B cuando se une al antígeno

La redisposición del gen en los lugares de la inmunoglobulina durante el desarrollo linfoide genera un repertorio de

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linfocitos B que expresan una diversidad de receptores antígenos La redisposición del gen, que es catalizada por la recombinasa del gen que activa la redisposición ("RAG"), integra los segmentos de gen V, D y J de inmunoglobulina para producir de manera productiva genes de inmunoglobulina productivamente redispuestos que codifican las cadena pesada y ligera de los anticuerpos IgM La diversidad de los anticuerpos IgM en este repertorio primario se logra a través de mecanismos combinatorios (la escogencia de los segmentos del gen V, D y J utilizada en un anticuerpo particular), asi como también la diversidad de unión La unión de los segmentos de V, D y J es un poco imprecisa, y los nucleótidos se pueden insertar en la unión de una manera no molde Existe por lo tanto un alto grado de diversidad resultante en los límites V-D-J Esto contribuye de una manera principal a la diversidad estructural de la tercera región de complementariedad determinante del anticuerpo, una región que a menudo juega un papel crítico en el reconocimiento de antígeno Este repertorio primario de los anticuerpos IgM comprende unos pocos millones de diferentes estructuras El tamaño de este repertorio significa que cualquier antígeno entrante es probablemente encontrado en un anticuerpo que lo reconoce con afinidad aceptable Un sitio de unión de alta afinidad es poco probable que esté disponible para la mayoría de los antígenos entrantes (el repertorio no es suficientemente grande), pero la afinidad de los anticuerpos

IgM disponibles en el repertorio primario variará de antígeno a antígeno Si el epítopo se reitera en alta afinidad sobre la superficie del antígeno (por ejemplo, una estructura repetida sobre la superficie de un virus o bacteria), entonces el anticuerpo IgM puede sin embargo ser efectivo para mediar la limpieza del organismo, a pesar de la baja afinidad de la interacción individual entre el epítopo antigénico y el sitio de combinación de inmunoglobulina La densidad de los epítopos también puede permitir las interacciones multivalentes con IgM, conduciendo a una interacción de alta avidez, siempre y cuando pueda ocurrir un espaciamiento adecuado de los epítopos antigénicos Sin embargo, para asegurar una repuesta efectiva y especifica, especialmente cuando la concentración de antígeno es baja (como puede ocurrir cuando el cuerpo está enfrentado con un número muy pequeño de partículas virales infectantes), sería preferible si los anticuerpos de alta afinidad estuvieran disponibles para neutralizar, por ejemplo, un mecanismo infectante El tamaño del repertorio primario milita contra la probabilidad de tales anticuerpos de alta afinidad que estén presentes en este repertorio El sistema inmune por lo tanto opera utilizando una estrategia de dos etapas El repertorio primario y los anticuerpos IgM se generan por un proceso de redisposición de gen y tienen lugar antes de que el antígeno se encuentre durante el desarrollo de linfocito temprano Sin embargo, una vez que el antígeno extraño se ha

encontrado, estas células B en el repertorio primario que codifican anticuerpos adecuados (aunque de baja afinidad) son selectivamente expandidos y sometidos en alternación iterativa de hipermutación dirigida y selección mediada por antígeno Esto permite una maduración significativa de la afinidad de los anticuerpos específicos de antígeno que son producidos El antígeno que se dispara también maneja recombinación conmutada de isotipo Así, en la ausencia de una estimulación de antígeno externa y cualquier inmunoglobulina maternalmente derivada, el suero solamente contendrá una diversidad de moléculas IgM no mutadas que han sido generadas por redisposición de gen Este repertorio cambia con la edad como resultado de la exposición continua de antígeno, de tal forma que la mayoría de la inmunoglobulina de suero en animales más viejos está compuesta de moléculas mutadas IgG (e IgA) cuyas especificidades se han desarrollado como consecuencia de la selección de antígeno

El IgA existe como un monómero H_2L_2 de aproximadamente 160kD en suero y, en secreciones, como un dimero del monómero H_2L_2 de aproximadamente 400 kD Como con el IgM, la polimerización (dimerización) es vía de una cadena J El IgA tiene dos subclases basadas en diferentes cadenas pesadas, IgA1 e IgA2 El IgA1 es producido en médula ósea y elabora mayoría del suero IgA Tanto el IgA1 como el IgA2 son sintetizados en

GALT (tejidos linfoides asociados a tripa) para ser secretados sobre las superficies de mucosa En razón a que el IgA puede ser sintetizado localmente y secretado en secreciones seromucosa del cuerpo, este algunas veces se denomina como anticuerpo secretorio o slgA Cuantitativamente, el IgA se sintetiza en cantidades mayores que el IgG, pero tiene una vida corta en el suero (6 días), y se pierde en los productos secretorios La concentración del IgA en el suero es aproximadamente 15% del anticuerpo total La secreción del IgA dimérico es mediada por una glicoproteína de 100 kD denominada componente secretorio Esta es la adición de la pieza secretoria a las moléculas IgA que cuenta por su capacidad para salir del cuerpo a las superficies de la mucosa por vía de las glándulas exocrinas El IgM se puede transportar de manera similar y hacer una proporción pequeña de los anticuerpos secretorios El IgA secretorio es la inmunoglobulina predominante presente en los fluidos gastrointestinales, las secreciones nasales, la saliva, las lágrimas y otras secreciones de mucosa del cuerpo Los anticuerpos IgA son importantes en la resistencia a la infección de las superficies de mucosa del cuerpo, particularmente el respiratorio, intestinal y los tractos urogenitales El IgA actúa como un recubrimiento protector para las superficies de mucosa contra la adherencia microbiana o la colonización inicial Este también puede neutralizar la actividad de toxinas sobre las superficies de

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mucosa Los receptores Fc para los microorganismos recubiertos con IgA encontrados sobre monocitos y neutrófilos derivados de la mucosa respiratoria sugieren que el IgA puede tener un papel en el pulmón, al menos, en la opsonización de patógenos El IgA secretorio también se transfiere por vía de la leche, es decir, el calostro, proveniente de la madre lactante al recién nacido, el cual le suministra inmunidad pasiva a muchos patógenos, especialmente a aquellos que entran por vía del tracto GI

El IgE es una inmunoglobulina de aproximadamente 190 kD que cuenta con aproximadamente 0,002% de las inmunoglobulinas del suero total Esta se produce por vía de las células de plasma por debajo del epitelio respiratorio e intestinal La mayoría del IgE está unido a las células de tejido, especialmente los mastocitos Si un agente infeccioso tiene éxito en penetrar la barrera IgA, esta viene contra la siguiente línea de defensa, el sistema MALT (tejidos linfoides asociados a mucosas) que se maneja por el IgE El IgE está unido muy firmemente a los receptores IgE Fc específicos sobre los mastocitos El contacto con los antígenos conducen a la liberación de mediadores de inflamación provenientes de células mástiles, que reclutan efectivamente a varios agentes de la respuesta inmune que incluye complemento, factores quimiotácticos para fagocitos, células T, etc Aunque una manifestación bien conocida de ésta reacción es un tipo de

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reacción de hipersensibilidad inmediata denominada alergia atópica (por ejemplo urticaria, asma, fiebre de heno etc) Las respuestas MALT actúan como un mecanismo de defensa por que ellos amplifican la respuesta inflamatoria y pueden facilitar el rechazo del patógeno

El IgE es una molécula de aproximadamente 175 kD que se semeja al IgG en su forma monomérica Los anticuerpos IgD se encuentran en la mayor parte de la superficie de linfocitos B Las mismas células pueden también llevar anticuerpo IgM Como se notó anteriormente, se cree que el IgD y el IgM funcionan como receptores de antígeno mutuamente interactuantes para controlar la activación y la supresión dé la célula B De esta manera, el Ig puede tener una función inmunoregulatoria

Además de la opsonización, la activación del complemento, y el ADCC los anticuerpos tienen otras funciones en la defensa del huésped incluyendo impedimento estérico, neutralización de toxina, aglutinación y precipitación Con relación al impedimento estérico, se entiende que los anticuerpos se combinan con las superficies de los microorganismos y pueden bloquear o evitar su unión a las células susceptibles o a las superficies de mucosa El anticuerpo contra un componente viral puede bloquear la unión del virus a las células huéspedes susceptibles y reducir de esta manera la

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inefectividad El IgA secretorio puede bloquear la unión de patógenos a la superficie de mucosa Los anticuerpos que neutralizan toxina (antitoxinas) pueden también reaccionar con la toxina bacterial soluble y bloquear la interacción con la toxina con su célula o sustrato objetivo específico Los anticuerpos también pueden combinarse con las superficies de los microorganismos o los antígenos solubles y hacer que ellos se aglutinen o precipiten Esto reduce el número de unidades infecciosas separadas y las hace más fácilmente fagocitosadas en razón a que el agrupamiento de partículas es mayor en tamaño Los fagocitos pueden remover flóculos o agregados de toxinas neutralizadas

Los anticuerpos han sido propuestos para uso en terapia Los animales, que incluyen humanos y ratones tienen la capacidad de hacer los anticuerpos capaces para reconocer (mediante la unión a) virtualmente cualquier determinante antigénico y discriminar entre epítopos similares No solamente hacen suministrar la base para protección contra organismos enfermos, sino también hacen a los candidatos atractivos apuntar a otros tipos de moléculas encontrados en el cuerpo, tales como receptores u otras proteínas presentes en la superficie de la célula normales y las moléculas presentes unicamente en la superficie de las células cancerosas Así la especificidad notoria de los anticuerpos los hace agentes promisorios para terapia humana

De las preparaciones de anticuerpo iniciales disponibles para uso, tal como gamaglobulinas intravenosas, incluyen antisuero animal y humano que fueron utilizados *in vivo* para destruir bacterias (tétano, neumococos) y neutralizar virus (hepatitis A y B, rabia, citomegalovirus, y varicela zoster) en la sangre de individuos infectados Posiblemente la aplicación temprana más importante es el uso de endotoxinas Sin embargo, existen problemas asociados con el uso de anticuerpos en terapia humana por que la respuesta del sistema inmune a cualquier antígeno aún la más simple es "policlonal", es decir, el sistema elabora anticuerpos de un gran rango de estructuras tanto en sus regiones de unión así como también en sus regiones efectuadoras El tratamiento de anticuerpo policlonal también estuvo asociado con efectos colaterales indeseados Además de la naturaleza policlonal de estas preparaciones de anticuerpo, existe el riesgo de infección proveniente de virus contaminantes La enfermedad de suero y anafilaxis son también considerados factores limitantes Adicionalmente, aún si uno fuera a aislar una célula secretora de anticuerpos simples, y la ubicara en cultivo, esta moriría después de unas pocas generaciones en razón del crecimiento limitado potencial de todas las células somáticas normales

Hasta finales de los 70, los anticuerpos policlonales obtenidos del suero sanguíneo de los animales inmunizados suministraban la única fuente de anticuerpos para investigación o tratamiento de enfermedad El aislamiento de anticuerpos específicos era esencialmente imposible hasta que Kohler y Milstein descubrieron como hacer "anticuerpos monoclonales" que tendrían una especificidad simple, que serían todos similares debido a la elaboración de un clon simple de células de plasma y que podrían crecer indefinidamente La técnica se describió en una publicación de 1975 (*Nature* 256 495-97), y Kohler y Milstein recibieron en 1984 el Premio Nobel de Medicina por este trabajo

La primera etapa en la técnica de Kohler y Milstein para producción de anticuerpos monoclonales involucra inmunizar un animal experimental con el antígeno de interés En la mayoría de sus experimentos, Kohler y Milstein inyectaron un ratón con células sanguíneas rojas de oveja El cuerpo del ratón inicia una respuesta inmune e inicia la producción de anticuerpos específicos para el antígeno El bazo del ratón es entonces removido y las células B que producen el anticuerpo de interés son aisladas Las células que producen el tumor que han crecido en cultivo son entonces fusionadas con los linfocitos B utilizando polietilenglicol con el fin de producir un "hibridoma" Solamente los hibridomas que resultan de la fusión sobrevivirán El linfocito del bazo

tiene un intervalo de vida limitado, y así cualquiera de las células B que no se fusionaron con un mieloma morirán en el cultivo Adicionalmente, aquellas células a las que les falta el aspecto de producir anticuerpos de la célula B no secretaran la enzima HGPRT, que se requiere para crecer en el medio hipoxatina-aminopterintimidina (HAT) El medio HAT sobre el cual crecen las células, y mide la senda para la síntesis de nucleótido Las sendas que producen HGPRT pueden desviarse de esta senda y continuar creciendo Al ubicar las células fusionadas en un medio HAT, los hibridomas verdaderos pueden ser aislados Las células de hibridoma aisladas son entonces seleccionadas para especificidad al antígeno deseado En razón a que cada hibridoma desciende de una célula B, este hace copias de solamente un anticuerpo El hibridoma que produce el anticuerpo de interés crece en cultivo para producir grandes cantidades de anticuerpos monoclonales, que son entonces aislados para uso adicional La técnica es denominada hibridización de células somáticas, y el hibridoma resultante (seleccionado tanto por inmortalidad como por producción del anticuerpo específico de interés) se puede cultivar indefinidamente, es decir, es una línea celular potencialmente inmortal

Los anticuerpos monoclonales no son ampliamente utilizados como reactivos de diagnóstico e investigación Sin embargo, su introducción a la terapia humana ha sido mucho más lenta

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Una dificultad principal en los anticuerpos de ratón son "vistos" por el sistema inmune humano como extraño El paciente humano monta una respuesta inmune contra estos, produciendo HAMA ("anticuerpos antiratón humano") Esto no solamente origina que los anticuerpos terapéuticos sean rápidamente eliminados del huésped, sino también de los complejos inmunes que originan daño a los riñones

Se han utilizado dos aproximaciones en un intento de reducir el problema del HAMA El primero es la producción de anticuerpos quiméricos en los cuales la parte de unión de antígeno (región variable) de un anticuerpo monoclonal de ratón que se fusiona con la parte efectuadora, región constante) del anticuerpo humano utilizando ingeniería genética En una segunda aproximación, los anticuerpos de roedor se han alterado a través de técnicas conocidas como injerto de región determinante de complementariedad (CDR) o "humanización" En este proceso, los sitios de unión de antígeno, que se forman mediante tres CDR de la cadena pesada y tres CDR de la cadena ligera, son extraídos de las células que secretan los roedores mAb e injertadas en el ADN que codifica para la estructura del anticuerpo humano En razón a que solamente el sitio de unión de antígeno CDR, el lugar del dominio de variable completo del anticuerpo de roedor es trasplantado, el anticuerpo humanizado resultante (una segunda generación o anticuerpo "hiperquimérico") es de

acuerdo con lo reportado menos inmunogénico que una primera generación de anticuerpo quimérico Este proceso ha sido adicionalmente mejorado para incluir cambios denominados como "reconformación" (Verhoeyen, et al , "Reshaping human antibodies grafting an anti-lysozyme activity", 1988 *Science* 239 1534-1536, Riechman, *et al* , "Reshaping antibodies for therapy", 1988 *Nature* 332 232-337, Tempest, *el al* , "Reshaping human monoclonal antibody to inhibit respiratory ayncitial virus infection in vivo", *Bio/Technol* 1991 9 266-271), "hiperquimerización" (Queen, et al , "A humanized antibody that binds to the human interleukin 2 receptor," 1989 *Proc Natl Acad Sci* USA 10029-10033, Co, *et al* , "Hmanized antibodies for antiviral Therapy," 1991 *Proc Natl Acad Sci* USA 88 2869-2873, Co, *et al* , "Chimeric and humanized antibodies with specificity for the CD33 antigen," 1992 *J Immunol* 148 1149-1154), y "revestimiento" (Mark, *et al* , "Derivation of therapeutically active humanized and veneered anti-CD18 antibodies In Metcalf BW, Dalton BJ, eds Cellular adhesion molecular definition to therapeutic potencial New York Plenum Press, 1994 291-312)

En el proceso de reconformación sobre la base de homología, la región variable del roedor es comparada con la secuencia consenso del subgrupo de la secuencia de proteína al cual éste pertenece De manera similar, la región constante humana seleccionada que acepta la estructura es comparada con su

secuencia consenso de familia Gussowal, *et al* , "Humanization of monoclonal antibodies " 1991 *Meth Enzymol* 201 99-121 El análisis de secuencia identifica los residuos, que pueden haber sufrido mutación durante el procedimiento de maduración de afinidad y pueden por lo tanto ser idiosincráticos La inclusión de los residuos humanos más comunes se dice que disminuye los problemas de inmunogenicidad al remplazar los residuos idiosincráticos aceptadores humanos Adicionalmente, el proceso de reconformación se dice que permite la comparación de las secuencias de consenso de humano y roedor para identificar las diferencias sistemáticas de las "especies" Los anticuerpos RSV19 son humanizados al emplear este procedimiento Taylor et al "Humanized monoclonal antibody to respiratory syncitial virus," 1991 *Lancet* 337 1411-1412, Tempest, *et al* , "Reshaping a human monoclonal antibody to inhibit human respiratory cyncitial virus infection in vivo," 1991 *Bio/Technol* 9 266-271

La hiperquimerización es un método alternativo para identificar las regiones CDR exteriores de los residuos que están probablemente involucradas en la construcción de la actividad de unión En este método, las secuencias humanas son comparadas con las secuencias de región variables de murino y aquella con homología más alta se selecciona como estructura aceptadora Como en el procedimiento de reconformación, los residuos "idiosincráticos" son

remplazados por residuos humanos de ocurrencia más comun Los residuos no CDR que pueden ser interactuantes con la secuencia CDR se identifican Finalmente, se determina cual de estos residuos va ser incluido en la estructura de región variable Los anticuerpos humanizados contra el antígeno CD33 se desarrollan de acuerdo con lo reportado por este método Co, *et al* , "Chimeric and humanized antibodies with specificity for the CD33 antigen," 1992 *J Immunol* 148 1149-154 Ver también Crter, et al , "Humanization of an anti-p185 HER2 antibody for human cancer therapy," 1992 *Proc Natl Acad Sci* USA 89 4285-4289

La superficie desplegada de la proteína es primariamente determinante en su inmunogenicidad Un anticuerpo de murino humanizado puede así ser hecho menos inmunogénico al remplazar los residuos expuestos que infieren de aquellos comúnmente encontrados en los anticuerpos humanos Este método de humanización se denomina como "revestimiento" El reemplazo apropiado de los residuos exteriores puede tener poco o ningún impacto en los dominios interiores o la estructura de inter dominio El revestimiento es un proceso de dos etapas En la primera etapa, las regiones variables humanas más homólogas se seleccionan y comparadas por cada uno de los residuos simples con las correspondientes regiones variables de ratón En la segunda etapa, los residuos presentes en el homólogo humano remplazan los residuos de

estructura de ratón, el cual difiere del homólogo humano Este reemplazo involucra solamente aquellos residuos que están sobre la superficie y al menos parcialmente expuesto

Sin embargo, le tomó más de un cuarto de siglo de investigación a la tecnología de anticuerpo monoclonal y a los métodos de ingeniería genética dar resultados en el desarrollo de moléculas de inmunoglobulina para el tratamiento de enfermedades humanas De hecho, no fue hasta pasados 5 años que los anticuerpos monoclonales se volvieron una clase expansiva de terapias Ver Glennie MJ y van de Winkel JG, *Drug Discov Today* 2003 Jun 1,8(11 503-10, Souriau C and Hudson PJ, "Recombinant antibodies for cancer diagnosis and therapy ," 2003 *Expert Opin Biol Ther* 3(2) 305-18 Ver también Pendley C, eyt al , "Immunogenicity of therapeutic monoclonal antibodies," 2003 *Curr Opin Mol Ther* 5(2) 172-9

Por lo mismo, un promedio de menos de un anticuerpo terapéutico por año se ha introducido en el mercado iniciando en 1986, 11 años después de la publicación de los anticuerpos monoclonales Cinco anticuerpos monoclonales de murino fueron introducidos en medicina humana durante un periodo de 20 años desde 1986-1995, incluyendo "muromonab CD3" (OrthoClone OKT3®), que se une a una molécula sobre las superficies de las células T y se lanzó en 1986 para evitar el rechazo agudo de trasplantes de órganos, "edrecolamb" (Panorex®), lanzado

en 1995 para el tratamiento de cáncer colorectal "odulimomab" (Antilfa®), lanzado en 1997 para uso en rechazo de trasplante, y, "ibritumomab" (Zevalin®yiuxetan), lanzado el 2002 para uso en linfoma no Hodgkin Adicionalmente, un Fab monoclonal "abciximab" (ReoPro®), fue lanzado en 1995 Este inhibe el apelmazamiento de plaquetas al unir lo receptores sobre su superficie que normalmente están ligadas por fibrinógeno y pueden ser de ayuda para prevenir el retaponamiento de las arterias coronarias en pacientes que han sufrido de angioplasia Tres anticuerpos monoclonales quiméricos también fueron lanzados "rituximab" (Rituxan®), en 1197, que se une a la molécula CD20 encontrada en la mayoría de las células B y se utiliza para tratar linfomas de célula B, "basilixímab" (Simulect®), en 1998 para rechazo de transplante, "infliximab" (Remicade®) que se une al factor de necrosis tumoral alfa (TNF-α), en 1998 para tratamiento de artritis reumatoide y enfermedad de Crohn's Adicionalmente, "abcixímab" (ReoPro®), un fragmento Fab de 47 6 kD del anticuerpo monoclonal humano-murino quimérico 7E3 que se une al receptor IIb/IIIa del hidroproteína (GP) de plaquetas humanas, se lanzo en 1995 como un adjunto para la intervención coronaria pericutánea para la prevención de complicaciones isquémicas cardiacas en pacientes que sufren de intervención coronaria percutánea Finalmente, siete monoclonales "humanizados" fueron lanzados desde 1997-2003 "daclizumab" (Zenapax®) en 1997, que unen una parte del

receptor IL-2 producido en al superficie de las células T activadas que se utilizan para evitar rechazo agudo de riñones transplantados, "palivizumab" (Synagis®) en 1998 para RSV, "trastuzumab" (Herceptin®) en 1998, el cual se une a HER-2, un receptor de factor de crecimiento encontrado en células cancerosa de mama, "gemtuzumab" (Mylotarg®) en 2000, el cual es un conjugado de un anticuerpo monoclonal que se une a CD33, una molécula de superficie celular expresada por la células cancerosas en leucemia mielogena aguda (AML) pero no encontrada en células madre normales necesitadas de repoblar la medula ósea, y "alemtuzumab" (MabCampath®) en 2001, que se une al CD52, una molécula encontrada en células sanguíneas blancas y que ha producido remisión temporal de leucemia linfocítica crónica, "adalimumab" (Humira® (D2E7)), un monoclonal anti-TNF humano que contiene regiones variables de cadena pesada y cadena ligera de derivados de humano que contienen monoclonal anti-TNF humano y regiones constantes IgG k humano se lanzaron en 2002 para el tratamiento de artritis reumatoide, y, "omalizumab" (Xolair®), que se une al IgE y que evita unirse a células mástiles fue aprobada en el 2003 para tratamiento de adultos y adolescentes sobre los doce años de edad con asma moderada a severa persistente que tienen un ensayo a una prueba de test positiva o una reactividad in vitro a alergeno perenne cuyo síntomas son inadecuadamente controlados con corticosteroides inhalados

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Así, la ingeniería de proteínas se ha fijado en un esfuerzo para disminuir problemas relacionados con la inmunogenicidad de polipéptidos de inmunoglobulina recombinantes administrados y para tratar de alterar las funciones efectuadoras de anticuerpos Sin embargo, los problemas permanecen Por ejemplo, la mayoria de los pacientes de cáncer tratados con rituximab recaen, generalmente a los 6-12, y se han reportado reacciones de infusión fatales a las 24 horas de la infusión de rituximab Esta reacciones fatales seguidas por complejo de reacción de infusión que incluyeron hipoxia, infiltrados pulmonares, a síndrome de zozobra respiratoria aguda, infarto al miocardio, fibrilación ventricular o choque cardiogénico La falla real aguda que requiere diálisis con casos de resultados fatales se ha reportado en el establecimiento del síndrome de lisis tumoral Luego de tratamiento con rituximab, por tener severa reacciones mucocutáneas, algunas con resultados fetales Adicionalmente, altas dosis de rituximab se requieren para inyección intravenosa porque la molécula es grande, aproximadamente 150 kDa, y la infusión esta limitada con los tejidos linfoides donde residen muchas células tumorales

La administración de trastuzumab puede dar como resultado un desarrollo de disfunción ventricular y falla cardiaca congestiva, y la incidencia y severidad de la disfunción cardiaca se ha reportado por ser particularmente alta en

pacientes que reciben trastuzumab en combinación con antraciclinas y ciclosfosfamida La administración de trastuzumab también puede dar como resultado severas reacciones hipertensivas (incluyendo anafilaxis), reacciones de infusión, y eventos pulmones

Los pacientes que reciben terapia inmunosupresiva de daclizumab están en riesgo creciente de desarrollar desórdenes linfoproliferativos e infecciones oportunistas, y no se conoce si el uso de daclizumab tendrá efectos a largo plazo sobre la capacidad del sistema inmune a responder a antígenos primero encontrados durante la inmunosupresión inducida por daclizumab

La hepatotoxicidad, incluyendo enfermedad venooclusiva hepática severa (VOD), también se ha reportado en asocio con el uso de gemtuzumab como un agente simple, como parte de un régimen de quimioterapia de combinación, y en pacientes sin una historia de enfermedad hepática o de transplante de célula madre hematopoyetica (HSCT) Los pacientes que reciben gemtuzumab bien sea antes o después de HSCT, pacientes que sufren enfermedad hepática o enfermedad o función hepática anormal, y pacientes que reciben gemtuzumab en combinaciones con otra quimioterapia pueden estar en riesgo creciente de desarrollar VOD severo La enfermedad proveniente de falla del hígado y de VOD ha sido reportada en pacientes que

recibieron gemtuzumab, y se ha advertido que a un monitoreo cuidadoso puede no identificar todos los pacientes en riesgo o evitar las complicaciones de la hepatotoxicidad

La hepatotoxicidad también se reporta en pacientes que recibieron alemtuzumab Han ocurrido casos serios y en algunos casos raros fatales, de pancitopenia/hipoplasia de la medula, trombositopenia idiopática autoinmune, y anemia hemolítica autoinmune en pacientes que recibieron terapia de alemtuzumab El alemtuzumab puede dar como resultado serias reacciones de infusión así como también infecciones oportunistas

En pacientes tratados con adalimumab, se han reportado infecciones serias y sepsis, incluyendo fatalidades, tales como la exacerbación de síntomas clínicos y/o evidencia radiográfica de enfermedad desmielinizante, y en pacientes tratados con adalimumab en ensayos clínico tuvieron una incidencia más alta de linfoma que la proporción esperada en la población general

Reacciones severas adversas severas en estudios clínicos con omalizumab han influido malignidades y anafilasis, en los cuales la incidencia observada de malignidades entre pacientes tratados con omalizumab (0 5%) es médicamente superior que entre pacientes en grupos de control (0 2%)

Las moléculas de inmunoglobulina más pequeñas se han construido en un esfuerzo para solucionar varios problemas asociados con la terapia de inmunoglobulina completa Los polipéptidos con fermento de región variable de inmunoglobulina de cadena simple (scFvs) son hechos de un dominio variable de cadena pesada de inmunoglobulina unidos por vía de un péptido ligador corto a un dominio variable de cadena ligera de inmunoglobulina Huston et al , 1988 Proc Natl Acad Sci USA, 85 5879-83 Se ha sugerido que el tamaño más pequeño de moléculas scFv puede conducir a una limpieza más rápida del plasma y a una penetración más efectiva dentro de los tejidos que las inmunoglobulinas completas Ver, por ejemplo, Jain, 1990 Cancer Res 50 814s-819s un scFv antitumoral se reporto por mostrar una penetración más rápida y una distribución más homogénea a través de la masa tumoral que el anticuerpo quimérico correspondiente Yolota et al , Cancer Res 52 3402-08 (1192)

A pesar de las ventajas de las moléculas scFv pueden tener con relación a las seroterapia, también existen inconvenientes para esta aproximación terapéutica Por ejemplo, la limpieza rápida de scFv puede evitar el suministro de una dosis efectiva mínima al tejido objetivo Adicionalmente, la elaboración de cantidades adecuadas de

scFv para administración a pacientes ha sido un reto debido a las dificultades de la expresión y aislamiento del scFv que afecta adversamente los rendimiento Durante la expresión, las moléculas de scFv les falta estabilidad y a menudo se agregan durante el pareado de regiones variables provenientes de moléculas diferentes Adicionalmente, los niveles de producción de moléculas scFv en sistema de expresión de mamífero se han reportado bajos, lo cual puede limitar el potencial para la elaboración eficiente de moléculas scFv para terapia Davis et al , 1990 J Biol Chem 265 10410-18, Traunecker et al , 1991 EMBO J 10 3655-59 Se han explorado estrategias para mejorar la producción, y se han aportado a la inclusión de la adición de glicosilación a regiones variables Ver, por ejemplo, U S Patent No 5,888,773, Jost et al , 1994 J Biol Chem 269 26267-73 otra desventaja para el uso de los scFv para terapia es la falta de una función efectuadora A un scFv que le falta la función citolitica, ADCC, y la citotoxicidad dependiente de complemento puede ser menos efectivo o inefectivo para tratar enfermedades Aunque el desarrollo de tecnología scFv inicio hace doce años, no existe en el presente ningún producto scFv aprobado para terapia

Alternativamente, se ha propuesto que la fusión de un scFv a otra molécula, tal como toxina, que pudiera tener la ventaja de la actividad de unión de antígeno específica y el tamaño

pequeño de un scFv para suministrar la toxina a un tejido objetivo Chaudary et al , 1989 Nature 339 394, batra et al , 1991 Mol Cell Biol 11 2200 La conjugación o fusión de las toxinas de los scFv ha sido así ofrecida como una estrategia alternativa para suministrar moléculas específicas de antígeno potente, pero dosificando con tales conjugados o quimeras se puede limitar por la toxicidad excesiva y/o no específica debido a la porción toxina de todas las preparaciones Los efectos tóxicos pueden incluir elevación suprafisiológica de las enzimas del hígado y síndrome de debilidad vascular, y otros efectos indeseables Además, las inmunotoxinas son en si mismas altamente inmunogénicas luego de administración a un huésped, y los anticuerpos del huésped generados contra la inmunotoxina limitan la utilidad potencial para tratamientos terapéuticos repetidos de un individuo

Las proteínas de fusión en las cuales las secuencias de polipéptido de la región constante de inmunoglobulina están presentes y las secuencias de no inmunoglobulina son sustituidas por las regiones variables del anticuerpo también se han investigado Por ejemplo, el CD4, y la proteína de superficie de célula T reconocidas por el HIV, fueron recombinantemente fusionados a un dominio efectuador Fc de inmunoglobulina, y una proteína de fusión IL-2-IgG1 que reportaron lisis mediada por complemento efectuada de células

que llevan receptor IL-2 Ver Sensel et al , Chem Immunol 65 129-158 (1997) Una introducción extensiva así como también la información detallada acerca de todos los aspectos de la tecnología de anticuerpo recombinante se puede encontrar en el texto "Recombinant Antibodies" (John Wiley & Sons, NY, 1999) Una recolección comprensiva de protocolos detallados de laboratorio de ingeniería de anticuerpos se puede encontrar en R Kontermann and S Dübel (eds) "The Antibody Engineering Lab Manual" (Springer Verlag, Heidelberg/New York, 2000)

Las enfermedades y desórdenes se creen que son los responsables de algún tipo de terapia con inmunoglobulina incluyen cáncer y desórdenes del sistema inmune El cáncer incluye un rango amplio de enfermedades, que afectan aproximadamente uno de cada cuatro individuos en el mundo La proliferación rápida y no regulada de células malignas una marca de muchos tipos de canceres, que incluyen las malignidades hematológicas Aunque los pacientes con condición maligna hematológica se han beneficiado de los avances en la terapia de cáncer en las pasadas dos décadas, Multani et al , 1998 J Clin Oncology 16 3691-3710, y se han incrementado los tiempos de remisión, la mayoría de los pacientes aun recaen y sucumben a su enfermedad Las barreras para curar con drogas citotóxicas incluyen, por ejemplo, la resistencia celular al tumor y la alta toxicidad de la

quimioterapia, que evita la dosificación óptima de muchos pacientes

Sin embargo, los pacientes han sido tratados con inmunoterapéuticos que apuntan a células malignas, es decir, a antígenos expresados sobre células tumorales Con relación a la selección de los antígenos de superficie de la célula tumoral adecuados para uso como objetivos de inmunoterapia, es preferible que tal antígeno objetivo no se exprese mediante tejidos normales, particularmente donde la preservación de tal tejido es importante a la supervivencia del huésped En el caso de malignidad hematológica, las células malignas expresan muchos antígenos que no están expresados sobre las superficies de las células madre u otras células esenciales El tratamiento de una condición maligna hematológica que utiliza un régimen terapéutico que vacía tanto las células normales como las malignas de origen hematológico ha sido aceptable cuando la generación de células normales provenientes de progenitores puede ocurrir después de que la terapia ha terminado Adicionalmente, el antígeno objetivo es deseablemente expresado en todas o virtualmente en todas las poblaciones clonogénicas de células tumorales, y es mejor que la expresión persista a pesar de la presión selectiva de la terapia de inmunoglobulina Las estrategias que emplean selección de un ideotipo de superficie celular (por ejemplo un ideotipo particular) como

un objetivo para terapia de malignidad de célula B se ha limitado por el crecimiento de las variantes de células tumorales con expresión de ideotipo de superficie alterada, aun cuando el antígeno exhibe un alto grado de selectividad tumoral Meeker et al , 1985 N Engl J Med 312 1658-65 El antígeno seleccionado debe traficar también adecuadamente después de una unión de inmunoglobulina a esta El vertimiento o internalización de un antígeno objetivo de superficie celular después de que una inmunoglobulina se une al antígeno pueden permitir a las células tumorales escapar de succión, limitando así la efectividad de la seroterapia Finalmente, la unión de una inmunoglobulina a antígenos con blanco de superficie celular que transmiten o trasducen señales de activación celular puede dar como resultado unas respuestas funcionales mejorada a inmunoterapia de células tumorales y puedan conducir al crecimiento de la detección y/o la apoptosis Mientras todas estas propiedades son importantes, el disparo de la apoptosis después de que una inmunoglobulina se une al antígeno objetivo también puede ser un factor crítico para la seroterapia exitosa

Los antígenos que han sido ensayados como objetivos para seropterapia de malignidades de célula B y T incluyen el ideotipo Ig (Brown et al , 1989 Blood 73 651-61), CD19 (Hekman et al , 1991 Cancer Immunol, Immunother 32 364-72), Vlasveld et al , 1995 Cancer Immunol Immunother 40 37-47),

CD20 (Press et al , 1987 Blood 69 584-91), Maloney et al , 1997 J Clin Oncol 15 3266-74), CD21 (Scheinberg et al , 1990 J Clin Oncol 8 792-803), CD5 (Dillman et al , 1986 J Biol Respn Mod 5 394-410), y CD52 (CAMPATH) (Pawson et al , 1997 J Clin Oncol 15 2667-72) De estos, el mayor beneficio para terapia de linfomas de célula T se ha tenido utilizando moléculas que apuntan al CD20 Otros blancos han estado limitados por las propiedades biológicas del antigeno Por ejemplo, el idiotipo de superficie se puede alterar a través de la mutación somática que permite el escape de la célula tumoral El CD5, CD21 y CD19 son internalizados rápidamente después de unión de anticuerpo monoclonal, que le permite a la célula tumoral escaparse de la destrucción a menos que los anticuerpos monoclonales estén conjugados con las moléculas de toxinas El CD22 se expresa sobre solamente un subconjunto de linfomas de célula T, limitando de esta manera su utilidad, aunque el CD52 se expresa tanto en células T como en células B y puede por lo tanto generar inmunosupresión contraproductiva por agotamiento

El tratamiento de pacientes con bajo grado o linfoma de célula B folicular que utiliza un anticuerpo monoclonal CD20 quimérico se ha reportado por introducir respuestas parciales o completas en pacientes McLaughlin et al , 1996 Blood 88 90a (abstract, suppl 1), Maloney et al , 1997 Blood 90 2188-95 Sin embargo, como se noto anteriormente, los

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lapsos tumorales ocurren comúnmente de los seis meses a un año Mejoras adicionales en la seroterapia son necesarias para inducir respuestas más durables, por ejemplo, en linfoma de célula B de bajo grado, y para permitir el tratamiento efectivo del linfoma de alto grado y otras enfermedades de célula B

Otra aproximación ha sido el blanco de radiosótopos a linfomas de célula B utilizando anticuerpos monoclonales específicos para CD20 Aunque la efectividad de la terapia se reporta como incrementada, la toxicidad asociada proveniente de la vida media in vivo larga del anticuerpo radioactivo incrementa también algunas veces el requerimiento de que el paciente necesite rescate de células madre Press et al , 1993 N Eng J Med 329 1219-1224, Kaminski et al , 1993 N Eng J Med 329 459-65 Los anticuerpos monoclonales al CD20 se han clivado con proteasas para producir $F(ab')_2$ o fragmentos Fab antes de la unión del radioisotopo Se ha reportado mejora en la penetración del conjugado de radioisotopo en el tumor y acortamiento en la vida media in vivo, reduciendo así la toxicidad en tejidos normales Sin embrago, a estas moléculas les falta la función efectuadora, que incluye la fijación de complemento y/o el ADCC

El CD20 fue la primer molécula de superficie de molécula específica de linaje de célula B identificada por un

anticuerpo monoclonal Esta es una fosfoproteína de transmembrana de célula B de 35 kDa hidrófoba no glicosilada que tiene tanto extremos aminos como carboxi en el citoplasma Einfeld et al , 1988 EMBO J 7 711-17 CD20 se expresa por medio de todas las células B maduras normales, pero no se expresa por medio de la las células B precursoras Los ligandos naturales para CD20 no se han identificado, y la función del CD20 en la biología de la célula B es aún entendida en forma incompleta

Los anticuerpos monoclonales antiCD20 efectúan la vialibilidad y el crecimiento de células B Clark et al , 1986 Proc Natl Acad Sci USA 83 4494-98 la reticulación extensiva del CD20 puede inducir la apoptosis en líneas celulares del linfoma B, Shan et al , 1998 Blood 91 1644-52, y la reticulación del CD20 sobre la superficie celular se ha reportado por incrementar la magnitud y mejorar la cinética de la trasducción de señal, por ejemplo, como se detecto al medir la fosforinación de tiroxina de sustratos celulares Deans et al , 1993 J Immunol 146 846-53 Por lo tanto, además del agotamiento celular mediante el complemento y mecanismos ADCC, la unión del receptor Fc por medio de anticuerpos monoclonales CD20 in vivo puede promover la apoptosis de células B malignas mediante reticulación de CD20, consistente con la teoría de que la efectividad de la terapia con CD20 del linfoma humano en un modelo de ratón

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SCID puede ser dependiente de la unión del receptor Fc por medio del anticuerpo monoclonal CD20 Funakoshi et al , 1996 J Immunotherapy 19 93-101 La presencia de dominios espaciados de membrana múltiple en el polipéptido CD20 (Einfeld et al , 1988 EMBO J 7 711-17, Stamenkovic et al , 1988 J Exp Med 167 1975-80, Tender et al , 1988 J Immunol 141 4388-4394), evita la internalización de CD20 después de la unión de anticuerpo, y este reconoció como una característica importante para la terapia de malignidad de célula B cuando un anticuerpo monoclonal de murino, 1F5, se inyecto en pacientes con linfoma de célula B, dando como resultado el agotamiento significativo de células malignas y respuestas clinicas parciales Press et al , 1987 Blood 69 584-91

En razón a que las células B maduras normales también expresan CD20, las células B normales se agotan mediante terapia de anticuerpo anti-CD20 Reff , M E et al , 1994 Blood 83 435-445 Después de que se completa el tratamiento, sin embrago, las células B normales se pueden regenerar de los precursores de célula B negativa CD20, por lo tanto, los pacientes tratados con terapia antiCD20 no experimentan inmunosupresión significativa El agotamiento de las células B normales también puede ser benéfico en enfermedades que involucran la producción inadecuada de anticuerpos u otras enfermedades donde las células B juegan un papel Un

anticuerpo monoclonal quimérico específico para CD20 que consiste de regiones variables de cadena pesada y ligera de origen de ratón fusionado a cadena pesada IgG1 humana y a regiones constantes de cadena ligera capa humana, de acuerdo con los reportes retuvieron la unión a CD20 y la capacidad de mediar ADCC y de fijar complemento Liu et al , 1987 J Immunol 139 3521-26 El mecanismo de actividad antitumoral de rituximab, discutido anteriormente, se cree que es una combinación de varias actividades, incluyendo ADCC, fijación de complemento, y disparo de señales que promueven la apoptosis en células B malignas, aunque el tamaño grande de rituximab evita la difusión óptima de la molécula en tejidos linfoides que contengan células malignas, limitando de esta manera estas actividades antitumorales Las enfermedades autoinmunes incluyen enfermedades anutoinmunes de tiroides, que incluyen la enfermedad de Graves' y la tiroiditis de Hashimoto' En los Estados Unidos solo, existen aproximadamente 20 millones de personas que tienen alguna forma de enfermedad autoinmune de tiroides La enfermedad autoinmune de tiroides da como resultado la producción de anticuerpos que estimulan la tiroides que originen hipertiroidismo (enfermedad de Graves') o destruyan la tiroides para originar hipotiroidismo (tiroiditis de Hashimoto's) La estimulación de la tiroides es causada por los anticuerpos que se unen y activan el receptor de hormona estimulante de tiroides (TSH) La destrucción de la tiroides

se origina por autoanticuerpos que reaccionan con otros antígenos de tiroides La terapia habitual para la enfermedad de Graves' incluye cirugía, yodo radiactivo, o una terapia de droga antitiroides El yodo reactivo es ampliamente utilizado, en razón a que los medicamentos antitiroides tienen efectos colaterales significativos y la recurrencia de la enfermedad es alta La cirugía de reserva a pacientes con grandes papadas o cuando existe necesidad de una normalización muy rápida de la función tiroides No existen terapias que apuntes a la producción de anticuerpos responsables para la estimulación del receptor TSH La terapia habitual para la tiroiditis de Hashimoto's es levotiroxina sodio, y la terapia es usualmente de larga duración por la baja probabilidad de remisión La terapia supresiva ha mostrado que encoge las papadas en la tiroiditis de Hashimoto's pero no existen terapias conocidas que reduzcan la producción de anticuerpos que apunten al mecanismo de la enfermedad

La artritis reumatoide (RA) es una enfermad crónica caracterizada por la inflamación de las articulaciones que conduce al hinchamiento, dolor, y a la pérdida de función La RA afecta un estimado de 2 5 millones de personas en los Estados Unidos La RA se origina por una combinación de eventos que incluyen una infección inicial o daño, una respuesta inmune anormal, y factores genéticos Aunque la

células T y células B autoreactivas están presentes en RA, la detección de altos niveles de anticuerpos que se reconectan en las articulaciones, denominados factor reumatoide, se utiliza para el diagnostico de RA La terapia habitual para RA incluye muchos medicamentos para manejar el dolor y disminuir la progresión de la enfermedad No se ha encontrado ninguna terapia que pueda curar la enfermedad Los medicamentos incluyen drogas antiinflamatorias no esteroides (NSAIDS), las drogas antirreumáticas que modifican la enfermedad (DMARDS) Los NSAIDS son útiles en la enfermedad benigna, pero fallan en evitar la progresión de la destrucción de las articulaciones y la debilidad en RA severo Tanto los NSAIDS como DMARDS están asociados con efectos colaterales significativos Solamente un nuevo DMARD, leflunomide, ha sido aprobado en casi diez años El leflunomide bloquea la producción de anticuerpos, reducen la inflamación, y diminuye la producción de RA Sin embargo, esta droga también causa severos efectos colaterales que incluyen náusea, diarrea, pérdida del pelo, salpullido, y sesiones del hígado

El Lupus Sistémico Eritematoso (SLE) es una enfermedad autoinmune originada por daños recurrentes a los vasos sanguíneos en múltiples órganos, incluyendo en riñón, la piel, y las articulaciones El SLE se estima que afecta más de 500 mil persona en los Estados Unidos En pacientes con

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SLE, una interacción fallida entre las células T y las células B da como resultado la producción de anticuerpos que atacan el núcleo de las células Estos incluyen el ADN de hebra antidoble y los anticuerpos anti-Sm Los anticuerpos que se unen a los fosfolípidos también se encuentran en aproximadamente la mitad de los complejos SLE, y son responsables por el daño a los vasos sanguíneos y los conteos sanguíneos bajos Los complejos inmunes se acumulan en los riñones, vasos sanguíneos, y articulaciones de pacientes SLE, donde ellos originan inflamación y daño de tejido Ningún tratamiento para SLE se ha encontrado para curar la enfermedad Los NSAIDS y DMARDS son utilizados para terapia dependiendo de la severidad de la enfermedad La plasmaferesis con intercambio de plasma para remover los anticuerpos puede originar la mejora temporal en pacientes SLE Existe un acuerdo general de que los anticuerpos son responsables por SLE, así que nuevas terapias que agoten el linaje de célula B, y que permitan al sistema inmune restablecerse como nuevas células B son generadas de los precursores, ofrecerían esperanza para beneficio a largo plazo en pacientes SLE

El síndrome de Sjogren's es una enfermedad autoinmune caracterizada por la destrucción de las glándulas que producen humedad del cuerpo El síndrome Sjogren's es uno de los desórdenes autoinmunes más prevalentes, que golpean a un

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estimado de 4 millones de personas en los Estados Unidos Aproximadamente la mitad de la persona golpeada con el síndrome Sjogren's también tiene una enfermedad de tejido colectiva, tal como RA, mientras que la otra mitad tiene síndrome de Sjogren's primario sin otra enfermedad autoinmune concurrente Los anticuerpos, que incluyen anticuerpos antinucleares, factor reumatoide, antifodrin, y receptor antimoscaninico están a menudo presentes en pacientes con síndrome de Sjogren's La terapia convencional incluye corticosteroides, y terapias adicionales más efectivas serían de beneficio

La purpura trombocitopenica inmune (ITP) se origina por anticuerpos que se unen a las plaquetas de la sangre y originan su destrucción Las drogas originan algunos casos de ITP, y otros están asociados con infección, embarazo, o enfermedad autoinmune tal como SLE Aproximadamente la mitad de todo los casos son clasificados "idiopáticos", significando que la causa es desconocida El tratamiento del ITP se determina por la severidad de los síntomas En algunos casos, no se requiere ninguna terapia aunque la mayoría de los casos drogas inmunosupresoras, que incluyen corticosteroides o infusiones intravenosas de globulina inmune para agotar las células T, son suministradas Otro tratamiento que usualmente da como resultado un numero creciente de plaquetas es la remoción del bazo, el órgano que

destruye las plaquetas recubiertas por anticuerpo Las drogas inmunosupresoras más potentes que incluyen ciclosforina, ciclofosfamida, o azatriopina son utilizadas por pacientes con casos severos La remoción de anticuerpos por paso de plasma de pacientes sobre una columna de proteína A se utiliza como una segunda línea de tratamiento en pacientes con enfermedad severa Se desean terapias adicionales más efectivas

Las esclerosis múltiple (MS) también es una enfermedad autoinmune Esta se caracteriza por la inflamación del sistema nervioso central y la destrucción de la mielina, que aisla las fibras de las células nerviosas en el cerebro, la columna vertebral, y el cuerpo Aunque la causa del MS es desconocida, se cree ampliamente T autoinmunes son los contribuyentes primarios a la patogénesis de la enfermedad Sin embargo, los altos niveles de anticuerpos están presentes en el fluido espinal cerebral de los pacientes con MS, y las teorías predicen que la respuesta de la célula B que conduce a la producción de anticuerpos es importante para mediar la enfermedad Ninguna terapia de agotamiento de célula B ha sido estudiada en pacientes con MS y no existe cura para el MS La terapia vital son corticosteroides, que pueden reducir la duración y la severidad de los ataques pero no afectan el curso del MS durante el tiempo Nuevas terapias de interferón

biotecnológico (IFN) para MS han sido recientemente aprobadas pero se desean terapias adicionales más efectivas

La Miastenia Gavis (MG) es un desorden neuromuscular autoinmune crónico que se caracteriza por la debilidad de los grupos de músculos voluntarios El MG afecta aproximadamente 40 mil personas en los Estados Unidos El MG es generado por anticuerpos que se unen a los receptores de acetilcolina expresados en las articulaciones neuromusculares Los autoanticuerpos reducen o bloquean los receptores de acetilcolina, evitando la transmisión de señales desde los nervios a los músculos No existe cura conocida para MG Los tratamientos comunes incluyen inmunosupresión con corticosteroides, ciclosforina, ciclofosfamida, o azatioprina La remoción quirurgica del timo se utiliza a menudo para calmar la respuesta autoinmune La plasmaferesis, utilizada, para reducir los niveles de anticuerpos en la sangre, es efectiva en MG, pero es de corta en vida en razón a que la producción de anticuerpos continúa La plasmaferesis es usualmente reservada para debilidad severa en musculos antes de cirugía Sería de beneficio nuevas y efectivas terapias

La soriasis afecta a aproximadamente a 5 millones de personas, y se caracteriza por la inflamación autoinmune en la piel La soriasis también está asociada con la artritis en

30% (artritis soriatica) Muchos tratamientos, que incluyen esteroides, retenoides de luz uv, derivados de vitamina D, ciclosporina, metotrexato se han utilizado pero es claro que la soriasis se beneficiaría con terapias nuevas y efectivas La escleroderma es una enfermedad autoinmune crónica del tejido conectivo que también se conoce como esclerosis sistémica El escleroserma se caracteriza por una sobreproducción de colágeno que resulta en el engrosamiento de la piel, y aproximadamente 300 personas en los Estados Unidos tienen escleroderma los cuales también se beneficiarían con terapias nuevas y efectivas

Existe una clara necesidad de composiciones y métodos mejorados para tratar malignidades, que incluyen malignidades de célula B y desórdenes que incluyen enfermedades autoinmunes, desórdenes, y condiciones, así como también otras enfermedades, desórdenes, y colisiones Las composiciones y métodos de la presente invención descritas y reivindicadas aquí suministran tales composiciones y métodos mejorados así como también otras ventajas

RESUMEN DE LA INVENCIÓN

Es un aspecto de la presente invención suministrar una proteína de fusión de inmunoglobulina con dominio de unión, que comprende un polipéptido con dominio de unión que se

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fusiona o se conecta de otra forma a un polipéptido de inmunoglobulina o a un polipéptido con región de acción de pivote, el cual a su vez está fusionado o conectado de otra forma a una región que comprende una o más regiones constantes nativas o tratadas con ingeniería provenientes de cadena pesada de inmunoglobulina, diferentes de CH1, por ejemplo, las regiones CH2y CH3 de IgG e IgA, o las regiones CH3 y CH4 de IgE La proteína de fusión de inmunoglobulina con dominio de unión comprende, consiste esencialmente de, o consiste de, un polipéptido de región constante de CH2 de cadena pesada de inmunoglobulina nativa o trabajada con ingeniería (o CH3 en el caso de una construcción derivada completamente o en parte de IgE) que es fusionada o conectada de otra forma al polipéptido de región de pivote y un polipéptido o región constante CH3 de cadena pesada de inmunoglobulina nativa o trabajada con ingeniería (o CH4 en el caso de una construcción derivada en todo o en parte del IgE) que es fusionada o conectada de otra manera a un polipéptido o región constante CH2 (o CH3 en el caso de una construcción derivada en todo o en parte de IgE) Tales proteínas de fusión de inmunoglobulina con dominio de unión son capaces de al menos una actividad inmunológica seleccionada del grupo que consiste de citotoxicidad mediada por célula dependiente de anticuerpo y fijación de complemento Tales polipéptidos con dominio de unión también

son capaces de unirse o unirse especificamente a un blanco, por ejemplo, un antigeno blanco

En ciertas modalidades, por ejemplo, el polipéptido con dominio de unión comprende al menos un polipéptido con región variable de inmunoglobulina que es seleccionado de un polipéptido con región variable de cadena ligera de inmunoglobulina nativo o trabajado con ingeniería y/o un polipéptido con región variable de cadena pesada de inmunoglobulina nativo o trabajado con ingeniería En ciertas modalidades adicionales las proteínas de fusión de inmunoglobulina con dominio de unión comprenden un polipéptido o región variable de cadena pesada de inmunoglobulina nativo o trabajado con ingeniería en donde el polipéptido o región variable de cadena pesada es un polipéptido o región variable de cadena pesada de inmunoglobulina humana trabajada con ingeniería (o un polipéptido o región variable de cadena pesada de inmunoglobulina trabajada con ingeniería de una especie no humana) se comprende una mutación, sustitución, o supresión de un o unos aminoácidos en un sitio que corresponde a uno cualquiera o más de las posiciones a aminoácido 9, 10, 11, 12, 108, 110 y /o 112 Las mutaciones, sustituciones, o supresiones de un o unos aminoácidos en el sitio que corresponden a uno o cualquiera o más de las posiciones de aminoácidos 9, 10 , 11 , 12 108, 110, y/o 112 la región

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variable de cadena pesada se pueden incluir dentro de una construcción tal como una construcción que corresponde a, por ejemplo, ID DE SEC NO __ En ciertas otras modalidades adicionales la proteína de fusión comprende un polipéptido que tiene una secuencia seleccionada de las ID DE SEC NO __o ID DE SEC NO __ En ciertas modalidades el polipéptido con región variable de inmunoglobulina se deriva de, por ejemplo, una inmunoglobulina humana, y en ciertas otras modalidades el polipéptido con región variable de inmunoglobulina comprende una secuencia de polipéptido de inmunoglobulina humanizado En ciertas modalidades el polipéptido con región variable de inmunoglobulina, sea o no humanizado, se deriva de una inmunoglobulina de murino, o se deriva de una inmunoglobulina proveniente de otras especies, que incluyen, por ejemplo, rata, cerdo, mono o camello

De acuerdo con ciertas modalidades de la presente invención, el polipéptido con dominio de unión comprende, consiste esencialmente de, o consiste de, (a) al menos un polipéptido con región variable de cadena ligera de inmunoglobulina nativo o trabajadas con ingeniería, (b) al menos un polipéptido de región variable de cadena pesada de inmunoglobulina nativo o trabajado con ingeniería, (c) al menos un polipéptido ligador que está fusionado o conectado de otra manera al polipéptido de (a) y el polipéptido de (b)

cadena ligera de inmunoglobulina y los polipéptidos de región variable de cadena pesada nativos o trabajados con ingeniería se derivan o son construidos de inmunoglobulinas humanas, y en ciertas otras modalidades adicionales el polipéptido ligador comprende al menos un polipéptido que incluye o tiene una secuencia de aminoácido Gly-Gly-Gly-Gly-Ser [ID DE SEC NO __] En otras modalidades el polipéptido ligador comprende al menos dos o tres repeticiones de un polipéptido que tiene una secuencia de aminoácido Gly-Gly-Gly-Gly-Ser [ID DE SEC NO __] En otras modalidades el ligador comprende un sitio de glicolización, que en ciertas modalidades adicionales es un sitio de glicolización ligado a asparagina, a un sitio de glicolización ligado a O, un sitio de manosilación C, un sitio de glipiasión o un sitio de fosfoglicación En otra modalidad al menos un polipéptido de región constante CH2 de cadena pesada de inmunoglobulina nativo o trabajado con ingeniería y un polipéptido de región constante CH3 de cadena pesada de inmunoglobulina nativo o trabajado con ingeniería se deriva de una cadena pesada de inmunoglobulina humana IgG o IgA En otra modalidad al menos un polipéptido de región constante CH3 de cadena pesada de inmunoglobulina nativo o trabajado con ingeniería y un polipéptido de región constante CH4 de cadena pesada de inmunoglobulina nativo o trabajado con ingeniería se deriva de una cadena pesada de inmunoglobulina humana IgE

Un polipéptido de región de pivote de inmunoglobulina puede comprender, consiste esencialmente o, por ejemplo, cualquiera de (1) un pivote o péptido que actúa con pivote o polipéptido que ocurre de manera natural por ejemplo, un polipéptido de región de pivote de inmunoglobulina que incluye, por ejemplo, pivote IgE humano tipo silvestre o una porción de ese, un pivote IgA humano tipo silvestre o una porción de este, pivote IgE de humano tipo silvestre o una porción de este, región de acción de pivote IgE humano tipo silvestre, es decir, IgE CH2, o una porción de este, una región de pivote de camello tipo silvestre o una porción de esta (que incluye región de pivote de llama IgG1 o una porción de este, una región de pivote de llama IgG2 o una porción de este, y una región de pivote de llama IgG3 o una porción de este), una región de pivote de tiburón nodriza o una porción de este, y/o una región de pivote de pez rata manchado o una porción de este, (2) un polipéptido de región de pivote alterado o trabajado con ingeniería que no contiene residuos de cisteína que se deriven o construidos de polipéptido de región de pivote de inmunoglobulina tipo silvestre que tiene uno o más residuos de cisteína, (3) un polipéptido de región de pivote alterado o trabajado con ingeniería que contiene un residuo de cisteína y que se deriva de un polipéptido de región de pivote de inmunoglobulina tipo silvestre que tiene uno o más residuos de cisteína, (4) un polipéptido de región de pivote que ha sido mutado o alterado de otra manera o trabajado por

ingeniería para contener o agregar uno o más sitios glicolización, por ejemplo, un sitio de glicolización ligado a aspargina, un sitio de glicolización ligado a aspargina, un sitio de manolización C, un sitio de glipiación o un sitio de fosfoglicación, (5) un polipéptido de región de pivote alterado o trabajado con ingeniería en el cual el número de residuos de cisteína se reduce mediante la sustitución o supresión de aminoácido, por ejemplo, una región de pivote IgG1 o IgG4 mutada o alterada de otra forma o trabajada con ingeniería que contiene por ejemplo cero, uno, o dos residuos de cisteína, o una región de pivote Ig2 mutada o alterada de otra forma o trabajada por ingeniería que contiene por ejemplo cero, uno, dos o tres residuos de cisteína, una región de pivote Ig3 mutada o alterada de otra forma o trabajada por ingeniería que contiene por ejemplo cero, uno, dos, tres, o de cuatro a diez residuos de cisteína o un polipéptido de región de pivote IgA1 o IgA2 humano mutado o alterado de otra forma o trabajado por ingeniería que contiene cero o solamente uno o dos residuos de cisteína (por ejemplo un pivote "SCC"), una región de pivote IgD mutada o alterada de otra forma o trabajada por ingeniería que no contiene residuos de cisteína, o una región de acción de pivote IgE humano mutado o alterado de otra forma o trabajado por ingeniería es decir, un polipéptido de región IgE CH2 que contiene cero o solamente uno, dos, tres o cuatro residuos de cisteína, o (6) cualquier otra molécula o región conectante

descrita o referenciada aquí o conocida de otra forma o posteriormente descubierta como útil para conectar dominios de inmunoglobulina adjuntos tales como, por ejemplo, un dominio CH1 y un dominio CH2 por ejemplo, un polipéptido de región de pivote se puede seleccionar del grupo que consiste (i) un polipéptido de regio de pivote de inmunoglobulina IgG1 humano tipo silvestre, por ejemplo, (ii) un Igg1 humano mutado o alterado de otra forma o trabajado por ingeniería u otro polipéptido de región de pivote de inmunoglobulina que se derive o se construya de un polipéptido de región de pivote de inmunoglobulina tipo silvestre que tenga tres o más residuos de cisteína, en donde dicho IgG1 humano mutado u otro polipéptido de región de pivote de inmunoglobulina contiene dos residuos de cisteína y en donde una primera cisteína de la región de pivote tipo silvestre no se muta, (iii) un IgG1 humano mutado o alterado de otra forma o trabajado por ingeniería u otro polipéptido de región de pivote de inmunoglobulina que se derive de un polipéptido de región de pivote de región de pivote de inmunoglobulina tipo silvestre que tenga tres o más residuos de cisteína, en donde dicho IgG1 humano mutado o polipéptido de región de pivote de inmunoglobulina contiene no más de un residuo de cisteína, y (iv) un IgG1 humano mutado o alterado de otra forma o trabajado por ingeniería u otro polipéptido de región de pivote de inmunoglobulina que se derive de un polipéptido de región de pivote de inmunoglobulina tipo silvestre que tenga

3 o más residuos de cisteína en donde dicho IgG1 humano mutado o alterado de otra manera o trabajado por ingeniería u otro polipéptido de región de pivote de inmunoglobulina no contiene residuos de cisteína En ciertas modalidades, por ejemplo, el polipéptido de región de pivote de inmunoglobulina es mutado de alterado de otra forma o el polipéptido de región de pivote trabajado por ingeniería y exhibe una capacidad reducida para dimerizarce, con relación a la inmunoglobulina G humana tipo silvestre o a otra región de pivote tipo silvestre o a un polipéptido de acción de pivote

Los polipéptidos de región constante de cadena pesada de inmunoglobulina pueden ser, por ejemplo, dominios CH2 y CH3 nativos o trabajados por ingeniería de un isotipo que es IgG humano o IgA humano Los polipéptidos CH3 y CH4 de región constante de cadena pesada de inmunoglobulina nativos o trabajados por ingeniería de un isotipo que es IgE humano

En ciertas otras modalidades el antígeno blanco puede ser, por ejemplo, CD19 (antígeno de linfocito B CD19 también denominado como antígeno de superficie de linfocito B4, o Leu-12), CD320 (antígeno de linfocito B 20, también denominado como antígeno de superficie de linfocito B B1, Leu-16 o Bp35), CD22 (receptor de célula B CD22 también denominado como Leu-14, molécula de adhesión de célula de

linfocito B, o BL-CAM)), CD37 (antígeno de leucocito CD37), CD40 (antígeno de superficie de célula B CD40, también denominado como miembro de de la superfamilia del receptor de de Factor de Necrosis Tumoral 5, el receptor CD40L, o BP50), CD80 (antígeno de activación de linfocito T CD80, también denominado como antígeno B7-1 de activación, B7, B7-1, o BB1 , CD86 (antígeno de activación de linfocito T, CD86, también denominado como antígeno B7-2 de activación, B70, FUN-1, o BU63), CD137 (también denominado como superfamilia del receptor del Factor de Necrosis Tumoral número 9), CD152 (también denominado como proteína de linfocito T citotóxico 4 o CTLA-4), CD45(antígeno común de leucocito, también denominado como L-CA, T 200, y EC3 1 3 48),CD45RA (una isoforma de CD45, y un antígeno expresado sobre linfocitos nativos inmaduros) CD45RB (una isoforma de CD45), CD45RO (una isoforma de CD45, y un antígeno de leucocito común expresado sobre células B y T memoria), L6 (antígeno asociado a tumor L6, también denominado como miembro de la superfamilia 4 de Transmembrana 1, marcador 1 de la superficie de componente de Membrana, o M3S1), CD2 (antígeno de superficie de célula T CD2, también denominado como antígeno de superficie de célula T22/Leu-5, LFA-2, receptor LFA-3, receptor de Eritrocito o un receptor Rocete), CD28 (proteína de superficie de homodímero especifica de célula T CD28, también denominada como Tp44), CD30 (antígeno de activación de linfocito CD30, también denominado como miembro 8 de la superfamilia del receptor

Factor de Necrosis Tumoral, receptor CD30L, o Ki-1), CD50 (también denominado como moléculas 3 de adhesión intracelular (ICAM3), O ICAM-R), CD54 (también denominado como molécula 1 de adhesión intracelular (ICAM1), o receptor de rinovirus del grupo principal), B7-H1 (ligando para un receptor de inmunoinhibitorio expresado mediante células T, células B y células mieloides activadas, también denominadas como PD-L1, ver Dong, *et al* , "B7-H1, a third member of the B7 family, co-stimulates T-cell proliferation and interleukin-10 secretion" 1999 *Nat Med* 5 1365-1369), CD134 (también denominado como miembro 4 de la superfamilia del receptor de Factor de Necrosis Tumoral, 4,0X40, receptor 0X40L, Antígeno ACT35, o receptor de proteína 1 activado transcripcionalmente TAX), 41BB (receptor ligando 4-1BB, antígeno de célula T 4-1BB, o antígeno de célula T ILA), CD153 (también denominado como miembro 8 de la superfamilia de ligando del Factor de Necrosis Tumoral, ligando CD30, o CD30-L), CD154 (también denominado como miembro 5 de la superfamilia de ligando del Factor de necrosis Tumoral, ligando CD40, CD40-L, proteína de activación relacionada con TNF, TRAP o antigeno de célula T Gp39) ICOS (Coestimulador Inducible), CD3, uno o más de las cadenas delta, epsilón, gama, eta, y /o zeta, sola o en combinación), CD4 (glicoproteína de superficie de célula T CD4, también denominada como antígeno de superficie de célula T4/Leu-3), CD25 (también denominado como cadena alfa de receptor de interleucina -2, sub unidad alfa de receptor IL-

2, p55, o antígeno Tac), CD8 alfa (cadena alfa CD8 de glicoproteína de superficie de célula T, también denominada como antígeno de diferenciación de linfocito T, T8/Leu-2, y Lyt-2, CD11b (también denominado como alfa-M Integrina sub unidad alfa MAC-1 de glicoproteína de superficie celular, cadena alfa CR-3, receptor Mo1 de adhesión de leucocito, o receptor de adherencia de Neutrófilo), CD14 (antígeno de diferenciación de monocito CD14, también denominado como glicoproteína rica en leucina específica de célula Mieloide o receptor LPS), CD56 (también denominado como molécula 1 de adhesión celular Neural), o CD69 (también denominado como antígeno de activación célula T Temprano, P60, Gp32/28, Leu-23, MLR-3, molécula inductora de Activación, O AIM), o factor TNF (por ejemplo TNF-α) La lista anterior de blancos de construcción y/o antígenos blanco son solamente ejemplos y no es exhaustiva

En otro aspecto, la invención incluye una construcción de unión (o un polinucleótido que que codifica tal construcción) que comprende un dominio extracelular CD154, o una porción funcional deseada de éste En una modalidad de éste aspecto de la invención, por ejemplo, la construcción de unión comprende un dominio extracelular CD54 fusionado o conectado de otra forma a un segundo dominio de unión El segundo dominio de unión, por ejemplo, puede comprender, y consiste esencialmente de, o consiste de al menos un polipéptido de

región variable de inmunoglobulina En al menos un polipéptido de región variable de inmunoglobulina puede ser un scFv nativo o trabajado por ingeniería El scFv nativo o trabajado por ingeniería puede ser un scFv nativo o trabajado por ingeniería descubierto o descrito aquí El segundo dominio de unión, que incluye un scFv nativo o trabajado por ingeniería, puede ser aquel que se una, por ejemplo, a cualquiera de los blancos, que incluyen, antígeno blanco descubiertos o descritos aquí, que incluyen pero no están limitados a, por ejemplo, cualquiera de B7-H1, ICOS, L6, CD2, CD3, CD8, CD4, CD11b, CD14, CD19, CD20, CD22, CD25, CD28, CD30, CD37, CD40, CD45, CD50, CD54, CD56, CD69, CD80, CD86, CD134, CD137, CD152, CD153,O CD154

En otra modalidad el polipéptido de dominio de unión comprende un dominio extracelular CTLA-4, o una porción funcional deseada de éste, y en modalidades adicionales al menos uno de los polipéptidos de región constante de cadena pesada de inmunoglobulina seleccionado de un polipéptido de región constante CH2 y un polipéptido de región constante CH3 que es un polipéptido de región constante IgG1 humano, bien sea nativo o trabajado por ingeniería

En otra modalidad adicional al menos uno de los polipéptidos de región constante de cadena pesada de inmunoglobulina seleccionado del polipéptido de región

constante CH2 y un polipéptido de región constante CH3 es un polipéptido de región constante IgA humano, bien sea nativo o trabajado por ingeniería

En otra modalidad adicional al menos uno de los polipéptidos de región constante de cadena pesada de inmunoglobulina seleccionado del polipéptido de región constante CH3 y un polipéptido de región constante CH4 es un polipéptido de región constante es un polipéptido de región constante IgE humano, bien sea nativo o trabajado por ingeniería

Regresando a otra modalidad, la presente invención suministra una proteína de fusión de inmunoglobulina con dominio de unión, que comprende o consiste esencialmente de, o consiste de, (a)un polipéptido con dominio de unión que está fusionado o conectado de otra forma a un polipéptido de región de pivote de inmunoglobulina, (b) un polipéptido de región constante CH2 (o IgE Ch3) de cadena pesada de inmunoglobulina nativo o trabajado por ingeniería que está fusionado o conectado de otra forma a un polipéptido de región de pivote, y (c) un polipéptido de región constante CH3 (o IgE Ch4) de cadena pesada inmunoglobulina nativo o trabajado por ingeniería que se fusiona o se conecta de otra forma al polipéptido de región constante CH2 (o IgCh3) en donde (1) el polipéptido con dominio de unión comprende un dominio extracelular CTL-4, una porción de éste, que es capaz de

unirse o de unirse específicamente al menos un ligando CTL-4 seleccionado del grupo que consiste de CD80 y CD86, (2) el polipéptido de región de pivote de inmunoglobulina puede ser como se describió anteriormente o aquí, y puede comprender, consiste esencialmente de, o consiste de, por ejemplo, un polipéptido que es seleccionado del grupo que consiste de un polipéptido de región de pivote IgA humano nativo o trabajado por ingeniería, un polipéptido de región de pivote IgG1 humano nativo o trabajado por ingeniería y un polipéptido de región CH2 IgE humano nativo o trabajado por ingeniería (3) un polipéptido de región constante de cadena pesada de inmunoglobulina que comprende, consiste esencialmente de, o consiste de, un polipéptido que se selecciona del grupo que consiste de un polipéptido de región constante CH2 de cadena pesada IgA humano nativo o trabajado por ingeniería, un polipéptido de región constante CH2 de cadena pesada IgG1 humano nativo trabajado por ingeniería, y un polipéptido de región constante CH3 de cadena pesada IgE humano nativo o trabajado por ingeniería (4) un polipéptido de región constante de cadena pesada inmunoglobulina que comprende, consiste esencialmente de, o consiste de, un polipéptido que se selecciona del grupo que consiste de un polipéptido de región constante CH3 de cadena pesada IgA humano nativo o trabajado por ingeniería, un polipéptido de región constante CH3 de cadena pesada IgG1 humano nativo o trabajado por ingeniería, y un polipéptido de región constante CH3 de

cadena pesada IgE humano nativo o trabajado por ingeniería, y (5) una proteína de fusión de inmunoglobulina con dominio de unión que es capaz de inducir al menos una actividad inmunológica seleccionada del grupo que consiste de citotoxicidad mediada por célula dependiente de anticuerpo, CDC, y fijación ce complemento En una modalidad adicional, la proteína de fusión de inmunoglobulina con dominio de unión es capaz de inducir dos actividades inmunológicas seleccionadas del grupo que consiste de citotoxicidad mediada por célula dependiente de anticuerpo, CDC, y fijación de complemento

En otra modalidad la presente invención suministra una proteína de fusión de inmunoglobulina con dominio de unión que comprende, consiste esencialmente de, o consiste de (a) un polipéptido con dominio de unión que se fusiona o se conecta de otra forma a un polipéptido de región de pivote de inmunoglobulina, en donde dicho polipéptido con región de pivote se pudo describir anteriormente o aquí, y puede comprender, consiste esencialmente de, o consiste de, por ejemplo una región de acción de pivote IgE humana nativa o trabajada por ingeniería, es decir, un polipéptido de región CH2 IgE, (b) un primer polipéptido de región constante de cadena pesada de inmunoglobulina nativo o trabajado por ingeniería que se fusiona o se conecta de otra forma al polipéptido de región de pivote, en donde dicho polipéptido

de región constante y nativo o trabajado por ingeniería, consiste esencialmente de, o consiste de, un polipéptido de región constante CH3 IgE humano nativo o trabajado por ingeniería, (e) un polipéptido de región constante de cadena pesada de inmunoglobulina nativo o trabajado por ingeniería que se fusiona o se conecta de otra forma al primer polipéptido de región constante nativo o trabajado por ingeniería, en donde dicho segundo polipéptido de región constante nativo o trabajado por ingeniería comprende, consiste esencialmente de, o consiste de, un polipéptido de región constante CH4 IgE humano nativo o trabajado por ingeniería y en donde (1) la proteína de fusión de inmunoglobulina con dominio de unión es capaz de inducir al menos una actividad inmunológica seleccionada de citotoxicidad e inducción mediada por célula dependiente de anticuerpo de un mecanismo de respuesta alérgico, y (2) el polipéptido con dominio de unión que es capaz de unirse o de unirse específicamente a un antígeno En una modalidad adicional el antígeno es un antígeno tumoral

En ciertas otras modalidades la presente invención suministra una proteína de fusión de inmunoglobulina con dominio de unión, que comprende, consiste esencialmente de, o consiste de, (a) un polipéptido con dominio de unión que se fusiona o se conecta de otra manera a un polipéptido de región de pivote de inmunoglobulina, en donde el polipéptido de dominio

de unión es capaz de unirse o de unirse específicamente al menos un antígeno que está presente en una célula efectuadora inmune y en donde el polipéptido con región de pivote puede ser como se describió anteriormente o aquí, y puede comprender, consiste esencialmente de, o consiste de, un ejemplo, un polipéptido seleccionado del grupo que consiste de un polipéptido con región de pivote IgA humano nativo o trabajado por ingeniería, un polipéptido con región de pivote IgG humano nativo o trabajado por ingeniería, es decir, el polipéptido de la región IgE CH2, (b) un primer polipéptido de región constante de cadena pesada de inmunoglobulina nativo o trabajado por ingeniería que se fusiona o se conecta de otra manera al polipéptido de región de pivote, en donde dicho primer polipéptido de región constante nativo o trabajado por ingeniería, comprende, consiste esencialmente de, o consiste de, un polipéptido seleccionado del grupo que consiste de un polipéptido de región constante IgA CH2 humano nativo o trabajado por ingeniería, un polipéptido de región constante IgG CH2 humano nativo o trabajado por ingeniería, y un polipéptido de región constante IgE CH3 humano nativo o trabajado por ingeniería, (c) un segundo polipéptido de región constante de pesada de inmunoglobulina nativo o trabajado por ingeniería que se fusiona o se conecta de otra manera al primer polipéptido de la región constante, en donde dicho segundo polipéptido de región constante comprende, consiste esencialmente de, o consiste de un polipéptido

seleccionado del grupo que consiste de un polipéptido de región constante IgA CH3 humano nativo o trabajado por ingeniería, un polipéptido de región constante IgG CH3 humano nativo o trabajado por ingeniería, y un polipéptido de región constante IgE CH4 humano nativo o trabajado por ingeniería, y (d) un polipéptido de dominio de ancla de membrana en plasma nativo o trabajado por ingeniería En un ejemplo de esta modalidad, el polipéptido de dominio de anclaje de la membrana de plasma se enlaza a un enlace de glicosil-fosfatidilinositol nativo o trabajado por ingeniería En una modalidad adicional el polipéptido con dominio de anclaje de membrana de plasma consiste esencialmente de, o consiste de, un polipéptido de dominio de transmembrana nativo o trabajado por ingeniería En otra modalidad adicional el polipéptido consiste esencialmente de, o consiste de, un polipéptido de dominio de transmembrana nativo o trabajado por ingeniería y un polipéptido de cola citoplasmática nativo o trabajado por ingeniería En aún una modalidad adicional el polipéptido con cola citoplasmática comprende, consiste esencialmente de, o consiste de, una secuencia de polipéptido que señala la apoptosis nativo o trabajado por ingeniería, la cual en una modalidad adicional se deriva o está construida de un polipéptido con dominio de muerte del receptor trabajado nativo o trabajado por ingeniería, un dominio de muerte, o una porción funcional o cualquiera En una modalidad adicional el polipéptido con dominio de muerte nativo o

trabajado por ingeniería comprende, consiste esencialmente de, o consiste de, por ejemplo, un polipéptido nativo o trabajado por ingeniería seleccionado de un dominio ITIM (motivo de inhibición basado en Tyr inmunoreceptor), un dominio ITAM (motivo de activación basado en Tyr inmunoreceptor), TRAF, RIP, CRADD, FADD (dominio de muerte asociado a Fas), TRADD (proteína de dominio asociada al tipo 1 de receptor de Factor de Necrosis Tumoral), RAIDD (también denominado como RAID, CD95 (miembro 6 de la superfamilia al receptor de Factor de Necrosis Tumoral, también denominado como receptor FASL, Un antígeno de superficie mediado por Apoptosis FAS, antígeno FAS y Apo-1), TNFR1, y/o DR5 (receptor de muerte-5) En otra modalidad la secuencia de polipéptido que señala la apoptosis nativo o trabajado por ingeniería comprende, consiste esencialmente de, o consiste de, por ejemplo, una secuencia derivada de un polipéptido caspasa nativo o trabajado por ingeniería que es la caspasa 3 o la caspasa 8 o la caspasa 10, que incluye la caspasa 8/FLICE/MACH-Mch5 y la caspasa 10/Flice 2/Mch4 En otra modalidad el polipéptido de dominio de anclaje de membrana de plasma comprende, consiste esencialmente de, o consiste de, por ejemplo, una secuencia de polipéptido con enlace de glicosil-fosfatidilinositol nativo o trabajado por ingeniería En otra modalidad el antígeno que está presente en una célula efectuadora inmune es, por ejemplo, CD2, CD216, CD28, CD30, CD32, CD40, CD50, CD54, CD64, CD80, CD86, B7-H1,

CD234, CD137, CD152, CD253, CD154, ICOS, CD19, CD20, CD22, CD37, L6, CD3, CD4, CD25, CD8, CD11b, CD14, CD56, CD69 En otra modalidad el IgG humano es un IgG1 humano nativo o trabajado con ingeniería Estas proteínas de fusión de inmunoglobulina con dominio de unión pueden ser capaces de inducir, por ejemplo, al menos una actividad inmunológica seleccionada de las citotoxicidad mediada por célula dependiente de anticuerpo y/o la fijación de complemento y/o CDC, y son capaces de unirse o unirse específicamente a un blanco, incluyendo, por ejemplo, un antígeno blanco En otras modalidades, las proteínas de fusión de inmunoglobulina con dominio de unión pueden ser capaces de inducir por ejemplo, dos actividades inmunológicas seleccionadas de citotoxicidad mediada por célula dependiente de anticuerpo y/o fijación de complemento y/o CDC, que son capaces de unirse o unirse específicamente a un blanco Las células efectuadoras inmunes incluyen, por ejemplo, granulocitos, células mástiles, monocitos, macrófagos, células dendríticas, neutrófilos, eosinófilos, vasófilos, células NK, células T (que incluyen células Th1, células Th2, células Tc, células T memoria, células nulas, y linfocitos granulares grandes, etc) y Células B Esta modalidad de la invención incluye además el uso de tales proteínas para terapia y, por ejemplo, el uso de tales vectores para terapia génica *in vivo y ex vivo* La lista anterior de componentes de construcción y de blancos no es exhaustiva, y puede incluir cualquier blanco deseado o

componente que pueda funcionar como, o ser útil para los propósitos, descritos aquí

En otra modalidad, la invención suministra una proteína que tiene un primer motivo de proteina que comprende, consiste esencialmente de, o consiste de, (1) una región de pivote de inmunoglobulina nativa o trabajada por ingeniería o una región de acción de pivote (por ejemplo IgE CH2) polipéptido que está fusionado o conectado de otra forma (2) o un polipéptido de región constante CH2 nativo o trabajado por ingenieria (o polipéptido de región constante IgE CH3 nativo o trabajado con ingeniería) Dicho primer motivo de proteina se puede fusionar o conectar de otra forma a una o más de otras de tales primeros motivos de proteína para formar un segundo motivo de proteína, el segundo motivo de proteína se fusiona conectado de otra forma a (3) una región constante CH3 nativa o trabajada con ingeniería (o una región constante IgE CH4 nativa o trabajada con ingeniería) para formar un tercer motivo de proteína Dicho primero, segundo o tercero motivos de proteína se pueden fusionar o conectar de otra forma a uno o más polipéptidos con dominio de anclaje de membrana de plasma nativo o trabajado con ingeniería descritos aquí que incluyen, por ejemplo, un polipéptido con dominio de transmembrana nativo o trabajado con ingenieria, y un polipéptido de dominio con dominio de transmembrana nativo o trabajado con ingeniería y un polipéptido de cola

citoplasmática nativo o trabajado con ingeniería, tal como por ejemplo, una secuencia de polipéptido que señala la apoptosis nativo o trabajado con ingeniería, que se puede derivar o construir un polipéptido con dominio de receptor muerte nativo o trabajado con ingeniería, un dominio de muerte, o una porción funcional o cualquiera Así, una proteína o un polinucleótido dentro de este aspecto de la invención, puede ser, por ejemplo, una construcción "Finge CH2-CH3-TransmembraneDomain-DeathDomain" También puede ser, por ejemplo, una construcción (Finge-CH2)x-CH3-TransmembraneDomain-DeathDomain, donde X va de 2 a 5, o tal otro número como se puede necesitar para lograr una longitud deseada o unión de receptor Fc y /o función o fusiones de fijación de complemento

Esta modalidad de la invención también incluye polinucleótidos que codifican tales proteínas, vectores que incluyen tales polinucleótidos, y células huéspedes que contienen tales polinucleótidos y vectores Esta modalidad de la invención incluye además el uso de tales proteínas para terapia, y, por ejemplo, el uso de tales polinucleótidos y/o vectores para terapia génica *in vivo* y *ex vivo* La invención suministra, en otra modalidad, una proteína de fusión de inmunoglobulina con dominio de unión, que comprende, consiste esencialmente de, o consiste de, (a) un polipéptido con dominio de unión que está fusionado o conectado de otra forma

a un polipéptido de región de pivote de inmunoglobulina, en donde el polipéptido con dominio de unión es capaz de unirse o unirse específicamente con al menos un antígeno que está presente sobre una superficie de célula cancerosa y en donde el polipéptido con región de pivote puede estar como se describió anteriormente o aquí, y puede comprender, consiste esencialmente de, consiste de, por ejemplo, un polipéptido seleccionado del grupo que consiste de un polipéptido con región de pivote IgA humano nativo o trabajado con ingeniería, un polipéptido con región de pivote IgG humano nativo o trabajado con ingeniería, y una región de pivote IgE humana nativa o trabajada con ingeniería, es decir, IgE CH2, polipéptido de región, (b) un primer polipéptido de región constante de cadena pesada de inmunoglobulina nativo trabajado con ingeniería que está fusionado o conectado de otra forma al polipéptido de región de pivote, en donde el primer polipéptido de región constante comprende, consiste esencialmente de, o consiste de, un polipéptido que es nativo o un polipéptido de región constante IgA CH2 humano nativo o trabajado con ingeniería, un polipéptido de región constante IgG CH2 humano nativo o trabajado con ingeniería, o un polipéptido con región constante IgE CH3 humano nativo o trabajado con ingeniería, y (c) un segundo polipéptido con región constante de cadena pesada de inmunoglobulina nativo o trabajado con ingeniería que está fusionado o conectado de otra forma al primer polipéptido de región constante, en

donde el segundo polipéptido de región constante comprende, consiste esencialmente de, o consiste de, un polipéptido que es un polipéptido con región constante IgA CH3 humano nativo o trabajado con ingeniería, un polipéptido con región constante IgG CH3 humano nativo o trabajado con ingeniería, o un polipéptido con región constante IgE CH4 humano nativo o trabajado con ingeniería En una modalidad adicional los polipéptidos IgG humanos son polipéptidos IgG1 humanos nativos o trabajados con ingeniería

En otra modalidad la presente invención suministra una proteína de fusión de inmunoglobulina con dominio de unión, que comprende, consiste esencialmente de, o consiste de, (a) un polipéptido con dominio de unión que está fusionado o conectado de otra forma a un polipéptido con región de pivote de inmunoglobulina, en donde dicho polipéptido con región de pivote puede ser como se describió anteriormente o aquí, y puede comprender, consiste esencialmente de, consiste de, por ejemplo, un polipéptido con región de pivote IgA humano tipo silvestre o trabajado con ingeniería, (b) un polipéptido con región constante CH2 de cadena pesada de inmunoglobulina nativo o trabajado con ingeniería que está fusionado o conectado de otra forma al polipéptido con región de pivote, en donde dicho polipéptido con región constante CH2 nativo o trabajado con ingeniería, consiste esencialmente de, consiste de, un polipéptido con región constante IgA CH2 humano nativo

o trabajado con ingeniería, y (c) un polipéptido con región constante CH3 de cadena pesada de inmunoglobulina nativo o trabajado con ingeniería que está fusionado o conectado de otra forma a un polipéptido con región constante CH2 nativo o trabajado con ingeniería, en donde el polipéptido con región constante CH3 nativo o trabajado con ingeniería comprende, consiste esencialmente de, o consiste de, un polipéptido que es (i) un polipéptido con región constante IgA CH3 humano tipo silvestre u otra región IgA, preferiblemente humana o humanizada, que es capaz de asociarse con Cadena J, (ii) un polipéptido con región constante IgA CH3 humano mutado, alterado o trabajado de otra manera con ingeniería que es, incapaz de asociarse con una cadena J, en donde (1) la proteína de fusión de inmunoglobulina con dominio de unión es capaz de al menos actividad inmunológica seleccionada del grupo que consiste de citotoxicidad mediada por célula dependiente de anticuerpo, CDC, y fijación de complemento, y (2) el polipéptido con dominio de unión es capaz de unirse o unirse específicamente a un objetivo tal como, por ejemplo un antígeno En ciertas modalidades adicionales el polipéptido con región constante IgA CH3 humano mutado que es capaz de asociarse con una cadena J es (i) un polipéptido que comprende, consiste esencialmente de, o consiste de, una secuencia de aminoácido como se estableció en la ID DE SEC NO _ o (ii) un polipéptido que comprende, consiste esencialmente de, o consiste de, una secuencia de aminoácido

como se estableció en la ID DE SEC NO _ En otras modalidades, el polipéptido con región de pivote IgA es un polipéptido con región de pivote IgA1 nativo o trabajado con ingeniería o un polipéptido con región de pivote IgA2 nativo o trabajado con ingeniería En aún otras modalidades, el polipéptido con región de pivote IgA es diferente del polipéptido con región de pivote IgA1 o IgA2 tipo silvestre mediante, por ejemplo, la alteración, sustitución, o supresión de uno o más residuos cisteína dentro de dicha región de pivote tipo silvestre

En ciertas otras modalidades la presente invención suministra una proteína de fusión de inmunoglobulina con dominio de unión, que comprende, consiste esencialmente de, o consiste de (a) un polipéptido con dominio de unión que está fusionado o conectado de otra forma a un polipéptido con región de pivote de inmunoglobulina, (b) un polipéptido con región constante CH2 con cadena pesada de inmunoglobulina nativo o trabajado con ingeniería que está fusionado o conectado de otra forma al polipéptido con región de pivote, en donde el polipéptido con región constante CH2 nativo o trabajado con ingeniería comprende, consiste esencialmente de, o consiste de, un polipéptido con región constante CH2 de llama nativo o trabajado con ingeniería que es un polipéptido con región constante IgG1 CH2 de llama nativo o trabajado con ingeniería, un polipéptido con región constante IgG2 CH2 de

llama nativo o trabajado con ingeniería, o un polipéptido con región constante IgG3 CH2 de llama nativo o trabajado con ingeniería, y (c) un polipéptido con región constante CH3 de cadena pesada de inmunoglobulina nativo o trabajado con ingeniería está fusionado o conectado de otra forma al polipéptido con región constante CH2 nativo o trabajado con ingeniería, en donde dicho polipéptido con región constante CH3 nativo o trabajado con ingeniería, consiste esencialmente de, o consiste de, un polipéptido con región constante CH3 de llama nativo o trabajado con ingeniería que se selecciona del grupo que consiste de un polipéptido con región constante IgG1 CH3 de llama nativo o trabajado con ingeniería, un polipéptido con región constante IgG2 CH3 de llama nativo o trabajado con ingeniería y un polipéptido con región constante IgG3 CH3 de llama nativo o trabajado con ingeniería en donde (1) la proteína de fusión de inmunoglobulina con dominio de unión de al menos una actividad inmunológica seleccionada del grupo que consiste de citotoxicidad mediada con célula dependiente de anticuerpo, fijación de complemento CDC, y (2) el polipéptido con dominio de unión es capaz de unirse o unirse específicamente a un objetivo, por ejemplo un antígeno objetivo En una modalidad adicional el polipéptido con región de pivote de inmunoglobulina, el polipéptido con región constante CH2 de llama nativo o trabajado con ingeniería y el polipéptido con región constante CH3 de llama nativo o trabajado con ingeniería comprende secuencias

derivadas de un polipéptido IgG1 de llama nativo o trabajado con ingeniería y la proteina de fusión no incluye un dominio IgG1 CH1 de llama nativo o trabajado con ingeniería En ciertas modalidades la invención suministra cualquiera de las proteínas de fusión de inmunoglobulina con dominio de unión descritas anteriormente en donde el polipéptido con región de pivote se muta, se trabaja con ingeniería, o se altera de otra forma para contener un sitio de glicosilación, el cual en ciertas modalidades adicionales es un sitio de glicosilación ligado a asparagina, un sitio de glicosilación ligado a O, un sitio de manosilación C, o un sitio de fosfoglicación

En ciertas modalidades la invención, suministran cualquiera de las construcciones de unión descritas anteriormente o aquí, que incluyen proteinas de fusión de inmunoglobulina con dominio de unión en donde el polipéptido con región de unión o dominio de unión comprende dos o más secuencias de polipéptido con dominio de unión en donde cada una de las secuencias de polipéptido con dominio de unión es capaz de unirse o unirse específicamente al o los objetivos tal como un o unos antígenos cuyo o cuyos objetivos antígeno o antigenos pueden ser iguales o diferentes Un nativo, más preferiblemente un pivote IgD trabajado con ingeniería es una región de conexión deseada entre los dominios de unión de una molécula biespecífica de la invención, es decir, con dos o

más dominios de unión, preferiblemente dos El pivote IgD humano tipo silvestre tiene una cisteína que forma un enlace disulfuro con la cadena liviana en la estructura IgD nativa Es deseable mutar o suprimir esta cisteína en el pivote IgD humano para uso como una región de conexión entre los dominios de unión de, por ejemplo, una molécula biespecífica Otros cambios de aminoácido o supresiones o alteraciones en un pivote IgD que no den como resultado una inflexibilidad de pivote indeseada están dentro del alcance de la invención La región de pivote IgD nativa o trabajada con ingeniería proveniente de otras especies está también dentro del alcance de la invención, como son las articulaciones IgD nativos o trabajados con ingenieria de especies no humanas La presente invención también suministra, en ciertas modalidades, una proteína de fusión de inmunoglobulina con dominio de unión, que comprende, consiste esencialmente de, o consiste de (a) un polipéptido con dominio de unión que está fusionado o conectado de otra forma a un polipéptido con región de pivote de inmunoglobulina, en donde el polipéptido con región de pivote puede ser como se describió anteriormente o aqui, y puede comprender, consiste esencialmente de, o consiste de, por ejemplo, una secuencia de polipéptido con región de pivote alternativa, (b) una región constante de cadena pesada de inmunoglobulina nativa o trabajada con ingeniería, tal como un polipéptido con región constante IgG o IgA CH2 (o un polipéptido con región constante IgE CH3) que está fusionado

o conectado de otra forma a un polipéptido con región de pivote, y (c) una segunda región constante de cadena pesada de inmunoglobulina nativa o trabajada con ingeniería, tal como un polipéptido con región constante IgG o IgA CH3 (o un polipéptido con región constante IgE CH4) que está fusionado o conectado de otra manera al primer polipéptido con región constante, en donde (1) la proteína de fusión de inmunoglobulina con dominio de unión es capaz de al menos una actividad inmunológica seleccionada de un grupo que consiste de citotoxicidad mediada por célula dependiente de anticuerpo, CDC, y fijación de complemento, y (2) el polipéptido con dominio de unión es capaz de unirse o unirse específicamente a un objetivo, tal como un antígeno

Regresando ahora a otra modalidad se suministra una proteína de fusión de inmunoglobulina con dominio de unión, que comprende, consiste esencialmente de, o consiste de (a) un polipéptido con dominio de unión que está fusionado o conectado de otra forma a un polipéptido con región de pivote de inmunoglobulina, en donde el polipéptido con dominio de unión es capaz de unirse o unirse específicamente a al menos un objetivo, tal como un antígeno, que está presente sobre una superficie de célula cancerosa y en donde el polipéptido con región de pivote puede ser como se describió anteriormente o aquí, y puede comprender, consiste esencialmente de, o consiste de, por ejemplo, una secuencia

de polipéptido con región de pivote alternativa, (b) un primer polipéptido con región constante de cadena pesada de inmunoglobulina nativo o trabajado con ingeniería que está fusionado o conectado de otra forma a un polipéptido con región de pivote, en donde dicho polipéptido con región constante nativo o trabajado con ingeniería comprende, consiste esencialmente de, o consiste de, un polipéptido seleccionado del grupo que consiste de un polipéptido con región constante IgA CH2 humano nativo o trabajado con ingeniería, un polipéptido con región constante IgG CH2 humano nativo o trabajado con ingeniería y un polipéptido con región constante IgE CH3 humano nativo o trabajado con ingeniería, y (c) un segundo polipéptido con región constante de cadena pesada de inmunoglobulina que está fusionado o conectado de otra forma al primer polipéptido con región constante, en donde el segundo polipéptido con región constante comprende, consiste esencialmente de, o consiste de, un polipéptido que es un polipéptido con región constante IgA CH3 humano nativo o trabajado con ingeniería, un polipéptido con región constante IgG CH3 humano nativo o trabajado con ingeniería, o un polipéptido con región constante IgE CH4 humano nativo o trabajado con ingeniería

En ciertas modalidades adicionales la secuencia de polipéptido con región de pivote alternativa comprende, consiste esencialmente de, o consiste de, una secuencia de

polipéptido de al menos diez aminoácidos continuos que están presentes en una secuencia seleccionada de la ID DE SEC NO _

En ciertas modalidades la presente invención suministra polinucleótidos o vectores (que incluyen vectores de clonación y vectores de expresión) o células transformadas o transfectadas, que incluyen polinucleótidos aislados o purificados o puros, vectores, y células aisladas transformadas o transfectadas, que codifican o que contienen uno cualquiera de los polipéptidos descritos anteriormente o aquí, o construcciones de proteína de la invención, por ejemplo, que incluyen proteínas de fusión de inmunoglobulina con dominio de unión Así, en varias modalidades la invención suministra una clonación recombinante o una construcción de expresión que comprende cualquiera de tales polinucleótidos que está operablemente ligado a un promotor

En otra modalidad se suministra una célula huésped transformada o transfectada con, o que contiene de otra forma cualquiera de tales clonaciones recombinantes o construcciones de expresión Las células huésped incluyen las células de un sujeto que sufre terapia celular *ex vivo* que incluye, por ejemplo, terapia génica *ex vivo*

En una modalidad relacionada se suministra un método para producir un polipéptido o proteína u otra construcción de la

invención, por ejemplo, que incluye una proteína de fusión de inmunoglobulina con dominio de unión, que comprende las etapas de (a) cultivar una célula huésped como se describió o se suministra lo presente bajo condiciones que permiten la expresión de la construcción, por ejemplo, una proteína de fusión de inmunoglobulina con dominio de unión, y (b) aislar la construcción, por ejemplo, la proteína de fusión de inmunoglobulina con dominio de unión de la célula huésped o el cultivo de célula huésped

En otra modalidad se suministra una composición farmacéutica que comprende uno cualquiera de los polipéptidos o proteínas anteriores o descritos aquí, u otras construcciones de la invención, por ejemplo, (incluyendo, por ejemplo, proteínas de fusión de inmunoglobulina con dominio de unión), en combinación con un portador fisiológicamente aceptable

En otra modalidad de la invención se suministra una composición farmacéutica que comprende, por ejemplo, un polinucleótido aislado, purificado o puro que codifica uno cualquiera de los polipéptidos o construcciones de proteína de la invención, por ejemplo (incluyendo, por ejemplo, proteinas de fusión de inmunoglobulina con dominio de unión), en combinación con un portador fisiológicamente aceptable, o por ejemplo, en combinación con, o en, un vehículo o vector de suministro de terapia de genes

En otra modalidad la invención suministra un método para tratar un sujeto que tiene o se sospecha que tiene una condición maligna o un desorden de célula B, que comprende administrar a un paciente una cantidad terapéuticamente efectiva de cualquiera de las composiciones farmacéuticas descritas o reivindicadas aquí

En ciertas modalidades adicionales la condición maligna o el desorden de célula B es un linfoma de célula B o una leucemia de célula B, o una enfermedad caracterizada por la producción de anticuerpo, y en ciertas otras modalidades el desorden de célula B es, por ejemplo, artritis reumatoide, miastenia gravis, enfermedad de Grave, diabetes melitus tipo I, esclerosis múltiple o una enfermedad autoinmune En ciertas otras modalidades la condición maligna es, por ejemplo, melanoma, mieloma, glioma, astrocitoma, linfoma, leucemia, carcinoma, o sarcoma, y así sucesivamente

Es un aspecto de la presente invención suministrar una proteína de fusión de inmunoglobulina con dominio de unión, que comprende, consiste esencialmente de, o consiste de, (a) un polipéptido con dominio de unión que está fusionado o conectado de otra forma a un polipéptido con región de pivote de inmunoglobulina, en donde dicho polipéptido con región de pivote es como se describió aquí, y se puede seleccionar del

grupo que consiste de (i) un polipéptido con región de pivote mutado, trabajado con ingeniería o alterado de otra forma que no contiene residuos de cisteína y que se deriva del polipéptido con región de pivote de inmunoglobulina tipo silvestre que tiene uno o más redichos de cisteína, (ii) un polipéptido con región de pivote mutado, trabajado con ingeniería o alterado de otra forma que contiene un residuo de cisteína que se deriva del polipéptido con región de pivote de inmunoglobulina tipo silvestre que tiene dos o más residuos de cisteína, (iii) un polipéptido con región de pivote IgA humano tipo silvestre, (iv) un polipéptido con región de pivote IgA humano mutado, trabajado con ingeniería o alterado de otra forma que no contiene residuos de cisteína, (v) un polipéptido con región de pivote IgA humano mutado, trabajado con ingeniería o alterado de otra forma que contiene un residuo de cisteina y (vi) un polipéptido con región de pivote IgA humano mutado, trabajado con ingeniería o alterado de otra manera que contiene dos residuos de cisteína, (b) un polipéptido con región constante CH2 de cadena pesada de inmunoglobulina nativo o trabajado con ingeniería que está fusionado o conectado de otra forma al polipéptido con región de pivote, y (c) un polipéptido con región constante CH3 de cadena pesada de inmunoglobulina nativo o trabajado con ingeniería que está fusionado o conectado de otra forma a un polipéptido con región constante CH2, en donde (1) la proteína de fusión de inmunoglobulina

con dominio de unión es capaz de al menos una actividad inmunológica seleccionada del grupo que consiste de citotoxicidad mediada por célula dependiente de anticuerpo y fijación de complemento y (2) el polipéptido con dominio de unión es capaz de unirse o unirse específicamente a un antígeno En una modalidad el polipéptido con región de pivote de inmunoglobulina es un polipéptido con región de pivote mutado, por ejemplo, y las construcciones resultantes exhiben una capacidad reducida de dimerizarse, con relación a una construcción que contiene un polipéptido con región de pivote de inmunoglobulina G humano tipo silvestre En otra modalidad el polipéptido con dominio de unión comprende, consiste esencialmente de, o consiste de, al menos un polipéptido con región variable de inmunoglobulina nativo o trabajado con ingeniería que es un polipéptido con región variable de cadena ligera de inmunoglobulina nativo o trabajado con ingeniería y/o un polipéptido con región variable de cadena pesada de inmunoglobulina nativo o trabajado con ingeniería En una modalidad adicional el polipéptido con región variable de inmunoglobulina nativo o trabajado con ingeniería se deriva de la inmunoglobulina humana y, por ejemplo, se puede humanizar

En otra modalidad, la invención suministra una proteína de fusión de inmunoglobulina con dominio de unión que incluye un polipéptido con dominio de unión que comprende, consiste

esencialmente de, o consiste de, (a) al menos un polipéptido con región variable de cadena liviana de inmunoglobulina nativo o trabajado con ingeniería, (b) al menos un polipéptido con región variable de cadena pesada de inmunoglobulina nativo o trabajado con ingeniería, y (c) al menos un péptido ligador que está fusionado o conectado de otra forma al polipéptido de (a) y al polipéptido de (b) En una modalidad adicional la región variable de cadena liviana de inmunoglobulina nativa o trabajada con ingeniería y los polipéptidos de región variable de cadena pesada nativos o trabajados con ingeniería se derivan de las inmunoglobulinas humanas y pueden, por ejemplo, ser humanizadas

En otra modalidad al menos un polipéptido con región constante CH2 (o IgE CH3) de cadena pesada de inmunoglobulina nativo o trabajado con ingeniería y el polipéptido de región constante CH3 (o IgE CH4) de cadena pesada de inmunoglobulina nativo o trabajado con ingeniería se deriva o se construye de una cadena pesada de inmunoglobulina humana En otra modalidad los polipéptidos CH2 y CH3 con región constante de cadena pesada de inmunoglobulina nativos o trabajados con ingeniería son de, o se derivan o se preparan de otra forma o se construyen de, un isotipo seleccionado del IgG humano y el IgA humano En otra modalidad el objetivo, por ejemplo, el antígeno objetivo se selecciona del grupo que consiste de CD16, CD19, CD20, CD37, CD40, CD45RO, CD80, CD86, CD137,

CD152 y L6 En ciertas modalidades adicionales de las construcciones de proteína de fusión descritas anteriormente, el dominio de unión comprende, consiste esencialmente de, o consiste de, un scFv y el scFv contiene un polipéptido ligador que comprende, consiste esencialmente de, o consiste de, al menos un polipéptido que comprende o que tiene una secuencia de aminoácido Gly-Gly-Gly-Gly-Ser [ID DE SEC NO _], y en ciertas otras modalidades el polipéptido ligador comprende, consiste esencialmente de, o consiste de, al menos tres repeticiones de un polipéptido que tiene una secuencia de aminoácido Gly-Gly-Gly-Gly-Ser [ID DE SEC NO _] En ciertas modalidades el polipéptido con región de pivote de inmunoglobulina comprende, consiste esencialmente de, o consiste de, un polipéptido con región de pivote IdG, IgA, IgD humano nativo o trabajado con ingeniería, o un polipéptido con región IgE CH2 nativo o trabajado con ingeniería En ciertas modalidades el polipéptido con dominio de unión comprende, consiste esencialmente de, o consiste de, un dominio extracelular CD154 nativo o trabajado con ingeniería En ciertas modalidades el polipéptido con dominio de unión comprende, consiste esencialmente de, o consiste de, un dominio extracelular CD154 nativo o trabajado con ingeniería y al menos un polipéptido con región variable de inmunoglobulina nativo o trabajado con ingeniería

En otras modalidades la invención suministra un polinucleótido aislado que codifica cualquiera de las construcciones de la invención, por ejemplo, la proteína o las construcciones de polipéptido de la invención que incluyen proteínas de fusión de inmunoglobulina con dominio de unión, y en modalidades relacionadas la invención suministra una construcción de expresión recombinante que comprende tal polinucleótido, y en ciertas modalidades adicionales la invención suministra una célula huésped transformada o transfectada con, o que contiene de otra forma tal construcción de expresión recombinante En otra modalidad la invención suministra un método para producir una construcción de la invención, por ejemplo, una proteína o una construcción de polipéptido de la invención tal como la proteína de fusión de inmunoglobulina con dominio de unión, que comprende las etapas de (a) cultivar una célula huésped que ha sido transformada o transfectada con, o hecha de otra forma para contener, una construcción de polinucleótido de la invención bajo condiciones que permitan la expresión de la construcción, por ejemplo, una construcción que codifica una proteína de fusión de inmunoglobulina con dominio de unión, y (b) aislar la construcción, por ejemplo, la proteína de fusión de inmunoglobulina con dominio de unión, del cultivo de célula huésped

La invención descrita y reivindica aquí incluye moléculas novedosas útiles, por ejemplo, como terapia y otros propósitos que incluyen propósitos de diagnóstico y de investigación Tales moléculas tienen, por ejemplo, unión de antígeno u otras funciones de unión y una o más funciones efectoras Las construcciones de ADN de la invención son utiles en, por ejemplo, terapias génicas, que incluyen terapias génicas *in vivo* y *ex vivo*

En un aspecto, las varias construcciones de moléculas de la invención incluyen moléculas que comprenden una "región de unión", una región "de cola", y una región "de conexión" que se une a una región de unión y a una región de cola

Las regiones de unión dentro de las moléculas de la invención pueden comprender, por ejemplo, dominios de unión para los objetivos deseados, que incluyen los objetivos de unión de antígeno Los dominios de unión para objetivos que se unen al antígeno pueden comprender, por ejemplo, una cadena sencilla Fvs y un dominio scFv En ciertas modalidades, las moléculas de la invención pueden comprender una región de unión que tiene al menos un polipéptido con región variable de inmunoglobulina, que puede ser un polipéptido con región variable de cadena liviana o cadena pesada En ciertas modalidades, las moléculas de la invención pueden comprender al menos una de tales regiones V de cadena liviana y una de

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tales regiones V de cadena pesada y al menos un péptido ligador que conecta las regiones V El ScFvs util en la invención también incluye aquellos con unión quimérica u otros dominios o secuencias Otros ScFvs utiles en la invención también incluyen aquellos con unión humanizada u otros dominios o secuencias En tales modalidades, toda o una porción de una unión de inmunoglobulina u otra secuencia que se deriva de la fuente no humana puede ser "humanizada" de acuerdo con procedimientos reconocidos para generar anticuerpos humanizados, es decir, secuencias de inmunoglobulina en las cuales las secuencias Ig humanas son introducidas para reducir el grado al cual el sistema inmune humano percibiría tales proteínas como extrañas

Ejemplos de scFvs útiles en la invención, sea incluidos como murino u otros scFvs (que incluyen scFvs humano), scFvs químérico, o scFvs humanizado, en todo o en parte incluyen scFvs CD20 antihumanos (por ejemplo, scFvs "2H7"), scFvs CD37 anti-humano (por ejemplo, scFvs "G28-1"), scFvs CD40 anti-humano (por ejemplo, scFvs "G28-5" y scFvs "40 2 220"), scFvs de antígeno asociados a carcinoma (por ejemplo, scFvs "L6"), scFvs anti-CTLA-4 (CD152) (por ejemplo, scFvs "10A8"), scFvs CD28 anti-humano (por ejemplo, scFvs "2E12"), scFvs CD3 anti-murino (por ejemplo, scFvs "500A2"), scFvs CD3 anti-humano (por ejemplo, scFvs G19-4), scFvs 4-1BB anti-murina (por ejemplo, scFvs "1D8"), scFvs 4-1BB anti-humano (por ejemplo,

scFvs "5B9"), CD45RO anti-humano (por ejemplo, scFvs "UCHL-1"), y CD16 anti-humano (por ejemplo, scFvs "Fc2")

Los scFvs útiles en la invención también incluyen scFvs que incluyen scFvs quiméricos y humanizados, que tienen una o más sustituciones de aminoácido Una sustitución de aminoácido preferida es la posición del aminoácido 11 en la cadena pesada variable (el V_H) Tal sustitución se puede preferir aquí como "XxxV_H11Zxx" Así, por ejemplo, cuando el aminoácido de ocurrencia normal en la posición V_H11 es una Leucina, y un residuo de aminoácido Serina es sustituido por lo tanto, la sustitución se identifica como "L V_H11S" o "Leu V_H11Ser" Otras modalidades preferidas de la invención incluyen moléculas que contienen scFvs en donde el residuo de aminoácido normalmente encontrado en la posición V_H11 se suprime Aún otras modalidades adicionales de la invención incluyen moléculas que contienen scFvs en donde los residuos de aminoácido normalmente encontrados en las posiciones V_H10 y/o V_H11 y/o V_H12 se sustituyen o se suprimen

Otras regiones de unión dentro de las moléculas de la invención pueden incluir dominios que comprenden sitios para glicosilación, por ejemplo, unión covalente de porciones carbohidrato tales como monosacáridos u oligosacáridos

Aún otras regiones de unión dentro de moléculas de la invención incluyen polipéptidos que pueden comprender proteínas o porciones de éstas que retienen la capacidad para unirse específicamente a otra molécula, incluyendo un antígeno Así, las regiones de unión pueden comprender o derivarse de hormonas, citoquinas, quimioquinas, y similares, la superficie de la célula o los receptores solubles para tales ligandos de polipéptido, lectinas, receptores de adhesión intercelular tales como integrinas de leucocito específicas, selectinas, miembros de la superfamilia de gen inmunoglobulina, moléculas de adhesión intercelular (ICAM-1, -2, -3) y similares Antígenos de histocompatibilidad, y asi sucesivamente Las regiones de unión derivadas de tales porciones generalmente incluirán porciones thoss de moléculas necesarias o deseadas para unión a un objetivo

Ciertas construcciones incluyen regiones de unión que comprenden receptores o dominios de unión de receptor Los dominios de receptor útiles para unirse a un objetivo incluyen, por ejemplo, un dominio extracelular CD154, o un dominio extracelular CTLA-4 En otro ejemplo, el dominio de unión puede incluir una primera porción que comprende, consiste esencialmente de, o consiste de, un dominio extracelular CD154 y una segunda porción que comprende, consiste esencialmente de, o consiste de, al menos un polipéptido de región variable de inmunoglobulina, dicha

segunda porción incluye, por ejemplo, un scFv o un V_H Ejemplos de otros receptores de superficie celular que pueden comprender, consiste esencialmente, o consiste de, o una porción de las cuales puede suministrar, una región de unión o un polipéptido con dominio de unión, incluyen, por ejemplo, HER1, HER2, HER3, HER4, receptor de factor de crecimiento epidérmico (EGFR), factor de crecimiento celular endotelial vascular, receptor de factor de crecimiento celular endotelial vascular, factor I de crecimiento similar a insulina, factor II de crecimiento similar a insulina, receptor de transferrina, receptor de estrógeno, receptor de progesterona, receptor de hormona estimulante de folículo (FSH-R), receptor de ácido retinoico, MUC-1, NY-ESO-1, Melan-A/MART-1, tirosinasa, Gp-100, MAGE, BAGE, GAGE, cualquiera de las clases CTA de los receptores que incluyen en particular el antígeno HOM-MEL-40 codificado mediante el gen SSX2, antígeno carcinoembiónico (CEA), y PyLT Los receptores de superficie celular adicionales que pueden ser fuentes de regiones de unión o polipéptidos con dominios de unión incluyen, por ejemplo, CD2, 4-1BB, ligando 4-1BB, CD5, CD10, CD27, CD28, CD152/CTLA-4, CD40, interferón γ (IFN-γ), interleuquina-4 (IL-4), interleuquina-17 (IL-17) y receptor de interleuquina-17 (IL-17R) Aún otros receptores de superficie celular que pueden ser fuentes de regiones de unión y/o polipéptidos con dominio de unión incluyen, por ejemplo, CD59, CD48, CD58/LFA-3, CD72, CD70, CD80/B7 1,

CD86/B7 2, B7-H1/B7-DC, IL-17, CD43, ICOS, CD3 (por ejemplo, subunidad gama, subunidad epsilon, subunidad delta), CD4, CD25, CD8, CD11b, CD14, CD56, CD69 y VLA-4 ($\alpha_4\beta_7$) Los siguientes receptores de superficie celular están típicamente asociados con células B CD19, CD20, CD22, CD30, CD153 (ligando CD30), CD37, CD50 (ICAM-3), CD106 (VCAM-1), CD54 (ICAM-1), interleuquina-12, CD134 (OX40), CD137 (41BB), CD83, y DEC-205 Estas listas no son exhaustivas Las regiones de unión tales como aquellas establecidas anteriormente pueden estar conectadas, por ejemplo, por un polipéptido de región de pivote IgD nativo o trabajado con ingeniería, preferiblemente un polipéptido con región de pivote IgD nativo o trabajado con ingeniería humano o humanizado La invención suministra adicionalmente así construcciones que comprenden, consisten esencialmente de, o consisten de, dos regiones de unión, por ejemplo un scFv y un receptor de superficie celular (o porción de éste), conectado mediante una tercera molécula, por ejemplo, un polipéptido con región de pivote IgD como se describió aquí

Varias moléculas de la invención descritas y reivindicadas aquí incluyen una región de conexión que une un extremo de la molécula a otro extremo Tales regiones de conexión pueden comprender, por ejemplo, polipéptidos con región de pivote de inmunoglobulina, que incluyen cualquier péptido de pivote o polipéptido de ocurrencia natural Una región de conexión

también puede incluir, por ejemplo, cualquier péptido artificial u otra molécula (que incluye, por ejemplo, moléculas no-péptido, moléculas de péptido parcial, y peptidomiméticos, etc) útiles para unir la región de cola y la región de unión Estos pueden incluir, por ejemplo, alteraciones de moléculas situadas en un polipéptido de cadena pesada de inmunoglobulina entre los residuos de aminoácido responsables por formar uniones disulfuro de dominio de inmunoglobulina de intracadena en regiones CH1 y CH2 Regiones de pivote de ocurrencia natural que incluyen aquellas localizadas entre unos dominios de región constante, CH1 y CH2, de una inmunoglobulina Polipéptidos útiles de región de pivote de inmunoglobulina incluyen, por ejemplo, polipéptidos de región de pivote de inmunoglobulina humana y polipéptidos de región de pivote de inmunoglobulina de llama u otro camélido Otros polipéptidos útiles de región de pivote de inmunoglobulina incluyen, por ejemplo, tiburón nodriza y polipéptidos de región de pivote de inmunoglobulina de pez rata manchado Los polipéptidos de región de pivote de inmunoglobulina humana incluyen, por ejemplo, articulaciones IgG tipo silvestre que incluyen articulaciones IgG1 humano tipo silvestre, polipéptidos de región de pivote de inmunoglobulina derivada de IgG humano, una porción de un pivote IgG humana o una región de pivote de inmunoglobulina derivada de IgG, polipéptidos de región de pivote IgA humana tipo silvestre, polipéptidos de región de pivote de

inmunoglobulina derivados de IgA humano, una porción de un polipéptido de región de pivote IgA humano o un polipéptido de región de pivote de inmunoglobulina derivado de IgA, polipéptidos de región de pivote IgD tipo silvestre, polipéptidos de región de pivote de inmunoglobulina derivados de IgD humano, una porción de un polipéptido de región de pivote IgD humano o un polipéptido de región de pivote de inmunoglobulina derivado de IgD, región de acción de pivote IgE humana tipo silvestre, es decir, polipéptidos de región IgE CH2 (que generalmente tienen 5 residuos de cisteina), polipéptidos de región de pivote de inmunoglobulina derivados de IgE humano, una porción de una región de acción de pivote IgE humana, es decir, un polipéptido de región IgE CH2 o un polipéptido de región de pivote de inmunoglobulina derivada de IgE, y así sucesivamente Un polipéptido "derivado de" o que es "una porción o fragmento de" una región de cadena de polipéptido de inmunoglobulina considerado por tener una función de pivote tiene uno o más aminoácidos en enlace peptídico, por ejemplo 15-115 aminoácidos, preferiblemente 95-110, 80-94, 60-80, o 5-65 aminoácidos, preferiblemente 10-50, más preferiblemente 15-35, aún más preferiblemente 18-32, aún más preferiblemente 20-30, aun más preferiblemente 21, 22, 23, 24, 25, 26, 27, 28, o 29 aminoácidos Los polipéptidos de región de pivote de inmunoglobulina de llama incluyen, por ejemplo, un pivote de llama IgG1 La región de conexión puede comprender una tira de aminoácidos

consecutivos de una región de pivote de inmunoglobulina Por ejemplo, la región de conexión puede comprender al menos cinco aminoácidos de región de pivote consecutivos, al menos diez aminoácidos de región de pivote consecutivos, al menos quince aminoácidos de región de pivote consecutivos, al menos 20 aminoácidos de región de pivote consecutivos, y al menos veinticinco o más aminoácidos de región de pivote consecutivos de pivote IgG humano, pivote IgA humano, pivote IgE humano, región de pivote de camélido, región de pivote de llama IgG1, región de pivote de tiburón nodriza, y región de pivote de pez rata manchado, que incluye por ejemplo una región de pivote IgG_1, una región de pivote IgG_2, una región de pivote IgG_3, una región de pivote IgG_3, y una región de pivote IgG_4

Tales regiones de conexión también incluyen, por ejemplo, polipéptidos de región de pivotes de inmunoglobulina mutados o alterados de otra forma o trabajados por ingeniería Un polipéptido con región de pivote de inmunoglobulina mutado o alterado de otra forma o trabajo por ingeniería puede comprender, consiste esencialmente de, o consiste de, una región de pivote que tiene su origen en una inmunoglobulina de unas especies, de un isotipo o clase de inmunoglobulina, o de una subclase de inmunoglobulina que es igual o diferente de aquella de cualquiera incluyendo el dominio CH2 y CH3 nativos o trabajados por ingeniería Los polipéptidos de

región de pivote de inmunoglobulina mutados o alterados de otra forma o trabajados con ingeniería incluyen aquellos derivados o construidos de, por ejemplo, región de pivote de inmunoglobulina tipo silvestre que contiene uno o más residuos cisteína, por ejemplo, una región de pivote IgG o IgA humana tipo silvestre que comprende naturalmente tres cisteínas En tales polipéptidos el número de residuos de cisteína se puede reducir mediante sustitución de aminoácido o supresión o truncamiento, por ejemplo Estos polipéptidos incluyen, por ejemplo, polipéptidos de región de pivote IgG1 o IgG4 humanos mutados u otros que contienen cero, uno, o dos residuos de cisteína, y polipéptidos de región de pivote IgA1 o IgA2 humanos mutados u otros que contienen cero, uno, o dos residuos de cisteína Los polipéptidos de región de pivote de inmunoglobulina mutados o alterados de otra forma o trabajados con ingeniería incluyen aquellos derivados o construidos de, por ejemplo, región de pivote de inmunoglobulina tipo silvestre que contiene tres o más residuos de cisteína, por ejemplo, una región de pivote IgG2 humana tipo silvestre (que tiene 4 cisteínas) o región de pivote IgG4 (que tiene 11 cisteínas) Polipéptidos de región de pivote de inmunoglobulina mutados o alterados de otra forma o trabajados con ingeniería incluye aquellos derivados o construidos de, por ejemplo, una región de inmunoglobulina tipo silvestre IgE CH2 que contiene generalmente cinco residuos de cisteína En tales polipéptidos el número de

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residuos de cisteína se puede reducir por uno o más residuos de cisteína por sustitución o supresión o truncamiento de aminoácido, por ejemplo También se incluyen polipéptidos de región de pivote alterados en los cuales los residuos de cisteína de la región de pivote son sustituidos con serina o uno o más aminoácidos que son menos polares, menos hidrófobos, más hidrofílicos, y/o neutros Tales polipéptidos de región de pivote de inmunoglobulina mutados incluyen, por ejemplo, polipéptidos de región de pivote mutada que contienen un residuo de cisteína que son derivados de polipéptido con región de pivote de inmunoglobulina tipo silvestre que tiene dos o más residuos de cisteína, tal como un polipéptido de región de pivote IgG o IgA humano mutado que contiene un residuo de cisteína que se deriva del polipéptido de región IgG o IgA humano tipo silvestre Los polipéptidos de región de conexión incluyen polipéptidos de región de pivote de inmunoglobulina que están comprometidos en su capacidad para formar uniones de disulfuro homodiméricas intercadena

Los polipéptidos de región de pivote de inmunoglobulina mutados también incluyen polipéptidos de región de pivote mutados que exhiben una capacidad reducida para dimerizarse, con relación al polipéptido de región de pivote G de inmunoglobulina humana tipo silvestre, y polipéptidos de región de pivote mutados que permiten la expresión de una

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mezcla de moléculas monoméricas y diméricas Los polipéptidos de región de pivote de inmunoglobulina mutados también incluyen polipéptidos de región de pivote trabajados con ingeniería para contener un sitio de glicosilación Los sitios de glicosilación incluyen, por ejemplo, un sitio de glicosilación ligado a asparagina, un sitio de glicosilación ligado a O, un sitio de manosilación C, un sitio de glipiación, y un sitio fosfoglicación

Las regiones de conexión específicas utiles en moléculas de la invención descritas y reivindicadas aquí incluyen, por ejemplo, las siguientes secuencias de 18 aminoácidos, DQEPKSCDKTHTCPPCPA, DQEPKSSDKTHTSPPSPA, Y DLEPKSCDKTHTCPPCPA Otras regiones de conexión específicas incluyen, por ejemplo, las articulaciones mutantes dentro de las secuencias denominadas aquí como "2H7 scFv (SSS-S)H WCH2 WCH3" y "2H7 scFv (CSS)H WCH2 WCH3", y el pivote derivado de IgA humano denominado aquí como "2H7 scFv IgAH WCH2 WCH3"

Las regiones de cola dentro de las moléculas de la invención pueden incluir secuencias de inmunoglobulina de región constante de cadena pesada Las regiones de cola pueden incluir, por ejemplo, un polipéptido que tiene al menos un polipéptido de región constante CH2 de cadena pesada de inmunoglobulina y un polipéptido de región constante CH3 de cadena pesada de inmunoglobulina Al menos uno de los

polipéptidos de región constante CH2 de cadena pesada de inmunoglobulina y el polipéptido de región constante CH3 de cadena pesada de inmunoglobulina derivado de una cadena pesada de inmunoglobulina humana Así, por ejemplo, los polipéptidos CH2 y/o CH3 se pueden derivar de moléculas de IgG humano, IgA humano, IgD humano Las regiones de cola también pueden incluir, por ejemplo, un polipéptido que tiene al menos un polipéptido de región constante CH3 de cadena pesada de inmunoglobulina y un polipéptido de región constante CH4 de cadena pesada de inmunoglobulina Al menos uno de los polipéptidos de región constante CH3 de cadena pesada de inmunoglobulina y el polipéptido de región constante CH4 de cadena pesada de inmunoglobulina se pueden derivar de una cadena pesada de inmunoglobulina humana Así, por ejemplo, los polipéptidos CH3 y/o CH4 se pueden derivar del IgE humano Un polipéptido de región CH2 de cadena pesada de inmunoglobulina incluido dentro de la molécula de la invención puede, por ejemplo, ser de las subclases IgG1, IgG2, IgG3 y/o IgG4 Un polipéptido de región CH3 de cadena pesada de inmunoglobulina incluido dentro de la molécula de la invención puede, por ejemplo, ser de las subclases IgG1, IgG2, IgG3 y/o IgG4 Adicionalmente, tanto el polipéptido de región CH2 de cadena pesada de inmunoglobulina como el polipéptido de región CH2 de cadena pesada de inmunoglobulina incluido dentro de una molécula de la invención pueden, por ejemplo, ser de las subclases IgG1, IgG2, IgG3 y/o IgG4 En

otras moléculas de la invención al menos uno de los polipéptidos de región constante de cadena pesada de inmunoglobulina seleccionado de un polipéptido de región constante CH2 y un polipéptido de región constante CH3 es un polipéptido de región constante IgA humano Un polipéptido de región CH2 de cadena pesada de inmunoglobulina incluido dentro de una molécula de la invención puede, por ejemplo, ser de las subclases IgA1 y/o IgA2 Un polipéptido de región CH3 de cadena pesada de inmunoglobulina incluido dentro de una molécula de la invención puede también, por ejemplo, ser de las subclases IgA1 y/o IgA2 Adicionalmente, tanto el polipéptido de región CH2 de cadena pesada de inmunoglobulina como el polipéptido de región CH2 de cadena pesada de inmunoglobulina incluido dentro de una molécula de la invención pueden, por ejemplo, ser de las subclases IgA1 y/o IgA2 En aun otras moléculas de la invención, la región de cola puede comprender o consiste esencialmente de un polipéptido de región constante CH2 y/o CH3 que comprende un polipéptido de IgA humano y/o IgE humano En otras modalidades, por ejemplo, la región de cola dentro de la molécula de la invención puede incluir un polipéptido de región constante CH2 y/o CH3 de cadena pesada de inmunoglobulina que es mutado (por ejemplo, un polipéptido de región constante IgA CH3 mutado que es capaz de asociarse con una cadena J en la cual, por ejemplo, el polipéptido de región constante IgA CH3 es de origen humano) La región de

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cola también puede comprender, consiste esencialmente de, o consiste de una porción extracelular de una proteína proveniente de la superfamilia TNF, por ejemplo, CD154

Para las moléculas de la invención destinadas a uso en humanos, estas regiones típicamente serán sustancial o completamente humanas para minimizar las respuestas inmunes humanas potenciales contra las moléculas y suministrar funciones efectoras apropiadas En ciertas modalidades de la invención, por ejemplo, la región de cola incluye una secuencia de región IgG1 CH3 humana, una secuencia de polipéptido de región constante de cadena pesada IgA tipo silvestre que es capaz o incapaz de asociarse con la cadena J

En las modalidades preferidas de la invención, un dominio CH1 no está incluido en la región de cola de la molécula, y el extremo carboxilo de la región de unión está unido a un amino terminal de la porción CH2 de la región de cola bien directa o indirectamente Una región de unión puede estar indirectamente unida a una región de cola, por ejemplo por vía de un polipéptido de región de conexión u otra molécula de conexión

La invención también incluye moléculas que tienen secuencias CH2 y/o CH3 mutadas dentro de una región de cola Por

ejemplo, una molécula de la invención puede incluir un dominio Fc mutado que tiene una o más mutaciones introducidas dentro de los dominios CH2, CH3 y/o CH4 En ciertas modalidades de la invención, las moléculas pueden incluir un polipéptido con región constante IgA CH3 tal como un polipéptido con región constante IgA CH3 humano en el cual dos o más residuos provenientes del C terminal se han suprimido para producir un polipéptido de región constante CH3 truncado En otras modalidades de la invención, las moléculas incluyen un polipéptido de región constante IgA CH3 humano mutando que es capaz de asociarse con una cadena J que comprende una supresión C terminal de cualquiera de cuatro o 18 aminoácidos Sin embargo, la invención necesita ser así limitada, de tal forma que las moléculas que contienen el polipéptido de región constante IgA CH3 mutado pueden comprender una supresión de 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21-25, 26-30 o más aminoácidos, en tanto que la proteína de fusión es capaz de unirse específicamente a un antigeno y capaz de al menos actividad inmunológica tal como ADCC, CDC o fijación de complemento La invención también incluye moléculas que contienen una región de cola que comprende un polipéptido de región constante IgA CH3 mutado que es incapaz de asociarse con una cadena J en virtud del reemplazo de la penultima cisteína, o mediante modificación química de ese residuo de

aminoácido, de una manera que evita la formación de unión disulfuro intercadena

Varias moléculas de la invención incluyen, por ejemplo, una proteína de fusión scFv con dominio de unión que tiene un polipéptido con dominio de unión que comprende, consiste esencialmente de, o consiste de (a) al menos un polipéptido con región variable de cadena liviana de inmunoglobulina, (b) al menos un polipéptido con región variable de cadena pesada de inmunoglobulina, y al menos un péptido ligador que se une al polipéptido de (a) y el polipéptido de (b) Tales polipéptidos pueden, por ejemplo, ser derivados de inmunoglobulinas humanas o inmunoglobulinas no humanas

Así, en un aspecto, la invención incluye una proteína de cadena sencilla de ocurrencia no natural y/o una proteína V_H y/o una proteína V_L, o una porción deseada de cualquiera de las anteriores, que incluye un primer polipéptido que comprende un polipéptido con dominio de unión capaz de unirse a una molécula objetivo, un segundo polipéptido comprende un ligador flexible u otro deseado unido a dicho primer polipéptido, un tercer polipéptido que comprende una región de cola, por ejemplo, un polipéptido de región constante de cadena pesada de inmunoglobulina truncado N-terminal (o porción deseada de éste) adherido a un segundo polipéptido El ligador flexible puede comprender, consiste esencialmente

de o consiste de, una región o porción de pivote de inmunoglobulina de esta que ha sido mutado o alterado de otra forma o trabajado por ingeniería, por ejemplo, uno que contiene un número de residuos cisteína que es menos que el número de residuos cisteína presentes en la región o porción de pivote de inmunoglobulina tipo silvestre (por ejemplo, cero, uno, o dos cisteínas en el caso de IgG1 o IgG4), y en donde dicha proteína de cadena sencilla de ocurrencia no natural es capaz de al menos una actividad inmunológica, por ejemplo, ADCC, CDC, y/o fijación de complemento La proteína de cadena simple puede ser capaz de dos actividades inmunológicas que incluyen, por ejemplo, ADCC, CDC, y/o fijación de complemento Esta proteína puede incluir un péptido con dominio de unión que es un Fv de cadena sencilla Adicionalmente, esta proteína puede incluir un péptido con dominio de unión que es un Fv de cadena sencilla en donde la región variable de cadena pesada del Fv de cadena sencilla tiene una supresión o sustitución de aminoácido en una o más de las posiciones aminoácido 9, 10, 11, 12, 108, 110, y 112 La proteína también puede incluir un polipéptido con dominio de unión que es un Fv de cadena sencilla en donde la región variable de cadena liviana del Fv de cadena sencilla tiene una supresión o sustitución de aminoácidos en una o más de las posiciones de aminoácido 12, 80, 81, 83, 105, 106, y 107

En otro aspecto, la invención incluye una proteína V_H de ocurrencia no natural, o una porción deseada de esta, que comprende, consiste esencialmente de, o consiste de, sola o en combinación con cualquier otra molécula o construcción, una región V_H o porción de ésta que tiene una supresión o sustitución de aminoácido en una o más de las posiciones de aminoácido 9, 10, 11, 12, 108, 110, y 112 de dicha región V_H Los aminoácidos pueden ser sustituidos con aminoácidos de ocurrencia natural o de ocurrencia no natural, o cualesquier otras moléculas útiles deseadas

También se describen o se reivindican usos de proteínas V_H, o porciones deseadas de ésta, que comprenden, consisten esencialmente de, o consisten de, solos o en combinación con cualquier otra molécula o construcción, una región V_H o porción de ésta que tiene una supresión o sustitución de aminoácido en una o más de las posiciones de aminoácido 9, 10, 11, 12, 108, 110, y 112 de dicha región V_H Tales usos incluyen usos en sistemas y métodos de despliegue de fago, despliegue de levadura, y despliegue de ribosoma

En aun otro aspecto, la invención incluye una proteína V_L de ocurrencia no natural, o una porción deseada de ésta, que comprende, consiste esencialmente de, o consiste de, sola o en combinación con cualquier otra molécula, una región V_L o porción de ésta que tiene una supresión o sustitución de

aminoácido en una o más de las posiciones de aminoácido 12, 80, 81, 83, 105, 106, y 107 de dicha región V_L Los aminoácidos se pueden sustituir con cualquier aminoácido de ocurrencia natural o no natural, o cualquier otra molécula útil deseada

También se describen y reivindican usos de proteínas V_L, o porciones deseadas de éstas, que comprenden, consisten esencialmente de, o consisten de, solas o en combinación con otra molécula, una región V_L o porción de ésta que tiene una supresión o sustitución de aminoácido en una o más de las posiciones de aminoácido 12, 80, 81, 83, 105, 106, y 107 de dicha región V_L Tales usos incluyen usos en sistemas y métodos de despliegue de fago, despliegue de levadura, y despliegue de ribosoma

En aún otro aspecto, la invención incluye una molécula que comprende, consiste esencialmente de, o consiste de, (1) una proteína V_H, o una porción deseada de ésta, en donde la proteína V_H o la porción de ésta tiene una supresión o sustitución de aminoácido en una o más de las posiciones de aminoácido 9, 10, 11, 12, 108, 110, y 112, y (2) una proteina V_H de ocurrencia no natural, o porción deseada de ésta, sola o en combinación con cualquiera otra molécula, en donde la proteína V_L o porción de ésta tiene una supresión o sustitución de aminoácido en una o más de las posiciones de

aminoácido 12, 80, 81, 83, 105, 106, y 107 Los aminoácidos se pueden sustituir con cualquiera de los aminoácidos de ocurrencia natural o no natural, o cualquiera otra molécula útil deseada

También se describen y se reivindican usos de una molécula que comprende, consiste esencialmente de, o consiste de, (1) una proteína V_H, o una porción deseada de ésta, en donde la proteína V_H o porción de ésta tiene una supresión o sustitución de aminoácido en una o más de las posiciones de aminoácido 9, 10, 11, 12, 108, 110, y 112, y (2) una proteína V_L de ocurrencia no natural, o porción deseada de ésta, sola o en combinación con cualquier otra molécula, en donde la proteína V_L o porción de ésta tiene una supresión o sustitución de aminoácido en una o más de las posiciones de aminoácido 12, 80, 81, 83, 105, 106, y 107 Tales usos incluyen usos en sistemas y métodos de despliegue de fago, despliegue de levadura, y despliegue de ribosoma

La invención también incluye construcciones moleculares en donde el dominio de unión es un Fv de cadena sencilla y la región variable de cadena pesada de dicho Fv de cadena sencilla tiene una sustitución de aminoácido en la posición de aminoácido 11 El aminoácido sustituido para el aminoácido de la posición de 11 de la región variable de cadena pesada del Fv de cadena sencilla se puede seleccionar del grupo que

consiste de serina, treonina, tirosina, asparagina, glutamina, ácido aspártico, ácido glutámico, lisina, arginina, e histidina La invención incluye así por ejemplo, una construcción en donde el dominio de unión es un Fv de cadena sencilla y la región variable de cadena pesada de dicho Fv de cadena sencilla tiene una sustitución de aminoácido serina en la posición de aminoácido 11 Otros cambios de posición de aminoácido, sustituciones, y supresiones, se anotan aquí

La invención también incluye, por ejemplo, una construcción en donde el dominio de unión es un Fv de cadena sencilla y el aminoácido en la posición 10 y/o 11 de la región variable de cadena pesada de dicho Fv de cadena sencilla se ha suprimido

En otro aspecto, la invención incluye construcciones en donde la región de unión se une a un tumor o a un antígeno asociado con tumor La región de unión de una construcción de la invención puede unirse, por ejemplo, a un antígeno de célula cancerígena Los antígenos de célula cancerígena a los cuales las construcciones de la invención se unen incluyen antígenos de superficie celular de cáncer y antígenos de célula cancerígena intracelular

En aun otro aspecto, la invención incluye una construcción en donde la región de unión se une a un antígeno en célula efectora inmune

En otro aspecto, la invención incluye una construcción en donde la región de unión se une a un antígeno de célula B que incluye, por ejemplo, un antígeno de célula B seleccionado del grupo que consiste de CD19, CD20, CD22, CD37, CD40, CD80, y CD86 Las construcciones de la invención que se unen a tales antígenos de célula B incluyen, por ejemplo, regiones de unión que comprenden un Fv de cadena sencilla Ejemplos de tales regiones de unión de Fv de cadena sencilla incluyen moléculas que comprenden o consisten esencialmente de Fvs de cadena sencilla seleccionados del grupo que consisten de Fv de cadena sencilla HD37, Fv de cadena sencilla 2H7, Fv de cadena sencilla G28-1, y Fv de cadena sencilla 4 4 220 Otros ejemplos incluyen una región de unión que comprende, consiste esencialmente de, o consiste de, un dominio extracelular de CTLA-4

En otro aspecto, la invención incluye una construcción en donde la región de unión se une a un antigeno de diferenciación de célula B Los antígenos de diferenciación de célula B incluyen, por ejemplo, CD19, CD20, CD21, CD22, CD23, CD37, CD40, CD45RO, CD80, CD86 y HLA clase II

En otro aspecto, la invención incluye una construcción en donde las regiones de unión se unen a un objetivo seleccionado del grupo que consiste de CD2, CD3, CD4, CD5, CD6, CD8, CD10, CD11b, CD14, CD19, CD20, CD21, CD22, CD23, CD24, CD25, CD28, CD30, CD37, CD40, CD43, CD50 (ICAM3), CD54 (ICAM1), CD56, CD69, CD80, CD86, CD134 (OX40), CD137 (41BB), CD152 (CTLA-4), CD153 (ligando CD30), CD154 (ligando CD40), ICOS, L6, B7-H1, y HLA clase II

La invención también incluye construcciones de proteína que tienen una región de unión, una región de cola, y una región de conexión, en donde la construcción de proteína es capaz de existir en solución como un monómero o en forma sustancialmente monomérica

La invención también incluye construcciones de proteína que tienen una región de unión, una región de cola, una región de conexión, en donde la construcción de proteína es capaz de formar un complejo que comprende dos o más de dichas construcciones de proteína que incluyen, por ejemplo, en donde dicho complejo es un dímero

En otro aspecto, las construcciones de la invención son capaces de participar en o inducir o elicitar o ayudar para inducir o elicitar, directa o indirectamente, al menos una actividad inmunológica seleccionada del grupo que consiste de

citotoxicidad mediada por célula dependiente de anticuerpo, citotoxicidad dependiente de complemento (o lisis mediada por complemento), fijación de complemento, inducción de apoptosis, inducción de una o más señales biológicamente activas, inducción de una o más células efectoras inmunes, activación de diferenciación celular, activación celular, liberación de una o más moléculas biológicamente activas, y neutralización de un agente o toxina infecciosa

En otro aspecto, las construcciones de unión de la invención son capaces de inducción de señales biológicamente activas mediante la activación o inhibición de una o más moléculas seleccionadas del grupo que consiste de proteínas quinasas, proteinas fosfatasas, G-proteínas, nucleótidos cíclicos u otros segundos mensajeros, canales de ion, y componentes de sendas secretoras Tales moléculas biológicamente activas son, por ejemplo, proteasas Otras moléculas biológicamente activas son, por ejemplo, citoquinas, que incluyen a manera de ejemplo monoquinas, linfoquinas, quimioquinas, factores de crecimiento, factores de estimulantes de colonia, interferonas, e interleuquinas

En otro aspecto, las construcciones de la invención son capaces de inducción, o participación en la inducción, de una o más células efectoras inmunes seleccionadas del grupo que

consiste de células NK, monocitos, macrófagos, células B, células T, mastocitos, neutrófilos, eosinófilos, y basófilos

En otro aspecto, las construcciones de la invención son capaces de inducción, o participación en la inducción, de una o más células efectoras inmunes que den como resultado citotoxicidad mediada por célula dependiente de anticuerpo o la liberación de una o más moléculas biológicamente activas

En otro aspecto, las construcciones de la invención son capaces de participar en y/o iniciar la apoptosis con células objetivo, por ejemplo, al activar uno o más mecanismos o moléculas de señalización

En otro aspecto, las construcciones de la invención son capaces de inducción, o participación en la inducción, de activación celular, en donde dicha activación conduce a cambios en la actividad transcripcional celular En una modalidad, la actividad transcripcional celular se incrementa En otra modalidad, la actividad transcripcional celular se disminuye

En otro aspecto, las construcciones de la invención que tienen regiones de cola que comprenden, consisten esencialmente de, o consisten de, regiones constantes de moléculas IgA o IgE, son capaces de inducción o participación

en la inducción de desgranulación de neutrófilos y/o mastocitos

En otro aspecto, las construcciones de la invención son capaces de promoción, o participación en la promoción de neutralización de un agente infeccioso, en donde dicho agente infeccioso es, por ejemplo, una bacteria, un virus, un parásito, o un hongo

En otro aspecto, las construcciones de la invención son capaces de promover, o participar en la promoción de, neutralización de una toxina, en donde dicha toxina se selecciona del grupo que consiste de endotoxinas y exotoxinas Tales toxinas incluyen, por ejemplo, exotoxinas seleccionadas del grupo que consiste de toxina de ántrax, toxina de cólera, toxina de difteria, toxina pertussis, toxina lábil al calor de *E coli* LT, toxina estable al calor de *E coli* ST, toxina shiga Exotoxina A de *Pseudomonas*, toxina botilinum, toxina de tétano, toxina AC de *Bordetella pertussis*, y toxina EF de *Bacillus anthracis* Otras toxinas incluyen, por ejemplo, saxitoxinas, tetrodotoxinas, toxinas de hongo (amatoxinas, giromitrina, orelanina, etc), aflatoxinas, alcaloides pirrolizidina, fitohemaglutininas, y grayanotoxinas

En otro aspecto, las construcciones de la invención son capaces de unirse aun objetivo intracelular a, por ejemplo, efectuar (o participar en efectuar) una función celular Tales construcciones incluyen, por ejemplo, construcciones que incluyen una región de cola que comprende, consiste esencialmente de, o consiste de, una región de dominio CH2 IgA nativa o trabajada por ingeniería y una región de dominio IgA CH3 nativa o trabajada por ingeniería, dicha región de cola es capaz de unirse a la cadena J Tal región de cola se encuentra, por ejemplo, en la construcción 2H7 scFv IgAH W1gACH2 WCH3 + Cadena J Así, la invención incluye construcciones que tienen, por ejemplo, un dominio de unión "Objetivo Anti-Intracelular" (por ejemplo, y scFv de "Objetivo Anti-Intracelular"), una región de conexión, y una región constante IgA nativa o trabajada por ingeniería capaz de unirse a la cadena J (por ejemplo, W1gACH2 WCH3)

En aún otro aspecto, las construcciones de la invención incluyen una molécula en donde un polipéptido de región constante de cadena pesada de inmunoglobulina N-terminal comprende un polipéptido de región constante IgG CH2 unido a un polipéptido de región constante IgG CH3 de cadena pesada de inmunoglobulina

En aún otro aspecto, la invención incluye un método para reducir una población de célula objetivo en un sujeto que

comprende administrar a dicho sujeto una cantidad terapéuticamente efectiva de una molécula de proteína que es menos de aproximadamente 120 kK, o menos de aproximadamente 150 kD, medida, por ejemplo, mediante HPLC y geles no reductores y que consiste esencialmente de (a) una primera proteína o molécula de péptido que es capaz de unirse a las células dentro de dicha población de célula objetivo, y (b) una segunda molécula de péptido o proteína que es capaz de (i) unirse a un receptor Fc y/o (ii) inducir apoptosis de célula objetivo y/o (iii) fijar complemento, en donde dicha primera molécula de proteína o péptido está directamente conectada a dicha segunda molécula de proteína o péptido, o, opcionalmente dicha primer molécula de proteína o péptido y dicha segunda molécula de proteina o péptido están ligadas por una tercer proteína o molécula de péptido, en donde dicha molécula de proteína no es un anticuerpo, un miembro de la familia TNF o la familia receptora TNF, y no está conjugado con una toxina de bacteria, una droga citotóxica, o un radioisótopo

En otro aspecto, la invención también incluye proteínas de cadena sencilla que comprenden, consisten esencialmente de, o consisten de, (i) un primer polipéptido que tiene un polipéptido de dominio de unión capaz de unirse a una molécula objetivo, (ii) un segundo polipéptido que comprende una región de conexión unida al terminal C de dicho primer

polipéptido, y (iii) un tercer polipéptido que comprende un polipéptido de región constante de cadena pesada de inmunoglobulina truncado N-terminal unido al terminal C de dicho segundo polipéptido, en donde dicha proteína de cadena simple es capaz de al menos una actividad inmunológica, y siempre y cuando que (a) cuando el polipéptido de región de conexión comprende un polipéptido de región de pivote IgG que no tiene residuos de cisteína, el objetivo de polipéptido de dominio de unión no es CD20 o L6, o (b) cuando el polipéptido de la región de conexión comprende un polipéptido de región de conexión IgG que no tiene residuos de cisteína, la proteína de cadena sencilla no es un 1F5 scFv capaz de unirse al CD20 La invención también incluye proteínas de cadena sencilla que comprenden, consisten esencialmente de, o consisten de, (i) un primer polipéptido que tiene un polipéptido de dominio de unión capaz de unirse a una molécula objetivo, (ii) un segundo polipéptido que comprende una región de conexión unida al terminal C de dicho primer polipéptido, y (iii) un tercer polipéptido que comprende un polipéptido de región constante de cadena pesada de inmunoglobulina truncada N-terminal unida al terminal C de dicho segundo polipéptido, en donde dicha segunda proteína de cadena es capaz de unirse a una molécula objetivo sobre o en una célula objetivo y disminuyendo el número de células objetivo *in vivo* y/o agotando una población de células objetivo *in vivo* La invención también incluye proteínas de

cadena sencilla que comprenden, consisten esencialmente de, o consisten de, (i) un primer polipéptido que tiene un polipéptido de dominio de unión capaz de unirse a una molécula objetivo, (ii) un segundo polipéptido que comprende una región de conexión unida al C-terminal del primer polipéptido, y (iii) un tercer polipéptido que comprende un polipéptido de región constante de cadena pesada de inmunoglobulina truncada N-terminal unida al C-terminal de dicho segundo polipéptido, en donde dicha proteína de cadena sencilla es capaz de inducir citotoxicidad mediada por célula dependiente de anticuerpo y fijación de complemento La invención también incluye proteínas de cadena sencilla que comprenden, consisten esencialmente de, o consisten de, (i) un primer polipéptido que tiene un polipéptido de dominio de unión capaz de unirse a una molécula objetivo, (ii) un segundo polipéptido que comprende una región de conexión unida al terminal C de dicho primer polipéptido, y (iii) un tercer polipéptido que comprende un polipéptido de región constante de cadena pesada de inmunoglobulina truncada N-terminal unida al terminal C de dicho segundo polipéptido, en donde dicha proteína de cadena sencilla es capaz de (1) inducir citotoxicidad mediada por célula dependiente de anticuerpo y fijación de complemento, y (2) unirse a una molécula objetivo sobre o en una célula objetivo y disminuir el número de células objetivo *in vivo* y/o agotando la población de células objetivo *in vivo* La invención también

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incluye proteínas de cadena sencilla que comprenden, consisten esencialmente de, o consisten de, (i) un primer polipéptido que tiene un polipéptido de dominio de unión capaz de unirse a una molécula objetivo, (ii) un segundo polipéptido que comprende una región de conexión unida al C-terminal de dicho primer polipéptido, y (iii) un tercer polipéptido que comprende un polipéptido de región constante de cadena pesada de inmunoglobulina truncada N-terminal unida al C-terminal de dicho segundo polipéptido, en donde cuando dicha región de conexión comprende un polipéptido de región de pivote IgG que tiene al menos primeros, segundos, y terceros residuos de cisteina, dichas primeras cisteínas son N-terminales a dicha segunda cisteína y dicha segunda cisteína es N-terminal a dicha tercer cisteína, uno o ambos de dichos segundo y terceros residuos de cisteína son sustituidos o suprimidos y en donde dicha proteína de cadena sencilla es capaz de al menos una actividad inmunológica La invención también incluye proteínas de cadena sencilla que comprenden, consisten esencialmente de, o consisten de, (i) un primer polipéptido que tiene un polipéptido de dominio de unión capaz de unirse a una molécula objetivo, (ii) un segundo polipéptido que comprende una región de conexión unida a un C-terminal de dicho primer polipéptido, y (iii) un tercer polipéptido que comprende un polipéptido de región constante de cadena pesada de inmunoglobulina truncada N-terminal unido al C-terminal de dicho segundo polipéptido, en

donde cuando dicha región de conexión comprende un polipéptido de región de pivote IgG que tiene al menos primeros, segundos, y terceros residuos de cisteína, dichas primeras cisteínas son N-terminales a dicha segunda cisteína y dicha segunda cisteína es N-terminal a dicha tercer cisteína, uno o ambos de dichos segundos y terceros residuos de cisteína se sustituyen o suprimen, y en donde dicha proteína de cadena sencilla es capaz de inducir al menos una actividad inmunológica seleccionada de (a) citotoxicidad mediada por célula dependiente de anticuerpo y (b) fijación de complemento La invención también incluye proteínas de cadena sencilla que comprenden, consisten esencialmente de, o consisten de, (i) un primer polipéptido que tiene un polipéptido de dominio de unión capaz de unirse a una molécula objetivo, (ii) un segundo polipéptido que comprende una región de conexión unida al C-terminal de dicho polipéptido, y (iii) un tercer polipéptido que comprende un polipéptido de región constante de cadena pesada de inmunoglobulina truncada N-terminal unida al terminal C de dicho segundo polipéptido, en donde cuando dicha región de conexión comprende un polipéptido de región de pivote IgG que tiene al menos primeros, segundos y terceros residuos de cisteína, dichas primeras cisteínas son N-terminales a dichas segundas cisteínas y dichas segundas cisteínas son N-terminales a dicha tercera cisteína, uno o ambos de dichos segundos y terceros residuos de cisteína son sustituidos o

suprimidos, y en donde dicha proteína de cadena sencilla es capaz de citotoxicidad mediada por célula dependiente de anticuerpo y fijación de complemento La invención también incluye proteína de cadena sencilla que comprende, consiste esencialmente de, o consiste de, (i) un primer polipéptido que tiene un polipéptido de dominio de unión capaz de unirse a una molécula objetivo, (ii) un segundo polipéptido que comprende una región de conexión unida al C-terminal de dicho primer polipéptido, y (iii) un tercer polipéptido que comprende un polipéptido de región constante de cadena pesada de inmunoglobulina truncada N-terminal unida al C-terminal de dicho segundo polipéptido, en donde cuando dicha región de conexión comprende un polipéptido de región de pivote IgG al menos primeros, segundo, y terceros residuos de cisteína, dicha primer cisteína es N-terminal a dicha segunda cisteína y dicha segunda cisteína es N-terminal a dicha tercer cisteína, uno o ambos de dichos segundos y terceros residuos de cisteína son sustituidos o suprimidos, y en donde dicha proteína de cadena sencilla es capaz de unirse a una molécula objetivo sobre o en una célula objetivo y disminuir el numero de células objetivo *in vivo* y/o agotando una población de células objetivo *in vivo* La invención también incluye proteína de cadena sencilla que comprende, consiste esencialmente de, o consiste de, (i) un primer polipéptido que tiene un polipéptido de dominio de unión capaz de unirse a una molécula objetivo, (ii) un segundo polipéptido que

comprende una región de conexión unida al C-terminal de dicho primer polipéptido, y (iii) un tercer polipéptido que comprende un polipéptido de región constante de cadena pesada de inmunoglobulina truncada N-terminal unido al C-terminal del segundo polipéptido, en donde cuando dicha región de conexión comprende un polipéptido de región de pivote IgG que tiene al menos primeros, segundos y terceros residuos de cisteína, dicha primer cisteína es N-terminal a dicha segunda cisteína y dicha segunda cisteina es N-terminal a dicha tercer cisteína, uno o ambos de dichos segundos y terceros residuos de cisteína son sustituidos o suprimidos, y en donde una proteína de cadena sencilla es capaz de inducir al menos una actividad inmunológica seleccionada de citotoxicidad mediada por célula dependiente de anticuerpo y fijación de complemento, y en donde dicha proteína de cadena sencilla es capaz de unirse a una molécula objetivo sobre o en una célula objetivo y disminuir el número de células objetivo *in vivo* y/o agotando una población de células objetivo *in vivo* La invención también incluye proteínas de cadena sencilla que comprenden, consisten esencialmente de, o consisten de, (i) un primer polipéptido que tiene un polipéptido de dominio de unión capaz de unirse a una molécula objetivo, (ii) un segundo polipéptido que comprende una región de conexión unida al C-terminal de dicho primer polipéptido, y (iii) un tercer polipéptido que comprende un polipéptido de región constante de cadena pesada de inmunoglobulina truncada N-

terminal unido al C-terminal de dicho segundo polipéptido, en donde cuando dicha región de conexión comprende un polipéptido de región de pivote IgG que tiene al menos primeros, segundos y terceros residuos de cisteína, dicha primer cisteína es N-terminal a dicha segunda cisteína y dicha segunda cisteína es N-terminal a dicha tercer cisteína, uno o ambos de dichos segundos y terceros residuos de cisteína son sustituidos o suprimidos, y en donde dicha proteína de cadena sencilla es capaz de inducir citotoxicidad mediada por células dependientes de anticuerpo y fijación de complemento, y en donde dicha proteína de cadena sencilla es capaz de unirse a una molécula objetivo sobre o en una célula objetivo y disminuir el numero de células objetivo *in vivo* y/o agotar una población de células objetivo *in vivo* La invención también incluye proteínas de cadena sencilla que comprenden, consisten esencialmente de, o consisten de, (i) un primer polipéptido que tiene un polipéptido de dominio de unión capaz de unirse a una molécula objetivo, dicho polipéptido de dominio de unión comprende una región variable de cadena pesada en donde la leucina en la posición 11 en la primera región marco de la región variable de cadena pesada se suprime o sustituye con otro aminoácido, (ii) un segundo polipéptido que comprende una región de conexión unida al C-terminal de dicho primer polipéptido, y (iii) un tercer polipéptido que comprende un polipéptido de región constante de cadena pesada de inmunoglobulina truncada N-terminal unido

al C-terminal de dicho segundo polipéptido, en donde dicha proteína de cadena sencilla es capaz de al menos una actividad inmunológica, y siempre y cuando el polipéptido de dominio de unión sea capaz de unirse a CD20 dicha región de conexión comprende tres residuos de cisteína en donde uno o dos de dichos tres residuos de cisteína se sustituyen o reemplazan con otro aminoácido La invención también incluye proteínas de cadena sencilla que comprenden, consisten esencialmente de, o consisten de, (i) un primer polipéptido que tiene un polipéptido de dominio de unión capaz de unirse a una molécula objetivo, dicho polipéptido de dominio de unión comprende una región variable de cadena pesada en donde la leucina en la posición 11 en la primera región marco de la región variable de cadena pesada se suprime o sustituye con otro aminoácido, (ii) un segundo polipéptido que comprende una región de conexión unida al C-terminal de dicho primer polipéptido, y (iii) un tercer polipéptido que comprende un polipéptido de región constante de cadena pesada de inmunoglobulina truncada N-terminal unida al C-terminal del segundo polipéptido, en donde dicha proteína de cadena sencilla es capaz de incluir al menos una actividad inmunológica, siempre y cuando la unión del polipéptido de dominio sea 2H7 scFv capaz de unirse a CD20 y dicha región de conexión comprenda un polipéptido de región de pivote IgG que tiene al menos primeros, segundos, y terceros residuos de cisteína, dicha primer cisteína es N-terminal a dicha segunda

cisteína y dicha segunda cisteína es N-terminal a dicha tercer cisteína, uno o dos de dichos residuos de cisteína se sustituyen o se suprimen La invención también incluye proteínas de cadena sencilla que comprenden, consisten esencialmente de, o consisten de, (i) un primer polipéptido que tiene un polipéptido con dominio de unión capaz de unirse a una molécula objetivo sobre o en una célula objetivo, dicho polipéptido con dominio de unión comprende una región variable de cadena pesada en donde la leucina en la posición 11 en la primera región marco de la región variable de cadena pesada se suprime o sustituye con otro aminoácido, (ii) un segundo polipéptido que comprende una región de conexión unida a dicho polipéptido, y (iii) un tercer polipéptido que comprende un polipéptido de región constante de cadena pesada de inmunoglobulina truncada N-terminal unido al segundo polipéptido, en donde dicha segunda proteína de cadena sencilla es capaz de al menos una actividad inmunológica, siempre y cuando dicha proteína de cadena sencilla no comprenda, consista esencialmente de, un polipéptido con dominio de unión capaz de unirse al CD20 y una región de conexión que comprenda (a) un pivote IgG que tiene tres residuos de cisteína o (b) un pivote IgG que comprende tres residuos de serinas (o aminoácidos similares) que han sido sustituidos por residuos de cisteína Existen muchas posibles variaciones y variantes de estas proteínas de cadena sencilla Por ejemplo, el polipéptido de dominio de unión

puede ser un anticuerpo de cadena sencilla o un scFv que incluye polipéptidos V_L y V_H de ocurrencia natural y/o ocurrencia no natural, y el polipéptido de dominio de unión puede unir cualquiera de un número de objetivos Los polipéptidos V_H de ocurrencia no natural incluyen, por vía de ejemplo y no de limitación, polipéptidos de región variable de cadena pesada humana que comprende una mutación, sustitución, o supresión de un o unos aminoácidos en la ubicación correspondiente con cualquiera de una o más de las posiciones de aminoácido 9, 10, 11, 12, 108, 110, y/o 112 Los polipéptidos V_L de ocurrencia no natural incluyen, por vía de ejemplo y no de limitación, polipéptidos de región variable de cadena liviana humana que comprenden una mutación, sustitución, o supresión de uno o varios aminoácidos en una ubicación correspondiente con cualquiera de una o más de las posiciones de aminoácido 12, 80, 81, 83, 105, 106, y 107 Los objetivos incluyen, por vía de ejemplo y no de limitación, CD19, CD20, CD28, CD30, CD37, CD40, L6, HER2, receptores de factor de crecimiento epidérmico (EGFR), factores de crecimiento celular endotelial (VEGF), factores de necrosis tumoral (por ejemplo, TNF-alfa), así como otros objetivos descritos o referidos aquí o de otra manera utiles, ya sea conocidos o descubiertos después Adicionalmente, el polipéptido de región de conexión puede ser cualquiera de un número de moléculas, ambas de ocurrencia natural y ocurrencia no natural Los polipéptidos de región de conexión incluyen,

por vía de ejemplo y no de limitación, polipéptidos de región de pivote de inmunoglobulina de ocurrencia natural y ocurrencia no natural Los polipéptidos de región de pivote de inmunoglobulina de ocurrencia natural incluyen polipéptidos de región de pivote de inmunoglobulina tipo silvestre tales como, por vía de ejemplo y no de limitación, polipéptidos de región de pivote IgG1 humano, polipéptidos de región de pivote IgA humano, polipéptidos de región de pivote IgD humano, regiones de acción de pivote IgE humano (por ejemplo, IgE CH2), regiones de pivote de inmunoglobulina de camélido, o cualesquier otros péptidos de región de pivote de ocurrencia natural descritos o referidos aquí o de otra manera útiles, ya sea conocidos o descubiertos después Los polipéptidos de región de pivote de inmunoglobulina de ocurrencia no natural incluyen, por vía de ejemplo y no de limitación, articulaciones de inmunoglobulina de ocurrencia natural mutadas, que incluyen polipéptidos de región de pivote de inmunoglobulina que contienen menos que el número de cisteinas tipo silvestre, por ejemplo, polipéptidos de región de pivote de inmunoglobulina de ocurrencia natural mutados que contienen cero, uno, o dos cisteínas, y cualquier otra molécula de región de conexión descrita o referida aquí o de otra manera útil o conocida o descubierta después como útil para la unión de conexión, por ejemplo, los dominios de inmunoglobulina tales como un dominio CH1 y un dominio CH2 Los polipéptidos de región constante de cadena pesada de

inmunoglobulina truncada N-terminal incluyen polipéptidos de región constante de cadena pesada de inmunoglobulina truncados N-terminal de ocurrencia natural y ocurrencia no natural que, con o sin otras porciones de la proteína de cadena sencilla, suministran una o más funciones efectoras tales como aquellas descritas aquí Los polipéptidos de región constante de cadena pesada de inmunoglobulina truncada N-terminal de ocurrencia natural incluyen, por vía de ejemplo y no de limitación, polipéptidos de región constante CH2CH3, que incluyen polipéptidos de región constante CH2CH3 tomados, juntos o separadamente, de IgG humano, IgA humano, e IgE humano, y cualquier otro polipéptido de región constante de cadena pesada de inmunoglobulina descrito o referenciado aquí o de otra manera conocido o descubierto después para ser útil Los polipéptidos de región constante de cadena pesada de inmunoglobulina truncada N-terminal de ocurrencia no natural incluyen, por vía de ejemplo y no de limitación, cualquier polipéptido de región constante de cadena pesada de ocurrencia natural mutada descrito o referenciado aquí o de otra manera conocido o descubierto después para ser útil

Varias construcciones específicas de la invención incluyen, solo por vía de ejemplo, las siguientes

1 2H7 scFv VH L11S (CSC-S) H WCH2 WCH3

2 2H7 scFv VH L11S IgE CH2 CH3 CH4

3 2H7 scFv VH L11S mIgE CH2 CH3 CH4

4 2H7 scFv VH L11S mIgAH WIgACH2 T4CH3

5 2H7 scFv VH L11S (SSS-S) H K322S CH2 WCH3

6 2H7 scFv VH L11S (CSS-S) H K322S CH2 WCH3

7 2H7 scFv VH L11S (SSS-S) H P331S CH2 WCH3

8 2HU scFv VH L11S (CSS-S) H P331S CH2 WCH3

9 2H7 scFv VH L11S (SSS-S) H T256N CH2 WCH3

10 2H7 scFv VH L11S (SSS-S) H RTPE/QNAK (255-258) CH2 WCH3

11 2H7 scFv VH L11S (SSS-S) H K290Q CH2 WCH3

12 2H7 scFv VH L11S (SSS-S) H A339P CH2 WCH3

13 G28-1 scFv (SSS-S) H WCH2 WCH3

14 G28-1 scFv IgAH WCH2 WCH3

15 G28-1 scFv VH L11S (SSS-S) H WCH2 WCH3

16 G28-1 scFv VH L11S (CSS-S) H WCH2 WCH3

17 G28-1 scFv VH L11S (CSC-S) H WCH2 WCH3

18 G28-1 scFv VH L11S (SSC-P) H WCH2 WCH3

19 CTLA4 (SSS-S) H P238SCH2 WCH32

20 CTLA4 (CCC-P) WH WCH2 WCH3

21 FC2-2 scFv (SSS-S) H WCH2 WCH3

22 FC2-2 scFv VHL11S (SSS-S) H WCH2 WCH3

23 UCHL-1 scFv (SSS-S) H WCH2 WCH3

24 UCHL-1 scFv VHL11S (SSS-S) H WCH2 WCH3

25 5B9 scFv (SSS-S) H WCH2 WCH3

26 5B9 scFv VHL11S (SSS-S) H WCH2 WCH3

27 2H7 scFv (SSS-S) H WCH2 WCH3

28 2H7 scFv (SSS-S) H P238SCH2 WCH3

29 2H7 scFv IgAH WCH2 WCH3

30 2H7 scFv IgAH WIgACH2 T4CH3

31 2H7 scFv IgAH WIgACH2 WCH3 + CadenaJ

32 2H7 scFv (CCC-P) WH WCH2 WCH3

33 2H7 scFv (SSS-S) H WCH2 F405YCH3

34 2H7 scFv (SSS-S) H WCH2 F405ACH3

35 2H7 scFv (SSS-S) H WCH2 Y407ACH3

36 2H4 scFv (SSS-S) HWCH2 F405A, Y407ACH3

37 2H7 scFv (CSS-S) H WCH2 WCH3

38 2H7 scFv (SCS-S) H WCH2 WCH3

39 2H7 scFv (SSC-P) H WCH2 WCH3

40 2H7 scFv (CSC-S) H WCH2 WCH3

41 2H7 scFv (CCS-P) H WCH2 WCH3

42 2H7 scFv (SCC-P) H WCH2 WCH3

43 2H7 scFv VH L11S (SSS-S) H WCH2 WCH3

44 2H7 scFv VH L11S (CSS-S) H WCH2 WCH3

45 G28-1 scFv VH L11S (SCS-S) H WCH2 WCH3

46 G28-1 scFv VH L11S (CCS-P) H WCH2 WCH3

47 G28-1 scFv VH L11S (SCC-P) H WCH2 WCH3

48 G28-1 scFv VH L11S mIgE CH2 CH3 CH4

49 G28-1 scFv VH L11S mIgAH WIgACH2 T4CH3

50 G28-1 scFv VH L11S hIgE CH2 CH3 CH4

51 G28-1 scFv VH L11S hIgAH WIgACH2 T4CH3

52 HD37 scFv IgAH WCH2 WCH3

53 HD37 scFv (SSS-S) H WCH2 WCH3

54 HD37 scFv VH L11S (SSS-S) H WCH2 WCH3

55 L6 scFv IGAH WCH2 WCH3

56 L6 scFv VHL11S (SSS-S) H WCH2 WCH3

57 2H7 scFv-llama IgG1

58 2H7 scFv-llama IgG2

59 2H7 scFv-llama IgG3

60 CD16-6 bajo (ED) (SSS-S) H P238SCH2 WCH3

61 CD16-9 alto (ED) (SSS-S) H P238SCH2 WCH3

62 2e12 scFv (SSS-S) H P238SCH2 WCH3-hCD80TM/CT

63 10A8 scFv (SSS-S) H P238SCH2 WCH3-hCD80TM/CT

64 40 2 36 scFv (SSS-S) H P238SCH2 WCH3-hCD80TM/CT

65 2H7 scFv (SSS-S) H P238SCH2 WCH3-hCD80TM/CT

66 G19-4 scFv (SSS-S) H P238SCH2 WCH3-hCD80TM/CT

67 2e12 scFv (SSS-S) H WCH2 WCH3-hCD80TM/CT

68 2e12 scFv IgAH IgACH2 T4CH3-hCD80TM/CT

69 2e12 scFv IgE CH2CH3CH4-hCD80TM/CT

70 2e12 scFv (SSS-S) H P238SCH2 WCH3-mFADD-TM/CT

71 2e12 scFv (SSS-S) H WCH2 WCH3-mFADD-TM/CT

72 2e12 scFv (SSS-S) H WCH2 WCH3-mcasp3-TM/CT

73 2e12 scFv (SSS-S) H P238SCH2 WCH3-mcasp3-TM/CT

74 2e12 scFv (SSS-S) H WCH2 WCH3-mcasp8--TM/CT

75 2e12 scFv (SSS-S) H P238SCH2 WCH3-mcasp8-TM/CT

76 2e12 scFv (SSS-S) H WCH2 WCH3-hcasp3--TM/CT

77 2e12 scFv (SSS-S) H P238SCH2 WCH3-hcasp3-TM/CT

78 2e12 scFv (SSS-S) H WCH2 WCH3-hcasp8--TM/CT

79 2e12 scFv (SSS-S) H P238SCH2 WCH3-hcasp8-TM/CT

80 1D8 scFv--hIgG1 (SSS-S) H P238SCH2 WCH3-hCD80TM/CT

858
847

81 1D8 scFv--hIgG1 (SSS-S) H WCH2 WCH3-hCD80TM/CT

82 1D8 scFv--mIgAT4-hCD80TM/CT

83 1D8 scFv--hIgE-hCD80TM/CT

84 1D8 scFv—hIgG1 (SSS-S) H P238SCH2 WCH3-mFADD-TM/CT

85 1D8 scFv—hIgG1 (SSS-S) H WCH2 WCH3-mFADD-TM/CT

86 1D8 scFv—hIgG1 (SSS-S) H WCH2 WCH3-mcasp3-TM/CT

87 1D8 scFv—hIgG1 (SSS-S) H P238SCH2 WCH3-mcasp3-TM/CT

88 1D8 scFv—hIgG1 (SSS-S) H WCH2 WCH3-mcasp8--TM/CT

89 1D8 scFv—hIgG1 (SSS-S) H P238SCH2 WCH3-mcasp8-TM/CT

90 1D8 scFv—hIgG1 (SSS-S) H WCH2 WCH3-hcasp3--TM/CT

91 1D8 scFv—hIgG1 (SSS-S) H P238SCH2 WCH3-hcasp3-TM/CT

92 1D8 scFv—hIgG1 (SSS-S) H WCH2 WCH3-hcasp8--TM/CT

93 1D8 scFv—hIgG1 (SSS-S) H P238SCH2 WCH3-]hcasp8-TM/CT L6 scFv (SSS-S) H WCH2 WCH3

94 2H7 scFv CD154 (L2)

95 2H7 scFv CD154 (S4)

96 CTLA4 IgAH IgACH2CH3

97 CTLA4 IgAH IgACH2 T4CH3

98 2H7 scFv IgAH IgACH2CH3

99 2H7 scFv IgAH IgAHCH2 T18CH3

100 2H&-40 2 220 scFv (SSS-S) H WCH2 WCH3 (anti-ccd20-anti-cd40 biespecifico)

101 2H7 scFv IgAH IgACH2 T4CH3-hCD89 TM/CT

102 G19-4 scFv (CCC-P) WH WCH2 WCH3-hCD89 TM/CT

103 2e12 scFv (CCC-P) WH WCH2 WCH3-hCD89 TM/CT

Estos y otros aspectos de la invención serán adicionalmente evidentes luego de la referencia de la siguiente descripción detallada y de los dibujos anexos Como se anotó aquí, todas las patentes referenciadas, artículos, documentos, y otros materiales descritos o identificados aquí se incorporan como referencia aquí en su totalidad como si cada uno se incorporara individualmente

BREVE DESCRIPCIÓN DE LOS DIBUJOS

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La Figura 24 ilustra la migración de proteínas de fusión IgG de llama 2H7 scFv purificadas en un gel de poliacrilamida SDS al 10% Las proteínas de fusión purificadas (5 µg por muestra) se prepararon en un amortiguador de muestra no reductora (carriles 2-5) y en un amortiguador de muestra reductora (carriles 6-9) Carril 1 marcadores de peso molecular (no reducido), Carriles 2 y 6 IgG1 de llama 2H7 scFv- (ID DE SEC NO _), Carriles 3 y 7 IgG2 de llama 2H7 scFv (ID DE SEC NO _), Carriles 4 y 8 IgG3 de llama 2H7 scFv (ID DE SEC NO _), y Carriles 5 y 9 Rituximab (anticuerpo anti-CD20 quimérico (región constante IgG1 humano)

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La Figura 26 describe actividad CDC de las proteínas de fusión IgG de llama 2H7 scFv, el IgG1 de llama 2H7 scFv (ID DE SEC NO _), IgG2 de llama 2H7 scFv (ID DE SEC NO _), y el IgG3 de llama 2H7 scFv (ID DE SEC NO _), y el IgG1 de humano 2H7 scFv (2H7 scFv IgG WTH WTCH2CH3) (ID DE SEC NO _) contra células BJAB en la presencia de complemento de conejo El Rituximab se incluyó como un control

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de llama) se combinaron con células objetivo (células BJAB) en tres diferentes proporciones, 1 25, 1 50, y 1 100 El Rituximab se incluyó como un control Cada punto de datos representa tres mediciones separadas

La Figura 29 describe actividad de citotoxicidad dependiente de complemento de células Reh (leucemia linfocítica aguda) que expresan proteínas de fusión scFv-Ig sobre la superficie celular Las células Reh fueron transfectadas con construcciones que codifican los anticuerpos scFv específicos para moléculas coestimulatorias humanas, CD152, CD28, CD40, y CD20, fusionadas al CH2-CH3-de pivote tipo silvestre IgG1 humano, que se fusionó a la transmembrana CD80 humana y a los dominios de cola citoplasmática La actividad citotóxica dependiente de complemento se midió en la presencia y ausencia de complemento de conejo (más C′ y no C′, respectivamente) Los datos representan el promedio de las muestras duplicadas Reh anti-hCD152 scFvIg células Reh transfectadas con polinucleótido 10A8 scFv IgG MTH (SSS) MT CH2CH3 (ID DE SEC NO _), Reh anti-hCD28scFvIg 2E12 scFv IgG MTH (SSS) MT CH2CH3 (ID DE SEC NO _), Reh anti-hCD40scFvIg 4 2 220 scFv IgG MTH (SSS) MT CH2CH3 (ID DE SEC NO _), y Reh anti-hCD20scFvIg 2H7 scFv IgG MTH (SSS) MT CH2CH3 (ID DE SEC NO _)

La Figura 30 presenta actividad de citotoxicidad mediada por célula dependiente de anticuerpo de células Reh que fueron transfectadas con construcciones que codifican anticuerpos scFv específicas para moléculas coestimulatorias humanas, CD152, CD28, CD40, y CD20, como se describió para la Figura 29, y CD3 de murino, fusionado a un pivote IgG1 de mutante humano y un CH2 mutante y CH3 tipo silvestre (Reh anti-mCD3scFv que designa células Reh transfectadas con polinucleótido 500A2 scFv IgG MTH (SSS) MTCH2WTCH3 ID DE SEC NO _)), que se fusionó a una transmembrana CD80 humana y a dominios de cola citoplasmáticas Los datos representan el promedio de las muestras cuadruplicadas

La Figura 31 lista construcciones de región constante de inmunoglobulina que fueron utilizadas en los experimentos ilustrados en las Figuras subsecuentes

La Figura 32 describe la actividad de citotoxicidad dependiente de complemento de las proteínas de fusión CTLA-4 Ig, CTLA-4 IgG WTH (CCC) WTCH2CH3 (ID DE SEC NO _) (2 µg/ml) y CTLA-4 IgG MTH MTCH2WTCH3 (ID DE SEC NO _) (2 µg/ml), en la presencia y ausencia de complemento de conejo (más C′ y no C′, respectivamente) Las células objetivo fueron células Reh y las células Reh transfectadas con CD80 (Reh CD80 10)

La Figura 33 muestra actividad de citotoxicidad mediada por célula dependiente de anticuerpo de proteínas de fusión CTLA-4 Ig, CTLA-4 IgG WTH (CCC) WTCH2CH3 (ID DE SEC NO _) (2 µg/ml) y CTLA-4 IgG MTH MTCH2WTCH3 (ID DE SEC NO _) (2 µg/ml) Las células efectoras, el PBMC humano, fueron agregadas a las células objetivo, Reh o Reh CD80 1, en las proporciones indicadas La Figura 33A presenta el nivel de muerte natural en células Reh CD80 1 en la ausencia de cualquier proteína de fusión Ig La Figura 33B presenta citotoxicidad mediada por célula dependiente de anticuerpo mediada por CTLA-4 IgG MTH MTCH2WTCH3, y la Figura 33C presenta citotoxicidad mediada por célula dependiente de anticuerpo mediada por CTLA-4 IgG WTH (CCC) WTCH2CH3 Cada punto de dato representa el promedio porcentual específico de muerte medido en cuatro pozos de muestra

La Figura 34 ilustra la unión de proteínas de fusión 2H7 (anti-CD20) scFv Ig a células (CD20+)CHO mediante inmunocitofluorimetría de flujo

La Figura 35 presenta una inmunoabsorción de las proteínas de fusión 2H7 scFv IgG e IgA Las células COS fueron transitoriamente transfectadas con varias construcciones de proteínas de fusión 2H7 scFv Ig Los polipéptidos expresados fueron inmunoprecipitados con proteína A, separados en un gel de poliacrilamida SDS no reductor, y luego transferidos a una

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membrana de polivinil fluoruro Las proteinas fueron detectadas utilizando un conjugado de peroxidasa de cola de caballo IgG (especifico Fc) anti-humano Carril 1 vector solamente, Carril 2 2H7 scFv IgG WTH (CCC) WTCH2CH3 (SEQ ID NO), Carril 3 2H7 scFv IgG MTH (CSS) WTCH2CH3 (ID DE SEC NO _), Carril 4 2H7 scFv IgG MTH (SCS) WTCH2CH3 (ID DE SEC NO _), Carril 5 2H7 scFv IgAH IgG WTCH2CH3 (ID DE SEC NO _), y Carril 6 2H7 scFv IgG MTH (SSS) WTCH2CH3 (ID DE SEC NO _)

La Figura 36 ilustra la unión del polipéptido 2H7 scFv IgAH IgACH2CH3 (ID DE SEC NO _) y 2H7 scFv IgAH IgAT4 (ID DE SEC NO _) a células (CD20+)CHO mediante inmunocitofluorimetria de flujo La fuente de los polipéptidos fue cultivar supernadantes de células COS transitoriamente transfectadas Las células COS transfectadas con un plásmido que comprende una secuencia que codifica 2H7 scFv IgAH IgACH2CH3 fueron co-transfectadas con una secuencia de nucleótido que contiene plásmido que codifica cadena J humana

La Figura 37 ilustra actividad de citotoxicidad mediada por célula dependiente de anticuerpo de proteínas de fusión anti-CD20 (2H7) scFv Ig contra células objetivo BJAB que utilizan sangre completa como la fuente de las células efectoras Las proteínas de fusión 2H7 scFv Ig purificadas fueron tituladas y combinadas con células BJAB etiquetadas con ^{51}Cr (5 x 10^4) y sangre completa (1 4 dilución final) Cada punto de datos

representa un porcentaje promedio específico de medición de muerte en cuatro pozos de muestra

La Figura 38 demuestra la actividad de citotoxicidad mediada por célula dependiente de anticuerpo de las proteínas de fusión 2H7 scFv Ig (5 µg/ml) células BJAB etiquetadas con ^{51}Cr a 0 25, 0 125, y 0 625 diluciones de la sangre completa Cada punto de datos representa el porcentaje promedio específico de muerte medida en cuatro pozos de muestra

La Figura 39 muestra una comparación de la actividad de citotoxicidad mediada por célula dependiente de anticuerpo de 2H7 scFv IgG MTH (SSS) WTCH2CH3 (5 µg/ml) y 2H7 scFv IgAH IgACH2CH3 (5 µg/ml) cuando el PBMC humano es la fuente de las células efectoras (Figura 39A) y cuando la sangre completa humana es la fuente de las células efectoras (Figura 39B)

La Figura 40 presenta una inmunoabsorción de las proteínas de fusión 2H7 scFv IgG Las células COS fueron transitoriamente transfectadas con varias construcciones de proteínas de fusión 2H7 scFv Ig Los supernadantes del cultivo que contienen los polipéptidos expresados fueron separados en un gel de poliacrilamida SDS no reductor, y luego fueron transferidos a una membrana de polivinil fluoruro Las proteínas fueron detectadas utilizando un conjugado de peroxidasa de cola de caballo IgG (Fc específico) anti-

humano Carriles 1-5 2H7 scFv IgG MTH (SSS) WTCH2CH3 purificado a 40 ng, 20 ng, 10 ng/5 ng, y 2 5 ng por carril, respectivamente Los supernadantes del cultivo fueron separados en los carriles 6-9 Carril 6 2H7 scFv IgG WTH (CCC) WTCH2CH3, Carril 7 2H7 scFv IgG MTH (CSS) WTCH2CH3, Carril 8 2H7 scFv IgG MTH (SCS) WTCH2CH3, y Carril 9 2H7 scFv VHSER11 IgG MTH (SSS) WTCH2CH3 El peso molecular (kDal) de las proteínas marcadoras se indica a la izquierda de la inmunoabsorción

La Figura 41 ilustra la expresión de superficie celular de 1D8 (anti-murino 4-1BB) proteína de fusión scFv IgG WTH WTCH2CH3-CD80sobre células de melanoma K1735 inmunofluorimetría de flujo (Fig 41A) La proteína de fusión scFv se detectó con IgG anti-humano de cabra $F(ab')_2$ ficoeritrin-conjugando La Fig 41B describe el crecimiento de tumores en ratones C3H naturales transplantados con inyección subcutánea con células de melanoma K1735 tipo silvestre (K1735-WT) o con células K1735 transfectadas con 1D8 scFv IgG WTH WTCH2CH3-CD80 (K1735-1D8) El crecimiento del tumor fue monitoreado al medir el tamaño del tumor La Fig 41C demuestra las cinéticas del crecimiento del tumor en ratones C3H naturales inyectados intraperitonealmente con anticuerpos monoclonales para remover células T CD8+, CD4+, o ambas CD4+ y CD8+ antes del transplante de los animales con las células K1735-1D8

La Figura 42 demuestra la terapia de los tumores K1735-WT establecidos utilizando K1735-1D8 como un inmunógeno Seis días después los ratones fueron transplantados con tumores K1735-WT, un grupo (cinco animales) fue inyectado subcutáneamente con células K1735-1D8 (círculos abiertos) o irradiada con células K1735-WT (cuadrados sólidos) sobre el lado contralateral Un grupo de control de ratones recibió PBS (cuadrados abiertos) Los tratamientos fueron repetidos en los días indicados por las flechas

La Figura 43 muestra el crecimiento de los tumores en los animales que fueron inyectados subcutáneamente con 2 x 10^6 células K1735-WT (cuadrados sólidos) y el crecimiento de los tumores en los animales que fueron inyectados subcutáneamente con 2x10^6 células K1735-WT más 2 x 10^5 células K1735-1D8 (triangulos abiertos)

La Figura 44 presenta un análisis de citometría de flujo de células tumorales de sarcoma de murino antígeno104 transfectadas con 1D8 scFv IgG WTH WTCH2CH3-CD80 aislado después de repetidas rondas de paneo contra IgG anti-humano Las células transfectadas que expresan 1D8 scFv IgG WTH WTCH2CH3-CD80 se detectó con IgG anti-humano de cabra conjugado con fluoroisotiocianato (FITC) (descrito en negro) Las células no transfectadas se muestran en gris

La Figura 45 ilustra la migración de varias proteínas de fusión 2H7 scFv Ig en un SDS-PAGE al 10% El 2H7 fue el anti-CD20 scFv y 40 2 220 fue el anti-CD40 scFv Carril 1 estándares de peso molecular preteñidos Bio-Rad, Carril 2 anti-CD20 scFv IgG MTH (SSS) MTCH2WTCH3, Carril 3 anti-CD20 scFv IgG MTH (SSS) WTCH2CH3, Carril 4 2H7 scFv IgAH IgG WTCH2CH3, Carril 5 anti-CD20-anti-CD40 scFv IgG MTH (SSS) MTCH2WTCH3, Carril 6 Rituximab, Carril 7 Novex Multimarkt estándares de peso molecular

La Figura 46 ilustra la función efectora medida en un anticuerpo dependiente de ensayo de citotoxicidad mediado por célula de las proteínas de fusión 2H7 Ig que contienen un dominio CH2 mutante o un dominio CH tipo silvestre El porcentaje específico de muerte de células objetivo BJAB en la presencia de células efectoras PBMC humano mediante 2H7 scFv IgG MTH (SSS) MTCH2WTCH3 (diamantes) se comparó con 2H7 scFv IgG MTH (SSS) WTCH2CH3 (cuadrados) y 2H7 scFv IgAH IgG WTCH2CH3 (triángulos) y Rituximab (círculos)

La Figura 47 muestra la expresión de superficie celular de una proteína de fusión CD3 scFv IgG WTH WTCH2CH3-CD80 anti-humana (ID DE SEC NO _) sobre células Reh (Fig 47A) y células linfoblastoides T51 (Fig 47B) al medir el

equivalente fluorescente lineal (LFE) utilizando inmunocitofluorometría de flujo

La Figura 48 presenta el porcentaje de muerte espeífica de Reh no transfectado y células T51 y el porcentaje específico de muerte de células Reh (Reh anti-hCD3) (Fig 48A) y células T51 (T51 anti-hCD3) (Fig 48B) que fueron transfectadas con una construcción que codifica anticuerpos scFv específicos para CD3 humano, fusionadas a CH2-CH3-pivote tipo silvestre IgG1 humano, que fueron fusionadas a transmembrana CD80 y a dominios de cola citoplasmática (CD3 anti-humano scFv IgG WTH WTCH2CH3-CD80 (ID DE SEC NO _) El PBMC humano (células efectoras) se combinó con células objetivo BJAB en las proporciones indicadas

La Figura 49 ilustra la unión de 5B9, un anticuerpo monoclonal CD137 anti-murino (4-1BB) y una proteína de fusión 5B9 scFv IgG (5B9 scFv IgG MTH (SSS) WTCH2CH3 (ID DE SEC NO _) a PBMC humano estimulado La unión de la proteína de fusión 5B9 scFv IgG se detectó mediante inmunocitofluorimetría de flujo utilizando IgG anti-humano de cabra conjugado con FITC La unión del anticuerpo monoclonal 5B9 se detectó con IgG anti-ratón de cabra conjugado con FITC

La Figura 50 ilustra el efecto de la mutación LVH11S sobre la expresión del 2H7 LVH111S scFv WCH2 WCH3 ("CytoxB scFv Ig", ID DE SEC NO _) en líneas de células CHO

La Figura 51 muestra un análisis SDS-PAGE semi-cuantitativo que examina la expresión de 2H7 LVH11S scFv WCH2 WCH3 (ID DE SEC NO _) cuando son transitoriamente transfectadas en células CHO Los carriles 2-5 son varias cantidades de 2H7 LVH11S scFv WCH2 WCH3 Carriles 6-10 son muestras de 10 µl de cinco diferentes clones que expresan 2H7 LVH11S scFv WCH2 WCH3

La Figura 52 muestra diferencias en la capacidad de unión entre una construcción G28-1 LV_H11S scFv Ig (ID DE SEC NO _) y una construcción de proteina de fusión de dominio de unión scFv Ig tipo silvestre G28-1 (ID DE SEC NO _), ambas obtenidas de células COS transitoriamente transfectadas La unión de células Ramos se determinó utilizando citometria de flujo Los datos ilustran un incremento significativo en la unión de la proteína V_H11S a las células Ramos CD37+

La Figura 53 ilustra niveles crecientes de la expresión de las construcciones G28-1 LVH11S scFv Ig (ID DE SEC NO _) comparadas con las construcciones scFv Ig tipo silvestre G28-1 en COS Los niveles de proteina se compararon utilizando análisis de inmunoabsorción Ambos geles de inmunoabsorción

tienen cantidades cuantificadas de una construcción G28-1 scFv Ig (SSS-S) H WCH2 WCH3 de la invención en los carriles 1-4 Los carriles 5-9 de la primera inmunoabsorción representan cinco diferentes clones cada uno transfectado con G28-1 scFv (SSS-S) H WCH2 WCH3, mientras que los carriles 5-9 de la segunda inmunoabsorción representan cinco diferentes clones transfectados con G28-1 LV_H11S scFv (SSS-S) H WCH2 WCH3 Las inmunoabsorciones ilustran que la forma LV_H11S origina que la construcción G28-1 scFv Ig se exprese a niveles muy altos

La Figura 54 ilustra la unión de derivados 2H7 scFv Ig con articulaciones alteradas (ID DE SEC NOS _,_,_,_,_,_,_,_) a células CHO que expresan CD20 (CD20+ CHO) mediante citometría de flujo, e indica que estas construcciones de pivote de región de conexión alteradas (incluyendo regiones de pivote (SSS-S), (CSS-S), (SCS-S) y (CSC-S)) retienen la función de unión a CD20

La Figura 55 muestra la capacidad para mediar citotoxicidad mediada por célula dependiente de anticuerpo de varias construcciones contra los objetivos Bjab (A) construcciones 2H7 scFv Ig de la invención que contienen regiones de onexión que comprenden articulaciones (CSS-S), (SCS-S), (CSC-S), y (SSS-S) (ID DE SEC NOS _,_,_,_,_,_,_,_) y (B) construcciones 2H7 scFv de la invención con varias regiones de conexión y

regiones de cola (ID DE SEC NOS _,_,_,_,_,_) El porcentaje específico de muerte se compara con el total de muerte inducida por un detergente Los controles son muerte natural en las células objetivo con efectores agregados y una construcción 2H7 con una región de conexión de pivote IgA y una región de cola derivada de IgA que no se une a los efectores PBMC

La Figura 56 ilustra la capacidad de varias construcciones 2H7 scFv Ig de la invención (ID DE SEC NO _,_,_,_,_,_) que incluyen regiones de conexión que tienen varias regiones de pivote (por ejemplo, (CSC-S), (SSS-S), (SCS-S), y (CSS-S)) para mediar la actividad de complemento en células Ramos El porcentaje específico de muerte es medido contra el control del complemento solamente, y el 100% de las muertes se determina mediante exposición de las células al detergente

La Figura 57 ilustra las muestras de unión de las construcciones 2H7 scFv Ig de la invención que contienen diferentes regiones de cola (ID DE SEC NO _,_,_) a CD20+ CHO utilizando inmunocitofluorimetría Las diferentes proteinas fueron detectadas utilizando FITC conjugado a anti-IgG, anti-IgA, y anti-IgE

La Figura 58A muestra la unión de 2H7 V_HL11S scFv IgECH2CH3CH4, se purificó utilizando cromatografía de

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inducción de carga hidrófoba (HCIC) y se eluyó con diferentes pHs 4 0 y 3 5, (ID DE SEC NO _) en células CD20+ CHO mediante citometría de flujo, indicando que las proteínas unidas a CD20 sean eluadas a un pH 4 0 o 3 5 La Figura 58B es una gráfica de datos que indica la capacidad de estas construcciones 2H7 VH L11S scFv IgE de la invención para mediar, por ejemplo, ADCC en células objetivo Bjab

La Figura 59 muestra la capacidad de unión de G28-1 VH L11S mIgECH2CH3CH4 (ID DE SEC NO _) (A) a células objetivo Bjab y Ramos y (B) a células CD20+ CHO mediante citometría de flujo

La Figura 60 muestra los perfiles Cromatografía Líquida de Alto Desempeño (HPLC) de varias construcciones de proteína de la invención (A) 2H7 scFv (SSS-S) H (P238S) CH2 WCH3 (ID DE SEC NO _) (B) 2H7 scFv (CSS-S) H WCH2 WCH3, (ID DE SEC NO _) (C) 2H7 scFv (SCS-S) H WCH2 WCH3, (ID DE SEC NO _) y (D) 2H7 scFv (SSS-S) H WCH2 (Y407A) CH3 (ID DE SEC NO _), que indica que la construcción A tiene formas de peso molecular aparente de 100kD y 75kD y que, al introducir ciertos cambios se obtiene un peso molecular predominante de 75 kD, como se ve en las construcciones B, C y D Ver Ejemplo 40

La Figura 61 muestra los perfiles HPLC de varias construcciones de proteína de la invención (A) 2H7 scFv (SSS-S) H WCH2 WCH3, (ID DE SEC NO _) (B) 2H7 scFv (CSC-S) H WCH2

WCH3, (ID DE SEC NO _) (C) 2H7 scFv (CCC-P) H WCH2 WCH3, (ID DE SEC NO _) y (D) 2H7 scFv IgAH WCH2 WCH3 (ID DE SEC NO _), indicando que la construcción A tiene formas de peso molecular aparente de 100kD y 75kD y que, al introducir ciertos cambios se obtiene una forma de peso molecular predominante de 75 kD, como se ve en las construcciones B y C, aunque la construcción D (que tiene una región de cola IgA) tiene un peso molecular aparente de 150 kD Ver Ejemplo 40

La Figura 62 muestra los perfiles HPLC de varias construcciones de proteína de la invención (A) 2H7 scFv (SSS-S) H WCH2 WCH3, (ID DE SEC NO _) (B) 2H7 scFv (SCS-S) H WCH2 WCH3, (ID DE SEC NO _) (C) 2H7 scFv IgA 3TCH2 WCH3, (ID DE SEC NO _) y (D) 2H7 scFv (SSS-S) H WCH2 (F405A Y407A) CH3 (ID DE SEC NO _, indicando que la construcción A tiene dos formas de peso molecular aparente de 100kD y 75kD, la construcción B tiene una forma predominante con un peso molecular aparente de 75 kD, aunque la construcción C con la mutación T4 conduce a tres formas con pesos moleculares aparentes cercanos a 600 kD y la construcción D con una mutación de punto doble en la región CH3 conduce a una forma predominante que tiene un peso molecular de menos de 44 kD Una mutación T4 se refiere aquí a una truncación de cuatro aminoácidos provenientes de una región CH3 Ver Ejemplo 40

La Figura 63 compara el efecto sobre la unión de las células CD20+CHO mediante las construcciones 2H7 VH L11S scFv Ig (ID DE SEC NOS _,_), con y sin las alteraciones F405A y Y407A en la región CH3, mediante citometría de flujo, indicando una pérdida de la capacidad de unión con este cambio de aminoácido doble Ver Ejemplo 41

La Figura 64 muestra la capacidad de unión de los derivados 2H7 VH L11S scFv Ig conjugados con FITC (ID DE SEC NOS _,_,_,_,) a células CH20+ CHO mediante citometría de flujo, que indica que estas construcciones no pierden capacidad de unión cuando se conjugan a un marcador fluorescente Ver Ejemplo 41

La Figura 65 muestra un análisis SDS-PAGE no reductor que examina 10 µg (por carril) de varias construcciones 2H7 VH L11S scFv Ig purificadas de la invención (ID DE SEC NOS _,_,_,_,_,_), que indican un peso molecular aparente para cada construcción en referencia al marcador de peso molecular estándar en el carril 1 Ver Ejemplo 41

La Figura 66 compara las secuencias de dominio CH2 de cuatro diferentes regiones IgG humanas, hIgG1, hIgG2, hIgG3, hIgG4, hIgG4, y una región de rata, rIgG2b Las mutaciones de punto que afectan el ADCC y CDC son marcadas con flechas Ver Ejemplo 52

La Figura 67 demuestra la capacidad de varias construcciones 2H7 VH L11S scFv Ig (ID DE SEC NOS _,_) para mediar ADCC en CHO y células transitoriamente transfectadas Lec13 CHO, que indican que las construcciones expresadas en las células Lec13 CHO tenían un 20% de incremento en la muerte específica sobre la misma construcción expresada en células CHO regulares Ver Ejemplo 42

La Figura 68 muestra el análisis SDS-PAGE, ambas reducidas y no reducidas, de alelos de alta y baja afinidad de CD16 (ED) (SSS-S) H P283S CH2 WCH3 soluble (ID DE SEC NOS _,_) Ver Ejemplo 43

La Figura 69 demuestra las diferentes capacidades de unión de las proteínas de fusión CD16 de alta y baja afinidad (ID DE SEC NOS _,_,) a 2H7 VH L11S scFv (CSC-S) WCH2 WCH3 (ID DE SEC NO _) o 2H7 VH L11S scFv (SSS-S) WCH2 WCH3 (ID DE SEC NO _), e indican una pérdida del alelo de afinidad alta y baja que se une utilizando construcciones P238S CH2 Ver Ejemplo 43

La Figura 70 muestra un diagrama de (A) un ensayo utilizado para detectar cambios en la unión de receptor Fc utilizando proteína de fusión Ig de dominio extracelular CD16 conjugada con FITC con una cola mutada, que elilmina auto-asociación y

(B) un sistema de despliegue de mamífero utilizando expresión de superficie celular de construcciones Ver Ejemplo 44

La Figura 71 muestra la inducción de apoptosis en células Bjab y Ramos mediante varios mAbs (ID DE SEC NOS _,_,_,_,_,_,_) y las construcciones scFvIg de la invención (ID DE SEC NOS _,_,_,_,_,_) Ver Ejemplo 45

La Figura 72 ilustra la capacidad de las construcciones 2H7 scFv (SSS-S) H WCH2 WCH3 y 2H7 scFv (SSS-S) H P238CH2 WCH3 para inducir apoptosis en células Ramos que son mediadas mediante la activación de caspasa 3 Los resultados indican que bajo estas condiciones, ambas construcciones se unen a CD20 e inducen apoptosis

La Figura 73 ilustra la capacidad de las construcciones 2H7 scFv (SSS-S) H WCH2 WCH3 y 2H7 scFv (SSS-S) H P238CH2 WCH3 para mediar la actividad CDC en células objetivo Bjab positivas CD20 Los resultados indican que ambas construcciones tienen la capacidad de mediar CDC

La Figura 74 compara la capacidad de las construcciones 2H7 scFv (SSS-S) H WCH2 WCH3 y 2H7 scFv (SSS-S) H P238CH2 WCH3 para mediar células objetivo ADCC en células objetivo Bjab Los resultados indican que la construcción 2H7 scFv (SSS-S) H WCH2 WCH3 fue muy efectiva para inducir altos niveles de

muerte específica, aunque la construcción 2H7 scFv (SSS-S) H P238CH2 WCH3 no fue efectiva en mediar ADCC

La Figura 75 compara la capacidad de las construcciones 2H7 scFv (SSS-S) H WCH2 WCH3 y 2H7 scFv (SSS-S) H P238CH2 WCH3 para unirse a CD16 soluble (formas de alta afinidad y baja afinidad) en células CHO positivas CD20 Los resultados indican que 2H7 scFv (SSS-S) H WCH2 WCH3 fue capaz de unirse a CD16 (ambas formas), aunque 2H7 scFv (SSS-S) H P238CH2 WCH3 no fue capaz de unirse a CD16 (cualquier forma)

La Figura 76 compara la capacidad de las construcciones 2H7 scFv (SSS-S) H WCH2 WCH3 y 2H7 scFv (SSS-S) H P238CH2 WCH3 para unirse a las células U937 positivas CD64 Los resultados indican que ambas construcciones tienen alta afinidad por el FcγRI, y que la mutación P238S reduce selectivamente la unión al FcγRIII

La Figura 77 ilustra un experimento *in vivo* en macacos para medir el efecto de las construcciones 2H7 scFv (SSS-S) H WCH2 WCH3 y 2H7 scFv (SSS-S) H P238CH2 WCH3 sobre el agotamiento de célula B Las construcciones fueron administradas con separación de una semana y las células B fueron medidas en los días -7, 0, 1, 3, 7, 8, 10, 14, 28 y 43 utilizando conteos de sangre completa y citometría de flujo de dos colores Los resultados indican que la construcción 2H7 scFv

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(SSS-S) H WCH2 WCH3 dio como resultado un agotamiento rápido y completo de células B, que duró hasta 28 dias después de la segunda inyección Por el contrario la construcción 2H7 scFv (SSS-S) H P238CH2 WCH3 dio como resultado una reducción lenta en las células B a aproximadamente 50% en las primeras dos semanas, sin embargo, las células B rápidamente iniciaron el retorno a su nivel regular poco después de esto Esto sugiere que el ADCC mediado por la interacción CD16 es probablemente necesario para un agotamiento rápido y sostenido de la célula B

La Figura 78 ilustra los perfiles SEC de las construcciones G28-1 VL11S scFv (SSS) H WCH2 WCH3 y G28-1 VL11S scFv (SSC) H WCH2 WCH3 La construcción con un pivote SSS generó un pico uniforme simple a aproximadamente 75-100 kD, mientras que la construcción con el pivote SSC generó una forma más pequeña y otras formas heterogéneas, que incluyen una a más de 200 kD

La Figura 79 ilustra la capacidad de unión de las construcciones G28-1 VL11S scFv (SSS) H WCH2 WCH3 y G28-1 VL11S scFv (SSC) H WCH2 WCH3 en células de linfoma de célula B Bjab, Ramos, WIL-2, Namalwa y Raji Los resultados en (a) indican que G28-1 VL11S scFv (SSS) H WCH2 WCH3 unido a las células Bjab y Ramos, y moderadamente a las células WIL-2, y a niveles inferiores a las células Namalwa y Raji Los resultados en (b) indican el G28-1 VL11S scFv (SSC) H WCH2

WCH3 unido a las células Bjab, y moderadamente a las células Ramos y WIL-2, y a niveles inferiores de las células Namalwa y Raji

La Figura 80 ilustra la unión de annexina y v-yoduro de propidio en células Ramos incubadas con construcciones G28-1 VL11S scFv (SSS) H WCH2 WCH3 y G28-1 VL11S scFv (SSC) H WCH2 WCH3 durante toda la noche Los resultados indican que ambas construcciones indujeron apoptosis, sin embargo, la construcción con el pivote (SSC) indujo más apoptosis que la construcción con el pivote (SSS)

La Figura 81 ilustra la capacidad de las construcciones G28-1 VL11S scFv (SSS) H WCH2 WCH3 y G28-1 VL11S scFv (SSC) H WCH2 WCH3 para inhibir la proliferación de las células Ramos Los resultados inmdican que ambas construcciones inhibieron la prolilferación

La Figura 82 ilustra la capacidad de las construcciones G28-1 VL11S scFv (SSS) H WCH2 WCH3 y G28-1 VL11S scFv (SSC) H WCH2 WCH3 y 2H7 scFv (CSS) H WCH2 WCH3 para inducir la apoptosis en las células B Ramos, solas y en combinaciones diferentes Los resultados mostrados en este experimento indican que ambas construcciones G28-1 fueron más eficientes que la construcción 2H7 Los resultados también indican que la construcción G28-1 VL11S scFv (SSS) H WCH2 WCH3 fue más

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eficiente que la construcción G28-1 VL11S scFv (SSC) H WCH2 WCH3 Sin embargo, la cantidad de apoptosis fue la más grande cujando las construcciones 2H7 y el G28-1 fueron utilizadas en combinación

La Figura 83 compara la capacidad de las construcciones G28-1 VL11S scFv (SSS) H WCH2 WCH3, G28-1 VL11S scFv (SSC) H WCH2 WCH3 y 2H7 scFv (CSS) H WCH2 WCH3 para mediar CDC en células B Ramos Los resultados indican que todas las construcciones mediaron CDC La construcción 2H7 fue más efectiva, seguida por G28-1 VL11scFv (SSC) H WCH2 WCH3, luego G28-1 VL11S scFv (SSS) H WCH2 WCH3

La Figura 84 compara la capacidad de las construcciones G28-1 VL11S scFv (SSS) H WCH2 WCH3, G28-1 VL11S scFv (SSC) H WCH2 WCH3 y 2H7 scFv (CSS) H WCH2 WCH3 para mediar ADCC en células B Ramos Los resultados indican que las construcciones G28-1 VL11S scFv (SSS) H WCH2 WCH3 y G28-1 VL11S scFv (SSC) H WCH2 WCH3 fueron capaces de mediar ADCC, mientras que la capacidad de la construcción 2H7 scFv (CSS) H WCH2 WCH3 para mediar ADCC fue inferior, pero aún mayor que el nivel de muerte natural

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DESCRIPCION DETALLADA DE LA INVENCIÓN

La presente invención está dirigida a moléculas novedosas útiles, por ejemplo, coo terapéuticos, así como también para otros propósitos que incluyen propósitos de diagnóstico e investigación Tales moléculas tienen, por ejemplo, unión de antígeno u otras funciones de unión y, por ejemplo, una o más funciones efectoras La invención incluye construcciones moleculares, que incluyen proteínas de fusión de inmunoglobulina con dominio de unión, y composiciones relacionadas y métodos, las cuales serán útiles en aplicaciones inmunoterapéuticas y de inmunodiagnóstico, y en métodos de investigación, y que ofrecen ciertas ventajas sobre los compuestos específicos de antígeno y los polipéptidos de la técnica anterior Las construcciones, incluyendo las proteínas de fusión, de la presente invención son preferiblemente cadenas de polipéptidos sencillos que comprenden, en la parte pertinente, los siguientes dominios o regiones fusionadas o conectadas de otra forma una construcción de región de unión, tal como un dominio de unión o un polipéptido, una construcción o región de conexión que incluye, por ejemplo, un polipéptido de región de pivote de inmunoglobulina nativo o trabajado por ingeniería, y una construcción de región de cola, que incluye, por ejemplo, una construcción que puede comprender, consiste esencialmente de, o consiste de, un polipéptido de región constante CH2 de

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cadena pesada de inmunoglobulina nativo o trabajado por ingeniería y un polipéptido de región constante CH3 de cadena pesada de inmunoglobulina nativo o trabajado por ingeniería De acuerdo con ciertas modalidades que son particularmente útiles para terapia génica, las construcciones, incluyendo las proteínas de fusión, de la presente invención pueden adicionalmente comprender un dominio de anclaje de membrana de plasma nativo o trabajado por ingeniería De acuerdo con ciertas otras modalidades preferidas las construcciones, que incluyen las proteínas de fusión, de la presente invención pueden adicionalmente incluir una región de cola que tiene un polipéptido de región constante CH4 de cadena pesada de inmunoglobulina nativo o trabajado por ingeniería En modalidades particularmente preferidas, las regiones de unión, tal como los dominios de polipéptido, de los cuales las construcciones, que incluyen las proteínas de fusión de inmunoglobulina con dominio de unión, están comprendidas, o se derivan de, polipéptidos que son los productos de las secuencias de gen humano, pero la invención no necesita ser así limitada y puede de hecho relacionar las construcciones, incluyendo las proteínas de fusión de inmunoglobulina con dominio de unión, como se suministra aquí que se derivan de cualquier fuente natural o artificial, que incluye polipéptidos trabajados con ingeniería genética y/o mutados

La presente invención se relaciona en parte con la observación sorprendente de que las construcines novedosas, que incluyen proteínas de fusión de inmunoglobulina con dominio de unión, descritas aquí son capaces de actividad inmunológica Más específicamente, estas proteínas retienen la capacidad de participar en actividades efectoras inmunológicas bien conocidas incluyendo, por ejemplo, citotoxicidad mediada por célula dependiente de anticuerpo (por ejemplo, subsecuente a la unión de antígeno sobre una superficie de célula, acoplamiento e inducción de células efectoras citotóxicas que llevan receptores Fc apropiados, tales como células Asesinas Naturales que llevan FcRγIII, bajo condiciones apropiadas) y/o fijación de complemento en citotoxicidad dependiente de complemento (por ejemplo, subsecuente a unión de antigeno sobre la superficie celular, reclutamiento y activación de proteínas citolíticas que son componentes de la cascada complemento sanguínea) a pesar de tener estructuras no se espera que sean capaces de promover tales actividades efectoras o de promoción de tales actividades como se describen aquí Para las revisiones de ADCC y CDC ver, por ejemplo, Carter, 2001 *Nat Rev Canc* 1 118, Sulica *et al* , 2001 *Int Rev Immunol* 20 371, Maloney *et al* , 2002 *Semin Oncol* 29 2, Sondel *et al* , 2001 *Hematol Oncol Clin North Am* 15 (4) 703-21, Maloney 2001 *Anticanc* Drugs 12 Suppl 2 1-4 La activación IgA de complemento mediante la trayectoria alternativa se describe, por ejemplo,

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en Schneiderman *et al* , 1990 *J Immunol* 145 233 Como se describe en mayor detalle adelante, el ADCC, la fijación de complemento, y el CDC son funciones inesperadas para las construcciones, incluyendo las proteínas de fusión, que comprenden por ejemplo regiones de cadena pesada de inmunoglobulina y que tienen las estructuras descritas aqui, y en particular para proteínas de fusión de inmunoglobulina que comprenden por ejemplo, polipéptidos de región de pivote de inmunoglobulina que están comprometidas en su capacidad para formar uniones disulfuro homodiméricas de intercadena

Otra ventaja lograda por la presente invención son las construcciones, que incluyen los polipéptidos de fusión de inmunoglobulina con dominio de unión, de la invención y que pueden ser producidas en cantidades sustanciales que son típicamente mayores de aquellas rutinariamente logradas con construcciones de anticuerpo de cadena sencilla de la técnica anterior, por ejemplo En las modalildades preferidas, las construcciones, incluyendo los polipéptidos de fusión de inmunoglobulina con dominio de unión, de la presente invención son recombinantemente expresadas en mamíferos u otros sistemas de expresión deseados y utiles, que ofrecen la ventaja de suministrar polipéptidos que son estables *in vivo* (por ejemplo, bajo condiciones fisiológicas) De acuerdo con teoría no limitante, tal estabilidad puede derivar en parte de modificaciones posttransduccionales, y específicamente

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glicosilación La producción de las construcciones, incluyendo construcciones de proteína de fusión de inmunoglobulina con dominio de unión, de la invención por vía de expresión de mamífero recombinante se han logrado en cultivos de célula estáticos a un nivel mayor de 50 mg de proteína por litro de supernadante de cultivo y han sido rutinariamente observadas en tales cultivos a 10-50 mg/litro, de tal forma que preferiblemente al menos 10-50 mg/litro se pueden producir bajo condiciones de cultivo estático, también se contempla la producción mejorada, en todo o en parte, de las construcciones de proteína de la invención utilizando metodologías a escala aceptadas por la técnica tales como la producción de "tanda de alimentación" (es decir, no estática), donde son obtenidos rendimientos de al menos 5-500 mg/l, y en algunos casos al menos 0 5-1 gm/l, dependiendo del producto de proteína particular

Una construcción, que incluye un polipéptido con dominio de unión, de acuerdo con la presente invención puede ser, por ejemplo, cualquier polipéptido que posea la capacidad de reconocer específicamente y unirse a una molécula biológica cognada o complejo de más de una molécula o montaje o agregado, sea estable o transitorio, de tal molécula Tales moléculas incluyen proteínas, polipéptidos, péptidos, aminoácidos, o derivados de estos, lípidos, ácidos grasos o similares, o derivados de estos, carbohidratos, sacáridos o

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similares o derivados de estos, ácidos nucleicos, nucleótidos, nucleósidos, purinas, pirimidinas o moléculas relacionadas, o derivados de estos, o similares, o cualquier combinación de estos tales como, por ejemplo, glicoproteínas, glicopéptidos, glicolípidos, y lipoproteínas, proteolípidos, o cualquier otra molécula biológica que puede estar presente en una muestra biológica Las muestras biológicas pueden ser suministradas, por ejemplo, al obtener una muestra de sangre, espécimen de biopsia, explante de tejido, cultivo de órgano, fluido biológico o cualquier otro tejido o célula u otra preparación de un sujeto o una fuente biológica El sujeto o fuente biológica puede, por ejemplo, ser un humano o animal no humano, un cultivo de célula primaria o una línea celular adaptada a cultivo que incluye pero no está limitada a lineas celulares trabajadas por ingeniería genética que pueden contener secuencias de ácido nucleico recombinante cromosomalmente integradas o episomales, líneas celulares inmortalizadas o inmortalizables, líneas celulares híbridas de célula somática, líneas celulares diferenciadas o diferenciables, líneas celulares transformadas y similares, etc En ciertas modalidades preferidas de la invención, el sujeto o fuente biológica se puede sospechar que tiene o está en riesgo de tener una enfermedad, o desorden o condición, que incluye una enfermedad maligna, desorden o condición de un desorden de célula B, que en ciertas modalidades adicionales puede ser una enfermedad autoinmune, y en ciertas

otras modalidades de la invención el sujeto o fuente biológica puede ser conocido por estar libre de un riesgo o presencia de tales enfermedades, desorden o condición

Una región de unión, que incluye el polipéptido con dominio de unión, por ejemplo puede ser cualquier compañero de unión producido por ocurrencia natural, sintético, semi-sintético, y/o recombinante para una molécula biológica u otra que es la estructura objetivo de interés, algunas veces denominada aquí como un "antígeno" pero destinada de acuerdo con la presente descripción a comprender cualquier objetivo biológico u otra molécula que sea deseable tener como objeto de la invención, por ejemplo, una proteína de fusión, unida o específicamente unida Las construcciones de la invención, incluyendo las proteínas de fusión de inmunoglobulina con dominio de unión, se definen por ser "inmunoespecíficas" o capaces de unirse a un grado deseado, incluyendo específicamente unión, si ellas se unen a una molécula objetivo deseada tal como un antígeno como se suministra aquí, a un nivel deseado, por ejemplo, con un K_a mayor o igual a aproximadamente 10^4 M^{-1}, preferiblemente mayor o igual de aproximadamente 10^5 M^{-1}, más preferiblemente mayor o igual a aproximadamente 10^6 M^{-1} y aún más preferiblemente mayor o igual a aproximadamente 10^7 M^{-1} Las afinidades aún mayores de aproximadamente 10^7 M^{-1} son aún más preferidas, tales como afinidades iguales a o mayores de aproximadamente 10^7 M^{-1}, aproximadamente 10^8 M^{-1}, y

aproximadamente 10^9 M^{-1}, y aproximadamente 10^{10} M^{-1} Las afinidades de las proteínas de fusión de inmunoglobulina con dominio de unión de acuerdo con la presente invención pueden ser fácilmente determinadas utilizando técnicas convencionales, por ejemplo aquellas descritas por Scatchard *et al* , 1949 *Ann N Y Acad Sci* 51 660 Tal determinación de proteína de fusión que se une a antígenos objetivo de interés también se pueden desarrollar utilizando cualquiera de un número de métodos conocidos para identificar y obtener proteínas que interactúan específicamente con otras proteínas o polipéptidos, por ejemplo, un sistema de selección de dos híbridos de levadura tal como el descrito en la Patente U S No 5,283,173 y la Patente U S No 5,468,614, o el equivalente

Las modalidades preferidas de las construcciones de la invención objeto, por ejemplo, las proteínas de fusión de inmunoglobulina con dominio de unión, comprenden regiones de unión o dominios de unión que pueden incluir, por ejemplo, al menos un polipéptido con región variable de inmunoglobulina nativo o trabajado con ingeniería, tal como todo o una porción o fragmento de una cadena pesada nativa o trabajada por ingeniería y/o una región de cadena liviana nativa o trabajada por ingeniería, siempre y cuando ésta sea capaz de unirse o unir específicamente un antígeno u otra estructura objetivo deseada de interés a un nivel deseado de unión y

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selectividad En otras modalidades preferidas la región de unión o el dominio de unión comprende, consiste esencialmente de, o consiste de, un producto Fv derivado de inmunoglobulina de cadena sencilla, por ejemplo, y scFv, que puede incluir todo o una porción de al menos una región V de cadena liviana de inmunoglobulina nativa o trabajada por ingeniería y toda o una porción de al menos una región V de cadena pesada de inmunoglobulina nativa o trabajada por ingeniería, y un ligador fusionado o conectado de otra forma a las regiones V, preparación y ensayo de tales construcciones como se describe con mayor detalle aquí Otra preparación y métodos de ensayo son bien conocidos en la técnica

Como se describe aquí y es conocido en la técnica, las inmunoglobulinas comprenden productos de una familia de genes los miembros los cuales exhiben un alto grado de conservación de secuencia Las secuencias de aminoácido de dos o más inmunoglobulinas o dominios de inmunoglobulina, o regiones o porciones de estas (por ejemplo, dominios V_H, dominios V_L, regiones de pivote, regiones constantes CH2, regiones constantes CH3) pueden ser alineadas y analizadas Las porciones de las secuencias que corresponden una a la otra se pueden identificar, por ejemplo, por homología de secuencia La determinación de la homología de secuencia se puede determinar con cualquiera de un número de alineamientos de secuencia y herramientas de análisis, que incluyen algoritmos

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de cómputo bien conocidos por aquellos expertos en la técnica, tal como el algoritmo Align o BLAST (Altschul, 1991 *J Mol Biol* 219 555-565, Henikoff y Henikoff, 1992 *Procesamiento Natl Acad Sci USA* 89 10915-10919), que está disponible en el sitio web de NCBI (http //www/ncbi nlm nih gov/cgi-bin/BLAST) Se pueden utilizar parámetros por defecto

Las porciones, por ejemplo, de una secuencia de referencia de inmunoglobulina particular y de una cualquiera o más de las secuencias de inmunoglobulina adicionales de interés que pueden ser comparadas con una secuencia de referencia Las secuencias, regiones, fragmentos "correspondientes" o similares, se pueden identificar con base en la convención para numerar posiciones de aminoácido de inmunoglobulina de acuerdo con Rabat, *Sequences of Proteins of immunological Interest*, (5ta ed Bethesda, MD Public Health Service, National Institutes of Health (1991)) Por ejemplo, de acuerdo con esta convención, la familia de inmunoglobulina a la cual una secuencia de inmunoglobulina de interés pertenece se determina con base en la conservación de los residuos de aminoácido invariantes de la secuencia de polipéptido de región variable, para identificar un sistema de numeración particular para la familia de la inmunoglobulina, y la o las secuencias de interés puedan entonces estar alineadas para traspasar números de posición de secuencia a los aminoácidos

individuales que comprenden tal o tales secuencias Preferiblemente al menos aproximadamente 70%, más preferiblemente al menos el 80%-85% o aproximadamente 86-89%, y aún más preferiblemente al menos aproximadamente 90%, aproximadamente 92%, aproximadamente 94%, aproximadamente 96%, aproximadamente 98% o aproximadamente 99% de los aminoácidos en una secuencia de aminoácido dada de al menos aproximadamente 1000, más preferiblemente aproximadamente 700-950, más preferiblemente aproximadamente 350-700, aún más preferiblemente aproximadamente 100-350, aún más preferiblemente aproximadamente 80-100, aproximadamente 70-80, aproximadamente 60-70, aproximadamente 50-60, aproximadamente 40-50 o aproximadamente 30-40 aminoácidos consecutivos de una secuencia, son idénticos a los aminoácidos localizados en las posiciones correspondientes en una secuencia de referencia tal como aquellas descritas en Rabat (1991) o en un compendio similar de secuencias de inmunoglobulina relacionadas, tales como pueden ser generadas de las bases de datos públicas (por ejemplo, Genbank, SwissProt, etc) utilizando herramientas de alineamiento de secuencia tales como, por ejemplo, aquellas descritas anteriormente En ciertas modalidades preferidas, una secuencia de inmunoglobulina de interés o una región, porción, derivado o fragmento de ésta es mayor que aproximadamente 95% idéntica a una secuencia de referencia correspondiente, y en ciertas modalidades preferidas tal

secuencia de interés puede diferir de una referencia correspondiente en no más de aproximadamente 1, 2, 3, 4, 5, 6, 7, 8, 9, o 10 posiciones de aminoácido

Por ejemplo, en ciertas modalidades la presente invención está dirigida a una construcción, que incluye una proteína de fusión de inmunoglobulina con dominio de unión, que comprende en la parte pertinente un polipéptido de región variable de cadena pesada de inmunoglobulina humana o de otras especies que comprende una mutación, alteración o supresión de un aminoácido en un sitio o sitios que corresponden a una o más de las posiciones de aminoácido 9, 10, 11, 12, 108, 110, 111 y 112 en, por ejemplo, la ID DE SEC NO _, que comprende, por ejemplo, una secuencia derivada de V_H de murino Con un número relativamente limitado de posiciones de semcuencia V_H de inmunoglobulina, por ejemplo, incluyendo la posición 11, la conservación del aminoácido se observa en la mayoría preponderante de secuencias V_H analizadas a través de las líneas de especies de mamífero (por ejemplo, Leu11, Val37, Gly44, Leu45, Trp47, Nguyen *et al* , 1998 *J Mol Biol* 275 413) Varios de tales residuos de aminoácido, y de esta manera sus cadenas laterales, están localizadas en la superficie del dominio variable (V_H) Ellos pueden contactar residuos de C_H1 (por ejemplo, Leu11) y los dominios V_L (por ejemplo, Val37, GLy44, Leu45, y Trp47) y pueden, en ausencia de cadenas livianas, contribuir a la estabilidad y

solubilidad de las proteínas (ver, por ejemplo, Chotia *et al* , 1985 *J Mol Biol* 186 651, Muyldermans *et al* , 1994 *Prot Engineer* 7 1129, Desmyter *et al* , 1996 *Nat Struct Biol* 3 803, Davies *et al* , 1994 *FEBS Lett* 339 285) En ciertas modalidades, por ejemplo, la presente invención también está dirigida a una construcción, que incluye una proteína de fusión de inmunoglobulina con dominio de unión, que comprende en la parte pertinente un polipéptido de región variable de cadena liviana de inmunoglobulina humana, o un polipéptido de región variable de cadena liviana de inmunoglobulina proveniente de otras especies que comprende una mutación, alteración o supresión en un aminoácido en un sitio o sitios que correspondan a una o más posiciones de aminoácido 12, 80, 81, 82, 83, 105, 106, 107 y 108 En aun otras ciertas modalidades, por ejemplo, la presente invención está dirigida a una construcción, que incluye una proteina de fusión de inmunoglobulina con dominio de unión, que comprende en la parte pertienente (1) un polipéptido de región variable de cadena pesada de inmunoglobulina humana, o un polipéptido con región variable de cadena liviana de inmunoglobulina de otras especies, que comprende, consiste esencialmente de, o consiste de, dicha secuencia de cadena pesada que tiene una mutación, alteración o supresión en un sitio o sitios que corresponden a una o más de las posiciones de aminoácido 9, 10, 11, 12, 108, 110, 111, y 112, y (2) un polipéptido de región variable de cadena liviana de inmunoglobulina humana,

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o un polipéptido con región variable de cadena liviana de inmunoglobulina de otras especies, que comprende, consiste esencialmente de, o consiste de, dichas secuencias de cadena liviana que tienen una mutación, alteración o supresión en un sitio o sitios que corresponden con una o más de las posiciones de aminoácido 12, 80, 81, 82, 83, 105, 106, 107, y 108

Como otro ejemplo, en referencia al compendio de secuencias de inmunoglobulina y las bases de datos tales como aquellas citadas anteriormente, por ejemplo, el relacionamiento de dos o más secuencias de inmunoglobulina una con la otra puede fácilmente y sin experimentación indebida ser establecido de una manera que permita la identificación de las especies animales de origen, la clase y la subclase (por ejemplo, isotipo) de una secuencia de polipéptido con inmunoglobulina particular o región de inmunoglobulina Cualquier secuencia de polipéptido con región variable de inmunoglobulina, que incluye V_H y/o V_L nativo o trabajado por ingeniería y/o secuencias de región variable de cadena sencilla (sFv) u otras secuencias derivadas de región V nativas o trabajadas por ingeniería o similares, se pueden utilizar como una región de unión o un dominio de unión Las secuencias trabajadas por ingeniería incluyen secuencias de inmunoglobulina provenientes de cualquier especie, preferiblemente humano o ratón, por ejemplo, que incluyen,

por ejemplo, una mutación, alteración o supresión en un aminoácido en un sitio o sitios que corresponden a una o más de las posiciones de aminoácido 9, 10, 11, 12, 108, 110, 111, y 112 en una secuencia de región variable de cadena pesada o un scFv, y/o una mutación, alteración o supresión en un sitio o sitios que corresponden a una o más de las posiciones de aminoácido 12, 80, 81, 82, 83, 105, 106, 107, y 108 en la secuencia de región variable de cadena liviana o un scFv

Varias modalidades preferidas incluyen, por ejemplo, secuencias de polipéptido de región V de inmunoglobulina nativa o trabajada por ingeniería derivadas, por ejemplo, de anticuerpos que incluyen anticuerpos monoclonales tales como murino y otros anticuerpos de roedor, o anticuerpos o anticuerpo monoclonales derivados de otra fuente tales como cabra, conejo, equino, bovino, camélido u otras especies, que incluyen animales transgénicos, y también incluyen anticuerpos humanizados o anticuerpos monoclonales Ejemplos no limitantes incluyen secuencias de polipéptido de region variable provenientes de anticuerpos monoclonales tales como aquellos referenciados aquí y/o descritos con mayor detalle en los Ejemplos que siguen, por ejemplo, anticuerpos monoclonales de murino específicos o de unión CD20 (por ejemplo, 2H7), otros anticuerpos monoclonales de murino espeíficos o de unión CD20 que no son anticuerpos 1F5, anticuerpo monoclonal L6 (específico para un epítopo definido

de carbohidrato y disponible del American Type Culture Collection, Manassas, VA, como hibridoma HB8677), y anticuerpos monoclonales que se unen a o son específicos para CD28 (por ejemplo, anticuerpo monoclonal 2E12), CD40, CD80, CD137 (por ejemplo, anticuerpo monoclonal 5B9 o anticuerpo monoclonal 1D8 que reconoce el homólogo de murino de CD137, 41BB) y CD152 (CTLA-4)

Otras regiones de unión, que incluyen polipéptidos con dominio de unión, pueden comprender cualquier proteína o porción de ésta que retenga la capacidad de unirse o de unirse específicamente a un antígeno como se suministra aquí, que no incluye inmunoglobulina De acuerdo con esto la invención contempla construcciones, incluyendo proteína de fusión, que comprenden la región de unión o los polipéptidos de dominio de unión que se derivan de los ligandos de polipéptido tales como hormonas, citoquinas, quimioquinas, y similares, superficie de célula o receptores solubles para tales ligandos de polipéptido, lectinas, receptores de adhesión intercelular tales como integrinas de leucocito específicas, selectinas, miembros de la superfamilia de gen de inmunoglobulina, moléculas de adhesión intercelular (ICAM-1, -2, -3) y similares, antígenos de histocompatibilidad, etc

Ejemplos de receptores de superficie celular útiles en la preparación de, o como, regiones de unión, o que pueden suministrar un polipe ptido con dominio de unión, y que también se pueden seleccionar como una molécula objetivo o antígeno al cual una construcción, incluyendo por ejemplo, una proteína de fusión con dominio Ig de unión de la presente invención deseablemente se une, incluye lo siguiente, o similares HER1 (por ejemplo, Acceso Genbank Nos U48722, SEG_HEGFREXS, KO3193), HER2 (Yoshino *et al* , 1994 *J Immunol* 152 2393, Disis *et al* , 1994 *Canc Res* 54 16, ver también, por ejemplo, Acceso GenBank Nos X03363, M17730, SEG HUMHER20), HER3 (por ejemplo, Acceso Genbank Nos U29339, M34309), HER4 (Plowman *et al* , 1993 *Nature* 366 473, ver también por ejemplo, Acceso Genbank Nos L07868, T64105), receptor de factor de crecimiento epidérmico (EGFR) (por ejemplo, Acceso Genbank Nos U48722, SEG_HEGFREXS, KO3193), factor de crecimiento celular endotelial vascular (por ejemplo, GenBank No M32977), receptor de factor de crecimiento celular endotelial vascular (VEGF) (por ejemplo, Acceso Genbank Nos AF022375, 1680143, U48801, X62568), factor I de crecimiento similar a insulina (por ejemplo, Acceso Genbank Nos X00173, X56774, X56773, X06043, ver también Patente Europea No GB 2241703), factor II de crecimiento similar a insulina (por ejemplo, Acceso Genbank Nos X03562, X00910, SEGHUMGFIA, SEGHUMGFI2, M17863, M17862), receptor de transferrina (Trowbridge y Omary, 1981 *Proc Nat*

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Acad USA 78 3039, ver también por ejemplo, Acceso Genbank Nos X01060, M11507), receptor de estrógeno (por ejemplo, Acceso Genbank Nos M38651, X03635, X99101, U47678, M12674), receptor de progesterona (por ejemplo, Acceso Genbank Nos X51730, X69068, M15716), receptor de hormona estimulante de folículo (FSH-R) (por ejemplo, Acceso Genbank Nos Z34260, M65085), receptor de ácido retinoico (por ejemplo, Acceso Genbank Nos L12060, M60909, X77664, X57280, X07282, X06538), MUC-1 (Barnes *et al* , 1989 *Proc Nat Acad Sci USA* 86 7159, ver también por ejemplo, Acceso Genbank Nos SEGMUSMUCIO, M65132, M64928) NY-ESO-1 (por ejemplo, Acceso Genbank Nos AJ003149, U87459), NA 17-A (por ejemplo, Patente Europea No WO 96140039), Melan-A/MART-1 (Kawakami *et al* , 1994 *Proc Nat Acad Sci USA* 91 3515, ver también por ejemplo, Acceso Genbank Nos U06654, U06452), tirosinasa (Topalian *et al* , 1994 *Proc Nat Acad Sci USA* 91 9461, ver también por ejemplo, Acceso Genbank Nos M26729, SEG HUMTYRO, ver también Weber *et al* , *J Clin Invest* (1998) 102 1258), Gp-100 (Kawakami *et al* , 1994 *Proc Nat Acad Sci USA* 91 3515, ver también por ejemplo, GenBank Ace No S73003, ver también Patente Europea No EP 668350, Adema *et al* , 1994 *J Biol Chem* 269 20126), MAGE (van den Bruggen *et al* , 1991 *Scieface* 254 1643, ver también por ejemplo, Acceso Genbank Nos U93163, AF064589, U66083, D32077, D32076, D32075, U10694, U10693, U10691, U10690, U10689, U10688, U10687, U10686, U10685, L18877, U10340, U10339, L18920,

U03735, M77481), BAGE (por ejemplo, GenBank Ace No U19180, ver también Patentes U S Nos 5,683,886 y 5,571,711), GAGE (por ejemplo, Acceso Genbank Nos AF055475, AF055474, AF055473, U19147, U19146, U19145, U19144, U19143, U19142), cualquiera de los clase CTA de recpeptores que incluyen en particular antígeno HOM-MEL-40 codificado mediante el gen SSX2 (por ejemplo, Acceso Genbank Nos X86175, U90842, U90841, X86174), antígeno carcinoembiónico (CEA, Gold y Freedman, 1985 *J Exp Med* 121 439, ver también por ejemplo, Acceso Genbank Nos SEGHUMCEA, M59710, M59255, M29540), y PyLT (por ejemplo, Acceso Genbank Nos J02289, J02038)

Receptores de superficie celular adicionales que pueden ser fuentes de región de unión o polipéptidos de dominio de unión o porciones de estos, o que pueden ser objetivos, incluyendo antígenos objetivo, incluyen lo siguiente, o similares CD2 (por ejemplo, Acceso Genbank Nos Y00023, SEGHUMCD2, M16336, M16445, SEG_MUSCD2, M14362), 4-1BB (CDw137, Kwon *et al* , 1989 *Proc Nat Acad Sci USA* 86 1963, ligando 4-1BB (Goodwin *et al* , 1993 *Eur J Immunol* 23 2361, Melero *et al* , 1998 *Eur J Immunol* 3 116), CD5 (por ejemplo, Acceso Genbank Nos X78985, X89405), CD10 (por ejemplo, Acceso Genbank Nos M81591, X76732) CD27 (por ejemplo, Acceso Genbank Nos M63928, L24495, L08096), CD28 (June *et al* , 1990 *Immunol Today* 11 211, ver también, por ejemplo, Acceso Genbank Nos

J02988, SEG_HUMCD28, M34563), CD152/CTLA-4 (por ejemplo, Acceso Genbank Nos L15006, X05719, SEGHUMIGCTL), CD40 (por ejemplo, Acceso Genbank Nos M83312, SEG_MUSC040A0, Y10507, X67878, X96710, U15637, L07414), interferon-γ (IFN-γ), ver, por ejemplo, Farrar *et al* 1993 *Ann Rev Immunol* 11 571 y referencias citadas aqui, Gray *et al* 1982 *Nature* 295 503, Rinderknecht *et al* 1984 *J Biol Chem* 259 6790, DeGrado *et al* 1982 *Nature* 300 379), interleuquina-4 (IL-4, ver, por ejemplo, 53mo Forum in Immunology, 1993 *Research in Immunol* 144 553-643, Banchereau *et al* , 1994 en *The Cytokine Handbook*, 2da ed , A Thomson, ed , *Academic Press*, NY, p 99, Keegan *et al* , 1994 *J Leukocyt Biol* 55 272, y referencias citadas allí), interleuquina-17 (IL-17) (por ejemplo, Acceso Genbank Nos U32659, U43088) y receptor de interleuquina-17 (IL-17R) (por ejemplo, Acceso Genbank Nos U31993, U58917) A pesar de lo mencionado anteriormente, la presente invención expresamente no comprende proteínas de fusión de inmunoglobulina descritas en U S 5,807,734, o U S 5,795,572

Los receptores de superfice celular adicionales que pueden ser fuentes de región de unión o polipéptidos de dominio de unión o porciones de estos, o que pueden servir como objetivos que incluyen antígenos objetivo o sitios de union incluyen los siguientes, o similares CD59 (por ejemplo, Acceso Genbank Nos SEG_HUMCD590, M95708, M34671), CD48 (por

ejemplo, Acceso Genbank Nos M59904), CD58/LFA-3 (por ejemplo, GenBank Ace No A25933, Y00636, E12817, ver también JP 1997075090-A), CD72 (por ejemplo, Acceso Genbank Nos AA311036, S40777, L35772), CD70 (por ejemplo, Acceso Genbank Nos Y13636, S69339), CD80/B7 1 (Freeman *et al* , 1989 *J Immunol* 43 2714, Freeman *et al* , 1991 *J Exp Med* 174 625, ver también por ejemplo, Acceso Genbank Nos U33208, I683379), CD86/B7 2 (Freeman *et al* , 1993 *J Exp Med* 178 2185, Borıello *et al* , 1995 *J Immunol* 155 5490, ver también, por ejemplo, Acceso Genbank Nos AF099105, SEGMMB72G, U39466, U04343, SEGHSB725, L25606, L25259), B7-H1/B7-DC (por ejemplo, Acceso Genbank Nos NM014143, AF177937, AF317088, Dong *et al* , 2002 *Nat Med Jun* 24 [epub ahead of prınt], PMID 12091876, Tseng *et al* , 2001 *J Exp Med* 193 839, Tamura *et al* , 2001 *Blood* 97 1809, Dong *et al* , 1999 *Nat Med* 5 1365), lıgando CD40 (por ejemplo, Acceso Genbank Nos SEGHUMCD40L, X67878, X65453, L07414), IL-17 (por ejemplo, Acceso Genbank Nos U32659, U43088), CD43 (por ejemplo, Acceso Genbank Nos X52075, J04536), ICOS (por ejemplo, Genbank Ace No AH011568), CD3 (por ejemplo, Acceso Genbank Nos NM-000073 (subunıdad gama), NM-000733 (subunıdad epsılon), X73617 (subunıdad delta)), CD4 (por ejemplo, Acceso Genbank No NM000616), CD25 (por ejemplo, Acceso Genbank No NM000417), CD8 (por ejemplo, Acceso Genbank No M12828), CD11b (por ejemplo, Acceso Genbank No J03925), CD14 (por ejemplo, Acceso Genbank No XM_039364), CD56 (por ejemplo,

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Acceso Genbank No U63041), CD69 (por ejemplo, Acceso Genbank No NM001781) y VLA-4 ($\alpha_4\beta_7$) (por ejemplo, Acceso Genbank Nos L12002, X16983, L20788, U97031, L24913, M68892, M95632) Los siguientes receptores de superficie celular están típicamente asociados con las células B CD19 (por ejemplo, Acceso GenBank Nos SEG_HUMCD19W0, M84371, SEG_MUSCD19W, M62542), CD20 (por ejemplo, Acceso Genbank Nos SEG_HUMCD20, M62541), CD22 (por ejemplo, Acceso Genbank Nos 1680629, Y10210, X59350, U62631, X52782, L16928), CD30 (por ejemplo, Acceso Genbank Nos M83554, D86042), CD153 (ligando CD30, por ejemplo, Acceso Genbank Nos L09753, M83554), CD37 (por ejemplo, Acceso Genbank Nos SEGMMCD37X, X14046, X53517), CD50 (ICAM-3, por ejemplo, Acceso Genbank No NM002162), CD106 (VCAM-1) (por ejemplo, Acceso Genbank Nos X53051, X67783, SEG_MMVCAM1C, ver también Patente U S No 5,596,090), CD54 (ICAM-1) (por ejemplo, Acceso Genbank Nos X84737, S82847, X06990, J03132, SEGMUSICAMO), interleuquina-12 (ver, por ejemplo, Reiter *et al*, 1993 *Crit Rev Immunol* 13 1, y referencias citadas allí), CD134 (OX40, por ejemplo, Acceso Genbank No AJ277151), CD137 (41BB, por ejemplo, Acceso GenBank No L12964, NM_001561), CD83 (por ejemplo, Acceso Genbank Nos AF001036, AL021918), DEC-205 (por ejemplo, Acceso Genbank Nos AF011333, U19271)

Las construcciones, que incluyen proteínas de fusión de inmunoglobulina de dominio de unión, de la presente invención

comprenden, por ejemplo, un dominio de unión, tal como un polipéptido de dominio de unión que, de acuerdo con ciertas modalidades particularmente preferidas, es capaz de unirse o unir específicamente al menos un objetivo, por ejemplo, un antígeno objetivo u otro sitio de unión que está presente en una célula efectora inmune De acuerdo con la teoría no limitante, tales construcciones, que incluyen por ejemplo proteínas de fusión de inmunoglobulina con dominio de unión, pueden ventajosamente reclutar la función o funciones de célullas efectoras inmunes deseadas en un contexto terapéutico, donde es bien conocido que las células efectoras inmunes que tienen diferentes funciones inmunes especializadas se pueden identificar o distinguir una de la otra sobre la base de su expresión diferencial de una amplia variedad de antígenos de superficie celular, que incluyen muchos de los antígenos descritos aquí a los cuales las construcciones de la invención que incluyen los polipéptidos con dominio de unión pueden unirse específicamente, como se anota aquí, las células efectoras inmunes incluyen cualquier célula que es capaz de mediar directamente una actividad que es un componente de la función del sistema inmune, que incluye células que tienen tal capacidad natural o como resultado de ingeniería genética

En ciertas modalidades una célula efectora inmune comprende un receptor de superficie celular para una inmunoglobulina u

otra molécula que se une a péptido, tal como un receptor para una región constante de inmunoglobulina y que incluye una clase de receptores comunmente denominado como "receptores Fc" ("FcR") Un número de los FcR ha sido estructural y/o funcionalmente caracterizado y son bien conocidos en la técnica, incluyendo los FcR que tienen capacidades específicas de interactuar con un subconjunto restringido de isotipos de cadena pesada de inmunoglobulina, o que interactúan con los dominios Fc con afinidades variantes, y/o que se pueden expresar sobre subconjuntos restringidos de células efectoras inmunes bajo ciertas condiciones (por ejemplo, Kijimoto- Ochichai *et al* , 2002 *Cell Mol Life Sci* 59 648, Davis *et al* , 2002 *Curr Top Microbiol Immunol* 266 85, Pawankar, 2001 *Curr Opin Allerg Clin Immunol* 1 3, Radaev *et al* , 2002 *Mol Immunol* 38 1073, Wurzburg *et al* , 2002 *Mol Immunol* 38 1063, Sulica *et al* , 2001 *Int Rev Immunol* 20 371, Underhill *et al* , 2002 *Ann Rev Immunol* 20 825, Coggeshall, 2002 *Curr Dir Autoimm* 5 1, Mimura *et al* , 2001 *Adv Exp Med Biol* 495 49, Baumann *et al* , 2001 *Adv Exp Med Biol* 495 219, Santoso *et al* , 2001 *Ital Heart J* 2 811, Novak *et al* , 2001 *Curr Opin Immunol* 13 721, Fossati *et al* , 2001 *Eur J Clin Invest* 31 821)

Las células que son capaces de mediar ADCC son ejemplos preferidos de células efectoras inmunes de acuerdo con la

presente invención Otros ejemplos preferidos incluyen células Asesinas Naturales, linfocitos T que infiltran tumor (TIL), linfocitos T citotóxicos, y células granulocíticas tales como células que comprenden mecanismos de respuesta alérgica Las células efectoras inmunes incluyen así, pero no están limitadas a, céluas de orígenes hematopoyéticos que incluyen células en varias etapas de diferenciación dentro de linajes mieloide y linfoide y las cuales pueden (pero no necesitan) expresar uno o más tipos de FcR de superficie celullar funcional, tal como los linfocitos T, linfocitos B, células NK, monocitos, macrófagos, células dendríticas, neutrófilos, basófilos, eosinófilos, mastocitos, plaquetas, eritrocitos, y precursores, progenitores (por ejemplo, células madre hematopoyéticas), así como formas quiescentes, activadas, y maduras de tales células Otras células efectoras inmunes pueden incluir células de orígen no hematopoyético que sean capaces de mediar funciones inmunes, por ejemplo, células endoteliales, queratinocitos, fibroblastos, osteoclastos, células epiteliales, y otras células Las céluas efectoras inmunes también pueden incluir células que median eventos citotóxicos o citostáticos, o eventos endocíticos, fagocíticos, o pinocitóticos, o que efectúan inducción de apoptosis, o que efectúan inmunidad microbiana o neutralización de infección microbiana, o células que median reacciones alérgicas, inflamatorias, de hipersensibilidad y/o autoinmunes

Los mecanismos de respuesta alérgica son bien conocidos en la técnica e incluyen un componente específico de antígeno (por ejemplo, alergeno) tal como una inmunoglobulina (por ejemplo, IgE), así como también las células y mediadores que comprenden secuelas a encontradores de alergenos-inmunoglobulina (por ejemplo, IgE) (por ejemplo, Ott *et al* , 2000 *J Allerg Clin Immunol* 106 429, Barnes, 2000 *J Allerg Clin Immunol* 106 5, Togias, 2000 *J Allerg Clin Immunol* 105 S599, Akdis *et al* , 2000 *Int Arch Allerg Immunol* 121 261, Beach, 2000 *Occup Med* 15 455) Particularmente con relación a las construcciones, que incluyen proteínas de fusión de inmunoglobulina con dominio de unión, de la presente invención que interactúan con FcR, ciertas modalidades de la presente invención contemplan construcciones que incluyen proteínas de fusión que comprenden uno o más dominios derivados de IgE que incluye, por ejemplo, aquellos que son capaces de inducir un mecanismo de respuesta alérgica que comprende FcR específico para IgE, o porciones de estos, cuyos FcR específicos para IgE incluyen aquellos anotados anteriormente y descritos o identificados en los artículos citados Sin querer estar ligado por una teoría o mecanismo particular, y como se describió aquí, las construcciones, que incluyen proteínas de fusión, de la presente invención pueden comprender porciones de polipéptidos de dominio Fc de cadena pesada IgE, por ejemplo,

dominios CH3 y CH4 IgE nativos o trabajados por ingeniería, sean suministrados o expresados como proteínas de superficie celular (por ejemplo, con un dominio de anclaje de membrana de plasma) o solubles o proteínas de otra forma no unidas a célula (por ejemplo, sin un dominio de anclaje de membrana de plasma) Además de acuerdo con la teoria no limitante, el reclutamiento e inducción de un mecanismo de respuesta alérgico (por ejemplo, una célula efectora inmune que expresa FcR-epsilon) puede proceder como resultado de cualquiera o ambas de la presencia de un dominio IgE Fc o porción de éste como se describió aquí (por ejemplo, uno que sea capaz de disparar un mecanismo alérgico mediante reticulación FcR) y la presencia de un objetivo tal como un antígeno al cual la región de unión, por ejemplo un dominio de unión, se une o se une específicamente La presente invención por lo tanto explota la inducción de mecanismos de respuesta enérgica en contextos en lo sucesivo no apreciados, tales como tratamiento de una condición maligna o desorden de célula B, que incluye aquellos descritos o referenciados aquí

Un polipéptido con región de pivote de inmunoglobulina incluye cualquier péptido de pivote o polipéptido que ocurra naturalmente, como un péptido artificial o como resultado de ingenieria genética que se situa, por ejemplo, en un polipéptido de cadena pesada de inmunoglobulina entre los residuos de aminoácido responsables de formar uniones

disulfuro con dominio de inmunoglobulina intracadena en las regiones CH1 y CH2 Los polipéptidos con región de pivote para uso en la presente invención también pueden incluir un polipéptido con región de pivote mutada o de otra manera alterada De acuerdo con esto, por ejemplo, un polipéptido con región de pivote de inmunoglobulina se puede derivar de, o puede ser una porción o fragmento de (es decir, uno o más aminoácidos en enlace de péptido, típicamente aproximadamente 15-115 aminoácidos, preferiblemente aproximadamente 95-110, aproximadamente 80-94, aproximadamente 60-80, o aproximadamente 5-65 aminoácidos, preferiblemente aproximadamente 10-50, más preferiblemente aproximadamente 15-35, aún más preferiblemente aproximadamente 18-32, aún más preferiblemente aproximadamente 20-30, aun más preferiblemente aproximadamente 21, 22, 23, 24, 25, 26, 27, 28, o 29 aminoácidos) un región de cadena de polipéptido de inmunoglobulina clásicamente considerada por tener función de pivote, que incluye aquellos descritos aquí, pero un polipéptido con región de pivote para uso en la presente invención no necesita estar restringido y puede incluir uno o más aminoácidos situados (de acuerdo con criterios estructurales para traspasar un residuo particular a un dominio particular que puede variar, como es conocido en la técnica) en un dominio de inmunoglobulina adjunto tal como un dominio CH1 y/o un dominio CH2 en los casos de IgG, IgA e IgD (o en un dominio de inmunoglobulina adjunto tal como un

dominio CH1 y/o un dominio CH3 en el caso de IgE), o en el caso de ciertas construcciones de inmunoglobulina artificialmente trabajadas con ingeniería, un dominio de región variable de inmunoglobulina

Los polipéptidos de región de pivote de inmunoglobulina tipo silvestre incluyen cualquier región de pivote de ocurrencia natural conocida o descubierta posteriormente que esté localizada entre los dominios de región constante, CH1 y CH2, de una inmunoglobulina, por ejemplo, una inmunoglobulina humana (o entre las regiones CH1 y CH3 de ciertos tipos de inmunoglobulinas, tales como IgE) Para uso en la construcción de un tipo de región de conexión, el polipéptido de región de pivote de inmunoglobulina tipo silvestre es preferiblemente un polipéptido con región de pivote de inmunoglobulina humana, preferiblemente que comprende una región de pivote proveniente de inmunoglobulina IgG, IgA, o IgD humana (o la región CH2 de una inmunoglobulina IgE), y más preferiblemente, por ejemplo, un polipéptido con región de pivote de un isotipo IgG1 humano tipo silvestre o mutado como se describió aquí

Como es conocido en la técnica, a pesar de la tremenda diversidad total en las secuencias de aminoácido de inmunoglobulina, la estructura primaria de inmunoglobulina exhibe un alto grado de conservación de secuencia en

porciones particulares de cadenas de polipéptido de inmunoglobulina, notablemente con relación a la ocurrencia de los residuos de cisteína los cuales, en virtud de sus grupos sulfihidrilo, ofrecen el potencial para formación de enlace disulfuro con otros grupos sulfidrilo disponibles De acuerdo con esto, en el contexto de la presente invención los polipéptidos de región de pivote de inmunoglobulina tipo silvestre para uso como regiones de conexión incluyen aquellos que caracterizan uno o más residuos de cisteína altamente conservados (por ejemplo, prevalentes en una población de una manera estadísticamente significativa), y en ciertas modalidades preferidas una región de conexión puede comprender, o consiste esencialmente de, o consiste de, un polipéptido con región de pivote mutado se puede seleccionar que contenga menos del número de cisteínas de ocurrencia natural, por ejemplo, cero o uno o dos residuos de cisteína en el caso de las regiones de pivote IgG1 e IgG4, y que se deriven o sean construidos de (o utilicen) tal secuencia con región de pivote tipo silvestre

En ciertas modalidades preferidas en donde la región de conexión es un polipéptido con región de pivote y el polipéptido con región de pivote es un polipéptido con región de pivote de inmunoglobulina IgG1 humano mutado, trabajado por ingeniería o alterado de otra forma que se derive o construya de (o utilice) una secuencia con región de pivote

tipo silvestre, se nota que la secuencia de polipéptido con región de pivote IgG1 humano tipo silvestre comprende tres residuos de cisteína no adyacentes, denominados como primera cisteína de la región de pivote tipo silvestre, una segunda cisteína de la región de pivote tipo silvestre y una tercera cisteína de la región de pivote tipo silvestre, respectivamente, que proceden junto con la secuencia de región de pivote proveniente del polipéptido N-terminal hacia el C-terminal Esto se puede denominar aquí como un pivote "CCC" (o un "WTH", es decir, pivote tipo silvestre) Ejemplos de regiones de pivote mutadas o trabajadas con ingeniería incluyen aquellas sin cisteínas, que pueden ser denominadas aquí como un pivote "XXX" (o, por ejemplo, como "MH-XXX", refiriéndose a un pivote mutante o trabajado por ingeniería con tres aminoácidos u otras moléculas en lugar de cisteínas de ocurrencia natural, tales como, por ejemplo, "MH-SSS", que se refieren a un pivote mutante con tres residuos de serina en lugar de los residuos de cisteína de ocurrencia natural Se entenderá que el término "mutante" se refiere solamente al hecho de que una molécula o moléculas diferentes están presentes, o ninguna molécula, en la posición de un residuo de ocurrencia natural y no se refiere a ningún método particular por el cual ha sido llevada a cabo tal sustitución, alteración, o supresión De acuerdo con esto, en ciertas modalidades de la presente invención, la región de conexión puede ser un polipéptido con región de pivote y el

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polipéptido con región de pivote es un polipéptido con región de pivote de inmunoglobulina IgG1 humano mutado que contiene dos residuos de cisteína y en el cual la primera cisteína de la región de pivote tipo silvestre no se ha cambiado o suprimido, por ejemplo Esto se puede denominar como un pivote "MH-CXX", por ejemplo, un pivote "MH-CSC", en cuyo caso el residuo de cisteína ha sido reemplazado con un residuo de serina En ciertas otras modalidades de la presente invención el polipéptido con región de pivote de inmunoglobulina IgG1 humano mutado contiene no más de un residuo de cisteína e incluye, por ejemplo, un pivote "MH-CSS" o un pivote "MH-SSC" o un pivote "MH-CSC", y en ciertas otras modalidades el polipéptido con región de pivote de inmunoglobulina IgG1 humana mutada no contiene residuos de cisteína tales como, por ejemplo, un pivote "MH-SSS"

Las construcciones, que incluyen proteínas de fusión de inmunoglobulina con dominio de unión, de la presente invención expresamente no contemplan ninguna proteína de fusión que se describa en la Patente U S No 5,892,019 La Patente U S No 5,892,019 se refiere a una región de pivote IgG1 humana en la cual el primer residuo de cisteína de la región de pivote IgG1 ha sido cambiado o suprimido, pero retenga los dos del segundo y tercer residuo de cisteína de la región de pivote IgG1 que corresponden a las segunda y tercera cisteínas de la secuencia de región de pivote IgG1

tipo silvestre La patente establece que el primer residuo de cisteína de la región de pivote IgG1 tipo silvestre es reemplazada para evitar la interferencia por el primer residuo de cisteína con ensamblaje adecuado del polipéptido descrito allí en un dímero La patente requiere que la segunda y tercera cisteínas de la región de pivote IgG1 sea retenida para suministrar enlace disulfuro intercadena entre dos regiones constantes de cadena pesada para promover la formación de dímero de tal forma que los contenidos de la molécula tengan función efectora tal como capacidad para mediar ADCC

Como contraste y como se describió aquí, las construcciones, que incluyen las proteínas de fusión de inmunoglobulina con dominio de unión, de la presente invención, varias de las cuales son capaces de ADCC, CDC y/o fijación de complemento, por ejemplo, no están así limitadas y pueden comprender, en la parte pertinente, por ejemplo, (i) un polipéptido con región de pivote de inmunoglobulina tipo silvestre, tal como un polipéptido con región de pivote de inmunoglobulina humana tipo silvestre, por ejemplo, un polipéptido con región de pivote de inmunoglobulina IgG1 humano, (ii) un polipéptido con región de pivote de inmunoglobulina mutada o de otra forma alterada, tal como un polipéptido con región de pivote de inmunoglobulina humana mutada o de otra manera alterada, por ejemplo, un polipéptido con región de pivote de

inmunoglobulina IgG1 humana mutada o de otra manera alterada que, por ejemplo, es o se haya derivado o construido de (o que utilice) un polipéptido con región de pivote de inmunoglobulina tipo silvestre o secuencia de aminoácido que tenga tres o más residuos de cisteína, en donde el polipéptido con región de pivote de inmunoglobulina IgG1 humano mutado o de otra forma alterado contenga dos residuos de cisteína y en donde la primera cisteína de la región de pivote tipo silvestre no se muta o suprimida, (iii) un polipéptido con región de pivote de inmunoglobulina mutada o de otra forma alterada, tal como un polipéptido con región de pivote de inmunoglobulina humana mutada o de otra forma alterada, por ejemplo, un polipéptido con región de pivote de inmunoglobulina IgG1 humana mutada o de otra forma alterada que, por ejemplo, sea o se haya derivado o construido de (o que utilice) un polipéptido con región de pivote de inmunoglobulina tipo silvestre o secuencia de ácido nucleico que tenga tres o más residuos de cisteína, en donde el polipéptido con región de pivote de inmunoglobulina IgG1 humano mutado o de otra forma alterado contenga no más de un residuo de cisteína, o (iv) un polipéptido con región de pivote de inmunoglobulina mutado o de otra forma alterado, tal como un polipéptido con región de pivote de inmunoglobulina humano mutado o de otra forma alterado, por ejemplo, un polipéptido con región de pivote de inmunoglobulina IgG1 humano mutado o de otra forma alterado,

que sea o se haya derivado o construido de (o que utilice) un polipéptido con región de pivote de inmunoglobulina tipo silvestre o secuencia de ácido nucleico que tenga tres o más residuos de cisteína, en donde el polipéptido con región de pivote de inmunoglobulina IgG1 humano mutado o de otra forma alterado (por ejemplo, mediante cambio o supresión de aminoácido) no contenga residuos de cisteína La presente invención ofrece ventajas inesperadas asociadas con la retención por medio de las construcciones, que incluyen las proteínas de fusión, descritas aquí de la capacidad de mediar ADCC y/o CDC y/o fijación de complemento no obstante la capacidad para dimerizarse por vía de las uniones disulfuro de intercadena de la región de pivote IgG1 son suprimidos o comprometidos mediante la remoción o reemplazo de uno, dos o tres residuos de cisteína de la región de pivote, y aún en construcciones donde la primera cisteína de una región de pivote IgG1, por ejemplo, no se muta o alterada de otra forma o suprimida

Una región de conexión puede comprender un polipéptido con región de pivote de inmunoglobulina mutada o alterada de otra forma, que en sí mismo puede comprender una región de pivote que tenga su orígen en una inmunoglobulina de una especie, de un isotipo o clase de inmunoglobulina, o de una subclase de inmunoglobulina que es diferente de aquella de la región de cola, por ejemplo, una región de cola que comprende, o

consiste esencialmente de, o consiste de, dominios CH2 y CH3 (o dominios IgE CH3 y CH4) Por ejemplo, en ciertas modalidades de la invención, una construcción, por ejemplo, una proteína de fusión de inmunoglobulina con dominio de unión, puede comprender una región de unión tal como un polipéptido con dominio de unión que se fusiona o se conecta de otra manera a un polipéptido con región de pivote de inmunoglobulina que comprende, o que consiste esencialmente de, o consiste de, un polipéptido con región de pivote IgA humano tipo silvestre, o un polipéptido con región de pivote IgA humano mutado o alterado de otra forma que contiene cero o solamente uno o más residuos de cisteína (pero menos del número de cisteínas tipo silvestre), como se describió aquí, o un polipéptido con región de pivote IgG humano tipo silvestre, tal como un pivote IgG1, o un polipéptido con región de acción de pivote IgE humano tipo silvestre, es decir, polipéptido con región IgE CH2, o un polipéptido con región de pivote IgG humano mutado o alterado de otra forma, tal como un pivote IgG1, que es o se ha mutado o alterado de otra manera para contener cero, uno o dos residuos de cisteína en donde la primer cisteína de la región de pivote tipo silvestre no se muta o alterada o suprimida, como también se describe aquí Tal polipéptido de región de pivote se puede fusionar o conectar de otra forma a, por ejemplo, una región de cola que comprende, o consiste esencialmente de, o consiste de, un polipéptido con región CH2 de cadena

pesada de inmunoglobulina de un isotipo o clase diferente, por ejemplo una subclase IgA o una IgD o una IgG (o una región CH3 de una subclase IgE), que en ciertas modalidades preferidas será la subclase IgG1 o IgA o IgE y en ciertas otras modalidades preferidas puede ser una de las subclases IgG2, IgG3 o IgG4

Por ejemplo, y como se describe con mayor detalle aquí, en ciertas modalidades de la presente invención una región de conexión se puede seleccionar por ser un polipéptido con región de pivote de inmunoglobulina, que es o se ha derivado de una región de pivote IgA tipo silvestre que comprende de manera natural tres cisteínas, donde el polipéptido con región de pivote seleccionado está truncado o alterado de otra forma o sustituido con relación a la región de pivote completa y/o ocurrencia natural de tal forma que solamente uno o dos residuos de cisteína permanecen (por ejemplo, ID DE SEC NO 35-36) De manera similar, en ciertas otras modalidades de la invención, la construcción puede ser una proteína de fusión de inmunoglobulina con dominio de unión que comprende un polipéptido con dominio de unión que se fusiona o se conecta de otra forma a un polipéptido con región de pivote de inmunoglobulina que comprende un polipéptido con región de pivote mutado o de otra forma alterado en el cual el número de residuos de cisteína se reduce mediante sustitución o supresión de aminoácidos, por

ejemplo una región de pivote IgG1 mutada o alterada de otra forma que contiene cero, uno o dos residuos de cisteína como se describió aquí, o una región de pivote IgD que contiene cero residuos de cisteína

Un polipéptido con región de pivote mutado o alterado de otra forma se puede así derivar o construir de (o utilizar) una región de pivote de inmunoglobulina tipo silvestre que contiene uno o más residuos de cisteína En ciertas modalidades, un polipéptido con región de pivote mutada o de otra forma alterada puede contener cero o solamente un residuo de cisteína, en donde el polipéptido con región de pivote mutado o alterado de otra forma es o se ha derivado de una región de pivote de inmunoglobulina tipo silvestre que contiene, respectivamente, uno o más o dos o más residuos de cisteína En el polipéptido con región de pivote mutado o de otra manera alterado, los residuos de cisteína de la región de pivote de inmunoglobulina tipo silvestre son preferiblemente suprimidos o sustituidos con aminoácidos que son incapaces de formar una unión disulfuro En una modalidad de la invención, un polipéptido con región de pivote mutada o de otra manera alterada es o se ha derivado de un polipéptido con región de pivote tipo silvestre IgG humano, que puede incluir cualquiera de cuatro subclases de isotipo IgG humano, IgG1, IgG2, IgG3, o IgG4 En ciertas modalidades preferidas, el polipéptido con región de pivote mutado o alterado de otra

manera es o se ha derivado de (o utiliza) un polipéptido con región de pivote tipo silvestre IgA o IgD Por vía de ejemplo, un polipéptido con región de pivote mutado o de otra forma alterado que se ha derivado de un polipéptido con región de pivote tipo silvestre IgG1 o IgA humano puede comprender mutaciones, alteraciones, o supresiones en dos de los residuos de cisteína en la región de pivote de inmunoglobulina tipo silvestre, o mutaciones, alteraciones, o supresiones en todos los tres residuos de cisteína

Los residuos de cisteína que están presentes en una región de pivote de inmunoglobulina tipo silvestre y que son removidos o alterados mediante mutagénesis o cualquier otra de las técnicas de acuerdo con modalidades particularmente preferidas de la presente invención incluyen residuos de cisteína que forman, o que son capaces de formar, uniones disulfuro intercadena Sin desear estar ligado por una teoría o mecanismo de acción, la presente invención contempla que la mutación, supresión, u otra alteración de tales residuos de cisteína de la región de pivote, que se cree que están involucrados en la formación de puentes disulfuro de intercadena, reducen la capacidad de la proteína de fusión de inmunoglobulina con dominio de unión objeto de la invención a dimerizarse (o formar oligómeros superiores) por vía de la formación de unión disulfuro de intercadena, aunque sorprendentemente no suprimiendo o comprometiendo

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indeseablemente la capacidad de una proteína de fusión u otra construcción a promover ADCC, y/o CDC y/o a fijar complemento En particular, los receptores Fc que median ADCC (por ejemplo, FcRIII, CD16) exhiben baja afinidad para los dominios Fc de inmunoglobulina, soportando la idea de que la unión funcional de Fc a FcR requiere estabilización de avidez del complejo Fc-FcR en virtud de la estructura dimérica de cadenas pesadas en un anticuerpo convencional, y/o la agregación FcR y reticulación por medio de una estructura Fc de anticuerpo convencional Sonderman *et al* , 2000 *Nature* 406 267, Radaev et *al* , 2001 *J Biol Chem* 276 16469, Radaev *et al* , 2001 *J Biol Chem* 276 16478, Koolwijk *et al* , 1989 *J Immunol* 143 1656, Kato *et al* , 2000 *Immunol Today* 21 310 De esta manera, las construcciones, que incluyen por ejemplo proteínas de fusión de inmunoglobulina con dominio de unión, de la presente invención suministran las ventajas asociadas con las construcciones de cadena sencilla que incluye proteinas de fusión de inmunoglobulina con cadena sencilla aunque también reteniendo inesperadamente una o más actividades inmunológicas De manera similar, la capacidad para fijar complemento se asocia típicamente con inmunoglobulinas que son diméricas con respecto a las regiones constantes de cadena pesada tal como aquellas que comprenden Fc, aunque varias construcciones, que incluyen proteínas de fusión de inmunoglobulina con dominio de unión, de la presente invención, las cuales pueden, debido al

reemplazo o supresión de residuos de cisteína con región de pivote o debido a otras modificaciones estructurales como se describen aquí, por ejemplo, han comprometido o suprimido capacidades para formar uniones disulfuro intercadena, exhiben la capacidad inesperada de fijar complemento Adicionalmente, de acuerdo con ciertas modallidades de la presente invención en donde una construcción, que incluye, por ejemplo, una proteína de fusión de inmunoglobulina con dominio de unión, puede comprender una región de conexión y una región de cola que comprenden, o consisten esencialmente de, o consisten de, una o más de una región de acción de pivote IgE humana, es decir, un polipéptido con región IgE CH2, un polipéptido con región constante IgE CH3 humana, y un polipéptido con región constante IgE CH4 humano, las construcciones de la invención incluyen proteínas de fusión que inesperadamente retienen la actividad inmunológica de mediar ADCC y/o de inducir un mecanismo de respuesta alérgica

La selección de un polipéptido con región de pivote de inmunoglobulina como una región de conexión de acuerdo con ciertas modalidades de las construcciones de la invención objeto, tal como las proteínas de fusión de inmunoglobulina con dominio de unión, se pueden relacionar con el uso de una secuencia de polipéptido de "región de pivote alternativa", que incluye una secuencia de polipéptido que no es

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necesariamente derivada de cualquier secuencia de región de pivote de inmunoglobulina *per se* En su lugar, una región de pivote alternativa se refiere a un polipéptido con región de pivote que comprende una secuencia de aminoácido, u otra secuencia molecular, de al menos aproximadamente diez aminoácidos o moléculas consecutivas, y en ciertas modalidades al menos aproximadamente 11, 12, 13, 14, , 15, 16, 17, 18, 19, 20, 21-25, 26-30, 31-50, 51-60, 71-80, 81-90, o 91-110 aminoácidos o moléculas que están presentes en una secuencia seleccionada de cualquiera una de las ID DE SEC NO _-_, por ejemplo una secuencia de polipéptido que es o se ha derivado de una región ubicada entre los dominios de aro similares a inmunoglobulina generados por disulfuro intracadena de miembros de la superfamilia del gen inmunoglobulina tales como CD2 (por ejemplo, Acceso Genbank No NM_001767), CD4 (por ejemplo, Acceso Genbank No NM_000616), CD5 (por ejemplo, Acceso Genbank No BC027901), cD6 (por ejemplo, Acceso Genbank No NM_006725), CD7 (por ejemplo, Acceso Genbank No XM_046782, BC009293, NM_006137) o CD8 (por ejemplo, Acceso Genbank No M12828), u otros miembros de la superfamilia Ig Por vía de ejemplo no limitante, una región de pivote alternativa utilizada como región de conexión, por ejemplo, puede suministrar un sitio de glicosilación como se suministra aquí, o puede suministrar una secuencia de polipéptido derivado de gen humano para propósitos de mejorar el grado de "humanización" de una

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proteína de fusión, o puede comprender, o consiste esencialmente de, o consiste de, una secuencia de aminoácidos que elimina o reduce la capacidad de una construcción de la invención, tal como una proteína de fusión, a formar multímeros u oligómeros o agregados o similares Ciertas secuencias de polipéptido con región de pivote alternativa, que incluyen aquellas descritas aquí, se pueden derivar o construir de (o utilizar) las secuencias de polipéptidos de los miembros de la superfamilia de gen de inmunoglobulina que no son de hecho inmunoglobulinas *per se* Por ejemplo y de acuerdo con la teoría no limitante, ciertas secuencias de polipéptido que están situadas entre el dominio de aro de inmunoglobulina generado por disulfuro intracadena del miembro de la superfamilia del gen de inmunoglobulina se puede utilizar en todo o en parte como los polipéptidos de la región de pivote alternativa como se suministra aquí, o pueden ser adicionalmente modificados para tal uso

Como se anotó anteriormente, las construcciones de la invención, que incluyen proteínas de fusión de inmunoglobulina con dominio de unión, se cree, de acuerdo con la teoría no limitante, están comprometidas con la capacidad para dimerizarse por vía de la formación de unión disulfuro de intercadena, y adicinalmente de acuerdo con la teoría, esta propiedad es una consecuencia, en todo o en parte, de una reducción en el número de residuos de cisteína que están

presentes en el polipéptido con región de pivote de inmunoglobulina seleccionado para inclusión en la construcción de la construcción, tal como una construcción de proteína de fusión La determinación de la capacidad relativa de un polipéptido a dimerizarse es del conocimiento de la técnica relevante, donde cualquier numero de metodologías establecidas se puede aplicar para detectar la dimerización de proteína (ver, por ejemplo, Scopes, *Protein Purification Principles and Practice*, 1987 Apringer-Verlag, New York) Por ejemplo, las técnicas de separación bioquímica para resolver proteínas sobre la base del tamaño molecular (por ejemplo, electrofóresis de gel, cromatografía de filtración de gel, ultracentrifugación analítica, etc), y/o la comparación de las propiedades físicoquímicas de las proteínas antes y después de la introducción del sulfidrilo-activo (por ejemplo, yodoacetamida, N-etilmaleimida) o agentes reductores de disulfuro (por ejemplo, 2-mercaptoetanol, ditiotreitol), u otras metodologias equivalentes, pueden ser todas empleadas para determinar el grado de dimerización u oligomerización de polipéptido, y para determinar la contribución posible de las uniones disulfuro a tales estructuras cuaternarias potenciales En ciertas modalidades, la invención se relaciona con una construcción, por ejemplo una proteína de fusión de inmunoglobulina con dominio de unión, que exhibe una capacidad reducida (es decir, de una manera estadísticamente significativa con relación a un control

derivado de IgG apropiado, por ejemplo) a dimerizarse, con relación a un polipéptido con región de pivote G de inmunoglobulina humana tipo silvestre como se suministra aquí Aquellos familiarizados con la técnica serán capaces de determinar fácilmente si una fusión particular de proteína despliega tal capacidad reducida para dimerizarse

Las composiciones y métodos para la prepaparación de las proteínas de fusión de inmunoglobulina, por ejemplo, son bien conocidas en la técnica Ver, por ejemplo, Patente U S No 5,892,019, que reporta proteínas recombinantes que son el producto de un polinucleótido sencillo codificante pero que no son construcciones, incluyendo proteínas de fusión de inmunoglobulina con dominio de unión, de acuerdo con la presente invención

Como contraste, por ejemplo, en una proteína de fusión de inmunoglobulina de la invención que está destinada para uso en humanos, cualquiera de las regiones constantes Ig incluidas será típicamente de orígen de secuencia humana, o humanizada, para minimizar una respuesta inmune anti-humana potencial y suministrar funciones efectoras apropiadas y/o deseadas La manipulación de las secuencias que codifican regiones constantes de anticuerpo se referencian en la publicación PCT de Morrison y Oi, WO 89/07142 En modalidades particulares preferidas, una región de cola se prepara de una

región constante de cadena pesada de inmunoglobulina en la cual el dominio CH1 es o se ha suprimido (las regiones CH1 y CH2 en el caso de IgE) y el extremo carboxilo del dominio de unión, o donde el dominio de unión comprende dos polipéptidos con región variable de inmunoglobulina, la segunda región variable (es decir, la más próxima al terminal C) se une al terminal amino de CH2 a través de una o más regiones de conexión, tal como una región de pivote o alterada Un diagrama esquemático que describe las estructuras de dos proteínas de fusión de inmunoglobulina con dominio de unión de ejemplo se muestra en la FIG 11 En modalidades particularmente preferidas no están presentes uniones disulfuro de intercadena, y en otras modalidades un número estricto de uniones disulfuro de intercadena pueden estar presentes con relación al número de tales uniones que estarían presentes si los polipéptidos con región de pivote tipo silvestre estuvieran en su lugar presentes En otras modalidades una construcción de la invención, tal como por ejemplo, una proteína de fusión, comprende, o consiste esencialmente de, o consiste de, un polipéptido con región de pivote mutado o alterado de otra manera que exhibe una capacidad reducida a dimerizarse, con relación a un polipéptido con región de pivote IgG humano tipo silvestre Así, una molécula de polinucleótido aislada que codifica para tal construcción de cadena sencilla, tal como una proteína de fusión de inmunoglobulina, tiene una región de unión, por

ejemplo, un dominio que suministra afinidad y selectividad de unió específica o deseada de otra forma para un objetivo, tal como un antígeno objetivo

La invención también contempla por ejemplo, en ciertas modalidades, construcciones que incluyen proteínas de fusión de inmunoglobulina con dominio de unión que comprenden secuencias de polipéptido fusionadas o de otra manera conectadas o porciones de estas derivadas o preparadas de una pluralidad de fuentes genéticas, por ejemplo, de acuerdo con paradigmas moleculares "intercambio de dominio" Aquellos que tienen familiaridad con la técnica apreciarán que la selección de tales secuencias de polipéptido para montaje en una construcción, tal como la proteína de fusión de inmunoglobulina con dominio de unión, por ejemplo, puede involucrar la determinación de las funciones apropiadas de cada secuencia de polipéptido componente, por ejemplo, basada en propiedades estructurales y/o funcionales de cada una de tales secuencias (ver, por ejemplo, Carayannopoulos *et al* , 1996 *J Exp Med* 183 1579, Harlow *et al* , Eds , *Antibodies A Laboratory Manual*, Cold Spring Harbor Laboratory, Cold Spring Harbor (1988)) Las secuencias de polipéptido componente de las cuales la construcción, tal como la proteína de fusión, está comprendida o preparada puede por lo tanto comprender dominio de unión intacto o de longitud completa, inmunoglobulina, ligador y/o secuencias de

polipéptido con dominio de anclaje de membrana de plasma, o versiones truncadas o variantes de estas tal como aquellas suministradas aquí De acuerdo con estas y modalidades relacionadas de la invención, cualquiera dos o más de los polipéptidos componentes candidatos de los cuales las construcciones de la invención objeto, por ejemplo, las proteínas de fusión, pueden estar comprendidas se derivarán o prepararán de fuentes independientes, tales como de secuencias de inmunoglobulina de diferente alotipo, isotipo, subclase, clase, o especie de orígen (por ejemplo, xenotipo) Así, en un ejemplo no limitante, un polipéptido con dominio de unión (o su polipéptido constituyente tal como uno o más polipéptidos con región variable y/o un polipéptido ligador), un polipéptido con región de pivote, polipéptidos con región constante CH2 y CH3 de cadena pesada de inmunoglobulina y opcionalmente un polipéptido con región constante CH4 de cadena pesada de inmunoglobulina como se puede obtener de la cadena pesada IgM o IgE, y un polipéptido con dominio de anclaje de membrana de plasma pueden todos ser separadamente obtenidos de diferentes fuentes genéticas y trabajados con ingeniería en una proteína quimérica o de fusión utilizando técnicas bien conocidas de acuerdo con la metodologías descritas aqui, por ejemplo

De acuerdo con esto, una construcción de la invención, por ejemplo una proteína de fusión de inmunoglobulina con dominio

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de unión de acuerdo con ciertas modalidades de la presente invención, pueden también por lo tanto comprender en la parte pertinente un polipéptido con región constante CH3 de cadena pesada de inmunoglobulina que es un polipéptido con región constante IgA CH3 tipo silvestre, o alternativamente, que es un polipéptido con región constante IgA CH3 mutado o alterado de otra manera o sustituido o truncado que es capaz de asociarse con una cadena J, o que no se asociará en un grado indeseado con una cadena J, preferiblemente los polipéptidos de región constante IgA CH3 utilizados en una porción de la región de cola de una construcción son de origen humano o son humanizados Por vía de breve antecedente, las moléculas IgA son conocidas por ser liberadas en los fluidos secretores por un mecanismo que involucra la asociación de IgA en los polímeros enlazados a disulfuro (por ejemplo, dímeros) por via de un polipéptido de cadena J (por ejemplo, Acceso Genbank Nos XM_059628, M12378, M12759, Johansen *et al* , 1999 *Eur J Immunol* 29 1701) y la interacción del complejo así formado con otra proteína que actua como un receptor para inmunoglobulina polimérica, y la cuales conocida como componente secretor de transmembrana (SC, Johansen *et al* , 2000 *Sc J Immunol* 52 240, ver también, por ejemplo, Sorensen *et al* , 2000 *Int Immunol* 12 19, Yoo *et al* , 1999 *J Biol Chem* 274 33771, Yoo *et al* , 2002 *J Immunol Meth* 261 1, Corthesy, 2002 *Trends Biotechnol* 20 65, Symersky *et al* , 2000 *Mol Immunol* 37 133, Crottet *et al* , 1999

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Biochem J 341 299) La formación de unión de disulfuro intercadena entre los dominios IgA Fc y la cadena J es mediada a través de un penultimo residuo de cisteína en una extensión C-terminal del aminoácido 18 que forma parte de un polipéptido de dominio CH3 de región constante de cadena pesada IgA (Yoo *et al* , 1999, Sorensen *et al* , 2000) Ciertas modalidades de las construcciones de la invención objeto, que incluyen por ejemplo proteínas de fusión, contemplan por lo tanto la inclusión de la secuencia de polipéptido de región constante de cadena pesada IgA tipo silvestre, que es capaz de asociarse con la cadena J Ciertas otras modalidades de la invención, sin embargo, contemplan proteínas de fusión que comprenden un polipéptido con región constante IgA CH3 mutado o alterado de otra forma, sustituido o truncado que es incapaz de asociarse con una cadena J De acuerdo con tales modalidades, por ejemplo, dos o más residuos del C-terminal de un polipéptido de región constante IgA CH3 tal como un polipéptido de región constante IgA CH3 humano se puede suprimir para producir un polipéptido de región constante CH3 truncado como se suministra aquí En las modalidades preferidas como se describe con mayor detalle aquí, un polipéptido con región constante IgA CH3 humano mutado que es incapaz de asociarse con una cadena J comprende tal supresión C-terminal de cualesquiera cuatro o 18 aminoácidos Sin embargo, la invención no necesita estar así limitada, de tal forma que el polipéptido con región constante IgA CH3 mutado

puede comprender una supresión de aproximadamente 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21-25, 26-30 o más aminoácidos, tan largas como la construcción, por ejemplo, la proteína de fusión, es capaz de unir específicamente un antígeno y de al menos una actividad inmunológica como se suministra aquí Alternativamente, la invención también contempla construcciones, por ejemplo, proteínas de fusión, que tienen una región de cola que comprende un polipéptido de región constante IgA CH3 mutado que es incapaz de asociarse con una cadena J en virtud del reemplazo de la penultima cisteína, o mediante modificación química del residuo aminoácido, de una manera que evita, o inhibe el nivel indeseado de, uniones disulfuro intercadena o formación de multímeros Los métodos para determinar si una construcción, por ejemplo, una proteína de fusión pueden asociarse con una cadena J serán conocidos por aquellos que tienen familiaridad con la técnica y son descritos o referenciados aquí

Como también se describió aquí de acuerdo con los procedimientos conocidos en la técnica, la construcción, por ejemplo, una proteína de fusión, puede además ser ensayada rutinariamente para actividad inmunológica,por ejemplo, en ensayos ADCC o CDC Como un ejemplo ilustrativo, una construcción, por ejemplo una proteína de fusión, de acuerdo con tal modalidad puede comprender un polipéptido con dominio

de unión derivado o construido de (o ue utiliza) una secuencia de polipéptido con región variable de cadena pesada humano nativo o trabajado con ingeniería, una secuencia de polipéptido con región de pivote de inmunoglobulina derivado de IgA humano nativo o trabajado con ingeniería, o una secuencia de polipéptido con región constante CH2 de cadena pesada de inmunoglobulina IgG1 humano nativo o trabajado con ingeniería, o una secuencia de polipéptido con región constante CH3 de cadena pesada de inmunoglobulina igG2 humano nativo o trabajado con ingeniería, y opcionalmente una secuencia de polipéptido con región constante CH4 de cadena pesada de inmunoglobulina IgE humano nativo o trabajado con ingeniería y/o una secuencia de polipéptido con dominio de anclaje de membrana de plasma TNF-α receptor tipo 1 (TNFR1) humano nativo o trabajado con ingeniería que comprende un polipéptido de cola citoplasmático que es capaz de señal apoptótica o de promover de otra manera la apoptosis La invención por lo tanto contempla estas y otras modalidades de acuerdo con la presente invención en la cual dos o más secuencias de polipéptido que están presentes en una construcción, por ejemplo, una proteína de fusión, tienen orígenes genéticos independientes

Como se anotó anteriormente, en ciertas modalidades la construcción, por ejemplo una proteína de fusión de inmunoglobulina con proteína de unión, comprenden al menos un

polipéptido con región variable de inmunoglobulina nativo o trabajado con ingeniería, que puede ser un polipéptido con región variable de cadena liviana nativo o trabajado con ingeniería o de cadena pesada nativo o trabajado con ingeniería, y en ciertas modalidades la proteína de fusión comprende al menos una de tales regiones V de cadena liviana nativa o trbajada con ingeniería y una de tales regiones V de cadena pesada nativa o trabajada con ingeniería y al menos un péptido ligador que está fusionado o conectado de otra manera a cada una de las regiones V nativas o trabajadas con ingeniería La construcción de tales dominios de unión, por ejemplo dominios Fv de cadena sencilla, se conoce en la técnica y se describe con mayor detalle en los ejemplos de adelante, y se ha descrito, por ejemplo, en varios documentos citados aquí, la selección y montaje de las regiones variables de cadena sencilla y de polipéptidos ligadores que se pueden fusionar o conectar de otra forma a cada una de las regiones V derivadas de cadena pesada y una región V derivada de cadena liviana (por ejemplo, para generar una región de unión, tal como un dominio de unión que comprende un polipéptido Fv de cadena sencilla) también es conocido en la técnica y se describe aquí Ver, por ejemplo, Patente U S No 5,869,620, 4,704,692, y 4,946,778 En ciertas modalidades toda o una porción o porciones de la secuencia de inmunoglobulina que se deriva de una fuente no humana puede ser "humanizada" de acuerdo con los procedimiento reconocidos

para generar anticuerpos humanizados, es decir, secuencias de inmunoglobulina en las cuales las secuencias Ig humanas son introducidas para reducir el grado al cual el sistema inmune humano percibiría tales proteínas como extrañas (ver, por ejemplo, Patentes U S Nos 5,693,762, 5,585,089, 4,816,567, 5,225539, 5,530,101, y documentos citados aquí)

Las construcciones de la invención, que incluyen proteínas de fusión de inmunoglobulina con dominio de unión, como se describe aquí pueden, de acuerdo con ciertas modalidades, comprender deseablemente sitios para glicosilación, por ejemplo, unión covalente de porciones carbohidrato tal como, por ejemplo, monosacxáridos u oligosacáridos La incorporación de secuencias de aminoácido que suministran sustratos para glicosilación de polipéptido está dentro del alcance de la técnica relevante, que incluye, por ejemplo, el uso de metodologías de ingeniería genética o de ingeniería de proteínas para obtener una secuencia de polipéptido que contiene, por ejemplo, el sitio clásico Asn-X-Ser/Thr para glicosilación ligada a N-(asparagina), o una secuencia que contiene residuos Ser o Thr que son sustratos adecuados para glicosilación ligada a O, o secuencias susceptibles de C-manosilación, modificación de glipiación/ glicosilfosfatidilinositol, o fosfoglicación, todas las cuales pueden ser idénticas de acuerdo con los criterios establecidos en la técnica (por ejemplo, Spiro, 2002

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Glybiology 12 43R) Sin desear estar ligado con ninguna teoría o mecanismo particular, las construcciones glicosiladas tales como las proteínas de fusión que tienen secuencias de aminoácido particulares pueden poseer beneficamente atributos asociados con una o más de solubilidad mejorada, estabilidad mejorada en solución, estabilidad fisiológica mejorada, biodisponibilidad mejorada que incluye biodistribución *in vivo*, y resistencia a proteasa superior, todas de manera estadísticamente significativa, con relación a las construcciones, que incluyen proteínas de fusión, que tienen secuencias de aminoácido iguales o altamente similares pero que les falta porciones de glicosilo En ciertas modalidades preferidas las construcciones de la invención objeto, tales como las construcciones de la proteína de fusión, pueden comprender un sitio de glicosilación que está presente en un ligador como se suministra aquí, y en ciertas otras modalidades preferidas la construcción de la invención objeto, por ejemplo, una proteína de fusión, comprende un sitio de glicosilación que está presente en una región de conexión, tal como una secuencia de polipéptido de región de pivote como se suministra aquí

En ciertas modalidades preferidas de la presente invención, tales como aquellas útiles para aplicaciones de terapia génica o en sistemas o ensayos de despliegue, tal como

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ensayos de selección (que incluyen sistemas de despliegue de librería y ensayos de selección de librería), la construcción, por ejemplo, una proteína de fusión de inmunoglobulina con dominio de unión, es una proteína o glicoproteína que es capaz de ser expresada por una célula huésped de tal forma que esta localiza la superficie celular Las construcciones, tales como las proteínas de fusión de inmunoglobulina con dominio de unión, que localizan la superficie celular pueden hacerlo así en virtud de tener características estructurales presentes naturalmente o artificialmente introducidas que dirigen la proteína de fusión a la superficie de la célula (por ejemplo, Nelson *et al* 2001 *Trends Cell Biol* 11 483, Ammon *et al* , 2002 *Arch Physiol Biochem* 110 137, Kasai *et al* , 2001 *J Cell Sci* 114 3115, Watson *et al* , 2001 *Am J Physiol Cell Physiol* 281 C215, Chatterjee *et al* , 200 *J Biol Chem* 275 24013) que incluye por vía de ilustración y no de limitación, secuencias de señal secretora, secuencias líderes, polipéptidos de dominio de anclaje de membrana de plasma y dominios de transmembrana tales como dominios de transmembrana hidrófoba (por ejemplo, Heuck *et al* , 2002 *Cell Biochem Biophys* 36 89, Sadlish *et al* , 2002 *Biochem J* 364 777, Phoenix *et al* , 2002 *Mol Membr Biol* 19 1, Minke *et al* , 2002 *Physiol Rev* 82 429) o sitios de unión de glicosilfosfatidilinositol (sitios de "glipiación", por ejemplo, Chatterjee *et al* , 2001 *Cell Mol Life Sci* 58

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1969, Hooper, 2001 *Proteomics* 1 748, Spiro, 2002 *Glycobiol* 12 43R), los dominios de unión de receptores de superficie celular, dominios de unión de matriz extracelular, o cualquier otra característica estructural que origine al menos una porción deseada de la población de proteína de fusión para localizar en todo o en parte, a la superficie celular Particularmente preferidas son las construcciones de proteína de fusión que comprenden un dominio de anclaje de membrana de plasma que incluye un dominio de polipéptido de transmembrana, típicamente que comprende un dominio de extensión de membrana que incluye una región hidrófoba capaz de favorecer energéticamente la interacción con las colas acilo graso de fosfolípido que forman el interior de la bicapa de membrana de plasma Tales características son conocidas por aquellos expertos en la técnica, quienes estarán adicionalmente familiarizados con los métodos para introducir secuencias de ácido nucleico que codifican estas características en las construcciones de expresión objeto mediante ingeniería genética, y con ensayos de rutina de tales construcciones para verificar la localización de la superficie celular del producto

De acuerdo con ciertas modalidades adicionales, un polipéptido de dominio de anclaje de membrana de plasma comprende tal polipéptido de dominio de transmembrana y también comprende un polipéptido de cola citoplasmático, que

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se refiere a una región o porción de la secuencia de polipéptido que entra en contacto con cara citoplasmática de la membrana de plasma y/o está en contacto con el citosol u otros componentes citoplasmáticos Un gran número de polipéptidos de cola citoplasmáticos son conocidos porque comprenden las porciones intracelulares de las proteínas de transmembrana de membrana de plasma, y las funciones discretas han sido identificadas por muchos de tales polipéptidos, que incluyen transmisión de señal biológica (por ejemplo, activación o inhibición de proteína quinasas, proteína fosfatasas, proteína G, nucleótidos cíclicos y otros segundos mensajeros, canales de ion, sendas secretoras), liberación de mediador biológicamente activo, asociación estable o dinámica con uno o más componentes citoesqueleto, diferenciación celular, activación celular, mitogénesis, citostásis, apoptosis y similares (por ejemplo, Maher *et al* , 2002 *Immunol Cell Biol* 80 131, El Far *et al* , 2002 *Biochem J* 365 329, Teng *et al* , 2002 *Genome Biol* 2REVIEWS 3012, Simons *et al* , 2001 *Cell Signal* 13 855, Furie *et al* , 2001 *Thromb Haemost* 86 214, Gaffen, 2001 *Cytokine* 14 63, Dittel, 2000 *Arch Immunol Ther Exp* (Warsz) 48 381, Parnes *et al* , 2000 *Immunol Rev* 176 75, Moretta *et al* , 2000 *Semin Immunol* 12 129, Ben Ze'ev, 1999 *Ann N Y Acad Sci* 886 37, Marsters *et al* , *Recent Programa Horm Res* 54 225)

La Figura 70 ilustra la unión, por ejemplo, de proteínas de fusión solubles FcRIII (CD16) conjugadas con fluoresceína a construcciones de dominio de unión 2H7 scFv que están unidas a CD20, expresadas por células, células CHO en este ejemplo La unión de CD16 a una construcción de la invención, por ejemplo, una construcción de dominio de unión scFv, suministra un ejemplo de una herramienta de selección que se puede utilizar para detectar y/o cuantificar cambios en la unión CD16 a construcciones alteradas de la invención, que incluyen construcciones de dominio de unión scFv, que contienen mutaciones específicas de objetivo o sitio, sustituciones, supresiones u otras alteraciones Los cambios en las propiedades de unión CD16 se pueden reflejar, por ejemplo, en cambios en la unión de cualquier proteína de afinidad alta CD16 (158V) o proteína de afinidad baja CD16 (158F) o ambas

Una representación esquemática de un ejemplo de tales procesos de selección se diagrama en el segundo dibujo de la Figura 70, en la cual las construcciones con dominio de unión scFv están desplegadas sobre la superficie celular de ñas células de mamífero Las moléculas con dominio de unión scFv en este ejemplo están desplegadas sobre la superficie celular a través de una molécula que sirve como un anclaje de dominio de transmembrana Estas moléculas pueden representar, por ejemplo, una construcción de dominio de unión scFv sencilla o

se pueden introducir en una población de células de mamífero como una librería de tales moléculas Las células transfectadas con propiedades de unión alteradas pueden entonces ser, por ejemplo, separadas, clasificadas o aisladas de otra forma de otras células al alterar la exigencia de las condiciones de selección o utilizar proteínas de fusión CD16 como sonda de unión Las células que expresan las moléculas scFv-Ig con unión alterada a cualquier alelo de alta afinidad CD16 (158V) o alelo de baja afinidad CD16 (158F) o ambos, por ejemplo, se apuede aislar

Este sistema de despliegue puede ser utillizado, por ejemplo, para crear una librería de construcciones de la invención con regiones de cola mutadas o alteradas de otra forma con tramos cortos de secuencia CH2 rremplazados con oligonucleótidos aleatorizados o, por ejemplo, aleatorización de residuo sencillo con todas las posibles sustituciones de aminoácido, natural o no natural, que incluye aminoácidos sintéticos Una vez tal librería sea construida, esta puede ser transfectada en células apropiadas, por ejemplo, células COS, mediante métodos conocidos en la técnica Los transfectantes pueden entonces estar unidos a, por ejemplo, construcciones CD16 marcadas, y separadas o clasificadas con base en sus propiedades de unión relativas o deseadas a múltiples alelotipos/isoformas Las células deseadas pueden ser cosechadas, y el ADN, por ejemplo, ADN de plásmido, aislado y

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luego transformado en, por ejemplo, bacterias Este proceso se puede repetir iterativamente múltiples veces hasta que los clones sencillos deseados son aislados de las células huésped de mamíferos Ver Seed B y Aruffo A, *Pro Nat'l Acad Sci USA* 1987 84 3365-3369, Aruffo A y Seed B, *Pro Nat'l Acad Sci USA* 1987 84 8573-8577

Uno de tales usos de este tipo de sistema de selección, por ejemplo, es para la identificación y/o aislamiento de construcciones de la invención que tienen regiones de cola, o regiones de cola, que se unen igualmente bien a ambos los alelos de alta y baja afinidad de CD16 con la meta de mejorar las funciones efectoras mediadas por las construcciones de dominio de unión scFv en múltiples subpoblaciones de pacientes Las construcciones de la invención que tienen regiones de cola, o regiones de cola con propiedades de unión alterada a otros receptores Fc también se pueden seleccionar utilizando un sistema de despliegue, por ejemplo, el sistema de despliegue descrito Otros sistemas de despliegue que no glicosilan proteínas, por ejemplo, aquellos que utilizan bacteriófago o levadura, no son generalmente deseados para la selección de construcciones de la invención que tienen regiones de cola basadas en Ig, o regiones de cola basadas en Ig, con propiedades de unión FcR alteradas La mayoría de los sistemas no mamífero, por ejemplo, no glicosilan proteínas

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La expresión de las construcciones de la invención, por ejemplo, las construcciones de dominio de unión scFv, expresadas sobre la superficie de una célula de mamífero mediante la incorporación de una molécula apropiada en la construcción, por ejemplo, la incorporación de un dominio de transmembrana o de una señal de anclaje GPI, también tienen utilidad en el sistema de despliegue que son útiles, por ejemplo, para la selección de construcciones de la invención, por ejemplo, moléculas de dominio de unión scFv alteradas que serán producidas a niveles superiores u otros deseados En una de tales modalidades, las células que son útiles en la producción de proteínas glicosiladas, por ejemplo, células de mamífero tales como células COS, son transfectads con una librería de construcciones de dominio de unión scFv en un plásmido que dirige su expresión a la superficie de la célula Las células, tales como las células COS, que expresan el nivel más alto u otro deseado de las moléculas de dominio de unión scFv se seleccionan mediante técnicas conocidas en el arte (por ejemplo separación, clasificación célula esteril, separación de glóbulo magnético, etc), y ADN, por ejemplo, ADN de plásmido, es aislado para transformación en otras células, por ejemplo, bacteria Después de una o más rondas de clones sencillos de selección son aislados que codifican moléculas de dominio de unión scFv capaces de un nivel más alto u otro deseado de expresión Los clones aislados pueden entonces ser alterados para remover el

anclaje de membrana y expresados en un sistema de célula apropiado, por ejemplo, un sistema de célula de mamífero, en donde las construcciones de dominio de unión scFv serán producidas, por ejemplo, mediante secreción en el fluido de cultivo a niveles deseados Sin desear estar unido por ningún mecanismo o teoría particular, se cree que resultan del requisito común de las glicoproteínas secretadas y las glicoproteínas de superficie celular para un péptido de señal y procesar a través de golgi para expresión Así, la selección de una molécula que muestra niveles de expresión mejorados sobre una superficie celular también dará como resultado la identificación de una molécula que tiene una mejora en los niveles de proteína secretada

Estos sistemas de despliegue que utilizan una construcción de la invención también se pueden utilizar para seleccionar y/o identificar y/o aislar variantes de afinidad del dominio de unión con una construcción

Particularmente preferidos son tales despliegues y/o sistemas de selección, por ejemplo, que incluyen o utilizan construcciones que incluyen (1) una secuencia de polipéptido con región variable de inmunoglobulina, que incluye V_H y/o V_L nativo o trabajado con ingeniería y/o secuencias de (sFv) de región variable de cadena sencilla, y que incluyen, por ejemplo, una mutación, alteración o supresión en un

aminoácido en un sitio o sitios que corresponden a una o más de las posiciones de aminoácido 9, 10, 11, 12, 108, 110, 111, y 112, en una secuencia de región V_H (que incluye en la secuencia de región V_H dentro de un scFv u otra construcción), y/o (2) una secuencia de polipéptido de región variable de inmunoglobulina, que incluye V_H y/o V_L nativo o trabajado con ingeniería y/o secuencias de región variable de cadena sencilla (sFv), y que incluyen, por ejemplo, una mutación, alteración o supresión en un sitio o sitios que corresponden a una o más de las posiciones de aminoácido 12, 80, 81, 82, 83, 105, 106, 107, y 108 en una secuencia de región variable de cadena liviana (que incluye en una secuencia de región V_L dentro del scFv u otra construcción) Especialmente preferidos son tales sistemas de despliegue y/o selección que incluyen o utilizan construcciones que incluyen una secuencia V_H trabajada por ingeniería (sea o no en asocio con uno o más de otras secuencias, que incluyen inmunoglobulina derivada u otras secuencias contenidas, por ejemplo, dentro de una construcción que contiene sFv o scFv), que incluye una mutación, alteración o supresión de un aminoácido en un sitio o sitios que corresponden a una posición de aminoácido 11 El aminoácido V_H11, si es sustituido, puede ser sustituido con otro aminoácido como se describió aquí, o por otra molécula según se desee

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En el contexto de otros métodos de utilizar construcciones de la invención, que incluyen proteínas de fusión de inmunoglobulina con dominio de unión, para el tratamiento de una condición maligna o de un desorden o desórdenes de célula B como se suministran aquí, incluyen, por ejemplo, mediante uno o más del número de métodos de terapia génica y técnicas de suministro de construcción relacionada, la presente invención también contempla ciertas modalidades en donde una construcción, por ejemplo, una proteina de fusión de inmunoglobulina con dominio de unión que comprende un polipéptido con dominio de anclaje de membrana de plasma se expresa (o es capaz de expresión) en una superficie celular y puede adicionalmente comprender un polipéptido de cola citoplasmático que comprende una secuencia de polipéptido señalando apoptosis Un número de secuencias de polipéptido señalando apoptosis son conocidas en la técnica, según se revisó, por ejemplo, en *When Cells Die A Comprehensive Evaluation of Apoptosis and Programmed Cell Death* (R A Lockshin *et al* , Eds , 1998 John Wiley & Sons, New York, ver también, por ejemplo, Green *et al* , 1998 *Science* 281 1309 y referencias citadas allí, Ferreira *et al* , 2002 *Clin Canc Res* 8 2024, Gurumurthy *et al* , 2001 *Cancer Metastas Rev* 20 225, Kanduc *et al* , 2002 *Int J Oncol* 21 165) Típicamente una secuencia de polipéptido señalando apoptosis comprende toda o una porción de, o se deriva o se construye de, un polipéptido con dominio de muerte receptor, por

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ejemplo, FADD (por ejemplo, Acceso Genbank Nos U24231, U43184, AF009616, AF009617, NM_012115), TRADD (por ejemplo, Acceso Genbank No NM003789), RAIDD (por ejemplo, Acceso Genbank No U87229), CD95 (FAS/Apo-1 por ejemplo, Acceso Genbank Nos X89101, NM003824, AF344850, AF344856), TNF-α-receptor-1 (TNFR1, por ejemplo, Acceso Genbank Nos S63368, AF040257), DR5 (por ejemplo, Acceso Genbank No AF020501, AF016268, AF012535), un dominio ITIM (por ejemplo, Acceso Genbank Nos AF081675, BC015731, N1VI 006840, NM006844, NM-006847, XM_017977, ver, por ejemplo, Billadeau *et al* , 2002 *J Clin Invest* 109 161), un dominio ITAM (por ejemplo, Acceso Genbank Nos NM005843, NM003473, BC030586, ver, por ejemplo, Billadeau *et al* , 2002), u otros polipéptidos de dominio de muerte receptores asociados a apoptosis conocidos en la técnica, por ejemplo, TNFR2 (por ejemplo, Acceso Genbank No L49431, L49432), caspasa/procaspasa-3 (por ejemplo, Acceso Genbank No XM_54686), caspasa/procaspasa-8 (por ejemplo, AF380342, NM_004208, NM 001228, NM-033355, NM 033356, NM-033357, NM 033358), caspasa/procaspasa-2 (por ejemplo, Acceso Genbank No AF314174, AF314175), etc

Las células en muestras biológicas que se sospecha que sufren de apoptosis se pueden examinar para cambios morfológicos, permeabilidad u otros cambios que son indicativos de un estado apoptótico Por ejemplo por vía de ilustración y no de limitación, la apoptosis en muchos tipos de células puede

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originar apariencia morfológica alterada tales como vejigas de membrana de plasma, cambios de forma de célula, pérdida de propiedades de adición de sustrato u otros morfológicos que pueden ser fácilmente detectados por una persona que tiene habilidad ordinaria en la técnica, por ejemplo al utilizar microscopía de luz Como otro ejemplo, las células que sufren apoptosis pueden exhibir fragmentación y desintegración de cromosomas, que pueden ser evidentes mediante microscopía y/o a través del uso de tintes específicos de cromatina o específicos de ADN que son conocidos en la técnica, incluyendo tintes fluorescentes Tales células pueden también exhibir propiedades de permeabilidad de membrana de plasma alterada o pueden ser fácilmente detectadas a través del uso de tintes vitales (por ejemplo, yoduro de propidio, azul trypan) o mediante la detección de fuga de lactato deshidrogenada en el medio extracelular Estos y otros medios para detectar células apoptóticas mediante criterio morfológico, permeabilidad de membrana de plasma alterada, y cambios relacionados serán evidentes para aquellos familiarizados con la técnica

En otra modalidad de la invención en donde una construcción, tal como la proteína de fusión de inmunoglobulina con dominio de unión, que se expresa en una superficie celular comprende un dominio de anclaje de membrana de plasma que tiene un dominio de transmembrana y una cola citoplasmática que

comprende un polipéptido de señalización de apoptosis, células en una muestra sencilla se pueden ensayar para transposición de fosfatidilserina (PS) de membrana de célula del interior a la hojuela exterior de la membrana de plasma, que se puede detectar, por ejemplo, al medir la hojuela exterior que se une a la proteína específica PS annexina Martin *et al* , *J Exp Med* 182 1545, 1995, Fadok *et al* , *J Immunol* 148 2207, 1992 En aún otras modalidades relacionadas de la invención, que incluyen las modalidades en donde una construcción, tal como una proteína de fusión de inmunoglobulina con dominio de unión, se expresan en la superficie celular y comprenden un dominio de anclaje de membrana de plasma que tiene un polipéptido que señala apoptosis y también que incluye modalidades en donde la construcción, tal como una proteína de fusión de inmunoglobulina con dominio de unión, es una proteína soluble a la que le falta un dominio de anclaje de membrana y que es capaz de inducir apoptosis, una respuesta celular a un apoptogen se determina mediante un ensayo para inducción de actividad de proteasa específica en cualquier miembro de una familia de proteasas activadas por apoptosis conocidas como caspasas (ver, por ejemplo, Green *et al* , 1998 *Science* 281 1309) Aquellos que son expertos en la técnica están familiarizados fácilmente con los métodos para determinar la actividad caspasa, por ejemplo determinar clivaje mediado por caspasa de sustratos de proteína específicamente reconocidos

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Estos sustratos pueden incluir, por ejemplo, poli-(ADP-ribosa) polimerasa (PARP) u otros péptidos de ocurrencia natural o sintéticos y proteínas clivadas mediante caspasas que son conocidas en la técnica (Ellerby *et al* , 1997 *J Neurosci* 17 6165) El péptido sintético Z-Tyr-Val-Ala-Asp-AFC (ID DE SEC NO __,), en donde "Z" indica una porción benzoil carbonilo y AFC indica 7-amino-4-trifluorometilcoumarina (Kluck *et al* , 1997 *Science* 275 1132, Nicholson *et al* , 1995 *Nature* 376 37), es uno de tales sustratos Otros ejemplos no limitantes de sustratos incluyen proteínas nucleares tales como U1-70 kDa y ADN-PKcs (Rosen and Casciola-Rosen, 1997 *J Cell Biochem* 64 50, Cohen, 1997 *Biochem J* 326 1) La apoptosis cellular también se puede detectar mediante la determinación de citocromo c que ha escapado de la mitocondria en células apoptóticas (por ejemplo, Liu *et al* , *Cell* 86 147, 1996) Tal detección de citocromo c se puede desarrollar espectrofotométricamente, inmunoquímicamente o mediante otros métodos bien establecidos para determinar la presencia de una proteína específica Las personas que son expertas habitualmente en la técnica apreciarán fácilmente que podría haber otras técnicas adecuadas para cuantificar la apoptosis

Modalidades particularmente preferidas de construcciones útiles para aplicaciones de terapia génica, que incluyen aquellas construcciones que incluyen un dominio de anclaje de

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membrana de plasma y/o polipéptido de cola citoplasmática (que incluye, por ejemplo, una secuencia que señala apoptosis), son tales construcciones que incluyen (1) una secuencia de polipéptido con región variable de inmunoglobulina, que incluye V_H y/o V_L nativo o trabajado por ingeniería y/o secuencias de región variable de cadena sencilla (sFv), y que incluyen, por ejemplo, una mutación, alteración o supresión de un aminoácido en un sitio o sitios que corresponden a una o más de las posiciones de aminoácido 9, 10, 11, 12, 108, 110, 111, y 112 en una secuencia de la región V_H (incluyendo en una secuencia de la región V_H dentro de un scFv u otra construcción), y/o (2) una secuencia de polipéptido o región variable de inmunoglobulina, que incluye V_H y/o V_L nativo o trabajado por ingeniería y/o secuencias de región variable de cadena sencilla (sFv), y las cuales incluyen, por ejemplo, una mutación, alteración o supresión en un sitio o sitios que corresponden a una o más de las posiciones de aminoácido 12, 80, 81, 82, 83, 105, 106, 107, y 108 en una secuencia de región variable de cadena liviana (incluyendo en una secuencia de región V_L dentro de un scFv u otra construcción) Son particularmente preferidas las construcciones que incluyen una secuencia V_H trabajada por ingeniería (estén o no asociadas con una o más de las otras secuencias, que incluyen inmunoglobulinas derivadas y otras secuencias contenidas, por ejemplo, dentro de una construcción que contiene sFv o scFv), la cual incluye una

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mutación, alteración, o supresión en un aminoácido en un sitio o sitios que corresponden a una posición de aminoácido 11 El aminoácido V_H11, si es sustituido, se puede sustituir con otro aminoácido como se describió aquí, o por medio de otra molécula según se desee

Una vez una construcción, tal como por ejemplo una proteína de fusión de inmunoglobulina con dominio de unión, como se suministra aqui se ha diseñado, los polinucleótidos que incluyen los ADN que codifican la construcción, donde este o una porción relevante de este es un polipéptido, se pueden sintetizar en todo o en parte por vía de síntesis de oligonucleótido como se describió por ejemplo, en Sinha *et al* , *Nucleic Acids Res* , 12 4539-4557 (1984), ensamblado por vía de PCR como se describió, por ejemplo, en Innis, Ed , *PCR Protocols*, Academic Press (1990) y también en Better *et al* *J Biol Chem* 267 16712-16118 (1992), clonado y expresado por vía de procedimientos estándar como se describió, por ejemplo, en Ausubel *et al* , Eds , *Current Protocols in Molecular Biology*, John Wiley & Sons, New York (1989) y también en Robinson *et al* , *Hum Antibod Hybridomas*, 2 84-93 (1991), y ensayado para la actividad deseada, por ejemplo, unido a un objetivo, o actividad de unión de antígeno específica, como se describió, por ejemplo, en Harlow *et al* , Eds , *Antibodies A Laboratory Manual*, Capítulo 14, Cold

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Spring Harbor Laboratory, Cold Spring Harbor (1988) y Munson *et al*, *Anal Biochem*, 107 220-239 (1980)

La preparación de moléculas de unión de cadena de polipéptido sencilla de la región Fv, moléculas Fv de cadena simple, son conocidas en la técnica Ver, por ejemplo, Patente U S No 4,946,778 En la presente invención, las moléculas similares a Fv de cadena simple que se pueden incluir en construcciones de la invención se pueden sintetizar al codificar una primera región variable de la cadena pesada o liviana, seguido por uno o más ligadores de la región variable de la cadena liviana o pesada correspondiente, respectivamente La selección de varios ligadores apropiados entre las dos regiones variables se describe en la Patente U S No 4,946,778 (ver también, por ejemplo, Huston *et al*, 1993 *Int Revestimiento Immunol* 10 195) Un ligador de ejemplo descrito es $(Gly-Gly-Gly-Gly-Ser)_3$, pero puede ser de cualquier longitud deseada El ligador es utilizado para convertir las cadenas livianas y pesadas naturalmente agregadas pero químicamente separadas en la porción de unión de antígeno amino terminal de una cadena de polipéptido sencilla, por ejemplo, en donde esta porción de unión de antígeno se doblará en una estructura similar a la estructura original hecha de dos cadenas de polipéptido, o aquella que tiene de otra forma la capacidad de unirse a un objetivo, por ejemplo, un antígeno objetivo Para aquellas construcciones

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que incluyen un scFv como una región de unión, un pivote de inmunoglobulina nativa o trabajada por ingeniería como una región de conexión, y una o más regiones constantes de cadena pesada nativas o trabajadas por ingeniería como una región de unión, secuencias de nucleótido que codifican las regiones variables de las cadenas pesada y liviana nativas o trabajadas por ingeniería, unidas a una secuencia que codifica a un ligador, están unidas a una secuencia de nucleótidos que codifica regiones constantes de anticuerpo nativas o trabajadas por ingeniería, según se desee Las regiones constantes pueden ser aquellas que permiten al polipéptido resultante formar uniones disulfuro de intercadena para formar un dimero, y que contienen las funciones efectoras deseadas, tales como la capacidad de mediar ADCC, CDC, o fijar complemento, aunque las regiones constantes nativas o trabajadas por ingeniería que no favorecen el dímero u otra función multímero o agregación son preferidas Para una construcción, tal como una molécula similar a inmunoglobulina, de la invención que se pretende para uso en humanos, incluidas secuencias que tienen regiones constantes y/o funciones de constantes deseadas serán típicamente humanas o substancialmente humanas o humanizadas para minimizar una respuesta inmune antihumana potencial y para suministrar funciones efectoras apropiadas o deseadas La manipulación de las secuencias que codifican las regiones constantes del anticuerpo se ha referenciado en la

publicación PCT de Morrison y Oi, WO 89/07142 En modalidades preferidas, el dominio CH1 es eliminado en totalidad o en parte desde una región de cola que incluye, o consiste esencialmente de, o consiste de, una región constante de inmunoglobulina nativa o creada por ingeniería (por ejemplo, la región constante nativa o creada por ingeniería CH2 y/o CH3, o la región constante nativa o creada por ingeniería CH2 y/o CH3 y/o CH4), y el extremo carboxilo de la región de ligado, por ejemplo, un polipéptido de dominio ligado tal como un polipéptido de región variable de inmunoglobulina, es unido al terminal amino de, por ejemplo, un CH2 por medio de una región de conexión, por ejemplo, un polipéptido de región de pivote nativo o creado por ingeniería según se suministra aqui

Como se describió anteriormente, la presente invención suministra construcciones de expresión recombinantes capaces de dirigir la expresión de construcciones de la invención incluyendo proteínas de fusión de inmunoglobulina y dominio de ligado, como se suministra aquí Los aminoácidos que ocurren en las diversas secuencias de aminoácidos referidas aquí, son identificados de acuerdo con sus abreviaciones de letra única o sus tres letras bien conocidas Los nucleótidos, que ocurren en las diversas secuencias de ADN o fragmentos de ella referidos aquí son designados con las designaciones de letra única estándar usadas rutinariamente

en la técnica Una secuencia de aminoácido dada puede también involucrar secuencias de aminoácidos similares pero cambiados, tal como aquellos que tienen solamente cambios menores, por ejemplo, por vía de ilustración y no como limitación modificaciones covalentes químicas, inserciones, eliminaciones y sustituciones, que pueden incluir adicionalmente sustituciones conservadoras o sustituciones con aminoácidos de ocurrencia no natural Las secuencias de aminoácidos que son similares unas a las otras pueden compartir regiones sustanciales de homología de secuencia De manera similar, las secuencias de nucleótidos pueden involucrar secuencias de nucleótidos similares substancialmente que tengan solamente cambios menores, por ejemplo como vía de ilustración y no como limitación, modificaciones, inserciones, eliminaciones y sustituciones químicas covalentes, que pueden incluir adicionalmente mutaciones silenciosas debido a la degeneración del código genético Las secuencias de nucleótidos que son similares una a otra pueden compartir regiones de homología de secuencias sustanciales

La presencia de una condición maligna en un sujeto se refiere a la presencia de células displásticas, cancerosas y/o transformadas en el sujeto, incluyendo, por ejemplo, células transformadas neoplácicas, de tumor, inhibidas en contacto u oncogeneticamente transformadas o similares (por ejemplo

melanoma, carcinomas tales como el adenocarcinoma, carcinoma de células escamosas, carcinoma de células pequeñas carcinoma de célula de avena etc , sarcomas tales como el chondrosarcoma, osteosarcoma, etc) que son conocidos en la técnica y para los cuales el criterio de diagnóstico y clasificación se han establecido En modalidades preferidas contempladas por la presente invención, por ejemplo, tales como células cancerosas son las células hematopoieticas malignas, tales como células transformadas del linaje linfoide y en particular linfomas de célula B y similares, las células cancerosas pueden en ciertas modalidades preferidas también ser células epiteliales tales como células de carcinoma La invención también contempla los desórdenes de las células B, que pueden incluir ciertas condiciones malignas que afectan las células B (por ejemplo linfoma de célula B) pero que no tiene el propósito de ser limitado a, y que también tiene el propósito de involucrar enfermedades autoinmune y en particular, enfermedades, desórdenes y condiciones que son caracterizadas por la producción de auto-anticuerpos, por ejemplo

Los auto-anticuerpos son anticuerpos que reaccionan contra sus propios antígenos Los auto-anticuerpos son detectados en diversas enfermedades autoinmunes (es decir, una enfermedad, desorden o condición en donde un sistema inmune anfitrión genera una reacción "auto" inmune anti e inapropiada) en

donde ellos están involucrados en la actividad de la enfermedad Los tratamientos actuales para diversas enfermedades autoinmunes incluyen drogas inmunosupresoras que requieren la administración continuada, carecen de especificidad, y causan efectos colaterales significativos Nuevas enseñanzas que pueden eliminar la producción de auto-anticuerpos con mínima toxicidad serán dirigidas a una necesidad médica a un no solucionada de un espectro de enfermedades que afectan a muchas personas Las construcciones de la invención objeto, que incluye proteínas de fusión de dominio de ligado-inmunoglobulina son diseñadas, por ejemplo, para una penetración mejorada en los tejidos linfoides El agotamiento de los linfocitos B interrumpe el ciclo de producción de auto-anticuerpos, y permite que el sistema inmune se reposicionen a medida que nuevos linfocitos B son producidos a partir de precursores en la médula del hueso

Un número de enfermedades, desórdenes, y condiciones han sido identificadas para las cuales los efectos beneficiosos se ha creído, de acuerdo con una teoría no limitativa, resultan de la terapia de agotamiento de células B Tales desórdenes, enfermedades, y condiciones incluyen pero no se limitan a, la enfermedad de Grave's, la enfermedad de Hashimoto's, artritis reumatoide, eritematoso lupus sistémico, Síndrome Inmune Trombocitopénica púrpura de Sjotrens, esclerosis múltiple,

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miastemia grave, escleroderma, soriasis, enfermedad de Inflamación del Intestino, incluyendo la enfermedad de Crohn's y la colitis Ulcerativa La enfermedad Inflamatoria del Intestino incluyendo la enfermedad de Crohn's y la colitis Ulcerativa, son enfermedades autoinmunes del sistema digestivo

La presente invención se relaciona además a construcciones de nucleótidos que codifican construcciones de la invención, por ejemplo, proteínas de fusión de ligado de dominio-inmunoglobulina y en particular métodos para administrar construcciones recombinantes que codifican tales proteínas para aplicaciones de terapia de gen que se puede expresar, por ejemplo, como fragmentos análogos y derivados de tales polipéptidos

Los términos "fragmentos", "derivados" y "análogos" cuando se refieren a construcciones de la invención incluyen, por ejemplo, polipéptidos de fusión o proteínas de fusión de dominio de ligado-inmunoglobulina, se refiere a cualquier construcción tal como un polipéptido de fusión o proteína de fusión de dominio de ligado- inmunoglobulina, que retiene esencialmente la misma función biológica o actividad biológica como tal polipéptido Asi, un análogo incluye una pro- o prepro-forma de una construcción, por ejemplo, una pro-proteína que puede ser activada por rompimiento de la

porción de pro-proteína para producir una construcción activa, tal como un polipéptido de fusión de ligado de dominio-inmunoglobulina

Un fragmento, derivado o análogo de una construcción de la invención, por ejemplo, un polipéptido de fusión o proteína de fusión de ligado de dominio-inmunoglobulina, incluye los polipéptidos de fusión o proteínas de fusión de ligado de dominio-inmunoglobulina codificados por los cADN referidos aquí, puede ser (i) uno en el cual uno o más de los residuos de aminoácidos se sustituyen con un residuo de aminoácido conservado o no conservado (preferiblemente un residuo de aminoácido conservado) y tal residuo de aminoácido sustituido puede ser o no ser codificado por el código genético, (ii) uno en el cual uno o más residuos de aminoácidos incluye un grupo sustituyente, o (iii) uno de los tales aminoácidos adicionales son fusionados o de otra manera conectados a la construcción, por ejemplo, un polipéptido de fusión de ligado de dominio-inmunoglobulina, incluyendo aminoácidos que son empleados para la detección o la alteración funcional específica de la construcción, incluyendo tales construcciones como un polipéptido de fusión de ligado de dominio-inmunoglobulina o un secuencia de proteína Tales fragmentos, derivados y análogos se espera que estén dentro del alcance de aquellos versados en la técnica a partir de las enseñanzas dadas aquí

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Las construcciones, incluyendo construcciones de polipéptidos, de la presente invención incluyen, por ejemplo, polipéptidos de fusión y proteínas de fusión de ligado de dominio-inmunoglobulina que tiene regiones de ligado tales como las secuencias de aminoácidos de ligado de dominio del polipéptido que son idénticas o similares a secuencias conocidas ene la técnica, o fragmentos o porciones de ella Por ejemplo como vía de ilustración adicional pero no de limitación, un dominio extracelular de molécula humana CD154 [ID de SEC No _] se contempla para uso de acuerdo con la presente invención, como son las porciones de tales polipéptidos y/o los polipéptidos que tengan por lo menos cerca de 70% de similaridad (preferiblemente mayor a 70% de identidad) y más preferiblemente cerca de 90% de similaridad (más preferiblemente mayor a 90% de identidad) al polipéptido reportado y todavía más preferiblemente cerca de 9% de similaridad (todavía más preferiblemente mayor a 95% de identidad) a los polipéptidos reportados y las porciones de tales polipéptidos, en donde tales porciones de un polipéptido de fusión de ligado de dominio-inmunoglobulina, por ejemplo, generalmente contiene por lo menos cerca de 30 aminoácidos y más preferiblemente cerca de 50 aminoácidos Los dominios extracelulares incluyen, por ejemplo, porciones de una molécula de superficie celular, y en modalidades particularmente preferidas las moléculas de superficie

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celular que son integrales con las proteínas de membrana o que comprenden un dominio de transmembrana de expansión de una membrana de plasma, que son construidas para extenderse más allá de la hojuela exterior de la bicapa de fosfolípidos de la membrana de plasma cuando la molécula se expresa en una superficie celular, preferiblemente de una manera que expone la porción de dominio extracelular de tal una molécula al ambiente externo de la célula, también conocido como medio extracelular Los métodos para determinar si una porción de una molécula de superficie celular comprende un dominio extracelular son bien conocidos en la técnica, e incluyen, por ejemplo, determinación experimental (por ejemplo, marcación directa o indirecta de la molécula, evaluación de si la molécula puede ser alterada estructuralmente por agentes en los cuales la membrana de plasma no es permeable tal como enzimas proteolíticas o lipolíticas) o predicción topológica con base en la estructura de la molécula (por ejemplo, análisis de la secuencia de aminoácido de un polipéptido) u otras metodologias

Como se usa aquí, un "aminoácido" es una molécula que tiene la estructura en donde un átomo de carbono central (el átomo (α)-carbono) es enlazado a un átomo de hidrógeno, un grupo de ácido carboxílico (el átomo de carbono del cual se refiere aquí como un a "átomo de carbono carboxilo"), un grupo amino (el átomo de nitrógeno al cual nos referimos aquí es un

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"átomo de aminonitrógeno"), y un grupo de cadena lateral, R Cuando se incorpora dentro de un péptido, polipéptido o proteína, un aminoácido pierde uno o más átomos de sus grupos amino y carboxílico en la reacción de deshidratación que enlaza un aminoácido con otro Como un resultado, cuando se ha incorporado dentro de una proteína, un aminoácido puede también ser llamado como un "residuo de aminoácido" En el caso de proteínas de ocurrencia natural, un grupo R del residuo de aminoácido se diferencia de los 20 aminoácidos de los cuales las proteínas son sintetizadas típicamente, aunque uno o más residuos de aminoácidos en una proteína puede ser derivado o modificado luego de la incorporación dentro de la proteína en sistemas biológicos (por ejemplo, por glicosilación y/o por la formación de cistina a través de la oxidación de las cadenas laterales de tiol de 2 residuos de aminoácido de cisteína no adyacentes, resultando en un enlace covalente de disulfuro que juega frecuentemente un papel importante en la estabilidad de la conformación plegada de una proteína, etc) Como aquellos versados en la técnica apreciarán, los aminoácidos de ocurrencia no natural también se pueden incorporar dentro de las proteínas, particularmente aquellos producidos por métodos sintéticos, incluyendo métodos de estado sólido y de síntesis automatizada Ejemplos de tales aminoácidos incluyen, sin limitación, ácido α-amino isobutírico, ácido 4-amino butírico, ácido L-amino butírico, ácido 6-amino hexanoico, ácido 2-amino isobutírico, ácido 3-

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amino propionico, ornitina, norlensina, norvalina, hidroxprolina, sarcosina, citralina, ácido cistéico, t-butilgliina, t-butilalanina, fenililicina, ciclohexilalanina, β-alanina, fluoro-aminoácidos, aminoácidos de diseñador (por ejemplo, aminoácidos de β-metilo, aminoácidos de α-metilo, aminoácidos de Nα-metilo) y análogos de aminoácidos en general, en adición cuando un átomo α-carbono tiene cuatro grupos diferentes (como es el caso con los 20 aminoácidos usados por los sistemas biológicos para sintetizar proteínas, excepto para la glicina, que tiene átomos de hidrógeno enlazados al átomo α-carbono), dos formas enantioméricamente de cada aminoácido existen designados D y L En mamíferos, solamente aminoácidos L son incorporados en polipéptidos de ocurrencia natural La presente invención visualiza proteínas que incorporan uno o más aminoácidos D- y L-lo mismo que proteínas comprendidas de solamente de residuos de D o L de aminoácidos

Aquí, las siguientes abreviaciones pueden ser usadas para los siguientes aminoácidos (y residuos de ellos) alanina (Ala, A), arginina (Arg, R), asparágina (Asn, N), ácido aspártico (Asp, D), citeína (Cys, C), glicina (Gly, G), ácido glutámico (Glu, E), glutamina (Gln, Q), histidina (His, H), isoleucina (Ile, I), leucina (Leu, L), lisina (Lys, K), metionina (Met, M), fenilalanina (Phe, F), prolina (Pro, P), serina (Ser, S), treonina (Thr, T), triptofan (Trp, W), tirosina (Tyr, Y), y

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valina (Val, V) Los aminoácidos no polares (hidrofóbicos) incluyen la alanina, leucina, isoleucina, valina, prolina, fenilalanina, triptofan, y metioninas Los aminoácidos neutrales incluyen la glicina, serina, treonina, cisteina, tirosina, esparagina, y glutamina Los aminoácidos básicos cargados positivamente incluyen arginina, lisina e histidina Los aminoácidos (acídicos) cargados negativamente incluyen al ácido aspártico y el ácido glutámico

"Proteínas" se refieren a cualquier polimero de dos o más aminoácidos individuales (sea de ocurrencia natural o no) enlazados por medio de enlace péptido, y que ocurren cuando el átomo del carbono carboxilo del grupo de ácido carboxílico enlazado al α-carbono de un aminoácido (o residuo de aminoácido) se vuelve ligado covalentemente al átomo aminonitrógeno del grupo amino enlazado al α-carbono de un aminoácido adyacente El término "proteínas" se entiende que incluye los términos "polipéptidos" y "péptidos" (que, a veces, pueden ser usados de manera intercambiable aquí) dentro de su significado En adición, las proteínas que comprenden múltiples subunidades de polipéptido u otros componentes también se entiende que quedan incluidos dentro del significado de "proteínas" como se usa aquí Similarmente, fragmentos de proteína, péptidos y polipéptidos están también dentro del alcance de la invención y pueden ser llamados aquí como "proteínas"

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En sistemas biológicos (sean ellos in vivo o in vitro, incluyendo sistemas libres de células) la secuencia de aminoácido particular de una proteína dada (es decir, la "estructura primaria" del polipéptido, cuando se escribe desde el terminal amino al terminal carboxi) se determina por la secuencia del nucleótido de la porción de codificación de un mARN, que a su vez está especificado por la información genética, típicamente el ADN genómico (que, para propósito de esta invención, se entiende que incluye el organelo de ADN, por ejemplo, el ADN mitocondrial y el ADN de cloroplasto) Por supuesto cualquier tipo de ácido nucleico que constituya en genoma de un organismo particular (por ejemplo, el ADN de doble hélice en el caso de la mayoría de animales y plantas, ARN de hélice unica, o doble hélice en el caso de algunos virus etc) se entiende que codifica para el producto de gen del organismo particular El ARN mensajero es traducido sobre un ribosoma, que cataliza la polimerización de un aminoácido libre, la identidad particular del cual es especificada por el codón particular (con respecto al mARN, tres ribonucleótidos A, G, C, o U adyacentes en la región de codificación del mARN) el mARN es entonces traducido, a un polipéptido naciente Las técnicas de ADN recombinante han permitido la síntesis a gran escala de proteínas y polipéptidos (por ejemplo la insulina humana, la hormona de crecimiento humano, erytropoietina, factor estimulante de

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colonias de granulocitos, etc) que tiene la misma secuencia principal que cuando se produjo naturalmente en organismos vivos Adicionalmente, tal tecnología ha permitido la síntesis de análogos de estos y otras proteínas, dichos análogos pueden contener una o más eliminaciones, inserciones, y/o sustituciones de aminoácidos cuando se ha comparado con las proteínas La tecnología de ADN recombinante también permite la síntesis de proteínas completamente nuevas

En sistemas no biológicos (por ejemplo, aquellos que emplean síntesis de estado sólido), la estructura primaria de una proteína (que también incluye localización de enlaces de disulfuro (cistina)) puede ser determinada por el usuario Como resultado, los polipéptidos que tienen una estructura primaria que duplica aquella de una proteína producida biológicamente se puede lograr, como pueden los análogos de tales proteínas En adición polipéptidos completamente novedosos también pueden ser sintetizados, como pueden ser proteínas que incorporan aminoácidos de ocurrencia no natural

Como se conoce en la técnica, "similaridad" entre dos polipéptidos puede ser determinada por medio de comparar las secuencias de aminoácidos (incluyendo los sustitutos de aminoácidos conservados allí) de un polipéptido con la

secuencia de un segundo polipéptido Los fragmentos o porciones de los acidos nucleicos que codifican los polipéptidos de la presente invención pueden ser usados para sintetizar ácidos nucleicos de longitud completa de la presente invención Como se usa aquí, "porcentaje de identidad" se refiere al porcentaje de aminoácidos idénticos situados en las posiciones de residuo de aminoácidos correspondientes cuando dos o más polipéptidos son alineados y sus secuencias son analizadas usando, por ejemplo, un algoritmo de BLAST espaciado (por ejemplo, Altschul et al , 1997 Nucl Ac Res 25 3389, Altschul et al , 1990 J Mol Biol , 215 403-410) el cual deja los espacios de las secuencias, y los errores de la secuencia de acuerdo con los pesos por defecto que se suministran por los Institutos Nacionales de Salud/ la base de datos NCBI (Betesda, MD, ver www ncbi nlm nih gov/cgi-bin/BLAST/nph-newblast) Otros métodos de alineamiento incluyen el BLITZ (MPsrch) (Sturrock & Collins, 1993), y FASTA (Pearson y Lipman, 1988 Proc Natl Acad Sci USA 85 2444-2448)

El término medios "aislados" significa en el caso de un material de ocurrencia natural, que el material está o ha sido removido de, o no está más asociado con, su entorno natural u original Por ejemplo, un ácido nucleico o proteína de ocurrencia natural o polipéptido de ocurrencia natural presente en un animal vivo no está aislado, pero el mismo

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ácido nucleico o polipéptido separado de algunos o todos de los materiales coexistentes en el sistema natural, está aislado Tales ácidos nucleicos podrían ser parte de un vector y/o tales ácidos nucleicos o polipéptidos podrían ser parte de una composición, y todavía estar aislados en que Tal vector o composición no es parte de su entorno natural El término "aislado", en el caso de materiales que no ocurren naturalmente, tal como una construcción fabricada recombinantemente de la invención, incluye material que está substancialmente o esencialmente libre de componentes que normalmente lo acompañan durante la fabricación, tal como, por ejemplo, proteínas y péptidos que han sido purificados a un grado deseado, preferiblemente, por ejemplo, de modo que ellos son por lo menos cerca del 80% puros, más preferiblemente por lo menos 90% y todavía más preferiblemente por lo menos 95% según se midió por técnicas conocidas en el arte

El término "gen" significa un segmento de ADN involucrado en la producción de una cadena de polipéptido, este también puede incluir regiones precedentes y subsiguientes a una región de codificación de polipéptido, por ejemplo, un "líder o colero" lo mismo que secuencias que intervienen (intrones) entre segmentos de codificación individual relevantes (exones)

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Como se describe aquí, la invención suministra construcciones que incluyen proteínas de fusión de ligado de dominio-inmunoglobulina, que pueden ser codificadas en parte o totalmente por ácidos nucleicos que tienen una secuencia de codificación de región de ligado tal que, por ejemplo, una secuencia de codificación de dominio de ligado se fusiona o de otra manera está conectada en el marco a una secuencia que codifica un dominio de inmunoglobulina nativa o producido por ingeniería para suministrar para la expresión de, por ejemplo, una secuencia de polipéptido de dominio de ligado fundida o de otra manera conectada a una secuencia de polipéptido funcional adicional que permite, por ejemplo por vía de ilustración y no de limitación, la detección, alteración funcional, aislamiento y/o purificación de la proteína de fusión Tales proteínas de fusión pueden permitir la alteración funcional de un dominio de ligado por medio de contener secuencias de polipéptido derivados de inmunoglobulina adicional que influencian el comportamiento del producto de fusión, por ejemplo (y como se describió anteriormente) por medio de reducir la disponibilidad de los grupos sulfidrilos para la participación en la formación de enlace de disulfuro, y por medio de conferir la habilidad para potenciar ADCC y/o CDC y/o complemento fijo

La modificación de un polipéptido puede ser efectuada por cualesquier medios conocidos por aquellos expertos en la

técnica Los métodos preferidos descansan aquí en la modificación de la codificación de ADN, por ejemplo, una proteína de fusión y expresión del ADN modificado, codificar ADN de una de las construcciones de la invención, por ejemplo, una de las fusiones de ligado de dominio-inmunoglobulina discutidas aquí, por ejemplo, puede ser alterada o mutagenizada usando metodologías estándar, incluyendo aquellas descritas más adelante Por ejemplo, los residuos de cisteína que pueden de otra manera facilitar la formación de multímeros o promover las conformaciones moleculares particulares pueden ser eliminadas de un polipéptido o reemplazadas, por ejemplo residuos de cisteína que son responsables para o participan en la formación de agregados Si es necesario, por ejemplo, la identidad de los residuos de cisteína que contribuye a la formación de agregados puede ser determinada empíricamente, por medio de eliminar y/o reemplazar un residuo de cisteína y asegurarse si la proteína se agrega en soluciones que contiene amortiguadores fisiológicamente aceptables y sales En adición, los fragmentos de fusiones de ligado de dominio-inmunoglobulina pueden ser construidos y usados Como se anotó anteriormente, los dominios de ligado de contra receptor/ligando para muchos candidatos de fusión de ligado de dominio-inmunoglobulina se han delineado, de modo que una persona que es experta en la técnica puede seleccionar fácilmente los dominios de polipéptido apropiados para la

inclusión en productos codificados en las construcciones de expresión presentes

Sustituciones conservadoras de aminoácidos son bien conocidos y pueden ser generalmente hechas sin alterar la actividad biológica de la molécula de proteína de fusión de ligado de dominio-inmunoglobulina Por ejemplo, tales sustituciones están hechas generalmente por medio de intercambiar dentro de los grupos de residuos polares, residuos cargados, residuos hidrofóbicos, residuos pequeños y similares Si es necesario, tales sustituciones pueden ser determinadas empíricamente meramente por medio de ensayar la proteina modificada resultante en cuanto habilidad para ligarse a los receptores de superficie celular apropiados en ensayos biológicos in vitro, o para ligar antígenos apropiados o moléculas objetivo deseadas

La presente invención se relaciona adicionalmente con ácidos nucleicos que hibridizan a construcciones de la invención, incluyendo por ejemplo, secuencias de polinucleótidos que codifican proteínas de fusión de ligado de dominio-inmunoglobulina según se suministra quimérica, o sus complementos, puede ser fácilmente visible para aquellos familiares con la técnica, si existe por lo menos cerca de 70%, preferiblemente cerca de 80 a 85%, más preferiblemente por lo menos cerca de 90%, y todavía más preferiblemente por

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lo menos cerca de 95%, 96%, 97%, 98% o 99% de identidad entre las secuencias

La presente invención se relaciona particularmente con ácidos nucleicos que hibridizan bajo condiciones astrictas a, por ejemplo, los ácidos nucleicos que codifican la fusión de ligado de dominio-inmunoglobulina como se ha referido aquí Como se usa aquí, para "hibridizar" bajo condiciones de un estricto especificado se usa para describir la estabilidad de híbridos formados entre dos moléculas de ácido nucleico de cadena única Lo estricto de la hibridización es típicamente expresado en condiciones de resistencia iónica y temperatura en la cual tales híbridos se templan y se lavan El término "condiciones estrictas" se refiere a condiciones que permiten la hibridización entre polinucleótidos Las condiciones estrictas pueden ser definidas por la concentración de sal, la concentración de disolvente orgánico (por ejemplo formamida), la temperatura, u otras condiciones bien conocidas en la técnica En particular, lo estricto puede ser incrementado por medio de reducir la concentración de sal, incrementar la concentración de disolventes orgánicos (por ejemplo formamida) o elevar la temperatura de hibridización Por ejemplo, la concentración de sal estricta será ordinariamente menos de cerca de 750 mM de NaCl y 75 mM de citrato de trisodio, preferiblemente menos de cerca de 500 mM de NaCl y 50 mM de citrato de trisodio, y más preferiblemente

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menos de cerca de 250 mM de NaCl y 25 mM de citrato de trisodio La hibridización de bajo estricto puede ser obtenida en ausencia de disolvente orgánico, por ejemplo, formamida, mientras que la hibridización muy estricta puede ser obtenida en la presencia de un disolvente orgánico, (por ejemplo, por lo menos cerca de 35% de formamida, más preferiblemente por lo menos 50% de formamida) Las condiciones de temperatura estrictas ordinariamente incluirán temperaturas de por lo menos de 30° C, más preferiblemente de por lo menos cerca de 37° C, y más preferiblemente por lo menos cerca de 42° C Variando parámetros adicionales, por ejemplo, tiempo de hibridización, la concentración del detergente, por ejemplo, dodecil sulfato de sodio (SDS), y la inclusión o exclusión del vehículo de ADN, son bien conocidos por aquellos versados en la técnica Diversos niveles de estricto se llevan a cabo por medio de combinar estas varias condiciones segun se necesite, y están dentro del experticio de la técnica Otras condiciones rigurosas típicas "alta", "media" y "baja" involucra las siguientes condiciones o condiciones equivalentes a ellas rigor alto 0,1 x SSPE o SSC, 0,1% de SDS, 65° C, rigor medio 0,2 x SSPE o SSC, 0,1% de SDS, 50° C , y rigor bajo 1,0 x SSPE o SSC, 0,1% de SDS, 50° C Como se conoce por aquellos versados en la técnica, las variaciones de rigor de condiciones de hibridización pueden ser logradas por medio de alterar el tiempo, la temperatura y/o la concentración de las soluciones que se

usan para la prehibridización, la hibridización y las etapas de lavado, y condiciones adecuadas también pueden depender en parte de la secuencia de nucleótidos particulares de la probeta usada, y del borrado, de la muestra de ácido nucleico de prueba De acuerdo con lo anterior, podrá apreciarse que las condiciones de rigor adecuadas pueden ser seleccionadas fácilmente sin experimentación indebida en donde una selectividad deseada de la probeta es identificada con base en su habilidad para hibridizar a una o más secuencias de algunos probandos mientras que no hibridiza a ciertas secuencias de otros probandos

Según se usa aquí, "las condiciones de rigor" preferidas generalmente se refieren a la hibridización que ocurrirá si hay por lo menos cerca de 90 a 95% y más preferiblemente por lo menos cerca de 97% de identidad entre las secuencias Las construcciones de ácido nucleico que hibridizan a, por ejemplo, los ácidos nucleicos que codifican la fusión de ligado de dominio-inmunoglobulina se refieren aquí, en modalidades preferidas, polipéptidos que codifican los cuales retienen substancialmente la misma función biológica o actividad que, por ejemplo, los polipéptidos de fusión de ligado de dominio-inmunoglobulina codificados por los cADN

Los ácidos nucleicos de la presente invención, también referidos aquí como polinucleótidos, pueden estar en la forma

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de ARN, por ejemplo, mARN, o en la forma de ADN, dicho ADN incluye cADN (también llamado "ADN complementario", el cual es una molécula de ADN que es complementaria a un ARN mensajero específico), ADN genómico, y ADN sintético El ADN puede ser de doble cadena o de una sola cadena, y si es de cadena única puede ser la cadena codificada o no codificada (antisentido) Una secuencia de codificación que codifica una construcción de la invención, por ejemplo, un polipéptido de fusión de ligado de dominio-inmunoglobulina para uso de acuerdo con la invención puede contener porciones que sin idénticas a la secuencia de codificación conocida en la técnica o descrita aquí por porciones de ella, o puede ser una secuencia de codificación diferente, que, como un resultado de la redundancia o degeneración del código genético, codifica la misma construcción o porción de la misma, incluyendo todo o una porción del polipéptido de fusión de ligado de dominio-inmunoglobulina

Los ácidos nucleicos que codifican construcciones de la invención, por ejemplo, polipéptidos de fusión de ligado de dominio-inmunoglobulina, para uso de acuerdo con la invención pueden incluir, sin limitarse a solamente las secuencias de codificación para la construcción tal como un polipéptido de fusión de ligado de dominio-inmunoglobulina, la secuencia de codificación para la construcción, tal como un polipéptido de fusión de ligado de dominio-inmunoglobulina y secuencia de

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codificación adicional, la secuencia de codificación para la construcción, tal como un polipéptido de fusión de ligado de dominio-inmunoglobulina (y opcionalmente secuencia de codificación adicional) y secuencia de no codificación, tal como intrones o secuencias 5' y/o 3' de no codificación de la secuencia de codificación para el polipéptido de fusión de ligado de dominio-inmunoglobulina o una porción o porciones de ellos, que por ejemplo pueden incluir adicionalmente pero sin necesidad de estar limitados a una o más secuencias de ácidos nucleicos regulatorias que pueden ser un promotor regulado o regulable, un mejorador, otras secuencias regulatorias de trascripción, secuencia de ligado represora, secuencia regulatoria de traducción o cualquier otra secuencia de ácido nucleico regulatoria Así, el término "ácido nucleico que codifica" o "polinucleótido que codifica" una construcción, por ejemplo, una proteína de fusión de ligado de dominio-inmunoglobulina, involucra un ácido nucleico que incluye solamente secuencia de codificación para, por ejemplo, un polipéptido de fusión de ligado de dominio-inmunoglobulina lo mismo que un ácido nucleico que incluye codificación adicional y/o secuencias de no codificación

Los ácidos nucleicos y oligonucleótidos para uso según se describió aquí pueden ser sintetizados por cualquier método conocido por aquellos versados en la técnica (ver, por

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ejemplo, WO 93/01286, solicitud de patente estadounidense serie No 07/723, 454, Patente U S No 5, 218, 088, Patente U S No 5, 175, 269, Patente U S No 5, 109, 124) La identificación de diversos oligonucleótidos y secuencias de ácido nucleico también involucran métodos conocidos en la técnica Por ejemplo, las propiedades deseadas, longitudes y otras características de los oligonucleótidos útiles para la clonación son bien conocidas En ciertas modalidades, los oligonucleótidos sintéticos y secuencias de ácidos nucleicos pueden ser diseñadas de modo que resistan la degradación por enzimas nucleolíticas de células anfitrionas endogenosas por medio de contener tales enlaces como fosforotioato, metilfosfonato, sulfona, sulfato, cetil, fosforoditioato, fosforamidato, ésteres de fosfato, y otros de tales enlaces que tien una utilidad probada en aplicaciones antisentido Ver, por ejemplo, Agrwal et al , Tetrehedron Lett 28 3539-3542 (1987) , Miller et al , J Am Chem Soc 93 6657-6665 (1971), Stec et al , Tetrehedron Lett 26 2191-2194 (1985), Moody et al , Nucl Acids Res 12 4769-4782 (1989, Uznanski et al , Nucl Acids Res (1989), Letsinger et al , Tetrahedron 40 137-143 (1984), Eckstein, Anne Rev Biochem 54 367-402 (1985), Eckstein, Trends Biol Sci 14 97-100 (1989), Stein In Oligodeoxynucleotides Antisense Inhibitors of Gene Expression, Cohen, Ed, Macmillan Press, London, pp 97-117 (1989), Jager et al , Biochemistry 27 7237-7246 (1988)

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En una modalidad, la presente invención suministra componentes truncados (por ejemplo polipéptidos de dominio de ligado, polipéptidos de región de pivote, enlazadores, etc) para uso en una construcción de la invención, por ejemplo, una proteína de fusión de ligado de dominio-inmunoglobulina En otra modalidad de la invención se suministran ácidos nucleicos que codifican una construcción de la invención, por ejemplo, una proteína de fusión de ligado de dominio-inmunoglobulina que tiene tales componentes truncados Una molécula truncada puede ser cualquier molécula que comprenda menos de la versión de longitud completa de la molécula de interés Las moléculas truncadas suministradas por la presente invención pueden incluir polímeros biológicos truncados y en modalidades preferidas de la invención tales moléculas pueden ser moléculas de ácido nucleico truncado o polipéptidos trucados Las moléculas de ácido nucleico truncadas tienen menos de la longitud completa de la secuencia de nucleótido como se ha conocido o descrito en las moléculas de ácido nucleico, en donde tal molécula de ácido nucleico conocida o descrita puede ser una molécula de ácido nucleico de ocurrencia natural, sintética, o recombinante, siempre que una persona versada en la técnica la mire como una molécula de longitud completa Así, por ejemplo, las moléculas de ácido nucleico truncadas que corresponden a una secuencia de gen que contiene menos de la longitud completa

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del gen en donde el gen comprende secuencias de codificación y de no codificación, promotores, mejoradores y otras secuencias regulatorias, secuencias de flanqueo y similares, u otras secuencias funcionales y no funcionales que son reconocidas como parte del gen En otro ejemplo, las moléculas de ácido nucleico truncadas que corresponden a una secuencia mARN contiene menos de la longitud completa dl trascripto mARN, el cual puede incluir diversas regiones traducidas o no conocidas lo mismo que otras secuencias funcionales y no funcionales

En otras modalidades preferidas, las moléculas truncadas son polipéptidos que comprenden menos de la longitud completa de la secuencia de aminoácido de una proteína particular o componente de polipéptido Como se usa aquí "eliminación" tiene el significado común como se entiende por aquellos versados en la técnica, y puede referirse a moléculas que carece de una o más porciones de una secuencia desde sea el terminal o desde una región no terminal, con relación a una molécula correspondiente de longitud completa, por ejemplo, como en el caso de moléculas truncadas suministradas aquí Las moléculas truncadas que son polímeros biológicos lineales tales como moléculas de ácido nucleico o polipéptidos pueden tener una o más de las eliminaciones desde sea el terminal de las moléculas y/o una o más eliminaciones desde una región no terminal de la molécula, en donde tales eliminaciones pueden

ser eliminaciones de entre 1 a 1,500 nucleótidos contiguos o residuos de aminoácido, preferiblemente entre cerca de 1 a 500 nucleótidos contiguos o residuo de aminoácido y más preferiblemente entre cerca de 1 a 300 nucleótidos contiguos o residuo de aminoácido, incluyendo eliminaciones de cerca de 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, 31-40, 41-50, 51-74, 75-100, 101-150, 151-200, 201-250 o 251-299 nucleótidos contiguos o residuos de aminoácido En ciertas modalidades preferidas particularmente las moléculas de ácido nucleico trucadas pueden tener una eliminación de cerca de 270 a 330 nucleótidos contiguos En ciertas modalidades más preferidas, las moléculas de polipéptido trucadas pueden tener una eliminación, de por ejemplo, cerca de 80 a 140 nucleótidos contiguos

La presente invención se relaciona a variantes de los ácidos nucleicos referenciados aquí que codifican fragmentos, análogos y/o derivados de una construcción de la invención, por ejemplo, un polipéptido de fusión de ligado de dominio-inmunoglobulina Las variantes de los ácidos nucleicos que codifican las construcciones de la invención, por ejemplo, proteínas de fusión de ligado de dominio-inmunoglobulina, pueden ser variantes alélicas de ocurrencia natural de una o más porciones de las secuencias de ácido nucleico incluidas allí, o variantes de ocurrencia no natural de tales

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secuencias o porciones o secuencias, que incluyen secuencias variadas por ingeniería molecular usando, por ejemplo, métodos conocidos en la técnica para variar las secuencias Como es conocido en la técnica, una variante alélica es una forma alterna de una secuencia de ácido nucleico la cual puede tener por lo meno una de una sustitución, una eliminación o una adición de uno o más nucleótidos, cualquiera de los cuales no altera substancialmente o de manera indeseable la función del polipéptido de fusión de ligado de dominio-inmunoglobulina codificada

Las variantes y derivados de las construcciones de la invención, por ejemplo, proteína de fusión de ligado de dominio-inmunoglobulina, puede ser obtenidas por mutaciones de secuencias de nucleótidos que codifican, por ejemplo, polipéptidos de fusión de ligado de dominio-inmunoglobulina o cualquier porción de ellos Las alteraciones de la secuencia de aminoácido nativa puede llevarse a cabo por cualquiera de un número de métodos convencionales Las mutaciones pueden ser introducidas en sitios, particulares, por ejemplo, por medio de sintetizar oligonucleótidos que contiene una secuencia mutante, flanqueado por sitios de restricción que permiten el ligado a fragmentos de la secuencia nativa Luego del ligado, la secuencia reconstruida resultante codifica un análogo que tiene la inserción de aminoácido deseada, la sustitución o la eliminación

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Alternativamente, por ejemplo, los procedimientos de mutagénesis específicos a sitio dirigidos al oligonucleótido pueden ser empleados para suministrar un gen alterado en donde los codones predeterminados pueden ser alterados por sustitución, eliminación, o inserción Métodos ejemplares de lograr tales alteraciones se han descrito por Walder et al , 1986 Gene 42 133, Bauer et al , 1985 Gene 37 73, Craik, January 1985 BioTechniques 12-19, Smith et al , Enero 1985 Genetic Engineering Principles and Methods BioTechniques 12-19, Costa GL, et al ,"Site-directed mutagenesis using a rapid PCR-based method,"1996 Methods Mol Biol 57 239-48, Rashtchian A ,"Novel methods for cloning and engineering genes using the polymerase chain reaction,"1995 Curr Opin Biotechraol 6 (1) 30-6, Sharon J, et al ,"Oligonucleotide-directed mutagenesis of antibody combining sites,"1993 Hist Rev Inziizunol 10 (2-3) 113-27, Kunkel, 1985 Proc Natl Acad Sci (JSA 82 488, Kunkel et al , 1987 Methods in Enzymol 154 367, y, Patente U S Nos 4,518,584 y 4,737,462

Como un ejemplo, la modificación del ADN puede ser llevada a cabo por mutagénesis dirigida al sitio del ADN que codifica a un proteína combinada con el uso de métodos de amplificación de ADN usando iniciadores para introducir y amplificar [illegible] en la plantilla de ADN, tal co[illegible]

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PCR por medio de sobreponer la extensión (SOE) La mutagénesis a sitio es típicamente adecuada usando un vector fago que tiene formas de cadena única o doble cadena, tal como los vectores fago M13, que son bien conocidos y disponibles comercialmente Otros vectores adecuados que contienen un origen de replicación fago de cadena única pueden ser usados Ver, por ejemplo, Veira et al , 1987 Meth Enzymol 15 3 En general, la mutagénesis dirigida a sitio se lleva a cabo por medio de preparar un vector de cadena única que codifica la proteína de interés (por ejemplo todo o una porción del componente de una proteína de fusión de ligado de dominio-inmunoglobulina dada) Un iniciador de oligonucleótido que contiene la mutación deseada dentro de una región de homología del ADN en el vector de cadena única es templado sobre el vector seguido por la adición de una polimerasa de ADN, tal como la polimerasa I del ADN de la E coli (fragmento Klenow), que usa la región de doble cadena como un iniciador para producir un heteroduplex en el cual una cadena codifica la secuencia alterada y la otra la secuencia original El heteroduplex es introducido dentro de las células bacteriana apropiadas y clones que incluyen la que mutación deseada sea seleccionada Las moléculas de ADN alteradas resultantes pueden ser expresadas de manera recombinante en células anfitrionas apropiadas para producir la proteína modificada

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Las construcciones de ADN equivalentes que incluyen el código para adiciones o sustituciones de residuos de aminoácidos o secuencias, o eliminaciones de terminal o residuos internos o secuencias no requeridas o deseadas para actividad biológica, por ejemplo, son también involucradas por la invención por ejemplo, como se discutió anteriormente, las secuencias que codifican residuos Cys que no son deseables o esenciales para la actividad biológica pueden ser alteradas para causar que los residuos Cys sean eliminados o reemplazados con otros aminoácidos por ejemplo, impidiendo así la formación de puentes de disulfuro intramoleculares incorrectos o indeseados luego de la síntesis o la renaturalización

Una "célula anfitriona" o "célula anfitriona recombinante" es una célula que contiene un vector, por ejemplo un vector de expresión, o una célula que de otra forma ha sido manipulada por técnicas recombinantes para expresar una proteína de interés Los organismos incluyen aquellos organismos en los cuales la producción recombinante de construcciones de la invención, por ejemplo, productos de fusión de ligado de dominio-inmunoglobulina codificados por las construcciones recombinantes de la presente invención pueden ocurrir, tales como bacterias (por ejemplo, E coli), levaduras (por ejemplo, Saccharomyces cerevisiae y Pichia pastoris), células de insectos, y células de mamíferos, incluyendo expresión in vitro e in vivo Organismos anfitriones por lo tanto pueden

incluir organismo para la construcción, propagación, expresión u otras etapas en la producción de composiciones suministradas aquí Los anfitriones incluyen sujetos en los cuales las respuestas inmunes tienen lugar, como se describió aquí Actualmente organismo anfitriones preferidos para la producción de construcciones de la invención que producen proteínas glicosiladas son células de mamíferos u otros sistemas de células que permitan la expresión y recuperación de las proteínas glicosiladas Otras líneas celulares incluyen cepas de murina consanguíneas y líneas celulares de murina, y líneas celulares de células de arena humanas

Una construcción de ADN que codifica una construcción deseada de la invención, por ejemplo, una proteína de fusión de ligado de dominio-inmunoglobulina es introducida dentro de un vector, por ejemplo, un plásmido, para la expresión en un anfitrión apropiado En modalidades preferidas, el anfitrión es un anfitrión mamífero, por ejemplo una línea celular de mamífero La secuencia que codifica la ligando o al dominio de ligado del ácido nucleico es preferiblemente optimizada en codón para la expresión del anfitrión particular Así, por ejemplo, si una construcción, por ejemplo, es una fusión de ligado de dominio-inmunoglobulina humano y es expresada en bacterias, los codones pueden ser optimizados para uso bacterial Para regiones de codificación pequeñas, el gen puede ser sintetizado como un oligonucleótido único Para

proteínas más grandes, la división de oligonucleótidos múltiples, mutagénesis u otras técnicas conocidas por aquellos expertos en la técnica pueden ser usadas Las secuencias de nucleótidos en plásmidos u otros vectores que son regiones regulatorias, tales como promotores y operadores, son asociados operacionalmente uno con otro para la trascripción La secuencia de nucleótidos que codifican una proteína de fusión de ligado de dominio-inmunoglobulina puede también incluir ADN que codifica una señal de secreción, mientras el péptido resultante sea una proteína precursora La proteina procesada resultante puede ser recuperada del espacio periplásmico o del medio de fermentación

En modalidades preferidas, los plásmidos de ADN pueden también incluir una secuencia terminadora de trascripción, como se usa aquí, "una región terminadora de trascripción es una secuencia" que señaliza la terminación de la trascripción El terminador de trascripción completo puede ser obtenido a partir de un gen que codifica la proteína, el cual puede ser el mismo o diferente del gen de la fusión de ligado de dominio-inmunoglobulina insertada que codifica al gen o la fuente del promotor Los terminadores de trascripción son componentes opcionales en los sistemas de expresión aquí, pero son empleados en las modalidades preferidas

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Los plásmidos u otros vectores usados a quimérica incluyen un promotor en asociación operativa con el ADN que codifica la proteína o polipéptido de interés y son designados para la expresión de las proteínas en anfitriones adecuados como se describió anteriormente (por ejemplo, bacterianos, murina o humano) dependiendo luego del uso deseado del plásmido (por ejemplo, administración de una vacuna que contiene secuencias que codifican la fusión de ligado de dominio-inmunoglobulina Los promotores adecuados para la expresión de las proteínas y polipéptidos dados aquí son ampliamente disponibles y son bien conocidos en la técnica Los promotores o promotores constitutivos inducibles que son ligados a las regiones regulatorias son los preferidos Tales promotores incluyen, por ejemplo, pero sin limitarse a, el promotor fago T7 y otros promotores fago similares a T7 tales como el T3, T5 y los promotores SP6, el trp, el lpp y los promotores lac, tale como el lacUV5, del E coli, el P10 o el promotor polihedrino de gen o los sistemas de expresión de células de baculovirus/insecto (ver, por ejemplo, Patentes estadounidenses Nos 5,243,041, 5,242,687, 5,266,317, 4,745,051, y 5,169,784) y promotores inducibles a partir de otorgamiento sistemas de expresión eucarióticos Para la expresión de las proteínas tales promotores son insertados en el plásmido en enlace operativo con una región de control tal como la lac operon

Regiones promotoras preferidas son aquellas que son inducibles y funcionales en células de mamíferos, por ejemplo Ejemplos de promotores inducibles y regiones promotoras adecuadas para expresión bacteriana incluyen, pero no se limita a El operador lac de la E coli responsable para la isopropil β-D-tiogalactopiranosida (IPTG, ver Nakamura et al , 1979 Cell 18 1109-1117), El promotor metalotioneina de elementos regulatorios de metal responsable para la inducción metales pesados (por ejemplo, zinc) (ver, por ejemplo, la patente estadounidense No 4,870,009), El promotor fago T7 lac responsable para el IPTG (ver, la patente estadounidense No 4,952,496, y Studier et al , 1990 Meth Enzymol 185 60-89) y el promotor TAC Dependiendo del sistema de anfitrión de expresión que vaya a ser usado, los plásmidos pueden incluir opcionalmente un gen marcador seleccionable o genes marcadores seleccionables que son funcionales en el anfitrión Así, por ejemplo, un gen marcador seleccionable incluye cualquier gen que confiere un fenotipo sobre la bacteria que permita que las células bacteriales transformadas sean identificadas y crezcan selectivamente entre una vasta mayoría de células no trasformadas Los genes marcadores seleccionables adecuados para anfitriones bacteriales, por ejemplo, incluyen el gen resistente a la ampicilina (Amp^r), el gen resistente a la tetraciclina (Tc^r) y el gen resistente a la kanamicina (Kan^r)

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El gen resistente a la kanamicina es actualmente el preferido para la expresión bacterial

En varios sistemas de expresión, los plásmidos u otros vectores pueden también incluir ADN que codifica una señal para la secreción de la proteína ligada operativamente Las señales de secreción adecuadas para uso son ampliamente disponibles y son bien conocidas en la técnica Las señales de secreción procarióticas y eucarióticas funcionales en la E coli pueden ser empleadas Dependiendo de los sistemas de expresión, presentes en las señales de secreción preferidas pueden incluir, pero no limitarse a, aquellas codificadas por los siguientes genes de E coli ompA, ompT, ompF, ompC, beta-lactamasa, y fosfatasa alcalina y similares (von Heijne, J Mol Biol 184 99-105, 1985) En adición, la señal de secreción de gen peIB bacterial (Lei et al , J Bacteriol 169 4379, 1987), la señal de secreción phoA, y la cek2 funcional en células de insectos pueden ser empleadas La señal de secreción más preferida para ciertos sistemas de expresión es la señal de secreción ompA de la E coli Otras señales de secreción procarióticas y eucarióticas conocidas por aquellos versados en la técnica también pueden ser empleadas (ver, por ejemplo, von Heijne, J Mol Biol 184 99-105, 1985) Usando los métodos descritos aquí, una persona versada en la técnica puede sustituir las señales de secreción que son funcionales, por ejemplo, en la levadura,

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en células de insectos o de mamíferos para secretar proteínas de aquellas células

Los plásmidos preferidos para la transformación de la célula E coli incluyen los vectores de expresión pET (por ejemplo, pET-11a, pET-12a-c, pET-15b, ver, la patente estadounidense No 4,952,496, disponible de Novagen, Madison, WI) Otros plásmidos preferidos incluyen los plásmidos pKK, particularmente el pKK 223-3, que contiene el promotor tac (Brosius et al , 1984 Proc Natl Acad Sci 81 6929, Ausubel et al , Current Protocols in Molecular Biology, patentes estadounidenses Nos 5, 22,463, 5,173,403, 5,187 153, 5,204,254, 5,212,058, 5,212,286, 5,215,907, 5,220,013, 5,223,483, y 5,229,279) El plásmido pKK ha sido modificado por reemplazo del gen con resistencia a la ampicilina con un gen con resistencia a la kanamicina (disponible de farmacia, obtenido del pUC4K, ver, por ejemplo, Vieira et al (1982 Gene 19 259-268, y la patente U S No 4,719,179) Los vectores de Baculovirus,tales como el pBlueBac (también llamado pJVETL y derivados de el), particularmente el pBlueBac III (ver, por ejemplo, la patentes U S Nos 5,278,050, 5,244,805, 5,243,041, 5,242,687, 5,266,317, 4,745,051, y 5,169,784, disponible de Invitrogen, San Diego) pueden también ser usadas para la expresión de los polipéptidos en células de insectos Otros plásmidos incluyen los plásmidos pIN-IIIompA (ver la patente U S No

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4,575,013, ver también Duffaud et al , Meth Enz 153 492-507, 1987), tal como el pIN-IIIompA2

Preferiblemente, si una o más moléculas de ADN son replicadas en células bacterianas, el anfitrión preferido es la E coli La molécula de ADN preferida en tal sistema también incluye un origen bacterial de replicación, para asegurar el mantenimiento de la molécula de ADN de la generación a generación de la bacteria De esta manera, grandes cantidades de la molécula de ADN pueden ser producidas por replicación en la bacteria En tale sistemas de expresión, los orígenes de replicación bacterial preferidos incluye, pero no se limitan a, el f1-ori y el col E1 como orígenes de replicación Los anfitriones preferidos para tales sistemas tiene contienen copias cromosomales del ADN que codifica la polimerasa T7 ARN ligada operativamente a un promotor inducible, tal como el promotor lacUV (ver Patente U S No 4,952,496) Tales anfitriones incluyen, pero no están limitados a, las cepas de lisogenes E coli HMS174 (DE3) pLysS, BL21 (DE3) pLysS, HMS174 (DE3) y BL21 (DE3) La cepa BL21 (DE3) es la preferida La cepa de pLys suministra bajos niveles de la lisozima T7, un inhibidor natural de la polimerasa T7 ARN

Las moléculas de ADN provistas también pueden contener un gen que codifica para una proteína represora La proteína

represora es capaz de represar la trascripción de un promotor que tienen secuencias de nucleótidos a los cuales la proteína represora se liga El promotor puede ser a su vez represado por medio de alterar las condiciones fisiológicas de la célula Por ejemplo, la alteración puede ser llevada a cabo agregando al medio de crecimiento una molécula que inhibe la habilidad para interactuar con el operador o con la proteina reguladora u otras regiones del ADN o por medio de alterar la temperatura del medio de crecimiento Las proteínas represoras preferidas incluyen, pero no se limitan al represor lacI de la E coli responsable para la inducción del IPTG, el represor λ cI857 sensible a la temperatura y similares El represor lacI de la E coli es el preferido

En general, las construcciones recombinantes de la invención objeto también contendrán elementos necesarios para la transcripción y la traducción En particular, tales elementos son preferidos en donde la construcción de expresión recombinante contenga secuencias de ácidos nucleicos que codifican proteínas de fusión de ligado de dominio-inmunoglobulina tienen el propósito de la expresión en una célula anfitriona u organismo anfitrión En ciertas modalidades de la presente invención, el tipo de célula preferido o tipo de célula específico de expresión de una célula de fusión de ligado de dominio-inmunoglobulina que codifica el gen se puede lograr por medio de colocar el gen

bajo la regulación de un promotor La escogencia del promotor dependerá del tipo de célula que va a ser transformada y el grado o tipo de control deseada Los promotores pueden ser constitutivos o activos y pueden adicionalmente ser específicos en el tipo de célula, específicos en el tejido, específicos en células individuales, o específicos en el evento, específicos temporalmente o inducibles Los promotores específicos en tipo de célula y los promotores específicos en tipo de eventos son los preferidos Ejemplos de promotores constitutivos o no específicos incluyen el promotor temprano SV40 (Patente U S No 5,118,627), el promotor perdido SV40 (Patente U S No 5,118,627), el promotor de gen temprano CMV (Patente U S No 5,168,062), y el promotor de adenovirus En adición a promotores virales, los promotores celulares son también llevables dentro del contexto de esta invención En particular los promotores celulares para los genes de monitorización son útiles Los promotores virales son preferidos, porque generalmente e ellos son promotores más fuertes que los promotores celulares Las regiones promotoras que han sido identificadas en los genes de muchos eucariotes incluyendo eucariotes altos, tal que los promotores adecuados para uso en una anfitrión particular pueden ser fácilmente seleccionados por aquellos versados en la técnica

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Los promotores inducibles también pueden ser usados Estos promotores incluyen el MMTV LR (PCT WO 91/13160), inducible por dexametasona, el promotor de metalotioneina, inducible por metales pesados, y promotores con elementos de respuesta de cAMP, inducibles por cAMP Por medio de usar un promotor inducible la secuencia de ácido nucleico que codifica una proteína de fusión de ligado de dominio-inmunoglobulina puede ser suministrada a una célula por la construcción de la expresión de la invención objeto y permanecerá calmada hasta la adición del inductor Esto permite un control adicional sobre la temporización de la producción del producto de gen

Los promotores específicos a tipo de evento son activos y regulados solamente sobre la ocurrencia de un evento, tal como una tumorigenicidad o infección viral El HIV LTR es un ejemplo bien conocido de un promotor específico de evento El promotor queda inactivo al menos que el producto gen *tat* este presente, lo que ocurre luego de una infección viral Algunos promotores de tipo evento también son específicos en tejido

Adicionalmente, los promotores que son regulados coordenadamente con un gen celular particular pueden ser usados Por ejemplo, promotores de genes que son expresados coordinadamente pueden ser usados cuando la expresión de una construcción de ligado particular de la invención, por ejemplo, un gen que codifica a una proteína de fusión de

ligado de dominio-inmunoglobulina es deseado en concierto con la expresión de uno o más genes introducidos endogenosos o exogenosamente adicionales Este tipo de promotor es especialmente útil cuando uno conoce el patrón de expresión de gen relevante para la inducción de una respuesta inmune de un tejido particular del sistema inmune, de modo que las células inmunocompetentes específicas dentro de ese tejido pueden ser activadas o de otra manera reclutadas para participar en la respuesta inmune

En adición al promotor, secuencias represoras, reguladores negativos o silenciadores específicos a tejido pueden ser insertados para reducir la expresión no específica de los genes que codifican proteínas de fusión de ligado de dominio-inmunoglobulina en ciertas situaciones, tales como, por ejemplo, un anfitrión que es inmunocomprometido transcientemente como parte de una estrategia terapéutica Múltiples elementos represores pueden ser insertados en la región promotora La represión de la trascripción es independiente de la orientación de los elementos represores o de la distancia desde el promotor Un pipo de secuencia represora es una secuencia aisladora Tales secuencias inhiben la trascripción (Dunaway et al , 1997 Mol Cell Biol 17 182-9, Gdula et al , 1996 Proc Natl Acad Sci USA 93 9378-83, Chan et al , 1996 J Virol 70 5312-28, Scott y Geyer, 1995 EMBO J 14 6258-67, Kalos y Fournier, 1995 Mol

Cell Biol 15 198-207, Chung et al , 1993 Cell 74 505-14) y silenciarán trascripciones de fondo no deseadas

Los elementos represores han sido también identificados en las regiones promotores en los genes para el colágeno tipo II (cartílago), la acetiltransferasa de colina, la albúmina (Hu et al , 1992 J Cell Growth Differ 3 (9) 577-588), quinasa de fosfoglicerato (PGK-2) (Misuno et al , 1992 Gene 119 (2) 293-297), y en el gen 6-fosfofructo-2-quinasa/fructosa-2,6-bifosfatasa (Lemaigre et al , Mol Cell Biol 11 (2) 1099-1106) Adicionalmente, el elemento regulatorio negativo Tse-1 ha sido identificado en un número de genes específicos del hígado, y se ha mostrado que bloquea el elemento de respuesta al cAMP (CRE) por inducción mediada de la activación del gen en hepatocitos (Boshart et al , 1990 Cell 61 (5) 905-916,)

En modalidades preferidas, los elementos que incrementan la expresión del producto deseado son incorporados dentro de la construcción Tales elementos incluyen sitios de ligado de ribosoma internos (IRES, Wang y Siddiqui, 1995 Curr Top Microbiol Immunol 203 99, Ehrenfeld and Semler, 1995 Curr Top Microbiol Immunol 203 65, Rees et al , 1996 Biotechniques 20 102, Sugimoto et al , 1994 Biotechnology 12 694) El IRES incrementa la eficiencia de la traducción Lo mismo que otras secuencias pueden promover la expresión Para algunos genes, las secuencias especialmente en el

extremo 5' inhiben la trascripción y/o la traducción Estas secuencias son palíndromos usualmente y pueden formar estructuras de horquilla Cualquiera de tales secuencias en el ácido nucleico para ser suministrados son generalmente eliminadas Los niveles de expresión de trascripto o del producto traducido son ensayados para confirmar o asegurar cuales secuencias afectan la expresión Los niveles de trascripción pueden ser ensayados por cualquier método conocido incluyendo la hibridización por manchado Northerm, la protección de probeta Rnasa y similares Los niveles de proteína pueden ser ensayados por cualquier método conocido incluyendo ELISA, manchado western, inmunocitoquimica y otras técnicas bien conocidas

Otros elementos pueden ser incorporados dentro de las construcciones de la invención, por ejemplo, dentro de construcciones que codifican la proteína de fusión de ligado de dominio-inmunoglobulina de la presente invención En modalidades preferidas, la construcción incluye una secuencia terminadora de trascripción, incluyendo una secuencia de poliadenilación, sitios aceptor y donor de división, y un mejorador Otros elementos útiles para la expresión y mantenimiento de la construcción en células de mamiferos u otras células eucarióticas pueden también ser incorporadas (por ejemplo, el origen de la replicación) Debido a que las construcciones se producen convenientemente en células

bacterıales, los elementos que son necesarios para, o que mejoran, la propagación en la bacterıa se incorporan Tales elementos ıncluyen un orıgen de replıcación, un marcador seleccionable y sımılares

Como se ha sumınıstrado aquí, un nıvel adıcıonal para controlar la expresión de las construcccıones que codıfıcan los ácıdos nucleıcos de la ınvención, por ejemplo, proteínas de fusión de lıgado de dominıo-ınmunoglobulına, sumınıstradas a células para terapıas de gen, por ejemplo, pueden ser sumınıstras por medıo de sumınıstrar sımultáneamente dos o más construccıones de ácıdo nucleıco dıferencıalmente reguladas El uso de tal construcción múltıple de ácıdo nucleıco puede permıtır la regulación coordınada de una respuesta ınmune tal como, por ejemplo, una coordınación espacıotemporal que depende del tıpo de célula y/o de la presencıa de otros componentes codıfıcados expresados Aquellos famılıarızados con la técnıca podrán aprecıar que nıveles múltıples de expresión de gen regulado pueden lograrse de una manera sımılar por medıo de la selección de secuencıas reguladoras adecuadas, ıncluyendo pero sın lımıtarse a, promotores, mejoradores y otros elementos regulatorios de gen bıen conocıdos

La presente ınvención también se relacıona con vectores, y a construcciones preparadas a partır de vectores conocıdos que

incluyen ácidos nucleicos de la presente invención, y en particular a "construcciones de expresión recombinante", incluyendo cualesquiera de varias construcciones conocidas, incluyendo construcciones de suministro, útiles para la terapia de gen, que incluye cualquier ácido nucleico que codifica, por ejemplo, proteínas de fusión de ligado de dominio-inmunoglobulina y polipéptidos de acuerdo con la invención según se ha provisto aquí, para las células anfitrionas que son generadas por ingeniería genética con vectores y/o a métodos de administrar la expresión u otras construcciones que comprenden secuencias de ácidos nucleicos que codifican, por ejemplo, polipéptidos y proteínas de fusión de ligado de dominio-inmunoglobulina de la invención, o fragmentos o variantes de ellas por medio de técnicas recombinantes

Varias construcciones de la invención, incluyendo por ejemplo, proteínas de ligado de dominio-inmunoglobulina, puede ser expresadas en virtualmente cualquier célula anfitriona, incluyendo células anfitrionas in vivo en el caso de uso en terapia de gen, bajo el control de promotores apropiados, dependiendo de la naturaleza de la construcción (por ejemplo, tipo de promotor, según se describió anteriormente), y sobre al naturaleza de las células anfitrionas deseadas (por ejemplo, si son terminalmente postmitóticas diferenciadas o divididas activamente, por

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ejemplo, si la construcción de expresión ocurre en la célula anfitriona como un episoma o es integrada dentro del genoma de la célula anfitriona)

Los vectores de expresión y clonación apropiados para uso con anfitriones procarióticos y eucarióticos son descritos, por ejemplo, por Sambrook, et al , Molecular Cloning A Laboratory Manual, Second Edition, Cold Spring Harbor, NY, (1989), como se ha anotado aquí, en modalidades particularmente preferidas de la invención, la expresión recombinante es conducida en células de mamíferos que han sido transfectadas o transformadas con las construcciones de expresión recombinante objeto de la invención Ver también, por ejemplo, Machida, CA ,"Viral Vectors for Gene Therapy Methods and Protocols", Wolff, JA,"Gene Therapeutics Methods and Applications of Direct Gene Transfer" (Birkhauser 1994), Stein, U and Walther, W (ediciones P, "Gene Therapy of Cancer Methods and Protocols" (Humana Press 2000), Robbins, PD (ed), "Gene Therapy Protocols" (Humana Press 1997), Morgan, JR (ed), "Gene Therapy Protocols" (Humana Press 2002), Meager, A (ed), "Gene Therapy Technologies, Applications and Regulations From Laboratory to Clinic" (John Wiley & Sons Inc 1999), MacHida, CA y Constant, y JG, "Viral Vectors for Gene Therapy Methods and Protocols" (Humana Press 2002), "New Methods Of Gene Therapy For Genetic Metabolic Diseases NIH Guide,"Volume 22, Número 35, Octubre

1, 1993 Ver también las patentes estadounidenses recientes relacionadas con terapia de genes, incluyendo vacunas, que incluyen las patentes U S Nos 6,384,210 ("Disolvente para síntesis de biopolímero, disolvente de microgotas y métodos de uso"), 6,384,203 ("Familia de inmunoreguladores designados como receptores similares a inmunoglobulina de leucocitos (LIR)"), 6,384,202 ("Compuestos activos específicos a células regulados por el ciclo de célula"), 6,384,018 ("Vacuna de polinucleótido para tuberculosis"), 6,383,814 ("Amfifilus catiónicos para suministro intracelular de moléculas terapéuticas"), 6,383,811 ("Poliamfolitos para el suministro de polliones a una célula"), 6,383,795 ("Purificación eficiente del adenovirus"), 6,383,794 ("Métodos para producir virus asociado adeno recombinante con alta titulación"), 6,383,785 ("Sistemas de expresión controlable automejorada farmacológicamente"), 6,383,753 ("Reguladores de levadura de mamíferos de proliferación celular"), 6,383,746 ("Promotor funcional para CCR5"), 6,383,743 ("Método para análisis serial de expresión de genes"), 6,383,738 ("El virus ORF P simples de Herpes es un represor de síntesis de proteína viral"), 6,383,737 ("Oxalil-CoA de Decarboxilasa humana"), 6,383,733 ("Métodos para tamizar compuestos farmacológicamente activos para el tratamiento de enfermedades tumorales"), 6,383,522 ("Composición de toxicidad reducida que contiene un agente antineoplácico y un extracto de cartílago de tiburón"), 6,383,512 ("Complejos

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vesiculares y métodos para hacerlos y usos de los mismos"), 6,383,481 ("Método para el trasplante de células de tallo hemopoietico"), 6,383,478 ("Sistema de encapsulación polimérica para promover la angiogénesis"), 6,383,138 ("Método para muestro transdérmico de analitos"), 6,380,382 ("Gen que codifica a una proteína que tiene diagnóstico, prevención, terapia, y otros usos"), 6,380,371 ("Construcción de ácido nucleico para el ciclo celular regulado por expresión de genes estructurales"), 6,380,369 ("Complejo metálico que contiene compuesto de partición de oligonucleósido y terapias"), 6,380,362 ("Polinucleótidos, polipéptidos expresados por los polinucleótidos y métodos para su uso"), 6,380,170 ("Construcción del ácido nucleico para el ciclo de la célula regula la expresión de genes estructurales"), 6,380,169 ("Metales complejos que contienen oligonucleósidos para el clivaje de compuestos y terapias"), 6,379,967 ("Herpesvirus saimiri como vector viral"), 6,379,966 ("Suministro intravascular de proteínas de proteasa de ácido nucleico no viral, y uso de los mismos")

Típicamente, por ejemplo, las construcciones de expresión se derivan de vectores de plásmidos Una construcción preferida es un vector pNASS (Clontech, Palo Alto, CA), que tiene secuencias de ácido nucleico que codifican un gen resistente a la ampicilina, una señal de polianidenilación y un sitio promotor T7 Otros vectores de expresión de mamíferos

adecuados son bien conocidos (ver, por ejemplo, Ausubel et al , 1995, Sambrook et al , supra, ver también por ejemplo, catálogos de Invitrogen, San Diego, CA , Novagen, Madison, WI, Farmacia, Piscataway, NJ, y otros) Actualmente las construcciones preferidas pueden ser preparadas de aquellas que incluyen una reductasa de dihidrofolato (DHFR) que codifica la secuencia bajo control regulatorio adecuado para promover o mejorar niveles de producción de la proteína de fusión de ligado de dominio-inmunoglobulina, dichos niveles resultan de la amplificación de los genes luego de la aplicación de un agente de selección apropiado (por ejemplo, metetrexato)

Generalmente, los vectores de expresión recombinantes incluirán orígenes de replicación y marcadores seleccionables que permiten la transformación de la célula anfitrión, y un promotor derivado de genes altamente expresados para dirigir la trascripción de una secuencia estructural aguas abajo, como se describió anteriormente La secuencia estructural heteróloga es ensamblada en la fase apropiada con la iniciación de la traducción y secuencias de terminación Así, por ejemplo, los ácidos nucleicos que codifica proteína de fusión de ligado de dominio-inmunoglobulina según se suministra aquí pueden incluir en una cualquiera de una variedad de construcciones de vector de expresión como una construcción de expresión recombinante para expresar un

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polipéptido de fusión de ligado de dominio-inmunoglobulina en una célula anfitriona En ciertas modalidades preferidas las construcciones son incluidas en formulaciones que son administradas in vivo Tales vectores y construcciones incluyen secuencias cromosomales, no cromosomales y sintéticas de ADN, por ejemplo, derivados de SV40, plásmidos bacteriales, ADN de fagos, plásmidos de levadura, vectores derivados de combinaciones de plásmidos y ADN de fago, ADN viral, tales como vacunas, adenovirus, virus de la difteroviruela aviar, y seudorabias o replicación deficiente de retrovirus compuestos se describe más adelante Sin embargo, cualquier otro vector puede ser usada para la preparación de una construcción de expresión recombinante, y en modalidades preferidas tal un vector puede ser replicable y viable en el anfitrión

Las secuencias de ADN apropiadas pueden ser insertadas dentro de un vector, por ejemplo, por una variedad de procedimientos En general, una secuencia de ADN es insertada dentro de un sitio de endonucleasa de restricción apropiado por procedimientos conocidos en la técnica Las técnicas estándar para la clonación, aislamiento de ADN, amplificación y purificación, para reacciones enzimáticas que involucran la ligasa de ADN, la polimerasa de ADN, endonucleasa de restricción y similares, y varias técnicas de separación son aquellas conocidas y comúnmente empleadas por aquellos

versados en la técnica Un número de técnicas estándar son descritas, por ejemplo, en Ausubel et al (1993 Current Protocols in Molecular Biology, Greene Publ Assoc Inc & John Wiley & Sons, Inc , Boston, MA), Sambrook et al (1989 Molecular Cloning, Second Ed , Cold Spring Harbor Laboratory, Plainview, NY), Maniatis et al (1982 Molecular Cloning, Cold Spring Harbor Laboratory, Plainview, NY), Glover (Ed) (1985 DNA Cloning Vol I and II, IRL Press, Oxford, UK), Hames and Higgins (Eds), (1985 Nucleic Acid Hybridization, IRL Press, Oxford, UK)

La secuencia de ADN en el vector de expresión está enlazada operativamente a por lo menos una secuencia de control de expresión apropiada (por ejemplo, un promotor constitutivo o un promotor regulado) para dirigir la síntesis de mARN Los ejemplos representativos de tales secuencias de control de expresión incluyen los promotores de células eucarióticas o sus virus, como se describió anteriormente Las regiones promotoras pueden ser seleccionadas de cualquier gen deseado usando vectores CAT (cloramfenicol tranferasa) u otros vectores con marcadores seleccionables Los promotores eucarióticos incluyen el CMV temprano inmediato, el HSV de timidina quinasa, el SV40 temprano y tardío, los LTR de retrovirus, y la metalotioneína-I de ratón La selección del vector y el promotor apropiados esta bien dentro del nivel del conocimiento de los versados en la técnica, y la

preparación de construcciones de expresión recombinantes preferidas particulares comprenden por lo menos un promotor o promotor regulado operablemente enlazado a un ácido nucleico que codifica un polipéptido de fusión de ligado de dominio-inmunoglobulina como se describió aquí

La trascripción del ADN que codifica las proteínas y polipéptidos incluidos dentro de la presente invención por eucariotes altos puede ser incrementada insertando una secuencia mejoradora dentro del vector Los mejoradores son elementos cis-actuantes del ADN, usualmente cerca de 10 a 300 bp que actúan sobre un promotor para incrementar su trascripción Ejemplos que incluyen el mejorador SV40 sobre el lado tardío del origen de replicación bp 100 a 270, un citomegalovirus temprano como mejorador y promotor, el mejorador de polioma sobre lado tardío del origen de replicación, y los mejoradores de adenovirus

La terapia de genes es el uso de material genético para tratar enfermedades Este comprende estrategias para reemplazar genes defectuosos o agregar nuevos genes a células y/o tejidos, y está siendo desarrollada para la aplicación en el tratamiento de cáncer, la corrección de desórdenes metabólicos y en el campo de la inmunoterapia Las terapias de genes de la invención incluyen el uso de varias construcciones de la invención, con o sin un vehículo

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separado o un vehículo de suministro o construcciones, para el tratamiento de enfermedades, desórdenes, y/o condiciones anotadas aquí Tales construcciones pueden también ser usadas como vacunas para tratamiento o prevención de enfermedades, desórdenes, y/o condiciones anotadas aquí Las vacunas de administración, por ejemplo, pueden hacer uso de polinucleótidos que codifican proteínas y ácidos nucleicos inmunogénicos determinantes para estimular el sistema inmune contra patógenos o células tumorales Tales estrategias puede estimular sea la inmunidad adquirida o innata o pueden involucrar la modificación de la función inmune por medio de la expresión de citoquina En la terapia de genes in vivo se involucra la inyección directa del material genético en un paciente o modelo animal de enfermedad humana Las vacunas y la modificación inmune son terapias sistémicas Con terapias in vivo específicas de tejido, tales como aquellas que buscan tratar el cáncer, el suministro de genes localizados y/o la expresión y sistemas de expresión/objetivos son los preferidos Los vectores para terapia de genes diversos que han sido designados para tejidos específicos como objetivo, y los procedimientos han sido desarrollados para tejidos específicos físicamente objetivados, por ejemplo, usando tecnologías con base en catéter, todas de las cuales se contemplan aquí Las enseñanzas ex vivo para la terapia de genes es también contemplada aquí e involucra la remoción, modificación genética, expansión y readministración de

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células propias de un paciente Ejemplos incluyen transplante de médula ósea para el tratamiento de cáncer o la modivación genética delas células progenitoras linfoides La terapia de genes ex vivo es preferiblemente aplicada al tratamiento de células que son fácilmente accesibles y pueden sobrevivir en cultivo durante el proceso de transferencia de genes (tal como células de sangre o de piel)

Los vectores para terapias de genes útiles incluyen vectores adenovirales, vectores lentivirales, vectores de virus asociado con adeno (AAV), vectores del virus simple Herpes (Hsv), y vectores retrovirales Las terapias de genes también pueden portar usando "ADN desnudo"suministro basado en lipsoma, y suministro basado en lípidos (incluyendo ADN sujetado a lípidos positivamente cargados), y electroporación

Como se ha provisto aquí, en ciertas modalidades, incluyendo pero sin limitarse las modalidades de terapia de genes, el vector puede ser un vector viral tal como, por ejemplo, un vector retroviral Miller et al , 1989 BioTechniques 7 980, Coffin and Varmus, 1996 Retroviruses, Cold Spring Harbor Laboratory Press, NY Por ejemplo retrovirus de los cuales los vectores de plámido retroviral pueden ser derivados, incluyen, pero no se limitan a, Virus de Leucemia Murino Moloney, virus de necrosis de bazo, retrovirus tales como el

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Virus de Sarcoma de Rous, el virus de Sarcoma de Harvey, el virus de leucosis aviar, el virus de inmunodeficiencia humana, adenovirus, Virus de Sarcoma Mieloproliferativo, y virus de tumor mamario

Los retrovirus son virus de ARN que pueden replicar e integrarse dentro del genoma de una célula anfitriona por medio del intermedio del ADN Este intermedio de ADN, o provirus puede ser integrado de manera estable dentro del ADN de la célula anfitriona De acuerdo con ciertas modalidades de la presente invención una construcción de expresión puede comprender un retrovirus dentro del cual un gen extraño que codifica una proteína extraña es incorporado en lugar del ARN retroviral normal Cuando el ARN retroviral ingresa en la célula anfitriona coincidente con la infección, el gen extraño es también introducido dentro de la célula, y puede entonces ser integrado dentro del ADN de la célula anfitriona como si fuera parte del genoma retroviral La expresión de este gen extraño dentro del anfitrión resulta en la expresión de la proteína extraña

La mayoría se los sistemas de vector retroviral que han sido desarrollados para terapia de genes son basada en retrovirus de murina Tales retrovirus existen en dos formas, como partículas virales libre referidas como viriones, o como provirus integrados en el ADN de la célula anfitriona La

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forma virión del virus contiene las proteínas estructurales y enzimáticas del retrovirus (incluyendo la transcriptasa inversa de la enzima), dos copias de ARN del genoma viral, y porciones de la membrana de plasma celular de fuente que contiene la glicoproteína de la envoltura viral El genoma retroviral está organizado en cuatro regiones principales, la repetición de terminal largo (LTR), que contiene elementos actuantes-cis necesarios para la iniciación y terminación de la trascripción y está situado tanto en el 5' yen el 3' de los genes de codificación, y los tres genes de codificación *gag, pol* y *env* Estos tres genes *gag, pol* y *env* codifican, respectivamente estructuras virales internas, proteínas enzimáticas (tales como la integrasa), y la glicoproteína de envoltura (designada como gp70 y p15e) que confiere infectividad y una especificidad de rango anfitrión del virus, lo mismo que el péptido "R" de función indeterminada

Líneas de célula de empaque separadas y vectores que producen las líneas de células han sido desarrollados debido a problemas de seguridad con respecto a los usos de los retrovirus incluyendo su uso en construcciones de expresión según se ha provisto por la presente invención Brevemente, esta metodología emplea el uso de dos componentes, un vector retroviral y una línea de células de empaque (PCL) El vector retroviral contiene repeticiones de terminales largos (LTR), el ADN extraño a ser transferido y una secuencia de empaque

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(y) Este vector retroviral no se reproducirá por si mismo porque los genes que codifican las proteínas estructurales y de envoltura no están incluidas dentro del genoma del vector El PCL que contiene genes que codifican el *gag, pol* y *env,* pero que no contiene la señal de en empaque "y" Así, un PCL puede solamente formar partículas de virión vacías por sí mismas Dentro de este método general, el vector retroviral es introducido dentro del PCL, creando así una línea de células productora de vector (VCL) Este VCL fabrica particulas de virión que contiene solamente el genoma retroviral del vector (extraño), y por lo tanto ha sido considerado previamente como un vector de retrovirus seguro para uso terapéutico

La "construcción de vector retroviral" se refiere a un conjunto que es, dentro de las modalidades preferidas de la invención, capaz de dirigir la expresión de una secuencia o gen de interés, tal como secuencias de ácido nucleico que codifican la fusión de ligado de dominio-inmunoglobulina Brevemente, la construcción del vector retroviral puede incluir un sitio de ligado 5'LTR, a tARN, una señal de empaque, un origen de una segunda síntesis de cadena de ADN y un 3'LTR Una amplia variedad de secuencias heterólogas pueden ser incluidas dentro de la construcción del vector, incluyendo por ejemplo, secuencias que codifican una proteína (por ejemplo, la proteína citotóxica, antígeno asociado a

enfermedad, una molécula inmune accesoria, o urgente reemplazado),los cuales con útiles como molécula misma (por ejemplo como una secuencia de ribozima o antisentido)

Las construcciones de vector retroviral de la presente invención pueden ser construidas fácilmente a partir de una amplia variedad de retrovirus, incluyendo por ejemplo retrovirus tipo B, C, y D lo mismo que espumavirus y lentivirus (ver, por ejemplo, RNA Virus de Tumor, Segunda Edición, Cold Spring Harbor Laboratory, 1985) Tales retrovirus pueden ser obtenidos fácilmente a través de depositario o colecciones tales como American Type Culture Collection ("ATCC", Rockville, Maryland) o aislados de fuentes conocidas usando técnicas comúnmente disponibles Cualquiera de los retrovirus anteriores puede se utiliza fácilmente para poder ensamblar o construir construcciones dd vector retroviral, células de empaque, o células productoras de la presente invención dada la descripción provista aquí, y las técnicas estándar recombinantes (por ejemplo, Sambrook et al, Molecular Cloning A Laboratory Manual, 2d ed , Cold Spring Harbor Laboratory Press, 1989, Kunkle, 1985 PNAS 82 488)

Promotores adecuados para uso en vectores virales generalmente pueden incluir, pero no se limitan a, el LTR retroviral, el promotor SV40, y el citomegalovirus humano

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(CMV) promotor descrito en Miller, et al , 1989 Biotechniques 7 980-990, o cualquier otro promotor (por ejemplo, promotores celulares tales como promotores eucarióticos incluyendo, pero no están limitados a, la histona, los promotores pol III, y β-actin) Otros promotores virales que pueden ser empleados incluyen, pero no se limitan a, promotores de adenovirus, timidina quinasa (TK), y promotores de parvovirus B19 La selección de un promotor será evidente para aquellos versados en la técnica a partir delas enseñanzas contenidas aquí, y puede estar entre promotores regulados o promotores según se describió anteriormente

Como se describió anteriormente, el vector de plásmido retroviral es empleado para traducir líneas de célula de empaque para formar líneas de células productoras Ejemplos de las células de empaque que pueden ser transfectadas incluye, pero no se limitan a, líneas de células de PE501, PA317,Ψ-2, Ψ-AM, PA12, T19- 14X, VT-19-17-H2, ΨCRE, ΨCRIP, GP+E-86, GP+envAml2, y DAN como se describe en Miller, Human Gene Therapy, 1 5-14 (1990) El vector puede traducir las células de empaque por medio de cualesquier medios conocidos en la técnica Tales medios incluyen, pero no se limitan a, electroporación, el uso de liposomas, y precipitación por $CaPO_4$ En una alternativa el vector de plásmido retroviral puede ser encapsulado en un liposoma, o acoplado a un lípido, y luego administrado a un anfitrión

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La línea de célula productora genera partículas de vector retrovıral ınfecciosas que ıncluyen secuencıas de ácidos nucleıcos que codıfıcan los polıpéptıdos o las proteínas de fusión de lıgado de dominıo-ınmunoglobulına Tales partículas de vector retrovıral pueden ser empleadas, para transducır células eucarıóticas, sea ın vıtro o ın vıvo Las células eucarióticas transducıdas expresarán la secuencıa de ácıdo nucleıco que codıfıca al polıpéptido o proteína de fusión de lıgado de dominıo-ınmunoglobulına Las células eucarıóticas que pueden ser transducıdas ıncluyen, pero no se lımıtan a, células de tallo embrıónico, lo mismo que células de tallo hematopıético, hepatocıtos, fıbroblastos, células mononucleares o polimorfonucleares de sangre de cırculación perıférica ıncluyendo células mıelomonocıtıcas, lınfocıtos, mıoblastos, macrófagos de tejıdo, células dendrítıcas, células Kupffer, células lınfoıdes y reticuloendotelıales de los nodos lınfáticos y del bazo, queratınocıtos, células endotelıales y células epıtelıales bronquıales

Como otro ejemplo de una modalıdad de la ınvención en la cual un vector vıral es usado para preparar, por ejemplo, una construcción de expresión de fusión de lıgado de dominıo-ınmunoglobulına, en una modalidad preferıda, las células anfıtrıonas transducıdas por una construcción vıral recombınante que dırıge la expresión de los polıpéptıdos o

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proteínas de fusión de ligado de dominio-inmunoglobulina puede producir partículas virales que contiene polipéptidos o proteínas de fusión de ligado de dominio-inmunoglobulina expresadas que se derivan a partir de porciones de una membrana de célula anfitriona incorporada por las partículas virales durante el brote viral

En otro aspecto, la presente invención se relaciona con células anfitrionas que contienen lo que aquí se ha descrito como construcciones de ácido nucleico, tales, como, por ejemplo, construcciones de expresión de fusión de ligado de dominio-inmunoglobulina recombinante Las células anfitrionas se producen por ingeniería genética (transducidas, transformadas o transfectadas) con los vectores y/o las construcciones de expresión de esta invención que puede ser, por ejemplo, un vector de clonación, un vector vehiculo, una construcción de expresión El vector o la construcción puede estar, por ejemplo, en la forma de un plásmido, una partícula viral, un fago, etc Las células anfitrionas generadas por ingeniería genética pueden ser cultivadas en un medio nutriente convencional modificado según sean apropiados para activar los promotores, los transformantes selectivos o los genes particulares de amplificación tales como genes que codifican polipéptidos de fusión de ligado de dominio-inmunoglobulina o proteínas de fusión de ligado de dominio-inmunoglobulina Las condiciones de cultivo para las células

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anfitrionas particulares seleccionadas para la expresión, tales como temperatura, pH y similares, serán fácilmente visibles a aquellos expertos en la técnica

La célula anfitriona para la producción o expresión de una construcción de la invención, por ejemplo, puede ser una célula eucariótica mayor, tal como una célula de un mamífero, o una célula eucariótica menor, tal como una célula de levadura, o la célula anfitriona puede ser una célula procariótica, tal como una célula bacterial Ejemplos representativos de células anfitrionas apropiadas de acuerdo con la presente invención incluyen, pero no necesitan ser limitadas a, células bacteriales tales como la *E coli*, *Streptomyces*, *Salmonella typhimurium*, células fungales, tales como levadura, Células de insectos, tales como *Drosophila S2* y *Spodoptera Sf9*, células de animales, tales como CHO, COS o 293, adenovirus, células de plantas o cualquier célula adecuada adaptada ya a propagación in vitro o establecida de nuevo La selección de un anfitrión apropiado se hace dentro del alcance de aquellos versados en la técnica a partir de las enseñanzas dadas aquí

Diversos sistemas de cultivo de células de mamífero también pueden ser empleados para expresar proteínas recombinantes Ejemplos de sistemas de expresión de mamíferos incluyen las líneas COS-7 de fibroblastos de riñón de mono, descritas por

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Gluzman, 1981 Cell 23 175, y otras líneas de células capaces de expresar un vector compatible, por ejemplo, las líneas de células C127, 3T3, CHO, HeLa y BHK los vectores de expresión de mamífero comprenden un origen de replicación y un promotor adecuado y mejorador adecuado y también cualesquier sitios de ligado de ribosoma, sitios de poliadenilación, sitios donores y aceptores de división, secuencias de terminación transcripcional, y flancos 5' de secuencias con transcritas, por ejemplo como se describe aquí con respecto a la preparación de las construcciones de expresión de fusión de ligado de dominio-inmunoglobulina Las secuencias de ADN derivadas de las divisiones SV40, y los sitios de poliadenilación pueden ser usados para suministrar los elementos genéticos no transcritos requeridos La introducción de la construcción dentro de la célula anfitriona puede ser efectuada por una variedad de métodos con los cuales aquellos versados en la técnica tienen familiaridad, incluyendo pero sin limitarse a, por ejemplo, transfección calcio fosfato, transfección mediada por DEAE-Dextran, o electroporación (Davis et al , 1986 Basic Methods in Molecular Biology)

Las construcciones de la presente invención, por ejemplo proteínas de fusión de ligado de dominio-inmunoglobulina, o composiciones comprende uno o más polinucleótidos codifican las mismas, como se describe aquí (por ejemplo, a ser

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administrado bajo condiciones y durante un tiempo suficiente para permitir la expresión de una proteína de fusión de ligado de dominio-inmunoglobulina en una célula anfitriona in vivo o in vitro, para terapia de genes, por ejemplo, entre otras cosas) pueden ser formulada en composiciones farmacéuticas para la administración de acuerdo con metodologías bien conocidas Las composiciones farmacéuticas generalmente comprenden una o más construcciones de expresión recombinante y/o productos de expresión de tales construcciones, en combinación con un vehículo farmacéuticamente aceptable, excipiente o diluyente farmacéuticamente aceptable Tales vehículos pueden ser no tóxicos para los recipientes en dosificaciones y concentraciones empleadas Para formulaciones con base en ácido nucleico, o para formulaciones que comprenden productos de expresión de las construcciones recombinantes objeto de la invención, cerca de 0,01 µg/kg a cerca de 100 mg/kg por peso del cuerpo serán administradas, por ejemplo, típicamente por una vía intradérmica, subcutánea, intramuscular o intravenosa, o por otras rutas Una dosificación preferida, por ejemplo es cerca de 1 µg/kg a cerca de 1 mg/kg con cerca de 5 µg/kg a cerca de 200 µg/kg particularmente preferido Será evidente para aquellos versados en la técnica que el número y frecuencia de la administración dependerá de la respuesta del anfitrión Los "vehículos farmacéuticamente aceptable" para uso terapéutico son bien conocidos en la

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técnica farmacéutica, y son descritos, como por ejemplo en las Remingtons Pharmaceutical Sciences, Mack Publishing Co (A R Gennaro edit 1985) Por ejemplo, se puede usar la salina estéril, y la salina amortiguada con fosfato en un pH fisiológico Los preservativos, estabilizadores, tintas y aun agentes saborizantes pueden ser provistos en la composición farmacéutica, por ejemplo, el benzoato de sodio, el ácido sórbico y ésteres da ácido p-hidroxibenzoico pueden ser agregados como preservativos Id en 1449 Se pueden usar en adición antioxidantes y agentes de suspensión Id

Las "sales farmacéuticamente aceptables" se refiere a sales de los compuestos de la presente invención derivados de la combinación de tales compuestos y un ácido orgánico o inorgánico (sales de adición ácida) o una base orgánica o inorgánica (sales de adición base) Los compuestos de la presente invención pueden ser usados sea en base libre o en forma de sales, tanto con ambas formas siendo consideradas dentro del alcance de la presente invención

Las composiciones farmacéuticas que contiene una o más de las construcciones de ácido nucleico de la invención, por ejemplo, construcciones que codifican la proteína de fusión de ligado de dominio-inmunoglobulina (sus productos expresados) pueden estar en cualquier forma que permita que la composición sea administrada a un paciente Por ejemplo,

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la composición puede estar en una forma sólida líquida o gas (aerosol) Rutas típicas de administración incluye sin limitación, las vías orales, tópica, parenteral (por ejemplo, sublingual o bucalmente), sublingual, rectal, vaginal e intranasal El término parenteral es usado como se usa aquí, incluye las inyecciones subcutáneas, intravenosas, intramusculares, intrasternales, intracavernosas, intratecales, intrameatales, intrauretrales o técnicas de infusión La composición farmacéutica es formulada de modo que se permita que los ingredientes activos contenidos en ella sean biodisponibles luego de la administración de la composición a un paciente Las composiciones que serán administradas a un paciente toman la forma de una o más unidades de dosificación, en donde, por ejemplo, una tableta puede ser una unidad de dosificación única, y un contenedor de uno o más compuestos de la invención en aerosol pueden contener una pluralidad de unidades de dosificación

Para la administración oral, un excipiente y/o aglomerante pueden estar presente Ejemplos son la sucrosa, caolín la glicerina, almidón, dextrinas, alginato de sodio, carboximetilcelulosa, y celulosa de etilo Agentes colorante y/o saborizantes también pueden estar presentes Una cáscara de recubrimiento puede ser empleada

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La composición puede estar en la forma de un líquido, es decir, un elíxir, un jarabe, una solución, una emulsión, o una suspensión El líquido puede ser para administración oral o para suministro por inyección, como dos ejemplos Cuando se propone para administración oral las composiciones preferidas contienen, en adición a uno o más construcciones de fusión de ligado de dominio-inmunoglobulina o productos expresados, uno o más de agentes endulzantes, preservativos, tintas/colorantes, y mejoradores del sabor En una composición a ser administrada por inyección, una o más de tensoactivos, preservativos, agentes humectantes, agentes dispersantes, agentes de suspensión, amortiguadores, estabilizadores y agentes isotónicos, por ejemplo, pueden ser incluidos

Una composición líquida farmacéutica como se usa aquí, sea en la forma de una solución, suspensión u otra forma similar, puede incluir uno o más de los siguientes adyuvantes diluyentes estériles tales como agua para inyección, solución salina, salina fisiológica preferiblemente, la solución de Ringer, cloruro de sodio isotónico, aceites fijos tales como mono o diglicéridos sintéticos que pueden servir como disolventes o medios de suspensión, polietilenglicoles, glicerina, propilen glicol, u otros disolventes, agentes tales antibacteriales tales como alcohol de bencilo o metil parabeno, antioxidantes tales como ácido ascórbico o

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disulfuro de sodio, agentes quelantes tales como ácido etilendiaminetetraacético, amortiguadores tales como acetatos, citratos o fosfatos y agentes para el ajuste de la tonicidad tales como cloruro de sodio o dextrosa La preparación parenteral puede ser encerrada en ampollas, jeringas desechables o frascos de dosis múltiples hechos de vidrio o plástico La salina fisiológica es un adyuvante preferido Una composición inyectable farmacéutica es preferiblemente estéril

También puede ser deseable incluir otros componentes en la preparación, tal como vehículos de suministro incluyendo pero sin limitarse a sales de aluminio, emulsiones de agua en aceite, vehículos de aceite biodegradable, emulsiones de aceite en agua, microcápsulas biodegradables, y liposomas Ejemplos de sustancias inmunoestimulatorias (adyuvantes) para uso en tales vehículos incluye la N-acetilmuramil-L-alanina-D-isoglutamina (MDP), lipopoli-sacáridos (LPS), glucan, IL-12, GM-CSF, gama interferón e IL-15

Mientras que cualquier vehículo conocido adecuado por aquellos versados en la técnica puede ser empleado en las composiciones farmacéuticas de esta invención, el tipo de vehículo variará dependiendo del modo de administración y si se desea una liberación sostenida Para la administración parenteral, tal como inyección subcutánea, el vehículo

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preferido comprende agua, salina, alcohol, una grasa, una cera o un amortiguador Para administración oral, cualesquiera de los anteriores vehículos o u vehículo sólido tal como manitol, lactosa, almidón, estearato de magnesio, sacarina de sodio, talco celulosa, glucosa, sucrosa y carbonato de magnesio, pueden ser empleados Las microesferas biodegradables (por ejemplo, galáctida polilláctica) pueden también ser empleados como vehículos para las composiciones farmacéuticas de esta invención Las microesferas biodegradables adecuadas se han descrito, en las patentes estadounidenses No 4,897,268 y 5,075,109 A este respecto, es preferible que la microesfera sea más grande a aproximadamente 25 micrones

Las composiciones farmacéuticas pueden también contener diluyentes tales como amortiguadores, antioxidantes tales como ácido ascórbico, polipéptidos de bajo peso molecular (menos a 10 residuos), proteínas, aminoácidos, carbohidratos que incluyen la glucosa, la sucrosa o dextrinas, agentes quelantes tales como EDTA, glutationa y otros estabilizadores y excipientes La salina neutral y amortiguada o salina mezclada con albúmina de suero no específico son diluyentes ilustrativos apropiados Preferiblemente, el producto está formulado como un liofilizato usando soluciones excipientes apropiadas (por ejemplo, sucrosa) como diluyentes

Como se ha descrito anteriormente, la invención objeto incluye composiciones capaces de suministrar moléculas de ácido nucleico que codifican proteínas de fusión de ligado de dominio-inmunoglobulina Tales composiciones incluyen vectores virales recombinantes (por ejemplo, retrovirus (ver WO 90/07936, WO 91/02805, WO 93/25234, WO 93/25698, y WO 94/03622), adenovirus (ver Berkner, 1988 Biotechniques 6 616-627, Li et al , 1993 Hum Gene tiller 4 403-409, Vincent et al , Nat Genet 5 130-134, y Kolls et al , 1994 Proc Natl Acad Sci USA 91 215-219), virus de viruela (ver patente U S No 4,769,330, patente U S No 5,017,487, y WO 89/01973)), moléculas de ácido nucleico de construcción de expresión recombinante complejada a una molécula policatiónica (ver WO 93/03709), y ácidos nucleicos asociados con liposomas (ver Wang et al , 1987 Proc Natl Acad Sci USA 84 7851) En algunas modalidades, el ADN puede estar ligado a adenovirus muerto o inactivado (ver Curiel et al , 1992 Hun Gene Ther 3 147-154, Cotton et al , 1992 Proc Natl Acad Sci USA 89 6094) Otras composiciones adecuadas incluyen ligando de ADN (ver Wu et al , 1989 J Biol Chem 264 16985-16987) y combinaciones de lipido-ADN (ver Felgner et al , 1989 Proc Natl Acad Sci USA 84 7413- 7417)

Además para dirigir procedimientos in vivo, procedimientos ex vivo pueden ser utilizados en los cuales las células con

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removidas de un anfitrión, modificadas, y colocadas en el mismo o en otro animal anfitrión Será evidente que uno puede utilizar cualquiera de las composiciones anotadas anteriormente para la introducción de las construcciones de la invención, por ejemplo, proteínas de fusión de ligado de dominio-inmunoglobulina o de moléculas de ácido nucleico que codifica la proteína de fusión de ligado de dominio-inmunoglobulina en células de tejido en un contexto ex vivo Los protocolos para métodos virales, físicos y químicos de toma son bien conocidos en la técnica

De acuerdo con lo anterior, la presente invención es útil para tratar a un paciente que tenga un desorden celular B o una condición maligna, o para tratar un cultivo de células derivados de tal paciente Como se ha usado aquí el término "paciente" se refiere a cualquier animal de sangre caliente, preferiblemente un humano Un paciente puede estar afligido con cáncer o con una condición maligna a tal como el linfoma de célula B, o puede estar normal (por ejemplo, libre de enfermedad e infección detectable) Un "cultivo de célula" incluye cualquier preparación llevable a un tratamiento ex vivo, por ejemplo una preparación que contiene células inmunocompetentes o células aisladas del sistema inmune (que incluyen, pero no se limitan a, células T, macrófagos, monocitos, células B y células dendríticas) Tales células pueden estar aisladas por cualquier de una variedad de

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técnicas bien conocidas por aquellos cercados en la técnica (por ejemplo, centrifugación por densidad Ficoll-hypaque) Las células pueden (pero no se necesita que) hayan sido aisladas de un paciente afligido con un desorden de célula B o una condición maligna, y pueden ser reintroducidos dentro de un paciente luego del tratamiento

Una composición líquida con el propósito de una administración parenteral u oral debería contener una cantidad de una construcción de la invención, por ejemplo, una construcción que codifica una proteína de fusión de ligado de dominio-inmunoglobulina o el producto expresado, tal que una dosificación adecuada sea obtenida Típicamente, esta cantidad es de por lo menos 0,01% en peso de una construcción de fusión de ligado de dominio-inmunoglobulina o del producto expresado en la composición Cuando el propósito es una administración oral, esta cantidad puede variar para estar entre 0,1 y cerca de 70% del peso de la composición Las composiciones orales preferidas contiene entre cerca de 4% y cerca de 50% de la construcción de fusión de ligado de dominio-inmunoglobulina o de los productos expresados Las composiciones preferidas y preparaciones de modo que, por ejemplo, una unidad de dosificación parenteral contiene entre 0,01 a 1% en peso del compuesto activo

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La composición farmacéutica puede tener el propósito de administración tópica, en cuyo caso el vehículo puede comprender adecuadamente una solución, emulsión, ungüento, o base de gel La base, por ejemplo, puede comprender uno o más de los siguientes petrolatun, lanolina polietilenglicoles, cera de abejas, aceites mineral, diluyentes tales como agua y alcohol, y emulsificadores y estabilizadores Agentes espesantes pueden estar frecuentes en una composición farmacéutica para administración tópica Si el propósito de está administración transdérmica, la composición puede incluir un parche transdérmico o un dispositivo de iontoforesis Las formulaciones tópicas pueden contener una concentración de una construcción de la invención, por ejemplo, una construcción de fusión de ligado de dominio-inmunoglobulina o producto expresado, entre cerca de 0,1 a 10% p/v (peso por unidad de volumen)

La composición puede tener el propósito de una administración rectal, en la forma, por ejemplo, de un supositorio que se fundirá en el recto y liberará la droga La composición para administración rectal puede contener una base oleaginosa como un excipiente adecuado no irritante Tales bases incluyen, sin limitación, lanolina, mantequilla de cocoa y polietilenglicol

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En los métodos de la invención, una construcción de la invención, por ejemplo, unas construcciones que codifican una fusión de ligado de dominio-inmunoglobulina o productos expresados, puede ser administrada por medio del uso de insertos, gránulos, formulaciones de liberación temporizada, parches o formulaciones de liberación rápida

Las construcciones de la invención, por ejemplo, construcciones de ligado por antígeno de la invención, pueden ser administradas o coadministradas a un animal o paciente en combinación con, o al mismo tiempo, o alrededor del mismo tiempo, con otros compuestos En otro aspecto, una o más construcciones, incluyendo por ejemplo una o más construcciones de ligado con antígeno, son administradas a un animal o paciente en conjunto con uno o más compuestos quimioterapeuticos tales como agentes alquilantes, análogos de nucleosido, y similares La administración o coadministración de una o más construcciones incluyendo una o más construcciones de ligado con antígeno, de la invención y uno o más agentes quimioterapeúticos puede ser usado para el tratamiento de tumores o cáncer en un animal o paciente Cánceres ilustrativos incluyen pero no se limitan a, cáncer de cabeza o cuello, cáncer de pecho, cáncer colorectal, cáncer gástrico, cáncer hepático, cáncer de vejiga, cáncer cervical, cáncer endometrial, cáncer de pulmon (células no pequeñas), cáncer de los ovarios, cáncer pancreático, cáncer

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de próstata, coriocarcinoma (cáncer de pulmón), leucemia celular de cabellos, leucemia linfótica crónica, leucemia linfocítica aguda (pecho y vejiga), leucemia mielogenosa aguda, leucemia meningeal, leucemia mielogenosa crónica, eritroleucemia Más comúnmente los cánceres tratado incluyen el linfoma no-Hodgking's (sarcoma osteogénico, sarcoma de tejido suave de adulto), linfoma de célula T, leucemia linfocitica crónica, linfomas no-Hodgking's de crecimiento lento, linfoma Hodgking's y cáncer de ovario

Ejemplos de un agente alquilante que puede ser coadministrado con una o más construcciones, incluyendo una o más construcciones de ligado con antígeno, de la invención, incluye mecloretamina, clorambucil, ifosfamida, melfalan, busulfan, carmustina, lomustina, procarbazina, dacardazina, cisplatina, carboplatina, mitomicina C, ciclofosfamida, isosfamide, hexametilmelamina, tiotepa, y dacarbazina, y análogos de ellos Ver por ejemplo la patente U S No 3,046,301 que describe la sintesis de clorambucil, lapatente U S No 3,732,340 que describe la síntesis de ifosfamida, la patente U S No 3,018,302 para la síntesis de ciclofosfamida, la patente U S No 3,032,584 que describe la síntesis de melfalan, y Braunwald et al ,"Harrison's Principles of Internal Medicin,"15t Ed , McGraw-Hill, New York, NY, pp 536-544 (2001) para aspectos clínicos de ciclofosfamida, clorambucil, melfalan, ifosfamida,

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procarbazina, hexametılmelamına, cısplatına, y carboplatına Ejemplos de análogos de nucleosıdo, ıncluyen, pero no se lımıtan a, fludarabına pentostatına, metotrexato, fluorouracıl, fluorodeoxıurıdına, CB3717, azacitıdına, cıtarabına, floxurıdına, mercaptopurına, 6-tıoguanına, cladrıbına y análogos de ellos Un ejemplo es la combınación de construcciones ıncluyendo construcciones de lıgado con antígeno que lıgan el CD20 Está construcción actúa como un agente quımıosensıble y trabaja junto con agentes quımıoterapéutıcos, tal que menos agentes quımıoterapéutıcos son necesarios para lograr efectos antıtumor o anticáncer Por ejemplo, la patente U S No 3, 923,785 descrıbe la síntesis de pentostatına, la patente U S No 4,080,325 descrıbe la sıntesıs del metotrexato, la patente U S No 2,802,005 descrıbe la síntesıs del fluorouracil, y Braunwald et al ,"Harrison's Prıncıples of Internal Medıcıne,"15th Ed , McGraw-Hıll, New York, NY, pp 536-544 (2001) para aspectos clínıcos del metotrexato, 5-fluorouracılo, cıtoquına arabınosıda, 6-mercaptopurına, 6-tıoguanina, y fosfato de fludarabina

En otro aspecto, una o más construcciones, ıncluyendo una o más construcciones de lıgado con antígeno de la ınvención pueden ser adminıstradas o coadminıstradas con compuestos que ınhıben la topoısomerasa II o compuestos que de alguna otra manera ınteractúan con ácıdos nucleıcos en las células Tales

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compuestos incluyen, por ejemplo, doxorrubicina, epirrubicina, etoposida, teniposida, mitoxantrona, y análogos de ellos En un ejemplo, está combinación es usada en tratamientos para reducir la contaminación de células tumorales delas células progenitores de sangre periférica (PBSC) en conjunto con quimioterapia de alta dosis y soporte de células de tallo autólogo (HDC-ASCT) Ver la patente U S 6,586,428 a Geroni et al

En otro aspecto, una o más construcciones, que incluyen una o más construcciones de ligado de antígeno, de la invención pueden ser administradas o coadministradas con drogas terapéuticas Por ejemplo, Virulizina (Lorus Therapeutics), que se cree que estimula la liberación del factor de necrosis tumoral, TNF-alfa, por células tumorales in vitro y activación estilumalato de células macrófagas Esto puede ser usado en combinación con una o más construcciones, incluyendo una o más construcciones de ligado con antígeno, de la invención para incrementar la apoptosis celular cancerosa y tratar diversos tipos de cánceres incluyendo el cáncer pancreático, melanoma maligno, el sarcoma de Kaposi (KS), el cáncer de pulmón, cáncer de pecho, cáncer uterino, de ovarios y cervical Otro ejemplo es CpG 7909 (Coley Pharmaceutical Group), que se cree que activa células NK y monocitos y promueve el ADCC Esta droga puede ser usada en combinación con construcciones específicas de cáncer o tumor, incluyendo

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construcciones de ligado con antígeno, de la invención tales como una construcción anti-CD20, para tratar el linfoma no-Hodgkin's u otros cánceres

Una o más construcciones, incluyendo una o más construcciones de ligado con antígeno de la invención también pueden ser combinadas con inhibidores de angiogénesis para incrementar los efectos antitumorales La angiogénesis es el crecimiento de nuevos vasos sanguíneos Este proceso permite que los tumores crezcan y logren metástasis La inhibición de la angiogénesis puede ayudar a prevenir la metástasis y detener el esparcimiento de las células tumorales Los inhibidores de angiogénesis incluyen, pero no se limitan a, angiostatina, endostatina, trombospondina, factor de plaquetas 4, inhibidor derivado de cartílago (CDI), retinoides, Interleucina-12, inhibido de tejidos de metaloproteinasa 1, 2 y 3 (TIMP-1, TIMP-2, y TIMP-3) y proteínas que bloquean la cascada de señalización de angiogénesis, tal como anti-VEGF (Vascular Endothelial Growt Factor) y IFN-alfa Los inhibidores de angiogénesis pueden ser administrados o coadministrados con construcciones específicas a tumor, incluyendo construcciones de ligado con antígeno capaces de mediar, por ejemplo, ADCC y/o fijación complementaria o ligado con antígeno conjugado con quimioterapia de la invención para combatir diversos tipos de cánceres, por ejemplo, cánceres de tumor sólido tales como cáncer del pulmón o de pecho

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En otro aspecto, una o más construcciones, incluyendo una o más construcciones de ligado con antígeno, de la invención pueden ser administradas o coadministrada con agentes antirreumáticos que modifican la enfermedad (agentes DMAR) para el tratamiento de artritis reumatoide, soriasis, colitis ulcerativa, lupus eritematosus sistémico (SLE), enfermedad de Crohn's, espondilitis anquilosa, y diversas procesos de enfermedad inflamatoria En tales tratamientos, las construcciones, por ejemplo, las construcciones de ligado con antigeno, de la invención son administradas comunmente en conjunto con compuestos tales como azatiofrina, ciclosforina, oro, hidroxicloroquina, metotrexato, fenicalamina, sulfasalazina, y similares

En otro aspecto, una o más construcciones, incluyendo una o más construcciones de ligado con antígeno, de la invención pueden ser administradas o coadministradas con agentes o compuestos que contractuan los efectos biológicos de la interleucina-1 incluyendo por ejemplo inhibidores de interleucina-1 y antagonistas de receptor de interleucina-1 Se ha pensado que la interleucina-1 tiene un papel en la generación de la artritis reumatoide (RA), inflamación, y la destrucción de las articulaciones Los inhibidores IL-1 también pueden ser en conjunto con las construcciones, incluyendo construcciones de ligado con antígeno, de la

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invención para tratar la artritis, la enfermedad de inflamación del intestino, choques de sepsis y sépticos, daño isquémico, reperfusión, daño isquémico cerebral tal como parálisis cerebral y esclerosis múltiple Ver la patente U S 6,159,460 otorgada a Thompson et al En otro aspecto, por ejemplo, una o más construcciones, que incluyen una o más construcciones de ligado con antígeno, de la invención pueden ser administradas o coadministradas a un animal o paciente en conjunto con uno o más glocorticoides por ejemplo, metilprednisilona, desametaxona, hidrocortisona, y similares Los glucocorticoides han sido usados para inducir apoptosis e inhibir el crecimiento, independiente de ADCC y CDC Estos compuestos pueden ser combinados con construcciones, incluyendo construcciones de ligado con antígeno, de la invención capaz de inducir apoptosis en células cancerosas En un ejemplo las construcciones de ligado con antígeno anti-CD20, y anti-CD40, que pueden ser usados para inducir la apoptosis en células B, son combinadas con glucocorticoides para tratar el linfoma Hodgkin's de las células B (NHL)

En otro aspecto, una o más construcciones, incluyendo una o más construcciones de ligado con antígeno, de la invención pueden ser administradas o coadministradas con inhibidores o antagonistas de p38 La vía de proteína quinasa activada por el mitogén p38 está involucrada en un número de procesos celulares instrumentales para el desarrollo de la artritis

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reumatoide Por ejemplo, la activación e infiltración de leucocitos lo mismo que el producto de citoquinas inflamatorias son procesos dependientes del p38

En otro aspecto, una o más construcciones, incluyendo una o más construcciones de ligado con antígeno, de la invención pueden ser administradas o coadministradas con compuestos que promueven la diferenciación y proliferación de las células B Las citoquinas tales como interleucina-4(IL-4) e interleucina-6 (IL-6), en adición a otras actividades biológicas, se han mostrado que estimulan la síntesis del anticuerpo y la secreción por medio de linfocitos B activados En un aspecto particular de la invención, las construcciones, incluyendo una o más construcciones de ligado con antígeno, que reconocen y ligan CD20 son coadministradas con una o más interleucina-4(IL-4) e interleucina-6 (IL-6)

En otro aspecto, una o más construcciones, incluyendo una o más construcciones de ligado con antígeno, de la invención pueden ser administradas o coadministradas con interleucina-2 (IL-2) La interleucina-2 (IL-2) es una linfocina que incrementa el producto de células efectoras, tales como las células ayudadoras a T CD4+, las células citotóxicas CD8, las células B de productores de anticuerpos, las células asesinas naturales (NK), y monocitos/macrófagos Las células T que

producen ayudas IL-2, que a su vez secretan más del IL-2 (un "lazo autocrino") El IL-2 puede ser usado para aumentar la citotoxicidad mediada por células dependiente de anticuerpo (ADCC) e inmunoterapia asociadas con construcciones de la invención En un ejemplo, una construcción anti-CD20 de la invención e IL-2 son usadas para tratar pacientes con linfoma no-Hodgkin's folicular recaimiento o refractuario En otro ejemplo IL-2 es administrado o coadministrado con inmunoterapia de HIV para ayudar con la recuperación de las células T

En otro aspecto, una o más construcciones, incluyendo una o más construcciones de ligado con antígeno, de la invención pueden ser administradas o coadministradas con interleucina-12 (IL-12) La (IL-12) se conoce porque promueve las respuestas de las células T citoliticas, promueve el desarrollo de las células T ayudadoras, promueve la actividad de las células asesinas naturales (NK), e induce la secreción de IFN-γ en las células T y NK El IL-12 también incrementa muchas células ayudadoras y efectoras que median la apoptosis En otro aspecto de la invención, una o más construcciones, incluyendo una o más construcciones de ligado con antígeno, son administradas o coadministradas con IL-12 en el tratamiento de un animal o paciente con un tumor o cáncer Por ejemplo, una construcción que incluye una construcción de ligado con antígeno, de la invención que liga

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el CD20 combinado con IL-2 para el tratamiento de un paciente con el linfoma no-Hodgkin's de célula B (NHL)

Una o más construcciones, incluyendo una o más construcciones de ligado con antígeno, de la invención también pueden ser combinadas con inmunomoduladores para potenciar la eficacia de las construcciones de ligado con antígeno de la invencion Los inmunomoduladores incluyen pero no se limitan a, Factores Estimulantes de Colonias (CSF), Factores de Necrosis Tumoral (TNF), e Interferones (IFN)

Los CSF pueden incluir CSF de granulocito-macrófago (GM-CSF), granulocito-CSF (G-CSF), y macrófago CSF (M-CSF) El GM-CSF se piensa que regula el desarrollo de los neutrofilos, macrófagos, monocitos y eosinofilos El G-CSF se ha mostrado que induce la producción de neutrofilos, y la producción de M-CSF El M-CSF se ha mostrado que estimula los macrófagos y los monocitos El uso de los CSF para tratar neutropenia en pacientes con cáncer ha sido ampliamente establecido En un ejemplo, las construcciones, incluyendo construcciones de ligado con antígeno, de la invención pueden ser combinadas construcción GN-CSF, G-CSF o combinaciones de ellos para poder acelerar la recuperación de la neutropenia en pacientes después de un trasplante de médula ósea y de quimioterapia Los neutrofilos juegan un papel principal en pelear contra microbios tales como bacterias, hongos y parásitos Los

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pacientes con neutropenia son particularmente susceptibles a las bacterias y a infecciones fungales de amplio esparcimiento En otro ejemplo, una construcción incluyendo una construcción de ligado con antígeno, de la invención pueden ser combinados con GM-CSF y neutrofilos tratados, monocitos y macrófagos para incrementar la actividad contra las bacterias, hongos, etc Incluyendo carinii Neumocistis del miedo

Un ejemplo de un IFN es el interferon alfa (IFN-α) El IFN-α está hecho naturalmente por algunos tipos de células sanguíneas blancas como parte de la respuesta inmune cuando el cuerpo reacciona contra cánceres o infecciones virales Este tiene dos modos principales de ataque, interfiriendo con el crecimiento y proliferación de células cancerosas y este potencia la producción de células t asesinas u otras células que atacan las células cancerosas El interferon también se ha pensado que facilita que las células cancerosas generen señales químicas que puedan hacerlas mejores objetivos para el sistema inmune, y ha sido usado en años recientes para diversos tipos diferentes de cáncer, particularmente cáncer de riñón, melanoma, mieloma múltiple, y algunos tipos de leucemia También se ha usado para tratar infecciones virales tales como la hepatitis El interferon-alfa2a, por ejemplo, promueve el ADCC y puede ser combinado con una o más construcciones, incluyendo construcciones de ligado con

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antígeno, de la invención para incrementar la eficiencia de la actividad de ADCC asociada con la construcción En otro ejemplo, una o más construcciones, incluyendo construcciones de ligado con antígeno, de la invención son administradas o coadministradas a un animal o paciente con interferon-gama(IFN-γ), que se ha mostrado que incrementa el numero de antígenos anti-CD20 en células B y en células de plasma de médula ósea(BMPC) Esto es particularmente útil en el tratamiento de pacientes con mielomas múltiples, que tienen una expresión reducida de CD20 en sus células B y en las células del plasma de la médula ósea (BMPC) De acuerdo con lo anterior, el tratamiento de pacientes con mieloma múltiple con construcciones, que incluyen construcciones de ligado con antígeno, de la invención, en particular construcciones que ligan el CD20 pueden ser útilmente coadministradas en conjunto con el IFN-γ

El TNF es una clase de químicos naturales con propiedades anticancerígenas Un ejemplo de un TNF es el TNF-alfa El TNF-alfa también se ha mostrado que tiene efectos sinérgicos con IFN-gama e IL-12 En otro ejemplo, el TBF puede ser administrado o coadministrado con una o más construcciones específicas a tumor, incluyendo una o más construcciones de ligado con antígeno, de la invención, e incluyen construcciones de ligado con antígeno conjugado con quimioterapia de la invención, junto con el IFN-gama, IL-12 o

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diversas combinaciones de ellos El TNF es también conocido como una molécula de regulación antinflamatoria Los anticuerpos TNF-alfa o antagonistas pueden ser combinados con construcciones de célula anti-T, incluyendo construcciones de ligado con antígeno, de la invención para tratar pacientes con artritis reumatoide, soriasis, colitis ulcerativa, lupus eritematosus sistémico (SLE), enfermedad de Crohn's, espondilitis ancilosia, y diversos procesos de enfermedad inflamatoria

En otro aspecto, una o más construcciones, incluyendo una o más construcciones de ligado con antígeno, de la invención pueden ser administradas o coadministradas con otras construcciones de anticuerpo o de ligado con antígeno de la invención Un ejemplo es una construcción, por ejemplo, una construcción de ligado con antígeno de la invención capaz de ligar el CD20 combinado con una construcción capaz de ligar el CD22, CD19 o combinaciones de ellos Esta combinación es efectiva como un tratamiento para formas indolentes y agresivas de linfomas de célula B, y formas agudas o crónicas de leucemias linfáticas Ver la patente U S 6,306,393 a Goldberg En otro ejemplo, las construcciones incluyendo construcciones de ligado con antígeno, de la invención son coadministradas con otras construcciones tales como construcciones de ligado con antígeno de la invención que ayudan a mediar la apoptosis Por ejemplo, una combinación de

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una o más construcciones, incluyendo una o más construcciones de ligado con antígeno de la invención capaces de ligar con CD28, CD3, CD20 o una combinación de ellos La combinación del anti-CD28 y CD3 suministran un método para la proliferación prolongada de células T Ver la patente U S 6,352,694 otorgada a June et al Esta proliferación prolongada de células T incrementa la citotoxidad de eficiencia inmuno dependiente, particularmente aquellas asociadas con anti-CD20

En otro aspecto, una o más construcciones, incluyendo una o más construcciones de ligado con antígeno, de la invención pueden ser administradas o coadministradas con una o más moléculas regulatorias de las células T Un ejemplo es una combinación con interleucina-12 (IL-12) La citoquina IL-12 estimula la inmunidad mediada por células, tiene actividad angiostática, y posee efectos antitumorales significativos en una variedad de modelos de tumor La IL-12 también se ha demostrado que estimula la producción de interferón-gama (IFN-γ) De acuerdo con lo anterior, el tratamiento de pacientes con mieloma múltiple con unas o más construcciones, incluyendo una o más las construcciones de ligado con antigeno, de la invención, en particular aquellas que ligan el CD20, se espera que sean más eficaces cuando son coadministradas en conjunto con el IL-12 En otro ejemplo, una o más construcciones, incluyendo una o más las

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construcciones de ligado con antígeno, de la invención pueden ser administradas o coadministradas con una construcción de ligado con dominio de la invención u otra proteína capaz de ligar CTLA-4 para promover la respuesta inmune antitumoral, por medio de inhibir la activación subregulada de células T

En otro aspecto, una o más construcciones, incluyendo una o más construcciones de ligado con antígeno, de la invención pueden ser combinadas con terapias e genes En un ejemplo, una construcción conjugada con quimioterapia de la invención es administrada o coadministrada con el oligonucleótido antisentido Bci-2 El Bcl-2 está asociado con la resistencia tumoral a terapias anticáncer, y se ha creído que bloquea la muerte celular inducida por quimioterapia En otro ejemplo una o más construcciones, incluyendo una o más construcciones de ligado con antígeno, de la invención es administrada o coadministrada con un adenovirus para suministrar un "gen suicida" Los adenovirus insertan el gen directamente en las células tumorales, lo que hace que estas células sensibles a una droga de otra manera inefectiva Los tratamientos con droga entonces destruyen las células tumorales, mientras dejan las células saludables sin tocar Sin embargo, UNA VEZ QUE LA terapia está completa las células cancerosas que escapan a la terapia pueden reestablecer y metastatisarze La terapia de gen combinada con una o más construcciones, incluyendo una o más construcciones de ligado con antígeno

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ayudarán a matar las células cancerosas permanentes y minimizan la reocurrencia del cáncer

Una combinación similar puede ser usada con operaciones paliativas (no radicales) a quirúrgicas de remoción de tumores En este ejemplo una o más construcciones, incluyendo una o más construcciones de ligado con antígeno, de la invención pueden ser administradas antes y después de extracción quirúrgica de tumores para poder incrementar la respuesta inmune y reducir la posibilidad de la reocurrencia por medio de matar cualesquier células cancerosas que no fueron removidas dentro de la cirugía

Otro aspecto combina una vacuna de cáncer o antígeno y moléculas reguladoras de células T Por ejemplo, la porción de ligado, por ejemplo, una porción de ligado con antígeno, de una construcción puede ser especifica para una célula cancerosa o antigeno O un fragmento de proteina de una célula cancerosa o antígeno Esto puede ayudar a mediar una respuesta inmune contra un tumor particular o antígeno Tales construcciones pueden ser combinadas con reguladores de células T para incrementar la eficiencia de la respuesta inmune

En otro ejemplo, una o más construcciones, incluyendo una o más construcciones de ligado con antigeno, de la invención

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son administradas o coadministradas con retinoides Los retinoides incluyen la vitamina A y sus derivados, que tienen la habilidad de detener que las células se dividan y cause que ellas se diferencien La vitamina a es combinada con una construcción anticáncer, incluyendo construcción de ligado con antígeno, de la invención para combatir diversas formas de cáncer

Los términos "construcción de ligado" y "construcción de ligado con antígeno" como se usan aquí pueden referirse a, por ejemplo, polipéptidos producidos por ingeniería genética, polipéptidos recombinantes, sintéticos, semisintéticos u otras proteínas de fusión que son capaces de ligar un objetivo, por ejemplo, un antígeno Las construcciones de ligado con antígeno de la invención pueden ser usadas en diversas aplicaciones, incluyendo aquellas dentro de la variedad de uso a las cuales los anticuerpos o construcciones tipo inmunoglobulina relacionados pueden ser colocadas Las construcciones, incluyendo construcciones de ligado con antígeno pueden ser usadas en experimentos in vivo o in vitro para propósitos terapéuticos, de diagnóstico, de investigación u otros propósitos Tales usos incluyen, los siguientes

Las construcciones, incluyendo construcciones de ligado con antigeno de la invención pueden ser usados para aplicaciones

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de inmunohistoquímica Por ejemplo, ellos pueden ser usados para la inmunolocalización de un antigeno particular o grupos de antígenos en de un tejido El tejido puede ser fijo o incubado con construcciones de ligado con antígeno de interés Estas construcciones pueden ser localizadas usando un anticuerpo secundario o construcción de ligado de la invención Acoplado A una etiqueta, por ejemplo, a una partícula de oro, o a una enzima que da una reacción química, similar a la peroxidasa del rábano o la beta-galactosidasa Un anticuerpo secundario o construcción de ligado es frecuentemente hecha reactiva contra, por ejemplo, una porción de la construcción de ligado primaria Así, por ejemplo, si la construcción de ligado primaria tiene una porción de cola humana, el anticuerpo secundario o la construcción de ligado podría ser, por ejemplo, un anticuerpo anti-ratón de conejo o construcción de ligado con antígeno que ha sido enlazado a beta-galactosidasa Alternativamente el anticuerpo o construcción de ligado de la invención puede ser purificado y entonces conjugados con otra molécula para producir una anticuerpo fluorescente o construcción de ligado

Las construcciones, incluyendo construcciones de ligado con antígeno de la invención también pueden ser usadas para detectar la localización de un antígeno o antígenos sobre las superficies de las células o para detectarla localización de

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materiales intracelulares usando, por ejemplo, microscopía de inmunoeléctrones Los materiales densos en electrones tales como ferritin u oro coloidal, por ejemplo, pueden ser conjugados a una construcción de ligado con antígeno El escaneo con microscopía de electrones puede ser usado para detectar la localización del complejo de construcción agente antigeno/ligado

Las construcciones, incluyendo las construcciones de ligado con antígeno de la invención también pueden ser usadas para cuantificar la presencia de un antígeno o antigenos usando entre una variedad de formatos de inmunoensayo, por ejemplo, un formato de radioinmunoensayo (RIA) o un formato de ensayo inmunosorbente ligado con enzimas (ELISA) Hay muchas variantes de estas enseñanzas, pero aquellas son basadas en la idea similar Por ejemplo, si un antígeno puede ser ligado a un soporte o superficie sólida, o está en solución, este puede ser detectado por medio de hacerlo reaccionar con una construcción de ligado con antígeno específica de la invención La presencia o cantidad de la construcción puede entonces ser detectada, o cuantificada por medio de hacerla reaccionar con, por ejemplo, un anticuerpo secundario o una segunda construcción de ligado con antígeno de la invención por medio de incorporar una marca o etiqueta directamente en el anticuerpo primario Alternativamente, por ejemplo, un polipéptido de ligado con antígeno de la invención puede ser

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ligado a una superficie sólida y se agrega el antígeno Un segundo anticuerpo o polipéptido de ligado con antígeno de la invención que reconoce un epítopo distinto sobre el antígeno puede entonces ser agregado y detectado Esta técnica se ha llamado comúnmente "ensayo en sándwich", lo que es frecuentemente usado para evitar problemas de alto fondo o reacciones no específicas, entre otras razones

Debido a que las construcciones de ligado de la invención pueden tener alta afinidad/afinidades y/o selectividad/selectividades para un epitopo o epítopos particulares, ellas pueden también ser usadas como reactivos de afinidad, por ejemplo, en proteínas o purificación de antígenos En un ejemplo de tal proceso, las construcciones de ligado con antígeno de la invención son inmovilizadas sobre un soporte adecuado, por ejemplo, resina Sephadex o papel de filtro La construcción inmovilizada es expuesta a una muestra que contiene, o se sospecha que contiene, una proteína objetivo o antígeno El soporte es enjuagado con un amortiguador adecuado que removerá los materiales no deseados El soporte se lava con otro amortiguador que liberará la proteína ligada o el antigeno ligado

Debido a que las construcciones de ligado particulares de la invención se pueden ligar a proteínas u otros antígenos con una alta afinidad y selectividad ellas también pueden ser

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usadas como un criterio para la importancia de una enzima particular o de otra macromolécula en una reacción particular Si una construcción de ligado con antígeno de la invención puede interferir con una reacción en una solución, esto indicará que la construcción puede ligarse específicamente a una proteína u otro material antigénico involucrado en esa reacción

Las construcciones, incluyendo las construcciones de ligado con antígeno de la invención también pueden ser usadas como bloqueadores de receptor o inhibidores o antagonistas

Las construcciones, incluyendo las construcciones de ligado con antígeno de la invención también pueden ser usadas en la identificación y el estudio de funciones de las proteínas Si una construcción de ligado con antígeno de la invención reacciona con una proteína específica, por ejemplo, esa proteína puede ser subsecuentemente ser precipitada a partir de una solución, por ejemplo La precipitación es típicamente llevada a cabo usando un anticuerpo secundario o una construcción de ligado con antígeno de la invención que se ligue a complejos primarios juntos Alternativamente, el complejo puede ser removido haciendo reaccionar la solución sea con una proteína A o, por ejemplo, dependiendo de la construcción, un anticuerpo anti-Fc, por ejemplo, que ha sido

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ligado a gránulos, por ejemplo, que pueden ser removidas fácilmente de la forma de la solución

Las construcciones, incluyendo construcciones de ligado con antígeno de la invención pueden también ser usadas en conjunto con experimentos de cambio de gel para identificar proteínas de ligado con ácido nucleico específico tales como proteínas de ligado con ADN Por ejemplo, las proteínas de ligado con ADN pueden ser ensayadas por su habilidad para ligarse con alta afinidad a un oligonucleótido particular La movilidad de un oligonucleótido asociado con la proteína es bien diferente de la movilidad de un oligonucleótido libre y resulta en un patrón de migración de gel y señal que es comúnmente llamado como cambio de gel La adición de la construcción al ensayo de ligado puede tener dos efectos Si la construcción se liga a una región de una proteína que no se involucrar en el ligado de ADN puede resultar en un complejo que tiene aun una movilidad mucho más lenta y es detectado como un gran cambio en movilidad (un súper cambio) Alternativamente, si la construcción se liga a una región de la proteína involucrada en reconocer el ADN entonces este puede romper el ligado y eliminar el cambio En cualquier caso los datos de estos experimentos pueden servir como un criterio para identificar la proteína de ligado ADN, por ejemplo

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También es posible usar construcciones, incluyendo construcciones de ligado con antígeno de la invención para detectar una proteína por manchado Western después del fraccionamiento por SDS-PAGE, por ejemplo Las proteínas fraccionadas son transferidas a una membrana tal como una lámina de nitrocelulosa, ellas con expuestas a una construcción de ligado con antígeno particular de la invención que reconoce específicamente, o reconoce hasta cierto grado de selectividad, las proteínas inmovilizadas en la mancha Esto permite que las proteínas particulares sean identificadas Esta enseñanza es particularmente util si la movilidad de la proteína cambia durante un experimento Por ejemplo, la incorporación de un fosfato o un carbohidrato, o la división de la proteína resulta en un cambio en la movilidad que puede ser seguido de manera directa por el análisis Western Con controles apropiados, esta enseñanza puede ser usada para medir la abundancia de una proteína en respuesta a manipulaciones experimentales

La combinación de geles SDS y la inmunoprecipitación también pueden ser extremadamente efectivas Si una proteína particular puede ser inmunoprecipitada en una solución, tanto el sobrenadante como las fracciones precipitadas pueden ser deparadas sobre un gel SDS y estudiadas usando construcciones de ligado con antígeno de la invención

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Algunas veces una construcción de ligado de la invención dirigida contra una proteína también precipita una segunda proteína que interactua con la primera proteína La segunda proteína, lo mismo que la primera, pueden verse por medio de manchar el gel o por autoradiografía Esta relación es frecuentemente la primera indicación de que una proteína funciona como parte de un complejo y también puede ser usada para demostrar una interacción física de dos proteínas que son hipotetizadas para interactuar sobre la base de otra evidencia (por ejemplo, dos tamizados híbridos o una mutación supresora) Esta enseñanza puede ser combinada con análisis de manchado Western de diversas maneras estimadamente efectivas

Así, por ejemplo, las construcciones de ligado con antigeno de la invención pueden ser usadas en una combinación de inmunoprecipitación y análisis Western en el estudio, por ejemplo, de la transducción de señal y el procesamiento de la proteina Por ejemplo, una proteína inmunoprecipitada puede ser estudiada subsecuentemente por análisis Western usando un anticuerpo o construcción de ligado con antígeno diferente de la invención que liga a la proteína Las más útiles son aquellas que son dirigidas contra determinantes estructurales particulares que pueden estar presentes en una proteína Asi, un anticuerpo o construcción de ligado con antígeno de la invención dirigida contra una región de la proteína que se

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somete a procesamiento proteolitico puede ser útil para seguir el procesamiento proteolítico Adicionalmente, una construcción de la invención o una mezcla de construcciones de ligado con antígeno de la invención que reconoce péptidos fosforilados (por ejemplo, anti PY (tirosina fosforilada) pueden ser usados para seguir el grado de fosforilación de una proteína (usando el análisis Western) después de que se precipita, o viceversa Las reacciones de glicosilación pueden también ser seguidas por construcciones de ligado con antígeno de la invención dirigidas contra el epítopo de carbohidrato (o por lectinas, es decir proteínas que reconocen a los carbohidratos) Similarmente, algunas construcciones de ligado con antígeno de la invención también se puede hacer que reconozcan específicamente un epítopo fosforilado, por ejemplo que reconozcan una tirosina o un residuo de serina después de la fosforilación, pero que no se liguen (o se liguen detectablemente) al epítopo en la ausencia de fosfato Esta enseñanza puede ser usada para determinar el estado de fosforilación de una proteína particular Por ejemplo, la fosforilación de CREB (elemento de respuesta cAMP de la proteína de ligado) puede ser seguido por un anticuerpo que reconozca específicamente un epítopo de una manera que es dependiente de la fosforilación de la serina 133

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Las construcciones, incluyendo construcciones de ligado con antígeno de la invención también pueden ser usadas para tamizar librerías de expresión para aislar polinucleótidos candidatos que expresen o presenten un epítopo particular, o que tengan una afinidad o característica de expresión particular

Las construcciones, incluyendo las construcciones de ligado con antígeno de la invención que liguen a una superficie de célula pueden también ser usadas como un marcador para cuantificar la fracción de células que expresan el marcador usando citometría de flujo Si las construcciones de ligado con antígeno diferentes de la invención/combinaciones con tintas fluorescentes son usadas, por ejemplo, la fracción de células que expresan diversos antígenos puede ser determinada

Las construcciones, incluyendo construcciones de ligado con antígeno de la invención que funcionan como anticuerpos anti-idiotipo, es decir, anticuerpos contra el dominio de ligado de otro anticuerpo, pueden ser usados en cualquier numero de métodos en los cuales sería deseable o útil mimetizar la estructura de un antígeno Tales usos incluyen por ejemplo, usos en vacunas de cáncer (incluyendo las construcciones de ligado con antígeno de la invención que incorporan un adyuvante molecular), como probetas para receptores, como

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agonistas de receptor como antagonistas de receptor, como bloqueadores o inhibidores de receptor, etc

En otro aspecto, las construcciones, incluyendo construcciones de ligado con antígeno de la invención pueden bioespecificar y por la tanto ser capaz de ligarse a dos epítopos distintos, lo que puede estar presente en tipos de células iguales o diferentes

Los usos in vivo de construcciones de la invención, incluyendo construcciones de ligado con antígeno, incluyen terapias, solas o en combinación con una o más de otras terapias, para diversas enfermedades incluyendo cánceres lo mismo que desórdenes de células B incluyendo enfermedades autoinmunes En algunos casos, las construcciones de la invención son administradas a un paciente En otros casos, las construcciones pueden ser acopladas a otra molécula por técnicas conocidas en la técnica, por ejemplo, una molécula fluorescente para ayudar a visualizar un objetivo, o una droga terapéutica y/o una toxina para ayudar a eliminar un objetivo

Por ejemplo, una moléculas marcada o un átomo marcado puede ser conjugado o de otra manera ligado a un construcción de ligado con antígeno de la invención para ayudar a visualizarlo o como un agente de diagnóstico Estos incluyen,

pero no se limitan a etiquetas enzimáticas, radioisótopos, o compuestos radioactivos o elementos radioactivos, compuestos o metales fluorescentes, compuestos quimioluminiscentes y compuestos bioluminiscentes Así, las construcciones de ligado o construcciones de ligado con antígeno de la invención pueden ser conjugadas con una droga, lo que permiten objetivar la droga específica e incrementar la eficiencia una vez que la droga alcance el objetivo Esto facilita las terapias con droga mientras que reduce la toxicidad sistémica y los efectos colaterales Esto permite el uso de drogas que de otra manera serían inaceptables cuando son administradas sistémicamente La dosificación depende de la potencia de la droga y la eficacia de la construcción del vehículo Otros ejemplos de usos in vivo incluyen el uso de construcciones de ligado o construcciones de ligado con antígeno de la invención en las cuales una toxina es ligada químicamente o conjugada a un polipéptido de la invención para formar, pe, moléculas que puedan ser llamadas "inmunoconjugadas" o "inmunotoxinas" Típicamente, por ejemplo, tal toxina puede incluir uno o más radioisótopos (por ejemplo, Iodo-131, Itrio-90, Renio-186, Cobre-67, y/o Bismuto-212), toxinas naturales, agentes de quimioterapia, modificadores de respuesta biológica u otras sustancias que sean capaces de ayudar a dañar o matarlas células objetivo, inhibir la replicación de las células objetivo o es efectiva en romper una función celular deseada en una célula objetivo

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La porción de toxina de la inmunotoxina puede ser derivada de diversas fuentes Las toxinas se derivan comúnmente de plantas o bacterias, pero las toxinas de origen humano o toxinas sintéticas puede ser usadas también, por ejemplo Ejemplos de toxinas derivadas de bacteria o plantas incluyen pero no se limitan a abrina, α-sarcina, toxina de difteria, ricina, saporina, y exotoxina *pseudomonas* Ejemplos de enzimas de mamíferos incluyen, pero no se limitan a, ribonucleasas (RNAse) y deoxiribunocleasas Las inmunotoxinas números que pueden ser usadas con una o más construcciones de la invención han sido descritas en la técnica Ver, por ejemplo, la patente U S No 4,753,894 otorgada a Frankel et al , la patente U S No 6,099,842 a Pastan et al , Nevelle, et al , 1982 Immunol Rev 62 75-91, Pastan et al , 1992 Aran Rev Biochem 61 331-354, Chaudary et al , 1989 Nature 339 394, y Batra et al , 1991 Mol Cell Biol 11 2200 Las toxinas modificadas descritas aquí y aquellas descritas en las diversas publicaciones también están dentro del alcance de la presente invención

Generalmente, las inmunotoxinas y otros agentes terapéuticos de esta in v son administrados en una concentración que es terapéuticamente efectiva para tratar o prevenir una enfermedad particular, desorden, o condición, tal como el tratamiento de tumores y malignas, el tratamiento de

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enfermedades autoinmune alergias e inflamación, etc Esta dosificación y modo efectivo de administración depende del animal o paciente que está siendo tratado, la enfermedad o condición que está siendo tratada, la resistencia de los inmunoconjugados o inmunotoxinas y le eficiencia del conjugado Para llevar a cabo esta tarea, las inmunotoxinas pueden ser formuladas usando una variedad de formulaciones aceptables y excipientes conocidas en la técnica Típicamente, por ejemplo, las inmunotoxinas son administradas por inyección, sea intravenosamente o intraperitonealmente Los métodos para llevar a cabo esta administración son conocidos por aquellos versados en la técnica En otro aspecto, la invención incluye composiciones administradas tópicamente u oralmente tal como un aerosol, una crema o un parche que pueda ser capaz de transmisión a través de las membranas mucosas

Los formulantes pueden ser agregados a unos inmunoconjugados o inmunotoxinas de la invención antes de la administración a un paciente que está siendo tratado Una formulación líquida es más común, pero otras formulaciones están dentro del alcance de la invención Los formulantes pueden incluir por ejemplo aceites, polímeros, vitaminas, carbohidratos, aminoácidos, sales amortiguadores, albúmina, tensoactivos o agentes de llenado Los carbohidratos pueden incluir azúcar o alcoholes de azúcar tales como mono, di, o plisacáridos, o

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glucanos solubles en agua Los sacáridos o glucanos pueden incluir por ejemplo fructosa, dextrosa, lactosa, glucosa, manosa, sorbosa, xilosa, maltosa, sucrosa, dextran, pululano, dextrina, alfa y beta ciclodextrina almidón soluble, almidón de hidroxetilo y carboximetilcelulosa, o mezclas de ellas "Alcohol de azúcar" puede ser definido como un hidrocarburo con 4 a 8 carbonos que tiene un grupo -OH e incluye, galactitol, inositol, manitol, xilitol, sorbitol, glicerol, y arabitol Estos azúcares o alcoholes de azúcar mencionados anteriormente pueden ser usados individualmente o en combinación No hay límite fijado a la cantidad que se usa siempre que el azúcar o el alcohol de azúcar sea soluble en la preparación acuosa En un aspecto, al azúcar o el alcohol de azúcar en su concentración está entre 0,5 p/v % y 15 p/v %, típicamente entre 1,5 p/v %, y 7 p/v %, más típicamente entre 2,0 p/v % y 6,0 p/v %

Aminoácidos ilustrativos incluyen las formas Levorotarias (L), de camitina, arginina, y betaína Sin embargo, otrao aminoácidos pueden ser agregados Los polímeros comúnmente usados incluyen el polivinilpirrolidona (PVP) con un peso molecular promedio entre 2,000 y 3,000, por ejemplo, o polietilenglicol (PEG) con un peso molecular promedio entre 3,000 y 5,000, por ejemplo Un amortiguador que puede ser usado en la composición para minimizar los cambios de pH en la solución antes de la liofilización o después de la

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reconstitución Cualquier amortiguador fisiológico puede ser usado, pero los más comúnmente utilizados son citrato, fosfato, succinato y amortiguadores de glutamato o mezclas de ellos La concentración puede estar, por ejemplo, entre 0,01 a 0,03 molar Concentraciones mayores o menores pueden ser usadas

Las inmunotoxinas de la invención pueden ser modificadas químicamente por conjugación covalente a un polímero para incrementar su vida promedio circulante, por ejemplo Polímeros ilustrativos y métodos para sujetarlos a péptidos se referencian en las patentes U S Nos 4,766,106 otorgada a Katre et al , 4,179,337 a Davis et al , 4,495,285 a Shimizu et al , y 4,609,546 a Hiratani

Los siguientes ejemplos se ofrecen como vía de ilustración y no como vía de limitación

EJEMPLO 1

CLONACIÓN DE LAS REGIONES VARIABLES 2H7 Y LA CONSTRUCCIÓN Y SECUENCIAMIENTO DEL 2H7SCFV-IG

Este ejemplo ilustra la clonación de las moléculas de cADN que codifican las regiones variables de la cadena pesada y la cadena liviana del anticuerpo monoclonal 2H7 Este ejemplo

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también demuestra la construcción, secuenciamiento, y expresión del 2H7scFv-Ig

Antes de cosechar, las células que expresan el anticuerpo 2H7 monoclonal que específicamente liga al CD20 se mantienen en un crecimiento de fase diaria durante varios día en un medio RPMI-1640 de Invitrogen/ Life Technologies, Gaithersburg, MD) suplementado con glutamina, piruvato, aminoácidos de DMEM no esenciales, y penicilina-estreptomicina Las células se granulan por centrifugación desde el medio de cultivo, y $2x10^7$ células se usan para preparar el ARN El ARN se aísla de las células del hidridoma productoras del 2H7 usando el kit de aislamiento de ARN total Pharmingen (San Diego, CA) (catálogo No 45520K) de acuerdo con las instrucciones del fabricante que vine con el kit Un microgramo (1 µg) del ARN total se usa como plantilla para preparar el cADN por trascripción inversa El ARN y 300 ng de iniciadores aleatorios se combinan y desnaturalizan a 72° C durante 10 minutos antes de la adición de la enzima La transcriptasa inversa Superscrip II (Life Technologies) se agrega al ARN más la mezcla de iniciador en un volumen total de 25 µl en la presencia de 5X de segunda carga y 0,1 M de DTT provista con la enzima La reacción de trascripción inversa se le permite proceder a 42° C durante una hora

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El cADN del 2H7 que se genera en la reacción de transcriptasa inversa cebada aleatoriamente y los iniciadores específicos de la región V fueron usados para amplificar por PCR las regiones variables para la cadena liviana y pesada del anticuerpo 2H7 Los iniciadores específicos de la región V fueron diseñados usando la secuencia publicada números de acceso Genbank M17954 para V_L y M17953 para V_H) con una guía Las dos cadenas variables fueron designadas con secuencias de extremos compatibles de modo que un scFv puede ser ensamblado por ligado de las dos regiones V después de la amplificación y la digestión de la enzima de restricción

Un enlazador de péptido $(Gly_4Ser)_3$ para ser insertado entre las dos regiones V se incorpora por medio de agregar extranucleótidos en el iniciador antisentido par al V_L del 2H7 Un sitio de restricción Sac I se introduce también en la unión entre las dos regiones V El iniciador de sentido usado para amplificar el V_L del 2H7, que incluye un sitio de restricción HindIII y el péptido líder de la cadena liviana es de 5'-gtc aag ctt gcc gcc atg gat ttt caa gtg cag att ttt cag c-3' (ID de SEC No _) El iniciador antisentido es de 5'-gtc gtc gag ctc cca cct cct cca gat cca cca ccg ccc gag cca ccg cca cct ttc agc tcc agc ttg gtc cc-3' (ID de SEC No _) El marco de lectura de la región V se indica en negrilla, y el codón subrayado Los sitios HindIII y SacI son

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indicados por secuencia subrayadas y se colocan en letras inclinadas

El dominio V_H se amplifica sin un péptido líder, pero se incluye un sitio de restricción 5? SacI para la fusión al V_L y un sitio de restricción BclI en el extremo 3' para la fusión con colas diversas, incluyendo el dominio IgG1 Fc humano y las formas truncadas del ligando CD40, CD154 El iniciador de sentido es 5'-gct gct gag ctc tca ggc tta tct aca gca agt ctg g-3' (ID de SEC No _) El sitio SacI se indica en fuente inclinada y subrayado, y el marco de lectura del codón para el primer aminoácido del dominio V_H se indica en negrilla, tipo subrayado El iniciador antisentido es 5'-gtt gtc tga tca gag acg gtg acc gtg gtc cc-3' (ID de SEC No _) El sitio BclI se indica en letra inclinada y subrayada y la última serina de la secuencia del dominio V_H se indica en negrilla y tipo subrayado

El scFv-Ig se ensambla por medio de insertar el fragmento scFv HindIII-BclI del 2H7 en PUC19 que contiene el pivote IgG1 humana, la regiones CH2, y CH3, que son digeridas con enzimas de restricción, HindIII-BclI Después de la ligación, los productos de ligación fueron transformados en la bacteria DH5α Clones positivos se tamizan o escogen para los fragmentos insertados apropiadamente usando el sitio SacI en la unión de V_L-V_H del 2H7 como un sitio de diagnóstico El

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cADN del 2h7scFv-Ig se somete a un ciclo de secuenciamiento en un termociclador PE9700 usando un programa de 25 ciclos por medio de desnaturalizar a 96° C durante 10 segundos, templar a 50° C durante 30 segundos, y extender a 72° C durante 4 minutos Los iniciadores de secuenciamiento son iniciadores pUC delantero directo e inverso y un iniciador interno que templa al dominio CH2 humano en la porción de región constante IgG Las reacciones de secuenciamiento son llevadas a cabo usando el mezclador Ready Sequencing Mix (PE-Applied Biosystems, Foster City, CA) de acuerdo con las instrucciones del fabricante Las muestras fueron purificadas subsecuentemente usando columnas Centrisep (Catálogo No CS-901, Princeton Separations, Adelphia, N J), los eluatos se secan en un en una secador a vació Savant, desnaturalizados en el Reactivo de Supresión de Plantilla (PE-ABI), y se analiza en una analizador genético ABI 310 Genetic Analyzer (PE-Applied Biosystems) La secuencia se edita, se traduce y se analiza usando la versión Vector Nti 6 0 (Informax, North Bethesda, MD) La figura 1 muestra el cADN y predice la secuencia de aminoácido de la construcción 2H7scFv-Ig

EJEMPLO 2

EXPRESIÓN DEL 2H7 SCFV-IG EN LÍNEAS DE CÉLULAS CHO ESTABLES

Este ejemplo ilustra la expresión del 2H7scFv-Ig en una línea de célula eucariótica y la caracterización del 2H7scFv-Ig

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expresado por SDS-PAGE y por ensayos funcionales, incluyendo ADCC y ión complementaria

El 2H7scFv-Ig HindIII-XbaI (aproximadamente 1,6 kb) como fragmento con la secuencia correcta se inserta dentro del vector de expresión pD18 de mamífero, y el ADN de localización de clones positivos se amplifica usando los kits de preparación DE plásmido QIAGEN (QIAGEN, Valencia, CA) El ADN del plásmido recombinante (100 µg) es entonces linearizado en una región no esencial por digestión con AscI, se purifica por extracción de fenol, y se resuspende en un medio de cultivo de tejido, Excell 302 (Catálogo No 14312-79P, JRH Biosciences, Lenexa, KS) Las células para la trascripción, las células CHO de DG44, se mantienen en un crecimiento logarítmico, y 10^7 células se cosecharon por cada reacción de transfección El ADN linearizado se agrega a las células CHO en un volumen total de 0,8 ml para la electroporación

El producto estable de la proteina de fusión scFv-Ig del 2H7 (ID de SEC No 10) se logra por electroporación de un plásmido amplificable seleccionable pD18, que contiene el cADN del scFv-Ig del 2H7 bajo el control del promotor CMV, en células (CHO) de Ovario de Hámster Chino (todas las líneas de células de la American Type Culture Collection, Manassas, VA, a menos que se diga de otra manera) El casete de expresión

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2H7 se subclona aguas abajo del promotor CMV dentro del sitio de clonación múltiple de vectores como un fragmento aproximadamente de 1,6 kb HindIII-XbaI El vector pD18 es una versión modificada del pcADN3 que codifica el marcador seleccionable DHFR con un promotor atenuado para incrementar la presión de selección para el plásmido El ADN del plásmido se prepara usando los kits Qiagen maxiprep y el plásmido puro se lineariza en un sitio AscI y unico antes de la extracción de fenol y la precipitación con etanol El ADN del esperma de salmón (Sigma- Aldrich, St Louis, MO) se agrega como ADN portador, y 100 µg de cada uno del ADN del plásmido y del portador se usan para transfectar 10^7 células DG44 del CHO por electroporación Las células se hacen crecer en fase logarítmica en un medio Excell 302 (JRH Biosciences) que contiene glutamina (4mM), piruvato, insulina recombinante, penicilina-estreptomicina, y aminoácidos no esenciales DMEM 2X (todos de Life Technologies, Gaithersburg, Maryland), llamado de aquí en adelante como el medio "Excel 302 completo" el medio para las células no transfectadas también contiene HT (diluido de una solución 100X de hipoxantina y timidina) (Invitrogen/Life Technologies) El medio para las transfecciones bajo selección contienen niveles varios de metotrexato (Sigma Aldrich) como agente selectivo en un rango entre 50 nM a 5 µM Las electroporaciones se llevan a cabo a 275 voltios, 950 µF A las células transfectadas se les permite recuperarse durante la noche en un medio no selectivo

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antes del plateado selectivo en placas de fondo plano de 96 pozos (Costar) en diluciones seriales varias que varían entre 125 células/pozo a 2,000 células/pozo El medio de cultivo para la clonación celular es Excel 302 completo, que contiene 100 nM de metotrexato Una vez el crecimiento clonal es suficiente, las diluciones seriales de los sobrenadantes del cultivo desde los pozos maestros se seleccionan en cuanto a ligad a células transfectadas CD20-CHO Los clones con la producción más alta de la proteína de fusión se expanden en frascos T25 y luego T75 para suministrar los numeros adecuados de células para congelamiento y para la medición de la producción del 2H7scFv-Ig Los niveles de producción se incrementan adicionalmente en cultivos desde tres clones por medio de amplificación progresiva en metotrexato que contiene el medio de cultivo En cada pasaje sucesivo de células, el medio Excel 302 completo contiene una concentración incrementada de metotrexato, de modo que solamente las células que amplificaron el plásmido DHFR pueden sobrevivir

Los sobrenadasntes se recolectan de las células CHO que expresan el 2H7scFv-Ig, se filtran a través de filtros PES expresos de 0,2 µm (Nalgene, Rochester, NY) y se pasan sobre una columna de proteína A-agarosa (IPA 300 de agarosa reticulada) (Repligen, Needham, NA) La columna se lava con PBS, y luego la proteína ligada se eluye usando 0,1 de amortiguador de citrato, pH 3,0 Las fracciones se recolectan

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y la proteína eluida se neutraliza usando un 1M Tris, pH 8,0, antes de la diálisis durante la noche en PBS La concentración del 2H7scFv-Ig puro (ID de SEC No _) se determina por absorción a 280 nm Un coeficiente de extinción de 1,77 se determina usando las herramientas de análisis de proteína en el paquete de software Vector de Nti y versión 6,0 (Informax, North Bethesda, MD) Este programa utiliza los datos de la composición de aminoácido para calcular los coeficientes de extinción

Los niveles de producción del 2H7scFv-Ig por células CHO transfectadas estables son analizados por citometría de flujo El 2H7scFv-Ig puro a las células CHO permite ligar a las células CHO que expresan CD20 (CD20 CHO) y se analiza por citometria de flujo usando un reactivo de segunda etapa de IgG anti-humano de fluoresceína conjugados (Catálogos No H10101 y H10501, CalTag, Burlingame, CA) La figura 2 (superior) muestra una curva estándar que se genera por titulación del 2H7scFv-Ig que liga al CD20 CHO En cada concentración de 2H7scFv-Ig, la brillantez media de la señal de fluoresceína en unidades lineales se muestra Los supernadantes que se recolectan de los frascos de T que contienen clones de célula CHO estables que expresan 2H7scFv-Ig de les permite entonces ligar al CD20 CHO y el ligado se analiza por citometría de flujo La señal de fluoresceína se genera por 2H7scFv-Ig contenida en los sobranadantes se mide

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y la concentración 2H7scFv-Ig en los sobrenadantes se calcula a partir de la curva estándar (figura 2, inferior)

El 2H7scFv-Ig purificado (ID de SEC No _) se analiza por electroforesis sobre geles SDS-Poliacrilamida Las muestras de 2H7scFv-Ig purificadas por las corridas en columna de proteína A-agarosa independiente, se hierven en un amortiguador de muestra SDS sin reducción de los enlaces de disulfuro y se aplicada al SDS 10% Tris-Bis geles (Catálogo No NP0301, Novex, Carlsbad, CA) Veinte microgramos de cada tanda purificada se cargan sobre los geles Las proteínas se visualizan después de la electroforesis por manchado Coomassie Azúl (Pierce Gel Code Blue Stain Reagent, Catálogo No 24590, Pierce, Rockford, IL), y se desmancha en agua destilada Los marcadores moleculares se incluyen en el mismo gel (Kaleidoscope Prestained Standards, Catálogo No 161-0324, Bio-Rad, Hercules, CA) Los resultados se presentan en la figura 3 Los números por encima de las pistas designan las tandas de purificación independientes Los pesos moleculares en kilodaltons de los marcadores de tamaño se indican en el lado izquierdo de la figura Experimentos adicionales con condiciones de preparación de muestra alternativa indican que la reducción de los enlaces de disulfuro por medio del hervido de la proteína en el amortiguador de muestra SDS que contiene DTT o 2-mercaptoetanol causa que el 2H7scFv-Ig se agregue

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Cualquier número de otros parámetros inmunológicos puede ser monitoreados usando ensayos de rutina que son bien conocidos en la técnica Estos pueden incluir, por ejemplo, citotoxicidad mediada por células dependiente del anticuerpo ADCC), respuestas secundarias de anticuerpo in vitro, análisis de flujo inmunocitofluorométricos y de diversas poblaciones celulares mononucleares linfoides o de sangre periférica usando sistemas de antígeno marcadores establecidos, inmunohistoquímica u otros ensayos relevantes Estos y otros ensayos pueden encontrarse, por ejemplo, en in Rose et al (Eds), Maslual of Cli72ical Laborato7-y Immunology, 5th Ed , 1997 American Society of Microbiology, Washington, DC

La habilidad del 2H7scFv-Ig para matar células positivas CD20 en la presencia del complemento, se ensaya usando lineas de células B Ramos y Bjab El complemento de conejo (Pel-Freez, Rogers, AK) se usa en el ensayo a una dilución final de 1/10 El 2H7scFv-Ig purificado se incuba con células B y el complemneto durante 45 minutos a 37° C, seguido por el conteo de células vivas y muertas por exclusión tripan azul Los resultados en la figura 4 muestran que en la presencia del complemento de conejo el 2H7scFv-Ig rompe las células B que expresaban el CD20

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La habilidad del 2H7scFv-Ig para matar células positivas CD20 en la presencia de células mononucleares de sangre periférica (PBMC) se ensaya midiendo la liberación de ^{51}Cr a partir de células Bjab marcadas en un ensayo de 4 horas usando una proporción de 100 1 de PBMC a células de Bjab Los resultados que se muestran en la figura 4B indican que 2H7scFv-Ig puede mediar la citotoxicidad celular dependiente del anticuerpo (ADCC) porque la liberación de ^{51}Cr es mayor en la presencia de tanto PBMC y 2H7scFv-Ig que en la presencia de cualquier PBMC o 2H7scFv-Ig solos

EJEMPLO 3

EFECTO DE LA LIGACIÓN SIMULTANEA DEL CD20 Y CD40 SOBRE EL CRECIMIENTO DE CELULAS B NORMALES, Y SOBRE LA EXPRESION CD95, Y INDUCCIÓN DE LA APOPTOSIS

Este ejemplo ilustra el efecto sobre la proliferación celular de la reticulación de CD20 y CD40 expresados sobre la superficie de la célula

Las células B densas restantes se aíslan a partir de amígdalas humanas por un gradiente de tapa Percoll y las células T se remueven por roseteado por E La proliferación de las células B de las amígdalas restantes densas se miden por una toma de $^{3}[H]]$-timidina durante las últimas 12 horas de un experimento de cuatro días La proliferación se mide en

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cultivos cuadruplicados con medias y desviaciones de estándar según se muestra El anticuerpo monoclonal CD20 de murina anti-humano (anti-CD20) se usa solo o reticulado con el anticuerpo K monoclonal anti-murina 187,1 (anti-CD20XL) La activación del CD40 se lleva a cabo usando CD154 humano soluble que se fusiona con CD8 de murina (CD154) (Hollenbaugh et al , EMBO J 11 4212-21 (1992)), y CD40 reticulado se lleva a cabo usando anticuerpo monoclonal CD8 53-6 anti-murina (CD154XL) Este procedimiento permite la reticulación simultáneas del CD20 y CD40 sobre la superficie celular Los resultados se dan en la figura 5

El efecto de la reticulación de CD20 y CD40 sobre células Ramos, una línea de células de linfoma B, se examina Las células Ramos se analizan en cuanto a expresión de CD95 (Fas) y porcentaje de apoptosis dieciocho horas después del tratamiento (ningún IgG anti-ratón de cabra (GAM)) y/o reticulación (+GAM) usando anticuerpos monoclonales de murina que ligan específicamente CD20 (IF5) y CD40 (G28-5) Las células de control se tratan con un control isotipo de no ligado (64,1) específico para CD3

Las células Ramos tratadas se cosecharon, se incubaron con FITC-anti-CD95, y se analizaron por citometría de flujo para determinar el nivel de expresión relativo del Fas sobre la superficie celular después de la reticulación del CD20 o

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CD40 Los datos se grafican como fluorescencia media de células luego del tratamiento con el estímulo indicado(figura 6A)

Las células Ramos tratadas del mismo experimento se cosecharon y se ligaron de anexina V se midieron para indicar el porcentaje de apoptosis en los cultivos tratados La apoptosis se mide por ligado de Anexina V 18 horas después de la reticulación de CD20 y CD40 usando 1F5 y G28-5 seguido por la reticulación con GAM El ligado de la Anexina V se mide usando un kit FITC-Annexin V (Catálogo No PN-IM2376, Immunotech, Marseille, France,) El ligado de la Anexina V se conoce que es un evento temprano en la progresión de las células hacia la apoptosis La apoptosis, o muerte celular programada es un proceso que se caracteriza por una cascada de reacciones catabólicas que llevan a la muerte celular por suicidio En la fase temprana de la apoptosis antes de que las células cambien su morfología e hidrolicen el ADN, la integridad de la membrana celular se mantiene pero las células pierden la asimetría de sus fosfolípidos de membrana, y exponiendo fosfolípidos cargados negativamente, tal como fosfatidilserina, en la superficie celular La Anexina V, una proteína de ligado en calcio y fosfolípido, se liga preferencialmente y con una alta afinidad a la fosfatidilserina Los resultados demuestran el efecto de la reticulación tanto de CD20 y CD40 sobre la expresión del

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receptor FAS (CD95) y se presentan en la figura 6B El efecto de la reticulación tanto de CD20 y CD40 sobre el ligado de Anexina V a las células se muestra en la figura 6B

EJEMPLO 4

CONSTRUCCIÓN Y CARACTERIZACIÓN DE LAS PROTEINAS DE FUSION ScFV-CD154 DEL 2H7

Para construir una molécula capaz de ligar tanto a CD20 como CD40, el cADN que codifica el scFv del 2H7 se fusiona con el cADN que codifica el CD154, y el ligando CD40 El cADNm del scFv del 2H7 que se codifica sobre el fragmento HindIII-BclI se remueve de la construcción scFvIg del 2H7, y se inserta dentro de un vector pD18 junto con un fragmento de cADN del BamHI-XbaI que codifica el dominio extracelular del CD154 humano El dominio extracelular se codifica en el terminal carboxi del CD154, similar al otro tipo II de proteínas de membrana

El dominio extracelular del CD154 humano se amplifica por PCR usando el cADN generado con iniciadores aleatorios y ARN de linfocitos T humanos activados con PHA (fitohemaglutinina) Los sistemas de iniciadores incluyen dos iniciadores 5' o sentido diferentes que crean uniones de fusión en dos posiciones diferente dentro del dominio extracelular del CD154 Las dos uniones de fusión diferentes se diseñan de

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modo que resulten en una forma corta o truncada (forma S4) que incluye aminoácidos 108 (Glu)-261 (Leu) + (Glu), y una forma larga o completa (forma 12) que incluye aminoácidos 48 (arg)-261 (leu) + (Glu), del dominio extracelular del CD154, construidos ambos como fragmentos de BamHI-XbaI El iniciador sentido que fusiona los dos dominios extracelulares truncados diferentes al 2H7scFv incluye un sitio BamHI para la clonación el iniciador de sentido para la forma S4 del cADN del CD154 se designa como identificador de secuencia No 11 o CD154BAM48 y codifica un mer 34 con la siguiente secuencia 5'-gtt gtc gga tcc aga aaa cag ctt tga aat gca a-3', mientras que el iniciador antisentido es designado como identificador de secuencia No 12 o CD154XBA y codifica un mer 44 con la siguiente secuencia 5'-gtt gtt tct aga tta tca ctc gag ttt gag taa gcc aaa gga cg-3'

Los iniciadores de oligonucleótidos usados en la amplificación de la forma larga (L2) del dominio extracelular CD154 codifican los aminoácidos 48 (Arg)-261 (Leu) + (Glu), que son como sigue El iniciador de sentido designado CD154BAM48 (ID de SEC No 13) codifica un 35-mer con la siguiente secuencia 5'-gtt gtc gga tcc aag aag gtt gga caa gat aga ag-3' El iniciador antisentido designado como CD154XBA (ID de SEC No _) codifica el 44-mer 5'-gtt gtt tct aga tta tca ctc gag ttt gag taa gcc aaa gga cg-3' Otras condiciones de la reacción PCR han sido idénticas a aquellas

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usadas para amplificar el scFv del 2H7 (ver el ejemplo 1) Los fragmentos PCR se purifican por kits rápidos PCR (QIAGEN, San Diego, CA) y se eluyen en 30 µl de ddH_2O, y se digieren con endonucleasas de restricción BamHI y XbaI (Roche) en un volumen de reacción de 40 µl e 37° C durante tres horas Los fragmentos se purifican por gel, purificados usando kits QIAEX de acuerdo con las instrucciones del fabricante (QIAGEN), y se ligan junto con el fragmento HindIII-BclI del 2H7 en el vector de expresión pD18 que se digiere con HindIII+XbaI Las reacciones de ligación se transforman en bacteria competente químicamente de DH5-alfa y plateadas en las placas LB que contiene 100 µg/ml de ampicilina Los transformantes se hicieron crecer durante la noche a 37° C, y las colonias aisladas usadas para inocular 3 ml de cultivos liquidos en Luria Broth contenían 100 µg/ml de ampicilina Los clones se tamizaron después de las preparaciones de miniplásmidos (QIAGEN) para la inserción tanto del scFv del 2H7 y los fragmentos de dominio extracelular CD154

Los cADN de la construcción 2H7scFv-CD154 se someten a ciclo de secuenciamiento sobre un termociclador Por ejemplo 9700 usando un programa de 25 ciclos que incluye la desnaturalización a 96° C 10 segundos, templado a 50° C durante 5 segundos, y extender a 60° C durante 4 minutos Los iniciadores de secuenciamiento usados fueron pD18 directo (ID de SEC No _ 5'-gtctatataagcagagctctggc-3') y pD18 inverso

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(ID de SEC No _ 5'-cgaggctgatcagcgagctctagca-3') En adición, un iniciador interno se usa el cual tiene homología a la secuencia CD154 humana (ID de SEC No _ 5'-ccgcaatttgaggattctgatcacc-3') Las reacciones de secuenciamiento incluyen iniciadores a 3,2 pmol, aproximadamente 200 ng de plantilla de ADN, y 8 µl de mezclado de secunciamiento Las reacciones de secuenciamiento se llevan a cabo usando el mezclador Big Dye Terminator Ready Sequencing Mix (PE- Applied Biosystems, Foster City, CA) de acuerdo con las instrucciones del fabricante Las muestras se purifican subsecuentemente usando columnas Centrisep (Princeton Separations, Adelphia, NJ) Los eluatos se secan en un secador al vacío de velocidad Savant, desnaturalizados en 20 µl de Reactivo de Supresión de Plantilla (ABI) a 95° C durante 2 minutos y se analiza sobre un Analizador Genético ABI 310 (PE-Applied Biosystems) La secuencia se edita, se traduce y se analiza usando la versión 6 0 del Vector Nti (Informax, North Bethesda, MD) La secuencia del cADN del 2H7scFv-CD154 L2 y a secuencia de aminoácido que se predice se presenta en la figura 7A, y la secuencia del cADN 2H7scFv-CD154 S4 y la secuencia de aminoácido que se predice se presentan en la figura 7B

La actividad de ligado de las proteínas de fusión de scFv-CD154 del 2H7 (ID de SEC No _y_) al CD20 y CD40 simultáneamente se determina por citometría de flujo El

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ensayo usa células objetivo CHO que expresan CD20 Después de una incubación de 45 minutos de las células CD20 CHO con sobrenadantes de las células transfectadas con el plásmido de expresión scFv-CD154 del 2H7, las células CD20CHO se lavan dos veces y se incuban con la proteína de fusión CD40-Ig conjugada con biotina en PBS/2% de FBS Después de 45 minutos, las células se lavan dos veces y se incuban con estrepavidina marcada-ficoeritrina (PE) a 1 100 en PBS/2% de FBS (Molecular Probes, Eugene OR) Después de una incubación de 30 minutos adicional las células se lavan 2X y se analizan por citometría de flujo Los resultados muestran que la molécula scFv-CD154 del 2H7 es capaz de ligarse al CD20 sobre la superficie celular y capturar al CD40 conjugado con biotina desde la solución (figura 8)

Para determinar el efecto del 2H7scFv-CD154 sobre el crecimiento y viabilidad del linfoma B y las líneas celulares linfoblastoides, las células se incuban con 2H7scFv-CD154 L2 (ID de SEC No _) durante 12 horas y luego se examinan en cuanto al ligado de al Adexina V El ligado de Adexina V es medido usando un kit FITC-Annexin V (Immunotech, Marseille, France, Catálogo No PN-IM2376) Las líneas de células B se incuban en cultivos de 1 ml con diluciones de sobrenadantes concentrados y dializados a partir de células que expresan formas secretadas de las proteínas de fusión 2H7scFv-CD154 Los resultados se dan en la figura 9

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La velocidad de crecimiento de las líneas de linfoma B Ramos en la presencia de 2H7scFv-CD154 se examina por una toma de ^{3}H-timidina durante las últimas 6 horas de un cultivo de 24 horas El efecto del 2H7scFv-CD154 sobre la proliferación celular se muestra en la figura 10

EJEMPLO 5

CONSTRUCCIÓN Y CARACTERIZACIÓN DE DERIVADOS DE ANTICUERPO CITOXB

Los anticuerpos CitoxB se preparan en el polipéptido scFv-IgG del 2H7 El scFv del 2H7 (ver ejemplo 1) se liga al dominio IgG1 Fc humano por vía de un dominio de pivote alterado (ver figua 11) Los residuos de cisteína en la región de pivote fueron sustituidos por residuos de serina por mutagénesis dirigida a sitio y otros métodos conocidos en la técnica El pivote mutante se funciona sea a un dominio Fc tipo natural o silvestre para crear una construcción, designada CitoxB-MHWTG1C o se fusiona con un dominio Fc mutado (CitoxB-MHWTG1C) que tiene mutaciones adicionales introducida en el dominio CH2 Los residuos de aminoácido en CH2 que están implicados en la función efectora son ilustrados en la figura 11 Las mutaciones de uno o más de estopas residuos pueden reducir el ligado al FcR y la mediación de las funciones efectoras En este ejemplo, el residuo de leucina 234

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conocida en la técnica como importante para el ligado del receptor Fc, se muta en la proteína de fusión scFv del 2H7, CitoxB-[MG1H/MG1C] En otra construcción, la región de pivote IgG1 humana se sustituye con una porción del pivote IgG1 humana, la cual se fusiona al dominio Fc humano tipo silvestre (CitoxB-IgAHWTHG1C) (Ver figura 11) Esta región de pivote mutada permite la expresión de una mezcla de moléculas monoméricas y diméricas que retiene propiedades funciones de los dominios CH2 y CH3 del IgG1 humano Los casetes de expresión del cADN recombinante sintético para estas moléculas se construyeron y los polipéptidos se expresaron en células CHODB44 de acuerdo con los métodos descritos en el ejemplo 2

Los derivados de proteína de fusión purificadas de moléculas CitoxB-scFvIg se analizan por SDS-PAGE de acuerdo con los métodos descritos en el ejemplo 2 Los geles de poliacrilamida se corren bajo condiciones de no reducción y de reducción Dos conjuntos de marcadores de peso de moléculas diferentes, marcadores pre-manchados Biorad, (BioRAD, Hercules, CA) y marcadores de peso molecular Novex Multimark se cargan en cada gel Los patrones de migración de las diferentes construcciones y del Rituximab™ se presentan en la figura 12

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La habilidad de los derivados diferentes de las moléculas CitoxB-scFvIg para mediar ADCC se miden usando las células de linfoma B Bjab como el objetivo y PBMC humano preparado frescamente como célula efectora (Ver ejemplo 2) Las proporciones de efectora a objetivo se varían como sigue 70 1, 35 1 y 18 1, con el número de células B Bjab por pozo que se mantiene constante pero el número de PBMC es variado Las células B Bjab se marcan durante dos horas con ^{51}Cr y se alicuotan a una densidad celular de 5 x 10^4 células/pozo para cada pozo de las placas de fondo plano de 96 pozos las proteínas de fusión purificadas o Rituximab se agregan a una concentración de 10 µg/ml a las diversas diluciones de PBMC La liberación espontánea se mide sin la adición de PBMC o de proteína de fusión, y la liberación m{axina se mide por la adición de detergente (1% de NP-40) a los pozos apropiados) Las reacciones se incuban durante horas y 100 µl de sobrenadante del cultivo se cosecha en un Lumaplate (Packard Instruments) y se le permite secarse durante la noche antes de contar los cpm liberados Los resultados se presentan en la figura 13

La actividad de citotoxicidad dependiente de complemento (CDC) de los derivados de CitoxB también se mide Las reacciones se llevaron a cabo esencialmente como se describen en el ejemplo 2 Los resultados se presentan en la figura 14

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como porcentajes de células muertas con respecto al total de células para cada concentración de la proteína de fusión

EJEMPLO 6

ESTUDIOS IN VIVO EN MACACO

Los estudios in vivo iniciales derivados de CitoxB han sido llevados a cabo en primates no humanos la figura 15 muestra los datos que caracterizan la vida promedio del suero de CitoxB en monos Las medidas se llevan a cabo sobre muestras de suero obtenidas de dos diferentes macacos (J99231 y K99334) después de que dosis de 6 mg/kg se administran a cada mono en los días que se indican por las flechas Para cada muestra, el nivel de 2H7scFvIg presente es estimado por comparación con una curva estándar generada por el ligado de la proteína de fusión CitoxB-(MHWTG1C)Ig purificada a células CD20 CHO (ver ejemplo 2) Los datos se tabula en el panel inferior de la figura 15

El efecto de la proteína de fusión CitoxB-(MHWTG1C)Ig sobre los niveles de células de CD40 más circulante en macacos se investigaron Cuentas de sangre completas se llevan a cabo en cada uno de los días que se indican en la figura 16 En adición, los ensayos FACS (clasificación de células activadas por fluorescencia) se llevan a cabo sobre linfocitos de sangre periférica usando fluoresceína específica al CD40

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conjugada con anticuerpo para detentar las células B dentro de la población celular El porcentaje de células positivas es entonces usado para calcular el número de células B en las muestras originales Los datos se grafican como miles de célula B por microlitros de sangre medida en lo días que se indican después de la inyección (figura 16)

EJEMPLO 7

CONSTRUCCIÓN Y EXPRESION DE UNA PROTEÍNA DE FUSION scFv-Ig ANTI-CD19

Una proteína de fusión scFv-Ig anti-CD19 se construye, se transfecta en células procarióticas y se expresa de acuerdo con los métodos presentados en los ejemplos 1, 2 y 5 y estándar en la técnica Las regiones de cadena pesada variable y de cadena liviana variable se clonan desde el ARN aislado a partir de células de hibridoma que producen el anticuerpo HD37, que específicamente se liga al CD19 Los niveles de expresión de un HD37 scFV-IgAHWTG1C y un HD37scFv-IgMHWTG1C que se miden y se comparan con una curva estándar generada usando scFvIg del HD37 purificado Los resultados se dan en la figura 17

EJEMPLO 8

CONSTRUCCIÓN Y EXPRESIÓN DE UNA PROTEÍNA DE FUSIÓN scFv-Ig ANTI-L6

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Una proteína de fusión scFv-Ig se construye usando regiones variables derivadas de un match monoclonal anticarsinoma L6 La proteína de fusión se construye, transfectando en células eucarióticas, y se expresa de acuerdo con los métodos presentados en los ejemplos 1, 2 y 5 y estándar de la técnica Los niveles de expresión de scFv-IgAH WCH2 CH3 L6 y L6 scFv-(SSS-S)H WCH2 WCH3 se miden y se comparan con una curva estándar generada usando el scFvIg L6 Los resultados se presentan en la figura 18

EJEMPLO 9

CARACTERIZACIÓN DE DIVERSAS PROTEINAS DE FUSIÓN scFV-Ig

En adición a la proteína de fusión scFv-Ig ya descrita, las proteínas de fusión G28-1 (anti-CD37) scFv-Ig se preparan esencialmente como se describió en los ejemplos 1 y 5 Las regiones variables de las cadenas pesada y liviana se clonan de acuerdo con métodos conocidos en la técnica La actividad ADCC del 2H7-MHWTG1C, 2H7-IgAHWTG1C, G28-1-MHWTG1C, G28-1 IgAHWTG1C, HD37-MEIWTG1C, y HD37-IgAHWTG1C se determina de acuerdo con los métodos descritos anteriormente (ver ejemplo 2) Los resultados se dan en la figura 19 La actividad ADCC de L6scFv-IgAHWTG1C y L6scFv-IgMHWTG1C se miden usando una línea celular carcinoma de pulmón humano 2981 Los resultados

[illegible] de llama, [illegible] medio de lisar

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se dan en la figura 20 El anticuerpo monoclonal L6 de murina se conoce que no exhibe la actividad ADCC

Las proteínas purificadas se analizan por SDS-PAGE bajo condiciones de reducción y de no reducción Las muestras se preparan y los geles se corren esencialmente como se describió en los ejemplos 2 y 5 Los resultados para la proteína de fusión L6 y 2H7 sdFv-Ig se presentan en la figura 21 y los resultados para la proteína de fusión G28-1 y HD37sdFv-Ig se presentan en la figura 22

EJEMPLO 10

CONSTRUCCIÓN Y EXPRESIÓN DE PROTEÍNAS DE FUSIÓN ANTI-CD20 scFv-LLAMAIG

Este ejemplo ilustra la clonación de los dominios de región constante llama IgG1, IgG2, e IgG3 y la construcción de proteínas de fusión de inmunoglobulina con cada una de las tres regiones constantes y anti-CD20scFv

Las regiones constantes de las inmunoglobulinas llama IgG1, IgG2, e IgG3 immunoglobulinas se clonan e insertan en construcciones de vector de mamíferos que contiene una cadena única anti-CD20 Fv, 2H7scFv El ARN total se aísla de las células mononucleares de sangre periférica (PBMC) de sangre de llama (Triple J Farms, Bellingham, WA) por medio de lisar

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los linfocitos en TRIzol® (Invitrogen Life Technologies, Carlsbad, CA) de acuerdo con las instrucciones del fabricante Un microgramo (1 µg) del ARN total se usa como plantilla para preparar cADN por trascripción inversa El ARN y 200 ng de iniciadores aleatorios fueron combinados y se desnaturalizan a 72 °C durante 10 minutos antes de la adición de la enzima La transcriptasa inversa Superscript II (Invitrogen Life Technologies) se agrega al ARN más la mezcla de iniciadores en un volumen total de 25 µl en la presencia de un amortiguador de segunda cadena 5X y 0,1 M de DTT provista con la enzima La reacción de trascripción inversa se le permite proceder a 42° C durante 1 hora El cADN se amplifica por PCR usando iniciadores específicos a secuencia Los iniciadores 5′ se designaron de acuerdo con las secuencias publicadas por los dominios V_{HH} y V_H de camélidas El iniciador 3′, que se usa para amplificar todos los tres isotipos se designa usando las secuencias del dominio CH3 de mamíferos como una guía Los iniciadores específicos siguientes se usan Los sitios BclI y XbaI están indicados por secuencia en letra inclinada subrayada

Cebador 5′ para región constante IgG1 de llama

LLG1-5′bgl 5′-gtt gt*t gat* caa gaa cca cat gga gga tgc acg tg-3′

(ID de SEC No _)

Cebador 5′ para región constante IgG2 de llama

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LLG2-5'bgl 5'-gtt gtt gat caa gaa ccc aag aca cca aaa cc-3'

(ID de SEC No _)

Cebador 5' para región constante IgG3 de llama

LLG3-5'bgl 5'-gtt gtt gat caa gcg cac cac agc gaa gac ccc-3'

(ID de SEC No _)

Cebador 3' para regiones constantes IgG1, IgG2, y IgG3 de llama

LLG123-3'X 5'-gtt gtt tct aga tta cta ttt acc cga aga ctg ggt gat gga-3'

(ID de SEC No _)

Los fragmentos PCR de tamaño esperado se clonaron en vectores de clonación TOPO® (Invitrogen Life Technologies) y luego se secuenciaron El iniciador de secuencia en sentido directo, LLseqsense, tiene la secuencia 5'ctg aga tcg agt tc gct g-3' (ID de SEC No _), y el iniciador antisentido, LLseqAS, tiene la secuencia 5'-cct cct ttg gct ttg tct c-3' (ID de SEC No _) El secuenciamiento se lleva a cabo como se describió en el ejemplo 1 La figura 23 compara la secuencia de aminoácido de los tres isotipos de regiones constantes de llama que contienen el pivote, los dominios CH2, y CH3 con la secuencia de aminoácido del pivote IgG1 humana y los CH2 y CH3

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Luego de verificar la secuencia, los productos amplificados que se digieren con enzimas de restricción BclI y Xbal para crear sitios de restricción compatibles Los fragmentos digeridos se purifican entonces con gel, y el ADN se elude usando un kit de extracción de gel QIAquick ((QIAGEN, Valencia, CA) La construcción de vector de expresión de mamífero 2H7scFv-Ig pD18 (ejemplo 2) se digiere con BclI y XbaI parta remover el pivote IgG humana, los dominios CH2 y CH3 El vector pD18 es un derivado modificado de pCDNA3 que contiene un gen de DHFR atenuado, que sirve como un marcador seleccionable para expresión de mamíferos (Hayden et al , Tissue Antigens 48 242-54 (1996)) Los productos PCR de la región constante de IgG1, IgG2, e IgG3 de llama purificados se ligan por ligasa de ADN T4 (Roche Molecular Biochemicals, Indianapolis, IN) en vector doblemente digerido 2H7 scFv-pD18 a temperatura ambiente durante la noche de acuerdo con las instrucciones del fabricante Después de la ligación, los productos de ligación se trasforman en bacterias DH5α de E coli (BD Biosciences, Palo Alto, CA) y plateadas de acuerdo con procedimiento de biologia molecular estándar e instrucciones del fabricante Las colonias aisladas se escogieron para tamizar los transformantes que contienen los insertos correctos

Para la expresión de los polipéptidos que se codifican, el ADN del plásmido de clones positivos se transfecta

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transientemente en células COS-7 usando DEAE-dextran (Hayden et al , Ther Immunol 1 3-15 (1994)) Las células COS-7 se siembran en aproximadamente 3 x 10^6 células por 150 mm de placa y se hacen crecer durante la noche haSTA que las células estén cerca de 75% confluentes Las celas son entonces lavadas una vez con DMEM libre de suero (Invitrogen Life Technologies, Grand Island, NY) El sobrenadante de transfección (10 ml) contiene 400 µg/ml de DEAE-dextran, 0,1 ml de cloroquina, y 5 µg/ml de construcciones de ADN se agregan a las células, las cuales se incuban entonces a 37° C durante 3 a 4 horas Luego de la incubación, las células se pulsan con 10 ml de 10% de sulfóxido de dimetilo (DMSO) en 1 x PBS a temperatura ambiente durante 2 minutos Las células entonces se colocan de nuevo en DMEM/10% de FBS completamente suplementado (1% de L-glutamina, 1% de pecinicilina/estreptomicina, 1% de piruvato de sodio, 1% de MEM de aminoácidos esenciales) (Invitrogen Life Technologies) Después de 245 horas el medio se reemplaza con DMEM completamente suplementado libre de suero(Invitrogen Life Technologies), y las células se mantiene hasta 21 días c on cambios en el medio cada 3 a 4 días

Las proteínas de fusión Ig se purifican por medio de pasar los sobrenadantes del cultivo de las células COS por la proteína A-agarosa ((Repligen, Cambridge, MA) en columnas de la pre A-agarosa Después de la aplicación del sobrenadante

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del cultivo las columnas de proteína A entonces se lavan con 1x PBS (Invitrogen Life Technologies) Las proteínas de fusión Ig ligadas se eluyeron con 0,1 M de ácido cítrico (pH 2,8), y las fracciones que se recolectan se neutralizan inmediatamente con base Tris (pH 10,85) Las fracciones que contienen la proteína se identifican por medio de medir la densidad óptica (A_{280}) y luego se mezclan, se dializan contra 1x PBS, (Invitrogen Life Technologies) y se filtran a través de un filtro de 0,2 µm

Las proteínas de fusión Ig purificadas se analizan por SDS-PAGE Alícuotas del IgG1 del scFv del 2H7 de llama, el IgG2 del scFv del 2H7 de llama, el IgG3 del scFv del 2H7 de llama, y Rituxan® (D (Rituximab, anti-CD20 antibody, Genentech, Inc and IDEC Pharmaceuticals Corp) (5 µg de proteína) que se combina con 25 µl 2x NuPAGE SDS como amortiguador de muestra (Invitrogen Life Technologies) (muestras no reducidas) Las muestras de cada proteína son también preparadas en amortiguador de muestra reducida que contiene 5% de 2-mercaptoetanol (Sigma-Aldrich, St Louis, MO) Los marcadores de peso molecular (Invitrogen Life Technologies) se aplican a los geles en amortiguadores no reducidos Las proteínas se fraccionan sobre NUAGE® 10% Bis-Tris geles (Invitrogen Life Technologies) Después de la electroforesis (aproximadamente 1 hora), los geles se lavan tres veces, 5 minutos cada una, con Agua Destilada (Invitrogen Life Technologies) y luego se

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manchan en 50 ml de la mancha Bio-Safe Coommassie (BioRad, Hércules, CA) durante la noche a temperatura ambiente Después de un lavado en agua destilada, los geles se fotografían El patrón de migración de cada proteína de fusión Ig se presenta en la figura 24

La habilidad de las proteínas de fusión Ig del scFv del 2H7 de llama para ligar a les células que expresan en CD20 se demuestra por citometría de flujo Diluciones seriales de partida a 25 µg/ml de IgG1 del scFv del 2H7 de llama purificada, y IgG2 del scFv de 2H7 de llama, y IgG3 de scFv de 2H7 de llama se preparan e incuban con células CHO (CD20+) CD20- transfectadas (del laboratorio del Dr S Skov, Institute of Medical Microbiology and Immunology, Copenhagen Dinamarca en 1% de FBS 1x medio de PBS (Invitrogen Life Technologies) durante una hora en hielo Después de la incubación, las células se centrifugan y se lavan con 1% de FBS en 1x PBS Para detectar el Ig del scFv de 2H7 de llama ligado, las células se incuban durante 1 hora sobre hielo con IgG anti-camélida de cabra conjugado con fluoresceína (cadena liviana y pesada) (1 100) (Triple J Farms) Las células son entonces se centrifugan y se resuspenden en 1% de FBS-1x PBS y se analizan usando un clasificador de células Coulter Epics XL (Beckman Coulter, Miami, FL) Los datos (porcentaje de brillantez máxima) se presentan en la figura 25

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EJEMPLO 11

FUNCIÓN EFECTORA DE LAS PROTEINAS DE FUSIÓN DEL Ig DEL SCFV ANTI-CD20 DE LLAMA

Este ejemplo demuestra la habilidad del IgG1, IgG2, e IgG3 como proteína de fusión anti-CD20 para mediar en la citotoxicidad dependiente del complemento (CDC) y las citotoxicidad mediada por célula dependiente del anticuerpo (ADCC)

La habilidad de las proteínas de fusión Ig del scFv del 2H7 de llama para matar células positivas CD20 en la presencia del complemento se ensaya usando la línea de células B humanas Bjab El complemento de conejo se obtiene a partir de conejos de 3 a 4 semanas de nacidos (Pel-Freez, Brown Deer, WI) Las células BJAB (2 x 10^6 células/ml) se combinan con el complemento de conejo (dilución final 1 10) y las proteínas de fusión Ig del 2H7 se purifican La IgG1 del scFv del 2H7 de llama purificada, la IgG2 del scFv de 2H7 de llama, la IgG3 de scFv de 2H7 de llama y la IgG1 del scFv humano del 2H7 tipo de pivote silvestre-Ch2CH3) (ejemplo 1) se agregan a diliciones seriales 1 3 iniciando una concentración de 30 µg/ml Después de 1 hora a 37° C la viabilidad celular se determina por medio de contar las células vivas y muertas por la exclusión tripan azul (0,4%) (Invitrogen Life Technologies) usando un hemacitómetro (Bright-line, Horsham,

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PA) El porcentaje de muertes se calcula dividendo el número de CESL muertas por el número total de células (muertas + células vivas) El dato que se presenta en la figura 26 muestra que todas las proteínas de fusión Ig tienen actividad CDC

La actividad ADCC de las proteínas de fusión del IgG del scFv de llama del 2H7 se determina usando células Bjab como células objetivo y célula mononucleares humanas o de llama de sangre periférica (PBMC) como células efectoras Las células Bjab se pre-incuban durante aproximadamente 2 horas con ^{51}Cr (100 µCi) (Amersham Biosciences, Piscataway, NJ) en IMDM completamente suplementado (Invitrogen Life Technologies) que contiene 15% de FBS Las células se mezclan intermíteteme durante el periodo de pre-incubación El PBMC human restante fresco se purifica a partir de sangre usando medio de separación de linfocitos (LSM) (ICN Pharmaceuticals, New York, NY) El PBMC se combina con células Bjab etiquetadas (5 x 10^4 células por pozo de una placa de cultivo de tejido de 96 pozos) en proporciones de 25 1, 50 1 y 100 1 A cada combinación se agrega 10 µg/ml de IgG1 del scFv de llama del 2H7 purificada, el IgG2 del scFv de llama del 2H7, y IgG3 del scFv de llama del 2H7, Rituximab, o anticuerpo no anti-CD20 Las mezclas se incuban durante 6 horas a 37° C El sobrenadante de cada reacción que contiene ^{51}Cr liberado de las células lisadas que se recolectaron en una placa de

filtro LumaPlate-96 (Packard, Meiden, CT), y que se secan durante la noche La cantidad de ^{51}Cr se mide por un lector de placa TopCount NXT (Packard) La FigurA 27 muestra que la proteína de fusión IgG2 de scFv de llama del 2H7 es la proteína de fusión de llama más efectiva en mediar al ADCC Cada punto de dato representa la medida promedio de pozos triplicados

La actividad ADCC es afectada por la fuente de las células efectoras EL PBMC de llama se aísla de la sangre de llama (Triple J Farms) usando LSM Las células efectoras de llama se agregan en las mismas proporciones a las células objetivo BJAB como se describe para el ensayo de ADCC usando células efectoras humanas Las células se combinan con 10 µg/ml de IgG1 de scFv de llama del 2H7 purificado, el IgG2 del scFv de llama del 2H7, IgG3 del scFv de llama del 2H7, Rituximab, o anticuerpo no anti-CD20 Los resultados se dan en la figura 28

EJEMPLO 12

CONSTRUCCIÓN Y CARACTERIZACIÓN DE LAS PROTEINAS DE FUSIÓN IG DEL SCFV EXPRESADAS SOBRE SUPERFICIES CELULARES

Este ejemplo describe un sistema de transfección retroviral para la expresión ectópica en superficies de receptores de superficie celular producidos por ingeniería genética

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compuestos de los scFv que ligan a los receptores coestimuladores El ejemplo también demuestra la función efectora de estas diversas proteínas de fusión Ig del scFv expresadas sobre la superficie de las células objetivo

Las regiones variables de las cadenas pesada y liviana se clonan a partir de anticuerpo de murina específicos para diversos receptores coestimuladores, y las construcciones Fv de cadena única se preparan esencialmente como se describió en el ejemplo 1 Los anticuerpos incluidos el 2H7, CD20 anti-humano, 40 2 220, CD40 anti-humano, el 2E12 CD28 anti-humano, 10A8, CD-152 anti-humano (anti-CTLA-4), y 500A2, CD3 anti-murina Las regiones variables de las cadenas pesada y liviana de cada anticuerpo se clonan de acuerdo con métodos estándar para la clonación de genes de inmunoglobulina y como se describió en el ejemplo 1 Las construcciones Fv de cadena única se preparan como se describió en el ejemplo 1 por medio de insertar una secuencia de nucleótido que codifica un enlazador de péptido $(gly_4ser)_3$ entre la región VL de la secuencia del nucleótido de 40 2 220, 2E12, 10A8, y 500A2 respectivamente (ID de SEC No _, respectivamente) y la región VH de la secuencia de nucleótido de 40 2 220, 2E12, 10A8, y 500A2, respectivamente (ID de SEC No respectivamente) La secuencia de polipéptido para VL del 40 2 220, 2E12, 10A8, y 500A2 se establece en la ID de SEC No _, respectivamente, y la secuencia de polipéptido para VH

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del 40 2 220, 2E12, 10A8, y 500A2 se establecen en la ID de SEC No _, respectivamente Cada polinucleótido scFV (ID de SEC No _ para 40 2 220, 2E12, 10A8, y 500A2, respectivamente) se fusiona a el pivote mutante IgG1 humana (CCC→SSS) y CH2 mutante (mutación de prolina a serina en el residuo 238 (238 se numera de acuerdo con la nomenclatura EU, Ward et al , 1995 Therap Immunol 2 77-94, el residuo 251 de acuerdo con Kabat et al) y los dominio CH3 tipo silvestre de acuerdo con lo métodos descritos en el ejemplo 5 y 11 Cada secuencia de polinucleótido de fusión IgG1 mutante de scFv entonces se fusiona en el marco a la secuencias que codifican el dominio de transmembrana y la cola citoplásmica del CD80 humano (ID de SEC No _), tal que cuando la proteína de fusión se expresa en la célula transfectada, el CD80 suministra un ancla para la expresión de superficie de la proteína de fusión Ig del scFv Los cADN qu codifican las proteínas de fusión scFv-IgG-CD80 (ID de SEC No _ para 4 2 220-, 2E12-, 10A8-, y 500A2 -scFv-IgG-CD80, respectivamente) de insertan en el vector retroviral pLNCX (BD Biosciences Clontech, Palo Alto, CA) de acuerdo con los procedimientos de biología molecular estándar y las instrucciones del vendedor Los cADN del scFv-Ig-CD80 se insertan entre las secuencias promotoras del gen CMV con resistencia al 5'LTR-neomicina y la secuencia 3'LTR Las construcciones retrovirales se transfectan en Reh, una línea de célula de leucemia linfocitica aguda (ATCC CRL-8286) Las

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celes transfectadas se tamızan para seleccıonar clones que expresaran las proteínas de fusıón scFV-Ig sobre la superfıcıe celular

Los ensayos CDC y ADCC se llevan a cabo con las células Reh transfectadas para determınar sı la expresión de los polıpéptıdos scFv-Ig sobre la superfıcıe celular aumentan la funcıón celular efectora Las células Reh que expresan el scFv-IgG mutante del CD152 antı-humano (ID de SEc No _), el IgG-CD80 mutante del scFv del Reh antı-humano CD28 (ID de SEC No _), el IgG mutante-CD80 del scFv del CD28 del Reh antı-humano (ID de SEC No _), el IgG mutante-CD80 scFv del CD40 del Reh antı-humano (ID de SEC No _), el IgG mutante-CD80 del scFv del CD20 antı-humano del Reh (ID de SEC No _) se combınan con PBMC humano (ver ejemplo 11) y complemento de conejo (10 µg/ml) durante una hora 37° C Las células Reh no trasfectadas se ıncluyen como una viabılıdad de control de las células que se determınan por exclusión tripan azul, y el porcentaje de células muertas es calculado (ver ejemplo 11) La figura 29 muestra la efectıvıdad de las proteınas de fusión scFv-IgG-CD80 cuando se expresan sobre la superfıcıe celular de las células tumorales para mediar la cıtotoxıcıdad dependiente de complemento

Las mismas células Reh transfectadas que se ensayan en el ensayo CDC más las células Reh transfectadas con la

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construcciones del polinucleótido que codifica el CD3 anti-murina-scFv-Ig-CD80 (ID de SEC No _) se analizan en cuanto a la actividad ADCC (ver ejemplo 11) Las células Reh no transfectadas y transfectadas se premarcan con ^{51}Cr (100, µCi) (Amersham) durante dos horas a 37° C El PBMC humano sirve como células efectoras y se agregan a las células objetivo Reh (5 x 10^4 células por pozo de la placa 96 pozos) en proporciones de 5 1, 2,5 1, y 1,25 1 Después de cinco horas a 37° C, los sobrenadantes del cultivo se recolectan y analizan como se describió en el ejemplo 11 El porcentaje específico de muertes se calcula de acuerdo con la ecuación siguiente ((Liberación del experimento menos liberación espontánea)/(liberación máxima menos liberación espontánea)) x 100 Los datos se presentan en la figura 30 Cada punto de dato representa el promedio de muestras cuadruplicadas

Usando los mismos procedimientos descritos anteriormente, los mismos resultados con otros dominios de ligado se obtienen usando los siguientes anticuerpo monoclonales como fuentes de scFv para CD20, 1F5 (Genbank AY 058907 y AY058906), para CD40, 2,36 y G28 5, para CD28, 9,3

La expresión de superficie celular de los dominios de ligado del anticuerpo se lleva a cabo por medio de fundir el anticuerpo scFvs al pivote IgA y la región que actúa del pivote IgE, es decir IgE CH2, y las regiones constantes Los

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polinucleótidos que codifican un scFv anti-4-1BB, 5B9 (4-1BB anti-humano) scFv, y 2el2 (CD40 anti-humano) se fusiona con IgAH IgA T4 (residuos CH3 de cuatro terminales eliminados) que se fusionan a la transmembrana CD80 y los dominios citoplásmicos y las regiones IgE Fc que se muestran en la ID de SEC No _ Los polipéptidos codificados se muestran en la ID de SEC No _

EJEMPLO 13

CONSTRUCCION Y SECUENCIA DE MUTANTES CH2-CH3-PIVOTE IG HUMANA Y MUTANTES DE REGIÓN VARIBLE 2H7

Este ejemplo describe la construcción de proteina de fusión scFv que contienen IgG1 humano mutante y regiones constantes IgA Este ejemplo también describe la construcción de un mutante de scFv de 2H7 con una mutación de punto único en la región de cadena pesada variable Las mutaciones se introducen en los dominios de región variable constante de acuerdo con los métodos descritos aquí y conocidos en la técnica de biología molecular La figura 312 presenta la nomenclatura para las construcciones de la región constante Ig

La región de pivote IgG1 humana de las proteinas de fusión CH2-CH3-pivote IgG1 humanas se mutan al sustituir residuos de cisteína que en una inmunoglobulina completa están

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involucrados en formar enlaces de disulfuro entre dos moléculas de cadena pesada Un mutante, scFv de 2H7 se funde a una región de pivote IgG1 humana en la cual todos lo0s tres residuos de cisteína se mutaron a residuos de serina ((MTH (SSS)), se prepara como se describe en el ejemplo 5 (designada en el ejemplo 5 como CitoxB-MHWTGIC (que incluye los dominios CH2 y CH3 de la IgG1 tipo silvestre)) (ahora se refiere como MTH (SSS) WTCH2CH3 del scFv del 2H7) y comprende la secuencia de polinucleótido ID de SEC No _ que codifica al polipéptido según se establece en la ID de SEC No _ La secuencia de polinucleótido que codifica este mutante (ID de SEC No _se usa como una plantilla para crear regiones de pivote mutantes en las cuales los primeros dos residuos de cisteína se sustituyen con residuos de serina (IgG MTH (SSC)) Un oligonucleótido se designa para sustituir el tercer residuo de serina con una cisteína y que tiene la siguiente secuencia 5'-gtt gtt gat cag gag ccc aaa tct tct gac aaa act cac aca tct cca ccg tgc cca gca cct g-3' (HuIgGMHncs3, ID de SEC No _) Un segundo mutante se prepara en el cual el pivote mutante tiene residuos de serina sustituyendo el primero y tercer residuos de (IgG MTH (SCS)) La secuencia del oligonucleótido para crear este mutante es como sigue 5'-gtt gtt gat cag gag ccc aaa tct tct gac aaa act cac aca tgc cca ccg-3' (HuIgGMHncs2, ID de SEC No _) Un tercer mutante se prepara con residuos de cisteína sustituidos en la segunda y tercera posición (IgG MTH (CSS)),

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también usando el mutante IgG MTH (SSS)como plantilla, y un oligonucleótido que tiene una de secuencia, 5'-gtt gtt gat cag gag ccc aaa tct tgt gac aaa act cac-3' (HuIgGMHncs1, ID de SEC No _)

Los oligonucleótidos que introducen las mutaciones dentro de la región de pivote son combinados con la plantilla y un 3' oligonucleótido que contiene un sitio XbaI (subrayado y en letras inclinadas (5'-gtt gtt *tct aga* tca ttt acc cgg aga cag gga gag gct ctt ctg cgt gta g-3' (ID de SEC No _)) para amplificar las secuencias (WT)-CH2-CH3 tipo silvestre por el pivote mutante por PCR Las secuencias mutantes IgG MTH CCS e IgG MTH SCS se amplifican durante 25 ciclos con un perfil de desnaturalización de 90° C, templado a 52° C durante 30 segundos y extensión a 72° durante 30 segundos La secuencia mutante IgG MTH SSC se amplifica bajo condiciones ligeramente diferentes perfil de desnaturalización de 94° C, templado a 45° C durante 30 segundos y extensión a 72° durante 45 segundos Los polinucleótidos amplificados se insertan en el vector de clonación TOPO® (Invitrogen Life Technologies) y luego se secuencian como se describe en el ejemplo 1 para confirmar la presencia de la mutación El vector pD18 que contiene el 2H7 scFV se digiere para remover las secuencias de región constante esencialmente como se describe en el ejemplo 10 Las regiones CH2-CH3 tipo silvestre del pivote mutante se inserta en el marco dentro del ADN del vector que

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se digiere para obtener los vectores que comprenden el MTH (CSS) WTCH2CH3 del scFv del 2H7 que codifica al ADN (ID de SEC No _), MTH (SCS) WTCH2CH3 del scFv del 2H7 que codifica el ADN (ID de SEC No _), y MTH (SSC) WTCH2CH3 del scFv del 2H7 que codifica al ADN (ID de SEC No _)

Una mutación de leucina a serina en la posición 11 en la primera región de estructura de la región variable de cadena pesada (numerada de acuerdo con Kabat et al , Sequences of Proteins of Immunological Interest, 5th ed Bethesda, MD Public Health Service, National Institutes of Health (1991)) se introduce en la fusión de proteína MTH (SSS) WTCH2CH3 del scFv del 2H7 (ID de SEC No _) El residuo de leucina tipo silvestre se sustituye con serina por mutagénesis que se dirige a sitio usando el oligonucleótido Vhser11 5'-gga ggt ggg agc tct cag gct tat cta cag cag tct ggg gct gag tcg gtg agg cc-3' (ID de SEC No _) (esta secuencia, o una secuencia de aminoácido que la codifica, puede ser excluida opcionalmente de las modalidades reivindicadas en particular de la presente invención) El iniciador 3'para PCR es huIgG1-3'que tiene la secuencia 5'-gtc *tct aga* cta tca ttt acc cgg aga cag-3' (ID de SEC No _) (el sitio XbaI subrayado y en letra inclinada) Después de la amplificación PCR, los fragmentos se insertan en el vector de clonación TOPO® y se secuencian para confirmar la presencia de la nutación de leucina VH11 a serina El ADN que codifica 2H7 scFv-IgG MTH

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(SSS) WTCH2CH3 se envıa en el vector de clonación PSL1180(farmacıa Bıotech, Inc , Piscataway, NJ) La construcción PSL1180-2H7 scFv-IgG MTH (SSS) WTCH2CH3 se dıgıere con Sac y XbaI para remover el dominıo VH tıpo sılvestre y los dominıos de pıvote y CH2 y CH3 El producto PCR que comprende el mutante VH11 se dıgıere con Sac y XbaI y luego se ınserta en la construcción PSL1180 que se dıgıere con procedımıentos estándar de bıología molecular La construcción es entonces dıgerıda con HındIII y XbaI, y se ınserta dentro del vector de expresión pD18 de mamífero (ver métodos descrıtos en el ejemplo 1 y ejemplo 10) El mutante se desıgna como 2H7 scFv VH11SERIgGMTH (SSS) WTCH2CH3 (Fıgura 31) La secuencıa de polınucleótıdo está provısta en la ID de SEC No _, y la secuencia de polipéptıdo codificada está provista en la ID de SEC No _ (estas secuencıas pueden ser excluıdas opcıonalmente de las modalıdades particulares reıvındıcadas de la presente ınvención)

Cuatro construcciones que contıenen dominıos de regıón constante IgA se preparan Una construcción contıene el pıvote IgA tıpo sılvestre fundıda al IgG1 CH2 y CH3 humanos (IgAH IgG WTCH2CH3) (Fıgura 31) Las amplificacıones PCR secuencıables se llevan a cabo para sustıtuır el pıvote IgG1 humana de la construcción 2H7 scFv con las secuencıas de nucleótıdo que codıfcan el pıvote IgA El ınıcıador de olıgonucleótıdo 5' (huIgA/Gchim5) para la prımera reacción

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PCR que tiene la secuencia, 5'-cca tct ccc tca act cca cct acc cca tct ccc tca tgc gca cct gaa ctc ctg-3' (ID de SEC No _) El iniciador (huIgAhg-5') para la segunda reacción PCR para agregar más secuencia de pivote específica IgA y agregar un sitio de enzima de restricción BclI (en letra inclinada y subrayado) tiene la secuencia, 5'-gtt gtt *gat cag* cca gtt ccc tca act cca cct acc cca tct ccc caa ct-3' (ID de SEC No _) El iniciador 3' para ambas etapas de amplificación es huIgG1-3' y que tiene la secuencia, 5'-gtc tct aga cta tca ttt acc cgg aga cag-3' (ID de SEC No _) La secuencia del producto PCR se confirma por clonación TOPO® como se describió anteriormente El fragmento purificado con gel se digiere con BclI y XbaI y entonces se inserta dentro del vector 2H7 scFv-pD18 que había sido digerido con BalI y XbaI para remover todos los dominios de región constante IgG1 la ligación se lleva a cabo como se describió en el ejemplo 10 para suministrar un vector de expresión de mamífero que comprende la secuencia de nucleótido (ID de SEC No _) que codifica un polipéptido 2H7 scFv IgA pivote-IgG1 CH2-CH3 (ID de SEC No _)

Un segundo vector de expresión de mamífero pD18 se construye el cual tiene una secuencia de polinucleótido (ID de SEC No _) que codifica un 2H7 scFv que se funde a el pivote IgA tipo silvestre, CH2, y CH3 como dominios (ID de SEC No _) Las secuencias de regiones constantes IgA humanas se obtienen

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usando iniciadores aleatorios para transcribir de manera inversa el ARN total aislado de las amígdalas humanas seguido por la amplificación PCR del cADN usando iniciadores específicos de secuencia, esencialmente como se describió en el ejemplo 10 La secuencia del nucleótido pivote IgA-CH2-CH3 humano (ID de SEC No _) que codifica el polipéptido IgA-CH2-CH3 (IgAH IgACH2CH3, Figura 31) (ID de SEC No _) se amplifica usando el oligonucleótido 5' huIgAhg-5' (ID de SEC No (lo mismo que antes_) y un oligonucleótido 3' huIgA3'que tiene la secuencia, 5'-gtt gtt tct aga tta tca gta gca ggt gcc gtc cac ctc cgc cat gac aac-3' (ID de SEC No _) La secreción de un polipéptido 2H7-IgA pivote-IgA CH2-CH3 de las células de mamífero transfectadas requiere la coexpresión de la cadena J humana que se liga covalentemente a dos dominios IgA CH3 vía enlaces de disulfuro El ARN total se aísla de las células B de amígdalas y se transcribe de manera inversa para generar el cADN segun se describió anteriormente La amplificación PCR de la secuencia de nucleótido que codifica la cadena J se lleva acabo con iniciadores específicos a cadena J El iniciador 5' PCR, HUJCH5n1, que tiene la secuencia, 5'-gtt gtt aga tct caa gaa gat gaa agg att gtt ctt-3' (ID de SEC No _), y la secuencia del iniciador 3', HUJCH3, que es de 5'-gtt gtt tct aga tta gtc agg ata gca ggc atc tgg-3' (ID de SEC No _) El cADN se clona en TOPO® para secuenciarlo como se describió en el ejemplo 10 El cADN que codifica la cadena J (ID de SEC No _) entonces se inserta en

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los vectores pD18 y dcDNA3-Hygro (+) (Invitrogen Life Technology) Para LA cotransfección con las construcciones 2H7 scFv IgA pivote-CH2-CH3 La cadena J tiene la secuencia de aminoácido predicha que se estable en la ID de SEC No _

La secreción de una región constante scFv IgA en la ausencia de la cadena se lleva a cabo por ingeniería genética de un dominio CH3 truncado con una eliminación de los cuatro aminoácidos del terminal carboxi (GTCY, ID de SEC No _) (IgAH IgA-T4, Figura 31), que incluye un residuo de cisteína que forma un enlace disulfuro con la cadena J La secuencia de nucleótido pivote IgA-CH2-CH3 que contiene la eliminación en CH3 (ID de SEC No _) se prepara un iniciador 5' PCR (huIg-Ahg-5') que tiene la secuencia 5'-gtt gtt *gat cag* cca gtt ccc tca act cca cct acc cca tct ccc tca act-3' (ID de SEC No _) (el sitio BclI está subrayado y puesto en letra inclinada), y un iniciador 3' PCR (HUIGA3T1) que tiene la secuencia 5'-gtt gtt tct aga tta tca gtc cac ctc cgc cat gac aac aga cac-3' (ID de SEC No _) Esta secuencia de nucleótido de región constante IgA mutada se inserta en un vector 2H7 scFv pD18 según se describió para la generación de las construcciones 2H7 scFv Ig previas (ver ejemplo 1 y ese ejemplo) que comprende la secuencia de polinucleótido (ID de SEC No _) que codifica un polinucleótido 2H7 IgAH IgAT4 (ID de SEC No _)

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Una cuarta construcción se prepara y que codifica un proteína de fusión de región constante 2H7 scFv-IgA con una eliminación de 14 aminoácido adicionales, la mayoría de los cuales son residuos hidrofóbicos, desde el terminal carboxi del IgA CH3 El polinucleótido que codifica el 2H7 scFv-IgAH IgA-T4 se usa como plantilla para producir por ingeniería una eliminación de la secuencia de nucleótido que codifica PTHVNVSVVMAEVD (ID de SEC No _) El iniciador de oligonucleótido 5' tiene la secuencia 5'-gtt gtt *gat cag* cca gtt ccc tca act cca cct acc cca tct ccc tca act- 3' (ID de SEC No _) (BclI se muestra como subrayado y en letra inclinada) La secuencia de oligonucleótido 3' es de 5'-gtt gtt tct aga tta tca ttt acc cgc caa gcg gtc gat ggt ctt-3' (ID de SEC No _) La región IgA CH3 eliminada se amplifica usando los oligonucleótidos anteriores para amplificar la región constante IfgA del ARN aislado de las amígdalas humanas tal que el cADN contenga la región que codifica al terminal carboxilo eliminado para los 18 aminoácidos La región constante IgAH IgA-T18 se inserta en el vector 2H7 scFv pD18 que comprende la secuencia de polinucleótido (ID de SEC No _) que codifica un polinucleótido 2H7 IgAH IgA-T18 (ID de SEC No _) como se describió anteriormente

EJEMPLO 14

FUNCIÓN EFECTORA DE LAS PROTEÍNAS DE FUSIÓN CTLA-4IGG

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El ejemplo compara las funciones efectoras de las proteínas de fusión CTLA-4 Ig en ensayos CDC y ADNN

Dos proteínas de fusión CTLA-4 Ig se construyen Una proteína de fusión comprende el dominio extracelular del CTLA-4 Ig fundido a el pivote tipo silvestre IgG1 humana, y los dominios CH2, CH3 y su designada CTLA-4 Ig WTH (CCC) WTCH2CH3 (ID de SEC No _) Un vector de expresión pD18 de mamífero que comprende una secuencia de polinucleótido que codifica CTLA-4 IgG WTH (CCC) WTCH2CH3 (ID de SEC No _) se prepara por medio de fundir en el marco la secuencia de nucleótido que codifica el dominio extracelular del CTLA-4 Ig (ID de SEC No _) (ver la patente U S No 5,844,095) a la secuencia de nucleótido que codifica IgG WTH (CCC) WTCH2CH3 (ID de SEC No _) de acuerdo con los métodos descritos en los ejemplos 1 y 10 La secuencia de nucleótido de dominio extracelular también comprende un sitio de enzima de restricción BclI en el extremo 3', y un secuencia de nucleótido de péptido líder (ID de SEC No _) que codifica un péptido oncoM líder (ID de SEC No _) Una segunda proteína de fusión CTLA-4 IgG, designada CTLA-4 IgG MTH (SSS) MTCH2WTCH3, contiene el dominio extracelular de CTLA-4 (mas la secuencia de péptido líder oncoM) fundida a un pivote IgG mutante en la cual todos los tres residuos de cisteína se reemplazan con residuos de serina La región de pivote fundida aun dominio IgG1 CH2 mutante que tiene una nutación en la posición isotipo 238

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(numeración EU, Ward et al , supra, (posición 251 usando la numeración de acuerdo con Kabat et al , supra, Posición 209 cuando la numeración comienza con el primer residuo de IgG1 CH1, es decir, PAPELLDGPS (ID de SEC No _) del IgG1 CH2 tipo silvestre se modifica aPAPELLDGSS (ID de SEC No _)), la cual se fusiona a IgG1 CH3 tipo silvestre (patente U S No 5,844,095) El polinucleótido CTLA-4 IgG MTH (SSS) MTCH2WTCH3 comprende la secuencia de nucleótido en la ID de SEC No _ y la secuencia de aminoácido deducida comprende la secuencias suministradas en la ID de SEC No _ Las proteínas de fusión CTLA-4 Ig son también preparadas usando secuencias de codificación de membrana extracelular CTLA-4 sin el peptido lider (ID de SEC No _)

Para medir la actividad CDC, el CTl,-4 IgG WTH (CCC) WTCH2CH3 purificado (2 µg/ml) o el CTLA-4 IgG MTH (SSS) MTCH2WTCH3 (2 µg/ml) se agrega a células Reh (ver Ejemplo 12) y a las células Reh transfectadas con la molécula coestimuladora CD80 de modo que el CD80 se expresa sobre la superficie celular (Reh CD80 10, ver Doty et al , 1998 J Immunol 161 2700, Doty et al , 1996 J Immunol 157 3270), en la presencia o ausencia de complemento de conejo (10 µg/ml) Las proteínas de fusión CTLA Ig se preparan a partir de sobrenadantes de cultivo de células CHO transientemente transfectadas de acuerdo con los métodos descritos en el ejemplo 10 Los ensayos se llevan a cabo esencialmente como se describe en

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los ejemplos 11 y 12 Los datos que se presentan en la figura 32 muestran que solamente las células Reh transfectadas con CD80 se asesinaron en la presencia del complemento y de la proteína de fusión CTLA-4 IgG WTH (CCC) WTCH2CH3

Las proteínas de fusión CTLA-4 Ig purificadas se ensayan también en ensayos ADCC PBMC humano, que sirve como células efectoras, se agregan al Reh o al Reh CD80 1 células objetivo a una proporción de 1,25 1, 2,5 1, 5,0 1 y 10 1 Las células se marcan y el ensayo se lleva a cabo a esencialmente como se describe en los ejemplos 11 y 12 Los resultados se dan en la figura 33 Cada punto de datos representa el promedio de cuatro pozos de cultivo independientes en cada proporción efector Célula objetivo Los datos muestran que solamente el CTLA-4 IgG WTH (CCC) WTCH2CH3 medio significativamente el ADCC de las células Reh CD80 10

EJEMPLO 15

FUNCION EFECTORA DE LAS PROTEINAS DE FUSIÓN CTLA-4 IgA

Las proteínas de fusión CTLA-4 IgA se preparan según se describe para las proteínas de fusión IgG (ver Ejemplos 1, 13, y 14) La secuencia de nucleótido de dominio extracelular del CTLA-4 (ID de SEC No _) se fusiona en un marco de lectura abierta a los nucleótidos que codifican IgAH IgACH2CH3 (ID de SEC No _) para suministrar la secuencia de

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nucleótido (ID de SEC No _) que codifica una proteína de fusión CTLA-4 IgAH IgACH2CH3 (ID de SEC No _) La proteína de fusión es expresada transcientemente en células COS (ver Ejemplo 10) o se expresan de manera estable en células CHO (ver Ejemplo 1) La secreción de la proteína de fusión CTLA-4 IgAH IgACH2CH3 requiere la cotransfección con una construcción que contiene una secuencia de polinucleótido (ID de SEC No _) que codifica la cadena J humana (ID de SEC No _) La proteína de CTLA-4 IgAH IgACH2CH3 se aísla segun se describe en los ejemplos 10 y 14 Para expresar una construcción CTLA-4 IgA sin la presencia de la cadena J, una construcción CTLA-4 IgAH IgA-T4 se prepara y se transfecta en células de mamífero De una manera similar como se describe para el fragmento extracelular CTLA-4 fundido a el pivote IgA-CH2CH3 tipo silvestre, la secuencia de nucleótido de dominio extracelular CTLA-4 (ID de SEC No _) se funde en un marco de lectura abierto a la secuencia de nucleótido (ID de SEC No _) que codifica un polipéptido IgAH IgA-T4 (ID de SEC No _) para suministrar una secuencia de nucleótido que comprende la (ID de SEC No _) que codifica un polipéptido CTLA-4 IGAH IgA-T4 (ID de SEC No _) La función efectora de cada construcción se evalua por CDC y ADCC según se describe en el ejemplo 14

EJEMPLO 16

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LIGADO DE PROTEÍNA DE FUSIÓN IG HUMANAS DEL ANTI-CD20 SCFV EN CÉLULAS CHO QUE EXPRESAN CD20

Este ejemplo describe el ligado de las proteínas de fusión 2H7 scFv Ig a células CHO que expresan CD20 El análisis se lleva acabo por citometría de flujo Los sobrenadantres el cultivo se recolectan de células de COS transcientemente transfectadas que expresan 2H7 scFv IgG WTH (CCC) WTCH2CH3 (ID de SEC No _), 2H7 scFv IgG MTH (CSS) WTCH2CH3 (ID de SEC No _), 2H7 scFv IgG MTH (SCS) WTCH2CH3 (ID de SEC No _), y 2H7 scFv VHSER11 WTH WTCH2CH3 (ID de SEC No _), y diluciones seriadas de dos veces que se preparan Las diluciones seriadas de dos veces de 2H7 scFv IgG MTH (SSC) WTCH2CH3 purificado (ID de SEC No _) se prepara a partir de una concentración de 5 µg/ml Los sobrenadantes de cultivo y las muestras de proteínas de fusión purificadas se incubaron con células (CD20+) CHO durante una hora sobre hielo Las células se lavan dos veces y entonces se incuban con IgG anti-humano de cabra conjugado FITC 1 100 (CalTag) durante 40 minutos El conjugado no ligado entonces se remueve por lavado de las células y se lleva acabo el análisis de citometría de flujo usando un clasificador de células Coulter Epics XL los resultados se dan en la Figura 34

EJEMPLO 17

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ANALISIS DE INMUNOMANCHADO DE LAS PROTEÍNAS DE FUSIÓN IGG E IGA HUMANAS ANTI-CD20 SCFV

Este ejemplo describe el análisis de inmunomanchado de las proteínas de fusión 2H7 scFv IgG y 2H7 scFv IgA que se inmunoprecipitan a partir de sobrenadantes de cultivo de células transfectadas

Las células COS se transfectaron transcientemente con plásmidos que comprenden las secuencias de nucleótidos para 2H7 scFv IgG WTH (CCC) WTCH2CH3 (ID de SEC No _), 2H7 scFv IgG MTH (CSS) WTCH2CH3 (ID de SEC No _), 2H7 scFv IgG MTH (SCS) WTCH2CH3 (ID de SEC No _), 2H7 scFv IgA H IgG WTCH2CH3 (ID de SEC No _), y scFv IgG MTH (SSS) WTCH2CH3 (ID de SEC No _) esencialmente se acuerdo con el método que se describe en el ejemplo 10 Las células también se transfectan con solamente vector Después de 48 a 72 horas a 37° C, los sobrenadantes del cultivo celular se recolectan y se combinan con bolitas de proteína A-agarosa (Repligen) durante una hora a 4° C Las bolitas se centrifugan y se lavan varias veces en TNEN [20 mM de Base Tris, 100 mM NaCl, 1 mM de EDTA, y 0,05% de NP-40, pH 8,0) Los inmunoprecipitados se combinan con 25 µl 2x NUPAGE® SDS como amortiguador de muestra (Invitrogen Life Technologies) (muestras no reducidas) Las proteínas se fraccionan sobre geles Bis-Tris 10% NUPAGE® (Invitrogen Life Technologies) Después de la electroforesis (aproximadamente

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1 hora), las proteínas se transfieren del gel hacia una membrana de fluoruro de polivinilideno Immobilon P (PVDF) (Millipore, Bedford, MA) usando un manchador semiseco (Ellard Instrumentation, Monroe, WA) La membrana PVDF se bloquea con PBS que contiene 5% de leche sin grasa y luego se ensaya con IgG anti-humano de cabra conjugado con HRP (específico a Fc) (CalTag) Después del lavado del inmunomanchado varias veces en PBS, el manchado se desarrolla usando ECL (Amersham Biosciences) Los resultados se muestran en la Figura 35

EJEMPLO 18

LIGADO DE LAS PROTEÍNAS DE FUSIÓN IGA HUMANAS DEL SCFV ANTI-CD20 A CÉLULAS CD20+CHO

Este ejemplo describe el análisis inmunocitofluorimetría de flujo de ligado de 2H7 scFv IgAH IgACH2CH3 (ID de SEC No _) y 2H7 scFv IgAH IgAT4 (ID de SEC No _) como proteínas de fusión a las células (CD20+) CHO

Las células COS se cotransfectan transcientemente según se describe en el ejemplo 10 con el ADN del plásmido que comprende una secuencia de polinucleótido (ID de SEC No _) que codifica al polipéptido 2H7 scFv IgAH IgACH2CH3 (ID de SEC No _) y con un plásmido separado que comprende una secuencia de polinucleótido (ID de SEC No _) que codifica un polipéptido de cadena J humana (ID de SEC No _) Las células

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COS también se transfectan con una secuencia de polinucleótido (ID de SEC No _) que codifica una proteína de fusión 2H7 scFv IgA que tiene una eliminación de cuatro aminoácidos en el terminal carboxi del CH3 (2H7 scFv IgAH IgA-T4, ID de SEC No _) Las transfecciones se llevan a cabo según se describe en el ejemplo 10 Los sobrenadantes del cultivo de las células COS se combinan con células (CD20+) CHO (ver Ejemplo 1) y se incuban durante una hora sobre hielo Las células se lavan dos veces con PBS-2% de FBS y entonces se combinan con la cadena IgA anti-humana de cabra conjugada con FITC (CalTag) (1 100) durante 40 minutos Las células se lavan nuevamente y luego se analizan por citometría de flujo usando un clasificador de célula Coulter Epics XL La Figura 36 muestra que la cotransfección con la cadena J no se requiere para la secreción de la proteína de fusión 2H7 scFv IgAH IgAT4, la proteína de fusión 2H7 IgA con el extremo carboxi CH3 (ID de SEC No _)

EJEMPLO 19

FUNCIÓN EFECTORA DE LAS PROTEÍNAS DE FUSIÓN IGA HUMANAS DEL SCFV ANTI-CD20

Este ejemplo ilustrará la actividad ADCC de las proteínas de fusión IgG y IgA del 2H7 contra células que expresan CD20 Células BJAB se premarcan con ^{51}Cr (100 J Ci) (Amersham) durante dos horas a 37° C Las células efectoras se obtiene

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de sangre completa humana en reposo, fresca, que se diluye en un volumen igual de solución Alsever's para impedir la coagulación Las proteínas de fusión 2H7 scFv IgG MTH (SSS) WTCH2CH3 (ID de SEC No _), 2H7 scFv IgG MTH (SCS) WTCH2CH3 (ID de SEC No _), 2H7 scFv IgG WTH (CCC) WTCH2CH3 (ID de SEC No _), y 2H7 scFv IgAH IgACH2CH3 (ID de SEC No _) se purifican de sobrenadantes de células COS que se transfectan transcientemente (100-200 ml) por cromatografía de proteina A como se describe en el ejemplo 10 Las células COS que se transfectan con el plásmido que codifica el 2H7 scFv IgAH IgACH2CH3 se cotransfectan con un plásmido que codifica la cadena J humana como se describe en el ejemplo 18 Diluciones seriales dobles de las proteínas de fusión 2H7 Ig purificadas se inician en 5 µg/ml se agregan a las células BJAB (5 x 10^4 por pozo de una placa de cultivo de 96 pozos) en la presencia de sangre completa (100 µl de sangre completa que se diluye en 1 1 en solución Alsever's solución final 1 4) y que se incuba durante cinco horas a 37° C Los sobrenadantes de cultivo se recolectan y analizan como se describe en el ejemplo 11 El porcentaje específico de muestres se calcula de acuerdo con la ecuación siguiente ((Liberación del experimento menos liberación espontánea)/(liberación máxima menos liberación espontánea)) x 100 Los datos se dan en la figura 37 Cada punto de dato representa el promedio de muestras cuadruplicadas

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En un segundo ensayo ADCC, el número de célula objetivo BJAB marcadas se mantienen constantes en cada muestra, y se agrega sangre completa a las diluciones de 0,25, 0,125, y 0,0625 Las proteínas de fusión IgG e IgA del 2H7 purificadas se agregan a una concentración de 5 µg/ml Las células BJAB, la sangre completa, y las proteínas de fusión se incuban, los sobrenadantes que se recolectan, y el porcentaje específico de muertes se calcula como se describió anteriormente El porcentaje específico de muertes para cada una de las proteínas de fusión 2H7 se presenta en la figura 38

La actividad ADCC de la 2H7 scFv IgG MTH (SSS) WTCH2CH3 purificada (5 µg/ml) y de 2H7 scFv IgAH IgACH2CH3 purificada (5 µg/ml) se compara en la presencia de diferentes poblaciones celulares efectoras El PBMC se aisla de sangre completa como se describe en los ejemplos 11 y 12 Los PBMC se combinan con células objetivo BJAB marcadas(5 x 10^4 por pozo de una palca de cultivo de tejidos de 96 pozos) en proporciones de 50 1, 25 1, y 12,5 1 El ensayo se lleva a cabo y los datos se analizan como se describió anteriormente La figura 39A muestra que solamente la proteína de fusión 2H7 scFv IgG MTH (SSS) WTCH2CH3 tiene actividad ADCC cuando los PBMC sirven como células efectoras La figura 39B muestra que tanto 2H7 scFv IgG MTH (SSS) WTCH2CH3 y 2H7 scFv IgAH IgACH2CH3 exhiben actividad ADCC cuando la sangre completa es

la fuente de células efectoras como se ilustra en la figura 38)

EJEMPLO 20

NIVEL DE EXPRESIÓN DE LA PROTEINA DE FUSION 2H7 SCFV VH11SEREGG MTH (SSS) WTCH2CH3

Este ejemplo compara el nivel de expresión de la proteína de fusión 2H7 scFv VH11Ser IgG MTH (SSS) WTCH2CH3 (ID de SEC No _) con otras construcciones 2H7 scFv IgG que no contienen la mutación en el dominio de cadena variable pesada El vecto pD18 de expresión de mamífero comprende la secuencias de nucleótido 2H7 scFv IgG MTH (SSS) WTCH2CH3 (ID de SEC No _), 2H7 scFv IgG MTH (CSS) WTCH2CH3 (ID de SEC No _), 2H7 scFv IgG MTH (SCS) WTCH2CH3 (ID de SEC No _), 2H7 scFv IgG WTH (CCC) WTCH2CH3 (ID de SEC No _), y 2H7 scFv VHSER11 IgG MTH (SSS) WTCH2CH3 (ver ejemplos 1 y 13) se transfectan transcientemente en células COS cells como se describe en el ejemplo 10 Después de 72 hoas a 37° C, los sobrenadantes del cultivo se recolectan y 1 µl de cada sobrenadante se combina con un amortiguador de muestra no reducto (ver método descrito en el ejemplo 10) Las muestras de sobrenadante de cultivo y las alícuotas de 2H7 scFv IgG MTH (SSS) WTCH2CH3 (40 ng, 20 ng, 10 ng/5 ng, y 2,5 ng) se fraccionan sobre geles de 10% Bis-Tris (MOPS) NuPAGE® (Invitrogen Life Technologies) Los estándares de proteína MultimarK®

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(Invitrogen Life Technologies) también se separan en el gel Las proteínas se transfieren a una membrana PDVF e inmunomanchadas como se describe en el ejemplo 17 La inmunomancha se presenta en la figura 40 Las cantidades de las proteínas de fusión se cuantifican por análisis de densitometría de las manchas usando el software ScionImage para Windows y se hace la comparación con la curva estándar La construcción 2H7 scFv IgG WTH (CCC) WTCH2CH3 produce aproximadamente 12 ng/ul o 12 microgramos/ml, y la 2H7 scFv IgG MTH (CSS) WTCH2CH3 produce aproximadamente 10 ng/ul o 10 microgramos/ml, la 2H7 scFv IgG MTH (SCS) WTCH2CH3 produce aproximadamente 1 ng/ul o 1 microgramo/ml, y la construcción 2H7 scFv VHSER11 IgG MTH (SSS) WTCH2CH3 produce aproximadamente 30 ng/ml o 30 microgramos/ml En modalidades particulares reivindicadas de la presente invención, una secuencia de aminoácido de 2H7 scFv VHSER11 IgG MTH (SSS) WTCH2CH3, o una secuencia de polinucleótido que codifica al 2H7 scFv VHSER11 IgG MTH (SSS) WTCH2CH3 puede ser excluido opcionalmente de la presente invención Similarmente, la secuencia de aminoácido de 2H7 scFv VHSER11 IgG WTH (CCC) WTCH2CH3, o una secuencia de polinucleótido que codifica 2H7 scFv VHSER11 IgG WTH (CCC) WTCH2CH3 puede ser excluida opcionalmente de las modalidades reivindicadas particulares de la presente invención Adicionalmente, una sustitución de aminoácido de una leucina en la posición 11 a serina en el dominio de cadena pesada variable, o polinucleótidos que

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codifican una sustitución de aminoácido de una leucina en la posición 11 a serina en el dominio de cadena pesada variable, puede ser excluida opcionalmente de las modalidades particulares reivindicadas de la presente invención

EJEMPLO 21

CONSTRUCCIÓN DE UNA PROTEINA DE FUSIÓN 2H7 SCFV IGG CON UN DOMINIO MUTANTE CH3

Las mutaciones de aminoácido se introducen en el dominio CH3 de una proteína de fusión 2H7 IgG El vector pD18 que comprende 2H7 scFv IgG MTH (SSS) WTCH2CH3 (ID de SEC No _) se digiere con BelI y XbaI para remover el fragmento MTH WTCH2CH3 (ID de SEC No _) el cual entonces se subclona dentro de una vector pvehículo (BD Biosciences Clontech, Palo Alto, CA) que se digiere doble vez con BelI y XbaI La subclonación se lleva a cabo en un vector resistente a la kanamicina porque el gen con resistencia a la ampicilina tiene un sitio XmnI, el cual se requiere para este procedimiento de clonación Cinco construcciones se preparan con las siguientes sustituciones (1) un residuo de fenilalanina en la posición 405 (numeración de acuerdo con Kabat et al supra) se sustituye con tirosina usando el oligonucleótido CH3Y405, (2) la fenilalanina de la posición 405 se sustituye con un residuo de alanina usando el oligonucleótido CH3A405, (3) el residuo de tirosina en la

posición 407 se sustituye con una alanina usando el oligonucleótido CH3A407, (4) tanto los aminoácidos tipo silvestre en las posiciones 405 y 407 se sustituyen con tirosina y alanina, respectivamente usando el oligonucleótido CH3Y405A407, y (5) ambos aminoácidos tipo silvestre en las posiciones 405 y 407 se sustituyen con alanina usando el oligonucleótido CH3A405A407 Los oligonucleótidos son iniciadores 3' para la amplificación PCR de una porción del dominio CH3 Las secuencias de nucleótidos para cada oligonucleótido 3' son las siguientes

CH3Y-405 5'-gtt gtt gaa gac gtt ccc ctg ctg cca cct gct ctt gtc cac ggt gag ctt gct gta gag gta gaa gga gcc-3' (ID de SEC No _)

CH3A405 5'-gtt gtt gaa gac gtt ccc ctg ctg cca cct gct ctt gtc cac ggt gag ctt gct gta gag ggc gaa gga gcc-3' (ID de SEC No _)

CH3A407 5'-gtt gtt gaa gac gtt ccc ctg ctg cca cct gct ctt gtc cac ggt gag ctt gct ggc gag gaa gaa gga gcc-3' (ID de SEC No _)

CH3Y405A407 5'-gtt gtt gaa gac gtt ccc ctg ctg cca cct gct ctt gtc cac ggt gag ctt gct ggc gag gta gaa gga gcc-3' (ID de SEC No _)

CH3A405A407 5'-gtt gtt gaa gac gtt ccc ctg ctg cca cct gct ctt gtc cac ggt gag ctt gct ggc gag ggc gaa gga gcc-3' (ID de SEC No _)

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La plantilla es la IgG1 humana con pivote MHWTCH2CH3 El iniciador de oligonucleótido 5' PCR es huIgGMHWC, [ID de SEC No _] Los productos amplificados se clonan con TOPO® y se secuencian como se describe en los ejemplos 1 y 10 El ADN de los clones con la secuencia correcta se digiere con BclI y SMI y se transfiere al pvehículo que contiene la secuencia MTH WTCH2CH3, que también se digiere con las mismas enzimas de restricción Las secuencias de IgG mutadas se remueven entonces por digestión con BelI y XbaI y se insertan en un vector pD18 que contiene 2H7 scFv que también se digiere con BelI y XbaI Las secuencias del polinucleótido para los dominios CH3 mutados, MTCH3 Y405, MTCH3 A405, MTCH3 A407, MTCH3 Y405A407, y MTCH3 A405A407 se muestran en la ID de SEC No -, respectivamente, y las secuencias de los polipéptidos para cada una se muestran en la ID de SEC No _, respectivamente Las secuencias del polinucleótido para el 2H7 scFv MTH WTCH2 MTCH3 Y405, 2H7 scFv MTH WTCH2 MTCH3 A405, scFv MTH WTCH2 MTCH3 A407, scFv MTH WTCH2 MTCH3 Y405A407, y scFv MTH WTCH2 MTCH3 A405A407, respectivamente, y las secuencias de aminoácidos deducidos se muestran en la ID de SEC NOs No _, respectivamente

EJEMPLO 22

CONSTRUCCIÓN DE LAS PROTEÍNAS DE FUSIÓN 2H7 SCFV IGG CON MUTACIONES DE PIVOTE

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Una proteína de fusión 2H7 scFv IgG se construye con el tercer resíduo de cisteína en la región de pivote IgG1 que se sustituye con un resíduo de serina La plantilla para la introducción de las mutaciones es un polinucleótido que codifica 2H7 scFv WTH WTCH2CH3 (ID de SEC No _) El oligonucleótido que introduce las mutaciones es un oligonucleótido iniciador HIgGMHcys3 con 5' PCR que tiene la secuencia 5'-gtt gtt gat cag gag ccc aaa tct tgt gac aaa act cac aca tgt cca ccg tcc cca gca cct-3' El oligonucleótido que introduce la mutación en la región de pivote es compuesto binado con la plantilla y un oligonucleótido 3' que contiene un sitio XbaI (subrayado y escrito con letras inclinadas) (5'-gtt gtt *tct aga* tca ttt acc cgg aga cag gga gag gct ctt ctg cgt gta g-3' (ID de SEC No _) para amplificar el tipo silvestre de pivote mutante (WT)-CH2-CH3 por PCR La secuencia mutante IgG MTH CCS se amplifica durante 30 ciclos con un perfil de desnaturalización de 94° C, templado de 50° C durante 30 segundos, y extensión a 72° C durante 30 segundos Los polinucleótidos amplificados se insertan en el vector de clonación TOPO® (Invitrogen Life Technologies) y luego se secuencian según se describe en el ejemplo 1 para confirmar la presencia de la mutación El vector pd18 que contiene 2H7 scFv se digiere para remover las secuencias de la región constante esencialmente como se describe en el ejemplo 10 Las regiones CH2-CH3 tipo silvestre de pivote

mutante se insertan en el marco dentro del ADN del vector digerido para obtener los vectores que comprenden ADN que codifica 2H7 scFv MTH (CCS) WTCH2CH3 (ID de SEC No _) La secuencia de polipéptido deducido se muestra en la ID de SEC No _

EJEMPLO 23

CONSTRUCCIÓN DE PROTEINAS DE FUSION ANTI-CD20 IGE

Un dominio de unión se fusiona a las secuencias de región constante IgE de tal forma que el polipéptido expresado es capaz de inducir un mecanismo de respuesta alérgico Las secuencia de nucleótido Fv de cadena sencilla de 40 2 220 (ID de SEC No _), un anticuerpo anti-CD20, se fusiona al IgE CH2-CH3-CH4 de acuerdo con los métodos descritos para otras construcciones de región constante de inmunoglobulina scFv (ver ejemplos 1, 5, 10, y 13) Para amplificación PCR se utilizan los dominios IgE CH2-CH3-CH4, un iniciador de oligonucleótido 5', hIgE5Bcl, que tiene la secuencia 5'-gtt gtt gat cac gtc tgc tcc agg gac ttc acc cc-3', y un iniciador de oligonucleótido 3', hIgE3 de parada, que tiene la secuencia 5'-gtt gtt tct aga tta act ttt acc ggg att tac aga cac cgc tcg ctg g-3'

El sistema de transfección retroviral para expresión de superficie ectópica de los receptores de superficie celular

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genéticamente construidos con ingeniería compuestos de scFvs que unen los receptores coestimulatorios descritos en el ejemplo 12 se utilizan para construir una proteína de fusión 40 2 220 scFv IgE-CD80 La secuencia de polinucleótido de fusión 40 2 220 scFv IgE se fusiona en estructura a secuencias que codifican la cadena citoplásmica y el dominio de transmembrana del CD80 humano(ID de SEC No _), de tal forma que cuando la proteina de fusión se expresa en la célula transfectada, el CD80 suministra un anclaje para la expresión de superficie de la proteína de fusión scFv Ig El cADN que codifica las proteínas de fusión anti-CD40 scFv-IgE-CD80 (ID de SEC No _) se inserta dentro del vector retroviral pLNCX (BD Biosciences Clontech) de acuerdo con procedimientos de biología molecular estándar e instrucciones del vendedor El 40 2 220 scFv-Ig-CD80 cADN se inserta dentro de la secuencia promotor del gen CMV de resistencia 5'LTR-neomicina y la secuencia 3'LTR Las construcciones retrovirales se transfectan dentro de una línea de célula de carcinoma, y las células transfectadas se escogen para seleccionar clones que expresan la proteína de fusión 40 2 220 scFv-Ig-CD80 en la superficie celular

EJEMPLO 24

CONSTRUCCIÓN DE MUTANTES IGA-T4 QUE SE EXPRESAN EN LA SUPERFICIE CELULAR

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El sistema de transfección retroviral para expresión de superficie ectópica de los receptores de superficie celular genéticamente construidos por ingeniería compuestos de scFv que unen los receptores coestimulatorios descritos en el ejemplo 12 se utilizan para construir una proteína de fusión IgA-T4-CD80 de pivote 2H7 scFv IgA La secuencia de polipéptido de fusión 2H7 scFv IgAH IgA-T4 (ID de SEC No _) se funciona en la estructura a las secuencias que codifican el dominio de transmembrana y la cola citoplasmática del CD80-humano (ID de SEC No _), de tal forma que cuando la proteína de fusión se expresa en la célula transfectada el CD80 suministra un anclaje para la expresión de superficie de la proteína de fusión scFv Ig El cADN que codifica la proteína de fusión 2H7 scFv IgAH IgA-T4-CD80 (ID de SEC No _) se inserta dentro del vector retroviral pLNCX (BD Biosciences Clontech) de acuerdo con procedimientos de biología molecular estándar en instrucciones del vendedor El cADN 2H7 scFv IgAH IgA-T4-CD80 se inserta dentro de las secuencias promotor de gen CMV de resistencia a la 5'LTR-neomicina y la secuencia 3'LTR La construcción retroviral se transfecta en Reh, una línea celular de leucemia linfocítica aguda (ATCC CRL-8286) Las células transfectadas se escogen para seleccionar clones que expresan proteínas de fusión 2H7 scFv-Ig en la superficie celular

EJEMPLO 25

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CARACTERIZACIÓN DE UNA PROTEINA DE FUSIÓN SCFV IG-CD80 ANTI-4-1BB EXPRESADA EN LA SUPERFICIE CELULAR DE CÉLULAS DE TUMOR Y CRECIMIENTO DE LAS CELULAS DE TUMOR IN VIVO

Este ejemplo describe la construcción de una proteína de fusión 4-1BB (CD137) scFv anti-murina que tiene una pivote tipo silvestre IgG y dominios CH2 y CH3 que se fusionan a los dominios citoplásmicos y de transmembrana CD80 El ejemplo también ilustra el efecto de la expresión de la superficie celular del polipéptido scFv IgG CD80-anti-4-1BB cuando las células de tumor transfectadas se transplantan en ratones

Las regiones de variable de cadena aliviana y pesada de un anticuerpo monoclonal (CD137) anti-4-1BB de rata (1D8) se clonan, y una construcción Fv de cadena sencilla se prepara esencialmente como se describe en el ejemplo 1 Las regiones variables de cadena liviana y pesada de cada anticuerpo se clonan de acuerdo con los métodos estándar para clonar genes de inmunoglobulina y como se describe en el ejemplo 1 La construcción Fv de cadena sencilla se prepara como se describe en el ejemplo 1 al insertar una secuencia de nucleótido que codifica un ligador de péptido $(gly_4ser)_3$ entre la secuencia de nucleótido de la región VL de 1D8 (ID de SEC No _) y la secuencia de nucleótido de la región VH de 1D8(ID de SEC No _) La secuencia de polipéptido para el 1D8 VL se muestra en la ID de SEC No _, y la secuencia de polipéptido

para el dominio VH se muestra en la ID de SEC No _ El polinucleótido scFv (ID de SEC No _) se fusiona luego a los dominios IgG1 de pivote-CH2-CH3 tipo silvestre de acuerdo con los métodos descritos en el ejemplo 1 La secuencia de polinucleótido de fusión IgG1 scFv se fusiona luego en estructura a las secuencias que codifican la cola citoplásmica y el dominio de transmembrana del CD80 humano(ID de SEC No _) esencialmente como se describe en el ejemplo 12, de tal forma que cuando la proteina de fusión se expresa en la célula transfectada, el CD(0 suministra un anclaje para la expresión de superficie de la región de proteína Ig scFv El cADN que codifica la proteina de fusión scFv-IgG-CD80 (ID de SEC No _) se inserta dentro del vector pLNCX retroviral (BD Biosciences Clontech) de acuerdo con procedimientos de biología molecular estándar en instrucciones del vendedor El cADN scFv-Ig-CD80 se inserta entre las secuencias de promotor de gen-CMV de resistencia 5'LTR-neomicina y la secuencia 3'LT

Las construcciones retrovirales se transfectan en el clon M2 metastático de K1735, una línea celular de melanoma suministrada por Dr I Hellstrom, PNRI, Seattle, WA Las células transfectadas se escogen para seleccionar clones que expresan las proteínas de fusión scFv-Ig sobre la superficie celular Para demostrar que la construcción 1D8 scFv IgG-CD80 se expresa en la superficie celular de las células de tumor,

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se analizan las células transfectadas mediante inmunocitofluorimetría de flujo Las células transfectadas (K1735-1D8) se incuban durante una hora en hielo en IgG antihumano de cabra ficoeritrin-conjugado $F(ab')_2$ El conjugado no unido se remueve luego mediante el lavado de las células y se desarrolla análisis de citometría de flujo que utiliza un clasificador de células Coulter Epics XL los resultados se presentan en la figura 41A

El crecimiento de las células transfectadas K1735-1D8 se examina in vivo Las células K1735-WT crecen progresivamente cuando se trasplantan subcutáneamente (s c) en ratones C3H nativos Aunque la misma dosis de células K1735-1D8 inicialmente forma tumores de una superficie de área de 30 mm^2 aproximadamente, los tumores empiezan a retroceder cerca del día 7 y ha desaparecido por el día 20 como se muestra en la figura 41B Las células de tumor que se transfectan como un vector similar codifica un scFv sin unión, una construcción anti-CD28 scFv humana, de crecimiento así como también células de tumor que no se han transfectado La presencia de una proteína extranjera, que es, dominio constante IgG1 Humano o regiones variables de rata, que no hacen transfección inmunogénica de las células K1735-WT, el crecimiento de las células K1735-1D8 en ratones C3H es idéntico a aquel de las células K1735-WT (no transfectadas)

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Para ınvestıgar los papeles de las células NK y los lınfocıtos T $CD4^+$ y $CD8^+$ en la regresıón de tumores K1735-1D8, ratones naïve se ınyectaron ıntraperıtonealmente (ı p) con antıcuerpos monoclonales (antıcuerpos monoclonales, típıcamente 50 µg en un volumen de 0,1 ml) para remover células T $CD8^+$, $CD4^+$ o ambas $CD4^+$ y $CD8^+$, o se ınyectan con antıcuerpos de conejo antı-asıalo-GM1 para remover células NK Doce días después, cuando el análisıs de cıtometría de flujo de células de bazo de ratones tratados ıdéntıcamente mostraron que las poblacıones de célula T objetıvo se agotaron, las células K1735-1D8 se transplantan s c a cada grupo agotado de célula T K1735-1D8 tiene crecimıento sımılar a los cınéticos en ratones que se han ınyectado con el antı-CD8 MAb o IgG de control de rata mientras la remoción de las células T $CD4^+$ resulta en el crecimıento de K1735-1D8 con los mısmos cınéticos K1735-WT Esta falla para ınhıbır el crecimıento del tumor después de la remoción de las células T $CD4^+$ se observa con respecto de la presencıa o ausencıa de células T $CD8^+$ El crecimıento de K1735-1D8 en todos los ratones NK agotados, aunque más lentamente que en el grupo CD4-agotado Los resultados se presentan en la fıgura 41C

EJEMPLO 26

EFECTO TERAPÉUTICO DE CELULAS DE TUMOR QUE EXPRESAN PROTEÍNA DE FUSIÓN ANTI-4-1BB SCFV IGG-CD80

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Este ejemplo examina la capacidad de las células de tumor transfectadas K1735-ID8 que expresan un anti-CD137 scFv sobre la superficie celular apara generar una respuesta inmune suficiente en ratones para mediar rechazo de tumores establecidos de tipo silvestre no transfectado Ratones C3H se transplantan con tumores K1I35-WT ($2x10^6$ células/animal) y con crecimiento de aproximadamente seis días El experimento se desarrolla utilizando ratones con tumores K1735-WT establecidos de superficie de área de 30 mm^2 Los ratones se vacunan mediante inyección s c de K1735-11D8 células K1735-WT irradiadas en el lado contralateral Se repiten inyecciones idénticas en los puntos de tiempo indicados en la figura 42 A un grupo de animales se les da cuatro inyecciones semanalmente de células K1735-1D8 De acuerdo con el mismo calendario, otro grupo dado se irradia (12,000 rads) con células K1735-WT y un tercer grupo se inyecta con PBS El área de datos se dibuja en la figura 42 Los tumores WT crecieron progresivamente en todo los ratones de control y en todos los ratones que recibieron células K1735-WT irradiadas En contraste, los tumores se redujeron en 4 de 5 ratones que se tratan mediante inmunización con K1735-1D8 Los animales permanecen libres de tumor y sin signos de toxicicidad cuando el experimento se termina tres meses después En el quinto ratón el nódulo de tumor se reduce de tamaño en la medida en que el ratón recibe células K1735-1D8, pero el crecimiento del tumor regresa después que se termina la terapia

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En otro experimento con 5 ratones/grupo, se inyectaron los ratones intravenosamente (i v) con 3 x 10^5 células K1735-WT para iniciar metástasis de pulmón Tres días después, las células K1735-1D8 se transplantan s c Este procedimiento se repite una vez semanalmente durante un mes, los ratones de control se inyectaron con PBS El experimento da por terminado cuando un ratón en el grupo de control ha muerto, 37 días después de que recibe las células K1735-WT En ese tiempo los pulmones de cada uno los ratones de control tienen >500 focos metastáticos Por el contrario menos de 10 focos metastáticos se presentan en los pulmones de los ratones inmunizados

En un tercer experimento, las mezclas de células K1735-WT y las células K1735-1D8 se inyectan enratones C3H sinérgicos inmunocomponentes Los ratones se inyectan subcutáneamente solo con células K7135-WT o con una mezcla de 2 x 10^6 células K1735-WT y 2 x 10^5 células K1735-1D8 El crecimiento del tumor se monitorea a intervalos de 5 días

EJEMPLO 27

EXPRESIÓN DE UNA PROTEÍNA DE FUSIÓN ANTI-4-1BB SCFV IGG-CD80 SOBRE LA SUPERFICIE CELULAR DE CÉLULAS DE SARCOMA

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Este ejemplo demuestra la expresión de un scFv anti-CD137 sobre la superficie celular de un segundo tipo de célula de tumor al transfectar una linea de célula de sarcoma de murina con una construcción anti-CD137 scFv IgG-CD80

El polinucleótido 1D8 scFv IgG WTH WTCH2CH3-CD80 (ID de SEC No _) se transfiere del vector pLNCX en el vector pCDNA3-higro que utiliza digestión de enzima de restricción y etapas de ligación de acuerdo con métodos de biología molecular estándar La construcción se corta con fragmentos HindIII + Cla1 y thesFv se llena con Klenow (Roche) y el fragmento de extremo romo se liga en el sitio EcoR5 de pcDNA3 Las células de tumor de sarcoma de murina Ag104 se transfectan con vectores pCDNA3-higro que contiene la proteína de fusión 1D8 scFv IgG CD80 Los clones resistentes a la higromicina se seleccionan mediante citometría de flujo que utiliza un anticuerpo IgG anti-humano FITC para detectar la expresión del transgen Solo aproximadamente el 15% de los clones resistentes tiene inicialmente proteina de fusión detectable Las células positivas identificadas por citometría de flujo se filtran repetidamente en frascos cubiertos con IgG anti-humano inmovilizado (10 µg/ml) de acuerdo con métodos estándar El filtrado se desarrolla mediante células de incubación en placas cubiertas durante 30 min a 37 C, las placas se lavan luego 2-3x 1 en verseno o PBS Después de cada round, las células se ensayan para expresión IgG por

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FACS El histograma en la figura 44 muestra el patrón de teñido después de cuatro rounds de filtro contra IgG anti-humano (negro) Las células no transfectadas se tiñen y se indican en gris Todas las células en la población son positivas

EJEMPLO 28

CONSTRUCCIÓN Y CARACTERIZACIÓN DE UNA PROTEÍNA DE FUSIÓN BISPECÍFICA SCFV IG Y PROTEINAS DE FUSIÓN SCFV IG CON UNA MUTACIÓN EN EL DOMINIO IGGI CH2

Se construye una proteína de fusión anti-CD20 (2H7) scFv IgG que tiene una pivote mutante (MT (SSS)) y un dominio CH2 mutante en el que la prolina en residuo (número de posición 238 de acuerdo con Ward et al , supra) se sustituye con una serina El polinucleótido que codifica 2H7 scFv IgG MTH (SSS) MTCH2WTCH3 (ID de SEC No _) se construye esencialmente de acuerdo con los métodos descritos en los ejemplos 1, 5, y 13 Los dominios CH3 de tipo silvestre CH2 mutantes de pivote IgG también se fusiona a un scFv biespecífico anti-CD20 (2H7)-anti-CD40 (40 2 220) La secuencia de polinucleótido de codificación anti-CD20-anti-CD40 scFv IgG MTH (SSS) MTCH2WTCH3 se muestra en la ID de SEC No _ y el polipéptido codificado se muestra en la ID de SEC No _

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Las células COS se transfectan transitoriamente con vectores que comprenden la secuencia de polinucleótido de codificación 2H7 scFv IgG MTH (SSS) MTCH2WTCH3 (ID de SEC No _), anti-CD20-anti-CD40 scFv IgG MTH (SSS) MTCH2WTCH3 (ID de SEC No _), 2H7 scFv IgG MTH (SSS) WTCH2CH3 (ID de SEC No _), y 2H7 scFv IgAH IgG WTCH2CH3 (ID de SEC No _) como se describe en el ejemplo 10 Los supernadantes de cultivo se recolectan y las proteínas de fusión se purifican mediante cromatografía de proteína A (ver ejemplo 10) Los polipéptidos purificados se fraccionan por SDS-PAGE de acuerdo con el método descrito en el ejemplo 10 El Rituximab (anticuerpo monoclonal anti-CD20), y los estándares de peso molecular de preteñido Bio-Rad (Bio-Rad, Hércules, CA), y estandares de peso molecular Multimark® (Invitrogen Life Technologies) también se aplican al gel Los resultados se presentan en la figura 45

La proteína de fusión 2H7 scFv Ig que contiene una mutación en el dominio CH2 se compara con las proteínas de fusión que tienen el dominio CH2 tipo silvestre en un ensayo ADCC Los ensayos se desarrollan esencialmente como se describen en los ejemplos 11 y 19 Se agrega PBMC (células efectoras) frescas en reposo a células ^{51}Cr-etiquetadas BJAB (células objetivo) en las proporciones indicadas en la figura 46 El 2H7 scFv IgG MTH (SSS) MTCH2WTCH3, 2H7 scFv IgG MTH (SSS) WTCH2CH3, 2H7 scFv IgAH IgG WTCH2CH3 purificado, y Rituximab, cada uno de 10 µg/ml se agregan a las mezclas de célula

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efectora/objetivo y se incuban durante cinco horas a 37° C Los supernadantes se cosechan y la cantidad de cromo libre se determina como se describe los ejemplos 11 y 19 El porcentaje específico de muertes para cada proteína de fusión se presenta en la figura 46

EJEMPLO 29

EXPRESIÓN DE SUPERFICIE CELULAR DE UNA PROTEÍNA DE FUSIÓN CD3 SCFV IGG ANTI-HUMANA

Se prepara una proteína de fusión CD3 scFv Ig CD80 anti-humana esencialmente como se describe en los ejemplos 1 y 12 La proteína de fusión comprende un CD3 scFv anti-humano fusionada a pivote IgG1 tipo silvestre(ID de SEC No _) y CH2 tipo silvestre (ID de SEC No _) y dominios CH3 (ID de SEC No _) fusionado a dominios citoplásmicos y de transmembrana CD80 (ID de SEC No _) para permitir la expresión de la superficie celular del anti-CD3 scFv El polinucleótido CD3 scFv IgG WTH WTCH2CH3-CD80 anti-humano (ID de SEC No _) que codifica al polipéptido (ID de SEC No _) se transfecta en células Reh y células T51 (línea de célula de linfoblastoides) La expresión de la proteína de fusión CD3 scFv IgG anti-humana se detecta mediante citometría de flujo que utiliza IgG anti-humano de cabra conjugado FITC (ver métodos en los ejemplos 4, 10, 16, 18) La figura 47A ilustra la expresión de la proteína de fusión CD3 anti-humana sobre

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la superficie celular de las células Reh, y la figura 47B muestra la expresión de la proteína de fusión sobre las células T41

Se desarrollan ensayos ADCC con células Reh y T51 transfectadas para determinar si la expresión de los polipéptidos scFv-Ig sobre la superficie aumenta la función de las células efectora Las células Reh transfectadas y no transfectadas y las células T51 transfectadas y no transfectadas se preetiquetan con ^{51} Cr (100 µCi) (Amersham) durante dos horas a 37° C El PBMC humano sirve como células efectoras y se agregan a las células objetivo (5 x 10^4 por pozo de placa de 96 pozos) en proporciones de 20 1, 10 1, 5 1, y 2,5 1 Después cuatro horas a 37° C, los supernadantes de cultivo se cosechan y analizan como se describe en los ejemplos 11 y 12 El porcentaje de muerte específica se calcula como se describe en el ejemplo 12 Los resultados se presentan en la figura 48

EJEMPLO 30

INDUCCIÓN DE EXPRESIÓN DE CITOQUINA EN CELULAS DE TUMOR QUE EXPRESAN ANTI-CD28 SCFV SOBRE LA SUPERFICIE CELULAR

Este ejemplo describe el efecto del scFv expresado en la superficie celular sobre la inducción de mARN de citoquina en linfocitos estimulados de cocultivo con células de tumor

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transfectadas con una proteína de fusión CD28 scFv IgG-CD80 anti-humano

Se desarrolla análisis PCR en tiempo real en muestra de ARN de PBMC humano estimulado con Reh, Reh-anti-CD28 (2el2) (ver ejemplo 12 para construcción de 2el2 scFv IgG WTH WHTCH3CH2-CD80 y transfección de células Reh), y Reh-CD80 (ver ejemplo 14) con el fin de medir los efectos del scFv expresado de superficie sobre la producción de citoquina por las células efectoras PBMC El ensayo PCR en tiempo real, se utiliza SYBR Green (QIAGEN) (Morrison et al , Biotechtziques 24 954-8, 960, 962 (1998)) y se mide mediante un Sistema de Detección de Secuencia ABI PRISM® 7000 (Applied Biosystems, Foster City, CA) que mide la formación de producto PCR después de cada ciclo de amplificación Las células se cosechan de cultivos y el ARN total se prepara utilizando kits de ARN QIAGEN, que incluye un sistema de purificación de columna granulador para homogenizar lisatos de células, y columnas mini Rneasy® para purificación de ARN Se reversa el cADN trascrito que utiliza cantidades iguales de ARN de cada tipo de célula y transcriptasa reversa Superscript II (Life Technologies) Se desarrolla análisis PCR en tiempo real SYBR Green que utiliza el cADN preparado como plantilla y pares específicos de iniciador para grupos de genes de citoquina El promedio de longitud de los productos PCR que se amplifican varían de 150-250 pares base Los niveles de cADN

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para muchas moléculas de respuesta de activación incluyen IFNγ, TNFα, GM-CSF, IL-4, IL-5, IL-6, IL-8, IL-10, IL-12, IL-15, ICOSL, CD80 y CD86 se ensayan El cADN de referencia de control para genes expresados constitutivamente, incluyen GAPDH, ß-actina, y CD3D se miden en cada ensayo La inducción más importante de mARN se observa para IFN-γ, y se observa inducción más modesta para el CTLA-4 y el ICOS

EJEMPLO 31

CLONACIÓN DE UN ANTICUERPO 4-1BB ANTI-HUMANO Y CONSTRUCCIÓN DE UNA PROTEÍNA DE FUSIÓN 4-1BB SCFV ANTI-HUMANA

Una línea de células de hibridoma que expresan un anticuerpo monmoclonal anti-humano de ratón(designado 5B9) se obtiene del Dr Robert Mittler, Emory University Vaccine Center, Atlanta Las regiones de cadena liviana y pesada variable se clonan de acuerdo con los métodos conocidos para clonación genes de inmunoglobulina y como se describe aquí-las células crecieron en medio FBS (Invitrogen Life Technologies) IMDM/15% durante varios días Las células con crecimiento logarítmico se cosechan de cultivos y el ARN total preparado que utiliza kits QIAGEN, que incluye un sistema de purificación de columna granulador QIA para homogenizar los lisatos de célula, y columnas mini Rneasy® para purificación de ARN de acuerdo con las instrucciones del fabricante El cADN reverso trascrito que utiliza iniciadores hexámeros

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aleatorios y transcriptasa Superscript II (Invitrogen Life Technologies)

El cADN se ancla de cola utilizando transferasa terminal y dGTP El PCR se desarrolla luego utilizando un iniciador complementario de anclaje de cola y un iniciador que recoce específicamente la hebra antisentido y la región constante de cualquier Ck (para amplificación de VL) o el isotipo CH1 de ratón apropiado (para amplificación de VH) Los fragmentos de región variable amplificado TOPO® se clonaron (Invitrogen Life Technologies), y los clones con insertos del tamaño correcto entonces se secuenciaron La secuencia consesus para cada dominio variable se determina de la secuencia de al menos cuatro clones independientes Las secuencias de polinucleótido 5B9 VL y VH se muestran en la ID de SEC Nos _ y _, y las secuencias de aminoácido deducido se muestran en las secuencias ID de SEC No _ y _ El scFv se construye mediante un método de cocer PCR que utiliza iniciadores traslapantes que contiene un dominio ligador sintético $(Gly_4Ser)_3$ entre las regiones variables de cadena liviana y pesada (ver ejemplo 1) El 5B9 scFv (ID de SEC No _)se codifica por la secuencia de polinucleótido que comprende la ID de SEC No _

Se fusiona la secuencia de polinucleótido 5B9 scFv en la estructura para la secuencia de polinucleótido codificada del

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pivote mutante IgG humana CH2 y CH3 tipo silvestre (MTH (SSS) WTCH2CH3, ID de SEC No _) de acuerdo con los métodos descritos en los ejemplos 5, 10, y 13 Se transfectaron transitoriamente las células COS con un vector que comprende la secuencia de polinucleótido 5B9 scFv IgG MTH (SSS) WTCH2CH3 (ID de SEC No _) El supernadante se recolecta en la unión del polipéptido 5B9 scFv IgG MTH (SSS) WTCH2CH3 (ID de SEC No _) se mide mediante inmunocitofluorimetría de flujo esencialmente como se describe en los ejemplos 4, 10, 16, y 18 El supernadante de cultivo de la línea de células de hibridoma 5B9 también se incluye en el ensayo de unión El PBMC humano fresco se incuba en la presencia de anti-CD3 inmovilizado durante cuatro días antes del experimento de unión para inducir la expresión del CD137 sobre la superficie de las células T activadas El PBMC estimulado se lava e incuba con el supernadante de cultivo de hibridoma o COS que contiene la proteína de fusión 5B9 scFv IgG o anticuerpo monoclonal de murina 5B9 respectivamente, durante una hora en hielo La unión de la proteina de fusión 5B9 scFv IgG o anticuerpo monoclonal de murina 5B9 se detecta con FITC conjugado IgG anti-humano o IgG anti-ratón, respectivamente Los resultados se presentan en la figura 49

EJEMPLO 32

CONSTRUCCIÓN DE PROTEINAS DE FUSION 2H7 SCFV IGG CON MUTACIONES DE ARTICULACIÓN

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Las proteínas de fusión 2H7 scFv IgG se construyen con el primer resíduo de cisteína y la segunda cisteína en la región de pivote IgG1 sustituida son un resíduo de serina para suministrar MTH (SCC) y MTH (CSC) La plantilla para la introducción de las mutaciones en un polinucleótido que codifica 2H7 scFv WTH WTCH2CH3 (ID de SEC No _) el oligonucleótido que introduce las mutaciones son los oligonucleótidos iniciadores 5' PCR HIgGMHcys1 (ID de SEC No _) y HIgGMHcys2 (ID de SEC No _) Las construcciones se preparan como se describe en la (ID de SEC No _) Los polipéptidos que codificados de los mutantes se expresan en la ID de SEC No _) y la secuencia de polipéptido se suministra en la ID de SEC No _)

EJEMPLO 33

CONSTRUCCIÓN DE 2H7 VtrL11S scFv (SSS-S) H WCH2 WCH3

Un cambio de leucina por serina en la posición 11 en la región de variable de cadena pesada (numeración de acuerdo con Kabat et al , Sequences of Proteins of Immunological Interest, 5th ed Bethesda, MD Public Health Service, National Institutes of Health (1991)) se introduce en la proteína de fusión 2H7 scFv MTH (SSS) WTCH2CH3 (ID de SEC No _) El resíduo de leucina tipo silvestre se sustituye serina mediante mutagénesis dirigida al sitio que utiliza el

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oligonucleótido Vhser11 5'-gga ggt ggg agc tct cag gct tat cta cag cag tct ggg gct gag tcg gtg agg cc-3' (ID de SEC No _) El iniciador 3' para el PCR es huIgG1-3'que tiene la secuencia 5'-gtc *tct aga* cta tca ttt acc cgg aga cag-3' (ID de SEC No _) (sitio XbaI subrayado y en itálicas) Después de amplificación PCR, los fragmentos se insertan en el vector de clonación TOPO® y se secuenciaron para confirmar la presencia de la leucina VH11 a mutación de serina El 2H7 scFv-IgG (SSS-S) H WCH2 WCH3 que codifica ADN se lanza en el vector de clonación PSL1180 (Pharmacia Biotech, Inc , Piscataway, NJ) La construcción PSL1180-2H7 scFv-IgG (SSS-S) H WCH2 WCH3 se digiere con Sac y XbaI para remover el dominio VH tipo silvestre y la región de conexión y los dominio CH2 y CH3 El producto PCR que comprende el mutante VH11 se digiere con Sac y XbaI y luego se inserta en la construcción PSL1180 de acuerdo con los procedimientos de biología molecular estándar La construcción luego se digiere con HindIII y XbaI, y se inserta dentro del vector de expresión mamífero pD18 (ver métodos descritos en el ejemplo 1 y ejemplo 10) El mutante se designa 2H7 scFv VH L11S IgG (SSS-S) H WCH2 WCH3 La secuencia de polinucleótido se suministra en la ID de SEC No _, y la secuencia de polipéptido codificado se suministra en la ID de SEC No _

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EJEMPLO 34

EXPRESION AND DE 2H7 SCFV VHL11S (SSS-S) H WCH2 WCH3 EN LÍNEAS CHO ESTABLES

Las células CHO DG44 se transfectaron mediante electroporación con aproximadamente 150 micrograms de plásmido de expresión linearizado que codifica el 2H7 VH L11S scFv (SSS-S) H WCH2 WCH3 Los cultivos se colocaron en placas en medio de selección que contiene 100 nM de metotrexato, en placas de cultivo de tejido de fondo plano de 96 pozos en varios numeros de células/pozo, que varían de 125 a 2000 Los clones resistentes al metotrexato se seleccionaron y los supernadantes de cultivo se seleccionaron para los expresores más altos de la proteína de fusión que utilizan un ensayo de unión CD20CHO similar a aquel descrito para la Figura 1 Los clones se amplificaron después de selección inicial en dosis gradualmente incrementadas de metotrexato Las células se pasaron por dos pasajes en una concentración mayor antes de ajustar la concentración a la siguiente dosis mayor Los clones se amplificaron para una concentración final de 1 micromolar de metotrexato

La Figura 50B ilustra los niveles de producción de 2H7 VH L11S scFv (SSS-S)H WCH2 WCH3 Los supernadantes gastados de las células CHO amplificadas que expresan esta molécula y que crecen en frascos T25 estacionarios se ensayaron para unión

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cuantitativa a las células CD20CHO mediante citometría de flujo La actividad se convirtió a concentración de proteína mediante la generación de una curva estándar que utiliza la misma molécula purificada de los supernadantes con cromatografía de afinidad de proteína A (Figura 50A) La concentración de la proteína purificada se determinó mediante A280 que utiliza un coeficiente de extinción suministrado por la composición de aminoácido de la proteina recombinante (Vector NIT) Aunque los niveles de producción varían entre los clones ensayados, múltiples clones producidos sobre 1 mg/ml Este nivel de expresión de proteína está sobre 10 veces mayor que la molécula idéntica excepto para el cambio de aminoácido en V_H

La Figura 51 ilustra los niveles de producción de 2H7 VHL11S scFv (SSS-S)H WCH2 WCH3 mediante análisis semi-cuantitativo en el SDS-PAGE Diez microlitros de supernadante gastado de las células CHO amplificadas que expresan esta molécula y crecen en frascos T25 estacionarios se mezclan con 10 microlitros de amortiguador de muestra SDS 2X sin reducción, que corren en geles SDS-PAGE, y teñidos con azul coomassie

EJEMPLO 35

CAPACIDAD DE UNION Y CONSTRUCCIÓN DE LAS CONSTRUCCIONES G28-1 scFv IG

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La construcción del G28-1 (anti-CD37) scFv se desarrolla utilizando aislamiento de ARN total del hibridoma G28-1 que utiliza reactivo Trizol (Invitrogen) de acuerdo con las instrucciones del fabricante El cADN se preparó utilizando iniciadores aleatorios y el protocolo previamente descrito para la clonación 2H7 en el Ejemplo 1 Los dominios de variable del scFv se clonaron utilizando uno o dos métodos el primer método utiliza una familia de oligonucleótido 5' degenerados específicos para cada familia de gen de región V y un iniciador específico 3' para cada región constante de cualquiera de los métodos que utilizan cadena liviana o pesada e iniciadores descritos en (Ig-Prime Kit Mouse Ig-Primer Set, Novagen) La segunda aproximación utiliza los métodos de anclaje de cola y los iniciadores descritos en (Gilliland LK et al, Tissue Antigens 47 1-20 (1995) En cualquier caso, los productos amplificados por PCR se clonan en el vector de clonación TOPO (Invitrogen) Los clones se digieren con EcoRI y se seleccionan para interés del tamaño apropiado Los clones positivos se secuencian como se describió anteriormente en el Ejemplo 1

Los iniciadores específicos se diseñan entonces para cada región V, uno con la secuencia líder y otro sin la secuencia líder Los iniciadores también se diseñan para incluir ligadores deseados y/o sitios de restricción en los extremos de iniciador Las reacciones PCR se desarrollan en el ADN

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clonado TOPO que utiliza un programa de 25 ciclos con el siguiente perfil 94C, 30 seg, 55C, 30 seg, 72C, 30 seg seguido por una extensión final de 72C durante 8 minutos Los productos PCR se purifican con gel y los fragmentos recuperados utilizan un kit de extracción de gel QIAQUICK (QIAGEN, Valencia, CA) Los fragmentos se diluyen 1 50 y 1 microlitro se utiliza para reacciones SEWING PCR de acuerdo con los métodos descritos en el Ejemplo 1 Los siguientes oligonucleótidos se utilizan para las reacciones PCR secundarias del dominio V_L para el G28-1 scFv

iniciadores 5' con el sitio SalI sin líder

5'-GTTGTTGTCGACATCCAGATGACTCAGTCTCCA3'(ID DE SEC NO _)

iniciador 5' con sitio HindIII y secuencia líder

5'-GTCAAGCTTGCCGCCATGGTATCCACAGCTCAGTTCCTTGG3'(ID DE SEC NO _)

iniciador 3'

5'-GCCACCCGACCCACCACCGCCCGAGCCACCGCCACCTTTGATCTCCAGTTCGGTGCC-3' (ID DE SEC NO_)

Los iniciadores que utilizan el dominio V_H se muestran adelante

Iniciador de sentido 5'

TCGGGCGGTGGTGGGTCGGGTGGCGGCGGATCGTCAGCGGTCCAGCTGCAGCAGTCTGGA-3' (ID DE SEC NO _)

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Iniciador antisentido 3' con sitio BclI

5'-TCAGTGCTGATCAGAAGAGACGGTGACTGAGGTTCCTTG-3' (ID DE SEC NO _)

Un cambio de leucina por serina en la posición 11 en la región variable de cadena pesada (numeración Rabat) se introduce en el G28-1 scFv mediante mutagénesis dirigida al sitio La forma tipo silvestre del G28-1 scFv se construyó inicialmente mediante coser/traslapar el PCR para insertar un ligador (gly4ser)3 entre los dominios VL y VH como se describió anteriormente Sin embargo, no se introdujo ningun sitio Sac I como una parte de esta fusión de los dominios variables, como alternativa, los sitios de restricción cercanos (HaeII y PvuII) cerca de la leucina 11 se utilizan para sintetizar el dominio VH mutado VL+ Los iniciadores se diseñan para contener uno de estos sitios y la secuencia de ADN que incluye el cambio L por S, seguido por los pares base tipo silvestre 12 Varios intentos en esta estrategia fallaron, así como una estrategia alternativa que utiliza el método Genetailor (Invitrogen) de mutagénesis dirigida al sitio como se utiliza para introducir la mutación deseada La mutagénesis se lleva a cabo de acuerdo con las instrucciones del fabricante En resumen el procedimiento involucra la metilación del plásmido de ADN con la metilasa ADN, la amplificación del ADN en una reacción de mutagénesis con dos iniciadores traslapantes, uno de los cuales contiene las

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mutaciones objetivo, transformación del plásmido E coli tipo silvestre que digiere todo el ADN metilado y deja solo el no metilado, el producto de amplificación mutado Ambos iniciadores son 30 nucleótidos aproximadamente de longitud (no incluye el sitio de mutación en el iniciador mutagénico, con una región traslapante en los extremos 5′ de 15-20 nucleótidos, para el fin de unión eficiente del producto de mutagénesis La plantilla de la reacción de mutagénesis era de 100 ng de un plásmido que contiene la construcción G28-1 scFvIg tipo silvestre, y los iniciadores utilizados para la mutagénesis G28-1 VH son como sigue

Iniciador delantero

5'-GCAGCAGTCTGGACCTGAGTCGGAAAAGCCTG-3′ (ID DE SEC NO _)

Iniciador inverso

5'-CTCAGGTCCAGACTGCTGCAGCTGGACCGC-3' (ID DE SEC NO_)

Las reacciones PCR se desarrollaron utilizando 15 ng de plantilla metilada, el iniciador anterior, y los componentes de reacción usuales como se describió previamente Un programa de 20 ciclos con el siguiente perfil se utiliza para la amplificación 94C, 30 seg, 55C, 30 seg, 68C, 8 min, seguido por una etapa de extensión final de 68C durante 10 minutos Los productos PCR se transforman en bacterias de tipo silvestre, y se seleccionan colonias para secuenciación Los clones con solo la mutación deseada se aíslan y se

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preparan los plásmidos como se describió previamente en el Ejemplo 33 El mutante se designa G28-1 scFv VH L11S (SSS-S) H WCH2 WCH3 La secuencia de polinucleótido se suministra en la ID DE SEC NO _, y la secuencia de polipéptido codificado se suministra en la ID DE SEC NO _

El nivel de expresión de las proteínas de fusión G28-1 se confirma utilizando análisis Immunblot de acuerdo con los métodos descritos en el Ejemplo 17 La Figura 53 ilustra un incremento grande en la expresión de proteína en las proteínas de fusión G28-1 mutante VH L11S comparado con la proteína de fusión G28-1 sin la mutación

Las proteínas de fusión G28-1 scFv Ig se transfectan transitoriamente y se expresan en las células COS de acuerdo con los métodos descritos en el Ejemplo 10 La Figura 52 ilustra la capacidad de las proteínas de fusión G28-1 scFv Ig de los supernadantes COS para unir el CD37 Las células Ramos y BJAB ambas expresan el CD37 humano, y por lo tanto se utilizan para la selección de los supernadantes G28-1 para actividad funcional La unión de G28-1 scFv (SSS-S) H WCH2 WCH3 y G28-1 scFv VH L11S (SSS-S) H WCH2 WCH3 a células Ramos CD37+ se utiliza mediante citometría de acuerdo con los métodos descritos en el Ejemplo 2 Cada punto en la gráfica representa el medio de cinco transfecciones replicadas La

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gráfica ilustra que el G28-1 scFv VH L11S (SSS-S)H WCH2 WCH3 es capaz de unir las células Ramos CD37+

Las construcciones de adición se hicieron con diferentes regiones de conexión El vector pD18 G28-1 scFv VHL11S (SSS-S) H WCH2 WCH3 se digiere con BclI y XbaI para remover la región de conexión CH2 y CH3 Estas se reemplazan con cada región de conexión diferente CH2 y CH3 de acuerdo con los métodos descritos en el Ejemplo 13 Las nuevas construcciones se designan G28-1 scFv VHL11S (CSS-S) H WH2 WH3 (ID DE SEC NO__), G28-1 scFv VHL11S (CSC-S) H WH2 WH3 (ID DE SEC NO_), G28-1 scFv VHL11S (CSS-S) H WH2 WH3 (ID DE SEC NO__), G28-1 scFv VHL11S (SSC-P) H WH2 WH3 (ID DE SEC NO_), G28-1 scFv VHL11S (SCS-S) H WH2 WH3 (ID DE SEC NO_), G28-1 scFv VHL11S (CCS-P) H WH2 WH3 (ID DE SEC NO_), y G28-1 scFv VHL11S (SCC-P) H WH2 WH3 (ID DE SEC NO_)

El G28-1 scFv también se adhiere a una región de conexión IgA, CH2, CH3 y un IgE CH2, CH3, CH4 El plásmido pD18 G28-1 scFv VHL11S (SSS-S) H WCH2 WCH3 se digiere utilizando los métodos anteriores para remover la región de conexión CH2 y CH3 Las regiones IgA se insertan utilizando métodos descritos en el Ejemplo 13 La construcción se designa G28-1 scFv VHL11S IgAH IgACH2 T4CH3 (ID DE SEC NO_) La región IgE CH2 CH3 CH4 se inserta en el vector pD18 digerido anterior que utiliza los métodos descritos en el Ejemplo 39 La

construcción se designa G28-1 scFv VHL11S IgECH2 CH3 CH4 (ID DE SEC NO_)

EJEMPLO 36

Caracterización de las proteínas de fusión mutante 2H7 scFv Ig

La Figura 54 ilustra la capacidad de unión de las construcciones 2H7 scFv Ig purificadas a células CD20+ CHO Las proteínas se transfectan en células CHO estables de acuerdo con los métodos descritos en el Ejemplo 2 La unión se determina utilizando citometria de flujo de acuerdo con los métodos descritos en el Ejemplo 2 La gráfica en la Figura 54 ilustra que estas proteínas retienen la función de unión al CD20 con regiones de conexión alterada Los resultados comparativos obtienen en cada uno de los mutantes 2H7 scFv VHL11S con cada tipo de región de conexión alterado (se omiten los resultados)

La capacidad de las construcciones 2H7 scFv-Ig con regiones de conexión mutadas para matar células CD20 positivas en la presencia de células mononucleares sanguíneas periféricas (PBMC) a través de ADCC se ensayan al medir la liberación del ^{51}Cr de células BJAB etiquetadas en un ensayo de 4 horas que utiliza 100 1 de proporción de PBMC a células BJAB Los resultados se mostrados en la Figura 55 indican que los

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mutantes 2H7 scFv-Ig pueden mediar citotoxicidad celular dependiente de anticuerpo (ADCC), en razón a que la liberación del ^{51}Cr era significativamente mayor en la presencia de ambos PBMC y 2H7scFv-Ig que en la presencia de PBMC o 2H7scFv-Ig solo Los resultados comparativos se obtienen en cada uno de los mutantes 2H7 scFv VHL11S con cada tipo de región de conexión alterada (se omiten los resultados)

La capacidad de las proteínas de fusión mutantes 2H7 scFv-Ig para matar células positivas CD20 en la presencia de complemento se ensaya al utilizar células objetivo Ramos de líneas de célula B El complemento de conejo se compró de Pel-Freez (Rogers, AK), y se utilizó en el ensayo en una concentración final de 1/10 El 2H7scFv-Ig purificado se incubó con células B y se complementa durante 45 minutos a 37° C, seguido por conteo de células vivas y muertas mediante exclusión de azul trypan Los resultados en la Figura 56 muestran que los mutantes 2H7scFv-Ig en la presencia de conejo complementan las células B lisadas que expresan CD20

EJEMPLO 37

UNION COMPARATIVA DE CONSTRUCCIONES IGA, IGG, E IGE 2H7 SCFV

La capacidad de unión de construcciones IgA, IgG e IgG se midió utilizando citometría de flujo de acuerdo con los

métodos descritos en el Ejemplo 2, que utiliza una segunda etapa específica disponible comercialmente (Caltag) para cada cola Ig Los resultados en la Figura 57 muestran que todas las construcciones IgE estaban disponibles para unirse a las células CD20+ CHO comparables con las capacidades de IgG e IgA Estos resultados también demuestran que las construcciones IgE se detectan con la segunda etapa IgE, pero no la segunda etapa IgA o IgG

EJEMPLO 38

CONSTRUCCIÓN Y CARACTERIZACION DE CONSTRUCCIONES 2H7 VH L11S IGE

El ARN de cola IgE se aisló de células SKO-007 (ATCC) utilizando minikits de ARN y de homogenización QIAGEN QIAshredder El cADN iniciado aleatoriamente se generó de acuerdo con el protocolo usual, con 4 microlitros de ARN eluado de las columnas QIAGEN El IgE humano del comienzo del CH1 hasta CH4 (aproximadamente 1 2 kb) se aisló mediante amplificación PCR de 5 microlitros de cADN, con un perfil de amplificación de 94C, 60 seg, 72C, 2 minutos durante 35 ciclos y los siguientes iniciadores

iniciador 5'

5-'ggatccacccgctgctgcaaaaacattccctccaatgccacctccgtgac-3' (ID DE SEC NO_)

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iniciador 3'

5'-tcatttaccgggatttacagacaccgctcgctggacggtctgtgaggggctcgctgc-3' (ID DE SEC NO _)

Los fragmentos de PCR se ligaron en el vector PCR 2 1-TOPO, y los transformantes seleccionados para insertos del tamaño correcto mediante digestión con EcoRI de acuerdo con los métodos descritos en el Ejemplo 1 Uno de los clones con la secuencia correcta se utiliza como plantilla para amplificar los dominios CH2-CH4 con los sitios de restricción apropiados adheridos para subclonación como formas de superficie celular o solubles (ORF) Los siguientes iniciadores se utilizaron con un perfil de amplificación de 94C, 60 seg, 55C, 60 seg, 72C, 2 min, durante 35 ciclos para amplificar un fragmento de aproximadamente 950 bp

iniciador 5' (adhiere el sitio BclI al extreme 5' del dominio CH2 de IgE)

5'-gttgttgatcacgtctgctccagggacttcacc-3' (ID DE SEC NO_)

iniciador 3' (adhiere el codón de parade y el sitio XbaI al extreme 3' del CH4 de IgE)

5'-gttgtttctagattatcatttaccaggatttacagacaccgctcgctg-3' (ID DE SEC NO_)

iniciador 3' (adhiere el SfuI y BamHI al extreme 3' el CH4 sin un codón de parada)

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5'-gttgttttcgaaggatccgctttaccagatttacagacaccgctcgctg-3' (ID DE SEC NO_)

La cola IgE CH2CH3CH4 con un codón de parada se digirió con BclI y XbaI e insertó dentro de un vector pD18 que contiene 2H7 VHL11S scFv Esta construcción se designó 2H7 IgECH2CH3CH4 La secuencia de polinucleótido se suministra en la ID DE SEC NO _, y la secuencia de polipéptido codificado se suministra en la ID DE SEC NO _ La cola IgECH2CH3CH4 sin codón de parada (ORF) se digirió con BclI y SfuI e insertó dentro de un vector pD18 que contiene 2H7 VHL11S scFv Esta construcción se designó 2H7 IgeCH2CH3CH4 (ORF) La secuencia de polinucleótido se suministra en la ID DE SEC NO _, y la secuencia de polipéptido codificado se suministra en la ID DE SEC NO _

El IgE humano también se amplifica como un fragmento de ambos dominios CH1 y CH2, solo con los dominios CH3 y CH4 adheridos a el pivote IgG1 humano Las reacciones PCR secuenciales que utilizan oligonucleótidos 5' traslapantes se utilizan para adherir el pivote IgG1 al dominio CH3 del IgE humano Los iniciadores para la primera etapa de la reacción PCR

iniciador 5'

5'-actcacacatccccaccgtccccagcatccaacccgagaggggtgagc-3' (ID DE SEC NO_)

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Iniciadores para la segunda etapa de cada reacción PCR

iniciador 5'

5'--tctgatcaggagcccaaatcttctgacaaaactcacacatccccaccg-3' (ID DE SEC NO_)

iniciador 3'

5'-gitttotagattatcatttaccaggatttacagacacogetogOtg-3' (ID DE SEC NO_)

El producto PCR se digirió con EcoRI y se secuenció de acuerdo con los métodos descritos en el Ejemplo 1 Los clones positivos se insertaron en plásmido pD18 que contiene 2H7 VHL11S scFv (SSS-S)H La construcción se designó 2H7 VHL11S scFv (SSS-S)H IgE CH3CH4 La secuencia de polinucleótido se suministra en la ID DE SEC NO _, y la secuencia de polipéptido codificado se suministra en la ID DE SEC NO _

La capacidad de unión de 2H7 scFv VH L11S IgECH2 CH3 CH4, se midió utilizando citometría de flujo, esencialmente de acuerdo con el Ejemplo 2 La proteína se purificó utilizando MEP HyperCel, (Cipergen, Catálogo #12035-010, Lote #200920/0271) resina de cromatografia y Cromatografía de Inducción de Carga Hidrófoba (HCIC) El HCIC absorbente es un absorbente de alta capacidad, alta selectividad, diseñado para capturar y purificación de anticuerpos monoclonales y

policlonales de varias fuentes que incluyen supernadantes de cultivo celular Las columnas se empacan con un volumen de 10 ml de lecho de MEP Hypercel, y equilibrado con PBS, pH 7 4 que contiene 0 1% NaN_3 Aproximadamente 1 litro de supernadante de cultivo 2H7 scFv VHL11S IgE CH2CH3CH4 CHO luego de correr sobre la columna Una serie de amortiguadores de citrato que varía de pH 3-6 se preparan para elusión de la fusión de proteína La columna se lavó en PBS La proteina se elúo en quince fracciones de 1 ml en pH 6, 5, y 4 Una fracción de 15 ml final se recolectó en pH 3 5 Las alícuotas de cada fracción se analizaron para A280 y también se sometieron a SDS-PAGE, cargando áspera de 10 microgramos/pozo basado en la lectura A280 Los resultados de estos dos análisis indican que la masa de la proteína no elua en amortiguadores de citrato en pH mayores, pero eluan en pH 4, y el lavado de post elusión en pH 3 5 también contiene cantidades importantes de proteína

La capacidad de estas proteínas purificadas 2H7 VH L11S IgE de unir células CD20+CHO se determina utilizando citometría de flujo de acuerdo con lo métodos descritos en el Ejemplo 2 que utilizan IgE de cabra anti-humano FITC-conjugado La Figura 58A ilustra que ambas proteínas purificadas son capaces de unir células CD20+ CHO

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La capacidad de estas proteínas purificadas 2H7 VH L11S IgE para mediar ADCC contra células objetivo BJAB con efectores PMRC se mide de acuerdo con los métodos descritos en el Ejemplo 2 La Figura 58B ilustra que ambas proteínas son capaces de mediar ADCC a niveles similares

EJEMPLO 39

CAPACIDAD DE UNIÓN Y CONSTRUCCIÓN DE MUTANTES scFv VH L11S CON REGIONES DE COLA IGA E IGE DE RATÓN

La murina IgA se clonó a partir de ARN de bazo de murina que utiliza esencialmente los mismos métodos utilizados para clonar las colas IgE humanas en el Ejemplo 38 Las reacciones PCR se desarrollan con 94C 60 seg, 52C 60 seg, 72C 2 min de perfil de amplificación durante 35 ciclos Los iniciadores de PCR utilizados para clonar las regiones CH1-CH4 fueron

iniciador 5'

5'-atctgttctcctcctactactcctcctccacct-3' (ID DE SEC NO__)

iniciador 3'

5'-tcagtagcagatgccatctccctctgacatgatgacagacacgct-3' (ID DE SEC NO_)

Iniciadores PCR utilizados para la eliminar la región CH1

iniciador 5'

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5'-gttgttgatcacatctgttctcctcctactactcctcctccacct-3' (ID DE SEC NO_)

iniciador 3' con un codón de parada, sitio XbaI en el extremo de la cola Ig, y la mutación T4 en la región CH3

5'-gttgtttctagattatcaatctccctctgacatgatgacagacac-3' (ID DE SEC NO_)

iniciador 3' para el ORF, un sitio SfuI y BamHI, y mutación T4 en la región CH3

5'-gttcttcgaaggatccgcatctccctctgacatgatgac-3' (ID DE SEC NO_)

La cola IgACH2 T4CH3 de ratón con un codón de parada se digirió con BclI y XbaI e insertó en un vector pD18 que contiene 2H7 VHL11S scfv y la IgAH Esta construcción se designó 2H7 VHL11S scFv IgAH mIgACH2 T4CH3 La secuencia de polinucleótido se suministra en la ID DE SEC NO _, y la secuencia de polipéptido codificado se suministra en la ID DE SEC NO _

La cola de IgACH2 T4CH3 de ratón sin codón de parada (ORF) no se digirió con BclI y SfuI e insertó en el vector pD18 que contiene 2H7 VHL11S scFv e IgAH Esta construcción se designó 2H7 VHL11S scFv IgAH mIgACH2 T4CH3 (ORF) La secuencia de polinucleótido se suministra en la ID DE SEC NO _, y la secuencia de polipéptido codificado se suministra en la ID DE SEC NO _

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La murina IgE se clonó IgE La2 (ATCC) ARN de murina esencialmente de acuerdo con los métodos descritos en el Ejemplo 38 Las reacciones PCR se desarrollaron con un 94C 60 seg, 54C 60 seg, 72C 2 min de perfil de amplificación durante 35 ciclos Los iniciadores PCR iniciales se utilizaron para clonar el CH1-CH4

iniciador 5'

5'-tctatcaggaaccctcagctctacccctgaagccctg-3' (ID DE SEC NO_)

iniciador 3'

5'-gttgtttctagattatcaggatggacggagggaggtgttaccaaggct-3' (ID DE SEC NO_)

Iniciadores PCR para remover la región CH1

iniciador 5'

5'-gttgttgatcacgttcgacctgtcaacatcactgagcccacc-3' (ID DE SEC NO_)

iniciador 3' con codón de parada en el sitio XbaI

5'-gttgtttctagattatcaggatggacggagggaggtgtaccaaggct-3' (ID DE SEC NO_)

iniciador 3' ORF, SfuI y BamHI

5'-gttgttttcgaaggatccgcggatggacggagggaggtgtta-3' (ID DE SEC NO_)

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La cola IgE CH2CH3CH4 con un codón de parada se digirió con BclI y XbaI e insertó en el vector pD18 que contiene 2H7 VHL11S scFv Esta construcción se designó 2H7 VHL11S mIgECH2CH3CH4 La secuencia de polinucleótido se suministra en la ID DE SEC NO _, y la secuencia de polipéptido codificado se suministra en la ID DE SEC NO _

La cola IgE CH2CH3CH4 de ratón sin codón de parada (ORF) se digirió con BclI y SfuI e insertó en el vector pD18 que contiene 2H7 VHL11S scFv Esta construcción se designó 2H7 VHL11S scFv mIgECH2CH3CH4 (ORF) La secuencia de polinucleótido se suministra en la ID DE SEC NO _, y la secuencia de polipéptido codificado se suministra en la ID DE SEC NO _

La capacidad de unión de 2H7 VH L11S mIgE y el mIgA (sin regiones de cola de ratón) a células CD20+CHO también se midieron mediante citometría de flujo de acuerdo con los métodos descritos en el Ejemplo 2 que utilizan reactivos (Caltag) de segunda etapa IgE o IgA disponibles comercialmente Cada punto en la Figura 59 representa la media de una población con brillo corregido por sustracción de la unión de la segunda etapa sola Esta Figura ilustra que estas construcciones tienen la capacidad de unir las células CD20+CHO

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EJEMPLO 40

PERFILES DE HPLC DE PROTEÍNAS DE FUSIÓN MUTANTE 2H7 scFv IG

El análisis HPLC de proteínas de fusión mutante 2H7 scFv-Ig purificadas con regiones CH3 y de conexión alterada Cada proteína se purifica mediante cromatografía de afinidad de Proteína A de supernadantes de células COS o CHO transfectadas Veinticinco a cincuenta microgramos de cada muestra se corre a 1 ml/min en PBS en una columna de 30 cm TSK-GEL G3000SW$_{XL}$ (Tosoh Biosep, Stuttgart, Alemania) La flecha cerca al inicio de cada perfil representa el punto de inyección de muestra Los estándares de filtración de gel (Bio-Rad) incluyen trioglobulina (670 kDa), gama globulina (158 kDa), ovalbúmina (44 kDa), mioglobina (12 5 kDa), y vitamina B-12 (1 35 kDa) Los estándares se corrieron en el inicio y final de cada experimento Las posiciones de migración de los estándares se muestran y no varían entre los experimentos (Figuras 60-62)

EJEMPLO 41

CAPACIDAD DE UNIÓN DE LAS PROTEÍNAS DE FUSIÓN MUTANTE 2H7 VHL11S

Los efectos de unión del CH3 mutante se comparan con la proteína de fusión CH3 no mutada Las construcciones se transfectan en células COS y purifican del supernadante

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utilizando técnicas de purificación de columna de Proteína A descritas en el Ejemplo 2 La Figura 63 ilustra los efectos diferenciales de las mutaciones CH3 sobre la unión que utiliza la citometría de flujo de acuerdo con los métodos descritos en el Ejemplo 2 Esta Figura ilustra que alguna capacidad de unión se pierde cuando el punto de mutación doble se introduce en la región CH3

La unión de las proteínas de fusión mutante 2H7 VH L11S conjugadas con fluoresceína isotiocianato (FITC) se determinó utilizando citometría de flujo Una solución de 1 mg/ml de FITC se preparó en DMSO Las proteínas de fusión se dializaron en amortiguador de bicarbonato de pH 9 3 durante la noche a 4C en un volumen de 2 litros La concentración de la proteína se ajustó a 1-5 mg/ml antes de la conjugación Una serie de tubos de 5 ml Falcon se configuró con FITC variante para proporciones de proteína, que varían de 15-60, pero proporciones mínimamente de 20 y 40 Las reacciones de conjugación se incubaron a 37C durante 30 minutos protegidos de la luz La proteína se separa de la fluorescencia libre en una columna G-25 Sephadex de 2 ml equilibrado con PBS, 0,5 NaCl, y 1% NaN3 La proteína se etiqueta con fluoresceina eluada de la primer columna se recolecta en un tubo de 5 ml El grado de etiqueta se determina al medir la absorbancia del conjugado diluido en 280 y 494 nm, y que utiliza las formas que se suministran por servicios técnicos en Molecular Probes

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(Eugene, OR) Los datos se han corregido para FITC índice de proteína La figura 64 ilustra que estas construcciones no pierden la capacidad de unión cuando se conjugan con un marcador fluorescente

Las construcciones 2H7 VH L11S purificadas corrieron sobre un gel SDS sin reducción de acuerdo con los métodos descritos en el ejemplo 2 Los patrones de migración se presentan en la figura 65

EJEMPLO 42

CARACTERIZACIÓN DE 2H7 scFv VH L11S (CSC-S)H WCH2 WCH3 EN CÉLULAS LEC13 CHO

2H7 scFv VH L11S (CSC-S)H WCH2 WCH3 se transfecta transitoriamente y se expresa en células Lecl3 CHO Se utilizaron células Lecl3CHO como huéspedes de células de mamífero para el 2H7 scFv VHS11 hIgG1 (CSS-S)H WCH2 WCH3 y (CSC-S)H WCH2 WCH3 que expresan plásmidos en transfecciones lado a lado Todas las transfecciones se desarrollaron en platos de cultivo de tejido de 100 mm Las células se transfectaron cuando aproximadamente el 90% de confluente utiliza lipofectamina 2000 (Invitrogen, Catalog # 11668-027, 0,75 ml), siguiendo las instrucciones del fabricante Ambas líneas celulares crecieron en la presencia de suero para promover y mantener la adherencia a los platos de cultivo

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celular, simplificando las manipulaciones de transfección, lavado, y cosechado del supernadante El ADN complejos de lipofectamina se les permite formarse en la ausencia de sueros y antibióticos, siguiendo el protocolo sugerido/condiciones registradas en el inserto del producto Los supernadantes de cultivo se cosecharon 72 horas después de la transfección, y luego de 72 horas después de la primera cosecha La proteína de fusión de las dos fuentes CHO se aísla mediante la purificación de proteína A como se describió previamente y se utilizo en los ensayos de unión CD20 y DAC

La habilidad de la proteína de fusión mutada para mediar DAC en células CD20 positivas se determina al utilizar los métodos descritos en el ejemplo 2 Las construcciones expresadas en las células Lec13 CHO exhiben mejor unión a las células objetivo CD20 CHO y también muestran actividad significativamente mejorada en los ensayos DAC relacionados con las proteínas derivadas de CHO DG44 en concentraciones equivalentes como se ilustra en la figura 67

EJEMPLO 43

CONSTRUCCIÓN DE ALELOS CD16 DE ALTA y BAJA AFINIDAD

Los alelos de baja (V) y alta (F) afinidad en la posición 158 del dominio extracelular CD16 humano se clonaron del cDNA

derivado del PBMC humano que utiliza un ensayo PCR Las reacciones PCR utilizan cADN iniciado aleatoriamente hecho de PBMC estimulado durante 3 días con anticuerpo anti-CD3 inmovilizado (64,1) antes de cosechar Las reacciones PCR incluyeron 2, 4, 6 u 8 microlitros de cADN, cada iniciador en 25 pmol, y un perfil de amplificación de 94C 60 seg, 55C 60 seg, 72C 2 min, durante 35 ciclos Los iniciadores PCR se listan adelante

Iniciador 5'- sin péptido líder 5'-
GTTGTTACCGGTGCAATGCGGACTGAAGATCTCCC
AAAGGCTGTG-3' (ID de SEC No _)
Iniciador 3'antisentido 5'-
GTTGTTTGATCAGCCAAACCTTGAGTGATGGTGATGTTCACA-3' (SEQ ID No _)

Los clones se secuencian e insertan dentro de un vector que contiene un péptido líder eficiente y la cola IgG humana (SSS-S)H P238SCH2 WCH3 Dos versiones diferentes de las proteínas de fusión CD16 ED se expresan La primera contiene el F158 (alta afinidad) y la segunda contiene el alelo V158 (baja afinidad) Las construcciones se clonan en un plásmido (SSS-S)H P238S CH2 WCH3 pD18 y se expresan en células COS y CHO como se describió anteriormente en los ejemplos 1 y 10 Los clones CHO se seleccionaron para expresión utilizando un ensayo ELISA intercalado IgG para determinar los niveles de expresión relativos de las proteínas de fusión en el

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supernadante de cultivo que utiliza el siguiente protocolo placas Immulon 1V se cubrieron en 4C con 0,4 microgramos/ml de IgG antihumano de cabra (ratón adsorbido), (Carrag, Catalog #, H10500) en amortiguador PBS Las placas fueron luego bloqueadas en PBS/1,5% de leche sin grasa a 4C durante la noche Las placas se lavaron tres veces en PBS/0,1% Twen 20, luego se incubaron con series de dilución de 100 microlitros de supernadante de cultivo de clon CHO a temperatura ambiente durante 3 horas Cuatro diluciones por clon se agregaron a pozos sucesivos, diluyendo en 5 veces incrementos de 1 5 a 1 375 En adición una curva estándar se deriva utilizando CTLA4 hIgG1 (SSS-S)H P238SCH2 WCH3 como una concentración estándar Las series de dilución utilizaron 5 veces diluciones partiendo en 0,5 microgramos/ml, un segundo conjunto de 8 pozos se utiliza para hacer series de dilución de 2 veces partiendo en 0,34 micrograms/ml Las placas se lavan tres veces en PBS 0,1% Twen 20 e incuban con IgG antihumano de cabra conjugado para peroxidasa de rábano (GAH IgG-HRP) a 1 5000, en PBS/0,5% BSA durante 1 hora Las placas se lavan cuatro veces con PBS/0,1% Tween 20, luego substrato de TMB cromagen (BD-Pharmingen) se agrega durante 10 minutos, y las reacciones se detienen mediante la adición de 100 microlitros de ácido sulfúrico 1N Las placas se leyeron luego a 415 nm sobre un lector de placa SpectraCount como aparece ahí Las concentraciones de la proteína de fusión se estiman por comparación de los OD en el rango

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lıneal a la carrera de la curva estándar CTLA4Ig en cada plato

Las proteínas se purıfican utılızando purıfıcacıón de proteína A y se conjugan directamente para ısotıocıanato de fluoresceína (FITC) como se descrıbe en el ejemplo 42 Estas proteínas se corren en geles SDS bajo condıcıones reducıdas y no reducidas de acuerdo con los métodos descrıtos en el ejemplo 2 La mıgracıón de estas proteínas se presenta en la fıgura 68

La habılidad de los alelos altos y bajos CD16 para unır 2H7 VH111S (CSC-S)H WCH2 WCH3 o unır 2H7 VH111S (SSS-S)H (P238S) CH2 WCH3 que se expresa en la superfıcıe celular de la célula objetıvo CD20+ CHO se determina al utılızar cıtometría de flujo de acuerdo con los métodos descritos en el ejemplo 2 Los resultados en la fıgura 69 demuestran la baja y alta afınıdad de los alelos que son capaces de unırse a 2H7 VHL11S (CSC-S)H WCH2 WCH3 (ID de SEC No _)y la pérdida de algunas capacıdades de unıón cuando la mutacıón P238S se ıntroduce dentro de la regıón CHO de la construcción (ID de SEC No _)

EJEMPLO 44

SISTEMA DE EXHIBICIÓN EN MAMÍFERO

Los diagramas de la figura 70a muestran conjugados FITC de FcRIII (CD16) proteínas de fusión soluble que se unen a construcciones 2H7 scFv-Ig que se adhieren a CD20 expresado por las células CHO La unión CD16 a un scFv-Ig suministra una herramienta de selección para detectar cambios en la unión CD16 a una construcción scFv-Ig alterada que contiene nutaciones objetivo o específicas de sitio Los cambios en las propiedades de unión CD16 pueden ser cambios en la unión de la proteína CD16 de alta afinidad (158F) o proteína de baja afinidad CD16 (158V) o ambas

Una representación esquemática de tal proceso de selección se diagrama en la figura 70b, en donde las construcciones scFv-Ig se despliegan sobre la superficie celular de las células de mamíferos Las moléculas scFv-Ig en este ejemplo se despliegan sobre la superficie celular debida a que ellas contienen un anclaje de dominio de transmembrana Estas moléculas pueden representar una construcción scFv-Ig sencilla o se pueden introducir dentro de una población de células de mamífero como una librería de tales moléculas Las células transfectadas con propiedades de unión alterada pueden luego ser filtradas, clasificadas, o de otra forma aisladas de otras células al alterar la restricción de las condiciones de selección y utilizar proteínas de fusión CD16 como la sonda de unión Las células que expresan las moléculas scFv-Ig con unión alterada al alelo de alta afinidad CD16 (158F) o alelo

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de baja afinidad CD16 (158V) o a ambos se puede aislar Por ejemplo, el sistema de exhibición se puede utilizar para crear una librería de colas Ig mutadas con estiramientos cortos de la secuencia CH2 reemplazada con oligonucleótidos aleatorizados o posible aleatorización de un residuo sencillo con todas las sustituciones de aminoácido posibles, que incluyen aminoácidos sintéticos Una vez se construye tal librería está se puede tranfectar en células CHO mediante métodos bien conocidos en la técnica Los transfectantes pueden entonces unirse a las construcciones CD16 etiquetadas, y filtradas o clasificadas basadas en sus propiedades de unión relativa a múltiples alelotipos /isoformas Las células filtradas y cosechadas, y los plásmidos de ADN se aíslan y luego se transforman en bacterias Este proceso se puede repetir iterativamente múltiples veces hasta que clones sencillos se aíslan de las células huésped de mamífero (ver Seed B y Aruffo A, PNAS 84 3365-3369 (1987) y Aruffo A y Seed B, PNAS 84 8573-8577 (1987)) Uno de tales usos de este tipo de selección puede aislar colas Ig que se unen igualmente bien a los alelos de alta y baja afinidad de CD16 con la meta de mejorar las funciones efectoras mediadas por construcciones scFv-Ig en múltiples sub-poblaciones de pacientes Las colas Ig con propiedades de unión alterada a otros receptores Fc también se pueden seleccionar utilizando el sistema de exhibición descrito Otros sistemas de exhibición por ejemplo aquellos que utilizan bacteriófagos o

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levadura no son adecuados para selección de colas Ig con propiedades de unión FcR alterado debida a los requerimientos de glicosilación en el dominio Ig CH2 que no puede ocurrir en los sistemas de no mamífero

Este sistema también es útil para la selección de moléculas scFv-Ig alteradas que se producirán en altos niveles En este ejemplo, las células de mamífero tales como las células COS se pueden transfectar con una librería de construcciones scFv-Ig en un plásmido que dirige su expresión a la superficie celular Las células COS que expresan los más altos niveles de las moléculas scFv-Ig se pueden seleccionar por técnicas bien conocidas en la técnica (por ejemplo filtración, clasificación de célula estéril, separación de lecho magnético, etc), y el ADN de plásmido se aísla para transformación en bacteria Después de varios rounds de selección de clones sencillo se aíslan codificando moléculas scFv-Ig capaces de alto nivel de expresión Cuando los clones aislados se alteran para remover el ancla de membrana y luego se expresan en células de mamífero, las construcciones scFv-Ig se secretarán dentro del fluido de cultivo en altos niveles Esto refleja el requerimiento común de glicoproteínas secretadas y glicoproteínas de superficie celular para un péptido señal y procesamiento a través del golgi para expresión, de tal forma que la selección para una molécula que ilustra una mejora en los niveles de expresión

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en la superficie celular también seleccionará para la molécula que ilustra una mejora en niveles de proteína secretada

EJEMPLO 45

CARACTERIZACIÓN DE G28-1 MABS Y scFVs

La capacidad del G28-1 mAbs y el scFvs para inducir apoptosis se mide mediante la unión Annexin V, que utiliza los métodos descritos en el ejemplo 3 Los resultados en la figura 71 demuestran que la unión de Annexin V de los anticuerpos G28-1 y scFV se incrementa cuando se tratan junto con anticuerpos 2H7 y construcciones scFv

EJEMPLO 46

CONSTRUCCIÓN DE CONSTRUCCIONES FC2-2 scFV

La construcción del FC2-2 (anti-CD16) scFv se desarrolla utilizando ARN aislado total del hibridoma FC2-2 y se clona utilizando los métodos descritos en el ejemplo 35 La secuencia de polinucleótido se suministra en la ID de SEC No _, y la secuencia de polipéptido codificada se provee en la ID de SEC No _ Los iniciadores específicos para la reacción PCR secundaria se listan adelante Los siguientes son iniciadores para la región de cadena variable liviana

Iniciador 5' con sitio HindIII sin líder 5'-

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GTTGTTAAGCTTGCCGCCATGGATTCAC

AGGCCCAGGTTCTT-3' (ID de SEC No _)

Iniciador 5' con sitio SalI y líder 5'-

GTTGTTGTCGACATTGTGATGTCACAGTCTCC

ATCCTCCCTA-3' (ID de SEC No _)

Iniciador 3'

5'-TCAGTGCTGATCATGAGGAGACGGTGACTGAGGTTCCTT-3' (ID de SEC No _)

Los siguientes son iniciadores para la región variable de cadena pesada

Iniciador 5' 5'-

TCGGGCGGTGGTGGGTCGGGTGGCGGCGGATCGTCACAGGTGCAGTTG

AAGGGAGTCAGGA-3' (ID de SEC No _)

Iniciador 3' 5'-

ACCCGACCCACCACCGCCCGAGCCACCGCCACCTTTTATTTCCAGCTTG

GTGCCACCTCCGAA-3' (ID de SEC No _)

Un cambio de leucina a serina en la posición 11 en la región de variable de cadena pesada (numeración Kabat) se introduce en el FC2-2 scFv mediante mutagénesis dirigida al sitio de acuerdo con los métodos descritos en el ejemplo 33 El scFv se adhiere a la cola (SSS-S)H WCH2 WCH3 IgG de acuerdo con los métodos descritos en el ejemplo 33 El mutante se designa FC2-2 scFv VH L11S (SSS-S)H WCH2 WCH3 La secuencia de polinucleótido se suministra en la ID de SEC No _, y la

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secuencia de polipéptido codificada se suministra en la ID de SEC No _

EJEMPLO 47

CONSTRUCCIÓN DE CONSTRUCCIONES 5B9 scFV

La construcción del 5B9 (anti-CD137) scFv se desarrolla utilizando ARN aislado total del hibridoma 5B9 y clonado utilizando los métodos descritos en el ejemplo 35 La secuencia de polinucleótido se suministra en la ID de SEC No _, y la secuencia de polipéptido codificado se suministra en la ID de SEC No _ Los iniciadores específicos para la reacción PCR secundaria se listan adelante Los siguientes son iniciadores de la región de cadena variable liviana

Iniciador 5' con sitio HindIII sin líder 5'-
GTTGTTAAGCTTGCCGCCATGAGGTTCT
CTGCTCAGCTTCT-3'(ID de SEC No _)

Iniciador 5'con el sitio SalI y líder 5'-
GTTGTTGTCGACATTTGTGATGACGCAGGCTG
CATTCTCCAATT -3' (ID de SEC No _)

Iniciador 3' 5'- TCAGTGCTGATCAGAGGAGGACGGTGACTGAGGTTCCTTG-3'(ID de SEC No _)

Los siguientes son iniciadores para la región de variable de cadena pesada

Iniciador 5' 5'-

1169

CGGGCGGTGGTGGGTCGGGTGGCGGCGGATCGTCACAGGTGCAGCTGA

AGCAGTCAGGA -3' (ID de SEC No _)

Iniciador 3' 5'-

CCCGACCCACCACCGCCCGAGCCACCGCCACCCTTCAGCTCCAGCTTG

GTGCCAGCACC-3'(ID de SEC No _)

Un cambio de la leucina a serina en la posición 11 en la región variable de cadena pesada (numeración Kabat) se introduce en el 5B9 scFv mediante mutagénesis dirigida al sitio y se adhiere al (SSS-S)H WCH2 WCH3 de acuerdo con los métodos descritos en el ejemplo 33 Esta construcción se designa 5B9 scFv VH L11S (SSS-S)H WCH2 WCH3 La secuencia de polinucleótido se suministra en la ID de SEC No _, y la secuencia de polipéptido codificada se suministra en la ID de SEC No _

EJEMPLO 48

CONSTRUCCIÓN DE CONSTRUCCIONES UCHL1 scFv

La construcción del UCHL1 (anti-CD45RO) scFv se desarrolla utilizando ARN aislado total del hibridoma UCHL1 y clonado utilizando los métodos descritos en el ejemplo 35 La secuencia de polinucleótido se suministra en la ID de SEC No _, y la secuencia de polipéptido codificada se provee en la ID de SEC No _ Los siguientes son iniciadores para la región de cadena variable liviana

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Iniciador 5' con sitio HindIII 5'-
GTTGTTAAGCTTGCCGCCATGAAGTTGCCTGTTAGGCTG
TTGGTGCTG-3'(ID de SEC No _)
Iniciador 3'con sitio Sac 5'-
AGAGCTCCCACCTCCTCCAGATCCACCACCGCCCGAGCCAC
CGCCATCTTTGATTTCCAGCTTGGT-3' (ID de SEC No _)

Los siguientes son iniciadores para la región variable de cadena pesada
Iniciador 5' 5'-
TTTCAGAGTAATCTGAGAGCTCCCACCTCCTCCAGATCCACCACCGCCCGA-3' (ID de SEC No _)
Iniciador 3' 5'-TCAGTGCTGATCATGCAGAGAGACAGTGACCAGAGTCCC-3'(ID de SEC No _)

Un cambio de leucina a serina en la posición 11 en la región de variable de cadena pesada (numeración Kabat) se introduce en el 5B9 scFv mediante mutagénesis dirigida al sitio y conectada a un (SSS-S)H WCH2 WCH3 de acuerdo con los métodos descritos en el ejemplo 33 El mutante se designa UCHL1 scFv VH L11S (SSS-S)H WCH2 WCH3 La secuencia de polinucleótido se suministra en la ID de SEC No _, y la secuencia de polipéptido codificada se suministra en la ID de SEC No _

EJEMPLO 49

L6 VHL11S scFv (SSS-S)H WCH2 WCH3

Un cambio de leucina a serina en la posición 11 en la región de variable de cadena pesada (numeración Kabat) se introduce en el L6 VHL11S scFv (SSS-S)H WCH2 WCH3 (construido de acuerdo con los métodos descritos en el ejemplo 106) mediante mutagénesis dirigida al sitio de acuerdo con los métodos descritos en el ejemplo 33 Se utiliza el plásmido L6scFvIg (SSS-S)H WCH2 WCH3 pD18 utilizado como plantilla Los clones positivos se insertaron dentro del plásmido pD18 que contiene (SSS-S)H WCH2 WCH3 de acuerdo con los métodos descritos en el ejemplo 33 El mutante se designa L6 scFv VH L11S (SSS-S)H WCH2 WCH3 La secuencia de polinucleótido se suministra en la ID de SEC No _, y la secuencia de polipéptido codificado se suministra en la ID de SEC No _

Los iniciadores PCR se listan adelante

Iniciador 5' con sitio de restricción PstI 5'-

ggcggatctctgcagatccagttggtgcagtctggacctgagtcgaagaagcct

ggagag-3' (ID de SEC No _)

inicador 3' 5' ggacagtgggagtggcacc-3' (ID de SEC No _)

EJEMPLO 50

CONSTRUCCIÓN DE LA CONSTRUCCIÓN HD37 scFv VHL11S

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Un cambio de leucina a serina en la posición 11 en la región de variable de cadena pesada (numeración Kabat) se introduce en el HD37 scFv mediante mutagénesis dirigida al sitio de acuerdo con los métodos descritos en el ejemplo 33 El plásmido HD37 scFv (SSS-S)H WCH2 WCH3 pD18 se utiliza como plantilla Los clones positivos se insertaron en el plásmido pD18 que contiene (SSS-S)H WCH2 WCH3 de acuerdo con los métodos descritos en el ejemplo 33 El mutante se designa HD37 scFv VH L11S (SSS-S)H WCH2 WCH3 La secuencia de polinucleótido se suministra en la ID de SEC No _, y la secuencia de polipéptido codificado se suministra en la ID de SEC No _ Los iniciadores PCR se listan adelante

Iniciador 5' 5'-caggttcagctgcagcagtctggggctgagtcggtgaggcctgg-3' (ID de SEC No _)

Iniciador 3' 5'-ggaggattcgtctgcagtcagagtggc-3' (ID de SEC No _)

EJEMPLO 51

CONSTRUCCIONES 2H7 scFv VHL11S

Se hicieron construcciones 2H7 VHL11S adicionales con diferentes regiones de conexión El vector pD18 2H7 scFv VHL11S (SSS-S)H WCH2 WCH3 se digiere con BelI y XbaI para remover la región de conexión, CH2 y CH3 Estas fueron reemplazadas con cada región de conexión diferente, CH2 y CH3 de acuerdo con los métodos descritos en el ejemplo 13 Las

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nuevas construcciones se designaron 2H7scFv VHL11S (CSS-S)H WH2 WH3 (ID de SEC No _), 2H7scFv VHL11S (CSC-S)H WH2 WH3 (ID de SEC No _) El 2H7 scFv VHL11S también se adhiere a una región de conexión IgA, CH2, CH3 y una IgE CH2, CH3, CH4 El plásmido pD18 2H7 scFv VHL11S (SSS-S)H WCH2 WCH3 se digiere utilizando los métodos anteriores para remover la región de conexión CH2, y CH3 Las regiones IgA se insertan utilizando los métodos descritos en el ejemplo 13 La construcción se designa 2H7 scFv VHLIIS IGAH IgACH2 T4CH3 (ID de SEC No _) La región IgE CH2 CH3 CH4 se inserta en el vector pD18 digerido anterior utilizando los métodos descritos en el ejemplo 39 La construcción se designa 2H7 scFv VHL IS IgECH2 CH3 CH4 (ID de SEC No _)

EJEMPLO 52

2H7 scFv VH L11S (CSC-S)H WCH2 WCH3

Esta construcción tiene una región de unión Fv de cadena sencilla 2H7 (anti-CD20) con un punto de mutación en el residuo de aminoácido 11 en la región variable de cadena pesada, en donde la leucina se ha cambiado a una serina, como se describe en el ejemplo 33 Esta región de unión se conecta a una región de conexión IgG1 humano, región CH2 y CH3, en donde la segunda cisteína y la prolina en la región de conexión se han cambiado a serinas (SSS-S) como se describe en el ejemplo 32 La secuencia de polinucleótido se

suministra en la ID de SEC No _, y la secuencia de polipéptido codificado se suministra en la ID de SEC No _

EJEMPLO 53

2H7 scFv VH L11S IGE CH2 CH3 CH4

Esta construcción tiene una región de unión Fv de cadena sencilla 2H7 (anti-CD20) con un punto de mutación en el residuo de aminoácido 11 en la región variable de cadena pesada, en donde la leucina se ha cambiado a una serina, como se describe en el ejemplo 33 Esta región se adhiere a la región de constante IgE humana que contiene CH2, CH3 y CH4 como se describe en el ejemplo 38 La secuencia de polinucleótido se suministra en la ID de SEC No _, y la secuencia de polipéptido codificado se suministra en la ID de SEC No _

EJEMPLO 54

2H7 scFv VH L11S MicE CH2 CH3 CH4

Esta construcción tiene una región de unión Fv de cadena sencilla 2H7 (anti-CD20) con un punto de mutación en el residuo de aminoácido 11 en la región variable de cadena pesada, en donde la leucina se ha cambiado a una serina, como se describe en el ejemplo 33 Esta región de unión se adhiere a una región constante IgE de ratón que contiene CH2, CH3 y

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CH4 como se describe en el ejemplo 39 La secuencia de polinucleótido se suministra en la ID de SEC No _, y la secuencia de polipéptido codificado se suministra en la ID de SEC No _

EJEMPLO 55

2H7 scFv VH L11S MIGAH WIGACH2 T4CH3

Esta construcción tiene una región de unión Fv de cadena sencilla 2H7 (anti-CD20) con un punto de mutación en el residuo de aminoácido 11 en la región de variable de cadena pesada, en donde la leucina se ha cambiado a una serina, como se describe en el ejemplo 33 Esta región de unión se adhiere a una región conexión del IgA como se describe en el ejemplo 5 Está región de conexión se adhiere a la región constante IgA de ratón de una región CH2 tipo silvestre y una región CH2 mutada en donde hay una truncación de los 4 residuos de aminoácido antes del codon de parada 3' como se describe en el ejemplo 39 La secuencia de polinucleótido se suministra en la ID de SEC No _, y la secuencia de polipéptido codificado se suministra en la ID de SEC No _

EJEMPLO 56

2H7 scFv VH L11S (SSS-S)H K322S CH2 WCH3

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Esta construcción tiene una región de unión Fv de cadena sencilla 2H7 (anti-CD20) con un punto de mutación en el residuo de aminoácido 11 en la región variable de cadena pesada, en donde la leucina se ha cambiado a una serina, como se describe en el ejemplo 33 Esta región de unión se conecta a una región de conexión IgG humana mutada, en donde todas las cisteínas y una prolina se han cambiado a serinas (SSS-S), de acuerdo con los métodos descritos en el ejemplo 5 La región de conexión se adhiere a una región IgG CH2 mutada y una región IgG CH3 tipo silvestre La mutación K322S en la región CH2 está en un residuo 322, en donde una lisina se ha cambiado a una serina que utiliza ensayo PCR traslapante Una plantilla (SSS-S)H WCH2 WCH3 IgG1 en el vector pD18 se utiliza para amplificación PCR, para crear derivados (SSS-S)H que contienen estas mutaciones CH2 Las reacciones PCR utilizan un perfil cíclico de 94C, 30 seg, 55C, 30 seg, 72C, 30 seg, durante 37 ciclos para completar las reacciones Este derivado IgG1 se construye al utilizar reacciones PCR secuenciales con oligonucleótidos traslapantes en las reacciones primarias y secundarias Los iniciadores de amplificación primarios introducen las mutaciones, pero eliminan un extremo del dominio Fc Los iniciadores de reacción secundaria readhieren estos extremos que utilizan iniciadores traslapantes El primer iniciador traslapante se agrega al comienzo del PCR, a las reacciones se les permite proceder para 12 ciclos, pausados y luego un segundo

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iniciador traslapante se agrega alas reacciones seguido por 25 ciclos más para completar las reacciones PCR de extensión de traslapo

Iniciadores para la primera reacción PCR

Iniciador 5' 5'-
ggagatggttttctcgatgggggctgggagggctttgttggagaccgagcacttgtactcc
-3' (ID de SEC No _)

Iniciador 3' 5'-ggacagtgggagtggcacc-3' (ID de SEC No _)

Los productos PCR se clonaron en el vector TOPO y secuenciados para verificación Los vectores positivos se utilizaron como plantillas para la segunda reacción PCR traslapante

Iniciador 5' 5'
ccgtctctgatcaggagcccaaatcttctgacaaaactcacacatccccaccgtccccagc
-3' (ID de SEC No _)

Iniciador 5' traslapante iniciador 5'-
tccccaccgtccccagcacctgaactcctgggggggatcgtcagtcttcctcttccccc
caaaacc-3' (ID de SEC No _)

Iniciador 3' 5'-caggaaacagctatgac-3' (ID de SEC No _)

El producto PCR se clona en el vector TOPO y se secuencia La secuencia de polinucleótido se suministra en la ID de SEC No _, y la secuencia de polipéptido codificado se suministra en la ID de SEC No _

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EJEMPLO 57

2H7 scFv VH LI IS (CSS-S)H K322S CH2 WCH3

Esta construcción tiene una región de unión Fv de cadena sencilla 2H7 (anti-CD20) con un punto de mutación en el residuo de aminoácido 11 en la región de variable de cadena pesada, en donde la leucina se ha cambiado a una serina, como se describe en el ejemplo 33 Esta región de unión se adhiere a una región de conexión IgG mutante, en donde la segunda y tercera cisteínas se han cambiado a serinas y la prolina se ha cambiado a serina (CSS-S), de acuerdo con los métodos descritos en el ejemplo 13 La región de conexión se adhiere a una región IgG CH2 mutada y a una región IgG CH3 tipo silvestre La mutación K322S en la región CH2 está en un residuo 322, en donde la lisina se ha cambiado por una serina que utiliza ensayo PCR traslapante La región se adhiere a una región IgG CH2 mutada y una región región tipo silvestre CH3 La mutación en la región CH2 se agrega mediante reacción PCR traslapante esencialmente de acuerdo con el ejemplo 57, con el vector (CSS-S)H WCH2 WCH3 IgG1 pD18 como una plantilla en la primer reacción PCR y diferentes iniciadores en la segunda reacción PCR que se listan adelante

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Iniciador 5'traslapante 5'-
tccccaccgtccccagcacctgaactcctgggggggatcgtcagtcttcctcttcccccaaa
acc-3'
Iniciador 3' 5'-caggaaacagctatgac-3' (ID de SEC No _)

Los productos PCR se clonan en el vector TOPO y se secuencian La secuencia de polinucleótido se suministra en la ID de SEC No _, y la secuencia de polipéptido codificado se suministra en la ID de SEC No _

EJEMPLO 58

2H7 scFvVH L11S (SSS-S)H P331S CH2 WCH3

Esta construcción tiene una región de unión Fv de cadena sencilla 2H7 (anti-CD20) con un punto de mutación en el residuo de aminoácido 11 en la región variable de cadena pesada, en donde la leucina se ha cambiado por una serina, como se describe en el ejemplo 33 Esta región de unión se conecta a una región de conexión IgG1 humana mutada, en donde todas las cisteínas y una prolina se han cambiado por serinas (SSS-S), de acuerdo con los métodos descritos en el ejemplo 5 La región de conexión se adhiere a una región IgG1 CH2 mutada y una región IgG CH3 tipo silvestre La mutación P331S en la región CH2 en donde la prolina en el residuo 331 se ha cambiado por una serina, se incorpora utilizando una reacción PCR sencilla que utiliza una plantilla (SSS-S)H WCH2 WCH3 y

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un perfil cíclico de 94C, 30 seg, 55C, 30 seg, 72C, 30 seg, durante 37 ciclos Los iniciadores específicos para reacción se listan adelante

Iniciador 5' 5'
ccgtctctgatcaggagcccaaatcttctgacaaaactcacacatccccaccgtccccagc
-3' (ID de SEC No _)
Iniciador 3' 5'-
gcagggtgtacacctgtggttctcggggctgccctttggctttggagatggttttctcgat
ggaggctgggagg-3' (ID de SEC No _)

La secuencia de polinucleótido se suministra en la ID de SEC No _, y la secuencia de polipéptido codificado se suministra en la ID de SEC No _

EJEMPLO 59

2E7 scFv VH L11S (CSS-S)H P331S CH2 WCH3

Esta región de unión se adhiere a una región de conexión IgG mutante, en donde la segunda y tercera cisteínas se han cambiado por serinas y la prolina se ha cambiado a serina (CSS-S), de acuerdo con los métodos descritos en el ejemplo 13 La región de conexión se adhiere a una región IgG CH2 mutada y a una región IgG CH3 tipo silvestre La mutación P331S en la región CH2, en donde la prolina en el residuo 331 se ha cambiado por una serina, se incorpora utilizando una

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región PCR sencilla que utiliza una palntilla (CSS-S)H WCH2 WCH3 pD18 y un perfil cíclico de 94C, 30 seg, 55C, 30 seg, 72C, 30 seg, durante 37 ciclos Los iniciadores específicos para reacción se listan adelante

Iniciador 5′ 5′
ccgtctctgatcaggaccccaaatcttgtgacaaaactcacacatccccaccgtccccagc
-3′ (ID de SEC No _)
Iniciador 3′ 5′-
gcagggtgtacacctgtggttctcggggctgccctttggctttggagatggttttctcgat
ggaggctgggagg-3′ (ID de SEC No _)

La secuencia de polinucleótido se suministra en la ID de SEC No _, y la secuencia de polipéptido codificado se suministra en la ID de SEC No _

EJEMPLO 60

2H7 scFv VH L11S (SSS-S)H T256N CH2 WCH3

Esta construcción tiene una región de unión Fv de cadena sencilla 2H7 (anti-CD20) con un punto de mutación en el residuo de aminoácido 11 en la región variable de cadena pesada, en donde la leucina se ha cambiado por una serina, como se describe en el ejemplo 33 Esta región de unión se conecta a una región de conexión IgG humana mutada, en donde

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todas las cisteínas y una prolina se han cambiado por serinas (SSS-S), de acuerdo con los métodos descritos en el ejemplo 5 La región de conexión se adhiere a una región IgG CH2 mutada y una región IgG CH3 tipo silvestre La mutación T256N en la región CH2, la treonina en el residuo 256 se ha cambiado por una asparagina, que utiliza los métodos PCR traslapantes descritos en el ejemplo 56 Los iniciadores específicos se listan adelante

Iniciadores para la primera reacción PCR

Iniciador 5' 5'-ttcctcttccccccaaaacccaaggacaccctcatgatctcccggaaccctgaggtcac-3' (ID de SEC No _)

Iniciador 3 5'-ggacagtgggagtggcacc-3' (ID de SEC No _)

El producto PCR se clona en el vector TOPO y se secuencia Este producto se utiliza como la plantilla en la segunda reacción PCR Los iniciadores para la segunda reacción PCR

Iniciador 5' 5'-ccgtctctgatcaggagcccaaatcttctgacaaaactcacacatccccaccgtccccagc-3' (ID de SEC No _)

Iniciador traslapante 5' 5'-tccccaccgtccccagcacctgaactcctgggggggatcgtcagtcttcctcttcccccaaaacc-3' (ID de SEC No _)

Iniciador 3 5'-caggaaacagctatgac-3' (ID de SEC No _)

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La secuencia de polinucleótido se suministra en la ID de SEC No _, y la secuencia de polipéptido codificado se suministra en la ID de SEC No _

EJEMPLO 61

2H7 scFv VH L11S (SSS-S)H RTPE/QNAK (255-258) CH2 WCH3

Esta construcción tiene una región de unión Fv de cadena sencilla 2H7 (anti-CD20) con un punto de mutación en el residuo de aminoácido 11 en la región variable de cadena pesada, en donde la leucina se ha cambiado por una serina, como se describe en el ejemplo 33 Esta región de unión se conecta a una región de conexión IgG humana mutada, en donde todas las cisteínas y una prolina se han cambiado por serinas (SSS-S), de acuerdo con los métodos descritos en el ejemplo 5 La región de conexión se adhiere a una región IgG CH2 mutada y a una región IgG CH3 tipo silvestre La mutación RTPE/QNAK en la región CH2, en donde los residuos 255-258 han mutado de arginina, treonina, prolina, ácido glutámico a glutamina, asparaginas, alanina y lisina respectivamente que utiliza las reacciones PCR traslapantes descritos en el ejemplo 56 Los iniciadores específicos se listan adelante

Iniciadores para la primera reacción PCR

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Iniciador 5' 5'-ttcctcttccccccaaaacccaaggacaccctcatgatctcccagaacgctaaggtcacatgc-3' (ID de SEC No _)

Inciador 3' 5'-ggacagtgggagtggcacc-3' (ID de SEC No _)

El producto PCR se clona en el vector TOPO, se secuencia y utiliza como una plantilla para la segunda reacción PCR Los iniciadores para la segunda reacción PCR

Iniciador 5' 5'-ccgtctctgatcaggagcccaaatcttctgacaaaactcacacatccccaccgtccccagc-3' (ID de SEC No _)

Iniciador traslapante 5' 5'-tccccaccgtccccagcacctgaactcctgggggatcgtcagtcttcctcttcccccaaaacc-3' (ID de SEC No _)

Iniciador 3' 5'-caggaaacagctatgac-3' (ID de SEC No _)

La secuencia de polinucleótido se suministra en la ID de SEC No _, y la secuencia de polipéptido codificado se suministra en la ID de SEC No _

EJEMPLO 62

2H7 scFv VH L11S (SSS-S)H K290Q CH2 WCH3

Esta construcción tiene una región de unión Fv de cadena sencilla 2H7 (anti-CD20) con un punto de mutación en el

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residuo de aminoácido 11 en la región variable de cadena pesada, en donde la leucina se ha cambiado por una serina, como se describe en el ejemplo 33 Esta región de unión se conecta a una región de conexión IgG1 humana mutada, en donde todas las cisteínas y una prolina se han cambiado por serinas (SSS-S), de acuerdo con los métodos descritos en el ejemplo 5 La región de conexión se adhiere a una región IgG1 CH2 mutada y a una región IgG1 CH3 tipo silvestre La mutación K290Q en la región CH2, en donde la lisina en el residuo 290 se ha cambiado a glutamina, que utiliza las reacciones PCR sencilla de acuerdo con los métodos descritos en el ejemplo 58 Los iniciadores específicos utilizados en esta reacción se listan adelante

Iniciador 5' 5'-ccgtctctgatcaggagcccaaatcttctgacaaaactcacacatccccaccgtccccagc-3' (ID de SEC No _)

Iniciador 3' 5'-gctcccgcggctgtgtcttggc-3' (ID de SEC No _)

Los productos PCR se clonan en el vector TOPO y se secuencian La secuencia de polinucleótido se suministra en la ID de SEC No _, y la secuencia de polipéptido codificado se suministra en la ID de SEC No _

EJEMPLO 63

2H7 scFv VH L11S (SSS-S)H A339P CH2 WCH3

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Esta construcción tiene una región de unión Fv de cadena sencilla 2H7 (anti-CD20) con un punto de mutación en el residuo de aminoácido 11 en la región variable de cadena pesada, en donde la leucina se ha cambiado por una serina, como se describe en el ejemplo 33 Esta región de unión se conecta a una región de conexión IgG1 humana mutada, en donde todas las cisteínas y una prolina se han cambiado por serinas (SSS-S), de acuerdo con los métodos descritos en el ejemplo 5 La región de conexión se adhiere a una región IgG1 CH2 humana mutada y a una región IgG1 CH3 humano tipo silvestre La mutación A339P en la región CH2, en donde la alanina en el residuo 339 se ha cambiado por una prolina, que utiliza una reacción PCR sencilla de acuerdo con los métodos descritos en el ejemplo 58 Los iniciadores específicos utilizados en esta reacción se listan adelante

Iniciador 5' 5'ccgtctctgatcaggagcccaaatcttctgacaaaactcacacat ccccaccgtccccagc-3' (ID DE SEC NO)
iniciador 3' 5'-ggaggtgggcagggtgtacacctgtggttctcggggctgccct ttgggtttggagatgg-3' (ID DE SEC NO)

Los productos PCR se clonaron en TOPO y se secuenciaron La secuencia de polinucleótido se suministra en la ID DE SEC NO _, y la secuencia de polipéptido codificado se suministra en la ID DE SEC NO _

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EJEMPLO 64

G28-1 scFv (SSS-S)H WCH2 WCH3

Esta construcción tiene una región de unión Fv de cadena sencilla G28-1 (anti-CD37) hecha de acuerdo con los métodos descritos en el Ejemplo 35 Esta región de unión se conecta con una región de conexión IgG1 humana mutada en donde todas las cisteínas y una prolina se han cambiado por serinas (SSS-S) de acuerdo con los métodos descritos en el Ejemplo 5 Esta región de conexión se conecta a una región IgG1 CH2 y CH3 humana tipo silvestre como se describe en el Ejemplo 1 Esta construcción ha sido previamente referida como G28-1-MHWTG1C y G28-1 scFv Ig, ambos tienen la misma secuencia como la construcción anterior La secuencia de polinucleótido se suministra en la ID DE SEC NO _, y la secuencia de polipéptido codificado se suministra en la ID DE SEC NO _

EJEMPLO 65

G28-1 scFv IGAH WCH2 WCH3

Esta construcción tiene una región de unión Fv de cadena sencilla G28-1 (anti-CD37) hecha de acuerdo con los métodos descritos en el Ejemplo 35 Esta región de unión se conecta con una región de conexión IgA humana y las regiones constantes IgG CH2 y CH3 humanas tipo silvestre como se

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describe en el Ejemplo 5 Esta construcción ha sido previamente referida como G28-1-IgAHWTG1C La secuencia de polinucleótido se suministra en la ID DE SEC NO _, y la secuencia de polipéptido codificado se suministra en la ID DE SEC NO _

EJEMPLO 66

G28-1 scFv VH L11S (SSS-S) H WCH2 WCH3

Esta construcción tiene una región de unión Fv de cadena sencilla G28-1 (anti-CD37) con un punto de mutación en el residuo de aminoácido 11 en la región variable de cadena pesada, en donde la leucina se ha cambiado por una serina como se describe en el Ejemplo 35 Esta región de unión se conecta con una región de conexión IgG1 humana mutada en donde todas las cisteínas y una prolina se han cambiado por serinas (SSS-S) de acuerdo con los métodos descritos en el Ejemplo 5 Esta región de conexión se adhiere a una región IgG1 CH2 y CH3 humana tipo silvestre como se describe en el Ejemplo 1 La secuencia de polinucleótido se suministra en la ID DE SEC NO _, y la secuencia de polipéptido codificado se suministra en la ID DE SEC NO _

EJEMPLO 67

G28-1 scFv VH L11S (CSS-S) H WCH2 WCH3

1189

Esta construcción tiene una región de unión Fv de cadena sencilla G28-1 (anti-CD37) con un punto de mutación en el residuo de aminoácido 11 en la región variable de cadena pesada, en donde la leucina se ha cambiado por una serina como se describe en el Ejemplo 35 Esta región de unión se adhiere a una región de conexión IgG1 humana mutante, en donde la segunda y tercera cisteínas se han cambiado por serinas y la prolina se ha cambiado por serina (CSS-S), de acuerdo con los métodos descritos en el Ejemplo 13 Esta región de conexión se adhiere a una región IgG1 CH2 y CH3 humana tipo silvestre como se describe en el Ejemplo 1 La secuencia de polinucleótido se suministra en la ID DE SEC NO _, y la secuencia de polipéptido codificado se suministra en la ID DE SEC NO _

EJEMPLO 68

G28-1 scFv VH L11S (CSC-S)H WCH2 WCH3

Esta construcción tiene una región de unión Fv de cadena sencilla G28-1 (anti-CD37) con un punto de mutación en el residuo aminoácido 11 en la región variable de cadena pesada, en donde la leucina se ha cambiado por una serina como se describe en el Ejemplo 35 Esta región de unión se adhiere a una región de conexión IgG1 humana mutante en donde la segunda cisteína y la prolina se han cambiado por serinas (CSC-S), de acuerdo con los métodos descritos en el Ejemplo

1190

32 Esta región de conexión se adhiere a una región IgG1 CH2 y CH3 humana tipo silvestre como se describe en el Ejemplo 1 La secuencia de polinucleótido se suministra en la ID DE SEC NO _, y la secuencia de polipéptido codificado se suministra en la ID DE SEC NO _

EJEMPLO 69

G28-1 scFv VH L11S (SSC-P)H WCH2 WCH3

Esta construcción tiene una región de unión Fv de cadena sencilla G28-1 (anti-CD37) con un punto de mutación en el residuo de aminoácido 11 en la región variable de cadena pesada, en donde la leucina se ha cambiado por una serina como se describe en el Ejemplo 35 Esta región de unión se adhiere a una región de conexión IgG1 humana mutante en donde la primera y segunda cisteínas se han cambiado por serinas (SSC-P), de acuerdo con los métodos descritos en el Ejemplo 13 Esta región de conexión se conecta con una región IgG1 CH2 y CH3 humana tipo silvestre como se describe en el Ejemplo 1 La secuencia de polinucleótido se suministra en la ID DE SEC NO _, y la secuencia de polipéptido codificado se suministra en la ID DE SEC NO _

EJEMPLO 70

CTLA4 (SSS-S) H P238SCH2 WCH3

1179
1191

Esta construcción tiene la región de unión CTLA-4 extracelular como se describe en el Ejemplo 14 Esta región de unión se conecta a una región de conexión IgG1 humana mutada en donde todas las cisteínas y una prolina se han cambiado a serina (SSS-S) de acuerdo con los métodos descritos en el Ejemplo 5 Esta región de pivote se adhiere a la región IgG1 CH2 humana mutada y una región IgG1 CH3 humana tipo silvestre La mutación P238S, en donde una prolina en el residuo 238 se cambia por una serina, se introduce utilizando un ensayo PCR Las reacciones PCR se desarrollan utilizando cADN iniciado aleatoriamente preparado de ARN de célula B tonsil humana Las amplificaciones PCR utilizan un perfil de amplificación de 94C 4 min, [94C 1 min, 55C 1 min, 72C 1 min, durante 30 ciclos seguido por una etapa de extensión final durante 6 minutos a 72C Los fragmentos de PCR fueron TOPO clonados y los clones con insertos EcoRI de aproximadamente 800 bp se secuenciaron como se describe en el Ejemplo 1 Los iniciadores utilizados para el PCR se listan adelante

Iniciador 5' 5'-gttgttgatcaggagcccaaatcttctgacaaaactcacaca tctccaccgtccccagcacctgaactcctgggtggaccgtcagtcttcc-3' (ID DE SEC NO)

Iniciador 3' 5'gttgtttctagattatcatttacccggagacag-3' (ID DE SEC NO)

Esta construcción ha sido previamente referida como CTLA-4 IgG MTH (SSS) MTCH2WTCH3, que tiene la misma secuencia como la construcción anterior La secuencia de polinucleótido se suministra en la ID DE SEC NO _, y la secuencia de polipéptido codificado se suministra en la ID DE SEC NO _

EJEMPLO 71

CTLA4 (CCC-P) WH WCH2 WCH3

Esta construcción tiene una región de unión CTLA-4 como se describe en el Ejemplo 14 Esta región de unión se adhiere a una región de conexión IgG1 humana tipo silvestre (CCC-P) como se describe en el Ejemplo 1 Esta región de conexión se conecta a una región IgG1 CH2 y CH3 humana tipo silvestre como se describe en el Ejemplo 1 Esta construcción ha sido previamente referida como CTLA-4 IgG WTH (CCC) WTCH2CH3, que tiene la misma secuencia como la construcción anterior La secuencia de polinucleótido se suministra en la ID DE SEC NO _, y la secuencia de polipéptido codificado se suministra en la ID DE SEC NO _

EJEMPLO 72

FC2-2 scFv (SSS-S) H WCH2 WCH3

Esta construcción tiene una Fv de cadena sencilla FC2-2 (anti-CD16) hecha de acuerdo con los métodos descritos en el

1193

Ejemplo 46 Esta región de unión se conecta con una región de conexión IgG1 humana mutada en donde todas las cisteínas y una prolina se han cambiado por serinas (SSS-S), de acuerdo con los métodos descritos en el Ejemplo 5 Esta región de conexión se conecta con una región IgG1 CH2 y CH3 humana tipo silvestre como se describe en el Ejemplo 1 La secuencia de polinucleótido se suministra en la ID DE SEC NO _, y la secuencia de polipéptido codificado se suministra en la ID DE SEC NO _

EJEMPLO 73

FC2-2 scFv VHL11S (SSS-S) H WCH2 WCH3

Esta construcción tiene una Fv de cadena sencilla FC2-2 (anti-CD16) con un punto de mutación en el residuo aminoácido 11 en la región variable de cadena pesada, en donde la leucina se ha cambiado a una serina como se describe en el Ejemplo 46 Esta región de unión se conecta con una región de conexión IgG1 humana mutada en donde todas las cisteínas y una prolina se han cambiado por serinas (SSS-S), de acuerdo con los métodos descritos en el Ejemplo 5 Esta región de conexión se conecta con una región IgG1 CH2 y CH3 humana tipo silvestre como se describe en el Ejemplo 1 La secuencia de polinucleótido se suministra en la ID DE SEC NO _, y la secuencia de polipéptido codificado se suministra en la ID DE SEC NO _

1182

1194

EJEMPLO 74

UCHL-1 scFv (SSS-S) H WCH2 WCH3

Esta construcción tiene una Fv de cadena sencilla UCHL-1 (anti-CD45RO) hecha de acuerdo con los métodos descritos en el Ejemplo 48 Esta región de unión se conecta con una región de conexión IgG1 humana mutada en donde todas las cisteínas y una prolina se han cambiado por serinas (SSS-S), de acuerdo con los métodos descritos en el Ejemplo 5 Esta región de conexión se conecta con una región IgG1 CH2 y CH3 humana tipo silvestre como se describe en el Ejemplo 1 La secuencia de polinucleótido se suministra en la ID DE SEC NO _, y la secuencia de polipéptido codificado se suministra en la ID DE SEC NO _

EJEMPLO 75

UCHL-1 scFv VHL11S (SSS-S) H WCH2 WCH3

Esta construcción tiene una Fv de cadena sencilla UCHL-1 (anti-CD45RO) con un punto de mutación en el residuo aminoácido 11 en la región variable de cadena pesada, en donde la leucina se ha cambiado a una serina como se describe en el Ejemplo 48 Esta región de unión se conecta con una región de conexión IgG1 humana mutada en donde todas las cisteínas y una prolina se han cambiado por serinas (SSS-S),

~~1183~~
1195

de acuerdo con los métodos descritos en el Ejemplo 5 Esta región de conexión se conecta con una región IgG1 CH2 y CH3 humana tipo silvestre como se describe en el Ejemplo 1 La secuencia de polinucleótido se suministra en la ID DE SEC NO _, y la secuencia de polipéptido codificado se suministra en la ID DE SEC NO _

EJEMPLO 76

5B9 scFv (SSS-S) H WCH2 WCH3

Esta construcción tiene una Fv de cadena sencilla 5B9 (anti-CD137) hecha de acuerdo con los métodos descritos en el Ejemplo 47 Esta región de unión se conecta con una región de conexión IgG1 humana mutada en donde todas las cisteínas y una prolina se han cambiado por serinas (SSS-S), de acuerdo con los métodos descritos en el Ejemplo 5 Esta región de conexión se conecta con una región constante IgG1 CH2 y CH3 humana tipo silvestre como se describe en el Ejemplo 1 Esta construcción ha sido previamente referida como 5B9 scFv IgG MTH (SSS) WTCH2CH3, que tiene la misma secuencia como la construcción anterior La secuencia de polinucleótido se suministra en la ID DE SEC NO _, y la secuencia de polipéptido codificado se suministra en la ID DE SEC NO _

EJEMPLO 77

5B9 scFv VHL11S (SSS-S) H WCH2 WCH3

1184
1196

Esta construcción tiene una Fv de cadena sencilla 5B9 (anti-CD137) con un punto de mutación en el residuo aminoácido 11 en la región variable de cadena pesada, en donde la leucina se ha cambiado a una serina como se describe en el Ejemplo 47 Esta región de unión se conecta con una región de conexión IgG1 humana mutada en donde todas las cisteínas y una prolina se han cambiado por serinas (SSS-S), de acuerdo con los métodos descritos en el Ejemplo 5 Esta región de conexión se conecta a una región constante IgG1 CH2 y CH3 humana tipo silvestre como se describe en el Ejemplo 1 Esta secuencia de polinucleótido se suministra en la ID DE SEC NO _, y la secuencia de polipéptido codificado se suministra en la ID DE SEC NO _

EJEMPLO 78

2H7 scFv(SSS-S)H WCH2 WCH3

Esta construcción tiene una región de unión Fv de cadena sencilla 2H7 (anti-CD20) como se describe en el Ejemplo 1 Esta región de unión se conecta con una región de conexión IgG1 humana mutada en donde todas las cisteínas y una prolina se han cambiado por serinas (SSS-S) de acuerdo con los métodos descritos en el Ejemplo 5 Esta región de conexión se conecta a una región constante IgG1 CH2 y CH3 tipo silvestre como se describe en el Ejemplo 1 Esta construcción ha sido

1197

previamente referida como 2H7-MHWTG1C, CytoxB-(MHWTG1C)-Ig, anti-CD20 scFv IgG MTH (SSS) WTCH2CH3, CytoxB-MHWTG1C, 2H7 scFv-IgG1 humano tipo silvestre pivote-CH2-CH3, y 2H7 scFv IgG MTH (SSS) WTCH2CH3, los cuales tienen todos la misma secuencia como la construcción anterior La secuencia de polinucleótido se suministra en la ID DE SEC NO _, y la secuencia de polipéptido codificado se suministra en la ID DE SEC NO _

EJEMPLO 79

2H7 scFv(SSS-S)H P238SCH2 WCH3

Esta construcción tiene una región de unión Fv de cadena sencilla 2H7 (anti-CD20) como se describe en el Ejemplo 1 Esta región de unión se conecta a una región de conexión IgG1 humana mutada en donde todas las cisteínas y una prolina se han cambiado por serinas (SSS-S) de acuerdo con los métodos descritos en el Ejemplo 5 Esta región de unión se conecta a una región de conexión IgG1 humana mutada en donde todas las cisteínas y una prolina se han cambiado a serinas (SSS-S) de acuerdo con los métodos descritos en el Ejemplo 5 Esta región de pivote se adhiere a una región IgG1 CH2 humana mutada y a una región IgG1 CH3 humana tipo silvestre La mutación P238S, en donde una prolina en el residuo 238 se cambia por una serina, se introduce de acuerdo con los métodos descritos en el Ejemplo 70 Esta construcción ha sido

1196
1198

previamente referida como 2H7 scFv IgG MTH (SSS) MTCH2WTCH3, anti-CD20 scFv IgG MTH (SSS) MTCH2CH3, y CytoxB-MHMG1C los cuales tienen todos la misma secuencia como la construcción anterior La secuencia de polinucleótido se suministra en la ID DE SEC NO _, y la secuencia de polipéptido codificado se suministra en la ID DE SEC NO _

EJEMPLO 80

2H7 scFv IgAH WCH2 WCH3

Esta construcción tiene una región de unión Fv de cadena sencilla 2H7 (anti-CD20) como se describe en el Ejemplo 1 Esta región de unión se conecta con una región de conexión IgA humano y a regiones constantes IgG1 CH2 y CH3 humana tipo silvestre como se describe en el Ejemplo 5 Esta construcción ha sido previamente referida como 2H7 scFv IgAH IgG WTCH2CH3, 2H7 scFv IgA pivote-IgG1 CH2-CH3, y CytoxB-IgAHWTHG1C, los cuales tienen todos la misma secuencia como la construcción anterior La secuencia de polinucleótido se suministra en la ID DE SEC NO _, y la secuencia de polipéptido codificado se suministra en la ID DE SEC NO _

EJEMPLO 81

2H7 scFv IgAH WIgACH2 T4CH3

1197
1199

Esta construcción tiene una región de unión Fv de cadena sencilla 2H7 (anti-CD20) como se describe en el Ejemplo 1 Esta región de unión se adhiere a una región de conexión del IgA humano como se describe en el Ejemplo 5 Esta región de conexión se adhiere a una región constante IgA humana que consiste de una región CH2 tipo silvestre y una región CH3 mutada en donde hay una truncación de 4 residuos de aminoácido antes del codón de parada 3' como se describe en el Ejemplo 13 Esta construcción ha sido previamente referida como 2H7 scFv IgAH IgAT4, la cual tiene la misma secuencia como la construcción anterior La secuencia de polinucleótido se suministra en la ID DE SEC NO _, y la secuencia de polipéptido codificado se suministra en la ID DE SEC NO _

EJEMPLO 82

2H7 scFv IGAH WIGACH2 WCH3 + CADENAJ

Esta construcción tiene una región de unión Fv de cadena sencilla 2H7 (anti-CD20) como se describe en el Ejemplo 1 Esta región de unión se adhiere a una región de conexión IgA humano tipo silvestre como se describe en el Ejemplo 5 Esta región de conexión se adhiere a una región constante IgA CH2 y CH3 humana tipo silvestre de acuerdo con los métodos descritos en el Ejemplo 13 La región constante se adhiere a una región de cadena-J como se describe en el Ejemplo 13 La secuencia de polinucleótido se suministra en la ID DE SEC

1180
1200

NO _, y la secuencia de polipéptido codificado se suministra en la ID DE SEC NO _

EJEMPLO 83

2H7 scFv (CCC-P) WH WCH2 WCH3

Esta construcción tiene una región de unión Fv de cadena sencilla 2H7 (anti-CD20) como se describe en el Ejemplo 1 Esta región de unión se adhiere a una región de conexión IgG1 humano tipo silvestre (CCC-P) como se describe en el Ejemplo 1 Esta región de conexión se adhiere a las regiones constantes IgG1 CH2 y CH3 humana tipo silvestre como se describe en el Ejemplo 1 Esta construcción ha sido previamente referida como 2H7 scFv Ig WTH (CCC) WTCH2CH3, 2H7 scFv IgG WTH WTCH2CH3, y 2H7 scFv-Ig, los cuales ambos tienen la misma secuencia como la construcción anterior La secuencia de polinucleótido se suministra en la ID DE SEC NO _, y la secuencia de polipéptido codificado se suministra en la ID DE SEC NO _

EJEMPLO 84

2H7 scFv (SSS-S) H WCH2 F405YCH3

Esta construcción tiene una región de unión Fv de cadena sencilla 2H7 (anti-CD20) como se describe en el Ejemplo 1 Esta región de unión se conecta con una región de conexión

1187
1201

IgG1 humana mutada en donde todas las cisteínas y una prolina se han cambiado por serinas (SSS-S) de acuerdo con los métodos descritos en el Ejemplo 5 Esta región de conexión se adhiere a un IgG1 CH2 humano tipo silvestre y una región IgG1 CH3 humana mutado La mutación F405Y, en donde la fenilalanina en el residuo 405 se ha cambiado a una tirosina, se introduce de acuerdo con los métodos descritos en el Ejemplo 21 Esta construcción ha sido previamente referida como 2H7 scFv MTH WTCH2 MTCH3 Y405, que tiene la misma secuencia como la construcción anterior La secuencia de polinucleótido se suministra en la ID DE SEC NO _, y la secuencia de polipéptido codificado se suministra en la ID DE SEC NO _

EJEMPLO 85

2H7 scFv (SSS-S)H WCH2 F405ACH3

Esta construcción tiene una región de unión Fv de cadena sencilla 2H7 (anti-CD20) como se describe en el Ejemplo 1 Esta región de unión se conecta con una región de conexión IgG1 humana mutada en donde todas las cisteínas y una prolina se han cambiado por serinas (SSS-S) de acuerdo con los métodos descritos en el Ejemplo 5 Esta región de conexión se adhiere a un IgG1 CH2 humano tipo silvestre y una región IgG1 CH3 humana mutada La mutación F405A, en donde la fenilalanina en el residuo 405 se ha cambiado a una alanina,

1202

se introduce de acuerdo con los métodos descritos en el Ejemplo 21 Esta construcción ha sido previamente referida como 2H7 scFv MTH WTCH2 MTCH3 A405, que tiene la misma secuencia como la construcción anterior La secuencia de polinucleótido se suministra en la ID DE SEC NO _, y la secuencia de polipéptido codificado se suministra en la ID DE SEC NO _

EJEMPLO 86

2H7 scFv (SSS-S) H WCH2 Y407ACH3

Esta construcción tiene una región de unión Fv de cadena sencilla 2H7 (anti-CD20) como se describe en el Ejemplo 1 Esta región de unión se conecta con una región de conexión IgG1 humana mutada en donde todas las cisteínas y una prolina se han cambiado por serinas (SSS-S) de acuerdo con los métodos descritos en el Ejemplo 5 Esta región de conexión se adhiere a un IgG1 CH2 humano tipo silvestre y una región IgG1 CH3 humana mutada La mutación Y407A, en donde la tirosina en el residuo 407 se ha cambiado a una alanina, se introduce de acuerdo con los métodos descritos en el Ejemplo 21 Esta construcción ha sido previamente referida como scFv MTH WTCH2 MTCH3 A407, que tiene la misma secuencia como la construcción anterior La secuencia de polinucleótido se suministra en la ID DE SEC NO _, y la secuencia de polipéptido codificado se suministra en la ID DE SEC NO _

1203

EJEMPLO 87

2H7 scFv(SSS-S)HWCH2 F405A, Y407ACH3

Esta construcción tiene una región de unión Fv de cadena sencilla 2H7 (anti-CD20) como se describe en el Ejemplo 1 Esta región de unión se conecta con una región de conexión IgG1 humana mutada en donde todas las cisteínas y una prolina se han cambiado por serinas (SSS-S) de acuerdo con los métodos descritos en el Ejemplo 5 Esta región de conexión se adhiere a un IgG1 CH2 humano tipo silvestre y una región IgG1 CH3 humana mutada La mutación F405A y Y407A, en donde la fenilalanina en el residuo 405 se ha cambiado por una alanina y la tirosina en el residuo 407 se ha cambiado por una alanina, se introduce de acuerdo con los métodos descritos en el Ejemplo 21 Esta construcción ha sido previamente referida como scFv MTH WTCH2 MTCH3 A405A407, que tiene la misma secuencia como la construcción anterior La secuencia de polinucleótido se suministra en la ID DE SEC NO _, y la secuencia de polipéptido codificado se suministra en la ID DE SEC NO _

EJEMPLO 88

2H7 scFv (CSS-S)H WCH2 WCH3

1192
1204

Esta construcción tiene una región de unión Fv de cadena sencilla 2H7 (anti-CD20) como se describe en el Ejemplo 1 Esta región de unión se adhiere a una región de conexión IgG1 humana mutada, en donde la segunda y tercera cisteínas se han cambiado por serinas y la prolina se ha cambiado por serina (CSS-S), de acuerdo con los métodos descritos en el Ejemplo 13 Esta región de conexión se adhiere a las regiones constantes IgG1 CH2 y CH3 humana tipo silvestre como se describe en el Ejemplo 1 Esta construcción ha sido previamente referida como 2H7 scFv MTH (CSS) WTCH2CH3, que tiene la misma secuencia como la construcción anterior La secuencia de polinucleótido se suministra en la ID DE SEC NO _, y la secuencia de polipéptido codificado se suministra en la ID DE SEC NO _

Example 89

2H7 scFv (SCS-S)H WCH2 WCH3

Esta construcción tiene una región de unión Fv de cadena sencilla 2H7 (anti-CD20) como se describe en el Ejemplo 1 Esta región de unión se adhiere a una región de conexión IgG1 humana mutante, en donde la primera y tercera cisteínas se han cambiado por serinas y la prolina se ha cambiado por serina (SCS-S), de acuerdo con los métodos descritos en el Ejemplo 13 Esta región de conexión se adhiere a las regiones constantes IgG1 CH2 y CH3 humana tipo silvestre como se

1205

describe en el Ejemplo 1 Esta construcción ha sido previamente referida como 2H7 scFv IgG MTH (SCS) WTCH2CH3, que tiene la misma secuencia como la construcción anterior La secuencia de polinucleótido se suministra en la ID DE SEC NO _, y la secuencia de polipéptido codificado se suministra en la ID DE SEC NO _

EJEMPLO 90

2H7 scFv (SSC-P) H WCH2 WCH3

Esta construcción tiene una región de unión Fv de cadena sencilla 2H7 (anti-CD20) como se describe en el Ejemplo 1 Esta región de unión se adhiere a una región de conexión IgG1 humana mutante, en donde la primera y segunda cisteínas se han cambiado por serinas (SSC-P), de acuerdo con los métodos descritos en el Ejemplo 13 Esta región de conexión se adhiere a las regiones constantes IgG1 CH2 y CH3 humana tipo silvestre como se describe en el Ejemplo 1 Esta construcción ha sido previamente referida como 2H7 scFv MTH (SSC) WTCH2CH3, que tiene la misma secuencia como la construcción anterior La secuencia de polinucleótido se suministra en la ID DE SEC NO _, y la secuencia de polipéptido codificado se suministra en la ID DE SEC NO _

EJEMPLO 91

2H7 scFv (CSC-S) H WCH2 WCH3

+99
1206

Esta construcción tiene una región de unión Fv de cadena sencilla 2H7 (anti-CD20) como se describe en el Ejemplo 1 Esta región de unión se adhiere a una región de conexión IgG1 humana mutante, en donde la segunda cisteína y la prolina se han cambiado por serina (CSC-S), de acuerdo con los métodos descritos en el Ejemplo 32 Esta región de conexión se adhiere a las regiones constantes IgG1 CH2 y CH3 humana tipo silvestre como se describe en el Ejemplo 1 Esta construcción ha sido previamente referida como 2H7 scFv MTH (CSC) WTCH2CH3, que tiene la misma secuencia como la construcción anterior La secuencia de polinucleótido se suministra en la ID DE SEC NO _, y la secuencia de polipéptido codificado se suministra en la ID DE SEC NO _

EJEMPLO 92

2H7 scFv (CCS-P) H WCH2 WCH3

Esta construcción tiene una región de unión Fv de cadena sencilla 2H7 (anti-CD20) como se describe en el Ejemplo 1 Esta región de unión se adhiere a una región de conexión IgG1 humana mutante, en donde la tercera cisteína se ha cambiado por una serina (CCS-P), de acuerdo con los métodos descritos en el Ejemplo 22 Esta región de conexión se adhiere a las regiones constantes IgG1 CH2 y CH3 humana tipo silvestre como se describe en el Ejemplo 1 Esta construcción ha sido

1195
1207

previamente referida como 2H7 scFv MTH (CCS) WTCH2CH3, que tiene la misma secuencia como la construcción anterior La secuencia de polinucleótido se suministra en la ID DE SEC NO _, y la secuencia de polipéptido codificado se suministra en la ID DE SEC NO _

EJEMPLO 93

2H7 scFv (SCC-P) H WCH2 WCH3

Esta construcción tiene una región de unión Fv de cadena sencilla 2H7 (anti-CD20) como se describe en el Ejemplo 1 Esta región de unión se adhiere a una región de conexión IgG1 humana mutante, en donde la primera cisteína se ha cambiado por una serina (SCC-P), de acuerdo con los métodos descritos en el Ejemplo 32 Esta región de conexión se adhiere a las regiones constantes IgG1 CH2 y CH3 humana tipo silvestre como se describe en el Ejemplo 1 Esta construcción ha sido previamente referida como 2H7 scFv MTH (SCC) WTCH2CH3, que tiene la misma secuencia como la construcción anterior La secuencia de polinucleótido se suministra en la ID DE SEC NO _, y la secuencia de polipéptido codificado se suministra en la ID DE SEC NO _

EJEMPLO 94

2H7 scFv VH L11S (SSS-S)H WCH2 WCH3

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Esta construcción tiene una región de unión Fv de cadena sencilla 2H7 (anti-CD20) con un punto de mutación en el residuo de aminoácido 11 en la región variable de cadena pesada, en donde la leucina se ha cambiado por una serina como se describe en el Ejemplo 33 Esta región de unión se conecta con una región de conexión IgG1 humana mutada, en donde todas las cisteínas y una prolina se han cambiado a serina (SSS-S), de acuerdo con los métodos descritos en el Ejemplo 5 Esta región de conexión se adhiere a las regiones constantes IgG1 CH2 y CH3 humana tipo silvestre como se describe en el Ejemplo 1 Esta construcción ha sido previamente referida como 2H7 scFv VH11SER IgG MTH (SSS) WTCH2CH3 y 2H7 scFv VHSER11 WTH WTCH2CH3, que tienen la misma secuencia como la construcción anterior La secuencia de polinucleótido se suministra en la ID DE SEC NO _, y la secuencia de polipéptido codificado se suministra en la ID DE SEC NO _

EJEMPLO 95

2H7 scFv VH L11S (CSS-S)H WCH2 WCH3

Esta construcción tiene una región de unión Fv de cadena sencilla 2H7 (anti-CD20) con un punto de mutación en el residuo de aminoácido 11 en la región variable de cadena pesada, en donde la leucina se ha cambiado por una serina como se describe en el Ejemplo 33 Esta región de unión se

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adhiere a una región de conexión IgG1 humana mutante, en donde la segunda y tercera cisteínas se han cambiado por serina y la prolina se ha cambiado por serina (CSS-S), de acuerdo con los métodos descritos en el Ejemplo 13 Esta región de conexión se adhiere a las regiones constantes IgG1 CH2 y CH3 humana tipo silvestre como se describe en el Ejemplo 1 La secuencia de polinucleótido se suministra en la ID DE SEC NO _, y la secuencia de polipéptido codificado se suministra en la ID DE SEC NO _

EJEMPLO 96

G28-1 scFv VH L11S (SCS-S)H WCH2 WCH3

Esta construcción tiene una región de unión Fv de cadena sencilla G28-1 (anti-CD37) con un punto de mutación en el residuo de aminoácido 11 en la región variable de cadena pesada, en donde la leucina se ha cambiado por una serina como se describe en el Ejemplo 35 Esta región de unión se adhiere a una región de conexión IgG1 humana mutante, en donde la primera y tercera cisteínas se han cambiado por serinas y la prolina se ha cambiado por serina (SCS-S), de acuerdo con los métodos descritos en el Ejemplo 13 Esta región de conexión se adhiere a las regiones constantes IgG1 CH2 y CH3 humana tipo silvestre como se describe en el Ejemplo 1 La secuencia de polinucleótido se suministra en la

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ID DE SEC NO _, y la secuencia de polipéptido codificado se suministra en la ID DE SEC NO _

EJEMPLO 97

G28-1 scFv VH L11S (CCS-P)H WCH2 WCH3

Esta construcción tiene una región de unión Fv de cadena sencilla G28-1 (anti-CD37) con un punto de mutación en el residuo de aminoácido 11 en la región variable de cadena pesada, en donde la leucina se ha cambiado por una serina como se describe en el Ejemplo 35 Esta región de unión se adhiere a una región de conexión IgG1 humana mutante, en donde la tercera cisteína se ha cambiado por una serina (CCS-P), de acuerdo con los métodos descritos en el Ejemplo 22 Esta región de conexión se adhiere a las regiones constantes IgG1 CH2 y CH3 humana tipo silvestre como se describe en el Ejemplo 1 La secuencia de polinucleótido se suministra en la ID DE SEC NO _, y la secuencia de polipéptido codificado se suministra en la ID DE SEC NO _

EJEMPLO 98

G28-1 scFv VH L11S (SCC-P)H WCH2 WCH3

Esta construcción tiene una región de unión Fv de cadena sencilla G28-1 (anti-CD37) con un punto de mutación en el residuo de aminoácido 11 en la región variable de cadena

pesada, en donde la leucina se ha cambiado por una serina como se describe en el Ejemplo 35 Esta región de unión se adhiere a una región de conexión IgG1 humana mutante, en donde la primera cisteína se ha cambiado por una serina (SCC-P), de acuerdo con los métodos descritos en el Ejemplo 32 Esta región de conexión se adhiere a las regiones constantes IgG1 CH2 y CH3 humana tipo silvestre como se describe en el Ejemplo 1 La secuencia de polinucleótido se suministra en la ID DE SEC NO _, y la secuencia de polipéptido codificado se suministra en la ID DE SEC NO _

EJEMPLO 99

G28-1 scFv VH L11S MIGE CH2 CH3 CH4

Esta construcción tiene una región de unión Fv de cadena sencilla G28-1 (anti-CD37) con un punto de mutación en el residuo de aminoácido 11 en la región variable de cadena pesada, en donde la leucina se ha cambiado por una serina como se describe en el Ejemplo 35 Esta región de unión se adhiere a una región IgE CH2 CH3 y CH4 de ratón tipo silvestre que utiliza los métodos descritos en el Ejemplo 39 La secuencia de polinucleótido se suministra en la ID DE SEC NO _, y la secuencia de polipéptido codificado se suministra en la ID DE SEC NO _

EJEMPLO 100

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G28-1 scFv VH L11S MIGAH WIGACH2 T4CH3

Esta construcción tiene una región de unión Fv de cadena sencilla G28-1 (anti-CD37) con un punto de mutación en el residuo de aminoácido 11 en la región variable de cadena pesada, en donde la leucina se ha cambiado por una serina como se describe en el Ejemplo 35 Esta región de unión se adhiere a una región de conexión IgA de ratón como se describe en el Ejemplo 39 Esta región de conexión se adhiere a una región constante IgA de ratón que consiste de una región CH2 tipo silvestre y una región CH3 mutada en donde hay una truncación de 4 aminoácidos en residuos como se describe en el Ejemplo 39 La secuencia de polinucleótido se suministra en la ID DE SEC NO _, y la secuencia de polipéptido codificado se suministra en la ID DE SEC NO _

EJEMPLO 101

G28-1 scFv VH L11S HIGE CH2 CH3 CH4

Esta construcción tiene una región de unión Fv de cadena sencilla G28-1 (anti-CD37) con un punto de mutación en el residuo de aminoácido 11 en la región variable de cadena pesada, en donde la leucina se ha cambiado por una serina como se describe en el Ejemplo 35 Esta región de unión se adhiere a una región constante IgE humana tipo silvestre que contiene CH2, CH3 y CH4 como se describe en el Ejemplo 38 La

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secuencia de polinucleótido se suministra en la ID DE SEC NO _, y la secuencia de polipéptido codificado se suministra en la ID DE SEC NO _

EJEMPLO 102

G28-1 scFv VH L11S HIGAH WIGACH2 T4CH3

Esta construcción tiene una región de unión Fv de cadena sencilla G28-1 (anti-CD37) con un punto de mutación en el residuo de aminoácido 11 en la región variable de cadena pesada, en donde la leucina se ha cambiado por una serina como se describe en el Ejemplo 35 Esta región de unión se adhiere a una región de conexión IgA humano como se describe en el Ejemplo 5 Esta región de conexión se adhiere a una región constante IgA humano que consiste de una región CH2 tipo silvestre y una región CH3 mutada en donde hay una truncación de 4 residuos de aminoácidos antes del codón de parada 3' como se describe en el Ejemplo 13 La secuencia de polinucleótido se suministra en la ID DE SEC NO _, y la secuencia de polipéptido codificado se suministra en la ID DE SEC NO _

EJEMPLO 103

HD37 scFv IGAH WCH2 WCH3

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El HD37 scFv se clonó a partir del hibridoma HD37 que utiliza los conjuntos iniciadores de familia Novagen-Ig, TOPO de clonación y secuenciación y ensayo PCR de tejido Para las reacciones PCR iniciales antes de tejer las plantillas de clon TOPO HD37 VH C-1 y HD37 KVL B-9 se utilizaron a 1 100 con un perfil de amplificación de 94C 30 seg, 55C, 30 seg, 72C, 30 segundos durante 25 ciclos Para suministrar plantillas para las reacciones SEWING PCR secundarias, los productos de reacción primaria fueron gel purificado QIAQUICK, y los eluados diluidos 50 veces Se agregan plantillas VL y VH traslapantes de un microlitro a las reacciones PCR con el perfil de amplificación siguiente 94C, 60 seg, SSC, 60 seg, 72C, 60 seg, durante 30 ciclos Después de dos ciclos, la máquina se pausó, y los iniciadores 5'VL y 3'VH de flanqueo se agregaron a las reacciones a 25 pmol cada uno, y las reacciones PCR se resumieron Los productos PCR fueron revisados en un gel para la presencia de un fragmento 750-800 bp, y los productos de las reacciones QIAQUICK purificados y digeridos con las enzimas de restricción apropiadas para inserción dentro de los vectores de expresión pD18 Ig

El PCR del dominio VL con péptido líder nativo y parte del ligador glyser

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Iniciador 5' 5'-gttgttaagettgccgccatggagacagacacactcct gctatgg-3' (ID DE SEC NO)

Iniciador 3' 5'gccacccgacccaccaccgcccgagccaccgccacctttgattt ccagcttggtgcctcc-3' (ID DE SEC NO)

El PCR del dominio VL sin péptido líder (Sitio SalI) y parte del ligador glyser

Iniciador 5' 5'-gttgttgtcgacattgtgctgacccaatctcca-3' (ID DE SEC NO)

Iniciador 3' 5'-gccacccgacccaccaccgcccgagccaccgccacctttgatt tccagcttggtgcctcc-3' (ID DE SEC NO)

El PCR de dominio VH con parte del ligador glyser y sitio BclI para fusión a colas -Ig

Iniciador 5' 5'-tcgggcggtggtgggtcgggtggcggcggatcgtcacaggttca gctgcagcagtctgg-3' (ID DE SEC NO)

Iniciador 3' 5'-tcagtgctgatcagaggagacggtgactgaggttccttg-3' (ID DE SEC NO)

Esta región de unión se conecta a una región de conexión IgA humano de tipo silvestre y a regiones constantes IgG CH2 y CH3 humana tipo silvestre como se describe en el Ejemplo 5 Esta región de conexión se adhiere a las regiones constantes IgG1 CH2 y CH3 humanas tipo silvestre como se describe en el

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Ejemplo 1 Esta construcción ha sido previamente referida como HD37 scFv-IgAHWTG1C y HD37-IgAHWTG1C, que tienen la misma secuencia como la construcción anterior La secuencia de polinucleótido se suministra en la ID DE SEC NO _, y la secuencia de polipéptido codificado se suministra en la ID DE SEC NO _

EJEMPLO 104

HD37 scFv (SSS-S) H WCH2 WCH3

Esta construcción tiene una cadena sencilla HD37 Fv como se describe en el Ejemplo 103 Esta región de unión se conecta a una región de conexión IgG1 humana mutada en donde todas las cisteínas y una prolina se han cambiado por serinas (SSS-S) de acuerdo con los métodos descritos en el Ejemplo 5 Esta región de conexión se adhiere a las regiones constantes IgG1 CH2 y CH3 humana tipo silvestre como se describe en el Ejemplo 1 Esta construcción se ha referido previamente como HD37-MHWTG1C y HD37 scFv-IgMHWTG1C, que tienen la misma secuencia como la construcción anterior La secuencia de polinucleótido se suministra en la ID DE SEC NO _, y la secuencia de polipéptido codificado se suministra en la ID DE SEC NO _

EJEMPLO 105

HD37 scFvVH L11S (SSS-S) H WCH2 WCH3

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Esta construcción tiene una cadena sencilla HD37 Fv con una mutación en la región variable de cadena pesada en el residuo de aminoácido 11, en donde la leucina se ha cambiado por serina de acuerdo con los métodos descritos en el Ejemplo 50 Esta región de unión se conecta con una región de conexión IgG1 humana mutada en donde todas las cisteínas y una prolina se han cambiado por serinas (SSS-S) de acuerdo con los métodos descritos en el Ejemplo 5 Esta región de conexión se adhiere a las regiones constantes IgG1 CH2 y CH3 humana tipo silvestre como se describe en el Ejemplo 1 La secuencia de polinucleótido se suministra en la ID DE SEC NO _, y la secuencia de polipéptido codificado se suministra en la ID DE SEC NO _

EJEMPLO 106

L6 scFv IGAH WCH2 WCH3

El L6 scFv se clonó a partir del hibridoma L6 (I Hellstrom) que utiliza el método de anclaje de cola descrito en el Tissue Antigens Paper de 1996 El perfil PCR era de 94C, 1 min, 50C, 2 min, 72C, 2 min, durante 35 ciclos Una secuencia concenso se obtuvo para las regiones VL y VH a partir de al menos 4 clones TOPO, los iniciadores se ordenaron para las reacciones PCR antes que las reacciones SEWING PCR como sigue Para las reacciones PCR iniciales antes de coser, las

plantillas clonadas TOPO L6VK y L6VH se utilizaron a 1 100 con un perfil de amplificación de 94C 30 seg, 55C, 30 seg, 72C, 30 segundos durante 25 ciclos Para suministrar plantillas para las reacciones PCR SEWING secundarias, los productos de reacción primaria eran gel purificado, QIAQUICK purificado, y los eluados diluidos 50 veces Se agregó un microlitro a cada plantilla traslapante VL y VH para reacciones PCR con el siguiente perfil de amplificación 94C, 60 seg, 55C, 60 seg, 72C, 60 seg, durante 30 ciclos Después de dos ciclos, la máquina se pausó, y los iniciadores 5'VL y 3'VH se agregaron a las reacciones en 25 pmol cada uno, y las reacciones PCR se reasumieron Los productos PCR se revisaron en un gel para la presencia de un fragmento 750-800 bp, y los productos de reacción QIAQUICK purificado y digerido con las enzimas de reacción apropiadas para la inserción dentro de los vectores de expresión pD18 Ig

El PCR de dominio VL con el péptido líder nativo y parte del ligador glyser

L6VLHindIII 5'-gttgttaagcttgccgccatggattttcaagtgcagattttcagc ttc-3' (ID DE SEC NO)

L6VLLK3 5'-gccacccgacccaccaccgcccgagccaccgccaccagagagctcttt cagctccagcttggt-3' (ID DE SEC NO)

PCR de dominio VL sin péptido líder (Sitio SaLI) y parte del ligador glyser

Iniciador 5' 5'-gttgttgtcgacattgttcttcccagtctccagcaat cctgtctg-3' (ID DE SEC NO)

Iniciador 3' 5'-gccaccccgacccaccaccgcccgagccaccgccaccaga gagctctttcagctccagcttggt-3' (ID DE SEC NO)

PCR de dominio VH con parte del ligador glyser y el sitio BclI para fusión a colas Ig

5' 5'-tcgggcggtggtgggtcgggtggcggcggatctctgcagatccagttggtgca gtct-3' (ID DE SEC NO)

3'Bcl 5'-tcagtgctgatcagaggagactgtgagagtggtgccttg-3 (ID DE SEC NO)

Esta región de unión se conecta a una región de conexión IgA humana y regiones constantes IgG1 CH2 y CH3 humana tipo silvestre como se describe en el Ejemplo 5 Esta región de conexión se adhiere a las regiones constantes IgG1 CH2 y CH3 humana tipo silvestre como se describe en el Ejemplo 1 Esta construcción ha sido previamente referida como L6 scFv-IgAHWTG1C, que TIENE la misma secuencia como la construcción anterior La secuencia de polinucleótido se suministra en la ID DE SEC NO _, y la secuencia de polipéptido codificado se suministra en la ID DE SEC NO _

EJEMPLO 107

L6 scFv VHL11S (SSS-S) H WCH2 WCH3

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Esta construcción tiene una cadena sencilla L6 Fv con una mutación en la región variable de cadena pesada en el residuo de aminoácido 11, en donde la leucina se ha cambiado por serina de acuerdo con los métodos descritos en el Ejemplo 49 Esta región de unión se conecta con una región de conexión IgG1 humana mutada en donde todas las cisteinas y una prolina se han cambiado por serinas (SSS-S) de acuerdo con los métodos descritos en el Ejemplo 5 Esta región de conexión se adhiere a las regiones constantes IgG1 CH2 y CH3 humana tipo silvestre como se describe en el Ejemplo 1 La secuencia de polinucleótido se suministra en la ID DE SEC NO _, y la secuencia de polipéptido codificado se suministra en la ID DE SEC NO _

EJEMPLO 108

2H7 scFv-LLAMA IGG1

Esta construcción tiene una región de unión Fv de cadena sencilla 2H7 (anti-CD20) como se describe en el Ejemplo 1 Esta región de unión se adhiere a regiones CH2 y CH3, pivote llama IgG1 de acuerdo con los métodos descritos en el Ejemplo 10 La secuencia de polinucleótido se suministra en la ID DE SEC NO _, y la secuencia de polipéptido codificado se suministra en la ID DE SEC NO _

EJEMPLO 109

2H7 scFv-LLAMA IGG2

Esta construcción tiene una región de unión Fv de cadena sencilla 2H7 (anti-CD20) como se describe en el Ejemplo 1 Esta región de unión se adhiere a regiones CH2 y CH3, pivote llama IgG2 de acuerdo con los métodos descritos en el Ejemplo 10 La secuencia de polinucleótido se suministra en la ID DE SEC NO _, y la secuencia de polipéptido codificado se suministra en la ID DE SEC NO _

EJEMPLO 110

2H7 scFv-LLAMA IGG3

Esta construcción tiene una región de unión Fv de cadena sencilla 2H7 (anti-CD20) como se describe en el Ejemplo 1 Esta región de unión se adhiere a regiones CH2 y CH3, pivote llama IgG3 de acuerdo con los métodos descritos en el Ejemplo 10 La secuencia de polinucleótido se suministra en la ID DE SEC NO _, y la secuencia de polipéptido codificado se suministra en la ID DE SEC NO _

EJEMPLO 111

CD16 BAJO (ED) (SSS-S) H P238SCH2 WCH3

HCT 1222

Esta construcción tiene el dominio de unión de alelo de baja afinidad CD16 extracelular como se describe en el Ejemplo 43 Esta región de unión se conecta a una región de conexión IgG1 humana mutada en donde todas las cisteínas y una prolina se han cambiado por serinas (SSS-S) de acuerdo con los métodos descritos en el Ejemplo 5 Esta región de pivote se adhiere a una región IgG1 CH2 humana mutada y a una región IgG1 CH3 humana tipo silvestre La mutación P238S, en donde una prolina en el residuo 238 se cambia por una serina, se introduce de acuerdo con los métodos descritos en el Ejemplo 70 La secuencia de polinucleótido se suministra en la ID DE SEC NO _, y la secuencia de polipéptido codificado se suministra en la ID DE SEC NO _

EJEMPLO 112

CD16-9 ALTO (ED) (SSS-S) H P238SCH2 WCH3

Esta construcción tiene el dominio de unión de alelo de alta afinidad CD16 extracelular como se describe en el Ejemplo 43 Esta región de unión se conecta a una región de conexión IgG1 humana mutada en donde todas las cisteínas y una prolina se han cambiado por serinas (SSS-S) de acuerdo con los métodos descritos en el Ejemplo 5 Esta región de pivote se adhiere a una región IgG1 CH2 humana mutada y a una región IgG1 CH3 humana tipo silvestre La mutación P238S, en donde una prolina en el residuo 238 se cambia por una serina, se

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introduce de acuerdo con los métodos descritos en el Ejemplo 70 La secuencia de polinucleótido se suministra en la ID DE SEC NO _, y la secuencia de polipéptido codificado se suministra en la ID DE SEC NO _

EJEMPLO 113

2e12 scFv (SSS-S)H P238SCH2 WCH3-hCD80TM/CT

Esta construcción tiene una región de unión Fv de cadena sencilla 2e12 (anti-CD28) descrita en el Ejemplo 12 Esta región de unión se conecta a una región de conexión IgG1 humana mutada en donde todas las cisteínas y una prolina se han cambiado por serinas (SSS-S) de acuerdo con los métodos descritos en el Ejemplo 5 Esta región de conexión se adhiere a una región IgG1 CH2 humana mutada y a una región IgG1 CH3 humana tipo silvestre La mutación P238S, en donde una prolina en el residuo 238 se cambia por una serina, se introduce de acuerdo con los métodos descritos en el Ejemplo 70 La región CH3 se adhiere a una región de cola citoplasmática y transmembrana CD80 humana (hCD80 TM/CT) El hCD80 TM/CT se clonó utilizando aleatoriamente cADN inicializado derivado de la línea celular BJAB de acuerdo con los métodos descritos en el Ejemplo 12 Esta región TM/CT se adhirió a una región Ig CH3 con un cuadro de lectura abierto (ORF) Las versiones de cuadro de lectura abierto de las construcciones scFvIg de interés se crearon por reemplazo de

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las versiones solubles de cada cola -Ig con ORF (versiones de cuadro de lectura abierto) de estas colas Los iniciadores PCR se diseñaron para los clones existentes de colas Ig solubles que eliminan el codón de parada y agregan uno o más sitios de restricción al extremo 3' del nuevo cassette -Ig Las secuencias de cola citoplasmática y de transmembrana deseados pueden entonces ser subclonados corriente debajo de estos nuevos cassettes -Ig Cada construcción utiliza el oligonucleótido 5' BCLI disponible existente utilizado en la amplificación de la versión soluble de las colas para las reacciones PCR Los oligonucleótidos 3' reemplazan el codón de parada sin los sitios de restricción de cuadro fusionados a la región de codificación para los dominios de proteina involucrados en la regulación de apoptosis

Las amplificaciones PCR se llevaron a cabo con 25 pmol de cada iniciador, reactivos PCR estándar, y volúmenes variantes de dominios clonados o cADN obtenido de PBMC, bazo, o ARN de timo Las reacciones utilizaron un perfil de ciclización de 94C, 60 seg, 55C, 60 seg, 72C, 2 min, durante 35 ciclos Los iniciadores para el IgG ORF se listan adelante

Iniciador 5' 5'-gttgtagatcaggagcccaaatcttctgacaaaactcac acatctccaccgtccccagcacctgaactcctgggggaccgtcagtcttcc-3' (ID DE SEC NO)

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Iniciador 3' 5'-gttgttttcgaaggatccgctttacccgggagcagggagaggct cttctgcgtgtagtg-3' (ID DE SEC NO)

La secuencia de polinucleótido se suministra en la ID DE SEC NO _, y la secuencia de polipéptido codificado se suministra enla ID DE SEC NO _

EJEMPLO 114

10A8 scFv (SSS-S)H P238SCH2 WCH3-HCD80TM/CT

Esta construcción tiene una región de unión Fv de cadena sencilla 10A8 (anti-CD2152) descrita en el Ejemplo 12 Esta región de unión se conecta con una región de conexión IgG1 humana mutada en donde todas las cisteínas y una prolina se han cambiado por serinas (SSS-S) de acuerdo con los métodos descritos en el Ejemplo 5 Esta región de pivote se adhiere a una región IgG1 CH2 humana mutada y a una región IgG1 CH3 humana tipo silvestre La mutación P238S, en donde una prolina en el residuo 238 se cambia por una serina, se introduce de acuerdo con los métodos descritos en el Ejemplo 70 Esta región CH3 se adhiere a un hCD80 TM/CT de acuerdo con los métodos descritos en el Ejemplo 113 La secuencia de polinucleótido se suministra en la ID DE SEC NO _, y la secuencia de polipéptido codificado se suministra en la ID DE SEC NO _

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EJEMPLO 115

40 2 220 scFv(SSS-S)H P238SCH2 WCH3-HCD80TM/CT

Esta construcción tiene una región de unión Fv de cadena sencilla 40 2 220 (anti-CD40) descrita en el Ejemplo 12 Esta región de unión se conecta con una región de conexión IgG1 humana mutada en donde todas las cisteínas y una prolina se han cambiado por serinas (SSS-S) de acuerdo con los métodos descritos en el Ejemplo 5 Esta región de pivote se adhiere a una región IgG1 CH2 humana mutada y a una región IgG1 CH3 humana tipo silvestre La mutación P238S, en donde una prolina en el residuo 238 se cambia por una serina, se introduce de acuerdo con los métodos descritos en el Ejemplo 70 Esta región CH3 se adhiere a un hCD80 TM/CT de acuerdo con los métodos descritos en el Ejemplo 113 La secuencia de polinucleótido se suministra en la ID DE SEC NO _, y la secuencia de polipéptido codificado se suministra en la ID DE SEC NO _

EJEMPLO 116

2H7 scFv (SSS-S)H P238SCH2 WCH3-HCD80TM/CT

Esta construcción tiene una región de unión Fv de cadena sencilla 2H7 (anti-CD20) como se describe en el Ejemplo 1 Esta región de unión se conecta con una región de conexión IgG1 humana mutada en donde todas las cisteínas y una prolina

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se han cambiado por serinas (SSS-S) de acuerdo con los métodos descritos en el Ejemplo 5 Esta región de pivote se adhiere a una región IgG1 CH2 humana mutada y a una región IgG1 CH3 humana tipo silvestre La mutación P238S, en donde una prolina en el residuo 238 se cambia por una serina, se introduce de acuerdo con los métodos descritos en el Ejemplo 70 Esta región CH3 se adhiere a un hCD80 TM/CT de acuerdo con los métodos descritos en el Ejemplo 113 Esta construcción se referido previamente como La secuencia de polinucleótido se suministra en la ID DE SEC NO _, y la secuencia de polipéptido codificado se suministra en la ID DE SEC NO _

EJEMPLO 117

G19-4 scFv (SSS-S)H P238SCH2 WCH3-HCD80TM/CT

Esta construcción tiene una región de unión Fv de cadena sencilla G19 (anti-CD3) descrita en el Ejemplo 29 Esta región de unión se conecta con una región de conexión IgG1 humana mutada en donde todas las cisteínas y una prolina se han cambiado por serinas (SSS-S) de acuerdo con los métodos descritos en el Ejemplo 5 Esta región de pivote se adhiere a una región IgG1 CH2 humana mutada y a una región IgG1 CH3 humana tipo silvestre La mutación P238S, en donde una prolina en el residuo 238 se cambia por una serina, se introduce de acuerdo con los métodos descritos en el Ejemplo

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70 Esta región CH3 se adhiere a un hCD80 TM/CT de acuerdo con los métodos descritos en el Ejemplo 113 La secuencia de polinucleótido se suministra en la ID DE SEC NO _, y la secuencia de polipéptido codificado se suministra en la ID DE SEC NO _

EJEMPLO 118

2E12 scFv (SSS-S)H WCH2 WCH3-ECD80TM/CT

Esta construcción tiene una región de unión Fv de cadena sencilla 2e12 (anti-CD28) descrita en el Ejemplo 12 Esta región de unión se conecta con una región de conexión IgG1 humana mutada en donde todas las cisteínas y una prolina se han cambiado por serinas (SSS-S) de acuerdo con los métodos descritos en el Ejemplo 5 Esta región de conexión se adhiere a regiones constantes IgG1 CH2 y CH3 humana tipo silvestre como se describe en el Ejemplo 1 Esta región CH3 se adhiere a un hCD80 TM/CT de acuerdo con los métodos descritos en el Ejemplo 113 Esta construcción ha sido previamente referida como 2e12 scFv IgG WTH WHTCH3CH2-CD80, que tiene la misma secuencia como la construcción anterior La secuencia de polinucleótido se suministra en la ID DE SEC NO _, y la secuencia de polipéptido codificado se suministra en la ID DE SEC NO _

EJEMPLO 119

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2E12 scFv IGAH IGACH2 T4CH3-HCD80TM/CT

Esta construcción tiene una región de unión Fv de cadena sencilla 2e12 (anti-CD28) descrita en el Ejemplo 12 Esta región de unión se adhiere a una región de conexión del IgA humano como se describe en el Ejemplo 5 Esta región de conexión se adhiere a una región constante IgA humana que consiste de una región CH3 mutada y una región CH2 tipo silvestre en donde hay una trucación de los cuatro residuos de aminoácido para el codón de parada 3' como se describe en el Ejemplo 13 Esta región CH3 se adhiere a un hCD80 TM/CT de acuerdo con los métodos descritos en el Ejemplo 113 Los iniciadores específicos utilizados para crear un IgA ORF se listan adelante

Iniciador 5' 5'-gttgttgatcagccagttccctcaactccacctacc-3' (ID DE SEC NO)
Iniciador 3' 5'-gttgttttcgaaggatccgcgtccacctccgccatgacaacaga (ID DE SEC NO)

La secuencia de polinucleótido se suministra en la ID DE SEC NO _, y la secuencia de polipéptido codificado se suministra en la ID DE SEC NO _

EJEMPLO 120

2E12 scFv IGE CH2CH3CH4-HCD80TM/CT

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Esta construcción tiene una región de unión Fv de cadena sencilla 2e12 (anti-CD28) descrita en el Ejemplo 12 Esta región se adhiere a la región constante IgE humana que contiene CH2, CH3 y CH4 como se describe en el Ejemplo 38 Esta región CH4 se adhiere a un hCD80 TM/CT esencialmente de acuerdo con los métodos descritos en el Ejemplo 113 Los iniciadores específicos utilizados para crear un IgE ORF se listan adelante

Iniciador 5' 5'-gttgttgatcacgtctgctccagggacttcacc-3' (ID DE SEC NO)
Iniciador 3' 5'-gttgttttcgaaggatccgctttaccagatttacagacaccg ctcgctg-3' (ID DE SEC NO)

La secuencia de polinucleótido se suministra en la ID DE SEC NO _, y la secuencia de polipéptido codificado se suministra en la ID DE SEC NO _

EJEMPLO 121

2E12 scFv (SSS-S)H P238SCH2 WCH3-MFADD-TM/CT

Esta construcción tiene una región de unión Fv de cadena sencilla 2E12 (anti-CD28) descrita en el Ejemplo 12 Esta región de unión se conecta con una región de conexión IgG1 humana mutada en donde todas las cisteínas y una prolina se

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han cambiado por serinas (SSS-S) de acuerdo con los métodos descritos en el Ejemplo 5 Esta región de pivote se adhiere a una región IgG1 CH2 humana mutada y a una región IgG1 CH3 humana tipo silvestre La mutación P238S, en donde una prolina en el residuo 238 se cambia por una serina, se introduce de acuerdo con los métodos descritos en el Ejemplo 70 La región CH3 se adhiere a una región de cola citoplasmática y transmembrana FADD de ratón (mFADD TM/CT) Esta región se clona utilizando esencialmente los mismos métodos descritos en el Ejemplo 113 El dominio fue PCR amplificado a partir de cADN inicializado aleatoriamente de ARN de bazo de ratón Los iniciadores específicos se listan adelante

Iniciador 5' 5'-gttgtggatccttcgaacccattcotggtgctgctgcac tcgctg-3' (ID DE SEC NO)
Iniciador 3' 5'-gttgttatcgatctcgagtcagggtgtttctgaggaagac acagt-3' (ID DE SEC NO)

Los iniciadores específicos utilizados para crear un IgG ORF de ratón se listan adelante

Iniciador 5' 5'-gttgtagatctggagcccagagggcccacaatcaagccctctcc tccaagcaaaagccca-3' (ID DE SEC NO
Iniciador 3' 5'-gttgttttcgaaggatccgctttacccggagtccgggagaag-3' (ID DE SEC NO)

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La secuencia de polinucleótido se suministra en la ID DE SEC NO _, y la secuencia de polipéptido codificado se suministra en la ID DE SEC NO _

EJEMPLO 122

2E12 scFv (SSS-S)H WCH2 WCH3-MFADD-TM/CT

Esta construcción tiene una región de unión Fv de cadena sencilla 2e12 (anti-CD28) descrita en el Ejemplo 12 Esta región de unión se conecta con una región de conexión IgG1 humana mutada en donde todas las cisteínas y una prolina se han cambiado por serinas (SSS-S) de acuerdo con los métodos descritos en el Ejemplo 5 Esta región de conexión se adhiere a las regiones constantes IgG1 CH2 y CH3 humana tipo silvestre como se describe en el Ejemplo 1 La región CH3 se adhiere a una región mFADD TM/TM de acuerdo con los métodos descritos en el Ejemplo 113 y 121 La secuencia de polinucleótido se suministra en la ID DE SEC NO _, y la secuencia de polipéptido codificado se suministra en la ID DE SEC NO _

EJEMPLO 123

2E12 scFv (SSS-S)H WCH2 WCH3-MCASP3-TM/CT

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Esta construcción tiene una región de unión Fv de cadena sencilla 2e12 (anti-CD28) descrita en el Ejemplo 12 Esta región de unión se conecta con una región de conexión IgG1 humana mutada en donde todas las cisteínas y una prolina se han cambiado por serinas (SSS-S) de acuerdo con los métodos descritos en el Ejemplo 5 Esta región de conexión se adhiere a regiones constantes IgG1 CH2 y CH3 humana tipo silvestre como se describe en el Ejemplo 1 La región CH3 se adhiere a una región de cola citoplasmática y transmembrana casp3 de ratón de acuerdo con los métodos descritos en los Ejemplos 113 y 121 Los iniciadores específicos utilizados para aislar la región mcasp3 TM/CT se listan adelante

Iniciador 5' 5'-gttgttggatccttcgaacatggagaacaacaaaacctcagt ggattca-3' (ID DE SEC NO)

Iniciador 3' 5'-gttgttatcgatctcgagctagtgataaaagtacagttc tttcgt-3' (ID DE SEC NO)

La secuencia de polinucleótido se suministra en la ID DE SEC NO _, y la secuencia de polipéptido codificado se suministra en la ID DE SEC NO _

EJEMPLO 124

2E12 scFv(SSS-S)H P238SCH2 WCH3-MCASP3-TM/CT

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Esta construcción tiene una región de unión Fv de cadena sencilla 2e12 (anti-CD28) descrita en el Ejemplo 12 Esta región de unión se conecta con una región de conexión IgG1 humana mutada en donde todas las cisteínas y una prolina se han cambiado por serinas (SSS-S) de acuerdo con los métodos descritos en el Ejemplo 5 Esta región de pivote se adhiere a una región IgG1 CH2 humana mutada y a una región IgG1 CH3 humana tipo silvestre La mutación P238S, en donde una prolina en el residuo 238 se cambia por una serina se introduce de acuerdo con los métodos descritos en el Ejemplo 70 La región CH3 se conecta con una región mcasp3 TM/CT de acuerdo con los métodos descritos en los Ejemplos 113, 121 y 123 La secuencia de polinucleótido se suministra en la ID DE SEC NO _, y la secuencia de polipéptido codificado se suministra en la ID DE SEC NO _

EJEMPLO 125

2E12 scFv(SSS-S)H WCH2 WCH3MCASP8-TM/CT

Esta construcción tiene una región de unión Fv de cadena sencilla 2e12 (anti-CD28) descrita en el Ejemplo 12 Esta región de unión se conecta con una región de conexión IgG1 humana mutada en donde todas las cisteínas y una prolina se han cambiado por serinas (SSS-S) de acuerdo con los métodos descritos en el Ejemplo 5 Esta región de conexión se adhiere a las regiones constantes IgG1 CH2 y CH3 humana tipo

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silvestre como se describe en el Ejemplo 1 La región CH3 se adhiere a una región de cola citoplasmática y de transmembrana casp8 de ratón (mcasp8 TM/CT) esencialmente de acuerdo con los métodos descritos en el Ejemplo 113 y 121 Los iniciadores específicos utilizados para clonar la región mcasp8 TM/CT se listan adelante

Iniciador 5' 5'-gttgtttcgaacatggatttccagagttgtctttatgctatt gctg-3' (ID DE SEC NO)
iniciador 3' 5'-gttgttatcgatctcgagtcattagggagggaagaagagctt cttccg-3' (ID DE SEC NO)

La secuencia de polinucleótido se suministra en la ID DE SEC NO _, y la secuencia de polipéptido codificado se suministra en la ID DE SEC NO _

EJEMPLO 126

2E12 scFv(SSS-S)H P238SCH2 WCH3-MCASP8-TM/CT

Esta construcción tiene una región de unión Fv de cadena sencilla 2e12 (anti-CD28) descrita en el Ejemplo 12 Esta región de unión se conecta con una región de conexión IgG1 humana mutada en donde todas las cisteínas y una prolina se han cambiado por serinas (SSS-S) de acuerdo con los métodos descritos en el Ejemplo 5 Esta región de pivote se adhiere a una región IgG1 CH2 humana mutada y a una región IgG1 CH3

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humana tipo silvestre La mutación P238S, en donde una prolina en el residuo 238 se cambia por una serina se introduce de acuerdo con los métodos descritos en el Ejemplo 70 La región CH3 se adhiere a una región mcasp8 TM/CT de acuerdo con los métodos descritos en los Ejemplos 113, 121 y 125 La secuencia de polinucleótido se suministra en la ID DE SEC NO _, y la secuencia de polipéptido codificado se suministra en la ID DE SEC NO _

EJEMPLO 127

2E12 scFv(SSS-S)H WCH2 WCH3-HCASP3-TM/CT

Esta construcción tiene una región de unión Fv de cadena sencilla 2e12 (anti-CD28) descrita en el Ejemplo 12 Esta región de unión se conecta con una región de conexión IgG1 humana mutada en donde todas las cisteínas y una prolina se han cambiado por serinas (SSS-S) de acuerdo con los métodos descritos en el Ejemplo 5 Esta región de conexión se adhiere a las regiones constantes IgG1 CH2 y CH3 humana tipo silvestre como se describe en el Ejemplo 1 La región CH3 se adhirió a una región de cola citoplasmática y de transmembrana casp3 humana (hcasp3 TM/CT) esencialmente de acuerdo con los métodos descritos en el Ejemplo 113 Los iniciadores específicos utilizados para clonar la región hcasp3 TM/CT se listan adelante

123C
123T

iniciador 5' 5'-gttgtggatccttcgaacatggagaacactgaaaact cagtggat-3' (ID DE SEC NO)

iniciador 3' 5'-gttgttatogatctogagttagtgataaaaatagagttct tttgtgag-S (ID DE SEC NO)

La secuencia de polinucleótido se suministra en la ID DE SEC NO _, y la secuencia de polipéptido codificado se suministra en la ID DE SEC NO _

EJEMPLO 128

2E12 scFv(SSS-S)H P238SCH2 WCH3-HCASP3-TM/CT

Esta construcción tiene una región de unión Fv de cadena sencilla 2e12 (anti-CD28) descrita en el Ejemplo 12 Esta región de unión se conecta con una región de conexión IgG1 humana mutada en donde todas las cisteínas y una prolina se han cambiado por serinas (SSS-S) de acuerdo con los métodos descritos en el Ejemplo 5 Esta región de pivote se adhiere a una región IgG1 CH2 humana mutada y a una región IgG1 CH3 humana tipo silvestre La mutación P238S, en donde una prolina en el residuo 238 se cambia por una serina se introduce de acuerdo con los métodos descritos en el Ejemplo 70 La región CH3 se adhiere a una región hcasp3 TM/CT de acuerdo con los métodos descritos en los Ejemplos 113 y 127 La secuencia de polinucleótido se suministra en la ID DE SEC

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NO _, y la secuencia de polipéptido codificado se suministra en la ID DE SEC NO _

EJEMPLO 129

2E12 scFv(SSS-S)H WCH2 WCH3-HCASP8--TM/CT

Esta construcción tiene una región de unión Fv de cadena sencilla 2e12 (anti-CD28) descrita en el Ejemplo 12 Esta región de unión se conecta con una región de conexión IgG1 humana mutada en donde todas las cisteínas y una prolina se han cambiado por serinas (SSS-S) de acuerdo con los métodos descritos en el Ejemplo 5 Esta región de conexión se adhiere a las regiones constantes IgG1 CH2 y CH3 humana tipo silvestre como se describe en el Ejemplo 1 La región CH3 se adhirió a una región de cola citoplasmática y de transmembrana casp8 humana (hcasp8 TM/CT) esencialmente de acuerdo con los métodos descritos en el Ejemplo 133 Los iniciadores específicos utilizados para clonar la región hcasp8 TM/CT se listan adelante

Iniciador 5' 5'-gttgtggatccttcgaacatggacttcagcagaaatctttatgat-3' (ID DE SEC NO)

iniciador 3' 5'-gttgttatcgatgcatgctcaatcagaagggaagacaagtttttttct-3' (ID DE SEC NO)

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La secuencia de polinucleótido se suministra en la ID DE SEC NO _, y la secuencia de polipéptido codificado se suministra en la ID DE SEC NO _

EJEMPLO 130

2E12 scFv(SSS-S)H P238SCH2 WCH3-HCASP8-TM/CT

Esta construcción tiene una región de unión Fv de cadena sencilla 2e12 (anti-CD28) descrita en el Ejemplo 12 Esta región de unión se conecta con una región de conexión IgG1 humana mutada en donde todas las cisteínas y una prolina se han cambiado por serinas (SSS-S) de acuerdo con los métodos descritos en el Ejemplo 5 Esta región de pivote se adhiere a una región IgG1 CH2 humana mutada y a una región IgG1 CH3 humana tipo silvestre La mutación P238S, en donde una prolina en el residuo 238 se cambia por una serina, se introduce de acuerdo con los métodos descritos en el Ejemplo 70 La región CH3 se adhiere a una región hcasp8 TM/CT de acuerdo con los métodos descritos en los Ejemplos 113 y 129 La secuencia de polinucleótido se suministra en la ID DE SEC NO _, y la secuencia de polipéptido codificado se suministra en la ID DE SEC NO _

EJEMPLO 131

1D8 scFv(SSS-S)H P238SCH2 WCH3-HCD80TM/CT

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Esta construcción tiene una región de unión Fv de cadena sencilla 1D8 (anti-4-1BB) descrita en el Ejemplo 25 Esta región de unión se conecta con una región de conexión IgG1 humana mutada en donde todas las cisteínas y una prolina se han cambiado por serinas (SSS-S) de acuerdo con los métodos descritos en el Ejemplo 5 Esta región de pivote se adhiere a una región IgG1 CH2 humana mutada y a una región IgG1 CH3 humana tipo silvestre La mutación P238S, en donde una prolina en el residuo 238 se cambia por una serina, se introduce de acuerdo con los métodos descritos en el Ejemplo 70 La región CH3 se adhiere a una región hCD80 TM/CT de acuerdo con los métodos descritos en el Ejemplo 113 La secuencia de polinucleótido se suministra en la ID DE SEC NO _, y la secuencia de polipéptido codificado se suministra en la ID DE SEC NO _

EJEMPLO 132

1D8 scFv(SSS-S)H WCH2 WCH3-HCD80TM/CT

Esta construcción tiene una región de unión Fv de cadena sencilla 1D8 (anti-4-1BB) descrita en el Ejemplo 25 Esta región de unión se conecta con una región de conexión IgG1 humana mutada en donde todas las cisteínas y una prolina se han cambiado por serinas (SSS-S) de acuerdo con los métodos descritos en el Ejemplo 5 Esta región de conexión se adhiere a las regiones constantes IgG1 CH2 y CH3 humana tipo

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silvestre como se describe en el Ejemplo 1 La región CH3 se adhiere a una región hCD80 TM/CT de acuerdo con los métodos descritos en el Ejemplo 113 Esta construcción se ha referido previamente como 1D8 scFv IgG WTH WTCH2CH3-CD80, que tiene la misma secuencia como la construcción anterior La secuencia de polinucleótido se suministra en la ID DE SEC NO _, y la secuencia de polipéptido codificado se suministra en la ID DE SEC NO _

EJEMPLO 133

1D8 scFv-MIGAH WIGA CH2 T4CH3-ECD80TM/CT

Esta construcción tiene una región de unión Fv de cadena sencilla 1D8 (anti-4-1BB) descrita en el Ejemplo 25 Esta región de unión se adhiere a una región de conexión del IgA humano como se describe en el Ejemplo 5 Esta región de conexión se adhiere a una región constante IgA de ratón que consiste de una región CH2 tipo silvestre y una región CH3 mutada en donde hay una truncación de 4 residuos de aminoácido antes del codón de parada 3' como se describe en el Ejemplo 39 La región CH3 se puede adherir a una región hCD80 TM/CT de acuerdo con los métodos descritos en los Ejemplos 113 que utilizan iniciadores que crean un IgA ORF

EJEMPLO 134

1D8 scFv IGE CH2CH3CH4-ECD80TM/CT

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Esta construcción tiene una región de unión Fv de cadena sencilla 1D8 (anti-4-1BB) descrita en el Ejemplo 25 Esta región de unión se adhiere a una región IgE constante humana que contiene CH2, CH3 y CH4 como se describe en el Ejemplo 38 La región CH4 se adhiere a un hCD80 TM/CT de acuerdo con los métodos descritos en los Ejemplos 113 y 120 La secuencia de polinucleótido se suministra en la ID DE SEC NO _, y la secuencia de polipéptido codificado se suministra en la ID DE SEC NO _

EJEMPLO 135

1D8 scFv(SSS-S)H P238SCH2 WCH3-MFADD-TM/CT

Esta construcción tiene una región de unión Fv de cadena sencilla 1D8 (anti-4-1BB) descrita en el Ejemplo 25 Esta región de unión se conecta con una región de conexión IgG1 humana mutada en donde todas las cisteínas y una prolina se han cambiado por serinas (SSS-S) de acuerdo con los métodos descritos en el Ejemplo 5 Esta región de pivote se adhiere a una región IgG1 CH2 humana mutada y a una región IgG1 CH3 humana tipo silvestre La mutación P238S, en donde una prolina en el residuo 238 se cambia por una serina, se introduce de acuerdo con los métodos descritos en el Ejemplo 70 La región CH3 se adhiere a una región mFADD TM/TM de acuerdo con los métodos descritos en el Ejemplo 113 y 121 La

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secuencia de polinucleótido se suministra en la ID DE SEC NO _, y la secuencia de polipéptido codificado se suministra en la ID DE SEC NO _

EJEMPLO 136

1D8 scFv (SSS-S)H WCH2 WCH3-MFADD-TM/CT

Esta construcción tiene una región de unión Fv de cadena sencilla 1D8 (anti-4-1BB) descrita en el Ejemplo 25 Esta región de unión se conecta con una región de conexión IgG1 humana mutada en donde todas las cisteínas y una prolina se han cambiado por serinas (SSS-S) de acuerdo con los métodos descritos en el Ejemplo 5 Esta región de conexión se adhiere a las regiones constantes IgG1 CH2 y CH3 humana tipo silvestre como se describe en el Ejemplo 1 La región CH3 se adhiere a una región mFADD TM/TM de acuerdo con los métodos descritos en el Ejemplo 113 y 121 La secuencia de polinucleótido se suministra en la ID DE SEC NO _, y la secuencia de polipéptido codificado se suministra en la ID DE SEC NO _

EJEMPLO 137

1D8 scFv (SSS-S)H WCH2 WCH3-mcasp3-TM/CT

Esta construcción tiene una región de unión Fv de cadena sencilla 1D8 (anti-4-1BB) descrita en el Ejemplo 25 Esta

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región de unión se conecta con una región de conexión IgG1 humana mutada en donde todas las cisteínas y una prolına se han cambiado por serinas (SSS-S) de acuerdo con los métodos descritos en el Ejemplo 5 Esta región de conexión se adhiere a las regiones constantes IgG1 CH2 y CH3 humana tipo sılvestre como se describe en el Ejemplo 1 La región CH3 se adhiere a una región mcasp3 TM/TM de acuerdo con los métodos descritos en el Ejemplo 113, 121 y 123 La secuencia de polınucleótido se suminıstra en la ID DE SEC NO _, y la secuencia de polipéptido codificado se suminıstra en la ID DE SEC NO _

EJEMPLO 138

1D8 scFv (SSS-S)H P238SCH2 WCH3-MCASP3-TM/CT

Esta construcción tiene una región de unión Fv de cadena sencılla 1D8 (antı-4-1BB) descrita en el Ejemplo 25 Esta región de unión se conecta con una región de conexión IgG1 humana mutada en donde todas las cisteínas y una prolına se han cambiado por serınas (SSS-S) de acuerdo con los métodos descritos en el Ejemplo 5 Esta región de pivote se adhiere a una región IgG1 CH2 humana mutada y a una región IgG1 CH3 humana tipo sılvestre La mutación P238S, en donde una prolina en el resıduo 238 se cambıa por una serına, se ıntroduce de acuerdo con los métodos descritos en el Ejemplo 70 La región CH3 se adhiere a una región mcasp3 TM/TM de

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acuerdo con los métodos descritos en el Ejemplo 113, 121 y 123 La secuencia de polinucleótido se suministra en la ID DE SEC NO _, y la secuencia de polipéptido codificado se suministra en la ID DE SEC NO _

EJEMPLO 139

1D8 scFv(SSS-S)H WCH2 WCH3-MCASP8--TM/CT

Esta construcción tiene una región de unión Fv de cadena sencilla 1D8 (anti-4-1BB) descrita en el Ejemplo 25 Esta región de unión se conecta con una región de conexión IgG1 humana mutada en donde todas las cisteínas y una prolina se han cambiado por serinas (SSS-S) de acuerdo con los métodos descritos en el Ejemplo 5 Esta región de conexión se adhiere a las regiones constantes IgG1 CH2 y CH3 humana tipo silvestre como se describe en el Ejemplo 1 La región CH3 se adhiere a una región mcasp8 TM/TM de acuerdo con los métodos descritos en el Ejemplo 113, 121 y 125 La secuencia de polinucleótido se suministra en la ID DE SEC NO _, y la secuencia de polipéptido codificado se suministra en la ID DE SEC NO _

EJEMPLO 140

1D8 scFv(SSS-S)H P238SCH2 WCH3-MCASP8-TM/CT

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Esta construcción tiene una región de unión Fv de cadena sencilla 1D8 (anti-4-1BB) descrita en el Ejemplo 25 Esta región de unión se conecta con una región de conexión IgG1 humana mutada en donde todas las cisteínas y una prolina se han cambiado por serinas (SSS-S) de acuerdo con los métodos descritos en el Ejemplo 5 Esta región de pivote se adhiere a una región IgG1 CH2 humana mutada y a una región IgG1 CH3 humana tipo silvestre La mutación P238S, en donde una prolina en el residuo 238 se cambia por una serina, se introduce de acuerdo con los métodos descritos en el Ejemplo 70 La región CH3 se adhiere a una región mcasp8 TM/TM de acuerdo con los métodos descritos en el Ejemplo 113, 121 y 125 La secuencia de polinucleótido se suministra en la ID DE SEC NO _, y la secuencia de polipéptido codificado se suministra en la ID DE SEC NO _

EJEMPLO 141

1D8 scFv (SSS-S)H WCH2 WCH3-HCASP3--TM/CT

Esta construcción tiene una región de unión Fv de cadena sencilla 1D8 (anti-4-1BB) descrita en el Ejemplo 25 Esta región de unión se conecta con una región de conexión IgG1 humana mutada en donde todas las cisteínas y una prolina se han cambiado por serinas (SSS-S) de acuerdo con los métodos descritos en el Ejemplo 5 Esta región de conexión se adhiere a las regiones constantes IgG1 CH2 y CH3 humana tipo

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silvestre como se describe en el Ejemplo 1 La región CH3 se adhiere a una región hcasp3 TM/CT de acuerdo con los métodos descritos en el Ejemplo 113, y 127 La secuencia de polinucleótido se suministra en la ID DE SEC NO _, y la secuencia de polipéptido codificado se suministra en la ID DE SEC NO _

EJEMPLO 142

1D8 scFv (SSS-S)H P238SCH2 WCH3-HCASP3-TM/CT

Esta construcción tiene una región de unión Fv de cadena sencilla 1D8 (anti-4-1BB) descrita en el Ejemplo 25 Esta región de unión se conecta con una región de conexión IgG1 humana mutada en donde todas las cisteínas y una prolina se han cambiado por serinas (SSS-S) de acuerdo con los métodos descritos en el Ejemplo 5 Esta región de pivote se adhiere a la región IgG1 CH2 humana mutada y a una región IgG1 CH3 humana tipo silvestre La mutación P238S, en donde una prolina en el residuo 238 se cambia por una serina, se introduce de acuerdo con los métodos descritos en el Ejemplo 70 La región CH3 se adhiere a una región hcasp3 TM/CT de acuerdo con los métodos descritos en el Ejemplo 113, y 127 La secuencia de polinucleótido se suministra en la ID DE SEC NO _, y la secuencia de polipéptido codificado se suministra en la ID DE SEC NO _

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EJEMPLO 143

1D8 scFv (SSS-S)H WCH2 WCH3-HCASP8--TM/CT

Esta construcción tiene una región de unión Fv de cadena sencilla 1D8 (anti-4-1BB) descrita en el Ejemplo 25 Esta región de unión se conecta con una región de conexión IgG1 humana mutada en donde todas las cisteínas y una prolina se han cambiado por serinas (SSS-S) de acuerdo con los métodos descritos en el Ejemplo 5 Esta región de conexión se adhiere a las regiones constantes IgG1 CH2 y CH3 humana tipo silvestre como se describe en el Ejemplo 1 La región CH3 se adhiere a una región hcasp8 TM/CT de acuerdo con los métodos descritos en el Ejemplo 113, y 129 La secuencia de polinucleótido se suministra en la ID DE SEC NO _, y la secuencia de polipéptido codificado se suministra en la ID DE SEC NO _

EJEMPLO 144

1D8 scFv (SSS-S)H P238SCH2 WCH3-HCASP8-TM/CT

Esta construcción tiene una región de unión Fv de cadena sencilla 1D8 (anti-4-1BB) descrita en el Ejemplo 25 Esta región de unión se conecta con una región de conexión IgG1 humana mutada en donde todas las cisteínas y una prolina se han cambiado por serinas (SSS-S) de acuerdo con los métodos descritos en el Ejemplo 5 Esta región de pivote se adhiere a

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la región IgG1 CH2 humana mutada y a una región IgG1 CH3 humana tipo silvestre La mutación P238S, en donde una prolina en el residuo 238 se cambia por una serina, se introduce de acuerdo con los métodos descritos en el Ejemplo 70 La región CH3 se adhiere a un hcasp8 TM/CT de acuerdo con los métodos descritos en el Ejemplo 113, y 129 La secuencia de polinucleótido se suministra en la ID DE SEC NO _, y la secuencia de polipéptido codificado se suministra en la ID DE SEC NO _

EJEMPLO 145

L6 scFv (SSS-S)H WCH2 WCH3

Esta construcción tiene un dominio de unión L6 scFv como se describe en el Ejemplo 105 Esta región de unión se conecta con una región de conexión IgG1 humana mutada en donde todas las cisteínas y una prolina se han cambiado por serinas (SSS-S) de acuerdo con los métodos descritos en el Ejemplo 5 Esta región de conexión se adhiere a las regiones constantes IgG1 CH2 y CH3 humana tipo silvestre como se describe en el Ejemplo 1 Esta construcción se ha referido previamente como L6 scFv-IgMHWTG1C, que tiene la misma secuencia como la construcción anterior La secuencia de polinucleótido se suministra en la ID DE SEC NO _, y la secuencia de polipéptido codificado se suministra en la ID DE SEC NO _

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EJEMPLO 146

2H7 scFv CD154(L2)

Esta construcción tiene una región de unión Fv de cadena sencilla 2H7 (anti-CD20) como se describe en el Ejemplo 1 Esta región de unión se ha adherido al dominio extracelular CD154 de acuerdo con los métodos descritos en el Ejemplo 4 La secuencia de polinucleótido se suministra en la ID DE SEC NO _, y la secuencia de polipéptido codificado se suministra en la ID DE SEC NO _

EJEMPLO 147

2H7 scFv CD154(84)

Esta construcción tiene una región de unión Fv de cadena sencilla 2H7 (anti-CD20) como se describe en el Ejemplo 1 Esta región de unión se ha adherido al dominio extracelular CD154 de acuerdo con los métodos descritos en el Ejemplo 4, tal como los métodos de adhesión resultan en una versión truncada comparada con la construcción descrita en el Ejemplo 146 La secuencia de polinucleótido se suministra en la ID DE SEC NO _, y la secuencia de polipéptido codificado se suministra en la ID DE SEC NO _

EJEMPLO 148

CTLA4 IGAH IGACH2CH3

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Esta construcción tiene la región de unión CTLA-4 extra celular como se describe en el Ejemplo 14 Esta región de unión se adhiere a una región de conexión IgA humana tipo silvestre como se describe en el Ejemplo 5 Esta región de conexión se adhiere a las regiones constantes IgA CH2 y CH3 humana tipo silvestre de acuerdo con los métodos descritos en el Ejemplo 13 Esta región constante se adhiere a una región de cadena J como se describe en el Ejemplo 13 Esta construcción se ha referido previamente como CTLA-4 IgAH IgACH2CH3, que tiene la misma secuencia como la construcción anterior La secuencia de polinucleótido se suministra en la ID DE SEC NO _, y la secuencia de polipéptido codificado se suministra en la ID DE SEC NO _

EJEMPLO 149

CTLA4 IGAH IGACH2 T4CH3

Esta construcción tiene una región de unión CTLA-4 extracelular como se describe en el Ejemplo 14 Esta región de unión se adhiere a una región de conexión IgA humana como se describe en el Ejemplo 5 Esta región de conexión se adhiere a la región constante IgA humana que consiste de una región CH2 tipo silvestre y una región CH3 mutada en donde existe una truncación de 4 residuos de aminoácido antes del codón de parada 3' como se describe en el Ejemplo 13 Esta

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construcción se ha referido previamente como CTLA-4 IgAH IgA-T4, que tiene la misma secuencia como la construcción anterior La secuencia de polinucleótido se suministra en la ID DE SEC NO _, y la secuencia de polipéptido codificado se suministra en la ID DE SEC NO _

EJEMPLO 150

2H7 scFv IGAH IGACH2CH3

Esta construcción tiene una región de unión Fv de cadena sencilla 2H7 (anti-CD20) como se describe en el Ejemplo 1 Esta región de unión se ha adherido a la región de conexión IgA humana tipo silvestre como se describe en el Ejemplo 5 Esta región de conexión se adhiere a una región constante IgA CH2 y CH3 humana tipo silvestre de acuerdo con los métodos descritos en el Ejemplo 13 La secuencia de polinucleótido se suministra en la ID DE SEC NO _, y la secuencia de polipéptido codificado se suministra en la ID DE SEC NO _

EJEMPLO 151

2H7 scFv IGAH IGAHCH2 T18CH3

Esta construcción tiene una región de unión Fv de cadena sencilla 2H7 (anti-CD20) como se describe en el Ejemplo 1 Esta región de unión se ha adherido a la región de conexión IgA humana como se describe en el Ejemplo 5 Esta región de

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conexión se adhiere a una región constante IgA humana que consiste de una región CH2 tipo silvestre y una región CH3 mutada en donde hay una truncación de 18 residuos de aminoácido antes del codón de parada 3' como se describe en el Ejemplo 13 La secuencia de polinucleótido se suministra en la ID DE SEC NO _, y la secuencia de polipéptido codificado se suministra en la ID DE SEC NO _

EJEMPLO 152

2H7-40 2 220 scFv (SSS-S)H WCH2 WCH3

Una proteína de fusión biespecífica se construye entre el 2H7 scFv (anti-CD20) y 40 2 220 (anti-CD40), que con ambos receptores de célula B objetivo La construcción 2H7 scFv hIgG1 (SSS-S)H WCH2 WCH3 en el vector de expresión pD18 se pasó a través de bacterias dam⁻ con el fin de permitir el clivaje en el sitio BclI El plásmido clivado se trató con fosfato alcalino antes de ligación a un fragmento scFv ligador-CD40 de corte BclI Este fragmento se sintetizó a partir del 40 2 220 scFv existente mediante reacciones PCR sucesivas con iniciadores traslapantes El ligador adherido es un ligador de tipo helicoidal patentado (patente BMS) con un número alto de residuos de ácido glutámico y glicina El scFv para el CD40 fue PCR amplificado sin el péptido líder como un fragmento SalI-BclI, pero incluye la cola en una construcción scFvIg existente para el CD20 (construcciones

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2H7 scFv hIgG1) El extremo 3' era sımılar a las otras moléculas scFv VH con una salıda del sıtıo de cuadro BclI fusıonado con la secuencia de tıpo VTVSS en el extremo del dominıo VH

Olıgos PCR

40 2 220 scFv

Inıcıador 5'--40 2 220S5 5'-gttgttgtcgacattgttctgactcagtct ccagccaccctgtc-3' (ID DE SEC NO)

Inıcıador 3'--40 2 220Bcl3 5'-gttgttgatcagagacagtgaccagtgt cccttgg-3' (ID DE SEC NO)

Inıcıadores Lıgadores

El frfagmento BclI-SalI creado medıante la hıbrıdızacıón complementarıa de olıgonucleótıdos Este fragmento se lıga luego dentro del vector dıgerıdo BclI con un SalI-BclI scFv para crear el (lıgador-scFv) fragmento BclI deseado para lanzadera

Inıcıador 5' 5'-gatcaatccaactctgaagaagcaaagaaagaggaggccaaaaa ggaggaagccaagaaatctaacagcg-3' (ID DE SEC NO)

Inıciador 3' 5'-tcgacgctgttagatttcttggcttcctccttttggcctcc tctttctttgcttcttcagagttggatt-3' (ID DE SEC NO)

Este fragmento BclI fue luego lıgado corrıente abajo del 2H7 scFv en pD18-Ig Los transformantes se seleccıonaron para la

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presencia de un inserto 2 4 kb HindIII-Xba y los clones positivos se secuenciaron antes para estudios adicionales Las transfecciones transitorias de célula COS se desarrollaron con esta construcción y los supernadantes de cultivo seleccionados para la presencia de proteína de tamaño predicho y para unirse a células CD20 y a células CHO transfectadas CD40

Nuevas construcciones biespecíficas se pueden crear al diseñar ligadores de tipo pivote que incorporan uno o preferiblemente dos sitios de restgricción en cualquier extremo del ligador, que facilita la digestión asimétrica y la transferencia de cassettes (ligador-scFv) o (scFv-ligador) entre diferentes construcciones Estas construcciones también incorporarfán las sustituciones de región VH L11S y otra región V, que presumiblemente facilitan apropiadamente el plegamiento y resultan en una expresión incrementada de las moléculas en las que ellas están insertas

Esta región de unión se conecta con una región de conexión IgG1 humana mutada en donde todas las cisteínas y una prolina se han cambiado por serinas (SSS-S) de acuerdo con los métodos descritos en el Ejemplo 5 Esta región de conexión se adhiere a una misma secuencia IgG1 CH2 humana de tipo silvestre como la construcción anterior La secuencia de polinucleótido se suministra en la ID DE SEC NO _, y la

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secuencia de polipéptido codificado se suministra en la ID DE SEC NO _

EJEMPLO 153

2H7 scFv IGAH IGACH2 T4CH3-HCD80 TM/CT

Esta construcción tiene una región de unión Fv de cadena sencilla 2H7 (anti-CD20) como se describe en el Ejemplo 1 Esta región de unión se adhiere a la región de conexión IgA humana como se describe en el Ejemplo 5 Esta región de conexión se adhiere a una región constante IgA humana que consiste de una región CH2 tipo silvestre y una región CH3 mutada en donde hay una truncación de 4 residuos de aminoácido antes del codón de parada 3' como se describe en el Ejemplo 13 Esta región CH3 se adhiere a un hCD80 TM/CT de acuerdo con los métodos descritos en el Ejemplo 113 y 119 Esta construcción se ha referido previamente como 2H7 scFv IgA pivote IgA-T4-CD80 y 2H7 scFv IgAH IgA-T4-CD80, las cuales tienen la misma secuencia como la construcción anterior La secuencia de polinucleótido se suministra en la ID DE SEC NO _, y la secuencia de polipéptido codificado se suministra en la ID DE SEC NO _

EJEMPLO 154

G19-4 scFv (CCC-P) WH WCH2 WCH3-HCD80 TM/CT

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Esta construcción tiene una región de unión Fv de cadena sencilla G19 (anti-CD3) descrita en el Ejemplo 29 Esta región de unión se adhiere a la región de conexión IgG1 humana tipo silvestre (CCC-P) como se describe en el Ejemplo 1 Esta región de conexión se adhiere a regiones constantes IgG1 CH2 y CH3 humana tipo silvestre como se describe en el Ejemplo 1 Esta región CH3 se adhiere a un hCD80 TM/CT de acuerdo con los métodos descritos en el Ejemplo 113 La secuencia de polinucleótido se suministra en la ID DE SEC NO _, y la secuencia de polipéptido codificado se suministra en la ID DE SEC NO _

EJEMPLO 155

2E12 scFv (CCC-P) WH WCH2 WCH3-HCD80 TM/CT

Esta construcción tiene una región de unión Fv de cadena sencilla 2e12 (anti-CD28) descrita en el Ejemplo 12 Esta región de unión se adhiere a la región de conexión IgG1 humana tipo silvestre (CCC-P) como se describe en el Ejemplo 1 Esta región de conexión se adhiere a regiones constantes IgG1 CH2 y CH3 humana tipo silvestre como se describe en el Ejemplo 1 Esta región CH3 se adhiere a un hCD80 TM/CT de acuerdo con los métodos descritos en el Ejemplo 113 La secuencia de polinucleótido se suministra en la ID DE SEC NO _, y la secuencia de polipéptido codificado se suministra en la ID DE SEC NO _

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EJEMPLO 156

2H7 VHL11S scFv (SSS-S) IGECH3CH4

Esta construcción tiene una región de unión Fv de cadena sencilla 2H7 (anti-CD20) con un punto de mutación en el residuo de aminoácido 11 en la región variable de cadena pesada, en donde la leucina se ha cambiado por una serina como se describe en el Ejemplo 33 Esta región de unión se conecta con una región conexión IgG1 humana mutada en donde todas las cisteínas y una prolina se han cambiado por serinas (SSS-S) de acuerdo con los métodos descritos en el Ejemplo 5 Esta región de conexión se adhiere a la región constante IgE CH3 y CH4 humana Esta región constante truncada se creó de acuerdo con los métodos descritos en el Ejemplo 38 La secuencia de polinucleótido se suministra en la ID DE SEC NO _, y la secuencia de polipéptido codificado se suministra en la ID DE SEC NO _

EJEMPLO 157

PIVOTE IGD

Una región de pivote alternativa se puede aislar de la región de pivote de inmunoglobulina IgD humana a utilizar ensayo PCR para aislar la región deseada La reacción PCR es la misma utilizada en el Ejemplo 1 Este pivote se truncó por los 6

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residuos de aminoácido en el extremo 3' Los iniciadores utilizados en esta reacción PCR se listan adelante

Iniciador 5' 5'-GTGGATCCAGGTTCGAAGTCTCCAAAGGCACAGGCC-3' (ID DE SEC NO)

Iniciador 3' 5'-GTTGI'CGACTGCACCGGTCTTTGTCTCTCTCTCTTC-3' (ID DE SEC NO)

La secuencia de polinucleótido se suministra en la ID DE SEC NO _, y la secuencia de polipéptido codificado se suministra en la ID DE SEC NO _

EJEMPLO 158

HCD28 TM/CT

Para algunas de las construcciones ORF de superficie celular, el dominio de transmembrana del CD80 se sustituyó con el dominio de transmembrana del CD28 humano en razón a que este forma un dímero sobre la superficie celular a diferencia de un monómero como lo es el CD80 Varias de las moléculas que dirigen el programa apoptótico requieren oligomerización/trimerización para formar un complejo de señalización, por lo tanto, es importante ser capaz de controlar la iniciación de la señalización al controlar el grado de oligomerización de estos receptores recombinantes sobre la superficie celular

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Los iniciadores utilizados en la amplificación PCR de la cola CD28 se dan adelante

iniciador 5' 5'-gttgtggatccttcgaaccccttttgggtgctggtgg tggttggtgga-3' (ID DE SEC NO)
iniciador 3' 5'-gttgttatcgatctcgagtcaggagcgataggctgcgaagtc-3' (ID DE SEC NO)

La secuencia de polinucleótido se suministra en la ID DE SEC NO _, y la secuencia de polipéptido codificado se suministra en la ID DE SEC NO _

EJEMPLO 159

2H7 scFv VH L11S (SSS-S)H K322L CH2 WCH3

Esta construcción tiene una región de unión Fv de cadena sencilla 2H7 (anti-CD20) con un punto de mutación en el residuo de aminoácido 11 en la región variable de cadena pesada, en donde la leucina se ha cambiado por una serina como se describe en el Ejemplo 33 Esta región de unión se conecta con una región conexión IgG humana mutada en donde todas las cisteínas y una prolina se han cambiado por serinas (SSS-S) de acuerdo con los métodos descritos en el Ejemplo 5 La región de conexión se adhiere a la región IgG CH2 mutada y una región IgG CH3 tipo silvestre La mutación K322L en la

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región CH2 está en un residuo 322, en donde una Lisina se ha cambiado por una leucina que utiliza PCR traslapante descrito en el Ejemplo 56, pero con diferentes iniciadores para la primera reacción PCR, que se listan adelante

Iniciador 5' 5'ttcctcttcccccaaaacccaaggacaccctcatgatctcccgg aaccctgaggtcac-3' (ID DE SEC NO)
Iniciador 3' 5'-ggacagtgggagtggcacc-3' (ID DE SEC NO)

El producto PCR se clonó en el vector TOPO y se secuenció La secuencia de polinucleótido se suministra en la ID DE SEC NO _, y la secuencia de polipéptido codificado se suministra en la ID DE SEC NO _

EJEMPLO 160

2H7 scFv VH L11S (CSS-S)H K322L CH2 WCH3

Esta construcción tiene una región de unión Fv de cadena sencilla 2H7 (anti-CD20) con un punto de mutación en el residuo de aminoácido 11 en la región variable de cadena pesada, en donde la leucina se ha cambiado por una serina como se describe en el Ejemplo 33 Esta región de unión se adhiere a una región conexión IgG mutante, en donde la segunda y tercera cisteínas se han cambiado por serinas y la prolina se ha cambiado por serina (CSS-S), de acuerdo con los métodos descritos en el Ejemplo 13 La región de conexión se

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adhiere a la región IgG CH2 mutada y a una región IgG CH3 tipo silvestre La mutación K322L en la región CH2 está en un residuo 322, en donde una Lisina se ha cambiado por una leucina que utiliza PCR traslapante descrito en el Ejemplo 56, que utiliza iniciadores del Ejemplo 159 en la primera reacción PCR y los iniciadores del Ejemplo 57 para la segunda reacción PCR

Los productos PCR se clonaron en el vector TOPO y se secuenciaron La secuencia de polinucleótido se suministra en la ID DE SEC NO _, y la secuencia de polipéptido codificado se suministra en la ID DE SEC NO _

EJEMPLO 161

AGOTAMIENTO DE LA CÉLULA B *IN VIVO* MEDIANTE 2H7 scFv (SSS-S)H WCH2 WCH3 Y 2H7 scFv (SSS-S)H P238SCH2 WCH3

Este Ejemplo describe experimentos que se relacionan con mecanismos efectores ADCC en el agotamiento de células B a través de la comparación de las actividades de una construcción anti-CD20 (2H7 scFv (SSS-S) H WCH2 WCH3) y una construcción anti-CD20 con una prolina para sustitución de aminoácido serina en el dominio CH2 (2H7 scFv (SSS-S)H P238SCH2 WCH3) La Figura 72 muestra los resultados de un experimento sobre la inducción de apoptosis mediante estas construcciones Los resultados indican que ambas moléculas

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unen el CD20 e inducen apoptosis que se media por la activación de la caspasa 3

La Figura 73 ilustra que las construcciones anti-CD20 median actividad CDC hacia las células objetivo positivas CD20 Una construcción 2H7 scFv (SSS-S)H WCH2 WCH3, una construcción 2H7 scFv (SSS-S)H P238SCH2 WCH3, o Rituximab se incubaron para incrementar las concentraciones con células 10^4 células objetivo Bjab y una dilución 1 10 de complemento de conejo (PelFreez) en un volumen de 100 microlitros durante 60 minutos Las alícuotas se tiñeron con trypan azul (Invitrogen), y contadas utilizando un hemacitómetro para determinar el porcentaje de población celular muerta durante el tratamiento Los controles negativos con células y solo un reactivo también se incluyen La Figura 74 ilustra que la construcción 2H7 scFv (SSS-S)H WCH2 WCH3 fue efectiva en el ADCC con células mononucleares sanguíneas periféricas La actividad ADCC del 2H7 scFv (SSS-S)H WCH2 WCH3 o Rituximab se midió *in vitro* contra la línea celular linfoma B BJAB como las células objetivo, y utilizando PBMC humano fresco como células efectoras El efector para proporciones objetivos se varió como sigue 100 1, 50 1, y 25 1, con el número de células BJAB por pozo constante remanente pero que varía el numero de PBMC Las células BJAB se etiquetaron durante 2 horas con ^{51}Cr y distribuido en alícuotas en una densidad celular de 5 x 10^4 células/pozo para cada pozo de placas de

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96 pozos de fondo plano Las proteínas de fusión purificadas o Rituximab se agregaron en una concentración de 10 µg/ml, y el PBMC se aggregó en 1 25 x 10^6 células/pozo (25 1), 2 5 x 10^6 células/pozo (50 1), o 5 x 10^6 células/pozo (100 1), en un volumen final de 200 µl La matanza natural se midió en cada proporción efector objetivo mediante la omisión de la construcción o MAb La liberación espontánea se midió sin la adición de PBMC o proteína de fusión, y la liberación máxima se midió mediante la adición de detergente (1% NP-40) para los pozos apropiados Las reacciones se incubaron durante 5 horas, y el supernadante de cultivo de 100 µl cosechado a un Lumaplate (Packard Instruments) y se le permitió secarse durante la noche antes de continuar la liberación de cpm sobre un Contador de Centelleo de Microplaca NXT de Conteo Superior Packard La Figura 75 ilustra que la construcción 2H7 scFv (SSS-S)H WCH2 WCH3 une la proteína de fusión CD16 soluble (construída a partir de alelos de alta o baja afinidad (158V/F)), mientras que la construcción 2H7 scFv (SSS-S)H P238SCH2 WCH3 no muestra unión detectable Las células CD20 CHO (10^6) se incuban con cantidades de saturación de 2H7 scFv (SSS-S)H WCH2 WCH3 o 2H7 scFv (SSS-S)H P238SCH2 WCH3 (10 ug/ml) durante una hora en hielo en FBS PBS/2% Las células se lavaron en FBS PBS/2% y se incubaron con diluciones seriales de 0 5 mg/ml de FITC-CD16 durante una hora en hielo Las células se lavaron y se midió la unión específica mediante citometría de flujo que utiliza una

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máquina Beckman-Coulter Epics C Los resultados se analizan utilizando software de análisis Expo y las unidades de fluorescencia normalizadas se graficaron como una función de la concentración La Figura 76 ilustra un experimento sobre la capacidad de las construcciones 2H7 scFv (SSS-S)H WCH2 WCH3 y 2H7 scFv (SSS-S)H P238SCH2 WCH3 para unirse a las células U937 Las células U937 (10^6) que expresan el CD64 se incuban en FBS PBS/2% durante una hora en hielo con 2H7 scFv (SSS-S)H WCH2 WCH3 o 2H7 scFv (SSS-S)H P238SCH2 WCH3 Las células se lavaron e incubaron durante una hora en hielo con IgG1 anti-FITC (Fc específico) (Caltag) en una dilución final de 1 100 Las células se lavaron y la fluorescencia se analizó en un citómetro de flujo Beckman-Coulter EpicsC Los datos se analizaron utilizando software de análisis Expo, y la intensidad de fluorescencia se graficó como una función de la concentración de 2H7 scFv (SSS-S)H WCH2 WCH3 o 2H7 scFv (SSS-S)H P238SCH2 WCH3 Esta Figura muestra que ambas la construcción 2H7 scFv (SSS-S) H WCH2 WCH3 y la construcción 2H7 scFv (SSS-S) H P238SCH2 WCH3 se une a las células U937 con curvas de titulación equivalente Los resultados indican que la construcción 2H7 scFv (SSS-S)H P238SCH2 WCH3 no se deteriora en su unión al receptor Fc de alta afinidad FcγRI (CD64) El hecho de que la unión del FcγRI de alta afinidad sobre las células U937 era similar para las dos moléculas en este experimento indica que el cambio de aminoácido P238S reduce selectivamente la unión al FcγRIII La Figura 77

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ılustra el agotamıento de la célula B en macacos medıado por la construcción antı-CD20 (2H7 scFv (SSS-S) H WCH2 WCH3) y un antı-CD20) scFv con una mutación de dominıo CH2 El 2H7 scFv (SSS-S) H WCH2 WCH3 o 2H7 scFv (SSS-S) H P238SCH2 WCH3 se adminıstraron a macacos (*M fasıcularıs*) mediante ınyección ıntravenosa en 6 mg/kg, con dos ınfusıones dadas una semana entre ellas El efecto de las células B cırculantes se mıdıó medıante la detección de células B CD40 posıtıvas en sangre perıférıca Las muestras de sangre se retıraron de los anımales ınyectados en los días 7, 0, 1, 3, 7, 8, 10, 14, 28 y 43 El agotamıento de las células B se estımó medıante la ejecución de CBC (conteo de sangre completo) y análısıs de citometrıa de flujo de dos colores en sangre de mono Los conjugados FITC o PE de los antıcuerpos contra CD40, CD19, CD20, IgG, CD3, CD8 se utılızaron en varıas combınacıones Los datos se dıagraman como el número de células B de sangre CD40 posıtivo tabuladas en mıles de células por mıcrolıtro durante el tıempo relatıvo al nıvel de punto de tıempo de preınyección ınıcıal de células B (máximo) Este experimento muestra que la ınyección de 2H7 scFv (SSS-S) H WCH2 WCH3 scFv resulta en agotamıento completo y rápıdo de las células B cırculantes que duró más de 28 días luego de la segunda ınyección La ınyección de 2H7 scFv (SSS-S) H P238SCH2 WCH3 scFv no agotó completamente las células B aunque se saturaron los epítopos CD20 Una lenta reducción en las células B para aproxımadamente 50% de los nıveles ınıcıales se observó

durante las primeras dos semanas, pero las células B retornaron rápidamente a los niveles de partida en estos animales Estos resultados indican que la capacidad para unir el FCγRI de alta afinidad y para mediar la citotoxicidad dependiente del complemento no es suficiente para una molécula CytoxB20G para agotar completamente las células B circulantes, y que el ADCC mediado por la interacción con CD16 es igualmente necesario para el agotamiento de la célula B rápido y sostenido

Estos experimentos indican que el 2H7 scFv (SSS-S) H WCH2 WCH3 scFvs es capaz de accionar las funciones de agotamiento de la célula B a través de tres diferentes mecanismos de acción (1) inducción de apoptosis, (2) mecanismos efectores CDC, y (3) mecanismos efectores ADCC Todos los tres mecanismos probablemente contribuyen al agotamiento de las células B *in vivo* Los datos indican que el agotamiento completo y sostenido de las células B requiere mecanismos efectores ADCC intactos Sin embargo, el agotamiento parcial obtenido con un ADCC negativo mutante también indica que la apoptosis y los mecanismos CDC contribuyen a mediar una porción del total de los efectos de agotamiento de célula B Los resultados apoyan el uso del 2H7 scFv (SSS-S) H WCH2 WCH3 scFv en pacientes con una enfermedad autoinmune o malignidad de célula B CD20 positiva

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EJEMPLO 162

INDUCCIÓN DE APOPTOSIS EN CÉLULAS B MEDIANTE CONSTRUCCIONES ANTI-CD37 GS-1 VL11S SCFV (SSS) H WCH2WCH3

En este Ejemplo, las construcciones anti-CD37 GS-1 VL11S scFv (SSS) H WCH2WCH3 se evalúa para su capacidad de unir células objetivo y para su capacidad de inducir apoptosis Las construcciones también se caracterizaron físicamente mediante la cromatografía de exclusión de tamaño (SEC) La Figura 78 ilustra el perfil SEC de las construcciones G28-1 (anti-CD37) que tiene formas de dominio de pivote SSC (GS-1 VL11S scFv (SSC) H WCH2WCH3) Las construcciones G28-1 (anti-CD37) se purificaron de los supernadantes de cultivo CHO mediante cromatografía de afinidad de proteína A Las alícuotas purificadas de 10-25 mg se sometieron a HPLC sobre un Tosoh Biosep, Inc TSK 3000 SWXL HPLC columna, tamaño de poro 5 mm El índice de flujo era de 1 ml/min, en PBS, pH 7 2 amortiguador de carrera Los índices de migración de los estándares de peso molecular se indican adelante del trazado La construcción GS-1 VL11S scFv (SSS) H WCH2WCH3 se indica en azul, mientras la construcción GS-1 VL11S scFv (SSC) H WCH2WCH3 se indica en rojo La construcción GS-1 VL11S scFv (SSS) H WCH2WCH3 genera un pico uniforme de aproximadamente 75-100 kDa, mientras la construcción GS-1 VL11S scFv (SSC) H WCH2WCH3 genera una forma más pequeña y otras formas heterogéneas, que incluyen una forma de alto peso molecular

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mayor de 200 KDa La Figura 79 ilustra la unión de las construcciones G28-1 (anti-CD37) a líneas de célula de linfoma de célula B Las diluciones de serie de GS-1 VL11S scFv (SSS) H WCH2WCH3 o GS-1 VL11S scFv (SSC) H WCH2WCH3 se incubaron con 10^6 células de cada tipo de célula durante 60 minutos en hielo en FBS PBS/2% Las muestras se lavaron dos veces, e incubaron con una mezcla de IgG anti-humano de cabra FITC e IgG F(ab')2 (CalTag) anti-humano de cabra FITC a 1 100 cada uno, en hielo durante 45 minutos Las muestras se lavaron y analizaron mediante citometría de flujo que utiliza un FACsCalibur (Becton-Dickinson) Los resultados de este experimento muestran que ambas construcciones GS-1 VL11S scFv (SSS) H WCH2WCH3 y GS-1 VL11S scFv (SSC) H WCH2WCH3 se unen a BJAB y células Ramos, y moderadamente a células WIL-2 Las moléculas de construcciones G28-1 (anti-CD37) (SSS)H G scFv o G28-1 (SSC)H G scFv unen las células Raji y Namalwa en niveles inferiores en estos experimentos La unión a todas las líneas fue significativa en razón al antecedente de fluorescencia que se ha sustraído de los datos mostrados en la Figura 79 La Figura 80 ilustra la unión de annexin y yoduro de v-propidio a células Ramous después de incubación durante la noche con formas GS-1 VL11S scFv (SSS) H WCH2WCH3 o GS-1 VL11S scFv (SSC) H WCH2WCH3 de scFvs El teñido de AnnexinV-PI de células Ramos se incubaron durante 24 horas con G28-1 (anti-CD37) scFvs Las células B Ramos se incubaron en 10^6 células/ml en 12 platos de pozo durante 24 horas con

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G28-1 (anti-CD37) scFvs en 10 mg/ml, en un volumen total de 2 ml Las células se tiñeron con annexinV y yoduro de propidio con un kit obtenido de Immunotech, de acuerdo con las instrucciones del fabricante Las muestras se analizaron mediante citometría de flujo de dos colores que utiliza un citómetro de flujo FACsCalibur (Becton-Dickinson) El % de células total en cada cuadrante se indica junto a cada punto de gráfica La Figura 83 muestra la unión del AnnexinV-yoduro de propidio a células después de incubación durante la noche con GS-1 VL11S scFv (SSS) H WCH2WCH3 o GS-1 VL11S scFv (SSC) H WCH2WCH3 Los resultados indicaron que ambas construcciones GS-1 VL11S scFv (SSS) H WCH2WCH3 y GS-1 VL11S scFv (SSC) H WCH2WCH3 fueron capaces de inducir apoptosis, pero que la forma SSC induce más apoptosis que la forma SSS La Figura 81 ilustra la inhibición de la proliferación de crecimiento de células Ramos en la presencia de construcciones G28-1 (anti-CD37) Las células B Ramos se incubaron con diluciones seriales de construcciones G28-1 (anti-CD37) purificada que contiene el pivote IgG1 identificado como (SSS)H o (SSC)H Los cultivos se incubaron en platos de cultivo de tejido de fondo plano de 96 pozos (Costar) a 37°C, 5% CO_2 durante 36 horas antes de pulsar con ^{3}H-timidina durante al menos 12 horas de una incubación de 48 horas (0 75 mCi/pozo) Las células se cosecharon en placas de 96 pozos GFC utilizando un cosechador Packard, se secaron, y se agregó 25 ml de fluido de centelleo Microscint a cada pozo antes de continuar con el

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conteo de centelleo (Packard) de microplaca TopCount NXT Los datos se grafican como concentración de proteína versus cpm incorporado Cada construcción muestra incremento de inhibición de proliferación con incremento de concentración de proteína La Figura 82 ilustra la inducción de apoptosis en las células B Ramos cultivadas en la presencia de construcciones 2H7 (anti-CD20) y G28-1 (anti-CD37) Las células B Ramos se incubaron con construcciones objetivo CD20 y/o CD37 (10 mg/ml) en solución durante 20 horas Las células fueron luego cosechadas, lavadas, e incubadas en annexinV y yoduro de propidio que utiliza un kit de teñido de Immunotech antes de citometría de flujo de dos colores que utiliza un citómetro de flujo FACsCalibur (Becton-Dickinson) La gráfica muestra el porcentaje de células positivas de annexin V identificadas por su teñido en el cuadrante derecho de la gráfica de puntos Los resultados de este experimento indican que ambas construcciones G28-1 eran más eficientes que la construcción 2H7 y que la construcción G28-1 VL11S scFv (SSS) H WCH2 WCH3 fue más eficiente que la construcción G28-1 VL11S scFv (SSC) H WCH2 WCH3 Sin embargo, la cantidad de apoptosis de células Ramos fue mayor cuando la construcción 2H7 y la G28-1 se utilizaron en combinación La Figura 83 ilustra la matanza de células Ramos mediante citotoxicidad dependiente de complemento (CDC) inducida por construcciones 2H7 (anti-CD20) y G28-1 (anti-CD37) con mutaciones de región de pivote Las 2H7 scFv (CSS-S)H WCH2 WCH3 (anti-CD20), G28-1 scFv

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(SSS)H G, G28-1 (SCS)H (anti-CD37-scFv), G28-1 (CSS)H (anti-CD37-scFv), o G28-1 (SSC)H (anti-CD37-scFv) se incubaron en 10 mg/ml con 10^4 Células Objetivo Ramos y una dilución 1 10 de complemento de conejo (PelFreez) en un volumen de 150 ml durante 90 minutos Las alícuotas se tiñeron con trypan azul (Invitrogen), y contadas utilizando un hemacitómetro para determinar el porcentaje de la población de células muertas durante tratamiento Los controles negativos con células y solo un reactivo también se incluyen Ambas G28-1 (SSS) (anti-CD37 scFv) y G28-1 (SSC) (anti-CD37 scFv) rápidamente mataron células Ramos en la presencia de complemento de conejo en este experimento La actividad del G28-1 (SSC) (anti-CD37 scFv) fue mayor que aquella de G28-1 (SSS) (anti-CD37 scFv), y fue cercanamente tan potente en este ensayo como la molécula CD20-dirigida 2H7 scFv (CSS-S)H WCH2 WCH3 scFv La Figura 84 ilustra la inducción de ADCC de células Ramos incubadas con G28-1 (anti-CD37) scFvs Las construcciones G28-1 (anti-CD37) en 10 mg/ml se incubaron en placas de 96 pozos de fondo plano con 10^4 células Ramos ^{51}Cr-etiquetado y PBMC humano en reposo en diferentets proporciones efector objetivo que varía de 0 a 100 Todas las incubaciones se desarrollaron en triplicado en cada proporción efector objetivo La matanza natural se midió en cada proporción efector objetivo mediante la omisión de las construcciones La liberación espontánea se midió sin la adición de PBMC o proteína de fusión, y la liberación máxima

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se midió mediante la adición de detergente (1% NP-40) a los pozos apropiados Las reacciones se incubaron durante 6 horas, y 100 ml del supernadante de cultivo cosechado a un Lumaplate (Packard Instruments) y se le permitió secarse durante la noche antes de conteo cpm liberado en un Contador de Centelleo de Microplaca Packard Top Count NXT Los resultados de estos experimentos indican que las construcciones G28-1 (anti-CD37) se unen a células objetivo, que ellas inducen apoptosis en las líneas de células B, y que ellas median funciones efectoras que incluyen CDC y ADCC

Secuencias o construcciones representativas no limitantes adicionales dentro, o utiles dentro, la presente invención es como sigue

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Articulación tipo silvestre HuIgGl, CH2, CH3 (secuencia de nucleótido) (ID DE SEC NO __)

tctgatcaggagcccaaatcttgtgacaaaactcacacatgcccaccgtgcccagcacctgaactcctgggggggaccgtcagtctt
cctcttccccccaaaacccaaggacaccctcatgatctcccggaccccctgaggtcacatgcgtggtggtggacgtgagccacgaa
gaccctgaggtcaagttcaactggtacgtggacggcgtggaggtgcataatgccaagacaaagccgcgggaggagcagtacaa
cagcacgtaccgtgtggtcagcgtcctcaccgtcctgcaccaggactggctgaatggcaaggagtacaagtgcaaggtctccaac
aaagccctcccagcccccatcgagaaaacaatctccaaagccaaagggcagccccgagaaccacaggtgtacaccctgcccccc
atcccgggatgagctgaccaagaaccaggtcagcctgacctgcctggtcaaaggcttctatcccagcgacatcgccgtggagtgg
gagagcaatgggcagccggagaacaactacaagaccacgcctcccgtgctggactccgacggctccttcttcctctacagcaag
ctcaccgtggacaagagcaggtggcagcaggggaacgtcttctcatgctccgtgatgcatgaggctctgcacaaccactacacgc
agaagagcctctccctgtctccgggtaaatgatctaga

Articulación tipo silvestre HuIsGl, CH2, CH3 (secuencia de aminoácido) (ID DE SEC NO __)

SDQEPKSCDKTtnrPPCPAPELLGGPSVFLFPPKPKDTLMlSRTPEVTCVVVDVSHE
DPEVKJFNWWDGVEVHNAKTKREEQYNSTYRVVSVLTVLHQDV/LNGKEYKCK
VSNEALPAPIEKTISKAKG (2PREPQVYTLPPSRDELTKN QVSLTCLVKGfiYPSDISV
EWESNGQPENNYKTTPPVI, DSDGSFFI, YSKLTVDKSRWQQGNVFSCSVMHEALH
NHYTQKSLSLSPGK

Articulación IgG1, CH2, CH3 de Llama (secuencia de nucleótido) (ID DE SEC NO __)

tgatcaagaaccacatggaggatgcacgtgcccncagtgcccncaatgcccngcnccngaactnccaggaggcccttctgtcttt
gtcttcccccgaaacccaaggacgtcctctccatttttggaggccgagtcacgtgcgttgtagtggacgtcggaaagaaagaccc
cgaggtcaatttcaactggtatattgatggcgttgaggtgcgaacggccaatacgaagccaaaagaggaacagttcaacagcacg
taccgcgtggtcagcgtcctgcccatccagcaccaggactggctgacggggaaggaattcaagtgcaaggtcaacaacaaagct
ctcccggcccccatcgagaggaccatctccaaggccaaagggcagacccgggagccgcaggtgtacaccctggccccacacc
gggaagaactggccaaggacaccgtgagcgtaacatgcctggtcaaaggcttctacccagctgacatcaacgttgagtggcaga
ggaacggtcagccggagtcagagggcacctacgccaacacgccgccacagctggacaacgacgggacctacttcctctacagc
aagctctcggtgggaaagaacacgtggcagcggggagaaaccttaacctgtgtggtgatgcatgaggccctgcacaaccactac
acccagaaatccatcacccagtcttcgggtaaatagtaatctaga

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EPHGGCTCPQCPAPELPGGPSVFVFPPKPKDVLSISGRPEVTCVVVDVGKEDPEVN
FNWYIDGVEVRTANTKPKEEQFNSTYRVVSVLPIQHQDWLTGKEFKCKVNNKAL
PAPIERTISKAKGQTREPQVYTLAPHREELAKDTVSVTCLVKGFYPADINVEWQRN
GQPESEGTYANTPPQLDNDGTYFLYSRLSVGKNTWQRGETLTGVVMHEALHNHY
TQKSITQSSGK

IgG2 de Llama (secuencia de nucleótido) (ID DE SEC NO __)

tgatcaagaacccaagacaccaaaaccacaaccaccaaccacaaccacaacccaatcctacaacagaatccaagtgtcccaaatgt
ccagccccctgagctcctgggagggccctcagtcttcatcttcccccccgaaacccaaggacgtccttctccatttctgggaggcccga
ggtcacgtgcgttgtggtagacgtgggccaggaagaccccgaggtcagttcaactggtacattgatggcgtgaggtgcgaacg
gccaacacgaggccaaaagaggaacagttcaacagcacgtaccgcgtggtcagcgtcctgcccatccagcaccaggactggct
gacgggaaggaattcaagtgcaaggtcaacaacaaagctctcccggcccccatcgagaagaccatctccaaggccaaagggc
agaccccgggagccgcaggtgtacaccctgggcccacacccgggaagagctggccaaggacacccgtgagcgtaacatgcctggt
caaaggcttctacccacctgatatcaacgtgagtggcagaggaatgggcagtccggagtcagagggcacytacgccaccagcc
caccccagctggacaacgacgggaccttacttcctctacagcaagctctcggtgggcaaagaacacgtggcagcagggagaaacc
ttcacctgtgtggtgatgcaccgaggccctgcacaaccactacacccagaaatccatcacccagtcttcgggtaaatagtaatctaga.

IgG2 de Llama (secuencia de aminoácido) (ID DE SEC NO __)

DQEPKTPKPQPQPQPQPNPTTESKCPKCPAPELLGGPSVFIFPPKPKDVLSISGRPEV
TCVVVDVGQEDPEVSFNWYIDGAEVRTANTRPKEEQFNSTYRVVSVLPIQHQDWL
TGKEFKCKVNNKALPAPIEKTISKAKGQTREPQVYTLAPHREELAKDTVSVTCLVK
GFYPPDINVEWQRNGQPESEGTYATTPPQLDNDGTYFLYSKLSVGKNTWQQGETF
TCVVMHEALHNHYTQKSITQSSGK

IgG3 Fc de Llama (secuencia de nucleótido) _(ID DE SEC NO __)

tgatcaagcgcaccacagcgaagaccccagctccaagtgtcccaaatgcccaggccctgaactcctggagggcccacggtctt
catcttcccccccgaaagccaaggacgtcctctccatcacccgaaaacctgaggtcacgtgcttgtggtggacgtgggtaaagaag
accctgagatcgagttcaagctggtccgtggatgacacagaggtacacacggctgagacaaagccaaaggaggaacagttcaac

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agcacgtaccgcgtggtcagcgtcctgcccatccagcaccaggactggctgacggggaaggaattcaagtgcaaggtcaacaa
caaagctctcccagcccccatcgagaggaccatctccaaggccaaagggcagaccccgggagccgcaggtgtacaccctgcc
ccacacccgggaagagctggccaaggcacaccgtgagcgtaacctgcctggtcaaaggcttcttcccagctgacatcaacgttgagt
ggcagaggaatgggcagccggagtcagagggcacctacgccaacacgccgccacagctggacaacgacgggacctacttcct
ctacagcaaactctccgtggacaagaacacgtggcagcaggggaacgtcttcacctgtgtggtgatgcacgaggctctacacaat
cactccacccagaaatccatcacccagtcttcgggtaaatagtaatctagagggccc

IgG3 Fc de Llama (secuencia de aminoácido) (ID DE SEC NO __)

DQAHHSEDPSSKCPKCPGPELLGGPTVFIFPPKAKDVLSITRKPEVTCLWWTWVKK
TLRSSSSWSVDDTEVHTAETKPKEEQFNSTYRVVSVLPIQHQDWLTGKEFKCKVN
NKALPAPIERTISKAKGQTREPQVYTLAPHREELAKDTVSVTCLVKGFFPADINVE
WQRNGQPESEGTYANTPPQLDNDGTYFLYSKLSVGKNTWQQGEVFTCVVMHEA
LHNHSTQKSITQSSGK

Articulación tipo silvestre HuIgG1 (secuencia de nucleótido) (ID DE SEC NO __)

gatcaggagcccaaatcttgtgacaaaactcacacatgcccaccgtgcccagca

Articulación tipo silvestre HuIgG1 (secuencia de aminoácido) (ID DE SEC NO __)

DQEPKSCDKTHTCPPCPA

HuIgG1 H2, articulación tipo silvestre con leu en la segunda posición (secuencia de nucleótido) (ID DE SEC NO __)

gatctggagcccaaatcttgtgacaaaactcacacatgcccaccgtgcccagca

HuIgG1 H2, articulación tipo silvestre con leu en la segunda posición (secuencia de aminoácido) (ID DE SEC NO __)

DLEPKSCDKTHTCPPCPA

NTHuIgG1 CH2 tipo silvestre (secuencia de nucleótido) (ID DE SEC NO __)

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cctgaactcctgggggggaccgtcagtcttcctcttccccccaaaacccaaggacaccctcatgatctcccggacccctgaggtcac
atgcgtggtggtggacgtgagccacgaagaccctgaggtcaagttcaactggtacgtggacggcgtggaggtgcataatgccaa
gacaaagccgcgcgggaggagcagtacaacagcacgtaccgtgtggtcagcgtcctcaccgtcctgcaccaggactggctgaatg
gcaaggagtacaagtgcaaggtctccaacaaagccctcccagcccccatcgagaaaaccatctccaaagccaaa

HuIgGl CH2 tipo silvestre (secuencia de aminoácido) (ID DE SEC NO __)

PELLGGPSVFLFPPKPKDTLMISRTPEVTCWVDVSHEDPEVKFNWYVDGVEVHNA
KTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAK

HuIgGl CH3 tipo silvestre (secuencia de nucleótido) (ID DE SEC NO __)

gggcagccccgagaaccacaggtgtacaccctgcccccatcccgggaggagatgaccaagaaccaggtcagcctgacctgcct
ggtcaaaggcttctatcccagcgacatcgccgtggagtgggagagcaatgggcagccggagaacaactacaagaccacgcctc
ccgtgctggactccgacggctccttcttcctctatagcaagctcaccgtggacaagagcaggtggcagcaggggaacgtcttctca
tgctccgtgatgcatgaggctctgcacaaccactacacgcagaagagcctctccctgtccccgggtaaatga

HuIgGl CH3 tipo silvestre (secuencia de aminoácido) (ID DE SEC NO __)

GQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPP
VLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

Articulación mutada HuIgGl (C-C-COS-S-S) (secuencia de nucleótido) (ID DE SEC NO __)

gatcaggagcccaaatcttctgacaaaactcacacatccccaccgtccccagca

Articulación mutada HuIgG1 (C-C-C→S-S-S) (secuencia de aminoácido) (ID DE SEC NO __)

DQEPKSSDKTHTSPPSPA

HIgG1MTH WTCH2CH3 (articulación mutante con CH2 y CH3 tipo silvestre (lectura de la articulación+cola Ig) (secuencia de nucleótido) (ID DE SEC NO __)

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tgatcaccccaaatcttctgacaaaactcacacatctccacogtcctcagcacctgaactcctgggtggacogtcagtcttcctcttcc
ccccaaaacccaaggacaccctcatgatctcccggaccoctgaggtcacatgcgtggtggtggacgtgagccacgaagaccctg
aggtcaagttcaactggtacgtggacggcgtggaggtgcataatgccaagacaaagccgcgggaggagcagtacaacagcacg
taccgtgtggtcagcgtcctcaccgtcctgcaccaggactggctgaatggcaaggagtacaagtgcaaggtctccaacaaagcoc
tcccagcccccatcgagaaaacaatctccaaagccaaagggcagccccgagaaccacaggtgtacaccctgcccccatcoogg
gatgagctgaccaagaaccaggtcagcctgacctgcctggtcaaaggcttctatcccagcgacatcgccgtggagtgggagagc
aatgggcagccggagaacaactacaagaccacgcctcccgtgctggactccgacggctccttcttcctctacagcaagctcaccg
tggacaagagcaggtggcagcaggggaacgtcttctcatgctccgtgatgcatgaggctctgcacaaccactacacgcagaaga
gcctctccctgtctccgggtaaatgataatctaga

Articulación mutante, pero CH2 y CH3 tipo silvestre (secuencia de aminoácido) (ID DE SEC NO __)

DHPKSSDKTHTSPPSSAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDP
EVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVS
NKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEW
ESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNH
YTQKSLSLSPGK

IgG1 de llama 2H7 scFv (secuencia de nucleótido) (ID DE SEC NO __)

aagcttgccgccatggatttttcaagtgcagattttcagcttcctgctaatcagtgcttcagtcataattgccagaggacaaattgttctct
cccagtctccagcaatcctgtctgcatctccaggggagaaggtcacaatgacttgcagggccagctcaagtgtaagttacatgcact
ggtaccagcagaagccaggatcctcccccaaaccctggatttatgccccatccaacctggcttctggagtccctgctcgcttcagtg
gcagtgggtctgggacctcttactctctcacaatcagcagagtggaggctgaagatgctgccactattactgccagcagtggagttt
taacccacccacgttcggtgctgggaccaagctggagctgaaagatggcggtggctcgggcggtggtgggtctggaggaggtg
ggagctctcaggcttatctacagcagtctggggctgagctggtgaggcctgggcctcagtgaagatgtcctgcaaggcttctggc
tacacatttaccagttacaatatgcactgggtaaagcagacacctagacagggcctggaatggattggagctatttatcccaggaaat
ggtgatacttcctacaatcagaagttcaagggcaaggccacactgactgtagacaaatcctccagcacagcctacatgcagctcag
cagcctgacatctgaagactctgcggtctatttctgtgcaagatggtgtactatagtaactcttactggtacttcgatgtctggggcac
agggaccacggtcaccgtctcttctgatcaagaaccacatggaggatgcacgtgcccncagtgcccncaatgoccngcnocng
aactnccaggaggcccttctgtcttttgtcttcccccccgaaacccaaggacgtcotctccatttttggaggccgagtcacgtgcgtgt
agtggacgtcggtaagaaagaccccgaggtcaatttcaactggtatatgatggcgtgaggtgcgaacggccaatacgaagcca
aaagaggaacagttcaacagcacgtacogcgtggtcagcgtcctgccatccagcaccaggactggctgacggggaaggaatt

1268
1279

caagtgcaaggtcaacaacaaagctctcccggcccccatcgagaggaccatctccaaggccaaagggcagaccccgggagccg
caggtgtacaccctgccccacacccgggaagaactggccaaggacaccgtgagcgtaacatgcctggtcaaaggcttctacccca
gctgacatcaacgtggagtggcagaggaacggtcagccggagtcagagggcacctacgccaacacgccgccacagctggaca
acgacgggacctacttcctctacagcaagctctcggtgggaaagaacacgtggcagcggggagaaacctaaccttgtggtgat
gcatgaggccctgcacaaccactacacccagaaatccatcacccagtcttcgggtaaatagtaatctaga

IgG1 de llama 2H7 scFv (secuencia de aminoácido) (ID DE SEC NO __)

MDFQVQIFSFLLISASVIIARGQIVLSQSPAILSASPGEKVTMTCRASSSVSYMHWY
QQKPGSSPKPWIYAPSNLASGVPARFSGSGSGTSYSLTISRVEAEDAATYYCQQWS
FNPPTFGAGTKLELKDGGGSGGGGSGGGGSSQAYLQQSGAELVRPGASVKMSCK
ASGYTFTSYNMHWVKQTPRQGLEWIGAIYPGNGDTSYNQKFKGKATLTVDKSSS
TAYMQLSSLTSEDSAVYFCARVVYYSNSYWYFDVWGTGTTVTVSSDQEPHGGCT
CPQCPAPELPGGPSVFVFPPKPKDVLSIFGGRVTCVVVDVGKKDPEVNFNWYIDGV
EVRTANTKPKEEQFNSTYRVVSVLPIQHQDWLTGKEFKCKVNNKALPAPIERTISK
AKGQTREPQVYTLAPHREELAKDTVSVTCLVKGFYPADINVEWQRNGQPESEGTY
ANTPPQLDNDGTYFLYSKLSVGKNTWQRGETLTCVVMHEALHNHYTQKSITQSS
GK

IgG2 de llama 2H7 scFv (secuencia de nucleótido) (ID DE SEC NO __)

aagcttgccgccatggatttttcaagtgcagatttcagcttcctgctaatcagtgcttcagtcataattgccagaggacaaattgttctct
cccagtctccagcaatcctgtctgcatctccaggggagaaggtcacaatgacttgcagggccagctcaagtgtaagttacatgcact
ggtaccagcagaagccaggatcctcccccaaaccctggatttatgccccatccaacctggcttctggagtccctgctcgcttcagtg
gcagtgggtctgggacctcttactctctcacaatcagcagagtggaggctgaagatgctgccacttattactgccagcagtggagttt
taacccacccacgttcggtgctgggaccaagctggagctgaaagtggcggtggctcgggcggtggtggatctggaggaggtg
ggagctctcaggcttatctacagcagtctggggctgagctggtgaggcctggggcctcagtgaagatgtcctgcaaggcttctggc
tacacatttaccagttacaatatgcactgggtaaagcagacacctagacagggcctggaatggattggagctatttatccaggaaat
ggtgatacttcctacaatcagaagttcaagggcaaggccacactgactgtagacaaatcctccagcacagcctacatgcagctcag
cagcctgacatctgaagactctgcggtctattttgtgcaagagtggtgtactatagtaactcttactggtacttcgatgtctggggcac
agggaccacggtcaccgtctcttctgatcaagaacccaagcaccaaaaccacaaccacaaccacaaccacaacccaatcctac
aacagaatccaagtgtcccaaatgtccagcccctgagcctcctgggagggccctcagtcttcatcttcccccccgaaacccaaggac
gtccttctccatttctgggaggccccgaggtcacgtgcgtggtggtagacgtgagccaggaagaccccgaggtcagttcaactggta

1269
1280

caltgatggcgctgaggtgcgaacggccaacacgaggccaaaagaggaacagttcaacagcacgtaccgtgtggtcagcgtcc
tgcccatccagcaccaggactggctgacggggaaggaattcaagtgcaaggtcaacaacaaagctctcccggcccccatcgag
aagaccatctccaaggccaaagggcagaccccgggagccgcaggtgtacaccctggcccccacaccgggaagagctggccaag
gacaccgtgagcgtaacatgcctggtcaaaggcttctacccaccgatatcaacgttgagtggcagaggaatgggcagccggagt
cagagggcacytacgccaccacgccacccccagctggacaacgacgggaccttcttcctctacagcaagctctcggtgggaaag
aacacgtggcagcagggagaaaccttcacctgtgtggtgatgcacgaggcctgcacaaccactacacccagaaatccatcacc
cagtctcgggtaaatagtaatctaga

IgG2 de llama AA2H7 scFv (secuencia de aminoácido) (ID DE SEC NO __)

MDFQVQIFSFLLISASVIIARGQIVLSQSPAILSASPGEKVTMTCRASSSVSYMHWY
QQKPGSSPKPWIYAPSNLASGVPARFSGSGSGTSYSLTISRVEAEDAATYYCQQWS
FNPPTFGAGTKLELKDGGGSGGGGSGGGGSSQAYLQQSGAELVRPGASVKMSCK
ASGYTFTSYNMHWVKQTPRQGLEWIGAIYPGNGDTSYNQKFKGKATLTVDKSSS
TAYMQLSSLTSEDSAVYFCARVVYYSNSYWYFDVWGTGTTVTVSSDQEPKTPKP
QPQPQPQPNPTTESKCPKCPAPELLGGPSVFIFPPKPKDVLSISGRPEVTCVVVDVG
QEDPEVSFNWYIDGAEVRTANTRPKEEQFNSTYRVVSVLPIQHQDWLTGKEFKCK
VNNKALPAPIEKTISKAKGQTREPQVYTLAPHREELAKDTVSVTCLVKGFYPPDIN
VEWQRNGQPESEGTYATTPPQLDNDGTYFLYSKLSVGKNTWQQGETFTCVVMHE
ALHNHYTQKSITQSSGK

IgG3 de llama NT2H7 scFv (secuencia de nucleótido) (ID DE SEC NO __)

aagcttgccgccatggatttcaagtgcagatttcagcttcctgctaatcagtgcttcagtcataattgccagaggacaaattgttctct
cccagtctccagcaatcctgtctgcatctccaggggagaaggtcacaatgacttgcagggccagctcaagtgtaagttacatgcact
ggtaccagcagaagccaggatcctccccaaaccctggatttatgcccatccaacctggcttctggagtccctgctcgcttcagtg
gcagtgggtctgggacctcttactctctcacaatcagcagagtggaggctgaagatgctgccactiattactgccagcagtggagttt
tacccacccacgttcggtgctgggaccaagctggagctgaaagatggcggtggctcgggcggtggtggatctggaggaggtg
ggagtctcaggcttatctacagcagtctgggggctgagctggtgaggcctggggcctcagtgaagatgtcctgcaaggcttctggc
tacacatttaccagttacaatatgcactgggtaaagcagacacctagacagggcctggaatggattggagctatttatcccggaaat
ggtgatacttcctacaatcagaagttcaagggcaaggccacactgactgtagacaaatcctccagcacagcctacatgcagctcag
cagcctgacatctgaagactctgcggtctatttctgtgcaagagtggtgtactatagtaactcttactggtacttcgatgtctgggggcac
agggaccacggtcaccgtctcttctgatcaagcgaaccacagcgaagacccccagctccaagtgtcccaaatgcccaggccctga
actccttggagggcccacggtcttcatcttccccccgaaagccaaggacgtcctctccatcacccgaaaacctgaggtcacgtgctt
gtggtggacgtgagtaagaagaccctgagatcgagttcaagctggtccgtggatgacacagaggtacacacggctgagacaaa

1270
1281

gccaaaggaggaacagttcaacagcacgtaccgcgtggtcagcgtcctgcccatccagcaccaggactggctgacggggaag
gaattcaagtgcaaggtcaacaacaaagctctcccagcccccatcgagaggaccatctccaaggccaaagggcagaccccgga
gccgcaggtgtacaccctgcccccatcccgggaagagctggccaaggacaccgtgagcgtaacctgcctggtcaaaggcttctt
cccagctgacatcaacgtggagtggcagaggaatgggcagccggagtcagagggcacctacgccaacacgccgccacagctg
gacaacgacgggacctacttcctctacagcaaactctccgtggggaaagaacacgtggcagcagggagaagtcttcacctgtgtgg
tgatgcacgaggctctacacaatcactcccacccagaaatccatcacccagtcttcgggtaaatagtaatctagagggccc

IgG3 de llama 2H7 scFv (secuencia de aminoácido) (ID DE SEC NO __)

MDFQVQIFSFLLISASVIIARGQIVLSQSPAILSASPGEKVTMTCRASSSVSYMHWY
QQKPGSSPKPWIYAPSNLASGVPARFSGSGSGTSYSLTISRVEAEDAATYYCQQWS
FNPPTFGAGTKLELKDGGGSGGGGSGGGGSSQAYLQQSGAELVRPGASVKMSCK
ASGYTFTSYNMHWVKQTPRQGLEWIGAIYPGNGDTSYNQKFKGKATLTVDKSSS
TAYMQLSSLTSEDSAVYFCARVVYYSNSYWYFDVWGTGTTVTVSSDQAHSHSED
PSSKCPKCPGPELLGGPTVFIFPPKAKDVLSITRKPEVTCLWWTWVKKTLRSSSSW
SVDDTEVHTAETKPKEEQFNSTYRVVSVLPIQHQDWLTGKEFKCKVNNKALPAPI
ERTISKAKGQTREPQVYTLAPHREELAKDTVSVTCLVKGFFPADINVEWQRNGQP
ESEGTYANTPPQLDNDGTYFLYSKLSVGKNTWQQGEVFTCVVMHEALHNHSTQK
SITQSSGK

<u>2H7 scFv WTH WTCH2CH3</u> (secuencia de nucleótido)

aagcttgccgccatggatttttcaagtgcagattttcagcttcctgctaatcagtgtcttcagtcataattgtccagaggacaaattgttctct
cccagtctccagcaatcctgtctgcatctccaggggagaaggtcacaatgacttgcagggccagctcaagtgtaagttacatgcact
ggtaccagcagaagccaggatcctcccccaaaccctggatttatgccccatccaacctggcttctggagtccctgctcgcttcagtg
gcagtgggtctgggacctcttactctctcacaatcagcagagtggaggctgaagatgctgccactattactgccagcagtggagtt
taacccaccacgttcggtgctgggaccaagctggagctgaaagtggcggtggctcgggcggtggtggatctggaggaggtg
ggagctctcaggcttatctacagcagtctggggctgagctggtgaggcctggggcctcagtgaagatgtcctgcaaggcttctggc
tacacatttaccagttacaatatgcactgggtaaagcagacacctagacagggcctggaatggattggagctatttatccaggaaat
ggtgatacttcctacaatcagaagttcaagggcaaggccacactgactgtagacaaatcctccagcacagcctacatgcagctcag
cagcctgacatctgaagactctgcggtctatttctgtgcaagagtggtgtactatagtaactcttactggtacttcgatgtctggggcac
agggaccacggtcaccgtctcttctgatcaggagccaaatcttgtgacaaaactcacacatgcccaccgtgcccagcacctgaac
tcctgaggggaccgtcagtcttcctcttccccccaaaacccaaggacaccctcatgatctcccggacccctgaggtcacatgcgtg

1271
1282

gtggtggacgtgagccacgaagaccctgaggtcaagttcaactggtacgtggacggcgtggaggtgcataatgccaagacaaag
ccgcgggaggagcagtacaacagcacgtaccgtgtggtcagcgtcctcaccgtcctgcaccaggactggctgaatggcaagga
gtacaagtgcaaggtctccaacaaagccctcccagcccccatcgagaaaacaatctccaaagccaaagggcagccccgagaac
cacaggtgtacaccctgcccccatcccgggatgagctgaccaagaaccaggtcagcctgacctgcctggtcaaaggcttctatcc
cagcgacatcgccgtggagtgggagagcaatgggcagccggagaacaactacaagaccacgcctcccgtgctggactccgac
ggctccttcttcctctacagcaagctcaccgtggacaagagcaggtggcagcaggggaacgtcttctcatgctccgtgatgcatga
ggctctgcacaaccactacacgcagaagagcctctccctgtctccgggtaaatgatctaga

2H7 scFv WTH WTCH2CH3 (secuencia de aminoácido) (ID DE SEC NO __)

MDFQVQIFSFLLISASVIIARGQIVLSQSPAILSASPGEKVTMTCRASSSVSYMHWY
QQKPGSSPKPWIYAPSNLASGVPARFSGSGSGTSYSLTISRVEAEDAATYYCQQWS
FNPPTFGAGTKLELKDGGGSGGGGSGGGGSSQAYLQQSGAELVRPGASVKMSCK
ASGYTFTSYNMHWVKQTPRQGLEWIGAIYPGNGDTSYNQKFKGKATLTVDKSSS
TAYMQLSSLTSEDSAVYFCARVVYYSNSYWYFDVWGTGTTVTVSSDQEPKSCDK
THTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWY
VDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPI
EKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPE
NNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSL
SPGK

Cola citoplasmática y dommio de transmembrana NTCD80 (+sitios de restricción) (secuencia de nucleótido) (ID DE SEC NO __)

gcggatccttcgaacctgctcccatcctgggccattaccttaatctcagtaaatggaatttttgtgatatgctgcctgacctactgctttg
ccccaagatgcagagagagaaggaggaatgagagattgagaagggaaagtgtacgccctgtataaatcgat

Cola citoplasmática y dominio de transmembrana CD80 (secuencia de aminoácido) (ID DE SEC NO __)

ADPSNLLPSWAITLISVNGIFVICCLTYCFAPRCRERRRNERLRRESVRPV

40 2 220 VL (CD40 scFv #1--VL anti-humano) (secuencia de nucleótido) (ID DE SEC NO __)

1272
1283

aagcttatggattttcaagtgcagattttcagcttcctgctaatcagtgttcagtcataatgtccagaggagtcgacattgttctgactc
agtctccagccaccctgtctgtgactccaggagatagagtctctctttcctgcagggccagccagagtattagcgactacttacactg
gtatcaacaaaaatcacatgagtctccaggcttctcatcaaatatgcttcccatttccatctctgggatcccctccaggttcagtggca
gtggatcagggtcagatttcactctcagtatcaacagtgtggaacctgaagatgttggaatttattactgtcaacatggtcacagcttc
cgtggacgttcggtggaggcaccaagctggaaatcaaacgg

40 2 220 VL (CD40 scFv #1--VL anti-humano) (secuencia de aminoácido) (ID DE SEC NO __)

MDFQVQIFSFLLISASVIMSRGVDIVLTQSPATLSVTPGDRVSLSCRASQSISDYLHW
YQQKSHESPRLLIKYASHSISGIPSRFSGSGSGSDFTLSINSVEPEDVGIYYCQHGHSF
PWTFGGGTKLEIKR

40 2 220 VH (para CD40 scFv #1--VH anti-humano) (secuencia de nucleótido) (ID DE SEC NO __)

cagatccagttggtgcaatctggacctgagctgaagaagcctggagagacagtcaggatctcctgcaaggcttctgggtatgcctt
cacaactactggaatgcagtgggtgcaagaggtgccaggaaagggttgaagtggatggctggataaacacccacactggagt
gccaaaatatgtagaagacttcaaggacggttttgccttctctttggaaacctctgccaacactgcatatttacagataagcaacctcaa
agatgaggacacggctacgtattctgtgtgagatccgggaatggtaactatgacctggcctactttgcttactggggccaagggac
actggtcactgtctctgatca

40 2 220 VH (para CD40 scFv #1--VH anti-humano) (secuencia de aminoácido) (ID DE SEC NO __)

QIQLVQSGPELKKPGETVRISCKASGYAFTTTGMQWVQEMPGKGLKWIGWINTPL
WSAKICRRLQGRFAFSLETSANTAYLQISNLKDEDTATYFCVRSGNGNYDLAYFA
YWGQGTLVTVS

40 2. 220 scFv (CD40 scFv #1 anti-humano) (secuencia de nucleótido) (ID DE SEC NO __)

aagcttatggattttcaagtgcagattttcagcttcctgctaatcagtgcttcagtcataatgtccagaggagtcgacattgttctgactc
agtctccagccaccctgtctgtgactccaggagatagagtctctctttcctgcagggccagccagagtattagcgactacttacactg
gtatcaacaaaaatcacatgagtctccaaggcttctcatcaaatatgcttcccatttccatctctgggatcccctccaggttcagtggca
gtggatcagggtcagatttcactctcagtatcaacagtgtggaacctgaagatgttggaatttattactgtcaacatggtcacagcttc

1273
1284

cgtggacgttcggtggaggcaccaagctggaaatcaaacgggtggcggtggctcgggcggaggtgggtcgggtggcggcg
gatctcagatccagttggtgcaatctggacctgagctgaagaagcctggagagacagtcaggatctcctgcaaggcttctgggtat
gccttcacaactactggatgcagtgggtgcaagaggcaggaaagggttgaagtggatggctggataaacacccacactg
gagtgccaaatatgtagaagacttcaaggacggtttgccttctctttggaaacctctgccaacactgcatatttacagataagcaacc
tcaaagatgaggacacggctacgtatttctgtgtgagatccgggaatggtaactatgacctggcctactttgcttactggggccaag
ggcactggtcactgtctctgatca

40 2 220 scFv (CD40 scFv #1 anti-humano) (secuencia de aminoácido) (ID DE SEC NO. __)

MDFQVQIFSFLLISASVIMSRGVDIVLTQSPATLSVTPGDRVSLSCRASQSISDYLHW
YQQKSHESPRLLIKYASHSISGIPSRFSGSGSGSDFTLSINSVEPEDVGIYYCQHGHSF
PWTFGGGTKLEIKRGGGGSGGGGSGGGGSQIQLVQSGPELKKPGETVRISCKASG
YAFTTTGMQWVQEMPGKGLKWIGWINTPLWSAKICRRLQGRFAFSLETSANTAY
LQISNLKDEDTATYFCVRSGNGNYDLAYFAYWGQGTLVTVS

2e12 VL (con péptido líder L6 VK) (secuencia de nucleótido) (ID DE SEC NO __)

atggatttcaagtgcagattttcagcttctgctaatcagtgtcacagtcataatgtccagaggagtcgacattgtgctcacccaatctc
cagcttctttggctgtgtctctagggcagagggccaccatctcctgcagagccagtgaaagttgttgaatattatgtcacaagtttaatgc
agtggtaccaacagaaaccaggacagccacccaaactcctcatctctgctgcatccaacgtagaatctggggtcctgccaggtt
agtggcagtgggtctgggacagacttcagcctcaacatccatcctgtggaggaggatgatattgcaatgtatttctgtcagcaaagta
ggaaggttccttggacgttcggtggaggcaccaagctggaaatcaaacgg

2el2 VL (con péptido líder L6 VK) (secuencia de aminoácido) (ID DE SEC NO __)

MDFQVQIFSFLLISASVIMSRGVDIVLTQSPASLAVSLGQRATISCRASESVEYYVTS
LMQWYQQKPGQPPKLLISAASNVESGVPARFSGSGSGTDFSLNIHPVEEDDIAMYF
CQQSRKVPWTFGGGTKLEIKR

2el2 VH (sin péptido líder) (secuencia de nucleótido) (ID DE SEC NO __)

caggtgcagctgaaggagtcaggacctggcctggtggcgccctcacagagcctgtccatcacatgcacggtctcagggttctcatt
aaccggctatggtgtaaactgggttcgccagcctccaggaaagggtctggagtggctgggaatgatatggggtgatggaagcac
agactataattcagctctcaaatccagactgagcatcaccaaggacaactccaagagccaagtttttcttaaaaatgaacagtctgcaa

1274
1285

actgatgacacagccagatactactgtgccagagatggttatagtaacttcattactatgttatggactactggggtcaaggaacctc
agtcaccgtctcctca(gatctg)

2el2 VH (secuencia de aminoácido) (ID DE SEC NO __)

QVQLKESGPGLVAPSQSLSITCTVSGFSLTGYGVNWVRQPPGKGLEWLGMIWGDG
STDYNSALKSRLSITKDNSKSQVFLKMNSLQTDDTARYYCARDGYSNFHYYVMD
YWGQGTSVTVSS

2e12scFv (+Sitios de restricción) (secuencia de nucleótido) (ID DE SEC NO __)

aagcttatggatttcaagtgcagattttcagcttcctgctaatcagtgcttcagtcataatgtccagaggagtcgacattgtgctcaccc
aatctccagcttctttggctgtgtctctaggtcagagagccaccatctcctgcagagccagtgaaagttgatattatgtcacaagtt
tatgcagtggtaccaacagaaaccaggacagccacccaaactcctcatctctgctgcatccaacgtagaatctggggtccctgcc
aggtttagtggcagtgggtctgggacagacttcagcctcaacatccatcctgtggaggaggatgatattgcaatgtatttctgtcagc
aaagtaggaaggttccttggacgttcggtggaggcaccaagctggaaatcaaacgggatggcggtggctcaggcggaggtggg
tcgagtggcggcaggatctcaggtgcagctgaaggagtcaggacctggcctggtggcgccctcacagagcctgtccatcacatgc
accgtctcagggttcttattaccggctatggtgtaaactgggttcgccagcctccaggaaagggtctggagtggctgggaatgat
atggggtgatggaagcacagactataattcagctctcaaatccagactgagcatcaccaaggacaactccaagagccaagtttctt
aaaatgaacagtctgcaaactgatgacacagccagatactactgtgccagagatggttatagtaacttttcattactatgttatggact
actggggtcaaggaacctcagtcaccgtctcctct(gatcag)

2el2scFv (secuencia de aminoácido) (ID DE SEC NO __)

MDFQVQIFSFLLISASVIMSRGVDIVLTQSPASLAVSLGQRATISCRASESVEYYVTS
LMQWYQQKPGQPPKLLISAASNVESGVPARFSGSGSGTDFSLNIHPVEEDDIAMYF
CQQSRKVPWTFGGGTKLEIKRGGGGSGGGGSGGGGSQVQLKESGPGLVAPSQSLS
ITCTVSGFSLTGYGVNWVRQPPGKGLEWLGMIWGDGSTDYNSALKSRLSITKDNS
KSQVFLKMNSLQTDDTARYYCARDGYSNFHYYVMDYWGQGTSVTVSS

10A8 es anti-CD152 (CTLA-4)

10A8 VL (con L6 VK péptido líder) (secuencia de nucleótido) (ID DE SEC NO __)

1275
1286

atggattttcaagtgcagattttcagcttcctgctaatcagtgcttcagtcataatgtccagaggagtcgacatccagatgacacagtct
ccatcctcactgtctgcatctctgggaggcaaagtcaccatcacttgcaaggcaagccaagacattaagaagtatataggttggtac
caacacaagcctggaaaaggtcccaggctgctcatatattacacatctacattacagccaggcatcccatcaggttcagtggaagt
gggtctgggagagattatccctcagcatcagaacctggagcctgaagatattgcaacttattattgtcaacagtatgataatcttcc
attgacgttcggctcggggacaaagttggaaataaaacgg

10A8 VL (secuencia de aminoácido) (ID DE SEC NO __)

MDFQVQIFSFLLISASVIMSRGVDIQMTQSPSSLSASLGGKVTITCKASQDIKKYIG
WYQHKPGKGPRLLIYYTSTLQPGIPSRFSGSGSGRDYSLSIRNLEPEDIATYYCQQY
DNLPLTFGSGTKLEIKR

10A8 VH (sin péptido líder) (secuencia de nucleótido) (ID DE SEC NO __)

gatgtacagcttcaggagtcaggacctggcctcgtgaaacctctcagtctctgtctctcacctgctctgtcactggctactccatcac
cagtggtttctactggaactggatccgacagtttccgggaaacaaactggaatggatgggctacataagctacgacggtaggaat
aactacaacccatctctcataaatcgaatctccatcactcgtgacacatctaagaaccagttttcctgaagttgagtctgtgactactg
aggacacagctacatattttgtgcaagacactacggtagtagcggagctatggactactggggtcaaggaacctcagtcaccgtc
tcctctgatca

10A8 VH (secuencia de aminoácido) (ID DE SEC NO __)

DVQLQESGPGLVKPSQSLSLTCSVTGYSITSGFYWNWIRQFPGNKLEWMGHISHD
GRNNYNPSLKNRISITRDTSKNQFFLKLSSVTTEDTATYFCARHYGSSGAMDYWGQ
GTSVTVSS

10A8 SCFV (secuencia de nucleótido) (ID DE SEC NO __)

aagcttatggattttcaagtgcagattttcagcttcctgctaatcagtgcttcagtcataatgtccagaggagtcgacatccagatgaca
cagtctccatcctcactgtctgcatctctgggaggcaaagtcaccatcacttgcaaggcaagccaagacattaagaagtatataggtt
ggtaccaacacaagcctggaaaaggtcccaggctgctcatatattacacatctacattacagccaggcatcccatcaggttcagtg
gaagtgggtctgggagagattatccctcagcatcagaacctggagcctgaagatattgcaacttattattgtcaacagtatgataat
cttccattgacgttcggctcggggacaaagttggaaataaaacgggggtggcggtggctcgggcggtggtgggtcgggtggcggc
ggatctgatgtacagcttcaggagtcaggacctggcctcgtgaaacctctcagtctctgtctctcacctgctctgtcactggctactc

1276
1287

catcaccagtggtttctactggaactggatccgacagtttccgggaaacaaactggaatggatgggccacataagccacgacggta
ggaataactacaacccatctctcataaatcgaatctccatcactcgtgacacatctagaaccagtttttcctgaagttgagttctgtga
ctactgaggacacagctacatatttctgtgcaagacactacggtagtagcggagctatggactactggggtcaaggaacctcagtc
accgtctcctctgatca

10A8 SCFV (secuencia de aminoácido) (ID DE SEC NO __)

MDFQVQIFSFLLISASVIMSRGVDIQMTQSPSSLSASLGGKVTITCKASQDIKKYIG
WYQHKPGKGPRLLIYYTSTLQPGIPSRFSGSGSGRDYSLSIRNLEPEDIATYYCQQY
DNLPLTPGSGTKLEIKRGGGGSGGGGSGGGGSDVQLQESGPGLVKPSQSLSLTCSV
TGYSITSGFYWNWIRQFPGNKLEWMGHISHDGRNNYNPSLINRISITRDTSKNQFFL
KLSSVTTEDTATYFCARHYGSSGAMDYWGQGTSVTVSSD

40_2_220-hmtIgG1-hCD80 (secuencia de nucleótido) (ID DE SEC NO __)

aagcttatggattttcaagtgcagattttcagcttcctgctaatcagtgcttcagtcataatgtccagaggagtcgacattgttctgactc
agtctccagccaccctgtctgtgactccaggagatagagtctctttcctgcagggccagccagagtattagcgactacttacactg
gtatcaacaaaaatcacatgagtctccaggcttctcatccaatatgcttcccattccatctgggatcccctccaggttcagtggca
gtggatcagggtcagatttcactctcagtatcaacagtgtggaacctgaagatgttggaatttattactgtcaacatggtcacagcttc
cgtggacgttcggtggaggcaccaagctggaaatcaaacgggatggcggtggctcgggcggaggtgggtcgggtggcggcgg
atctcagatccagttggtgcaatctggacctgagctgaagaagcctggagagacagtcaggatctcctgcaaggcttctgggtat
gccttcacaactactggaatgcagtgggtgcaagaggatgccaggaaagggttgaagtggatgggctggataaacaccccactctg
gagtgccaaaatatgtagaagacttcaaggacggtttgccttctctttggaaacctctgccaacactgcatatttacagataagcaacc
tcaaagatgaggacacggctacgtatttctgtgtgagatccgggaatggtaactatgacctggcctactttgcttactggggccaag
ggacactggtcactgtctctgatctggagcccaaatcttctgacaaaactcacacatcccccaccgtcccagcacctgaactcctgg
ggggatcgtcagtcttcctcttccccccaaaacccaaggacaccctcatgatctcccggacccctgaggtcacatgcgtggtggtg
gacgtgagccacgaagaccctgaggtcaagttcaactggtacgtggacggcgtggaggtgcataatgccaagacaaagccgcg
ggaggagcagtacaacagcacgtaccgtgtggtcagcgtcctcaccgtcctgcaccaggactggctgaatggcaaggagtacaa
gtgcaaggtctccaacaaagccctcccagcccccatcgagaaaaccatctccaaagccaaagggcagccccgagaaccacagg
tgtacaccctgcccccatcccgggatgagctgaccaagaaccaggtcagcctgacctgcctggtcaaaggcttctatcccagcga
catcgccgtggagtgggagagcaatgggcagccggagaacaactacaagaccacgcctcccgtgctggactccgacggctcct
tcttcctctacagcaagctcaccgtggacaagagcaggtggcagcaggggaacgtcttctcatgctccgtgatgcatgaggctctg
cacaaccactacacgcagaagagcctctccctgtctccgggtaaagcggatccttcgaaccgtccccatcctgggccatcacctta
atctcagtaaatggaatttttgtgtatgctgcctgacctactgcttgccccaagatgcagagagaggaaggaggaatgagagattga
gaaggaaagtgtacgccctgtataaatcgat

1277
1288

40.2.220-hmtIgG1-hCD80 (secuencia de aminoácido) (ID DE SEC NO __)

MDFQVQIFSFLLISASVIMSRGVDIVLTQSPATLSVTPGDRVSLSCRASQSISDYLHW
YQQKSHESPRLLIKYASHSISGIPSRFSGSGSGSDFTLSINSVEPEDVGIYYCQHGHSF
PWTFGGGTKLEIKRGGGGSGGGGSGGGGSQIQLVQSGPELKKPGETVRISCKASG
YAFTTTGMQWVQEMPGKGLKWIGWINTPLWSAKICRRLQGRFAFSLETSANTAY
LQISNLKDEDTATYFCVRSGNGNYDLAYFAYWGQGTLVTVSDLEPKSSDKTHTSP
PSPAPELLGGSSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVE
VHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISK
AKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKT
TPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGKA
DPSNLLPSWAITLISVNGIFVICCLTYCFAPRCRERRRNERLRRESVRPV

2e12scFv-hmtIgG1-proteína de fusión CD80 (secuencia de nucleótido) (ID DE SEC NO __)

aagcttatggattttcaagtgcagattttcagcttcctgctaatcagtgcttcagtcataatgtccagaggagatcggacattgttgctcaccc
aatctccagcttctttggctgtgtctctaggtcagagagccaccatctcctgcagagccagtgaaagttgtgaatattatgtcacaagtt
tatgcagtggtaccaacagaaaccaggacagccacccaaactcctcatctatctgcttgcatccaacgtagaatctggggtccctgcc
aggtttagtggcagtgggtctgggacagacttcagcctcaacatccatcctgtggaggaggatgatattgcaatgtatttctgtcagc
aaagtaggaaggttccttggacgttcggtggaggcaccaagctggaaatcaaacgggggtggcggttcgggcggaggtggg
tcgggtggcggcggatctcaggtgcagctgaaggagtcaggacctggcctggtgcgcctcacagagcctgtccatcacatgc
accgtctcagggttctcattaaccggctatggtgtaaactgggttcgccagcctccaggaaagggtctggagtggctgggaatgat
atggggtgatggaagcacagactataattcagctctcaaatccagactgagcatcaccaaggacaactccaagagccaagttttctt
aaaaatgaacagtctgcaaactgatgacacagccagatactactgtgccagaggtggttatagtaacttttcattactatgttatggact
actggggtcaaggaacctcagtcaccgtctcctcagatctggagcccaaatcttctgacaaaactcacacatcccccaccgtcccca
gcacctgaactcctgggggggatcgtcagtcttcctcttccccccaaaacccaaggacaccctcatgatctcccggacccctgaggt
cacatgcgtggtggtggacgtgagccacgaagaccctgaggtcaagttcaactggtacgtggacggcgtggaggtgcataatgc
caagacaaagccgcgcgggaggagcagtacaacagcacgtaccgtgtggtcagcgtcctcaccgtcctgcaccaggactggctga
atggcaaggagtacaagtgcaaggtctccaacaaagccctcccagcccccatcgagaaaaccatctccaaagccaaagggcag
ccccgagaaccacaggtgtacaccctgcccccatcccgggatgagctgaccaagaaccaggtcagcctgacctgcctggtcaaa
ggcttctatcccagcgacatcgccgtggagtgggagagcaatgggcagccggagaacaactacaagaccacgcctcccgtgct
ggactccgacggctccttcttcctctacagcaagctcaccgtggacaagagcaggtggcagcaggggaacgtcttctcatgctcc
gtgatgcatgaggctctgcacaaccactacacgcagaagagcctctccctgtctccgggtaaagcggatccttcgaacctgctcca
atcctgggccattacttaatctcagtaaatggaattttgtgatatgctgcctgaccactgcttgccccaagatgcagagagagaa
ggaggaatgagagattgagaagggaaagtgtacgcctgtataaaatcgat

1289

2el2scFv-hmtIgG1-CD80 proteína de fusión (secuencia de aminoácido) (ID DE SEC NO __)

MDFQVQIFSFLLISASVIMSRGVDIVLTQSPASLAVSLGQRATISCRASESVEYYVTS
LMQWYQQKPGQPPKLLISAASNVESGVPARFSGSGSGTDFSLNIHPVEEDDIAMYF
CQQSRKVPWTFGGGTKLEIKRGGGGSGGGGSGGGGSQVQLKESGPGLVAPSQSLS
ITCTVSGFSLTGYGVNWVRQPPGKGLEWLGMIWGDGSTDYNSALKSRLSITKDNS
KSQVFLKMNSLQTDDTARYYCARDGYSNFHYYVMDYWGQGTSVTVSSDLEPKS
SDKTHTSPPSPAPELLGGSSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFN
WYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALP
APIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQ
PENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSL
SLSPGKADPSNLLPSWAITLISVNGIFVICCLTYCFAPRCRERRRNERLRRESVRPV

10A8 scFv-hmtIgG1-CD80 (secuencia de nucleótido) (ID DE SEC NO __)

aagcttatggattttcaagtgcagattttcagcttcctgctaatcagtgcttcagtcataatgtccagaggagtcgacatccagatgaca
cagtctccatcctcactgtctgcatctctgggaggcaaagtcaccatcacttgcaaggcaagccaagacattaagaagtatataggtt
ggtaccaacacaagcctggaaaaggtcccaggctgctcatatattacacatctacattacagccaggcatcccatcaaggttcagtg
gaagtgggtctgggagagattattccctcagcatcagaaacctggagcctgaagatattgcaacttattattgtcaacagtatgataat
cttccattgacgttcggctcggggacaaagttggaaataaaacggggtggcggtggctcgggcggtggtgggtcgggtggcggc
ggatctgatgtacagcttcaggagtcaggacctggcctcgtgaaaccttctcagtctctgtctctcacctgctctgtcactggctactc
catcaccagtggtttctactggaactggatccgacagtttccgggaaacaaactggaatggatgggccacataagccacgacggta
ggaataactacaacccatctctcataaatcgaatctccatcactcgtgacacatctaagaaccagtttttcctgaagttgagttctgtga
ctactgaggacacagctacatatttctgtgcaagacactacggtagtagcggagctatggactactggggtcaaggaacctcagtc
accgtctcctctgatctggagcccaaatcttctgacaaaactcacacatccccaccgtccccagcacctgaactcctgggggggatc
gtcagtcttcctcttcccccaaaacccaaggacaccctcatgatctcccggaccccctgaggtcacatgcgtggtggtggacgtga
gccacgaagaccctgaggtcaagttcaactggtacgtggacggcgtggaggtgcataatgccaagacaaagccgcgggagga
gcagtacaacagcacgtaccgtgtggtcagcgtcctcaccgtcctgcaccaggactggctgaatggcaaggagtacaagtgcaa
ggtctccaacaaagccctcccagcccccatcgagaaaaccatctccaaagccaaagggcagccccgagaaccacaggtgtaca
ccctgcccccatcccgggatgagctgaccaagaaccaggtcagcctgacctgcctggtcaaaggcttctatcccagcgacatcgc
cgtggagtgggagagcaatgggcagccggagaacaactacaagaccacgcctcccgtgctggactccgacggctccttcttcct

~~1279~~ 1290

ctacagcaagctcaccgtggacaagagcaggtggcagcaggggaacgtcttctcatgctccgtgatgcatgaggctctgcacaac
cactacacgcagaagagcctctccctgtctccgggtaaagcggatccttcgaacctgctcccatcctgggccattaccttaatctca
gtaaatggaatttttgtgatatgctgcctgacctactgctttgccccaagatgcagagagagaaggaggaatgagagattgagaagg
gaaagtgtacgccctgtataaatcgat

10A8 scFv-hmtIgG1-CD80 (secuencia de aminoácido) (ID DE SEC NO __)

MDFQVQIFSFLLISASVIMSRGVDIQMTQSPSSLSASLGGKVTITCKASQDIKKYIG
WYQHKPGKGPRLLIYYTSTLQPGIPSRFSGSGSGRDYSLSIRNLEPEDIATYYCQQY
DNLPLTFGSGTKLEIKRGGGGSGGGGSGGGGSDVQLQESGPGLVKPSQSLSLTCSV
TGYSITSGFYWNWIRQFPGNKLEWMGHISHDGRNNYNPSLINRISITRDTSKNQFFL
KLSSVTTEDTATYFCARHYGSSGAMDYWGQGTSVTVSSDLEPKSSDKTHTSPPSP
APELLGGSSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVH
NAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAK
GQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPP
VLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGKADPS
NLLPSWAITLISVNGIFVICCLTYCFAPRCRERRRNERLRRESVRPV

500A2-hmtIgG1-CD80 (secuencia de nucleótido) (ID DE SEC NO __)

atgttgtatacatctcagctccttgggcttttactcttctggatttcagcctccagaagtgacatagtgctgactcagactccagccactc
tgtctctaattcctggagaaagagtcacaatgacctgtaagaccagtcagaatattggcacaatcttacactggtatcaccaaaaacc
aaaggaggctccaagggctctcatcaagtatgcttcgcagtccattcctgggatcccctccagattcagtggcagtggttcggaaac
agatttcactctcagcatcaataacctggagcctgatgatatcggaatttattactgtcaacaaagtagaagctggcctgtcacgttcg
gtcctggcaccaagctggagataaaacggggtggcggtggctcgggcggaggtgggtcgggtggcggcggatctcaggtcaa
gctgcagcagtccggttctgaactagggaaacctggggcctcagtgaaactgtcctgcaagacttcaggctacatattcacagatc
actatatttcttgggtgaaacagaagcctggagaaagcctgcagtggataggaaatgtttatggtggaaatggtggtacaagctaca
atcaaaaattccagggcaaggccacactgactgtagataaaatctctagcacagcctacatggaactcagcagcctgacatctgag
gattctgccatctattactgtgcaagaaggccggtagcgacgggccatgctatggactactggggtcaggggatccaagttaccgt
ctcctctgatctggagcccaaatcttctgacaaaactcacacatcccccaccgtccccagcacctgaactcctgggggggatcgtcagt
cttcctcttcccccaaaacccaaggacaccctcatgatctcccggaccccctgaggtcacatgcgtggtggtggacgtgagccacg
aagaccctgaggtcaagttcaactggtacgtggacggcgtggaggtgcataatgccaagacaaagccgcgggaggagcagtac
aacagcacgtaccgtgtggtcagcgtcctcaccgtcctgcaccaggactggctgaatggcaaggagtacaagtgcaaggtctcca
acaaagccctcccagcccccatcgagaaaaccatctccaaagccaaagggcagccccgagaaccacaggtgtacaccctgccc
ccatcccgggatgagctgaccaagaaccaggtcagcctgacctgcctggtcaaaggcttctatcccagcgacatcgccgtggagt
gggagagcaatgggcagccggagaacaactacaagaccacgcctcccgtgctggactccgacggctccttcttcctctacagca

1286
1291

agctcaccgtggacaagagcaggtggcagcaggggaacgtcttctcatgctccgtgatgcatgaggctctgcacaaccactacac
gcagaagagcctctccctgtctccgggtaaagcggatccttcgaacctgctcccatcctgggccattaccttaatctcagtaaatgga
atttttgtgatatgctgcctgacctactgctttgccccaagatgcagagagagaaggaggaatgagagattgagaagggaaagtgta
cgccctgtataaatcgat

500A2-hmtIgG1-CD80 (secuencia de aminoácido) (ID DE SEC NO __)

MLYTSQLLGLLLFWISASRSDIVLTQTPATLSLIPGERVTMTCKTSQNIGTILHWYH
QKPKEAPRALIKYASQSIPGIPSRFSGSGSETDFTLSINNLEPDDIGIYYCQQSRSWPV
TFGPGTKLEIKRGGGGSGGGGSGGGGSQVKLQQSGSELGKPGASVKLSCKTSGYIF
TDHYISWVKQKPGESLQWIGNVYGGNGGTSYNQKFQGKATLTVDKISSTAYMEL
SSLTSEDSAIYYCARRPVATGHAMDYWGQGIQVTVSSDLEPKSSDKTHTSPPSPAP
ELLGGSSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNA
KTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQ
PREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVL
DSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGKADPSNL
LPSWAITLISVNGIFVICCLTYCFAPRCRERRRNERLRRESVRPV

2H7 scFv MTH (SSS) WTCH2CH3 (secuencia de nucleótido) (ID DE SEC NO __)

aagcttgccgccatggatttttcaagtgcagattttcagcttcctgctaatcagtgcttcagtcataattgtccagaggacaaattgttctct
cccagtctccagcaatcctgtctgcatctccaggggagaaggtcacaatgacttgcagggccagctcaagtgtaagttacatgcact
ggtaccagcagaagccaggatcctcccccaaaccctggatttatgccccatccaacctggcttctggagtccctgctcgcttcagtg
gcagtgggtctgggacctcttactctctcacaatcagcagagtggaggctgaagatgctgccactattactgccagcagtggagtit
tacccacccacgttcggtgctgggaccaagctggagctgaaagatggcggtggctcgggcggtggtggatctggaggaggtg
ggagtctcaggcttatctacagcagtctggggctgagctggtgaggcctggggcctcagtgaagatgtcctgcaaggcttctggc

1287
1292

tacacattaccagttacaatatgcactgggtaaagcagacacctagacagggcctggaatggattggagctatttatccaggaaat
ggtgatacttcctacaatcagaagttcaagggcaaggccacactgactgtagacaaatcctccagcacagcctacatgcagctcag
cagcctgacatctgaagactctgcggtctatttctgtgcaagagtggtgtactatagtaactcttactggtacttcgatgtctggggcac
agggaccacggtcaccgtctcttctgatcaggagcccaaatcttctgacaaaactcacacatcccccaccgtcccccagcacctgaac
tcctgggggggaccgtcagtcttcctcttccccccaaaacccaaggacaccctcatgatctcccggacccctgaggtcacatgcgtg
gtggtggacgtgagccacgaagaccctgaggtcaagttcaactggtacgtggacggcgtggaggtgcataatgccaagacaaag
ccgcgggaggagcagtacaacagcacgtaccgtgtggtcagcgtcctcaccgtcctgcaccaggactggctgaatggcaagga
gtacaagtgcaaggtctccaacaaagccctcccagccccatcgagaaaacaatctccaaagccaaagggcagccccgagaac
cacaggtgtacaccctgcccccatcccgggatgagctgaccaagaaccaggtcagcctgacctgcctggtcaaaggcttctatcc
cagcgacatcgccgtggagtgggagagcaatgggcagccggagaacaactacaagaccacgcctcccgtgctggactccgac
ggctccttcttcctctacagcaagctcaccgtggacaagagcaggtggcagcaggggaacgtcttctcatgctccgtgatgcatga
ggctctgcacaaccactacacgcagaagagcctctccctgtctccgggtaaatgatctaga

2H7 scFv MTH (SSS) WTCH2CH3 (secuencia de aminoácido) (ID DE SEC NO __)

MDFQVQIFSFLLISASVIIARGQIVLSQSPAILSASPGEKVTMTCRASSSVSYMHWY
QQKPGSSPKPWIYAPSNLASGVPARFSGSGSGTSYSLTISRVEAEDAATYYCQQWS
FNPPTFGAGTKLELKDGGGSGGGGSGGGGSSQAYLQQSGAELVRPGASVKMSCK
ASGYTFTSYNMHWVKQTPRQGLEWIGAIYPGNGDTSYNQKFKGKATLTVDKSSS
TAYMQLSSLTSEDSAVYFCARVVYYSNSYWYFDVWGTGTTVTVSSDQEPKSSDK
THTSPPSPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYV
DGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIE
KTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPEN
NYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLS
PGK

2H7 scFv IGAH IGG WT CH2CH3 (2H7 scFv con articulación IgA y WT CH2 y CH3) (secuencia de nucleótido) (ID DE SEC NO __)

aagcttgccgccatggattttcaagtgcagattttcagcttcctgctaatcagtgcttcagtcataattgccagaggacaaattgttctct
cccagtctccagcaatcctgtctgcatctccaggggagaaggtcacaatgacttgcagggccagctcaagtgtaagttacatgcact
ggtaccagcagaagccaggatcctccccaaaccctggatttatgccccatccaacctggcttctggagtccctgctcgcttcagtg
gcagtgggtctgggacctcttactctctcacaatcagcagagtggaggctgaagatgctgccacttattactgccagcagtggagttt
taacccacccacgttcggtgctgggaccaagctggagctgaaagatggcggtggctcgggcggtggtggatctggaggaggtg
ggagctctcaggcttatctacagcagtctggggctgagctggtgaggcctggggcctcagtgaagatgtcctgcaaggcttctggc

1292
1293

tacacatttaccagttacaatatgcactgggtaaagcagacacctagacagggcctggaatggattggagctatttatccaggaaat
ggtgatacttcctacaatcagaagttcaagggcaaggccacactgactgtagacaaatcctccagcacagcctacatgcagctcag
cagcctgacatctgaagactctgcggtctatttctgtgcaagagtggtgtactatagtaactcttactggtacttcgatgtctggggcac
agggaccacggtcaccgtctctgatcagccagttccctcaactccacctaccccatctccctcaactccacctaccccatctcctca
tgcgcacctgaactcctgggggggaccgtcagtcttcctcttcccccaaaacccaaggacaccctcatgatctcccggaccccctga
ggtcacatgcgtggtggtggacgtgagccacgaagaccctgaggtcaagttcaactggtacgtggacggcgtggaggtgcataa
tgccaagacaaagccgcgggaggagcagtacaacagcacgtaccgtgtggtcagcgtcctcaccgtcctgcaccaggactggc
tgaatggcaaggagtacaagtgcaaggtctccaacaaagccctcccagccccatcgagaaaacaatctccaaagccaaagggc
agccccgagaaccacaggtgtacaccctgcccccatcccgggatgagctgaccaagaaccaggtcagcctgacctgcctggtca
aaggcttctatcccagcgacatcgccgtggagtgggagagcaatgggcagccggagaacaactacaagaccacgcctcccgtg
ctggactccgacggctccttcttcctctacagcaagctcaccgtggacaagagcaggtggcagcaggggaacgtcttctcatgctc
cgtgatgcatgaggctctgcacaaccactacacgcagaagagcctctccctgtctccgggtaaatgatctaga

2H7 scFv IgAH 1GG WT CH2CH3 (secuencia de aminoácido) (ID DE SEC NO __)

MDFQVQIFSFLLISASVIIARGQIVLSQSPAILSASPGEKVTMTCRASSSVSYMHWY
QQKPGSSPKPWIYAPSNLASGVPARFSGSGSGTSYSLTISRVEAEDAATYYCQQWS
FNPPTFGAGTKLELKDGGGSGGGGSGGGGSSQAYLQQSGAELVRPGASVKMSCK
ASGYTFTSYNMHWVKQTPRQGLEWIGAIYPGNGDTSYNQKFKGKATLTVDKSSS
TAYMQLSSLTSEDSAVYFCARVVYYSNSYWYFDVWGTGTTVTVSDQPVPSTPPTP
SPSTPPTPSPSCAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFN
WYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALP
APIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQ
PENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSL
SLSPGK

<u>2H7 scFv IsAH IgACH2CH3</u> (2H7 scFv articulación IgA e IgA CH2 y CH3) (secuencia de nucleótido) (ID DE SEC NO __)

aagcttgccgccatggattttcaagtgcagattttcagcttcctgctaatcagtgcttcagtcataattgccagaggacaaattgttctct
cccagtctccagcaatcctgtctgcatctccaggggagaaggtcacaatgacttgcagggccagctcaagtgtaagttacatgcact
ggtaccagcagaagccaggatcctccccaaaccctggatttatgccccatccaacctggcttctggagtcctgctcgcttcagtg
gcagtgggtctgggacctcttactctctcacaatcagcagagtggaggctgaagatgctgccacttattactgccagcagtggagttt
taacccacccacgttcggtgctgggaccaagctggagctgaaagatggcggtggctcgggcggtggtggatctggaggaggtg
ggagctctcaggcttatctacagcagtctggggctgagctggtgaggcctggggcctcagtgaagatgtcctgcaaggcttctggc
tacacatttaccagttacaatatgcactgggtaaagcagacacctagacagggcctggaatggattggagctatttatccaggaaat

1283
1299

ggtgatacttcctacaatcagaagttcaagggcaaggccacactgactgtagacaaatcctccagcacagcctacatgcagctcag
cagcctgacatctgaagactctgcggtctatttctgtgcaagagtggtgtactatagtaactcttactggtacttcgatgtctggggcac
agggaccacggtcaccgtctcttctgatcagccagttccctcaactccacctaccccatctccctcaactccacctaccccatctccc
tcatgctgccaccccccgactgtcactgcaccgaccggccctcgaggacctgctcttaggttcagaagcgatcctcacgtgcacact
gaccggcctgagagatgcctcaggtgtcaccttcacctggacgccctcaagtgggaagagcgctgttcaaggaccacctgaccgt
gacctctgtggctgctacagcgtgtccagtgtcctgccgggctgtgccgagccatggaaccatgggaagaccttcacttgcactgc
tgcctaccccgagtccaagaccccgctaaccgccaccctctcaaaatccggaaacacattccggcccgaggtccacctgctgccg
ccgccgtcggaggagctggccctgaacgagctggtgacgctgacgtgcctggcacgtggcttcagccccaaggatgtgctggtt
cgctggctgcaggggtcacaggagctgccccgcgagaagtacctgacttgggcatcccggcaggagcccagccagggcacca
ccaccttcgctgtgaccagcatactgcgcgtggcagccgaggactggaagaagggggacaccttctcctgcatggtgggccacg
aggccctgccgctggccttcacacagaagaccatcgaccgcttggcgggtaaacccacccatgtcaatgtgtctgttgtcatggcg
gaggtggacggcacctgctactgataatctaga

2H7 scFv IgAH IgACH2CH3 (2H7 scFv articulación IgA e IgA CH2 y CH3) (secuencia de aminoácido) (ID DE SEC NO __)

MDFQVQIFSFLLISASVIIARGQIVLSQSPAILSASPGEKVTMTCRASSSVSYMHWY
QQKPGSSPKPWIYAPSNLASGVPARFSGSGSGTSYSLTISRVEAEDAATYYCQQWS
FNPPTFGAGTKLELKDGGGSGGGGSGGGGSSQAYLQQSGAELVRPGASVKMSCK
ASGYTFTSYNMHWVKQTPRQGLEWIGAIYPGNGDTSYNQKFKGKATLTVDKSSS
TAYMQLSSLTSEDSAVYFCARVVYYSNSYWYFDVWGTGTTVTVSSDQPVPSTPPT
PSPSTPPTPSPSCCHPRLSLHRPALEDLLLGSEAILTCTLTGLRDASGVTFTWTPSSG
KSAVQGPPDRDLCGCYSVSSVLPGCAEPWNHGKTFTCTAAYPESKTPLTATLSKS
GNTFRPEVHLLPPPSEELALNELVTLTCLARGFSPKDVLVRWLQGSQELPREKYLT
WASRQEPSQGTTTFAVTSILRVAAEDWKKGDTFSCMVGHEALPLAFTQKTIDRLA
GKPTHVNVSVVMAEVDGTCY

Articulación IgA -CH2-CH3 (Human IgA tail, full length) (secuencia de nucleótido) (ID DE SEC NO __)

tgatcagccagttccctcaactccacctaccccatctccctcaactccacctaccccatctccctcatgctgccacccccgactgtca
ctgcaccgaccggccctcgaggacctgctcttaggttcagaagcgatcctcacgtgcacactgaccggcctgagagatgcctcag
gtgtcaccttcacctggacgccctcaagtgggaagagcgctgttcaaggaccacctgaccgtgacctctgtggctgctacagcgtg
tccagtgtcctgccgggctgtgccgagccatggaaccatgggaagaccttcacttgcactgctgcctaccccgagtccaagaccc
cgctaaccgccaccctctcaaaatccggaaacacattccggcccgaggtccacctgctgccgccgccgtcggaggagctggccc
tgaacgagctggtgacgctgacgtgcctggcacgtggcttcagccccaaggatgtgctggttcgctggctgcaggggtcacagg

agctgccccgcgagaagtacctgacttgggcatcccggcaggagcccagccagggcaccaccaccttcgctgtgaccagcata
ctgcgcgtggcagccgaggactggaagaaggggacaccttctcctgcatggtgggccacgaggccctgccgctggccttcac
acagaagaccatcgaccgcttggcgggtaaacccacccatgtcaatgtgtctgttgtcatggcggaggtggacggcacctgctact
gataatctaga

Articulación-CH2-CH3 IgA (cola IgA humana, longitud completa) (secuencia de aminoácido) (ID DE SEC NO __)

DQPVPSTPPTPSPSTPPTPSPSCCHPRI SLHRPALEDLLLGSEAILTCTLTGLRDASGV
TFTWTPSSGKSAVQGPPDRDLCGCYSVSSVLPGCAEPWNHGKTFTCTAAYPESKT
PLTATLSKSGNTFRPBVHLLPPPSEELALNELVTLTCLARGFSPKDVLVRWLGSQ
BLPREKYLTWASRQEPSQGTTTFAVTSILRVAAEDWKKGDTFSCMVGHEALPLAF
TQKTIDRLAGKPTHVNVSVVMAEVDGTCY

Cadena J Humana (secuencia de nucleótido) (ID DE SEC NO __)

agatctcaagaagatgaaaggattgttcttgttgacaacaaatgtaagtgtgcccggattacttccaggatcatccgttcttccgaaga
tcctaatgaggacattgtggagagaaacatccgaattattgttcctctgaacaacagggagaatatctctgatcccacctcaccattga
gaaccagatttgtgtaccatttgtctgacctcagctgtaaaaaatgtgatcctacagaagtggagctggataatcagatagttactgct
acccagagcaatatctgtgatgaagacagtgctacagaggacctgctacacttatgacagaaacaagtgctacacagctgtggtccc
actcgtatatggtggtgagaccaaaatggtggaaacagccttaaccccagatgcctgctatcctgactaatctaga

Cadena J humana (secuencia de aminoácido) (ID DE SEC NO __)

RSQEDERIVLVDNKCKCARITSRIIRSSEDPNEDIVERNIRIIVPLNNRENISDPTSPLR
TRFVYHLSDLSCKKCDPTEVELDNQIVTATQSNICDEDSATETCYTYDRNKCYTAV
VPLVYGGETKMVETALTPDACYP

Amino ácidos 4 carboxy terminal eliminados de IgA CH3 (secuencia de aminoácido) (ID DE SEC NO __)

GTCY

IgAH IgAT4 (cola IgA humana, truncada (3T1)-(desaparece últimos 4 amino ácidos del terminal carboxy) (secuencia de nucleótido) (ID DE SEC NO __)

1285
1296

tgatcagccagttccctcaactccacctaccccatctccctcaactccacctaccccatctccctcatgctgccacccccgactgtca
ctgcaccgaccggccctcgaggacctgctcttaggttcagaagcgatcctcacgtgcacactgaccggcctgagagatgcctcag
gtgtcaccttcacctggacgccctcaagtgggaagagcgctgttcaaggaccacctgaccgtgacctctgtggctgctacagcgtg
tccagtgtcctgccgggctgtgccgagccatggaaccatgggaagaccttcacttgcactgctgcctaccccgagtccaagaccc
cgctaaccgccacccctctcaaaatccggaaacacattccggcccgaggtccacctgctgccgccgccgtcggaggagctggccc
tgaacgagotggtgacgctgacgtgcctggcacgtggcttcagccccaaggatgtgctggttcgctggctgcaggggtcacagg
agctgccccgcgagaagtacctgacttgggcatcccggcaggagcccagccagggcaccaccaccttcgctgtgaccagcata
ctgcgcgtggcagccgaggactggaagaaggggacaccttctcctgcatggtgggccacgaggccctgccgctggccttcac
acagaagaccatcgaccgcttggcgggtaaacccacccatgtcaatgtgtctgttgtcatggcggaggtggactgataatctaga

IgAH IgAT4 (secuencia de aminoácido) (ID DE SEC NO __)

DQPVPSTPPTPSPSTPPTPSPSCCHPRLSLHRPALEDLLLGSEAILTCTLTGLRDASGV
TFTWTPSSGKSAVQGPPDRDLCGCYSVSSVLPGCAEPWNHGKTFTCTAAYPESKT
PLTATLSKSGNTFRPEVHLLPPPSEELALNELVTLTCLARGFSPKDVLVRWLQGSQ
ELPREKYLTWASRQEPSQGTTTFAVTSILRVAAEDWKKGDTFSCMVGHEALPLAF
TQKTIDRLAGKPTHVNVSVVMAEVD

2H7 scFv IgAH IgAT4 (construct 2H7 scFv IgA 3T1, trunca el dominio CH3 en el extremo 3') (secuencia de nucleótido) (ID DE SEC NO __)

aagcttgccgccatggattttcaagtgcagattttcagcttcctgctaatcagtgcttcagtcataattgccagaggacaaattgttctct
cccagtctccagcaatcctgtctgcatctccaggggagaaggtcacaatgacttgcagggccagctcaagtgtaagttacatgcact
ggtaccagcagaagccaggatcctcccccaaaccctggatttatgccccatccaacctggcttctggagtccctgctcgcttcagtg
gcagtgggtctgggacctcttactctctcacaatcagcagagtggaggctgaagatgctgccacttattactgccagcagtggagttt
taacccacccacgttcggtgctgggaccaagctggagctgaaagatggcggtggctcgggcggtggtggatctggaggaggtg
ggagctctcaggcttatctacagcagtctggggctgagctggtgaggcctggggcctcagtgaagatgtcctgcaaggcttctggc
tacacatttaccagttacaatatgcactgggtaaagcagacacctagacagggcctggaatggattggagctatttatccaggaaat
ggtgatacttcctacaatcagaagttcaagggcaaggccacactgactgtagacaaatcctccagcacagcctacatgcagctcag
cagcctgacatctgaagactctgcggtctatttctgtgcaagagtggtgtactatagtaactcttactggtacttcgatgtctggggcac
agggaccacggtcaccgtctcttctgatcagccagttccctcaactccacctaccccatctccctcaactccacctaccccatctccc
tcatgctgccacccccgactgtcactgcaccgaccggccctcgaggacctgctcttaggttcagaagcgatcctcacgtgcacact
gaccggcctgagagatgcctcaggtgtcaccttcacctggacgccctcaagtgggaagagcgctgttcaaggaccacctgaccgt
gacctctgtggctgctacagcgtgtccagtgtcctgccgggctgtgccgagccatggaaccatgggaagaccttcacttgcactgc
tgcctaccccgagtccaagaccccgctaaccgccacccctctcaaaatccggaaacacattccggcccgaggtccacctgctgccg
ccgccgtcggaggagctggccctgaacgagctggtgacgctgacgtgcctggcacgtggcttcagccccaaggatgtgctggtt

1296
1297

cgctggctgcaggggtcacaggagctgccccgcgagaagtacctgacttgggcatcccggcaggagcccagccagggcacca
ccaccttcgctgtgaccagcatactgcgcgtggcagccgaggactggaagaaggggggacaccttctcctgcatggtgggccacg
aggccctgccgctggccttcacacagaagaccatcgaccgcttggcgggtaaacccacccatgtcaatgtgtctgttgtcatggcg
gaggtggactgataatctaga

2H7 scFv IgAH-T4 (secuencia de aminoácido) (ID DE SEC NO __)

MDFQVQIFSFLLISASVIIARGQIVLSQSPAILSASPGEKVTMTCRASSSVSYMHWY
QQKPGSSPKPWIYAPSNLASGVPARFSGSGSGTSYSLTISRVEAEDAATYYCQQWS
FNPPTFGAGTKLELKDGGGSGGGGSGGGGSSQAYLQQSGAELVRPGASVKMSCK
ASGYTFTSYNMHWVKQTPRQGLEWIGAIYPGNGDTSYNQKFKGKATLTVDKSSS
TAYMQLSSLTSEDSAVYFCARVVYYSNSYWYFDVWGTGTTVTVSSDQPVPSTPPT
PSPSTPPTPSPSCCHPRLSLHRPALEDLLLGSEAILTCTLTGLRDASGVTFTWTPSSG
KSAVQGPPDRDLCGCYSVSSVLPGCAEPWNHGKTFTCTAAYPESKTPLTATLSKS
GNTFRPEVHLLPPPSEELALNELVTLTCLARGFSPKDVLVRWLQGSQELPREKYLT
WASRQEPSQGTTTFAVTSILRVAAEDWKKGDTFSCMVGHEALPLAFTQKTIDRLA
GKPTHVNVSVVMAEVD

14 aminoácidos eliminados de IgAH-T4 (de tal forma que 18 amino ácidos eliminados de IgA CH3 tipo silvestre) (secuencia de aminoácido) (ID DE SEC NO __)

PTHVNVSVVMAEVD

IgAH IgA-T18 (cola IgA truncada, 3T2) (secuencia de nucleótido) (ID DE SEC NO __)

tgatcagccagttccctcaactccacctaccccatctccctcaactccacctaccccatctccctcatgctgccacccccgactgtca
ctgcaccgaccggccctcgaggacctgctcttaggttcagaagcgatcctcacgtgcacactgaccggcctgagagatgcctcag
gtgtcaccttcacctggacgccctcaagtgggaagagcgctgttcaaggaccacctgaccgtgacctctgtggctgctacagcgtg
tccagtgtcctgccgggctgtgccgagccatggaaccatgggaagaccttcacttgcactgctgcctaccccgagtccaagaccc
cgctaaccgccacccctctcaaaatccggaaacacattccggcccgaggtccacctgctgccgccgccgtcggaggagctggccc
tgaacgagctggtgacgctgacgtgcctggcacgtggcttcagccccaaggatgtgctggttcgctggctgcaggggtcacagg
agctgccccgcgagaagtacctgacttgggcatcccggcaggagcccagccagggcaccaccaccttcgctgtgaccagcata
ctgcgctggcagccgaggactggaagaaggggggacaccttctcctgcatggtgggcacgaggccctgccgctggccttcac
acagaagaccatcgaccgcttggcgggtaaa

IgAH IgA-T18 (secuencia de aminoácido) (ID DE SEC NO __)

1287
1298

DQPVPSTPPTPSPSTPPTPSPSCCHPRLSLHRPALEDLLLGSEAILTCTLTGLRDASGV
TFTWTPSSGKSAVQGPPDRDLCGCYSVSSVLPGCAEPWNHGKTFTCTAAYPESKT
PLTATLSKSGNTFRPEVHLLPPPSEELALNELVTLTCLARGFSPKDVLVRWLQGSQ
ELPREKYLTWASRQEPSQGTTTFAVTSILRVAAEDWKKGDTFSCMVGHEALPLAF
TQKTIDRLAGK

2H7 scFv IgAH IgAT18 (cola IgA humana truncada, 3T2) (secuencia de nucleótido) (ID DE SEC NO __)

aagcttgccgccatggattttcaagtgcagattttcagcttcctgctaatcagtgcttcagtcataattgccagaggacaaattgttctct
cccagtctccagcaatcctgtctgcatctccaggggagaaggtcacaatgacttgcagggccagctcaagtgtaagttacatgcact
ggtaccagcagaagccaggatcctcccccaaaccctggatttatgccccatccaacctggcttctggagtccctgctcgcttcagtg
gcagtgggtctgggacctcttactctctcacaatcagcagagtggaggctgaagatgctgccacttattactgccagcagtggagttt
taacccacccacgttcggtgctgggaccaagctggagctgaaagatggcggtggctcgggcggtggtggatctggaggaggtg
ggagctctcaggcttatctacagcagtctggggctgagctggtgaggcctggggcctcagtgaagatgtcctgcaaggcttctggc
tacacatttaccagttacaatatgcactgggtaaagcagacacctagacagggcctggaatggattggagctatttatccaggaaat
ggtgatacttcctacaatcagaagttcaagggcaaggccacactgactgtagacaaatcctccagcacagcctacatgcagctcag
cagcctgacatctgaagactctgcggtctatttctgtgcaagagtggtgtactatagtaactcttactggtacttcgatgtctggggcac
agggaccacggtcaccgtctcttctgatcagccagttccctcaactccacctaccccatctccctcaactccacctaccccatctccc
tcatgctgccaccccccgactgtcactgcaccgaccggccctcgaggacctgctcttaggttcagaagcgatcctcacgtgcacact
gaccggcctgagagatgcctcaggtgtcaccttcacctggacgccctcaagtgggaagagcgctgttcaaggaccacctgaccgt
gacctctgtggctgctacagcgtgtccagtgtcctgccgggctgtgccgagccatggaaccatgggaagaccttcacttgcactgc
tgcctaccccgagtccaagaccccgctaaccgccaccctctcaaaatccggaaacacattccggcccgaggtccacctgctgccg
ccgccgtcggaggagctggccctgaacgagctggtgacgctgacgtgcctggcacgtggcttcagccccaaggatgtgctggtt
cgctggctgcaggggtcacaggagctgccccgcgagaagtacctgacttgggcatcccggcaggagcccagccagggcacca
ccaccttcgctgtgaccagcatactgcgcgtggcagccgaggactggaagaaggggacaccttctcctgcatggtgggccacg
aggccctgccgctggccttcacacagaagaccatcgaccgcttggcgggtaaa

<u>2H7 scFv IgAH IgAT18</u> (secuenciade aminoácido) (ID DE SEC NO __)

1288
1299

MDFQVQIFSFLLISASVIIARGQIVLSQSPAILSASPGEKVTMTCRASSSVSYMHWY
QQKPGSSPKPWIYAPSNLASGVPARFSGSGSGTSYSLTISRVEAEDAATYYCQQWS
FNPPTFGAGTKLELKDGGGSGGGGSGGGGSSQAYLQQSGAELVRPGASVKMSCK
ASGYTFTSYNMHWVKQTPRQGLEWIGAIYPGNGDTSYNQKFKGKATLTVDKSSS
TAYMQLSSLTSEDSAVYFCARVVYYSNSYWYFDVWGTGTTVTVSSDQPVPSTPPT
PSPSTPPTPSPSCCHPRLSLHRPALEDLLLGSEAILTCTLTGLRDASGVTFTWTPSSG
KSAVQGPPDRDLCGCYSVSSVLPGCAEPWNHGKTFTCTAAYPESKTPLTATLSKS
GNTFRPEVHLLPPPSEELALNELVTLTCLARGFSPKDVLVRWLQGSQELPREKYLT
WASRQEPSQGTTTFAVTSILRVAAEDWKKGDTFSCMVGHEALPLAFTQKTIDRLA
GK

CTLA-4 IgG WTH WTCH2CH3 (-CTLA4EC-hIgGWT oncoMLP humano) (secuencia de nucleótido) (ID DE SEC NO __)

gcaacctacatgatggggaatgagttgaccttcctagatgattccatctgcacgggcacctccagtggaaatcaagtgaacctcact
atccaaggactgagggccatggacacgggactctacatctgcaaggtggagctcatgtacccaccgccatactacctgggcatag
gcaacggaacccagatttatgtaattgatccagaaccgtgcccagattctgatcaacccaaatcttgtgacaaaactcacacatgcc
caccgtgcccagcacctgaactcctgggggggaccgtcagtcttcctcttcccccaaaacccaaggacaccctcatgatctcccgg
acccctgaggtcacatgcgtggtggtggacgtgagccacgaagaccctgaggtcaagttcaactggtacgtggacggcgtggag
gtgcataatgccaagacaaagccgcgggaggagcagtacaacagcacgtaccgtgtggtcagcgtcctcaccgtcctgcaccag
gactggctgaatggcaaggagtacaagtgcaaggtctccaacaaagccctcccagcccccatcgagaaaacaatctccaaagcc
aaagggcagccccgagaaccacaggtgtacaccctgcccccatcccgggatgagctgaccaagaaccaggtcagcctgacctg
cctggtcaaaggcttctatcccagcgacatcgccgtggagtgggagagcaatgggcagccggagaacaactacaagaccacgc
ctcccgtgctggactccgacggctccttcttcctctacagcaagctcaccgtggacaagagcaggtggcagcaggggaacgtctt
ctcatgctccgtgatgcatgaggctctgcacaaccactacacgcagaagagcctctccctgtctccgggtaaatga

CTLA-4 IgG WTH WTCH2CH3 (secuencia de aminoácido) (ID DE SEC NO __)

MGVLLTQRTLLSLVLALLFPSMASMAMHVAQPAVVLASSRGIASFVCEYASPGKA
TEVRVTVLRQADSQVTEVCAATYMMGNELTFLDDSICTGTSSGNQVNLTIQGLRA
MDTGLYICKVELMYPPPYYLGIGNGTQIYVIDPEPCPDSDQPKSCDKTHTCPPCPAP
ELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNA
KTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQ
PREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVL
DSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

1289
1300

péptido líder+CTLA4 EC OncoM Human (BclI) (secuencia de nucleótido) (ID DE SEC NO __)

atggggtactgctcacacagaggacgctgctcagtctggtccttgcactcctgtttccaagcatggcgagcatggcaatgcacgt
ggcccagcctgctgtggtactggccagcagccgaggcatcgccagctttgtgtgtgagtatgcatctccaggcaaagccactgag
gtccgggtgacagtgcttcggcaggctgacagccaggtgactgaagtctgtgcggcaacctacatgatggggaatgagttgacct
tcctagatgattccatctgcacgggcacctccagtggaaatcaagtgaacctcactatccaaggactgagggccatggacacggg
actctacatctgcaaggtggagctcatgtacccaccgccatactacctgggcataggcaacggaacccagatttatgtaattgatcc
agaaccgtgcccagattctgatcaa

Péptido líder+CTLA4 EC OncoM humano (secuencia de aminoácido) (ID DE SEC NO __)

MGVLLTQRTLLSLVLALLFPSMASMAMHVAQPAVVLASSRGIASFVCEYASPGKA
TEVRVTVLRQADSQVTEVCAATYMMGNELTFLDDSICTGTSSGNQVNLT1QGLRA
MDTGLYICKVELMYPPPYYLGIGNGTQIYVIDPEPCPDSDQ

Líder OncoM humano (secuencia de nucleótido) (ID DE SEC NO __)

atgggggtactgctcacacagaggacgctgctcagtctggtccttgcactcctgtttccaagcatggcgagcatg

Líder OncoM humano (secuencia de aminoácido) (ID DE SEC NO __)

MGVLLTQRTLLSLVLALLFPSM

CTLA4 EC humano (sin LP) (secuencia de nucleótido) (ID DE SEC NO __)

gcaatgcacgtggcccagcctgctgtggtactggccagcagccgaggcatcgccagctttgtgtgtgagtatgcatctccaggca
aagccactgaggtccgggtgacagtgcttcggcaggctgacagccaggtgactgaagtctgtgcggcaacctacatgacgggga
atgagttgaccttcctagatgattccatctgcacgggcacctccagtggaaatcaagtgaacctcactatccaaggactgagggcca
tggacacgggactctacatctgcaaggtggagctcatgtacccaccgccatactacctgggcataggcaacggaacccagatttat
gtaattgatccagaaccgtgcccagattct

CTLA4 EC humano (sin LP) (secuencia de aminoácido) (ID DE SEC NO __)

1290
1301

AMHVAQPAVVLASSRGIASFVCEYASPGKATEVRVTVLRQADSQVTEVCAATYM
TGNELTFLDDSICTGTSSGNQVNLTIQGLRAMDTGLYICEZVELMYPPPYYLGIGNG
TQIYVIDPEPCPDS

<u>CTLA4 IgG MTH (SSS) MTCH2CH3 humano</u> (secuencia de nucleótido) (ID DE SEC NO _)

atgggggtactgctcacacagaggacgctgctcagtctggtccttgcactcctgtttccaagcatggcgagcatggcaatgcacgt
ggcccagcctgctgtggtactggccagcagccgaggcatcgccagctttgtgtgtgagtatgcatctccaggcaaagccactgag
gtccgggtgacagtgcttcggcaggctgacagccaggtgactgaagtctgtgcggcaacctacatgatggggaatgagttgacct
tcctagatgattccatctgcacgggcacctccagtggaaatcaagtgaacctcactatccaaggactgagggccatggacacggg
actctacatctgcaaggtggagctcatgtacccaccgccatactacctgggcataggcaacggaacccagatttatgtaattgatcc
agaaccgtgcccagattctgatcaacccaaatcttctgacaaaactcacacatccccaccgtccccagcacctgaactcctggggg
gatcgtcagtcttcctcttcccccaaaacccaaggacaccctcatgatctcccggaccccctgaggtcacatgcgtggtggtggac
gtgagccacgaagaccctgaggtcaagttcaactggtacgtggacggcgtggaggtgcataatgccaagacaaagccgcggga
ggagcagtacaacagcacgtaccgtgtggtcagcgtcctcaccgtcctgcaccaggactggctgaatggcaaggagtacaagtg
caaggtctccaacaaagccctcccagccccatcgagaaaacaatctccaaagccaaagggcagccccgagaaccacaggtgt
acaccctgcccccatcccgggatgagctgaccaagaaccaggtcagcctgacctgcctggtcaaaggcttctatcccagcgacat
cgccgtggagtgggagagcaatgggcagccggagaacaactacaagaccacgcctcccgtgctggactccgacggctccttctt
cctctacagcaagctcaccgtggacaagagcaggtggcagcaggggaacgtcttctcatgctccgtgatgcatgaggctctgcac
aaccactacacgcagaagagcctctccctgtctccgggtaaatga

<u>CTLA4 IgG MTH (SSS) MTCH2CH3 humano</u> (secuencia de aminoácido) (SEQ ID NO _)

MGVILLTQRTLLSLVLALLFPSMASMAMHVAQPAVVLASSRGIASFVCEYASPGK
ATEVRVTVLRQADSQVTEVCAATYMMGNELTFLDDSICTGTSSGNQVNLTIQGLR
AMDTGLYICKVELMYPPPYYLGIGNGTQIWIDPEPCPDSDQPKSSDKTHTSPPSPAP
ELLGGSSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNA
KTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQ
PREPQVYTLPPSRDELTKNQVSLTCLKGFYPSDIAVEWESNGQPENNYKTTPPVL
DSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

CTLA-4 IgAH IgACH2CH3 (oncoMLP-CTLA4EC-IgA humano) (secuencia de nucleótido) (ID DE SEC NO _)

1297
1302

atggggtactgctcacacagaggacgctgctcagtctggtccttgcactcctgtttccaagcatggcgagcatggcaatgcacgt
ggcccagcctgctgtggtactggccagcagccgaggcatcgccagctttgtgtgtgagtatgcatctccaggcaaagccactgag
gtccgggtgacagtgcttcggcaggctgacagccaggtgactgaagtctgtgcggcaacctacatgatggggaatgagttgacct
tcctagatgattccatctgcacgggcacctccagtggaaatcaagtgaacctcactatccaaggactgagggccatggacacggg
actctacatctgcaaggtggagctcatgtacccaccgccatactacctgggcataggcaacggaacccagatttatgtaattgatcc
agaaccgtgcccagattctgatcagccagttccctcaactccacctaccccatctccctcaactccacctaccccatctccctcatgct
gccacccccgactgtcactgcaccgaccggccctcgaggacctgctcttaggttcagaagcgatcctcacgtgcacactgaccgg
cctgagagatgcctcaggtgtcaccttcacctggacgccctcaagtgggaagagcgctgttcaaggaccacctgaccgtgacctct
gtggctgctacagcgtgtccagtgtcctgccgggctgtgccgagccatggaaccatgggaagaccttcacttgcactgctgcctac
cccgagtccaagaccccgctaaccgccaccctctcaaaatccggaaacacattccggcccgaggtccacctgctgccgccgccg
tcggaggagctggccctgaacgagctggtgacgctgacgtgcctggcacgtggcttcagccccaaggatgtgctggttcgctgg
ctgcaggggtcacaggagctgccccgcgagaagtacctgacttgggcatcccggcaggagcccagccagggcaccaccaccctt
cgctgtgaccagcatactgcgcgtggcagccgaggactggaagaaggggacaccttctcctgcatggtgggccacgaggccc
tgccgctggccttcacacagaagaccatcgaccgcttggcgggtaaacccacccatgtcaatgtgtctgttgtcatggcggaggtg
gacggcacctgctactgataatctaga

CTLA-4 IsAH lgACH2CH3 (secuencia de aminoácido) (ID DE SEC NO __)

MGVLLTQRTLLSLVLALLFPSMASMAMHVAQPAVVLASSRGIASFVCEYASPGKA
TEVRVTVLRQADSQVTEVCAATYMMGNELTFLDDSICTGTSSGNQVNLTIQGLRA
MDTGLYICKVELMYPPPYYLGIGNGTQIYVIDPEPCPDSDQPVPSTPPTPSPSTPPTP
SPSCCMPRLSLHRPALEDLLLGSEAILTCTLTGLRDASGVTFTWTPSSGKSAVQGPP
DRDLCGCYSVSSVLPGCAEPWNHGKTFTCTAAYPESKTPLTATLSKSGNTFRPEVH
LLPPPSEELALNELVTLTCLARGFSPKDVLVRWLQGSQELPREKYLTWASRQEPSQ
GTTTFAVTSILRV AAEDWKKGDTFSCMVGHEALPLAFTQKTIDRLAGKPTHVNVS
VVMAEVDGTCY

CTLA-4 IsAH IgA-T4 (oncoMLP-CTLA4EC-IgA3Tl humano) (secuencia de nucleótido) (ID de SEC NO)

atggggtactgctcacacagaggacgctgctcagtctggtccttgcactcctgtttccaagcatgggagcatggcaatgcacgt
ggcccagcctgctgtggtactggccagcagccgaggcatcgccagctttgtgtgtgagtatgcatctccaggcaaagccactgag
gtccgggtgacagtgcttcggcaggctgacagccaggtgactgaagtctgtgcggcaacctacatgatggggaatgagttgacct
tcctagatgattccatctgcacgggcacctccagtggaaatcaagtgaacctcactatccaaggactgagggccatggacacggg
actctacatctgcaaggtggagctcatgtacccaccgccatactacctgggcataggcaacggaacccagatttatgtaattgatcc
agaaccgtgcccagattctgatcagccagttccctcaactccacctaccccatctccctcaactccacctaccccatctccctcatgct

1292
1303

gccacccccgactgtcactgcaccgaccggccctcgaggacctgctcttaggttcagaagcgatcctcacgtgcacactgaccgg
cctgagagatgcctcaggtgtcaccttcacctggacgccctcaagtgggaagagcgctgttcaaggaccacctgaccgtgacctct
gtggctgctacagcgtgtccagtgtcctgccgggctgtgccgagccatggaaccatgggaagaccttcacttgcactgctgcctac
cccgagtccaagaccccgctaaccgccaccctctcaaaatccggaaacacattccggcccgaggtccacctgctgccgccgccg
tcggaggagctggccctgaacgagctggtgacgctgacgtgcctggcacgtggcttcagccccaaggatgtgctggttcgctgg
ctgcaggggtcacaggagctgccccgcgagaagtacctgacttgggcatcccggcaggagcccagccagggcaccaccaccctt
cgctgtgaccagcatactgcgcgtggcagccgaggactggaagaaggggacaccttctcctgcatggtgggccacgaggccc
tgccgctggccttcacacagaagaccatcgaccgcttggcgggtaaacccacccatgtcaatgtgtctgttgtcatggcggaggtg
gactgataatctaga

CTLA-4 Ig A-T4 (secuencia de aminoácido) (ID DE SEC NO __)

MGVLLTQRTLLSLVLALLFPSMASMAMHVAQPAVVLASSRGIASFVCEYASPGKA
TEVRVTVLRQADSQVTEVCAATYMMGNELTFLDDSICTGTSSGNQVNLTIQGLRA
MDTGLYICKVELMYPPPYYLGIGNGTQIYVIDPEPCPDSDQPVPSTPPTPSPSTPPTP
SPSCCHPRLSLHRPALEDLLLGSEAILTCTLTGLRDASGVTFTWTPSSGKSAVQGPP
DRDLCGCYSVSSVLPGCAEPWNHGKTFTCTAAYPESKTPLTATLSKSGNTFRPEVH
LLPPPSEELALNELVTLTCLARGFSPKDVLVRWLQGSQELPREKYLTWASRQEPSQ
GTTTFAVTSILRVAAEDWKKGDTFSCMVGHEALPLAFTQKTIDRLAGKPTHVNVS
VVMAEVD

IsG1 CH2 humano con 238 mutación pro→ser (secuencia de nucleótido) (ID DE SEC NO __)

cctgaactcctgggggga tcgtcagtcttcctcttccccccaaaacccaaggacaccctcatgatctcccggacccctgaggtcac
atgcgtggtggtggacgtgagccacgaagaccctgaggtcaagttcaactggtacgtggacggcgtggaggtgcataatgccaa
gacaaagccgcgggaggagcagtacaacagcacgtaccgtgtggtcagcgtcctcaccgtcctgcaccaggactggctgaatg
gcaaggagtacaagtgcaaggtctccaacaaagccctcccagcccccatcgagaaaaccatctccaaagccaaag

IgG1 CH2 humano con 238 mutation pro→ser (secuencia de aminoácido) (ID DE SEC NO __)

PELLGGSSVFLFPPKPKDTLMISRTPEVTCVWDVSHEDPEVKFNWYVDGVEVHN
AKTKPRFEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNXALPAPIEKTISKAK

1293
1304

Amino acidos que rodean Pro a Ser en CH2 (secuencia de aminoácido) (ID DE SEC NO __)

PAPELLGGPS

Amino acidos que rodean Pro a Ser en CH2 (secuencia de aminoácido) (ID DE SEC NO __)

PAPELLGGSS

hIgE3BB (deja una estructura abierta en el exremo del gen para leer dentro del dominio de cola citoplásmico y de transmembrana adherido a los BamHI o SfuI) (secuencia de nucleótido) (ID DE SEC NO __)

gtt gtt ttc gaa gga tcc gct tta ccg gga ttt aca gac acc gct cgc tgg

IgE Fc (CH2-CH3-CH4) ORF humano (secuencia de nucleótido) (ID DE SEC NO __)

tgatcacgtctgctccagggacttcaccccgcccaccgtgaagatcttacagtcgtcctgcgacggcggcgggcacttccccccg
accatccagctcctgtgcctcgtctcgggtacaccccagggactatcaacatcacctggctggaggacgggcaggtcatggacg
tggacttgtccaccgcctctaccacgcaggaggggtgagctggcctccacacaaagcgagctcaccctcagccagaagcactggc
tgtcagaccgcacctacacctgccaggtcacctatcaaggtcacaccttgaggacagcaccaagaagtgtgcagattccaaccc
gagaggggtgagcgcctacttaagccggccagccccgttcgacctgtccatccgcaagtcgcccacgatcacctgtctggtggtg
gacctggcacccagcaaggggaccgtgaacctgacctggtcccgggccagtgggaagcctgtgaaccatccaccagaaagg
aggagaagcagcgcaatggcacgttaccgtcacgtccaccctgccggtgggcacccgagactggatcgaggggaagaccta
ccagtgcagggtgacccaccccccacctgcccagggccctcatgcggtccacgaccaagaccagcggccccgcgtgctgcccg
gaagtctatgcgtttgcgacgccgggagtggccggggagccggacaagcgcaccctcgcctgcctgatccagaacttcatgcct
gaggacatctcggtgcagtggctgcacaacgaggtgcagctcccggacgcccggcacagcacgacgcagccccgcaagacc
aagggctccggcttcttcgtcttcagccgcctggaggtgaccagggccgaatgggagcagaaagatgagttcatctgccgtgcag
tccatgaggcagcgagccctcacagaccgtccagcgagcggtgtctgtaaatccggtaaagcggatcctcgaa

IgE Fc (CH2-CH3-CH4) ORF humano (secuencia de aminoácido) (ID DE SEC NO __)

DHVCSRDFTPPTVKILQSSCDGGGHFPPTIQLLCLVSGYTPGTINITWLEDGQVMDV
DLSTASTTQEGELASTQSELTLSQKHWLSDRTYTCQVTYQGHTFEDSTKKCADSN
PRGVSAYLSRPSPFDLFIRKSPTITCLVVDLAPSKGTVNLTWSRASGKPVNHSTRKE

1294
1305

EKQRNGTLTVTSTLPVGTRDWIEGETYQCRVTHPHLPRALMRSTTKTSGPRAAPE
VYAFATPEWPGSRDILRTLACLIQNFMPEDISVQWLHNEVQLPDARHSTTQPRKTK
GSGFFVFSRLEVTRAEWEQKDEFICRAVHEAASPSQTVQRAVSVNPGKADPS

IFhIgGwtBcl5 (secuencia de nucleótido) (ID DE SEC NO __)

gtt gtt tga tea gga gcc caa atc ttg tga caa aac tca cac atg ccc acc gtg ccc agc acc (63 mer)

HuIgGMHWC (sentido, iniciador 5' para mutación de articulación tipo silvestre CCC para SSS mutante) (secuencia de nucleótido) (ID DE SEC NO __)

gtt gtt gat cag gag ccc aaa tct tct gac aaa act cac aca tct cca ccg tcc cca gca cct gaa ctc ctg ggt gga ccg tca gtc ttc c

1D8 VH (secuencia de nucleótido) (ID DE SEC NO __

caggtgcagctgaaggaggcaggacctggcctggtgcaaccgacacagaccctgtccctcacatgcactgtctctgggttctcatt
aaccagcgatggtgtacactggattcgacagcctccaggaaagggtctggaatggatgggaataatatattatgatggaggcaca
gattataattcagcaattaaatccagactgagcatcagcagggacacctccaagagccaagttttcttaaaaatcaacagtctgcaaa
ctgatgacacagccatgtattactgtgccagaatccactttgattactggggccaaggagtcatggtcacagtctcctct

1D8 VH (sin líder) (secuencia de aminoácido) (ID DE SEC NO __)

QVQLKJBAGPGLVQPTQTLSLTCTVSGFSLTSDGVHWIRQPPGKGLEWMGUYYDG
GTDYNSAIKSKLSISRDTSKSQVFLKINSLQTDDTAMYYCARIHFDYWGQGVMVT
VSS

1D8 VL (sin leader) (secuencia de nucleótido) (ID DE SEC NO __)

gacattgtgctcactcagtctccaacaaccatagctgcatctccaggggagaaggtcaccatcacctgccgtgccagctccagtgt
aagttacatgtactggtaccagcagaagtcaggcgcctcccctaaactctggatttatgacacatccaagctggcttctggagttcca
aatcgcttcagtggcagtgggtctgggacctcttattctctcgcaatcaacaccatggagactgaagatgctgccacttattactgtca
gcagtggagtagtactccgctcacgttcgggtctgggaccaagctggagatcaaacgg

1D8 VL (secuencia de aminoácido) (ID DE SEC NO __)

1295
1306

DIVLTQSPTTIAASPGEKVTITCRASSSVSYMYWYQQKSGASPKLWIYDTSKLASG
VPNRFSGSGSGTSYSLAINTMETEDAATYYCQQWSSTPLTFGSGTKLEIKR

1D8 scFv (secuencia de nucleótido) (ID DE SEC NO __)

aagcttatggattttcaagtgcagattttcagcttcctgctaatcagtgcttcagtcataatgtccagaggagtcgacattgtgctcactc
agtctccaacaaccatagctgcatctccaggggagaaggtcaccatcacctgccgtgccagctccagtgtaagttacatgtactggt
accagcagaagtcaggcgcctcccctaaactctggatttatgacacatccaagctggcttctggagttccaaatcgcttcagtggca
gtgggtctgggacctcttattctctcgcaatcaacaccatggagactgaagatgctgccacttattactgtcagcagtggagtagtact
ccgctcacgttcgggtctgggaccaagctggagatcaaacggggtggcggtggctcgggcggtggtgggtcgggtggcggcg
gatctcaggtgcagctgaaggaggcaggacctggcctggtgcaaccgacacagaccctgtccctcacatgcactgtctctgggtt
ctcattaaccagcgatggtgtacactggattcgacagcctccaggaaagggtctggaatggatgggaataatatattatgatggagg
cacagattataattcagcaattaaatccagactgagcatcagcagggacacctccaagagccaagttttcttaaaaatcaacagtctg
caaactgatgacacagccatgtattactgtgccagaatccactttgattactggggccaaggagtcatggtcacagtctcctctgatca

1D8 scFv (secuencia de aminoácido) (ID DE SEC NO __)

MDFQVQIFSFLLISASVIMSRGVDIVLTQSPTTIAASPGEKVTITCRASSSVSYMYWY
QQKSGASPKLWIYDTSKLASGVPNRFSGSGSGTSYSLAINTMETEDAATYYCQQW
SSTPLTFGSGTKLEIKRGGGGSGGGGSGGGGSQVQLKEAGPGLVQPTQTLSLTCTV
SGFSLTSDGVHWIRQPPGKGLEWMGIIYYDGGTDYNSAIKSRLSISRDTSKSQVFLK
INSLQTDDTAMYYCARIHFDYWGQGVMVTVSS

1D8 scFv IsG WTH WTCH2CH3 (secuencia de nucleótido) (ID DE SEC NO __)

aagcttatggattttcaagtgcagattttcagcttcctgctaatcagtgcttcagtcataatgtccagaggagtcgacattgtgctcactc
agtctccaacaaccatagctgcatctccaggggagaaggtcaccatcacctgccgtgccagctccagtgtaagttacatgtactggt
accagcagaagtcaggcgcctcccctaaactctggatttatgacacatccaagctggcttctggagttccaaatcgcttcagtggca
gtgggtctgggacctcttattctctcgcaatcaacaccatggagactgaagatgctgccacttattactgtcagcagtggagtagtact
ccgctcacgttcgggtctgggaccaagctggagatcaaacggggtggcggtggctcgggcggtggtgggtcgggtggcggcg
gatctcaggtgcagctgaaggaggcaggacctggcctggtgcaaccgacacagaccctgtccctcacatgcactgtctctgggtt
ctcattaaccagcgatggtgtacactggattcgacagcctccaggaaagggtctggaatggatgggaataatatattatgatggagg
cacagattataattcagcaattaaatccagactgagcatcagcagggacacctccaagagccaagttttcttaaaaatcaacagtctg
caaactgatgacacagccatgtattactgtgccagaatccactttgattactggggccaaggagtcatggtcacagtctcctctgatc
aggagcccaaatcttgtgacaaaactcacacatgcccaccgtgcccagcacctgaactcctgggggggaccgtcagtcttcctcttc
ccccaaaacccaaggacaccctcatgatctcccggaccccctgaggtcacatgcgtggtggtggacgtgagccacgaagaccct

1296
1307

gaggtcaagttcaactggtacgtggacggcgtggaggtgcataatgccaagacaaagccgcgggaggagcagtacaacagcac
gtaccgtgtggtcagcgtcctcaccgtcctgcaccaggactggctgaatggcaaggagtacaagtgcaaggtctccaacaaagcc
ctcccagccccatcgagaaaacaatctccaaagccaaagggcagccccgagaaccacaggtgtacaccctgcccccatcccg
ggatgagctgaccaagaaccaggtcagcctgacctgcctggtcaaaggcttctatcccagcgacatcgccgtggagtgggagag
caatgggcagccggagaacaactacaagaccacgcctcccgtgctggactccgacggctccttcttcctctacagcaagctcacc
gtggacaagagcaggtggcagcaggggaacgtcttctcatgctccgtgatgcatgaggctctgcacaaccactacacgcagaag
agcctctccctgtctccgggtaaatgatctaga

1D8 scFv IsG WTH WTCH2CH3 (secuencia de aminoácido) (ID DE SEC NO __)

MDFQVQIFSFLLISASVIMSRGVDIVLTQSPTTIAASPGEKVTITCRASSSVSYMYWY
QQKSGASPKLWIYDTSKLASGVPNRFSGSGSGTSYSLAINTMETEDAATYYCQQW
SSTPLTFGSGTKLEIKRGGGGSGGGGSGGGGSQVQLKEAGPGLVQPTQTLSLTCTV
SGFSLTSDGVHWIRQPPGKGLEWMGIIYYDGGTDYNSAIKSRLSISRDTSKSQVFLK
INSLQTDDTAMYYCARIHFDYWGQGVMVTVSSDQEPKSCDKTHTCPPCPAPELLG
GPSVFLFPPKPKDTLMISRTPEVTCWVDVSHEDPEVKFNWYVDGVEVHNAKTKP
REEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREP
QVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDG
SFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

1D8 scFv leG MTH MTCH2CH3-CD80 (secuencia de nucleótido) (ID DE SEC NO __)

aagcttatggattttcaagtgcagattttcagcttcctgctaatcagtgcttcagtcataatgtccagaggagtcgacattgtgctcactc
agtctccaacaaccatagctgcatctccaggggagaaggtcaccatcacctgccgtgccagctccagtgtaagttacatgtactggt
accagcagaagtcaggcgcctccctaaactctggatttatgacacatccaagctggcttctggagttccaaatcgcttcagtggca
gtgggtctgggacctcttattctctcgcaatcaacaccatggagactgaagatgctgccacttattactgtcagcagtggagtagtact
ccgctcacgttcgggtctgggaccaagctggagatcaaacggggtggcggtggctcgggcggtggtgggtcgggtggcggcg
galctcaggtgcagctgaaggaggcaggacctggcctggtgcaaccgacacagaccctgtccctcacatgcactgtctctgggtt
ctcattaaccagcgatggtgtacactggattcgacagcctccaggaaagggtctggaatggatgggaataatatattatgatggagg
cacagattataattcagcaattaaatccagactgagcatcagcagggacacctccaagagccaagttttcttaaaaatcaacagtctg
caaactgatgacacagccatgtattactgtgccagaatccactttgattactggggccaaggagtcatggtcacagtctcctctgatct
ggagcccaaatcttctgacaaaactcacacaagcccaccgagcccagcacctgaactcctgggggggatcgtcagtcttcctcttcc
ccccaaaacccaaggacaccctcatgatctcccggaccccctgaggtcacatgcgtggtggtggacgtgagccacgaagaccctg
aggtcaagttcaactggtacgtggacggcgtggaggtgcataatgccaagacaaagccgcgggaggagcagtacaacagcacg
taccgtgtggtcagcgtcctcaccgtcctgcaccaggactggctgaatggcaaggagtacaagtgcaaggtctccaacaaagccc
tcccagccccatcgagaaaaccatctccaaagccaaagggcagccccgagaaccacaggtgtacaccctgcccccatcccgg
gatgagctgaccaagaaccaggtcagcctgacctgcctggtcaaaggcttctatcccagcgacatcgccgtggagtgggagagc

1297
1308

aatgggcagccggagaacaactacaagaccacgcctcccgtgctggactccgacggctccttcttcctctacagcaagctcaccg
tggacaagagcaggtggcagcaggggaacgtcttctcatgctccgtgatgcatgaggctctgcacaaccactacacgcagaaga
gcctctccctgtctccgggtaaagcggatccttcgaacctgctcccatcctgggccattaccttaatctcagtaaatggaatttttgtga
tatgctgcctgacctactgctttgccccaagatgcagagagagaaggaggaatgagagattgagaagggaaagtgtacgccctgt
ataaatcgata

1D8 scFv IgG MTH MTCH2CH3-CD80 (secuencia de ammoácido) (ID DE SEC NO __)

MDFQVQIFSFLLISASVIMSRGVDIVLTQSPTTIAASPGEKVTITCRASSSVSYMYWY
QQKSGASPKIWIYDTSKLASGVPNRFSGSGSGTSYSLAINTMETEDAATYYCQQW
SSTPLTFGSGTKLEIKRGGGGSGGGGSGGGGSQVQLKEAGPGLVQPTQTLSLTCTV
SGFSLTSDGVHWIRQPPGKGLEWMGIIYYDGGTDYNSAIKSRLSISRDTSKSQVFLK
INSLQTDDTAMYYCARIHFDYWGQGVMVTVSSDLEPKSSDKTHTSPPSPAPELLG
GSSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKP
REEQYNSTYRVVSVLTVLHQDWLNGKFEYKCKVSNKATPAPIEKTISKAKGQPRE
PQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSD
GSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGKADPSNLLPS
WAITLISVNGIFVICCLTYCFAPRCRERRRNERLRRESVRPV

1D8 scFv IgG WTH WTCH2CH3-CD80 (secuencia de nucleótido) (SEQ ID NO __)

aagcttatggattttcaagtgcagattttcagcttcctgctaatcagtgcttcagtcataatgtccagaggagtcgacattgtgctcactc
agtctccaacaaccatagctgcatctccaggggagaaggtcaccatcacctgccgtgccagctccagtgtaagttacatgtactggt
accagcagaagtcaggcgcctccctaaactctggatttatgacacatccaagctggcttctggagttccaaatcgcttcagtggca
gtgggtctgggacctcttattctctcgcaatcaacaccatggagactgaagatgctgccacttattactgtcagcagtggagtagtact
ccgctcacgttcgggtctgggaccaagctggagatcaaacggggtggcggtggctcgggcggtggtgggtcgggtggcggcg
gatctcaggtgcagctgaaggaggcaggacctggcctggtgcaaccgacacagaccctgtccctcacatgcactgtctctgggtt
ctcattaaccagcgatggtgtacactggattcgacagcctccaggaaagggtctggaatggatgggaataatatattatgatggagg
cacagattataattcagcaattaaatccagactgagcatcagcagggacacctccaagagccaagttttcttaaaaatcaacagtctg
caaactgatgacacagccatgtattactgtgccagaatccactttgattactggggccaaggagtcatggtcacagtctcctctgatct
ggagcccaaatcttgtgacaaaactcacacatgcccaccgtgcccagcacctgaactcctgggggggaccgtcagtcttcctcttcc
ccccaaaacccaaggacaccctcatgatctcccggaccccctgaggtcacatgcgtggtggtggacgtgagccacgaagaccctg
aggtcaagttcaactggtacgtggacggcgtggaggtgcataatgccaagacaaagccgcgggaggagcagtacaacagcacg
taccgtgtggtcagcgtcctcaccgtcctgcaccaggactggctgaatggcaaggagtacaagtgcaaggtctccaacaaagccc
tcccagcccccatcgagaaaaccatctccaaagccaaagggcagccccgagaaccacaggtgtacaccctgcccccatcccgg

1298
1309

gatgagctgaccaagaaccaggtcagcctgacctgcctggtcaaaggcttctatcccagcgacatcgccgtggagtgggagagc
aatgggcagccggagaacaactacaagaccacgcctcccgtgctggactccgacggctccttcttcctctacagcaagctcaccg
tggacaagagcaggtggcagcaggggaacgtcttctcatgctccgtgatgcatgaggctctgcacaaccactacacgcagaaga
gcctctccctgtctccgggtaaagcggatccttcgaacctgctcccatcctgggccattaccttaatctcagtaaatggaatttttgtga
tatgctgcctgacctactgctttgccccaagatgcagagagagaaggaggaatgagagattgagaagggaaagtgtacgccctgt
ataaatcgata

1D8 scFv IgG WTH WTCH2CH3-CD80 (secuencia de aminoácido) (ID DE SEC NO __)

MDFQVQIFSFLLISASVIMSRGVDIVLTQSPTTIAASPGEKVTITCRASSSVSYMYWY
QQKSGASPKLWIYDTSKLASGVPNRFSGSGSGTSYSLAINTMETEDAATYYCQQW
SSTPLTFGSGTKLEIKRGGGGSGGGGSGGGGSQVQLKESGPGLVQPTQTLSLTCTV
SGFSLTSDGVHWIRQPPGKGLEWMGIIYYDGGTDYNSAIKSRLSISRDTSKSQVFLK
INSLQTDDTAMYYCARIHFDYWGQGVMVTVSSDLEPKSCDKTHTCPPCPAPELLG
GPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKP
REEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREP
QVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDG
SFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGKADPSNLLPSW
AITLISVNGIFVICCLTYCFAPRCRERRRNERLRRESVRPV

CD3 scFv WTH WTCH2CH3 anti-humano (secuencia de nucleótido) (ID DE SEC NO __)

aagcttatggattttcaagtgcagattttcagcttcctgctaatcagtgcttcagtcataatgtccagaggagtcgacatccagatgaca
cagactacatcctccctgtctgcctctctgggagacagagtcaccatcagttgcagggcaagtcaggacattcgcaattatttaaact
ggtatcagcagaaaccagatggaactgttaaactcctgatctactacacatcaagattacactcaggagtcccatcaaggttcagtg
gcagtgggtctggaacagattattctctcaccattgccaacctgcaaccagaagatattgccacttacttttgccaacagggtaatac
gcttccgtggacgttcggtggaggcaccaaactggtaaccaaacgggagctcggtggcggtggctcgggcggtggtgggtcgg
gtggcggcggatctatcgatgaggtccagctgcaacagtctggacctgaactggtgaagcctggagcttcaatgtcctgcaaggc
ctctggttactcattcactggctacatcgtgaactggctgaagcagagccatggaaagaaccttgagtggattggacttattaatccat
acaaaggtcttactacctacaaccagaaattcaagggcaaggccacattaactgtagacaagtcatccagcacagcctacatggag
ctcctcagtctgacatctgaagactctgcagtctattactgtgcaagatctgggtactatggtgactcggactggtacttcgatgtctgg
ggcgcagggaccacggtcaccgtctcctctgatcaggagcccaaatcttgtgacaaaactcacacatgcccaccgtgcccagcac
ctgaactcctgggggggaccgtcagtcttcctcttcccccaaaacccaaggacaccctcatgatctcccggaccccctgaggtcaca
tgcgtggtggtggacgtgagccacgaagaccctgaggtcaagttcaactggtacgtggacggcgtggaggtgcataatgccaag

1310

acaaagccgcgggaggagcagtacaacagcacgtaccgtgtggtcagcgtcctcaccgtcctgcaccaggactggctgaatgg
caaggagtacaagtgcaaggtctccaacaaagccctcccagccccccatcgagaaaaacaatctccaaagccaaagggcagcccc
gagaaccacaggtgtacaccctgccccccatcccgggatgagctgaccaagaaccaggtcagcctgacctgcctggtcaaaggct
tctatcccagcgacatcgccgtggagtgggagagcaatgggcagccggagaacaactacaagaccacgcctcccgtgctggac
tccgacggctccttcttcctctacagcaagctcaccgtggacaagagcaggtggcagcaggggaacgtcttctcatgctccgtgat
gcatgaggctctgcacaaccactacacgcagaagagcctctccctgtctccgggtaaatgatctaga

CD3 scFv WTH WTCH2CH3 Anti-humano (secuencia de aminoácido) (ID DE SEC NO __)

MDFQVQIFSFLLISASVIMSRGVDIQMTQTTSSLSASLGDRVTISCRASQDIRNYLN
WYQQKPDGTVKLLIYYTSRLHSGVPSRFSGSGSGTDYSLTIANLQPEDIATYFCQQ
GNTLPWTFGGGTKLVTKRELGGGGSGGGGSGGGGSIDEVQLQQSGPELVKPGAS
MSCKASGYSFTGYTVNWLKQSHGKNLEWIGLINPYKGLTTYNQKFKGKATLTVD
KSSSTAYMELLSLTSEDSAVYYCARSGYYGDSDWYFDVWGAGTTVTVSSDQEPK
SCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKF
NWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKA
LPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESN
GQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQ
KSLSLSPGK

2H7-antiCD40 scFv MTH (SSS) MTCH2WTCH3 (2h7-40 2 220Ig + sitios de restricción) (secuencia de nucleótido) (ID DE SEC NO __)

aagcttgccgccatggattttcaagtgcagattttcagcttcctgctaatcagtgcttcagtcataattgccagaggacaaattgttctct
cccagtctccagcaatcctgtctgcatctccaggggagaaggtcacaatgacttgcagggccagctcaagtgtaagttacatgcact
ggtaccagcagaagccaggatcctccccaaaccctggatttatgccccatccaacctggcttctggagtccctgctcgcttcagtg
gcagtgggtctgggacctcttactctctcacaatcagcagagtggaggctgaagatgctgccacttattactgccagcagtggagttt
taacccacccacgttcggtgctgggaccaagctggagctgaaaggtggcggtggctcgggcggtggtggatctggaggaggtg
ggagctctcaggcttatctacagcagtctggggctgagctggtgaggcctggggcctcagtgaagatgtcctgcaaggcttctggc
tacacatttaccagttacaatatgcactgggtaaagcagacacctagacagggcctggaatggattggagctatttatccaggaaat
ggtgatacttcctacaatcagaagttcaagggcaaggccacactgactgtagacaaatcctccagcacagcctacatgcagctcag
cagcctgacatctgaagactctgcggtctatttctgtgcaagagtggtgtactatagtaactcttactggtacttcgatgtctggggcac
agggaccacggtcaccgtctcttctgatcaatccaactctgaagaagcaaagaaagaggaggccaaaaaggaggaagccaaga
aatctaacagcgtcgacattgttctgactcagtctccagccaccctgtctgtgactccaggagatagagtctctctttcctgcagggcc
agccagagtattagcgactacttacactggtatcaacaaaaatcacatgagtctccaaggcttctcatcaaatatgcttcccattccatc

1300
1311

tctgggatcccctccaggttcagtggcagtggatcagggtcagatttcactctcagtatcaacagtgtggaacctgaagatgttggaa
tttattactgtcaacatggtcacagctttccgtggacgttcggtggaggcaccaagctggaaatcaaacggggtggcggtggctcg
ggcggaggtgggtcgggtggcggcggatctcagatccagttggtgcaatctggacctgagctgaagaagcctggagagacagt
caggatctcctgcaaggcttctgggtatgccttcacaactactggaatgcagtgggtgcaagagatgccaggaaagggtttgaagt
ggattggctggataaacaccccactctggagtgccaaaatatgtagaagacttcaaggacggtttgccttctctttggaaacctctgc
caacactgcatatttacagataagcaacctcaaagatgaggacacggctacgtatttctgtgtgagatccgggaatggtaactatga
cctggcctactttgcttactggggccaagggacactggtcactgtctctgatcaggagcccaaatcttctgacaaaactcacacatcc
ccaccgtccccagcacctgaactcctgggggggatcgtcagtcttcctcttcccccaaaacccaaggacaccctcatgatctcccg
gacccctgaggtcacatgcgtggtggtggacgtgagccacgaagaccctgaggtcaagttcaactggtacgtggacggcgtgga
ggtgcataatgccaagacaaagccgcgggaggagcagtacaacagcacgtaccgtgtggtcagcgtcctcaccgtcctgcacca
ggacgctgaatggcaaggagtacaagtgcaaggtctccaacaaagccctcccagccccatcgagaaaacaatctccaaagc
caaagggcagccccgagaaccacaggtgtacaccctgccccatcccgggatgagctgaccaagaaccaggtcagcctgacct
gcctggtcaaaggcttctatcccagcgacatcgccgtggagtgggagagcaatgggcagccggagaacaactacaagaccacg
cctcccgtgctggactccgacggctccttcttcctctacagcaagctcaccgtggacaagagcaggtggcagcaggggaacgtct
tctcatgctccgtgatgcatgaggctctgcacaaccactacacgcagaagagcctctccctgtctccgggtaaatgatctaga

2H7-antiCD40 scFv MTH (SSS) MTCH2WTCH3 (2H7-40 2-220Ig) (secuencia de aminoácido) (ID DE SEC NO __)

MDPQVQIFSFLLISASVIIARGQIVLSQSPAILSASPGEKVTMTCRASSSVSYMHWY
QQKPGSSPKPWIYAPSNLASGVPARFSGSGSGTSYSLTISRVEAEDAATYYCQQWS
FNPPTFGAGTKLELKGGGGSGGGGSGGGGSSQAYLQQSGAELVRPGASVKMSCK
ASGYTFTSYNMHWVKQTPRQGLEWIGAIYPGNGDTSYNQKFKGKATLTVDKSSS
TAYMQLSSLTSEDSAVYFCARVVYYSNSYWYFDVWGTGTTVTVSSDQSNSEEAK
KEEAKKEEAKKSNSVDIVLTQSPATLSVTPGDRVSLSCRASQSISDYLHWYQQKSH
ESPRLLIKYASHSISGIPSRFSGSGSGSDFTLSINSVEPEDVGIYYCQHGHSFPWTFGG
GTKLEIKRGGGGSGGGGSGGGGSQIQLVQSGPELKKPGETVRISCKASGYAFTTTG
MQWVQEMPGKGLKWIGWINTPLWSAKICRRLQGRFAFSLETSANTAYLQINNLKD
EDTATYFCVRSGNGNYDLAYFAYWGQGTLVTVSDQEPKSSDKTHTSPPSPAPELL
GGSSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTK
PREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPRE
PQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSD
GSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

5B9 VH (incluye el VH péptido líder) (secuencia de nucleótido) (ID DE SEC NO __)

1307
1312

atggctgtcttggggctgctcttctgcctggtgacatttccaagctgtgtcctatcccaggtgcagctgaagcagtcaggacctggcc
tagtgcagtcctcacagagcctgtccatcacctgcacagtctctggtttctcattaactacctatgctgtacactgggttcgccagtctc
caggaaagggtctggagtggctgggagtgatatggagtggtggaatcacagactataatgcagctttcatatccagactgagcatc
accaaggacgattccaagagccaagttttctttaaaatgaacagtctgcaacctaatgacacagccatttattactgtgccagaaatg
ggggtgataactacccttattactatgctatggactactggggtcaaggaacctcagtcaccgtctcctca

5B9 VH (menos el líder) (secuencia de nucleótido) (ID DE SEC NO __)

caggtgcagctgaagcagtcaggacctggcctKgtgcagtcctcacagagcctgtccatcacctgcacagtctctggtttctcatta.
actacctatgctgtacactgggttcgccagtctccaggaaagggtctggagtggctgggagtgatatggagtggtggaatcacaga
ctataatgcagctttcatatccagactgagcatcaccaaggacgattccaagagccaagttttctttaaaatgaacagtctgcaaccta
atgacacagccatttattactgtgccagaaatgggggtgataactacccttattactatgctatggactactggggtcaaggaacctca
gtcaccgtctcctca

5B9 VH (incluye péptido líder) (secuencia de aminoácido) (ID DE SEC NO __)

MAVLGLIFCLVTFPSCVLSQVQLKQSGPGLVQSSQSLSITCTVSGFSLTTYAVHWV
RQSPGKGLEWLGVIWSGGITDYNAAFISRLSITKDDSKSQVFFKMNSLQPNDTAIY
YCARNGGDNYPYYYAMDYWGQGTSVTVSS

5B9 VH (sin péptido líder) (secuencia de aminoácido) (ID DE SEC NO __)

QVQLKQSGPGLVQSSQSLSITCTVSGFSLTTYAVHWVRQSPGKGLEWLGVIWSGGI
TDYNAAFISRLSITKDDSKSQVFFKMNSLQPNDTAIYYCARNGGDNYPYYYAMDY
WGQGTSVTVSS

5B9 VL (secuencia de nucleótido) (ID DE SEC NO __)

atgaggttctctgctcagcttctggggctgcttgtgctctggatccctggatccactgcagatattgtgatgacgcaggctgcattctc
caatccagtcactcttggaacatcagcttccatctcctgcaggtctagtaagagtctcctacatagtaatggcatcacttatttgtattgg
tatctgcagaagccaggccagtctcctcagctcctgatttatcagatgtccaaccttgcctcaggagtcccagacaggttcagtagca
gtgggtcaggaactgatttcacactgagaatcagcagagtggaggctgaggatgtgggtgtttattactgtgctcaaaatctagaact
tccgctcacgttcggtgctgggaccaagctggagctgaaacgg

5B9 VL (secuencia de aminoácido) (ID DE SEC NO __)

1313

MRFSAQLLGLLVLWIPGSTADIVMTQAAFSNPVTLGTSASISCRSSKSLLHSNGITY
LYWYLQKPGQSPQLLIYQMSNLASGVPDRFSSSGSGTDFTLRISRVEAEDVGVYYC
AQNLELPLTFGAGTKLELKR

5B9 scFv (secuencia de nucleótido) (ID DE SEC NO __)

aagcttgccgccatgaggttctctgctcagcttctggggctgcttgtgctctggatccctggatccactgcagatattgtgatgacgca
ggctgcattctccaatccagtcactcttggaacatcagcttccatctcctgcaggtctagtaagagtctcctacatagtaatggcatca
cttatttgtattggtatctgcagaagccaggccagtctcctcagctcctgatttatcagatgtccaaccttgcctcaggagtcccagaca
ggttcagtagcagtgggtcaggaactgatttcacactgagaatcagcagagtggaggctgaggatgtgggtgtttattactgtgctc
aaaatctagaacttccgctcacgttcggtgctgggaccaagctggagctgaaacggggtggcggtggctcgggcggtggtgggt
cgggtggcggcggatcgtcacaggtgcagctgaagcagtcaggacctggcctagtgcagtcctcacagagcctgtccatcacct
gcacagtctctggtttctcattaactacctatgctgtacactgggttcgccagtctccaggaaagggtctggagtggctgggagtgat
atggagtggtggaatcacagactataatgcagctttcatatccagactgagcatcaccaaggacgattccaagagccaagttttcttt
aaaatgaacagtctgcaacctaatgacacagccatttattactgtgccagaaatgggggtgataactacccttattactatgctatgga
ctactggggtcaaggaacctcagtcaccgtctcctct

5B9 scFv (secuencia de aminoácido) (ID DE SEC NO __)

MRFSAQLLGLLVLWIPGSTADIVMTQAAFSNPVTLGTSASISCRSSKSLLHSNGITY
LYWYLQKPGQSPQLLIYQMSNLASGVPDRFSSSGSGTDFTLRISRVEAEDVGVYYC
AQNLELPLTFGAGTKLELIRGGGGSGGGGSGGGGSSQVQLKQSGPGLVQSSQSLS
ITCTVSGFSLTTYAVHWVRQSPGKGLEWLGVIWSGGITDYNAAFISRLSITKDDSK
SQVFFKMNSLQPNDTAIYYCARNGGDNYPYYYAMDYWGQGTSVTVSS

5B9 scFv-hmtIgG1-hCD80 (secuencia de nucleótido) (ID DE SEC NO __)

1303
1314

aagcttgccgccatgaggttctctgctcagctttctggggctgctgtgctctggatcctggatccactgcagatattgtgatgacgca
ggctgcattctccaatccagtcactcttggaacatcagcttccatctcctgcaggtctagtaagagtctcctacatagtaatggcatca
cttatttgtattggtatctgcagaagccaggccagtctcctcagctcctgatttatcagatgtccaacctgcctcaggagtcccagaca
ggttcagtagcagtgggtcaggaactgatttcacactgagaatcagcagagtggaggctgaggatgtgggtgtttattactgtgctc
aaaatctagaacttccgctcacgttcggtgctgggaccaagctggagctgaaacgggtggcggtggctcgggcggtggtgggt
cgggtggcggcggatctgtcacaggtgcagctgaagcagtcaggacctggcctagtgcagtcctcacagagcctgtccatcacct
gcacagtctctggtttctcattaactacctatgctgtacactgggttcgccagtctccaggaaagggtctggagtggctgggagtgat
atggagtggtggaatcacagactataatgcagctttcatatccagactgagcatcaccaaggacgattccaagagccaagtttttctt
aaaatgaacagtctgcaacctaatgacacagccatttattactgtgccagaaatgggggtgataactacccttattactatgctatgga
ctactggggtcaaggaacctcagtcaccgtctcctctgatctggagccaaatcttctgacaaaactcacacatgcccaccgagcc
cagcacctgaactcctgggggggatcgtcagtcttcctcttccccccaaaacccaaggacaccctcatgatctcccggacccctgag
gtcacatgcgtggtggtggacgtgagccacgaagaccctgaggtcaagttcaactggtacgtggacggcgtggaggtgcataat
gccaagacaaagccgcgcgggaggagcagtacaacagcacgtaccgtgtggtcagcgtcctcaccgtcctgcaccaggactggct
gaatggcaaggagtacaagtgcaaggtctccaacaaagccctcccagcccccatcgagaaaaccatctccaaagccaaagggc
agccccgagaaccacaggtgtacaccctgcccccatcccgggatgagctgaccaagaaccaggtcagcctgacctgcctggtca
aaggcttctatcccagcgacatcgccgtggagtgggagagcaatgggcagccggagaacaactacaagaccacgcctcccgtg
ctggactccgacggctccttcttcctctacagcaagctcaccgtggacaagagcaggtggcagcaggggaacgtcttctcatgctc
cgtgatgcatgaggctctgcacaaccactacacgcagaagagcctctccctgtctccgggtaaagcggatccttcgaacctgctcc
catcctgggccattacttaatctcagtaaatggaatttttgtgtatgtgtgcctgacctactgcttttgccccaagatgcagagagaga
aggaggaatgagagattgagaagggaaagtgtacgccctgtataaatcgatactcgag

5B9 scFv-hmtIgG1-hCD80 (secuencia de aminoácido) (ID DE SEC NO __)

MRFSAQLLGLLVLWIPGSTADIVMTQAAFSNPVTLGTSASISCRSSKSLLHSNGITY
LYWYLQKPGQSPQLLIYQMSNLASGVPDRFSSSGSGTDFTLRISRVEAEDVGVYYC
AQNLELPLTFGAGTKLELKRGGGGSGGGGSGGGGSSQVQLKQSGPGLVQSSQSLS
ITCTVSGFSLTTYAVHWVRQSPGKGLEWLGVIWSGGITDYNAAFISRLSITKDDSK
SQVFFKMNSLQPNDTAIYYCARNGGDNYPYYYAMDYWGQGTSVTVSSDLEPKSS
DKTHTSPPSPAPELLGGSSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNW
YVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKBYKCKSNKALPA
PIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQP
ENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLS
LSPGKADPSNLLPSWAITLISVNGIFVICCLTYCFAPRCRERRRNERLRRESVRPV

1304
1315

2el2 scFv WTH CH2 CH3 (2el2 scFv-WthIgG-CD80) (secuencia de nucleótido) (ID DE SEC NO __)

aagcttatggattttcaagtgcagattttcagcttcctgctaatcagtgcttcagtcataatgtccagaggagatcgacattgtgctcaccc
aatctccagcttcttttggctgtgtctctaggtcagagagccaccatctcctgcagagccagtgaaagttgtgaatattatgtcacaagtt
taatgcagtggtaccaacagaaaccaggacagccacccaaactcctcatctgctgcatccaacgtagaatctggggtccctgcc
aggtttagtggcagtgggtctgggacagacttcagcctcaacatccatcctgtggaggaggatgatattgcaatgtatttctgtcagc
aaagtaggaaggttccttggacgttcggtggaggcaccaagctggaaatcaaacgggatggcggtggctcgggcggaggtggg
tcgggtggcggcggatctcaggtgcagctgaaggagtcaggacctggcctggtggcgccctcacagagcctgtccatcacatgc
accgtctcagggttctcattaaccggctatggtgtaaactgggttcgccagcctccaggaaagggtctggagtggctgggaatgat
atggggtgatggaagcacagactataattcagctctcaaatccagactgagcatcaccaaggacaactccaagagccaagttttctt
aaaaatgaacagtctgcaaactgatgacacagccagatactactgtgccagagatggttatagtaacttttcattactatgttatggact
actggggtcaaggaacctcagtcaccgtctcctcagatctggagcccaaatcttgtgacaaaactcacacatgcccaccgtgccca
gcacctgaactcctgggggaccgtcagtcttcctcttccccccaaaacccaaggacaccctcatgatctcccggacccctgaggt
cacatgcgtggtggtggacgtgagccacgaagaccctgaggtcaagttcaactggtacgtggacggcgtggaggtgcataatgc
caagacaaagccgcgggaggagcagtacaacagcacgtaccgtgtggtcagcgtcctcaccgtcctgcaccaggactggctga
atggcaaggagtacaagtgcaaggtctccaacaaagccctcccagcccccatcgagaaaaccatctccaaagccaaagggcag
ccccgagaaccacaggtgtacaccctgcccccatcccgggatgagctgaccaagaaccaggtcagcctgacctgcctggtcaaa
ggcttctatcccagcgacatcgccgtggagtgggagagcaatgggcagccggagaacaactacaagaccacgcctcccgtgct
ggactccgacggctccttcttcctctacagcaagctcaccgtggacaagagcaggtggcagcaggggaacgtcttctcatgctcc
gtgatgcatgaggctctgcacaaccactacacgcagaagagcctctccctgtctccgggtaaagcggatccttcgaacctgctccc
atcctgggccattacttaatctcagtaaatggaatttttgtgatatgctgcctgacctactgcttgcccccaagatgcagagagagaa
ggaggaatgagagattgagaagggaaagtgtacgccctgtataaatcgat

2el2 scFv WTH CH2 CH3 (2el2 scFv-WthIgG-CD80) (secuencia de aminoácido) (ID DE SEC NO __)

MDFQVQIFSFLLISASVIMSRGVDIVLTQSPASLAVSLGQRATISCRASESVEYYVTS
LMQWYQQKPGQPPKLLISAASNVESGVPARFSGSGSGTDFSLIHPVEEDDIAMYF
CQQSRKVPWTFGGGTKLEIKRGGGGSGGGGSGGGGSQVQLKESGPGLVAPSQSLS
ITCTVSGFSLTGYGVNWVRQPPGKGLEWLGMIWGDGSTDYNSALKSRLSITKDNS
KSQVFLKMNSLQTDDTARYYCARDGYSNFHYYVMDYWGQGTSVTVSSDLEPKS
CDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVWDVSHEDPEVKFN
WYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALP
APIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQ

1305
1316

PENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSL
SLSPGKADPSNLLPSWAITLISVNGIFVICCLTYCFAPRCRERRRNERLRRESVRPV

IgE Fc 2H7-humano (CH2-CH3-CH4) (secuencia de nucleótido) (ID DE SEC NO __)

aagcttgccgccatggattttcaagtgcagattttcagcttcctgctaatcagtgcttcagtcataattgtccagaggacaaattgttctct
cccagtctccagcaatcctgtctgcatctccaggggagaaggtcacaatgacttgcagggccagctcaagtgtaagttacatgcact
ggtaccagcagaagccaggatcctcccccaaaccctggatttatgccccatccaacctggcttctggagtccctgctcgcttcagtg
gcagtgggtctgggacctcttactctctcacaatcagcagagtggaggctgaagatgctgccactttattactgccagcagtggagttt
taacccaccccacgttcggtgctgggaccaagctggagctgaaaggtggcggtggctcgggcggtggtgggatctggaggaggtg
ggagctcaggcttatctacagcagtctggggctgagctggtgaggcctggggcctcagtgaagatgtcctgcaaggcttctggc
tacacatttaccagttacaatatgcactgggtaaagcagacacctagacagggcctggaatggattggagctatttatcccaggaaat
ggtgatacttcctacaatcagaagttcaagggcaaggccacactgactgtagacaaatcctccagcacagcctacatgcagctcag
cagcctgacatctgaagactctgcggtctattttgtgcaagagtggtgtactatagtaactcttactggtacttcgatgtctggggcac
agggaccacggtcaccgtctctgatcacgtctgctccagggacttcaccccgccccaccgtgaagatcttacagtcgtcctgcgacg
gcggcgggcacttccccccgaccatccagctcctgtgcctcgtctctgggtacacccccagggactatcaacatcacctggctgga
ggacgggcaggtcatggacgtggacttgtccaccgcctctaccacgcaggagggtgagctggcctccacacaaagcgagctca
ccctcagccagaagcactggctgtcagaccgcacctacacctgccaggtcacctatcaaggtcacaccttgaggacagcaccaa
gaagtgtgcagatccaacccgagaggggtgagcgcctacctaagccggcccagcccgttcgaccttgttatccgtaagtcgcc
cacgatcacctgtctggtggtggacctggcacccagcaaggggaccgtgaacctgaactggtcccgggccagtgggaagcctgt
gaaccactccaccagaaaggaggagaagcagcgcaatggcacgttaaccgtcacgtccaccctgccggtgggcacccgagact
ggatcgaggggggagaactaccagtgcagggtgacccaccccccacctgcccagggccctatgcggtccacgaccaagaccag
cggcccgcgtgctgcccccggaagtctatgcgtttgcgacgccggagtggccgggggagccgggacaagcgcacccctcgcctgc
ctgatccagaacttcatgcctgaggacatctcggtgcagtggctgcacaacgaggtgcagctcccggacgcccggcacagcacg
acgcagcccccgcaagaccaagggctccggcttcttcgtcttcagccgcctggaggtgaccagggccgaatgggagcagaaaga
tgagtcatctgccgtgcagtccatgaggcagcgagccccicacagaccgtccagcgagcggtgtctgtaaatcccggtaaatgat
aatctaga

2H7 scFv IgE (CH2-CH3-CH4) (secuencia de aminoácido) (ID DE SEC NO __)

1306
1317

MDFQVQIFSFLLISASVIIARGQIVLSQSPAILSASPGEKVTMTCRASSSVSYMHWY
QQKPGSSPKPWIYAPSNLASGVPARFSGSGSGTSYSLTISRVEAEDAATYYCQQWS
FNPPTFGAGTKLELKGGGGSGGGGSGGGGSSQAYLQQSGAELVRPGASVKMSCK
ASGYTFTSYNMHWVKQTPRQGLEWIGAIYPGNGDTSYNQKFKGKATLTVDKSSS
TAYMQLSSLTSEDSAVYFCARVVYYSNSYWYFDVWGTGTTVTVSDHVCSRDFTP
PTVKILQSSCDGGGHFPPTIQLLCLVSGYTPGTINITWLEDGQVMDVDLSTASTTQE
GELASTQSELTLSQKHWLSDRTYTCQVTYQGHTFEDSTKKCADSNPRGVSAYLSR
PSPFDLFIRKSPTITCLVVDLAPSKGTVNLTWSRASGKPVNHSTRKEEKQRNGTLTV
TSTLPVGTRDWIEGETYQCRVTHPHLPRALMRSTTKTSGPRAAPEVYAFATPEWP
GSRDKRTLACLIQNFMPEDISVQWLHNEVQLPDARHSTTQPRKTKGSGFFVFSRLE
VTRAEWEQKDEFICRAVHEAASPSQTVQRAVSVNPGK

2H7 scFv MH (SSS) MCH2WTCH3 (secuencia de nucleótido) (ID DE SEC NO __)

aagcttgccgccatggatttttcaagtgcagattttcagcttcctgctaatcagtgcttcagtcataattgccagaggacaaattgttctct
cccagtctccagcaatcctgtctgcatctccagggagagaaggtcacaatgacttgcagggccagctcaagtgtaagttacatgcact
ggtaccagcagaagccaggatcctcccccaaaccctggatttatgcccatccaacctggcttctggagtccctgctcgcttcagtg
gcagtgggtctgggacctcttactctctcacaatcagcagagtggaggctgaagatgctgccactattactgccagcagtggagtt
tnaccccacccacgttcggtgctgggaccaagctggagctgaaagatggcggtggctcgggcggtggtgggtctggaggaggtg
ggaggtctcaggcttatctacagcagtctggggctgagctggtgaggcctggggcctcagtgaagatgtcctgcaaggcttctggc
tacacatttaccagttacaatatgcactgggtaaagcagacacctagacagggcctggaatggattggagctatttatccaggaaat
ggtgatacttcctacaatcagaagttcaagggcaaggccacactgactgtagacaaatcctccagcacagcctacatgcagctcag
cagcctgacatctgaagactctgcggtctatttctgtgcaagagtggtgtactatagtaactcttactggtacttcgatgtctggggcac
agggaccacggtcaccgtctcttctgatcaggagcccaaatcttctgacaaaactcacacatcccccaccgtcccccagcacctgaac
tcctgggggggatcgtcagtcttcctcttccccccaaaacccaaggacaccctcatgatctcccggacccctgaggtcacatgcgtg
gtggtggacgtgagccacgaagaccctgaggtcaagttcaactggtacgtggacggcgtggaggtgcataatgccaagacaaag
ccgcgggaggagcagtacaacagcacgtaccgtgtggtcagcgtcctcaccgtcctgcaccaggactggctgaatggcaagga
gtacaagtgcaaggtctccaacaaagccctcccagcccccatcgagaaaacaatctccaaagccaaagggcagccccgagaac
cacaggtgtacaccctgcccccatcccgggatgagctgaccaagaaccaggtcagcctgacctgcctggtcaaaggcttctatcc
cagcgacatcgccgtggagtgggagagcaatgggcagccggagaacaactacaagaccacgcctcccgtgctggactccgac
ggctccttctcctctacagcaagctcaccgtggacaagagcaggtggcagcaggggaacgtcttctcatgctccgtgatgcatga
ggctctgcacaaccactacacgcagaagagcctctccctgtctccgggtaaatgatctaga

2H7 scFv MH (SSS) MCH2WTCH3 (secuencia de aminoácido) (ID DE SEC NO __)

~~1307~~
1318

MDFQVQIFSFLLISASVIIARGQIVLSQSPAILSASPGEKVTMTCRASSSVSYMHWY
QQKPGSSPKPWIYAPSNLASGVPARFSGSGSGTSYSLTISRVEAEDAATYYCQQWS
FNPPTFGAGTKLELKDGGGSGGGGSGGGGSSQAYLQQSGAELVRPGASVKMSCK
ASGYTFTSYNMHWVKQTPRQGLEWIGAIYPGNGDTSYNQKFKGKATLTVDKSSS
TAYMQLSSLTSEDSAVYFCARVVYYSNSYWYFDVWGTGTTVTVSSDQEPKSSDK
THTSPPSPAPELLGGSSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYV
DGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKBYKCKVSNKALPAPIE
KTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPEN
NYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLS
PGK

5B9 scFv MTHWTCH2CH3 (secuencia de nucleótido) (ID DE SEC NO __)

aagcttgccgccatgaggttctctgctcagcttictggggctgcttgtgctctggatcctggatccactgcagatattgtgatgacgca
ggctgcattctccaatccagtcactcttggaacatcagcttccatctcctgcaggtctagtaagagtctcctacatagtaatggcatca
cttatttgtattggtatctgcagaagccaggccagtctcctcagctcctgatttatcagatgtccaaccttgcctcaggagtcccagaca
ggttcagtagcagtgggtcaggaactgatttcacactgagaatcagcagagtggaggctgaggatgtgggtgtttattactgtgctc
aaaatctagaacttccgctcacgttcggtgctgggaccaagctggagctgaaacgggggtggcggtggctcgggcggtggtgggt
cgggtggcggcggatcgtcacaggtgcagctgaagcagtcaggacctggcctagtgcagtcctcacagagcctgtccatcacct
gcacagtctctggtttctcattaactacctatgctgtacactgggttcgccagtctccaggaaagggtctggagtggctgggagtgat
atggagtggtgggaatcacagactataatgcagctttcatatccagactgagcatcaccaaggacgattccaagagccaagtttttctt
aaaatgaacagtctgcaacctaatgacacagccatttattactgtgccagaaatgggggtgataactacccttattactatgctatgga
ctactggggtcaaggaacctcagtcaccgtctcctctgatcaggagcccaaatcttctgacaaaactcacacatcccccaccgtcccc
agcacctgaactcctgggggggaccgtcagtcttcctcttccccccaaaacccaaggacaccctcatgatctcccggacccctgag
gtcacatgcgtggtggtggacgtgagccacgaagaccctgaggtcaagttcaactggtacgtggacggcgtggaggtgcataat
gccaagacaaagccgcgcgggaggagcagtacaacagcacgtaccgtgtggtcagcgtcctcaccgtcctgcaccaggactggct
gaatggcaaggagtacaagtgcaaggtctccaacaaagccctcccagccccccatcgagaaaacaatctccaaagccaaagggc
agccccgagaaccacaggtgtacaccctgcccccatcccgggatgagctgaccaagaaccaggtcagcctgacctgcctggtca
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ctggactccgacggctccttcttcctctacagcaagctcaccgtggacaagagcaggtggcagcaggggaacgtcttctcatgctc
cgtgatgcatgaggctctgcacaaccactacacgcagaagagcctctccctgtctccgggtaaatgatctaga

5B9 scFv MTHWTCH2CH3 (secuencia de aminoácido) (ID DE SEC NO __)

MRFSAQLLGLLVLWIPGSTADIVMTQAAFSNPVTLGTSASISCRSSKSLLHSNGITY
LYWYLQKPGQSPQLLIYQMSNLASGVPDRFSSSGSGTDFTLRISRVEAEDVGVYYC
AQNLELPLTFGAGTKLELKRGGGGSGGGGSGGGGSSQVQLKQSGPGLVQSSQSLS
ITCTVSGFSLTTYAVHWVRQSPGKGLEWLGVIWSGGITDYNAAFISRLSITKDDSK
SQVFFKMNSLQPNDTAIYYCARNGGDNYPYYYAMDYWGQGTSVTVSSDQEPKSS
DKTHTSPPSPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNW
YVDGVEVHNAKTKPREEQYNSTYRWSVLTVLHQDWLNGKEYKCKVSNKALPA
PIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQP
ENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLS
LSPGK

Mutaciones de articulación IgG1 humana

<u>2H7 scFv-MTH (CSS) WTCH2CH3</u> (secuencia de nucleótido) (ID DE SEC NO __)

aagcttgccgccatggatttcaagtgcagattttcagcttcctgctaatcagtgcttcagtcataattgccagaggacaaattgttctct
cccagtctccagcaatcctgtctgcatctccaggggagaaggtcacaatgacttgcagggccagctcaagtgtaagttacatgcact
ggtaccagcagaagccaggatcctccccaaaccctggatttatgccccatccaacctggcttctggagtccctgctcgcttcagtg
gcagtgggtctgggacctcttactctctcacaatcagcagagtggaggctgaagatgctgccacttattactgccagcagtggagttt
taacccacccacgttcggtgctgggaccaagctggagctgaaagatggcggtggctcgggcggtggtggatctggaggaggtg
ggagctctcaggcttatctacagcagtctggggctgagctggtgaggcctggggcctcagtgaagatgtcctgcaaggcttctggc
tacacatttaccagttacaatatgcactgggtaaagcagacacctagacagggcctggaatggattggagctatttatccaggaaat
ggtgatacttcctacaatcagaagttcaagggcaaggccacactgactgtagacaaatcctccagcacagcctacatgcagctcag
cagcctgacatctgaagactctgcggtctatttctgtgcaagagtggtgtactatagtaactcttactggtacttcgatgtctggggcac
agggaccacggtcaccgtctcttctgatcaggagcccaaatcttgtgacaaaactcacacatccccaccgtccccagcacctgaac
tcctgggggggaccgtcagtcttcctcttcccccaaaacccaaggacaccctcatgatctcccggacccctgaggtcacatgcgtg
gtggtggacgtgagccacgaagaccctgaggtcaagttcaactggtacgtggacggcgtggaggtgcataatgccaagacaaag
ccgcgggaggagcagtacaacagcacgtaccgtgtggtcagcgtcctcaccgtcctgcaccaggactggctgaatggcaagga
gtacaagtgcaaggtctccaacaaagccctcccagccccatcgagaaaacaatctccaaagccaaagggcagccccgagaac
cacaggtgtacaccctgcccccatcccgggatgagctgaccaagaaccaggtcagcctgacctgcctggtcaaaggcttctatcc
cagcgacatcgccgtggagtgggagagcaatgggcagccggagaacaactacaagaccacgcctcccgtgctggactccgac
ggctccttcttcctctacagcaagctcaccgtggacaagagcaggtggcagcaggggaacgtcttctcatgctccgtgatgcatga
ggctctgcacaaccactacacgcagaagagcctctccctgtctccgggtaaatgatctaga

<u>2H7 scFv-MTH (CSS) WTCH2CH3</u> (secuencia de aminoácido) (ID DE SEC NO __)

1309
1320

MDFQVQIFSFLLISASVIIARGQIVLSQSPAILSASPGEKVTMTCRASSSVSYMHWY
QQKPGSSPKPWIYAPSNLASGVPARFSGSGSGTSYSLTISRVEAEDAATYYCQQWS
FNPPTFGAGTKLELKDGGGSGGGGSGGGGSSQAYLQQSGAELVRPGASVKMSCK
ASGYTFTSYNMHWVKQTPRQGLEWIGAIYPGNGDTSYNQKFKGKATLTVDKSSS
TAY'IQLSSLTSEDSAVYFCARWYYSNSYWYFDVWGTGTTVTVSSDQEPKSCDK
THTSPPSPAPELLGGPSVFLFPPKPEDTLMISRTPEVTCVVVDVSHEDPEVKFNWYV
DGVBVHNAKTKPREEQYNSTYRWSVLTVLHQDWLNGKEYKCKVSNKALPAPIE
KTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPEN
NYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLS
PGK

2H7 scFv-MTH (SCS) WTCH2CH3 (secuencia de nucleótido) (ID DE SEC NO __)

aagcttgccgccatggattttcaagtgcagattttcagcttcctgctaatcagtgcttcagtcataattgccagaggacaaattgttctct
cocagtctccagcaatcctgtctgcatctccaggggagaaggtcacaatgacttgcagggccagctcaagtgtaagttacatgcact
ggtaccagcagaagccaggatcctcccccaaaccctggatttatgccccatccaacctggcttctggagtccctgctcgcttcagtg
gcagtgggtctgggacctcttactctctcacaatcagcagagtggaggctgaagatgctgccacttattactgccagcagtggagttt
taacccacccacgttcggtgctgggaccaagctggagctgaaagatggcggtggctcgggcggtggtggatctggaggaggtg
ggagctctcaggcttatctacagcagtctggggctgagctggtgaggcctggggcctcagtgaagatgtcctgcaaggcttctggc
tacacatttaccagttacaatatgcactgggtaaagcagacacctagacagggcctggaatggattggagctatttatccaggaaat
ggtgatacttcctacaatcagaagttcaagggcaaggccacactgactgtagacaaatcctccagcacagcctacatgcagctcag
cagcctgacatctgaagactctgcggtctatttctgtgcaagagtggtgtactatagtaactcttactggtacttcgatgtctggggcac
agggaccacggtcaccgtctcttctgatcaggagcccaaatcttctgacaaaactcacacatgcccaccgtccccagcacctgaac
tcctgggggggaccgtcagtcttcctcttccccccaaaacccaaggacaccctcatgatctcccggacccctgaggtcacatgcgtg
gtggtggacgtgagccacgaagaccctgaggtcaagttcaactggtacgtggacggcgtggaggtgcataatgccaagacaaag
ccgcgggaggagcagtacaacagcacgtaccgtgtggtcagcgtcctcaccgtcctgcaccaggactggctgaatggcaagga
gtacaagtgcaaggtctccaacaaagccctcccagccccatcgagaaaacaatctccaaagccaaagggcagccccgagaac
cacaggtgtacaccctgccccatcccgggatgagctgaccaagaaccaggtcagcctgacctgcctggtcaaaggcttctatcc
cagcgacatcgccgtggagtgggagagcaatgggcagccggagaacaactacaagaccacgcctcccgtgctggactccgac
ggctccttcttcctctacagcaagctcaccgtggacaagagcaggtggcagcaggggaacgtcttctcatgctccgtgatgcatga
ggctctgcacaaccactacacgcagaagagcctctccctgtctccgggtaaatgatctaga

2H7 sc-MTH (SCS) WTCH2CH3 (secuencia de aminoácido) (SEQ ID NO __)

MDFQVQIFSFLLISASVIIARGQIVLSQSPAILSASPGEKVTMTCRASSSVSYMHWY
QQKPGSSPKPWIYAPSNLASGVPARFSGSGSGTSYSLTISRVEAEDAATYYCQQWS

1310
1321

FNPPTFGAGTKLELKDGGGSGGGGSGGGGSSQAYLQQSGAELVRPGASVKMSCK
ASGYTFTSYMMdHWVKQTPRQGLEWIGAIYPGNGDTSYNQKFKGKATLTVDKSSS
TAYMQLSSLTSEDSAVYFCARVVYYSNSYWYFDVWGTGTTVTVSSDQEPKSSDK
THTCPPSPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWY
VDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPI
EKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPE
INYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSL
SPGK

2H7 scFv-MTH (SSC) WTCH2CH3 (nucleotide sequence) (SEQ ID NO)

aagcttgccgccatggattttcaagtgcagattttcagcttcctgctaatcagtgcttcagtcataattgccagaggacaaattgttctct
cccagtctccagcaatcctgtctgcatctccaggggagaaggtcacaatgacttgcagggccagctcaagtgtaagttacatgcact
ggtaccagcagaagccaggatcctccccaaaccctggatttatgccccatccaacctggcttctggagtccctgctcgcttcagtg
gcagtgggtctgggacctcttactctctcacaatcagcagagtggaggctgaagatgctgccacttattactgccagcagtggagttt
taacccacccacgttcggtgctgggaccaagctggagctgaaagatggcggtggctcgggcggtggtggatctggaggaggtg
ggagctctcaggcttatctacagcagtctggggctgagctggtgaggcctggggcctcagtgaagatgtcctgcaaggcttctggc
tacacatttaccagttacaatatgcactgggtaaagcagacacctagacagggcctggaatggattggagctatttatccaggaaat
ggtgatacttcctacaatcagaagttcaagggcaaggccacactgactgtagacaaatcctccagcacagcctacatgcagctcag
cagcctgacatctgaagactctgcggtctatttctgtgcaagagtggtgtactatagtaactcttactggtacttcgatgtctggggcac
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gtggtggacgtgagccacgaagaccctgaggtcaagttcaactggtacgtggacggcgtggaggtgcataatgccaagacaaag
ccgcgggaggagcagtacaacagcacgtaccgtgtggtcagcgtcctcaccgtcctgcaccaggactggctgaatggcaagga
gtacaagtgcaaggtctccaacaaagccctcccagcccccatcgagaaaacaatctccaaagccaaagggcagccccgagaac
cacaggtgtacaccctgcccccatcccgggatgagctgaccaagaaccaggtcagcctgacctgcctggtcaaaggcttctatcc
cagcgacatcgccgtggagtgggagagcaatgggcagccggagaacaactacaagaccacgcctcccgtgctggactccgac
ggctccttcttcctctacagcaagctcaccgtggacaagagcaggtggcagcaggggaacgtcttctcatgctccgtgatgcatga
ggctctgcacaaccactacacgcagaagagcctctccctgtctccgggtaaatgatctaga

2H7 scFv-MTH (SSC) WTCH2CH3 (secuencia de aminoácido) (ID DE SEC NO __)

1344
1322

MDFQVQIFSFLLISASVIIARGQIVLSQSPAILSASPGEKVTMTCRASSSVSYMHWY
QQKPGSSPKPWIYAPSNLASGVPARFSGSGSGTSYSLTISRVEAEDAATYYCQQWS
FNPPTFGAGTKLELKDGGGSGGGGSGGGGSSQAYLQQSGAELVRPGASVKMSCK
ASGYTFTSYNMHWVKQTPRQGLEWIGAIYPGNGDTSYNQKFKGKATLTVDKSSS
TAYMQLSSLTSEDSAVYFCARVVYYSNSYWYFDVWGTGTTVTVSSDQEPKSSDK
THTSPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWY
VDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPI
EKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPE
NNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSL
SPGK

HIgGMHcys1 (secuencia de nucleótido) (ID DE SEC NO __)

gtt gtt gat cag gag ccc aaa tct tct gac aaa act cac aca tg

HIgGMHcys2 (secuencia de nucleótido) (ID DE SEC NO __)

gtt gtt gat cag gag ccc aaa tct tgt gac aaa act cac aca tct cca ccg tgc

HIgGMHcys3 (secuencia de nucleótido) (ID DE SEC NO __)

gtt gtt gat cag gag ccc aaa tct tgt gac aaa act cac aca tgt cca ccg tec cca gca cct

HuIgG1 MTCH3Y405 (secuencia de nucleótido) (ID DE SEC NO __)

gggcagccccgagaaccacaggtgtacaccctgcccccatcccgggaggagatgaccaagaaccaggtcagcctgacctgcct
ggtcaaaggcttctatcccagcgacatcgccgtggagtgggagagcaatgggcagccggagaacaactacaagaccacgcctc
ccgtgctggactccgacggctccttctacctctatagcaagctcaccgtggacaagagcaggtggcagcaggggaacgtcttctc
atgctccgtgatgcatgaggctctgcacaaccactacacgcagaagagcctctccctgtccccgggtaaatga

HuIgG1 MTCH3Y405 (secuencia de aminoácido) (ID DE SEC NO __)

GQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPP
VLDSDGSFYLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

HuIgG1 MTCH3A405 (secuencia de nucleótido) (ID DE SEC NO __)

T312
1323

gggcagccccgagaaccacaggtgtacaccctgccccatcccgggaggagatgaccaagaaccaggtcagcctgacctgcct
ggtcaaaggcttctatcccagcgacatcgccgtggagtgggagagcaatgggcagccggagaacaactacaagaccacgcctc
ccgtgctggactccgacggctccttcgccctctatagcaagctcaccgtggacaagagcaggtggcagcaggggaacgtcttctc
atgctccgtgatgcatgaggctctgcacaaccactacacgcagaagagcctctccctgtccccgggtaaatga

HuIgGl MTCH3A405 (secuencia de aminoácido) (ID DE SEC NO __)

GQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPP
VLDSDGSFALYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

HuIgGl MTCH3A407 (secuencia de nucleótido) (ID DE SEC NO __)

gggcagccccgagaaccacaggtgtacaccctgccccatcccgggaggagatgaccaagaaccaggtcagcctgacctgcc
tggtcaaaggcttctatcccagcgacatcgccgtggagtgggagagcaatgggcagccggagaacaactacaagaccacgcct
cccgtgctggactccgacggctccttcttcctcgccagcaagctcaccgtggacaagagcaggtggcagcaggggaacgtcttct
catgctccgtgatgcatgaggctctgcacaaccactacacgcagaagagcctctccctgtccccgggtaaatga

HuIgG1 MTCH3A407 (secuencia de aminoácido) (ID DE SEC NO __)

GQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPP
VLDSDGSFFLASKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

HuIgGl MTCH3Y405A407 (secuencia de nucleótido) (ID DE SEC NO __)

gggcagccccgagaaccacaggtgtacaccctgccccatcccgggaggagatgaccaagaaccaggtcagcctgacctgcct
ggtcaaaggcttctatcccagcgacatcgccgtggagtgggagagcaatgggcagccggagaacaactacaagaccacgcctc
ccgtgctggactccgacggctccttctacctcgccagcaagctcaccgtggacaagagcaggtggcagcaggggaacgtcttctc
atgctccgtgatgcatgaggctctgcacaaccactacacgcagaagagcctctccctgtccccgggtaaatga

HuIgGl MTCH3Y405A407 (secuencia de aminoácido) (ID DE SEC NO __)

GQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPP
VLDSDGSFYLASKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

HuIG1 MTCH3A405A407 (secuencia de nucleótido) (ID DE SEC NO __)

1315
1329

gggcagccccgagaaccacaggtgtacaccctgccccatcccgggaggagatgaccaagaaccaggtcagcctgacctgcct
ggtcaaaggcttctatcccagcgacatcgccgtggagtgggagagcaatgggcagccggagaacaactacaagaccacgcctc
ccgtgctggactccgacggctccttcgccctcgccagcaagctcaccgtggacaagagcaggtggcagcaggggaacgtcttct
catgctccgtgatgcatgaggctctgcacaaccactacacgcagaagagcctctccctgtccccgggtaaatga

HuIgG1 MTCH3A405A407 (secuencia de aminoácido) (ID DE SEC NO __)

GQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPP
VLDSDGSFALASKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

2H7 scFv MTH (SSS) WTCH2MTCH3Y405 (secuencia de nucleótido) (ID DE SEC NO __)

aagcttgccgccatggattttcaagtgcagattttcagcttcctgctaatcagtgcttcagtcataattgccagaggacaaattgttctct
cccagtctccagcaatcctgtctgcatctccaggggagaaggtcacaatgacttgcagggccagctcaagtgtaagttacatgcact
ggtaccagcagaagccaggatcctccccaaaccctggatttatgccccatccaacctggcttctggagtccctgctcgcttcagtg
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taacccacccacgttcggtgctgggaccaagctggagctgaaagatggcggtggctcgggcggtggtggatctggaggaggtg
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tcctgggggggaccgtcagtcttcctcttcccccaaaacccaaggacaccctcatgatctcccggacccctgaggtcacatgcgtg
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ccgcgggaggagcagtacaacagcacgtaccgtgtggtcagcgtcctcaccgtcctgcaccaggactggctgaatggcaagga
gtacaagtgcaaggtctccaacaaagccctcccagccccatcgagaaaacaatctccaaagccaaagggcagccccgagaac
cacaggtgtacaccctgcccccatcccgggaggagatgaccaagaaccaggtcagcctgacctgcctggtcaaaggcttctatcc
cagcgacatcgccgtggagtgggagagcaatgggcagccggagaacaactacaagaccacgcctcccgtgctggactccgac
ggctccttctacctctatagcaagctcaccgtggacaagagcaggtggcagcaggggaacgtcttctcatgctccgtgatgcatga
ggctctgcacaaccactacacgcagaagagcctctccctgtccccgggtaaatgatctaga

2H7 scFv MTH (SSS) WTCH2MTCH3Y405 (secuencia de aminoácido) (ID DE SEC NO __)

BTY
1325

MDFQVQIFSFLLISASVIIARGQIVLSQSPAILSASPGEKVTMTCRASSSVSYMHWY
QQKPGSSPKPWIYAPSNLASGVPARFSGSGSGTSYSLTISRVEAEDAATYYCQQWS
FNPPTFGAGTKLELKDGGGSGGGGSGGGGSSQAYLQQSGAELVRPGASVKMSCK
ASGYTFTSYNMHWVKQTPRQGLEWIGAIYPGNGDTSYNQKFKGKATLTVDKSSS
TAYMQLSSLTSEDSAVYFCARVVYYSNSYWYFDVWGTGTTVTVSSDQEPKSSDK
THTSPPSPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYV
DGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIE
KTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPEN
NYKTTPPVLDSDGSFYLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLS
PGK

2H7 scFv MTH (SSS) WTCH2MTCH3A405 (secuencia de nucleótido) (ID DE SEC NO __)

aagcttgccgccatggattttcaagtgcagattttcagcttcctgctaatcagtgcttcagtcataattgccagaggacaaattgttctct
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ggtaccagcagaagccaggatcctccccaaaccctggatttatgccccatccaacctggcttctggagtccctgctcgcttcagtg
gcagtgggtctgggacctcttactctctcacaatcagcagagtggaggctgaagatgctgccacttattactgccagcagtggagttt
taacccacccacgttcggtgctgggaccaagctggagctgaaagatggcggtggctcgggcggtggtggatctggaggaggtg
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ggtgatacttcctacaatcagaagttcaagggcaaggccacactgactgtagacaaatcctccagcacagcctacatgcagctcag
cagcctgacatctgaagactctgcggtctatttctgtgcaagagtggtgtactatagtaactcttactggtacttcgatgtctggggcac
agggaccacggtcaccgtctcttctgatcaggagcccaaatcttctgacaaaactcacacatccccaccgtccccagcacctgaac
tcctgggggggaccgtcagtcttcctcttcccccaaaacccaaggacaccctcatgatctcccggaccccctgaggtcacatgcgtg
gtggtggacgtgagccacgaagaccctgaggtcaagttcaactggtacgtggacggcgtggaggtgcataatgccaagacaaag
ccgcgggaggagcagtacaacagcacgtaccgtgtggtcagcgtcctcaccgtcctgcaccaggactggctgaatggcaagga
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cacaggtgtacaccctgcccccatcccgggaggagatgaccaagaaccaggtcagcctgacctgcctggtcaaaggcttctatcc
cagcgacatcgccgtggagtgggagagcaatgggcagccggagaacaactacaagaccacgcctcccgtgctggactccgac
ggctccttcgccctctatagcaagctcaccgtggacaagagcaggtggcagcaggggaacgtcttctcatgctccgtgatgcatga
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2H7 scFv MTH (SSS) WTCH2MTCH3A405 (secuencia de nucleótido) (ID DE SEC NO __)

~~1315~~
1326

mdfqvqifsflliaasvmargqivlsqspailaaspgekvtmtcraasvsymhwyqqkpgsspkpwiyatsnlasgvparf
sgsgsgtsysltisrveaedaatyycqqwsfnpptfgagtkleikdggggsggggsggggsqvylqqsgaelvrpgasvkmsc
kasgytftsymmhwvkqtprqglewigaiypgngdtsynqkfkgkatltvdkssstaymqlsaltsedsavyfcarvvyysn
sywyfdvwgtgttvtvsadqepksadkthtsppspapellggpsvflfppkpkdtlmisrtpevtcvvvdvshedpevkfnw
yvdgvevhnaktkpreeqynstyrvvsvltvlhqdwlngkeykckvsnkalpapiektiskakgqprepqvytlppsreemt
knqvsltclvkgfypsdiavewesngqpennykttppvldsdgsfflyskltvdksrwqqgnvfscsvmhealhnhytqksl
slspgk

2H7 scFv MTH (SSS) WTCH2MTCH3A407 (secuencia de nucleótido) (ID DE SEC NO _)

aagcttgccgccatggattttcaagtgcagattttcagcttcctgctaatcagtgcttcagtcataattgccagaggacaaattgttctct
cccagtctccagcaatcctgtctgcatctccaggggagaaggtcacaatgacttgcagggccagctcaagtgtaagttacatgcact
ggtaccagcagaagccaggatcctccccaaaccctggatttatgccccatccaacctggcttctggagtccctgctcgcttcagtg
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taacccacccacgttcggtgctgggaccaagctggagctgaaagatggcggtggctcgggcggtggtggatctggaggaggtg
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ggtgatacttcctacaatcagaagttcaagggcaaggccacactgactgtagacaaatcctccagcacagcctacatgcagctcag
cagcctgacatctgaagactctgcggtctatttctgtgcaagagtggtgtactatagtaactcttactggtacttcgatgtctggggcac
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gtacaagtgcaaggtctccaacaaagccctcccagccccatcgagaaaacaatctccaaagccaaagggcagccccgagaac
cacaggtgtacaccctgcccccatcccgggaggagatgaccaagaaccaggtcagcctgacctgcctggtcaaaggcttctatcc
cagcgacatcgccgtggagtgggagagcaatgggcagccggagaacaactacaagaccacgcctcccgtgctggactccgac
ggctccttcttcctctcgccagcaagctcaccgtggacaagagcaggtggcagcaggggaacgtcttctcatgctccgtgatgcatga
ggctctgcacaaccactacacgcagaagagcctctccctgtccccgggtaaatga

2H7 scFv MTH (SSS) WTCH2MTCH3A407 (secuencia de aminoácido) (SEQ ID NO.)

1316
1327

MDFQVQIFSFLLISASVIIARGQIVLSQSPAILSASPGEKVTMTCRASSSVSYMHWY
QQKPGSSPKPWIYAPSNLASGVPARFSGSGSGTSYSLTISRVEAEDAATYYCQQWS
FNPPTFGAGTKLELKDGGGSGGGGSGGGGSSQAYLQQSGAELVRPGASVKMSCK
ASGYTFTSYNMHWVKQTPRQGLEWIGAIYPGNGDTSYNQKFKGKATLTVDKSSS
TAYMQLSSLTSEDSAVYFCARVVYYSNSYWYFDVWGTGTTVTVSSDQEPKSSDK
THTSPPSPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYV
DGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIE
KTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPEN
NYKTTPPVLDSDGSFFLASKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLS
PGK

2H7 scFv MTH (SSS) WTCH2MTCH3Y405A407 (secuencia de nucleótido) (ID DE SEC NO __)

aagcttgccgccatggatttttcaagtgcagattttcagcttcctgctaatcagtgcttcagtcataattgccagaggacaaattgttctct
cccagtctccagcaatcctgtctgcatctccaggggagaaggtcacaatgacttgcagggccagctcaagtgtaagttacatgcact
ggtaccagcagaagccaggatcctcccccaaaccctggatttatgccccatccaacctggcttctggagtccctgctcgcttcagtg
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taacccaccccacgttcggtgctgggaccaagctggagctgaaagatggcggtaggctcgggcggtggtggatctggaggaggtg
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ggtgatacttcctacaatcagaagttcaagggcaaggccacactgactgtagacaaatcctccagcacagcctacatgcagctcag
cagcctgacatctgaagactctgcggtctatttctgtgcaagagtggtgtactatagtaactcttactggtacttcgatgtctggggcac
agggaccacggtcaccgtctcttctgatcaggagcccaaatcttctgacaaaactcacacatcccccaccgtcccagcacctgaac
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ccgcgggaggagcagtacaacagcacgtaccgtgtggtcagcgtcctcaccgtcctgcaccaggactggctgaatggcaagga
gtacaagtgcaaggtctccaacaaagccctcccagccccatcgagaaaacaatctccaaagccaaagggcagccccgagaac
cacaggtgtacaccctgcccccatcccgggaggagatgaccaagaaccaggtcagcctgacctgcctggtcaaaggcttctatcc
cagcgacatccgcgtggagtgggagagcaatgggcagccggagaacaactacaagaccacgcctcccgtgctggactccgac
ggctccttcttcctctacagcaagctcaccgtggacaagagcaggtggcagcaggggaacgtcttctcatgctccgtgatgcatg
aggctctgcacaaccactacacgcagaagagcctctccctgtccccgggtaaatga

2H7 scFv MTH (SSS) WTCH2MTCH3Y405A407 (secuencia de aminoácido) (ID DE SEC NO __)

~~1317~~
1328

MDFQVQIFSFLLISASVIIARGQIVLSQSPAILSASPGEKVTMTCRASSSVSYMHWY
QQKPGSSPKPWIYAPSNLASGVPARFSGSGSGTSYSLTISRVEAEDAATYYCQQWS
FNPPTFGAGTKLELKDGGGSGGGGSGGGGSSQAYLQQSGAELVRPGASVKMSCK
ASGYTFTSYNMHWVKQTPRQGLEWIGAIYPGNGDTSYNQKFKGKATLTVDKSSS
TAYMQLSSLTSEDSAVYFCARVVYYSNSYWYFDVWGTGTTVTVSSDQEPKSSDK
THTSPPSPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYV
DGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIE
KTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPEN
NYKTTPPVLDSDGSFYLASKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLS
PGK

2H7 scFv MTH (SSS) WTCH2MTCH3A405A407 (secuencia de nucleótido) (ID DE SEC NO __)

aagcttgccgccatggatttttcaggtgcagatttcagcttcctgctaatcagtgtttcagtcataattgtccagaggacaaattgttctct
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ggtaccagcagaagccaggatcctccccaaaccctggatttatgccccatccaacctggcttctggagtcctgctcgcttcagtg
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cacaggtgtacaccctgcccccatcccgggaggagatgaccaagaaccaggtcagcctgacctgcctggtcaaaggcttctatcc
cagcgacatcgccgtggagtgggagagcaatgggcagccggagaacaactacaagaccacgcctcccgtgctggactccgac
ggctctttcgcctcgccagcaagctaccgtggacaagagcaggtggcagcaggggaacgtcttctcatgctccgtgatgcatg
aggctctgcacaaccactacacgcagaagagcctctccctgtccccgggtaaatga

2H7 scFv MTH (SSS) WTCH2MTCH3A405A407 (secuencia de aminoácido) (ID DE SEC NO __)

1328
1329

MDFQVQIFSFLLISASVIIARGQIVLSQSPAILSASPGEKVTMTCRASSSVSYMHWY
QQKPGSSPKPWIYAPSNLASGVPARFSGSGSGTSYSLTISRVEAEDAATYYCQQWS
FNPPTFGAGTKLELKDGGGSGGGGSGGGGSSQAYLQQSGAELVRPGASVKMSCK
ASGYTFTSYNMHWVKQTPRQGLEWIGAIYPGNGDTSYNQKFKGKATLTVDKSSS
TAYMQLSSLTSEDSAVYFCARVVYYSNSYWYFDVWGTGTTVTVSSDQEPKSSDK
THTSPPSPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYV
DGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIE
KTISKAKGQPREPQVYTLPPSRREMTKNQVSLTCLVKGFYPSDIAVEWESNGQPEN
NYKTTPPVLDSDGSFALASKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLS
PGK

2H7 scFv MTH (SCC) WTCH2CH3 (secuencia de nucleótido)

aagcttgccgccatggattttcaagtgcagattttcagcttcctgctaatcagtgcttcagtcataattgccagaggacaaattgttctct
cccagtctccagcaatcctgtctgcatctccaggggagaaggtcacaatgacttgcagggccagctcaagtgtaagttacatgcact
ggtaccagcagaagccaggatcctccccaaaccctggatttatgccccatccaacctggcttctggagtccctgctcgcttcagtg
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tcctgggggggaccgtcagtcttcctcttcccccaaaacccaaggacaccctcatgatctcccggaccctgaggtcacatgcgtg
gtggtggacgtgagccacgaagaccctgaggtcaagttcaactggtacgtggacggcgtggaggtgcataatgccaagacaaag
ccgcgggaggagcagtacaacagcacgtaccgtgtggtcagcgtcctcaccgtcctgcaccaggactggctgaatggcaagga
gtacaagtgcaaggtctccaacaaagccctcccagccccatcgagaaaacaatctccaaagccaaagggcagccccgagaac
cacaggtgtacaccctgcccccatcccgggatgagctgaccaagaaccaggtcagcctgacctgcctggtcaaaggcttctatcc
cagcgacatcgccgtggagtgggagagcaatgggcagccggagaacaactacaagaccacgcctcccgtgctggactccgac
ggctccttcttcctctacagcaagctcaccgtggacaagagcaggtggcagcaggggaacgtcttctcatgctccgtgatgcatga
ggctctgcacaaccactacacgcagaagagcctctccctgtctccgggtaaatgatctaga

2H7 scFv-MTH (SCC) WTCH2CH3 (secuencia de aminoácido)

1379
1330

MDFQVQIFSFLLISASVnARGQIVLSQSPAILSASPGEKVTMTCRASSSVSYMHWY
QQKPGSSPKPWIYAPSNLASGVPARFSGSGSGTSYSLTISRVEAEDAATYYCQQWS
F'NPPTFGAGTKLELKDGGGSGGGGSGGGGSSQAYLQQSGAELVRPGASVKMSCK
ASGYTFTSYNMHWVKQTPRQGLEWIGAIYPGNGDTSYNQKFKGKATLTVDKSSS
TAYMQLSSLTSEDSAVYFCARVVYYSNSYWYFDVWGTGTTVTVSSDQEPKSSDK
THTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWY
VDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPI
EKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPE
NNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSL
SPGK

2H7 scFv MTH (CSC) WTCH2CH3 (secuencia de nucleótido)

aagcttgccgccatggattttcaagtgcagattttcagcttcctgctaatcagtgcttcagtcataattgccagaggacaaattgttctct
cccagtctccagcaatcctgtctgcatctccaggggagaaggtcacaatgacttgcagggccagctcaagtgtaagttacatgcact
ggtaccagcagaagccaggatcctcccccaaaccctggatttatgccccatccaacctggcttctggagtccctgctcgcttcagtg
gcagtgggtctgggacctcttactctctcacaatcagcagagtggaggctgaagatgctgccacttattactgccagcagtggagttt
taacccacccacgttcggtgctgggaccaagctggagctgaaagatggcggtggctcgggcggtggtggatctggaggaggtg
ggagctctcaggcttatctacagcagtctggggctgagctggtgaggcctggggcctcagtgaagatgtcctgcaaggcttctggc
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ggtgatacttcctacaatcagaagttcaagggcaaggccacactgactgtagacaaatcctccagcacagcctacatgcagctcag
cagcctgacatctgaagactctgcggtctatttctgtgcaagagtggtgtactatagtaactcttactggtacttcgatgtctggggcac
agggaccacggtcaccgtctcttctgatcaggagcccaaatcttgtgacaaaactcacacatctccaccgtgcccagcacctgaac
tcctgggggggaccgtcagtcttcctcttccccccaaaacccaaggacaccctcatgatctcccggacccctgaggtcacatgcgtg
gtggtggacgtgagccacgaagaccctgaggtcaagttcaactggtacgtggacggcgtggaggtgcataatgccaagacaaag
ccgcgggaggagcagtacaacagcacgtaccgtgtggtcagcgtcctcaccgtcctgcaccaggactggctgaatggcaagga
gtacaagtgcaaggtctccaacaaagccctcccagccccatcgagaaaacaatctccaaagccaaagggcagccccgagaac
cacaggtgtacaccctgcccccatcccgggatgagctgaccaagaaccaggtcagcctgacctgcctggtcaaaggcttctatcc
cagcgacatcgccgtggagtgggagagcaatgggcagccggagaacaactacaagaccacgcctcccgtgctggactccgac
ggctccttcttcctctacagcaagctcaccgtggacaagagcaggtggcagcaggggaacgtcttctcatgctccgtgatgcatga
ggctctgcacaaccactacacgcagaagagcctctccctgtctccgggtaaatgatctaga

2H7 scFv MTH (CSC) WTCH2CH3 (amino acM sequence)

1331

MDFQVQIFSFLLISASVIIARGQIVLSQSPAILSASPGEKVTMTCRASSSVSYMHWY
QQKPGSSPKPWIYAPSNLASGVPARFSGSGSGTSYSLTISRVEAEDAATYYCQQWS
FNPPTFGAGTKLELKDGGGSGGGGSGGGGSSQAYLQQSGAELVRPGASVKMSCK
ASGYTFTSYNMHWVKQTPRQGLEWIGAIYPGNGDTSYNQKFKGKATLTVDKSSS
TAYMQLSSLTSEDSAVYFCARVVYYSNSYWYFDVWGTGTTVTVSSDQEPKSCDK
THTSPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWY
VDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPI
EKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPE
NNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSL
SPGK

2H7 scFv MTH (CCS) WTCH2CH3 (secuencia de nucleótido)

aagcttgccgccatggattttcaagtgcagattttcagcttcctgctaatcagtgcttcagtcataattgccagaggacaaaattgttctct
cccagtctccagcaatcctgtctgcatctccaggggagaaggtcacaatgacttgcagggccagctcaagtgtaagttacatgcact
ggtaccagcagaagccaggatcctccccaaaccctggatttatgccccatccaacctggcttctggagtccctgctcgcttcagtg
gcagtgggtctgggacctcttactctctcacaatcagcagagtggaggctgaagatgctgccacttattactgccagcagtggagttt
taacccacccacgttcggtgctgggaccaagctggagctgaaagatggcggtggctcgggcggtggtggatctggaggaggtg
ggagctctcaggcttatctacagcagtctggggctgagctggtgaggcctggggcctcagtgaagatgtcctgcaaggcttctggc
tacacatttaccagttacaatatgcactgggtaaagcagacacctagacagggcctggaatggattggagctatttatccaggaaat
ggtgatacttcctacaatcagaagttcaagggcaaggccacactgactgtagacaaatcctccagcacagcctacatgcagctcag
cagcctgacatctgaagactctgcggtctatttctgtgcaagagtggtgtactatagtaactcttactggtacttcgatgtctggggcac
agggaccacggtcaccgtctcttctgatcaggagcccaaatcttgtgacaaaactcacacatgtccaccgtccccagcacctgaac
tcctgggggggaccgtcagtcttcctcttcccccaaaacccaaggacaccctcatgatctcccggacccctgaggtcacatgcgtg
gtggtggacgtgagccacgaagaccctgaggtcaagttcaactggtacgtggacggcgtggaggtgcataatgccaagacaaag
ccgcgggaggagcagtacaacagcacgtaccgtgtggtcagcgtcctcaccgtcctgcaccaggactggctgaatggcaagga
gtacaagtgcaaggtctccaacaaagccctcccagccccatcgagaaaacaatctccaaagccaaagggcagccccgagaac
cacaggtgtacaccctgcccccatcccgggatgagctgaccaagaaccaggtcagcctgacctgcctggtcaaaggcttctatcc
cagcgacatcgccgtggagtgggagagcaatgggcagccggagaacaactacaagaccacgcctcccgtgctggactccgac
ggctccttcttcctctacagcaagctcaccgtggacaagagcaggtggcagcaggggaacgtcttctcatgctccgtgatgcatga
ggctctgcacaaccactacacgcagaagagcctctccctgtctccgggtaaatgatctaga

2H7 scFv MTH (CCS) WTCH2CH3 (secuencia de aminoácido)

MDFQVQIFSFLLISASVIIARGQIVLSQSPAILSASPGEKVTMTCRASSSVSYMHWY
QQKPGSSPKPWIYAPSNLASGVPARFSGSGSGTSYSLTISRVEAEDAATYYCQQWS

1327
1332

FNPPTFGAGTKLELKDGGGSGGGGSGGGGSSQAYLQQSGAELVRPGASVKMSCK
ASGYTFTSYNMHWVKQTPRQGLEWIGAIYPGNGDTSYNQKFKGKATLTVDKSSS
TAYMQLSSLTSEDSAVYFCARVVYYSNSYWYFDVWGTGTTVTVSSDQEPKSCDK
THTCPPSPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWY
VDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPI
EKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPE
NNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSL
SPGK

HuIgAHIgA-T4-ORF (secuencia de nucleótido)

tgatcagccagttccctcaactccacctaccccatctccctcaactccacctaccccatctccctcatgctgccacccccgactgtca
ctgcaccgaccggccctcgaggacctgctcttaggttcagaagcgatcctcacgtgcacactgaccggcctgagagatgcctcag
gtgtcaccttcacctggacgccctcaagtgggaagagcgctgttcaaggaccacctgaccgtgacctctgtggctgctacagcgtg
tccagtgtcctgccgggctgtgccgagccatggaaccatgggaagaccttcacttgcactgctgcctaccccgagtccaagaccc
cgctaaccgccaccctctcaaaatccggaaacacattccggcccgaggtccacctgctgccgccgccgtcggaggagctggccc
tgaacgagctggtgacgctgacgtgcctggcacgtggcttcagccccaaggatgtgctggttcgctggctgcaggggtcacagg
agctgccccgcgagaagtacctgacttgggcatcccggcaggagcccagccagggcaccaccaccttcgctgtgaccagcata
ctgcgcgtggcagccgaggactggaagaaggggggacaccttctcctgcatggtgggccacgaggccctgccgctggccttcac
acagaagaccatcgaccgcttggcgggtaaacccacccatgtcaatgtgtctgttgtcatggcggaggtggacgcggatccttcga
ac

HuIg A-T4-0B (secuencia de aminoácido)

DQPVPSTPPTPSPSTPPTPSPSCCHPRLSLHRPALEDLLLGSEAILTCTLTGLRDASGV
TFTWTPSSGKSAVQGPPDRDLCGCYSVSSVLPGCAEPWNHGKTFTCTAAYPESKT
PLTATLSKSGNTFRPEVHLLPPPSEELALNELVTLTCLARGFSPKDVLVRWLQGSQ
ELPREKYLTWASRQEPSQGTTTFAVTSILRVAAEDWKKGDTFSCMVGHEALPLAF
TQKTIDRLAGKPTHVNVSVVMAEVDADPSN

1D8-IgAH IgA-T4-CD80 (secuencia de nucleótido)

aagcttatggattttcaagtgcagattttcagcttcctgctaatcagtgcttcagtcataatgtccagaggagtcgacattgtgctcactc
agtctccaacaaccatagctgcatctccaggggagaaggtcaccatcacctgccgtgccagctccagtgtaagttacatgtactggt
accagcagaagtcaggcgcctcccctaaactctggatttatgacacatccaagctggcttctggagttccaaatcgcttcagtggca
gtgggtctgggacctcttattctctcgcaatcaacaccatggagactgaagatgctgccacttattactgtcagcagtggagtagtact

1322
1333

ccgctcacgttcgggtctgggaccaagctggagatcaaacggggtggcggtggctcgggcggtggtgggtcgggtggcggcg
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ctcattaaccagcgatggtgtacactggattcgacagcctccaggaaagggtctggaatggatgggaataatatattatgatggagg
cacagattataattcagcaattaaatccagactgagcatcagcagggacacctccaagagccaagttttcttaaaaatcaacagtctg
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ccgaccggccctcgaggacctgctcttaggttcagaagcgatcctcacgtgcacactgaccggcctgagagatgcctcaggtgtc
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gccccgcgagaagtacctgacttgggcatcccggcaggagcccagccagggcaccaccaccttcgctgtgaccagcatactgc
gcgtggcagccgaggactggaagaaggggacaccttctcctgcatggtgggccacgaggccctgccgctggccttcacacag
aagaccatcgaccgcttggcgggtaaacccacccatgtcaatgtgtctgttgtcatggcggaggtggacgcggatccttcgaacaa
cctgctcccatcctgggccattaccttaatctcagtaaatggaatttttgtgatatgctgcctgacctactgctttgccccaagatgcag
agagagaaggaggaatgagagattgagaagggaaagtgtacgccctgtataaatcgatac

AA

1D8 scFv IgAH IgA-T4-CD80 (secuencia de aminoácido)

MDFQVQIFSFLLISASVIMSRGVDIVLTQSPTTIAASPGEKVTITCRASSSVSYMYWY
QQKSGASPKLWIYDTSKLASGVPNRFSGSGSGTSYSLAINTMETEDAATYYCQQW
SSTPLTFGSGTKLEIKRGGGGSGGGGSGGGGSQVQLKESGPGLVQPTQTLSLTCTV
SGFSLTSDGVHWIRQPPGKGLEWMGIIYYDGGTDYNSAIKSRLSISRDTSKSQVFLK
MNSLQTDDTAMYYCARIHFDYWGQGVMVTVSSDQPVPSTPPTPSPSTPPTPSPSCC
HPRLSLHRPALEDLLLGSEAILTCTLTGLRDASGVTFTWTPSSGKSAVQGPPDRDL
CGCYSVSSVLPGCAEPWNHGKTFTCTAAYPESKTPLTATLSKSGNTFRPEVHLLPP
PSEELALNELVTLTCLARGFSPKDVLVRWLQGSQELPREKYLTWASRQEPSQGTTT
FAVTSILRVAAEDWKKGDTFSCMVGHEALPLAFTQKTIDRLAGKPTHVNVSVVM
AEVDADPSNNLLPSWAITLISVNGIFVICCLTYCFAPRCRERRRNERLRRESVRPV

IgE Fc (CH2-CH3-CH4) ORF humano (secuencia de nucleótido)

tgatcacgtctgctccagggacttcaccccgcccaccgtgaagatcttacagtcgtcctgcgacggcggcgggcacttccccccg
accatccagctcctgtgcctcgtctctgggtacaccccagggactatcaacatcacctggctggaggacgggcaggtcatggacg

~~13C3~~
1334

tggacttgtccaccgcctctaccacgcaggagggtgagctggcctccacacaaagcgagctcaccctcagccagaagcactggc
tgtcagaccgcacctacacctgccaggtcacctatcaaggtcacacctttgaggacagcaccaagaagtgtgcagattccaaccc
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aggagaagcagcgcaatggcacgttaaccgtcacgtccaccctgccggtgggcacccgagactggatcgaggggggagacctа
ccagtgcagggtgacccacccccacctgcccagggccctcatgcggtccacgaccaagaccagcggcccgcgtgctgccccg
gaagtctatgcgtttgcgacgccggagtggccggggagccgggacaagcgcaccctcgcctgcctgatccagaacttcatgcct
gaggacatctcggtgcagtggctgcacaacgaggtgcagctcccggacgcccggcacagcacgacgcagccccgcaagacc
aagggctccggcttcttcgtcttcagccgcctggaggtgaccagggccgaatgggagcagaaagatgagttcatctgccgtgcag
tccatgaggcagcgagcccctcacagaccgtccagcgagcggtgtctgtaaatcccggtaaagcggatccttcgaa

AA

IgE Fc (CH2-CH3-CH4) ORF humano (secuencia de aminoácido)

DHVCSRDFTPPTVKILQSSCDGGGHFPPTIQLLCLVSGYTPGTINITWLEDGQVMDV
DLSTASTTQEGELASTQSELTLSQKHWLSDRTYTCQVTYQGHTFEDSTKKCADSN
PRGVSAYLSRPSPFDLFIRKSPTITCLVVDLAPSKGTVNLTWSRASGKPVNHSTRKE
EKQRNGTLTVTSTLPVGTRDWIEGETYQCRVTHPHLPRALMRSTTKTSGPRAAPE
VYAFATPEWPGSRDKRTLACLIQNFMPEDISVQWLHNEVQLPDARHSTTQPRKTK
GSGFFVFSRLEVTRAEWEQKDEFICRAVHEAASPSQTVQRAVSVNPGKADPS

IgE Fc ID8 scFv-humano (CH2-CH3-CH4)-CD80 (secuencia de nucleótido)

ccgctcacgttcgggtctgggaccaagctggagatcaaacggggtggcggtggctcgggcggtggtgggtcgggtggcggcg
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ctcattaaccagcgatggtgtacactggattcgacagcctccaggaaagggtctggaatggatgggaataatatattatgatggagg
cacagattataattcagcaattaaatccagactgagcatcagcagggacacctccaagagccaagttttcttaaaaaatcaacagtctg
caaactgatgacacagccatgtattactgtgccagaatccactttgattactggggccaaggagtcatggtcacagtctcctctgatc
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ggcacccagcaaggggaccgtgaacctgacctggtcccgggccagtgggaagcctgtgaaccactccaccagaaaggaggag
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1324
1335

cagggtgacccacccccacctgcccagggccctcatgcggtccacgaccaagaccagcggcccgcgtgctgccccggaagtct
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ccggcttcttcgtcttcagccgcctggaggtgaccagggccgaatgggagcagaaagatgagttcatctgccgtgcagtccatga
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cattaccttaatctcagtaaatggaatttttgtgatatgctgcctgacctactgctttgccccaagatgcagagagagaaggaggaatg
agagattgagaagggaaagtgtacgccctgtataaatcgata

IgE Fc ID8-scFv-humano (CH2-CH3-CH4)-CD80 (secuencia de aminoácido)

MDFQVQIFSFLLISASVIMSRGVDIVLTQSPTTIAASPGEKVTITCRASSSVSYMYWY
QQKSGASPKLWIYDTSKLASGVPNRFSGSGSGTSYSLAINTMETEDAATYYCQQW
SSTPLTFGSGTKLEIKRGGGGSGGGGSGGGGSQVQLKEAGPGLVQPTQTLSLTCTV
SGFSLTSDGVHWIRQPPGKGLEWMGIIYYDGGTDYNSAIKSRLSISRDTSKSQVFLK
INSLQTDDTAMYYCARIHFDYWGQGVMVTVSSDHVCSRDFTPPTVKILQSSCDGG
GHFPPTIQLLCLVSGYTPGTINITWLEDGQVMDVDLSTASTTQEGELASTQSELTLS
QKHWLSDRTYTCQVTYQGHTFEDSTKKCADSNPRGVSAYLSRPSPFDLFIRKSPTI
TCLVVDLAPSKGTVNLTWSRASGKPVNHSTRKEEKQRNGTLTVTSTLPVGTRDWI
EGETYQCRVTHPHLPRALMRSTTKTSGPRAAPEVYAFATPEWPGSRDKRTLACLI
QNFMPEDISVQWLHNEVQLPDARHSTTQPRKTKGSGFFVFSRLEVTRAEWEQKDE
FICRAVHEAASPSQTVQRAVSVNPGKADPSKLPSWAITLISVNGIFVICCLTYCFAP
RCRERRRNERLRRESVRPV

5B9-IvAH IgA-T4-CD80 (secuencia de nucleótido)

1325
1336

aagcttgccgccatgaggttctctgctcagcttctggggctgcttgtgctctggatccctggaiccactgcgatatttgtgatgacgca
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ggttcagtagcagtgggtcaggaactgatttcacactgagaatcagcagagtggaggctgaggatgtgggtgtttattactgtgctc
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cgggtggcggcggatcgtcacaggtgcagctgaagcagtcaggacctggcctagtgcagtcctcacagagcctgtccatcacct
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ccacctgaccgtgaccicigtggctgctacagcgtgtccagtgtcctgccgggctgtgccgagccatggaaccatgggaagacctt
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gccagggcaccacccacctcgctgtgaccagcatactgcgcgtggcagccgaggactggaagaggggacacctictcctgc
atggtgggccacgaggccctgccgctggccticacacagaagccatcgaccgcttggcgggtaaacccacccatgtcaatgtgt
ctgttgtcatggcggaggtggacgcggatcctccgaacaacctgctcccatcctgggccattacctaatctcagtaaatggaatttit
gtgatatgctgcctgacctactgcttigcccaagatgcagagagagaaggaggaatgagagattgagaagggaaagtgtacgcc
ctgtataaatcgatac

5B9-Ig A-T4-CD80 (secuencia de aminoácido)

MRFSAQLLGLLVLWIPGSTADIVMTQAAFSNPVTLGTSASISCRSSKSLLHSNGITY
LYWYLQKPGQSPQLLIYQMSNLASGVPDRFSSSGSGTDFTLRISRVEAEDVGVYYC
AQNLELPLTFGAGTKLELKRGGGGSGGGGSGGGGSSQVQLKQSGPGLVQSSQSLS
ITCTVSGFSLTTYAVHWVRQSPGKGLEWLGVIWSGGITDYNAAFISRLSITKDDSK
SQVFFKMNSLQPNDTAIYYCARNGGDNYPYYYAMDYWGQGTSVTVSSDQPVPST
PPTPSPSTPPTPSPSCCHPRLSLHRPALEDLLLGSEAILTCTLTGLRDASGVTFTWTPS
SGKSAVQGPPDRDLCGCYSVSSVLPGCAEPWNHGKTFTCTAAYPESKTPLTATLS
KSGNTFRPEVHLLPPPSEELALNELVTLTCLARGFSPKDVLVRWLQGSQELPREKY
LTWASRQEPSQGTTTFAVTSILRVAAEDWKKGDTFSCMVGHEALPLAFTQKTIDR
LAGKPTHVNVSVVMAEVDADPSNNLLPSWAITLISVNGIFVICCLTYCFAPRCRER
RRNERLRRESVRPV

1326
1337

IgE Fc 5B9-scFv-humano (CH2-CH3-CH4)-CD80 (secuencia de nucleótido)

aagcttgccgccatgaggttctctgctcagcttctggggctgcttgtgctctggatccctggatccactgcagatattgtgatgacgca
ggctgcattctccaatccagtcactcttggaacatcagccttccatctcctgcaggtctagtaagagtctcctacatagtaatggcatca
cttatttgtattggtatctgcagaagccaggccagtctcctcagctcctgatttatcagatgtccaacctTgcctcaggagtcccagaca
ggttcagtagcagtgggtcaggaactgatttcacactgagaatcagcagagtggaggctgaggatgttgggttttattactgtgctc
aaaatctagaacttccgctcacgttcggtgctgggaccaagctggagctgaaacgggggtggcggtggctcgggcggtggtgggt
cgggtggcggcggatcgtcacaggtgcagctgaagcagtcaggacctggcctagtgcagtcctcacagagcctgtccatcacct
gcacagtctctggtttctcattaactacctatgctgtacactgggttcgccagtctccaggaaagggtctggagtggctgggagtgat
atggagtggtggaatcacagactataatgcagcttttatatccagactgagcatcaccaaggacgattccaagagccaagtttttctt
aaaatgaacagtctgcaacctaatgacacagccatttattactgtgccagaaatgggggtgataactacccttattactatgctatgga
ctactggggtcaaggaacctcagtcaccgtctcctctgatcacgtctgctcagggacttcaccccgcccaccgtgaagatcttaca
gtcgtcctgcgacggcggcgggcacttccccccgaccatccagctcctgtgcctcgtctctgggtacaccccagggactatcaac
atcacctggctggaggacgggcaggtcatggacgtggagttgtccaccgcctctaccacgaaggaggggtgagctggcctccaca
caaagcgagctcaccctcagccagaagcactggctgtcagaccgcacctacacctgccaggtcacctatcaaggtcacaccttg
aggacagcaccaagaagtgtgcagattccaacccgagaggggtgagcgcctacctaagccggcccagccccgttcgacctgttca
tccgcaagtcgcccacgatcaccTgtctggtggtggacctggcacccagcaaggggaccgtgaacctgacctggtcccgggcca
gtgggaagcctgtgaccactccaccagaaaggaggagcagcgcaatggcacgttaccgtcacgtccaccctgccggtg
ggcacccgagactggatcgagggggagacctaccagtgcagggtgacccacccccacctgcccagggccctcatgcggtcca
cgaccaagaccagcggcccgcgtgctgcccggaagtctatgcgtttgcgacgccgggagtggccgggggagccgggacaagc
gcaccctcgcctgcctgatccagaacttcatgcctgaggacatctcggtgcagtggctgcacaacgaggtgcagctcccggacgc
ccggcacagcacgacgcagcccccgcaagaccaagggctccggcttcttcgtcttcagccgcctggaggtgaccagggccgaat
gggagcagaaagatgagttcatctgccgtgcagtccatgaggcagcgagccccctcacagaccgtccagcgagcggtgtctgtaa
atcccggtaaagcgggatccttcgaagctcccatcctgggccatttacctaatctcagtaaatggaatttttgtgtatatgctgcctgacct
actgctttgccccaagatgcagagagagaaggaggaatgagagattgagaagggaaagtgtacgccctgtataaatcgata

IgE Fc X 5B9-scFv-humano (CH2-CH3-CH4)-CD80 (secuencia de aminoácido)

MRFSAQLLGLLVLWIPGSTADIVMTQAAFSNPVTLGTSASISCRSSKSLLHSNGITY
LYWYLQKPGQSPQLLIYQMSNLASGVPDRFSSSGSGTDFTLRISRVEAEDVGVYYC
AQNLELPLTFGAGTKLELKRGGGGSGGGGSGGGGSSQVQLKQSGPGLVQSSQSLS
ITCTVSGFSLTTYAVHWVRQSPGKGLEWLGVIWSGGITDYNAAFISRLSITKDDSK
SQVFFKMNSLQPNDTAIYYCARNGGDNYPYYYAMDYWGQGTSVTVSSDHVCSR
DFTPPTVKILQSSCDGGGHFPPTIQLLCLVSGYTPGTINITWLEDGQVMDVDLSTAS
TTQEGELASTQSELTLSQKHWLSDRTYTCQVTYQGHTFEDSTKKCADSNPRGVSA
YLSRPSPFDLFIRKSPTITCLVVDLAPSKGTVNLTWSRASGKPVNHSTRKEEKQRNG
TLTVTSTLPVGTRDWIEGETYQCRVTHPHLPRALMRSTTKTSGPRAAPEVYAFATP
EWPGSRDKRTLACLIQNFMPEDISVQWLHNEVQLPDARHSTTQPRKTKGSGFFVFS
RLEVTRAEWEQKDEFICRAVHEAASPSQTVQRAVSVNPGKADPSKLPSWAITLISV
NGIFVICCLTYCFAPRCRERRRNERLRRESVRPV

2el2-scFv-IgAH IgA-T4-CD80 (secuencia de nucleótido)

aagcttatggatttcaagtgcagattttcagcttcctgctaatcagtgcttcagtcataatgtccagaggagtcgacatgtgctcaccc
agtctccagcttctttggctgtgtctctaggtcagagagccaccatctcctgcagagccagtgaaagttgttgaatatttatgtcacaagtt
tatgcagtggtaccaacagaaaccaggacagccacccaaactcctcatctatctgcttgcatccaacgtagaaatctggggtccctgcc
aggtttagtggcagtgggtctgggacagacttcagcctcaacatccatcctgtggaggaggatgatattgcaatgtatttctgtcagc
aaagtaggaaggttccttggacgttcggtggaggcaccaagctggaaatcaaacgggtggcggtggctcgggcggaggtggg
tcgggtggcggcggatctcaggtgcagctgaaggagtcaggacctggcctggtggcgccctcacagagcctgtccatcacatgc
accgtctcagggttctcattaccggctatggtgtaaactgggttcgccagcctccaggaaagggtctggagtggctgggaatgat
atggggtgatggaagcacagactataattcagctctcaaatccagactgagcatcaccaaggacaactccaagagccaagttttctt
aaaatgaacagtctgcaaactgatgacacagccagatactactgtgccagaggtggttatagtaacttcattactatgttatggact
actggggtcaaggaacctcagtcaccgtctcctcagatcagccagttccctcaactccacctaccccatctccctcaactccaccta
ccccatctccctcatgctgccacccccgactgtcactgcaccgacggccctcgaggacctgctcttaggttcagaagcgatcctc
acgtgcacactgaccggcctgagagatgccttcaggtgtcaccttcacctggacgccctcagtgggaagagcgctgttcaaggac
cacctgaccgtgaccttctgtggctgctacagcgtgtccagtgtcctgccgggctgtgccgagccatggaaccatgggaagacctte
acttgcactgctgcctaccccgagtccaagaccccgctaaccgccaccctttcaaaatccgtaaacacatttccggccgaggtcc
acctgctgccgccgccgtcggaggagctggccctgaacgagctggtgacgctgacgtgcctggcacgtggcttcagccccaag
gatgtgctggttcgctggctgcagggtcacaggagctgccccgcgagaagtacctgacttgggcatcccggcaggagcccag
ccagggcaccacaccacttcgctgtgaccagcatactgcgcgtggcagccgaggactggaagaagggggacaccttctcctgcat
ggtgggccacgaggccctgccgctggccttcacacagaagaccatcgaccgcttggcgggtaaacccaccatgtcaatgtgtct
gtgtcatggcggaggtggacgcggatcctcgaacaacctgctccatcctgggccatcaccttaatctcagtaaatggaatttttgt
gatatgctgcctgacctactgcttttgccccaagatgcagagagagaaggaggaatgagagatgagaagggaaagtgtacgccct
gtataaatcgatac

1328
1339

2el2-scFv-IgAH IvA-T4-CD80 (secuencia de aminoácido)

MDFQVQIFSFLLISASVIMSRGVDIVLTQSPASLAVSLGQRATISCRASESVEYYVTS
LMQWYQQKPGQPPKLLISAASNVESGVPARFSGSGSGTDFSLNIHPVEEDDIAMYF
CQQSRKVPWTFGGGTKLEIKRGGGGSGGGGSGGGGSQVQLKESGPGLVAPSQSLS
ITCTVSGFSLTGYGVNWVRQPPGKGLEWLGMIWGDGSTDYNSALKSRLSITKDNS
KSQVFLKMNSLQTDDTARYYCARDGYSNFHYYVMDYWGQGTSVTVSSDQPVPS
TPPTPSPSTPPTPSPSCCHPRLSLHRPALEDLLLGSEAILTCTLTGLRDASGVTFTWTP
SSGKSAVQGPPDRDLCGCYSVSSVLPGCAEPWNHGKTFTCTAAYPESKTPLTATLS
KSGNTFRPEVHLLPPPSEELALNELVTLTCLARGFSPKDVLVRWLQGSQELPREKY
LTWASRQEPSQGTTTFAVTSILRVAAEDWKKGDTFSCMVGHEALPLAFTQKTIDR
LAGEXPTHVNVSVVMAEVDADPSNNLLPSATAITLISVNGIFVICCLTYCFAPRCRE
RRRNERLRRESVRPV

IgE Fc 2el2-scFv-humano (CH2-CH3-CH4)-CD80 (secuencia de nucleótido)

TS09
1390

agcttatggattttcaagtgcagattttcagcttcctgctaatcagtgcttcagtcataatgtccagaggagtcgacattgtgctcaccc
aatctccagcttctttggctgtgtctctaggtcagagagccacaatctcctgcagagccagtgaaagtgttgaatattatgtcacaagtt
taatgcagtggtaccaacagaaaccaggacagccacccaaactcctcatctctgctgcatccaacgtagaatctggggtcoctgcc
aggtttagtggcagtgggtctgggacagacttcagcctcaacatccatcctgtggaggaggatgatattgcaatgtatttctgtcagc
aaagtaggaaggttccttggacgttcggtggaggcaccaagctggaaatcaaacgggtagc-ggtggctcgggcggaggtggg
tcgggtggcggcggatctcaggtgcagctgaaggagtcaggacctggcctggtggcgccctcacagagcctgtccatcacatgc
accgtctcagggttctcattaaccggctatggtgtaaactgggtccgccagcctccaggaaagggtctggagtggctgggaatgat
atggggtgatggaagcacagactataattcagtctcaaatccagactgagcatcaccaaggacaactccaagagccaagttttctt
aaaaatgaacagtctgcaaactgatgacacagccagatactactgtgccagagatggttatagtaactttcattacttatgttatggact
actggggtcaaggaacctcagtcaccgtctcctcagatcacgtctgctccagggacttcaccccgcccaccgtgaagatcttacag
tcgtcctgcgacggcggcgggcacttcccccgaccatccagctcctgtgcctcgtctctgggtacaccccagggactatcaacat
cacctggctggaggacgggcaggtcatggacgtggacttgtccaccgcctctaccacgcaggagggtgagctggcctccacac
aaagcgagctcaccctcagccagaagcactggctgtcagaccgcacctacacctgccaggtcacctatcaaggtcacaccttga
ggacagcaccaagaagtgtgcagattccaaccccgagagggggtgagcgcctacctaagccggccccagcccgttcgaccigttcat
ccgtaagtcgcccacgatcacctgtctggtggtggacctggcacccagcaaggggaccgtgaacctgacctggtcccgggcca
gtgggaagcctgtgaaccactccaccagaaaggaggagaagcagcgcaatggcacgttaaccgtcacgtccaccctgccggtg
ggcacccgagactggatcgaggggagacctaccagtgcagggtgacccaccccccacctgcccagggccctcatgcggtcca
cgaccaagaccagcggcccgcgtgctgccccggaagtctatgcgtttgcgacgccggagtggccgggggagccgggacaagc
gcacctcgcctgcctgatccagaacttcatgcctgaggacatctcggtgcagtggctgcacaacgaggtgcagctccggacgc
ccggcacagcacgacgcagccccgcaagaccaagggctccggcttcttcgtcttcagccgcctggaggtgaccagggccgaat
gggagcagaaagatgagttcatctgccgtgcagtccatgaggcagcgagccccctcacagaccgtccagcgagcggtgtctgtaa
atcccggtaaagcggatccttcgaagctcccatcctgggccattaccttaatctcagtaaatggaattttgtgataigctgcctgacct
actgctttgcccaagatgcagagagagaaggaggaatgagagattgagaagggaaagtgtacgccctgtataaatcgata

IgE Fc 2el2-scFv-humano IgE Fc (CH2-CH3-CH4)-CD80 (secuencia de aminoácido)

1350
1391

MDFQVQIFSFLLISASVIMSRGVDIVLTQSPASLAVSLGQRATISCRASESVEYYVTS
LMQWYQQKPGQPPKLLISAASNVESGVPARFSGSGSGTDFSLNIHPVEEDDIAMYF
CQQSRKVPWTFGGGTKLEIKRGGGGSGGGGSGGGGSQVQLKESGPGLVAPSQSLS
ITCTVSGFSLTGYGVNWVRQPPGKGLEWLGMIWGDGSTDYNSALKSRLSITKDNS
KSQVFLKMNSLQTDDTARYYCARDGYSNFHYYVMDYWGQGTSVTVSSDHVCSR
DFTPPTVKILQSSCDGGGHFPPTIQLLCLVSGYTPGTINITWLEDGQVMDVDLSTAS
TTQEGELASTQSELTLSQKHWLSDRTYTCQVTYQGHTFEDSTKKCADSNPRGVSA
YLSRPSPFDLFIRKSPTITCLVVDLAPSKGTVNLTWSRASGKPVNHSTRKEEKQRNG
TLTVTSTLPVGTRDWIEGETYQCRVTHPHLPRALMRSTTKTSGPRAAPEVYAFATP
EWPGSRDKRTLACLIQNFMPEDISVQWLHNEVQLPDARHSTTQPRKTKGSGFFVFS
RLEVTRAEWEQKDEFICRAVHEAASPSQTVQRAVSVNPGKADPSKLPSWAITLISV
NGIFVICCLTYCFAPRCRERRRNERLRRESVRPV

500A2 scFv (secuencia de nucleótido)

atgttgtatacatctcagctccttgggctttttactcttctggatttcagcctccagaagtgacatagtgctgactcagactccagccactt
tgtctctaatttccttggagaaagagtcacaatgacctgtaaggccagtcagaatattggcacaatcttacactggtatcaccaaaaatcc
aaaggaggctccaagggctctcatcaagtatgcttccgcagtccatcctgggatcccctccagatttcagtggcagtggttcgggaaac
agatttcactctcagcatcaatagcctggagcctgaagatattgcaatttattactgtcaacaaagtagaaggctggccttgtcacgttcg
gtcctggcaccaagctggagataaaacgggggtggcggttggctcgggcggaggtgggtcgggtggcggcggatctcaggtcaa
gctgcagcagtccggttctgaactagggaaacctggggcctcagtgaaactgtcctgcaagacttcaggctacatatttcacagatc
actatatttcttgggtgaaacagaagcctggacaaggcttgcagtggataggaaatgtttatggtggaaatggtggtacaagctaca
atcaaaaatttccagggcaaggccacactgactgtagataaatcctctagcacagcctacatggaactcagcagcctgacatctgag
gattctgcgtctattactgtgcaagaaggctcggtagcgacgggcgtatgctatggactactggggtcaggggatccaagttaccgt
ctcctctgatc

500A2 scFv (secuencia de aminoácido)

MLYTSQLLGLLLFWISASRSDIVLTQTPATLSLIPGERVTMTCKTSQNIGTILHWYH
QKPKEAPRALIKYASQSIPGIPSRFSGSGSETDFTLSINNLEPDDIGIYYCQQSRSWPV
TFGPGTKLEIKRGGGGSGGGGSGGGGSQVKLQQSGSELGKPGASVKLSCKTSGYIF
TDHYISWVKQKPGESLQWIGNVYGGNGGTSYNQKFQGKATLTVDKISSTAYMEL
SSLTSEDSAIYYCARRPVATGHAMDYWGQGIQVTVSSD

NT

~~1331~~
1392

oligo 5'

Nombre IgGWT3

GTTGTTTTCGAAGGATCCGCTTTACCCGGAGACAGGGAGAGGCTCTT

NT
oligo 3'

Nombre hIgGWT5

GTTGTTAGATCTGGAGCCCAAATCTTGTGACAAAACTCACACATG

NT

Oligo 3'

Nombre FADD5

Secuencia

GTTGTGGATCCTTCGAACCCGTTCCTGGTGCTGCTGCACTCGGTGTCG

NT

Oligo 3'

Nombre FADD3

Secuencia

GTTGTTATCGATCTCGAGTTATCAGGACGCTTCGGAGGTAGATGCGTC

NT

FADD CSSCFV (nucleotide sequence)

1350
1343

gtggatccttcgaacccgttcctggtgctgctgcactcggtgtcgtccagcctgtcgagcagcgagctgaccgagctcaagttccta
tgcctcgggcgcgtgggcaagcgcaagctggagcgcgtgcagagcggcctagacctcttctccatgctgctggagcagaacga
cctggagcccgggcacaccgagctcctgcgcgagctgctcgcctccctgcggcgccacgacctgctgcggcgcgtcgacgact
tcgaggcgggggcggcggccggggccgcgcctggggaagaagacctgtgtgcagcatttaacgtcatatgtgataatgtgggg
aaagattggagaaggctggctcgtcagctcaaagtctcagacaccaagatcgacagcatcgaggacagatacccccgcaacctg
acagagcgtgtgcgggagtcactgagaatctggaagaacacagagaaggagaacgcaacagtggcccacctggtgggggctc
tcaggtcctgccagatgaacctggtggctgacctggtacaagaggttcagcaggcccgtgacctccagaacaggagtggggcca
tgtccccgatgtcatggaactcagacgcatctacctccgaagcgtcctgataactcgagatcgataacaac

FADD-CSSCFV (secuencia de aminoácido)

VDPSNPFLVLLHSVSSSLSSSELTELKFLCLGRVGKRKLERVQSGLDLFSMLLEQND
LEPGHTELLRELLASLRRHDLLRRVDDFEAGAAAGAAPGEEDLCAAFNVICDNVG
KDWRRLARQLKVSDTKIDSIEDRYPRNLTERVRESLRIWKNTEKENATVAHLVGA
LRSCQMNLVADLVQEVQQARDLQNRSGAMSPMSWNSDASTSEAS

HCD28tm5B (secuencia de nucleótido)

GTTGTGGATCCTCCCTTTTGGGTGCTGGTGGTGGTTGGTGTCCTGGCTTGCTAT
AGCTTG

HCD28tm3S (secuencia de nucleótido)

GTTGTTTCGAACCCAGAAAATAATAAAGGCCACTGTTACTAGCAAGCTATAGC
AAGCCAG

HCD2Stm5' (secuencia de nucleótido)

GTTGTGGATCCTCCCTTTTGGGTGCTGGTGGT

HCD28tm3' (secuencia de nucleótido)

GTTGTTTCGAACCCAGAAAATAATAAAGGCCAC

HCD80tm5' (secuencia de nucleótido)

1333
1349

GTTGTGGATCCTCCTGCTCCCATCCTGG

HCD80tm3' (secuencia de nucleótido)

GTTGTTTCGAACGGCAAAGCAGTAGGTCAGGC

MFADD5BB (secuencia de nucleótido)

GTTGTGGATCCTTCGAACCCATTCCTGGTGCTGCTGCACTCGCTG

MFADD3XC (secuencia de nucleótido)

GTTGTTATCGATCTCGAGTCAGGGTGTTTCTGAGGAAGACAC

Secuencia de nucleótido FADD de Murina (longitud completa pero sin flanquear las secuencias de transmemebrana -Ig) (secuencia de nucleótido)

gtggatccttcgaacatggacccattcctggtgctgctgcactcgctgtccggcagcctgtcgggcaacgatctgatggagctcaa
gttcttgtgccgcgagcgcgtgagcaaacgaaagctggagcgcgtgcagagtggcctggacctgttcacggtgctgctggagca
gaacgacctggagcgcgggcacaccgggctgctgcgcgagttgctggcctcgctgcgccgacacgatctactgcagcgcctgg
acgacttcgaggcggggacggcgaccgctgcgcccccgggggaggcagatctgcaggtggcatttgacattgtgtgtgacaatg
tggggagagactggaaaagactggcccgcgagctgaaggtgtctgaggccaagatggatgggattgaggagaagtacccccg
aagtctgagtgagcgggtaagggagagtctgaaagtctggaagaatgctgagaagaagaacgcctcggtggccggactggtca
aggcgctgcggacctgcaggctgaatctggtggctgacctggtggaagaagcccaggaatctgtgagcaagagtgagaatatgt
ccccagtactaagggattcaactgtgtcttcctcagaaacaccctgactcgagatcgat

FADD de Murina (secuencia de aminoácido)

VDPSNMDPFLVLLHSLSGSLSGNDLMELKFLCRERVSKRKLERVQSGLDLFTVLLE
QNDLERGHTGLLRELLASLRRHDLLQRLDDFEAGTATAAPPGEADLQVAFDIVCD
NVGRDWKRLARELKVSEAKMDGIEEKYPRSLSERVRESLKVWKNAEKKNASVA
GLVKALRTCRLNLVADLVEEAQESVSKSENMSPVLRDSTVSSSETP

MCASP3-5 (secuencia de nucleótido)

1354
1395

GTTGTGGATCCTTCGAACATGGAGAACAACAAAACCTCAGTGGATTCA

MCASP3-3 (secuencia de nucleótido)

GTTGTTATCGATCTCGAGCTAGTGATAAAAGTACAGTTCTTTCGT

MCASP8-5 (secuencia de nucleótido)

GTTGTTTCGAACATGGATTTCCAGAGTTGTCTTTATGCTATTGCTG

MCASP8-3 (secuencia de nucleótido)

GTTGTTATCGATCTCGAGTCATTAGGGAGGGAAGAAGAGCTTCTTCCG

hcasp3-5 (secuencia de nucleótido)

GTTGTGGATCCTTCGAACATGGAGAACACTGAAAACTCAGTGGAT

hcasp3-3 (secuencia de nucleótido)

GTTGTTATCGATCTCGAGTTAGTGATAAAAATAGAGTTCTTTTGTGAG

hcasp8-5 (secuencia de nucleótido)

GTTGTGGATCCTTCGAACATGGACTTCAGCAGAAATCTTTATGAT

hcasp8-3 (secuencia de nucleótido)

GTTGTTATCGATGCATGCTCAATCAGAAGGGAAGACAAGTTTTTTCT

1 2H7 scFv con mutaciones VHL11 alternativas

Secuencia de nucleótido

aagcttgccgccatggattttcaagtgcagattttcagcttcctgctaatcagtgcttcagtcataattgccagaggacaaattgttctc
tcccagtctccagcaatcctgtctgcatctccaggggagaaggtcacaatgacttgcagggccagctcaagtgtaagttacatgcac

1335
1346

tggtaccagcagaagccaggatcctcccccaaaccctggatttatgccccatccaacctggcttctggagtccctgctcgcttcagt
ggcagtgggtctgggacctcttactctctcacaatcagcagagtggaggctgaagatgctgccacttattactgccagcagtggagt
tttaacccacccacgttcggtgctgggaccaagctggagctgaaagatggcggtggctcgggcggtggtggatctggaggaggt
gggagctctcaggcttatctacagcagtctggggctgag (**uno de los siguienes ten, acn, gan, can, aan, cgn, agn**)
gtgaggcctggggcctcagtgaagatgtcctgcaaggcttctggctacacatttaccagttacaatatgcactgggtaaagcagaca
cctagacagggcctggaatggattggagctatttatccaggaaatggtgatacttcctacaatcagaagttcaagggcaaggccac
actgactgtagacaaatcctccagcacagcctacatgcagctcagcagcctgacatctgaagactctgcggtctatttctgtgcaag
agtggtgtactatagtaactcttactggtacttcgatgtctggggcacagggaccacggtcaccgtctcttctgatcag

Secuencia de aminoácido

MDFQVQIFSFLLISASVIIARGQIVLSQSPAILSASPGEKVTMTCRASSSVSYMHWY
QQKPGSSPKPWIYAPSNLASGVPARFSGSGSGTSYSLTISRVEAEDAATYYCQQWS
FNPPTFGAGTKLELKDGGGSGGGGSGGGGSSQAYLQQSGAE (**one of the following S, T, D, E, Q, N, R, K, H**)
VRPGASVKMSCKASGYTFTSYNMHWVKQTPRQGLEWIGAIYPGNGDTSYNQKFK
GKATLTVDKSSSTAYMQLSSLTSEDSAVYFCARVVYYSNSYWYFDVWGTGTTVT
VSSDQ

2 eliminación de VHL11

Secuencia de nucleótido

aagcttgccgccatggattttcaagtgcagattttcagcttcctgctaatcagtgcttcagtcataattgccagaggacaaattgttctc
tcccagtctccagcaatcctgtctgcatctccaggggagaaggtcacaatgacttgcagggccagctcaagtgtaagttacatgcac
tggtaccagcagaagccaggatcctcccccaaaccctggatttatgccccatccaacctggcttctggagtccctgctcgcttcagt
ggcagtgggtctgggacctcttactctctcacaatcagcagagtggaggctgaagatgctgccacttattactgccagcagtggagt
tttaacccacccacgttcggtgctgggaccaagctggagctgaaagatggcggtggctcgggcggtggtggatctggaggaggt
gggagctctcaggcttatctacagcagtctggggctgaggtgaggcctggggcctcagtgaagatgtcctgcaaggcttctggct
acacatttaccagttacaatatgcactgggtaaagcagacacctagacagggcctggaatggattggagctatttatccaggaaatg
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gggaccacggtcaccgtctcttctgatcag

Secuencia de aminoácido

~~1336~~
1347

MDFQVQIFSFLLISASVIIARGQIVLSQSPAILSASPGEKVTMTCRASSSVSYMHWY
QQKPGSSPKPWIYAPSNLASGVPARFSGSGSGTSYSLTISRVEAEDAATYYCQQWS
FNPPTFGAGTKLELKDGGGSGGGGSGGGGSSQAYLQQSGAEVRPGASVKMSCKA
SGYTFTSYNMHWVKQTPRQGLEWIGAIYPGNGDTSYNQKFKGKATLTVDKSSST
AYMQLSSLTSEDSAVYFCARVVYYSNSYWYFDVWGTGTTVTVSSDQ

3 2H7 VL L106 conmutaciones alternativas

Secuencia de nucleótido

aagcttgccgccatggattttcaagtgcagattttcagcttcctgctaatcagtgcttcagtcataattgccagaggacaaattgttctct
cccagtctccagcaatcctgtctgcatctccaggggagaaggtcacaatgacttgcagggccagctcaagtgtaagttacatgcact
ggtaccagcagaagccaggatcctccccaaaccctggatttatgccccatccaacctggcttctggagtccctgctcgcttcagtg
gcagtgggtctgggacctcttactctctcacaatcagcagagtggaggctgaagatgctgccacttattactgccagcagtggagttt
taacccacccacgttcggtgctgggaccaagctggag (tcn, agn, aan, cgn, can, gan, y derivados no
naturales de estos codones) aaagatggcggtggctcgggcggtggtggatctggaggaggtgggagctc

Secuencia de aminoácido

MDFQVQIFSFLLISASVIIARGQIVLSQSPAILSASPGEKVTMTCRASSSVSYMHWY
QQKPGSSPKPWIYAPSNLASGVPARFSGSGSGTSYSLTISRVEAEDAATYYCQQWS
FNPPTFGAGTKLE **(S, T, R, K, H, Q, N, D, E, y derivados no naturales de estos
aminoácidos en la posición 106)** KDGGGSGGGGSGGGGSS

4 Eliminación VL L106

Secuencia de nucleótido

aagcttgccgccatggattttcaagtgcagattttcagcttcctgctaatcagtgcttcagtcataattgccagaggacaaattgttctc
tcccagtctccagcaatcctgtctgcatctccaggggagaaggtcacaatgacttgcagggccagctcaagtgtaagttacatgcac
tggtaccagcagaagccaggatcctccccaaaccctggatttatgccccatccaacctggcttctggagtccctgctcgcttcagt
ggcagtgggtctgggacctcttactctctcacaatcagcagagtggaggctgaagatgctgccacttattactgccagcagtggagt
ttaacccacccacgttcggtgctgggaccaagctggagaaagatggcggtggctcgggcggtggtggatctggaggaggtgg
gagctc

~~1337~~
1348

Secuencia de aminoácido

MDFQVQIFSFLLISASVIIARGQIVLSQSPAILSASPGEKVTMTCRASSSVSYMHWY
QQKPGSSPKPWIYAPSNLASGVPARFSGSGSGTSYSLTISRVEAEDAATYYCQQWS
FNPPTFGAGTKLEKDGGGSGGGGSGGGGSS

5 IgE CH3 CH4

Secuencia de nucleótido

tccaacccgagaggggtgagcgcctacctaagccggcccagcccgttcgacctgttcatccgcaagtcgcccacgatcacctgtc
tggtggtggacctggcacccagcaaggggaccgtgaacctgacctggtcccgggccagtgggaagcctgtgaaccactccacc
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aagaccaagggctccggcttcttcgtcttcagccgcctggaggtgaccagggccgaatgggagcagaaagatgagttcatctgcc
gtgcagtccatgaggcagcgagcccctcacagaccgtccagcgagcggtgtctgtaaatcccggtaaatgataatctagaa

Secuencia de aminoácido

SNPRGVSAYLSRPSPFDLFIRKSPTITCLVVDLAPSKGTVNLTWSRASGKPVNHSTR
KEEKQRNGTLTVTSTLPVGTRDWIEGETYQCRVTHPHLPRALMRSTTKTSGPRAA
PEVYAFATPEWPGSRDKRTLACLIQNFMPEDISVQWLHNEVQLPDARHSTTQPRK
TKGSGFFVFSRLEVTRAEWEQKDEFICRAVHEAASPSQTVQRAVSVNPGK

6. hIgGlHAgE WCH3 WCH4

Secuencia de nucleótido

tgatcaggagcccaaatcttctgacaaaactcacacatcccccaccgtcccagcatccaaccccgagaggggtgagcgcctacctc
agccggcccagccccgttcgacctgttcatccgcaagtcgcccacgatcacctgtctggtggtggacctggcacccagcaaggggg
accgtgaacctgacctggtcccgggccagtgggaagcctgtgaaccactccaccagaaaggaggagaagcagcgcaatggca
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gccgcctggaggtgaccagggccgaatgggagcagaaagatgagttcatctgccgtgcagtccatgaggcagcgagcccctca
cagaccgtccagcgagcggtgtctgtaaatcccggtaaatgataatctagaa

1348
1349

Secuencia de aminoácido

DQEPKSSDKTHTSPPSPASNPRGVSAYLSRPSPFDLFIRKSPTITCLVVDLAPSKGTV
NLTWSRASGKPVNHSTRKEEKQRNGTLTVTSTLPVGTRDWIEGETYQCRVTHPHL
PRALMRSTTKTSGPRAAPEVYAFATPEWPGSRDKRTLACLIQNFMPEDISVQWLH
NEVQLPDARHSTTQPRKTKGSGFFVFSRLEVTRAEWEQKDEFICRAVHEAASPSQT
VQRAVSVNPGK

7 IgE WCH2 WCH3 WCH4

Secuencia de nucleótido

tgatcacgtctgctccagggacttcaccccgcccaccgtgaagatcttacagtcgtcctgcgacggcggcgggcacttccccccg
accatccagctcctgtgcctcgtctctgggtacaccccagggactatcaacatcacctggctggaggacgggcaggtcatggacg
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gaggacatctcggtgcagtggctgcacaacgaggtgcagctcccggacgcccggcacagcacgacgcagccccgcaagacc
aagggctccggcttcttcgtcttcagccgcctggaggtgaccagggccgaatgggagcagaaagatgagttcatctgccgtgcag
tccatgaggcagcgagcccctcacagaccgtccagcgagcggtgtctgtaaatcccggtaaatgataatctaga

Secuencia de aminoácido

DHVCSRDFTPPTVKILQSSCDGGGHFPPTIQLLCLVSGYTPGTINITWLEDGQVMDV
DLSTASTTQEGELASTQSELTLSQKHWLSDRTYTCQVTYQGHTFEDSTKKCADSN
PRGVSAYLSRPSPFDLFIRKSPTITCLVVDLAPSKGTVNLTWSRASGKPVNHSTRKE
EKQRNGTLTVTSTLPVGTRDWIEGETYQCRVTHPHLPRALMRSTTKTSGPRAAPE
VYAFATPEWPGSRDKRTLACLIQNFMPEDISVQWLHNEVQLPDARHSTTQPRKTK
GSGFFVFSRLEVTRAEWEQKDEFICRAVHEAASPSQTVQRAVSVNPGK

8. hIgGlH/IgE CH3 CH4 (ORF)

1339
1350

Secuencia de nucleótido

tgatcaggagcccaaatcttctgacaaaactcacacatcccccaccgtccccagcatccaacccgagaggggtgagcgcctaccta
agccggcccagcccgttcgacctgttcatccgcaagtcgcccacgatcacctgtctggtggtggacctggcacccagcaagggg
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cgttaaccgtcacgtccaccctgccggtgggcacccgagactggatcgaggggggagacctaccagtgcagggtgacccacccc
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gccgcctggaggtgaccagggccgaatgggagcagaaagatgagttcatctgccgtgcagtccatgaggcagcgagcccctca
Cagaccgtccagcgagcggtgtctgtaaatcccggtaaagcggatccttcgaa

Secuencia de aminoácido

DQEPKSSDKTHTSPPSPASNPRGVSAYLSRPSPFDLFIRKSPTITCI VVDLAPSKGTV
NLTWSRASGKPVNHSTRKEEKQRNGTLTVTSTLPVGTRDWIEGETYQCRVTHPHL
PRALMRSTTKTSGPRAAPEVYAFATPEWPGSRDKRTLACLIQNFMPEDISVQWH
NEVQLPDARHSTTQPRKTKGSGFFVFSRLEVTRAEWEQKDEFICRAVHEAASPSQT
VQRA VSVNPGKSGSFE

9 2H7 VHL11S scFv hIgG1 (SSS-S) H hIgE WCH3 WCH4

Secuencia de nucleótido

1340
1351

aagcttgccgccatggattttcaagtgcagattttcagcttcctgctaatcagtgcttcagtcataattgccagaggacaaattgttctct
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aagggctccggcttcttcgtcttcagccgcctggaggtgaccagggccgaatgggagcagaaagatgagttcatctgccgtgcag
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Secuencia de aminoácido

MDFQVQIFSFLLISASVIIARGQIVLSQSPAILSASPGEKVTMTCRASSSVSYMHWY
QQKPGSSPKPWIYAPSNLASGVPARFSGSGSGTSYSLTISRVEAEDAATYYCQQWS
FNPPTFGAGTKLELKDGGGSGGGGSGGGGSSQAYLQQSGAESVRPGASVKMSCK
ASGYTFTSYNMHWVKQTPRQGLEWIGAIYPGNGDTSYNQKFKGKATLTVDKSSS
TAYMQLSSLTSEDSAVYFCARVVYYSNSYWYFDVWGTGTTVTVSSDQEPKSSDK
THTSPPSSASNPRGVSAYLSRPSPFDLFIRKSPTITCLVVDLAPSKGTVNLTWSRASG
KPVNHSTRKEEKQRNGTLTVTSTLPVGTRDWIEGETYQCRVTHPHLPRALMRSTT
KTSGPRAAPEVYAFATPEWPGSRDKRTLACLIQNFMPEDISVQWLHNEVQLPDAR
HSTTQPRKTKGSGFFVFSRLEVTRAEWEQKDEFICRAVHEAASPSQTVQRAVSVNP
GK

10 2H7 VHL11S scFv hIgG1 (SSS-P) H hIgE WCH3 WCH4

Secuencia de nucleótido

1341
1352

aagcttgccgccatggattttcaagtgcagattttcagcttcctgctaatcagtgcttcagtcataattgccagaggacaaattgttctct
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ggtgatacttcctacaatcagaagttcaaggcaaggccacactgactgtagacaaatcctccagcacagcctacatgcagctcag
cagcctgacatctgaggactctgcggtctatttctgtgcaagagtggtgtactatagtaactcttactggtacttcgatgtctgggcac
agggaccacggtcaccgtctctcagtcaggagcccaaatcttctgacaaaactcacacatcccaccgtcccccagcatccaacc
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gaggagaagcagcgcaatggcacgttaaccgtcacgtccaccctgccggtgggcaccccgagacttgatcgagggggagacct
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aagggctccggcttcttcgtcttcagccgcctggaggtgaccagggccgaatgggagcagaaagatgagttcatctgccgtgcag
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Secuencia de aminoácido

MDFQVQIFSFLLISASVIIAR. GQIVLSQSPAILSASPGEKVTMTCRASSSVSYMHWY
QQKPGSSPKPWIYAPSNLASGVPARFSGSGSGTSYSLTISRVEAEDAATYYCQQWS
FNPPTFGAGTKLELKDGGGSGGGGSGGGGSSQAYLQQSGAESVRPGASVKMSCK
ASGYTFTSYNMHWVKQTPRQGLEWIGAIYPGNGDTSYNQKFKGKATLTVDKSSS
TAYMQLSSLTSEDSAVYFCARVVYYSNSYWYFDVWGTGTTVTVSSDQEPKSSDK
THTSPPSPASNPRGVSAYLSRPSPFDLFIRKSPTITCLVVDLAPSKGTVNLTWSRASG
KPVNHSTRKEEKQRNGTLTVTSTLPVGTRDWEGETYQCRVTHPHLPRALMRSTT
KTSGPRAAPEVYAFATPEWPGSRDKRTLACLIQNFMPEDISVQWLHNEVQLPDAR
HSTTQPRKTKGSGFFVFSRLEVTRAEWEQKDEFICRAVHEAASPSQTVQRAVSVNP
GK

10 2H7 VL L106S

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tacccacccacgttcggtgctgggaccaagctggagctgaaagatggcggtggctcgggcggtggtggatctggaggaggtg
gaagctc

Secuencia de aminoácido

1352
1353

MDFQVQIFSFLLISASVIIARGQIVLSQSPAILSASPGEKVTMTCRASSSVSYMHWY
QQKPGSSPKPWIYAPSNLASGVPARFSGSGSGTSYSLTISRVEAEDAATYYCQQWS
FNPPTFGAGTKLESKDGGGSGGGGSGGGGSS

11 2H7 VL L106S scFv

Secuencia de nucleótido

aagcttgccgccatggattttcaagtgcagattttcagcttcctgctaatcagtgcttcagtcataattgccagaggacaaattgttctct
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ggtgatacttcctacaatcagaagttcaagggcaaggccacactgactgtagacaaatcctccagcacagcctacatgcagctcag
cagcctgacatctgaagactctgcggtctatttctgtgcaagagtggtgtactatagtaactcttactggtacttcgatgtctggggcac
agggaccacggtcaccgtctcttctgatcag

Secuencia de aminoácido

MDFQVQIFSFLLISASVIIARGQIVLSQSPAILSASPGEKLVTMTCRASSSVSYMHWY
QQKPGSSPKPWIYAPSNLASGVPARFSGSGSGTSYSLTISRVEAEDAATYYCQQWS
FNPPTFGAGTKLESKDGGGSGGGGSGGG (SSQAYLQQSGAELVRPGASVKIvfSCK
ASGYFTSYMmWKQTPRQGLEWIGAIYPGNGDTSYNQK FKGKATLTVDKSSS
TAYMQLSSLTSEDSAVYFCARVVYYSNSYWYFDVWGTGTTVTVSL) Q

12 2H7 scFs VL L106S VHLlIS sc

Secuencia de nucleótido

aagcttgccgccatggattttcaagtgcagattttcagcttcctgctaatcagtgcttcagtcataattgccagaggacaaattgttctc
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tggtaccagcagaagccaggatcctccccaaaccctggatttatgccccatccaacctggcttctggagtccctgctcgcttcagt
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1345
1354

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acagggaccacggtcaccgtctcttctgatcag

Secuencia de aminoácido

MDFQVQIFSFLLISASVIIARGQIVLSQSPAILSASPGEKVTMTCRASSSVSYMHWY
QQKPGSSPKPWIYAPSNLASGVPARFSGSGSGTSYSLTISRVEAEDAATYYCQQWS
FNPPTFGAGTKLESKDGGGSGGGGSGGGGSSQAYLQQSGAESVRPGASVKMSCK
ASGYTFTSYNMHWVKQTPRQGLEWIGAIYPGNGDTSYNQKFKGKATLTVDKSSS
TAYMQLSSLTSEDSAVYFCARVVYYSNSYWYFDVWGTGTTVTVSSDQ

10 Ligador de articulación IgD humano con sitios de restricción adheridos

Nucleótido

gtggatccaggttcgaagtctccaaaggcacaggcctcctccgtgcccactgcacaaccccaagcagagggcagcctcgccaa
ggcaaccacagccccagccaccacccgtaacacaggaagaggaggagaagagaagaagaaggagaaggagaaagaggaa
caagaagagagagagacaaagaccggtgcagtcgacg

Aminoácido

VDPGSKSPKAQASSVPTAQPQAEGSLAKATTAPATTRNTGRGGEEKKKEKEKEEQ
EERETKTGAVD

Secuencia de dominio de articulación IgD nativo

(Incluye un residuo de cisteína – truncamos la anterior articulación para ese residuo para estas construcciones)

Nucleótido

1344
1355

gagtctccaaaggcacaggcctcctccgtgcccactgcacaaccccaagcagagggcagcctcgccaaggcaaccacagccc
cagccaccacccgtaacacaggaagaggaggagaagagaagaagaaggagaaggagaaagaggaacaagaagagagaga
gacaaagacaccagagtgtccgagccacacccagcctcttggcgtctacctgctaaccccct

Secuencia de aminoácido

ESPKAQASSVPTAQPQAEGSLAKATTAPATTRNTGRGGEEKKKEKEKEEQEERET
KTPECPSHTQPLGVYLLTP

12 2H7 VH L11S

Secuencia de nucleótido

caggcttatctacagcagtctggggctgagtcggtgaggcctggggcctcagtgaagatgtcctgcaaggcttctggctacacatt
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Secuencia de aminoácido

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13 2H7 VH L11S scFv

Secuencia de nucleótido

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Secuencia de aminoácido

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14 2H7 scFv VH L11S hIgG1 (CSC-S) H WCH2 WCH3

Secuencia de nucleótido

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Secuencia de aminoácido

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15 2H7 scFv VH L11S IgE WCH2 WCH3 WCH4

Secuencia de nucleótido

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Secuencia de aminoácido

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16 2H7 scFv VH L11S mIgE WCH2 WCH3 WCH4

Secuencia de nucleótido

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1359

Secuencia de aminoácido

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17 2H7 scFv VH L11S hIgA WH WCH2 T4CH3

Secuencia de nucleótido

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Secuencia de aminoácido

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18 2H7 scFv VH L11S mIgA WH WCH2 T4 CH3

Secuencia de nucleótido

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Secuencia de aminoácido

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Secuencia de nucleótido

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Secuencia de aminoácido

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20 Región K322S CH2

Secuencia de nucleótido

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Secuencia de aminoácido

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21 K322S CH2 WCH3

Secuencia de nucleótido

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Secuencia de aminoácido

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1 K322L CH2 WCH3

Secuencia de nucleótido

1362
1363

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Secuencia de aminoácido

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22 2H7 scFv VHL11S hIgG1 (SSS-S)H K322SCH2 WCH3

Secuencia de nucleótido

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1353
1369

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Secuencia de aminoácido

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PGK

23 ZH7 sch VHLlIS hIgGl (SSS-S) H B22I, H2 WCHS"-

Secuencia de nucleótido

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Secuencia de aminoácido

1354
1365

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PGK

24 2H7 scFv VHLlIS hIgGl (CSS-S) H K322SCH2 WCH3

Secuencia de nucleótido

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Secuencia de aminoácido

1385
1366

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PGK

25. P331S CH2

Secuencia de nucleótido

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Secuencia de aminoácido

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26 P331S CH2 WCH3

Secuencia de nucleótido

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Secuencia de aminoácido

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27 2H7 scFv VH L11S (SSS-S)H P331S CH2 WCH3

Secuencia de nucleótido

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Secuencia de aminoácido

~~1357~~
1368

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PGK

28 2H7 scFv VH L11S (CSS-S) H P331S CH2 WCH3

Secuencia de nucleótido

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Secuencia de aminoácido

~~1358~~
1369

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PGK

29 Región T256N CH2

Secuencia de nucleótido

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Secuencia de aminoácido

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30 T256N CH2 WCH3

Secuencia de nucleótido

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Secuencia de aminoácido

1359
1370

PELLGGPSVFLFPPKPKDTLMISRNPEVTCVVVDVSHEDPEVKFNWYVDGVEVHN
AKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKG
QPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPV
LDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

31 2H7 scFv VH L11S (SSS-S) H T256N CH2 WCH3

Secuencia de nucleótido

agcttgccgccatggatttccaagtgcagattttcagcttcctgctaatcagtgcttcagtcataattgccagaggacaaattgttctct
cccagtctccagcaatcctgtctgcatctccaggggagaagggtcacaatgacttgcagggccagctcaagtgtaagttacatgcact
ggtaccagcagaagccaggatcctcccccaaaccctggatttatgccccatccaacctggcttctggagtccctgctcgcttcagtg
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Secuencia de aminoácido

MDFQVQIFSFLLISASVIIARGQIVLSQSPAILSASPGEKVTMTCRASSSVSYMHWY
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FNPPTFGAGTKLELKDGGGSGGGGSGGGGSSQAYLQQSGAESVRPGASVKMSCK
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EKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPE
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SPGK

~~1360~~
1371

32 2H7 scFv VH L11S (CSS-S) H T256N CH2 WCH3

Secuencia de nucleótido

aagcttgccgccatggattttcaagtgcagattttcagcttcctgctaatcagtgcttcagtcataattgccagaggacaaattgttctct
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Secuencia de aminoácido

MDFQVQIFSFLLISASVIIARGQIVLSQSPAILSASPGEKVTMTCRASSSVSYMHWY
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EKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPE
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SPGK

1361
1372

33 RTPE/QNAK (255-258) CH2

Secuencia de nucleótido

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Secuencia de aminoácido

PELLGGPSVFLFPPKPKDTLMISQNAKVTCVVVDVSHEDPEVKFNWYVDGVEVHN
AXTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAK

34. RTPE/QNAK (255-258) CH2 WCH3

Secuencia de nucleótido

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Secuencia de aminoácido

PELLGGPSVFLFPPKPKDTLMISQNAKVTCVVVDVSHEDPEVKFNWYVDGVEVHN
AKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKG
QPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPV
LDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

35 2H7 scFv VH L11S (SSS-S) H RTPE/QNAK (255-258) CH2 WCH3

1362
1373

Secuencia de nucleótido

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Secuencia de aminoácido

NIDFQVQIFSFLLISASVIIARGQIVLSQSPAILSASPGEKVTMTCRASSSVSY1XEWY
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FNPPTFGAGTKLELKDGGGSGGGGSGGGGSSQAYLQQSGAESVRPGASVKMSCK
ASGYTFTSYNMHWVKQTPRQGLEWIGAIYPGNGDTSYNQKFKGKATLTVDKSSS
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VDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPI
EKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPE
NNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSL
SPGK

36 2H7 scFv VH L11S (CSS-S) H RTPE/QNAK (255-258) CH2 WCH3

1365
1379

Secuencia de nucleótido

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Secuencia de aminoácido

MDFQVQIFSFLLISASVIIARGQIVLSQSPAILSASPGEKVTMTCRASSSVSYMHWY
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FNPPTFGAGTKLELKDGGGSGGGGSGGGGSSQAYLQQSGAESVRPGASVKMSCK
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EKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPE
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SPGK

36 región K290Q CH2

Secuencia de nucleótido

cctgaactcctgggggggaccgtcagtcttcctcttccccccaaaacccaaggacaccctcatgatctcccggacccctgaggtcac
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~~1364~~
1375

Secuencia de aminoácido

PELLGGPSVFLFPPKPKDTLMISRTPEVTCVWDVSHEDPEVKFNWYVDGVEVHN
AKTQPREEQYNSTYRWSVLTVLHQDWLNGKBYKCKVSNKALPAPIEKTISKAK

37 K290Q CH2 WCH3

Secuencia de nucleótido

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Secuencia de aminoácido

PELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHN
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38 2H7 scFv VH L11S (SSS-S) H K290Q CH2 WCH3

Secuencia de nucleótido

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1366
1376

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Secuencia de aminoácido

MDFQVQIFSFLLISASVIIARGQIVLSQSPAILSASPGEKVTMTCRASSSVSYMHWY
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THTSPPSSAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYV
DGVEVHNAKTQPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIE
KTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPEN
NYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLS
PGK

39 2H7 scfv VH L11S (CSS-S) H K290Q CH2 WCH3

Secuencia de nucleótido

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1366
1377

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Secuencia de aminoácido

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PGK

40 A339PCH2

Secuencia de nucleótido

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Secuencia de aminoácido

PELLGGPSVFLFPPKPKDTLMISRTPEVTCVWDVSHEDPEVKFNWYVDGVEVHN
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1367
1378

41 A339P2CHZ WCH3

Secuencia de nucleótido

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Secuencia de aminoácido

PELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHN
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QPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPV
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42 2H7 scFv VHL11S (SSS-S) H A339P CH2 WCH3

Secuencia de nucleótido

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1368
1379

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Secuencia de aminoácido

MDFQVQIFSFLLISASVIIARGQIVLSQSPAILSASPGEKVTMTCRASSSVSYMHWY
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PGK

43 2H7 scFv VHL11S (CSS-S) H A339P CH2 WCH3

Secuencia de nucleótido

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1369
1380

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Secuencia de aminoácido

MDFQVQIFSFLLISASVIIARGQIVLSQSPAILSASPGEKVTMTCRASSSVSYMHWY
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PGK

44. G28-1VH

Secuencia de nucleótido

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gatcag

Secuencia de aminoácido

AVQLQQSGPELEKPGASVKISCKASGYSFTGYNMNWVKQNNGKSLEWIGNIDPY
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1370
1381

45 G28-1VL

Secuencia de nucleótido

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cgtca

Secuencia de aminoácido

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46 G28-1 scFv

Secuencia de nucleótido

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Secuencia de aminoácido

MVSTAQFLGLLLLWLTGGRCDIQMTQSPASLSASVGETVTITCRTSENVYSYLAW
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DNPWTFGGGTELEIKGGGGSGGGGSGGGGSSAVQLQQSGPELEKPGASVKISCKA

1371
1382

SGYSFTGYNMNWVKQNNGKSLEWIGNIDPYYGGTTYNRKFKGKATLTVDKSSST
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47 G28-1 VHL11S

Secuencia de nucleótido

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gatcag

Secuencia de aminoácido

AVQLQQSGPESEKPGASVKISCKASGYSFTGYNMNWVKQNNGKSLEWIGNIDPYY
GGTTYNRKFKGKATLTVDKSSSTAYMQLKSLTSEDSAVYYCARSVGPMDYWGQ
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48 G28-1 VHL11S scFv

Secuencia de nucleótido

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Secuencia de aminoácido

1383

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49 G28-1 scFv (SSS-S) H WCH2 WCH3

Secuencia de nucleótido

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Secuencia de aminoácido

MVSTAQFLGLLLLWLTGGRCDIQMTQSPASLSASVGETVTITCRTSENVYSYLAW
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1384

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50 G28-1 scFv IgAW H IgGlWCH2 WCH3

Secuencia de nucleótido

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Secuencia de aminoácido

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AYMQLKSLTSEDSAVYYCARSVGPMDYWGQGTSVTVSSDQPVPSTPPTPSPSTPPT
PSPSCAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVILFNWYVDG

1374
1385

VEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTI
SKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNY
KTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG
K

51 G28-1 scFv VHL11S (SSS-S) H WCH2 WCH3

Secuencia de nucleótido

aagcttgccgccatggtatccacagctcagttccttgggttgctgctgctgtggcttacaggtggcagatgtgacatccagatgactc
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Secuencia de aminoácido

MVSTAQPLGLLLLWLTGGRCDIQMTQSPASLSASVOETVTfTCRTSENVYSYLAW
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DNPWTFGGGTELEIKGGGGSGGGGSGGGGSSAVQLQQSGPESEKPGASVKISCKA
SGYSFTGYNMNWVKQNNGKSLEWIGNIDPYYGGTTYNRKFKGKATLTVDKSSST
AYMQLKSLTSEDSAVYYCARSVGPMDYWGQGTSVTVSSDHDQEPKSSDKTHTSP
PSSAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVE

1385
1386

VHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISK
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52 G28-1 scFv VHI, 11S (CSS-S) H WCH2 WCH3

Secuencia de nucleótido

aagcttgccgccatggtatccacagctcagttccttgggttgctgctgctgtggcttacaggtggcagatgtgacatccagatgactc
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Secuencia de aminoácido

MVSTAQFLGLLLLWLTGGRCDIQMTQSPASLSASVGETVTITCRTSENVYSYLAW
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DNPWTFGGGTELEIKGGGGSGGGGSGGGGSSAVQLQQSGPESEKPGASVKISCKA
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1387

GQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPP
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53 G28-1 scFv VH L11S (CSC-S) H WCH2 WCH3

Secuencia de nucleótido

aagcttgccgccatggtatccacagctcagttccttgggttgctgctgctgtggcttacaggtggcagatgtgacatccagatgactc
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Secuencia de aminoácido

MVSTAQFLGLLLLWLTGGRCDIQMTQSPASLSASVGETVTITCRTSENVYSYLAW
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DNPWTFGGGTELEIKGGGGSGGGGSGGGGSSAVQLQQSGPESEKPGASVKISCKA
SGYSFTGYNMNWVKQNNGKSLEWIGNIDPYYGGTTYNRKFKGKATLTVDKSSST
AYMQLKSLTSEDSAVYYCARSVGPMDYWGQGTSVTVSSDQEPKSCDKTHTSPPC
SAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEV
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1377
1388

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54. G28-1 scFv VH L11S (SSC-P) H WCH2 WCH3

Secuencia de nucleótido

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Secuencia de aminoácido

MVSTAQFLGLLLLWLTGGRCDIQMTQSPASLSASVGETVTITCRTSENVYSYLAW
YQQKQGKSPQLLVSFAKTLAEGVPSRFSGSGSGTQFSLKISSLQPEDSGSYFCQHHS
DNPWTFGGGTELEIKGGGGSGGGGSGGGGSSAVQLQQSGPESEKPGASVKTSCKA
SGYSFTGYNMNWVKQNNGKSLEWIGNIDPYYGGTTYNRKFKGKATLTVDKSSST
AYMQLKSLTSEDSAVYYCARSVGPMDYWGQGTSVTVSSDQEPKSSDKTHTSPPCP
PELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVH
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1378
1389

GQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKT'lPP
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IL 54. HCTLA4 HIGG1 (SSS-S) H P238SCH2 WCH3

Secuencia de nucleótido

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Secuencia de aminoácido

MACLGFQRHKAQLNLAARTWPCTLLFFLLFIPVFCKAMHVAQPAVVLASSRGIAS
FVCEYASPGKATEVRVTVLRQADSQVTEVCAATYMTGNELTFLDDSICTGTSSGN
QVNLTIQGLRAMDTGLYICKVELMYPPPYYLGIGNGTQIYVIDPEPCPDSDQPKSSD
KTHTSPPSSAPELLGGSSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWY
VDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPI
EKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPE
NNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSL
SPGK

55 Fc2-2 VL

Secuencia de nucleótido

1379
1390

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Secuencia de aminoácido

MDSQAQVLMLLLLWVSGTCGDIVMSQSPSSLAVSVGEKVSMSCKSSQSLLYNHN
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56 FC2-2VH

Secuencia de nucleótido

gggagctcgcaggtgcagttgaaggagtcaggacctggcctggtggcgccctcacagagcctgtccatcacatgcaccgtctca
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Secuencia de aminoácido

GSSQVQLKESGPGLVAPSQSLSITCTVSGFSLTVYGVNWVRQPPGKGLDWLGMIW
GDGSTDYNSALKSRLSISKDNSKSQVFLKMDSLQTDDTARYYCARDHYGTHYAM
DYWGQGTSVTVSSDQ

57 FC2-2scFv

Secuencia de nucleótido

gttgttaagcttgccgccatggattcacaggcccaggttcttatgttactgctgctatgggtatctggtacctgtggggacattgtgatg
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1380
1391

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Secuencia de aminoácido

MDSQAQVLMLLLLWVSGTCGDIVMSQSPSSLAVSVGEKVSMSCKSSQSLLYNHN
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SLSITCTVSGFSLTVYGVNWVRQPPGKGLDWLGMIWGDGSTDYNSALKSRLSISK
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58. FC2-2 VHL11S

Secuencia de nucleótido

gggagctctcaggtgcagttgaaggagtcaggacctggctcggtggcgccctcacagagcctgtccatcacatgcaccgtctcag
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Secuencia de aminoácido

(GSS)QVQLKESGPGSVAPSQSLSITCTVSGFSLTVYGVNWVRQPPGKGLDWLGMI
WGDGSTDYNSALKSRLSISKDNSKSQVFLKMDSLQTDDTARYYCARDHYGTHYA
MDYWGQGTSVTVSSDQ

59 FC2-2 VH L11S scFv

Secuencia de nucleótido

1392

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Secuencia de aminoácido

MDSQAQVLMLLLLWVSGTCGDIVMSQSPSSLAVSVGEKVSMSCKSSQSLLYNHN
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60 FC2-2 (SSS-S) H WCH2 WCH3

Secuencia de nucleótido

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1393

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aminoácido

MDSQAQVLMLLLLWVSGTCGDIVMSQSPSSLAVSVGEKVSMSCKSSQSLLYNHN
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61 FC2-2 VHL11S (SSS-S) H WCH2 WCH3

Secuencia de nucleótido

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Secuencia de aminoácido

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62 UCHL-1 VH

Secuencia de nucleótido

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Secuencia de aminoácido

MGRLTSSFLLLIVPAYVLSQITLKESGPGILQPSQTLSLTCSFSGFSLTTYGIGVGWIR
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63 UCHL-1 VL

1324
1395

Secuencia de nucleótido

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Secuencia de aminoácido

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64 UCHL-1 scFv

Secuencia de nucleótido

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Secuencia de aminoácido

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1385
1396

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65 UCHL-1 VH I11SL12S

Secuencia de nucleótido

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gatca

Secuencia de aminoácido

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66 UCHL-1 scFv VH L11S

Secuencia de nucleótido

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Secuencia de aminoácido

1386
1397

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67 UCHL-1 scFv (SSS-S) H WCH2 WCH3

Secuencia de nucleótido

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Secuencia de aminoácido

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1387
1398

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68 UCHL-1 scFv VHL11S (SSS-S) H WCH2 WCH3

Secuencia de nucleótido

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Secuencia de aminoácido

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1388
1399

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69 5B9 VHL11S

Secuencia de nucleótido

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Secuencia de aminoácido

(GSS)QVQLKQSGPGSVQSSQSLSITCTVSGFSLTTYAVHWVRQSPGKGLEWLGVI
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YAMDYWGQGTSVTVSS

73 5B9 VH L11S scFv

Secuencia de nucleótido

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Secuencia de aminoácido

1389
1400

MRFSAQLLGLLVLWIPGSTADIVMTQAAFSNPVTLGTSASISCRSSKSLLHSNGITY
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70 5B9 scFv VHL11S (SSS-S) H WCH2 WCH3

Secuencia de nucleótido

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Secuencia de aminoácido

MRFSAQLLGLLVLWIPGSTADIVMTQAAFSNPVTLGTSASISCRSSKSLLHSNGITY
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1390
1401

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76. 2H7 scFv VH L11S (SSS-S) H P238SC H2 WCH3

Secuencia de nucleótido

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Secuencia de aminoácido

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PGK

78 2H7 scFv VH L11S (SSS S) H WCH2 WCH3

1397
1402

Secuencia de nucleótido

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Secuencia de aminoácido

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PGK

79 ZH7 scFv VH L11S (CSS-S) H WCH2 WCH3

Secuencia de nucleótido

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Secuencia de aminoácido

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77 G8-1 scFv VH L11S (SCS-S) H WCH2 WCH3

Secuencia de nucleótido

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Secuencia de aminoácido

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79 G28-1 scFv VHL11S (CCS-P) H WCH2 WCH3

Secuencia de nucleótido

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Secuencia de aminoácido

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80 G28-1 scFv VH LI IS (SCC-P) H WCH2 WCH3

Secuencia de nucleótido

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Secuencia de aminoácido

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81 G28-1 scFv VH L11S mIgE CH2 CH3 CH4

Secuencia de nucleótido

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Secuencia de aminoácido

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82 G28-1 scFv VH L11S mIgA WH WCH2 T4CH3

Secuencia de nucleótido

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Secuencia de aminoácido

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83 G28-1 scFv Vh LI 1 S MgE CH2 CH3 CH4

Secuencia de nucleótido

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Secuencia de aminoácido

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84. G28-1 scFv VH L11S MgA WH WCH2 T4CH3

Secuencia de nucleótido

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Secuencia de aminoácido

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85 HD37 VL

Secuencia de nucleótido

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Secuencia de aminoácido

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86. HD37 VH

Secuencia de nucleótido

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Secuencia de aminoácido

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87 HD37 scFv

Secuencia de nucleótido

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Secuencia de aminoácido

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88 HD37 VHL11S

Secuencia de nucleótido

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Secuencia de aminoácido

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89 HD37 SCFV VHL11S

Secuencia de nucleótido

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Secuencia de aminoácido

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90 HD37 scF'e IgAH hIgG1 WCH2 T4CH3

Secuencia de nucleótido

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Secuencia de aminoácido

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1405
1414

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REKYLTWASRQEPSQGTTTFAVTSILRVAAEDWKKGDTFSCMVGHEALPLAFTQK
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91 HD37 scFv (SSS-S) H WCH2 WCH3

Secuencia de nucleótido

aagcttgccgccatggagacagacacactcctgctatgggtgctgctgctctgggttccaggctccactggtgacattgtgctgacc
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Secuencia de aminoácido

METDTLLLWVLLLWVPGSTGDIVLTQSPASLAVSLGQRATISCKASQSVDYDGDS
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1403
1415

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92 HD37 scFv VH L11S (SSS-S) H WCH2 WCH3

Secuencia de nucleótido

aagcttgccgccatggagacagacacactcctgctatgggtgctgctgctctgggttccaggctccactggtgacattgtgctgacc
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Secuencia de aminoácido

METDTLLLWVLLLWVPGSTGDIVLTQSPASLAVSLGQRATISCKASQSVDYDGDS
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1409
1416

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91 L6 VL

Secuencia de nucleótido

atggattttcaagtgcagattttcagcttcctgctaatcagtgcttcagtcataatgtccagaggacaaattgttctctcccagtctccag
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ct

Secuencia de aminoácido

MDFQVQIFSFLLISASVIMSRGQIVLSQSPAILSASPGEKVTLTCRASSSVSFMNWYQ
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92 L6 VH

Secuencia de nucleótido

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aaaatgaggacatggctacatatttctgtgcaagatttagctatggtaactcacgttacgctgactactggggccaaggcaccactct
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Secuencia de aminoácido

1405
1417

QIQLVQSGPELKKPGETVKISCKASGYTFTNYGMNWVKQAPGKGLKWMGWINTY
TGQPTYADDFKGRFAFSLETSAYTAYLQINNLKNEDMATYFCARFSYGNSRYADY
WGQGTTLTVSSD

93 L6 scFv

Secuencia de nucleótido

atggattttcaagtgcagattttcagcttcctgctaatcagtgcttcagtcataatgtccagaggacaaattgttctctcccagtctccag
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Secuencia de aminoácido

MDFQVQIFSFLLISASVIMSRGQIVLSQSPAILSASPGEKVTLTCRASSSVSFMNWYQ
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94 L6 VHL11S

Secuencia de nucleótido

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1406
1418

Secuencia de aminoácido

QIQLVQSGPESKXPGETVmSCKASGYTFTNYGMNWVKQAPGKGLKWMGWINTY
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95 L6 VH L11S scFv

Secuencia de nucleótido

Aagcttgttgttatggattttcaagtgcagattttcagcttcctgctaatcagtgcttcagtcataatgtccagaggacaaattgttctctc
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Secuencia de aminoácido

MDFQVQIFSFLLISASVIMSRGQIVLSQSPAILSASPGEKVTLTCRASSSVSFMNWYQ
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YTAYLQINNLKNEDMATYFCARFSYGNSRYADYWGQGTTLTVSSD

96 L6 Or L6 VHL11S scFv IgAH hIgG1 WCH2 WCH3

Secuencia de nucleótido

(se muestra L6 VHL1 1S)

1407
1919

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Secuencia de aminoácido

MDFQVQIFSFLLISASVIMSRGQIVLSQSPAILSASPGEKVTLTCRASSSVSFMNWYQ
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SLSPGK

97 L6 scFv VHL11S (SSS-S) H WCH2 WCH3

Secuencia de nucleótido

1408
1420

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Secuencia de aminoácido

MDFQVQIFSFLLISASVIMSRGQIVLSQSPAILSASPGEKVTLTCRASSSVSFMNWYQ
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NPLTFGAGTKLELKELSGGGGSGGGGSGGGGSLQIQLVQSGPESKKPGETVKISCK
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YTAYLQINNLKNEDMATYFCARFSYGNSRYADYWGQGTTLTVSSDQEPKSSDKT
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DGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIE
KTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPEN
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PGK

98 P238 CH2 WCH3

a. P238S CH2 WCH3

1409
1421

Secuencia de nucleótido

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Secuencia de aminoácido

PELLGGSSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHN
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QPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPV
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b. (SSS-S) H P238S CH2 WCH3

Secuencia de nucleótido

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Secuencia de aminoácido

DQEPKSSDKTHTSPPSSAPELLGGSSVFLFPPKPKDTLMISRTPEVTCVVVDVSHED
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1470
1422

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HYTQKSLSLSPGK*

99 a.CD16-6 bajo (ED)+NL+(SSS-S) H P238S CH2 WCH3

Secuencia de nucleótido

ggagcccaaatcttctgacaaaactcacacatccccaccgtcctcagcacctgaactcctgggggatcgtcagtcttcctcttccc
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Secuencia de aminoácido

MWQLLLPTALLLLVSAGMRTEDLPKAVVFLEPQWYRVLEKDSVTLKCQGAYSPE
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LYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

100 b CD16-6 bajo (ED)+HE4LP+ (SSS-S) H P238S CH2 WCH3

Secuencia de nucleótido

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1423

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Secuencia de aminoácido

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101 CD16-9 alto (ED) (SSS-S) H P238S CH2 CH3

a CD16-9 alto (ED) NL+ (SSS-S) H P238S CH2 CH3

Secuencia de nucleótido

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1442

1429

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Secuencia de aminoácido

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CD16-9 alto (ED) +HE4LP+hIgG1 (SSS-S) H P238S CH2 CH3

Secuencia de nucleótido

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1425

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Secuencia de aminoácido

MPACRLGPLAAALLLSLLLFGFTLVSGTGAMRTEDLPKAVVFLEPQWYRVLEKDS
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102 a. CD16 ED bajo (líder nativo)

Secuencia de nucleótido

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Secuencia de aminoácido

mwqlllptallllvsagmrtedlpkavvflepqwyrvlekdsvtlkcqgayspednstqwfhneslissqassyfidaatvdds
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1426

b CD16 ED bajo (HE4 líder)

Secuencia de nucleótido

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Secuencia de aminoácido

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103 a. CD16 ED alto (líder nativo)

Secuencia de nucleótido

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Secuencia de aminoácido

1415
1427

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b CD16 ED alto (HE4 lıder)

Secuencıa de nucleótıdo

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Secuencıa de amınoácıdo

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104 2el2 scFv (SSS-S) H P238S CH2 WCH3-hCD80TM/CT

Secuencıa de nucleótıdo

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1416
1428

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Secuencia de aminoácido

MDFQVQIFSFLLISASVIMSRGVDIVLTQSPASLAVSLGQRATISCRASESVEYYVTS
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105 10A8 scFv (SSS-S) H P238SCH2 WCH3-hCD80TM/CT

Secuencia de nucleótido

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1429

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Secuencia de aminoácido

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106. 2H7scFvVHL11S (SSS-P) H P238SCH2CH3-hCD80TM/CT

Secuencia de nucleótido

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Secuencia de aminoácido

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107 G19-4 scFv (SSS-P) H P238SCH2 WCH3-hCD80TM/CT

Secuencia de nucleótido

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Secuencia de aminoácido

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108. 2el2 scFv IgA WH WCH2 T4 CH3-hCD80TM/CT

Secuencia de nucleótido

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Secuencia de aminoácido

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Secuencia de nucleótido

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Secuencia de aminoácido

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Secuencia de nucleótido

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Secuencia de aminoácido

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111 measp3-TM/CT

Secuencia de nucleótido

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Secuencia de aminoácido

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112 2el2 scFv (SSS-P) H WCH2WCH3-mcasp3-TM/CT

Secuencia de nucleótido

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Secuencia de aminoácido

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113 2el2 scFv (SSS-P) H P238SCH2WCH3-mcasp3-TM/CT

Secuencia de nucleótido

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Secuencia de aminoácido

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114 measp8-TM/CT

Secuencia de nucleótido

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Secuencia de aminoácido

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Secuencia de aminoácido

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116 2el2 scFv hTgGl (SSS-P) H P238SCH2 WCH3-mcasp8-TM/CT

Secuencia de nucleótido

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Secuencia de aminoácido

1437
1493

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LKSICDQVNKSLLGKIEDYERSSTERRMSLEGREELPPSVLDEMSLKMAELCDSPR
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117 hcasp3-TM/CT

Secuencia de nucleótido

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Secuencia de aminoácido

1432
1444

DPSNMENTENSVDSKSIKNLEPKIIHGSESMDSGISLDNSYKMDYPEMGLCIIINNK
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118. 2el2 scFv hIgGl (SSS-P) H WCH2 WCH3-hcasp3-TM/CT

Secuencia de nucleótido

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1435
1495

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Secuencia de aminoácido

MDFQVQIFSFLLISASVIMSRGVDIVLTQSPASLAVSLGQRATISCRASESVEYYVTS
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TKELYFYH

123 2el2 scFv hIgGl (SSS-P) H P238SCH2 WCH3-hcasp3-TM/CT

Secuencia de nucleótido

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1439
1496

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Secuencia de aminoácido

MDFQVQIFSFLLISASVIMSRGVDIVLTQSPASLAVSLGQRATISCRASESVEYYVTS
LMQWYQQKPGQPPKLLISAASNVESGVPARFSGSGSGTDFSLNIHPVEEDDIAMYF
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KSQVFLKMNSLQTDDTARYYCARDGYSNFHYYVMDYWGQGTSVTVSSDLEPKS
SDKTHTSPPSPAPELLGGSSVFLFPPKPKDTLMISRTPEVTCWVDVSHEDPEVKFN
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1435
1447

124. hcasp8-TM/CT hCaspase8B

Secuencia de nucleótido

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Secuencia de aminoácido

DPSNMDFSRNLYDIGEQLDSEDLASLKFLSLDYIPQRKQEPIKDALMLFQRLQEKR
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hCaspasa8C

1436
1448

Secuencia de nucleótido

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Secuencia de aminoácido

DPSNMDFSRNLYDIGEQLDSEDLASLKFLSLDYIPQRKQEPIKDALMLFQRLQEKR
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125. 2el2 scFv hIgG1 (SSS-P) H WCH2 WCH3-hcasp8-TM/CT

Secuencia de nucleótido

1437
1949

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1438
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Secuencia de aminoácido

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Secuencia de nucleótido

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Secuencia de aminoácido

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Secuencia de nucleótido

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Secuencia de aminoácido

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Secuencia de nucleótido

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Secuencia de aminoácido

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Secuencia de nucleótido

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Secuencia de aminoácido

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Secuencia de nucleótido

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Secuencia de aminoácido

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Secuencia de nucleótido

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Secuencia de aminoácido

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Secuencia de nucleótido

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Secuencia de aminoácido

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Secuencia de nucleótido

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Secuencia de aminoácido

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Secuencia de nucleótido

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Secuencia de aminoácido

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Secuencia de aminoácido

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Secuencia de nucleótido

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Secuencia de aminoácido

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Secuencia de aminoácido

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Secuencia de nucleótido

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Secuencia de aminoácido

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Secuencia de nucleótido

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Secuencia de aminoácido

1457
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Secuencia de nucleótido

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Secuencia de aminoácido

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145 hCTLA4 IgAH IgACH2CH3

Secuencia de nucleótido

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Secuencia de aminoácido

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146 hCTLA4 IgA WH WCH2 T4CH3 (hCTLA4 IgAH IgACH2CH3)

Secuencia de nucleótido

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1460
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Secuencia de aminoácido

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147 hIGA WH WCH2 T18CH3

Secuencia de nucleótido

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1473

Secuencia de aminoácido

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Secuencia de nucleótido

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Secuencia de aminoácido

1479

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Secuencia de nucleótido

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a tcattctttccacctgggtaccaagtctctttctgcttggtgatggtactccttttgcagtggacacaggactatatttctctgtgaaga
caaacattcgaagctcaacaagagactggaaggaccataaatttaaatggagaaaggaccctcaagacaaatgaccc

Secuencia de aminoácido

MWQLLLPTALLLLVSAGMRTEDLPKAVVFLEPQWYRVLEKDSVTLKCQGAYSPE
DNSTQWFHNESLISSQASSYFIDAATVDDSGEYRCQTNLSTLSDPVQLEVHIGWLL
LQAPRWVFKEEDPIHLRCHSWKNTALHKVTYLQNGKGRKYFHHNSDFYIPKATL
KDSGSYFCRGLVGSKNVSSETVNITITQGLAVSTISSFFPPGYQVSFCLVMVLLFAV
DTGLYFSVKTNIRSSTRDWKDHKFKWRKDPQDK

1463
1475

Articulación tipo silvestre HuIgG1, CH2, CH3 (secuencia de nucleótido) (ID DE SEC NO __)

tctgatcaggagcccaaatcttgtgacaaaactcacacatgcccaccgtgcccagcacctgaactcctgggggggaccgtcagtctt
cctcttccccccaaaacccaaggacaccctcatgatctcccggacccctgaggtcacatgcgtggtggtggacgtgagccacgaa
gaccctgaggtcaagttcaactggtacgtggacggcgtggaggtgcataatgccaagacaaagccgcgggaggagcagtacaa
cagcacgtaccgtgtggtcagcgtcctcaccgtcctgcaccaggactggctgaatggcaaggagtacaagtgcaaggtctccaac
aaagccctcccagcccccatcgagaaaacaatctccaaagccaaagggcagccccgagaaccacaggtgtacaccctgcccccc
atcccgggatgagctgaccaagaaccaggtcagcctgacctgcctggtcaaaggcttctatcccagcgacatcgccgtggagtgg
gagagcaatgggcagccggagaacaactacaagaccacgcctcccgtgctggactccgacggctccttcttcctctacagcaag
ctcaccgtggacaagagcaggtggcagcaggggaacgtcttctcatgctccgtgatgcatgaggctctgcacaaccactacacgc
agaagagcctctccctgtctccgggtaaatgatctaga

Articulación tipo silvestre HuIsG1, CH2, CH3 (secuencia de aminoácido) (ID DE SEC NO __)

SDQEPKSCDKTtnrPPCPAPELLGGPSVFLFPPKPKDTLMlSRTPEVTCVVVDVSHE
DPEVKJFNWWDGVEVHNAKTKREEQYNSTYRVVSVLTVLHQDV/LNGKEYKCK
VSNEALPAPIEKTISKAKG (2PREPQVYTLPPSRDELTKN QVSLTCLVKGfiYPSDISV
EWESNGQPENNYKTTPPVI, DSDGSFFI, YSKLTVDKSRWQQGNVFSCSVMHEALH
NHYTQKSLSLSPGK

Articulación IgG1, CH2, CH3 de Llama (secuencia de nucleótido) (ID DE SEC NO __)

tgatcaagaaccacatggaggatgcacgtgcccncagtgcccncaatgcccngcnccngaactnccaggaggcccttctgtctttt
gtcttcccccgaaacccaaggacgtcctctccatttttggaggccgagtcacgtgcgttgtagtggacgtcggaaagaaagaccc
cgaggtcaatttcaactggtatattgatggcgttgaggtgcgaacggccaatacgaagccaaaagaggaacagttcaacagcacg
taccgcgtggtcagcgtcctgcccatccagcaccaggactggctgacggggaaggaattcaagtgcaaggtcaacaacaaagct
ctcccggcccccatcgagaggaccatctccaaggccaaagggcagacccgggagccgcaggtgtacaccctggccccacacc
gggaagaactggccaaggacaccgtgagcgtaacatgcctggtcaaaggcttctacccagctgacatcaacgttgagtggcaga
ggaacggtcagccggagtcagagggcacctacgccaacacgccgccacagctggacaacgacgggacctacttcctctacagc
aagctctcggtgggaaagaacacgtggcagcggggagaaaccttaacctgtgtggtgatgcatgaggccctgcacaaccactac
acccagaaatccatcacccagtcttcgggtaaatagtaatctaga

Articulación IgG1, CH2, CH3 de Llama (en la figura 23 como IgG1 de Llama) (secuencia de aminoácido) (ID DE SEC NO __)

1464
1476

EPHGGCTCPQCPAPELPGGPSVFVFPPKPKDVLSISGRPEVTCVVVDVGKEDPEVN
FNWYIDGVEVRTANTKPKEEQFNSTYRVVSVLPIQHQDWLTGKEFKCKVNNKAL
PAPIERTISKAKGQTREPQVYTLAPHREELAKDTVSVTCLVKGFYPADINVEWQRN
GQPESEGTYANTPPQLDNDGTYFLYSRLSVGKNTWQRGETLTGVVMHEALHNHY
TQKSITQSSGK

IgG2 de Llama (secuencia de nucleótido) (ID DE SEC NO __)

tgatcaagaacccaagacaccaaaaccacaaccacaacccacaaccacaacccaatcctacaacagaatccaagtgtcccaaatgt
cccgcccctgagctcctgggagggccctcagtcttcatcttcccccccgaaacccaaggacgtccttctccatttctgggaggcccga
ggtcacgtgcgttgtggtagacgtgagccaggaagaccccgaggtcagtttcaactggtacattgatggcgctgaggtgcgaacg
gccaacacgaggccaaagaggaacagttcaacagcacgtaccgcgtggtcagcgtcctgccatccagcaccaggactggct
gacgggaaggaattcaagtgcaaggtcaacaacaaagctttcccggcccccatcgagaagaccatctccaaggccaaagggc
agaccccgagaccgcaggtgtacaccctgcccccacacccgggaagagctgaccaaggacaccgtgagcgtaacatgcctggt
caaaggcttctacccaccgatatcaacgtgagtggcagaggaatgggcagccggagtcagagggcacytacgccaccagcc
cacccccagctggacaacgacgggacctacttcctctacagcaagctctcggtgggaaagaacacgtggcagcagggagaaacc
ttcacctgtgtggtgatgcacgaggccctgcacaaccactacacccagaaatccatcacccagtcttcgggtaaatagtaatctaga

IgG2 de Llama (secuencia de aminoácido) (ID DE SEC NO __)

DQEPKTPKPQPQPQPQPNPTTESKCPKCPAPELLGGPSVFIFPPKPKDVLSISGRPEV
TCVVVDVGQEDPEVSFNWYIDGAEVRTANTRPKEEQFNSTYRVVSVLPIQHQDWL
TGKEFKCKVNNKALPAPIEKTISKAKGQTREPQVYTLAPHREELAKDTVSVTCLVK
GFYPPDINVEWQRNGQPESEGTYATTPPQLDNDGTYFLYSKLSVGKNTWQQGETF
TCVVMHEALHNHYTQKSITQSSGK

IgG3 Fc de Llama (secuencia de nucleótido) _(ID DE SEC NO __)

tgatcaagcgcaccacagcgaagacccagctccagtgtcccaaatgcccaggccctgaactcctggagggcccacggtctt
catcttcccccccgaaagccaaggacgtcctctccatcacccgaaaacctgaggtcacgtgcttgtggtggacgtgggtaaagaag
accctgagatcgagttcaagctggtccgtggatgacacagaggtacacacggctgagacaaagccaaaggaggaacagttcaac

1465
1477

agcacgtaccgcgtggtcagcgtcctgcccatccagcaccaggactggctgacggggaaggaattcaagtgcaaggtcaacaa
caaagctctcccagcccccatcgagaggaccatctccaaggccaaagggcagaccccgggagccgcaggtgtacaccctgcc
ccacacccgggaagagctggccaaggcaccgtgagcgtaacctgcctggtcaaaggcttctttcccagctgacatcaacgttgagt
ggcagaggaatgggcagccggagtcagagggcacctacgccaacacgccgccacagctggacaacgacgggacctacttcct
ctacagcaaactctccgtgggaagaacacgtggcagcaggggagaagtcttcacctgtgtggtgatgcacgaggctctacacaat
cactccacccagaaatccatcacccagtcttcgggtaaatagtaatctagagggccc

IgG3 Fc de Llama (secuencia de aminoácido) (ID DE SEC NO __)

DQAHHSEDPSSKCPKCPGPELLGGPTVFIFPPKAKDVLSITRKPEVTCLWWTWVKK
TLRSSSSWSVDDTEVHTAETKPKEEQFNSTYRVVSVLPIQHQDWLTGKEFKCKVN
NKALPAPIERTISKAKGQTREPQVYTLAPHREELAKDTVSVTCLVKGFFPADINVE
WQRNGQPESEGTYANTPPQLDNDGTYFLYSKLSVGKNTWQQGEVFTCVVMHEA
LHNHSTQKSITQSSGK

Articulación tipo silvestre HuIsG1 (secuencia de nucleótido) (ID DE SEC NO __)

gatcaggagcccaaatcttgtgacaaaactcacacatgcccaccgtgcccagca

Articulación tipo silvestre HuIgG1 (secuencia de aminoácido) (ID DE SEC NO __)

DQEPKSCDKTHTCPPCPA

HuIgG1 H2, articulación tipo silvestre con leu en la segunda posición (secuencia de nucleótido) (ID DE SEC NO __)

gatctggagcccaaatcttgtgacaaaactcacacatgcccaccgtgcccagca

HuIgG1 H2, articulación tipo silvestre con leu en la segunda posición (secuencia de aminoácido) (ID DE SEC NO __)

DLEPKSCDKTHTCPPCPA

NTHutsG1 CH2 tipo silvestre (secuencia de nucleótido) (ID DE SEC NO __)

cctgaactcctgggggggaccgtcagtcttcctcttccccccaaaacccaaggacaccctcatgatctcccggacccctgaggtcac
atgcgtggtggtggacgtgagccacgaagaccctgaggtcaagttcaactggtacgtggacggcgtggaggtgcataatgccaa
gacaaagccgcgggaggagcagtacaacagcacgtaccgtgtggtcagcgtcctcaccgtcctgcaccaggactggctgaatg
gcaaggagtacaagtgcaaggtctccaacaaagccctcccagcccccatcgagaaaaccatctccaaagccaaa

HuIgG1 CH2 tipo silvestre (secuencia de aminoácido) (ID DE SEC NO __)

PELLGGPSVFLFPPKPKDTLMISRTPEVTCWVDVSHEDPEVKFNWYVDGVEVHNA
KTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAK

HuIgG1 CH3 tipo silvestre (secuencia de nucleótido) (ID DE SEC NO __)

gggcagccccgagaaccacaggtgtacaccctgcccccatcccgggaggagatgaccaagaaccaggtcagcctgacctgcct
ggtcaaaggcttctatcccagcgacatcgccgtggagtgggagagcaatgggcagccggagaacaactacaagaccacgcctc
ccgtgctggactccgacggctccttcttcctctatagcaagctcaccgtggacaagagcaggtggcagcaggggaacgtcttctca
tgctccgtgatgcatgaggctctgcacaaccactacacgcagaagagcctctccctgtccccgggtaaatga

HuIgG1 CH3 tipo silvestre (secuencia de aminoácido) (ID DE SEC NO __)

GQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPP
VLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

Articulación mutada HuIgG1 (C-C-COS-S-S) (secuencia de nucleótido) (ID DE SEC NO __)

gatcaggagcccaaatcttctgacaaaactcacacatccccaccgtccccagca

Articulación mutada HuIgG1 (C-C-C→S-S-S) (secuencia de aminoácido) (ID DE SEC NO __)

DQEPKSSDKTHTSPPSPA

HIgG1MTH WTCH2CH3 (articulación mutante con CH2 y CH3 tipo silvestre (lectura de la articulación+cola Ig) (secuencia de nucleótido) (ID DE SEC NO __)

1467
1479

tgatcaccccaaatcttctgacaaaactcacacatctccaccgtcctcagcacctgaactcctgggtggaccgtcagtcttcctcttcc
ccccaaaacccaaggacaccctcatgatctcccggacccctgaggtcacatgcgtggtggtggacgtgagccacgaagaccctg
aggtcaagttcaactggtacgtggacggcgtggaggtgcataatgccaagacaaagccgcgggaggagcagtacaacagcacg
taccgtgtggtcagcgtcctcaccgtcctgcaccaggactggctgaatggcaaggagtacaagtgcaaggtctccaacaaagccc
tcccagccccatcgagaaaacaatctccaaagccaaagggcagccccgagaaccacaggtgtacaccctgccccatcccgg
gatgagctgaccaagaaccaggtcagcctgacctgcctggtcaaaggcttctatcccagcgacatcgccgtggagtgggagagc
aatgggcagccggagaacaactacaagaccacgcctcccgtgctggactccgacggctccttcttcctctacagcaagctcaccg
tggacaagagcaggtggcagcaggggaacgtcttctcatgctccgtgatgcatgaggctctgcacaaccactacacgcagaaga
gcctctccctgtctccgggtaaatgataatctaga

Articulación mutante, pero CH2 y CH3 tipo silvestre (secuencia de aminoácido) (ID DE SEC NO __)

DHPKSSDKTHTSPPSSAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDP
EVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVS
NKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEW
ESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNH
YTQKSLSLSPGK

IgG1 de llama 2H7 scFv (secuencia de nucleótido) (ID DE SEC NO __)

aagcttgccgccatggatttttcaagtgcagattttcagcttcctgctaatcagtgcttcagtcataattgccagaggacaaattgttctct
cccagtctccagcaatcctgtctgcatctccaggggagaaggtcacaatgacttgcagggccagctcaagtgtaagttacatgcact
ggtaccagcagaagccaggatcctcccccaaaccctggatttatgccccatccaacctggcttctggagtccctgctcgcttcagtg
gcagtgggtctgggacctcttactctctcacaatcagcagagtggaggctgaagatgctgccactattactgccagcagtggagttt
taacccacccacgttcggtgctgggaccaagctggagctgaaagatggcggtggctcgggcggtggtggatctggaggaggtg
ggagctctcaggcttatctacagcagtctggggctgagctggtgaggcctggggcctcagtgaagatgtcctgcaaggcttctggc
tacacatttaccagttacaatatgcactgggtaaagcagacacctagacagggcctggaatggattggagctatttatccaggaaat
ggtgatacttcctacaatcagaagttcaagggcaaggccacactgactgtagacaaatcctccagcacagcctacatgcagctcag
cagcctgacatctgaagactctgcggtctattttgtgcaagagtggtgtactatagtaactcttactggtacttcgatgtctggggcac
agggaccacggtcaccgtctcttcagatcaagaaccacatggaggatgcacgtgcccccagtgcccccaatgcccgcccccg
aactcccaggaggcccttcgtctttgtcttcccccccgaaacccaaggacgtccttctccatttttggaggccgagtcacgtgcgttgt
agtggacgtcggaaagaaagacccccgaggtcaatttcaactggtatattgatggcgttgaggtgcgaacggccaatacgaagcca
aaagaggaacagttcaacagcacgtaccgcgtggtcagcgtcctgcccatccagcaccaggactggctgacggggaaggaatt

1468
1480

caagtgcaaggtcaacaacaaagctctcccggcccccatcgagaggaccatctccaaggccaaagggcagaccccgggagccg
caggtgtacaccctgccccacacccgggaagaactgaccaaggacaccgtgagcgtaacatgcctggtcaaaggcttctaccca
gctgacatcaacgtgagtggcagaggaacggtcagccggagtcagagggcacctacgccaacacgccgccacagctggaca
acgacgggacctacttcctctacagcaagctctcggtgggaaagaacacgtggcagcggggagaaacctaacctgtgtggtgat
gcatgaggccctgcacaaccactacacccagaaatccatcacccagtcttcgggtaaatagtaatctaga

IgG1 de llama 2H7 scFv (secuencia de aminoácido) (ID DE SEC NO. __)

MDFQVQIFSFLLISASVIIARGQIVLSQSPAILSASPGEKVTMTCRASSSVSYMHWY
QQKPGSSPKPWIYAPSNLASGVPARFSGSGSGTSYSLTISRVEAEDAATYYCQQWS
FNPPTFGAGTKLELKDGGGSGGGGSGGGGSSQAYLQQSGAELVRPGASVKMSCK
ASGYTFTSYNMHWVKQTPRQGLEWIGAIYPGNGDTSYNQKFKGKATLTVDKSSS
TAYMQLSSLTSEDSAVYFCARVVYYSNSYWYFDVWGTGTTVTVSSDQEPHGGCT
CPQCPAPELPGGPSVFVFPPKPKDVLSITGGRVTCVVVDVGKKDPEVNFNWYIDGV
EVRTANTKPKEEQFNSTYRVVSVLPIQHQDWLTGKEFKCKVNNKALPAPIERTISK
AKGQTREPQVYTLAPHREELAKDTVSVTCLVKGFYPADINVEWQRNGQPESEGTY
ANTPPQLDNDGTYFLYSKLSVGKNTWQRGETLTCVVMHEALHNHYTQKSITQSS
GK

IgG2 de llama 2H7 scFv (secuencia de nucleótido) (ID DE SEC NO __)

aagcttgccgccatggatttcaagtgcagatttcagcttcctgctaatcagtgcttcagtcataattgccagaggacaaattgttctct
cccagtctccagcaatcctgtctgcatctccaggggagaaggtcacaatgacttgcagggccagctcaagtgtaagttacatgcact
ggtaccagcagaagccaggatcctcccccaaaccctggatttatgccccatccaacctggcttctggagtccctgctcgcttcagtg
gcagtgggtctgggacctcttactctctcacaatcagcagagtggaggctgaagatgctgccacttattactgccagcagtggagttt
tacccacccacgttcggtgctgggaccaagctggagctgaaagatggcggtgctcgggcggtggtggatctggaggaggtg
ggagctctcaggcttatctacagcagtctggggctgagctggtgaggcctggggcctcagtgaagatgtcctgcaaggcttctggc
tacacatttaccagttacaatatgcactgggtaaagcagacacctagacagggcctggaatggattggagctatttatccaggaaat
ggtgatacttcctacaatcagaagttcaagggcaaggccacactgactgtagacaaatcctccagcacagcctacatgcagctcag
cagcctgacatctgaagactctgcggtctatttctgtgcaagagtggtgtactatagtaactcttactggtacttcgatgtctggggcac
agggaccacggtcaccgtctcttctgatcaagacccaagcaccaacaaccacaaccacaaccacaaccacaaccacaaccaatcctac
aacagaatccaagtgtcccaaatgtccagccctgagcttctgggagggccctcagtcttcatcttcccccccgaaacccaaggac
gtcctctccatttctgggaggccctgaggtcacgtgcgttgtggtagacgtgggccaggaagaccccgaggtcagttcaactggta

1469
1481

catgatggcgctgaggtgcgaacggccaacacgaggccaaaagaggaacagttcaacagcacgtaccgcgtggtcagcgtcc
tgcccatccagcaccaggactggctgacggggaaggaattcaagtgcaaggtcaacaacaaagctctcccggcccccatcgag
aagaccatctccaaggccaaagggcagaccccgggagccgcaggtgtacaccctggccccacaccgggaagagctggccaag
gacaccgtgagcgtaacatgcctggtcaaggcttctacccaccgatatcaacgttgagtggcagaggaatgggcagccggagt
cagagggcacytacgccaccacgccacccccagctggacaacgacgggaccttcttcctctacagcaagctctcggtgggaaag
aacacgtggcagcagggagaaaccttcacctgtgtggtgatgcacgaggccctgcacaaccactacacccagaaatccatcacc
cagtcttcgggtaaatagtaatctaga

IgG2 de llama AA2H7 scFv (secuencia de aminoácido) (ID DE SEC NO __)

MDFQVQIFSFLLISASVIIARGQIVLSQSPAILSASPGEKVTMTCRASSSVSYMHWY
QQKPGSSPKPWIYAPSNLASGVPARFSGSGSGTSYSLTISRVRAEDAATYYCQQWS
FNPPTFGAGTKLELKDGGGSGGGGSGGGGSSQAYLQQSGAELVRPGASVKMSCK
ASGYTFTSYNMHWVKQTPRQGLEWIGAIYPGNGDTSYNQKFKGKATLTVDKSSS
TAYMQLSSLTSEDSAVYFCARVVYYSNSYWYFDVWGTGTTVTVSSDQEPKTPKP
QPQPQPQPNPTTESKCPKCPAPELLGGPSVFIFPPKPKDVLSISGRPEVTCVVVDVG
QEDPEVSFNWYIDGAEVRTANTRPKEEQFNSTYRVVSVLPIQHQDWLTGKEFKCK
VNNKALPAPIEKTISKAKGQTREPQVYTLAPHREELAKDTVSVTCLVKGFYPPDIN
VEWQRNGQPESEGTYATTPPQLDNDGTYFLYSKLSVGKNTWQQGETFTCVVMHE
ALHNHYTQKSITQSSGK

IgG3 de llama NT2H7 scFv (secuencia de nucleótido) (ID DE SEC NO __)

aagcttgccgccatggatttttcaagtgcagattttcagcttcctgctaatcagtgcttcagtcataattgccagaggacaaattgttctct
cccagtctccagcaatcctgtctgcatctccaggggagaaggtcacaatgacttgcagggccagctcaagtgtaagttacatgcact
ggtaccagcagaagccaggatcctccccaaaccctggatttatgccccatccaacctggcttctggagtccctgctcgcttcagtg
gcagtgggtctgggacctcttactctctcacaatcagcagagtggaggctgaagatgctgccactattactgccagcagtggagttt
tnaccnaccccacgttcggtgctgggaccaagctggagctgaaagatggcggtggctcgggcggtggtggatctggaggaggtg
ggagtctcaggcttatctacagcagtctggggctgagctggtgaggcctggggcctcagtgaagatgtcctgcaaggcttctggc
tacacatttaccagttacaatatgcactgggtaaagcagacacctagacagggcctggaatggattggagctatttatcccaggaaat
ggtgatacttcctacaatcagaagttcaagggcaaggccacactgactgtagacaaatcctccagcacagcctacatgcagctcag
cagcctgacatctgaagactctgcggtctatttctgtgcaagagtggtgtactatagtaactcttactggtacttcgatgtctggggcac
agggaccacggtcaccgtctcttctgatcaagcgaaccacagcgaagaccccagctccaagtgtcccaaatgcccaggccctga
actccttggagggcccacggtcttcatcttccccccgaaagccaaggacgtccttctccatcacccgaaaacctgaggtcacgtgctt
gtggtggacgtgagtaaagaagaccctgagatcgagttcaagctggtccgtggatgacacagaggtacacacggctgagacaaa

1470
1982

gccaaggaggaacagttcaacagcacgtaccgcgtggtcagcgtcctgcccatccagcaccaggactggctgacggggaag
gaattcaagtgcaaggtcaacaacaaagctctcccagccccccatcgagaggaccatctccaaggccaaagggcagaccccgga
gccgcaggtgtacaccctggcccccacccgggaagagctggccaaggaaccgtgagcgtaacctgcctggtcaaaggcttctt
cccagctgacatcaacgttgagtggcagaggaatgggcagccggagtcagagggcacctacgccaacacgccgccacagctg
gacaacgacgggacctacttcctctacagcaaactctccgtgggtaaagaacacgtggcagcagggagaagtcttcacctgtgtgg
tgatgcacgaggctctacacaatcactccacccagaaatccatcacccagtcttcgggtaaatagtaatctagagggccc

IgG3 de llama 2H7 scFv (secuencia de aminoácido) (ID DE SEC NO __)

MDFQVQIFSFLLISASVIIARGQIVLSQSPAILSASPGEKVTMTCRASSSVSYMHWY
QQKPGSSPKPWIYAPSNLASGVPARFSGSGSGTSYSLTISRVEAEDAATYYCQQWS
FNPPTFGAGTKLELKDGGGSGGGGSGGGGSSQAYLQQSGAELVRPGASVKMSCK
ASGYTFTSYNMHWVKQTPRQGLEWIGAIYPGNGDTSYNQKFKGKATLTVDKSSS
TAYMQLSSLTSEDSAVYFCARVVYYSNSYWYFDVWGTGTTVTVSSDQAHSHSED
PSSKCPKCPGPELLGGPTVFIFPPKAKDVLSITRKPEVTCLWWTWVKKTLRSSSSW
SVDDTEVHTAETKPKEEQFNSTYRVVSVLPIQHQDWLTGKEFKCKVNNKALPAPI
ERTISKAKGQTREPQVYTLAPHREELAKDTVSVTCLVKGFFPADINVEWQRNGQP
ESEGTYANTPPQLDNDGTYFLYSKLSVGKNTWQQGEVFTCVVMHEALHNHSTQK
SITQSSGK

<u>2H7 scFv WTH WTCH2CH3</u> (secuencia de nucleótido)

aagcttgccgccatggatttttcaagtgcagattttcagcttcctgctaatcagtgtttcagtcatcattgccagaggacaaattgttctct
cccagtctccagcaatcctgtctgcatctccaggggagaaggtcacaatgacttgcagggccagctcaagtgtaagttacatgcact
ggtaccagcagaagccaggatcctcccccaaacccttggatttatgccccatccaacctggcttctggagtccctgctcgcttcagtg
gcagtgggtctgggacctcttactctctcacaatcagcagagtggaggctgaagatgctgccacttatttactgccagcagtggagtt
tacccacccacgttcggtgctgggaccaagctggagctgaaagtggcggtggctcgggcggtggtggatctggaggaggtg
gtggctctcaggcttatctacagcagtctggggctgagctggtgaggcctggggcttcagtgaagatgtcctgcaaggcttctggc
tacacatttaccagttacaatatgcactgggtaaagcagacacctagacagggcctggaatggattggagctatttatccaggaaat
ggtgatacttcctacaatcagaagttcaagggcaaggccacactgactgtagacaaatcctccagcacagcctacatgcagctcag
cagcctgacatctgaagactctgcggtctatttctgtgcaagagtggtgtactatagtaactcttactggtacttcgatgtctgggcac
agggaccacggtcaccgtctcttctgatcaggagcccaaatcttgtgacaaaactcacacatgcccaccgtgcccagcacctgaac
tcctgggggggaccgtcagtcttcctcttccccccaaaacccaaggacaccctcatgatctcccggacccctgaggtcacatgcgtg

1483

gtggtggacgtgagccacgaagaccctgaggtcaagttcaactggtacgtggacggcgtggaggtgcataatgccaagacaaag
ccgcgggaggagcagtacaacagcacgtaccgtgtggtcagcgtcctcaccgtcctgcaccaggactggctgaatggcaagga
gtacaagtgcaaggtctccaacaaagccctcccagcccccatcgagaaaacaatctccaaagccaaagggcagccccgagaac
cacaggtgtacaccctgcccccatcccgggatgagctgaccaagaaccaggtcagcctgacctgcctggtcaaaggcttctatcc
cagcgacatcgccgtggagtgggagagcaatgggcagccggagaacaactacaagaccacgcctcccgtgctggactccgac
ggctccttcttcctctacagcaagctcaccgtggacaagagcaggtggcagcaggggaacgtcttctcatgctccgtgatgcatga
ggctctgcacaaccactacacgcagaagagcctctccctgtctccgggtaaatgatctaga

2H7 scFv WTH WTCH2CH3 (secuencia de aminoácido) (ID DE SEC NO __)

MDFQVQIFSFLLISASVIIARGQIVLSQSPAILSASPGEKVTMTCRASSSVSYMHWY
QQKPGSSPKPWIYAPSNLASGVPARFSGSGSGTSYSLTISRVEAEDAATYYCQQWS
FNPPTFGAGTKLELKDGGGSGGGGSGGGGSSQAYLQQSGAELVRPGASVKMSCK
ASGYTFTSYNMHWVKQTPRQGLEWIGAIYPGNGDTSYNQKFKGKATLTVDKSSS
TAYMQLSSLTSEDSAVYFCARVVYYSNSYWYFDVWGTGTTVTVSSDQEPKSCDK
THTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWY
VDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPI
EKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPE
NNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSL
SPGK

Cola citoplasmática y dominio de transmembrana NTCD80 (+sitios de restricción) (secuencia de nucleótido) (ID DE SEC NO __)

gcggatccttcgaacctgctcccatcctgggccattaccttaatctcagtaaatggaatttttgtgatatgctgcctgacctactgctttg
ccccaagatgcagagagagaaggaggaatgagagattgagaagggaaagtgtacgccctgtataaatcgat

Cola citoplasmática y dominio de transmembrana CD80 (secuencia de aminoácido) (ID DE SEC NO __)

ADPSNLLPSWAITLISVNGIFVICCLTYCFAPRCRERRRNERLRRESVRPV

40 2 220 VL (CD40 scFv #1–VL anti-humano) (secuencia de nucleótido) (ID DE SEC NO __)

1472
1484

aagcttatggatttcaagtgcagattttcagcttcctgctaatcagtgttcagtcataatgtccagaggagtcgacattgttctgactc
agtctccagccaccctgtctgtgactccaggagatagagtctctttcctgcagggccagccagagtattagcgactacttacactg
gtatcaacaaaaatcacatgagtctccaggcttctcatcaaatatgcttcccatccatctctgggatcccctccaggttcagtggca
gtggatcagggtcagatttcactctcagtatcaacagtgtggaacctgaagatgttggaattattactgtcaacatggtcacagcttc
cgtggacgttcggtggaggcaccaagctggaaatcaaacgg

40 2. 220 VL (CD40 scFv #1--VL anti-humano) (secuencia de aminoácido) (ID DE SEC NO __)

MDFQVQIFSFLLISASVIMSRGVDIVLTQSPATLSVTPGDRVSLSCRASQSISDYLHW
YQQKSHESPRLLIKYASHSISGIPSRFSGSGSGSDFTLSINSVEPEDVGIYYCQHGHSF
PWTFGGGTKLEIKR

40 2 220 VH (para CD40 scFv #1--VH anti-humano) (secuencia de nucleótido) (ID DE SEC NO __)

cagatccagttggtgcaatctggacctgagctgaagaagcctggagagacagtcaggatctcctgcaaggcttctgggtatgccti
cacaactactggaatgcagtgggtgcaagaggatgccaggaaagggttgaagtggatggctggataaacaccccacictggagt
gccaaaatatgtagaagacttcaaggacggttgccttctctttggaacctctgccaacactgcatatttacagataagcaacctcaa
agatgaggacacggctacgtatttctgtgtgagatccgggaatggtaactatgacctggcctactttgcttactggggccaagggac
actggtcactgtctctgatca

40 2 220 VH (para CD40 scFv #1--VH anti-humano) (secuencia de aminoácido) (ID DE SEC NO __)

QIQLVQSGPELKKPGETVRISCKASGYAFTTTGMQWVQEMPGKGLKWIGWINTPL
WSAKICRRLQGRFAFSLETSANTAYLQISNLKDEDTATYFCVRSGNGNYDLAYFA
YWGQGTLVTVS

40 2 220 scFv (CD40 scFv #1 anti-humano) (secuencia de nucleótido) (ID DE SEC NO __)

aagcttatggatttcaagtgcagattttcagcttcctgctaatcagtgcttcagtcataatgtccagaggagtcgacattgttctgactc
agtctccagccaccctgtctgtgactccaggagatagagtctctttcctgcagggccagccagagtattagcgactacttacactg
gtatcaacaaaaatcacatgagtctccaggcttctcatcaaatatgcttcccatccatctctgggatcccctccaggttcagtggca
gtggatcagggtcagatttcactctcagtatcaacagtgtggaacctgaagatgttggaattattactgtcaacatggtcacagcttc

1473
1485

cgtggacgttcggtggaggcaccaagctggaaatcaaacgggctgatgctgcaccaactgtatccatcttcccaccatccagtgagcagttaacatctggaggtgcctcagtcgtgtgcttctacaacaacttctaccccagagacatcaatgtcaagtggaagattgatggcagtgaacgacaaaatggcgtcctgaacagttggactgatcaggacagcaaggacagcacctacagcatgagcagcaccctcacgttgaccaaggacgagtatgaacgacataacagctatacctgtgaggccactcacaagacatcaacttcatcaagagcttcaacaggaatgagtgttag

40 2 220 scFv (CD40 scFv #1 anti-humano) (secuencia de aminoácido) (ID DE SEC NO __)

MDFQVQIFSFLLISASVIMSRGVDIVLTQSPATLSVTPGDRVSLSCRASQSISDYLHW
YQQKSHESPRLLIKYASHSISGIPSRFSGSGSGSDFTLSINSVEPEDVGIYYCQHGHSF
PWTFGGGTKLEIKRGGGGSGGGGSGGGGSQIQLVQSGPELKKPGETVRISCKASG
YAFTTTGMQWVQEMPGKGLKWIGWINTPLWSAKICRRLQGRFAFSLETSANTAY
LQISNLKDEDTATYFCVRSGNGNYDLAYFAYWGQGTLVTVS

2e12 VL (con péptido líder L6 VK) (secuencia de nucleótido) (ID DE SEC NO __)

atggatttcaagtgcagattttcagcttctgctaatcagtgtcacagtcatatgtccagaggagtcgacattgtgctcacccaatctc
cagcttctttggctgtgtctctagggcagagggccaccatctcctgcagagccagtgaaagtgttgaatatttatgtcacaagtttaatgc
agtggtaccaacagaaaccaggacagccacccaaactcctcatctctgctgcatccaacgtagaatctggggtccctgccaggttt
agtggcagtgggtctgggacagacttcagcctcaacatccatcctgtggaggaggatgatattgcaatgtatttctgtcagcaaagta
ggaaggttccttggacgttcggtggaggcaccaagctggaaatcaaacgg

2e12 VL (con péptido líder L6 VK) (secuencia de aminoácido) (ID DE SEC NO __)

MDFQVQIFSFLLISASVIMSRGVDIVLTQSPASLAVSLGQRATISCRASESVEYYVTS
LMQWYQQKPGQPPKLLISAASNVESGVPARFSGSGSGTDFSLNIHPVEEDDIAMYF
CQQSRKVPWTFGGGTKLEIKR

2e12 VH (sin péptido líder) (secuencia de nucleótido) (ID DE SEC NO __)

caggtgcagctgaaggagtcaggacctggcctggtggcgccctcacagagcctgtccatcacatgcaccgtctcagggttctcatt
aaccggctatggtgtaaactgggttcgccagcctccaggaaagggtctggagtggctgggaatgatatggggtgatggaagcac
agactataattcagctctcaaatccagactgagcatcaccaaggacaactccaagagccaagttttcttaaaaatgaacagtctgcaa

1479
1486

actgatgacacaagccagatactactgtgccagagatggttatagtaacttttcattactatgttatggactactggggtcaaggaacctc
agtcaccgtctcctca(gatctg)

2el2 VH (secuencia de aminoácido) (ID DE SEC NO __)

QVQLKESGPGLVAPSQSLSITCTVSGFSLTGYGVNWVRQPPGKGLEWLGMIWGDG
STDYNSALKSRLSITKDNSKSQVFLKMNSLQTDDTARYYCARDGYSNFHYYVMD
YWGQGTSVTVSS

2e12scFv (+Sitios de restricción) (secuencia de nucleótido) (ID DE SEC NO __)

aagcttatggatttcaagtgcagattttcagcttcctgctaatcagtgcttcagtcataatgtccagaggagtcgacattgtgctcaccc
aatctccagcttctttggctgtgtctctaggtcagagagccaccatctcctgcagagccagtgaaagtgttgaatattatgtcacaagtt
taatgcagtggtaccaacagaaaccaggacagccacccaaactcctcatctctgctgcatccaacgtagaatctggggtccctgcc
aggtttagtggcagtgggtctgggacagacttcagcctcaacatccatcctgtggaggaggatgatattgcaatgtatttctgtcagc
aaagtaggaaggttccttggacgttcggtggaggcaccaagctggaaatcaaacgggatgcggctgcaccatctgtcttcatcttc
ccgccatctgatgagcagttgaaatctggaactgcctctgttgtgtgcctgctgaataacttctatcccagagaggccaaagtacag
tggaaggtggataacgccctccaatcgggtaactcccaggagagtgtcacagagcaggacagcaaggacagcacctacagcctca
gcagcaccctgacgctgagcaaagcagactacgagaaacacaaagtctacgcctgcgaagtcacccatcagggcctgagctcgcc
cgtcacaaagagcttcaacaggggagagtgttag(gatcag)

2el2scFv (secuencia de aminoácido) (ID DE SEC NO __)

MDFQVQIFSFLLISASVIMSRGVDIVLTQSPASLAVSLGQRATISCRASESVEYYVTS
LMQWYQQKPGQPPKLLISAASNVESGVPARFSGSGSGTDFSLNIHPVEEDDIAMYF
CQQSRKVPWTFGGGTKLEIKRGGGGSGGGGSGGGGSQVQLKESGPGLVAPSQSLS
ITCTVSGFSLTGYGVNWVRQPPGKGLEWLGMIWGDGSTDYNSALKSRLSITKDNS
KSQVFLKMNSLQTDDTARYYCARDGYSNFHYYVMDYWGQGTSVTVSS

10A8 es anti-CD152 (CTLA-4)

10A8 VL (con L6 VK péptido líder) (secuencia de nucleótido) (ID DE SEC NO __)

1475
1487

atggattttcaagtgcagattttcagcttcctgctaatcagtgcttcagtcataatgtccagaggagtcgacatccagatgacacagtct
ccatcctcactgtctgcatctctgggaggcaaagtcaccatcacttgcaaggcaagccaagacattaagaagtatataggttggtac
caacacaagcctggaaaaggtccctaggctgctcatatattacacatctacattacagccaggcatcccatcaggttcagtgggagt
gggtctgggagagattattccctcagcatcagaacctggagcctgaagatattgcaacttattattgtcaacagtatgataatcttcc
attgacgttcggctcggggacaaagttggaaataaaacgg

10A8 VL (secuencia de aminoácido) (ID DE SEC NO __)

MDFQVQIFSFLLISASVIMSRGVDIQMTQSPSSLSASLGGKVTITCKASQDIKKYIG
WYQHKPGKGPRLLIYYTSTLQPGIPSRFSGSGSGRDYSLSIRNLEPEDIATYYCQQY
DNLPLTFGSGTKLEIKR

10A8 VH (sin péptido líder) (secuencia de nucleótido) (ID DE SEC NO __)

gatgtacagcttcaggagtcaggacctggcctcgtgaaacctctcagtctctgtctctcacctgctctgtcactggctactccatcac
cagtggttctactggaactggatccgacagtttccgggaaacaaactggaatggatgggctacataagctacgacggtaggaat
aactacaacccatctctcaaaatcgaatctccatcactcgtgacacatctaagaaccagttttcctgaagttgagtctgtgactactg
aggacacagctacatattttgtgcaagacactacggtagtagctggagctatggactactggggtcaaggaacctcagtcaccgtc
tcctctgatca

10A8 VH (secuencia de aminoácido) (ID DE SEC NO __)

DVQLQESGPGLVKPSQSLSLTCSVTGYSITSGFYWNWIRQFPGNKLEWMGHISHD
GRNNYNPSLINRISITRDTSKNQFFLKLSSVTTEDTATYFCARHYGSSGAMDYWGQ
GTSVTVSS

10A8 SCFV (secuencia de nucleótido) (ID DE SEC NO __)

aagcttatggattttcaagtgcagattttcagcttcctgctaatcagtgcttcagtcataatgtccagaggagtcgacatccagatgaca
cagtctccatcctcactgtctgcatctctgggaggcaaagtcaccatcacttgcaaggcaagccaagacattaagaagtatataggtt
ggtaccaacacaagcctggaaaaggtccctaggctgctcatatattacacatctacattacagccaggcatcccatcaggttcagtg
gaagtgggtctgggagagattattccctcagcatcagaacctggagcctgaagatattgcaacttattattgtcaacagtatgataat
cttccattgacgttcggctcggggacaaagttggaaataaaacggggtggcggtggctcgggcggtggtgggtcgggtggcggc
ggatctgatgtacagcttcaggagtcaggacctggcctcgtgaaacctctcagtctctgtctctcacctgctctgtcactggctactc

1988

catcaccagtggttctactggaactggatccgacagttccgggaaacaaactggatggatgggccacataagccacgacggta
ggaataactacaacccatctctcataaatcgaatctccatcactcgtgacacatctaagaaccagttttcctgaagttgagttctgtga
ctaatgaggacacagctacataatttctgtgcaagacactacggtagtagcggagctatggactactggggtcaaggaacctcagtc
accgtctcctctgatca

10A8 SCFV (secuencia de aminoácido) (ID DE SEC NO __)

MDFQVQIFSFLLISASVIMSRGVDIQMTQSPSSLSASLGGKVTITCKASQDIKKYIG
WYQHKPGKGPRLLIYYTSTLQPGIPSRFSGSGSGRDYSLSIRNLEPEDIATYYCQQY
DNLPLTFGSGTKLEIKRGGGGSGGGGSGGGGSDVQLQESGPGLVKPSQSLSLTCSV
TGYSITSGFYWNWIRQFPGNKLEWMGHISHDGRNNYNPSLINRISITRDTSKNQFFL
KLSSVTTEDTATYFCARHYGSSGAMDYWGQGTSVTVSSD

40_2_220-hmtIgG1-hCD80 (secuencia de nucleótido) (ID DE SEC NO __)

aagcttatggattttcaagtgcagattttcagcttcctgctaatcagtgcttcagtcataatgtccagaggagtcgacattgttctgactc
agtctccagccaccctgtctgtgactccaggagatagagtctctctttcctgcagggccagccagagtattagcgactacttacactg
gtatcaacaaaaatcacatgagtctccaggcttctcatcaaatatgcttcccattccatctctgggatcccctcaggttcagtggca
gtggatcagggtcagatttcactctcagtatcaacagtgtggaacctgaagatgttggaatttattactgtcaacatggtcacagcttc
cgtggacgttcggtggaggcaccaagctggaaatcaaacgggggtggcggtggctcgggcggaggtgggtcgggtggcggcg
gatctcagatccagttggtgcaatctggacctgagctgaagaagcctggagagacagtcaggatctcctgcaaggcttctgggtat
gccttcacaactactggaatgcagtgggtgcaagaggatgccaggaaagggttgaagtggatggctggataaacacccccactctg
gagtgccaaaatatgtagaagacttcaaggacggattgccttctctttggaaacctctgccaacactgcatatttacagataagcaacc
tcaaagatgaggacacggctacgtatttctgtgtgagatccgggaatggtaactatgacctggcctacttttgctactgggccaag
ggcactggtcactgtctctgatctggagcccaaatcttctgacaaaactcacacatcccccaccgtcccagcacctgaacttcctgg
ggggatcgtcagtcttcctcttccccccaaaacccaaggacaccctcatgatctcccggacccctgaggtcacatgcgtggtggtg
gacgtgagccacgaagaccctgaggtcaagttcaactggtacgtggacggcgtggaggtgcataatgccaagacaaagccgcg
ggaggagcagtacaacagcacgtaccgtgtggtcagcgtcctcaccgtcctgcaccaggactggctgaatggcaaggagtacaa
gtgcaaggtctccaacaaagccctcccagcccccatcgagaaaaccatctccaaagccaaagggcagccccgagaaccacagg
tgtacaccctgcccccatcccgggatgagctgaccaagaaccaggtcagcctgacctgcctggtcaaaggcttctatcccagcga
catagccgtggagtgggagagcaatgggcagccggagaacaactacaagaccacgcctcccgtgctggactccgacggctcct
tcttcctctacagcaagctcaccgtggacaagagcaggtggcagcaggggaacgtcttctcatgctccgtgatgcatgaggctctg
cacaaccactacacgcagaagagcctctccctgtctccgggtaaagcggatccttcgaacctgctccaatcctgggccattacctta
atctagtaaatggaatttttgtgatatgctgcctgacctactgcttgccccaagatgcagagagagaaggaggaatgagagattga
gaagggaaagtgtacgccctgtataaatcgat

1477
1989

40.2.220-hmtIgG1-hCD80 (secuencia de aminoácido) (ID DE SEC NO __)

MDPQVQIFSFLLISASVIMSRGVDIVLTQSPATLSVTPGDRVSLSCRASQSISDYLHW
YQQKSHESPRLLIKYASHSISGIPSRFSGSGSGSDFTLSINSVEPEDVGIYYCQHGHSF
PWTFGGGTKLEIKRGGGGSGGGGSGGGGSQIQLVQSGPELKKPGETVRISCKASG
YAFTTTGMQWVQEMPGKGLKWIGWINTPLWSAKICRRLQGRFAFSLETSANTAY
LQISNLKDEDTATYFCVRSGNGNYDLAYFAYWGQGTLVTVSDLEPKSSDKTHTSP
PSPAPELLGGSSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVE
VHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISK
AKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKT
TPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGKA
DPSNLLPSWAITLISVNGIFVICCLTYCFAPRCRERRRNERLRRESVRPV

2e12scFv-hmtIgG1-proteína de fusión CD80 (secuencia de nucleótido) (ID DE SEC NO __)

aagcttatggattttcaagtgcagattttcagcttcctgctaatcagtgcttcagtcatcatgtccagaggagatcgacattgtgatcacgc
aatctccagcttctttggctgtgtctctaggtcagagagccaccatctcctgcagagccagtgaaagtgttgatagttatggcaacagtt
tatgcagtggtaccaacagaaaccaggacagccacccaaactcctcatctatcttgcatccaacctagaatctggggtccctgcc
aggtttagtggcagtgggtctgggacagacttcagcctcaacatccatcctgtggaggaggatgatattgcaatgtatttctgtcagc
aaagtaggaaggttccttggacgttcggtggaggcaccaagctggaaatcaaacgggatggcggtggctcgggcggaggtggc
tcgggtggcggcggatctcaggtgcagctgaaggagtcaggacctggcctggtggcgccctcacagagcctgtccatcacatgc
accgtctcagggttctcattaccggctatggtgtaaactgggttcgccagcctccaggaaagggtctggagtggctgggaatgat
atggggtgatggaagcacagactataattcagctctcaaatccagactgagcatcaccaaggacaactccaagagccaagttttctt
taaaatgaacagtctgcaaactgatgacacagccagatactactgtgccagaggtggttatagtaacttttcattactatgttatggact
actggggtcaaggaacctcagtcaccgtctcctcagatctggagcccaaatcttctgacaaaactcacacatcccccaccgtcccca
gcacctgaactcctgggggggatcgtcagtcttcctcttccccccaaaacccaaggacaccctcatgatctcccggacccctgaggt
cacatgcgtggtggtggacgtgagccacgaagaccctgaggtcaagttcaactggtacgtggacggcgtggaggtgcataatgc
caagacaaagccgcgggaggagcagtacaacagcacgtaccgtgtggtcagcgtcctcaccgtcctgcaccaggactggctga
atggcaaggagtacaagtgcaaggtctccaacaaagccctcccagcccccatcgagaaaaccatctccaaagccaaagggcag
ccccgagaaccacaggtgtacaccctgcccccatcccgggatgagctgaccaagaaccaggtcagcctgacctgcctggtcaaa
ggcttctatcccagcgacatcgccgtggagtgggagagcaatgggcagccggagaacaactacaagacacgcctcccgtgct
ggactccgacggctccttcttcctctacagcaagctcaccgtggacaagagcaggtggcagcaggggaacgtcttctcatgctcc
gtgatgcatgaggctctgcacaaccactacacgcagaagagcctctccctgtctccgggtaaagcggatccttcgaacctgctccc
atcctgggccaattaccttaatctcagtaaatggaattttgtgtatgctgcctgacctactgcttgccccaagatgcagagagagaa
ggaggaatgagagattgagaagggaaagtgtacgccctgtataaatcgat

1428
1490

2el2scFv-hmtIgG1-CD80 proteína de fusión (secuencia de aminoácido) (ID DE SEC NO __)

MDFQVQIFSFLLISASVIMSRGVDIVLTQSPASLAVSLGQRATISCRASESVEYYVTS
LMQWYQQKPGQPPKLLISAASNVESGVPARFSGSGSGTDFSLNIHPVEEDDIAMYF
CQQSRKVPWTFGGGTKLEIKRGGGGSGGGGSGGGGSQVQLKESGPGLVAPSQSLS
ITCTVSGFSLTGYGVNWVRQPPGKGLEWLGMIWGDGSTDYNSALKSRLSITKDNS
KSQVFLKMNSLQTDDTARYYCARDGYSNFHYYVMDYWGQGTSVTVSSDLEPKS
SDKTHTSPPSPAPELLGGSSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFN
WYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALP
APIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQ
PENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSL
SLSPGKADPSNLLPSWAITLISVNGIFVICCLTYCFAPRCRERRRNERLRRESVRPV

10A8 scFv-hmtIgGl-CD80 (secuencia de nucleótido) (ID DE SEC NO __)

aagcttatggattttcaagtgcagattttcagcttcctgctaatcagtgcttcagtcataatgtccagaggagtcgacatccagatgaca
cagtctccatcctcactgtctgcatctctgggaggcaaagtcaccatcacttgcaaggcaagccaagacattaagaagtatataggtt
ggtaccaacacaagcctggaaaaggtcccaggctgctcatatattacacatctacattacagccaggcatcccatcaaggttcagtg
gaagtgggtctgggagagattattccctcagcatcagaaacctggagcctgaagatattgcaacttattattgtcaacagtatgataat
cttccattgacgttcggctcggggacaaagttggaaataaaacggggtggcggtggctcgggcggtggtgggtcgggtggcggc
ggatctgatgtacagcttcaggagtcaggacctggcctcgtgaaaccttctcagtctctgtctctcacctgctctgtcactggctactc
catcaccagtggtttctactggaactggatccgacagtttccgggaaacaaactggaatggatgggccacataagccacgacggta
ggaataactacaacccatctctcataaatcgaatctccatcactcgtgacacatctaagaaccagtttttcctgaagttgagttctgtga
ctactgaggacacagctacatatttctgtgcaagacactacggtagtagcggagctatggactactggggtcaaggaacctcagtc
accgtctcctctgatctggagcccaaatcttctgacaaaactcacacatccccaccgtccccagcacctgaactcctgggggggatc
gtcagtcttcctcttcccccaaaacccaaggacaccctcatgatctcccggacccctgaggtcacatgcgtggtggtggacgtga
gccacgaagaccctgaggtcaagttcaactggtacgtggacggcgtggaggtgcataatgccaagacaaagccgcgggaggga
gcagtacaacagcacgtaccgtgtggtcagcgtcctcaccgtcctgcaccaggactggctgaatggcaaggagtacaagtgcaa
ggtctccaacaaagccctcccagccccatcgagaaaaccatctccaaagccaaagggcagccccgagaaccacaggtgtaca
ccctgccccatcccgggatgagctgaccaagaaccaggtcagcctgacctgcctggtcaaaggcttctatcccagcgacatcgc
cgtggagtgggagagcaatgggcagccggagaacaactacaagaccacgcctcccgtgctggactccgacggctccttcttcct

1479
1491

ctacagcaagctcaccgtggacaagagcaggtggcagcaggggaacgtcttctcatgctccgtgatgcatgaggctctgcacaac
cactacacgcagaagagcctctccctgtctccgggtaaagcggatccttcgaacctgctcccatcctgggccattaccttaatctca
gtaaatggaatttttgtgatatgctgcctgacctactgctttgccccaagatgcagagagagaaggaggaatgagagattgagaagg
gaaagtgtacgccctgtataaatcgat

10A8 scFv-hmtIgG1-CD80 (secuencia de aminoácido) (ID DE SEC NO __)

MDFQVQIFSFLLISASVIMSRGVDIQMTQSPSSLSASLGGKVTITCKASQDIKKYIG
WYQHKPGKGPRLLIYYTSTLQPGIPSRFSGSGSGRDYSLSIRNLEPEDIATYYCQQY
DNLPLTFGSGTKLEIKRGGGGSGGGGSGGGGSDVQLQESGPGLVKPSQSLSLTCSV
TGYSITSGFYWNWIRQFPGNKLEWMGHISHDGRNNYNPSLINRISITRDTSKNQFFL
KLSSVTTEDTATYFCARHYGSSGAMDYWGQGTSVTVSSDLEPKSSDKTHTSPPSP
APELLGGSSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVH
NAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAK
GQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPP
VLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGKADPS
NLLPSWAITLISVNGIFVICCLTYCFAPRCRERRRNERLRRESVRPV

500A2-hmtIgG1-CD80 (secuencia de nucleótido) (ID DE SEC NO __)

atgttgtatacatctcagctccttgggcttttactcttctggatttcagcctccagaagtgacatagtgctgactcagactccagccactc
tgtctctaattcctggagaaagagtcacaatgacctgtaagaccagtcagaatattggcacaatcttacactggtatcaccaaaaacc
aaaggaggctccaagggctctcatcaagtatgcttcgcagtccattcctgggatcccctccagattcagtggcagtggttcggaaac
agatttcactctcagcatcaataacctggagcctgatgatatcggaatttattactgtcaacaaagtagaagctggcctgtcacgttcg
gtcctggcaccaagctggagataaaacggggtggcggtggctcgggcggaggtgggtcgggtggcggcggatctcaggtcaa
gctgcagcagtccggttctgaactagggaaacctggggcctcagtgaaactgtcctgcaagacttcaggctacatattcacagatc
actatatttcttgggtgaaacagaagcctggagaaagcctgcagtggataggaaatgtttatggtggaaatggtggtacaagctaca
atcaaaaattccagggcaaggccacactgactgtagataaaatctctagcacagcctacatggaactcagcagcctgacatctgag
gattctgccatctattactgtgcaagaaggccggtagcgacgggccatgctatggactactggggtcaggggatccaagttaccgt
ctcctctgatctggagcccaaatcttctgacaaaactcacacatcccccaccgtccccagcacctgaactcctgggggggatcgtcagt
cttcctcttcccccaaaacccaaggacaccctcatgatctcccggaccccctgaggtcacatgcgtggtggtggacgtgagccacg
aagaccctgaggtcaagttcaactggtacgtggacggcgtggaggtgcataatgccaagacaaagccgcgggaggagcagtac
aacagcacgtaccgtgtggtcagcgtcctcaccgtcctgcaccaggactggctgaatggcaaggagtacaagtgcaaggtctcca
acaaagccctcccagcccccatcgagaaaaccatctccaaagccaaagggcagccccgagaaccacaggtgtacaccctgccc
ccatcccgggatgagctgaccaagaaccaggtcagcctgacctgcctggtcaaaggcttctatcccagcgacatcgccgtggagt
gggagagcaatgggcagccggagaacaactacaagaccacgcctcccgtgctggactccgacggctccttcttcctctacagca

~~1480~~ 1492

agctcaccgtggacaagagcaggtggcagcaggggaacgtcttctcatgctccgtgatgcatgaggctctgcacaaccactacac
gcagaagagcctctccctgtctccgggtaaagcggatccttcgaacctgctcccatcctgggccattaccttaatctcagtaaatgga
attttgtgatatgctgcctgacctactgctttgccccaagatgcagagagagaaggaggaatgagagattgagaagggaaagtgta
cgccctgtataaatcgat

500A2-hmtIgG1-CD80 (secuencia de aminoácido) (ID DE SEC NO __)

MLYTSQLLGLLLFWISASRSDIVLTQTPATLSLIPGERVTMTCKTSQNIGTILHWYH
QKPKEAPRALIKYASQSIPGIPSRFSGSGSETDFTLSINNLEPDDIGIYYCQQSRSWPV
TFGPGTKLEIKRGGGGSGGGGSGGGGSQVKLQQSGSELGKPGASVKLSCKTSGYIF
TDHYISWVKQKPGESLQWIGNVYGGNGGTSYNQKFQGKATLTVDKISSTAYMEL
SSLTSEDSAIYYCARRPVATGHAMDYWGQGIQVTVSSDLEPKSSDKTHTSPPSPAP
ELLGGSSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNA
KTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQ
PREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVL
DSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGKADPSNL
LPSWAITLISVNGIFVICCLTYCFAPRCRERRRNERLRRESVRPV

2H7 scFv MTH (SSS) WTCH2CH3 (secuencia de nucleótido) (ID DE SEC NO __)

aagcttgccgccatggatttttcaagtgcagattttcagcttctgctaatcagtgtcttcagtcataattgtccagaggacaaattgttctct
cccagtctccagcaatcctgtctgcatctccaggggagaaggtcacaatgacttgcagggccagctcaagtgtaagttacatgcact
ggtaccagcagaagccaggatcctcccccaaacctggatttatgccccatccaacctggcttctggagtcctgctcgcttcagtg
gcagtgggtctgggacctcttactctctcacaatcagcagagtggaggctgaagatgctgccactattactgccagcagtggagttt
tacccacccacgttcggtgctgggaccaagctggagctgaaagatggcggtggctcgggcggtggtggatctggaggaggtg
gaagtctcaggcttatctacagcagtctggggctgagctggtgaggcctggggcctcagtgaagatgtcctgcaaggcttctggc

1487
1493

tacacatttaccagttacaatatgcactgggtaaagcagacacctagacagggcctggaatggattggagctatttatccaggaaat
ggtgatacttcctacaatcagaagttcaagggcaaggccacactgactgtagacaaatcctccagcacagcctacatgcagctcag
cagcctgacatctgaagactctgcggtctatttctgtgcaagagtgggtgtactatagtaactcttactggtacttcgatgtctgggg
agggaccacggtcaccgtctcttctgatcaggagcccaaatcttctgacaaaactcacacatcccccaccgtcccagcacctgaac
tcctgggggggaccgtcagtcttcctcttccccccaaaacccaaggacacccttcatgatctcccggacccctgaggtcacatgcgtg
gtggtggacgtgagccacgaagaccctgaggtcaagttcaactggtacgtggacggcgtggaggtgcataatgccaagacaaag
ccgcgggaggagcagtacaacagcacgtaccgtgtggtcagcgtcctcaccgtcctgcaccaggactggctgaatggcaagga
gtacaagtgcaaggtctccaacaaagccctcccagcccccatcgagaaaacaatctccaaagccaaagggcagccccgagaac
cacaggtgtacaccctgcccccatcccgggatgagctgaccaagaaccaggtcagcctgacctgcctggtcaaaggcttctatcc
cagcgacatcgccgtggagtgggagagcaatgggcagccggagaacaactacaagaccacgcctcccgtgctggactccgac
ggctccttcttcctctacagcaagctcaccgtggacaagagcaggtggcagcaggggaacgtcttctcatgctccgtgatgcatga
ggctctgcacaaccactacacgcagaagagcctctccctgtctccgggtaaatgatctaga

2H7 scFv MTH (SSS) WTCH2CH3 (secuencia de aminoácido) (ID DE SEC NO __)

MDFQVQIFSFLLISASVIIARGQIVLSQSPAILSASPGEKVTMTCRASSSVSYMHWY
QQKPGSSPKPWIYAPSNLASGVPARFSGSGSGTSYSLTISRVEAEDAATYYCQQWS
FNPPTFGAGTKLELKDGGGSGGGGSGGGGSSQAYLQQSGAELVRPGASVKMSCK
ASGYTFTSYNMHWVKQTPRQGLEWIGAIYPGNGDTSYNQKFKGKATLTVDKSSS
TAYMQLSSLTSEDSAVYFCARVVYYSNSYWYFDVWGTGTTVTVSSDQEPKSSDK
THTSPPSPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYV
DGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIE
KTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPEN
NYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLS
PGK

2H7 scFv IGAH IGG WT CH2CH3 (2H7 scFv con articulación IgA y WT CH2 y CH3) (secuencia de nucleótido) (ID DE SEC NO __)

aagcttgccgccatggattttcaagtgcagattttcagcttcctgctaatcagtgcttcagtcataattgccagaggacaaattgttctct
cccagtctccagcaatcctgtctgcatctccaggggagaaggtcacaatgacttgcagggccagctcaagtgtaagttacatgcact
ggtaccagcagaagccaggatcctccccaaaccctggatttatgccccatccaacctggcttctggagtcctgctcgcttcagtg
gcagtgggtctgggacctcttactctctcacaatcagcagagtggaggctgaagatgctgccacttattactgccagcagtggagttt
taacccacccacgttcggtgctgggaccaagctggagctgaaagatggcggtggctcgggcggtggtggatctggaggaggtg
ggagctctcaggcttatctacagcagtctggggctgagctggtgaggcctggggcctcagtgaagatgtcctgcaaggcttctggc

1482
1994

tacacatttaccagttacaatatgcactgggtaaagcagacacctagacagggcctggaatggattggagctatttatccaggaaat
ggtgatacttcctacaatcagaagttcaagggcaaggccacactgactgtagacaaatcctccagcacagcctacatgcagctcag
cagcctgacatctgaagactctgcggtctatttctgtgcaagagtggtgtactatagtaactcttactggtacttcgatgtctggggcac
agggaccacggtcaccgtctctgatcagccagttccctcaactccacctaccccatctccctcaactccacctaccccatctccctca
tgcgcacctgaactcctgggggggaccgtcagtcttcctcttcccccaaaacccaaggacaccctcatgatctcccggaccccctga
ggtcacatgcgtggtggtggacgtgagccacgaagaccctgaggtcaagttcaactggtacgtggacggcgtggaggtgcataa
tgccaagacaaagccgcgggaggagcagtacaacagcacgtaccgtgtggtcagcgtcctcaccgtcctgcaccaggactggc
tgaatggcaaggagtacaagtgcaaggtctccaacaaagccctcccagccccatcgagaaaacaatctccaaagccaaagggc
agccccgagaaccacaggtgtacaccctgcccccatcccgggatgagctgaccaagaaccaggtcagcctgacctgcctggtca
aaggcttctatcccagcgacatcgccgtggagtgggagagcaatgggcagccggagaacaactacaagaccacgcctcccgtg
ctggactccgacggctccttcttcctctacagcaagctcaccgtggacaagagcaggtggcagcaggggaacgtcttctcatgctc
cgtgatgcatgaggctctgcacaaccactacacgcagaagagcctctccctgtctccgggtaaatgatctaga

2H7 scFv IgAH 1GG WT CH2CH3 (secuencia de aminoácido) (ID DE SEC NO __)

MDFQVQIFSFLLISASVIIARGQIVLSQSPAILSASPGEKVTMTCRASSSVSYMHWY
QQKPGSSPKPWIYAPSNLASGVPARFSGSGSGTSYSLTISRVEAEDAATYYCQQWS
FNPPTFGAGTKLELKDGGGSGGGGSGGGGSSQAYLQQSGAELVRPGASVKMSCK
ASGYTFTSYNMHWVKQTPRQGLEWIGAIYPGNGDTSYNQKFKGKATLTVDKSSS
TAYMQLSSLTSEDSAVYFCARVVYYSNSYWYFDVWGTGTTVTVSDQPVPSTPPTP
SPSTPPTPSPSCAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFN
WYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALP
APIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQ
PENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSL
SLSPGK

<u>2H7 scFv IsAH IgACH2CH3</u> (2H7 scFv articulación IgA e IgA CH2 y CH3) (secuencia de nucleótido) (ID DE SEC NO __)

aagcttgccgccatggattttcaagtgcagattttcagcttcctgctaatcagtgcttcagtcataattgccagaggacaaattgttctct
cccagtctccagcaatcctgtctgcatctccaggggagaaggtcacaatgacttgcagggccagctcaagtgtaagttacatgcact
ggtaccagcagaagccaggatcctccccaaaccctggatttatgccccatccaacctggcttctggagtccctgctcgcttcagtg
gcagtgggtctgggacctcttactctctcacaatcagcagagtggaggctgaagatgctgccacttattactgccagcagtggagttt
taacccacccacgttcggtgctgggaccaagctggagctgaaagatggcggtggctcgggcggtggtggatctggaggaggtg
ggagctctcaggcttatctacagcagtctggggctgagctggtgaggcctggggcctcagtgaagatgtcctgcaaggcttctggc
tacacatttaccagttacaatatgcactgggtaaagcagacacctagacagggcctggaatggattggagctatttatccaggaaat

1483
1495

ggtgatacttcctacaatcagaagttcaagggcaaggccacactgactgtagacaaatcctccagcacagcctacatgcagctcag
cagcctgacatctgaagactctgcggtctatttctgtgcaagagtggtgtactatagtaactcttactggtacttcgatgtctggggcac
agggaccacggtcaccgtctcttctgatcagccagttccctcaactccacctaccccatctccctcaactccacctaccccatctccc
tcatgctgccaccccccgactgtcactgcaccgaccggccctcgaggacctgctcttaggttcagaagcgatcctcacgtgcacact
gaccggcctgagagatgcctcaggtgtcaccttcacctggacgccctcaagtgggaagagcgctgttcaaggaccacctgaccgt
gacctctgtggctgctacagcgtgtccagtgtcctgccgggctgtgccgagccatggaaccatgggaagaccttcacttgcactgc
tgcctaccccgagtccaagaccccgctaaccgccaccctctcaaaatccggaaacacattccggcccgaggtccacctgctgccg
ccgccgtcggaggagctggccctgaacgagctggtgacgctgacgtgcctggcacgtggcttcagccccaaggatgtgctggtt
cgctggctgcaggggtcacaggagctgccccgcgagaagtacctgacttgggcatcccggcaggagcccagccagggcacca
ccaccttcgctgtgaccagcatactgcgcgtggcagccgaggactggaagaagggggacaccttctcctgcatggtgggccacg
aggccctgccgctggccttcacacagaagaccatcgaccgcttggcgggtaaacccacccatgtcaatgtgtctgttgtcatggcg
gaggtggacggcacctgctactgataatctaga

2H7 scFv IgAH IgACH2CH3 (2H7 scFv articulación IgA e IgA CH2 y CH3) (secuencia de aminoácido) (ID DE SEC NO ·__)

MDFQVQIFSFLLISASVIIARGQIVLSQSPAILSASPGEKVTMTCRASSSVSYMHWY
QQKPGSSPKPWIYAPSNLASGVPARFSGSGSGTSYSLTISRVEAEDAATYYCQQWS
FNPPTFGAGTKLELKDGGGSGGGGSGGGGSSQAYLQQSGAELVRPGASVKMSCK
ASGYTFTSYNMHWVKQTPRQGLEWIGAIYPGNGDTSYNQKFKGKATLTVDKSSS
TAYMQLSSLTSEDSAVYFCARVVYYSNSYWYFDVWGTGTTVTVSSDQPVPSTPPT
PSPSTPPTPSPSCCHPRLSLHRPALEDLLLGSEAILTCTLTGLRDASGVTFTWTPSSG
KSAVQGPPDRDLCGCYSVSSVLPGCAEPWNHGKTFTCTAAYPESKTPLTATLSKS
GNTFRPEVHLLPPPSEELALNELVTLTCLARGFSPKDVLVRWLQGSQELPREKYLT
WASRQEPSQGTTTFAVTSILRVAAEDWKKGDTFSCMVGHEALPLAFTQKTIDRLA
GKPTHVNVSVVMAEVDGTCY

Articulación IgA -CH2-CH3 (Human IgA tail, full length) (secuencia de nucleótido) (ID DE SEC NO __)

tgatcagccagttccctcaactccacctaccccatctccctcaactccacctaccccatctccctcatgctgccacccccgactgtca
ctgcaccgaccggccctcgaggacctgctcttaggttcagaagcgatcctcacgtgcacactgaccggcctgagagatgcctcag
gtgtcaccttcacctggacgccctcaagtgggaagagcgctgttcaaggaccacctgaccgtgacctctgtggctgctacagcgtg
tccagtgtcctgccgggctgtgccgagccatggaaccatgggaagaccttcacttgcactgctgcctaccccgagtccaagaccc
cgctaaccgccaccctctcaaaatccggaaacacattccggcccgaggtccacctgctgccgccgccgtcggaggagctggccc
tgaacgagctggtgacgctgacgtgcctggcacgtggcttcagccccaaggatgtgctggttcgctggctgcaggggtcacagg

1484
1496

agctgccccgcgagaagtacctgacttgggcatcccggcaggagcccagccagggcaccaccaccttcgctgtgaccagcata
ctgcgcgtggcagccgaggactggaagaaggggacaccttctcctgcatggtgggccacgaggccctgccgctggccttcac
acagaagaccatcgaccgcttggcgggtaaacccacccatgtcaatgtgtctgttgtcatggcggaggtggacggcacctgctact
gataatctaga

Articulación-CH2-CH3 IgA (cola IgA humana, longitud completa) (secuencia de aminoácido) (ID DE SEC NO __)

DQPVPSTPPTPSPSTPPTPSPSCCHPRI SLHRPALEDLLLGSEAILTCTLTGLRDASGV
TFTWTPSSGKSAVQGPPDRDLCGCYSVSSVLPGCAEPWNHGKTFTCTAAYPESKT
PLTATLSKSGNTFRPBVHLLPPPSEELALNELVTLTCLARGFSPKDVLVRWLGSQ
BLPREKYLTWASRQEPSQGTTTFAVTSILRVAAEDWKKGDTFSCMVGHEALPLAF
TQKTIDRLAGKPTHVNVSVVMAEVDGTCY

Cadena J Humana (secuencia de nucleótido) (ID DE SEC NO __)

agatctcaagaagatgaaaggattgttcttgttgacaacaaatgtaagtgtgcccggattacttccaggatcatccgttcttccgaaga
tcctaatgaggacattgtggagagaaacatccgaattattgttcctctgaacaacagggagaatatctctgatcccacctcaccattga
gaaccagatttgtgtaccatttgtctgacctcagctgtaaaaaatgtgatcctacagaagtggagctggataatcagatagttactgct
acccagagcaatatctgtgatgaagacagtgctacagagacctgctacacttatgacagaaacaagtgctacacagctgtggtccc
actcgtatatggtggtgagaccaaaatggtggaaacagccttaaccccagatgcctgctatcctgactaatctaga

Cadena J humana (secuencia de aminoácido) (ID DE SEC NO __)

RSQEDERIVLVDNKCKCARITSRIIRSSEDPNEDIVERNIRIIVPLNNRENISDPTSPLR
TRFVYHLSDLSCKKCDPTEVELDNQIVTATQSNICDEDSATETCYTYDRNKCYTAV
VPLVYGGETKMVETALTPDACYP

Amino ácidos 4 carboxy terminal eliminados de IgA CH3 (secuencia de aminoácido) (ID DE SEC NO __)

GTCY

IgAH IgAT4 (cola IgA humana, truncada (3T1)-(desaparece últimos 4 amino ácidos del terminal carboxy) (secuencia de nucleótido) (ID DE SEC NO __)

1485
1497

tgatcagccagttccctcaactccacctaccccatctccctcaactccacctaccccatctccctcatgctgccacccccgactgtca
ctgcaccgaccggccctcgaggacctgctcttaggttcagaagcgatcctcacgtgcacactgaccggcctgagagatgcctcag
gtgtcaccttcacctggacgccctcaagtgggaagagcgctgttcaaggaccacctgaccgtgacctctgtggctgctacagcgtg
tccagtgtcctgccgggctgtgccgagccatggaaccatgggaagaccttcacttgcactgctgcctaccccgagtccaagaccc
cgctaaccgccacccctctcaaaatccggaaacacattccggcccgaggtccacctgctgccgccgccgtcggaggagctggccc
tgaacgagctggtgacgctgacgtgcctggcacgtggcttcagccccaaggatgtgctggttcgctggctgcaggggtcacagg
agctgccccgcgagaagtacctgacttgggcatcccggcaggagcccagccagggcaccaccaccttcgctgtgaccagcata
ctgcgcgtggcagccgaggactggaagaaggggacaccttctcctgcatggtgggccacgaggccctgccgctggccttcac
acagaagaccatcgaccgcttggcgggtaaacccacccatgtcaatgtgtctgttgtcatggcggaggtggactgataatctaga

IgAH IgAT4 (secuencia de aminoácido) (ID DE SEC NO __)

DQPVPSTPPTPSPSTPPTPSPSCCHPRLSLHRPALEDLLLGSEAILTCTLTGLRDASGV
TFTWTPSSGKSAVQGPPDRDLCGCYSVSSVLPGCAEPWNHGKTFTCTAAYPESKT
PLTATLSKSGNTFRPEVHLLPPPSEELALNELVTLTCLARGFSPKDVLVRWLQGSQ
ELPREKYLTWASRQEPSQGTTTFAVTSILRVAAEDWKKGDTFSCMVGHEALPLAF
TQKTIDRLAGKPTHVNVSVVMAEVD

2H7 scFv IgAH IgAT4 (construct 2H7 scFv IgA 3T1, trunca el dominio CH3 en el extremo 3') (secuencia de nucleótido) (ID DE SEC NO __)

aagcttgccgccatggattttcaagtgcagattttcagcttcctgctaatcagtgcttcagtcataattgccagaggacaaattgttctct
cccagtctccagcaatcctgtctgcatctccaggggagaaggtcacaatgacttgcagggccagctcaagtgtaagttacatgcact
ggtaccagcagaagccaggatcctcccccaaaccctggatttatgccccatccaacctggcttctggagtccctgctcgcttcagtg
gcagtgggtctgggacctcttactctctcacaatcagcagagtggaggctgaagatgctgccacttattactgccagcagtggagttt
taacccacccacgttcggtgctgggaccaagctggagctgaaagatggcggtggctcgggcggtggtggatctggaggaggtg
ggagctctcaggcttatctacagcagtctggggctgagctggtgaggcctggggcctcagtgaagatgtcctgcaaggcttctggc
tacacatttaccagttacaatatgcactgggtaaagcagacacctagacagggcctggaatggattggagctatttatccaggaaat
ggtgatacttcctacaatcagaagttcaagggcaaggccacactgactgtagacaaatcctccagcacagcctacatgcagctcag
cagcctgacatctgaagactctgcggtctatttctgtgcaagagtggtgtactatagtaactcttactggtacttcgatgtctggggcac
agggaccacggtcaccgtctcttctgatcagccagttccctcaactccacctaccccatctccctcaactccacctaccccatctccc
tcatgctgccacccccgactgtcactgcaccgaccggccctcgaggacctgctcttaggttcagaagcgatcctcacgtgcacact
gaccggcctgagagatgcctcaggtgtcaccttcacctggacgccctcaagtgggaagagcgctgttcaaggaccacctgaccgt
gacctctgtggctgctacagcgtgtccagtgtcctgccgggctgtgccgagccatggaaccatgggaagaccttcacttgcactgc
tgcctaccccgagtccaagaccccgctaaccgccacccctctcaaaatccggaaacacattccggcccgaggtccacctgctgccg
ccgccgtcggaggagctggccctgaacgagctggtgacgctgacgtgcctggcacgtggcttcagccccaaggatgtgctggtt

1486
1498

cgctggctgcaggggtcacaggagctgccccgcgagaagtacctgacttgggcatcccggcaggagcccagccagggcacca
ccaccttcgctgtgaccagcatactgcgcgtggcagccgaggactggaagaaggggacaccttctcctgcatggtgggccacg
aggccctgccgctggccttcacacagaagaccatcgaccgcttggcgggtaaacccacccatgtcaatgtgtctgttgtcatggcg
gaggtggactgataatctaga

2H7 scFv IgAH-T4 (secuencia de aminoácido) (ID DE SEC NO __)

MDFQVQIFSFLLISASVIIARGQIVLSQSPAILSASPGEKVTMTCRASSSVSYMHWY
QQKPGSSPKPWIYAPSNLASGVPARFSGSGSGTSYSLTISRVEAEDAATYYCQQWS
FNPPTFGAGTKLELKDGGGSGGGGSGGGGSSQAYLQQSGAELVRPGASVKMSCK
ASGYTFTSYNMHWVKQTPRQGLEWIGAIYPGNGDTSYNQKFKGKATLTVDKSSS
TAYMQLSSLTSEDSAVYFCARVVYYSNSYWYFDVWGTGTTVTVSSDQPVPSTPPT
PSPSTPPTPSPSCCHPRLSLHRPALEDLLLGSEAILTCTLTGLRDASGVTFTWTPSSG
KSAVQGPPDRDLCGCYSVSSVLPGCAEPWNHGKTFTCTAAYPESKTPLTATLSKS
GNTFRPEVHLLPPPSEELALNELVTLTCLARGFSPKDVLVRWLQGSQELPREKYLT
WASRQEPSQGTTTFAVTSILRVAAEDWKKGDTFSCMVGHEALPLAFTQKTIDRLA
GKPTHVNVSVVMAEVD

14 aminoácidos eliminados de IgAH-T4 (de tal forma que 18 amino ácidos eliminados de IgA CH3 tipo silvestre) (secuencia de aminoácido) (ID DE SEC NO __)

PTHVNVSVVMAEVD

IgAH IgA-T18 (cola IgA truncada, 3T2) (secuencia de nucleótido) (ID DE SEC NO __)

tgatcagccagttccctcaactccacctaccccatctccctcaactccacctaccccatctccctcatgctgccacccccgactgtca
ctgcaccgaccggccctcgaggacctgctcttaggttcagaagcgatcctcacgtgcacactgaccggcctgagagatgcctcag
gtgtcaccttcacctggacgccctcaagtgggaagagcgctgttcaaggaccacctgaccgtgacctctgtggctgctacagcgtg
tccagtgtcctgccgggctgtgccgagccatggaaccatgggaagaccttcacttgcactgctgcctaccccgagtccaagaccc
cgctaaccgccaccctctcaaaatccggaaacacattccggcccgaggtccacctgctgccgccgccgtcggaggagctggccc
tgaacgagctggtgacgctgacgtgcctggcacgtggcttcagccccaaggatgtgctggttcgctggctgcaggggtcacagg
agctgccccgcgagaagtacctgacttgggcatcccggcaggagcccagccagggcaccaccaccttcgctgtgaccagcata
ctgcgctggcagccgaggactggaagaaggggacaccttctcctgcatggtgggcacgaggccctgccgctggccttcac
acagaagaccatcgaccgcttggcgggtaaa

IgAH IgA-T18 (secuencia de aminoácido) (ID DE SEC NO __)

1487
1499

DQPVPSTPPTPSPSTPPTPSPSCCHPRLSLHRPALEDLLLGSEAILTCTLTGLRDASGV
TFTWTPSSGKSAVQGPPDRDLCGCYSVSSVLPGCAEPWNHGKTFTCTAAYPESKT
PLTA'ILSKSGNTFRPEVHLLPPPSEELALNELVTLTCLARGFSPKDVLVRWLQGSQ
ELPREKYLTWASRQEPSQGTTTFAVTSILRVAAEDWKKGDTFSCMVGHEALPLAF
TQKTIDRLAGK

2H7 scFv IgAH IgAT18 (cola IgA humana truncada, 3T2) (secuencia de nucleótido) (ID DE SEC NO __)

aagcttgccgccatggattttcaagtgcagattttcagcttcctgctaatcagtgcttcagtcataattgccagaggacaaaattgttctct
cccagtctccagcaatcctgtctgcatctccaggggagaaggtcacaatgacttgcagggccagctcaagtgtaagttacatgcact
ggtaccagcagaagccaggatcctcccccaaaccctggatttatgccccatccaacctggcttctggagtccctgctcgcttcagtg
gcagtgggtctgggacctcttactctctcacaatcagcagagtggaggctgaagatgctgccacttattactgccagcagtggagttt
taacccacccacgttcggtgctgggaccaagctggagctgaaagatggcggtggctcgggcggtggtggatctggaggaggtg
ggagctctcaggcttatctacagcagtctggggctgagctggtgaggcctggggcctcagtgaagatgtcctgcaaggcttctggc
tacacatttaccagttacaatatgcactgggtaaagcagacacctagacagggcctggaatggattggagctatttatccaggaaat
ggtgatacttcctacaatcagaagttcaagggcaaggccacactgactgtagacaaatcctccagcacagcctacatgcagctcag
cagcctgacatctgaagactctgcggtctatttctgtgcaagagtggtgtactatagtaactcttactggtacttcgatgtctggggcac
agggaccacggtcaccgtctcttctgatcagccagttccctcaactccacctaccccatctccctcaactccacctaccccatctccc
tcatgctgccaccccgactgtcactgcaccgaccggccctcgaggacctgctcttaggttcagaagcgatcctcacgtgcacact
gaccggcctgagagatgcctcaggtgtcaccttcacctggacgccctcaagtgggaagagcgctgttcaaggaccacctgaccgt
gacctctgtggctgctacagcgtgtccagtgtcctgccgggctgtgccgagccatggaaccatgggaagaccttcacttgcactgc
tgcctaccccgagtccaagaccccgctaaccgccaccctctcaaaatccggaaacacattccggcccgaggtccacctgctgccg
ccgccgtcggaggagctggccctgaacgagctggtgacgctgacgtgcctggcacgtggcttcagccccaaggatgtgctggtt
cgctggctgcaggggtcacaggagctgccccgcgagaagtacctgacttgggcatcccggcaggagcccagccagggcacca
ccaccttcgctgtgaccagcatactgcgcgtggcagccgaggactggaagaaggggacaccttctcctgcatggtgggccacg
aggccctgccgctggccttcacacagaagaccatcgaccgcttggcgggtaaa

<u>2H7 scFv IgAH IgAT18</u> (secuencia de aminoácido) (ID DE SEC NO __)

1488
1500

MDFQVQIFSFLLISASVIIARGQIVLSQSPAILSASPGEKVTMTCRASSSVSYMHWY
QQKPGSSPKPWIYAPSNLASGVPARFSGSGSGTSYSLTISRVEAEDAATYYCQQWS
FNPPTFGAGTKLELKDGGGSGGGGSGGGGSSQAYLQQSGAELVRPGASVKMSCK
ASGYTFTSYNMHWVKQTPRQGLEWIGAIYPGNGDTSYNQKFKGKATLTVDKSSS
TAYMQLSSLTSEDSAVYFCARVVYYSNSYWYFDVWGTGTTVTVSSDQPVPSTPPT
PSPSTPPTPSPSCCHPRLSLHRPALEDLLLGSEAILTCTLTGLRDASGVTFTWTPSSG
KSAVQGPPDRDLCGCYSVSSVLPGCAEPWNHGKTFTCTAAYPESKTPLTATLSKS
GNTFRPEVHLLPPPSEELALNELVTLTCLARGFSPKDVLVRWLQGSQELPREKYLT
WASRQEPSQGTTTFAVTSILRVAAEDWKKGDTFSCMVGHEALPLAFTQKTIDRLA
GK

CTLA-4 IgG WTH WTCH2CH3 (-CTLA4EC-hIgGWT oncoMLP humano) (secuencia de nucleótido) (ID DE SEC NO __)

gcaacctacatgatggggaatgagttgaccttcctagatgattccatctgcacgggcacctccagtggaaatcaagtgaacctcact
atccaaggactgagggccatggacacgggactctacatctgcaaggtggagctcatgtacccaccgccatactacctgggcatag
gcaacggaacccagatttatgtaattgatccagaaccgtgcccagattctgatcaacccaaatcttgtgacaaaactcacacatgcc
caccgtgcccagcacctgaactcctgggggggaccgtcagtcttcctcttcccccaaaacccaaggacaccctcatgatctcccgg
acccctgaggtcacatgcgtggtggtggacgtgagccacgaagaccctgaggtcaagttcaactggtacgtggacggcgtggag
gtgcataatgccaagacaaagccgcgggaggagcagtacaacagcacgtaccgtgtggtcagcgtcctcaccgtcctgcaccag
gactggctgaatggcaaggagtacaagtgcaaggtctccaacaaagccctcccagcccccatcgagaaaacaatctccaaagcc
aaagggcagccccgagaaccacaggtgtacaccctgcccccatcccgggatgagctgaccaagaaccaggtcagcctgacctg
cctggtcaaaggcttctatcccagcgacatcgccgtggagtgggagagcaatgggcagccggagaacaactacaagaccacgc
ctcccgtgctggactccgacggctccttcttcctctacagcaagctcaccgtggacaagagcaggtggcagcaggggaacgtctt
ctcatgctccgtgatgcatgaggctctgcacaaccactacacgcagaagagcctctccctgtctccgggtaaatga

CTLA-4 IgG WTH WTCH2CH3 (secuencia de aminoácido) (ID DE SEC NO __)

MGVLLTQRTLLSLVLALLFPSMASMAMHVAQPAVVLASSRGIASFVCEYASPGKA
TEVRVTVLRQADSQVTEVCAATYMMGNELTFLDDSICTGTSSGNQVNLTIQGLRA
MDTGLYICKVELMYPPPYYLGIGNGTQIYVIDPEPCPDSDQPKSCDKTHTCPPCPAP
ELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNA
KTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQ
PREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVL
DSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

1484
1501

péptido líder+CTLA4 EC OncoM Human (BclI) (secuencia de nucleótido) (ID DE SEC NO __)

atgggggtactgctcacacagaggacgctgctcagtctggtccttgcactcctgtttccaagcatggcgagcatggcaatgcacgt
ggcccagcctgctgtggtactggccagcagccgaggcatcgccagctttgtgtgtgagtatgcatctccaggcaaagccactgag
gtccgggtgacagtgcttcggcaggctgacagccaggtgactgaagtctgtgcggcaacctacatgatggggaatgagttgacct
tcctagatgattccatctgcacgggcacctccagtggaaatcaagtgaacctcactatccaaggactgagggccatggacacggg
actctacatctgcaaggtggagctcatgtacccaccgccatactacctgggcataggcaacggaacccagatttatgtaattgatcc
agaaccgtgcccagattctgatcaa

Péptido líder+CTLA4 EC OncoM humano (secuencia de aminoácido) (ID DE SEC NO __)

MGVLLTQRTLLSLVLALLFPSMASMAMHVAQPAVVLASSRGIASFVCEYASPGKA
TEVRVTVLRQADSQVTEVCAATYMMGNELTFLDDSICTGTSSGNQVNLT1QGLRA
MDTGLYICKVELMYPPPYYLGIGNGTQIYVIDPEPCPDSDQ

Líder OncoM humano (secuencia de nucleótido) (ID DE SEC NO __)

atgggggtactgctcacacagaggacgctgctcagtctggtccttgcactcctgtttccaagcatggcgagcatg

Líder OncoM humano (secuencia de aminoácido) (ID DE SEC NO __)

MGVLLTQRTLLSLVLALLFPSM

CTLA4 EC humano (sin LP) (secuencia de nucleótido) (ID DE SEC NO __)

gcaatgcacgtggcccagcctgctgtggtactggccagcagccgaggcatcgccagctttgtgtgtgagtatgcatctccaggca
aagccactgaggtccgggtgacagtgcttcggcaggctgacagccaggtgactgaagtctgtgcggcaacctacatgacgggga
atgagttgaccttcctagatgattccatctgcacgggcacctccagtggaaatcaagtgaacctcactatccaaggactgagggcca
tggacacgggactctacatctgcaaggtggagctcatgtacccaccgccatactacctgggcataggcaacggaacccagatttat
gtaattgatccagaaccgtgcccagattct

CTLA4 EC humano (sin LP) (secuencia de aminoácido) (ID DE SEC NO __)

1490
1502

AMHVAQPAVVLASSRGIASFVCEYASPGKATEVRVTVLRQADSQVTEVCAATYM
TGNELTFLDDSICTGTSSGNQVNLTIQGLRAMDTGLYICEZVELMYPPPYYLGIGNG
TQIYVIDPEPCPDS

CTLA4 IgG MTH (SSS) MTCH2CH3 humano (secuencia de nucleótido) (ID DE SEC NO __)

atgggggtactgctcacacagaggacgctgctcagtctggtccttgcactcctgtttccaagcatggcgagcatggcaatgcacgt
ggcccagcctgctgtggtactggccagcagccgaggcatcgccagctttgtgtgtgagtatgcatctccaggcaaagccactgag
gtccgggtgacagtgcttcggcaggctgacagccaggtgactgaagtctgtgcggcaacctacatgatggggaatgagttgacct
tcctagatgattccatctgcacgggcacctccagtggaaatcaagtgaacctcactatccaaggactgagggccatggacacggg
actctacatctgcaaggtggagctcatgtacccaccgccatactacctgggcataggcaacggaacccagatttatgtaattgatcc
agaaccgtgcccagattctgatcaacccaaatcttctgacaaaactcacacatccccaccgtccccagcacctgaactcctggggg
gatcgtcagtcttcctcttcccccaaaacccaaggacaccctcatgatctcccggaccccctgaggtcacatgcgtggtggtggac
gtgagccacgaagaccctgaggtcaagttcaactggtacgtggacggcgtggaggtgcataatgccaagacaaagccgcggga
ggagcagtacaacagcacgtaccgtgtggtcagcgtcctcaccgtcctgcaccaggactggctgaatggcaaggagtacaagtg
caaggtctccaacaaagccctcccagccccatcgagaaaacaatctccaaagccaaagggcagccccgagaaccacaggtgt
acaccctgcccccatcccgggatgagctgaccaagaaccaggtcagcctgacctgcctggtcaaaggcttctatcccagcgacat
cgccgtggagtgggagagcaatgggcagccggagaacaactacaagaccacgcctcccgtgctggactccgacggctccttctt
cctctacagcaagctcaccgtggacaagagcaggtggcagcaggggaacgtcttctcatgctccgtgatgcatgaggctctgcac
aaccactacacgcagaagagcctctccctgtctccgggtaaatga

CTLA4 IgG MTH (SSS) MTCH2CH3 humano (secuencia de aminoácido) (SEQ ID NO __)

MGVILLTQRTLLSLVLALLFPSMASMAMHVAQPAVVLASSRGIASFVCEYASPGK
ATEVRVTVLRQADSQVTEVCAATYMMGNELTFLDDSICTGTSSGNQVNLTIQGLR
AMDTGLYICKVELMYPPPYYLGIGNGTQIWIDPEPCPDSDQPKSSDKTHTSPPSPAP
ELLGGSSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNA
KTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQ
PREPQVYTLPPSRDELTKNQVSLTCLKGFYPSDIAVEWESNGQPENNYKTTPPVL
DSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

CTLA-4 IgAH IgACH2CH3 (oncoMLP-CTLA4EC-IgA humano) (secuencia de nucleótido) (ID DE SEC NO _)

1497
1503

atgggggtactgctcacacagaggacgctgctcagtctggtccttgcactcctgtttccaagcatggcgagcatggcaatgcacgt
ggcccagcctgctgtggtactggccagcagccgaggcatcgccagctttgtgtgtgagtatgcatctccaggcaaagccactgag
gtccgggtgacagtgcttcggcaggctgacagccaggtgactgaagtctgtgcggcaacctacatgatggggaatgagttgacct
tcctagatgattccatctgcacgggcacctccagtggaaatcaagtgaacctcactatccaaggactgagggccatggacacggg
actctacatctgcaaggtggagctcatgtacccaccgccatactacctgggcataggcaacggaacccagatttatgtaattgatcc
agaaccgtgcccagattctgatcagccagttccctcaactccacctaccccatctccctcaactccacctaccccatctccctcatgct
gccacccccgactgtcactgcaccgaccggccctcgaggacctgctcttaggttcagaagcgatcctcacgtgcacactgaccgg
cctgagagatgcctcaggtgtcaccttcacctggacgccctcaagtgggaagagcgctgttcaaggaccacctgaccgtgacctct
gtggctgctacagcgtgtccagtgtcctgccgggctgtgccgagccatggaaccatgggaagaccttcacttgcactgctgcctac
cccgagtccaagaccccgctaaccgccaccctctcaaaatccggaaacacattccggcccgaggtccacctgctgccgccgccg
tcggaggagctggccctgaacgagctggtgacgctgacgtgcctggcacgtggcttcagccccaaggatgtgctggttcgctgg
ctgcaggggtcacaggagctgccccgcgagaagtacctgacttgggcatcccggcaggagcccagccagggcaccaccaccctt
cgctgtgaccagcatactgcgcgtggcagccgaggactggaagaaggggacaccttctcctgcatggtgggccacgaggccc
tgccgctggccttcacacagaagaccatcgaccgcttggcgggtaaacccacccatgtcaatgtgtctgttgtcatggcggaggtg
gacggcacctgctactgataatctaga

CTLA-4 IsAH lgACH2CH3 (secuencia de aminoácido) (ID DE SEC NO __)

MGVLLTQRTLLSLVLALLFPSMASMAMHVAQPAVVLASSRGIASFVCEYASPGKA
TEVRVTVLRQADSQVTEVCAATYMMGNELTFLDDSICTGTSSGNQVNLTIQGLRA
MDTGLYICKVELMYPPPYYLGIGNGTQIYVIDPEPCPDSDQPVPSTPPTPSPSTPPTP
SPSCCMPRLSLHRPALEDLLLGSEAILTCTLTGLRDASGVTFTWTPSSGKSAVQGPP
DRDLCGCYSVSSVLPGCAEPWNHGKTFTCTAAYPESKTPLTATLSKSGNTFRPEVH
LLPPPSEELALNELVTLTCLARGFSPKDVLVRWLQGSQELPREKYLTWASRQEPSQ
GTTTFAVTSILRV AAEDWKKGDTFSCMVGHEALPLAFTQKTIDRLAGKPTHVNVS
VVMAEVDGTCY

CTLA-4 IsAH IgA-T4 (oncoMLP-CTLA4EC-IgA3Tl humano) (secuencia de nucleótido) (ID de SEC NO)

atgggggtactgctcacacagaggacgctgctcagtctggtccttgcactcctgtttccaagcatgggagcatggcaatgcacgt
ggcccagcctgctgtggtactggccagcagccgaggcatcgccagctttgtgtgtgagtatgcatctccaggcaaagccactgag
gtccgggtgacagtgcttcggcaggctgacagccaggtgactgaagtctgtgcggcaacctacatgatggggaatgagttgacct
tcctagatgattccatctgcacgggcacctccagtggaaatcaagtgaacctcactatccaaggactgagggccatggacacggg
actctacatctgcaaggtggagctcatgtacccaccgccatactacctgggcataggcaacggaacccagatttatgtaattgatcc
agaaccgtgcccagattctgatcagccagttccctcaactccacctaccccatctccctcaactccacctaccccatctccctcatgct

1495
1509

gccacccccgactgtcactgcaccgaccggccctcgaggacctgctcttaggttcagaagcgatcctcacgtgcacactgaccgg
cctgagagatgcctcaggtgtcaccttcacctggacgccctcaagtgggaagagcgctgttcaaggaccacctgaccgtgacctct
gtggctgctacagcgtgtccagtgtcctgccgggctgtgccgagccatggaaccatgggaagaccttcacttgcactgctgcctac
cccgagtccaagaccccgctaaccgccaccctctcaaaatccggaaacacattccggcccgaggtccacctgctgccgccgccg
tcggaggagctggccctgaacgagctggtgacgctgacgtgcctggcacgtggcttcagccccaaggatgtgctggttcgctgg
ctgcaggggtcacaggagctgccccgcgagaagtacctgacttgggcatcccggcaggagcccagccagggcaccaccaccctt
cgctgtgaccagcatactgcgcgtggcagccgaggactggaagaaggggacaccttctcctgcatggtgggccacgaggccc
tgccgctggccttcacacagaagaccatcgaccgcttggcgggtaaacccacccatgtcaatgtgtctgttgtcatggcggaggtg
gactgataatctaga

CTLA-4 Ig A-T4 (secuencia de aminoácido) (ID DE SEC NO __)

MGVLLTQRTLLSLVLALLFPSMASMAMHVAQPAVVLASSRGIASFVCEYASPGKA
TEVRVTVLRQADSQVTEVCAATYMMGNELTFLDDSICTGTSSGNQVNLTIQGLRA
MDTGLYICKVELMYPPPYYLGIGNGTQIYVIDPEPCPDSDQPVPSTPPTPSPSTPPTP
SPSCCHPRLSLHRPALEDLLLGSEAILTCTLTGLRDASGVTFTWTPSSGKSAVQGPP
DRDLCGCYSVSSVLPGCAEPWNHGKTFTCTAAYPESKTPLTATLSKSGNTFRPEVH
LLPPPSEELALNELVTLTCLARGFSPKDVLVRWLQGSQELPREKYLTWASRQEPSQ
GTTTFAVTSILRVAAEDWKKGDTFSCMVGHEALPLAFTQKTIDRLAGKPTHVNVS
VVMAEVD

IsGl CH2 humano con 238 mutación pro→ser (secuencia de nucleótido) (ID DE SEC NO __)

cctgaactcctgggggggatcgtcagtcttcctcttccccccaaaacccaaggacaccctcatgatctcccggacccctgaggtcac
atgcgtggtggtggacgtgagccacgaagaccctgaggtcaagttcaactggtacgtggacggcgtggaggtgcataatgccaa
gacaaagccgcgggaggagcagtacaacagcacgtaccgtgtggtcagcgtcctcaccgtcctgcaccaggactggctgaatg
gcaaggagtacaagtgcaaggtctccaacaaagccctcccagccccccatcgagaaaaccatctccaaagccaaag

IgG1 CH2 humano con 238 mutation pro→ser (secuencia de aminoácido) (ID DE SEC NO __)

PELLGGSSVFLFPPKPKDTLMISRTPEVTCVWDVSHEDPEVKFNWYVDGVEVHN
AKTKPRFEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNXALPAPIEKTISKAK

1493
1505

Amino acidos que rodean Pro a Ser en CH2 (secuencia de aminoácido) (ID DE SEC NO __)

PAPELLGGPS

Amino acidos que rodean Pro a Ser en CH2 (secuencia de aminoácido) (ID DE SEC NO __)

PAPELLGGSS

hIgE3BB (deja una estructura abierta en el exremo del gen para leer dentro del dominio de cola citoplásmico y de transmembrana adherido a los BamHI o SfuI) (secuencia de nucleótido) (ID DE SEC NO __)

gtt gtt ttc gaa gga tcc gct tta ccg gga ttt aca gac acc gct cgc tgg

IgE Fc (CH2-CH3-CH4) ORF humano (secuencia de nucleótido) (ID DE SEC NO __)

tgatcacgtctgctccagggacttcacccctgcccaccgtgaagatcttacagtcgtcctgcgacggcggcgggcacttccccccg
accatccagctcctgtgctctgtctctgggtacaccccagggactatcaacatcacctggctggaggacgggcaggtcatggacg
tggacttgtccacgcctctaccacgaggagggtgagctggcctccacacaaagcgagctcaccctcagccagaagcactggc
tgtcagaccgcacctacacctgccaggtcacctatcaaggtcacaccttgaggacagcaccaagaagtgtgcagatttccaaccc
gagaggggtgagcgcctacctaagccggcccagccccgttcgacctgttcatccgcaagtcgcccacgatcacctgtctggtggtg
gacctggcacccagcaaggggaccgtgaacctgacctggtcccgggccagtgggaagcctgtgaaccactccaccagaaagg
aggagaagcagcgcaatggcacgttaccgtcacgtccaccctgccggtgggcacccgagactggatcgaggggagacctca
ccagtgcagggtgacccaccccccacctgcccagggccctcatgcggtccacgaccaagacagcggccgcgtgctgcccg
gaagtctatgcgtttgcgacgccggagtggccggggagccgggacaagcgcaccctcgcctgcctgatccagaacttcatgcct
gaggacatctcggtgcagtggctgcacaacgaggtgcagctcccggacgcccggcacagcacgacgcagcccgcaagacc
aagggctccggctcttcgtcttcagccgcctggaggtgaccagggccgaatgggagcagaaagatgagttcatctgccgtgcag
tccatgaggcagcgagccccttcacagaccgtccagcgagcggtgtctgtaaatcccggtaaagcggatccttcgaa

IgE Fc (CH2-CH3-CH4) ORF humano (secuencia de aminoácido) (ID DE SEC NO __)

DHVCSRDFTPPTVKILQSSCDGGGHFPPTIQLLCLVSGYTPGTINITWLEDGQVMDV
DLSTASTTQEGELASTQSELTLSQKHWLSDRTYTCQVTYQGHTFEDSTKKCADSN
PRGVSAYLSRPSPFDLFIRKSPTITCLVVDLAPSKGTVNLTWSRASGKPVNHSTRKE

1499
1506

EKQRNGTLTVTSTLPVGTRDWIEGETYQCRVTHPHLPRALMRSTTKTSGPRAAPE
VYAFATPEWPGSRDILRTLACLIQNFMPEDISVQWLHNEVQLPDARHSTTQPRKTK
GSGFFVFSRLEVTRAEWEQKDEFICRAVHEAASPSQTVQRAVSVNPGKADPS

IFhIgGwtBcl5 (secuencia de nucleótido) (ID DE SEC NO __)

gtt gtt tga tca gga gcc caa atc ttg tga caa aac tca cac atg ccc acc gtg ccc agc acc (63 mer)

HuIgGMHWC (sentido, iniciador 5' para mutación de articulación tipo silvestre CCC para SSS mutante) (secuencia de nucleótido) (ID DE SEC NO __)

gtt gtt gat cag gag ccc aaa tct tct gac aaa act cac aca tct cca ccg tcc cca gca cct gaa ctc ctg ggt gga ccg tca gtc ttc c

1D8 VH (secuencia de nucleótido) (ID DE SEC NO __

caggtgcagctgaaggaggcaggacctggcctggtgcaaccgacacagaccctgtccctcacatgcactgtctctgggttctcatt
aaccagcgatggtgtacactggattcgacagcctccaggaaagggtctggaatggatgggaataatatattatgatggaggcaca
gattataattcagcaattaaatccagactgagcatcagcagggacacctccaagagccaagttttcttaaaaatcaacagtctgcaaa
ctgatgacacagccatgtattactgtgccagaatccactttgattactggggccaaggagtcatggtcacagtctcctct

1D8 VH (sin líder) (secuencia de aminoácido) (ID DE SEC NO __)

QVQLKJBAGPGLVQPTQTLSLTCTVSGFSLTSDGVHWIRQPPGKGLEWMGUYYDG
GTDYNSAIKSKLSISRDTSKSQVFLKINSLQTDDTAMYYCARIHFDYWGQGVMVT
VSS

1D8 VL (sin leader) (secuencia de nucleótido) (ID DE SEC NO __)

gacattgtgctcactcagtctccaacaaccatagctgcatctccaggggagaaggtcaccatcacctgccgtgccagctccagtgt
aagttacatgtactggtaccagcagaagtcaggcgcctcccctaaactctggatttatgacacatccaagctggcttctggagttcca
aatcgcttcagtggcagtgggtctgggacctcttattctctcgcaatcaacaccatggagactgaagatgctgccacttattactgtca
gcagtggagtagtactccgctcacgttcgggtctgggaccaagctggagatcaaacgg

1D8 VL (secuencia de aminoácido) (ID DE SEC NO __)

1495
1507

DIVLTQSPTTIAASPGEKVTITCRASSSVSYMYWYQQKSGASPKLWIYDTSKLASG
VPNRFSGSGSGTSYSLAINTMETEDAATYYCQQWSSTPLTFGSGTKLEIKR

<u>1D8 scFv</u> (secuencia de nucleótido) (ID DE SEC NO __)

aagcttatggattttcaagtgcagattttcagcttcctgctaatcagtgcttcagtcataatgtccagaggagtcgacattgtgctcactc
agtctccaacaaccatagctgcatctccaggggagaaggtcaccatcacctgccgtgccagctccagtgtaagttacatgtactggt
accagcagaagtcaggcgcctcccctaaactctggatttatgacacatccaagctggcttctggagttccaaatcgcttcagtggca
gtgggtctgggacctcttattctctcgcaatcaacaccatggagactgaagatgctgccacttattactgtcagcagtggagtagtact
ccgctcacgttcgggtctgggaccaagctggagatcaaacggggtggcggtggctcgggcggtggtgggtcgggtggcggcg
gatctcaggtgcagctgaaggaggcaggacctggcctggtgcaaccgacacagaccctgtccctcacatgcactgtctctgggtt
ctcattaaccagcgatggtgtacactggattcgacagcctccaggaaagggtctggaatggatgggaataatatattatgatggagg
cacagattataattcagcaattaaatccagactgagcatcagcagggacacctccaagagccaagttttcttaaaaatcaacagtctg
caaactgatgacacagccatgtattactgtgccagaatccactttgattactggggccaaggagtcatggtcacagtctcctctgatca

1D8 scFv (secuencia de aminoácido) (ID DE SEC NO __)

MDFQVQIFSFLLISASVIMSRGVDIVLTQSPTTIAASPGEKVTITCRASSSVSYMYWY
QQKSGASPKLWIYDTSKLASGVPNRFSGSGSGTSYSLAINTMETEDAATYYCQQW
SSTPLTFGSGTKLEIKRGGGGSGGGGSGGGGSQVQLKEAGPGLVQPTQTLSLTCTV
SGFSLTSDGVHWIRQPPGKGLEWMGIIYYDGGTDYNSAIKSRLSISRDTSKSQVFLK
INSLQTDDTAMYYCARIHFDYWGQGVMVTVSS

<u>1D8 scFv IsG WTH WTCH2CH3</u> (secuencia de nucleótido) (ID DE SEC NO __)

aagcttatggattttcaagtgcagattttcagcttcctgctaatcagtgcttcagtcataatgtccagaggagtcgacattgtgctcactc
agtctccaacaaccatagctgcatctccaggggagaaggtcaccatcacctgccgtgccagctccagtgtaagttacatgtactggt
accagcagaagtcaggcgcctcccctaaactctggatttatgacacatccaagctggcttctggagttccaaatcgcttcagtggca
gtgggtctgggacctcttattctctcgcaatcaacaccatggagactgaagatgctgccacttattactgtcagcagtggagtagtact
ccgctcacgttcgggtctgggaccaagctggagatcaaacggggtggcggtggctcgggcggtggtgggtcgggtggcggcg
gatctcaggtgcagctgaaggaggcaggacctggcctggtgcaaccgacacagaccctgtccctcacatgcactgtctctgggtt
ctcattaaccagcgatggtgtacactggattcgacagcctccaggaaagggtctggaatggatgggaataatatattatgatggagg
cacagattataattcagcaattaaatccagactgagcatcagcagggacacctccaagagccaagttttcttaaaaatcaacagtctg
caaactgatgacacagccatgtattactgtgccagaatccactttgattactggggccaaggagtcatggtcacagtctcctctgatc
aggagcccaaatcttgtgacaaaactcacacatgcccaccgtgcccagcacctgaactcctgggggggaccgtcagtcttcctcttc
cccccaaaacccaaggacaccctcatgatctcccggaccccctgaggtcacatgcgtggtggtggacgtgagccacgaagaccct

1496
1508

gaggtcaagttcaactggtacgtggacggcgtggaggtgcataatgccaagacaaagccgcgggaggagcagtacaacagcac
gtaccgtgtggtcagcgtcctcaccgtcctgcaccaggactggctgaatggcaaggagtacaagtgcaaggtctccaacaaagcc
ctcccagcccccatcgagaaaacaatctccaaagccaaagggcagccccgagaaccacaggtgtacaccctgcccccatcccg
ggatgagctgaccaagaaccaggtcagcctgacctgcctggtcaaaggcttctatcccagcgacatcgccgtggagtgggagag
caatgggcagccggagaacaactacaagaccacgcctcccgtgctggactccgacggctccttcttcctctacagcaagctcacc
gtggacaagagcaggtggcagcaggggaacgtcttctcatgctccgtgatgcatgaggctctgcacaaccactacacgcagaag
agcctctccctgtctccgggtaaatgatctaga

1D8 scFv IsG WTH WTCH2CH3 (secuencia de aminoácido) (ID DE SEC NO __)

MDFQVQIFSFLLISASVIMSRGVDIVLTQSPTTIAASPGEKVTITCRASSSVSYMYWY
QQKSGASPKLWIYDTSKLASGVPNRFSGSGSGTSYSLAINTMETEDAATYYCQQW
SSTPLTFGSGTKLEIKRGGGGSGGGGSGGGGSQVQLKEAGPGLVQPTQTLSLTCTV
SGFSLTSDGVHWIRQPPGKGLEWMGIIYYDGGTDYNSAIKSRLSISRDTSKSQVFLK
INSLQTDDTAMYYCARIHFDYWGQGVMVTVSSDQEPKSCDKTHTCPPCPAPELLG
GPSVFLFPPKPKDTLMISRTPEVTCWVDVSHEDPEVKFNWYVDGVEVHNAKTKP
REEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREP
QVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDG
SFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

1D8 scFv leG MTH MTCH2CH3-CD80 (secuencia de nucleótido) (ID DE SEC NO __)

aagcttatggattttcaagtgcagattttcagcttcctgctaatcagtgcttcagtcataatgtccagaggagtcgacattgtgctcactc
agtctccaacaaccatagctgcatctccaggggagaaggtcaccatcacctgccgtgccagctccagtgtaagttacatgtactggt
accagcagaagtcaggcgcctccctaaactctggatttatgacacatccaagctggcttctggagttccaaatcgcttcagtggca
gtgggtctgggacctcttattctctcgcaatcaacaccatggagactgaagatgctgccacttattactgtcagcagtggagtagtact
ccgctcacgttcgggtctgggaccaagctggagatcaaacggggtggcggtggctcgggcggtggtgggtcgggtggcggcg
galctcaggtgcagctgaaggaggcaggacctggcctggtgcaaccgacacagaccctgtccctcacatgcactgtctctgggtt
ctcattaaccagcgatggtgtacactggattcgacagcctccaggaaagggtctggaatggatgggaataatatattatgatggagg
cacagattataattcagcaattaaatccagactgagcatcagcagggacacctccaagagccaagttttcttaaaaatcaacagtctg
caaactgatgacacagccatgtattactgtgccagaatccactttgattactggggccaaggagtcatggtcacagtctcctctgatct
ggagcccaaatcttctgacaaaactcacacaagcccaccgagcccagcacctgaactcctgggggggatcgtcagtcttcctcttcc
ccccaaaacccaaggacaccctcatgatctcccggaccccctgaggtcacatgcgtggtggtggacgtgagccacgaagaccctg
aggtcaagttcaactggtacgtggacggcgtggaggtgcataatgccaagacaaagccgcgggaggagcagtacaacagcacg
taccgtgtggtcagcgtcctcaccgtcctgcaccaggactggctgaatggcaaggagtacaagtgcaaggtctccaacaaagccc
tcccagcccccatcgagaaaaccatctccaaagccaaagggcagccccgagaaccacaggtgtacaccctgcccccatcccgg
gatgagctgaccaagaaccaggtcagcctgacctgcctggtcaaaggcttctatcccagcgacatcgccgtggagtgggagagc

1497
1509

aatgggcagccggagaacaactacaagaccacgcctcccgtgctggactccgacggctccttcttcctctacagcaagctcaccg
tggacaagagcaggtggcagcaggggaacgtcttctcatgctccgtgatgcatgaggctctgcacaaccactacacgcagaaga
gcctctccctgtctccgggtaaagcggatccttcgaacctgctcccatcctgggccattaccttaatctcagtaaatggaatttttgtga
tatgctgcctgacctactgctttgccccaagatgcagagagagaaggaggaatgagagattgagaagggaaagtgtacgccctgt
ataaatcgata

1D8 scFv IgG MTH MTCH2CH3-CD80 (secuencia de aminoácido) (ID DE SEC NO __)

MDFQVQIFSFLLISASVIMSRGVDIVLTQSPTTIAASPGEKVTITCRASSSVSYMYWY
QQKSGASPKIWIYDTSKLASGVPNRFSGSGSGTSYSLAINTMETEDAATYYCQQW
SSTPLTFGSGTKLEIKRGGGGSGGGGSGGGGSQVQLKEAGPGLVQPTQTLSLTCTV
SGFSLTSDGVHWIRQPPGKGLEWMGIIYYDGGTDYNSAIKSRLSISRDTSKSQVFLK
INSLQTDDTAMYYCARIHFDYWGQGVMVTVSSDLEPKSSDKTHTSPPSPAPELLG
GSSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKP
REEQYNSTYRVVSVLTVLHQDWLNGKFEYKCKVSNKATPAPIEKTISKAKGQPRE
PQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSD
GSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGKADPSNLLPS
WAITLISVNGIFVICCLTYCFAPRCRERRRNERLRRESVRPV

1D8 scFv IgG WTH WTCH2CH3-CD80 (secuencia de nucleótido) (SEQ ID NO __)

aagcttatggattttcaagtgcagattttcagcttcctgctaatcagtgcttcagtcataatgtccagaggagtcgacattgtgctcactc
agtctccaacaaccatagctgcatctccaggggagaaggtcaccatcacctgccgtgccagctccagtgtaagttacatgtactggt
accagcagaagtcaggcgcctcccctaaactctggatttatgacacatccaagctggcttctggagttccaaatcgcttcagtggca
gtgggtctgggacctcttattctctcgcaatcaacaccatggagactgaagatgctgccacttattactgtcagcagtggagtagtact
ccgctcacgttcgggtctgggaccaagctggagatcaaacggggtggcggtggctcgggcggtggtgggtcgggtggcggcg
gatctcaggtgcagctgaaggaggcaggacctggcctggtgcaaccgacacagaccctgtccctcacatgcactgtctctgggtt
ctcattaaccagcgatggtgtacactggattcgacagcctccaggaaagggtctggaatggatgggaataatatattatgatggagg
cacagattataattcagcaattaaatccagactgagcatcagcagggacacctccaagagccaagttttcttaaaaatcaacagtctg
caaactgatgacacagccatgtattactgtgccagaatccactttgattactggggccaaggagtcatggtcacagtctcctctgatct
ggagcccaaatcttgtgacaaaactcacacatgcccaccgtgcccagcacctgaactcctgggggggaccgtcagtcttcctcttcc
ccccaaaacccaaggacaccctcatgatctcccggaccccctgaggtcacatgcgtggtggtggacgtgagccacgaagaccctg
aggtcaagttcaactggtacgtggacggcgtggaggtgcataatgccaagacaaagccgcgggaggagcagtacaacagcacg
taccgtgtggtcagcgtcctcaccgtcctgcaccaggactggctgaatggcaaggagtacaagtgcaaggtctccaacaaagccc
tcccagcccccatcgagaaaaccatctccaaagccaaagggcagccccgagaaccacaggtgtacaccctgcccccatcccgg

1498
1510

gatgagctgaccaagaaccaggtcagcctgacctgcctggtcaaaggcttctatcccagcgacatcgccgtggagtgggagagc
aatgggcagccggagaacaactacaagaccacgcctcccgtgctggactccgacggctccttcttcctctacagcaagctcaccg
tggacaagagcaggtggcagcaggggaacgtcttctcatgctccgtgatgcatgaggctctgcacaaccactacacgcagaaga
gcctctccctgtctccgggtaaagcggatccttcgaacctgctcccatcctgggccattaccttaatctcagtaaatggaatttttgtga
tatgctgcctgacctactgctttgccccaagatgcagagagagaaggaggaatgagagattgagaagggaaagtgtacgccctgt
ataaatcgata

1D8 scFv IgG WTH WTCH2CH3-CD80 (secuencia de aminoácido) (ID DE SEC NO. __)

MDFQVQIFSFLLISASVIMSRGVDIVLTQSPTTIAASPGEKVTITCRASSSVSYMYWY
QQKSGASPKLWIYDTSKLASGVPNRFSGSGSGTSYSLAINTMETEDAATYYCQQW
SSTPLTFGSGTKLEIKRGGGGSGGGGSGGGGSQVQLKESGPGLVQPTQTLSLTCTV
SGFSLTSDGVHWIRQPPGKGLEWMGIIYYDGGTDYNSAIKSRLSISRDTSKSQVFLK
INSLQTDDTAMYYCARIHFDYWGQGVMVTVSSDLEPKSCDKTHTCPPCPAPELLG
GPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKP
REEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREP
QVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDG
SFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGKADPSNLLPSW
AITLISVNGIFVICCLTYCFAPRCRERRRNERLRRESVRPV

CD3 scFv WTH WTCH2CH3 anti-humano (secuencia de nucleótido) (ID DE SEC NO __)

aagcttatggattttcaagtgcagattttcagcttcctgctaatcagtgcttcagtcataatgtccagaggagtcgacatccagatgaca
cagactacatcctccctgtctgcctctctgggagacagagtcaccatcagttgcagggcaagtcaggacattcgcaattatttaaact
ggtatcagcagaaaccagatggaactgttaaactcctgatctactacacatcaagattacactcaggagtcccatcaaggttcagtg
gcagtgggtctggaacagattattctctcaccattgccaacctgcaaccagaagatattgccacttacttttgccaacagggtaatac
gcttccgtggacgttcggtggaggcaccaaactggtaaccaaacgggagctcggtggcggtggctcgggcggtggtgggtcgg
gtggcggcggatctatcgatgaggtccagctgcaacagtctggacctgaactggtgaagcctggagcttcaatgtcctgcaaggc
ctctggttactcattcactggctacatcgtgaactggctgaagcagagccatggaaagaaccttgagtggattggacttattaatccat
acaaaggtcttactacctacaaccagaaattcaagggcaaggccacattaactgtagacaagtcatccagcacagcctacatggag
ctcctcagtctgacatctgaagactctgcagtctattactgtgcaagatctgggtactatggtgactcggactggtacttcgatgtctgg
ggcgcagggaccacggtcaccgtctcctctgatcaggagcccaaatcttgtgacaaaactcacacatgcccaccgtgcccagcac
ctgaactcctgggggggaccgtcagtcttcctcttcccccaaaacccaaggacaccctcatgatctcccggaccctgaggtcaca
tgcgtggtggtggacgtgagccacgaagaccctgaggtcaagttcaactggtacgtggacggcgtggaggtgcataatgccaag

1499
1511

acaaagccgcgggaggagcagtacaacagcacgtaccgtgtggtcagcgtcctcaccgtcctgcaccaggactggctgaatgg
caaggagtacaagtgcaaggtctccaacaaagccctcccagccccccatcgagaaaacaatctccaaagccaaagggcagcccc
gagaaccacaggtgtacaccctgcccccatcccgggatgagctgaccaagaaccaggtcagcctgacctgcctggtcaaaggct
tctatcccagcgacatcgccgtggagtgggagagcaatgggcagccggagaacaactacaagaccacgcctcccgtgctggac
tccgacggctccttcttcctctacagcaagctcaccgtggacaagagcaggtggcagcaggggaacgtcttctcatgctccgtgat
gcatgaggctctgcacaaccactacacgcagaagagcctctccctgtctccgggtaaatgatctaga

CD3 scFv WTH WTCH2CH3 Anti-humano (secuencia de aminoácido) (ID DE SEC NO __)

MDFQVQIFSFLLISASVIMSRGVDIQMTQTTSSLSASLGDRVTISCRASQDIRNYLN
WYQQKPDGTVKLLIYYTSRLHSGVPSRFSGSGSGTDYSLTIANLQPEDIATYFCQQ
GNTLPWTFGGGTKLVTKRELGGGGSGGGGSGGGGSIDEVQLQQSGPELVKPGAS
MSCKASGYSFTGYTVNWLKQSHGKNLEWIGLINPYKGLTTYNQKFKGKATLTVD
KSSSTAYMELLSLTSEDSAVYYCARSGYYGDSDWYFDVWGAGTTVTVSSDQEPK
SCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKF
NWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKA
LPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESN
GQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQ
KSLSLSPGK

2H7-antiCD40 scFv MTH (SSS) MTCH2WTCH3 (2h7-40 2 220Ig + sitios de restricción) (secuencia de nucleótido) (ID DE SEC NO __)

aagcttgccgccatggattttcaagtgcagattttcagcttcctgctaatcagtgcttcagtcataattgccagaggacaaattgttctct
cccagtctccagcaatcctgtctgcatctccaggggagaaggtcacaatgacttgcagggccagctcaagtgtaagttacatgcact
ggtaccagcagaagccaggatcctccccaaaccctggatttatgccccatccaacctggcttctggagtccctgctcgcttcagtg
gcagtgggtctgggacctcttactctctcacaatcagcagagtggaggctgaagatgctgccacttattactgccagcagtggagttt
taacccacccacgttcggtgctgggaccaagctggagctgaaaggtggcggtggctcgggcggtggtggatctggaggaggtg
ggagctctcaggcttatctacagcagtctggggctgagctggtgaggcctggggcctcagtgaagatgtcctgcaaggcttctggc
tacacatttaccagttacaatatgcactgggtaaagcagacacctagacagggcctggaatggattggagctatttatccaggaaat
ggtgatacttcctacaatcagaagttcaagggcaaggccacactgactgtagacaaatcctccagcacagcctacatgcagctcag
cagcctgacatctgaagactctgcggtctatttctgtgcaagagtggtgtactatagtaactcttactggtacttcgatgtctggggcac
agggaccacggtcaccgtctcttctgatcaatccaactctgaagaagcaaagaaagaggaggccaaaaaggaggaagccaaga
aatctaacagcgtcgacattgttctgactcagtctccagccaccctgtctgtgactccaggagatagagtctctctttcctgcagggcc
agccagagtattagcgactacttacactggtatcaacaaaaatcacatgagtctccaaggcttctcatcaaatatgcttcccattccatc

1500
1512

tctgggatcccctccaggttcagtggcagtggatcagggtcagatttcactctcagtatcaacagtgtggaacctgaagatgttggaa
tttattactgtcaacatggtcacagctttccgtggacgttcggtggaggcaccaagctggaaatcaaacggggtggcggtggctcg
ggcggaggtgggtcgggtggcggcggatctcagatccagttggtgcaatctggacctgagctgaagaagcctggagagacagt
caggatctcctgcaaggcttctgggtatgccttcacaactactggaatgcagtgggtgcaagagatgccaggaaagggtttgaagt
ggattggctggataaacaccccactctggagtgccaaaatatgtagaagacttcaaggacggtttgccttctctttggaaacctctgc
caacactgcatatttacagataagcaacctcaaagatgaggacacggctacgtatttctgtgtgagatccgggaatggtaactatga
cctggcctactttgcttactggggccaagggacactggtcactgtctctgatcaggagcccaaatcttctgacaaaactcacacatcc
ccaccgtccccagcacctgaactcctgggggggatcgtcagtcttcctcttcccccaaaacccaaggacaccctcatgatctcccg
gacccctgaggtcacatgcgtggtggtggacgtgagccacgaagaccctgaggtcaagttcaactggtacgtggacggcgtgga
ggtgcataatgccaagacaaagccgcgggaggagcagtacaacagcacgtaccgtgtggtcagcgtcctcaccgtcctgcacca
ggacgctgaatggcaaggagtacaagtgcaaggtctccaacaaagccctcccagccccatcgagaaaacaatctccaaagc
caaagggcagccccgagaaccacaggtgtacaccctgccccatcccgggatgagctgaccaagaaccaggtcagcctgacct
gcctggtcaaaggcttctatcccagcgacatcgccgtggagtgggagagcaatgggcagccggagaacaactacaagaccacg
cctcccgtgctggactccgacggctccttcttcctctacagcaagctcaccgtggacaagagcaggtggcagcaggggaacgtct
tctcatgctccgtgatgcatgaggctctgcacaaccactacacgcagaagagcctctccctgtctccgggtaaatgatctaga

2H7-antiCD40 scFv MTH (SSS) MTCH2WTCH3 (2H7-40 2-220Ig) (secuencia de aminoácido) (ID DE SEC NO __)

MDPQVQIFSFLLISASVIIARGQIVLSQSPAILSASPGEKVTMTCRASSSVSYMHWY
QQKPGSSPKPWIYAPSNLASGVPARFSGSGSGTSYSLTISRVEAEDAATYYCQQWS
FNPPTFGAGTKLELKGGGGSGGGGSGGGGSSQAYLQQSGAELVRPGASVKMSCK
ASGYTFTSYNMHWVKQTPRQGLEWIGAIYPGNGDTSYNQKFKGKATLTVDKSSS
TAYMQLSSLTSEDSAVYFCARVVYYSNSYWYFDVWGTGTTVTVSSDQSNSEEAK
KEEAKKEEAKKSNSVDIVLTQSPATLSVTPGDRVSLSCRASQSISDYLHWYQQKSH
ESPRLLIKYASHSISGIPSRFSGSGSGSDFTLSINSVEPEDVGIYYCQHGHSFPWTFGG
GTKLEIKRGGGGSGGGGSGGGGSQIQLVQSGPELKKPGETVRISCKASGYAFTTTG
MQWVQEMPGKGLKWIGWINTPLWSAKICRRLQGRFAFSLETSANTAYLQISNLKD
EDTATYFCVRSGNGNYDLAYFAYWGQGTLVTVSDQEPKSSDKTHTSPPSPAPELL
GGSSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTK
PREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPRE
PQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSD
GSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

5B9 VH (incluye el VH péptido líder) (secuencia de nucleótido) (ID DE SEC NO __)

1507
1513

atggctgtcttggggctgctcttctgcctggtgacatttccaagctgtgtcctatcccaggtgcagctgaagcagtcaggacctggcc
tagtgcagtcctcacagagcctgtccatcacctgcacagtctctggtttctcattaactacctatgctgtacactgggttcgccagtctc
caggaaagggtctggagtggctgggagtgatatggagtggtggaatcacagactataatgcagctttcatatccagactgagcatc
accaaggacgattccaagagccaagttttctttaaaatgaacagtctgcaacctaatgacacagccatttattactgtgccagaaatg
ggggtgataactacccttattactatgctatggactactggggtcaaggaacctcagtcaccgtctcctca

5B9 VH (menos el líder) (secuencia de nucleótido) (ID DE SEC NO __)

caggtgcagctgaagcagtcaggacctggcctKgtgcagtcctcacagagcctgtccatcacctgcacagtctctggtttctcatta
actacctatgctgtacactgggttcgccagtctccaggaaagggtctggagtggctgggagtgatatggagtggtggaatcacaga
ctataatgcagctttcatatccagactgagcatcaccaaggacgattccaagagccaagttttctttaaaatgaacagtctgcaaccta
atgacacagccatttattactgtgccagaaatgggggtgataactacccttattactatgctatggactactggggtcaaggaacctca
gtcaccgtctcctca

5B9 VH (incluye péptido líder) (secuencia de aminoácido) (ID DE SEC NO __)

MAVLGLIFCLVTFPSCVLSQVQLKQSGPGLVQSSQSLSITCTVSGFSLTTYAVHWV
RQSPGKGLEWLGVIWSGGITDYNAAFISRLSITKDDSKSQVFFKMNSLQPNDTAIY
YCARNGGDNYPYYYAMDYWGQGTSVTVSS

5B9 VH (sin péptido líder) (secuencia de aminoácido) (ID DE SEC NO __)

QVQLKQSGPGLVQSSQSLSITCTVSGFSLTTYAVHWVRQSPGKGLEWLGVIWSGGI
TDYNAAFISRLSITKDDSKSQVFFKMNSLQPNDTAIYYCARNGGDNYPYYYAMDY
WGQGTSVTVSS

5B9 VL (secuencia de nucleótido) (ID DE SEC NO __)

atgaggttctctgctcagcttctggggctgcttgtgctctggatccctggatccactgcagatattgtgatgacgcaggctgcattctc
caatccagtcactcttggaacatcagcttccatctcctgcaggtctagtaagagtctcctacatagtaatggcatcacttatttgtattgg
tatctgcagaagccaggccagtctcctcagctcctgatttatcagatgtccaaccttgcctcaggagtcccagacaggttcagtagca
gtgggtcaggaactgatttcacactgagaatcagcagagtggaggctgaggatgtgggtgtttattactgtgctcaaaatctagaact
tccgctcacgttcggtgctgggaccaagctggagctgaaacgg

5B9 VL (secuencia de aminoácido) (ID DE SEC NO __)

1502
1519

MRFSAQLLGLLVLWIPGSTADIVMTQAAFSNPVTLGTSASISCRSSKSLLHSNGITY
LYWYLQKPGQSPQLLIYQMSNLASGVPDRFSSSGSGTDFTLRISRVEAEDVGVYYC
AQNLELPLTFGAGTKLELKR

5B9 scFv (secuencia de nucleótido) (ID DE SEC NO __)

aagcttgccgccatgaggttctctgctcagcttctggggctgcttgtgctctggatccctggatccactgcagatattgtgatgacgca
ggctgcattctccaatccagtcactcttggaacatcagcttccatctcctgcaggtctagtaagagtctcctacatagtaatggcatca
cttatttgtattggtatctgcagaagccaggccagtctcctcagctcctgatttatcagatgtccaaccttgcctcaggagtcccagaca
ggttcagtagcagtgggtcaggaactgatttcacactgagaatcagcagagtggaggctgaggatgtgggtgtttattactgtgctc
aaaatctagaacttccgctcacgttcggtgctgggaccaagctggagctgaaacggggtggcggtggctcgggcggtggtgggt
cgggtggcggcggatcgtcacaggtgcagctgaagcagtcaggacctggcctagtgcagtcctcacagagcctgtccatcacct
gcacagtctctggtttctcattaactacctatgctgtacactgggttcgccagtctccaggaaagggtctggagtggctgggagtgat
atggagtggtggaatcacagactataatgcagctttcatatccagactgagcatcaccaaggacgattccaagagccaagttttcttt
aaaatgaacagtctgcaacctaatgacacagccatttattactgtgccagaaatgggggtgataactacccttattactatgctatgga
ctactggggtcaaggaacctcagtcaccgtctcctct

5B9 scFv (secuencia de aminoácido) (ID DE SEC NO __)

MRFSAQLLGLLVLWIPGSTADIVMTQAAFSNPVTLGTSASISCRSSKSLLHSNGITY
LYWYLQKPGQSPQLLIYQMSNLASGVPDRFSSSGSGTDFTLRISRVEAEDVGVYYC
AQNLELPLTFGAGTKLELIRGGGGSGGGGSGGGGSSQVQLKQSGPGLVQSSQSLS
ITCTVSGFSLTTYAVHWVRQSPGKGLEWLGVIWSGGITDYNAAFISRLSITKDDSK
SQVFFKMNSLQPNDTAIYYCARNGGDNYPYYYAMDYWGQGTSVTVSS

5B9 scFv-hmtIgG1-hCD80 (secuencia de nucleótido) (ID DE SEC NO __)

1515

aagcttgccgccatgaggttctctgctcagcttctggggctgcttgtgctctggatcctggatccactgcagatattgtgatgacgca
ggctgcattctccaatccagtcactcttggaacatcagccttccatctcctgcaggtctagtaagagtctcctacatagtaatggcatca
cttatttgtattggtatctgcagaagccaggccagtctcctcagctcctgatttatcagatgtccaacttgcctcaggagtcccagaca
ggttcagtagcagtgggtcaggaactgatttcacactgagaatcagcagagtggaggctgaggatgtgggtgtttattactgtgctc
aaaatctagaacttccgctcacgttcggtgctgggaccaagctggagctgaaacgggtggcggtggctcgggcggtggtgggt
cgggtggcggcggatcgtcacaggtgcagctgaagcagtcaggacctggcctagtgcagtcctcacagagcctgtccatcacct
gcacagtctctggtttctcattaactacctatgctgtacactgggttcgccagtctccaggaaagggtctggagtggctgggagtgat
atggagtggtggaatcacagactataatgcagctttcatatccagactgagcatcaccaaggacgattccaagagccaagtttcttt
aaaatgaacagtctgcaacctaatgacacagccatttattactgtgccagaaatgggggtgataactacccttattactatgctatgga
ctactggggtcaaggaacctcagtcaccgtctcctctgatctggagccccaaatcttctgacaaaactcacacatgcccaccgtgccc
cagcacctgaactcctgggggggatcgtcagtcttcctcttccccccaaaacccaaggacaccctcatgatctcccggacccctgag
gtcacatgcgtggtggtggacgtgagccacgaagaccctgaggtcaagttcaactggtacgtggacggcgtggaggtgcataat
gccaagacaaagccgcgcgggaggagcagtacaacagcacgtaccgtgtggtcagcgtcctcaccgtcctgcaccaggactggct
gaatggcaaggagtacaagtgcaaggtctccaacaaagccctcccagcccccatcgagaaaaccatctccaaagccaaagggc
agccccgagaaccacaggtgtacaccctgcccccatcccgggatgagctgaccaagaaccaggtcagcctgacctgcctggtca
aaggcttctatcccagcgacatcgccgtggagtgggagagcaatgggcagccggagaacaactacaagaccacgcctcccgtg
ctggactccgacggctccttcttcctctacagcaagctcaccgtggacaagagcaggtggcagcaggggaacgtcttctcatgctc
cgtgatgcatgaggctctgcacaaccactacacgcagaagagcctctccctgtctccgggtaaagcggatccttcgaacctgctcc
catcctgggccattacctaatctcagtaaatgggaatttttgtgtatgctgcctgacctactgcttgcccaagatgcagagagaga
aggaggaatgagagattgagaagggaaagtgtacgccctgtataaatcgatactcgag

5B9 scFv-hmtIgG1-hCD80 (secuencia de aminoácido) (ID DE SEC NO __)

MRFSAQLLGLLVLWIPGSTADIVMTQAAFSNPVTLGTSASISCRSSKSLLHSNGITY
LYWYLQKPGQSPQLLIYQMSNLASGVPDRFSSSGSGTDFTLRISRVEAEDVGVYYC
AQNLELPLTFGAGTKLELKRGGGGSGGGGSGGGGSSQVQLKQSGPGLVQSSQSLS
ITCTVSGFSLTTYAVHWVRQSPGKGLEWLGVIWSGGITDYNAAFISRLSITKDDSK
SQVFFKMNSLQPNDTAIYYCARNGGDNYPYYYAMDYWGQGTSVTVSSDLEPKSS
DKTHTSPPSPAPELLGGSSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNW
YVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKBYKCKSNKALPA
PIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQP
ENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLS
LSPGKADPSNLLPSWAITLISVNGIFVICCLTYCFAPRCRERRRNERLRRESVRPV

~~1504~~ 1516

2el2 scFv WTH CH2 CH3 (2el2 scFv-WthIgG-CD80) (secuencia de nucleótido) (ID DE SEC NO __)

aagcttatggattttcaagtgcagattttcagcttcctgctaatcagtgcttcagtcataatgtccagaggagatcgacattgtgctcaccc
aatctccagcttctttggctgtgtctctaggtcagagagccaccatctcctgcagagccagtgaaagttgtgaatattatgtcacaagtt
taatgcagtggtaccaacagaaaccaggacagccacccaaactcctcatctatgctgcatccaacgtagaatctggggtccctgcc
aggtttagtggcagtgggtctgggacagacttcagcctcaacatccatcctgtggaggaggatgatattgcaatgtatttctgtcagc
aaagtaggaaggttccttggacgttcggtggaggcaccaagctggaaatcaaacgggatggcggtggctcgggcggaggtggg
tcgggtggcggcggatctcaggtgcagctgaaggagtcaggacctggcctggtggcgccctcacagagcctgtccatcacatgc
accgtctcagggttctcattaccggctatggtgtaaactgggttcgccagcctccaggaaagggtctggagtggctgggaatgat
atgggtgatggaagcacagactataattcagctctcaaatccagactgagcatcaccaaggacaactccaagagccaagttttctt
aaaaatgaacagtctgcaaactgatgacacagccagatactactgtgccagagatggttatagtaactttcattactatgttatggact
actggggtcaaggaacctcagtcaccgtctcctcagatctggagcccaaatcttgtgacaaaactcacacatgcccaccgtgccca
gcacctgaactcctgggggggaccgtcagtcttcctcttccccccaaaacccaaggacaccctcatgatctcccggacccctgaggt
cacatgcgtggtggtggacgtgagccacgaagaccctgaggtcaagttcaactggtacgtggacggcgtggaggtgcataatgc
caagacaaagccgcgcgggaggagcagtacaacagcacgtaccgtgtggtcagcgtcctcaccgtcctgcaccaggactggctga
atggcaaggagtacaagtgcaaggtctccaacaaagccctcccagccccatcgagaaaaccatctccaaagccaaagggcag
ccccgagaaccacaggtgtacaccctgcccccatcccgggatgagctgaccaagaaccaggtcagcctgacctgcctggtcaaa
ggcttctatcccagcgacatcgccgtggagtgggagagcaatgggcagccggagaacaactacaagaccacgcctcccgtgct
ggactccgacggctccttcttcctctacagcaagctcaccgtggacaagagcaggtggcagcaggggaacgtcttctcatgctcc
gtgatgcatgaggctctgcacaaccactacacgcagaagagcctctccctgtctccgggtaaagcggatccttcgaacctgctccc
atcctgggccattaccttaatctcagtaaatggaatttttgtgatatgctgcctgacctactgcttgccccaagatgcagagagagaa
ggaggaatgagagattgagaagggaaagtgtacgccctgtataaatcgat

2el2 scFv WTH CH2 CH3 (2el2 scFv-WthIgG-CD80) (secuencia de aminoácido) (ID DE SEC NO __)

MDFQVQIFSFLLISASVIMSRGVDIVLTQSPASLAVSLGQRATISCRASESVEYYVTS
LMQWYQQKPGQPPKLLISAASNVESGVPARFSGSGSGTDFSLIHPVEEDDIAMYF
CQQSRKVPWTFGGGTKLEIKRGGGGSGGGGSGGGGSQVQLKESGPGLVAPSQSLS
ITCTVSGFSLTGYGVNWVRQPPGKGLEWLGMIWGDGSTDYNSALKSRLSITKDNS
KSQVFLKMNSLQTDDTARYYCARDGYSNFHYYVMDYWGQGTSVTVSSDLEPKS
CDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVWDVSHEDPEVKFN
WYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALP
APIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQ

1505
1519

PENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSL
SLSPGKADPSNLLPSWAITLISVNGIFVICCLTYCFAPRCRERRRNERLRRESVRPV

IgE Fc 2H7-humano (CH2-CH3-CH4) (secuencia de nucleótido) (ID DE SEC NO __)

aagcttgccgccatggatttttcaagtgcagattttcagcttcctgctaatcagtgcttcagtcataattgccagaggacaaattgttctct
cccagtctccagcaatcctgtctgcatctccaggggagaaggtcacaatgacttgcagggccagctcaagtgtaagttacatgcact
ggtaccagcagaagccaggatcctcccccaaaccctggatttatgccccatccaacctggcttctggagtccctgctcgcttcagtg
gcagtgggtctgggacctcttactctctcacaatcagcagagtggaggctgaagatgctgccacttattactgccagcagtggagttt
taacccaccccacgttcggtgctgggaccaagctggagctgaaaggtggcggtggctcgggcggtggtgggatctggaggaggtg
ggagctctcaggcttatctacagcagtctggggctgagctggtgaggcctggggcctcagtgaagatgtcctgcaaggcttctggc
tacacatttaccagttacaatatgcactgggtaaagcagacacctagacagggcctggaatggattggagctatttatcccggaaat
ggtgatacttcctacaatcagaagttcaagggcaaggccacactgactgtagacaaatcctccagcacagcctacatgcagctcag
cagcctgacatctgaagactctgcggtctattactgtgcaagatggttgtactatagtaactcttactggtacttcgatgtctggggcac
agggaccacggtcaccgtctctgatcacgtctgctccagggacttcaccccgccccaccgtgaagatcttacagtcgtcctgcgacg
gcggcgggcacttccccccgaccatccagctcctgtgcctcgtctcgggtacaccccagggactatcaacatcacctggctgga
ggacgggcaggtcatggacgtggacttgtccaccgcctctaccacgcaggagggtgagctggcctccacacaaagcgagctca
cctcagccagaagcactggctgtcagaccgcacctacacctgccaggtcacctatcaaggtcacaccttgaggacagcaccaa
gaagtgtgcagattccaaccccgagaggggtgagcgctacctaagccggcccagcccgtcgacctgttcatccgcaagtcgcc
cacgatcacctgtctggtggtggacctggcacccagcaaggggaccgtgaacctgacctggtcccgggccagtgggaagcctgt
gaaccactccaccagaaaggaggagaagcagcgcaatggcacgttaaccgtcacgtccacccctgccggtgggcacccgagact
ggatcgaggggagaactacaagtgcagggtgacccaccccccacctgcccagggcctcatgcggtccacgaccaagaccag
cggcccgcgtgctgccccggaagtctatgcgttgcgacgccgggagtggccgggggagccgggacaagcgcacccctgcctgc
ctgatccagaacttcatgcctgaggacatctcggtgcagtggctgcacaacgaggtgcagctcccggacgcccggcacagcacg
acgcagccccgcaagaccaagggctccggcttcttcgtcttcagccgcctggaggtgaccagggccgaatgggagcagaaaga
tgagttcatctgccgtgcagtccatgaggcagcgagccccttacagaccgtccagcgagcggtgtctgtaaatcccggtaaatgat
aatctaga

2H7 scFv IgE (CH2-CH3-CH4) (secuencia de aminoácido) (ID DE SEC NO __)

1518

MDFQVQIFSFLLISASVIIARGQIVLSQSPAILSASPGEKVTMTCRASSSVSYMHWY
QQKPGSSPKPWIYAPSNLASGVPARFSGSGSGTSYSLTISRVEAEDAATYYCQQWS
FNPPTFGAGTKLELKGGGGSGGGGSGGGGSSQAYLQQSGAELVRPGASVKMSCK
ASGYTFTSYNMHWVKQTPRQGLEWIGAIYPGNGDTSYNQKFKGKATLTVDKSSS
TAYMQLSSLTSEDSAVYFCARVVYYSNSYWYFDVWGTGTTVTVSDHVCSRDFTP
PTVKILQSSCDGGGHFPPTIQLLCLVSGYTPGTINITWLEDGQVMDVDLSTASTTQE
GELASTQSELTLSQKHWLSDRTYTCQVTYQGHTFEDSTKKCADSNPRGVSAYLSR
PSPFDLFIRKSPTITCLVVDLAPSKGTVNLTWSRASGKPVNHSTRKEEKQRNGTLTV
TSTLPVGTRDWIEGETYQCRVTHPHLPRALMRSTTKTSGPRAAPEVYAFATPEWP
GSRDKRTLACLIQNFMPEDISVQWLHNEVQLPDARHSTTQPRKTKGSGFFVFSRLE
VTRAEWEQKDEFICRAVHEAASPSQTVQRAVSVNPGK

2H7 scFv MH (SSS) MCH2WTCH3 (secuencia de nucleótido) (ID DE SEC NO __)

aagcttgccgccatggatttttcaagtgcagattttcagcttcctgctaatcagtgcttcagtcataattgtccagaggacaaattgttctct
cccagtctccagcaatcctgtctgcatctccaggggagaaggtcacaatgacttgcagggccagctcaagtgtaagttacatgcact
ggtaccagcagaagccaggatcctcccccaaaccctggatttatgcccatccaacctggcttctggagtccctgctcgcttcagtg
gcagtgggtctgggacctcttactctctcacaatcagcagagtggaggctgaagatgctgccacttattactgtcaagcagtggagttt
tacccacccacgttcggtgctgggaccaagctggagctgaaagatggcggtggctcgggcggtggtggatctggaggaggtg
ggagctctcaggttaatctacagcagtctggggctgagctggtgaggcctggggcctcagtgaagatgtcctgcaaggcttctggc
tacacatttaccagttacaatatgcactgggtaaagcagacacctagacagggcctggaatggattggagctatttatccaggaaat
ggtgatacttcctacaatcagaagttcaagggcaaggccacactgactgtagacaaatcctccagcacagcctacatgcagctcag
cagcctgacatctgaagactctgcggtctatttctgtgcaagagtggtgtactatagtaactcttactggtacttcgatgtctggggcac
agggaccacggtcaccgtctcttcagtcaggagcccaaatcttctgacaaaactcacacatcccaccgtcccccagcacctgaac
tcctgggggggatcgtcagtcttcctcttccccccaaaacccaaggacaccctcatgatctcccggacccctgaggtcacatgcgtg
gtggtggacgtgagccacgaagaccctgaggtcaagttcaactggtacgtggacggcgtggaggtgcataatgccaagacaaag
ccgcgggaggagcagtacaacagcacgtaccgtgtggtcagcgtcctcaccgtcctgcaccaggactggctgaatggcaagga
gtacaagtgcaaggtctccaacaaagccctcccagccccatcgagaaaacaatctccaaagccaaagggcagccccgagaac
cacaggtgtacaccctgcccccatcccgggatgagctgaccaagaaccaggtcagcctgacctgcctggtcaaaggcttctatcc
cagcgacatcgccgtggagtgggagagcaatgggcagccggagaacaactacaagaccacgcctcccgtgctggactccgac
ggctccttcttcctctacagcaagctcaccgtggacaagagcaggtggcagcaggggaacgtcttctcatgctccgtgatgcatga
ggctctgcacaaccactacacgcagaagagcctctccctgtctccgggtaaatgatctaga

2H7 scFv MH (SSS) MCH2WTCH3 (secuencia de aminoácido) (ID DE SEC NO __)

1519

MDFQVQIFSFLLISASVIIARGQIVLSQSPAILSASPGEKVTMTCRASSSVSYMHWY
QQKPGSSPKPWIYAPSNLASGVPARFSGSGSGTSYSLTISRVEAEDAATYYCQQWS
FNPPTFGAGTKLELKDGGGSGGGGSGGGGSSQAYLQQSGAELVRPGASVKMSCK
ASGYTFTSYNMHWVKQTPRQGLEWIGAIYPGNGDTSYNQKFKGKATLTVDKSSS
TAYMQLSSLTSEDSAVYFCARVVYYSNSYWYFDVWGTGTTVTVSSDQEPKSSDK
THTSPPSPAPELLGGSSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYV
DGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKBYKCKVSNKALPAPIE
KTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPEN
NYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLS
PGK

5B9 scFv MTHWTCH2CH3 (secuencia de nucleótido) (ID DE SEC NO __)

aagcttgccgccatgaggttctctgctcagcttctggggctgcttgtgctctggatcctggatccactgcagatattgtgatgacgca
ggctgcattctccaatccagtcactcttggaacatcagcttccatctcctgcaggtctagtaagagtctcctacatagtaatggcatca
cttatttgtattggtatctgcagaagccaggccagtctcctcagctcctgatttatcagatgtccaacctgcctcaggagtcccagaca
ggttcagtagcagtgggtcaggaactgatttcacactgagaatcagcagagtggaggctgaggatgtgggtgtttattactgtgctc
aaaatctagaacttccgctcacgttcggtgctgggaccaagctggagctgaaacgggggtggcggtggctcgggcggtggtgggt
cgggtggcggcggatcgtcacaggtgcagctgaagcagtcaggacctggcctagtgcagtcctcacagagcctgtccatcacct
gcacagtctctgggtttctcattaactacctatgctgtacactgggttcgccagtctccaggaaagggtctggagtggctgggagtgat
atggagtggtggaatcacagactataatgcagctttcatatccagactgagcatcaccaaggacgattccaagagccaagtttcttt
aaaatgaacagtctgcaacctaatgacacagccatttattactgtgccagaaatgggggtgatactacccttattactatgctatgga
ctactggggtcaaggaacctcagtcaccgtctcctctgatcagggagcccaaatcttctgacaaaactcacacatcccccaccgtcccc
agcacctgaactcctgggggggaccgtcagtcttcctcttccccccaaaacccaaggacaccctcatgatctcccggacccctgag
gtcacatgcgtggtggtggacgtgagccacgaagaccctgaggtcaagttcaactggtacgtggacggcgtggaggtgcataat
gccaagacaaagccgcgggaggagcagtacaacagcacgtaccgtgtggtcagcgtcctcaccgtcctgcaccaggactggct
gaatggcaaggagtacaagtgcaaggtctccaacaaagccctcccagccccccatcgagaaaacaatctccaaagccaaagggc
agccccgagaaccacaggtgtacaccctgcccccatcccgggatgagctgaccaagaaccaggtcagcctgacctgcctggtca
aaggcttctatcccagcgacatcgccgtggagtgggagagcaatgggcagccggagaacaactacaagaccacgcctcccgtg
ctggactccgacggctccttcttcctctacagcaagctcaccgtggacaagagcaggtggcagcaggggaacgtcttctcatgctc
cgtgatgcatgaggctctgcacaaccactacacgcagaagagcctctccctgtctccgggtaaatgatctaga

5B9 scFv MTHWTCH2CH3 (secuencia de aminoácido) (ID DE SEC NO __)

1520

MRFSAQLLGLLVLWIPGSTADIVMTQAAFSNPVTLGTSASISCRSSKSLLHSNGITY
LYWYLQKPGQSPQLLIYQMSNLASGVPDRFSSSGSGTDFTLRISRVEAEDVGVYYC
AQNLELPLTFGAGTKLELKRGGGGSGGGGSGGGGSSQVQLKQSGPGLVQSSQSLS
ITCTVSGFSLTTYAVHWVRQSPGKGLEWLGVIWSGGITDYNAAFISRLSITKDDSK
SQVFFKMNSLQPNDTAIYYCARNGGDNYPYYYAMDYWGQGTSVTVSSDQEPKSS
DKTHTSPPSPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNW
YVDGVEVHNAKTKPREEQYNSTYRWSVLTVLHQDWLNGKEYKCKVSNKALPA
PIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQP
ENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLS
LSPGK

Mutaciones de articulación IgG1 humana

<u>2H7 scFv-MTH (CSS) WTCH2CH3</u> (secuencia de nucleótido) (ID DE SEC NO __)

aagcttgccgccatggatttcaagtgcagattttcagcttcctgctaatcagtgcttcagtcataattgccagaggacaaattgttctct
cccagtctccagcaatcctgtctgcatctccaggggagaaggtcacaatgacttgcagggccagctcaagtgtaagttacatgcact
ggtaccagcagaagccaggatcctccccaaaccctggatttatgccccatccaacctggcttctggagtccctgctcgcttcagtg
gcagtgggtctgggacctcttactctctcacaatcagcagagtggaggctgaagatgctgccacttattactgccagcagtggagttt
taacccacccacgttcggtgctgggaccaagctggagctgaaagatggcggtggctcgggcggtggtggatctggaggaggtg
ggagctctcaggcttatctacagcagtctggggctgagctggtgaggcctggggcctcagtgaagatgtcctgcaaggcttctggc
tacacatttaccagttacaatatgcactgggtaaagcagacacctagacagggcctggaatggattggagctatttatccaggaaat
ggtgatacttcctacaatcagaagttcaagggcaaggccacactgactgtagacaaatcctccagcacagcctacatgcagctcag
cagcctgacatctgaagactctgcggtctatttctgtgcaagagtggtgtactatagtaactcttactggtacttcgatgtctggggcac
agggaccacggtcaccgtctcttctgatcaggagcccaaatcttgtgacaaaactcacacatcccccaccgtccccagcacctgaac
tcctgggggggaccgtcagtcttcctcttcccccaaaacccaaggacaccctcatgatctcccggacccctgaggtcacatgcgtg
gtggtggacgtgagccacgaagaccctgaggtcaagttcaactggtacgtggacggcgtggaggtgcataatgccaagacaaag
ccgcgggaggagcagtacaacagcacgtaccgtgtggtcagcgtcctcaccgtcctgcaccaggactggctgaatggcaagga
gtacaagtgcaaggtctccaacaaagccctcccagcccccatcgagaaaacaatctccaaagccaaagggcagccccgagaac
cacaggtgtacaccctgcccccatcccgggatgagctgaccaagaaccaggtcagcctgacctgcctggtcaaaggcttctatcc
cagcgacatcgccgtggagtgggagagcaatgggcagccggagaacaactacaagaccacgcctcccgtgctggactccgac
ggctccttcttcctctacagcaagctcaccgtggacaagagcaggtggcagcaggggaacgtcttctcatgctccgtgatgcatga
ggctctgcacaaccactacacgcagaagagcctctccctgtctccgggtaaatgatctaga

<u>2H7 scFv-MTH (CSS) WTCH2CH3</u> (secuencia de aminoácido) (ID DE SEC NO __)

1524

MDFQVQIFSFLLISASVIIARGQIVLSQSPAILSASPGEKVTMTCRASSSVSYMHWY
QQKPGSSPKPWIYAPSNLASGVPARFSGSGSGTSYSLTISRVEAEDAATYYCQQWS
FNPPTFGAGTKLELKDGGGSGGGGSGGGGSSQAYLQQSGAELVRPGASVKMSCK
ASGYTFTSYNMHWVKQTPRQGLEWIGAIYPGNGDTSYNQKFKGKATLTVDKSSS
TAY'1QLSSLTSEDSAVYFCARWYYSNSYWYFDVWGTGTTVTVSSDQEPKSCDK
THTSPPSPAPELLGGPSVFLFPPKPEDTLMISRTPEVTCVVVDVSHEDPEVKFNWYV
DGVBVHNAKTKPREEQYNSTYRWSVLTVLHQDWLNGKEYKCKVSNKALPAPIE
KTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPEN
NYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLS
PGK

2H7 scFv-MTH (SCS) WTCH2CH3 (secuencia de nucleótido) (ID DE SEC NO __)

aagcttgccgccatggattttcaagtgcagattttcagcttcctgctaatcagtgcttcagtcataattgccagaggacaaaattgttctct
cocagtctccagcaatcctgtctgcatctccaggggagaaggtcacaatgacttgcagggccagctcaagtgtaagttacatgcact
ggtaccagcagaagccaggatcctcccccaaaccctggatttatgccccatccaacctggcttctggagtccctgctcgcttcagtg
gcagtgggtctgggacctcttactctctcacaatcagcagagtggaggctgaagatgctgccacttattactgccagcagtggagttt
taacccacccacgttcggtgctgggaccaagctggagctgaaagatggcggtggctcgggcggtggtggatctggaggaggtg
ggagctctcaggcttatctacagcagtctggggctgagctggtgaggcctggggcctcagtgaagatgtcctgcaaggcttctggc
tacacatttaccagttacaatatgcactgggtaaagcagacacctagacagggcctggaatggattggagctatttatccaggaaat
ggtgatacttcctacaatcagaagttcaagggcaaggccacactgactgtagacaaatcctccagcacagcctacatgcagctcag
cagcctgacatctgaagactctgcggtctatttctgtgcaagagtggtgtactatagtaactcttactggtacttcgatgtctggggcac
agggaccacggtcaccgtctcttctgatcaggagcccaaatcttctgacaaaactcacacatgcccaccgtccccagcacctgaac
tcctgggggggaccgtcagtcttcctcttcccccaaaacccaaggacaccctcatgatctcccggacccctgaggtcacatgcgtg
gtggtggacgtgagccacgaagaccctgaggtcaagttcaactggtacgtggacggcgtggaggtgcataatgccaagacaaag
ccgcgggaggagcagtacaacagcacgtaccgtgtggtcagcgtcctcaccgtcctgcaccaggactggctgaatggcaagga
gtacaagtgcaaggtctccaacaaagccctcccagcccccatcgagaaaacaatctccaaagccaaagggcagccccgagaac
cacaggtgtacaccctgcccccatcccgggatgagctgaccaagaaccaggtcagcctgacctgcctggtcaaaggcttctatcc
cagcgacatcgccgtggagtgggagagcaatgggcagccggagaacaactacaagaccacgcctcccgtgctggactccgac
ggctccttcttcctctacagcaagctcaccgtggacaagagcaggtggcagcaggggaacgtcttctcatgctccgtgatgcatga
ggctctgcacaaccactacacgcagaagagcctctccctgtctccgggtaaatgatctaga

2H7 sc-MTH (SCS) WTCH2CH3 (secuencia de aminoácido) (SEQ ID NO __)

MDFQVQIFSFLLISASVIIARGQIVLSQSPAILSASPGEKVTMTCRASSSVSYMHWY
QQKPGSSPKPWIYAPSNLASGVPARFSGSGSGTSYSLTISRVEAEDAATYYCQQWS

FNPPTFGAGTKLELKDGGGSGGGGSGGGGSSQAYLQQSGAELVRPGASVKMSCK
ASGYTFTSYMdHWVKQTPRQGLEWIGAIYPGNGDTSYNQKFKGKATLTVDKSSS
TAYMQLSSLTSEDSAVYFCARVVYYSNSYWYFDVWGTGTTVTVSSDQEPKSSDK
THTCPPSPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWY
VDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPI
EKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPE
INYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSL
SPGK

2H7 scFv-MTH (SSC) WTCH2CH3 (nueteotide sequence) (SEQ ID NO)

aagcttgccgccatggattttcaagtgcagattttcagcttcctgctaatcagtgcttcagtcataattgccagaggacaaattgttctct
cccagtctccagcaatcctgtctgcatctccaggggagaaggtcacaatgacttgcagggccagctcaagtgtaagttacatgcact
ggtaccagcagaagccaggatcctccccccaaaccctggatttatgccccatccaacctggcttctggagtccctgctcgcttcagtg
gcagtgggtctgggacctcttactctctcacaatcagcagagtggaggctgaagatgctgccacttattactgccagcagtggagttt
taacccacccacgttcggtgctgggaccaagctggagctgaaagatggcggtggctcgggcggtggtggatctggaggaggtg
ggagctctcaggcttatctacagcagtctggggctgagctggtgaggcctggggcctcagtgaagatgtcctgcaaggcttctggc
tacacatttaccagttacaatatgcactgggtaaagcagacacctagacagggcctggaatggattggagctatttatccaggaaat
ggtgatacttcctacaatcagaagttcaagggcaaggccacactgactgtagacaaaatcctccagcacagcctacatgcagctcag
cagcctgacatctgaagactctgcggtctatttctgtgcaagagtggtgtactatagtaactcttactggtacttcgatgtctggggcac
agggaccacggtcaccgtctcttctgatcaggagcccaaatcttctgacaaaactcacacatccccaccgtgcccagcacctgaac
tcctgggggggaccgtcagtcttcctcttcccccaaaacccaaggacaccctcatgatctcccggacccctgaggtcacatgcgtg
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ccgcgggaggagcagtacaacagcacgtaccgtgtggtcagcgtcctcaccgtcctgcaccaggactggctgaatggcaagga
gtacaagtgcaaggtctccaacaaagccctcccagcccccatcgagaaaacaatctccaaagccaaagggcagccccgagaac
cacaggtgtacaccctgcccccatcccgggatgagctgaccaagaaccaggtcagcctgacctgcctggtcaaaggcttctatcc
cagcgacatcgccgtggagtgggagagcaatgggcagccggagaacaactacaagaccacgcctcccgtgctggactccgac
ggctccttcttcctctacagcaagctcaccgtggacaagagcaggtggcagcaggggaacgtcttctcatgctccgtgatgcatga
ggctctgcacaaccactacacgcagaagagcctctccctgtctccgggtaaatgatctaga

<u>2H7 scFv-MTH (SSC) WTCH2CH3</u> (secuencia de aminoácido) (ID DE SEC NO __)

MDFQVQIFSFLLISASVIIARGQIVLSQSPAILSASPGEKVTMTCRASSSVSYMHWY
QQKPGSSPKPWIYAPSNLASGVPARFSGSGSGTSYSLTISRVEAEDAATYYCQQWS
FNPPTFGAGTKLELKDGGGSGGGGSGGGGSSQAYLQQSGAELVRPGASVKMSCK
ASGYTFTSYNMHWVKQTPRQGLEWIGAIYPGNGDTSYNQKFKGKATLTVDKSSS
TAYMQLSSLTSEDSAVYFCARVVYYSNSYWYFDVWGTGTTVTVSSDQEPKSSDK
THTSPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWY
VDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPI
EKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPE
NNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSL
SPGK

HIgGMHcys1 (secuencia de nucleótido) (ID DE SEC NO __)

gtt gtt gat cag gag ccc aaa tct tct gac aaa act cac aca tg

HIgGMHcys2 (secuencia de nucleótido) (ID DE SEC NO __)

gtt gtt gat cag gag ccc aaa tct tgt gac aaa act cac aca tct cca ccg tgc

HIgGMHcys3 (secuencia de nucleótido) (ID DE SEC NO __)

gtt gtt gat cag gag ccc aaa tct tgt gac aaa act cac aca tgt cca ccg tec cca gca cct

HuIgG1 MTCH3Y405 (secuencia de nucleótido) (ID DE SEC NO __)

gggcagccccgagaaccacaggtgtacaccctgcccccatcccgggaggagatgaccaagaaccaggtcagcctgacctgcct
ggtcaaaggcttctatcccagcgacatcgccgtggagtgggagagcaatgggcagccggagaacaactacaagaccacgcctc
ccgtgctggactccgacggctccttctacctctatagcaagctcaccgtggacaagagcaggtggcagcaggggaacgtcttctc
atgctccgtgatgcatgaggctctgcacaaccactacacgcagaagagcctctccctgtccccgggtaaatga

HuIgG1 MTCH3Y405 (secuencia de aminoácido) (ID DE SEC NO __)

GQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPP
VLDSDGSFYLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

HuIgG1 MTCH3A405 (secuencia de nucleótido) (ID DE SEC NO __)

1512
1529

gggcagccccgagaaccacaggtgtacaccctgccccatcccgggaggagatgaccaagaaccaggtcagcctgacctgcct
ggtcaaaggcttctatcccagcgacatcgccgtggagtgggagagcaatgggcagccggagaacaactacaagaccacgcctc
ccgtgctggactccgacggctccttcgccctctatagcaagctcaccgtggacaagagcaggtggcagcaggggaacgtcttctc
atgctccgtgatgcatgaggctctgcacaaccactacacgcagaagagcctctccctgtccccgggtaaatga

HuIgGl MTCH3A405 (secuencia de aminoácido) (ID DE SEC NO __)

GQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPP
VLDSDGSFALYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

HuIgGl MTCH3A407 (secuencia de nucleótido) (ID DE SEC NO __)

gggcagccccgagaaccacaggtgtacaccctgccccatcccgggaggagatgaccaagaaccaggtcagcctgacctgcc
tggtcaaaggcttctatcccagcgacatcgccgtggagtgggagagcaatgggcagccggagaacaactacaagaccacgcct
cccgtgctggactccgacggctccttcttcctcgccagcaagctcaccgtggacaagagcaggtggcagcaggggaacgtcttct
catgctccgtgatgcatgaggctctgcacaaccactacacgcagaagagcctctccctgtccccgggtaaatga

HuIgG1 MTCH3A407 (secuencia de aminoácido) (ID DE SEC NO __)

GQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPP
VLDSDGSFFLASKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

HuIgGl MTCH3Y405A407 (secuencia de nucleótido) (ID DE SEC NO __)

gggcagccccgagaaccacaggtgtacaccctgccccatcccgggaggagatgaccaagaaccaggtcagcctgacctgcct
ggtcaaaggcttctatcccagcgacatcgccgtggagtgggagagcaatgggcagccggagaacaactacaagaccacgcctc
ccgtgctggactccgacggctccttctacctcgccagcaagctcaccgtggacaagagcaggtggcagcaggggaacgtcttctc
atgctccgtgatgcatgaggctctgcacaaccactacacgcagaagagcctctccctgtccccgggtaaatga

HuIgGl MTCH3Y405A407 (secuencia de aminoácido) (ID DE SEC NO __)

GQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPP
VLDSDGSFYLASKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

HuIG1 MTCH3A405A407 (secuencia de nucleótido) (ID DE SEC NO __)

1515
1525

gggcagccccgagaaccacaggtgtacaccctgccccatcccgggaggagatgaccaagaaccaggtcagcctgacctgcct
ggtcaaaggcttctatcccagcgacatcgccgtggagtgggagagcaatgggcagccggagaacaactacaagaccacgcctc
ccgtgctggactccgacggctccttcgccctcgccagcaagctcaccgtggacaagagcaggtggcagcaggggaacgtcttct
catgctccgtgatgcatgaggctctgcacaaccactacacgcagaagagcctctccctgtccccgggtaaatga

HuIgG1 MTCH3A405A407 (secuencia de aminoácido) (ID DE SEC NO __)

GQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPP
VLDSDGSFALASKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

2H7 scFv MTH (SSS) WTCH2MTCH3Y405 (secuencia de nucleótido) (ID DE SEC NO __)

aagcttgccgccatggattttcaagtgcagattttcagcttcctgctaatcagtgcttcagtcataattgccagaggacaaattgttctct
cccagtctccagcaatcctgtctgcatctccaggggagaaggtcacaatgacttgcagggccagctcaagtgtaagttacatgcact
ggtaccagcagaagccaggatcctccccaaaccctggatttatgccccatccaacctggcttctggagtccctgctcgcttcagtg
gcagtgggtctgggacctcttactctctcacaatcagcagagtggaggctgaagatgctgccacttattactgccagcagtggagttt
taacccacccacgttcggtgctgggaccaagctggagctgaaagatggcggtggctcgggcggtggtggatctggaggaggtg
ggagctctcaggcttatctacagcagtctggggctgagctggtgaggcctggggcctcagtgaagatgtcctgcaaggcttctggc
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cagcctgacatctgaagactctgcggtctatttctgtgcaagagtggtgtactatagtaactcttactggtacttcgatgtctggggcac
agggaccacggtcaccgtctcttctgatcaggagcccaaatcttctgacaaaactcacacatcccccaccgtccccagcacctgaac
tcctgggggggaccgtcagtcttcctcttcccccaaaacccaaggacaccctcatgatctcccggaccccctgaggtcacatgcgtg
gtggtggacgtgagccacgaagaccctgaggtcaagttcaactggtacgtggacggcgtggaggtgcataatgccaagacaaag
ccgcgggaggagcagtacaacagcacgtaccgtgtggtcagcgtcctcaccgtcctgcaccaggactggctgaatggcaagga
gtacaagtgcaaggtctccaacaaagccctcccagcccccatcgagaaaacaatctccaaagccaaagggcagccccgagaac
cacaggtgtacaccctgcccccatcccgggaggagatgaccaagaaccaggtcagcctgacctgcctggtcaaaggcttctatcc
cagcgacatcgccgtggagtgggagagcaatgggcagccggagaacaactacaagaccacgcctcccgtgctggactccgac
ggctccttctacctctatagcaagctcaccgtggacaagagcaggtggcagcaggggaacgtcttctcatgctccgtgatgcatga
ggctctgcacaaccactacacgcagaagagcctctccctgtccccgggtaaatgatctaga

2H7 scFv MTH (SSS) WTCH2MTCH3Y405 (secuencia de aminoácido) (ID DE SEC NO __)

1514
1526

MDFQVQIFSFLLISASVIIARGQIVLSQSPAILSASPGEKVTMTCRASSSVSYMHWY
QQKPGSSPKPWIYAPSNLASGVPARFSGSGSGTSYSLTISRVEAEDAATYYCQQWS
FNPPTFGAGTKLELKDGGGSGGGGSGGGGSSQAYLQQSGAELVRPGASVKMSCK
ASGYTFTSYNMHWVKQTPRQGLEWIGAIYPGNGDTSYNQKFKGKATLTVDKSSS
TAYMQLSSLTSEDSAVYFCARVVYYSNSYWYFDVWGTGTTVTVSSDQEPKSSDK
THTSPPSPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYV
DGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIE
KTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPEN
NYKTTPPVLDSDGSFYLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLS
PGK

2H7 scFv MTH (SSS) WTCH2MTCH3A405 (secuencia de nucleótido) (ID DE SEC NO __)

aagcttgccgccatggattttcaagtgcagattttcagcttcctgctaatcagtgcttcagtcataattgccagaggacaaattgttctct
cccagtctccagcaatcctgtctgcatctccaggggagaaggtcacaatgacttgcagggccagctcaagtgtaagttacatgcact
ggtaccagcagaagccaggatcctccccaaaccctggatttatgccccatccaacctggcttctggagtccctgctcgcttcagtg
gcagtgggtctgggacctcttactctctcacaatcagcagagtggaggctgaagatgctgccacttattactgccagcagtggagttt
taacccacccacgttcggtgctgggaccaagctggagctgaaagatggcggtggctcgggcggtggtggatctggaggaggtg
ggagctctcaggcttatctacagcagtctggggctgagctggtgaggcctggggcctcagtgaagatgtcctgcaaggcttctggc
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cagcctgacatctgaagactctgcggtctatttctgtgcaagagtggtgtactatagtaactcttactggtacttcgatgtctggggcac
agggaccacggtcaccgtctcttctgatcaggagcccaaatcttctgacaaaactcacacatccccaccgtccccagcacctgaac
tcctgggggggaccgtcagtcttcctcttcccccaaaacccaaggacaccctcatgatctcccggaccctgaggtcacatgcgtg
gtggtggacgtgagccacgaagaccctgaggtcaagttcaactggtacgtggacggcgtggaggtgcataatgccaagacaaag
ccgcgggaggagcagtacaacagcacgtaccgtgtggtcagcgtcctcaccgtcctgcaccaggactggctgaatggcaagga
gtacaagtgcaaggtctccaacaaagccctcccagccccatcgagaaaacaatctccaaagccaaagggcagccccgagaac
cacaggtgtacaccctgcccccatcccgggaggagatgaccaagaaccaggtcagcctgacctgcctggtcaaaggcttctatcc
cagcgacatcgccgtggagtgggagagcaatgggcagccggagaacaactacaagaccacgcctcccgtgctggactccgac
ggctccttcgccctctatagcaagctcaccgtggacaagagcaggtggcagcaggggaacgtcttctcatgctccgtgatgcatga
ggctctgcacaaccactacacgcagaagagcctctccctgtccccgggtaaatga

2H7 scFv MTH (SSS) WTCH2MTCH3A405 (secuencia de nucleótido) (ID DE SEC NO __)

1515
1527

mdfqvqifsfllisasvmisrgqivlsqspailsaspgekvtmtcrasssvsymhwyqqkpgsspkpwiyatsnlasgvparf
sgsgsgtsysltisrveaedaatyycqqwsfnpptfgagtkleikdgggsggggsggggsqsqylqqsgaelvrpgasvkmsc
kasgytftsymmhwvkqtprqglewigaiypgngdtsynqkfkgkatltvdkssstaymqlsaltsedsavyfcarvvyysn
sywyfdvwgtgttvtvsadqepksadkthtsppspapellggpsvflfppkpkdtlmisrtpevtcvvvdvshedpevkfnw
yvdgvevhnaktkpreeqynstyrvvsvltvlhqdwlngkeykckvsnkalpapiektiskakgqprepqvytlppsreemt
knqvsltclvkgfypsdiavewesngqpennyktppvldsdgsfflyskltvdksrwqqgnvfscsvmhealhnhytqksl
slspgk

2H7 scFv MTH (SSS) WTCH2MTCH3A407 (secuencia de nucleótido) (ID DE SEC NO __)

aagcttgccgccatggattttcaagtgcagattttcagcttcctgctaatcagtgcttcagtcataattgccagaggacaaattgttctct
cccagtctccagcaatcctgtctgcatctccaggggagaaggtcacaatgacttgcagggccagctcaagtgtaagttacatgcact
ggtaccagcagaagccaggatcctcccccaaaccctggatttatgccccatccaacctggcttctggagtccctgctcgcttcagtg
gcagtgggtctgggacctcttactctctcacaatcagcagagtggaggctgaagatgctgccacttattactgccagcagtggagttt
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2H7 scFv MTH (SSS) WTCH2MTCH3A407 (secuencia de aminoácido) (SEQ ID NO)

1516
1528

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ASGYTFTSYNMHWVKQTPRQGLEWIGAIYPGNGDTSYNQKFKGKATLTVDKSSS
TAYMQLSSLTSEDSAVYFCARVVYYSNSYWYFDVWGTGTTVTVSSDQEPKSSDK
THTSPPSPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYV
DGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNRALPAPIE
KTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPEN
NYKTTPPVLDSDGSFFLASKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLS
PGK

2H7 scFv MTH (SSS) WTCH2MTCH3Y405A407 (secuencia de nucleótido) (ID DE SEC NO __)

aagcttgccgccatggatttttcaagtgcagatttttcagcttcctgctaatcagtgcttcagtcataattgccagaggacaaattgttctct
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2H7 scFv MTH (SSS) WTCH2MTCH3Y405A407 (secuencia de aminoácido) (ID DE SEC NO __)

1512/1529

MDFQVQIFSFLLISASVIIARGQIVLSQSPAILSASPGEKVTMTCRASSSVSYMHWY
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TAYMQLSSLTSEDSAVYFCARVVYYSNSYWYFDVWGTGTTVTVSSDQEPKSSDK
THTSPPSPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYV
DGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIE
KTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPEN
NYKTTPPVLDSDGSFYLASKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLS
PGK

2H7 scFv MTH (SSS) WTCH2MTCH3A405A407 (secuencia de nucleótido) (ID DE SEC NO __)

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2H7 scFv MTH (SSS) WTCH2MTCH3A405A407 (secuencia de aminoácido) (ID DE SEC NO __)

1578
1530

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FNPPTFGAGTKLELKDGGGSGGGGSGGGGSSQAYLQQSGAELVRPGASVKMSCK
ASGYTFTSYNMHWVKQTPRQGLEWIGAIYPGNGDTSYNQKFKGKATLTVDKSSS
TAYMQLSSLTSEDSAVYFCARVVYYSNSYWYFDVWGTGTTVTVSSDQEPKSSDK
THTSPPSPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYV
DGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIE
KTISKAKGQPREPQVYTLPPSRREMTKNQVSLTCLVKGFYPSDIAVEWESNGQPEN
NYKTTPPVLDSDGSFALASKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLS
PGK

2H7 scFv MTH (SCC) WTCH2CH3 (secuencia de nucleótido)

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2H7 scFv-MTH (SCC) WTCH2CH3 (secuencia de aminoácido)

~~1517~~ 1531

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F'NPPTFGAGTKLELKDGGGSGGGGSGGGGSSQAYLQQSGAELVRPGASVKMSCK
ASGYTFTSYNMHWVKQTPRQGLEWIGAIYPGNGDTSYNQKFKGKATLTVDKSSS
TAYMQLSSLTSEDSAVYFCARVVYYSNSYWYFDVWGTGTTVTVSSDQEPKSSDK
THTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWY
VDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPI
EKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPE
NNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSL
SPGK

2H7 scFv MTH (CSC) WTCH2CH3 (secuencia de nucleótido)

aagcttgccgccatggattttcaagtgcagattttcagcttcctgctaatcagtgcttcagtcataattgccagaggacaaattgttctct
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2H7 scFv MTH (CSC) WTCH2CH3 (amino acM sequence)

1500
1532

MDFQVQIFSFLLISASVIIARGQIVLSQSPAILSASPGEKVTMTCRASSSVSYMHWY
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FNPPTFGAGTKLELKDGGGSGGGGSGGGGSSQAYLQQSGAELVRPGASVKMSCK
ASGYTFTSYNMHWVKQTPRQGLEWIGAIYPGNGDTSYNQKFKGKATLTVDKSSS
TAYMQLSSLTSEDSAVYFCARVVYYSNSYWYFDVWGTGTTVTVSSDQEPKSCDK
THTSPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWY
VDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPI
EKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPE
NNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSL
SPGK

2H7 scFv MTH (CCS) WTCH2CH3 (secuencia de nucleótido)

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2H7 scFv MTH (CCS) WTCH2CH3 (secuencia de aminoácido)

MDFQVQIFSFLLISASVIIARGQIVLSQSPAILSASPGEKVTMTCRASSSVSYMHWY
QQKPGSSPKPWIYAPSNLASGVPARFSGSGSGTSYSLTISRVEAEDAATYYCQQWS

1533

FNPPTFGAGTKLELKDGGGSGGGGSGGGGSSQAYLQQSGAELVRPGASVKMSCK
ASGYTFTSYNMHWVKQTPRQGLEWIGAIYPGNGDTSYNQKFKGKATLTVDKSSS
TAYMQLSSLTSEDSAVYFCARVVYYSNSYWYFDVWGTGTTVTVSSDQEPKSCDK
THTCPPSPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWY
VDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPI
EKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPE
NNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSL
SPGK

HuIgAHIgA-T4-ORF (secuencia de nucleótido)

tgatcagccagttccctcaactccacctaccccatctccctcaactccacctaccccatctccctcatgctgccacccccgactgtca
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ac

HuIg A-T4-0B (secuencia de aminoácido)

DQPVPSTPPTPSPSTPPTPSPSCCHPRLSLHRPALEDLLLGSEAILTCTLTGLRDASGV
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ELPREKYLTWASRQEPSQGTTTFAVTSILRVAAEDWKKGDTFSCMVGHEALPLAF
TQKTIDRLAGKPTHVNVSVVMAEVDADPSN

1D8-IgAH IgA-T4-CD80 (secuencia de nucleótido)

aagcttatggattttcaagtgcagattttcagcttcctgctaatcagtgcttcagtcataatgtccagaggagtcgacattgtgctcactc
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1522
1534

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AA

1D8 scFv IgAH IgA-T4-CD80 (secuencia de aminoácido)

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IgE Fc (CH2-CH3-CH4) ORF humano (secuencia de nucleótido)

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1523
1535

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AA

IgE Fc (CH2-CH3-CH4) ORF humano (secuencia de aminoácido)

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VYAFATPEWPGSRDKRTLACLIQNFMPEDISVQWLHNEVQLPDARHSTTQPRKTK
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IgE Fc ID8 scFv-humano (CH2-CH3-CH4)-CD80 (secuencia de nucleótido)

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1524
1536

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IgE Fc ID8-scFv-humano (CH2-CH3-CH4)-CD80 (secuencia de aminoácido)

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5B9-IvAH IgA-T4-CD80 (secuencia de nucleótido)

~~1525~~
1537

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5B9-Ig A-T4-CD80 (secuencia de aminoácido)

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1538

IgE Fc 5B9-scFv-humano (CH2-CH3-CH4)-CD80 (secuencia de nucleótido)

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IgE Fc X 5B9-scFv-humano (CH2-CH3-CH4)-CD80 (secuencia de aminoácido)

1527
1539

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2el2-scFv-IgAH IgA-T4-CD80 (secuencia de nucleótido)

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1528
1540

2el2-scFv-IgAH IvA-T4-CD80 (secuencia de aminoácido)

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<u>IgE Fc 2el2-scFv-humano</u> (CH2-CH3-CH4)-CD80 (secuencia de nucleótido)

1529
1541

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actgcttgccccaagatgcagagagagaggaggaatgagagattgagaggggaaagtgtacgccctgtataaatcgata

IgE Fc 2el2-scFv-humano IgE Fc (CH2-CH3-CH4)-CD80 (secuencia de aminoácido)

1530
1592

MDFQVQIFSFLLISASVIMSRGVDIVLTQSPASLAVSLGQRATISCRASESVEYYVTS
LMQWYQQKPGQPPKLLISAASNVESGVPARFSGSGSGTDFSLNIHPVEEDDIAMYF
CQQSRKVPWTFGGGTKLEIKRGGGGSGGGGSGGGGSQVQLKESGPGLVAPSQSLS
ITCTVSGFSLTGYGVNWVRQPPGKGLEWLGMIWGDGSTDYNSALKSRLSITKDNS
KSQVFLKMNSLQTDDTARYYCARDGYSNFHYYVMDYWGQGTSVTVSSDHVCSR
DFTPPTVKILQSSCDGGGHFPPTIQLLCLVSGYTPGTINITWLEDGQVMDVDLSTAS
TTQEGELASTQSELTLSQKHWLSDRTYTCQVTYQGHTFEDSTKKCADSNPRGVSA
YLSRPSPFDLFIRKSPTITCLVVDLAPSKGTVNLTWSRASGKPVNHSTRKEEKQRNG
TLTVTSTLPVGTRDWIEGETYQCRVTHPHLPRALMRSTTKTSGPRAAPEVYAFATP
EWPGSRDKRTLACLIQNFMPEDISVQWLHNEVQLPDARHSTTQPRKTKGSGFFVFS
RLEVTRAEWEQKDEFICRAVHEAASPSQTVQRAVSVNPGKADPSKLPSWAITLISV
NGIFVICCLTYCFAPRCRERRRNERLRRESVRPV

500A2 scFv (secuencia de nucleótido)

atgttgtatacatctcagctccttgggcttttactcttctggattcagcctccagaagtgacatagtgctgactcagactccagccact
gtctcttctattcctggagaaagagtcacaatgacctgtaaggccagtcagaatattggcacaaatcttacactggtatcaccaaaaacc
aaaggaggctccaagggctctcatcaagtatgcttccgagtccatctcctggaatcccctccagattcagtggcagtgggtctgggaca
agatttcactctcagcatcaataacctggagcctgaagatattgcagaatttattactgtcaacaaagtaggaagctggccttgtcacgttcg
gtcctggcaccaagctggagataaaacgggggtggcggttggctcgggcggaggtgggtcgggtggcggcggatctcaggtcaa
gttgcagcagtctggttctgaactaggggaaacctggggcctcagtgaaactgtcctgcaagacttcaggctacatattcacagatc
actatatttcttgggtgaaacagaagcctggagaaggcttgcagtggataggaaatgtttatggtggaaatggtggtacaagctaca
atcaaaaatttcagggcaaggccacactgactgtagataaatcttctagcacagcctacatggaactcagcagcctgacatctgag
gattctgcgatctattactgtgcaagaggccggtagcgacgggccatgctatggactactggggtcaggggatccaagttaccgt
ctcctctgatc

500A2 scFv (secuencia de aminoácido)

MLYTSQLLGLLLFWISASRSDIVLTQTPATLSLIPGERVTMTCKTSQNIGTILHWYH
QKPKEAPRALIKYASQSIPGIPSRFSGSGSETDFTLSINNLEPDDIGIYYCQQSRSWPV
TFGPGTKLEIKRGGGGSGGGGSGGGGSQVKLQQSGSELGKPGASVKLSCKTSGYIF
TDHYISWVKQKPGESLQWIGNVYGGNGGTSYNQKFQGKATLTVDKISSTAYMEL
SSLTSEDSAIYYCARRPVATGHAMDYWGQGIQVTVSSD

NT

4531
1543

oligo 5'

Nombre IgGWT3

GTTGTTTTCGAAGGATCCGCTTTACCCGGAGACAGGGAGAGGCTCTT

NT

oligo 3'

Nombre hIgGWT5

GTTGTTAGATCTGGAGCCCAAATCTTGTGACAAAACTCACACATG

NT

Oligo 3'

Nombre FADD5

Secuencia

GTTGTGGATCCTTCGAACCCGTTCCTGGTGCTGCTGCACTCGGTGTCG

NT

Oligo 3'

Nombre FADD3

Secuencia

GTTGTTATCGATCTCGAGTTATCAGGACGCTTCGGAGGTAGATGCGTC

NT

FADD CSSCFV (aucleotide sequence)

1532
1599

gtggatccttcgaacccgttcctggtgctgctgcactcggtgtcgtccagcctgtcgagcagcgagctgaccgagctcaagttccta
tgcctcgggcgcgtgggcaagcgcaagctggagcgcgtgcagagcggcctagacctcttctccatgctgctggagcagaacga
cctggagcccgggcacaccgagctcctgcgcgagctgctcgcctccctgcggcgccacgacctgctgcggcgcgtcgacgact
tcgaggcgggggcggcggccggggccgcgcctggggaagaagacctgtgtgcagcatttaacgtcatatgtgataatgtgggg
aaagattggagaaggctggctcgtcagctcaaagtctcagacaccaagatcgacagcatcgaggacagataccccgcaacctg
acagagcgtgtgcgggagtcactgagaatctggaagaacacagagaaggagaacgcaacagtggcccacctggtgggggctc
tcaggtcctgccagatgaacctggtggctgacctggtacaagaggttcagcaggcccgtgacctccagaacaggagtggggcca
tgtccccgatgtcatggaactcagacgcatctacctccgaagcgtcctgataactcgagatcgataacaac

FADD-CSSCFV (secuencia de aminoácido)

VDPSNPFLVLLHSVSSSLSSSELTELKFLCLGRVGKRKLERVQSGLDLFSMLLEQND
LEPGHTELLRELLASLRRHDLLRRVDDFEAGAAAGAAPGEEDLCAAFNVICDNVG
KDWRRLARQLKVSDTKIDSIEDRYPRNLTERVRESLRIWKNTEKENATVAHLVGA
LRSCQMNLVADLVQEVQQARDLQNRSGAMSPMSWNSDASTSEAS

HCD28tm5B (secuencia de nucleótido)

GTTGTGGATCCTCCCTTTTGGGTGCTGGTGGTGGTTGGTGTCCTGGCTTGCTAT
AGCTTG

HCD28tm3S (secuencia de nucleótido)

GTTGTTTCGAACCCAGAAAATAATAAAGGCCACTGTTACTAGCAAGCTATAGC
AAGCCAG

HCD2Stm5' (secuencia de nucleótido)

GTTGTGGATCCTCCCTTTTGGGTGCTGGTGGT

HCD28tm3' (secuencia de nucleótido)

GTTGTTTCGAACCCAGAAAATAATAAAGGCCAC

HCD80tm5' (secuencia de nucleótido)

1533
1545

GTTGTGGATCCTCCTGCTCCCATCCTGG

HCD80tm3' (secuencia de nucleótido)

GTTGTTTCGAACGGCAAAGCAGTAGGTCAGGC

MFADD5BB (secuencia de nucleótido)

GTTGTGGATCCTTCGAACCCATTCCTGGTGCTGCTGCACTCGCTG

MFADD3XC (secuencia de nucleótido)

GTTGTTATCGATCTCGAGTCAGGGTGTTTCTGAGGAAGACAC

Secuencia de nucleótido FADD de Murina (longitud completa pero sin flanquear las secuencias de transmemebrana -Ig) (secuencia de nucleótido)

gtggatccttcgaacatggacccattcctggtgctgctgcactcgctgtccggcagcctgtcgggcaacgatctgatggagctcaa
gttcttgtgccgcgagcgcgtgagcaaacgaaagctggagcgcgtgcagagtggcctggacctgttcacggtgctgctggagca
gaacgacctggagcgcgggcacaccgggctgctgcgcgagttgctggcctcgctgcgccgacacgatctactgcagcgcctgg
acgacttcgaggcggggacggcgaccgctgcgccccgggggaggcagatctgcaggtggcatttgacattgtgtgtgacaatg
tggggagagactggaaaagactggcccgcgagctgaaggtgtctgaggccaagatggatgggattgaggagaagtacccccg
aagtctgagtgagcgggtaagggagagtctgaaagtctggaagaatgctgagaagaagaacgcctcggtggccggactggtca
aggcgctgcggacctgcaggctgaatctggtggctgacctggtggaagaagcccaggaatctgtgagcaagagtgagaatatgt
ccccagtactaagggattcaactgtgtcttcctcagaaacaccctgactcgagatcgat

FADD de Murina (secuencia de aminoácido)

VDPSNMDPFLVLLHSLSGSLSGNDLMELKFLCRERVSKRKLERVQSGLDLFTVLLE
QNDLERGHTGLLRELLASLRRHDLLQRLDDFEAGTATAAPPGEADLQVAFDIVCD
NVGRDWKRLARELKVSEAKMDGIEEKYPRSLSERVRESLKVWKNAEKKNASVA
GLVKALRTCRLNLVADLVEEAQESVSKSENMSPVLRDSTVSSSETP

MCASP3-5 (secuencia de nucleótido)

1534
1546

GTTGTGGATCCTTCGAACATGGAGAACAACAAAACCTCAGTGGATTCA

MCASP3-3 (secuencia de nucleótido)

GTTGTTATCGATCTCGAGCTAGTGATAAAAGTACAGTTCTTTCGT

MCASP8-5 (secuencia de nucleótido)

GTTGTTTCGAACATGGATTTCCAGAGTTGTCTTTATGCTATTGCTG

MCASP8-3 (secuencia de nucleótido)

GTTGTTATCGATCTCGAGTCATTAGGGAGGGAAGAAGAGCTTCTTCCG

hcasp3-5 (secuencia de nucleótido)

GTTGTGGATCCTTCGAACATGGAGAACACTGAAAACTCAGTGGAT

hcasp3-3 (secuencia de nucleótido)

GTTGTTATCGATCTCGAGTTAGTGATAAAAATAGAGTTCTTTTGTGAG

hcasp8-5 (secuencia de nucleótido)

GTTGTGGATCCTTCGAACATGGACTTCAGCAGAAATCTTTATGAT

hcasp8-3 (secuencia de nucleótido)

GTTGTTATCGATGCATGCTCAATCAGAAGGGAAGACAAGTTTTTTCT

1 2H7 scFv con mutaciones VHL11 alternativas

Secuencia de nucleótido

aagcttgccgccatggattttcaagtgcagattttcagcttcctgctaatcagtgcttcagtcataattgccagaggacaaattgttctc
tcccagtctccagcaatcctgtctgcatctccaggggagaaggtcacaatgacttgcagggccagctcaagtgtaagttacatgcac

~~1535~~
1547

tggtaccagcagaagccaggatcctcccccaaaccctggatttatgccccatccaacctggcttctggagtccctgctcgcttcagt
ggcagtgggtctgggacctcttactctctcacaatcagcagagtggaggctgaagatgctgccacttattactgccagcagtggagt
ttaacccacccacgttcggtgctgggaccaagctggagctgaaagatggcggtggctcgggcggtggtggatctggaggaggt
gggagctctcaggcttatctacagcagtctggggctgag **(uno de los siguienes ten, acn, gan, can, aan, cgn, agn)**
gtgaggcctggggcctcagtgaagatgtcctgcaaggcttctggctacacatttaccagttacaatatgcactgggtaaagcagaca
cctagacagggcctggaatggattggagctatttatccaggaaatggtgatacttcctacaatcagaagttcaagggcaaggccac
actgactgtagacaaatcctccagcacagcctacatgcagctcagcagcctgacatctgaagactctgcggtctatttctgtgcaag
agtggtgtactatagtaactcttactggtacttcgatgtctggggcacagggaccacggtcaccgtctcttctgatcag

Secuencia de aminoácido

MDFQVQIFSFLLISASVIIARGQIVLSQSPAILSASPGEKVTMTCRASSSVSYMHWY
QQKPGSSPKPWIYAPSNLASGVPARFSGSGSGTSYSLTISRVEAEDAATYYCQQWS
FNPPTFGAGTKLELKDGGGSGGGGSGGGGSSQAYLQQSGAE **(one of the following S, T, D, E, Q, N, R, K, H)**
VRPGASVKMSCKASGYTFTSYNMHWVKQTPRQGLEWIGAIYPGNGDTSYNQKFK
GKATLTVDKSSSTAYMQLSSLTSEDSAVYFCARVVYYSNSYWYFDVWGTGTTVT
VSSDQ

2 eliminación de VHL11

Secuencia de nucleótido

aagcttgccgccatggattttcaagtgcagattttcagcttcctgctaatcagtgcttcagtcataattgccagaggacaaattgttctc
tcccagtctccagcaatcctgtctgcatctccaggggagaaggtcacaatgacttgcagggccagctcaagtgtaagttacatgcac
tggtaccagcagaagccaggatcctcccccaaaccctggatttatgccccatccaacctggcttctggagtccctgctcgcttcagt
ggcagtgggtctgggacctcttactctctcacaatcagcagagtggaggctgaagatgctgccacttattactgccagcagtggagt
ttaacccacccacgttcggtgctgggaccaagctggagctgaaagatggcggtggctcgggcggtggtggatctggaggaggt
gggagctctcaggcttatctacagcagtctggggctgaggtgaggcctggggcctcagtgaagatgtcctgcaaggcttctggct
acacatttaccagttacaatatgcactgggtaaagcagacacctagacagggcctggaatggattggagctatttatccaggaaatg
gtgatacttcctacaatcagaagttcaagggcaaggccacactgactgtagacaaatcctccagcacagcctacatgcagctcagc
agcctgacatctgaagactctgcggtctatttctgtgcaagagtggtgtactatagtaactcttactggtacttcgatgtctggggcaca
gggaccacggtcaccgtctcttctgatcag

Secuencia de aminoácido

1536
1598

MDFQVQIFSFLLISASVIIARGQIVLSQSPAILSASPGEKVTMTCRASSSVSYMHWY
QQKPGSSPKPWIYAPSNLASGVPARFSGSGSGTSYSLTISRVEAEDAATYYCQQWS
FNPPTFGAGTKLELKDGGGSGGGGSGGGGSSQAYLQQSGAEVRPGASVKMSCKA
SGYTFTSYNMHWVKQTPRQGLEWIGAIYPGNGDTSYNQKFKGKATLTVDKSSST
AYMQLSSLTSEDSAVYFCARVVYYSNSYWYFDVWGTGTTVTVSSDQ

3 2H7 VL L106 conmutaciones alternativas

Secuencia de nucleótido

aagcttgccgccatggattttcaagtgcagattttcagcttcctgctaatcagtgcttcagtcataattgccagaggacaaattgttctct
cccagtctccagcaatcctgtctgcatctccaggggagaaggtcacaatgacttgcagggccagctcaagtgtaagttacatgcact
ggtaccagcagaagccaggatcctccccaaaccctggatttatgccccatccaacctggcttctggagtccctgctcgcttcagtg
gcagtgggtctgggacctcttactctctcacaatcagcagagtggaggctgaagatgctgccacttattactgccagcagtggagttt
taacccacccacgttcggtgctgggaccaagctggag (tcn, agn, aan, cgn, can, gan, y derivados no
naturales de estos codones) aaagatggcggtggctcgggcggtggtggatctggaggaggtgggagctc

Secuencia de aminoácido

MDFQVQIFSFLLISASVIIARGQIVLSQSPAILSASPGEKVTMTCRASSSVSYMHWY
QQKPGSSPKPWIYAPSNLASGVPARFSGSGSGTSYSLTISRVEAEDAATYYCQQWS
FNPPTFGAGTKLE **(S, T, R, K, H, Q, N, D, E, y derivados no naturales de estos
aminoácidos en la posición 106)** KDGGGSGGGGSGGGGSS

4. Eliminación VL L106

Secuencia de nucleótido

aagcttgccgccatggattttcaagtgcagattttcagcttcctgctaatcagtgcttcagtcataattgccagaggacaaattgttctc
tcccagtctccagcaatcctgtctgcatctccaggggagaaggtcacaatgacttgcagggccagctcaagtgtaagttacatgcac
tggtaccagcagaagccaggatcctccccaaaccctggatttatgccccatccaacctggcttctggagtccctgctcgcttcagt
ggcagtgggtctgggacctcttactctctcacaatcagcagagtggaggctgaagatgctgccacttattactgccagcagtggagt
ttaacccacccacgttcggtgctgggaccaagctggagaaagatggcggtggctcgggcggtggtggatctggaggaggtgg
gagctc

1537
1549

Secuencia de aminoácido

MDFQVQIFSFLLISASVIIARGQIVLSQSPAILSASPGEKVTMTCRASSSVSYMHWY
QQKPGSSPKPWIYAPSNLASGVPARFSGSGSGTSYSLTISRVEAEDAATYYCQQWS
FNPPTFGAGTKLEKDGGGSGGGGSGGGGSS

5. IgE CH3 CH4

Secuencia de nucleótido

tccaacccgagaggggtgagcgcctacctaagccggcccagcccgttcgacctgttcatccgcaagtcgcccacgatcacctgtc
tggtggtggacctggcacccagcaaggggaccgtgaacctgacctggtcccgggccagtgggaagcctgtgaaccactccacc
agaaaggaggagaagcagcgcaatggcacgttaaccgtcacgtccaccctgccggtgggcacccgagactggatcgaggggg
agacctaccagtgcagggtgacccacccccacctgcccagggccctcatgcggtccacgaccaagaccagcggcccgcgtgct
gccccggaagtctatgcgtttgcgacgccggagtggccggggagccgggacaagcgcaccctcgcctgcctgatccagaactt
catgcctgaggacatctcggtgcagtggctgcacaacgaggtgcagctcccggacgcccggcacagcacgacgcagccccgc
aagaccaagggctccggcttcttcgtcttcagccgcctggaggtgaccagggccgaatgggagcagaaagatgagttcatctgcc
gtgcagtccatgaggcagcgagcccctcacagaccgtccagcgagcggtgtctgtaaatcccggtaaatgataatctagaa

Secuencia de aminoácido

SNPRGVSAYLSRPSPFDLFIRKSPTITCLVVDLAPSKGTVNLTWSRASGKPVNHSTR
KEEKQRNGTLTVTSTLPVGTRDWIEGETYQCRVTHPHLPRALMRSTTKTSGPRAA
PEVYAFATPEWPGSRDKRTLACLIQNFMPEDISVQWLHNEVQLPDARHSTTQPRK
TKGSGFFVFSRLEVTRAEWEQKDEFICRAVHEAASPSQTVQRAVSVNPGK

6. hIgGIHAgE WCH3 WCH4

Secuencia de nucleótido

tgatcaggagcccaaatcttctgacaaaactcacacatcccccaccgtcccagcatccaaccccgaggaggggtgagcgcctacctc
agccggcccagccccgttcgacctgttcatccgcaagtcgcccacgatcacctgtctggtggtggacctggcacccagcaaggggg
accgtgaacctgacctggtcccgggccagtgggaagcctgtgaaccactccaccagaaaggaggagaagcagcgcaatggca
cgttaaccgtcacgtccaccctgccggtgggcacccgagactggatcgaggggagacctaccagtgcagggtgacccacccc
cacctgcccagggccctcatgcggtccacgaccaagaccagcggcccgcgtgctgccccggaagtctatgcgtttgcgacgcc
ggagtggccgggggagccgggacaagcgcacctcgcctgcctgatccagaacttcatgcctgaggacatctcggtgcagtggc
gcacaacgaggtgcagctcccggacgcccggcacagcacgacgcagccccgcaagaccaagggctccggcttcttcgtcttca
gccgcctggaggtgaccagggccgaatgggagcagaaagatgagttcatctgccgtgcagtccatgaggcagcgagccccta
cagaccgtccagcgagcggtgtctgtaaatcccggtaaatgataatctagaa

~~1538~~
1550

Secuencia de aminoácido

DQEPKSSDKTHTSPPSPASNPRGVSAYLSRPSPFDLFIRKSPTITCLVVDLAPSKGTV
NLTWSRASGKPVNHSTRKEEKQRNGTLTVTSTLPVGTRDWIEGETYQCRVTHPHL
PRALMRSTTKTSGPRAAPEVYAFATPEWPGSRDKRTLACLIQNFMPEDISVQWLH
NEVQLPDARHSTTQPRKTKGSGFFVFSRLEVTRAEWEQKDEFICRAVHEAASPSQT
VQRAVSVNPGK

7 IgE WCH2 WCH3 WCH4

Secuencia de nucleótido

tgatcacgtctgctccagggacttcaccccgcccaccgtgaagatcttacagtcgtcctgcgacggcggcgggcacttccccccg
accatccagctcctgtgcctcgtctctgggtacaccccagggactatcaacatcacctggctggaggacgggcaggtcatggacg
tggacttgtccaccgcctctaccacgcaggagggtgagctggcctccacacaaagcgagctcaccctcagccagaagcactggc
tgtcagaccgcacctacacctgccaggtcacctatcaaggtcacacctttgaggacagcaccaagaagtgtgcagattccaaccc
gagaggggtgagcgcctacctaagccggcccagcccgttcgacctgttcatccgcaagtcgcccacgatcacctgtctggtggtg
gacctggcacccagcaaggggaccgtgaacctgacctggtcccgggccagtgggaagcctgtgaaccactccaccagaaagg
aggagaagcagcgcaatggcacgttaaccgtcacgtccaccctgccggtgggcacccgagactggatcgaggggggagaccta
ccagtgcagggtgacccacccccacctgcccagggccctcatgcggtccacgaccaagaccagcggcccgcgtgctgccccg
gaagtctatgcgtttgcgacgccggagtggccggggagccgggacaagcgcaccctcgcctgcctgatccagaacttcatgcct
gaggacatctcggtgcagtggctgcacaacgaggtgcagctcccggacgcccggcacagcacgacgcagccccgcaagacc
aagggctccggcttcttcgtcttcagccgcctggaggtgaccagggccgaatgggagcagaaagatgagttcatctgccgtgcag
tccatgaggcagcgagcccctcacagaccgtccagcgagcggtgtctgtaaatcccggtaaatgataatctaga

Secuencia de aminoácido

DHVCSRDFTPPTVKILQSSCDGGGHFPPTIQLLCLVSGYTPGTINITWLEDGQVMDV
DLSTASTTQEGELASTQSELTLSQKHWLSDRTYTCQVTYQGHTFEDSTKKCADSN
PRGVSAYLSRPSPFDLFIRKSPTITCLVVDLAPSKGTVNLTWSRASGKPVNHSTRKE
EKQRNGTLTVTSTLPVGTRDWIEGETYQCRVTHPHLPRALMRSTTKTSGPRAAPE
VYAFATPEWPGSRDKRTLACLIQNFMPEDISVQWLHNEVQLPDARHSTTQPRKTK
GSGFFVFSRLEVTRAEWEQKDEFICRAVHEAASPSQTVQRAVSVNPGK

8 hIgGlH/IgE CH3 CH4 (ORF)

~~1537~~ 1551

Secuencia de nucleótido

tgatcaggagcccaaatcttctgacaaaactcacacatccccaccgtccccagcatccaacccgagaggggtgagcgcctaccta
agccggcccagcccgttcgacctgttcatccgcaagtcgcccacgatcacctgtctggtggtggacctggcacccagcaagggg
accgtgaacctgacctggtcccgggccagtgggaagcctgtgaaccactccaccagaaaggaggagaagcagcgcaatggca
cgttaaccgtcacgtccaccctgccggtgggcacccgagactggatcgaggggggagacctaccagtgcagggtgacccacccc
cacctgcccagggccctcatgcggtccacgaccaagaccagcggcccgcgtgctgccccggaagtctatgcgtttgcgacgcc
ggagtggccggggagccgggacaagcgcaccctcgcctgcctgatccagaacttcatgcctgaggacatctcggtgcagtggct
gcacaacgaggtgcagctcccggacgcccggcacagcacgacgcagccccgcaagaccaagggctccggcttcttcgtcttca
gccgcctggaggtgaccagggccgaatgggagcagaaagatgagttcatctgccgtgcagtccatgaggcagcgagcccctca
Cagaccgtccagcgagcggtgtctgtaaatcccggtaaagcggatccttcgaa

Secuencia de aminoácido

DQEPKSSDKTHTSPPSPASNPRGVSAYLSRPSPFDLFIRKSPTITCI VVDLAPSKGTV
NLTWSRASGKPVNHSTRKEEKQRNGTLTVTSTLPVGTRDWIEGETYQCRVTHPHL
PRALMRSTTKTSGPRAAPEVYAFATPEWPGSRDKRTLACLIQNFMPEDISVQWH
NEVQLPDARHSTTQPRKTKGSGFFVFSRLEVTRAEWEQKDEFICRAVHEAASPSQT
VQRA VSVNPGKSGSFE

9 2H7 VHL11S scFv hIgG1 (SSS-S) H hIgE WCH3 WCH4

Secuencia de nucleótido

1540
1552

aagcttgccgccatggatttttcaagtgcagattttcagcttcctgctaatcagtgcttcagtcataattgccagaggacaaattgttctct
cccagtctccagcaatcctgtctgcatctccaggggagaaggtcacaatgacttgcagggccagctcaagtgtaagttacatgcact
ggtaccagcagaagccaggatcctcccccaaaccctggatttatgccccatccaacctggcttctggagtcctgctcgcttcagtg
gcagtgggtctgggacctcttactctctcacaatcagccgagtggaggctgaagatgctgccacttattactgccagcagtggagttt
taacccacccacgttcggtgctgggaccaagctggagctgaaagatggcggtggctcgggcggtggtggatctggaggaggtg
ggaggtctcaggcttatttacagcagtctggggctgagtcggtgaggcctggggcctcagtgaagatgtcctgcaaggcttctggc
tacacatttaccagttacaatatgcactgggtaaagcagacacctagacagggcctggaatggattggagctatttatccaggaaat
ggtgatacttcctacaatcagaagttcaagggcaaggccacactgactgtagacaaatcctccagcacagcctacatgcagctcag
cagcctgacatctgaagactctgcggtctattttctgtgcaagagtggtgtactatagtaactcttactggtacttcgatgtctggggcac
agggaccacggtcaccgtctcttctgatcagggagcccaaatcttctgacaaaactcacacatcccccaccgtcctcagcatccaacc
cgaggggggtgagcgcctacctaagccggcccagccccgttcgacctgttcatccgcaagtcgcccacgatcacctgtctggtggt
ggacctggcacccagcaaggggtacccgtgaacctgacctggtcccgggccagtgggtaagcctgtgaaccactccaccagaaag
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Secuencia de aminoácido

MDFQVQIFSFLLISASVIIARGQIVLSQSPAILSASPGEKVTMTCRASSSVSYMHWY
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10 2H7 VHL11S scFv hIgG1 (SSS-P) H hIgE WCH3 WCH4

Secuencia de nucleótido

1547
1553

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Secuencia de aminoácido

MDFQVQIFSFLLISASVIIAR GQIVLSQSPAILSASPGEKVTMTCRASSSVSYMHWY
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FNPPTFGAGTKLELKDGGGSGGGGSGGGGSSQAYLQQSGAESVRPGASVKMSCK
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10 2H7 VL L106S

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Secuencia de aminoácido

1547
1559

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11 2H7 VL L106S scFv

Secuencia de nucleótido

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Secuencia de aminoácido

MDFQVQIFSFLLISASVIIARGQIVLSQSPAILSASPGEKLVTMTCRASSSVSYMHWY
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FNPPTFGAGTKLESKDGGGSGGGGSGGG (SSQAYLQQSGAELVRPGASVKIvfSCK
ASGYFTSYMmWKQTPRQGLEWIGAIYPGNGDTSYNQK. FKGKATLTVDKSSS
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12 2H7 scFs VL L106S VHLllS sc

Secuencia de nucleótido

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1543
1555

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Secuencia de aminoácido

MDFQVQIFSFLLISASVIIARGQIVLSQSPAILSASPGEKVTMTCRASSSVSYMHWY
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10 Ligador de articulación IgD humano con sitios de restricción adheridos

Nucleótido

gtggatccaggttcgaagtctccaaaggcacaggcctcctccgtgcccactgcacaaccccaagcagagggcagcctcgccaa
ggcaaccacagccccagccaccacccgtaacacaggaagaggaggagaagagaagaagaaggagaaggagaaagaggaa
caagaagagagagagacaaagaccggtgcagtcgacg

Aminoácido

VDPGSKSPKAQASSVPTAQPQAEGSLAKATTAPATTRNTGRGGEEKKKEKEKEEQ
EERETKTGAVD

Secuencia de dominio de articulación IgD nativo

(Incluye un residuo de cisteína – truncamos la anterior articulación para ese residuo para estas construcciones)

Nucleótido

1546
1556

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Secuencia de aminoácido

ESPKAQASSVPTAQPQAEGSLAKATTAPATTRNTGRGGEEKKKEKEKEEQEERET
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12 2H7 VH L11S

Secuencia de nucleótido

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Secuencia de aminoácido

QAYLQQSGAESVRPGASVKMSCKASGYTFTSYNMHWKQTPRQGLEWIGAIYPG
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13 2H7 VH L11S scFv

Secuencia de nucleótido

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1545
1557

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Secuencia de aminoácido

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14 2H7 scFv VH L11S hIgG1 (CSC-S) H WCH2 WCH3

Secuencia de nucleótido

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Secuencia de aminoácido

1546
1558

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SPGK

15. 2H7 scFv VH L11S IgE WCH2 WCH3 WCH4

Secuencia de nucleótido

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1577
1559

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tgataatctaga

Secuencia de aminoácido

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16 2H7 scFv VH L11S mIgE WCH2 WCH3 WCH4

Secuencia de nucleótido

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1560

Secuencia de aminoácido

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17 2H7 scFv VH L11S hIgA WH WCH2 T4CH3

Secuencia de nucleótido

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Secuencia de aminoácido

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18. 2H7 scFv VH L11S mIgA WH WCH2 T4 CH3

Secuencia de nucleótido

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Secuencia de aminoácido

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A mIgA. WCH2 T4CH3

Secuencia de nucleótido

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Secuencia de aminoácido

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20 Región K322S CH2

Secuencia de nucleótido

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1563

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Secuencia de aminoácido

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21 K322S CH2 WCH3

Secuencia de nucleótido

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Secuencia de aminoácido

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1 K322L CH2 WCH3

Secuencia de nucleótido

1564

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Secuencia de aminoácido

DQEPKSSDKTHTSPPSSAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHED
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22 2H7 scFv VHL11S hIgGl (SSS-S)H K322SCH2 WCH3

Secuencia de nucleótido

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1565

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Secuencia de aminoácido

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23 ZH7 sch VHLlIS hIgGl (SSS-S) H B22I, H2 WCHS"-

Secuencia de nucleótido

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Secuencia de aminoácido

1554
1566

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24- 2H7 scFv VHLlIS hIgGl (CSS-S) H K322SCH2 WCH3

Secuencia de nucleótido

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Secuencia de aminoácido

~~1555~~
1567

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FNPPTFGAGTKLELKDGGGSGGGGSGGGGSSQAYLQQSGAESVRPGASVKMSCK
ASGYTFTSYNMHWVKQTPRQGLEWIGAIYPGNGDTSYNQKFKGKATLTVDKSSS
TAYMQLSSLTSEDSAVYFCARVVYYSNSYWYFDVWGTGTTVTVSSDQEPKSCDK
THTSPPSSAPELLGGPSVFLFPPKPKDTLMISRTPEVTCWVDVSHEDPEVKFNWYV
DGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCSVSNKALPAPIE
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PGK

25 P331S CH2

Secuencia de nucleótido

cctgaactcctgggggaccgtcagtcttcctcttcccccaaaacccaaggacaccctcatgatctcccggacccctgaggtcac
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Secuencia de aminoácido

PELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHN
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26 P331S CH2 WCH3

Secuencia de nucleótido

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1556
1568

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Secuencia de aminoácido

PELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHN
AKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPASIEKTISKAKG
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27 2H7 scFv VH L11S (SSS-S)H P331S CH2 WCH3

Secuencia de nucleótido

aagcttgccgccatggattttcaagtgcagattttcagcttcctgctaatcagtgcttcagtcataattgccagaggacaaattgttctct
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Secuencia de aminoácido

1557
1569

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KTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPEN
NYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLS
PGK

28 2H7 scFv VH L11S (CSS-S) H P331S CH2 WCH3

Secuencia de nucleótido

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Secuencia de aminoácido

1558
1570

MDPQVQIFSFLLISASVIIARGQIVLSQSPAILSASPGEKVTMTCRASSSVSYMHWY
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FNPPTFGAGTKLELKDGGGSGGGGSGGGGSSQAYLQQSGAELVRPGASVKMSCK
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TAYMQLSSLTSEDSAVYFCARVVYYSNSYWYFDVWGTGTTVTVSSDQEPKSCDK
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PGK

29 Región T256N CH2

Secuencia de nucleótido

cctgaactcctgggggggaccgtcagtcttcctcttcccccaaaacccaaggacaccctcatgatctcccggaaccctgaggtca
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Secuencia de aminoácido

PELLGGPSVFLFPPKPKDTLMISRNPEVTCVVVDVSHEDPEVKFNWYVDGVEVHN
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30 T256N CH2 WCH3

Secuencia de nucleótido

cctgaactcctgggggggaccgtcagtcttcctcttcccccaaaacccaaggacaccctcatgatctcccggaaccctgaggtcac
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Secuencia de aminoácido

1559
1591

PELLGGPSVFLFPPKPKDTLMISRNPEVTCVVVDVSHEDPEVKFNWYVDGVEVHN
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LDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

31 2H7 scFv VH L11S (SSS-S) H T256N CH2 WCH3

Secuencia de nucleótido

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Secuencia de aminoácido

MDFQVQIFSFLLISASVIIARGQIVLSQSPAILSASPGEKVTMTCRASSSVSYMHWY
QQKPGSSPKPWIYAPSNLASGVPARFSGSGSGTSYSLTISRVEAEDAATYYCQQWS
FNPPTFGAGTKLELKDGGGSGGGGSGGGGSSQAYLQQSGAESVRPGASVKMSCK
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EKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPE
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SPGK

1560
1572

32 2H7 scFv VH L11S (CSS-S) H T256N CH2 WCH3

Secuencia de nucleótido

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Secuencia de aminoácido

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EKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPE
NNYRTTPPVLDSDGSFFLYSKLTVDKSRWQQGNFSCSVMHEALHNHYTQKSLSL
SPGK

1573

33 RTPE/QNAK (255-258) CH2

Secuencia de nucleótido

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Secuencia de aminoácido

PELLGGPSVFLFPPKPKDTLMISQNAKVTCVVVDVSHEDPEVKFNWYVDGVEVHN
AXTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAK

34. RTPE/QNAK (255-258) CH2 WCH3

Secuencia de nucleótido

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Secuencia de aminoácido

PELLGGPSVFLFPPKPKDTLMISQNAKVTCVVVDVSHEDPEVKFNWYVDGVEVHN
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QPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPV
LDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

35. 2H7 scFv VH L11S (SSS-S) H RTPE/QNAK (255-258) CH2 WCH3

1600
1579

Secuencia de nucleótido

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Secuencia de aminoácido

NIDFQVQIFSFLLISASVIIARGQIVLSQSPAILSASPGEKVTMTCRASSSVSY1XEWY
QQKPGSSPKPWIYAPSNLASGVPARFSGSGSGTSYSLTISRVEAEDAATYYCQQWS
FNPPTFGAGTKLELKDGGGSGGGGSGGGGSSQAYLQQSGAESVRPGASVKMSCK
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TAYMQLSSLTSEDSAVYFCARVVYYSNSYWYFDVWGTGTTVTVSSDQEPKSSDK
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EKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPE
NNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSL
SPGK

36 2H7 scFv VH L11S (CSS-S) H RTPE/QNAK (255-258) CH2 WCH3

1595

Secuencia de nucleótido

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Secuencia de aminoácido

MDFQVQIFSFLLISASVIIARGQIVLSQSPAILSASPGEKVTMTCRASSSVSYMHWY
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EKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPE
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SPGK

36 región K290Q CH2

Secuencia de nucleótido

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1569
1576

Secuencia de aminoácido

PELLGGPSVFLFPPKPKDTLMISRTPEVTCVWDVSHEDPEVKFNWYVDGVEVHN
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37 K290Q CH2 WCH3

Secuencia de nucleótido

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Secuencia de aminoácido

PELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHN
AKTQPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKG
QPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPV
LDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

38. 2H7 scFv VH L11S (SSS-S) H K290Q CH2 WCH3

Secuencia de nucleótido

aagcttgccgccatggattttcaagtgcagattttcagcttcctgctaatcagtgcttcagtcataattgccagaggacaaattgttctct
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1565
1577

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Secuencia de aminoácido

MDFQVQIFSFLLISASVIIARGQIVLSQSPAILSASPGEKVTMTCRASSSVSYMHWY
QQKPGSSPKPWIYAPSNLASGVPARFSGSGSGTSYSLTISRVEAEDAATYYCQQWS
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THTSPPSSAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYV
DGVEVHNAKTQPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIE
KTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPEN
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PGK

39 2H7 scfv VH L11S (CSS-S) H K290Q CH2 WCH3

Secuencia de nucleótido

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1566

1578.

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Secuencia de aminoácido

MDFQVQIFSFLLISASVIIARGQIVLSQSPAILSASPGEKVTMTCRASSSVSYMHWY
QQKPGSSPKPWIYAPSNLASGVPARFSGSGSGTSYSLTISRVEAEDAATYYCQQWS
FNPPTFGAGTKLELKDGGGSGGGGSGGGGSSQAYLQQSGAESVRPGASVKMSCK
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TAYMQLSSLTSEDSAVYFCARVVYYSNSYWYFDVWGTGTTVTVSSDQEPKSCDK
THTSPPSSAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYV
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KTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPEN
NYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLS
PGK

40 A339PCH2

Secuencia de nucleótido

cctgaactcctgggggggaccgtcagtcttcctcttcccccaaaacccaaggacaccctcatgatctcccggaccccctgaggtcac
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Secuencia de aminoácido

PELLGGPSVFLFPPKPKDTLMISRTPEVTCVWDVSHEDPEVKFNWYVDGVEVHN
AKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKPK

1579

41 A339P2CHZ WCH3

Secuencia de nucleótido

cctgaactcctgggggggaccgtcagtcttcctcttcccccaaaacccaaggacaccctcatgatctcccggacccctgaggtcac
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Secuencia de aminoácido

PELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHN
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42 2H7 scFv VHL11S (SSS-S) H A339P CH2 WCH3

Secuencia de nucleótido

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1568
1580

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Secuencia de aminoácido

MDFQVQIFSFLLISASVIIARGQIVLSQSPAILSASPGEKVTMTCRASSSVSYMHWY
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ASGYTFTSYNMHWVKQTPRQGLEWIGAIYPGNGDTSYNQKFKGKATLTVDKSSS
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THTSPPSSAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYV
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KTISKPKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPEN
NYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLS
PGK

43 2H7 scFv VHL11S (CSS-S) H A339P CH2 WCH3

Secuencia de nucleótido

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4569
1581

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Secuencia de aminoácido

MDFQVQIFSFLLISASVIIARGQIVLSQSPAILSASPGEKVTMTCRASSSVSYMHWY
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TAYMQLSSLTSEDSAVYFCARVVYYSNSYWYFDVWGTGTTVTVSSDQEPKSCDK
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KTISKPKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPEN
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PGK

44 G28-1VH

Secuencia de nucleótido

gcggtccagctgcagcagtctggacctgagctggaaaagcctggcgcttcagtgaagatttcctgcaaggcttctggttactcattc
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gatcag

Secuencia de aminoácido

AVQLQQSGPELEKPGASVKISCKASGYSFTGYNMNWVKQNNGKSLEWIGNIDPY
YGGTTYNRKFKGKATLTVDKSSSTAYMQLKSLTSEDSAVYYCARSVGPMDYWG
QGTSVTVSSDQ

1582

45 G28-1VL

Secuencia de nucleótido

aagcttgccgccatggtatccacagtcagttccttgggttgctgctgtggcttacaggtggcagatgtgacatccagatgactc
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cgtca

Secuencia de aminoácido

MVSTAQFLGLLLLWLTGGRCDIQMTQSPASLSASVGETVTITCRTSENVYSYLAW
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46 G28-1 scFv

Secuencia de nucleótido

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Secuencia de aminoácido

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1583

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47 G28-1 VHL11S

Secuencia de nucleótido

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Secuencia de aminoácido

AVQLQQSGPESEKPGASVKISCKASGYSFTGYNMNWVKQNNGKSLEWIGNIDPYY
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48. G28-1 VHL11S scFv

Secuencia de nucleótido

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Secuencia de aminoácido

1589

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49 G28-1 scFv (SSS-S) H WCH2 WCH3

Secuencia de nucleótido

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Secuencia de aminoácido

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1585

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50 G28-1 scFv IgAW H IgGIWCH2 WCH3

Secuencia de nucleótido

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Secuencia de aminoácido

MVSTAQFLGLLLLWLTGGRCDIQMTQSPASLSASVGETVTITCRTSENVYSYLAW
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1586

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51 G28-1 scFv VHL11S (SSS-S) H WCH2 WCH3

Secuencia de nucleótido

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Secuencia de aminoácido

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1587

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52 G28-1 scFv VHI, 11S (CSS-S) H WCH2 WCH3

Secuencia de nucleótido

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Secuencia de aminoácido

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1576
1588

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53 G28-1 scFv VH L11S (CSC-S) H WCH2 WCH3

Secuencia de nucleótido

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Secuencia de aminoácido

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54 G28-1 scFv VH L11S (SSC-P) H WCH2 WCH3

Secuencia de nucleótido

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Secuencia de aminoácido

MVSTAQFLGLLLLWLTGGRCDIQMTQSPASLSASVGETVTITCRTSENVYSYLAW
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AYMQLKSLTSEDSAVYYCARSVGPMDYWGQGTSVTVSSDQEPKSSDKTHTSPPCP
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1578
1590

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II. 54. HCTLA4 HIGG1 (SSS-S) H P238SCH2 WCH3

Secuencia de nucleótido

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Secuencia de aminoácido

MACLGFQRHKAQLNLAARTWPCTLLFFLLFIPVFCKAMHVAQPAVVLASSRGIAS
FVCEYASPGKATEVRVTVLRQADSQVTEVCAATYMTGNELTFLDDSICTGTSSGN
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KTHTSPPSSAPELLGGSSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWY
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SPGK

55. Fc2-2 VL

Secuencia de nucleótido

1591

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Secuencia de aminoácido

MDSQAQVLMLLLLWVSGTCGDIVMSQSPSSLAVSVGEKVSMSCKSSQSLLYNHN
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56 FC2-2VH

Secuencia de nucleótido

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Secuencia de aminoácido

GSSQVQLKESGPGLVAPSQSLSITCTVSGFSLTVYGVNWVRQPPGKGLDWLGMIW
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DYWGQGTSVTVSSDQ

57 FC2-2scFv

Secuencia de nucleótido

gttgttaagcttgccgccatggattcacaggcccaggttcttatgttactgctgctatgggtatctggtacctgtggggacattgtgatg
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1500
1592

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Secuencia de aminoácido

MDSQAQVLMLLLLWVSGTCGDIVMSQSPSSLAVSVGEKVSMSCKSSQSLLYNHN
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SLSITCTVSGFSLTVYGVNWVRQPPGKGLDWLGMIWGDGSTDYNSALKSRLSISK
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58 FC2-2 VHL11S

Secuencia de nucleótido

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Secuencia de aminoácido

(GSS)QVQLKESGPGSVAPSQSLSITCTVSGFSLTVYGVNWVRQPPGKGLDWLGMI
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59 FC2-2 VH L11S scFv

Secuencia de nucleótido

1593

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Secuencia de aminoácido

MDSQAQVLMLLLLWVSGTCGDIVMSQSPSSLAVSVGEKVSMSCKSSQSLLYNHN
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60 FC2-2 (SSS-S) H WCH2 WCH3

Secuencia de nucleótido

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1582
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aminoácido

MDSQAQVLMLLLLWVSGTCGDIVMSQSPSSLAVSVGEKVSMSCKSSQSLLYNHN
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VYYCQQYYTYPPTFGGGTKLEIKGGGGSGGGGSGGGGSSQVQLKESGPGLVAPSQ
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61 FC2-2 VHL11S (SSS-S) H WCH2 WCH3

Secuencia de nucleótido

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1585
1595

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Secuencia de aminoácido

MDSQAQVLMLLLLWVSGTCGDIVMSQSPSSLAVSVGEKVSMSCKSSQSLLYNHN
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62 UCHL-1 VH

Secuencia de nucleótido

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Secuencia de aminoácido

MGRLTSSFLLLIVPAYVLSQITLKESGPGILQPSQTLSLTCSFSGFSLTTYGIGVGWIR
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63 UCHL-1 VL

~~1580~~
1596

Secuencia de nucleótido

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Secuencia de aminoácido

4KLPVRLLVLMFWIPASISDVVMTQTPLSLPVSLGDQASISCRSSQSLLYSNGNTYL
HWYLQKPGQSPKI LIYKLSNRFSGVPDRFSGSGSGTDFTLKISRVEAEDLGVYFCS
QS'PWTFGGGTLEE

64 UCHL-1 scFv

Secuencia de nucleótido

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Secuencia de aminoácido

MKLPVRLLVLMFWIPASISDVVMTQTPLSLPVSLGDQASISCRSSQSLLYSNGNTYL
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1585
1597

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65. UCHL-1 VH I11SL12S

Secuencia de nucleótido

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gatca

Secuencia de aminoácido

(GSS)QITLKESGPGSSQPSQTLSLTCSFSGFSLTTYGIGVGWIRQPPGKGLEWLTHIW
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66. UCHL-1 scFv VH L11S

Secuencia de nucleótido

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Secuencia de aminoácido

1586
1598

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67 UCHL-1 scFv (SSS-S) H WCH2 WCH3

Secuencia de nucleótido

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Secuencia de aminoácido

MKLPVRLLVLMFWIPASISDVVMTQTPLSLPVSLGDQASISCRSSQSLLYSNGNTYL
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1599

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68 UCHL-1 scFv VHL11S (SSS-S) H WCH2 WCH3

Secuencia de nucleótido

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Secuencia de aminoácido

MKLPVRLLVLMFWIPASISDVVMTQTPLSLPVSLGDQASISCRSSQSLLYSNGNTYL
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1588
1600

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69 5B9 VHL11S

Secuencia de nucleótido

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Secuencia de aminoácido

(GSS)QVQLKQSGPGSVQSSQSLSITCTVSGFSLTTYAVHWVRQSPGKGLEWLGVI
WSGGITDYNAAFISRLSITKDDSKSQVFFKMNSLQPNDTAIYYCARNGGDNYPYY
YAMDYWGQGTSVTVSS

73 5B9 VH L11S scFv

Secuencia de nucleótido

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Secuencia de aminoácido

1601

MRFSAQLLGLLVLWIPGSTADIVMTQAAFSNPVTLGTSASISCRSSKSLLHSNGITY
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70 5B9 scFv VHL11S (SSS-S) H WCH2 WCH3

Secuencia de nucleótido

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Secuencia de aminoácido

MRFSAQLLGLLVLWIPGSTADIVMTQAAFSNPVTLGTSASISCRSSKSLLHSNGITY
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AQNLELPLTFGAGTKLELKRGGGGSGGGGSGGGGSSQVQLKQSGPGSVQSSQSLSI
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QVFFKMNSLQPNDTAIYYCARNGGDNYPYYYAMDYWGQGTSVTVSSDQEPKSS
DKTHTSPPSSAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNW

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76 2H7 scFv VH L11S (SSS-S) H P238SC H2 WCH3

Secuencia de nucleótido

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Secuencia de aminoácido

MDFQVQIFSFLLISASVIIARGQIVLSQSPAILSASPGEKVTMTCRASSSVSYMHWY
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PGK

78 2H7 scFv VH L11S (SSS S) H WCH2 WCH3

1594
1603

Secuencia de nucleótido

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Secuencia de aminoácido

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PGK

79 ZH7 scFv VH L11S (CSS-S) H WCH2 WCH3

Secuencia de nucleótido

1597
1604

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Secuencia de aminoácido

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77 G8-1 scFv VH L11S (SCS-S) H WCH2 WCH3

Secuencia de nucleótido

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Secuencia de aminoácido

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79 G28-1 scFv VHL11S (CCS-P) H WCH2 WCH3

Secuencia de nucleótido

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Secuencia de aminoácido

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80 G28-1 scFv VH LI IS (SCC-P) H WCH2 WCH3

Secuencia de nucleótido

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Secuencia de aminoácido

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81 G28-1 scFv VH L11S mIgE CH2 CH3 CH4

Secuencia de nucleótido

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Secuencia de aminoácido

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82 G28-1 scFv VH L11S mIgA WH WCH2 T4CH3

Secuencia de nucleótido

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Secuencia de aminoácido

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83 G28-1 scFv Vh LI 1 S MgE CH2 CH3 CH4

Secuencia de nucleótido

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Secuencia de aminoácido

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84. G28-1 scFv VH L11S MgA WH WCH2 T4CH3

Secuencia de nucleótido

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Secuencia de aminoácido

MVSTAQFLGLLLLWLTGGRCDIQMTQSPASLSASVGETVTITCRTSENVYSYLAW
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85. HD37 VL

Secuencia de nucleótido

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Secuencia de aminoácido

1600
1612

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86 HD37 VH

Secuencia de nucleótido

gggagctcgcaggttcagctgcagcagtctggggctgagctggtgaggcctgggtcctcagtgaagatttcctgcaaggcttctg
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Secuencia de aminoácido

(GSS)QVQLQQSGAELVRPGSSVKISCKASGYAFSSYWMNWVKQRPGQGLEWIGQI
WPGDGDTNYNGKFKGKATLTADESSSTAYMQLSSLASEDSAVYFCARRETTTVG
RYYYAMDYWGQGTSVTVSSDQ

87 HD37 scFv

Secuencia de nucleótido

aagcttgccgccatggagacagacacactcctgctatgggtgctgctgctctgggttccaggctccactggtgacattgtgctgacc
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Secuencia de aminoácido

1607
1613

METDTLLLWVLLLWVPGSTGDIVLTQSPASLAVSLGQRATISCKASQSVDYDGDS
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88. HD37 VHL11S

Secuencia de nucleótido

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Secuencia de aminoácido

(GSS)QVQLQQSGAESVRPGSSVKISCKASGYAFSSYWMNWVKQRPGQGLEWIGQI
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89 HD37 SCFV VHL11S

Secuencia de nucleótido

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1602

1614

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Secuencia de aminoácido

METDTLLLWVLLLWVPGSTGDIVLTQSPASLAVSLGQRATISCKASQSVDYDGDS
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90 HD37 scF'e IgAH hIgG1 WCH2 T4CH3

Secuencia de nucleótido

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Secuencia de aminoácido

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1605
1615

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91 HD37 scFv (SSS-S) H WCH2 WCH3

Secuencia de nucleótido

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Secuencia de aminoácido

METDTLLLWVLLLWVPGSTGDIVLTQSPASLAVSLGQRATISCKASQSVDYDGDS
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SCKASGYAFSSYWMNWVKQRPGQGLEWIGQIWPGDGDTNYNGKFKGKATLTAD
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1604
1616

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QKSLSLSPGK

92 HD37 scFv VH L11S (SSS-S) H WCH2 WCH3

Secuencia de nucleótido

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Secuencia de aminoácido

METDTLLLWVLLLWVPGSTGDIVLTQSPASLAVSLGQRATISCKASQSVDYDGDS
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1605
1617

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QKSLSLSPGK

91 L6 VL

Secuencia de nucleótido

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ct

Secuencia de aminoácido

MDFQVQIFSFLLISASVIMSRGQIVLSQSPAILSASPGEKVTLTCRASSSVSFMNWYQ
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92 L6 VH

Secuencia de nucleótido

cagatccagttggtgcagtctggacctgagctgaagaagcctggagagacagtcaagatctcctgcaaggcttctgggtatacctt
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Secuencia de aminoácido

1606
1618

QIQLVQSGPELKKPGETVKISCKASGYTFTNYGMNWVKQAPGKGLKWMGWINTY
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93 L6 scFv

Secuencia de nucleótido

atggattttcaagtgcagattttcagcttcctgctaatcagtgcttcagtcataatgtccagaggacaaattgttctctcccagtctccag
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Secuencia de aminoácido

MDFQVQIFSFLLISASVIMSRGQIVLSQSPAILSASPGEKVTLTCRASSSVSFMNWYQ
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94. L6 VHL11S

Secuencia de nucleótido

ctgcagatccagttggtgcagtctggacctgagtcgaagaagcctggagagacagtcaagatctcctgcaaggcttctgggtatac
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1619

Secuencia de aminoácido

QIQLVQSGPESKXPGETVmSCKASGYTFTNYGMNWVKQAPGKGLKWMGWINTY
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WGQGTTLTVSSD

95. L6 VH L11S scFv

Secuencia de nucleótido

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Secuencia de aminoácido

MDFQVQIFSFLLISASVIMSRGQIVLSQSPAILSASPGEKVTLTCRASSSVSFMNWYQ
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96. L6 Or L6 VHL11S scFv IgAH hIgG1 WCH2 WCH3

Secuencia de nucleótido

(se muestra L6 VHL1 1S)

1605
1620

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Secuencia de aminoácido

MDFQVQIFSFLLISASVIMSRGQIVLSQSPAILSASPGEKVTLTCRASSSVSFMNWYQ
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97 L6 scFv VHL11S (SSS-S) H WCH2 WCH3

Secuencia de nucleótido

~~1609~~ 1621

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Secuencia de aminoácido

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PGK

98. P238 CH2 WCH3

a. P238S CH2 WCH3

1610
1622

Secuencia de nucleótido

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Secuencia de aminoácido

PELLGGSSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHN
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b. (SSS-S) H P238S CH2 WCH3

Secuencia de nucleótido

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Secuencia de aminoácido

DQEPKSSDKTHTSPPSSAPELLGGSSVFLFPPKPKDTLMISRTPEVTCVVVDVSHED
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1623

SNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVE
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HYTQKSLSLSPGK*

99 a CD16-6 bajo (ED)+NL+(SSS-S) H P238S CH2 WCH3

Secuencia de nucleótido

ggagcccaaatcttctgacaaaactcacacatcccccaccgtcctcagcacctgaactcctgggggggatcgtcagtcttcctcttccc
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Secuencia de aminoácido

MWQLLLPTALLLLVSAGMRTEDLPKAVVFLEPQWYRVLEKDSVTLKCQGAYSPE
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LYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

100 b CD16-6 bajo (ED)+HE4LP+ (SSS-S) H P238S CH2 WCH3

Secuencia de nucleótido

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1624

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Secuencia de aminoácido

MPACRLGPLAAALLLSLLLFGFTLVSGTGAMRTEDLPKAVVFLEPQWYRVLEKDS
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101 CD16-9 alto (ED) (SSS-S) H P238S CH2 CH3

a. CD16-9 alto (ED) NL+ (SSS-S) H P238S CH2 CH3

Secuencia de nucleótido

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1615
1625

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Secuencia de aminoácido

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CD16-9 alto (ED) +HE4LP+hIgG1 (SSS-S) H P238S CH2 CH3

Secuencia de nucleótido

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1626

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Secuencia de aminoácido

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TPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

102 a. CD16 ED bajo (líder nativo)

Secuencia de nucleótido

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Secuencia de aminoácido

mwqlllptalllvsagmrtedlpkavvflepqwyrvlekdsvtlkcqgayspednstqwfhneslissqassyfidaatvdds
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1615
1627

b CD16 ED bajo (HE4 lider)

Secuencia de nucleótido

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Secuencia de aminoácido

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103 a CD16 ED alto (lider nativo)

Secuencia de nucleótido

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a

Secuencia de aminoácido

1616
1628

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b CD16 ED alto (HE4 lider)

Secuencia de nucleótido

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Secuencia de aminoácido

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104. 2el2 scFv (SSS-S) H P238S CH2 WCH3-hCD80TM/CT

Secuencia de nucleótido

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1627
1629

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Secuencia de aminoácido

MDFQVQIFSFLLISASVIMSRGVDIVLTQSPASLAVSLGQRATISCRASESVEYYVTS
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105. 10A8 scFv (SSS-S) H P238SCH2 WCH3-hCD80TM/CT

Secuencia de nucleótido

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1630

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Secuencia de aminoácido

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106. 2H7scFvVHL11S (SSS-P) H P238SCH2CH3-hCD80TM/CT

Secuencia de nucleótido

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~~1619~~
1631

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Secuencia de aminoácido

MDFQVQIFSFLLISASVIIARGQIVLSQSPAILSASPGEKVTMTCRASSSVSYMHWY
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107 G19-4 scFv (SSS-P) H P238SCH2 WCH3-hCD80TM/CT

Secuencia de nucleótido

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FIG 10
1632

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Secuencia de aminoácido

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108 2el2 scFv IgA WH WCH2 T4 CH3-hCD80TM/CT

Secuencia de nucleótido

1621
1633

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Secuencia de aminoácido

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1622

1634

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Secuencia de nucleótido

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Secuencia de aminoácido

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110 2el2 scFv (SSS-P) H WCH2WCH3-mFADD-TM/CT

Secuencia de nucleótido

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Secuencia de aminoácido

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111 measp3-TM/CT

Secuencia de nucleótido

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1625

1637

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Secuencia de aminoácido

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112 2el2 scFv (SSS-P) H WCH2WCH3-mcasp3-TM/CT

Secuencia de nucleótido

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Secuencia de aminoácido

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113 2el2 scFv (SSS-P) H P238SCH2WCH3-mcasp3-TM/CT

Secuencia de nucleótido

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Secuencia de aminoácido

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1628
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114 mcasp8-TM/CT

Secuencia de nucleótido

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Secuencia de aminoácido

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115 2el2 scFv hIgGl (SSS-P) H WCH2 WCH3-mcasp8-TM/CT

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Secuencia de aminoácido

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116 2el2 scFv hTgGl (SSS-P) H P238SCH2 WCH3-mcasp8-TM/CT

Secuencia de nucleótido

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Secuencia de aminoácido

1632
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117 heasp3-TM/CT

Secuencia de nucleótido

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Secuencia de aminoácido

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118 2el2 scFv hIgGl (SSS-P) H WCH2 WCH3-hcasp3-TM/CT

Secuencia de nucleótido

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Secuencia de aminoácido

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123 2el2 scFv hIgG1 (SSS-P) H P238SCH2 WCH3-heasp3-TM/CT

Secuencia de nucleótido

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Secuencia de aminoácido

MDFQVQIFSFLLISASVIMSRGVDIVLTQSPASLAVSLGQRATISCRASESVEYYVTS
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1635
1698

124 hcasp8-TM/CT hCaspase8B

Secuencia de nucleótido

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Secuencia de aminoácido

DPSNMDFSRNLYDIGEQLDSEDLASLKFLSLDYIPQRKQEPIKDALMLFQRLQEKR
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hCaspase8C

1635
1649

Secuencia de nucleótido

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Secuencia de aminoácido

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Secuencia de nucleótido

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1639
1651

Secuencia de aminoácido

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Secuencia de nucleótido

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Secuencia de aminoácido

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1644
1653

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128. 2el2 scFv-hIgGl (SSS-P) H WCH2 WCH3-hFADD-TM/CT

Secuencia de nucleótido

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Secuencia de aminoácido

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Secuencia de nucleótido

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Secuencia de aminoácido

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Secuencia de nucleótido

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Secuencia de aminoácido

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Secuencia de nucleótido

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Secuencia de aminoácido

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Secuencia de nucleótido

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Secuencia de aminoácido

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Secuencia de nucleótido

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Secuencia de aminoácido

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134. 1D8 scFv hIgG1 (SSS-P) H WCH2 WCH3-mcasp3-TM/CT

Secuencia de nucleótido

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Secuencia de aminoácido

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135 1D8 scFv hIgG1 (SSS-P) H P238S CH2 WCH3-mcasp3-TM/CT

Secuencia de nucleótido

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Secuencia de aminoácido

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136. 1D8 scFv hIgGl (SSS-P) H WCH2 WCH3-mcasp8-TM/CT

Secuencia de nucleótido

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1669

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Secuencia de aminoácido

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Secuencia de nucleótido

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Secuencia de aminoácido

1654
1666

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138. 1D8 scFv hIgG1 (SSS-P) H WCH2 WCH3-hcasp3-TM/CT

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Secuencia de aminoácido

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Secuencia de nucleótido

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Secuencia de aminoácido

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Secuencia de nucleótido

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Secuencia de aminoácido

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Secuencia de nucleótido

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Secuencia de aminoácido

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Secuencia de nucleótido

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caacggaacccagatttatgtaattgatccagaaccgtgcccagattctgatcagccagttccctcaactccacctaccccatctccc
tcaactccacctaccccatctccctcatgctgccaccccccgactgtcactgcaccgaccggccctcgaggacctgctcttaggttca
gaagcgatcctcacgtgcacactgaccggcctgagagatgcctcaggtgtcaccttcacctggacgccctcaagtgggaagagc
gctgttcaaggaccacctgaccgtgacctctgtggctgctacagcgtgtccagtgtcctgccgggctgtgccgagccatggaacca
tgggaagaccttcacttgcactgctgcctaccccgagtccaagaccccgctaaccgccaccctctcaaaatccggaaacacattcc
ggcccgaggtccacctgctgccgccgccgtcggaggagctggccctgaacgagctggtgacgctgacgtgcctggcacgtggc
ttcagccccaaggatgtgctggttcgctggctgcaggggtcacaggagctgccccgcgagaagtacctgacttgggcatcccgg
caggagcccagccagggcaccaccaccttcgctgtgaccagcatactgcgcgtggcagccgaggactggaagaaggggggaca
ccttctcctgcatggtgggccacgaggccctgccgctggccttcacacagaagaccatcgaccgcttggcgggtaaacccaccca
tgtcaatgtgtctgttgtcatggcggaggtggacggcacctgctactgataatctaga

Secuencia de aminoácido

MACLGFQRHKAQLNLAARTWPCTLLFFLLFIPVFCKAMHVAQPAVVLASSRGIAS
FVCEYASPGKATEVRVTVLRQADSQVTEVCAATYMTGNELTFLDDSICTGTSSGN
QVNLTIQGLRAMDTGLYICKVELMYPPPYYLGIGNGTQIYVIDPEPCPDSDQPVPST
PPTPSPSTPPTPSPSCCHPRLSLHRPALEDLLLGSEAILTCTLTGLRDASGVTFTWTPS
SGKSAVQGPPDRDLCGCYSVSSVLPGCAEPWNHGKTFTCTAAYPESKTPLTATLS
KSGNTFRPEVHLLPPPSEELALNELVTLTCLARGFSPKDVLVRWLQGSQELPREKY
LTWASRQEPSQGTTTFAVTSILRVAAEDWKKGDTFSCMVGHEALPLAFTQKTIDR
LAGKPTHVNVSVVMAEVDGTCY

146. hCTLA4 IgA WH WCH2 T4CH3 (hCTLA4 IgAH IgACH2CH3)

Secuencia de nucleótido

atggcttgccttggatttcagcggcacaaggctcagctgaacctggctgccaggacctggccctgcactctcctgttttttcttctcttc
atccctgtcttctgcaaagcaatgcacgtggcccagcctgctgtggtactggccagcagccgaggcatcgccagctttglgtgtga
gtatgcatctccaggcaaagccactgaggtccgggtgacagtgcttcggcaggctgacagccaggtgactgaagtctgtgcggc
aacctacatgacggggaatgagttgaccttcctagaigattccatctgcacgggcacctccagtggaaatcaagtgaacctcactat
ccaaggactgagggccatggacacgggactctacatctgcaaggtggagctcatgtacccaccgccatactacctgggcatagg

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caacggaacccagatttatgtaattgatccagaaccgtgcccagattctgatcagccagttccctcaactccacctaccccatctccc
tcaactccacctaccccattccctcatgctgccacccccgactgtcactgcaccgaccggccctcgaggacctgctcttaggttca
gaagcgatcctcacgtgcacactgaccggcctgagagatgcctcaggtgtcaccttcacctggacgccctcaagtgggaagagc
gctgttcaaggaccacctgaccgtgacctctgtggctgctacagcgtgtccagtgtcctgccgggctgtgccgagccatggaacca
tgggaagaccttcacttgcactgctgcctaccccgagtccaagaccccgctaaccgccaccctctcaaaatccggaaacacattcc
ggcccgaggtccacctgctgccgccgccgtcggaggagctggccctgaacgagctggtgacgctgacgtgcctggcacgtggc
ttcagccccaaggatgtgctggttcgctggctgcaggggtcacaggagctgccccgcgagaagtacctgacttgggcatcccgg
caggagcccagccagggcaccaccaccttcgctgtgaccagcatactgcgcgtggcagccgaggactggaagaagggggaca
ccttctcctgcatggtgggccacgaggccctgccgctggccttcacacagaagaccatcgaccgcttggcgggtaaacccaccca
tgtcaatgtgtctgttgtcatggcggaggtggactgataatctaga

Secuencia de aminoácido

MACLGFQRHKAQLNLAARTWPCTLLFFLLFIPVFCKAMHVAQPAVVLASSRGIAS
FVCEYASPGKATEVRVTVLRQADSQVTEVCAATYMTGNELTFLDDSICTGTSSGN
QVNLTIQGLRAMDTGLYICKVELMYPPPYYLGIGNGTQIYVIDPEPCPDSDQPVPST
PPTPSPSTPPTPSPSCCHPRLSLHRPALEDLLLGSEAILTCTLTGLRDASGVTFTWTPS
SGKSAVQGPPDRDLCGCYSVSSVLPGCAEPWNHGKTFTCTAAYPESKTPLTATLS
KSGNTFRPEVHLLPPPSEELALNELVTLTCLARGFSPKDVLVRWLQGSQELPREKY
LTWASRQEPSQGTTTFAVTSILRVAAEDWKKGDTFSCMVGHEALPLAFTQKTIDR
LAGKPTHVNVSWMAEVD

147 hIGA WH WCH2 T18CH3

Secuencia de nucleótido

tgatcagccagttccctcaactccacctaccccatctccctcaactccacctaccccatctccctcatgctgccacccccgactgtca
ctgcaccgaccggccctcgaggacctgctcttaggttcagaagcgatcctcacgtgcacactgaccggcctgagagatgcctcag
gtgtcaccttcacctggacgccctcaagtgggaagagcgctgttcaaggaccacctgaccgtgacctctgtggctgctacagcgtg
tccagtgtcctgccgggctgtgccgagccatggaaccatgggaagaccttcacttgcactgctgcctaccccgagtccaagaccc
cgctaaccgccaccctctcaaaatccggaaacacattccggcccgaggtccacctgctgccgccgccgtcggaggagctggccc
tgaacgagctggtgacgctgacgtgcctggcacgtggcttcagccccaaggatgtgctggttcgctggctgcaggggtcacagg
agctgccccgcgagaagtacctgacttgggcatcccggcaggagcccagccagggcaccaccaccttcgctgtgaccagcata
ctgcgcgtggcagccgaggactggaagaagggggacaccttctcctgcatggtgggccacgaggccctgccgctggccttcac
acagaagaccatcgaccgcttggcgggtaaatgataatctaga

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Secuencia de aminoácido

DQPVPSTPPTPSPSTPPTPSPSCCHPRLSLHRPALEDLLLGSEAILTCTLTGLRDASGV
TFTWTPSSGKSAVQGPPDRDLCGCYSVSSVLPGCAEPWNHGKTFTCTAAYPESKT
PLTATLSKSGNTFRPEVHLLPPPSEELALNELVTLTCLARGFSPKDVLVRWLQGSQ
ELPREKYLTWASRQEPSQGTTTFAVTSILRVAAEDWKKGDTFSCMVGHEALPLAF
TQKTIDRLAGK

150 G19-4 scFv (SSS-P) WH WCH2 WCH3-hCD80TMCT

Secuencia de nucleótido

aagcttatggattttcaagtgcagattttcagcttcctgctaatcagtgcttcagtcataatgtccagaggagtcgacatccagatgaca
cagactacatcctccctgtctgcctctctgggagacagagtcaccatcagttgcagggcaagtcaggacattcgcaattatttaaact
ggtatcagcagaaaccagatggaactgttaaactcctgatctactacacatcaagattacactcaggagtcccatcaaggttcagtg
gcagtgggtctggaacagattattctctcaccattgccaacctgcaaccagaagatattgccacttacttttgccaacagggtaatac
gcttccgtggacgttcggtggaggcaccaaactggtaaccaaacgggagctcggtggcggtggctcgggcggtggtgggtcgg
gtggcggcggatctatcgatgaggtccagctgcaacagtctggacctgaactggtgaagcctggagcttcaatgtcctgcaaggc
ctctggttactcattcactggctacatcgtgaactggctgaagcagagccatggaaagaaccttgagtggattggacttattaatccat
acaaaggtcttactacctacaaccagaaattcaagggcaaggccacattaactgtagacaagtcatccagcacagcctacatggag
ctcctcagtctgacatctgaagactctgcagtctattactgtgcaagatctgggtactatggtgactcggactggtacttcgatgtctgg,
ggcgcagggaccacggtcaccgtctcctctgatctggagcccaaatcttctgacaaaactcacacatccccaccgtccccagcac
ctgaactcctgggggggaccgtcagtcttcctcttccccccaaaacccaaggacaccctcatgatctcccggacccctgaggtcaca
tgcgtggtggtggacgtgagccacgaagaccctgaggtcaagttcaactggtacgtggacggcgtggaggtgcataatgccaag
acaaagccgcgcgggaggagcagtacaacagcacgtaccgtgtggtcagcgtcctcaccgtcctgcaccaggactggctgaatgg
caaggagtacaagtgcaaggtctccaacaaagccctcccagccccatcgagaaaaccatctccaaagccaaagggcagccccc
gagaaccacaggtgtacaccctgccccatcccgggatgagctgaccaagaaccaggtcagcctgacctgcctggtcaaaggct
tctatcccagcgacatcgccgtggagtgggagagcaatgggcagccggagaacaactacaagaccacgcctcccgtgctggac
tccgacggctccttcttcctctacagcaagctcaccgtggacaagagcaggtggcagcaggggaacgtcttctcatgctccgtgat
gcatgaggctctgcacaaccactacacgcagaagagcctctccctgtctccgggtaaagcggatccttcgaacctgctcccatcct
gggccattaccttaatctcagtaaatggaatttttgtgatatgctgcctgacctactgctttgccccaagatgcagagagagaaggag
gaatgagagattgagaagggaaagtgtacgccctgtataaatcgat

Secuencia de aminoácido

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MDFQVQIFSFLLISASVIMSRGVDIQMTQTTSSLSASLGDRVTISCRASQDIRNYLN
WYQQKPDGTVKLLIYYTSRLHSGVPSRFSGSGSGTDYSLTIANLQPEDIATYFCQQ
GNTLPWTFGGGTKLVTKRELGGGGSGGGGSGGGGSIDEVQLQQSGPELVKPGAS
MSCKASGYSFTGYIVNWLKQSHGKNLEWIGLINPYKGLTTYNQKFKGKATLTVD
KSSSTAYMELLSLTSEDSAVYYCARSGYYGDSDWYFDVWGAGTTVTVSSDLEPK
SSDKTHTSPPSPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKF
NWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKA
LPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVICGFYPSDIAVEWESN
GQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQ
KSLSLSPGKADPSNLLPSWAITLISVNGIFVICCLTYCFAPRCRERRRNERLRRESVR
PV

HCD16bajoFL+NL

Secuencia de nucleótido

aagcttgccgccatgtggcagctgctcctcccaactgctctgctacttctagtttcagctggcatgcggactgaagatctcccaaagg
ctgtggtgttcctggagcctcaatggtacagggtgctcgagaaggacagtgtgactctgaagtgccagggagcctactcccctgag
gacaattccacacagtggtttcacaatgagagcctcatctcaagccaggcctcgagctacttcattgacgctgccacagtcgacgac
agtggagagtacaggtgccagacaaacctctccaccctcagtgacccggtgcagctagaagtccatatcggctggctgttgctcca
ggcccctcggtgggtgttcaaggaggaagaccctattcacctgaggtgtcacagctggaagaacactgctctgcataaggtcacat
atttacagaatggcaaaggcaggaagtattttcatcataattctgacttctacattccaaaagccacactcaaagacagcggctcctac
ttctgcaggggcttgttgggagtaaaaatgtgtcttcagagactgtgaacatcaccatcactcaaggtttggcagtgtcaaccatctc
a. tcattctttccacctgggtaccaagtctctttctgcttggtgatggtactccttttgcagtggacacaggactatatttctctgtgaaga
caaacattcgaagctcaacaagagactggaaggaccataaatttaaatggagaaaggaccctcaagacaaatgaccc

Secuencia de aminoácido

MWQLLLPTALLLLVSAGMRTEDLPKAVVFLEPQWYRVLEKDSVTLKCQGAYSPE
DNSTQWFHNESLISSQASSYFIDAATVDDSGEYRCQTNLSTLSDPVQLEVHIGWLL
LQAPRWVFKEEDPIHLRCHSWKNTALHKVTYLQNGKGRKYFHHNSDFYIPKATL
KDSGSYFCRGLVGSKNVSSETVNITITQGLAVSTISSFFPPGYQVSFCLVMVLLFAV
DTGLYFSVKTNIRSSTRDWKDHKFKWRKDPQDK

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De lo anterior, se apreciará que, aunque modalidades específicas de la invención se han descrito aquí para propósito de la ilustración, varias modificaciones se pueden hacer sin desviarse del espíritu y alcance de la invención De acuerdo con esto no se espera que la presente invención se limite por las reivindicaciones adjuntas

Todas las patentes, solicitud de patente, publicaciones, artículos científicos, sitios web, y otros documentos y materiales referenciados o mencionados aquí son indicadores de los niveles de experiencia de aquellos expertos en la técnica a los cuales pertenece la invención, y cada uno de tales documentos referidos y material se incorpora aquí como referencia para el mismo alcance como sistemas este se hubiera incorporado como referencia en su totalidad individualmente o establecido aquí completamente Adicionalmente, todas las reivindicaciones en esta solicitud, y todas las solicitudes de prioridad, que incluyen pero no están limitados a las reivindicaciones originales, se incorporan aquí completamente dentro y forman parte de, la descripción escrita de la invención Los solicitantes reservan el derecho de incorporar físicamente dentro de esta especificación cualquiera y todos los materiales e información de cualquiera de tales patentes, solicitudes, publicaciones, artículos científicos, sitios web, información

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disponible electrónicamente, y otros materiales de referencia o documentos Los solicitantes reservan el derecho a incorporar físicamente dentro de cualquier parte de este documento, que incluye cualquier parte de la descripción escrita las reivindicaciones referidas anteriormente que incluyen pero no están limitadas a cualesquier reivindicaciones originales

Los métodos específicos y composiciones descritas aquí son representativos de las modalidades preferidas y son de ejemplo y no se pretenden que sean limitaciones del alcance de la invención Otros objetos, aspectos, y modalidades ocurrirán por aquellos expertos en la técnica luego de la consideración de esta descripción, y se abarcan dentro del espíritu de la invención como se define mediante el alcance de las reivindicaciones Será fácilmente evidente para un experto en la técnica que varias sustituciones y modificaciones se pueden hacer a la invención descrita aqui sin apartarse del alcance y el espíritu de la invención La invención descrita en forma ilustrativa aquí adecuada se puede practicar en la ausencia de cualquier elemento o elementos, o limitación o limitaciones, que no se describen específicamente aquí como esenciales, así por ejemplo, en cada instancia aquí, en las modalidades o ejemplos de la presente invención, cualquiera de los términos "que comprende", "que consiste esencialmente de", y "que consiste

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de" se puede reemplazar con cualquiera de otros dos términos en la descripción También los términos "que comprende", "que incluye", "que contiene", etc , son para ser leídos ampliamente y sin limitación Los métodos y procesos ilustrativos descritos aquí adecuadamente se pueden practicar en diferentes ordenes o etapas, y ellos no son necesariamente restrictivos a los órdenes o etapas indicados aquí o en las reivindicaciones También como se utiliza aquí y en las reivindicaciones adjuntas las formas singulares "un", "uno", y "el" incluyen referencia plural a menos que el contexto claramente dicte lo contrario Así, por ejemplo, a una "célula huésped" incluye una pluralidad (por ejemplo, un cultivo o población) de tales células huésped y así sucesivamente De acuerdo con las circunstancias la patente se puede interpretar por ser limitada a los ejemplos específicos o modalidades o métodos específicamente descritos aquí Bajo ninguna circunstancia se puede interpretar como limitada por cualquier declaración hecha por cualquier examinador o por cualquier otro funcionario o empleado de la oficina de marcas y patentes a amenos que tal declaración sea específica y sin calificación o reserva expresa adoptada en una respuesta escrita por los solicitantes

Los términos y expresiones que se han empleado se utilizan como términos de descripción y no de limitación, y no hay intento en el uso de tales términos y expresiones para

excluir cualquier equivalente de las características mostradas y descritas o porciones de esta Pero se reconoce que varias modificaciones son posibles dentro del alcance de la invención como se reivindica Así, se entenderá que la presente invención ha sido descrita específicamente mediante métodos preferidos y características opcionales, modificaciones o variaciones de los conceptos aquí descritos se pueden reclasificar por aquellos expertos en la técnica, y que tales modificaciones y variaciones se consideran están dentro del alcance de esta invención como se define por las reivindicaciones adjuntas

La invención se ha descrito ampliamente y en forma general aquí Cada una de las especies más estrechas y las agrupaciones subgenéricas caen dentro de la descripción genérica también forman parte de la invención Esto incluye la descripción de la invención con una condición o limitación negativa de remover cualquier materia objeto del género, con respecto de si o no el material es excisido se describe específicamente aquí Por ejemplo, la materia objeto de la presente invención puede opcionalmente excluir cualquier materia objeto o secuencias descritas o incluidas en la solicitud relacionada publicada como U S S N 2003/0118592A1 de junio 26, 2003 (y la lista de secuencia de la ID de SEC No 1-427 publicada por la USPTO), por Ledbetter et al Titulada "Binding Domain-Immunoglobulin Fusion Proteins"

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Otras modalidades están dentro de las siguientes reivindicaciones Adicionalmente, en donde las características o aspectos de la invención se describen en términos de grupos Markush, aquellos expertos en la técnica reconocerán que la invención también se describe en términos de cualquier miembro individual o subgrupo de miembros del grupo Markush

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REIVINDICACIONES

1 Una proteína de cadena sencilla de ocurrencia no natural que comprende i) un primer polipéptido que tiene un polipéptido de dominio de unión capaz de unir una molécula objetivo, dicho polipéptido de dominio de unión comprende una región variable de cadena pesada, dicha región variable de cadena pesada comprende una sustitución de aminoácido o agotamiento en uno o más residuo de aminoácido, ii)un segundo polipéptido que comprende una región de conexión adherida a dicho primer polipéptido, y iii) un tercer polipéptido que comprende un polipéptido de región constante de cadena pesada de inmunoglobulina truncada N-terminal adherida al segundo polipéptido, en donde dicha proteína de cadena sencilla de ocurrencia no natural es capaz de al menos una actividad inmunológica

2 Una proteína de la reivindicación 1 en donde dicho polipéptido de dominio de unión Fv una cadena sencilla

3 Una proteína de la reivindicación 1 en donde la sustitución o eliminación de uno o más aminoácidos en dicha región variable de cadena pesada es efectiva para incrementar la expresión o estabilidad de dicha

proteína relacionada con una proteína sin dicho agotamiento o sustitución

4 Una proteína de la reivindicación 1 en donde dicho polipéptido de dominio de unión comprende un polipéptido de región variable de cadena liviana de inmunoglobulina y un polipéptido de región variable de cadena pesada de inmunoglobulina

5 Una proteína de la reivindicación 4 que comprende además un segundo polipéptido de dominio de unión capaz de unir una segunda molécula objetivo, dicho segundo polipéptido de dominio de unión comprende un polipéptido de región variable de cadena liviana de inmunoglobulina y un polipéptido de región variable de cadena pesada de inmunoglobulina

6 Una proteína de la reivindicación 5 en donde la primer molécula objetivo y la segunda molécula objetivo son diferentes

7 Una proteína de la reivindicación 5 en donde la primer molécula objetivo y la segunda molécula objetivo son la misma

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8 Una proteína de la reivindicación 1 en donde dicho polipéptido de dominio de unión Fv es una cadena sencilla que comprende una o más sustituciones de aminoácido en las posiciones 9, 10, 11, 12, 108, 110, 112 en dicha región variable de cadena pesada

9 Una proteína de la reivindicación 1 en donde dicho polipéptido de dominio de unión Fv es una cadena sencilla que comprende una sustitución de aminoácido en la posición 11 en dicha región variable de cadena pesada

10 Una proteína de la reivindicación 9 en donde el aminoácido sustituido para el aminoácido en la posición de 11 de la región variable de cadena pesada Fv cadena sencilla se selecciona de serina, treonina, cisteína, tirosina, asparagina, glutamina, ácido aspártico, ácido glutámico, lisina, arginina, e histidina

11 Una proteína de la reivindicación 9 en donde una leucina en la posición 11 de la región de variable de cadena pesada Fv de cadena sencilla se reemplaza con un aminoácido diferente de serina

12 Una proteína de la reivindicación 9 en donde la leucina se reemplaza por serina en la posición 11, y en donde

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dicha proteína es capaz de citotoxicidad mediada por célula dependiente de anticuerpo y fijación de complemento, y es capaz de unir dicha molécula objetivo reduciendo un número de células objetivo

13 Una proteína de la reivindicación 9 en donde la leucina se reemplaza por des-leucina en la posición 11

14 Una proteína de la reivindicación 12 que tiene una expresión recombinante incrementada o estable relacionada con dicha proteina no tiene una sustitución de aminoácido en la posición 11

15 Una proteína de la reivindicación 14 en donde la expresión de dicha proteína tiene una sustitución de aminoácido en la posición 11 es 10-100 veces mayor que dicha proteína sin una sustitución en la posición 11

16 Una proteína de la reivindicación 14 en donde dicha expresión está en células de mamífero

17 Una proteína de la reivindicación 1 en donde dicho polipéptido de dominio de unión Fv es una cadena sencilla y el aminoácido en la posición 11 de la región variable de cadena pesada de dicha Fv de cadena sencilla se ha eliminado

18 Una proteína de la reivindicación 1 en donde dicho polipéptido de dominio de unión Fv es una cadena sencilla y dicho polipéptido de dominio de unión comprende una región variable de cadena liviana en donde dicha región variable de cadena liviana tiene una eliminación de aminoácido o sustitución en una o más de las posiciones de aminoácido 12, 80, 81, 83, 105,106 y 107

19 Una proteína de la reivindicación 18 en donde el aminoácido de la posición 106 se ha sustituido o eliminado

20 Una proteína de la reivindicación 2 en donde dicho polipéptido de dominio de unión se une a un antígeno de tumor

21 Una proteína de la reivindicación 2 en donde dicho polipéptido de dominio de unión se une a un antígeno o a una célula detectora inmune

22 Una proteína de la reivindicación 2 en donde dicho polipéptido de dominio de unión se une a un antígeno de célula de cáncer

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23 Una proteína de la reivindicación 22 en donde dicho antígeno de célula de cáncer es un antígeno de superficie

24 Una proteína de la reivindicación 22 en donde dicho antígeno de célula de cáncer es un antígeno intracelular

25 Una proteína de la reivindicación 1 en donde dicho polipéptido de dominio de unión se une a un antígeno de célula B

26 Una proteína de la reivindicación 25 en donde dicho antígeno de célula B se selecciona a partir de CD19, CD20, CD22, CD37, CD40, CD80 Y CD86

27 Una proteína de la reivindicación 2 en donde dicha Fv de cadena sencilla se une a un antígeno de célula B

28 Una proteína de la reivindicación 27 en donde dicho antígeno de célula B se selecciona de CD19, CD20, CD22, CD37, CD40, CD80 Y CD86

29 Una proteína de la reivindicación 28 en donde dicho Fv de cadena sencilla se selecciona de Fv de cadena

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sencilla HD37, Fv de cadena sencilla 2H7, Fv de cadena sencilla G28-1, y Fv de cadena sencilla 4 4 220

30 Una proteína de la reivindicación 2 en donde dicho Fv de cadena sencilla se selecciona de Fv de cadena sencilla HD37, Fv de cadena sencilla 2H7, Fv de cadena sencilla G28-1, FC2-2, UCHL-1, 5B9, LÑ6, 10*8, 2e12, 40 2 36, G19-4, 1D8, y Fv de cadena sencilla 4 4 220

31 Una proteína de la reivindicación 1 en donde dicho polipéptido de dominio de unión es un scFv que se une a un antígeno de diferenciación de célula B

32 Una proteína de la reivindicación 31 en donde dicho antigeno de célula B se selecciona de CD19, CD20, CD22, CD37, y CD40

33 Una proteína de la reivindicación 1 en donde dicho polipéptido de dominio de unión se une a un objetivo seleccionado de CD2, CD3, CD4, CD5, CD6, CD8, CD10, CD11b, CD14, CD19, CD20, CD21, CD22, CD23, CD24, CD25, CD28, CD30, CD37, CD40, CD43, CD50 (ICAM3), CD54 (ICAM1), CD56, CD69, CD80, CD86, CD134 (OX40), CD137 (41BB), CD152 (CTLA-4), CD153 (ligando CD30), CD154 (ligando CD40), ICOS, L6, B7-H1, y HLA clase II

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34 Una proteína de la reivindicación 1 en donde dicha proteína es capaz de formar un complejo que comprende dos o más de dichas proteínas

35 Una proteína de la reivindicación 34 en donde dicho complejo es in dímero

36 Una proteína de la reivindicación 1 en donde dicha proteina es un monómero

37 Una proteína de la reivindicación 1 acoplada a una droga, toxina, inmunomodulador, efector de polipéptido, isótopo, etiqueta o porción efectora

38 Una proteína de la reivindicación 1 en donde dicha al menos actividad inmunológica se selecciona de citotoxicidad mediada por célula dependiente de anticuerpo, fijación de complemento, inducción de apoptosis, inducción una o más señales activas biológicas, inducción de una o más células efectoras inmunes, activación de diferenciación celular, activación celular, liberación de una o más moléculas activas biológicamente, y neutralización de un agente infeccioso o toxina

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39 Una proteína de la reivindicación 38 en donde dicha actividad inmunológica comprende dos actividades inmunológicas seleccionadas de citotoxicidad mediada por célula dependiente de anticuerpo, fijación de complemento, inducción de apoptosis, inducción una o más señales activas biológicamente, inducción de una o más células efectoras inmunes, activación de diferenciación celular, activación celular, liberación de una o más moléculas activas biológicamente, y neutralización de un agente infeccioso o toxina

40 Una proteína de la reivindicación 38 que es capaz de inducción de señales activas biológicamente por activación o inhibición de una o más moléculas seleccionadas de proteína quinasa, proteína fosfatas, G-proteínas, nucleótidos cíclicos u otros segundos mensajeros, canales de ión, y componentes de trayectoria secretora, o que es capaz de inducción de una o más células efectoras inmunes seleccionadas de células NK, monocitos, macrófagos, células B, células T, mastocitos, neutrófilos, eosinófilos, y basofilos

41 Una proteína de la reivindicación 40 en donde dicha inducción de una o más células efectoras inmunes conduce a citotoxicidad mediada por célula dependiente

de anticuerpo o la liberación de una o más moléculas activas biológicamente

42 Una proteína de la reivindicación 38 que es capaz de activación celular, en donde dicha activación conduce a cambios en la actividad transcripcional celular

43 Una proteína de la reivindicación 42 en donde dicha actividad transcripcional celular se incremeta

44 Una proteína de la reivindicación 42 en donde dicha actividad transcripcional celular se reduce

45 Una proteína de la reivindicación 38 en donde dicha una o más moléculas activas biológicamente es una proteasa

46 Una proteína de la reivindicación 38 en donde dicha una o más moléculas activas biológicamente es una citoquina

47 Una proteína de la reivindicación 46 en donde dicha citoquina se selecciona a partir de monoquinas, linfoquinas, quemoquinas, factores de crecimiento, factores estimuladores de colonia, interferones, e interleuquinas

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48 Una proteína de la reivindicación 38 que es capaz de neutralización de un agente infeccioso, en donde dicho agente infeccioso es una bacteria, un virus, un parásito, p un hongo

49 Una proteína de la reivindicación 38 que es capaz de neutralización de una toxina, en donde dicha toxina se selecciona a partir de endotoxinas y exotoxinas

50 Una proteína de la reivindicación 38 que es capaz de neutralización de una toxina, en donde dicha toxina es una exotoxina seleccionada de toxina ántrax, toxina cólera, toxina difteria, toxina perthusis, toxina labile-calor E coli LT, toxina estable al calor E Coli ST, toxina shiga Pseudomofaas Exotoxina A, toxina botilinum, toxina tetanus, toxina Bordetella pertussis AC, y Bacillus anthracis EF

51 Una proteína de la reivindicación 38 que es capaz de neutralización de una toxina, en donde dicha toxina es una endotoxina seleccionada de saxitoxinas, tetrodotoxina, toxinas hongo, aflatoxinas, alcaloides pirrolizidina, fitofemagglutininas, y grayanotoxinas

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52 Una proteína de la reivindicación 1 en donde dicha proteína es capaz de unirse a un objetivo intracelular para efectuar una función celular

53 Una proteína de la reivindicación 1 en donde dicho polipéptido de dominio de unión comprende una región variable de cadena liviana adherida a dicha región variable de cadena liviana mediante un ligador de dominio de unión en donde dicho ligador de dominio de unión comprende uno o más péptidos que tiene una secuencia Gly-Gly-Gly-Gly-Ser

54 Una proteína de la reivindicación 53 que comprende tres péptidos Gly-Gly-Gly-Gly-Ser

55 Una proteína de la reivindicación 1 en donde dicho polipéptido de dominio de unión comprende región variable de inmunoglobulina tipo silvestre o construida por ingeniería obtenida de las especies seleccionadas a partir de humanos, murina, rata, cerdo, y mono

56 Una proteína de la reivindicación 1 en donde dicho polipéptido de dominio de unión comprende una región variable de inmunoglobulina humanizada

57 Una proteína de la reivindicación 2 en donde dicho polipéptido de región constante de cadena pesada de inmunoglobulina truncado N-terminal comprende un polipéptido de región constante IgG CH2 adherido a un polipéptido de región constante IgG CH3 de cadena pesada de inmunoglobulina

58 Una proteína de la reivindicación 2 en donde dicho polipéptido de región constante de cadena pesada de inmunoglobulina truncado N-terminal consiste esencialmente de un polipéptido de región constante IgG CH2 adherido a un polipéptido de región constante IgG CH3 de cadena pesada de inmunoglobulina

59 Una proteína de la reivindicación 2 en donde dicho polipéptido de región constante de cadena pesada de inmunoglobulina truncado N-terminal comprende un polipéptido de región constante IgG CH2 adherido a un polipéptido de región constante IgG CH3 de cadena pesada de inmunoglobulina

60 Una proteína de la reivindicación 2 en donde dicho polipéptido de región constante de cadena pesada de inmunoglobulina truncado N-terminal consiste esencialmente de un polipéptido de región constante IgG

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CH2 adherido a un polipéptido de región constante IgG CH3 de cadena pesada de inmunoglobulina

61 Una proteína de la reivindicación 1 en donde dicho polipéptido de dominio de unión es un Fv de cadena sencilla que comprende al menos una porción de una región constante humana

62 Una proteína de la reivindicación 2 en donde dicho polipéptido de dominio de unión es un Fv de cadena sencilla que comprende al menos una porción de una región constante humana

63 Una proteína de la reivindicación 1 en donde dicha región de conexión comprende una región de pivote de ocurrencia natural seleccionada de un pivote humano o porción de este, pivote IgG humano o una porción de este, pivote IgA humano o una porción de este, pivote IgE humano o una porción de este, región de pivote camelida o una porción de esta, región de pivote de llama IgG1 o una porción de esta, región de pivote de tiburón nodriza o porción de esta, y región de pivote de pez rata o una porción de esta

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64 Una proteína de la reivindicación 1 en donde dicha región de conexión comprende un pivote IgE humano o una porción de este

65 Una proteína de la reivindicación 1 en donde dicha región de conexión comprende una región de pivote IgG1, IgG2, IgG3 o IgG4 que tiene cualquier residuo de cisteína uno o cero

66 Una proteína de la reivindicación 1 en donde dicha región de conexión comprende una región de pivote IgG4 que tiene entre cero y dos residuos de cisteína

67 Una proteína de la reivindicación 1 en donde dicha región de conexión comprende una región de pivote de inmunoglobulina IgG1 humana tipo silvestre

68 Una proteína de la reivindicación 1 en donde dicha región de conexión comprende un sitio de glicosilación

69 Una proteína de la reivindicación 1 en donde dicha región de conexión no tiene residuos de cisteína capaces de formar enlaces de disulfuro

70 Una proteína de la reivindicación 1 en donde dicha región de conexión tiene un residuo cisteína

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71 Una proteína de la reivindicación 1 en donde dicha región de conexión comprende un polipéptido de región de pivote de inmunoglobulina tipo silvestre mutada que comprende no más de un residuo de cisteína

72 Una proteína de la reivindicación 1 en donde dicha región de conexión se altera de tal forma que dicha proteína tiene una capacidad reducida para dimerizar

73 Una proteína de la reivindicación 1 en donde dicha región de conexión comprende tres residuos de cisteína y un residuo de prolina, en donde uno o más de dichos residuos de cisteína se eliminan o sustituyen y dicho residuo de prolina se sustituye o elimina

74 Una proteína de la reivindicación 1 en donde dicha región de conexión comprende un polipéptido de región de pivote de inmunoglobulina tipo silvestre mutada que comprende primero, segundo, y tercero residuos de cisteína, en donde dicho primer residuo de cisteína es N-terminal a dicha segunda cisteína y dicha segunda cisteína es N-terminal a dicha tercera cisteína en donde dicho primer residuo de cisteína se sustituye o elimina

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75 Una proteína de la reivindicación 74 en donde polipéptido de región de pivote tipo silvestre es de IgG1 humano

76 Una proteína de cadena sencilla de ocurrencia no natural comprende i) un primer polipéptido que tiene un polipéptido de dominio de unión capaz de unirse a una molécula objetivo, dicho polipéptido de dominio de unión comprende una región variable de cadena pesada que comprende uno o más eliminaciones de aminoácidos o sustitución en las posiciones 9, 10, 11, 12, 108, 110, 112, ii)un segundo polipéptido que comprende una región de conexión adherida a dicho primer polipéptido, y iii) un tercer polipéptido que comprende un polipéptido de región constante de cadena pesada de inmunoglobulina truncado N-terminal adherida al segundo polipéptido, en donde dicha proteína de cadena sencilla de ocurrencia no natural es capaz de al menos una actividad inmunológica, y en donde dicha proteina tiene una expresión recombinante incrementada o estabilidad relativa a dicha proteína no tiene una eliminación o sustitución de aminoácido

77 Una proteína Fv de cadena sencilla de ocurrencia no natural que comprende i) un primer polipéptido que tiene un polipéptido de dominio de unión capaz de

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unirse a una molécula objetivo en una célula objetivo, dicho polipéptido de dominio de unión comprende una región variable de cadena pesada en donde la leucina en la posición 11 en la primer región de estructura en la región variable de cadena pesada se elimina o sustituye con otro aminoácido, ii) un segundo polipéptido que comprende una región de conexión adherida a dicho primer polipéptido, y iii) un tercer polipéptido que comprende un polipéptido de región constante de cadena pesada de inmunoglobulina truncada N-terminal adherida al segundo polipéptido, en donde dicha proteína de cadena sencilla de ocurrencia no natural (1) es capaz de unirse a dicha molécula objetivo, y (2) es capaz de citotoxicidad mediada por célula dependiente de anticuerpo y fijación de complemento, y (3) es capaz de reducir el número de células objetivo

78 Una proteína de la reivindicación 77 que comprende además una sustitución o eliminación del aminoácido en la posición 10 en la primera región de estructura en la región variable de cadena pesada

79 Una proteína de la reivindicación 77 en donde la sustitución de aminoácido en la posición 11 es efectiva para incrementar la expresión o estabilidad de dicha proteína Fv de cadena sencilla relacionada con una

proteína Fv de cadena sencilla sin dicha eliminación o sustitución

80 Una proteína de la reivindicación 77 en donde el número de residuos es el índice EU de acuerdo con Kabat

81 Una proteína de la reivindicación 77 en donde dicha región de conexión comprende una prolina y primeros, segundos, y terceros residuos de cisteína, en donde dicho primer residuo de cisteína es N-terminal a dicho segunda cisteína y dicha segunda cisteína es N-terminal a dicha tercera cisteína dicho primer residuo de cisteína es N-terminal a dicho residuo de prolina

82 Una proteína de la reivindicación 77 en donde dicha región de conexión comprende un pivote IgA o una porción de este

83 Una proteína de la reivindicación 81 en donde dicha proteína de cadena sencilla comprende un dominio de unión Fv de cadena sencilla de un hibridoma 2H7, en donde dicho segundo residuo de cisteína se reemplaza por serina y dicho residuo prolina se reemplaza por serina en la región de conexión, y dicha región constante de cadena pesada comprende dominios CH2 y CH3 de IgG_1

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84 Una proteína de la reivindicación 82 en donde dicha proteína de cadena sencilla comprende un dominio de unión Fv de cadena sencilla de un hibridoma 2H7, dicha región de conexión comprende un pivote IgA de murina o porción de este, y dicha región constante de cadena pesada comprende dominios CH2 y CH3 de IgA de murina, en donde dicho CH3 comprende una eliminación o sustitución en cuatro aminoácidos que da la región constante de cadena pesada IgA incapaz de asociar con un polipéptido de cadena J

85 Una proteína de la reivindicación 81 en donde dicha proteína de cadena sencilla comprende un dominio de unión Fv de cadena sencilla 2H7, en donde dicho primer, segundo, y tercero residuos de cisteína en la región de conexión se reemplazan por serina, y dicho residuo de prolina en la región de conexión se reemplaza por serina, dicha región constante de cadena pesada comprende dominios CH2 y CH3 de IgG_1 en donde la lisina se reemplaza por serina en la posición 322 en dicha región CH2

86 Una proteína de la reivindicación 81 en donde dicha proteína de cadena sencilla comprende un dominio de unión Fv de cadena sencilla de un hibridoma 2H7, en

donde la región de conexión dicho segundo y tercer residuo de cisteína se reemplaza por serina, y dicho residuo de prolina se reemplaza por serina, dicha región constante de cadena pesada comprende los dominios CH2 y CH3 de IgG_1 en donde la lisina se reemplaza por serina en la posición 322 en dicha región CH2

87 Una proteína de la reivindicación 81 en donde dicha proteína de cadena sencilla comprende un dominio de unión Fv de cadena sencilla de un hibridoma 2H7, en donde la región de conexión dicho primero, segundo, y tercer residuo de cisteína se reemplaza por serina, y dicho residuo de prolina se reemplaza por serina, dicha región constante de cadena pesada comprende los dominios CH2 y CH3 de IgG_1 en donde la prolina se reemplaza por serina en la posición 331 en dicha región CH2

88 Una proteína de la reivindicación 81 en donde dicha proteína de cadena sencilla comprende un dominio de unión Fv de cadena sencilla de un hibridoma 2H7, en donde la región de conexión dicho segundo, y tercer residuo de cisteína se reemplaza por serina, y dicho residuo de prolina se reemplaza por serina, dicha región constante de cadena pesada comprende los

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dominios CH2 y CH3 de IgG_1 en donde la prolina se reemplaza por serina en la posición 331 en dicha región CH2

89 Una proteína de la reivindicación 81 en donde dicha proteína de cadena sencilla comprende un dominio de unión Fv de cadena sencilla 2H7, en donde en la región de conexión dicho primero, segundo, y tercero residuos de cisteína se reemplazan por serina, y dicho residuo de prolina se reemplaza por serina, dicha región constante de cadena pesada comprende los dominios CH2 y CH3 de IgG_1 en donde la treonina se reemplaza por asparagina en la posición 256 en dicha región CH2

90 Una proteína de la reivindicación 81 en donde dicha proteína de cadena sencilla comprende un dominio de unión Fv de cadena sencilla 2H7, en donde en la región de conexión dicho primero, segundo, y tercero residuos de cisteína se reemplazan por serina, y dicho residuo de prolina se reemplaza por serina, dicha región constante de cadena pesada comprende los dominios Fv CH2 y CH3 de cadena sencilla 2H7 de IgG_1 en donde en el dominio CH2 la arginina se reemplaza por glutamina en la posición 255, la treonina se reemplaza por asparagina en la posición 256, la prolina se reemplaza

por alanina en la posición 25, y el ácido glutámico se reemplaza por lisina en la posición 258

91 Una proteína de la reivindicación 81 en donde dicha proteína de cadena sencilla comprende un dominio de unión Fv de cadena sencilla de un hibridoma 2H7, en donde en la región de conexión dicho primero, segundo, y tercero residuos de cisteína se reemplazan por serina, y dicho residuo de prolina se reemplaza por serina, dicha región constante de cadena pesada comprende los dominios CH2 y CH3 de IgG_1 en donde la lisina se reemplaza por glutamina en la posición 290 en dicha región CH2

92 Una proteína de la reivindicación 81 en donde dicha proteína de cadena sencilla comprende un dominio de unión Fv de cadena sencilla de un hibridoma 2H7, en donde en la región de conexión dicho primero, segundo, y tercero residuos de cisteína se reemplazan por serina, y dicho residuo de prolina se reemplaza por serina, dicha región constante de cadena pesada que comprende los dominios CH2 y CH3 de IgG_1 en donde la alanina se reemplaza por prolina en la posición 339 en dicha región CH2

93 Una proteína de la reivindicación 81 en donde dicha proteína de cadena sencilla comprende un dominio de unión Fv de cadena sencilla de un hibridoma G28-1, en donde en la región de conexión dicho primero, segundo, y tercero residuos de cisteína se reemplazan por serina, y dicho residuo de prolina se reemplaza por serina, dicha región constante de cadena pesada comprende dominios CH2 y CH3 de IgG_1

94 Una proteína de la reivindicación 81 en donde dicha proteína de cadena sencilla comprende un dominio de unión Fv de cadena sencilla de un hibridoma G28-1, en donde en la región de conexión dicho segundo, y tercero residuos de cisteína se reemplazan por serina, y dicho residuo de prolina se reemplaza por serina, dicha región constante de cadena pesada comprende dominios CH2 y CH3 de IgG_1

95 Una proteína de la reivindicación 81 en donde dicha proteina de cadena sencilla comprende un dominio de unión Fv de cadena sencilla de un hibridoma G28-1, en donde en la región de conexión dicho segundo residuo de cisteína se reemplaza por serina, y dicho residuo de prolina se reemplaza por serina, dicha región constante de cadena pesada comprende dominios CH2 y CH3 de IgG_1

96 Una proteína de la reivindicación 81 dicha proteína de cadena sencilla comprende un dominio de unión Fv de cadena sencilla de un hibridoma G28-1, en donde en la región de conexión dicho primero, y segundo residuos de cisteína se reemplazan por serina, dicha región constante de cadena pesada comprende dominios CH2 y CH3 de IgG_1

97 Una proteína de la reivindicación 81 dicha proteína de cadena sencilla comprende un dominio de unión Fv de cadena sencilla de un hibridoma FC2-2, en donde en la región de conexión dicho primero, segundo, y tercero residuos de cisteína se reemplazan por serina, y dicho residuo de prolina se reemplaza por serina, dicha región constante de cadena pesada comprende dominios CH2 y CH3 de IgG_1

98 Una proteína de la reivindicación 81 en donde dicha proteína de cadena sencilla comprende un dominio de unión Fv de cadena sencilla de un hibridoma UCHL-1, en donde en la región de conexión dicho primero, segundo, y tercero residuos de cisteína se reemplazan por serina, y dicho residuo de prolina se reemplaza por serina, dicha región constante de cadena pesada comprende dominios CH2 y CH3 de IgG_1

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99 Una proteína de la reivindicación 81 en donde dicha proteína de cadena sencilla comprende un dominio de unión Fv de cadena sencilla de un hibridoma 5B9, en donde en la región de conexión dicho primero, segundo, y tercero residuos de cisteina se reemplazan por serina, y dicho residuo de prolina se reemplaza por serina, en donde dicha región constante de cadena pesada comprende dominios CH2 y CH3 de IgG_1

100 Una proteína de la reivindicación 81 en donde dicha proteína de cadena sencilla comprende un dominio de unión Fv de cadena sencilla de un hibridoma 2H7, en donde en la región de conexión dicho primero, segundo, y tercero residuos de cisteína se reemplazan por serina, y dicho residuo de prolina se reemplaza por serina, dicha región constante de cadena pesada comprende dominios CH2 y CH3 de IgG_1

101 Una proteína de la reivindicación 81 en donde dicha proteína de cadena sencilla comprende un dominio de unión Fv de cadena sencilla de un hibridoma 2H7, en donde en la región de conexión dicho segundo, y tercero residuos de cisteína se reemplazan por serina, y dicho residuo de prolina se reemplaza por serina, en donde dicha región constante de cadena pesada comprende dominios CH2 y CH3 de IgG_1

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102 Una proteína de la reivindicación 81 en donde dicha proteína de cadena sencilla comprende un dominio de unión Fv de cadena sencilla de un hibridoma G28-1, en donde en la región de conexión dicho primero, y tercero residuos de cisteína se reemplazan por serina, y dicho residuo de prolina se reemplaza por serina, dicha región constante de cadena pesada comprende dominios CH2 y CH3 de IgG_1

103 Una proteína de la reivindicación 81 en donde dicha proteína de cadena sencilla comprende un dominio de unión Fv de cadena sencilla de un hibridoma G28-1, que comprende una región de conexión en donde dicho tercero residuo de cisteína se reemplazan por serina, y dicho residuo de prolina se reemplaza por serina, dicha región constante de cadena pesada comprende dominios CH2 y CH3 de IgG_1

104 Una proteína de la reivindicación 81 en donde dicha proteína de cadena sencilla comprende un dominio de unión Fv de cadena sencilla de un hibridoma G28-1, en donde en la región de conexión dicho primer residuo de cisteína se reemplaza por serina, dicha región constante de cadena pesada comprende dominios CH2 y CH3 de IgG_1

105 Una proteína de la reivindicación 82 en donde dicha proteína de cadena sencilla comprende un dominio de unión Fv de cadena sencilla de un hibridoma G28-1, en donde dicha región de conexión comprende una región de pivote IgA de murina, dicha región constante de cadena pesada comprende dominios CH2 y CH3 de IgA de murina, y dicho CH3 comprende una eliminación o sustitución en los cuatro aminoácidos que da la región constante de cadena pesada IgA incapaz de asociarse con un polipéptido de cadena J

106 Una proteína de la reivindicación 82 en donde dicha proteína de cadena sencilla comprende un dominio de unión Fv de cadena sencilla de un hibridoma G28-1, en donde dicha región de conexión comprende una región de pivote IgA humano,y dicha región constante de cadena pesada comprende dominios CH2 y CH3 de IgA de humano, en donde dicho CH3 comprende una eliminación o sustitución en los cuatro aminoácidos que da la región constante de cadena pesada IgA incapaz de asociarse con un polipéptido de cadena J

107 Una proteína de la reivindicación 81 en donde dicha proteína de cadena sencilla comprende un dominio de unión Fv de cadena sencilla de un hibridoma HD37, en

donde en la región de conexión dicho primero, segundo, y tercero resíduos de cisteína se reemplaza por serina, y dicho resíduo de prolina se reemplaza por serina, y dicha región constante de cadena pesada comprende dominios CH2 y CH3 de IgG_1

108 Una proteína de la reivindicación 81 en donde dicha proteína de cadena sencilla comprende un dominio de unión Fv de cadena sencilla de un hibridoma L6, en donde en la región de conexión dicho primero, segundo, y tercero residuos de cisteína se reemplaza por serina, y dicho resíduo de prolina se reemplaza por serina, y dicha región constante de cadena pesada comprende dominios CH2 y CH3 de IgG_1

109 Una proteína Fv de cadena sencilla de ocurrencia no natural comprende i) un primer polipéptido que tiene un polipéptido de dominio de unión capaz de unirse a una molécula objetivo, dicho polipéptido de dominio de unión comprende una región variable de cadena pesada en donde la leucina se reemplaza por serina en la posición 11 en la primer región de estructura de la región variable de cadena pesada en donde dicha proteína tiene una expresión incrementada o estabilidad en las células de mamífero relacionadas con una proteína que no tiene dicha sustitución de aminoácido, ii)un segundo

polipéptido que comprende una región de conexión adherida a dicho primer polipéptido, y iii) un tercer polipéptido que comprende un polipéptido de región constante de cadena pesada de inmunoglobulina truncado N-terminal adherido al segundo polipéptido, en donde dicha proteína Fv de cadena sencilla de ocurrencia no natural es capaz de al menos una actividad inmunológica

110 Una proteína Fv de cadena sencilla de ocurrencia no natural comprende i) un primer polipéptido que tiene un polipéptido de dominio de unión capaz de unirse a una molécula objetivo, dicho polipéptido de dominio de unión comprende una región variable de cadena pesada en donde la leucina se reemplaza por serina en la posición 11 en la primer región de estructura de la región variable de cadena pesada, y ii) un segundo polipéptido que comprende un polipéptido de región constante de cadena pesada de inmunoglobulina IgE truncado N-terminal adherido al segundo polipéptido, en donde dicha proteína de cadena sencilla de ocurrencia no natural es capaz de al menos una actividad inmunológica

111 Una proteína de la reivindicación 110 en donde la sustitución de aminoácido en la posición 11 es efectiva

para incrementar la expresión o etabilidad de dicha proteína Fv de cadena sencilla relacionada con una proteína Fv de cadena sencilla sin dicha eliminación o sustitución

112 Una proteína de la reivindicación 110 en donde dicha proteína de cadena sencilla comprende un dominio de unión Fv de cadena sencilla de un hibridoma 2H7 y dicha región constante de cadena pesada comprende dominios CH2 y CH3 de IgE

113 Una proteína de la reivindicación 110 en donde dicha proteína de cadena sencilla comprende un dominio de unión Fv de cadena sencilla de un hibridoma 2H7 y dicha región constante de cadena pesada comprende dominios CH2, CH3, y CH4 de murina IgE

114 Una proteína de la reivindicación 110 en donde dicha proteína de cadena sencilla comprende un dominio de unión Fv de cadena sencilla de un hibridoma G28-1, en donde dicha región constante de cadena pesada comprende dominios CH2, CH3, y CH4 de murina IgE

115 Una proteína de la reivindicación 110 en donde dicha proteína de cadena sencilla comprende un dominio de unión Fv de cadena sencilla de un hibridoma G28-1, en

donde dicha región constante de cadena pesada comprende dominios CH2, CH3, y CH4 de IgE humano

116 Una proteína de cadena sencilla de ocurrencia no natural, que incluye un primer polipéptido que comprende un polipéptido de dominio de unión capaz de unirse a una molécula objetivo, un segundo polipéptido que comprende una región de conexión adherida a dicho primer polipéptido, un tercer polipéptido que comprende un polipéptido de región constante de cadena pesada de inmunoglobulina truncada N-terminal adherida al segundo polipéptido, en donde dicha región de conexión comprende primero, segundo, y tercero residuos de cisteína, en donde dicho primer residuo de cisteína es N-terminal a dicho segundo residuo de cisteína y dicha segunda cisteína es N-terminal a dicha tercera cisteína, en donde uno o ambos de dichos segundos y terceros residuos de cisteína se sustituye o elimina, y en donde dicha proteína de cadena sencilla de ocurrencia no natural es capaz de al menos una actividad inmunológica

117 Una proteína de la reivindicación 116 en donde dicho polipéptido de dominio de unión es un Fv de cadena sencilla

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118 Una proteína de la reivindicación 116 en donde dicho polipéptido de dominio de unión comprende un polipéptido de región variable de cadena liviana de inmunoglobulina y un polipéptido de región variable de cadena pesada de inmunoglobulina

119 Una proteína de la reivindicación 118 que comprende además segundo polipéptido de dominio de unión capaz de unir una segunda molécula objetiva, dicho segundo polipéptido de dominio de unión comprende un polipéptido de región variable de cadena liviana de inmunoglobulina y un polipéptido de región variable de cadena pesada de inmunoglobulina

120 Una proteína de la reivindicación 116 en donde dicho polipéptido de dominio de unión es un Fv de cadena sencilla que comprende una región variable de cadena pesada, en donde dicha región variable de cadena pesada tiene una sustitución o eliminación de aminoácido en uno o más de las posiciones aminoácido 9, 10, 11, 12, 108, 110, 112

121 Una proteína de la reivindicación 117 en donde dicho polipéptido de dominio de unión es un Fv de cadena sencilla que comprende una región variable de cadena liviana, en donde dicha región variable de cadena

liviana tiene una eliminación de aminoácido o sustitución en uno o más de las posiciones aminoácido 12, 80, 81, 83, 105, 106, y 107

122 Una proteína de la reivindicación 116 en donde dicho polipéptido de dominio de unión es un Fv de cadena sencilla que comprende una región variable de cadena pesada, en donde dicha región variable de cadena pesada tiene una sustitución de aminoácido en la aminoácido posición 11

123 Una proteína de la reivindicación 122 en donde el ácido en la posición de 11 de la región variable de cadena pesada Fv cadena sencilla se selecciona de serina, treonina, cisteína, tirosina, asparagina, glutamina, ácido aspártico, ácido glutámico, lisina, arginina, e histidina

124 Una proteína de la reivindicación 122 en donde el aminoácido sustituido para el aminoácido en la posición 11 de la región variable de cadena pesada Fv de cadena sencilla se selecciona de serina, treonina, cisteina, tirosina, esparagina, y glutamina

125 Una proteína de la reivindicación 122 en donde en donde la leucina se reemplaza por serina en la posición 11

126 Una proteína de la reivindicación 122 en donde en donde la leucina se reemplaza por des-leucina en la posición 11

127 Una proteína de la reivindicación 116 en donde dicho polipéptido de dominio de unión es un Fv de cadena sencilla y el aminoácido en la posición 11 en la región variable de cadena pesada de dicho Fv de cadena sencilla se ha eliminado

128 Una proteína de la reivindicación 121 en donde en donde la leucina se reemplaza por serina en la posición 106

129 Una proteína de la reivindicación 117 en donde dicho polipéptido de dominio de unión se une a un antígeno de tumor

130 Una proteína de la reivindicación 117 en donde dicho polipéptido de dominio de unión se une a un antígeno en una célula detectora inmune

131 Una proteína de la reivindicación 117 en donde dicho polipéptido de dominio de unión se une a un antígeno de célula de cáncer

132 Una proteína de la reivindicación 131 en donde dicho antígeno de célula de cáncer es un antígeno de superficie

133 Una proteína de la reivindicación 131 en donde dicho antígeno de célula de cáncer es un antígeno intracelular

134 Una proteína de la reivindicación 116 en donde dicho polipéptido de dominio de unión se une a un antígeno de célula B

135 Una proteína de la reivindicación 135 en donde dicho antígeno de célula B se selecciona de CD19, CD20, CD22, CD37, CD40, CD80, y CD86

136 Una proteína de la reivindicación 117 en donde dicho Fv de cadena sencilla se une a un antígeno de célula B

137 Una proteína de la reivindicación 136 en donde dicho antígeno de célula B se selecciona de CD19, CD20, CD22, CD37, CD40, CD80, y CD86

138 Una proteína de la reivindicación 137 en donde dicho Fv de cadena sencilla se selecciona de Fv de cadena

sencilla HD37, Fv de cadena sencilla 2H7, Fv de cadena sencilla G28-1, y Fv de cadena sencilla 4 4 220

139 Una proteína de la reivindicación 117 en donde dicho Fv de cadena sencilla se selecciona de Fv de cadena sencilla HD37, Fv de cadena sencilla 2H7, Fv de cadena sencilla G28-1, FC2-2, UCHL-1, 5B9, L6, 10A8, 2e12, 40 2 36, G19-4, 1D8, y Fv de cadena sencilla 4 4 220

140 Una proteína de la reivindicación 116 en donde dicho polipéptido de dominio de unión es un scFv que se une a un antígeno de diferenciación de célula B

141 Una proteína de la reivindicación 136 en donde dicho antígeno de célula B se selecciona de CD19, CD20, CD22, CD37, y CD40

142 Una proteína de la reivindicación 116 en donde dicho polipéptido de dominio de unión se une a un objetivo seleccionado de CD2, CD3, CD4, CD5, CD6, CD8, CD10, CD11b, CD14, CD19, CD20, CD21, CD22, CD23, CD24, CD25, CD28, CD30, CD37, CD40, CD43, CD50 (ICAM3), CD54 (ICAM1), CD56, CD69, CD80, CD86, CD134 (OX40), CD137 (41BB), CD152 (CTLA-4), CD153 (ligando CD30), CD154 (ligando CD40), ICOS, L6, B7-H1, y HLA clase II

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143 Una proteína de la reivindicación 116 en donde dicha proteína es capaz de formar un complejo que comprende dos o más de dichas proteínas

144 Una proteína de la reivindicación 143 en donde dicho complejo es un dímero

145 Una proteína de la reivindicación 116 en donde dicha proteína es un monómero

146 Una proteína de la reivindicación 116 acoplada a una droga, toxina, inmunomodulador, efector polipéptido, isótopo, etiqueta, o porción efectora

147 Una proteína de la reivindicación 116 en donde dicha actividad inmunológica se selecciona de citotoxicidad mediada por célula dependiente de anticuerpo, fijación de complemento, inducción de apoptosis, inducción de una o más señales activas biológicamente, inducción de una o más células efectoras inmunes, activación de diferenciación celular, activación celular, liberación de una o más moléculas biológicamente activas, y neutralización de un agente infeccioso o toxina

148 Una proteína de la reivindicación 147 que es capaz de inducción de señales activas biológicamente mediante

activación o inhibición de una o más moléculas seleccionadas de proteína quinasa, proteína fosfatasa, G-proteínas, nucleótidos cíclicos u otros segundos mensajeros, canales de ion, y componentes de trayectoria secretora

149 Una proteína de la reivindicación 147 que es capaz de inducción de una o más células efectoras inmunes seleccionadas de Células NK, monocitos, macrófagos, Células B, células T, mastocitos, neutrófilos, eosinófilos, y basófilos

150 Una proteína de la reivindicación 149 en donde dicha inducción de una o más células efectoras inmunes conduce a citotoxicidad mediada por célula dependiente de anticuerpo o la liberación de una o más moléculas biológicamente activas

151 Una proteína de la reivindicación 147 que es capaz de activación celular, en donde dicha activación conduce a cambios en la actividad transcripcional celular

152 Una proteina de la reivindicación 151 en donde dicha actividad transcripcional celular se incrementa

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153 Una proteína de la reivindicación 151 en donde dicha actividad transcripcional celular se reduce

154 Una proteína de la reivindicación 147 en donde dicha una o más moléculas biológicamente activas es una proteasa

155 Una proteína de la reivindicación 147 en donde dicha una o más moléculas biológicamente activas es una citoquina

156 Una proteína de la reivindicación 155 en donde dicha citoquina se selecciona de monoquinas, linfoquinas, quemoquinas, factores de crecimiento, factores de estimulación de colonia, interferones, e interleuquinas

157 Una proteína de la reivindicación 147 que es capaz de neutralización de un agente infeccioso, en donde dicho agente infeccioso es una bacteria, un virus, un parásito, o un hongo

158 Una proteína de la reivindicación 147 proteina A que es capaz de neutralización de una toxina, en donde dicha toxina se selecciona de endotoxinas y exotoxinas

159 Una proteína de la reivindicación 147 que es capaz de neutralización de una toxina, en donde dicha toxina es una exotoxina seleccionada de toxina antrax, toxina cólera, toxina difteria, toxina pertusis, Toxina lábil-calor *E coli* LT, Toxina estable al calor *E coli* ST, Exotoxina *Pseudomonas* toxina shiga A, toxina botulinum, toxina tétano, Toxina *Bordetella pertussis* AC, y *Bacillus anthracis* EF

160 Una proteína de la reivindicación 147 que es capaz de neutralización de una toxina, en donde dicha toxina es una endotoxina seleccionada de saxitoxinas, tetrodotoxina, toxinas de hongo, aflatoxinas, alcaloides pirrolizidina, fitohemaglutininas, y grayanotoxinas

161 Una proteína de la reivindicación 116 en donde dicho polipéptido de dominio de unión comprende una región variable de cadena liviana adherida a dicha región variable de cadena pesada mediante un ligador de dominio de unión, en donde dicho ligador de dominio de unión comprende uno o más péptidos que tienen una secuencia Gly-Gly-Gly-Gly-Ser

162 Una proteína de la reivindicación 116 que comprende tres péptidos Gly-Gly-Gly-Gly-Ser

163 Una proteína de la reivindicación 116 en donde dicho polipéptido de dominio de unión comprende región variable de inmunoglobulina construida por ingeniería o tipo silvestre obtenido de especies seleccionadas de humano, murina, rata, cerdo, y mono

164 Una proteína de la reivindicación 116 en donde dicha proteína es capaz de unirse a un objetivo intracelular para efectuar una función celular

165 Una proteína de la reivindicación 116 en donde dicho polipéptido de región constante de cadena pesada de inmunoglobulina truncado N-terminal comprende un polipéptido de región constante IgG CH2 adherido a un polipéptido de región constante IgG CH3 de cadena pesada de inmunoglobulina

166 Una proteína de la reivindicación 116 en donde dicho polipéptido de región constante de cadena pesada de inmunoglobulina está sin un dominio CH1 activo funcionalmente

167 Una proteína de la reivindicación 116 dicho polipéptido de región constante de cadena pesada de inmunoglobulina truncado N-terminal consiste esencialmente de un

polipéptido de región constante IgG CH2 adherido a un polipéptido de región constante IgG CH3 de cadena pesada de inmunoglobulina

168 Una proteína de la reivindicación 116 que comprende al menos una porción de una región constante humana

169 Una proteína de la reivindicación 116 en donde dicha región de conexión comprende una región de pivote de ocurrencia natural seleccionada de un pivote humano o porción de éste, pivote IgG humana o una porción de ésta, pivote IgA humana o una porción de ésta, pivote IgE humana o una porción de ésta, región de pivote de camélido o una porción de ésta, Región de pivote de llama IgG1 o porción de ésta, región de pivote de tiburón nodriza o porción de ésta, y región de pivote de pez rata o una porción de ésta

170 Una proteína de la reivindicación 116 en donde dicha región de conexión comprende un pivote IgE humano o una porción de ésta

171 Una proteína de la reivindicación 116 en donde dicha región de conexión comprende una región de pivote IgG1, IgG2, IgG3 o IgG4 humana que tiene residuo de cisteína uno o cero

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172 Una proteína de la reivindicación 116 en donde dicha región de conexión comprende una región de pivote IgGA humana que tiene entre cero y dos residuos de cisteína

173 Una proteína de la reivindicación 116 en donde dicha región de conexión comprende una región de pivote de inmunoglobulina IgG1 humana tipo silvestre

174 Una proteína de la reivindicación 116 en donde dicha región de conexión comprende un sitio de glicosilación

175 Una proteína de la reivindicación 116 en donde dicha región de conexión no tiene residuos de cisteína capaces de formar enlaces de disulfuro

176 Una proteína de la reivindicación 116 en donde dicha región de conexión tiene un residuo de cisteína

177 Una proteína de la reivindicación 116 en donde dicha región de conexión comprende un polipéptido de región de pivote de inmunoglobulina tipo silvestre mutado que comprende no más de un residuo de cisteína

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178 Una proteína de la reivindicación 116 en donde dicha región de conexión se altera de modo que dicha proteína tiene una capacidad reducida para dimerizar

179 Una proteína de la reivindicación 116 en donde dicha región de conexión comprende una prolina y primero, segundo, y tercero residuos de cisteína, en donde dicho primer residuo de cisteína es N-terminal a dicha segunda cisteína y dicha segunda cisteína es N-terminal a dicha tercera cisteína, dicho tercer residuo de cisteína es N-terminal a dicho residuo de prolina

180 Una proteína de la reivindicación 179 en donde dicha proteína de cadena sencilla comprende un dominio de unión Fv de cadena sencilla CTLA-4, en donde en la región de conexión dichos primero, segundo, y tercero residuos de cisteína se reemplazan por serina y dicho residuo de prolina se reemplaza por serina, dicha región constante de cadena pesada comprende dominios CH2 y CH3 de IgGI en donde la prolina se reemplaza por serina en la posición 238 en dicho dominio CH2

181 Una proteína de la reivindicación 179 en donde dicha proteína de cadena sencilla comprende un dominio de unión Fv de cadena sencilla CTLA-4 y dicha región

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constante de cadena pesada comprende dominios CH2 y CH3 de IgGI

182 Una proteína de la reivindicación 179 en donde dicha proteína de cadena sencilla comprende un dominio de unión Fv de cadena sencilla FC2-2, en donde en la región de conexión dichos primero, segundo, y tercero residuos de cisteína se reemplazan por serina y dicho residuo de prolina se reemplaza por serina, dicha región constante de cadena pesada comprende dominios CH2 y CH3 de IgG_1

183 Una proteína de la reivindicación 179 en donde dicha proteína de cadena sencilla comprende un dominio de unión Fv de cadena sencilla UCHL-1, en donde en la región de conexión dichos primero, segundo, y tercero residuos de cisteína se reemplazan por serina y dicho residuo de prolina se reemplaza por serina, dicha región constante de cadena pesada comprende dominios CH2 y CH3 de IgG_1

184 Una proteína de la reivindicación 179 en donde dicha proteína de cadena sencilla comprende un dominio de unión Fv de cadena sencilla 5B9, en donde en la región de conexión dichos primero, segundo, y tercero residuos de cisteína se reemplazan por serina y dicho residuo de

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prolina se reemplaza por serina, dicha región constante de cadena pesada comprende dominios CH2 y CH3 de IgC_1

185 Una proteína de la reivindicación 179 en donde dicha proteína de cadena sencilla comprende un dominio de unión Fv de cadena sencilla 2H7 scFv, en donde en la región de conexión dichos primero, segundo, y tercero residuos de cisteína se reemplazan por serina y dicho residuo de prolina se reemplaza por serina, dicha región constante de cadena pesada comprende dominios CH2 y CH3 de IgG_1

186 Una proteína de la reivindicación 179 en donde dicha proteína de cadena sencilla comprende un dominio de unión Fv de cadena sencilla 2H7, en donde en la región de conexión dicho residuo de prolina se reemplaza por serina, dicha región constante de cadena pesada comprende dominios CH2 y CH3 de IgG_1

187 Una proteína de la reivindicación 179 en donde dicha proteína de cadena sencilla comprende un Fv de cadena sencilla 2H7 que comprende una región de conexión en donde dichos primero, segundo, y tercero residuos de cisteína se reemplazan por serina y dicho residuo de prolina se reemplaza por serina, dicha región constante de cadena pesada comprende dominios CH2 y CH3 de IgG_1

en donde fenilalanina se reemplaza por tirosina en la posición 405 en dicha región CH3

188 Una proteína de la reivindicación 179 en donde dicha proteína de cadena sencilla comprende un dominio de unión Fv de cadena sencilla 2H7, en donde en la región de conexión dichos primero, segundo, y tercero residuos de cisteína se reemplazan por serina y dicho residuo de prolina se reemplaza por serina, dicha región constante de cadena pesada comprende dominios CH2 y CH3 de IgG_1 en donde fenilalanina se reemplaza por alanina en la posición 405 en dicha región CH3

189 Una proteína de la reivindicación 179 en donde dicha proteína de cadena sencilla comprende un dominio de unión Fv de cadena sencilla 2H7, en donde en la región de conexión dichos primero, segundo, y tercero residuos de cisteína se reemplazan por serina y dicho residuo de prolina se reemplaza por serina, dicha región constante de cadena pesada comprende dominios CH2 y CH3 de IgG_1 en donde la tirosina se reemplaza por alanina en la posición 407 en dicha región CH3

190 Una proteína de la reivindicación 189 que tiene un peso molecular aparente de cerca de 75 kDa

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191 Una proteína de la reivindicación 179 en donde dicha proteína de cadena sencilla comprende un dominio de unión Fv de cadena sencilla 2H7, en donde en la región de conexión dichos primero, segundo, y tercero residuos de cisteína se reemplazan por serina y dicho residuo de prolina se reemplaza por serina, dicha región constante de cadena pesada comprende dominios CH2 y CH3 de IgG_1 en donde la fenilalanina se reemplaza por alanina en la posición 405 y la tirosina se reemplaza por alanina en la posición 407 en dicha región CH3

192 Una proteína de la reivindicación 179 en donde dicha proteína de cadena sencilla comprende un dominio de unión Fv de cadena sencilla 2H7, en donde en la región de conexión dichos segundo y tercero residuos de cisteina se reemplazan por serina y dicho residuo de prolina se reemplaza por serina, dicha región constante de cadena pesada comprende dominios CH2 y CH3 de IgG_1

193 Una proteína de la reivindicación 179 en donde dicha proteína de cadena sencilla comprende un dominio de unión Fv de cadena sencilla 2H7, en donde en la región de conexión dichos primero y tercero residuos de cisteina se reemplazan por serina y dicho residuo de prolina se reemplaza por serina, dicha región constante de cadena pesada comprende dominios CH2 y CH3 de IgG_1

194 Una proteína de la reivindicación 179 en donde dicha proteína de cadena sencilla comprende un dominio de unión Fv de cadena sencilla 2H7, en donde en la región de conexión dichos primero y segundo residuos de cisteína se reemplazan por serina, dicha región constante de cadena pesada comprende dominios CH2 y CH3 de IgG_1

195 Una proteína de la reivindicación 179 en donde dicha proteína de cadena sencilla comprende un Fv de cadena sencilla 2H7 que comprende una región de conexión en donde dicho segundo residuo de cisteína se reemplaza por serina y dicho residuo de prolina se reemplaza por serina, dicha región constante de cadena pesada comprende dominios CH2 y CH3 de IgG_1

196 Una proteína de la reivindicación 179 en donde dicha proteina de cadena sencilla comprende un Fv de cadena sencilla 2H7 que comprende una región de conexión en donde dicho tercer residuo de cisteína se reemplaza por serina, dicha región constante de cadena pesada comprende dominios CH2 y CH3 de IgG_1

197 Una proteína de la reivindicación 179 en donde dicha proteína de cadena sencilla comprende un dominio de

unión Fv de cadena sencilla 2H7, en donde en la región de conexión dicho primer residuo de cisteína se reemplaza por serina, y en donde dicha región constante de cadena pesada comprende los dominios Fv CH2 y CH3 de cadena sencilla 2H7

198 Una proteína de la reivindicación 179 en donde dicha proteína de cadena sencilla comprende un dominio de unión Fv de cadena sencilla HD37, en donde en la región de conexión dichos primero, segundo y tercero residuos de cisteína se reemplazan por serina y dicho residuo de prolina se reemplaza por serina, dicha región constante de cadena pesada comprende dominios CH2 y CH3 de IgG_1

199 Una proteína de la reivindicación 179 en donde dicha proteína de cadena sencilla comprende un dominio de unión Fv de cadena sencilla 2H7 y dicha región constante de cadena pesada comprende polipéptidos de región constante CH2 y CH3 de llama IgG_1

200 Una proteína de la reivindicación 179 en donde dicha proteína de cadena sencilla comprende un dominio de unión Fv de cadena sencilla 2H7 y dicha región constante de cadena pesada comprende polipéptidos de región constante CH2 y CH3 de IgG1 de llama

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201 Una proteína de la reivindicación 179 en donde dicha proteína de cadena sencilla comprende un dominio de unión Fv de cadena sencilla 2H7 y dicha región constante de cadena pesada comprende polipéptidos de región constante CH2 y CH3 de IgG_3 de llama

202 Una proteína de la reivindicación 179 en donde dicha proteína de cadena sencilla comprende un dominio de unión Fv de cadena sencilla CD-16-6, en donde en la región de conexión dichos primero, segundo, y tercero residuos de cisteína se reemplazan por serina y dicho residuo de prolina se reemplaza por serina, dicha región constante de cadena pesada comprende dominios CH2 y CH3 de IgG_1 en donde la prolina se reemplaza por serina en la posición 238 en dicha región CH2

203 Una proteína de la reivindicación 179 en donde dicha proteína de cadena sencilla comprende un dominio de unión de receptor CD-16, en donde en la región de conexión dichos primero, segundo, y tercero residuos de cisteína se reemplazan por serina y dicho residuo de prolina se reemplaza por serina, dicha región constante de cadena pesada comprende dominios CH2 y CH3 de IgG_1 en donde la prolina se reemplaza por serina en la posición 238 en dicha región CH2

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204 Una proteína de la reivindicación 179 en donde dicha proteína de cadena sencilla comprende un dominio de unión Fv de cadena sencilla 2e12, en donde en la región de conexión dichos primero, segundo, y tercero residuos de cisteína se reemplazan por serina y dicho residuo de prolina se reemplaza por serina, dicha región constante de cadena pesada comprende dominios CH2 y CH3 de IgG_1 en donde la prolina se reemplaza por serina en la posición 238 en dicha región CH2, y en donde dicho dominio CH3 se adhiere a un polipéptido que comprende dominios de cola citoplásmico y transmembrana CD80

205 Una proteína de la reivindicación 179 en donde dicha proteína de cadena sencilla comprende un dominio de unión Fv de cadena sencilla 10A8 scFv, en donde en la región de conexión dichos primero, segundo, y tercero residuos de cisteína se reemplazan por serina y dicho residuo de prolina se reemplaza por serina, dicha región constante de cadena pesada comprende dominios CH2 y CH3 de IgG_1 en donde la prolina se reemplaza por serina en la posición 238 en dicha región CH2, y en donde dicho dominio CH3 se adhiere a un polipéptido que comprende dominios de cola citoplásmico y transmembrana CD80

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206 Una proteína de la reivindicación 179 en donde dicha proteína de cadena sencilla comprende un dominio de unión Fv de cadena sencilla de un hibridoma 40 2 36, en donde en la región de conexión dichos primero, segundo y tercero residuos de cisteína se reemplazan por serina y dicho residuo de prolina se reemplaza por serina, dicha región constante de cadena pesada comprende dominios CH2 y CH3 de IgG_1 en donde la prolina se reemplaza por serina en la posición 238 en dicha región CH2, y en donde dicho dominio CH3 se adhiere a un polipéptido que comprende dominios de cola citoplásmico y transmembrana CD80

207 Una proteína de la reivindicación 179 en donde dicha proteína de cadena sencilla comprende un dominio de unión Fv de cadena sencilla de un hibridoma 2H7, en donde en la región de conexión dichos primero, segundo y tercero residuos de cisteína se reemplazan por serina y dicho residuo de prolina se reemplaza por serina, dicha región constante de cadena pesada comprende dominios CH2 y CH3 de IgG_1 en donde la prolina se reemplaza por serina en la posición 238 en dicha región CH2, y en donde dicho dominio CH3 se adhiere a un polipéptido que comprende dominios de cola citoplásmico y transmembrana CD80

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208 Una proteína de la reivindicación 179 en donde dicha proteína de cadena sencilla comprende un dominio de unión Fv de cadena sencilla de un hibridoma G19-4, en donde en la región de conexión dichos primero, segundo y tercero residuos de cisteína se reemplazan por serina y dicho residuo de prolina se reemplaza por serina, dicha región constante de cadena pesada comprende dominios CH2 y CH3 de IgG_1 en donde la prolina se reemplaza por serina en la posición 238 en dicha región CH2, y en donde dicho dominio CH3 se adhiere a un polipéptido que comprende dominios de cola citoplásmico y transmembrana CD80

209 Una proteína de la reivindicación 179 en donde dicha proteína de cadena sencilla comprende un dominio de unión Fv de cadena sencilla de un hibridoma G19-4, dicha región constante de cadena pesada comprende dominios CH2 y CH3 de IgG_1 en donde dicho dominio CH3 se adhiere a un polipéptido que comprende dominios de cola citoplásmico y transmembrana CD80

210 Una proteína de la reivindicación 179 en donde dicha proteína de cadena sencilla comprende un dominio de unión Fv de cadena sencilla de un hibridoma 2e12, en donde en la región de conexión dichos primero, segundo y tercero residuos de cisteina se reemplazan por serina

y dicho residuo de prolina se reemplaza por serina, dicha región constante de cadena pesada comprende dominios CH2 y CH3 de IgG_1, en donde dicho dominio CH3 se adhiere a un polipéptido que comprende dominios de cola citoplásmico y transmembrana CD80

211 Una proteína de la reivindicación 179 en donde dicha proteína de cadena sencilla comprende un dominio de unión Fv de cadena sencilla de un hibridoma 2e12, en donde en la región de conexión dichos primero, segundo y tercero residuos de cisteína se reemplazan por serina y dicho residuo de prolina se reemplaza por serina, dicha región constante de cadena pesada comprende dominios CH2 y CH3 de IgG_1 en donde la prolina se reemplaza por serina en la posición 238 en dicha región CH2 y dicho dominio CH3 se adhiere a un polipéptido que comprende los dominios de cola citoplásmico y de transmembrana mFADD

212 Una proteína de la reivindicación 179 en donde dicha proteína de cadena sencilla comprende un dominio de unión Fv de cadena sencilla de un hibridoma 2e12, en donde en la región de conexión dichos primero, segundo y tercero residuos de cisteína se reemplazan por serina y dicho residuo de prolina se reemplaza por serina, dicha región constante de cadena pesada comprende

dominios CH2 y CH3 de IgG_1, en donde dicho dominio CH3 se adhiere a un polipéptido que comprende los dominios de cola citoplásmico y de transmembrana mFADD

213 Una proteína de la reivindicación 179 en donde dicha proteína de cadena sencilla comprende un dominio de unión Fv de cadena sencilla de un hibridoma 2e12, en donde en la región de conexión dichos primero, segundo y tercero residuos de cisteína se reemplazan por serina y dicho residuo de prolina se reemplaza por serina, dicha región constante de cadena pesada comprende dominios CH2 y CH3 de IgG_1, en donde dicho dominio CH3 se adhiere a un polipéptido que comprende los dominios de cola citoplásmicos y de transmembrana mcasp3

214 Una proteína de la reivindicación 179 en donde dicha proteína de cadena sencilla comprende un dominio de unión Fv de cadena sencilla de un hibridoma 2e12, en donde en la región de conexión dichos primero, segundo y tercero residuos de cisteína se reemplazan por serina y dicho residuo de prolina se reemplaza por serina, dicha región constante de cadena pesada comprende dominios CH2 y CH3 de IgG_1 en donde la prolina se reemplaza por serina en la posición 238 en dicha región CH2 y dicho dominio CH3 se adhiere a un polipéptido que

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comprende los dominios de cola citoplásmicos y de transmembrana mcasp3

215 Una proteína de la reivindicación 179 en donde dicha proteína de cadena sencilla comprende un dominio de unión Fv de cadena sencilla de un hibridoma 2e12, en donde en la región de conexión dichos primero, segundo y tercero residuos de cisteína se reemplazan por serina y dicho residuo de prolina se reemplaza por serina, dicha región constante de cadena pesada comprende dominios CH2 y CH3 de IgG_1, en donde dicho dominio CH3 se adhiere a un polipéptido que comprende los dominio de cola citoplásmicos y de transmembrana mcasp8

216 Una proteína de la reivindicación 179 en donde dicha proteína de cadena sencilla comprende un dominio de unión Fv de cadena sencilla de un hibridoma 2e12, en donde en la región de conexión dichos primero, segundo y tercero residuos de cisteína se reemplazan por serina y dicho residuo de prolina se reemplaza por serina, dicha región constante de cadena pesada comprende dominios CH2 y CH3 de IgG_1 en donde la prolina se reemplaza por serina en la posición 238 en dicha región CH2 y dicho dominio CH3 se adhiere a un polipéptido que comprende los dominio de cola citoplásmicos y de transmembrana mcasp8

217 Una proteína de la reivindicación 179 en donde dicha proteína de cadena sencilla comprende un dominio de unión Fv de cadena sencilla de un hibridoma 2e12, en donde en la región de conexión dichos primero, segundo y tercero residuos de cisteína se reemplazan por serina y dicho residuo de prolina se reemplaza por serina, dicha región constante de cadena pesada comprende dominios CH2 y CH3 de IgG_1, en donde dicho dominio CH3 se adhiere a un polipéptido que comprende los dominios de cola citoplásmicos y de transmembrana hcasp3

218 Una proteína de la reivindicación 179 en donde dicha proteína de cadena sencilla comprende un dominio de unión Fv de cadena sencilla de un hibridoma 2e12, en donde en la región de conexión dichos primero, segundo y tercero residuos de cisteína se reemplazan por serina y dicho residuo de prolina se reemplaza por serina, dicha región constante de cadena pesada comprende dominios CH2 y CH3 de IgG_1 en donde la prolina se reemplaza por serina en la posición 238 en dicha región CH2 y dicho dominio CH3 se adhiere a un polipéptido que comprende los dominios de cola citoplásmicos y de transmembrana hcasp3

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219 Una proteína de la reivindicación 179 en donde dicha proteína de cadena sencilla comprende un dominio de unión Fv de cadena sencilla de un hibridoma 2e12, en donde en la región de conexión dichos primero, segundo y tercero residuos de cisteína se reemplazan por serina y dicho residuo de prolina se reemplaza por serina, dicha región constante de cadena pesada comprende dominios CH2 y CH3 de IgG_1, en donde dicho dominio CH3 se adhiere a un polipéptido que comprende los dominios de cola citoplásmicos y de transmembrana hcasp8

220 Una proteína de la reivindicación 179 en donde dicha proteína de cadena sencilla comprende un dominio de unión Fv de cadena sencilla de un hibridoma 2e12, en donde en la región de conexión dichos primero, segundo y tercero residuos de cisteína se reemplazan por serina y dicho residuo de prolina se reemplaza por serina, dicha región constante de cadena pesada comprende dominios CH2 y CH3 de IgG_1 en donde la prolina se reemplaza por serina en la posición 238 en dicha región CH2 y dicho dominio CH3 se adhiere a un polipéptido que comprende los dominios de cola citoplásmicos y de transmembrana hcasp8

221 Una proteína de la reivindicación 179 en donde dicha proteína de cadena sencilla comprende un dominio de

unión Fv de cadena sencilla de un hibridoma 2e12, dicha región constante de cadena pesada comprende los dominios CH2 y CH3 de IgG_1 en donde dicho dominio CH3 se adhiere a un polipéptido que comprende dominios de cola citoplásmico y transmembrana CD80

222 Una proteína de la reivindicación 179 en donde dicha proteína de cadena sencilla comprende un dominio de unión Fv de cadena sencilla de un hibridoma 1D8, en donde en la región de conexión dichos primero, segundo y tercero residuos de cisteína se reemplazan por serina y dicho residuo de prolina se reemplaza por serina, dicha región constante de cadena pesada comprende dominios CH2 y CH3 de IgG_1 en donde la prolina se reemplaza por serina en la posición 238 en dicha región CH2 y dicho dominio CH3 se adhiere a un polipéptido que comprende dominios de cola citoplásmico y transmembrana CD80

223 Una proteína de la reivindicación 179 en donde dicha proteína de cadena sencilla comprende un dominio de unión Fv de cadena sencilla de un hibridoma 1D8 scFv, en donde en la región de conexión dichos primero, segundo y tercero residuos de cisteína se reemplazan por serina y dicho residuo de prolina se reemplaza por serina, dicha región constante de cadena pesada

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comprende dominios CH2 y CH3 de IgG_1, en donde dicho dominio CH3 se adhiere a un polipéptido que comprende dominios de cola citoplásmico y transmembrana CD80

224 Una proteína de la reivindicación 179 en donde dicha proteína de cadena sencilla comprende un dominio de unión Fv de cadena sencilla de un hibridoma 1D8, en donde en la región de conexión dichos primero, segundo y tercero residuos de cisteína se reemplazan por serina y dicho residuo de prolina se reemplaza por serina, dicha región constante de cadena pesada comprende dominios CH2 y CH3 de IgG_1 en donde la prolina se reemplaza por serina en la posición 238 en dicha región CH2 y dicho dominio CH3 se adhiere a un polipéptido que comprende los dominios de cola citoplásmico y de transmembrana mFADD

225 Una proteína de la reivindicación 179 en donde dicha proteína de cadena sencilla comprende un dominio de unión Fv de cadena sencilla de un hibridoma 1D8, en donde en la región de conexión dichos primero, segundo y tercero residuos de cisteína se reemplazan por serina y dicho residuo de prolina se reemplaza por serina, dicha región constante de cadena pesada comprende dominios CH2 y CH3 de IgG_1, en donde dicho dominio CH3

se adhiere a un polipéptido que comprende los dominios de cola citoplásmico y de transmembrana mFADD

226 Una proteína de la reivindicación 179 en donde dicha proteína de cadena sencilla comprende un dominio de unión Fv de cadena sencilla de un hibridoma 1D8, en donde en la región de conexión dichos primero, segundo y tercero residuos de cisteína se reemplazan por serina y dicho residuo de prolina se reemplaza por serina, dicha región constante de cadena pesada comprende dominios CH2 y CH3 de IgG_1 en donde dicho dominio CH3 se adhiere a un polipéptido que comprende los dominios de cola citoplásmicos y de transmembrana mcasp3

227 Una proteína de la reivindicación 179 en donde dicha proteína de cadena sencilla comprende un dominio de unión Fv de cadena sencilla de un hibridoma 1D8, en donde en la región de conexión dichos primero, segundo y tercero residuos de cisteína se reemplazan por serina y dicho residuo de prolina se reemplaza por serina, dicha región constante de cadena pesada comprende dominios CH2 y CH3 de IgG_1 en donde la prolina se reemplaza por serina en la posición 238 en dicha región CH2 y dicho dominio CH3 se adhiere a un polipéptido que comprende los dominios de cola citoplásmicos y de transmembrana mcasp3

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228 Una proteína de la reivindicación 179 en donde dicha proteína de cadena sencilla comprende un dominio de unión Fv de cadena sencilla de un hibridoma 1D8, en donde en la región de conexión dichos primero, segundo y tercero residuos de cisteína se reemplazan por serina y dicho residuo de prolina se reemplaza por serina, dicha región constante de cadena pesada comprende dominios CH2 y CH3 de IgG_1, en donde dicho dominio CH3 se adhiere a un polipéptido que comprende los dominio de cola citoplásmicos y de transmembrana mcasp8

229 Una proteína de la reivindicación 179 en donde dicha proteína de cadena sencilla comprende un dominio de unión Fv de cadena sencilla de un hibridoma 1D8, en donde en la región de conexión dichos primero, segundo y tercero residuos de cisteína se reemplazan por serina y dicho residuo de prolina se reemplaza por serina, dicha región constante de cadena pesada comprende dominios CH2 y CH3 de IgG_1 en donde la prolina se reemplaza por serina en la posición 238 en dicha región CH2 y dicho dominio CH3 se adhiere a un polipéptido que comprende los dominio de cola citoplásmicos y de transmembrana mcasp8

230 Una proteína de la reivindicación 179 en donde dicha proteína de cadena sencilla comprende un dominio de unión Fv de cadena sencilla de un hibridoma 1D8, en donde en la región de conexión dichos primero, segundo y tercero residuos de cisteína se reemplazan por serina y dicho residuo de prolina se reemplaza por serina, dicha región constante de cadena pesada comprende dominios CH2 y CH3 de IgG_1, en donde dicho dominio CH3 se adhiere a un polipéptido que comprende los dominios de cola citoplásmicos y de transmembrana hcasp3

231 Una proteína de la reivindicación 179 en donde dicha proteína de cadena sencilla comprende un dominio de unión Fv de cadena sencilla de un hibridoma 1D8, en donde en la región de conexión dichos primero, segundo y tercero residuos de cisteína se reemplazan por serina y dicho residuo de prolina se reemplaza por serina, dicha región constante de cadena pesada comprende dominios CH2 y CH3 de IgG_1 en donde la prolina se reemplaza por serina en la posición 238 en dicha región CH2 y dicho dominio CH3 se adhiere a un polipéptido que comprende los dominios de cola citoplásmicos y de transmembrana hcasp3

232 Una proteína de la reivindicación 179 en donde dicha proteína de cadena sencilla comprende un dominio de

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unión Fv de cadena sencilla de un hibridoma 1D8, en donde en la región de conexión dichos primero, segundo y tercero residuos de cisteína se reemplazan por serina y dicho residuo de prolina se reemplaza por serina, dicha región constante de cadena pesada comprende dominios CH2 y CH3 de IgG_1, en donde dicho dominio CH3 se adhiere a un polipéptido que comprende los dominios de cola citoplásmicos y de transmembrana hcasp8

233 Una proteína de la reivindicación 179 en donde dicha proteína de cadena sencilla comprende un dominio de unión Fv de cadena sencilla de un hibridoma 1D8, en donde en la región de conexión dichos primero, segundo y tercero residuos de cisteína se reemplazan por serina y dicho residuo de prolina se reemplaza por serina, dicha región constante de cadena pesada comprende dominios CH2 y CH3 de IgG_1 en donde la prolina se reemplaza por serina en la posición 238 en dicha región CH2 y dicho dominio CH3 se adhiere a un polipéptido que comprende los dominios de cola citoplásmicos y de transmembrana hcasp8

234 Una proteína de la reivindicación 179 en donde dicha proteína de cadena sencilla comprende un dominio de unión Fv de cadena sencilla L6, en donde en la región de conexión dichos primero, segundo y tercero residuos

de cisteína se reemplazan por serina y dicho resíduo de prolina se reemplaza por serina, dicha región constante de cadena pesada comprende dominios CH2 y CH3 de IgG_1

235 Una proteína de la reivindicación 179 en donde dicha proteína de cadena sencilla comprende un Fv de cadena sencilla G28-1 que comprende una región de conexión en donde dichos primero, segundo, y tercero resíduos de cisteína se reemplazan por serina y dicho resíduo de prolina se reemplaza por serina, en donde dicha región constante de cadena pesada comprende los dominios Fv CH2 y CH3 de cadena sencilla G28-1

236 Un método para reducir una población de células objetivo en un sujeto que comprende administrar a dicho sujeto una cantidad terapéuticamente efectiva de una proteína que es menos de cerca de 150kD que comprende las etapas de a) tratar la población de células objetivo con una primer proteína o péptido que se une a las células dentro de dicha población de célula objetivo, y b) tratar la población de célula objetivo con una segunda proteína o péptido que es capaz de al menos una de i) unir un receptor Fc, ii) inducir apoptosis de célula objetivo, o iii) fijar complementos, en donde dicha primera molécula de proteína o péptido se conecta directamente a dicha

segunda molécula de proteína o péptido o, opcionalmente, dicha primera molécula de proteína o péptido y dicha segunda molécula de proteína o péptido se ligan por una tercera molécula de proteína o péptido, y en donde dicha molécula de proteína no es un anticuerpo, un miembro de la familia TNF o la familia de receptor TNF, y no se conjuga con una toxina bacteriana, una droga citotóxica, o un radioisótopo

237 Un método para reducir una población de células objetivo en un sujeto que comprende administrar a dicho sujeto una cantidad terapéuticamente efectiva de una proteína que es menos de cerca de 150kD que consiste esencialmente de las etapas de a) tratar la población de células objetivo con una primer proteína o péptido que se une a las células dentro de dicha población de célula objetivo, y b) tratar la población de célula objetivo con una segunda proteína o péptido que es capaz de al menos uno de i) unir un receptor Fc, ii) inducir apoptosis de célula objetivo, o iii) fijar complementos, en donde dicha primera molécula de proteína o péptido se conecta directamente a dicha segunda molécula de proteína o péptido o, opcionalmente, dicha primer molécula de proteína o péptido y dicha segunda molécula de proteína o péptido se ligan por una tercera molécula de proteína o

péptido, y en donde dicha molécula de proteína no es un anticuerpo, un miembro de la familia TNF o la familia de receptor TNF, y no se conjuga con una toxina bacteriana, una droga citotóxica, o un radioisótopo

238 Una proteína de cadena sencilla de ocurrencia no natural que comprende i) un primer polipéptido que tiene un polipéptido de dominio de unión capaz de unirse a una molécula objetivo, dicho polipéptido de dominio de unión comprende una región variable de cadena liviana que comprende una o más eliminaciones o sustituciones de aminoácido, ii) un segundo polipéptido que comprende una región de conexión adherida a dicho primer polipéptido, y iii) un tercer polipéptido que comprende un polipéptido de región constante de cadena pesada de inmunoglobulina truncado N-terminal adherido al segundo polipéptido, en donde dicha proteína de cadena sencilla de ocurrencia no natural es capaz de al menos una actividad inmunológica

239 Una proteína de cadena sencilla de ocurrencia no natural que comprende i) un primer polipéptido que tiene un polipéptido de dominio de unión capaz de unirse a una molécula objetivo, dicho polipéptido de dominio de unión comprende una región variable de cadena liviana que comprende una o más eliminaciones o

sustituciones de aminoácido en las posiciones 12, 80, 81, 83, 105, 106, y 107, ii) un segundo polipéptido que comprende una región de conexión adherida a dicho primer polipéptido, y iii) un tercer polipéptido que comprende un polipéptido de región constante de cadena pesada de inmunoglobulina truncado N-terminal adherido al segundo polipéptido, en donde dicha proteína de cadena sencilla de ocurrencia no natural es capaz de al menos una actividad inmunológica

240 Una proteína de la reivindicación 238 en donde una o más eliminaciones o sustituciones de aminoácido en dicha región variable de cadena liviana es efectiva para incrementar la expresión o estabilidad de dicha proteína relacionada con una proteína sin dicha elilminación o sustitución

241 Una proteína de la reivindicación 238 en donde dicho polipéptido de dominio de unión es un Fv de cadena sencilla

242 Una proteína de la reivindicación 238 en donde dicho polipéptido de dominio de unión comprende un polipéptido de región variable de cadena liviana de inmunoglobulina y un polipéptido de región variable de cadena pesada de inmunoglobulina

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243 Una proteína de la reivindicación 242 que comprende además un segundo polipéptido de dominio de unión capaz de unir una segunda molécula objetivo, dicho segundo polipéptido de dominio de unión comprende un polipéptido de región variable de cadena liviana de inmunoglobulina y un polipéptido de región variable de cadena pesada de inmunoglobulina

244 Una proteína de la reivindicación 241 en donde dicho polipéptido de dominio de unión es un Fv de cadena sencilla y dicha región variable de cadena liviana de dicho Fv de cadena sencilla tiene una eliminación o sustitución de aminoácido en la posición 106

245 Una proteína de la reivindicación 241 en donde dicho polipéptido de dominio de unión es un Fv de cadena sencilla y el aminoácido en la posición 106 de la región variable de cadena pesada de dicho Fv de cadena sencilla se ha eliminado

246 Una proteína de la reivindicación 238 en donde dicho polipéptido de dominio de unión es un Fv de cadena sencilla y dicha región variable de cadena liviana de dicho Fv de cadena sencilla tiene una sustitución de aminoácido en la posición 106

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247 Una proteína de la reivindicación 244 en donde la leucina se reemplaza por serina en la posición de 106

248 Una proteína de la reivindicación 244 en donde la leucina se reemplaza por des-leucina en la posición de 106

249 Una proteína de la reivindicación 244 en donde el aminoácido se sustituyó por el aminoácido en la posición 106 de la región variable de cadena liviana Fv de cadena sencilla se selecciona de serina, treonina, cisteína, tirosina, asparagina, glutamina, ácido aspártico, ácido glutámico, lisina, arginina, e histidina

250 Una proteína de la reivindicación 244 en donde dicho polipéptido de dominio de unión es un Fv de cadena sencilla que comprende una región variable de cadena pesada, en donde dicha región variable de cadena pesada tiene una sustitución de aminoácido en la posición de aminoácido 11

251 Una proteína de la reivindicación 241 en donde dicho polipéptido de dominio de unión se une a un antígeno de tumor

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252 Una proteína de la reivindicación 241 en donde dicho polipéptido de dominio de unión se une a un antígeno en una célula efectora inmune

253 Una proteína de la reivindicación 241 en donde dicho polipéptido de dominio de unión se une a un antigeno de célula de cáncer

254 Una proteína de la reivindicación 253 en donde dicho antígeno de célula de cáncer es un antígeno de superficie

255 Una proteína de la reivindicación 253 en donde dicho antígeno de célula de cáncer is un antígeno intracelular

256 Una proteína de la reivindicación 238 en donde dicho polipéptido de dominio de unión se une a un antígeno de célula B

257 Una proteína de la reivindicación 256 en donde dicho antígeno de célula B se selecciona de CD19, CD20, CD22, CD37, CD40, CD80, y CD86

258 Una proteína de la reivindicación 241 en donde dicho Fv de cadena sencilla se une a un antígeno de célula B

259 Una proteína de la reivindicación 258 en donde dicho antígeno de célula B se selecciona de CD19, CD20, CD22, CD37, CD40, CD80, y CD86

260 Una proteína de la reivindicación 259 en donde dicho Fv de cadena sencilla se selecciona de Fv de cadena sencilla HD37, Fv de cadena sencilla 2H7, Fv de cadena sencilla G28-1, y Fv de cadena sencilla 4 4 220

261 Una proteína de la reivindicación 238 en donde dicho polipéptido de dominio de unión es un scFv que se une a un antígeno de diferenciación de célula B

262 Una proteína de la reivindicación 261 en donde dicho antígeno de célula B se selecciona de CD19, CD20, CD22, CD37, y CD40

263 Una proteína de la reivindicación 238 en donde dicho polipéptido de dominio de unión se une a un objetivo seleccionado de CD2, CD3, CD4, CD5, CD6, CD8, CD10, CD11b, CD14, CD19, CD20, CD21, CD22, CD23, CD24, CD25, CD28, CD30, CD37, CD40, CD43, CD50 (ICAM3), CD54 (ICAM1), CD56, CD69, CD80, CD86, CD134 (OX40), CD137

(41BB) CD152 (CTLA-4), CD153 (ligando CD30), CD154 (ligando CD40), ICOS, L6, B7-H1, y HLA clase II

264 Una proteína de la reivindicación 238 en donde dicha proteína es capaz de formar un complejo que comprende dos o más de dichas proteínas

265 Una proteína de la reivindicación 264 en donde dicho complejo es un dímero

266 Una proteína de la reivindicación 238 en donde dicha proteína es un monómero

267 Una proteína de la reivindicación 238 en donde dicha actividad inmunológica se selecciona de citotoxicidad mediada por célula dependiente de anticuerpo, fijación de complemento, inducción de apoptosis, inducción de una o más señales activas biológicamente, inducción de una o más células efectoras inmunes, activación de diferenciación celular, activación celular, liberación de una o más moléculas biológicamente activas, y neutralización de un agente infeccioso o toxina

268 Una proteína de la reivindicación 267 que es capaz de inducción de señales activas biológicamente mediante activación o inhibición de una o más moléculas

seleccionadas de proteína quinasa, proteína fosfatasa, G-proteínas, nucleótidos cíclicos u otros segundos mensajeros, canales de ion, y componentes de trayectoria secretora

269 Una proteína de la reivindicación 267 que es capaz de inducción de una o más células efectoras inmunes seleccionadas de células NK, monocitos, macrófagos, células B, células T, mastocitos, neutrófilos, eosinófilos, y basófilos

270 Una proteína de la reivindicación 267 en donde dicha inducción de una o más células efectoras inmunes conduce a citotoxicidad mediada por célula dependiente de anticuerpo o la liberación de una o más moléculas biológicamente activas

271 Una proteína de la reivindicación 267 que es capaz de activación celular, en donde dicha activación conduce a cambios en la actividad transcripcional celular

272 Una proteína de la reivindicación 271 en donde dicha actividad transcripcional celular se incrementa

273 Una proteína de la reivindicación 271 en donde dicha actividad transcripcional celular se reduce

274 Una proteína de la reivindicación 267 en donde dicha una o más moléculas biológicamente activas es una proteasa

275 Una proteína de la reivindicación 267 en donde dicha una o más moléculas biológicamente activas es una citoquina

276 Una proteína de la reivindicación 275 en donde dicha citoquina se selecciona de monoquinas, linfoquinas, quemoquinas, factores de crecimiento, factores de estimulación de colonia, interferones, e interleuquinas

277 Una proteína de la reivindicación 267 que es capaz de neutralización de un agente infeccioso, en donde dicho agente infeccioso es una bacteria, un virus, un parásito, o un hongo

278 Una proteína de la reivindicación 267 que es capaz de neutralización de una toxina, en donde dicha toxina se selecciona de endotoxinas y exotoxinas

279 Una proteína de la reivindicación 267 que es capaz de neutralización de una toxina, en donde dicha toxina es

una exotoxina seleccionada de toxina antrax, toxina cólera, toxina difteria, toxina pertussis, Toxina LT lábil-calor *E coli*, Toxina estable al calor *E coli* ST, shiga toxina *Pseudomonas* Exotoxina A, toxina botulinum, toxina tétano, Toxina *Bordetella pertussis* AC, y *Bacillus anthracis* EF

280 Una proteína de la reivindicación 267 que es capaz de neutralización de una toxina, en donde dicha toxina es una endotoxina seleccionada de saxitoxinas, tetrodotoxina, toxinas de hongos, aflatoxinas, alcaloides pirrolizidina, fitohemaglutininas, y grayanotoxinas

281 Una proteína de la reivindicación 238 en donde dicha proteína es capaz de unirse a un objetivo intracelular para efectuar una función celular

282 Una proteína de la reivindicación 238 en donde dicho polipéptido de región constante de cadena pesada de inmunoglobulina truncado N-terminal comprende un polipéptido de región constante IgG CH2 adherido a un polipéptido de región constante IgG CH3 de cadena pesada de inmunoglobulina

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283 Una proteína de la reivindicación 238 en donde dicho polipéptido de región constante de cadena pesada de inmunoglobulina truncado N-terminal consiste esencialmente de un polipéptido de región constante IgG CH2 adherido a un polipéptido de región constante IgG CH3 de cadena pesada de inmunoglobulina

284 Una proteína de la reivindicación 238 que comprende al menos una porción de una región constante humana

285 Una proteína de la reivindicación 238 en donde dicha región de conexión comprende una región de pivote de ocurrencia natural seleccionada de un pivote humano o porción de ésta, pivote IgG humana o una porción de ésta, pivote IgA humana o una porción de ésta, pivote IgE humana o una porción de ésta, región de pivote de camélido o una porción de ésta, región de pivote de llama IgG1 o porción de ésta, región de pivote de tiburón nodriza o porción de ésta, y región de pivote de pez rata o una porción de ésta

286 Una proteína de la reivindicación 238 en donde dicha región de conexión comprende un pivote IgE humana o una porción de ésta

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287 Una proteína de la reivindicación 238 en donde dicha región de conexión comprende una región de pivote IgG1, IgG2, IgG3 o IgG4 humana que tiene ya sea cero o un residuo de cisteína

288 Una proteína de la reivindicación 238 en donde dicha región de conexión comprende una región de pivote IgGA humana que tienen entre cero y dos residuos de cisteína

289 Una proteína de la reivindicación 238 en donde dicha región de conexión comprende una región de pivote de inmunoglobulina IgG1 humana tipo silvestre

290 Una proteína de la reivindicación 238 en donde dicha región de conexión comprende un sitio de glicosilación

291 Una proteína de la reivindicación 238 en donde dicha región de conexión no tiene residuos de cisteína capaces de formar enlaces de disulfuro

292 Una proteína de la reivindicación 238 en donde dicha región de conexión tiene un residuo de cisteína

293 Una proteína de la reivindicación 238 en donde dicha región de conexión comprende un polipéptido de región

de pivote de inmunoglobulina tipo silvestre mutado que comprende no más de un residuo de cisteína

294 Una proteína de la reivindicación 238 en donde dicha región de conexión se altera de modo que dicha proteína tiene una capacidad reducida para dimerizar

295 Una proteína de la reivindicación 238 en donde dicha región de conexión comprende una prolina y primero, segundo, y tercero residuos de cisteína, en donde dicho primer residuo de cisteína es N-terminal a dicha segunda cisteína y dicha segunda cisteina es N-terminal a dicha tercera cisteína, dicho tercer residuo de cisteína es N-terminal a dicho residuo de prolina

296 Una proteina de cadena sencilla de ocurrencia no natural, que incluye un primer polipéptido que comprende un polipéptido de dominio de unión capaz de unirse a una molécula objetivo, un segundo polipéptido que comprende una región de conexión adherida a dicho primer polipéptido, dicha región de conexión comprende al menos una porción de una región de pivote IgA, un tercer polipéptido que comprende un polipéptido de región constante de cadena pesada de inmunoglobulina truncado N-terminal adherido al segundo polipéptido, y en donde dicha proteína de cadena sencilla de

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ocurrencia no natural es capaz de al menos una actividad inmunológica

297 Una proteína de la reivindicación 296 en donde dicho polipéptido de dominio de unión comprende un polipéptido de región variable de cadena liviana de inmunoglobulina y un polipéptido de región variable de cadena pesada de inmunoglobulina

298 Una proteína de la reivindicación 297 que comprende además un segundo polipéptido de dominio de unión capaz de unir una segunda molécula objetivo, dicho segundo polipéptido de dominio de unión comprende un polipéptido de región variable de cadena liviana de inmunoglobulina y un polipéptido de región variable de cadena pesada de inmunoglobulina

299 Una proteína de la reivindicación 296 en donde dicha región de conexión consiste esencialmente de al menos una porción de una región de pivote IgA

300 Una proteína de la reivindicación 296 en donde dicha región de conexión consiste esencialmente de una región de pivote IgA

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301 Una proteína de la reivindicación 300 que se expresa como una proteína que tiene un molecular aparente de al menos cerca de 70 kDa

302 Una proteína de la reivindicación 300 que se expresa como una proteina que tienen un molecular aparente de entre cerca de 70 kDa y cerca de 700 kDa

303 Una proteína de la reivindicación 296 en donde dicha región de conexión comprende entre 10 a 50 aminoácidos

304 Una proteína de la reivindicación 296 en donde dicha región de conexión comprende un polipéptido de región de pivote de inmunoglobulina IgA humano modificado

305 Una proteína de la reivindicación 296 en donde dicha región de conexión tiene solo un residuo de cisteína

306 Una proteína de la reivindicación 296 en donde dicha región de conexión tiene dos residuos de cisteína

307 Una proteína de la reivindicación 296 en donde dicho polipéptido de región constante de cadena pesada de inmunoglobulina truncado N-terminal en el tercer polipéptido comprende regiones constantes de cadena pesada de inmunoglobulina IgA

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308 Una proteína de la reivindicación 307 comprende dominios CH2 y CH3 de regiones constantes de cadena pesada de inmunoglobulina IgA

309 Una proteína de la reivindicación 308 en donde dicho dominio de región constante IgA CH3 comprende una sustitución o eliminación en uno o más aminoácidos que da la región constante de cadena pesada IgA incapaz de asociarse con un polipéptido de cadena J

310 Una proteína de la reivindicación 308 en donde dicha región constante de cadena pesada IgA es capaz de asociarse con un polipéptido de cadena J

311 Una proteína de la reivindicación 309 en donde dicho dominio de región IgA CH3 comprende una eliminación entre cuatro y veinte aminoácidos

312 Una proteína de la reivindicación 309 en donde dicho dominio de región IgA comprende una eliminación de cuatro aminoácidos

313 Una proteína de la reivindicación 309 en donde dicho dominio de región IgA comprende una eliminación de veinte aminoácidos

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314 Una proteína de la reivindicación 296 en donde dicha proteína de cadena sencilla comprende un dominio de unión Fv de cadena sencilla L6, en donde la región de conexión comprende una región de pivote IgA o porción de ésta, dicha región constante de cadena pesada comprende dominios CH2 y CH3 de IgG_1

315 Una proteína de la reivindicación 296 en donde dicha proteína de cadena sencilla comprende un dominio de unión Fv de cadena sencilla de un hibridoma L6, en donde la región de conexión comprende una región de pivote IgA o porción de ésta, dicha región constante de cadena pesada comprende dominios CH2 y CH3 de IgG_1

316 Una proteína de la reivindicación 296 en donde dicha proteína de cadena sencilla comprende un dominio de unión Fv de cadena sencilla de un hibridoma CTLA-4, en donde la región de conexión comprende una región de pivote IgA o porción de ésta, dicha región constante de cadena pesada comprende dominios CH2 y CH3 de IgA de murina

317 Una proteína de la reivindicación 296 en donde dicha proteína de cadena sencilla comprende un dominio de unión Fv de cadena sencilla de un hibridoma CTLA-4, en

donde la región de conexión comprende una región de pivote IgA o porción de ésta, dicha región constante de cadena pesada comprende dominios CH2 y CH3 de IgA de murina y dicho CH3 comprende una eliminación o sustitución en cuatro aminoácidos que da la región constante de cadena pesada IgA incapaz de asociarse con un polipéptido de cadena J

318 Una proteína de la reivindicación 296 en donde dicha proteína de cadena sencilla comprende un dominio de unión Fv de cadena sencilla de un hibridoma 2H7, en donde la región de conexión comprende una región de pivote IgA o porción de ésta, dicha región constante de cadena pesada comprende dominios CH2 y CH3 de IgA de murina

319 Una proteína de la reivindicación 296 en donde dicha proteína de cadena sencilla comprende un dominio de unión Fv de cadena sencilla de un hibridoma 2H7, dicha región de conexión comprende un pivote IgA o porción de ésta, y dicha región constante de cadena pesada comprende dominios CH2 y CH3 de IgA, en donde dicho CH3 comprende una eliminación o sustitución en dieciocho aminoácidos que da la región constante de cadena pesada IgA incapaz de asociarse con un polipéptido de cadena J

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320 Una proteína de la reivindicación 296 en donde dicha proteína de cadena sencilla comprende un dominio de unión Fv de cadena sencilla de un hibridoma 2e12, dicha región de conexión comprende un pivote IgA o porción de ésta, y dicha región constante de cadena pesada comprende dominios CH2 y CH3 de IgA, en donde dicho CH3 comprende una eliminación o sustitución en cuatro aminoácidos que da la región constante de cadena pesada IgA incapaz de asociarse con un polipéptido de cadena J y dicho dominio CH3 se adhiere a un polipéptido que comprende dominios de cola citoplásmico y transmembrana CD80

321 Una proteína de la reivindicación 296 en donde dicha proteína de cadena sencilla comprende un dominio de unión Fv de cadena sencilla de un hibridoma 2e12, dicha región de conexión comprende un pivote IgA o porción de ésta, dicha región constante de cadena pesada comprende dominios CH2 y CH3 de IgA, en donde dicha CH3 comprende una eliminación o sustitución en cuatro aminoácidos que da la región constante de cadena pesada IgA incapaz de asociarse con un polipéptido de cadena J y dicho dominio CH3 se adhiere a un polipéptido que comprende dominios de cola citoplásmico y transmembrana CD80

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322 Una proteína de la reivindicación 296 en donde dicha proteína de cadena sencilla comprende un dominio de unión Fv de cadena sencilla de un hibridoma 1D8, dicha región de conexión comprende un pivote IgA o porción de ésta, dicha región constante de cadena pesada comprende dominios CH2 y CH3 de IgA, en donde dicho CH3 comprende una eliminación o sustitución en cuatro aminoácidos que da la región constante de cadena pesada IgA incapaz de asociarse con un polipéptido de cadena J y dicho dominio CH3 se adhiere a un polipéptido que comprende dominios de cola citoplásmico y transmembrana CD80

323 Una proteína de la reivindicación 296 en donde dicha proteína de cadena sencilla comprende un dominio de unión Fv de cadena sencilla de un hibridoma G28-1, en donde la región de conexión comprende una región de pivote IgA o porción de ésta, dicha región constante de cadena pesada comprende dominios CH2 y CH3 de IgG_1

324 Una proteína de la reivindicación 296 en donde dicha proteína de cadena sencilla comprende un dominio de unión Fv de cadena sencilla de un hibridoma 2H7, en donde la región de conexión comprende una región de pivote IgA o porción de ésta, dicha región constante de cadena pesada comprende dominios CH2 y CH3 de IgG_1

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325 Una proteína de la reivindicación 296 en donde dicha proteína de cadena sencilla comprende un dominio de unión Fv de cadena sencilla de un hibridoma 2H7, en donde la región de conexión comprende una región de pivote IgA o porción de ésta, dicha región constante de cadena pesada comprende dominios CH2 y CH3 de IgA, en donde dicho CH3 comprende una eliminación o sustitución en cuatro aminoácidos que da la región constante de cadena pesada IgA incapaz de asociarse con un polipéptido de cadena J y dicho dominio CH3 se adhiere a un polipéptido que comprende dominios de cola citoplásmico y transmembrana CD80

326 Una proteína de la reivindicación 296 en donde dicha proteína de cadena sencilla comprende un dominio de unión Fv de cadena sencilla de un hibridoma 2H7, en donde la región de conexión comprende una región de pivote IgA o porción de ésta, dicha región constante de cadena pesada comprende dominios CH2 y CH3 de IgA, en donde la región constante de cadena pesada IgA es capaz de asociarse con un polipéptido de cadena J

327 Una proteína de la reivindicación 296 en donde dicha proteína de cadena sencilla comprende un dominio de unión Fv de cadena sencilla de un hibridoma HD37, en donde la región de conexión comprende una región de

pivote IgA o porción de ésta, dicha región constante de cadena pesada comprende dominios CH2 y CH3 de IgA

328 Una proteína de cadena sencilla de ocurrencia no natural que comprende i) un primer polipéptido que comprende un polipéptido de dominio de unión capaz de unirse a una molécula objetivo, y ii) un segundo polipéptido que comprende al menos una porción de un polipéptido de región constante de cadena pesada de inmunoglobulina IgE adherido al segundo polipéptido, en donde dicha proteína de cadena sencilla de ocurrencia no natural es capaz de al menos una actividad inmunológica

329 Una proteína de la reivindicación 328 en donde dicho polipéptido de dominio de unión comprende una región variable no-IgE

330 Una proteína de la reivindicación 328 en donde dicho polipéptido de dominio de unión es un Fv de cadena sencilla seleccionado de Fv de cadena sencilla HD37, Fv de cadena sencilla 2H7, Fv de cadena sencilla G28-1, FC2-2, UCHL-1, 5B9, L6, 10A8, 2e12, 40 2 36, G19-4, 1D8, y Fv de cadena sencilla 4 4 220

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331 Una proteína de la reivindicación 328 en donde dicho polipéptido de dominio de unión comprende un polipéptido de región variable de cadena liviana de inmunoglobulina y un polipéptido de región variable de cadena pesada de inmunoglobulina

332 Una proteína de la reivindicación 331 que comprende además un segundo polipéptido de dominio de unión capaz de unir una segunda molécula objetivo, dicho segundo polipéptido de dominio de unión que comprende un polipéptido de región variable de cadena liviana de inmunoglobulina y un polipéptido de región variable de cadena pesada de inmunoglobulina

333 Una proteína de la reivindicación 332 en donde dichas cadenas pesadas y livianas de inmunoglobulina comprenden cadenas pesadas y livianas de inmunoglobulina IgG o una porción de éstas

334 Una proteína de la reivindicación 328 en donde dicho polipéptido de dominio de unión comprende al menos una porción de una región variable de inmunoglobulina IgG

335 Una proteína de la reivindicación 334 en donde dicha región variable IgG comprende una región variable de cadena pesada y una región variable de cadena liviana

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336 Una proteína de la reivindicación 328 que es capaz de unirse a un receptor específico Fc para IgE

337 Una proteína de la reivindicación 328 que no tiene región de cadena variable IgE

338 Una proteína de la reivindicación 328 en donde el dominio de unión consiste esencialmente de una región de cadena liviana de inmunoglobulina IgG y una región de cadena pesada y liviana de inmunoglobulina IgG

339 Una proteína de la reivindicación 328 en donde dicho polipéptido de región constante de cadena pesada de inmunoglobulina IgE comprende dominios CH2 y CH3

340 Una proteína de la reivindicación 328 en donde dicho polipéptido de región constante de cadena pesada de inmunoglobulina IgE comprende los dominios CH2, CH3 y CH4

341 Una proteína de la reivindicación 328 en donde dicho polipéptido de dominio de unión es un Fv de cadena sencilla que comprende una región variable de cadena pesada, en donde dicha región variable de cadena pesada

tiene una sustitución de aminoácido en la posición de aminoácido 11

342 Una proteína de la reivindicación 328 en donde dicha proteína de cadena sencilla es un Fv de cadena sencilla seleccionada de Fv de cadena sencilla HD37, Fv de cadena sencilla 2H7, Fv de cadena sencilla G28-1, y Fv de cadena sencilla 4 4 220

343 Una proteína de la reivindicación 328 en donde dicha proteína de cadena sencilla es un Fv de cadena sencilla que comprende un Fv de cadena sencilla 2H7 o un Fv de cadena sencilla G28-1

344 Un método para reducir una población de células objetivo en un sujeto que comprende administrar a dicho sujeto una cantidad terapéuticamente efectiva de una proteína de cadena sencilla de ocurrencia no natural, dicha proteína comprende un primer polipéptido que comprende un polipéptido de dominio de unión, dicho polipéptido de dominio de unión comprende al menos una porción de una región variable IgG y es capaz de unirse a una molécula objetivo, un segundo polipéptido comprende una región de conexión adherida a dicho primer polipéptido, un tercer polipéptido comprende un polipéptido de región constante de cadena pesada de

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inmunoglobulina IgE adherido al segundo polipéptido, en donde dicha proteína de cadena sencilla de ocurrencia no natural es capaz de al menos una actividad inmunológica

345 El método de la reivindicación 344 en donde dicha proteína se administra dentro del torrente sanguíneo a un paciente

346 El método de la reivindicación 344 en donde los monocitos se activan pero los mastocitos no se activan como un resultado de la administración de la proteína

347 El método de la reivindicación 344 en donde la proteína se administra o co-administra con un bloqueador de liberación de histamina

348 Un método para agotar células en un animal que comprende administrar una proteína IgE modificada dentro del torrente sanguíneo de un animal

349 El método de la reivindicación 348 en donde la proteína IgE modificada se administra o co-administra con un bloqueador de liberación de histamina

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350 Una proteína de la reivindicación 328 en donde dicha región de conexión comprende un pivote IgG o porción de este, y dicha región constante de cadena pesada de IgE y comprende los dominios CH2, CH3, y CH4 sin un dominio CH2

351 Una proteína de la reivindicación 328 en donde dicha proteína de cadena sencilla comprende un dominio de unión Fv de cadena sencilla de un 2e12, y dicha región constante de cadena pesada comprende los dominios IgE CH2, CH3, y CH4, y en donde dicha región constante de cadena pesada se adhiere a un polipéptido comprende los dominios de cola citoplasmático y de transmembrana CD80

352 Una proteína de cadena sencilla de ocurrencia no natural comprende i) un primer polipéptido que tiene un polipéptido de dominio de unión capaz de unirse una molécula objetivo, ii)un segundo polipéptido que comprende una región de conexión adherida a dicho primer polipéptido, dicha región de conexión comprende tres residuos de cisteína y un residuo de prolina, en donde uno o más de dichos residuos de cisteína se eliminan o sustituyen, y iii) un tercer polipéptido que comprende un polipéptido de región constante de cadena pesada de inmunoglobulina truncado N-terminal adherido

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al segundo polipéptido, en donde dicha proteína de cadena sencilla de ocurrencia no natural es capaz de al menos una actividad inmunológica

353 Una proteína de la reivindicación 352 en donde dicha región de conexión comprende primeros, segundos, y terceros residuos de cisteína, en donde dicho primer residuo de cisteina es N-terminal a dicha segunda cisteína y dicha segunda cisteína es N-terminal a dicha tercera cisteína, en donde dicho tercer residuo de cisteína no se sustituye o elimina, y en donde dicho segunda y tercera cisteína se sustituyen o eliminan

354 Una proteína de la reivindicación 353 en donde dicho segundo y tercero residuos de cisteína se sustituyen por otro aminoácido

355 Una proteína de la reivindicación 354 en donde dicho segundo y tercero residuos de cisteína se sustituyen por residuos de serina

356 Una proteína de la reivindicación 352 que se expresa como una proteína homogénea que tiene un peso molecular aparente de cerca de 75 kDa

357 Una proteína de la reivindicación 352 en donde dicha región de conexión comprende primeros, segundos, y terceros residuos de cisteína, en donde dicho primer residuo de cisteína es N-terminal a dicha segunda cisteína y dicha segunda cisteína es N-terminal a dicha tercera cisteína, en donde dicho primer residuo de cisteína se sustituyen o eliminan

358 Una proteína de la reivindicación 352 en donde dicho segundo y tercer residuos de cisteína en dicha región de conexión no se sustituyen o eliminan

359 Una proteína de la reivindicación 352 en donde dicha región de conexión comprende primeros, segundos, y terceros residuos de cisteína, en donde dicho primer residuo de cisteína es N-terminal a dicha segunda cisteína y dicha segunda cisteína es N-terminal a dicha tercera cisteína, en donde dicho segundo residuo de cisteína se sustituye o elimina

360 Una proteína de la reivindicación 352 en donde dicho primer y tercer residuos de cisteína en dicha región de conexión no se sustituyen o eliminan

361 Una proteína de la reivindicación 352 en donde dicha región de conexión comprende primeros, segundos, y

terceros residuos de cisteina, en donde dicho primer residuo de cisteína es N-terminal a dicha segunda cisteína y dicha segunda cisteína es N-terminal a dicha tercera cisteína, en donde dicho tercer residuo de cisteína se sustituye o elimina

362 La proteína de la reivindicación 10 en donde dicho primer y segundo residuos de cisteína en dicha región de conexión no se sustituyen o eliminan

363 Una proteína de la reivindicación 352 en donde dicha región de conexión comprende entre 5 y 65 aminoácidos

364 Una proteína de la reivindicación 352 en donde dicha región de conexión comprende entre 10 y 50 aminoácidos

365 Una proteína de la reivindicación 352 en donde dicha región de conexión comprende entre 15 y 35 aminoácidos

366 Una proteína de la reivindicación 352 en donde dicha región de conexión comprende al menos cinco aminoácidos de región de pivote consecutivo

367 Una proteína de la reivindicación 352 en donde dicha región de conexión comprende al menos diez aminoácidos de región de pivote consecutivo

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368 Una proteína de la reivindicación 352 en donde dicha región de conexión comprende al menos quince aminoácidos de región de pivote consecutivo

369 Una proteína de la reivindicación 353 en donde dicha segunda y tercera cisteínas se reemplazan por residuos de serina y dicha región de conexión comprende al menos una porción de una región de pivote IgG_1

370 Una proteína de la reivindicación 369 en donde dicha región de conexión comprende al menos 5 aminoácidos consecutivos de una región de pivote IgG_1

371 Una proteína de la reivindicación 353 en donde dicha segunda y tercera cisteínas se reemplazan por residuos de serina y dicha región de conexión comprende al menos una porción de una región de pivote IgG_2

372 Una proteína de la reivindicación 371 en donde dicha región de conexión comprende al menos 5 aminoácidos consecutivos de una región de pivote IgG_2

373 Una proteína de la reivindicación 353 en donde dicha segunda y tercera residuos de cisteína se reemplazan por residuos de serina y dicha región de conexión

comprende al menos una porción de una región de pivote IgG_3

374 Una proteína de la reivindicación 373 en donde dicha región de conexión comprende al menos 5 aminoácidos consecutivos de una región de pivote IgG_3

375 Una proteína de la reivindicación 353 en donde dichos segundo y tercero residuos de cisteína se reemplazan por residuos de serina y dicha región de conexión comprende al menos una porción de una región de pivote IgG_4

376 Una proteina de la reivindicación 375 en donde dicha región de conexión comprende al menos 5 aminoácidos consecutivos de una región de pivote IgG_4

377 Una proteína de la reivindicación 352 en donde dichos segundo y tercer residuos de cisteína se sustituyen o eliminan y dicho residuo de prolina se sustituye o elimina, en donde dicho polipéptido de región constante de cadena pesada comprende un dominio CH3 en donde la tirosina se remplaza por alanina en la posición 407, y en donde dicha proteína tiene un peso molecular aparente de cerca de 75kDa

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378 Una proteína de la reivindicación 352 en donde dichos primer y tercer residuos de cisteína se sustituyen o eliminan y dicho residuo de prolina se sustituye o elimina, en donde dicho polipéptido de región constante de cadena pesada comprende un dominio CH3 en donde la tirosina se remplaza por alanina en la posición 407, y en donde dicha proteína tiene un peso molecular aparente de cerca de 75kDa

379 Una proteína de cadena sencilla de ocurrencia no natural, que incluye un primer polipéptido que comprende un polipéptido de dominio de unión capaz de unirse a una molécula objetivo, un segundo polipéptido que comprende una región de conexión adherida a dicho primer polipéptido, un tercer polipéptido que comprende un polipéptido de región constante de cadena pesada de inmunoglobulina truncada N-terminal adherida al segundo polipéptido, en donde dicha región de conexión comprende primero, segundo, y tercero residuos de cisteína, en donde dicho primer residuo de cisteina es N-terminal a dicho segundo residuo de cisteína y dicha segunda cisteína es N-terminal a dicha tercera cisteína, en donde uno de ambos de dichos segundos y terceros residuos de cisteína se sustituye o elimina, y en donde dicha proteína de cadena sencilla de

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ocurrencia no natural es capaz de al menos una actividad inmunológica

380 Una proteína de la reivindicación 352 en donde dicha región de conexión comprende un polipéptido de región de pivote de inmunoglobulina IgG1 humano modificado

381 Una proteína de la reivindicación 352 en donde dicha región de conexión solo tiene un residuo de cisteína

382 Una proteína de la reivindicación 381 en donde dicha región de conexión comprende un polipéptido de región de pivote de inmunoglobulina IgG1 humano modificado

383 Una proteína de la reivindicación 352 en donde dicho polipéptido de región constante de cadena pesada de inmunoglobulina truncado N-terminal comprende un polipéptido de región constante IgG CH2 adherido a un polipéptido de región constante IgG CH3 de cadena pesada de inmunoglobulina

384 Una proteína de la reivindicación 352 en donde dicho polipéptido de región constante de cadena pesada de inmunoglobulina truncado N-terminal consiste esencialmente de un polipéptido de región constante IgG

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CH2 adherido a un polipéptido de región constante IgG CH3 de cadena pesada de inmunoglobulina

385 Una proteína de cadena sencilla de ocurrencia no natural, que incluye un primer polipéptido que comprende un polipéptido de dominio de unión capaz de unirse a una molécula objetivo, un segundo polipéptido que comprende una región de conexión adherida a dicho primer polipéptido, y un tercer polipéptido que comprende un polipéptido de región constante CH2 de cadena pesada de inmunoglobulina y un polipéptido de región constante CH3 de cadena pesada de inmunoglobulina, dicho polipéptido de región constante CH3 comprende uno o más sustituciones de aminoácido o eliminación que inhibe dos de dichas proteínas de asociarse juntas para formar un dímero no covalente, en donde dicha proteina de cadena sencilla de ocurrencia no natural es capaz de al menos una actividad inmunológica

386 Una proteína de cadena sencilla de ocurrencia no natural, incluye un primer polipéptido que comprende un polipéptido de dominio de unión capaz de unirse a una molécula objetivo, un segundo polipéptido que comprende una región de conexión adherida a dicho primer polipéptido, un tercer polipéptido que comprende un

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polipéptido de región constante CH2 de cadena pesada de inmunoglobulina y un polipéptido de región constante CH3 de cadena pesada de inmunoglobulina, dicho polipéptido de región constante CH3 comprende uno o más sustituciones de aminoácido o eliminación en las pocisiones 405 y 407, en donde dicha proteína de cadena sencilla de ocurrencia no natural es capaz de al menos una actividad inmunológica

387 Una proteína de la reivindicación 252 o 253 que comprende sustituciones de aminoácido en la posición 405 y 407

388 Una proteína de la reivindicación 387 en donde la fenilananina se reemplaza con alanina en la posición 405 y la tirosina se sustituye con alanina en la posición 407

389 Una proteína de la reivindicación 387 que tiene un peso molecular aparente de entre cerca de 40 kDa y cerca de 50 kDa

390 Una proteína de la reivindicación 387 que tiene una actividad biológica de un agonista o antagonista

391 Una proteína de la reivindicación 390 que tiene una actividad biológica de un antagonista

392 Una proteína de la reivindicación 387 en donde solo un aminoácido en las posiciones 405 y 407 se sustituye

393 Una proteína de la reivindicación 392 que tiene un peso molecular aparente de entre cerca de 70 kDa y cerca de 80 kDa

394 Una proteína de la reivindicación 387 en donde la fenilananina se reemplaza por alanina en la posición 405

395 Una proteína de la reivindicación 387 en donde la fenilananina se reemplaza por tirosina en la posición 405

396 Una proteína de la reivindicación 386 en donde la tirosina se reemplaza por alanina en la posición 407

397 Una proteína de la reivindicación 386 que comprende sustituciones de aminoácidos en la posición 405 y 407

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398 Una proteína de la reivindicación 393 en donde la fenilananina se reemplaza por alanina en la posición 405 y la tirosina se reemplaza por alanina en la posición 407

399 Un polinucléotido que codifica una proteína de acuerdo con una cualquiera de las reivindicaciones 1, 8, 9, 12, 18, 30, y 112-109

400 Un polinucléotido que codifica una proteína de acuerdo con una cualquiera de las reivindicaciones 116, 120, 121, y 180-235

401 Un polinucléotido que codifica una proteína de acuerdo con una cualquiera de las reivindicaciones 238, 239, 296, 328, 352, 353, 385, y 386

402 Un polinucléotido que codifica una proteína de acuerdo con una cualquiera de las reivindicaciones 399-401 que se liga operablemente a un promotor u otra secuencia que mejora la expresión del polinucleótido en una célula

403 Una célula que contiene un polinucleótido de una cualquiera de las reivindicaciones 399-401

404 Un vector recombinante capaz de expresar una proteína de acuerdo con cualquiera de las reivindicaciones 1, 8, 9, 12, 18, 30, y 112-109

405 Un vector recombinante capaz de expresar una proteína de acuerdo con cualquiera de las reivindicaciones 116, 120, 121, y 180-235

406 Un método para expresar una proteína de acuerdo con una cualquiera de las reivindicaciones 1, 8, 9, 12, 18, 30, y 1 12-109 de acuerdo con condiciones en las que se expresa la proteína

407 Un método para expresar una proteína de acuerdo con una cualquiera de las reivindicaciones 116, 120, 121, y 180-235 bajo condiciones en las que la proteína se expresa

408 Un método para expresar una proteína de acuerdo con una cualquiera de las reivindicaciones 238, 239, 296, 328, 352, 353, 385, y 386 de acuerdo con condiciones en las que la proteína se expresa

409 Una composición que comprende una proteína de acuerdo con una cualquiera de las reivindicaciones 238, 239,

296, 328, 352, 353, 385, y 386 en combinación con uno o más compuestos terapéuticos adicionales

410 Un método para tratar un sujeto que comprende las etapas de administrar a dicho sujeto uno o más primeros compuestos de acuerdo con una cualquiera de las reivindicaciones 238, 239, 296, 328, 352, 353, 385, y 386, administrar o coadministrar un segundo compuesto al sujeto o en combinación con dicho primer compuesto, en donde el tratamiento es efectivo para tratar o prevenir una enfermedad, insuficiencia, o condición indeseada

411 El método de acuerdo con la reivindicación 410 en donde el segundo compuesto se selecciona a partir de un compuesto quimioterapéutico, droga terapéutica, inhibidores de angiogénesis, esteroides activadores de célula B, activadores de célula T, factores de estimulación de colonia, factores de necrosis de tumor, interferones, anticuerpo, construcción de unión de la invención, terapia de gen, retinoides, procedimientos quirúrgicos, agentes de alquilación, análogos de nucleosido, topoisomerasa II, VEGF, IFN-alfa, agentes de DMAR, interluquina-1, glucocorticoides, inhibidores p38, interluquina-4, interluquina-6, interluquina-2, interluquina-12, IFN-γ, GM-CSF, G-CSF, M-CSF, anti-

CTLA-4, Bcl-2 antisentido, vitamina A, anti-CD19, anti-CD20, anti-CD22, anti-CD28 o anti-CD3

412 Un método para exhibir moléculas recombinantes, tales moléculas incluyen una región variable de cadena pesada de inmunoglobulina construida por ingeniaría o nativa, la mejora comprende una región de cadena pesada de inmunoglobulina que incluye una o más mutaciones, sustituciones, alteraciones y/o eliminaciones en uno o más residuos de aminoácido que corresponde a las posiciones 9, 10, 11, 12, 108, 110, y 112 en dicha región variable de cadena pesada

413 Una proteína de unión de antígeno de cadena sencilla de ocurrencia no natural comprende proteína que tiene una fórmula seleccionada de 2H7 scFv VH LI IS (CSC-S) H WCH2 WCH3, 2H7 scFv VH LI 1 S IgE CH2 CH3 CH4, 2H7 scFv VH L11 S mlgE CH2 CH3 CH4, 2H7 scFv VH L11S mIgAH WIgACH2 T4CH3, 2H7 scFv VH LI 1S (SSS-S) H K322S CH2 WCH3, 2H7 scFv ßIH L1 I S-(CSS-S) H K322S CH2 WCH3, 2H7 scFv VH L11S (SSS-S) H P331S CH2 WCH3, 2HU scFv VH L11S (CSS-S) H P331S CH2 WCH3, 2H7 scFv VH L11S (SSS-S) H T256N CH2 WCH3, 2H7 scFv VH LI IS (SSS-S) H RTPE/QNAK (255-258) CH2 WCH3, 2H7 scFv VH L11S (SSS-S) H K290Q CH2 WCH3, 2H7 scFv VH LI IS (SSS-S) H A339P CH2 WCH3, G28-1 scFv (SSS-S) H WCH2 WCH3, G28-1 scFv IgAH WCH2

WCH3, G28-1 scFv VH L11S (SSS-S) H WCH2 WCH3, G28-1 scFv VH LI IS (CSS-S) H WGH2 WCH3, G28-1 scFv VH LI IS (CSC-S) H WCH2 WCH3, G28-1 scFv VH L11S (SSC-P) H WCH2 WCH3, CTLA4 (SSS-S) H P238SCH2 WCH32, CTLA4 (CCC-P) WH WCH2 WCH3, FC2-2 scFv (SSS-S) H WCH2 WCH3, FC2-2 scFv VHL11S (SSS-S) H WCH2 WCH3, UCHL-1 scFv (SSS-S) H WCH2 WCH3, UCHL-1 scFv VHL11S (SSS-S) H WCH2 WCH3, 5B9 scFv (SSS-S) H WCH2 WCH3, 5B9 scFv VHL11S (SSS-S) H WCH2 WCH3, 2H7 scFv (SSS-S) H WCH2 WCH3, 2H7 scFv (SSS-S) H P238SCH2 WCH3, 2H7 scFv IgAH WCH2 WCH3, 2H7 scFv IgAH WIgACH2 T4CH3, 2H7 scFv IgAH WIgACH2 WCH3 + JChaın, 2H7 scFv (CCC-P) WH WCH2 WCH3, 2H7 scFv (SSS-S) H WCH2 F405YCH3, 2H7 scFv (SSS-S) H WCH2 F405ACH3, 2H7 scFv (SSS-S) H WCH2 Y407ACH3, 2H4 scFv (SSS-S) HWCJH2 F405A, Y407ACH3, 2H7 scFv (CSS-S) H WCH2 WCH3, 2H7 scFv (SCS-S) H WCH2 WCH3, 2H7 scFv (SSC-P) H WCH2 WCH3, 2H7 scFv (CSC-S) H WCH2 WCH3, 2H7 scFv (CCS-P) H WCH2 WCH3, 2H7 scFv (SCC-P) H WCH2 WCH3, 2H7 scFv VH LI IS (SSS-S) H WCH2 WCH3, 2H7 scFvVHL1 IS (CSS-S) H WCH2 WCH3, G28-1 scFv VH L11S (SCS-S) H WCH2 WCH3, G28-1 scFv VH L11S (CCS-P) H WCH2 WCH3, G28-1 scFv VH LI IS (SCC-P) H WCH2 WCH3, G28-1 scFv VH L11S mIgE CH2 CH3 CH4, G28-1 scFv VH LI IS mIgAH WIgACH2 T4CH3, G28-1 scFv VH LI IS hIgE CH2 CH3 CH4, G28-1 scFv VH L11S hIgAH WIgACH2 T4CH3, HD37 scFv IgAH WCH2 WCH3, HD37 scFv (SSS-S) H WCH2 WCH3, HD37 scFv VH L11S (SSS-S) H WCH2 WCH3, L6 scFv

IGAH WCH2 WCH3, L6 scFv VHL11S (SSS-S) H WCH2 WCH3, 2H7 scFv-llama IgGl, 2H7 scFv-llama IgG2, 2H7 scFv-llama IgG3, CD16-6 bajo (ED) (SSS-S) H P238SCH2 WCH3, CD16-9 alto (ED) (SSS-S) H P238SCH2 WCH3, 2el2 scFv (SSS-s) H P238SCH2 WCH3-hCD80TM/CT, 10A8 scFv (SSS-s) H P238SCH2 WCH3-hCD80TM/CT, 40 2 36 scFv (SSS-s) H P238SCH2 WCH3-hCD80TM/CT, 2H7 scFv (SSS-s) H P238SCH2 WCH3-hCD80TM/CT, G19-4 scFv (SSS-s) H P238SCH2 WCH3-hCD80TM/CT, 2el2 scFv (SSS-s) H WCH2 WCH3-hCD80TM/CT, 2el2 scFv IgAH IgACH2 T4CH3-hCD80TM/CT, 2e12 scFv IgE CH2CH3CH4-bCD80TM/CT, 2el2 scFv (SSS-s) H P238SCH2 WCH3-mFADD-TM/CT, 2e12 scFv (SSS-s)H WCH2 WCH3-mFADD-TM/CT, 2el2 scFv (SSS-s) H WCH2 WCH3--mcasp3-TM/CT, 2el2 scFv (SSS-s) H P238SCH2 WCH3-mcasp3-TM/CT, 2el2 scFv (SSS-s) H WCH2 WCH3-mcasp8--TM/CT, 2el2 scFv (SSS-s) H P238SCH2 WCH3-mcasp8-TM/CT, 2el2 scFv (SSS-s) H WCH2 WCH3-hcasp3--TM/CT, 2el2 scFv (SSS-s) H P238SCH2 WCH3-hcasp3-TM/CT, 2el2 scFv (SSS-s) H WCH2 WCH3-hcasp8--TM/CT, 2el2 scFv (SSS-s) H P238SCH2 WCH3hcasp8-TM/CT, 1D8 scFv--hIgGl (SSS-s) H P238SCH2 WCH3-hCD80TM/CT, 1D8 scFv--hIgG1 (SSS-s) H WCH2 WCH3-hCD80TM/CT, 1D8 scFv-mIgAT4-hCD80TM/CT, 1D8 scFv--hIgE-hCD80TM/CT, 1D8 scFv--hIgGl (SSS-s) H P238SCH2 WCH3-mFADD-TM/CT, 1D8 scFv--hIgGl (SSS-s) H WCH2 WCH3-mFADD-TM/CT, 1D8 scFv--hIgGl (SSS-s) H WCH2 WCH3- mcasp3 TM/CT, 1D8 scFv--hIgGl (SSS-s) H P238SCH2 WCH3-mcasp3-

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TM/CT, 1D8 scFv--hIgG1 (SSS-s) H WCH2 WCH3-mcasp8--
TM/CT, 1D8 scFv--hIgG1 (SSS-s) H P238SCH2 WCH3-mcasp8-
TM/CT, 1D8 scFv--hIgG1 (SSS-s) H WCH2 WCH3- hcasp3--
TM/CT, 1D8 scFv--hIgG1 (SSS-s) H P238SCH2 WCH3-hcasp3-
TM/CT, 1D8 scFv--hIgG1 (SSS-s) H WCH2 WCH3-hcasp8-
TM/CT, 1D8 scFv--hIgG1 (SSS-s) H P238SCH2 WCH3-hcasp8-
TM/CT, L6 scFv (SSS-S) H WCH2 WCH3, 2H7 scFv CD154 (L2), 2H7 scFv CD154 (S4), CTLA4 IgAH IGACH2CH3, CTLA4 IgAH IgACH2 T4CH3, 2H7 scFv IgAH IgACH2CH3, 2H7 scFv IgAH IgAHCH2 T18CH3, 2H&-40 2 220 scFv (SSS-S) H WCH2 WCH3 (anti-ccd20-anti-cd40 biespécifico), 2H7 scFv IgAH IgACH2 T4CH3-hCD89 TM/CT, G19-4 scFv (CCC-P) WH WCH2 WCH3-hCD89 TM/CT, y 2e12 scFv (CCC-P) WH WCH2 WCH3-hCD89 TM/CT

PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant s or agent s file reference 49076 000004	FOR FURTHER ACTION	see Noufication of Transmitul of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below
International application No. PCT/US03/41600	International filing date (*day/month/year*) 24 December 2003 (24 12 2003)	(Earliest) Priority Date (*day/month/year*) 26 July 2003 (26 07.2003)
Applicant TRUBION PHARMACEUTICALS, INC		

This international search report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18 A copy is being transmitted to the International Bureau.

This international search report consists of a total of 4 sheets

☒ It is also accompanied by a copy of each prior art document cited in this report.

1 **Basis of the Report**

a With regard to the language, the international search was carried out on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

☐ the international search was carried out on the basis of a translation of the international application furnished to this Authority (Rule 23 1(b)).

b With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of the sequence listing

☒ contained in the international application in written form.

☒ filed together with the international application in computer readable form.

☐ furnished subsequently to this Authority in written form.

☐ furnished subsequently to this Authority in computer readable form.

☐ the statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

☐ the statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

2 ☐ Certain claims were found unsearchable (See Box I)

3 ☐ Unity of invention is lacking (See Box II)

4 With regard to the title,

☒ the text is approved as submitted by the applicant.

☐ the text has been established by this Authority to read as follows

5 With regard to the abstract,

☒ the text is approved as submitted by the applicant.

☐ the text has been established according to Rule 38 2(b) by this Authority as it appears in Box III The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority

6 The figure of the drawings to be published with the abstract is Figure No ____

☐ as suggested by the applicant. ☒ None of the figures

☐ because the applicant failed to suggest a figure

☐ because this figure better characterizes the invention.

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INTERNATIONAL SEARCH REPORT

International application No
PCT/US03/41600

A CLASSIFICATION OF SUBJECT MATTER
IPC(7) C12N 15/00, A61K 39/395, C07K 16/00
US CL. 530/387 3, 388 85, 391 3, 424/130 1, 536/23 4, 435/320 1, 69 6
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)
U S 530/387 3, 388 85, 391 3, 424/130 1, 536/23.4 435/320 1, 69.6

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 practicable, search terms used)
Please See Continuation Sheet

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication where appropriate of the relevant passages	Relevant to claim No.
Y	HAYDEN et al Single-chain mono- and bispecific antibody derivatives with novel biological properties and antitumor activity from COS cell transient expression system. Therapeutic Immunology 1994, Vol 94 pages 3-15 especially Figure 1, Methods.	1-7, 20-28, 31-40, 53-57, 59, 62-63, 65-75, 116-119 129-137, 140-150, 161-169, 171-181, 238, 240-243, 251-259, 261 267 282 285, 287-295, 399-411
Y	US 6,147,203 A (PASTAN et al) 14 November 200 (14 11 2000), see entire document, especially abstract, column5-6.	1-7, 20-28, 31-40, 53-57, 59, 62-63, 65-75, 116-119, 129-137, 140-150, 161-169, 171-181, 238, 240-243, 251-259, 261-267, 282-285, 287-295, 399-411

☒ Further documents are listed in the continuation of Box C ☐ 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 application or patent 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
29 October 2004 (29 10 2004)

Date of mailing of the international search report
02 NOV 2004

Name and mailing address of the ISA/US
Mail Stop PCT Attn. ISA/US
Commissioner for Patents
P O Box 1450
Alexandria Virginia 22313 1450
Facsimile No (703) 305-3230

Authorized officer
Lynn R Helms
Telephone No 571-272-1600

orm PCT/ISA/210 (second sheet) (July 1998)

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PCT/US03/41600

INTERNATIONAL SEARCH REPORT

C (Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate of the relevant passages	Relevant to claim No.
Y	US 6 074,644 A (PASTAN et al) 13 June 2000 (13 06 2000), see entire document, especially column 20.	26 28, 32, 135, 137, 142 257, 259, 262
Y	US 5,677,425 A (BODMER et al) 14 October 1997 (14 10 1997), see entire document, especially abstract, column 3-4	65-75, 116, 172-180, 288-295
Y	US 6,482,919 B2 (LEDBETTER et al) 19 November 2002 (19 11 2002), see entire document.	180

Continuation of B FIELDS SEARCHED Item 3:
CAPLUS MEDLINE, WEST, BIOSIS
Search terms inventor name, scfv hinge, cysteine fusion protein, CD19, CD3, deleted hinge, altered hinge, IgG1, IgA IgE, disulfide stabilized constant region.

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NOTES TO FORM PCT/ISA/220

These Notes are intended to give the basic instructions concerning the filing of amendments under Article 19. The Notes are based on the requirements of the Patent Cooperation Treaty, the Regulations and the Administrative Instructions under that Treaty. In case of discrepancy between these Notes and those requirements, the latter are applicable. For more detailed information, see also the *PCT Applicant's Guide*, a publication of WIPO.

In these Notes, "Article", "Rule" and "Section" refer to the provisions of the PCT, the PCT Regulations and the PCT Administrative Instructions, respectively.

INSTRUCTIONS CONCERNING AMENDMENTS UNDER ARTICLE 19

The applicant has, after having received the international search report, one opportunity to amend the claims of the international application. It should however be emphasized that, since all parts of the international application (claims, description and drawings) may be amended during the international preliminary examination procedure, there is usually no need to file amendments of the claims under Article 19 except where, e.g. the applicant wants the latter to be published for the purposes of provisional protection or has another reason for amending the claims before international publication. Furthermore, it should be emphasized that provisional protection is available in some States only.

What parts of the international application may be amended?

Under Article 19, only the claims may be amended.

During the international phase, the claims may also be amended (or further amended) under Article 34 before the International Preliminary Examining Authority. The description and drawings may only be amended under Article 34 before the International Preliminary Examining Authority.

Upon entry into the national phase, all parts of the international application may be amended under Article 28 or, where applicable, Article 41.

When? Within 2 months from the date of transmittal of the international search report or 16 months from the priority date, whichever time limit expires later. It should be noted, however, that the amendments will be considered as having been received on time if they are received by the International Bureau after the expiration of the applicable time limit but before the completion of the technical preparations for international publication (Rule 46.1).

Where not to file the amendments?

The amendments may only be filed with the International Bureau and not with the receiving Office or the International Searching Authority (Rule 46.2).

Where a demand for international preliminary examination has been/is filed, see below.

How? Either by cancelling one or more entire claims, by adding one or more new claims or by amending the text of one or more of the claims as filed.

A replacement sheet must be submitted for each sheet of the claims which, on account of an amendment or amendments, differs from the sheet originally filed.

All the claims appearing on a replacement sheet must be numbered in Arabic numerals. Where a claim is cancelled, no renumbering of the other claims is required. In all cases where claims are renumbered, they must be renumbered consecutively (Administrative Instructions, Section 205(b)).

The amendments must be made in the language in which the international application is to be published.

What documents must/may accompany the amendments?

Letter (Section 205(b)):

The amendments must be submitted with a letter.

The letter will not be published with the international application and the amended claims. It should not be confused with the "Statement under Article 19(1)" (see below, under "Statement under Article 19(1)").

The letter must be in English or French, at the choice of the applicant. However, if the language of the international application is English, the letter must be in English. If the language of the international application is French, the letter must be in French.

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NOTES TO FORM PCT/ISA/220 (continued)

The letter must indicate the differences between the claims as filed and the claims as amended. It must, in particular indicate in connection with each claim appearing in the international application (it being understood that identical indications concerning several claims may be grouped), whether

(i) the claim is unchanged,
(ii) the claim is cancelled;
(iii) the claim is new
(iv) the claim replaces one or more claims as filed,
(v) the claim is the result of the division of a claim as filed.

The following examples illustrate the manner in which amendments must be explained in the accompanying letter"

1 [Where originally there were 48 claims and after amendment of some claims there are 51]
"Claims 1 to 29, 31 32, 34 35 37 to 48 replaced by amended claims bearing the same numbers; claims 30, 33 and 36 unchanged, new claims 49 to 51 added."

2. [Where originally there were 15 claims and after amendment of all claims there are 11]
"Claims 1 to 15 replaced by amended claims 1 to 11 "

3 [Where originally there were 14 claims and the amendments consist in cancelling some claims and in adding new claims]
"Claims 1 to 6 and 14 unchanged claims 7 to 13 cancelled, new claims 15 16 and 17 added." or
"Claims 7 to 13 cancelled, new claims 15 16 and 17 added, all other claims unchanged."

4. [Where various kinds of amendments are made]
"Claims 1-10 unchanged claims 11 to 13, 18 and 19 cancelled, claims 14 15 and 16 replaced by amended claim 14, claim 17 subdivided into amended claims 15 16 and 17 new claims 20 and 21 added."

"Statement under Article 19(1)" (Rule 46.4)

The amendments may be accompanied by a statement explaining the amendments and indicating any impact that such amendments might have on the description and the drawings (which cannot be amended under Article 19(1)).

The statement will be published with the international application and the amended claims.

It must be in the language in which the international application is to be published.

It must be brief not exceeding 500 words if in English or if translated into English.

It should not be confused with and does not replace the letter indicating the differences between the claims as filed and as amended. It must be filed on a separate sheet and must be identified as such by a heading, preferably by using the words "Statement under Article 19(1)."

It may not contain any disparaging comments on the international search report or the relevance of citations contained in that report. Reference to citations, relevant to a given claim, contained in the international search report may be made only in connection with an amendment of that claim.

Consequence if a demand for international preliminary examination has already been filed

If at the time of filing any amendments and any accompanying statement under Article 19 a demand for international preliminary examination has already been submitted, the applicant must preferably, at the time of filing the amendments (and any statement) with the International Bureau, also file with the International Preliminary Examining Authority a copy of such amendments (and of any statement) and where required, a translation of such amendments for the procedure before that Authority (see Rules 55.3(a) and 62.2, first sentence). For further information, see the Notes to the demand form (PCT/IPEA/401).

Consequence with regard to translation of the international application for entry into the national phase

The applicant's attention is drawn to the fact that, upon entry into the national phase a translation of the claims as amended under Article 19 may have to be furnished to the designated/elected Offices, instead of, or in addition to, the translation of the claims as filed.

For further details on the requirements of each designated/elected Office see the *PCT Applicant's Guide* Volume II.

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PATENT COOPERATION TREATY

From the
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

To:
BRADFORD J DUFT
BUCHANAN INGERSOLL PC
ONE OXFORD CENTRE, 20TH FLOOR
PITTSBURGH, PA 15219-1410

PCT

WRITTEN OPINION

(PCT Rule 66)

Date of Mailing (day/month/year)	19 AUG 2005
Applicant s or agent s file reference 49076.000004	REPLY DUE within 1 months/days from the above date of mailing

International application No.	International filing date (day/month/year)	Priority date (day/month/year)
PCT/US03/41600	24 December 2003 (24 12 2003)	26 July 2003 (26.07 2003)

International Patent Classification (IPC) or both national classification and IPC

IPC(7): C12N 15/00, A61K 39/395; C07K 16/00 and US Cl. 530/387 3, 388.85, 391.3, 424/130 1, 536/23.4; 435/320 1, 69 6

Applicant

TRUBION PHARMACEUTICALS, INC.

DOCKETED: 9/19/05

1 This written opinion is the first (first, etc.) drawn by this International Preliminary Examining Authority.

2. This opinion contains indications relating to the following items:

- I ☒ Basis of the opinion
- II ☐ Priority
- III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- IV ☐ Lack of unity of invention
- V ☒ Reasoned statement under Rule 66.2 (a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- VI ☐ Certain documents cited
- VII ☐ Certain defects in the international application
- VIII ☐ Certain observations on the international application

3 The applicant is hereby invited to reply to this opinion.

When? See the time limit indicated above ~~The applicant may, before the expiration of that time limit, request the Authority to grant an extension. See rule 66.2(d).~~

How? By submitting a written reply, accompanied, where appropriate, by amendments, according to Rule 66.3. For the form and the language of the amendments, see Rules 66.8 and 66.9

Also For an additional opportunity to submit amendments, see Rule 66.4.
For the examiner s obligation to consider amendments and/or arguments, see Rule 66.4 *bis*
For an informal communication with the examiner, see Rule 66.6

If no reply is filed, the international preliminary examination report will be established on the basis of this opinion.

4. The final date by which the international preliminary examination report must be established according to Rule 69 2 is: 26 November 2005 (26.11.2005)

Name and mailing address of the IPEA/US Mail Stop PCT Attn. IPEA/ US Commissioner for Patents P O. Box 1450 Alexandria Virginia 22313-1450 Facsimile No. (703) 305-3230	Authorized Officer Larry R. Helms Telephone No. 703-308-0196

Form PCT/IPEA/408 (cover sheet)(July 1998)

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WRITTEN OPINION

International application No.
PCT/US03/41600

I Basis of the opinion

1 With regard to the *elements of the international application:**

☒ the international application as originally filed

☒ the description.
pages 1-449, as originally filed
pages NONE, filed with the demand
pages NONE, filed with the letter of _____

☒ the claims
pages 450-498, as originally filed
pages NONE as amended (together with any statement) under Article 19
pages NONE, filed with the demand
pages NONE, filed with the letter of _____

☒ the drawings
pages 1-88, as originally filed
pages NONE filed with the demand
pages NONE, filed with the letter of _____

☒ the sequence listing part of the description.
pages none, as originally filed
pages NONE, filed with the demand
pages NONE filed with the letter of _____

2 With regard to the language, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.
These elements were available or furnished to this Authority in the following language _____ which is

☐ the language of a translation furnished for the purposes of international search (under Rule23 1(b))

☐ the language of publication of the international application (under Rule 48 3(b))

☐ the language of the translation furnished for the purposes of international preliminary examination(under Rules 55 2 and/or 55 3)

3 With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the written opinion was drawn on the basis of the sequence listing.

☐ contained in the international application in printed form.

☐ filed together with the international application in computer readable form.

☐ furnished subsequently to this Authority in written form.

☐ furnished subsequently to this Authority in computer readable form.

☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4 ☐ The amendments have resulted in the cancellation of:

☐ the description, pages NONE

☐ the claims, Nos NONE

☐ the drawings, sheets/fig NONE

5 ☐ This opinion has been drawn as if (some of) the amendments had not been made since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70 2(c))

* *Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this opinion as "originally filed."*

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WRITTEN OPINION

International application No.
PCT/US03/41600

V Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1 STATEMENT

Novelty (N)	Claims Please See Continuation Sheet	YES
	Claims Please See Continuation Sheet	NO
Inventive Step (IS)	Claims Please See Continuation Sheet	YES
	Claims Please See Continuation Sheet	NO
Industrial Applicability (IA)	Claims Please See Continuation Sheet	YES
	Claims Please See Continuation Sheet	NO

2 CITATIONS AND EXPLANATIONS

Claims 1 7 20-28 31-40, 53-57, 59 62-63 65-75, 116-119 129-137 140-150, 161-169, 171-181 238 240-243, 251 259 261-267 282-285 287-295, 399-411 lack an inventive step under PCT Article 33(3) as being obvious over Hayden et al in view of Pastan et al (203) and Pastan et al ('644) and Bodmer et al and Ledbetter et al.

The claims are summarized as a single chain protein comprising a binding domain polypeptide, a second polypeptide connecting region and a N-terminal truncated heavy chain constant region attached to the second polypeptide, wherein the protein has a substitution to increase stability, wherein the protein binds CD20, wherein the hinge is IgG1 with one cysteine, and the protein binds CTLA4.

Hayden et al teach a molecule comprising a binding polypeptide fused to a linker and the linker is connected to a protein and the protein is connected to the hinge CH2 CH3 of a constant region. Hayden et al does not teach the connecting region directly to the hinge or a substitution of an amino acid to obtain a stable molecule or one cystein in the hinge or binding to CD20 or CTLA4. these deficiencies are made up for in the teachings of Pastan et al (203) and Pastan et al ('644) and Bodmer et al and Ledbetter et al

Pastan ('203) teach disulfide stabilized scfv molecules.

Pastan (644) teach binding of antibodies to CD20.

Bodmer et al teach reducing the cysteines in the IgG1 hinge to one.

Ledbetter et al teach reducing the IgG1 hinge to one cysteine.

It would have been obvious to produce a single chain protein with a SCfv binding domain and a linker linking the scFv to the IgG1 hinge with one cysteine and the molecule binds CD20 or CTLA-4.

It would have been obvious because Hayden et al teach a scfv and a linker to a scfv and this linked to the hinge CH2, CH3 and it would have been obvious to link the scfv directly because Bodmer teach mutating to one cysteine in the hinge to promote dimerization and thus one could obtain a bifunctional antibody In addition it would have been obvious to produce a single chain protein to bind to CD20 or CTLA-4 because the prior art teaches the relevance of antigen binding fragments to these antigens In addition, it would have been obvious to substitute a cysteine in the VH and VL for stabilization because Pastan (203) teach that substitution leads to a more stabilized molecule

Therefore, claims 1-7 20-28 31-40 53-57 59 62-63 65-75 116-119 129-137, 140-150 161 169 171 181 238 240-243, 251 259 261 267, 282-285 287 295 399-411 lack an inventive step under PCT Article 33(3) as being obvious over Hayden et al in view of Pastan et al (203) and Pastan et al (644) and Bodmer et al and Ledbetter et al

Claims 8-19 29-30, 41-52 58 60-61, 64, 76-115, 120-128 138-139, 151 160, 170, 182-237, 239 244-250, 260, 268-281, 286, 296-398 412-413 meet the criteria set out in PCT Article 33(2)-(3) because the prior art does not teach or fairly suggest the claimed invention.

NEW CITATIONS

none

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WRITTEN OPINION

International application No.
PCT/US03/41600

Supplemental Box
(To be used when the space in any of the preceding boxes is not sufficient)

TIME LIMIT
The time limit set for response to a Written Opinion may not be extended. 37 CFR 1 484(d) Any response received after the expiration of the time limit set in the Written Opinion will not be considered in preparing the International Preliminary Examination Report.

V 1 Reasoned Statements
The opinion as to Novelty was positive (Yes)with respect to claims 1-413
The opinion as to Novelty was negative (No) with respect to claims NONE
The opinion as to Inventive Step was positive (Yes)with respect to claims 8-19, 29-30, 41 52 58, 60-61 64, 76-115, 120-128 138-139, 151 160, 170, 182-237, 239 244-250 260, 268-281, 286, 296-398 412-413
The opinion as to Inventive Step was negative(NO) with respect to claims 1 7 20-28, 31 40, 53-57 59, 62-63, 65-75, 116-119, 129-137, 140-150, 161 169 171 181 238 240-243 251-259, 261-267, 282-285 287 295 399-411
The opinion as to Industrial Applicability was positive (YES) with respect to claims NONE
The opinion as to Industrial Applicability was negative(NO) with respect to claims NONE

PCT

REQUEST

The undersigned requests that the present international application be processed according to the Patent Cooperation Treaty

For receiving Office use only

International Application No.

International Filing Date

Name of receiving Office and "PCT International Application"

Applicant's or agent's file reference *(if desired) (12 characters maximum)* 49076 000004 PCT3

Box No. I TITLE OF INVENTION

BINDING CONSTRUCTS AND METHODS FOR USE THEREOF

Box No. II APPLICANT ☐ This person is also inventor

Name and address *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)*

Trubion Pharmaceuticals, Inc
2401 Fourth Avenue, Suite 1050
Seattle, WA 98121
United States of America

Telephone No. 206/838-0500

Facsimile No. 206/838-0503

Teleprinter No.

Applicant's registration No. with the Office

State *(that is, country)* of nationality: US

State *(that is, country)* of residence: US

This person is applicant for the purposes of: ☐ all designated States ☒ all designated States except the United States of America ☐ the United States of America only ☐ the States indicated in the Supplemental Box

Box No. III FURTHER APPLICANT(S) AND/OR (FURTHER) INVENTOR(S)

Name and address: *(Family name followed by given name, for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)*

Ledbetter, Jeffrey A.
18798 Ridgefield Road Northwest
Shoreline, WA 98177
United States of America

This person is:
☐ applicant only
☒ applicant and inventor
☐ inventor only *(If this check-box is marked, do not fill in below.)*

Applicant's registration No. with the Office

State *(that is, country)* of nationality: US

State *(that is, country)* of residence: US

This person is applicant for the purposes of: ☐ all designated States ☐ all designated States except the United States of America ☒ the United States of America only ☐ the States indicated in the Supplemental Box

☒ Further applicants and/or (further) inventors are indicated on a continuation sheet.

Box No. IV AGENT OR COMMON REPRESENTATIVE, OR ADDRESS FOR CORRESPONDENCE

The person identified below is hereby/has been appointed to act on behalf of the applicant(s) before the competent International Authorities as: ☒ agent ☐ common representative

Name and address *(Family name followed by given name, for a legal entity, full official designation. The address must include postal code and name of country.)*

Duft, Bradford J
Buchanan Ingersoll PC
One Oxford Centre, 20th Floor
Pittsburgh, PA 15219-1410
United States of America

Telephone No. 619/578-5000

Facsimile No. 619/578-5940

Teleprinter No.

Agent's registration No. with the Office 32,219

☐ *Address for correspondence* Mark this check-box where no agent or common representative is/has been appointed and the space above is used instead to indicate a special address to which correspondence should be sent.

1804

Continuation of Box No. III FURTHER APPLICANT(S) AND/OR (FURTHER) INVENTOR(S)

If none of the following sub-boxes is used, this sheet should not be included in the request.

Name and address *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)*

Hayden-Ledbetter, Martha S
18798 Ridgefield Road Northwest
Shoreline, WA 98177
United States of America

This person is:
- ☐ applicant only
- ☒ applicant and inventor
- ☐ inventor only *(If this check-box is marked, do not fill in below.)*

Applicant's registration No. with the Office

State *(that is, country)* of nationality: US

State *(that is, country)* of residence: US

This person is applicant for the purposes of: ☐ all designated States ☐ all designated States except the United States of America ☒ the United States of America only ☐ the States indicated in the Supplemental Box

Name and address *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)*

Thompson, Peter A.
14075 30th Place N E
Bellevue, WA 98007
United States of America

This person is:
- ☐ applicant only
- ☒ applicant and inventor
- ☐ inventor only *(If this check-box is marked, do not fill in below.)*

Applicant's registration No. with the Office

State *(that is, country)* of nationality: US

State *(that is, country)* of residence: US

This person is applicant for the purposes of: ☐ all designated States ☐ all designated States except the United States of America ☒ the United States of America only ☐ the States indicated in the Supplemental Box

Name and address *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)*

This person is:
- ☐ applicant only
- ☐ applicant and inventor
- ☐ inventor only *(If this check-box is marked, do not fill in below.)*

Applicant's registration No. with the Office

State *(that is, country)* of nationality:

State *(that is, country)* of residence:

This person is applicant for the purposes of: ☐ all designated States ☐ all designated States except the United States of America ☐ the United States of America only ☐ the States indicated in the Supplemental Box

Name and address *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)*

This person is:
- ☐ applicant only
- ☐ applicant and inventor
- ☐ inventor only. *(If this check-box is marked, do not fill in below.)*

Applicant's registration No. with the Office

State *(that is, country)* of nationality:

State *(that is, country)* of residence:

This person is applicant for the purposes of: ☐ all designated States ☐ all designated States except the United States of America ☐ the United States of America only ☐ the States indicated in the Supplemental Box

☐ Further applicants and/or (further) inventors are indicated on another continuation sheet.

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Box No. V DESIGNATION OF STATES *Mark the applicable check-boxes below: at least one must be marked.*

The following designations are hereby made under Rule 4.9(a):

Regional Patent

☒ **AP** **ARIPO Patent** GH Ghana, GM Gambia, KE Kenya, LS Lesotho, MW Malawi, MZ Mozambique, SD Sudan, SL Sierra Leone SZ Swaziland, TZ United Republic of Tanzania, UG Uganda, ZM Zambia, ZW Zimbabwe, and any other State which is a Contracting State of the Harare Protocol and of the PCT *(If other kind of protection or treatment desired, specify on dotted line)*

☒ **EA** **Eurasian Patent** AM Armenia, AZ Azerbaijan BY Belarus, KG Kyrgyzstan, KZ Kazakhstan, MD Republic of Moldova, RU Russian Federation, TJ Tajikistan, TM Turkmenistan, and any other State which is a Contracting State of the Eurasian Patent Convention and of the PCT

☒ **EP** **European Patent:** AT Austria, BE Belgium BG Bulgaria, CH & LI Switzerland and Liechtenstein, CY Cyprus, CZ Czech Republic, DE Germany DK Denmark, EE Estonia, ES Spain, FI Finland, FR France, GB United Kingdom, GR Greece, HU Hungary, IE Ireland, IT Italy, LU Luxembourg, MC Monaco, NL Netherlands, PT Portugal, RO Romania, SE Sweden, SI Slovenia, SK Slovakia, TR Turkey and any other State which is a Contracting State of the European Patent Convention and of the PCT

☒ **OA** **OAPI Patent** BF Burkina Faso BJ Benin, CF Central African Republic, CG Congo CI Côte d'Ivoire, CM Cameroon, GA Gabon, GN Guinea GQ Equatorial Guinea, GW Guinea-Bissau, ML Mali, MR Mauritania, NE Niger, SN Senegal, TD Chad, TG Togo, and any other State which is a member State of OAPI and a Contracting State of the PCT *(If other kind of protection or treatment desired, specify on dotted line)*

National Patent *(If other kind of protection or treatment desired, specify on dotted line)*

☒ AE United Arab Emirates
☒ AG Antigua and Barbuda
☒ AL Albania
☒ AM Armenia
☒ AT Austria
☒ AU Australia
☒ AZ Azerbaijan
☒ BA Bosnia and Herzegovina
☒ BB Barbados
☒ BG Bulgaria
☒ BR Brazil
☒ BY Belarus
☒ BZ Belize
☒ CA Canada
☒ CH & LI Switzerland and Liechtenstein
☒ CN China
☒ CO Colombia
☒ CR Costa Rica
☒ CU Cuba
☒ CZ Czech Republic
☒ DE Germany
☒ DK Denmark
☒ DM Dominica
☒ DZ Algeria
☒ EC Ecuador
☒ EE Estonia
☒ ES Spain
☒ FI Finland
☒ GB United Kingdom
☒ GD Grenada
☒ GE Georgia
☒ GH Ghana
☒ GM Gambia
☒ HR Croatia
☒ HU Hungary
☒ ID Indonesia
☒ IL Israel
☒ IN India
☒ IS Iceland
☒ JP Japan
☒ KE Kenya
☒ KG Kyrgyzstan
☒ KP Democratic People's Republic of Korea
☒ KR Republic of Korea
☒ KZ Kazakhstan
☒ LC Saint Lucia
☒ LK Sri Lanka
☒ LR Liberia
☒ LS Lesotho
☒ LT Lithuania
☒ LU Luxembourg
☒ LV Latvia
☒ MA Morocco
☒ MD Republic of Moldova
☒ MG Madagascar
☒ MK The former Yugoslav Republic of Macedonia
☒ MN Mongolia
☒ MW Malawi
☒ MX Mexico
☒ MZ Mozambique
☒ NI Nicaragua
☒ NO Norway
☒ NZ New Zealand
☒ OM Oman
☒ PG Papua New Guinea
☒ PH Philippines
☒ PL Poland
☒ PT Portugal
☒ RO Romania
☒ RU Russian Federation
☒ SC Seychelles
☒ SD Sudan
☒ SE Sweden
☒ SG Singapore
☒ SK Slovakia
☒ SL Sierra Leone
☒ SY Syrian Arab Republic
☒ TJ Tajikistan
☒ TM Turkmenistan
☒ TN Tunisia
☒ TR Turkey
☒ TT Trinidad and Tobago
☒ TZ United Republic of Tanzania
☒ UA Ukraine
☒ UG Uganda
☒ US United States of America
☒ UZ Uzbekistan
☒ VC Saint Vincent and the Grenadines
☒ VN Viet Nam
☒ YU Serbia and Montenegro
☒ ZA South Africa
☒ ZM Zambia
☒ ZW Zimbabwe

Check-boxes below reserved for designating States which have become party to the PCT after issuance of this sheet:

☐ ☐ ☐

Precautionary Designation Statement In addition to the designations made above the applicant also makes under Rule 4.9(b) all other designations which would be permitted under the PCT except any designation(s) indicated in the Supplemental Box as being excluded from the scope of this statement. The applicant declares that those additional designations are subject to confirmation and that any designation which is not confirmed before the expiration of 15 months from the priority date is to be regarded as withdrawn by the applicant at the expiration of that time limit. *(Confirmation (including fees) must reach the receiving Office within the 15-month time limit.)*

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Supplemental Box *If the Supplemental Box is not used, this sheet should not be included in the request.*

1 If in any of the Boxes except Boxes Nos. VIII(i) to (v) for which a special continuation box is provided, the space is insufficient to furnish all the information, in such case, write "Continuation of Box No...." (indicate the number of the Box) and furnish the information in the same manner as required according to the captions of the Box in which the space was insufficient, in particular:

(i) If more than two persons are to be indicated as applicants and/or inventors and no "continuation sheet" is available: in such case, write "Continuation of Box No. III" and indicate for each additional person the same type of information as required in Box No. III. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below;

(ii) If in Box No. II or in any of the sub-boxes of Box No. III, the indication "the States indicated in the Supplemental Box" is checked: in such case, write "Continuation of Box No. II" or "Continuation of Box No. III" or "Continuation of Boxes No. II and No. III" (as the case may be), indicate the name of the applicant(s) involved and, next to (each) such name, the State(s) (and/or where applicable, ARIPO, Eurasian, European or OAPI patent) for the purposes of which the named person is applicant;

(iii) If in Box No. II or in any of the sub-boxes of Box No. III, the inventor or the inventor/applicant is not inventor for the purposes of all designated States or for the purposes of the United States of America, in such case, write "Continuation of Box No. II" or "Continuation of Box No. III" or "Continuation of Boxes No. II and No. III" (as the case may be), indicate the name of the inventor(s) and, next to (each) such name, the State(s) (and/or where applicable, ARIPO, Eurasian, European or OAPI patent) for the purposes of which the named person is inventor;

(iv) If, in addition to the agent(s) indicated in Box No. IV, there are further agents, in such case, write "Continuation of Box No. IV" and indicate for each further agent the same type of information as required in Box No. IV;

(v) If in Box No. V the name of any State (or OAPI) is accompanied by the indication "patent of addition," or "certificate of addition," or if, in Box No. V the name of the United States of America is accompanied by an indication "continuation" or "continuation-in-part", in such case, write "Continuation of Box No. V" and the name of each State involved (or OAPI), and after the name of each such State (or OAPI), the number of the parent title or parent application and the date of grant of the parent title or filing of the parent application;

(vi) If in Box No. VI, there are more than five earlier applications whose priority is claimed: in such case, write "Continuation of Box No. VI" and indicate for each additional earlier application the same type of information as required in Box No. VI.

2. If, with regard to the precautionary designation statement contained in Box No. V, the applicant wishes to exclude any State(s) from the scope of that statement, in such case, write "Designation(s) excluded from precautionary designation statement" and indicate the name or two-letter code of each State so excluded.

CONTINUATION OF BOX NO IV

additional agents with same address as first named agent
Collett, James W (46,636)
Opalko, Bryan H (40 751)

1795
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Box No. VI PRIORITY CLAIM

The priority of the following earlier application(s) is hereby claimed.

Filing date of earlier application *(day/month/year)*	Number of earlier application	Where earlier application is:		
		national application: country or Member of WTO	regional application:* regional Office	international application: receiving Office
item (1) 26/07/2003	10/627,556	US		
item (2)				
item (3)				
item (4)				
item (5)				

☐ Further priority claims are indicated in the Supplemental Box.

The receiving Office is requested to prepare and transmit to the International Bureau a certified copy of the earlier application(s) *(only if the earlier application was filed with the Office which for the purposes of this international application is the receiving Office)* identified above as:

☒ all items ☐ item (1) ☐ item (2) ☐ item (3) ☐ item (4) ☐ item (5) ☐ other, see Supplemental Box

* *Where the earlier application is an ARIPO application, indicate at least one country party to the Paris Convention for the Protection of Industrial Property or one Member of the World Trade Organization for which that earlier application was filed (Rule 4.10(b)(ii)).*

Box No. VII INTERNATIONAL SEARCHING AUTHORITY

Choice of International Searching Authority (ISA) *(if two or more International Searching Authorities are competent to carry out the international search, indicate the Authority chosen, the two-letter code may be used)*:

ISA / US

Request to use results of earlier search, reference to that search *(if an earlier search has been carried out by or requested from the International Searching Authority)*.

Date *(day/month/year)* Number Country *(or regional Office)*

Box No. VIII DECLARATIONS

The following declarations are contained in Boxes Nos VIII (i) to (v) *(mark the applicable check-boxes below and indicate in the right column the number of each type of declaration)*:

Number of declarations

☐ Box No. VIII (i) Declaration as to the identity of the inventor

☐ Box No. VIII (ii) Declaration as to the applicant's entitlement, as at the international filing date, to apply for and be granted a patent

☐ Box No. VIII (iii) Declaration as to the applicant's entitlement, as at the international filing date, to claim the priority of the earlier application

☐ Box No. VIII (iv) Declaration of inventorship (only for the purposes of the designation of the United States of America)

☐ Box No. VIII (v) Declaration as to non-prejudicial disclosures or exceptions to lack of novelty

1397
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Box No. IX CHECK LIST; LANGUAGE OF FILING

This international application contains

(a) In paper form, the following number of sheets.

request (including declaration sheets)	6
description (excluding sequence listings and/or tables related thereto)	449
claims	49
abstract	1
drawings	88
Sub-total number of sheets	593
sequence listings	
tables related thereto	
(for both, actual number of sheets if filed in paper form, whether or not also filed in computer readable form, see (c) below)	
Total number of sheets	593

(b) ☐ only in computer readable form (Section 801(a)(i))
- (i) ☐ sequence listings
- (ii) ☐ tables related thereto

(c) ☐ also in computer readable form (Section 801(a)(ii))
- (i) ☐ sequence listings
- (ii) ☐ tables related thereto

Type and number of carriers (diskette, CD-ROM, CD-R or other) on which are contained the
- ☐ sequence listings:
- ☐ tables related thereto:

(additional copies to be indicated under items 9(ii) and/or 10(ii), in right column)

This international application is accompanied by the following item(s) *(mark the applicable check-boxes below and indicate in right column the number of each item)*. — Number of items

1	☒	fee calculation sheet	1
2	☐	original separate power of attorney	
3	☐	original general power of attorney	
4	☐	copy of general power of attorney, reference number, if any	
5	☐	statement explaining lack of signature	
6	☐	priority document(s) identified in Box No VI as item(s)	
7	☐	translation of international application into *(language)*	
8	☐	separate indications concerning deposited microorganism or other biological material	
9	☐	sequence listings in computer readable form *(indicate type and number of carriers)*	
	(i) ☐	copy submitted for the purposes of international search under Rule 13*ter* only (and not as part of the international application)	
	(ii) ☐	*(only where check-box (b)(i) or (c)(i) is marked in left column)* additional copies including, where applicable, the copy for the purposes of international search under Rule 13*ter*	
	(iii) ☐	together with relevant statement as to the identity of the copy or copies with the sequence listings mentioned in left column	
10	☐	tables in computer readable form related to sequence listings *(indicate type and number of carriers)*	
	(i) ☐	copy submitted for the purposes of international search under Section 802(b-*quater*) only (and not as part of the international application)	
	(ii) ☐	*(only where check-box (b)(ii) or (c)(ii) is marked in left column)* additional copies including, where applicable, the copy for the purposes of international search under Section 802(b-*quater*)	
	(iii) ☐	together with relevant statement as to the identity of the copy or copies with the tables mentioned in left column	
11	☒	other *(specify)* postcard	

Figure of the drawings which should accompany the abstract:

Language of filing of the international application: English

Box No. X SIGNATURE OF APPLICANT, AGENT OR COMMON REPRESENTATIVE

Next to each signature, indicate the name of the person signing and the capacity in which the person signs (if such capacity is not obvious from reading the request).

Bryan H. Opelka 12-24-2003

Bryan H. Opelka
Registration No. 40,751

For receiving Office use only

1 Date of actual receipt of the purported international application:	2. Drawings. ☐ received. ☐ not received.
3 Corrected date of actual receipt due to later but timely received papers or drawings completing the purported international application.	
4 Date of timely receipt of the required corrections under PCT Article 11(2):	
5 International Searching Authority (if two or more are competent): ISA /	6 ☐ Transmittal of search copy delayed until search fee is paid

For International Bureau use only

Date of receipt of the record copy by the International Bureau.

1809 1798

The demand must be filed directly with the competent International Preliminary Examining Authority or if two or more Authorities are with the one chosen by the applicant. The full name or two-letter code of that Authority may be indicated by the applicant on the line

IPEA/ US

PCT

CHAPTER II

DEMAND

under Article 31 of the Patent Cooperation Treaty

The undersigned requests that the international application specified below be the subject of international preliminary examination according to the Patent Cooperation Treaty and hereby elects all eligible States (except where otherwise indicated).

For International Preliminary Examining Authority use only

Identification of IPEA	Date of receipt of DEMAND

Box No. I IDENTIFICATION OF THE INTERNATIONAL APPLICATION

Applicant's or agent's file reference: 49076 000004 PCT3

International application No	International filing date *(day/month/year)*	(Earliest) Priority date *(day/month/year)*
PCT/US2003/041600	24 December 2003 (24 12 2003)	26 July 2003 (26 07.2003)

Title of invention: BINDING CONSTRUCTS AND METHODS FOR USE THEREOF

Box No. II APPLICANT(S)

Name and address *(Family name followed by given name for a legal entity, full official designation. The address must include postal code and name of country.)*

Trubion Pharmaceuticals Inc.
2401 Fourth Avenue, Suite 1050
Seattle WA 98121
United State of America

Telephone No. 206/838-0500
Facsimile No 206/838-0503
Teleprinter No.
Applicant's registration No with the Office

State *(that is country)* of nationality	State *(that is country)* of residence
US	US

Name and address *(Family name followed by given name for a legal entity, full official designation. The address must include postal code and name of country.)*

Ledbetter Jeffrey A.
18798 Ridgefield Road Northwest
Shoreline, WA98177
United States of America

State *(that is country)* of nationality	State *(that is country)* of residence
US	US

Name and address *(Family name followed by given name for a legal entity full official designation. The address must include postal code and name of country.)*

Hayden-Ledbetter, Martha S
18798 Ridgefield Road Northwest
Shoreline WA 98177
United States of America

State *(that is country)* of nationality	State *(that is country)* of residence
US	US

☒ Further applicants are indicated on a continuation sheet.

Express Mail Label No. ED534724545US

ED534724545US

1810

Sheet No. 2

International application No.
PCT/US2003/041800

Continuation of Box No. II APPLICANT(S)

If none of the following sub-boxes is used, this sheet should not be included in the demand.

Name and address *(Family name followed by given name for a legal entity, full official designation. The address must include postal code and name of country.)*

Thompson, Peter A.
14075 30th Place N E
Bellevue, WA 98007
United States of America

State *(that is, country)* of nationality	State *(that is country)* of residence
US	US

Name and address *(Family name followed by given name for a legal entity, full official designation. The address must include postal code and name of country.)*

State *(that is, country)* of nationality	State *(that is country)* of residence

Name and address. *(Family name followed by given name for a legal entity, full official designation. The address must include postal code and name of country.)*

State *(that is country)* of nationality	State *(that is country)* of residence:

Name and address: *(Family name followed by given name for a legal entity full official designation. The address must include postal code and name of country.)*

State *(that is country)* of nationality	State *(that is country)* of residence:

☐ Further applicants are indicated on another continuation sheet.

1811 1800

Sheet No. 3

International application No
PCT/US2003/041800

Box No. III AGENT OR COMMON REPRESENTATIVE, OR ADDRESS FOR CORRESPONDENCE

The following person is ☒ agent ☐ common representative

and ☒ has been appointed earlier and represents the applicant(s) also for international preliminary examination.

☐ is hereby appointed and any earlier appointment of (an) agent(s) /common representative is hereby revoked.

☐ is hereby appointed specifically for the procedure before the International Preliminary Examining Authority, in addition to the agent(s)/common representative appointed earlier

Name and address *(Family name followed by given name for a legal entity, full official The address must include postal code and name of country.)*

Duft, Bradford J et al
Buchanan Ingersoll
One Oxford Centre 20th Floor
Pittsburgh, PA 15219-1410
United States of America

Telephone No.
619/578-5000

Facsimile No.
619/578-5940

Teleprinter No.

Agent's registration No. with the Office
32,219

☐ Address for correspondence Mark this check-box where no agent or common representative is/has been appointed and the space above is used instead to indicate a special address to which correspondence should be sent.

Box No. IV BASIS FOR INTERNATIONAL PRELIMINARY EXAMINATION

Statement concerning amendments *

1 The applicant wishes the international preliminary examination to start on the basis of:

☒ the international application as originally filed.

the description ☒ as originally filed
☐ as amended under Article 34

the claims ☒ as originally filed
☐ as amended under Article 19 (together with any accompanying statement)
☐ as amended under Article 34

the drawings ☒ as originally filed
☐ as amended under Article 34

2. ☐ The applicant wishes any amendment to the claims under Article 19 to be considered as reversed.

3 ☐ The applicant wishes the start of the international preliminary examination to be postponed until the expiration of 20 months from the priority date unless the International Preliminary Examining Authority receives a copy of any amendments made under Article 19 or a notice from the applicant that he does not wish to make such amendments (Rule 69 1(d)) *(This check-box may be marked only where the time limit under Article 19 has not yet expired.)*

* Where no check box is marked, international preliminary examination will start on the basis of the international application as originally filed or where a copy of amendments to the claims under Article 19 and/or amendments of the international application under Article 34 are received by the International Preliminary Examining Authority before it has begun to draw up a written opinion or the international preliminary examination report, as so amended.

Language for the purposes of international preliminary examination English

☒ which is the language in which the international application was filed.
☐ which is the language of a translation furnished for the purposes of international search.
☐ which is the language of publication of the international application.
☐ which is the language of the translation (to be) furnished for the purposes of international preliminary examination.

Box No. V ELECTION OF STATES

The applicant hereby elects all eligible States *(that is all States which have been designated and which are bound by Chapter II of the PCT)*

excluding the following States which the applicant wishes not to elect:

1812 1804

Sheet No. 4

International application No.
PCT/US2003/041800

Box No. VI CHECK LIST

The demand is accompanied by the following elements in the language referred to in Box No IV, for the purposes of international preliminary examination		For International Preliminary Examining Authority use only: received	not received
1 translation of international application	sheets	☐	☐
2. amendments under Article 34	sheets	☐	☐
3 copy (or, where required, translation) of amendments under Article 19	sheets	☐	☐
4 copy (or, where required, translation) of statement under Article 19	sheets	☐	☐
5 letter	sheets	☐	☐
6. other *(specify)*	sheets	☐	☐

The demand is also accompanied by the item(s) marked below:

1 ☒ fee calculation sheet
2 ☐ original separate power of attorney
3 ☐ original general power of attorney
4 ☐ copy of general power of attorney; reference number, if any:
5 ☐ statement explaining lack of signature
6 ☐ sequence listing in computer readable form
7 ☒ other *(specify)* Return receipt Postcard

Box No VII SIGNATURE OF APPLICANT, AGENT OR COMMON REPRESENTATIVE

Next to each signature, indicate the name of the person signing and the capacity in which the person signs (if such capacity is not obvious from reading the demand).

James W Colletti, Ph D (Reg No 46,636)
Buchanan Ingersoll

For International Preliminary Examining Authority use only

1 Date of actual receipt of DEMAND

2 Adjusted date of receipt of demand due to CORRECTIONS under Rule 60 1(b).

3 ☐ The date of receipt of the demand is AFTER the expiration of 19 months from the priority date and item 4 or 5, below does not apply ☐ The applicant has been informed accordingly

4 ☐ The date of receipt of the demand is WITHIN the period of 19 months from the priority date as extended by virtue of Rule 80.5

5 ☐ Although the date of receipt of the demand is after the expiration of 19 months from the priority date, the delay in arrival EXCUSED pursuant to Rule 82.

For International Bureau use only

Demand received from IPEA on.

1813 1802

SUPERINDUSTRIA Y COMERCIO
Radicacion : 06019270 00000000 Folios: 1001
Fecha (AMD): 2006-02-27 16:51:10
Tramite 011 PCT-PATENT 378 FASE RNCI 411 PRESENTAC
Actuacion 2020 DIVISION DE NUEVAS CREACIONES

Industria y Comercio

SUPERINTENDENCIA

Delegatura de Propiedad Industrial
División de Nuevas Creaciones

REQUISITOS NECESARIOS PARA PRESENTACIÓN Y AGILIZACIÓN DEL TRAMITE

PCT

	USUARIO	RADICADOR
PATENTE DE INVENCIÓN (X) MODELO DE UTILIDAD ( )	( )	( )
-Indicación de que se solicita la concesión de una patente.	( )	X
-Datos de identificación del solicitante o de la persona que se presenta la solicitud	( )	X
Descripción de la invención	( )	X
Dibujos de ser estos pertinentes.	( )	X
-Comprobante de Pago de las tasas establecidas.	( )	X
COMPLETA (X) INCOMPLETA ( )	( )	( )
DISEÑO INDUSTRIAL ( )		
-Indicación de que se solicita el registro de Diseño Industrial	( )	( )
Datos de identificación del solicitante o de la persona que se 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 Diseño Industrial	( )	( )
Datos de identificación del solicitante o de la persona que se presenta la solicitud	( )	( )
-Representación gráfica del Esquema Trazado	( )	( )
-Comprobante de Pago de las tasas establecidas.	( )	( )
COMPLETA ( ) INCOMPLETA ( )	( )	( )

Firma Responsable _____

Fecha _____

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PETITORIO

Industria y Comercio
SUPERINTENDENCIA

SUPERINDUSTRIA Y COMERCIO
Radicacion 06019270 00000000 Folios 1001
Fecha (AMD) 2006-02-27 16 51:10
Tramite 011 PCT-PATENT 376 FASE NACI 411 PRESENTAC
Funcionario POPO BTUTSTON DE MIRUAR CONANTINES

FORMULARIO UNICO DE SOLIC PCT
ENTRADA EN FASE NACIONAL 26-02-2006

1) SOLICITUD DE

☒ Patente de invención ☐ Patente de Modelo de Utilidad

☐ Capitulo I ☒ Capitulo II

2) SOLICITANTE (71)	**Nombre TRUBION PHARMACEUTICALS , INC** **Direccion 2401 Fourth Avenue, Suite 1050, Seattle, WA 98121, Estados Unidos** **Nacionalidad o domicilio Seattle, WA, Estados Unido s** **Lugar de constitución WA, Estados Unidos**	**IDENTIFICACIÓN** **C C** ☐ **NIT** ☐ **C E** ☐ **OTRO** ☐ **Cual** _____ **NUMERO** _____
	Teléfono Fax **E. Mail**	
3) REPRESENTANTE APODERADO	Nombre DILIA MARÍA RODRÍGUEZ D ALEMAN Dirección Carrera 11 N° 86-53 Piso 6 Bogotá D C Teléfono 6181088 Fax 6350824 E Mail info@clarkemodet com co	**IDENTIFICACIÓN** **C C** ☒ **NIT** ☐ **C E** ☐ **OTRO** ☐ **NUMERO** 41 654 882 **TP** 20824 CSJ
4) INVENTOR (ES) (72)	**Nombre 1 LEDBETTER, Jeffrey, A , 2 HAYDEN-LEDBETTER, Martha, 3 THOMPSON, Meter, A** **Dirección 1 18798 Ridgefield Road Northwest, Shoreline, WA 98177, USA, 2 18798 Ridgefield Road Northwest, Shoreline, WA 98177 USA, 3 14075 30th Place, N E , Bellevue, WA 98007, USA** **Nacionalidad o Domicilio Todos los anteriores, estadounidenses**	

5) PCT (86)
Solicitud Internacional No PCT/US2003/041600 Fecha DIC 24, 2003

Publicación Internacional No WO2005/017148 A1 Fecha Feb 24, 2005

6)Titulo (54) CONSTRUCCIONES DE UNIÓN Y MÉTODOS PARA USO DE ELLAS

7) Clasificacion Internacional C12N 15/00, A61K 39/395,C07K 16/00 , A61P37/00, 29/00

8) PRIORIDAD	**33) Pais de Origen**	**(32) Fecha**	**(31) Numero de Solicitud**
SI ☒ **N** ☐	**Estados Unidos**	**Julio 26, 2003**	**10/627,556**

9) Comprobante de pago N° 06-7,292 Fecha Enero 27, 2006

Instrucciones para el diligenciamiento del presente formulario Ver reverso de esta pagina

SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO
DIVISIÓN DE NUEVAS CREACIONES

2020/
Bogotá, D C ,

Doctor(a)
Alicia Lloreda Ricaurte **Oficio N°**
Apoderado(a)
ASTRAZENECA AB

REFERENCIA	**Radicación**	**00 78740**
	Trámite	**002**
	Evento	**001**
	Actuación	**430**
	Folios	**011**

Como resultado del examen de patentabilidad de la solicitud de patente de invención N° 00 78740, realizado segun el artículo 45 de la decisión 486 de la comisión de la Comunidad Andina, se notifica

1 DOCUMENTOS ESTUDIADOS

- El resumen y el capítulo descriptivo de la solicitud de los folios 5 a 72
- Los ejemplos que ilustran la síntesis de los compuestos de la invención en los folios 105 a 129
- Las preparaciones que ilustran los procesos de síntesis de intermedianos en los folios 73 a 104
- Las cláusulas 1 a 73 relacionadas en el capítulo reivindicatorio de la solicitud y que se encuentran en los folios 130 a 165
- El primer examen de forma realizado a la solicitud No 1895 que se encuentra en los folios 171 a 174
- La respuesta al primer examen de forma, allegada mediante memorial de fecha 15/12/2000 que se encuentra en los folios 175 a 336 y que contiene la copia de la solicitud mediante la cual se reivindica prioridad
- La copia de la solicitud original mediante la cual se reivindica prioridad Sueca SE9903759-0 de 18/10/1999 en los folios 208 a 324, junto con la correspondiente traducción del capítulo reivindicatorio en los folios 177 a 207
- El memorial de fecha 29/12/2000 que contiene la modificación al capítulo reivindicatorio y a ciertas secciones del aparte descriptivo <u>El nuevo capítulo consta de 73 cláusulas, presentes en los folios 348 a 383</u>

2 OBJETO DE LA INVENCIÓN

La solicitud hace referencia a ***"Nuevos compuestos oxabispidina útiles en el tratamiento de arritmias cardiacas"***

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REIVINDICACIONES

1 Una proteína de cadena sencilla de ocurrencia no natural que comprende i) un primer polipéptido que tiene un polipéptido de dominio de unión capaz de unir una molécula objetivo, dicho polipéptido de dominio de unión comprende una region variable de cadena pesada, dicha región variable de cadena pesada comprende una sustitución de aminoácido o agotamiento en uno o más residuo de aminoácido, ii)un segundo polipéptido que comprende una región de conexión adherida a dicho primer polipéptido, y iii) un tercer polipéptido que comprende un polipéptido de región constante de cadena pesada de inmunoglobulina truncada N-terminal adherida al segundo polipeptido, en donde dicha proteína de cadena sencilla de ocurrencia no natural es capaz de al menos una actividad inmunológica

2 Una proteína de la reivindicación 1 en donde dicho polipéptido de dominio de unión Fv una cadena sencilla

3 Una proteína de la reivindicación 1 en donde la sustitución o eliminación de uno o más aminoácidos en dicha región variable de cadena pesada es efectiva para incrementar la expresión o estabilidad de dicha

REPUBLICA DE COLOMBIA
SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO

RESOLUCION
Por la cual se decide una revocatoria directa

Rad Exp Nº 02075481

el recurso de reposición ante el Superintendente Delegado para la Propiedad Industrial al momento de notificarse o dentro de los 5 días hábiles siguientes "

ARTICULO SEGUNDO Notificar al doctor Jorge E Vera Vargas, en su calidad de apoderado de la sociedad Industria Americana de Colchones Induamercol S A, y al doctor Helmer Zuluaga Vargas, en su calidad de apoderado de la sociedad opositora Industrias Spring S A , o a quienes hagan sus veces, el contenido de la presente resolución, entregándoles copia de la misma, advirtiéndoles que contra ella no procede recurso alguno

NOTIFIQUESE Y CUMPLASE
Dada en Bogotá D C , a los

EL SUPERINTENDENTE DELEGADO PARA LA PROPIEDAD INDUSTRIAL,

GIANCARLO MARCENARO JIMENEZ

Doctor
JORGE E VERA VARGAS
Calle 70 A Nº 11-43
Bogotá D C

Doctor
HELMER ZULUAGA VARGAS
Calle 90 Nº 11-44 Piso 6º
Bogotá D C

4670
1816

proteína relacionada con una proteína sin dicho agotamiento o sustitución

4 Una proteína de la reivindicación 1 en donde dicho polipéptido de dominio de unión comprende un polipéptido de región variable de cadena liviana de inmunoglobulina y un polipéptido de región variable de cadena pesada de inmunoglobulina

5 Una proteína de la reivindicación 4 que comprende además un segundo polipeptido de dominio de unión capaz de unir una segunda molécula objetivo, dicho segundo polipéptido de dominio de unión comprende un polipéptido de región variable de cadena liviana de inmunoglobulina y un polipéptido de región variable de cadena pesada de inmunoglobulina

6 Una proteína de la reivindicación 5 en donde la primer molécula objetivo y la segunda molécula objetivo son diferentes

7 Una proteína de la reivindicación 5 en donde la primer molécula objetivo y la segunda molécula objetivo son la misma

The examination is being carried out on the following application documents

Description, Pages

1-105 as published

Sequence listings part of the description, Pages

1-50 as published

Claims, Numbers

1-58 received on 18.04.2001 with letter of 17 04.2001

Drawings, Sheets

1/17-17/17 as published

1 The applicant is asked to provide a basis for amendments made with letter dated 17 04 2001

2 The following documents (D3-D4) were found in a search of the state of the art in accordance with Article 54(3) EPC Copies of the documents are annexed to the communication and the numbering will be adhered to in the rest of the procedure

D3 EP 1103564
D4 WO 00/26249

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1817

REPUBLICA DE COLOMBIA
SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO

Bogotá,
2020

Doctor(a)
RODRIGUEZ DILIA MARIA

REFERENCIA	Radicación No	6 19270
	Solicitud internacional	US2003/041600
	Fecha inicio fase nacional	26/02/2006
	Trámite	11
	Evento	378
	Actuación	430
	Oficio No	4323

Como resultado del examen de forma, realizado con fundamento en el artículo 38 de la Decisión 486 de la Comisión de la Comunidad Andina, artículo 27 del Tratado de Cooperación en Materia de Patentes (PCT), regla 51 bis del Reglamento y Circular Única de la Superintendencia de Industria y Comercio, se le comunica que

1 Si fuere el caso, el solicitante debe adjuntar la traducción de los anexos del reporte de examen preliminar internacional (Art 36 2 b) del tratado PCT

2 La solicitud no contiene la copia del documento en que consta la cesión del derecho a la patente del inventor al solicitante o a su causante (Art 26-k) Decisión 486

3 La solicitud no contiene el poder debidamente otorgado por la sociedad TRUBION PHARMACEUTICALS, INC , con el lleno de los requisitos exigidos por los artículos 63 y subsiguientes del Código de Procedimiento Civil Adicionalmente, deberá presentar el documento que acredita la existencia y representación legal de la sociedad solicitante

4 El solicitante debe presentar un nuevo titulo que permita identificar la materia de la cual trata la solicitud, ya que el titulo relacionado no se encuentra con la precisión necesaria para identificar claramente el objeto de la solicitud de patente (Regla 4 3 PCT, Art 26-27 D-486, Circular única 5 2 9)

5 Debe aclarar el nombre del inventor THOMPSON, Peter, por cuanto difiere en la publicación internacional y el petitorio presentado en fase nacional

En cumplimiento de lo dispuesto por el artículo 39 de la Decisión 486, se le requiere para que dentro del término de dos meses siguientes a la fecha de notificación del presente oficio, complete los requisitos anteriormente enunciados En caso de no dar cumplimiento al presente requerimiento, la solicitud se considerará abandonada y perderá su prelación

Notifíquese el presente oficio conforme a lo dispuesto en el numeral 5 2, literal e), del capítulo quinto del título primero de la Circular Unica No 10 de 2001

Continua en la página siguiente

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1618

REPUBLICA DE COLOMBIA

SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO

Radicación No 6 19270

ALIX CARMENZA CESPEDES DE VERGEL
Jefe de la división de Nuevas Creaciones

El anterior requerimiento fue notificado por fijación en lista de fecha 21 ABR 2006
jramos