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(54) Titre : FORME HUMANISEE DE L'ANTICORPS DE SOURIS 14.18 (H14.18) SE LIANT AU GD2 ET FUSION DE CELLE-CI AVEC IL-2

(54) Title: HUMANIZED ANTIBODY (H14.18) OF THE MOUSE 14.18 ANTIBODY BINDING TO GD2 AND ITS FUSION WITH IL-2

(57) Abrégé/Abstract:
The invention provides humanized antibody H14.18 binding the human cell surface glycosphingolipid GD2. The antibody comprises modified variable regions, more specially, modified framework regions, which reduce their immunogenitity when administered to a human. The antibody may be coupled to the therapeutic agent such as IL-2 and used in the treatment of cancer.

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(54) Title: HUMANIZED ANTIBODY (H14.18) OF THE MOUSE 14.18 ANTIBODY BINDING TO GD2 AND ITS FUSION WITH IL-2

(57) Abstract: The invention provides humanized antibody H14.18 binding the human cell surface glycosphingolipid GD2. The antibody comprises modified variable regions, more specially, modified framework regions, which reduce their immunogenitity when administered to a human. The antibody may be coupled to the therapeutic agent such as IL-2 and used in the treatment of cancer.

HUMANIZED ANTIBODY (H14.18) OF THE MOUSE 14.18 ANTIBODY BINDING TO GD2 AND ITS FUSION WITH IL-2

This invention relates generally to modified antibodies. More particularly, the invention relates to modified antibodies with reduced immunogenicity that specifically bind the human cell surface glycosphingolipid GD2, and their use as therapeutic agents.

BACKGROUND OF THE INVENTION

There has been significant progress in the development of antibody-based therapies over the years. For example, investigators have identified not only a variety of cancer-specific markers but also a variety of antibodies that specifically bind to those markers. Antibodies can be used to deliver certain molecules, for example, a toxin or an immune stimulatory moiety, for example, a cytokine, to a cancer cell expressing the marker so as to selectively kill the cancer cell.

The 14.18 antibody is a mouse-derived monoclonal antibody directed against the cell surface glycosphingolipid GD2. GD2 is a disialoganglioside that is normally only expressed at a significant level on the outer surface membranes of neuronal cells, where its exposure to the immune system is limited by the blood brain barrier.

Many tumor cells, in contrast, have abnormal levels of glycosphingolipid cell surface expression. For example, GD2 is expressed on the surfaces of a wide range of tumor cells including neuroblastomas, medulloblastomas, astrocytomas, melanomas, small-cell lung cancer, osteosarcomas and other soft tissue sarcomas. Thus, GD2 is a convenient tumor-specific marker for targeting immune-stimulatory protein domains to tumor cells for the purpose of raising an effective immune response against the tumor cells to destroy them. While the 14.18 mouse antibody (m14.18 antibody) may assist the targeting of these protein domains to tumor cells, its mouse-derived amino acid sequences can impair the desired therapeutic effect.

When administered to a patient, antibodies can have an associated immunogenicity in the host mammal. This is more likely to occur when the antibodies are not autologous. Consequently, the effectiveness of antibody-based therapies often is limited by an immunogenic response directed against the therapeutic antibody. This immunogenic response typically is increased when the antibody is derived in whole or in part from a mammal different than the host mammal, *e.g.*, when the antibody is derived from a mouse and the recipient is a human.

For clinical use in humans, it may be helpful to modify mouse-derived antibodies to more closely resemble human antibodies, so as to reduce or minimize the immunogenicity of the mouse-derived antibody. The immunogenicity of the mouse-derived antibody can be reduced by the creation of a chimeric antibody in which the constant regions of a human antibody are fused to mouse variable domains. However, the remaining mouse variable domains are generally still immunogenic in humans, and can thus impair the efficacy of an antibody-based therapy.

Some approaches to reducing immunogenicity, such as "veneering" and "humanization" involve the introduction of many amino acid substitutions and may disrupt the binding of an antibody to an antigen. The m14.18 antibody binds to GD2 with moderate affinity. Therefore, mutations that significantly lower the affinity of m14.18 for GD2 are expected to make it less effective for therapeutic purposes in humans. Accordingly, there is a need in the art for therapeutic antibodies that can effectively target GD2 and have reduced immunogenicity when administered to a human.

SUMMARY OF THE INVENTION

Generally, the present invention provides a modified form of the m14.18 antibody that is less immunogenic in humans, but still maintains the binding affinity of m14.18 for human GD2.

More particularly, the invention provides a humanized form of the m14.18 antibody (hu14.18 antibody) in which several mouse-specific amino acids in one or more of the framework regions have been substituted with different amino acids to reduce their immunogenicity in humans. The invention also provides fusions of the hu14.18 antibody to one or more non-immunoglobulin moieties for enhancing the effects of targeted immune therapy.

In one aspect, the present invention provides an antibody variable region including the amino acid sequence set forth in SEQ ID NO: 1, which defines an immunoglobulin light chain variable region (V_L region). In another aspect, the invention relates to an antibody variable region including the amino acid sequence set forth in SEQ ID NO: 2, which defines an immunoglobulin heavy chain variable region (V_H region). In one embodiment, the invention provides an antibody variable region in which the amino acid sequence of SEQ ID NO: 1 is linked to the amino acid sequence set forth in SEQ ID NO:

2. The amino acid sequences can be linked, such as by a disulfide bond or a peptide bond.

In another aspect, the invention relates to an antibody variable region that specifically binds to GD2 and includes at least amino acids 1-23 of SEQ ID NO: 1, amino acids 1-25 of SEQ ID NO: 2, or amino acids 67-98 of SEQ ID NO: 2. These sequences define framework regions in the immunoglobulin variable regions of the hu14.18 antibody. Framework regions are described in greater detail below.

One aspect of the invention relates to a method for targeting a cell with GD2 on its surface and includes administering an antibody variable region of the present invention to a patient. In one embodiment, the targeted cell is a tumor cell. Further aspects of the invention include a nucleic acid encoding the antibody variable region or a cell that includes this nucleic acid, either of which can be administered to a patient or used for *in vitro* protein production.

The invention also provides a polypeptide that includes an antibody variable region of the invention and an Fc portion comprising at least a CH2 domain, nucleic acids encoding the polypeptide, cells including the nucleic acids, and methods for targeting a cell with GD2 on its surface by administering the polypeptide, nucleic acid, or cell to a patient. In some embodiments of the invention, the Fc portion is derived from IgG1.

The antibody variable region can be linked, with or without an intervening Fc portion, to a non-immunoglobulin moiety. Specifically, the non-immunoglobulin moiety can be a cytokine, such as an interleukin, a hematopoietic factor, a lymphokine, an interferon, or a chemokine. The interleukin can be, for example, interleukin-2 or interleukin-12. The hematopoietic factor and lymphokine can be, for example, granulocyte-macrophage colony stimulating factor (GM-CSF) and a lymphotoxin, respectively. The interferon can be, for example, interferon-α, interferon-β, or interferon-γ. In some embodiments of the invention, the fusion protein includes a second non-immunologlobulin moiety, such as a second cytokine. In a particular embodiment, the fusion protein includes the antibody variable region, IL-2, and IL-12.

In one aspect, the invention provides a humanized antibody-IL2 fusion protein designated as hu14.18-IL2 that specifically binds GD2 and stimulates immune function comprising the light chain of SEQ ID NO. 5 and the heavy chain of SEQ ID NO. 6.

In another aspect, the invention provides a vector comprising the nucleotide sequence of SEQ ID NO. 4 containing the nucleic acid sequences that code for the fusion protein as described above.

In another aspect, the invention provides pharmaceutical composition comprising the fusion protein as described above and a pharmaceutical carrier or excipient.

In another aspect, the invention provides use of the fusion protein as described above for the manufacture of a medicament for stabilizing disease progression in GD2 positive cancer patients.

In another aspect, the invention provides use of the fusion protein as described above for the manufacture of a medicament for increasing ADCC and NK-lysis activity in GD2 positive cancer patients.

In another aspect, the invention provides a pharmaceutical composition as described above, for use in stabilizing disease progression in GD2 positive cancer patients.

In another aspect, the invention provides a pharmaceutical composition as described above, for use in increasing ADCC and NK-lysis activity in GD2 positive cancer patients.

It is to be understood that the features of the various embodiments described herein are not mutually exclusive and can exist in various combinations and permutations.

Description of the Drawings

Figure 1A shows the amino acid sequence of an immunoglobulin light chain variable region in accordance with the invention.

Figure 1B shows the amino acid sequence of an immunoglobulin heavy chain variable region in accordance with the invention.

Figure 2A-D show the nucleotide sequence of an expression vector, including the nucleic acid constructs encoding an immunoglobulin light chain and an immunoglobulin heavy chain-IL-2 fusion protein in accordance with the invention.

Figure 3A shows the amino acid sequence of an immunoglobulin light chain in accordance with the invention.

Figure 3B shows the amino acid sequence of an immunoglobulin heavy chain in accordance with the invention.

DETAILED DESCRIPTION OF THE INVENTION

The present invention provides a modified form of the m14.18 antibody that is less immunogenic in humans, but is still able to specifically bind human GD2. The reduced immunogenicity is provided by one or more altered amino acid sequences in the immunoglobulin variable domains. The antibody is useful for treating GD2-positive tumors, particularly when fused to a cytokine or other immune modulator.

As used herein, the terms "antibody" and "immunoglobulin" are understood to mean (i) an intact antibody (for example, a monoclonal antibody or polyclonal antibody), (ii) antigen binding portions thereof, including, for example, an Fab fragment, an Fab' fragment, an $(Fab')_2$ fragment, an Fv fragment, a single chain antibody binding site, an sFv, (iii) bi-specific antibodies and antigen binding portions thereof, and (iv) multi-specific antibodies and antigen binding portions thereof.

As used herein, the terms "bind specifically," "specifically bind" and "specific binding" are understood to mean that the antibody has a binding affinity for a particular antigen of at least about 10^6 M^{-1}, more preferably, at least about 10^7 M^{-1}, more preferably at least about 10^8 M^{-1}, and most preferably at least about 10^{10} M^{-1}.

As used herein, the terms "Framework Regions" and "FRs" are understood to mean the regions of an immunoglobulin variable region adjacent to the Complementarity-Determining Regions (CDRs). CDRs are the portions of an immunoglobulin variable region that interact primarily with an antigen. As shown in FIG.1, the V_H and V_L regions both contain four FRs and are located within the boxed portions of the amino acid sequences.

In particular, with reference to the amino acid sequence shown in FIG. 1A (SEQ ID NO: 1), the light chain FRs are defined by the amino acid sequences from Asp1 to

Cys23 (huV$_L$FR1), from His39 to His54 (huV$_L$FR2), from Gly62 to Cys93 (huV$_L$FR3), and from Phe104 to Lys113 (huV$_L$FR4). With reference to the amino acid sequence shown in FIG. 1B (SEQ ID NO: 2), the heavy chain FRs are defined by the amino acid sequences from Glu1 to Ser25 (huV$_H$FR1), from Trp36 to Gly49 (huV$_H$FR2), from Arg67 to Ser98 (huV$_H$FR3), and from Trp103 to Ser113 (huV$_H$FR4).

Protein sequences of the invention

The present invention features antibodies that bind, preferably specifically, to the human cell surface glycosphingolipid GD2 and have modified regions derived from the m14.18 antibody. The V_H or V_L amino acid sequences (or both) are modified or humanized to reduce their immunogenicity when administered to a human. In accordance with the invention, the m14.18 antibody can be humanized, for example, by using deimmunization methods in which potential T cell epitopes are eliminated or weakened by introduction of mutations that reduce binding of a peptide epitope to an MHC Class II molecule (see, for example, WO98/52976 and WO00/34317). Alternatively, non-human T cell epitopes are mutated so that they correspond to human self epitopes that are present in human antibodies (see, for example, U.S. Patent No. 5,712,120). The present invention provides GD2 antibodies having V_L and V_H regions that include at least one humanized FR sequence, thereby reducing immunogenicity when administered to a human.

I. Heavy and Light Chains Variable Regions

As mentioned above, the hu14.18 includes humanized variable regions derived from the m14.18 antibody that maintain specific binding of human GD2 antigen. In some embodiments of the invention, the V_L region of the hu14.18 antibody includes the following polypeptide:

D-V-V-M-T-Q-T-P-L-S-L-P-V-T-P-G-E-P-A-S-I-S-C-R-S-S-Q-S-L-V-H-R-N-G-N-T-Y-L-H-W-Y-L-Q-K-P-G-Q-S-P-K-L-L-I-H-K-V-S-N-R-F-S-G-V-P-D-R-F-S-G-S-G-S-G-T-D-F-T-L-K-I-S-R-V-E-A-E-D-L-G-V-Y-F-C-S-Q-S-T-H-V-P-P-L-T-F-G-A-G-T-K-L-E-L-K (SEQ ID NO: 1).

In particular embodiments, the hu14.18 antibody includes a light chain FR1 that is defined by residues 1 to 23 of SEQ ID NO: 1, namely, D-V-V-M-T-Q-T-P-L-S-L-P-V-T-P-G-E-P-A-S-I-S-C (huV$_L$FR1).

In other embodiments of the invention, the V_H region of the hu14.18 antibody includes the following polypeptide:

E-V-Q-L-V-Q-S-G-A-E-V-E-K-P-G-A-S-V-K-I-S-C-K-A-S-G-S-S-F-T-G-Y-N-M-N-W-V-R-Q-N-I-G-K-S-L-E-W-I-G-A-I-D-P-Y-Y-G-G-T-S-Y-N-Q-K-F-

K-G-R-A-T-L-T-V-D-K-S-T-S-T-A-Y-M-H-L-K-S-L-R-S-E-D-T-A-V-Y-Y-C-V-S-G-M-E-Y-W-G-Q-G-T-S-V-T-V-S-S (SEQ ID NO: 2).

In particular embodiments, the hu14.18 antibody includes a heavy chain FR1 that is defined by residues 1 to 25 of SEQ ID NO: 2, namely E-V-Q-L-V-Q-S-G-A-E-V-E-K-P-G-A-S-V-K-I-S-C-K-A-S (huV$_H$FR1).

In further embodiments of the invention, the hu14.18 antibody includes a heavy chain FR3 that is represented by residues 67 to 98 of SEQ ID NO: 2, namely R-A-T-L-T-V-D-K-S-T-S-T-A-Y-M-H-L-K-S-L-R-S-E-D-T-A-V-Y-Y-C-V-S (huV$_H$FR3).

Various combinations of the foregoing embodiments are also within the scope of the present invention. For example, the hu14.18 antibody may include the V_L sequence set forth in SEQ ID NO: 1 and the V_H sequence set forth in SEQ ID NO: 2. The V_L and V_H regions can be linked by a disulfide bond or a peptide bond, depending on how their nucleic acid sequences are constructed. In general, V regions are linked by a disulfide bond when their sequences are encoded on separate DNA constructs. In contrast, the V regions are typically linked by a peptide bond when their sequences are encoded on a single-chain DNA construct.

The present invention also contemplates an antibody that specifically binds GD2 and includes at least a portion of the humanized V regions. For example, the hu14.18 antibody can include a V_L region as defined by SEQ ID NO:1 and a V_H region having at least one humanized FR, such as huV$_H$FR1 or huV$_H$FR2. Alternatively, the antibody of the present invention can include a V_H region as defined by SEQ ID NO: 2 and a V_L region having at least one humanized FR, such as huV$_L$FR1. The hu14.18 antibody can also include a V_H region having at least one humanized FR and/or a V_L region having at least one humanized FR.

In certain embodiments of the invention, the light chain variable region and the heavy chain variable region can be coupled, respectively, to a light chain constant region and a heavy chain constant region of an immunoglobulin. The immunoglobulin light chains have constant regions that are designated as either kappa or lambda chains. In a particular embodiment of the invention, the light chain constant region is a kappa chain. The heavy chain constant regions, and various modification and combinations thereof are discussed below in detail.

II. Fc portion

The antibody variable domains of the present invention are optionally fused to an Fc portion. As used herein, the Fc portion encompasses domains derived from the heavy chain constant region of an immunoglobulin, preferably a human immunoglobulin, including a fragment, analog, variant, mutant or derivative of the constant region. The constant region of an immunoglobulin heavy chain is defined as a naturally-occurring or synthetically produced polypeptide homologous to at least a portion of the C-terminal region of the heavy chain, including the CH1, hinge, CH2, CH3, and, for some heavy chain classes, CH4 domains. The "hinge" region joins the CH1 domain to the CH2-CH3 region of an Fc portion. The constant region of the heavy chains of all mammalian immunoglobulins exhibit extensive amino acid sequence similarity. DNA sequences for these immunoglobulin regions are well known in the art. (See, *e.g.*, Gillies *et al.* (1989) J. Immunol. Meth. 125:191).

In the present invention, the Fc portion typically includes at least a CH2 domain. For example, the Fc portion can include the entire immunoglobulin heavy chain constant region (CH1-hinge-CH2-CH3). Alternatively, the Fc portion can include all or a portion of the hinge region, the CH2 domain and the CH3 domain.

The constant region of an immunoglobulin is responsible for many important antibody effector functions, including Fc receptor (FcR) binding and complement fixation. There are five major classes of the heavy chain constant region, classified as IgA, IgG, IgD, IgE, and IgM, each with characteristic effector functions designated by isotype.

IgG, for example, is separated into four γ isotypes: γ1, γ2, γ3, and γ4, also known as IgG1, IgG2, IgG3, and IgG4, respectively. IgG molecules can interact with multiple classes of cellular receptors including three classes of Fcγ receptors (FcγR) specific for the IgG class of antibody, namely FcγRI, FcγRII, and FcγRIII. The sequences important for the binding of IgG to the FcγR receptors have been reported to be in the CH2 and CH3 domains.

The serum half-life of an antibody is influenced by the ability of that antibody to bind to an Fc receptor (FcR). Similarly, the serum half-life of immunoglobulin fusion proteins is also influenced by the inability to bind to such receptors (Gillies *et al.*, Cancer Research (1999) 59:2159-66). The CH2 and CH3 domains of IgG2 and IgG4 have undetectable or reduced binding affinity to Fc receptors compared to those of IgG1. Accordingly, the serum half-life of the featured antibody can be increased by using the

CH2 and/or CH3 domain from IgG2 or IgG4 isotypes. Alternatively, the antibody can include a CH2 and/or CH3 domain from IgG1 or IgG3 with modification in one or more amino acids in these domains to reduce the binding affinity for Fc receptors (see, *e.g.*, U.S. patent application 09/256,156, published as U.S. patent application publication 2003-0105294-A1).

The hinge region of the Fc portion normally adjoins the C-terminus of the CH1 domain of the heavy chain constant region. When included in the proteins of the present invention, the hinge is homologous to a naturally-occurring immunoglobulin region and typically includes cysteine residues linking two heavy chains via disulfide bonds as in natural immunoglobulins. Representative sequences of hinge regions for human and mouse immunoglobulin can be found in ANTIBODY ENGINEERING, a PRACTICAL GUIDE, (Borrebaeck, ed., W. H. Freeman and Co., 1992).

Suitable hinge regions for the present invention can be derived from IgG1, IgG2, IgG3, IgG4, and other immunoglobulin isotypes. The IgG1 isotype has two disulfide bonds in the hinge region permitting efficient and consistent disulfide bonding formation. Therefore, a preferred hinge region of the present invention is derived from IgG1. Optionally, the first, most N-terminal cysteine of an IgG1 hinge is mutated to enhance the expression and assembly of antibodies or antibody fusion proteins of the invention (see, *e.g.*, U.S. patent application 10/093,958, published as U.S. patent application publication 2003-0044423-A1).

In contrast to IgG1, the hinge region of IgG4 is known to form interchain disulfide bonds inefficiently (Angal *et al.*, (1993), Mol. Immunol. 30:105-8). Also, the IgG2 hinge region has four disulfide bonds that tend to promote oligomerization and possibly incorrect disulfide bonding during secretion in recombinant systems. One suitable hinge region for the present invention can be derived from the IgG4 hinge region, preferentially containing a mutation that enhances correct formation of disulfide bonds between heavy chain-derived moieties (Angal *et al.*, (1993), Mol. Immunol. 30(1):105-8). Another preferred hinge region is derived from an IgG2 hinge in which the first two cysteines are each mutated to another amino acid, such as, in order of general preference, serine, alanine, threonine, proline, glutamic acid, glutamine, lysine, histidine, arginine, asparagine, aspartic acid, glycine, methionine, valine, isoleucine, leucine, tyrosine, phenylalanine, tryptophan or selenocysteine (see, *e.g.*, U.S. patent application publication 2003-0044423-A1).

An Fc portion fused to an antibody variable region of the invention can contain CH2 and/or CH3 domains and a hinge region that are derived from different antibody isotypes. For example, the Fc portion can contain CH2 and/or CH3 domains of IgG2 or IgG4 and a hinge region of IgG1. Assembly of such hybrid Fc portions has been described in U.S. patent application publication 2003-0044423-A1.

When fused to an antibody variable region of the invention, the Fc portion preferably contains one or more amino acid modifications that generally extend the serum half-life of an Fc fusion protein. Such amino acid modifications include mutations substantially decreasing or eliminating Fc receptor binding or complement fixing activity. For example, one type of such mutation removes the glycosylation site of the Fc portion of an immunoglobulin heavy chain. In IgG1, the glycosylation site is Asn297 (see, for example, U.S. patent application 10/310,719, published as U.S. patent application publication 2003-0166163-A1).

III. Fusion junction region

The antibody variable regions of the present invention can optionally be linked or fused to a non-immunoglobulin moiety directly or indirectly, such as through a linker peptide (*e.g.*, $(Gly_4\text{-}Ser)_3$ (SEQ ID NO: 3)). The immunogenicity of the disclosed fusion proteins can be reduced by impairing the ability of the fusion junction or junctional epitope to interact with a T-cell receptor, as described in U. S. patent application publication 2003-0166877-A1. Even in a fusion between two human proteins, *e.g.*, human Fc and human IL-2, the region surrounding the fusion junction or junctional epitope includes a peptide sequence that is not normally present in the human body and, thus, that can be immunogenic. The immunogenicity of the junctional epitope can be reduced, for example, by introducing one or more glycosylation sites near the fusion junction, or by identifying a candidate T-cell epitope spanning the junction as described in U.S. patent application publication 2003-0166877-A1 and changing an amino acid near the junction to reduce the ability of the candidate T-cell epitope to interact with a T-cell receptor.

The serum half-life of the protein can also be increased by introducing mutations into the fusion junction region. For example, in a protein including a CH3 domain fused to a non-immunoglobulin moiety, the C-terminal lysine of the CH3 domain can be changed to another amino acid, such as alanine, which can provide a substantial increase in serum half-life of the resulting fusion protein.

In certain embodiments, proteolytic cleavage of the fusion junction is desirable. Accordingly, the intergenic region can include a nucleotide sequence encoding a proteolytic cleavage site. This site, interposed between the immunoglobulin and the cytokine, can be designed to provide for proteolytic release of the cytokine at the target site. For example, it is well known that plasmin and trypsin cleave after lysine and arginine residues at sites that are accessible to the proteases. Other site-specific endoproteases and the amino acid sequences they recognize are well-known.

IV. Treatment of human disease with hu14.18 antibody fusion proteins

The antibody variable regions of the invention can be attached to a diagnostic and/or a therapeutic agent. The agent can be fused to the antibody to produce a fusion protein. Alternatively, the agent can be chemically coupled to the antibody to produce an immuno-conjugate. The agent can be, for example, a toxin, radiolabel, imaging agent, immunostimulatory moiety or the like.

The antibody variable region of the invention can be attached to a cytokine. Preferred cytokines include interleukins such as interleukin-2 (IL-2), IL-4, IL-5, IL-6, IL-7, IL-10, IL-12, IL-13, IL-14, IL-15, IL-16 and IL-18, hematopoietic factors such as granulocyte-macrophage colony stimulating factor (GM-CSF), granulocyte colony stimulating factor (G-CSF) and erythropoeitin, tumor necrosis factors (TNF) such as TNF , lymphokines such as lymphotoxin, regulators of metabolic processes such as leptin, interferons such as interferon α, interferon ß, and interferon γ and chemokines. Preferably, the antibody-cytokine fusion protein or immunoconjugate displays cytokine biological activity. In one embodiment, the antibody variable domain is fused to IL-2. Preferably, several amino acids within the IL-2 moiety are mutated to reduce toxicity, as described in U.S. patent application publication 2003-0166163-A1.

For example, FIGS. 3A and 3B show the amino acid sequences of a particular embodiment of an antibody fusion protein in accordance with the invention. Specifically, FIG. 3A shows the peptide sequence of a humanized immunoglobulin light chain that includes a variable and constant region. FIG. 3B shows the peptide sequence of a humanized immunoglobulin heavy chain linked to IL-2. The polypeptides provide a humanized antibody fusion protein capable of specifically binding to GD2 and stimulating the immune system.

Optionally, the protein complexes can further include a second agent, such as a second cytokine. In one embodiment, a hu14.18 antibody fusion protein includes IL-12

and IL-2. The construction of protein complexes containing an immunoglobulin domain and two, different cytokines is described in detail in U.S. Patent No. 6,617,135.

Fusion proteins of the present invention are useful in treating human disease, such as cancer. When treating human tumors, it is particularly useful to administer an antibody-IL-2 fusion protein comprising the V regions of the invention by infusion or subcutaneous injection, using doses of 0.1 to 100 milligrams/meter^2/patient. In a preferred embodiment, it is particularly useful to administer an antibody-IL-2 fusion protein comprising the V regions of the invention by infusion or subcutaneous injection, using doses of 1 to 10 milligrams/meter^2/patient, and more preferably about 3 to 6 milligrams/meter^2/patient.

Clinical studies have shown that following administration of hu14.18-IL-2, the fusion protein retains its ability to activate IL-2 responsive cells through the IL-2 receptor and retains its ability to bind to GD2-positive tumor cells and to deliver IL-2 to their surface. Furthermore, administration of hu14.18-IL-2 fusion protein to a cancer patients resulted in stabilization of disease progression in a surprisingly large number of patients (see Example 1).

Pharmaceutical compositions of the invention may be used in the form of solid, semisolid, or liquid dosage forms, such as, for example, pills, capsules, powders, liquids, suspensions, or the like, preferably in unit dosage forms suitable for administration of precise dosages. The compositions include a conventional pharmaceutical carrier or excipient and, in addition, may include other medicinal agents, pharmaceutical agents, carriers, adjuvants, etc. Such excipients may include other proteins, such as, for example, human serum albumin or plasma proteins. Actual methods of preparing such dosage forms are known or will be apparent to those skilled in the art. The composition or formulation to be administered will, in any event, contain a quantity of the active component(s) in an amount effective to achieve the desired effect in the subject being treated.

Administration of the compositions hereof can be via any of the accepted modes of administration for agents that exhibit such activity. These methods include oral, parenteral, or topical administration and otherwise systemic forms. Intravenous injection in a pharmaceutically acceptable carrier is a preferred method of administration (see Example 1).

The amount of active compound administered will, of course, be dependent on the subject being treated, the severity of the affliction, the manner of administration, and the judgment of the prescribing physician.

Nucleic acids of the invention

I. hu14.18 antibody constructs

The invention also features nucleic acids capable of expressing each of the above types of proteins. These include, for example, nucleic acids encoding the amino acid sequence set forth in SEQ ID NO: 1; the amino acid sequence set forth in SEQ ID NO: 2; a hu14.18 antibody V_L region that includes the huV_LFR1 amino acid sequence; a hu14.18 antibody V_H region that includes the huV_HFR1 amino acid sequence; a hu14.18 antibody V_H region that includes huV_HFR3 amino acid sequence; and fusion proteins comprising a hu14.18 antibody including at least one of the foregoing humanized FR sequences and one or more therapeutic agents.

The hu14.18 antibodies of this invention can be produced by genetic engineering techniques; *i.e.*, by forming a nucleic acid construct encoding an GD2 specific antibody containing the desired FRs of the present invention. In one embodiment, the gene construct encoding the featured antibody includes, in 5' to 3' orientation, a DNA segment which encodes a heavy chain variable region including at least one humanized FR therein and a DNA segment encoding a heavy chain constant region. In another embodiment, another DNA segment encoding a cytokine is fused to the 3' end of the DNA segment encoding the heavy chain constant region. In a different embodiment, the gene construct includes, in 5' to 3' orientation, a DNA segment encoding a heavy chain variable region including at least one humanized FR and a DNA segment encoding a cytokine. Alternatively, a nucleic acid of the invention can include, in 5' to 3' orientation, a DNA segment encoding a light chain variable region including at least one humanized FR therein and a DNA segment encoding a cytokine. In some embodiments, a nucleic acid encoding a cytokine is joined in frame to the 3' end of a gene encoding a constant region (*e.g.*, CH3 exon), either directly or through an intergenic region (*e.g.*, by appropriate linkers, such as by DNA encoding $(Gly_4\text{-}Ser)_3$ (SEQ ID NO: 3)).

II. Expression of hu14.18 antibody constructs

Nucleic acid encoding proteins of the present invention can be assembled or inserted into one or more expression vectors for introduction into an appropriate recipient cell where it is expressed. The introduction of nucleic acids into expression vectors can

be accomplished by standard molecular biology techniques. Preferred expression vectors include those from which the encoded protein can be expressed in either bacteria or mammalian cells.

In accordance with the invention, a heavy chain of an antibody variable region is preferably co-expressed in the same cell with a corresponding light chain. For fusion proteins that comprise multiple polypeptide chains, more than one expression vector can be used. Co-transfection methods using, for example, two expression vectors, frequently result in both vectors being delivered to a target cell. Alternatively, it is sometimes useful to use a single vector encoding a plurality of polypeptides for co-expression in the same cell.

For example, FIGS. 2A-D show the nucleic acid sequence of a single vector encoding both the heavy and light chains of an immunoglobulin in accordance with the invention. The vector also includes a nucleic acid encoding IL-2 fused to the 3' end of the immunoglobulin heavy chain. Thus, when introduced into a cell, this vector alone can provide a humanized antibody-IL-2 fusion protein that specifically binds GD2 and stimulates immune function.

Furthermore, it can be convenient to express the proteins of the present invention as single-chain molecules. For example, an antibody variable region can be expressed as a single chain antibody or sFv optionally fused to a non-immunoglobulin protein. In another embodiment, a heavy chain (with or without a fused cytokine) is combined with a light (or heavy) chain counterpart (with or without a fused cytokine) to form monovalent and divalent immunoconjugates.

Recipient cell lines are preferably lymphoid cells, such as a myeloma (or hybridoma). Myelomas can synthesize, assemble, and secrete immunoglobulins encoded by transfected genes and can glycosylate proteins. A particularly preferred recipient cell is the Sp2/0 myeloma, which normally does not produce endogenous immunoglobulin. When transfected, the cell will produce only immunoglobulins encoded by the transfected gene constructs. Transfected myelomas can be grown in culture or in the peritonea of mice where secreted immunoconjugates can be recovered from ascites fluid. Other lymphoid cells such as B lymphocytes can also be used as recipient cells.

There are several methods for transfecting lymphoid cells with vectors containing the nucleic acid constructs encoding the chimeric Ig chain. A preferred way of introducing a vector into lymphoid cells is by spheroblast fusion. (see, *e.g.*, Gillies *et al.* (1989) Biotechnol. 7:798-804). Alternative methods include electroporation or calcium

phosphate precipitation. Other useful methods of producing the immunoconjugates include the preparation of an RNA sequence encoding the construct and its translation in an appropriate *in vivo* or *in vitro* system. Once expressed, the proteins of the invention can be harvested by standard protein purification procedures (see, *e.g.*, U.S. Patent No. 5,650,150).

III. Treatment of cancer by gene therapy

The nucleic acids of the invention can be used as gene therapy agents for treatment of cancer and other diseases in which it is desirable to target the immune system to a specific cell type. For example, cells can be withdrawn from a human or animal, and one or more nucleic acids encoding an antibody of the present invention can be transfected into the cells. The cells are then reintroduced into the human or animal. The transfected cells can be normal or cancer cells. Alternatively, a nucleic acid can be introduced into cells *in situ*. The human or animal then mounts an immune response to the cancer cells, which can cure or lessen the severity of the cancer. An antibody variable region of the invention, coupled to appropriate regulatory elements to promote expression in mammalian cells, can be transfected into the cells by any of a variety of techniques, including via calcium phosphate, a "gene gun", adenovirus vectors, cationic liposomes, retroviral vectors, or any other efficient transfection method.

In a particular embodiment of the invention, a hu14.18 antibody is used to selectively deliver a cytokine to a target cell *in vivo* so that the cytokine can exert a localized biological effect such as a local inflammatory response, stimulation of T cell growth and activation, or ADCC activity. A therapeutically effective amount of the antibody is administered into the circulatory system of a subject harboring the target cell.

The invention is illustrated further by the non-limiting examples.

EXAMPLES

Example 1

Purification and formulation of hu14.18-IL2

In one study, hu14.18-IL2 was expressed from NS/0 cells, tissue culture supernatant was harvested, and the hu14.18-IL2 protein was purified using, in sequence, Abx Mixed Resin column chromatography, recombinant Protein A chromatography, and Q Sepharose™ column chromatography, followed by Pellicon™ 2 tangential flow diafiltration

for buffer exchange into formulation buffer. Details of these purification steps are described below. Virus inactivation and removal steps were interdigitated into these steps as described below. The virus inactivation and removal steps were not necessary for purification per se, but were used to satisfy regulatory considerations.

Two liters of NS/0 tissue culture supernatant containing hu14.18-IL2 was pH-adjusted to 5.9 with 1M acetic acid and was applied to an Abx column (J. T. Baker); washed with 10 mM MES, 100 mM sodium acetate pH 6.2; and eluted with 500 mM sodium acetate pH 7. This material was loaded onto a recombinant Protein A column (Pharmacia); washed with 100 mM sodium phosphate, 150 mM NaCl pH 7; washed with 100 mM sodium phosphate, 150 mM NaCl pH 6; washed with 10 mM sodium phosphate pH 7; and eluted with 100 mM sodium phosphate, 150 mM NaCl pH 3.5. The pH of the eluted material was 4.2. To promote virus inactivation, this pH was reduced to 3.8 and the preparation was incubated for 30 minutes, after which the pH was neutralized to 7 with 1M NaOH. To remove nucleic acid, this material was loaded onto a Q sepharose column (Pharmacia) and washed with 100 mM sodium phosphate, 150 mM NaCl pH 7. Nucleic acid bound to the column, while the protein was found in the flow through and washes, which were repeated until the A280 returned to baseline. Pellicon 2 diafiltration (Millipore) was performed according to the manufacturer's instructions, so that the final hu14.18-IL2 material was placed in the following formulation.

1.	Mannitol	4%
2.	Arginine Hydrochloride USP/NF	100 mM
3.	Citric Acid USP-FCC	5 mM
4.	Polysorbate 80	0.01% (w.v)

The pH of the formulation buffer was adjusted to 7 with 1 M NaOH.

As a final step, the preparation was filtered through a Viresolve 180 membrane (Millipore), which has a molecular weight cutoff of 180,000 Daltons. This had the effect of 'polishing' the material so that as a result, aggregated dimers and higher-order oligomers were removed.

Example 2

Anti-tumor Activity of the hu14.18-IL-2 Fusion Protein
Observed in Phase I Clinical Trials

To evaluate the safety and efficacy of hu14.18-IL-2, a Phase I clinical trial was performed. Eligible patients had histologically confirmed melanoma that was considered

surgically and medically incurable. These patients could have either measurable or evaluable metastatic disease, or they could have no evidence of disease following surgical resection of either distant metastases or regionally recurrent disease. Patients with multiple (two or more) local or regional recurrences were included only if they had prior evidence of lymph node involvement and if each recurrence was separated in time by at least 2 months. All patients needed to have adequate bone marrow function (defined by total white blood cells (WBC) > 3,500/ml, or total granulocytes > 2000/ml, platelets > 100,000/ml, and hemoglobin >10.0 g/dl), adequate liver function [defined by an aspartate aminotransferase (AST) < 3 x normal and a total bilirubin < 2.0 mg/dl], and adequate renal function (defined by a serum creatinine <2.0 mg/dl or a creatinine clearance of >60 ml/minute). All patients had an electrocorticography (ECOG) performance status of 0 or 1 and a life expectancy of at least 12 weeks. Patients who had previously received chemotherapy, radiation therapy, or other immunosuppressive therapy within 4 weeks prior to study were excluded. Patients could have prior central nervous system (CNS) metastases if treated and stable for at least 4 weeks prior to starting the study. Informed consent was obtained from all patients.

This phase I trial was designed as an open-label, nonrandomized dose escalation study in which groups of 3 to 6 patients received hu14.18-IL-2 at one of the following dose levels: 0.8, 1.6, 3.2, 4.8, 6.0 or 7.5 mg/m^2/day. The hu14.18-IL-2 was administered on an inpatient basis as a 4-hour intravenous (IV) infusion over 3 consecutive days during the first week of each course. The hu14.18-IL-2 fusion protein was administered to patients in a formulation comprising 4% Mannitol; Arginine HCl, 100 mM; Citrate, 5 mM; and 0.01% Tween 80™, at pH 7. Patients were discharged from the hospital, if stable, approximately 24 hours following the completion of the third infusion. Adverse events and toxicities were graded as per NCI Common Toxicity Criteria (version 2.0) and the University of Wisconsin Comprehensive Cancer Center Toxicity Grading Scale for IL-2 (performance status, weight gain, and temperature). Dose-limiting toxicity (DLT) was defined as the occurrence of grade 3 or 4 toxicity other than grade 3 lymphopenia, hyperbilirubinemia, hypophosphatemia or hyperglycemia. The maximal tolerated dose (MTD) was defined as the dose level at which two of six patients had DLT during course 1. Patients with grade 3 treatment-related toxicities were required to recover to at least grade 1 before they could resume treatment at a 50% dose reduction for course 2. Patients with ≥25% disease progression were removed from the study. Patients with stable disease were administered course 2.

The pharmacokinetic properties of hu14.18-IL-2 were evaluated in the patients. When hu14.18-IL-2 levels were evaluated in serial samples from all 33 patients immediately following the first 4-hour infusion (day 1, course 1), the half-life was found to be 3.7 hours (+/- SD of 0.9 h). This is intermediate between the half-lives of its 2 components (approximately 45 minutes for IL-2 and 3 days for the chimeric m14.18 antibody), and comparable to that which was observed for the half-life of chimeric m14.18-IL-2 in mice. Following the clearance of hu14.18-IL-2 from the serum of these patients, neither the IL-2 nor hu14.18 antibody components could be detected. The peak serum and area under the curve (AUC) during course 1 showed a significant dose-dependent increase ($p<0.001$).

Thirty-three patients were treated in this study. Table 1 lists clinical outcomes. Two patients (6%) completed only the first 2 of 3 days for course 1. One of these patients (dose level 3) had a grade 3 hyperbilirubinemia on day 2 of treatment, and the other patient (dose level 6) had grade 3 hypoxia and hypotension requiring treatment to be held. Both of these patients had progression of disease and did not receive a second course of therapy. Nineteen patients (58%) had stable disease following the first course of therapy and received a second course of therapy. Five patients (15% of all patients) required a 50% dose reduction for course 2 secondary to adverse events in course 1. Seventeen patients (52% of all patients) completed course 2. One patient (dose level 4) declined to receive the final infusion during course 2, and one patient (dose level 6) had the final infusion during course 2 held due to hypotension. Eight patients (24% of all patients) had stable disease following the second course of treatment. The results indicate that hu14.18-IL-2 caused stabilization of disease progression in a surprisingly large number of patients.

Eight of the 33 patients maintained stable disease after 2 courses of therapy, and 4 of these 8 patients continue with no evidence of progressive disease (1 with stable disease and 3 with no evidence of disease) for 20-52 months since completing protocol therapy.

Five of the 33 patients entered the study with no measurable disease following surgical resection of recurrences or metastases. Two of these five patients had disease progression, while the remaining 3 patients continued with no evidence of disease (20-52 months). These findings are consistent with the hypothesis that clinical benefit from an immunotherapeutic intervention is most likely in a patient with a low tumor burden. One additional patient had an objective decrease in a lung nodule following two courses of therapy, but the overall disease response was scored as disease progression due to growth

in a distant node. The node was resected following hu14.18-IL-2 therapy and the patient remained free from disease progression for over 3 years.

TABLE 1

Clinical Outcomes

	Number of Patients
Patients completing course 1	31
Stable disease following course 1	19
50% dose reduction for course 2	5
Patients completing course 2	17
Stable disease following course 2	8

Immune stimulation *in vivo* by hu14.18-IL-2 in a Phase I clinical trial.

Patients treated with hu14.18-IL-2 were also examined for indications of immune stimulation. A peripheral blood lymphopenia occurred on days 2-4, and this was followed by a rebound lymphocytosis on days 5-22. Both of these changes were dose-dependent ($p<0.01$ and $p<0.05$, respectively). The lymphocyte counts on days 5, 8, 15 and 22 were significantly greater than baseline for course 1. The baseline lymphocyte count for course 2 (day 29 of course 1) was increased over the baseline lymphocyte count for course 1, indicating that effects of the first course of treatment are still present on day 29. In addition, the lymphocyte counts during course 2 on days 5, 8 and 15 are greater than the corresponding values for days 5, 8, and 15 during course 1 for these 12 patients.

Lymphocyte cell surface phenotype showed an expansion of CD16+ and CD56+ lymphocytes (natural killer (NK) cell markers) following the first week of hu14.18-IL-2 therapy. This effect was still present on day 29 of course 1 (day 1, course 2). For patients 19-33 (receiving 4.8-7.5 mg/m^2/day), lymphocyte cell surface phenotype was determined on days 15 and 22 in addition to days 1 and 8. This analysis demonstrated that the augmentation of CD56 and CD56/CD16 co-expressing cells remained significantly elevated ($p<0.01$) on days 8, 15 and 22.

As a measure of immune activation, C-reactive protein (CRP) levels for patients 13-33 and soluble IL-2 receptor (sIL-2R) levels for the 31 patients completing course 1,

were obtained. A significant increase in mean CRP was present on treatment days 3-5 in both course 1 and course 2 compared to baseline for each course. This increase in CRP returned to baseline levels by day 8 of each treatment course. The sIL-2R level was significantly increased over baseline starting 24 hours after the hu14.18-IL-2 infusion during both course 1 and course 2, which persisted through day 8. The increase in sIL-2R was found to be dose dependent ($p = 0.014$). sIL-2R values for course 2 were increased compared to corresponding values in course 1 for days 1-5 for patients receiving the same dose in both courses ($p < 0.05$).

The LA-N-5 neuroblastoma cell line that expresses GD2 and binds hu14.18-IL-2 was used to evaluate IL-2 activated NK function and antibody dependent cellular cytotoxicity (ADCC) on peripheral blood mononuclear cells (PBMC) from 31 patients completing course 1. There was a significant increase in killing mediated by lymphocytes from day 8 when compared with day 1 for these two assays. The 12 patients that received course 2 at the same dose as in course 1, showed ADCC results that were very similar to those obtained during course 1. The only parameter that was found to be different for course 2 from course 1 was increased killing in the presence of IL-2 on day 1, indicating that augmented killing in this assay remained elevated on day 29 (day 1, course 2).

Because the LA-N-5 target is relatively resistant to fresh NK cells, it is useful for measuring IL-2 augmented killing, and ADCC. However, the weak killing of LA-N-5 mediated by fresh PBMC in medium (without supplemental IL-2 *in vitro*) was not significantly greater on day 8 than on day 1.

For patients 19-33, standard NK assays were performed on days 1, 8, 15 and 22, using the NK susceptible K562 target cell line. A significant increase in NK lysis of K562 target cells, when tested either in medium or in the presence of IL-2, was observed on days 8 and 22 when compared with day 1. Serum samples from selected patients were also evaluated to determine functional IL-2 activity and functional anti-GD2 antibody.

The IL-2 responsive Tf-1b cell line demonstrated IL-2-induced proliferation with patient serum obtained following infusion of hu14.18-IL-2. A progressive increase in proliferation was seen during the first 4 hours following the 4-hour infusion. Values returned to baseline by 16 hours after this infusion, consistent with the serum half-life for hu14.18-IL-2 of approximately 4 hours. Serum samples from these time-points were also examined by flow cytometry for the presence of intact hu14.18-IL-2 immunocytokine (IC) that retains its IL-2 component and its anti-GD2 antibody activity. hu14.18-IL-2 capable of binding to the M21 cell line (GD2 positive) was detectable in patient serum

samples following an infusion of IC. The amount of IC able to bind to M21 progressively increased during the first 4 hours following the 4-hour infusion, and decreased after that, again consistent with the half-life of approximately 4 hours. Finally, *in vitro* assays were performed with specimens from patients to determine whether administration of hu14.18-IL-2 results in conditions *in vivo* consistent with those needed to achieve ADCC. PBMCs from day 8 show augmented ADCC on GD2+ target cells when hu14.18-IL-2 is added to the cytotoxic assay. This same ADCC assay was performed with PBMC from day 8, however instead of adding hu14.18-IL-2 to the assay, serum from the patient, obtained before or after hu14.18-IL-2 administration, was added. PBMC obtained from patients on day 8 of course 2 were able to mediate augmented killing of the LA-N-5 cell line in the presence of serum obtained following hu14.18-IL-2 administration, compared to that observed with serum obtained prior to infusion. Thus the hu14.18-IL-2 circulating in patients after IV administration is able to facilitate ADCC with PBMCs activated *in vivo* by hu14.18-IL-2 from that same patient.

In summary, these results indicate that there were immunological changes associated with this hu14.18-IL-2 therapy including an increase in lymphocyte count, an increase in the percentage of CD16+ and CD56+ PBMC, an increase in NK lysis, and an increase in ADCC. Additional evidence for immune activation included an increase in serum levels of CRP and of sIL-2R. Laboratory analyses of serum and PBMC showed that the hu14.18-IL-2 molecule circulating in patient serum following IV administration retained its ability to activate IL-2 responsive cells through the IL-2 receptor and retained its ability to bind to GD2 positive tumor cells, and deliver IL-2 to their surface, as detected by flow cytometry. NK cells were activated *in vivo* based on their ability to mediate NK and ADCC function *in vitro*. Furthermore, the NK cells activated *in vivo* by the hu14.18-IL-2 administered to these patients were able to mediate ADCC facilitated by the hu14.18-IL-2 circulating in the serum of those same patients. Thus, conditions to achieve immune activation were achieved in all patients in this study.

SEQUENCE LISTING

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			20					25				30			
Asn	Gly	Asn	Thr	Tyr	Leu	His	Trp	Tyr	Leu	Gln	Lys	Pro	Gly	Gln	Ser
		35					40					45			
Pro	Lys	Leu	Leu	Ile	His	Lys	Val	Ser	Asn	Arg	Phe	Ser	Gly	Val	Pro
	50					55					60				
Asp	Arg	Phe	Ser	Gly	Ser	Gly	Ser	Gly	Thr	Asp	Phe	Thr	Leu	Lys	Ile
65					70					75					80
Ser	Arg	Val	Glu	Ala	Glu	Asp	Leu	Gly	Val	Tyr	Phe	Cys	Ser	Gln	Ser
				85					90					95	
Thr	His	Val	Pro	Pro	Leu	Thr	Phe	Gly	Ala	Gly	Thr	Lys	Leu	Glu	Leu
			100					105					110		
Lys	Arg	Thr	Val	Ala	Ala	Pro	Ser	Val	Phe	Ile	Phe	Pro	Pro	Ser	Asp
		115					120					125			
Glu	Gln	Leu	Lys	Ser	Gly	Thr	Ala	Ser	Val	Val	Cys	Leu	Leu	Asn	Asn
	130					135					140				

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Ser Thr Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr
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Glu Lys His Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser
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            20                  25                  30
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        35                  40                  45
Gly Ala Ile Asp Pro Tyr Tyr Gly Gly Thr Ser Tyr Asn Gln Lys Phe
    50                  55                  60
Lys Gly Arg Ala Thr Leu Thr Val Asp Lys Ser Thr Ser Thr Ala Tyr
65                  70                  75                  80
Met His Leu Lys Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
                85                  90                  95
Val Ser Gly Met Glu Tyr Trp Gly Gln Gly Thr Ser Val Thr Val Ser
            100                 105                 110
Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Ser Ser
        115                 120                 125
Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys Leu Val Lys Asp
    130                 135                 140
Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr
145                 150                 155                 160
Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr
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Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu Gly Thr Gln
            180                 185                 190
Thr Tyr Ile Cys Asn Val Asn His Lys Pro Ser Asn Thr Lys Val Asp
        195                 200                 205
Lys Arg Val Glu Pro Lys Ser Cys Asp Lys Thr His Thr Cys Pro Pro
    210                 215                 220
Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro
225                 230                 235                 240
Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr
                245                 250                 255
Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn
            260                 265                 270
Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg
        275                 280                 285
Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val
    290                 295                 300
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Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser
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325 330 335
Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Glu
340 345 350
Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe
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Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu
370 375 380
Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe
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Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly
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Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Ala Pro Thr Ser Ser Ser
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Thr Lys Lys Thr Gln Leu Gln Leu Glu His Leu Leu Leu Asp Leu Gln
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Met Ile Leu Asn Gly Ile Asn Asn Tyr Lys Asn Pro Lys Leu Thr Arg
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Ser Asn Ile Asn Val Ile Val Leu Glu Leu Lys Gly Ser Glu Thr Thr
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Phe Met Cys Glu Tyr Ala Asp Glu Thr Ala Thr Ile Val Glu Phe Leu
545 550 555 560
Asn Arg Trp Ile Thr Phe Cys Gln Ser Ile Ile Ser Thr Leu Thr
565 570 575

CLAIMS:

1. A humanized antibody-IL2 fusion protein designated as hu14.18-IL2 that specifically binds GD2 and stimulates immune function comprising the light chain of SEQ ID NO. 5 and the heavy chain of SEQ ID NO. 6.

2. A vector comprising the nucleotide sequence of SEQ ID NO. 4 containing the nucleic acid sequences that code for the fusion protein of claim 1.

3. Pharmaceutical composition comprising the fusion protein of claim 1 and a pharmaceutical carrier or excipient.

4. Use of the fusion protein of claim 1 for the manufacture of a medicament for stabilizing disease progression in GD2 positive cancer patients.

5. Use of the fusion protein of claim 1 for the manufacture of a medicament for increasing ADCC and NK-lysis activity in GD2 positive cancer patients.

6. A pharmaceutical composition as claimed in claim 3, for use in stabilizing disease progression in GD2 positive cancer patients.

7. A pharmaceutical composition as claimed in claim 3, for use in increasing ADCC and NK-lysis activity in GD2 positive cancer patients.

Humanized Immunoglobulin Light Chain Variable Region

D-V-V-M-T-Q-S-P-L-S-L-P-V-T-P-G-E-P-A-S-I-S-C-R-S-S-Q-S-L-V-H-I-

huV_LFR1

N-G-N-T-Y-L-H-W-Y-L-Q-K-P-G-Q-S-P-K-L-L-I-H-K-V-S-N-R-F-S-

huV_LFR2

G-V-P-D-R-F-S-G-S-G-S-G-T-D-F-T-L-K-I-S-R-V-E-A-E-D-L-G-V-Y-F-C-S-

huV_LFR3

Q-S-T-H-V-P-P-L-T-F-G-A-G-T-K-L-E-L-K (SEQ ID NO: 1)

huV_LFR4

FIG. 1A

Humanized Immunoglobulin Heavy Chain Variable Region

huV$_H$FR1 → E-V-Q-L-V-Q-S-G-A-E-V-K-K-P-G-A-S-V-K-I-S-C-K-A-S-G-Y-T-F-T-G-Y-Y-M-N

huV$_H$FR2 → W-V-R-Q-N-I-G-K-S-L-E-W-I-G-A-I-D-P-Y-Y-G-G-T-S-Y-N-Q-K-F-K-G

huV$_H$FR3 → R-A-T-L-T-V-D-K-S-T-S-T-A-Y-M-H-L-K-S-L-R-S-E-D-T-A-V-Y-Y-C-V-S-Y

huV$_H$FR4 → W-G-Q-G-T-S-V-T-V-S-S (SEQ ID NO: 2)

FIG. 1B

Expression Vector Nucleotide Sequence

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CCAAAGTACAGTGGAAGGTGGATAACGCCCTCCAATCGGGTAACTCCCAGGAGAGTGTCACAGAG
CAGGACAGCAAGGACAGCACCTACAGCCTCAGCAGCACCCTGACGCTGAGCAAAGCAGACTACG
AGAAACACAAAGTCTACGCCTGCGAAGTCACCCATCAGGGCCTGAGCTCGCCCGTCACAAAGAGC
TTCAACAGGGGAGAGTGTTAGAGGGAGAAGTGCCCCCACCTGCTCCTCAGTTCCAGCCTGACCCCC
TCCCATCCTTTGGCCTCTGACCCTTTTTCCACAGGGGACCTACCCCTATTGCGGTCCTCCAGCTCAT
CTTTCACCTCACCCCCCTCCTCCTCCTTGGCTTTAATTATGCTAATGTTGGAGGAGAATGAATAAAT
AAAGTGAATCTTTGCACCTGTGGTTTCTCTCTTTCCTCAATTTAATAATTATTATCTGTTGTTTACCA
ACTACTCAATTTCTCTTATAAGGGACTAAATATGTAGTCATCCTAAGGCGCATAACCATTTATAAA
AATCATCCTTCATTCTATTTTACCCTATCATCCTCTGCAAGACAGTCCTCCCTCAAACCCACAAGCC
TTCTGTCCTCACAGTCCCCTGGGCCATGGTAGGAGAGACTTGCTTCCTTGTTTTCCCCTCCTCAGCA
AGCCCTCATAGTCCTTTTTAAGGGTGACAGGTCTTACGGTCATATATCCTTTGATTCAATTCCCTGG
GAATCAACCAAGGCAAATTTTTCAAAAGAAGAAACCTGCTATAAAGAGAATCATTCATTGCAACA
TGATATAAAATAACAACACAATAAAAGCAATTAAATAAACAAACAATAGGGAAATGTTTAAGTTC
ATCATGGTACTTAGACTTAATGGAATGTCATGCCTTATTTACATTTTTAAACAGGTACTGAGGGAC
TCCTGTCTGCCAAGGGCCGTATTGAGTACTTTCCACAACCTAATTTAATCCACACTATACTGTGAG
ATTAAAAACATTCATTAAAATGTTGCAAAGGTTCTATAAAGCTGAGAGACAAATATATTCTATAAC
TCAGCAATCCCACTTCTAGGGTCGATCGACGTTGACATTGATTATTGACTAGTTATTAATAGTAATC
AATTACGGGGTCATTAGTTCATAGCCCATATATGGAGTTCCGCGTTACATAACTTACGGTAAATGG
CCCGCCTGGCTGACCGCCCAACGACCCCCGCCCATTGACGTCAATAATGACGTATGTTCCCATAGT
AACGCCAATAGGGACTTTCCATTGACGTCAATGGGTGGAGTATTTACGGTAAACTGCCCACTTGGC
AGTACATCAAGTGTATCATATGCCAAGTACGCCCCCTATTGACGTCAATGACGGTAAATGGCCCGC

FIG. 2A

CTGGCATTATGCCCAGTACATGACCTTATGGGACTTTCCTACTTGGCAGTACATCTACGTATTAGTC
ATCGCTATTACCATGGTGATGCGGTTTTGGCAGTACATCAATGGGCGTGGATAGCGGTTTGACTCA
CGGGGATTTCCAAGTCTCCACCCCATTGACGTCAATGGGAGTTTGTTTTGGCACCAAAATCAACGG
GACTTTCCAAAATGTCGTAACAACTCCGCCCCATTGACGCAAATGGGCGGTAGGCGTGTACGGTG
GGAGGTCTATATAAGCAGAGCTCTCTGGCTAACTACAGAACCCACTGCTTAACTGGCTTATCGAAA
TTAATACGACTCACTATAGGGAGACCCAAGCTCCTCGAGGCTAGAATGAAGTTGCCTGTTAGGCTG
TTGGTGCTGATGTTCTGGATTCCTGGTGAGGAGAGAGGGAAGTGAGGGAGGAGAATGGACAGGGA
GCAGGAGCACTGAATCCCATTGCTCATTCCATGTATCTGGCATGGGTGAGAAGATGGGTCTTATCC
TCCAGCATGGGGCCTCTGGGGTGAATACTTGTTAGAGGGAGGTTCCAGATGGGAACATGTGCTAT
AATGAAGATTATGAAATGGATGCCTGGGATGGTCTAAGTAATGCCTTAGAAGTGACTAGACACTT
GCAATTCACTTTTTTTGGTAAGAAGAGATTTTTAGGCTATAAAAAAATGTTATGTAAAAATAAACG
ATCACAGTTGAAATAAAAAAAAAAATATAAGGATGTTCATGAATTTTGTGTATAACTATGTATTTCT
CTCTCATTGTTTCAGCTTCCTTAAGCGAGGTGCAGCTGGTGCAGTCCGGCGCCGAGGTGGAGAAGC
CCGGCGCCTCCGTGAAGATCTCCTGCAAGGCCTCCGGCTCCTCCTTCACCGGCTACAACATGAACT
GGGTGCGCCAGAACATCGGCAAGTCCCTGGAGTGGATCGGCGCCATCGACCCCTACTACGGCGGC
ACCTCCTACAACCAGAAGTTCAAGGGCCGCGCCACCCTGACCGTGGACAAGTCCACCTCCACCGC
CTACATGCACCTGAAGTCCCTGCGCTCCGAGGACACCGCCGTGTACTACTGCGTGTCCGGCATGGA
GTACTGGGGCCAGGGCACCTCCGTGACCGTGTCCTCCGGTAAGCTTTTCTGGGGCAGGCCAGGCCT
GACCTTGGCTTTGGGGCAGGGAGGGGGCTAAGGTGAGGCAGGTGGCGCCAGCCAGGTGCACACCC
AATGCCCATGAGCCCAGACACTGGACGCTGAACCTCGCGGACAGTTAAGAACCCAGGGGCCTCTG
CGCCCTGGGCCCAGCTCTGTCCCACACCGCGGTCACATGGCACCACCTCTCTTGCAGCCTCCACCA
AGGGCCCATCGGTCTTCCCCCTGGCACCCTCCTCCAAGAGCACCTCTGGGGGCACAGCGGCCCTGG
GCTGCCTGGTCAAGGACTACTTCCCCGAACCGGTGACGGTGTCGTGGAACTCAGGCGCCCTGACCA
GCGGCGTGCACACCTTCCCGGCTGTCCTACAGTCCTCAGGACTCTACTCCCTCAGCAGCGTGGTGA
CCGTGCCCTCCAGCAGCTTGGGCACCCAGACCTACATCTGCAACGTGAATCACAAGCCCAGCAAC
ACCAAGGTGGACAAGAGAGTTGGTGAGAGGCCAGCACAGGGAGGGAGGGTGTCTGCTGGAAGCC
AGGCTCAGCGCTCCTGCCTGGACGCATCCCGGCTATGCAGTCCCAGTCCAGGGCAGCAAGGCAGG
CCCCGTCTGCCTCTTCACCCGGAGGCCTCTGCCCGCCCCACTCATGCTCAGGGAGAGGGTCTTCTG
GCTTTTTCCCCAGGCTCTGGGCAGGCACAGGCTAGGTGCCCCTAACCCAGGCCCTGCACACAAAGG
GGCAGGTGCTGGGCTCAGACCTGCCAAGAGCCATATCCGGGAGGACCCTGCCCCTGACCTAAGCC
CACCCCAAAGGCCAAACTCTCCACTCCCTCAGCTCGGACACCTTCTCTCCTCCCAGATTCCAGTAA
CTCCCAATCTTCTCTCTGCAGAGCCCAAATCTTGTGACAAAACTCACACATGCCCACCGTGCCCAG
GTAAGCCAGCCCAGGCCTCGCCCTCCAGCTCAAGGCGGGACAGGTGCCCTAGAGTAGCCTGCATC
CAGGGACAGGCCCCAGCCGGGTGCTGACACGTCCACCTCCATCTCTTCCTCAGCACCTGAACTCCT
GGGGGGACCGTCAGTCTTCCTCTTCCCCCCAAAACCCAAGGACACCCTCATGATCTCCCGGACCCC
TGAGGTCACATGCGTGGTGGTGGACGTGAGCCACGAAGACCCTGAGGTCAAGTTCAACTGGTACG
TGGACGGCGTGGAGGTGCATAATGCCAAGACAAAGCCGCGGGAGGAGCAGTACAACAGCACGTA
CCGTGTGGTCAGCGTCCTCACCGTCCTGCACCAGGACTGGCTGAATGGCAAGGAGTACAAGTGCA
AGGTCTCCAACAAAGCCCTCCCAGCCCCCATCGAGAAAACCATCTCCAAAGCCAAAGGTGGGACC
CGTGGGGTGCGAGGGCCACATGGACAGAGGCCGGCTCGGCCCACCCTCTGCCCTGAGAGTGACCG
CTGTACCAACCTCTGTCCCTACAGGGCAGCCCCGAGAACCACAGGTGTACACCCTGCCCCCATCAC
GGGAGGAGATGACCAAGAACCAGGTCAGCCTGACCTGCCTGGTCAAAGGCTTCTATCCCAGCGAC
ATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAGAACAACTACAAGACCACGCCTCCCGTGCT
GGACTCCGACGGCTCCTTCTTCCTCTATAGCAAGCTCACCGTGGACAAGAGCAGGTGGCAGCAGG
GGAACGTCTTCTCATGCTCCGTGATGCATGAGGCTCTGCACAACCACTACACGCAGAAGAGCCTCT
CCCTGTCCCCGGGTAAAGCCCCAACTTCAAGTTCTACAAAGAAAACACAGCTGCAACTGGAGCAT
CTCCTGCTGGATCTCCAGATGATTCTGAATGGAATTAACAACTACAAGAATCCCAAACTCACCAGG
ATGCTCACATTCAAGTTCTACATGCCCAAGAAGGCCACAGAGCTCAAACATCTCCAGTGTCTAGAG
GAGGAACTCAAACCTCTGGAGGAAGTGCTAAACCTCGCTCAGAGCAAAAACTTCCACTTAAGACC
TAGGGACTTAATCAGCAATATCAACGTAATAGTTCTGGAACTAAAGGGATCCGAAACAACATTCA
TGTGTGAATATGCTGATGAGACAGCAACCATTGTAGAATTTCTGAACAGATGGATTACCTTTTGTC

FIG. 2B

AAAGCATCATCTCAACACTAACTTGATAATTAAGTGCTCGAGGGATCCAGACATGATAAGATACA
TTGATGAGTTTGGACAAACCACAACTAGAATGCAGTGAAAAAAATGCTTTATTTGTGAAATTTGTG
ATGCTATTGCTTTATTTGTAACCATTAGAAGCTGCAATAAACAAGTTAACAACAACAATTGCATTC
ATTTTATGTTTCAGGTTCAGGGGGAGGTGTGGGAGGTTTTTTAAAGCAAGTAAAACCTCTACAAAT
GTGGTATGGCTGATTATGATCCTGCCTCGCGCGTTTCGGTGATGACGGTGAAAACCTCTGACACAT
GCAGCTCCCGGAGACGGTCACAGCTTGTCTGTAAGCGGATGCCGGGAGCAGACAAGCCCGTCAGG
GCGCGTCAGCGGGTGTTGGCGGGTGTCGGGGCGCAGCCATGACCCAGTCACGTAGCGATAGCGGA
GTGTATACTGGCTTAACTATGCGGCATCAGAGCAGATTGTACTGAGAGTGCACCATATGCGGTGTG
AAATACCGCACAGATGCGTAAGGAGAAAATACCGCATCAGGCGCTCTTCCGCTTCCTCGCTCACTG
ACTCGCTGCGCTCGGTCGTTCGGCTGCGGCGAGCGGTATCAGCTCACTCAAAGGCGGTAATACGGT
TATCCACAGAATCAGGGGATAACGCAGGAAAGAACATGTGAGCAAAAGGCCAGCAAAAGGCCAG
GAACCGTAAAAAGGCCGCGTTGCTGGCGTTTTTCCATAGGCTCCGCCCCCCTGACGAGCATCACAA
AAATCGACGCTCAAGTCAGAGGTGGCGAAACCCGACAGGACTATAAAGATACCAGGCGTTTCCCC
CTGGAAGCTCCCTCGTGCGCTCTCCTGTTCCGACCCTGCCGCTTACCGGATACCTGTCCGCCTTTCT
CCCTTCGGGAAGCGTGGCGCTTTCTCAATGCTCACGCTGTAGGTATCTCAGTTCGGTGTAGGTCGTT
CGCTCCAAGCTGGGCTGTGTGCACGAACCCCCCGTTCAGCCCGACCGCTGCGCCTTATCCGGTAAC
TATCGTCTTGAGTCCAACCCGGTAAGACACGACTTATCGCCACTGGCAGCAGCCACTGGTAACAGG
ATTAGCAGAGCGAGGTATGTAGGCGGTGCTACAGAGTTCTTGAAGTGGTGGCCTAACTACGGCTA
CACTAGAAGGACAGTATTTGGTATCTGCGCTCTGCTGAAGCCAGTTACCTTCGGAAAAAGAGTTGG
TAGCTCTTGATCCGGCAAACAAACCACCGCTGGTAGCGGTGGTTTTTTTGTTTGCAAGCAGCAGAT
TACGCGCAGAAAAAAAGGATCTCAAGAAGATCCTTTGATCTTTTCTACGGGGTCTGACGCTCAGTG
GAACGAAAACTCACGTTAAGGGATTTTGGTCATGAGATTATCAAAAAGGATCTTCACCTAGATCCT
TTTAAATTAAAAATGAAGTTTTAAATCAATCTAAAGTATATATGAGTAAACTTGGTCTGACAGTTA
CCAATGCTTAATCAGTGAGGCACCTATCTCAGCGATCTGTCTATTTCGTTCATCCATAGTTGCCTGA
CTCCCCGTCGTGTAGATAACTACGATACGGGAGGGCTTACCATCTGGCCCCAGTGCTGCAATGATA
CCGCGAGACCCACGCTCACCGGCTCCAGATTTATCAGCAATAAACCAGCCAGCCGGAAGGGCCGA
GCGCAGAAGTGGTCCTGCAACTTTATCCGCCTCCATCCAGTCTATTAATTGTTGCCGGGAAGCTAG
AGTAAGTAGTTCGCCAGTTAATAGTTTGCGCAACGTTGTTGCCATTGCTGCAGGCATCGTGGTGTC
ACGCTCGTCGTTTGGTATGGCTTCATTCAGCTCCGGTTCCCAACGATCAAGGCGAGTTACATGATC
CCCCATGTTGTGCAAAAAAGCGGTTAGCTCCTTCGGTCCTCCGATCGTTGTCAGAAGTAAGTTGGC
CGCAGTGTTATCACTCATGGTTATGGCAGCACTGCATAATTCTCTTACTGTCATGCCATCCGTAAGA
TGCTTTTCTGTGACTGGTGAGTACTCAACCAAGTCATTCTGAGAATAGTGTATGCGGCGACCGAGT
TGCTCTTGCCCGGCGTCAACACGGGATAATACCGCGCCACATAGCAGAACTTTAAAAGTGCTCATC
ATTGGAAAACGTTCTTCGGGGCGAAAACTCTCAAGGATCTTACCGCTGTTGAGATCCAGTTCGATG
TAACCCACTCGTGCACCCAACTGATCTTCAGCATCTTTTACTTTCACCAGCGTTTCTGGGTGAGCAA
AAACAGGAAGGCAAAATGCCGCAAAAAAGGGAATAAGGGCGACACGGAAATGTTGAATACTCAT
ACTCTTCCTTTTTCAATATTATTGAAGCATTTATCAGGGTTATTGTCTCATGAGCGGATACATATTT
GAATGTATTTAGAAAAATAAACAAATAGGGGTTCCGCGCACATTTCCCCGAAAAGTGCCACCTGA
CGTCTAAGAAACCATTATTATCATGACATTAACCTATAAAAATAGGCGTATCACGAGGCCCTTTCG
TCTTCAAGAATTCCGATCCAGACATGATAAGATACATTGATGAGTTTGGACAAACCACAACTAGA
ATGCAGTGAAAAAAATGCTTTATTTGTGAAATTTGTGATGCTATTGCTTTATTTGTAACCATTAGAA
GCTGCAATAAACAAGTTAACAACAACAATTGCATTCATTTTATGTTTCAGGTTCAGGGGGAGGTGT
GGGAGGTTTTTTAAAGCAAGTAAAACCTCTACAAATGTGGTATGGCTGATTATGATCTAAAGCCAG
CAAAAGTCCCATGGTCTTATAAAAATGCATAGCTTTCGGAGGGGAGCAGAGAACTTGAAAGCATC
TTCCTGTTAGTCTTTCTTCTCGTAGACCTTAAATTCATACTTGATTCCTTTTTCCTCCTGGACCTCAG
AGAGGACGCCTGGGTATTCTGGGAGAAGTTTATATTTCCCCAAATCAATTTCTGGGAAAAACGTGT
CACTTTCAAATTCCTGCATGATCCTTGTCACAAAGAGTCTGAGGTGGCCTGGTTGATTCATGGCTTC
CTGGTAAACAGAACTGCCTCCGACTATCCAAACCATGTCTACTTTACTTGCCAATTCCGGTTGTTCA
ATAAGTCTTAAGGCATCATCCAAACTTTTGGCAAGAAAATGAGCTCCTCGTGGTGGTTCTTTGAGT
TCTCTACTGAGAACTATATTAATTCTGTCCTTTAAAGGTCGATTCTTCTCAGGAATGGAGAACCAG

FIG. 2C

GTTTTCCTACCCATAATCACCAGATTCTGTTTACCTTCCACTGAAGAGGTTGTGGTCATTCTTTGGA
AGTACTTGAACTCGTTCCTGAGCGGAGGCCAGGGTCGGTCTCCGTTCTTGCCAATCCCCATATTTTG
GGACACGGCGACGATGCAGTTCAATGGTCGAACCATGAGGGCACCAAGCTAGCTTTTTGCAAAAG
CCTAGGCCTCCAAAAAAGCCTCCTCACTACTTCTGGAATAGCTCAGAGGCCGAGGCGGCCTCGGCC
TCTGCATAAATAAAAAAAATTAGTCAGCCATGGGGCGGAGAATGGGCGGAACTGGGCGGAGTTAG
GGGCGGGATGGGCGGAGTTAGGGGCGGGACTATGGTTGCTGACTAATTGAGATGCATGCTTTGCA
TACTTCTGCCTGCTGGGGAGCCTGGGGACTTTCCACACCTGGTTGCTGACTAATTGAGATGCATGC
TTTGCATACTTCTGCCTGCTGGGGAGCCTGGGGACTTTCCACACCCTAACTGACACACATTCCACA
(SEQ ID NO: 4)

FIG. 2D

Humanized Immunoglobulin Light chain

D-V-V-M-T-Q-T-P-L-S-L-P-V-T-P-G-E-P-A-S-I-S-C-R-S-S-Q-S-L-V-H-R-N-G-N-T-Y-

L-H-W-Y-L-Q-K-P-G-Q-S-P-K-L-L-I-H-K-V-S-N-R-F-S-G-V-P-D-R-F-S-G-S-G-S-G-T-

D-F-T-L-K-I-S-R-V-E-A-E-D-L-G-V-Y-F-C-S-Q-S-T-H-V-P-P-L-T-F-G-A-G-T-K-L-E-

L-K-R-T-V-A-A-P-S-V-F-I-F-P-P-S-D-E-Q-L-K-S-G-T-A-S-V-V-C-L-L-N-N-F-Y-P-R-

E-A-K-V-Q-W-K-V-D-N-A-L-Q-S-G-N-S-Q-E-S-V-T-E-Q-D-S-K-D-S-T-Y-S-L-S-S-T-

L-T-L-S-K-A-D-Y-E-K-H-K-V-Y-A-C-E-V-T-H-Q-G-L-S-S-P-V-T-K-S-F-N-R-G-E-C

(SEQ ID NO: 5)

FIG. 3A

Humanized Immunoglobulin Heavy Chain-IL-2

E-V-Q-L-V-Q-S-G-A-E-V-E-K-P-G-A-S-V-K-I-S-C-K-A-S-G-S-S-F-T-G-Y-N-M-N-W-V-R-Q-N-I-G-K-S-L-E-W-I-G-

A-I-D-P-Y-Y-G-G-T-S-Y-N-Q-K-F-K-G-R-A-T-L-T-V-D-K-S-T-S-T-A-Y-M-H-L-K-S-L-R-S-E-D-T-A-V-Y-Y-C-V-S-

G-M-E-Y-W-G-Q-G-T-S-V-T-V-S-S-A-S-T-K-G-P-S-V-F-P-L-A-P-S-S-K-S-T-S-G-G-T-A-A-L-G-C-L-V-K-D-Y-F-P-

E-P-V-T-V-S-W-N-S-G-A-L-T-S-G-V-H-T-F-P-A-V-L-Q-S-S-G-L-Y-S-L-S-S-V-V-T-V-P-S-S-S-L-G-T-Q-T-Y-I-C-N-

V-N-H-K-P-S-N-T-K-V-D-K-R-V-E-P-K-S-C-D-K-T-H-T-C-P-P-C-P-A-P-E-L-L-G-G-P-S-V-F-L-F-P-P-K-P-K-D-T-L-

M-I-S-R-T-P-E-V-T-C-V-V-V-D-V-S-H-E-D-P-E-V-K-F-N-W-Y-V-D-G-V-E-V-H-N-A-K-T-K-P-R-E-E-Q-Y-N-S-T-Y-

R-V-V-S-V-L-T-V-L-H-Q-D-W-L-N-G-K-E-Y-K-C-K-V-S-N-K-A-L-P-A-P-I-E-K-T-I-S-K-A-K-G-Q-P-R-E-P-Q-V-Y-

T-L-P-P-S-R-E-E-M-T-K-N-Q-V-S-L-T-C-L-V-K-G-F-Y-P-S-D-I-A-V-E-W-E-S-N-G-Q-P-E-N-N-Y-K-T-T-P-P-V-L-D-

S-D-G-S-F-F-L-Y-S-K-L-T-V-D-K-S-R-W-Q-Q-G-N-V-F-S-C-S-V-M-H-E-A-L-H-N-H-Y-T-Q-K-S-L-S-L-S-P-G-A-P-

T-S-S-S-T-K-K-T-Q-L-Q-L-E-H-L-L-L-D-L-Q-M-I-L-N-G-I-N-N-Y-K-N-P-K-L-T-R-M-L-T-F-K-F-Y-M-P-K-K-A-T-

E-L-K-H-L-Q-C-L-E-E-E-L-K-P-L-E-E-V-L-N-L-A-Q-S-K-N-F-H-L-R-P-R-D-L-I-S-N-I-N-V-I-V-L-E-L-K-G-S-E-T-T-

F-M-C-E-Y-A-D-E-T-A-T-I-V-E-F-L-N-R-W-I-T-F-C-Q-S-I-I-S-T-L-T (SEQ ID NO: 6)

FIG. 3B

WO 2004/055056 PCT/EP2003/014295

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