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Methods and compositions for modifying the immune response

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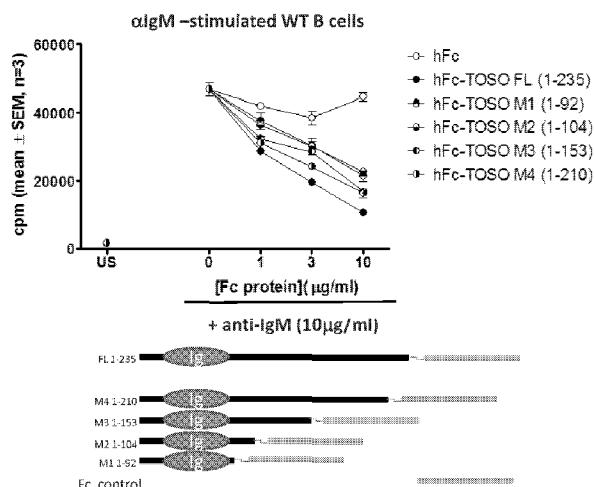
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FIG. 5



(57) **Abstract:** The present invention is directed to methods and compositions for modulating proteins involved in the immune response, specifically by modulating the expression and/or activity of the Toso protein. The present invention further provides methods and compositions for treatment of autoimmune disease and cancer by modulating the expression and/or activity of the Toso protein.

METHODS AND COMPOSITIONS FOR MODIFYING THE IMMUNE RESPONSE

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] The present application claims the benefit of priority to U.S. Provisional Application No. 61/949,927, filed March 7, 2014, the content of which is expressly incorporated herein by reference in its entirety for all purposes.

BACKGROUND OF THE INVENTION

[0002] Tuning of the immune response can be of use in treatment of autoimmune disease or cancer. By driving down drivers of the inflammatory response, symptoms of autoimmune disease can be ameliorated or completely subdued. Manipulations of drivers of the immune response may also be used to treat cancer by inducing the body's own immune system to attack cancer cells.

[0003] There is a need for characterization of different pathways in the immune response to identify targets for therapeutics for both autoimmune disease and cancer.

SUMMARY OF THE INVENTION

[0004] Accordingly, the present invention provides methods and compositions for modulating the immune response to either dampen the inflammatory response and thereby treat autoimmune disease or induce the body's own immune system to attack cancer cells.

[0005] In one aspect, the present invention provides a method for the treatment of cancer, the method comprising administering to a subject in need thereof a combination of a pharmaceutically effective amount of (i) at least one immune checkpoint inhibitor and (ii) an inhibitor of Toso activity. In an exemplary embodiment, the at least one immune checkpoint inhibitor is a member selected from the group consisting of: a PD-1 inhibitor, a CTLA-4 inhibitor, a LAG3 inhibitor, and a TIM3 inhibitor.

[0006] In a further embodiment and in accordance with the above, the inhibitor of Toso activity is a soluble Toso protein or an antibody to Toso.

[0007] In a still further embodiment and in accordance with any of the above, the combination therapy for treatment of cancer comprises a PD-1 inhibitor, a CTLA-4 inhibitor, and a soluble Toso protein. In a yet further embodiment, the PD-1 inhibitor is an anti-PD-1 antibody and the CTLA-4 inhibitor is an anti-CTLA-4 antibody.

[0008] In one aspect, the present invention provides a method for increasing a level of infiltrating lymphocytes in a tumor, the method comprising treating the tumor with a soluble Toso protein, wherein the level of infiltrating lymphocytes that results from the treating is higher than the level without the treating. In one embodiment, the infiltrating lymphocytes comprise CD3+ cells, CD8+ cells, or a combination of CD3+ cells and CD8+ cells. In a further embodiment, the method further comprises treating the tumor with one or more of a member selected from a PD-1 inhibitor, a CTLA-4 inhibitor, and a PDL-1 inhibitor.

[0009] In one aspect, the present invention provides a method of dampening the immune response, the method comprising decreasing expression and/or activity of Toso. In one embodiment, the method of dampening the immune response further comprises increasing expression and/or activity of IgM. In a further embodiment, the method further comprises decreasing expression and/or activity of PAMPs and/or DAMPs.

[0010] In one aspect, the present invention provides a method of treating autoimmune disease, the method comprising one or more of the following steps: (a) decreasing the expression and/or activity of Toso; (b) increasing the expression and/or activity of IgM; and (c) decreasing the expression and/or activity of PAMPs and/or DAMPs.

[0011] In one aspect, the present invention provides a method of inducing the immune response, the method comprising increasing expression and/or activity of Toso. In one embodiment, the method further comprises decreasing expression and/or activity of IgM. In further embodiment, the method further comprises increasing expression and/or activity of PAMPs and/or DAMPs. In a still further embodiment, the method further comprises modulating activity of a scramblase protein.

[0012] In one aspect, the present invention provides a method of treating cancer, the method comprising one or more of the following steps: (a) increasing the expression and/or activity of Toso; (b) decreasing the expression and/or activity of IgM; (c) increasing the expression and/or activity of PAMPs and/or DAMPs; and (d) modulating the activity of a scramblase protein.

BRIEF DESCRIPTION OF THE DRAWINGS

[0013] **FIG. 1** provides sequences described herein.

[0014] **FIG. 2** illustrates embodiments of truncation mutants of Toso proteins.

[0015] **FIG. 3** shows data from a B cell proliferation study of the effects of soluble Toso proteins of the invention.

[0016] **FIG. 4** shows data from a B cell proliferation study of the effects of soluble and full length Toso proteins of the invention.

[0017] **FIG. 5** shows data from a B cell proliferation assay with truncated forms of soluble hToso.

[0018] **FIG. 6** shows data from an IgM binding assay.

[0019] **FIG. 7** shows data from an IgM binding assay.

[0020] **FIG. 8** shows data from an in vitro assay of chronic lymphocytic leukemia (CLL) cell proliferation.

[0021] **FIG. 9** shows data from a study on tumor area upon treatment with soluble Toso protein as compared to controls.

[0022] **FIG. 10** shows survival data from a study in mice treated with soluble Toso protein.

[0023] **FIG. 11** shows data from a study of lymphocyte infiltration in tumors after treatment with soluble Toso protein.

[0024] **FIG. 12** shows data from a study of combination therapies including both soluble Toso protein and anti-PD-1 antibody.

[0025] **FIG. 13** shows data from a study of combination therapies including both soluble Toso protein and anti-PD-1 antibody.

[0026] **FIG. 14** shows data from a study of combination therapies including both soluble Toso protein and anti-CTLA-4 antibody.

[0027] **FIG. 15** shows data from a study of combination therapies including soluble Toso protein, anti-PD-1 antibody and anti-CTLA-4 antibody.

[0028] **FIG. 16** shows data from a study of combination therapies including soluble Toso protein, anti-PD-1 antibody and anti-CTLA-4 antibody.

DETAILED DESCRIPTION OF THE INVENTION

[0029] The practice of the present invention may employ, unless otherwise indicated, conventional techniques and descriptions of organic chemistry, polymer technology, molecular biology (including recombinant techniques), cell biology, biochemistry, and immunology, which are within the skill of the art. Such conventional techniques include polymer array synthesis, hybridization, ligation, phage display, and detection of hybridization using a label. Specific illustrations of suitable techniques can be had by reference to the example herein below. However, other equivalent conventional procedures can, of course, also be used. Such conventional techniques and descriptions can be found in standard laboratory manuals such as *Genome Analysis: A Laboratory Manual Series* (Vols. I-IV), *Using Antibodies: A Laboratory Manual*, *Cells: A Laboratory Manual*, *PCR Primer: A Laboratory Manual*, and *Molecular Cloning: A Laboratory Manual* (all from Cold Spring Harbor Laboratory Press), Stryer, L. (1995) *Biochemistry* (4th Ed.) Freeman, New York, Gait, "Oligonucleotide Synthesis: A Practical Approach" 1984, IRL Press,

London, Nelson and Cox (2000), *Lehninger, Principles of Biochemistry* 3rd Ed., W. H. Freeman Pub., New York, N.Y. and Berg et al. (2002) *Biochemistry*, 5th Ed., W. H. Freeman Pub., New York, N.Y., all of which are herein incorporated in their entirety by reference for all purposes.

[0030] Note that as used herein and in the appended claims, the singular forms "a," "an," and "the" include plural referents unless the context clearly dictates otherwise. Thus, for example, reference to "a polymerase" refers to one agent or mixtures of such agents, and reference to "the method" includes reference to equivalent steps and methods known to those skilled in the art, and so forth.

[0031] Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. All publications mentioned herein are incorporated herein by reference for the purpose of describing and disclosing devices, compositions, formulations and methodologies which are described in the publication and which might be used in connection with the presently described invention.

[0032] Where a range of values is provided, it is understood that each intervening value, to the tenth of the unit of the lower limit unless the context clearly dictates otherwise, between the upper and lower limit of that range and any other stated or intervening value in that stated range is encompassed within the invention. The upper and lower limits of these smaller ranges may independently be included in the smaller ranges is also encompassed within the invention, subject to any specifically excluded limit in the stated range. Where the stated range includes one or both of the limits, ranges excluding either both of those included limits are also included in the invention.

[0033] In the following description, numerous specific details are set forth to provide a more thorough understanding of the present invention. However, it will be apparent to one of skill in the art that the present invention may be practiced without one or more of these specific details. In other instances, well-known features and procedures well known to those skilled in the art have not been described in order to avoid obscuring the invention.

[0034] As used herein, the term "comprising" is intended to mean that the compositions and methods include the recited elements, but not excluding others. "Consisting essentially of" when used to define compositions and methods, shall mean excluding other elements of any essential significance to the composition or method. "Consisting of" shall mean excluding more than trace elements of other ingredients for claimed compositions and substantial method steps. Embodiments defined by each of these transition terms are within the scope of this invention. Accordingly, it is intended that the methods and compositions can include additional steps and components (comprising) or alternatively including steps and compositions of no significance

(consisting essentially of) or alternatively, intending only the stated method steps or compositions (consisting of).

[0035] All numerical designations, e.g., pH, temperature, time, concentration, and molecular weight, including ranges, are approximations which are varied (+) or (-) by increments of 0.1. It is to be understood, although not always explicitly stated that all numerical designations are preceded by the term "about". The term "about" also includes the exact value "X" in addition to minor increments of "X" such as "X + 0.1" or "X - 0.1." It also is to be understood, although not always explicitly stated, that the reagents described herein are merely exemplary and that equivalents of such are known in the art.

[0036] A "composition" may include any substance comprising an agent or compound and is also intended to encompass any combination of an agent or compound and other substances, including a carrier, e.g., compound or composition, inert (for example, a detectable agent or label) or active, such as an adjuvant, diluent, binder, stabilizer, buffers, salts, lipophilic solvents, preservative, adjuvant or the like. Carriers also include pharmaceutical excipients and additives proteins, peptides, amino acids, lipids, and carbohydrates (e.g., sugars, including monosaccharides, di-, tri-, tetra-, and oligosaccharides; derivatized sugars such as alditols, aldonic acids, esterified sugars and the like; and polysaccharides or sugar polymers), which can be present singly or in combination, comprising alone or in combination 1-99.99% by weight or volume. Exemplary protein excipients include serum albumin such as human serum albumin (HSA), recombinant human albumin (rHA), gelatin, casein, and the like. Representative amino acid/antibody components, which can also function in a buffering capacity, include alanine, glycine, arginine, betaine, histidine, glutamic acid, aspartic acid, cysteine, lysine, leucine, isoleucine, valine, methionine, phenylalanine, asparagine, and the like. Carbohydrate excipients are also intended within the scope of this invention, examples of which include but are not limited to monosaccharides such as fructose, maltose, galactose, glucose, D-mannose, sorbose, and the like; disaccharides, such as lactose, sucrose, trehalose, cellobiose, and the like; polysaccharides, such as raffinose, melezitose, maltodextrins, dextrins, starches, and the like; and alditols, such as mannitol, xylitol, maltitol, lactitol, xylitol sorbitol (glucitol) and myoinositol.

[0037] The term pharmaceutically acceptable carrier (or medium), which may be used interchangeably with the term biologically compatible carrier or medium, refers to reagents, cells, compounds, materials, compositions, and/or dosage forms that are not only compatible with the cells and other agents to be administered therapeutically, but also are, within the scope of sound medical judgment, suitable for use in contact with the tissues of human beings and animals without excessive toxicity, irritation, allergic response, or other complication commensurate with a reasonable benefit/risk ratio. Pharmaceutically acceptable carriers suitable for use in the present invention include liquids, semi-solid (e.g., gels) and solid materials (e.g., cell scaffolds and

matrices, tubes sheets and other such materials as known in the art and described in greater detail herein). These semi-solid and solid materials may be designed to resist degradation within the body (non-biodegradable) or they may be designed to degrade within the body (biodegradable, bioerodable). A biodegradable material may further be bioresorbable or bioabsorbable, i.e., it may be dissolved and absorbed into bodily fluids (water-soluble implants are one example), or degraded and ultimately eliminated from the body, either by conversion into other materials or breakdown and elimination through natural pathways.

[0038] As used herein, the term “patient” or “subject” intends an animal, a mammal or yet further a human patient. For the purpose of illustration only, a mammal includes but is not limited to a human, a simian, a murine, a bovine, an equine, a porcine or an ovine.

[0039] As used herein, the term “oligonucleotide” or “polynucleotide” refers to a short polymer composed of deoxyribonucleotides, ribonucleotides or any combination thereof. Oligonucleotides are generally at least about 10, 15, 20, 25, 30, 40, 50, 60, 70, 80, 90, 100 or more nucleotides in length. An oligonucleotide may be used as a primer or as a probe.

[0040] The term “amino acid” refers to naturally occurring and synthetic amino acids, as well as amino acid analogs and amino acid mimetics that function in a manner similar to the naturally occurring amino acids. Naturally occurring amino acids are those encoded by the genetic code, as well as those amino acids that are later modified, e.g., hydroxyproline, γ -carboxyglutamate, and O-phosphoserine. Amino acid analogs refers to compounds that have the same basic chemical structure as a naturally occurring amino acid, i.e., an α carbon that is bound to a hydrogen, a carboxyl group, an amino group, and an R group, e.g., homoserine, norleucine, methionine sulfoxide, methionine methyl sulfonium. Such analogs have modified R groups (e.g., norleucine) or modified peptide backbones, but retain the same basic chemical structure as a naturally occurring amino acid. Amino acid mimetics refers to chemical compounds that have a structure that is different from the general chemical structure of an amino acid, but that function in a manner similar to a naturally occurring amino acid.

[0041] The term “isolated” as used herein refers to molecules or biological or cellular materials being substantially free from other materials, e.g., greater than 70%, or 80%, or 85%, or 90%, or 95%, or 98%. In one aspect, the term “isolated” refers to nucleic acid, such as DNA or RNA, or protein or polypeptide, or cell or cellular organelle, or tissue or organ, separated from other DNAs or RNAs, or proteins or polypeptides, or cells or cellular organelles, or tissues or organs, respectively, that are present in the natural source and which allow the manipulation of the material to achieve results not achievable where present in its native or natural state, e.g., recombinant replication or manipulation by mutation. The term “isolated” also refers to a nucleic acid or peptide that is substantially free of cellular material, viral material, or culture medium when produced by

recombinant DNA techniques, or chemical precursors or other chemicals when chemically synthesized. Moreover, an “isolated nucleic acid” is meant to include nucleic acid fragments which are not naturally occurring as fragments and would not be found in the natural state. The term “isolated” is also used herein to refer to polypeptides which are isolated from other cellular proteins and is meant to encompass both purified and recombinant polypeptides, e.g., with a purity greater than 70%, or 80%, or 85%, or 90%, or 95%, 98%, or 99%. The term “isolated” is also used herein to refer to cells or tissues that are isolated from other cells or tissues and is meant to encompass both cultured and engineered cells or tissues.

[0042] A “recombinant” nucleic acid refers an artificial nucleic acid that is created by combining two or more sequences that would not normally occur together. In one embodiment, it is created through the introduction of relevant DNA into an existing organismal DNA, such as the plasmids of bacteria, to code for or alter different traits for a specific purpose, such as antibiotic resistance. A “recombinant” polypeptide is a polypeptide that is derived from a recombinant nucleic acid.

[0043] As used herein, the term “promoter” refers to a nucleic acid sequence sufficient to direct transcription of a gene. Also included in the invention are those promoter elements which are sufficient to render promoter dependent gene expression controllable for cell type specific, tissue specific or inducible by external signals or agents.

[0044] In some embodiments, a promoter is an inducible promoter or a discrete promoter. Inducible promoters can be turned on by a chemical or a physical condition such as temperature or light. Examples of chemical promoters include, without limitation, alcohol-regulated, tetracycline-regulated, steroid-regulated, metal-regulated and pathogenesis-related promoters. Examples of discrete promoters can be found in, for examples, Wolfe et al. Molecular Endocrinology 16(3): 435-49.

[0045] As used herein, the term “regulatory element” refers to a nucleic acid sequence capable of modulating the transcription of a gene. Non-limiting examples of regulatory element include promoter, enhancer, silencer, poly-adenylation signal, transcription termination sequence. Regulatory element may be present 5' or 3' regions of the native gene, or within an intron.

[0046] Various proteins are also disclosed herein with their GenBank Accession Numbers for their human proteins and coding sequences. However, the proteins are not limited to human-derived proteins having the amino acid sequences represented by the disclosed GenBank Accession Nos, but may have an amino acid sequence derived from other animals, particularly, a warm-blooded animal (e.g., rat, guinea pig, mouse, chicken, rabbit, pig, sheep, cow, monkey, etc.).

[0047] As used herein, the term “Toso”, “FAIM3” or “Fas apoptotic inhibitory molecule 3” refers to a protein having an amino acid sequence substantially identical to any of the representative Toso sequences, including any and all versions of GenBank Accession Nos. NP_001135945 (human

isoform b), NP_001180267 (human isoform c), NP_005440 (human isoform a), NP_081252 (mouse) or NP_001014843 (rat). Suitable cDNA encoding Toso are provided at GenBank Accession Nos. NM_001142473, NM_001193338, NM_005449, NM_026976, and NM_001014843.

[0048] As used herein, the term “biological activity of Toso” or “Toso activity” refers to any biological activity associated with the full length native Toso protein. In some embodiments, the biological activity of Toso refers to binding to an IgM antibody. In further embodiments, the biological activity of Toso refers to inhibiting CD11b or CD18 activity. In yet further embodiments, the biological activity of Toso refers to increasing the activation threshold of granulocytes. Activation threshold can be measured by number of activated granulocytes from bone marrow. In further embodiments, the biological activity of Toso includes the activation of dendritic cells and their ability to present antigen to T cells. In further embodiments, the biological activity of Toso includes inhibition of apoptosis or enhancement of TNF signaling. In some embodiments, the Toso biological activity is equivalent to the activity of a protein having an amino acid sequence represented by GenBank Accession No. NP_001135945, NP_001180267, NP_005440, NP_081252 or NP_001014843, including any and all versions of these accession numbers. As will be appreciated, other forms of Toso, including deletion mutants and amino acid variants as discussed herein, may also show Toso biological activity.

[0049] As used herein, the term “treating” refers to administering a pharmaceutical composition for the purpose of improving the condition of a patient by reducing, alleviating, reversing, or preventing at least one adverse effect or symptom of a disease or disorder.

[0050] As used herein, the term “preventing” refers to identifying a subject (i.e., a patient) having an increased susceptibility to a disease or disorder but not yet exhibiting symptoms of the disease or disorder, and administering a therapy according to the principles of this disclosure. The preventive therapy is designed to reduce the likelihood that the susceptible subject will later become symptomatic or that the disease will be delayed in onset or progress more slowly than it would in the absence of the preventive therapy. A subject may be identified as having an increased likelihood of developing the disease/disorder by any appropriate method including, for example, by identifying a family history of the disease/disorder, or having one or more diagnostic markers indicative of disease/disorder or susceptibility to disease/disorder.

[0051] As used herein, the term “sample” or “test sample” refers to any liquid or solid material containing nucleic acids. In suitable embodiments, a test sample is obtained from a biological source (i.e., a “biological sample”), such as cells in culture or a tissue sample from an animal, most preferably, a human.

[0052] As used herein, the term “substantially identical”, when referring to a protein or polypeptide, is meant one that has at least 80%, 85%, 90%, 95%, or 99% sequence identity to a reference amino acid sequence. The length of comparison is preferably the full length of the polypeptide or protein, but is generally at least 10, 15, 20, 25, 30, 40, 50, 60, 80, or 100 or more contiguous amino acids. A “substantially identical” nucleic acid is one that has at least 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% sequence identity to a reference nucleic acid sequence. The length of comparison is preferably the full length of the nucleic acid, but is generally at least 20 nucleotides, 30 nucleotides, 40 nucleotides, 50 nucleotides, 75 nucleotides, 100 nucleotides, 125 nucleotides, or more.

[0053] As used herein, an “amino acid substitution” or “substitution” refers to the replacement of an amino acid at a particular position in a starting polypeptide sequence with another amino acid. For example, the substitution M23Y refers to a variant polypeptide in which the methionine at position 23 is replaced with a tyrosine.

[0054] A “biological equivalent” of a protein or nucleic acid refers to a protein or nucleic acid that is substantially identical to the protein or nucleic acid by amino acid or nucleic acid sequence or that has an equivalent biological activity.

[0055] As used herein, the term “effective amount” refers to a quantity of compound (e.g., a Toso protein or biologically active fragment thereof) delivered with sufficient frequency to provide a medical benefit to the patient. In one embodiment, an effective amount of a protein is an amount sufficient to treat or ameliorate a symptom of a disease.

[0056] A population of cells intends a collection of more than one cell that is identical (clonal) or non-identical in phenotype and/or genotype.

[0057] “Substantially homogeneous” describes a population of cells in which more than about 50%, or alternatively more than about 60 %, or alternatively more than 70 %, or alternatively more than 75 %, or alternatively more than 80%, or alternatively more than 85 %, or alternatively more than 90%, or alternatively, more than 95 %, of the cells are of the same or similar phenotype. Phenotype can be determined by a pre-selected cell surface marker or other marker.

[0058] The terms autologous transfer, autologous transplantation, autograft and the like refer to treatments wherein the cell donor is also the recipient of the cell replacement therapy. The terms allogeneic transfer, allogeneic transplantation, allograft and the like refer to treatments wherein the cell donor is of the same species as the recipient of the cell replacement therapy, but is not the same individual. A cell transfer in which the donor's cells and have been histocompatibly matched with a recipient is sometimes referred to as a syngeneic transfer. The terms xenogeneic transfer, xenogeneic transplantation, xenograft and the like refer to treatments wherein the cell donor is of a different species than the recipient of the cell replacement therapy.

[0059] As used herein, an “antibody” includes whole antibodies and any antigen binding fragment or a single chain thereof. Thus the term “antibody” includes any protein or peptide containing molecule that comprises at least a portion of an immunoglobulin molecule. Examples of such include, but are not limited to a complementarity determining region (CDR) of a heavy or light chain or a ligand binding portion thereof, a heavy chain or light chain variable region, a heavy chain or light chain constant region, a framework (FR) region, or any portion thereof, or at least one portion of a binding protein. In general, the term “antibody” includes any polypeptide that includes at least one constant domain, including, but not limited to, CH1, CH2, CH3 and CL. Antibodies that find use in the present invention can take on a number of formats as described herein, including traditional antibodies as well as antibody derivatives, fragments and mimetics.

[0060] The antibodies can be polyclonal or monoclonal and can be isolated from any suitable biological source, e.g., murine, rat, sheep and canine.

[0061] A monoclonal antibody is an antibody produced by a single clone of cells or a hybridoma, and therefore is a single pure homogeneous type of antibody.

[0062] A hybridoma is a cell that is produced in the laboratory from the fusion of an antibody-producing lymphocyte and a non-antibody producing cancer cell, usually a myeloma or lymphoma. A hybridoma proliferates and produces a continuous supply of a specific monoclonal antibody.

[0063] The term “human antibody” as used herein, is intended to include antibodies having variable and constant regions derived from human germline immunoglobulin sequences. The human antibodies of the invention may include amino acid residues not encoded by human germline immunoglobulin sequences (e.g., mutations introduced by random or site-specific mutagenesis *in vitro* or by somatic mutation *in vivo*). However, the term “human antibody” as used herein, is not intended to include antibodies in which CDR sequences derived from the germline of another mammalian species, such as a mouse, have been grafted onto human framework sequences. Thus, as used herein, the term “human antibody” refers to an antibody in which substantially every part of the protein (e.g., CDR, framework, C_L, C_H domains (e.g., C_{H1}, C_{H2}, C_{H3}), hinge, (VL, VH)) is substantially non-immunogenic in humans, with only minor sequence changes or variations. Similarly, antibodies designated primate (monkey, baboon, chimpanzee, etc.), rodent (mouse, rat, rabbit, guinea pig, hamster, and the like) and other mammals designate such species, sub-genus, genus, sub-family, family specific antibodies. Further, chimeric antibodies include any combination of the above. Such changes or variations optionally and preferably retain or reduce the immunogenicity in humans or other species relative to non-modified antibodies. Thus, a human antibody is distinct from a chimeric or humanized antibody. It is pointed out that a human antibody can be produced by a non-human animal or prokaryotic or eukaryotic cell that is capable of expressing functionally rearranged human immunoglobulin (e.g., heavy chain and/or light chain)

genes. Further, when a human antibody is a single chain antibody, it can comprise a linker peptide that is not found in native human antibodies. For example, an Fv can comprise a linker peptide, such as two to about eight glycine or other amino acid residues, which connects the variable region of the heavy chain and the variable region of the light chain. Such linker peptides are considered to be of human origin.

[0064] As used herein, a human antibody is “derived from” a particular germline sequence if the antibody is obtained from a system using human immunoglobulin sequences, e.g., by immunizing a transgenic mouse carrying human immunoglobulin genes or by screening a human immunoglobulin gene library. A human antibody that is “derived from” a human germline immunoglobulin sequence can be identified as such by comparing the amino acid sequence of the human antibody to the amino acid sequence of human germline immunoglobulins. A selected human antibody typically is at least 90% identical in amino acids sequence to an amino acid sequence encoded by a human germline immunoglobulin gene and contains amino acid residues that identify the human antibody as being human when compared to the germline immunoglobulin amino acid sequences of other species (e.g., murine germline sequences). In certain cases, a human antibody may be at least 95%, or even at least 96%, 97%, 98%, or 99% identical in amino acid sequence to the amino acid sequence encoded by the germline immunoglobulin gene. Typically, a human antibody derived from a particular human germline sequence will display no more than 10 amino acid differences from the amino acid sequence encoded by the human germline immunoglobulin gene. In certain cases, the human antibody may display no more than 5, or even no more than 4, 3, 2, or 1 amino acid difference from the amino acid sequence encoded by the germline immunoglobulin gene.

[0065] The term “recombinant human antibody”, as used herein, includes all human antibodies that are prepared, expressed, created or isolated by recombinant means, such as antibodies isolated from an animal (e.g., a mouse) that is transgenic or transchromosomal for human immunoglobulin genes or a hybridoma prepared therefrom, antibodies isolated from a host cell transformed to express the antibody, e.g., from a transfectoma, antibodies isolated from a recombinant, combinatorial human antibody library, and antibodies prepared, expressed, created or isolated by any other means that involve splicing of human immunoglobulin gene sequences to other DNA sequences. Such recombinant human antibodies have variable and constant regions derived from human germline immunoglobulin sequences. In certain embodiments, however, such recombinant human antibodies can be subjected to *in vitro* mutagenesis (or, when an animal transgenic for human Ig sequences is used, *in vivo* somatic mutagenesis) and thus the amino acid sequences of the VH and VL regions of the recombinant antibodies are sequences that, while derived from and related to human germline VH and VL sequences, may not naturally exist within

the human antibody germline repertoire *in vivo*. Methods to making these antibodies are described herein.

[0066] “Isotype” as used herein is meant any of the subclasses of immunoglobulins defined by the chemical and antigenic characteristics of their constant regions. It should be understood that therapeutic antibodies can also comprise hybrids of isotypes and/or subclasses.

[0067] The terms “polyclonal antibody” or “polyclonal antibody composition” as used herein refer to a preparation of antibodies that are derived from different B-cell lines. They are a mixture of immunoglobulin molecules secreted against a specific antigen, each recognizing a different epitope.

[0068] The terms “monoclonal antibody” or “monoclonal antibody composition” as used herein refer to a preparation of antibody molecules of single molecular composition. A monoclonal antibody composition displays a single binding specificity and affinity for a particular epitope.

[0069] As used herein, the term “label” intends a directly or indirectly detectable compound or composition that is conjugated directly or indirectly to the composition to be detected, e.g., N-terminal histidine tags (N-His), magnetically active isotopes, e.g., ¹¹⁵Sn, ¹¹⁷Sn and ¹¹⁹Sn, a non-radioactive isotopes such as ¹³C and ¹⁵N, polynucleotide or protein such as an antibody so as to generate a "labeled" composition. The term also includes sequences conjugated to the polynucleotide that will provide a signal upon expression of the inserted sequences, such as green fluorescent protein (GFP) and the like. The label may be detectable by itself (e.g. radioisotope labels or fluorescent labels) or, in the case of an enzymatic label, may catalyze chemical alteration of a substrate compound or composition which is detectable. The labels can be suitable for small scale detection or more suitable for high-throughput screening. As such, suitable labels include, but are not limited to magnetically active isotopes, non-radioactive isotopes, radioisotopes, fluorochromes, chemiluminescent compounds, dyes, and proteins, including enzymes. The label may be simply detected or it may be quantified. A response that is simply detected generally comprises a response whose existence merely is confirmed, whereas a response that is quantified generally comprises a response having a quantifiable (e.g., numerically reportable) value such as an intensity, polarization, and/or other property. In luminescence or fluorescence assays, the detectable response may be generated directly using a luminophore or fluorophore associated with an assay component actually involved in binding, or indirectly using a luminophore or fluorophore associated with another (e.g., reporter or indicator) component.

[0070] Examples of luminescent labels that produce signals include, but are not limited to bioluminescence and chemiluminescence. Detectable luminescence response generally comprises a change in, or an occurrence of, a luminescence signal. Suitable methods and luminophores for luminescently labeling assay components are known in the art and described for

example in Haugland, Richard P. (1996) *Handbook of Fluorescent Probes and Research Chemicals* (6th ed.). Examples of luminescent probes include, but are not limited to, aequorin and luciferases.

[0071] Examples of suitable fluorescent labels include, but are not limited to, fluorescein, rhodamine, tetramethylrhodamine, eosin, erythrosin, coumarin, methyl-coumarins, pyrene, Malacite green, stilbene, Lucifer Yellow, Cascade BlueTM, and Texas Red. Other suitable optical dyes are described in the Haugland, Richard P. (1996) *Handbook of Fluorescent Probes and Research Chemicals* (6th ed.).

[0072] In another aspect, the fluorescent label is functionalized to facilitate covalent attachment to a cellular component present in or on the surface of the cell or tissue such as a cell surface marker. Suitable functional groups, including, but not are limited to, isothiocyanate groups, amino groups, haloacetyl groups, maleimides, succinimidyl esters, and sulfonyl halides, all of which may be used to attach the fluorescent label to a second molecule. The choice of the functional group of the fluorescent label will depend on the site of attachment to a linker, the agent, the marker, or the second labeling agent.

[0073] Although the present invention is described primarily with reference to specific embodiments, it is also envisioned that other embodiments will become apparent to those skilled in the art upon reading the present disclosure, and it is intended that such embodiments be contained within the present inventive methods.

I. Overview of the invention

[0074] The present invention is directed to methods and compositions for modulating immune system pathways, particularly any pathways involving the Toso protein (which is also interchangeably referred to herein as “Toso” or “Toso receptor” or “Faim3” or “FCMR”). The terms “immune system pathways,” and “immune pathways” as used herein refer to the coordinated action by cellular and soluble components in a network of tissues and circulating systems that combats pathogens, injury by inert materials and cancers. Modulation of immune system pathways may involve changing the expression or the functional activity in one or more components of the pathway to thereby modulate the response by the immune system (the “immune response”). The present invention provides methods and compositions for tuning the activity or expression of these immune system pathway components to either dampen or increase the immune response. In certain exemplary embodiments, dampening the immune response serves to treat or ameliorate the symptoms of autoimmune diseases. In other exemplary embodiments, increasing the immune response serves to treat, ameliorate or prevent cancer.

[0075] In one aspect, the present invention provides methods and compositions for decreasing Toso expression and/or function. This decrease may be accomplished by any method known in the art and described herein, including without limitation administration of an antibody that binds to Toso, administration of a soluble Toso protein (also referred to herein as “sToso”) to interfere with Toso’s ability to interact with one or more binding partners, and administration of compositions to inhibit protein expression, such siRNA. Toso expression and/or function may also be decreased indirectly by modulating expression or function of other proteins, particularly proteins that themselves directly bind to or otherwise interact with Toso. Note that as used herein, “soluble Toso protein” and all grammatical equivalents generally refers to a protein that is not membrane bound.

[0076] In some embodiments, decrease of Toso expression or activity is accompanied by an increase in the expression or functional activity of other proteins with which Toso interacts through direct binding or other association (physical and/or functional). Exemplary proteins whose activity or expression levels may be affected by a decrease in Toso expression or activity include without limitation IgM, TSG6 and B7H3.

[0077] In certain aspects, the present invention provides methods and compositions for altering the immune pathway by modulating Toso activity as well as the activity of immune checkpoint inhibitors. In particular embodiments, the present invention provides methods and compositions for increasing the immune response through the use of a combination therapy that includes a modulator of Toso activity and an inhibitor of an immune checkpoint protein. In certain examples, the combination therapy includes the use of a soluble Toso receptor in combination with an immune checkpoint inhibitor. In further examples, the combination therapy includes the use of a soluble Toso protein and one or more of a CTLA-4 inhibitor, a PD-1 inhibitor, a TIM3 inhibitor, a LAG3 inhibitor, a PD-1 ligand (such as PDL-1), an inhibitor of a PD-1 ligand, a B7-H3 protein, an inhibitor of a B7-H3 protein, a B7-H4 protein, and an inhibitor of a B7-H4 protein. In certain examples, the combination therapy includes a soluble Toso protein plus a PD-1 inhibitor or a soluble Toso protein plus a PD-1 inhibitor plus a CTLA-4 inhibitor. In further examples, the soluble Toso protein comprises an amino acid sequence according to any one of SEQ ID NOs. 1-25 as well as any of the soluble Toso proteins described in USSN 13/831,031, filed March 14, 2013, which is hereby incorporated by reference in its entirety for all purposes and in particular for all teachings, written description, sequences, and figures directed to soluble Toso proteins. In yet further examples, the PD-1 inhibitor is an anti-PD-1 antibody such as nivolumab or lambrolizumab, and the CTLA-4 inhibitor is an antibody such as ipilimumab or tremelimumab.

[0078] In further embodiments and in accordance with any of the above, decrease of Toso expression or activity results in a decrease of the expression or activity of molecules associated with the inflammatory response (also referred to herein as pathogen-associated molecular patters

or “PAMPs” and damage-associated molecular patterns or “DAMPs”). Such molecules are known in the art and discussed herein. The decrease in such PAMPs or DAMPs may result from a decrease in the expression or activity of Toso directly or through an effect on an intermediary protein, such as an increase in IgM and/or B7H3 expression or activity.

[0079] In still further embodiments and in accordance with any of the above, the present invention provides methods and compositions for dampening the immune response and thereby treating an autoimmune disease by altering the expression or function of one or more proteins in a pathway. In an exemplary embodiment, expression or function of Toso is decreased in order to thereby increase expression or function of IgM and/or B7H3, which then decreases the expression or function of PAMPs and/or DAMPs to ultimately dampen the immune response and treat or ameliorate the symptoms of the autoimmune disease. In further exemplary embodiments, Toso expression is not altered, but IgM and/or B7H3 expression or function is increased in an alternate way to thereby decrease expression or function of PAMPs and/or DAMPs. In still further exemplary embodiments, the PAMPs and/or DAMPs are depressed directly through direct action or through action on expression or function of Toso without changing the expression of other intermediary proteins such as IgM, TSG6 and/or B7H3.

[0080] In another aspect, Toso expression and/or function is increased. This increase may be accomplished by any method known in the art and described herein, including without limitation administration of an agonist to Toso and administration of compositions to increase protein expression.

[0081] In some embodiments, increase of Toso expression or activity is accompanied by a decrease in the expression or functional activity of other proteins with which Toso interacts through direct binding or other association (physical and/or functional). Exemplary proteins whose activity or expression levels may be affected by an increase in Toso expression or activity include without limitation IgM, TSG6 and/or B7H3.

[0082] In further embodiments and in accordance with any of the above, an increase of Toso expression and/or activity is accompanied by a decrease in the expression or functional activity of IgM, which then results in the increase in the function or activity of PAMPs and/or DAMPs. In other embodiments, an increase in Toso expression and/or activity results in the increase in the function or activity of PAMPs and/or DAMPs without the involvement of IgM, either through direct action or indirectly through another protein.

[0083] In yet further embodiments and in accordance with any of the above, the present invention provides methods and compositions for increasing the immune response by altering the expression or function of one or more proteins in a pathway. This increase is conducted such that it leads to the death of one or more cancer cells. In an exemplary embodiment, expression or

function of Toso is increased in order to thereby decrease expression or function of IgM and/or B7H3, which then increases the expression or function of PAMPs and/or DAMPs to ultimately increase the immune response and treat or ameliorate the symptoms of cancer. In further exemplary embodiments, Toso expression is not altered, but IgM and/or B7H3 expression or function is decreased in an alternate way to thereby increase expression or function of PAMPs and/or DAMPs. In still further exemplary embodiments, the PAMPs and/or DAMPs are increased directly through direct action or through action on expression or function of Toso without changing the expression of other proteins such as IgM, TSG6 and/or B7H3. The manipulation of one or more of these proteins serves to increase the immune response, thereby leading to the death of cancer-associated cells, including tumor cells.

[0084] In still further aspects and in accordance with any of the above, methods and compositions of the invention further involve one or more scramblase proteins, which are responsible for the translocation of phospholipids between the two monolayers of a lipid bilayer of a cell membrane. In certain embodiments, methods and compositions of the invention directly or indirectly modulate scramblase activity, leading to targeted apoptosis of cancer cells.

[0085] In certain aspects, proteins that can be used to dampen the immune response are identified through one or more screens. The proteins identified in such screens may in some embodiments interact directly or indirectly with one or more of the above identified proteins, particularly Toso, to affect the immune response.

II. Methods and compositions for modulating activity and expression for immune response drivers

[0086] As will be discussed in further detail herein, the present invention provides methods and compositions for modulating the activity of proteins involved in the immune response, including Toso, B7H3, PAMPs and DAMPs, scramblase, and immune checkpoint inhibitors such as PD-1 and CTLA-4.

[0087] Compositions of the invention may in certain embodiments include agents that modulate Toso activity. Such compositions, as will be discussed in further detail below, include without limitation a soluble form of the Toso protein or an antibody to Toso. Compositions and methods for modulating Toso activity are also described in PCT/IB13/01179, filed on March 14, 2013, which is hereby incorporated by reference in its entirety for all purposes, and in particular for all teachings related to methods and compositions for modulating Toso activity, including any written description, examples, figures, and figures legends.

[0088] Modulating the activity or expression of any combination of these proteins may include without limitation the use of antibodies, aptamers, binding partners, small or large molecule agonists, small or large molecule inhibitors, as well as manipulations of gene expression, such as

inhibitory RNA (including siRNA) or any other methods or compositions known in the art to inhibit or upregulate gene expression of a targeted protein.

[0089] As discussed in further detail herein, different combinations of these proteins may be manipulated to increase or decrease activity and/or expression in different combinations. For example, methods and compositions for decreasing Toso activity and/or expression may be combined with methods and compositions for increasing increase in IgM activity and/or expression, which may also in certain embodiments be further combined with methods and compositions for decreasing PAMPs/DAMPs activity and/or expression. In another embodiment, only methods and compositions for decreasing Toso activity and/or expression are used, and this decrease in activity is accompanied by a change in the activity and/or expression of other proteins, including without limitation one or more of IgM, B7H3, PAMPs, DAMPs, scramblase, and immune checkpoint inhibitors such as PD-1 and CTLA-4.

IIA. Inhibition of expression

[0090] In certain aspects of the present invention, the expression of one or more proteins involved in the immune response is inhibited. Such proteins may include without limitation Toso, IgM, B7H3, PAMPs, DAMPs, scramblase, and immune checkpoint inhibitors such as PD-1 and CTLA-4.

[0091] In certain embodiments, the invention provides methods of delivering interfering RNA to inhibit the expression of a target mRNA thus decreasing target mRNA levels in patients with target mRNA-related disorders.

[0092] The phrase "attenuating expression" with reference to a gene or an mRNA as used herein means administering or expressing an amount of interfering RNA (e.g., an siRNA) to reduce translation of a target mRNA into protein, either through mRNA cleavage or through direct inhibition of translation. The terms "inhibit," "silencing," and "attenuating" as used herein refer to a measurable reduction in expression of a target mRNA or the corresponding protein as compared with the expression of the target mRNA or the corresponding protein in the absence of an interfering RNA of the invention. The reduction in expression of the target mRNA or the corresponding protein is commonly referred to as "knock-down" and is reported relative to levels present following administration or expression of a non-targeting control RNA (e.g., a non-targeting control siRNA). Knock-down of expression of an amount including and between 50% and 100% is contemplated by embodiments herein. However, it is not necessary that such knock-down levels be achieved for purposes of the present invention.

[0093] Knock-down is commonly assessed by measuring the mRNA levels using quantitative polymerase chain reaction (qPCR) amplification or by measuring protein levels by western blot or enzyme-linked immunosorbent assay (ELISA). Analyzing the protein level provides an assessment

of both mRNA cleavage as well as translation inhibition. Further techniques for measuring knock-down include RNA solution hybridization, nuclease protection, northern hybridization, gene expression monitoring with a microarray, antibody binding, radioimmunoassay, and fluorescence activated cell analysis.

[0094] Attenuating expression of a target gene by an interfering RNA molecule of the invention can be inferred in a human or other mammal by observing an improvement in symptoms of the disorder and/or by observing a change in a characteristic, whether that characteristic be behavioral or physiological (including a change in the expression level of another protein).

[0095] In one embodiment, a single interfering RNA is delivered to decrease target mRNA levels. In other embodiments, two or more interfering RNAs targeting the mRNA are administered to decrease target mRNA levels.

[0096] As used herein, the terms "interfering RNA" and "interfering RNA molecule" refer to all RNA or RNA-like molecules that can interact with RISC and participate in RISC-mediated changes in gene expression. Examples of other interfering RNA molecules that can interact with RISC include short hairpin RNAs (shRNAs), single-stranded siRNAs, microRNAs (miRNAs), picoRNAs (piRNAs), and dicer-substrate 27-mer duplexes. Examples of "RNA-like" molecules that can interact with RISC include siRNA, single-stranded siRNA, miRNA, piRNA, and shRNA molecules that contain one or more chemically modified nucleotides, one or more non-nucleotides, one or more deoxyribonucleotides, and/or one or more non-phosphodiester linkages. Thus, siRNAs, single-stranded siRNAs, shRNAs, miRNAs, piRNA, and dicer-substrate 27-mer duplexes are subsets of "interfering RNAs" or "interfering RNA molecules."

[0097] The term "siRNA" as used herein refers to a double-stranded interfering RNA unless otherwise noted. Typically, an siRNA used in a method of the invention is a double-stranded nucleic acid molecule comprising two nucleotide strands, each strand having about 19 to about 28 nucleotides (i.e. about 19, 20, 21, 22, 23, 24, 25, 26, 27, or 28 nucleotides). Typically, an interfering RNA used in a method of the invention has a length of about 19 to 49 nucleotides. The phrase "length of 19 to 49 nucleotides" when referring to a double-stranded interfering RNA means that the antisense and sense strands independently have a length of about 19 to about 49 nucleotides, including interfering RNA molecules where the sense and antisense strands are connected by a linker molecule.

[0098] The interfering RNA used in a delivery system and method of the invention can be unmodified or can be chemically stabilized to prevent degradation in the lysosome or other compartments in the endocytic pathway.

[0099] Single-stranded interfering RNA has been found to effect mRNA silencing. Therefore, embodiments of the present invention also provide for administration of a single-stranded

interfering RNA. The single-stranded interfering RNA has a length of about 19 to about 49 nucleotides as for the double-stranded interfering RNA cited above. The single-stranded interfering RNA has a 5' phosphate or is phosphorylated in situ or in vivo at the 5' position. The term "5' phosphorylated" is used to describe, for example, polynucleotides or oligonucleotides having a phosphate group attached via ester linkage to the C5 hydroxyl of the sugar (e.g., ribose, deoxyribose, or an analog of same) at the 5' end of the polynucleotide or oligonucleotide.

[0100] Single-stranded interfering RNAs can be synthesized chemically or by in vitro transcription or expressed endogenously from vectors or expression cassettes as described herein in reference to double-stranded interfering RNAs. 5' Phosphate groups may be added via a kinase, or a 5' phosphate may be the result of nuclease cleavage of an RNA. A hairpin interfering RNA is a single molecule (e.g., a single oligonucleotide chain) that comprises both the sense and antisense strands of an interfering RNA in a stem-loop or hairpin structure (e.g., a shRNA). For example, shRNAs can be expressed from DNA vectors in which the DNA oligonucleotides encoding a sense interfering RNA strand are linked to the DNA oligonucleotides encoding the reverse complementary antisense interfering RNA strand by a short spacer. If needed for the chosen expression vector, 3' terminal T's and nucleotides forming restriction sites may be added. The resulting RNA transcript folds back onto itself to form a stem-loop structure.

[0101] Interfering RNAs may differ from naturally-occurring RNA by the addition, deletion, substitution or modification of one or more nucleotides. Non-nucleotide material may be bound to the interfering RNA, either at the 5' end, the 3' end, or internally. Such modifications are commonly designed to increase the nuclease resistance of the interfering RNAs, to improve cellular uptake, to enhance cellular targeting, to assist in tracing the interfering RNA, to further improve stability, to reduce off-target effects, or to reduce the potential for activation of the interferon pathway. For example, interfering RNAs may comprise a purine nucleotide at the ends of overhangs. Conjugation of cholesterol to the 3' end of the sense strand of an siRNA molecule by means of a pyrrolidine linker, for example, also provides stability to an siRNA.

[0102] Further modifications include a biotin molecule, a peptidomimetic, a fluorescent dye, or a dendrimer, for example.

[0103] Nucleotides may be modified on their base portion, on their sugar portion, or on the phosphate portion of the molecule and function in embodiments of the present invention. Modifications include substitutions with alkyl, alkoxy, amino, deaza, halo, hydroxyl, thiol groups, or a combination thereof, for example. Nucleotides may be substituted with analogs with greater stability such as replacing a ribonucleotide with a deoxyribonucleotide, or having sugar

modifications such as 2' OH groups replaced by 2' amino groups, 2' O-methyl groups, 2' methoxyethyl groups, or a 2'-O, 4'-C methylene bridge, for example. Examples of a purine or pyrimidine analog of nucleotides include a xanthine, a hypoxanthine, an azapurine, a methylthioadenine, 7-deaza-adenosine and O- and N-modified nucleotides. The phosphate group of the nucleotide may be modified by substituting one or more of the oxygens of the phosphate group with nitrogen or with sulfur (phosphorothioates). Modifications are useful, for example, to enhance function, to improve stability or permeability, to reduce off-target effects, or to direct localization or targeting.

[0104] In certain embodiments, an interfering molecule of the invention comprises at least one of the modifications as described above.

[0105] The phrases "target sequence" and "target mRNA" as used herein refer to the mRNA or the portion of the mRNA sequence that can be recognized by an interfering RNA used in a method of the invention, whereby the interfering RNA can silence gene expression as discussed herein. Techniques for selecting target sequences for siRNAs are provided, for example, by Tuschl, T. et al., "The siRNA User Guide," revised May 6, 2004, available on the Rockefeller University web site; by Technical Bulletin #506, "siRNA Design Guidelines," Ambion Inc. at Ambion's web site; and by other web-based design tools at, for example, the Invitrogen, Dharmacon, Integrated DNA Technologies, or Genscript web sites. Initial search parameters can include G/C contents between 35% and 55% and siRNA lengths between 19 and 27 nucleotides. The target sequence may be located in the coding region or in the 5' or 3' untranslated regions of the mRNA. The target sequences can be used to derive interfering RNA molecules, such as those described herein.

[0106] Interfering RNA target sequences (e.g., siRNA target sequences) within a target mRNA sequence can be selected using available design tools as discussed above. Interfering RNAs corresponding to a target sequence are then tested in vitro by transfection of cells expressing the target mRNA followed by assessment of knockdown as described herein. The interfering RNAs can be further evaluated in vivo using animal models as described herein.

[0107] In certain embodiments, an interfering RNA delivery system comprises an interfering RNA molecule that targets a gene associated with the immune response, including without limitation Toso, IgM, B7H3, PAMPs, DAMPs, scramblase, and immune checkpoint inhibitors such as PD-1 and CTLA-4.

IIB. Antibodies

[0108] In one aspect, the present invention provides an antibody that binds to one or more proteins involved in the immune response, including without limitation Toso, IgM, B7H3, PAMPs, DAMPs, scramblase, and immune checkpoint inhibitors such as PD-1 and CTLA-4. In some embodiments,

antibodies of the invention increase the activity of these proteins. In other embodiments, antibodies of the invention decrease their activity.

[0109] Methods of preparing antibodies are generally known in the art. For example, United States Patent No. 6,727,350 discloses an antibody directed to Toso, which is hereby incorporated by reference in its entirety for all purposes and in particular for all teachings related to antibodies directed to the proteins discussed herein.

[0110] Antibodies to other components of the immune system pathway are also known in the art and are of use alone or in combination with any of the other molecules described herein. For example, antibodies to PD-1 include without limitation nivolumab, pembrolizumab, MK-3475 (lambrolizumab), and BMS-936558 (described for example in *N Engl J Med* 2012; 366:2443-2454). Antibodies to CTLA-4 include without limitation ipilimumab and tremelimumab.

[0111] An antibody of use in the methods of the present invention may be a polyclonal antibody, monoclonal antibody, chimeric antibody, humanized antibody or a derivative or fragment thereof as defined below. In one aspect, a fragment comprises, or alternatively consists essentially of, or yet further consists of the CDR of an antibody. In one aspect, an antibody of the invention is detectably labeled or further comprises a detectable label conjugated to it. Also provided is a hybridoma cell line that produces a monoclonal antibody of this invention. Compositions comprising one or more of the above embodiments are further provided herein.

[0112] Also provided is a composition comprising the antibody and a carrier. Further provided is a biologically active fragment of the antibody, or a composition comprising the antibody fragment. Suitable carriers are defined *supra*.

[0113] Further provided is an antibody-peptide complex comprising, or alternatively consisting essentially of, or yet alternatively consisting of, the antibody and a polypeptide specifically bound to the antibody. In one aspect, the polypeptide is the chimeric polypeptide against which the antibody is raised.

[0114] This invention also provides an antibody capable of specifically forming a complex with Toso, which are useful in the therapeutic methods of this invention. Antibodies of the invention include, but are not limited to mouse, rat, and rabbit or human antibodies. Antibodies can be produced in cell culture, in phage, or in various animals, including but not limited to cows, rabbits, goats, mice, rats, hamsters, guinea pigs, sheep, dogs, cats, monkeys, chimpanzees, apes, etc. The antibodies are also useful to identify and purify therapeutic polypeptides.

[0115] This invention also provides an antibody-peptide complex comprising, or alternatively consisting essentially of, or yet alternatively consisting of, antibodies described above and an antigen (or a portion of an antigen) specifically bound to the antibody. The antigen may include without limitation a polypeptide (including full length and portions of full length proteins), lipid

antigens, and carbohydrate antigens. In one aspect the complex is an isolated complex. In a further aspect, the antibody of the complex is, but not limited to, a polyclonal antibody, a monoclonal antibody, a humanized antibody or an antibody derivative described herein. Either or both of the antibody or antigen of the antibody-antigen complex can be detectably labeled or further comprises a detectable label conjugated to it. In one aspect, the antibody-antigen complex of the invention can be used as a control or reference sample in diagnostic or screening assays.

[0116] Polyclonal antibodies of the invention can be generated using conventional techniques known in the art and are well-described in the literature. Several methodologies exist for production of polyclonal antibodies. For example, polyclonal antibodies are typically produced by immunization of a suitable vertebrate animal such as, but not limited to, chickens, goats, guinea pigs, hamsters, horses, lamas, mice, rats, and rabbits. An antigen is injected into the mammal, which induces the B-lymphocytes to produce IgG immunoglobulins specific for the antigen. This IgG is purified from the mammal's serum. Variations of this methodology include modification of adjuvants, routes and site of administration, injection volumes per site and the number of sites per animal for optimal production and humane treatment of the animal. For example, adjuvants typically are used to improve or enhance an immune response to antigens. Most adjuvants provide for an injection site antigen depot, which allows for a slow release of antigen into draining lymph nodes. Other adjuvants include surfactants which promote concentration of protein antigen molecules over a large surface area and immunostimulatory molecules. Non-limiting examples of adjuvants for polyclonal antibody generation include Freund's adjuvants, Ribi adjuvant system, and Titermax. Polyclonal antibodies can be generated using methods described in U.S. Patent Nos. 7,279,559; 7,119,179; 7,060,800; 6,709,659; 6,656,746; 6,322,788; 5,686,073; and 5,670,153, which are hereby incorporated by reference in their entirety for all purposes and in particular for all teachings related to antibodies.

[0117] The monoclonal antibodies of the invention can be generated using conventional hybridoma techniques known in the art and well-described in the literature. For example, a hybridoma is produced by fusing a suitable immortal cell line (e.g., a myeloma cell line such as, but not limited to, Sp2/0, Sp2/0-AG14, NSO, NS1, NS2, AE-1, L.5, >243, P3X63Ag8.653, Sp2 SA3, Sp2 MA1, Sp2 SS1, Sp2 SA5, U397, MLA 144, ACT IV, MOLT4, DA-1, JURKAT, WEHI, K-562, COS, RAJI, NIH 3T3, HL-60, MLA 144, NAMAIWA, NEURO 2A, CHO, PerC.6, YB2/O) or the like, or heteromyelomas, fusion products thereof, or any cell or fusion cell derived therefrom, or any other suitable cell line as known in the art (see, e.g., www.atcc.org, www.lifetech.com., last accessed on November 26, 2007, and the like), with antibody producing cells, such as, but not limited to, isolated or cloned spleen, peripheral blood, lymph, tonsil, or other immune or B cell containing cells, or any other cells expressing heavy or light chain constant or variable or

framework or CDR sequences, either as endogenous or heterologous nucleic acid, as recombinant or endogenous, viral, bacterial, algal, prokaryotic, amphibian, insect, reptilian, fish, mammalian, rodent, equine, ovine, goat, sheep, primate, eukaryotic, genomic DNA, cDNA, rDNA, mitochondrial DNA or RNA, chloroplast DNA or RNA, hnRNA, mRNA, tRNA, single, double or triple stranded, hybridized, and the like or any combination thereof. Antibody producing cells can also be obtained from the peripheral blood or, preferably the spleen or lymph nodes, of humans or other suitable animals that have been immunized with the antigen of interest. Any other suitable host cell can also be used for expressing-heterologous or endogenous nucleic acid encoding an antibody, specified fragment or variant thereof, of the present invention. The fused cells (hybridomas) or recombinant cells can be isolated using selective culture conditions or other suitable known methods, and cloned by limiting dilution or cell sorting, or other known methods.

[0118] In one embodiment, the antibodies described herein can be generated using a Multiple Antigenic Peptide (MAP) system. The MAP system utilizes a peptidyl core of three or seven radially branched lysine residues, on to which the antigen peptides of interest can be built using standard solid-phase chemistry. The lysine core yields the MAP bearing about 4 to 8 copies of the peptide epitope depending on the inner core that generally accounts for less than 10% of total molecular weight. The MAP system does not require a carrier protein for conjugation. The high molar ratio and dense packing of multiple copies of the antigenic epitope in a MAP has been shown to produce strong immunogenic response. This method is described in U.S. Patent No. 5,229,490 and is herein incorporated by reference in its entirety for all purposes and in particular for all teachings related to the MAP system.

[0119] Other suitable methods of producing or isolating antibodies of the requisite specificity can be used, including, but not limited to, methods that select recombinant antibody from a peptide or protein library (e.g., but not limited to, a bacteriophage, ribosome, oligonucleotide, RNA, cDNA, or the like, display library; e.g., as available from various commercial vendors such as Cambridge Antibody Technologies (Cambridgeshire, UK), MorphoSys (Martinsreid/Planegg, Del.), Biovation (Aberdeen, Scotland, UK) BioInvent (Lund, Sweden), using methods known in the art. See U.S. Patent Nos. 4,704,692; 5,723,323; 5,763,192; 5,814,476; 5,817,483; 5,824,514; 5,976,862, which are hereby incorporated by reference in their entirety for all purposes and in particular for all teachings related to methods related to antibodies. Alternative methods rely upon immunization of transgenic animals (e.g., SCID mice, Nguyen et al. (1997) *Microbiol. Immunol.* 41:901-907; Sandhu et al. (1996) *Crit. Rev. Biotechnol.* 16:95-118; Eren et al. (1998) *Immunol.* 93:154-161 that are capable of producing a repertoire of human antibodies, as known in the art and/or as described herein. Such techniques, include, but are not limited to, ribosome display (Hanes et al. (1997) *Proc. Natl. Acad. Sci. USA* 94:4937-4942; Hanes et al. (1998) *Proc. Natl. Acad. Sci. USA*

95:14130-14135); single cell antibody producing technologies (e.g., selected lymphocyte antibody method ("SLAM") (U.S. Patent No. 5,627,052, Wen et al. (1987) *J. Immunol.* 17:887-892; Babcock et al. (1996) *Proc. Natl. Acad. Sci. USA* 93:7843-7848); gel microdroplet and flow cytometry (Powell et al. (1990) *Biotechnol.* 8:333-337; One Cell Systems, (Cambridge, Mass); Gray et al. (1995) *J. Imm. Meth.* 182:155-163; and Kenny et al. (1995) *Bio. Technol.* 13:787-790); B-cell selection (Steenbakkers et al. (1994) *Molec. Biol. Reports* 19:125-134, which are hereby incorporated by reference in their entirety for all purposes and in particular for all teachings related to methods for generating antibodies.

[0120] Antibody derivatives of the present invention can also be prepared by delivering a polynucleotide encoding an antibody of this invention to a suitable host such as to provide transgenic animals or mammals, such as rodents, goats, cows, horses, sheep, and the like, that produce such antibodies in their milk or serum. These methods are known in the art and are described for example in U.S. Patent Nos. 5,827,690; 5,849,992; 4,873,316; 5,849,992; 5,994,616; 5,565,362; and 5,304,489, which are hereby incorporated by reference in their entirety for all purposes and in particular for all teachings related to generating antibodies.

[0121] The term "antibody derivative" includes post-translational modification to linear polypeptide sequence of the antibody or fragment. For example, U.S. Patent No. 6,602,684 B1, which is hereby incorporated by reference in its entirety for all purposes and in particular for all teachings related to modifications of antibodies, describes a method for the generation of modified glycoforms of antibodies, including whole antibody molecules, antibody fragments, or fusion proteins that include a region equivalent to the Fc region of an immunoglobulin, having enhanced Fc-mediated cellular toxicity, and glycoproteins so generated.

[0122] Antibody derivatives also can be prepared by delivering a polynucleotide of this invention to provide transgenic plants and cultured plant cells (e.g., but not limited to tobacco, maize, and duckweed) that produce such antibodies, specified portions or variants in the plant parts or in cells cultured there from. For example, Cramer et al. (1999) *Curr. Top. Microbiol. Immunol.* 240:95-118 and references cited therein, describe the production of transgenic tobacco leaves expressing large amounts of recombinant proteins, e.g., using an inducible promoter. Transgenic maize have been used to express mammalian proteins at commercial production levels, with biological activities equivalent to those produced in other recombinant systems or purified from natural sources. See, e.g., Hood et al. (1999) *Adv. Exp. Med. Biol.* 464:127-147 and references cited therein. Antibody derivatives have also been produced in large amounts from transgenic plant seeds including antibody fragments, such as single chain antibodies (scFv's), including tobacco seeds and potato tubers. See, e.g., Conrad et al. (1998) *Plant Mol. Biol.* 38:101-109 and reference

cited therein. Thus, antibodies of the present invention can also be produced using transgenic plants, according to known methods.

[0123] Antibody derivatives also can be produced, for example, by adding exogenous sequences to modify immunogenicity or reduce, enhance or modify binding, affinity, on-rate, off-rate, avidity, specificity, half-life, or any other suitable characteristic. Generally part or all of the non-human or human CDR sequences are maintained while the non-human sequences of the variable and constant regions are replaced with human or other amino acids.

[0124] In general, the CDR residues (an example of an antibody fragment) are directly and most substantially involved in influencing antigen binding. Humanization or engineering of antibodies of the present invention can be performed using any known method such as, but not limited to, those described in U.S. Patent Nos. 5,723,323; 5,976,862; 5,824,514; 5,817,483; 5,814,476; 5,763,192; 5,723,323; 5,766,886; 5,714,352; 6,204,023; 6,180,370; 5,693,762; 5,530,101; 5,585,089; 5,225,539; and 4,816,567, which are hereby incorporated by reference in their entirety for all purposes and in particular for all teachings related to humanization or engineering of antibodies.

[0125] Techniques for making partially to fully human antibodies are known in the art and any such techniques can be used. According to one embodiment, fully human antibody sequences are made in a transgenic rodent, such as a mouse, which has been engineered to express human heavy and light chain antibody genes. Multiple strains of such transgenic mice have been made which can produce different classes of antibodies. B cells from transgenic mice which are producing a desirable antibody can be fused to make hybridoma cell lines for continuous production of the desired antibody. (See for example, Russel et al. (2000) Infection and Immunity 68(4):1820-1826; Gallo et al. (2000) European J. of Immun. 30:534-540; Green (1999) J. of Immun. Methods 231:11-23; Yang et al. (1999A) J. of Leukocyte Biology 66:401-410; Yang (1999B) Cancer Research 59(6):1236-1243; Jakobovits (1998) Advanced Drug Delivery Reviews 31:33-42; Green & Jakobovits (1998) J. Exp. Med. 188(3):483-495; Jakobovits (1998) Exp. Opin. Invest. Drugs 7(4):607-614; Tsuda et al. (1997) Genomics 42:413-421; Sherman-Gold (1997) Genetic Engineering News 17(14); Mendez et al. (1997) Nature Genetics 15:146-156; Jakobovits (1996) Weir's Handbook of Experimental Immunology, The Integrated Immune System Vol. IV, 194.1-194.7; Jakobovits (1995) Current Opinion in Biotechnology 6:561-566; Mendez et al. (1995) Genomics 26:294-307; Jakobovits (1994) Current Biology 4(8):761-763; Arbones et al. (1994) Immunity 1(4):247-260; Jakobovits (1993) Nature 362(6417):255-258; Jakobovits et al. (1993) Proc. Natl. Acad. Sci. USA 90(6):2551-2555; and U.S. Patent No. 6,075,181.)

[0126] The antibodies of this invention also can be modified to create chimeric antibodies. Chimeric antibodies are those in which the various domains of the antibodies' heavy and light chains are coded for by DNA from more than one species. See, e.g., U.S. Patent No. 4,816,567.

[0127] Alternatively, the antibodies of this invention can also be modified to create veneered antibodies. Veneered antibodies are those in which the exterior amino acid residues of the antibody of one species are judiciously replaced or “veneered” with those of a second species so that the antibodies of the first species will not be immunogenic in the second species thereby reducing the immunogenicity of the antibody. Since the antigenicity of a protein is primarily dependent on the nature of its surface, the immunogenicity of an antibody could be reduced by replacing the exposed residues which differ from those usually found in another mammalian species’ antibodies. This judicious replacement of exterior residues should have little, or no, effect on the interior domains, or on the interdomain contacts. Thus, ligand binding properties should be unaffected as a consequence of alterations which are limited to the variable region framework residues. The process is referred to as “veneering” since only the outer surface or skin of the antibody is altered, the supporting residues remain undisturbed.

[0128] The procedure for “veneering” makes use of the available sequence data for human antibody variable domains compiled by Kabat et al. (1987) Sequences of Proteins of Immunological Interest, 4th ed., Bethesda, Md., National Institutes of Health, updates to this database, and other accessible U.S. and foreign databases (both nucleic acid and protein). Non-limiting examples of the methods used to generate veneered antibodies include EP 519596; U.S. Patent No. 6,797,492; and described in Padlan et al. (1991) Mol. Immunol. 28(4-5):489-498.

[0129] The term “antibody derivative” also includes “diabodies” which are small antibody fragments with two antigen-binding sites, wherein fragments comprise a heavy chain variable domain (VH) connected to a light chain variable domain (VL) in the same polypeptide chain. (See for example, EP 404,097; WO 93/11161; and Hollinger et al. (1993) Proc. Natl. Acad. Sci. USA 90:6444-6448.) By using a linker that is too short to allow pairing between the two domains on the same chain, the domains are forced to pair with the complementary domains of another chain and create two antigen-binding sites. (See also, U.S. Patent No. 6,632,926 to Chen et al. which discloses antibody variants that have one or more amino acids inserted into a hypervariable region of the parent antibody and a binding affinity for a target antigen which is at least about two fold stronger than the binding affinity of the parent antibody for the antigen.)

[0130] The term “antibody derivative” further includes “linear antibodies”. The procedure for making linear antibodies is known in the art and described in Zapata et al. (1995) Protein Eng. 8(10):1057-1062. Briefly, these antibodies comprise a pair of tandem Fd segments (V_H -C_H 1-VH -C_H1) which form a pair of antigen binding regions. Linear antibodies can be bispecific or monospecific.

[0131] The antibodies of this invention can be recovered and purified from recombinant cell cultures by known methods including, but not limited to, protein A purification, ammonium sulfate or

ethanol precipitation, acid extraction, anion or cation exchange chromatography, phosphocellulose chromatography, hydrophobic interaction chromatography, affinity chromatography, hydroxylapatite chromatography and lectin chromatography. High performance liquid chromatography ("HPLC") can also be used for purification.

[0132] Antibodies of the present invention include naturally purified products, products of chemical synthetic procedures, and products produced by recombinant techniques from a eukaryotic host, including, for example, yeast, higher plant, insect and mammalian cells, or alternatively from a prokaryotic cells as described above.

[0133] If a monoclonal antibody being tested binds with protein or polypeptide, then the antibody being tested and the antibodies provided by the hybridomas of this invention are equivalent. It also is possible to determine without undue experimentation, whether an antibody has the same specificity as the monoclonal antibody of this invention by determining whether the antibody being tested prevents a monoclonal antibody of this invention from binding the protein or polypeptide with which the monoclonal antibody is normally reactive. If the antibody being tested competes with the monoclonal antibody of the invention as shown by a decrease in binding by the monoclonal antibody of this invention, then it is likely that the two antibodies bind to the same or a closely related epitope. Alternatively, one can pre-incubate the monoclonal antibody of this invention with a protein with which it is normally reactive, and determine if the monoclonal antibody being tested is inhibited in its ability to bind the antigen. If the monoclonal antibody being tested is inhibited then, in all likelihood, it has the same, or a closely related, epitopic specificity as the monoclonal antibody of this invention.

[0134] The term "antibody" also is intended to include antibodies of all isotypes. Particular isotypes of a monoclonal antibody can be prepared either directly by selecting from the initial fusion, or prepared secondarily, from a parental hybridoma secreting a monoclonal antibody of different isotype by using the sib selection technique to isolate class switch variants using the procedure described in Steplewski et al. (1985) Proc. Natl. Acad. Sci. USA 82:8653 or Spira et al. (1984) J. Immunol. Methods 74:307.

[0135] The isolation of other hybridomas secreting monoclonal antibodies with the specificity of the monoclonal antibodies of the invention can also be accomplished by one of ordinary skill in the art by producing anti-idiotypic antibodies. Herlyn et al. (1986) Science 232:100. An anti-idiotypic antibody is an antibody which recognizes unique determinants present on the monoclonal antibody produced by the hybridoma of interest.

[0136] Idiotypic identity between monoclonal antibodies of two hybridomas demonstrates that the two monoclonal antibodies are the same with respect to their recognition of the same epitopic determinant. Thus, by using antibodies to the epitopic determinants on a monoclonal antibody it is

possible to identify other hybridomas expressing monoclonal antibodies of the same epitopic specificity.

[0137] It is also possible to use the anti-idiotype technology to produce monoclonal antibodies which mimic an epitope. For example, an anti-idiotypic monoclonal antibody made to a first monoclonal antibody will have a binding domain in the hypervariable region which is the mirror image of the epitope bound by the first monoclonal antibody. Thus, in this instance, the anti-idiotypic monoclonal antibody could be used for immunization for production of these antibodies.

[0138] In some aspects of this invention, it will be useful to detectably or therapeutically label the antibody. Methods for conjugating antibodies to these agents are known in the art. For the purpose of illustration only, antibodies can be labeled with a detectable moiety such as a radioactive atom, a chromophore, a fluorophore, or the like. Such labeled antibodies can be used for diagnostic techniques, either *in vivo*, or in an isolated test sample.

[0139] The coupling of antibodies to low molecular weight haptens can increase the sensitivity of the antibody in an assay. The haptens can then be specifically detected by means of a second reaction. For example, it is common to use haptens such as biotin, which reacts avidin, or dinitrophenol, pyridoxal, and fluorescein, which can react with specific anti-hapten antibodies. See, Harlow & Lane (1988) *supra*.

[0140] The antibodies of the invention also can be bound to many different carriers. Thus, this invention also provides compositions containing the antibodies and another substance, active or inert. Examples of well-known carriers include glass, polystyrene, polypropylene, polyethylene, dextran, nylon, amyloses, natural and modified celluloses, polyacrylamides, agaroses and magnetite. The nature of the carrier can be either soluble or insoluble for purposes of the invention. Those skilled in the art will know of other suitable carriers for binding monoclonal antibodies, or will be able to ascertain such, using routine experimentation.

[0141] In certain embodiments, antibodies of the invention include mutations in the constant region that improve pharmacokinetic properties of the antibodies as compared to antibodies without such mutations. Such antibodies will in certain embodiments include an Fc domain that is derived from human IgG1 at the C-terminus, which in yet further embodiments include mutations that diminish or ablate antibody-dependent and complement dependent cytotoxicity. In a still further embodiment, such mutations include one or more of the following mutations singly or in any combination: E233P; L234V; L235A; ΔG236; A327G; A330S; P331S. In a yet further embodiment, the Fc domain comprises a sequence according to SEQ ID NO: 3, which is shown in FIG. 10. In a still further embodiment, the Fc domain comprises a sequence with a sequence identity of about 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% and 100% identity to SEQ ID NO: 3.

[0142] In further embodiments, one or more amino acid modifications are made in one or more of the CDRs of the antibody. In general, only 1 or 2 or 3 amino acids are substituted in any single CDR, and generally no more than from 4, 5, 6, 7, 8 9 or 10 changes are made within a set of CDRs. However, it should be appreciated that any combination of no substitutions, 1, 2 or 3 substitutions in any CDR can be independently and optionally combined with any other substitution.

[0143] In some cases, amino acid modifications in the CDRs are referred to as "affinity maturation". An "affinity matured" antibody is one having one or more alteration(s) in one or more CDRs which results in an improvement in the affinity of the antibody for antigen, compared to a parent antibody which does not possess those alteration(s). In some cases, although rare, it may be desirable to decrease the affinity of an antibody to its antigen, but this is generally not preferred.

[0144] Affinity maturation can be conducted to increase the binding affinity of the antibody for the antigen by at least about 10% to 50-100-150% or more, or from 1 to 5 fold as compared to the "parent" antibody. Preferred affinity matured antibodies will have nanomolar or even picomolar affinities for the target antigen. Affinity matured antibodies are produced by known procedures. See, for example, Marks et al., 1992, *Biotechnology* 10:779-783 that describes affinity maturation by variable heavy chain (VH) and variable light chain (VL) domain shuffling. Random mutagenesis of CDR and/or framework residues is described in: Barbas, et al. 1994, *Proc. Nat. Acad. Sci. USA* 91:3809-3813; Shier et al., 1995, *Gene* 169:147-155; Yelton et al., 1995, *J. Immunol.* 155:1994-2004; Jackson et al., 1995, *J. Immunol.* 154(7):3310-9; and Hawkins et al, 1992, *J. Mol. Biol.* 226:889-896, for example.

[0145] Alternatively, amino acid modifications can be made in one or more of the CDRs of the antibodies of the invention that are "silent", e.g. that do not significantly alter the affinity of the antibody for the antigen. These can be made for a number of reasons, including optimizing expression (as can be done for the nucleic acids encoding the antibodies of the invention).

[0146] Thus, included within the definition of the CDRs and antibodies of the invention are variant CDRs and antibodies; that is, the antibodies of the invention can include amino acid modifications in one or more of the CDRs of Ab79 and Ab19. In addition, as outlined below, amino acid modifications can also independently and optionally be made in any region outside the CDRs, including framework and constant regions.

[0147] In some embodiments, the antibodies of the invention are conjugated with drugs to form antibody-drug conjugates (ADCs). In general, ADCs are used in oncology applications, where the use of antibody-drug conjugates for the local delivery of cytotoxic or cytostatic agents allows for the targeted delivery of the drug moiety to tumors, which can allow higher efficacy, lower toxicity, etc. An overview of this technology is provided in Ducry et al., *Bioconjugate Chem.*, 21:5-13 (2010),

Carter et al., *Cancer J.* 14(3):154 (2008) and Senter, *Current Opin. Chem. Biol.* 13:235-244 (2009), all of which are hereby incorporated by reference in their entirety for all purposes and in particular for all teachings related to antibody drug conjugates.

[0148] Thus, in some embodiments, the invention provides Toso antibodies conjugated to drugs. Generally, conjugation is done by covalent attachment to the antibody and generally relies on a linker, often a peptide linkage (which, as is known in the art, may be designed to be sensitive to cleavage by proteases at the target site or not). In addition, as described above, linkage of the linker-drug unit (LU-D) can be done by attachment to cysteines within the antibody. As will be appreciated by those in the art, the number of drug moieties per antibody can change, depending on the conditions of the reaction, and can vary from 1:1 to 10:1 drug:antibody. As will be appreciated by those in the art, the actual number is an average.

[0149] The drug of the ADC can be any number of agents, including but not limited to cytotoxic agents such as chemotherapeutic agents, growth inhibitory agents, toxins (for example, an enzymatically active toxin of bacterial, fungal, plant, or animal origin, or fragments thereof), or a radioactive isotope (that is, a radioconjugate) are provided. In other embodiments, the invention further provides methods of using the ADCs.

[0150] Drugs for use in antibody-drug conjugates of the present invention include cytotoxic drugs, particularly those which are used for cancer therapy. Such drugs include, in general, DNA damaging agents, anti-metabolites, natural products and their analogs. Exemplary classes of cytotoxic agents include the enzyme inhibitors such as dihydrofolate reductase inhibitors, and thymidylate synthase inhibitors, DNA intercalators, DNA cleavers, topoisomerase inhibitors, the anthracycline family of drugs, the vinca drugs, the mitomycins, the bleomycins, the cytotoxic nucleosides, the pteridine family of drugs, diynenes, the podophyllotoxins, dolastatins, maytansinoids, differentiation inducers, and taxols.

[0151] Members of these classes include, for example, methotrexate, methopterin, dichloromethotrexate, 5-fluorouracil, 6-mercaptopurine, cytosine arabinoside, melphalan, leurosine, leurosine, actinomycin, daunorubicin, doxorubicin, mitomycin C, mitomycin A, caminomycin, aminopterin, tallysomycin, podophyllotoxin and podophyllotoxin derivatives such as etoposide or etoposide phosphate, vinblastine, vincristine, vindesine, taxanes including taxol, taxotere retinoic acid, butyric acid, N8-acetyl spermidine, camptothecin, calicheamicin, esperamicin, ene-diynes, duocarmycin A, duocarmycin SA, calicheamicin, camptothecin, maytansinoids (including DM1), monomethylauristatin E (MMAE), monomethylauristatin F (MMAF), and maytansinoids (DM4) and their analogues.

[0152] Toxins may be used as antibody-toxin conjugates and include bacterial toxins such as diphtheria toxin, plant toxins such as ricin, small molecule toxins such as geldanamycin (Mandler et

al (2000) *J. Nat. Cancer Inst.* 92(19):1573-1581; Mandler et al (2000) *Bioorganic & Med. Chem. Letters* 10:1025-1028; Mandler et al (2002) *Bioconjugate Chem.* 13:786-791), maytansinoids (EP 1391213; Liu et al., (1996) *Proc. Natl. Acad. Sci. USA* 93:8618-8623), and calicheamicin (Lode et al (1998) *Cancer Res.* 58:2928; Hinman et al (1993) *Cancer Res.* 53:3336-3342). Toxins may exert their cytotoxic and cytostatic effects by mechanisms including tubulin binding, DNA binding, or topoisomerase inhibition.

[0153] Conjugates of a Toso antibody and one or more small molecule toxins, such as a maytansinoids, dolastatins, auristatins, a trichothecene, calicheamicin, and CC1065, and the derivatives of these toxins that have toxin activity, are contemplated.

[0154] In accordance with any of the above, another type of modification that can be made to antibodies of the invention is alterations in glycosylation. In another embodiment, the antibodies disclosed herein can be modified to include one or more engineered glycoforms. By "engineered glycoform" as used herein is meant a carbohydrate composition that is covalently attached to the antibody, wherein said carbohydrate composition differs chemically from that of a parent antibody. Engineered glycoforms may be useful for a variety of purposes, including but not limited to enhancing or reducing effector function. An exemplary form of engineered glycoform is afucosylation, which has been shown to be correlated to an increase in ADCC function, presumably through tighter binding to the Fc_YRIIIa receptor. In this context, "afucosylation" means that the majority of the antibody produced in the host cells is substantially devoid of fucose, e.g. 90-95-98% of the generated antibodies do not have appreciable fucose as a component of the carbohydrate moiety of the antibody (generally attached at N297 in the Fc region). Defined functionally, afucosylated antibodies generally exhibit at least a 50% or higher affinity to the Fc_YRIIIa receptor.

[0155] Engineered glycoforms may be generated by a variety of methods known in the art (Umana et al., 1999, *Nat Biotechnol* 17:176-180; Davies et al., 2001, *Biotechnol Bioeng* 74:288-294; Shields et al., 2002, *J Biol Chem* 277:26733-26740; Shinkawa et al., 2003, *J Biol Chem* 278:3466-3473; U.S. Pat. No. 6,602,684; U.S. Ser. No. 10/277,370; U.S. Ser. No. 10/113,929; PCT WO 00/61739A1; PCT WO 01/29246A1; PCT WO 02/31140A1; PCT WO 02/30954A1, all entirely incorporated by reference in their entirety for all purposes and in particular for all teachings related to engineered glycoforms. Many of these techniques are based on controlling the level of fucosylated and/or bisecting oligosaccharides that are covalently attached to the Fc region, for example by expressing an IgG in various organisms or cell lines, engineered or otherwise (for example Lec-13 CHO cells or rat hybridoma YB2/0 cells, by regulating enzymes involved in the glycosylation pathway (for example FUT8 [~~β~~1,6-fucosyltransferase] and/or β 1-4-N-acetylglucosaminyltransferase III [GnTIII]), or by modifying carbohydrate(s) after the IgG has been

expressed. For example, the "sugar engineered antibody" or "SEA technology" of Seattle Genetics functions by adding modified saccharides that inhibit fucosylation during production; see for example 20090317869, hereby incorporated by reference in its entirety. Engineered glycoform typically refers to the different carbohydrate or oligosaccharide; thus an antibody can include an engineered glycoform.

[0156] Alternatively, engineered glycoform may refer to a variant that comprises the different carbohydrate or oligosaccharide. As is known in the art, glycosylation patterns can depend on both the sequence of the protein (e.g., the presence or absence of particular glycosylation amino acid residues, discussed below), or the host cell or organism in which the protein is produced. Particular expression systems are known in the art and discussed herein.

[0157] Glycosylation of polypeptides is typically either N-linked or O-linked. N-linked refers to the attachment of the carbohydrate moiety to the side chain of an asparagine residue. The tri-peptide sequences asparagine-X-serine and asparagine-X-threonine, where X is any amino acid except proline, are the recognition sequences for enzymatic attachment of the carbohydrate moiety to the asparagine side chain. Thus, the presence of either of these tri-peptide sequences in a polypeptide creates a potential glycosylation site. O-linked glycosylation refers to the attachment of one of the sugars N-acetylgalactosamine, galactose, or xylose, to a hydroxyamino acid, most commonly serine or threonine, although 5-hydroxyproline or 5-hydroxylysine may also be used.

[0158] Addition of glycosylation sites to an antibody (or to any other polypeptide, such as the soluble Toso protein discussed above) is conveniently accomplished by altering the amino acid sequence such that it contains one or more of the above-described tri-peptide sequences (for N-linked glycosylation sites). The alteration may also be made by the addition of, or substitution by, one or more serine or threonine residues to the starting sequence (for O-linked glycosylation sites). For ease, the antibody amino acid sequence is preferably altered through changes at the DNA level, particularly by mutating the DNA encoding the target polypeptide at preselected bases such that codons are generated that will translate into the desired amino acids.

[0159] Another means of increasing the number of carbohydrate moieties on an antibody or another protein is by chemical or enzymatic coupling of glycosides to the protein. These procedures are advantageous in that they do not require production of the protein in a host cell that has glycosylation capabilities for N- and O-linked glycosylation. Depending on the coupling mode used, the sugar(s) may be attached to (a) arginine and histidine, (b) free carboxyl groups, (c) free sulphydryl groups such as those of cysteine, (d) free hydroxyl groups such as those of serine, threonine, or hydroxyproline, (e) aromatic residues such as those of phenylalanine, tyrosine, or tryptophan, or (f) the amide group of glutamine. These methods are described in WO 87/05330 and in Aplin and Wriston, 1981, CRC Crit. Rev. Biochem., pp. 259-306, both entirely incorporated

by reference herein in their entirety for all purposes and in particular for all teachings related to coupling carbohydrate moieties to proteins.

[0160] Removal of carbohydrate moieties present on the starting antibody (e.g. post-translationally) may be accomplished chemically or enzymatically. Chemical deglycosylation requires exposure of the protein to the compound trifluoromethanesulfonic acid, or an equivalent compound. This treatment results in the cleavage of most or all sugars except the linking sugar (N-acetylglucosamine or N-acetylgalactosamine), while leaving the polypeptide intact. Chemical deglycosylation is described by Hakimuddin et al., 1987, Arch. Biochem. Biophys. 259:52 and by Edge et al., 1981, Anal. Biochem. 118:131, both entirely incorporated by reference. Enzymatic cleavage of carbohydrate moieties on polypeptides can be achieved by the use of a variety of endo- and exo-glycosidases as described by Thotakura et al., 1987, Meth. Enzymol. 138:350, entirely incorporated by reference. Glycosylation at potential glycosylation sites may be prevented by the use of the compound tunicamycin as described by Duskin et al., 1982, J. Biol. Chem. 257:3105, entirely incorporated by reference. Tunicamycin blocks the formation of protein-N-glycoside linkages.

[0161] Another type of covalent modification of the antibody comprises linking the antibody to various nonproteinaceous polymers, including, but not limited to, various polyols such as polyethylene glycol, polypropylene glycol or polyoxyalkylenes, in the manner set forth in, for example, 2005-2006 PEG Catalog from Nektar Therapeutics (available at the Nektar website) U.S. Pat. Nos. 4,640,835; 4,496,689; 4,301,144; 4,670,417; 4,791,192 or 4,179,337, all entirely incorporated by reference for all purposes and in particular for all teachings related to linking antibodies to polymers. In addition, as is known in the art, amino acid substitutions may be made in various positions within the antibody to facilitate the addition of polymers such as PEG. See for example, U.S. Publication No. 2005/0114037A1, entirely incorporated by reference.

[0162] The present invention further includes the nucleic acids encoding the Toso antibodies of the invention. In the case where both a heavy and light chain constant domains are included in the antibody, generally these are made using nucleic acids encoding each, that are combined into standard host cells (e.g. CHO cells, etc.) to produce the tetrameric structure of the antibody. If only one constant domain is being made, only a single nucleic acid will be used.

[0163] Formulations of the antibodies used in accordance with the present invention can be prepared for storage by mixing an antibody having the desired degree of purity with optional pharmaceutically acceptable carriers, excipients or stabilizers (Remington's Pharmaceutical Sciences 16th edition, Osol, A. Ed. [1980]), in the form of lyophilized formulations or aqueous solutions. Acceptable carriers, excipients, or stabilizers are nontoxic to recipients at the dosages and concentrations employed, and include buffers such as phosphate, citrate, and other organic

acids; antioxidants including ascorbic acid and methionine; preservatives (such as octadecyldimethylbenzyl ammonium chloride; hexamethonium chloride; benzalkonium chloride, benzethonium chloride; phenol, butyl or benzyl alcohol; alkyl parabens such as methyl or propyl paraben; catechol; resorcinol; cyclohexanol; 3-pentanol; and m-cresol); low molecular weight (less than about 10 residues) polypeptides; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids such as glycine, glutamine, asparagine, histidine, arginine, or lysine; monosaccharides, disaccharides, and other carbohydrates including glucose, mannose, or dextrans; chelating agents such as EDTA; sugars such as sucrose, mannitol, trehalose or sorbitol; salt-forming counter-ions such as sodium; metal complexes (e.g. Zn-protein complexes); and/or non-ionic surfactants such as TWEEN™, PLURONICS™ or polyethylene glycol (PEG).

[0164] The formulations of the invention may also contain more than one active compound as necessary for the particular indication being treated, preferably those with complementary activities that do not adversely affect each other. For example, it may be desirable to provide antibodies with other specificities. Alternatively, or in addition, the composition may comprise a cytotoxic agent, cytokine, growth inhibitory agent and/or small molecule antagonist. Such molecules are suitably present in combination in amounts that are effective for the purpose intended.

[0165] The active ingredients may also be entrapped in microcapsules prepared, for example, by coacervation techniques or by interfacial polymerization, for example, hydroxymethylcellulose or gelatin-microcapsules and poly-(methylmethacrylate) microcapsules, respectively, in colloidal drug delivery systems (for example, liposomes, albumin microspheres, microemulsions, nano-particles and nanocapsules) or in macroemulsions. Such techniques are disclosed in Remington's Pharmaceutical Sciences 16th edition, Osol, A. Ed. (1980).

[0166] The formulations to be used for in vivo administration should be sterile, or nearly so. This is readily accomplished by filtration through sterile filtration membranes.

[0167] Sustained-release preparations may be prepared. Suitable examples of sustained-release preparations include semipermeable matrices of solid hydrophobic polymers containing the antibody, which matrices are in the form of shaped articles, e.g. films, or microcapsules. Examples of sustained-release matrices include polyesters, hydrogels (for example, poly(2-hydroxyethyl-methacrylate), or poly(vinylalcohol)), polylactides (U.S. Pat. No. 3,773,919), copolymers of L-glutamic acid and .gamma. ethyl-L-glutamate, non-degradable ethylene-vinyl acetate, degradable lactic acid-glycolic acid copolymers such as the LUPRON DEPOT® (injectable microspheres composed of lactic acid-glycolic acid copolymer and leuprolide acetate), and poly-D-(-)-3-hydroxybutyric acid. While polymers such as ethylene-vinyl acetate and lactic acid-glycolic acid

enable release of molecules for over 100 days, certain hydrogels release proteins for shorter time periods.

[0168] When encapsulated antibodies remain in the body for a long time, they may denature or aggregate as a result of exposure to moisture at 37°C, resulting in a loss of biological activity and possible changes in immunogenicity. Rational strategies can be devised for stabilization depending on the mechanism involved. For example, if the aggregation mechanism has been shown to be intermolecular S–S bond formation through thio-disulfide interchange, stabilization may be achieved by modifying sulfhydryl residues, lyophilizing from acidic solutions, controlling moisture content, using appropriate additives, and developing specific polymer matrix compositions.

IIC. Soluble Toso proteins

[0169] A soluble Toso protein of the invention (also referred to interchangeably herein as the “soluble Toso receptor,” “Toso-Fc”, and “soluble Toso polypeptide”) includes all or part of an extracellular domain of a Toso receptor. The soluble Toso proteins of the invention in further embodiments include a signal domain and/or an Fc domain. As will be discussed in further detail herein, these components of the soluble Toso protein may be combined in any way with or without additional components and/or modifications to provide a soluble Toso protein of the invention.

[0170] In one aspect, the soluble Toso protein of the invention comprises an extracellular domain of Toso. In a still further embodiments, the soluble Toso protein comprises the extracellular domain of human Toso isoform a. The extracellular domain of human Toso is predicted to span amino acids P21 to G251 of NP_005440.1, human Toso isoform a (see Shima et al., *Int. Immunol.*, 2010, which is hereby incorporated in its entirety for all purposes and in particular for all teachings related to the extracellular domain of Toso). For the sake of clarity, the majority of the discussion herein is directed to soluble Toso proteins comprising all or part of an extracellular domain of a human Toso protein. However, it will be appreciated that the extracellular domain of a Toso protein from any species can be used to produce soluble Toso proteins in accordance with the description herein, and it would be well within the ability of one of skill in the art to identify the regions of the Toso protein from another species that correspond to the regions of the human Toso protein discussed herein.

[0171] In one embodiment, the soluble Toso protein comprises an extracellular domain sequence according to SEQ ID NO: 1, which is shown in FIG. 1. In a further embodiment, the soluble Toso protein has a sequence identity of about 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% and 100% identity to SEQ ID NO: 1. In a still further embodiment, the soluble Toso protein comprises a polypeptide with 1-75, 2-70, 3-65, 4-60, 5-55, 6-50, 7-45, 8-40, 9-35, 10-30, 11-25, 12-20, 13-15, 5-20, 6-18, 8-16, 10-14 amino acid substitutions in SEQ ID NO: 1. In a yet further embodiments, the soluble Toso protein comprises a polypeptide with 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 amino acid substitutions in SEQ ID NO: 1.

[0172] In one embodiment, the soluble Toso protein comprises an extracellular domain sequence according to SEQ ID NO: 8, which is shown in FIG. 1. In a further embodiment, the soluble Toso protein has a sequence identity of about 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% and 100% identity to SEQ ID NO: 8. In a still further embodiment, the soluble Toso protein comprises a polypeptide with 1-75, 2-70, 3-65, 4-60, 5-55, 6-50, 7-45, 8-40, 9-35, 10-30, 11-25, 12-20, 13-15, 5-20, 6-18, 8-16, 10-14 amino acid substitutions in SEQ ID NO: 8. In a yet further embodiment, the soluble Toso protein comprises a polypeptide with 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 amino acid substitutions in SEQ ID NO: 8.

[0173] In a further embodiment and in accordance with any of the above, the soluble Toso protein of the invention includes amino acids 18 to 253 of SEQ ID NO: 7. In a still further embodiment, the soluble Toso protein of the invention includes amino acids 21 to 253 of SEQ ID NO: 7. In a still further embodiment, the soluble Toso protein includes amino acids 21 to 251 of SEQ ID NO: 7. In a yet further embodiment, the soluble Toso protein includes any of the following ranges of amino acids from SEQ ID NO: 7: 1-255, 5-245, 10-235, 15-225, 20-215, 25-205, 30-195, 35-185, 40-175, 45-165, 50-155, 45-145, 40-135, 35-125, 30-115, 35-105, 40-95, 45-85, 50-75, 55-65. In a still further embodiment, the soluble Toso protein includes a sequence with a sequence identity of about 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% and 100% identity to amino acids 18 to 253 or 21 to 253 of SEQ ID NO: 7.

[0174] In a still further embodiment and in accordance with any of the above, the soluble Toso protein of the invention includes all or a portion of SEQ ID NO: 8, pictured in FIG. 1. In still further embodiments, the soluble Toso protein includes amino acids 1-231, 6-221, 11-211, 16-201, 21-191, 26-181, 31-171, 36-161, 41-151, 46-141, 51-131, 56-121, 61-111, 66-101, 71-91, 76-81 of SEQ ID NO: 8. In yet further embodiments, the soluble Toso protein includes a polypeptide with at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% and 100% sequence identity to the amino acid regions 1-231, 6-221, 11-211, 16-201, 21-191, 26-181, 31-171, 36-161, 41-151, 46-141, 51-131, 56-121, 61-111, 66-101, 71-91, 76-81 of SEQ ID NO: 8.

[0175] In a yet further embodiment, the soluble Toso protein of the invention comprises any one of SEQ ID NOs: 8, 9, 11, 13, 15, 17, 19, 21, and 23. In a still further embodiment, the soluble Toso protein includes a sequence with a sequence identity of about 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% and 100% identity to SEQ ID NOs: 8, 9, 11, 13, 15, 17, 19, 21, and 23. These sequences include deletion variants of the extracellular domain of the Toso receptor.

[0176] In still further embodiments and in accordance with any of the above, the soluble Toso protein of the invention comprises a deletion variant of the full extracellular domain of the Toso protein. In exemplary embodiments, the deletion variants that are a component of a soluble Toso protein of the invention include a polypeptide in which one or more of the following amino acids have been deleted from SEQ ID NO:8: 1-21, 1-35, 1-87, both regions 1-21 and 211-231, 211-231,154-231, 105-231, and 93-231. In further exemplary embodiments, the deletion variants that are a component of a soluble Toso protein of the invention include a polypeptide with a sequence identity of about 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% to a polypeptide according to SEQ ID NO:8 with one or more of the following amino acid regions deleted: 1-21, 1-35, 1-87, both regions 1-21 and 211-231, 211-231,154-231, 105-231, and 93-231.

[0177] In further embodiments and in accordance with any of the above, the soluble Toso protein of the invention includes an extracellular domain component that comprises regions that bind to a ligand of the Toso receptor. In an exemplary embodiment, the soluble Toso protein of the invention includes an extracellular domain component that binds to IgM. In a still further embodiment, the soluble Toso protein of the invention includes amino acids 35 to 87 of SEQ ID NO: 8. In further exemplary embodiments, the soluble Toso protein of the invention includes amino acids 25-100, 29-95, 33-90, 37-85, 41-80, 45-75, 49-70, 53-65, or 57-60 of SEQ ID NO: 8.

[0178] In a further aspect and in accordance with any of the above, the soluble Toso protein of the invention includes an extracellular domain component as is discussed above and further includes a signal sequence. In an exemplary embodiment, the signal sequence enhances secretion from host cells. In a yet further embodiment, the signal sequence includes without limitation a member selected from an IL-2 signal sequence, α -mating factor pre-sequence from *Saccharomyces cerevisiae*, α -amylase signal sequence from *Aspergillus niger*, Glucoamylase signal sequence from *Aspergillus awamori*, Serum albumin signal sequence from *Homo sapiens*, Inulinase signal sequence from *Kluyveromyces maxianus*, Invertase signal sequence from *Saccharomyces cerevisiae*, Killer protein signal sequence from *Saccharomyces cerevisiae*, Lysozyme signal sequence from *Gallus gallus*. In a still further embodiment, the signal sequence comprises a sequence according to SEQ ID NO: 2, which is shown in FIG. 1. In a still further embodiment, the signal sequence comprises a sequence with a sequence identity of about 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% and 100% identity to SEQ ID NO: 2. In specific embodiments, the soluble Toso protein of the invention comprises any one of SEQ ID NOs: 1, 8, 9, 11, 13, 15, 17, 21 and 23 or variants of those sequences as a fusion protein with SEQ ID NO: 2 or with a sequence with an identity of about 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% and 100% identity to SEQ ID NO: 2.

[0179] In a further aspect and in accordance with any of the above, a soluble Toso protein of the invention comprises an extracellular domain of a Toso receptor and an Fc domain. In an exemplary embodiment, the soluble Toso protein is a fusion protein comprising the extracellular domain of isoform A of the human Toso protein and an Fc domain. In a further embodiment, the Fc domain includes any domain that enhances the half-life of the protein as compared to the protein without the Fc domain. In a still further embodiment, the Fc domain includes any domain that improves the pharmacokinetic profile of the protein as compared to the protein without the Fc domain. In a still further embodiment, the Fc domain is derived from human IgG1 at the C-terminus, which in a yet further embodiment includes mutations that diminish or ablate antibody-dependent and complement dependent cytotoxicity. In a still further embodiment, such mutations include one or more of the following mutations singly or in any combination: E233P; L234V; L235A; ΔG236; A327G; A330S; P331S. In a yet further embodiment, the Fc domain comprises a sequence according to SEQ ID NO: 3, which is shown in FIG. 1. In a still further embodiment, the Fc domain comprises a sequence with a sequence identity of about 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% and 100% identity to SEQ ID NO: 3.

[0180] In further exemplary embodiments, the soluble Toso protein of the invention comprises a fusion protein comprising both an extracellular domain component and an Fc domain component. In still further exemplary embodiments, the soluble Toso protein of the invention comprises SEQ ID NOs: 1, 8, 9, 11, 13, 15, 17, 21 and 23 or variants of those sequences as a fusion protein with SEQ ID NO: 3 or with a sequence with an identity of about 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% and 100% identity to SEQ ID NO: 3.

[0181] In one aspect and in accordance with any of the above, the soluble Toso protein of the invention comprises an extracellular Toso domain, a signal sequence and an Fc domain – each of those components may comprise any of the above described versions of these components in any combination. In a still further embodiment, the soluble Toso protein of the invention comprises a sequence according to SEQ ID NO: 5, which is shown in FIG. 1. In a still further embodiment, the soluble Toso protein of the invention comprises a sequence with a sequence identity of about 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% and 100% identity to SEQ ID NO: 5.

[0182] In further embodiments and in accordance with any of the above, the soluble Toso protein of the invention comprises a sequence according to any one of SEQ ID NOs. 10, 12, 14, 16, 18, 20, 22 and 24, which comprise an extracellular domain component, a signal sequence, and an Fc domain. In further embodiments, the soluble Toso protein of the invention comprises a sequence with a sequence identity of about 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% and 100% to any one of SEQ ID NOs. 10, 12, 14, 16, 18, 20, 22 and 24.

[0183] In further aspects and in accordance with any of the above, a soluble Toso protein of the invention may further include a linker between the Toso extracellular domain component and the Fc domain, between the Toso extracellular domain component and the signal sequence, or between both the Toso extracellular domain component and the Fc domain and the Toso extracellular domain component and the signal sequence. In exemplary embodiments, such a linker may be an amino acid linker, a polymeric linker, or any other linker known in the art to be effective for joining two amino acid sequences together. In further exemplary embodiments, the linker is an amino acid linker. In still further embodiments, the linker is the amino acid sequence: ISAMVRS (SEQ ID NO: 25). In yet further embodiments, the linker is a variant of SEQ ID NO: 25 containing 1, 2, 3, 4, 5, or 6 amino acid substitutions.

[0184] In further embodiments and in accordance with any of the above, variants of the soluble Toso proteins of the invention can be made through modification of the amino acid sequences of any of the soluble Toso proteins discussed herein, including SEQ ID NOs. 5, 6, or 8-24. Such modifications can be achieved using any known technique in the art e.g., site-directed mutagenesis or PCR based mutagenesis. Such techniques are described for example in Sambrook et al., *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Press, Plainview, N.Y., 1989 and Ausubel et al., *Current Protocols in Molecular Biology*, John Wiley & Sons, New York, N.Y., 1989, each of which is incorporated by reference in its entirety for all purposes and in particular for all teachings related to forming protein variants.

[0185] In still further embodiments and in accordance with any of the above, soluble Toso proteins of the invention may be in monomeric or multimeric forms, wherein each monomer of the multimer comprises a single extracellular domain sequence. In further embodiments, the soluble Toso proteins of the invention are in multimers of 2, 3, 4, 5, 6, 7, 8, 9, 10 or more monomers. In a specific embodiment, soluble Toso proteins are multimers of 6 monomers. In a further embodiment, a hexameric Toso multimer is formed of monomers comprising a hexamerization tag. In a further embodiment, the hexamerization tag comprises a 21 amino acid tail piece from human IgA alpha heavy chain constant region as described in Hirano et al., *Blood* (2006), which is hereby incorporated by reference for all purposes and in particular for all teachings related to hexamerization or other multimerization tags. In yet a further embodiment, the hexamerization tag of the invention includes a sequence according to SEQ ID NO: 4, shown in FIG. 1.

[0186] In further embodiments and in accordance with any of the above, the soluble Toso protein of the invention is modified to alter one or more functional properties of the protein. In exemplary embodiments, the soluble Toso protein is chemically modified. For example, the soluble Toso protein may be modified with one or more polymers to improve its stability in vivo and/or alter its pharmacokinetic profile. Such polymers include without limitation one or more of a variety of

nonproteinaceous polymers, e.g., polyethylene glycol, polypropylene glycol, or polyoxyalkylenes, in the manner set forth in U.S. Pat. Nos. 4,640,835; 4,496,689; 4,301,144; 4,670,417; 4,791,192; or 4,179,337, which are all hereby incorporated by reference in their entirety for all purposes and in particular for all teachings related to linking proteins to polymers.

[0187] Modifications of the Toso soluble protein included within the scope of this invention include reacting targeted amino acid residues of a Toso polypeptide in accordance with any of the sequences and soluble Toso proteins discussed above with an organic derivatizing agent that is capable of reacting with selected side chains or the N-or C-terminal residues of a Toso polypeptide. Commonly used crosslinking agents include, e.g., 1,1-bis(diazoacetyl)-2-phenylethane, glutaraldehyde, N-hydroxysuccinimide esters, for example, esters with 4-azidosalicylic acid, homobifunctional imidoesters, including disuccinimidyl esters such as 3,3'-dithiobis(succinimidylpropionate), bifunctional maleimides such as bis-N-maleimido-1,8-octane and agents such as methyl-3-[(p-azidophenyl)dithio]propioimidate.

[0188] Other modifications include deamidation of glutamyl and asparaginyl residues to the corresponding glutarnyl and aspartyl residues, respectively, hydroxylation of proline and lysine, phosphorylation of hydroxyl groups of seryl or threonyl residues, methylation of the "-amino groups of lysine, arginine, and histidine side chains [T. E. Creighton, *Proteins; Structure and Molecular Properties*, W. H. Freeman & Co., San Francisco, pp. 79-86 (1983)], acetylation of the N-terminal amine, and amidation of any C-terminal carboxyl group.

[0189] Another type of modification of the soluble Toso protein included within the scope of this invention comprises altering the native glycosylation pattern of the polypeptide. "Altering the native glycosylation pattern" is intended for purposes herein to mean deleting one or more carbohydrate moieties found in native sequence Toso polypeptide, and/or adding one or more glycosylation sites that are not present in the native sequence Toso polypeptide.

[0190] Addition of glycosylation sites to Toso soluble protein may be accomplished by altering the amino acid sequence thereof. The alteration may be made, for example, by the addition of, or substitution by, one or more serine or threonine residues to the native sequence the soluble Toso protein (for O-linked glycosylation sites). The soluble Toso protein amino acid sequence may optionally be altered through changes at the DNA level, particularly by mutating the DNA encoding the Toso soluble protein at preselected bases such that codons are generated that will translate into the desired amino acids.

[0191] Another means of increasing the number of carbohydrate moieties on the Toso soluble protein is by chemical or enzymatic coupling of glycosides to the polypeptide. Such methods are described in the art, e.g., in WO 87/05330 published Sep. 11, 1987, and in Aplin and Wriston, *CRC Crit. Rev. Biochem.*, pp. 259-306 (1981), which are hereby incorporated by reference in their

entirety for all purposes and in particular for all teachings related to altering carbohydrate moieties on a protein.

[0192] Removal of carbohydrate moieties present on the soluble Toso protein may be accomplished chemically or enzymatically or by mutational substitution of codons encoding for amino acid residues that serve as targets for glycosylation. Chemical deglycosylation techniques are known in the art and described, for instance, by Hakimuddin, et al., *Arch. Biochem. Biophys.*, 259:52 (1987) and by Edge, et al., *Anal. Biochem.*, 118:131 (1981). Enzymatic cleavage of carbohydrate moieties on polypeptides can be achieved by the use of a variety of endo-and exo-glycosidases as described by Thotakura, et al., *Meth. Enzymol.*, 138:350 (1987), which are hereby incorporated by reference in their entirety for all purposes and in particular for all teachings related to altering carbohydrate moieties on a protein.

[0193] The soluble Toso protein of the present invention may also be modified in a way to form chimeric molecules comprising the protein fused to another, heterologous polypeptide or amino acid sequence. In one embodiment, such a chimeric molecule comprises a fusion of a soluble Toso polypeptide with a tag polypeptide which provides an epitope to which an anti-tag antibody can selectively bind. The epitope tag is generally placed at the amino- or carboxyl-terminus of the Toso polypeptide. The presence of such epitope-tagged forms of a Toso polypeptide can be detected using an antibody against the tag polypeptide. Also, provision of the epitope tag enables the Toso polypeptide to be readily purified by affinity purification using an anti-tag antibody or another type of affinity matrix that binds to the epitope tag. In an alternative embodiment, the chimeric molecule may comprise a fusion of a Toso polypeptide with an immunoglobulin or a particular region of an immunoglobulin. For a bivalent form of the chimeric molecule, such a fusion could be to the Fc region of an IgG molecule or GST fusions.

[0194] Various tag polypeptides and their respective antibodies are well known in the art. Examples include poly-histidine (poly-his) or poly-histidine-glycine (poly-his-gly) tags; the flu HA tag polypeptide and its antibody 12CA5 [Field, et al., *Mol. Cell Biol.*, 8:2159-2165 (1988)]; the c-myc tag and the 8F9, 3C7, 6E10, G4, B7, and 9E10 antibodies thereto [Evan, et al., *Molecular and Cellular Biology*, 5:3610-3616 (1985)]; and the Herpes Simplex virus glycoprotein D (gD) tag and its antibody [Paborsky, et al., *Protein Engineering*, 3(6):547-553 (1990)]. Other tag polypeptides include the Flag-peptide [Hopp, et al., *BioTechnology*, 6:1204-1210 (1988)]; the KT3 epitope peptide [Martin, et al., *Science*, 255:192-194 (1992)]; tubulin epitope peptide [Skinner, et al., *J. Biol. Chem.*, 266:15163-15166 (1991)]; and the T7 gene 10 protein peptide tag [Lutz-Freyermuth, et al., *Proc. Natl. Acad. Sci. USA*, 87:6393-6397 (1990)].

[0195] In further embodiments and in accordance with any of the above, a soluble Toso protein of the invention is fused with a cell penetrating peptide.

[0196] In further aspects, the present invention encompasses nucleic acids encoding one or more soluble Toso proteins as well as host cells comprising such nucleic acids. In certain embodiments, host cells used in accordance with the present invention include without limitation HEK293F, HEK298T, Cos7, HeLa, and CHO-DHFR deficient cells. In still further embodiments, soluble Toso proteins of the invention are stably expressed in a cell line that has been modified to grow in a serum-free suspension.

[0197] In further aspects, compositions of the invention may include any of the soluble Toso proteins discussed herein along with additives and pharmaceutically acceptable carriers. As used herein, "pharmaceutically acceptable carrier" includes any material, which when combined with the conjugate retains the conjugates' activity and is non-reactive with the subject's immune systems. Examples include, but are not limited to, any of the standard pharmaceutical carriers such as a phosphate buffered saline solution, water, emulsions such as oil/water emulsion, and various types of wetting agents. Other carriers may also include sterile solutions, tablets including coated tablets and capsules. Typically such carriers contain excipients such as starch, milk, sugar, certain types of clay, gelatin, stearic acid or salts thereof, magnesium or calcium stearate, talc, vegetable fats or oils, gums, glycols, or other known excipients. Such carriers may also include flavor and color additives or other ingredients. Compositions comprising such carriers are formulated by well known conventional methods.

[0198] In one aspect, the present invention is directed to methods of modulating Toso activity. In one embodiment, methods of modulating Toso activity comprise inhibiting Toso activity. In other embodiments, methods of modulating Toso activity comprise increasing Toso activity.

[0199] In some embodiments, methods of the present invention involve directly modulating Toso activity. In an exemplary embodiment, such methods include applying an agent that binds to Toso, such as an antibody.

[0200] In other embodiments, Toso activity is modulated indirectly, for example by binding cognate ligands of Toso. In an exemplary embodiment, Toso activity is modulated by administering a soluble Toso protein.

[0201] In further embodiments, Toso activity is modulated by a combination of mechanisms, for example by administering a composition comprising an agent that binds to Toso in combination with a composition comprising an agent that binds to cognate ligands of Toso. In an exemplary embodiment, such a combination may include without limitation a Toso antibody and a soluble Toso protein.

[0202] As will be appreciated, methods of modulating Toso activity can include the use of any of the compositions described herein in any combination, including any one or more of SEQ ID NOs. 1-25 as well as any variants or modifications thereof as described herein.

IID. Combination therapies

[0203] In accordance with any of the above, methods of the present invention includes methods of treating cancer or autoimmune diseases by treating a subject in need thereof with a combination of molecules that modulate components of the immune system pathway. In general, these combination therapies include administering soluble Toso protein with one or more other modulators of the immune system pathway. The soluble Toso protein may be administered prior to, simultaneously with, or subsequent to administering of the one or more other modulators of the immune system pathway. In certain aspects, the one or more other modulators of the immune system pathway include without limitation IgM, B7H3, PAMPs, DAMPs, scramblase, and immune checkpoint inhibitors.

[0204] In further aspects, the combination therapies comprise administering soluble Toso protein with one or more immune checkpoint inhibitors. In certain embodiments, the combination therapies comprise administering Toso protein with inhibitors of PD-1, inhibitors of CTLA-4, or inhibitors of both PD-1 and CTLA-4. In further embodiments, the combination therapies comprise administering Toso protein with anti-PD-1 antibodies or anti-CTLA-4 antibodies. In yet further embodiments, the combination therapies comprise administering Toso protein with both anti-PD-1 and anti-CTLA-4 antibodies. In still further embodiments, the combination therapies comprise administering Toso protein comprising any one of SEQ ID Nos: 1-25 with one or more immune checkpoint inhibitors.

[0205] While preferred embodiments of the present invention have been shown and described herein, it will be obvious to those skilled in the art that such embodiments are provided by way of example only. Numerous variations, changes, and substitutions will now occur to those skilled in the art without departing from the invention. It should be understood that various alternatives to the embodiments of the invention described herein may be employed in practicing the invention. It is intended that the following claims define the scope of the invention and that methods and structures within the scope of these claims and their equivalents be covered thereby.

Example 1: Inhibition of B Cell Proliferation by Soluble Toso Proteins

[0206] FIG. 3 shows the results of an assay of B cell proliferation. The target cell was purified wildtype B cells. The stimulus was BCR crosslinking with anti-mouse IgM or TLR stimulation with LPS. The assay was to determine whether proliferation was modified by human or mouse soluble Toso proteins. As shown in FIG. 3, both hToso-Fc and mToso-Fc inhibit the proliferation of mouse B cells in culture. Fc proteins alone (control) do not influence proliferation.

[0207] FIG. 4 shows that the inhibitory activity of full length hToso is equivalent in WT and Toso KO B cells, therefore an interaction of sToso-Fc with endogenous mToso is not required for the anti-proliferative effect. Removing the putative Ig domain from full length Toso abrogates inhibitory

activity in this model, suggesting the Ig domain is important for this specific activity of Toso in this model.

[0208] FIG. 5 shows that truncated forms of soluble hToso containing the Ig domain inhibit mouse B cell proliferation to varying degrees. In this assay, full length hToso showed the greatest effect.

Example 2: IgM binding assays

[0209] FIG. 6 shows the results of an IgM binding assay. For the assay, soluble TOSO proteins were immobilized on 96 well plates (Nunc Maxisorb). After washing and blocking, mouse IgM was incubated on plate. After washing, bound IgM was detected by incubation with anti-mouse IgM-HRP, followed by washing and addition of HRP substrate. As shown in FIG. 6, immobilized full length hToso binds mouse IgM in a dose dependent fashion.

[0210] FIG. 7 shows mutant hTOSO binding to IgM: truncated/deletion mutants of hTOSO were immobilized as described above and IgM binding assessed. Mutant forms M1, M3, M4 and M7 (M7 is minus Ig domain) fail to bind IgM, while mutant M2 (1-104) appears to bind IgM more strongly.

Example 3: Toso inhibition of CLL cell proliferation

[0211] FIG. 8 shows the results of a study of CLL activation in-vitro from four independent patient samples. This assay provides an in-vitro model of proliferation centers found in CLL lymphoid organs. Peripheral blood CD19+ cells were purified from CLL patients, and activated in-vitro with Resiquimod (R-848, a TLR7/8 agonist) + IL-2 in a serum free system. These cells are termed "2S". The effect of soluble hTOSO and exogenous human IgM on proliferation was assessed. hTOSO, and hIgM to a lesser extent, appear to have an inhibitory effect on CLL growth as estimated by 3H Thy incorporation.

Example 4: Treatment with soluble Toso protein attenuates tumorigenesis and enhances survival

[0212] C57Bl/6 mice were obtained from The Jackson Laboratory. B16F10 murine melanoma cell line was grown in Iscove's modified Dulbecco's medium supplemented with 10% FBS, 2mM L-glutamine, and antibiotics (50 U/ml penicillin and 50µg/ml streptomycin).

[0213] Mice of 8 - 14 weeks of age (n = 5 - 10) were subcutaneously inoculated with 2×10^5 B16F10 melanoma cells. Mice which did not receive the full injection, or whose injection did not result in a visible subcutaneous bleb without leakage, were not used in the experiment and sacrificed immediately. Tumor measurements were taken every other day using digital calipers.

[0214] C57Bl/6 mice were treated with Toso-Fc alone. For the treatment of B16F10 melanoma, mice received intraperitoneal injections (i.p.) of 50µg of Toso-Fc, or control Fc protein, or PBS (vehicle control) every other day starting day 10 after B16F10 inoculation.

[0215] FIG. 9 shows that treatment of the mice with the soluble Toso protein resulted in an attenuation of tumorigenesis with a significant decrease in tumor area. n = 10 Fc, 10 PBS, and 15 Toso-Fc.

[0216] FIG. 10 shows that Toso-Fc treated mice also showed prolonged survival.

Example 5: Treatment with soluble Toso protein increases tumor infiltrating lymphocytes

[0217] FIG. 11 shows that Toso-Fc treatment increases the percent of tumors that contain tumor infiltrating lymphocytes. Mice were inoculated with B16F10 as described above. Fifteen days after B16F10 melanoma inoculation mice were sacrificed, tumor infiltrating lymphocyte (TIL) analysis was performed. Subcutaneous melanoma tumors were surgically removed and weighed. Tumors were then cut into 1mm³ pieces with a scalpel and placed in digestion media (RPMI supplemented with 10% FBS, 2mM L-glutamine, antibiotics [50 U/ml penicillin and 50µg/ml streptomycin], 1mg/ml collagenase IV, and 20µg/ml DNase I). Tumor samples in digestion media were subsequently vortexed every 10 minutes during a 30-minute incubation at 37°C. To obtain a single cell suspension, tumors were then passed through a 70µm nylon filter and washed three times with RPMI supplemented with 1% FBS. Whole tumor single cell suspensions were counted, and 6x10⁶ cells were stained with MAbs for T cell markers (CD3, CD8, CD4) and analyzed by flow cytometry.

Example 6: Combination therapies with soluble Toso protein and immune checkpoint inhibitors show increased effects on tumorigenesis attenuation and survival

[0218] C57Bl/6 mice were obtained from The Jackson Laboratory. B16F10 murine melanoma cell line was grown in Iscove's modified Dulbecco's medium supplemented with 10% FBS, 2mM L-glutamine, and antibiotics (50 U/ml penicillin and 50µg/ml streptomycin).

[0219] Mice of 8 - 14 weeks of age (n = 5 - 10) were subcutaneously inoculated with 2 x 10⁵ B16F10 melanoma cells. Mice which did not receive the full injection, or whose injection did not result in a visible subcutaneous bleb without leakage, were not used in the experiment and sacrificed immediately. Tumor measurements were taken every other day using digital calipers.

[0220] C57Bl/6 mice were treated with Toso-Fc alone, or in combination with immune checkpoint blockade antibodies. For the treatment of B16F10 melanoma, mice received intraperitoneal injections (i.p.) of 50µg of Toso-Fc, or control Fc protein, or PBS (vehicle control) every other day starting day 10 after B16F10 inoculation. Immune checkpoint blockade antibodies αCTLA-4 (9D9) and αPD-1 (RPM1-14) were used in combination with Toso-Fc treatments. αCTLA-4 (100µg) and αPD-1 (250µg) antibodies were also administered i.p. starting on day 10. Three treatments of checkpoint blockade antibody were given, and treatments were administered every three days (days 10, 13, and 16).

[0221] FIG. 12 shows that a combined treatment with Toso-Fc and anti-PD-1 antibody shows attenuates tumorigenesis to a greater degree than PD-1 antibody or Toso-Fc alone. FIG. 13 shows that this combination therapy also enhances survival over the application of the individual components alone.

[0222] While a combination of Toso-Fc and anti-CTLA-4 antibody did not seem to increase survival (FIG. 14), the triple therapy of Toso-Fc with anti-PD-1 and anti-CTLA-4 attenuated tumorigenesis (FIG. 15) and enhanced survival (FIG. 16) over treatments with Toso alone or with a double therapy involving only the immune checkpoint inhibitors (the anti-PD-1 and anti-CTLA-4 antibodies).

[0223] The present specification provides a complete description of the methodologies, systems and/or structures and uses thereof in example aspects of the presently-described technology. Although various aspects of this technology have been described above with a certain degree of particularity, or with reference to one or more individual aspects, those skilled in the art could make numerous alterations to the disclosed aspects without departing from the spirit or scope of the technology hereof. Since many aspects can be made without departing from the spirit and scope of the presently described technology, the appropriate scope resides in the claims hereinafter appended. Other aspects are therefore contemplated. Furthermore, it should be understood that any operations may be performed in any order, unless explicitly claimed otherwise or a specific order is inherently necessitated by the claim language. It is intended that all matter contained in the above description and shown in the accompanying drawings shall be interpreted as illustrative only of particular aspects and are not limiting to the embodiments shown. Unless otherwise clear from the context or expressly stated, any concentration values provided herein are generally given in terms of admixture values or percentages without regard to any conversion that occurs upon or following addition of the particular component of the mixture. To the extent not already expressly incorporated herein, all published references and patent documents referred to in this disclosure are incorporated herein by reference in their entirety for all purposes. Changes in detail or structure may be made without departing from the basic elements of the present technology as defined in the following claims.

THE CLAIMS DEFINING THE INVENTION ARE AS FOLLOWS:

1. A method for the treatment of cancer, the method comprising administering to a subject in need thereof a combination of a pharmaceutically effective amount of (i) at least one immune checkpoint inhibitor and (ii) an inhibitor of Toso activity.
2. The method of claim 1, wherein the at least one immune checkpoint inhibitor is a member selected from the group consisting of: a PD-1 inhibitor, a CTLA-4 inhibitor, a LAG3 inhibitor, and a TIM3 inhibitor.
3. The method of claim 1 or claim 2, wherein the inhibitor of Toso activity is a soluble Toso protein.
4. The method of claim 1 or claim 2, wherein the inhibitor of Toso activity is an antibody to Toso.
5. The method of claim 1 or claim 2, wherein the combination comprises a PD-1 inhibitor, a CTLA-4 inhibitor, and a soluble Toso protein.
6. The method of claim 5, wherein the PD-1 inhibitor is an anti-PD-1 antibody and the CTLA-4 inhibitor is an anti-CTLA-4 antibody.
7. A method for increasing a level of infiltrating lymphocytes in a tumor, the method comprising treating the tumor with a soluble Toso protein and one or more of a member selected from a PD-1 inhibitor, a CTLA-4 inhibitor, and a PDL-1 inhibitor, wherein the level of infiltrating lymphocytes that results from the treating is higher than the level without the treating.
8. The method of claim 7, wherein the infiltrating lymphocytes comprise CD3+ cells, CD8+ cells, or a combination of CD3+ cells and CD8+ cells.

FIGURE 1

SEQ ID NO: 1

Ile Ser Ala Met Val Arg Ser Arg Ile Leu Pro Glu Val Lys Val Glu
Gly Glu Leu Gly Gly Ser Val Thr Ile Lys Cys Pro Leu Pro Glu Met
His Val Arg Ile Tyr Leu Cys Arg Glu Met Ala Gly Ser Gly Thr Cys
Gly Thr Val Val Ser Thr Thr Asn Phe Ile Lys Ala Glu Tyr Lys Gly
Arg Val Thr Leu Lys Gln Tyr Pro Arg Lys Asn Leu Phe Leu Val Glu
Val Thr Gln Leu Thr Glu Ser Asp Ser Gly Val Tyr Ala Cys Gly Ala
Gly Met Asn Thr Asp Arg Gly Lys Thr Gln Lys Val Thr Leu Asn Val
His Ser Glu Tyr Glu Pro Ser Trp Glu Glu Gln Pro Met Pro Glu Thr
Pro Lys Trp Phe His Leu Pro Tyr Leu Phe Gln Met Pro Ala Tyr Ala
Ser Ser Ser Lys Phe Val Thr Arg Val Thr Thr Pro Ala Gln Arg Gly
Lys Val Pro Pro Val His His Ser Ser Pro Thr Thr Gln Ile Thr His
Arg Pro Arg Val Ser Arg Ala Ser Ser Val Ala Gly Asp Lys Pro Arg
Thr Phe Leu Pro Ser Thr Thr Ala Ser Lys Ile Ser Ala Leu Glu Gly
Leu Leu Lys Pro Gln Thr Pro Ser Tyr Asn His His Thr Arg Leu His
Arg Gln Arg Ala Leu Asp Tyr Gly Ser Gln Ser Gly Arg Glu Gly Gln
Gly Phe His

SEQ ID NO: 2

Met Tyr Arg Met Gln Leu Leu Ser Cys Ile Ala Leu Ser Leu Ala Leu
Val Thr Asn Ser

SEQ ID NO: 3

Arg Ser Val Glu Cys Pro Pro Cys Pro Ala Pro Pro Val Ala Gly Pro

Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser
Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp
Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn
Ala Lys Thr Lys Pro Arg Glu Tyr Asn Ser Thr Tyr Arg Val Val
Gln Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu
Tyr Lys Cys Lys Val Ser Asn Lys Gly Leu Pro Ser Ser Ile Glu Lys
Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr
Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr
Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu
Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu
Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys
Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu
Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly

SEQ ID NO: 4

Ala Gly Lys Pro Thr His Val Asn Val Ser Val Val Met Ala Glu Val
Asp Gly Thr Cys Tyr

SEQ ID NO: 5

Met Tyr Arg Met Gln Leu Leu Ser Cys Ile Ala Leu Ser Leu Ala Leu
Val Thr Asn Ser Ile Ser Ala Met Val Arg Ser Arg Ile Leu Pro Glu
Val Lys Val Glu Gly Glu Leu Gly Gly Ser Val Thr Ile Lys Cys Pro
Leu Pro Glu Met His Val Arg Ile Tyr Leu Cys Arg Glu Met Ala Gly
Ser Gly Thr Cys Gly Thr Val Val Ser Thr Thr Asn Phe Ile Lys Ala
Glu Tyr Lys Gly Arg Val Thr Leu Lys Gln Tyr Pro Arg Lys Asn Leu

FIG. 1 (cont.)

Phe Leu Val Glu Val Thr Gln Leu Thr Glu Ser Asp Ser Gly Val Tyr
Ala Cys Gly Ala Gly Met Asn Thr Asp Arg Gly Lys Thr Gln Lys Val
Thr Leu Asn Val His Ser Glu Tyr Glu Pro Ser Trp Glu Glu Gln Pro
Met Pro Glu Thr Pro Lys Trp Phe His Leu Pro Tyr Leu Phe Gln Met
Pro Ala Tyr Ala Ser Ser Lys Phe Val Thr Arg Val Thr Thr Pro
Ala Gln Arg Gly Lys Val Pro Pro Val His His Ser Ser Pro Thr Thr
Gln Ile Thr His Arg Pro Arg Val Ser Arg Ala Ser Ser Val Ala Gly
Asp Lys Pro Arg Thr Phe Leu Pro Ser Thr Thr Ala Ser Lys Ile Ser
Ala Leu Glu Gly Leu Leu Lys Pro Gln Thr Pro Ser Tyr Asn His His
Thr Arg Leu His Arg Gln Arg Ala Leu Asp Tyr Gly Ser Gln Ser Gly
Arg Glu Gly Gln Gly Phe His Arg Ser Val Glu Cys Pro Pro Cys Pro
Ala Pro Pro Val Ala Gly Pro Ser Val Phe Pro Pro Lys Pro
Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val
Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val
Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln
Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln
Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Gly
Leu Pro Ser Ser Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro
Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met Thr
Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser
Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr
Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr
Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe
Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys
Ser Leu Ser Leu Ser Pro Gly Lys

SEQ ID NO: 6

Ser Tyr Arg Met Gln Leu Leu Ser Cys Ile Ala Leu Ser Leu Ala Leu
Val Thr Asn Ser Pro Glu Val Lys Val Glu Gly Glu Leu Gly Gly Ser
Val Thr Ile Lys Cys Pro Leu Pro Glu Met His Val Arg Ile Tyr Leu
Cys Arg Glu Met Ala Gly Ser Gly Thr Cys Gly Thr Val Val Ser Thr
Thr Asn Phe Ile Lys Ala Glu Tyr Lys Gly Arg Val Thr Leu Lys Gln
Tyr Pro Arg Lys Asn Leu Phe Leu Val Glu Val Thr Gln Leu Thr Glu
Ser Asp Ser Gly Val Tyr Ala Cys Gly Ala Gly Met Asn Thr Asp Arg
Gly Lys Thr Gln Lys Val Thr Leu Asn Val His Ser Glu Tyr Glu Pro
Ser Trp Glu Glu Gln Pro Met Pro Glu Thr Pro Lys Trp Phe His Leu
Pro Tyr Leu Phe Gln Met Pro Ala Tyr Ala Ser Ser Lys Phe Val
Thr Arg Val Thr Thr Pro Ala Gln Arg Gly Lys Val Pro Pro Val His
His Ser Ser Pro Thr Thr Gln Ile Thr His Arg Pro Arg Val Ser Arg
Ala Ser Ser Val Ala Gly Asp Lys Pro Arg Thr Phe Leu Pro Ser Thr
Thr Ala Ser Lys Ile Ser Ala Leu Glu Gly Leu Leu Lys Pro Gln Thr
Pro Ser Tyr Asn His His Thr Arg Leu His Arg Gln Arg Ala Leu Asp
Tyr Gly Ser Gln Ser Gly Arg Glu Gly Gln Gly Phe His Arg Ser Val
Glu Cys Pro Pro Cys Pro Ala Pro Pro Val Ala Gly Pro Ser Val Phe
Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro
Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu Val
Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr
Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val
Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys
Lys Val Ser Asn Lys Gly Leu Pro Ser Ser Ile Glu Lys Thr Ile Ser
Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro

FIG. 1 (cont.)

Ser Arg Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val
Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly
Gln Pro Glu Asn Asn Tyr Lys Thr Pro Pro Val Leu Asp Ser Asp
Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp
Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His
Asn His Tyr Thr Gln Lys Ser Leu Ser Pro Gly Lys Ala Gly
Lys Pro Thr His Val Asn Val Ser Val Val Met Ala Glu Val Asp Gly
Thr Cys Tyr

SEQ ID NO: 7

Met Asp Phe Trp Leu Trp Pro Leu Tyr Phe Leu Pro Val Ser Gly Ala
Leu Arg Ile Leu Pro Glu Val Lys Val Glu Gly Glu Leu Gly Gly Ser
Val Thr Ile Lys Cys Pro Leu Pro Glu Met His Val Arg Ile Tyr Leu
Cys Arg Glu Met Ala Gly Ser Gly Thr Cys Gly Thr Val Val Ser Thr
Thr Asn Phe Ile Lys Ala Glu Tyr Lys Gly Arg Val Thr Leu Lys Gln
Tyr Pro Arg Lys Asn Leu Phe Leu Val Glu Val Thr Gln Leu Thr Glu
Ser Asp Ser Gly Val Tyr Ala Cys Gly Ala Gly Met Asn Thr Asp Arg
Gly Lys Thr Gln Lys Val Thr Leu Asn Val His Ser Glu Tyr Glu Pro
Ser Trp Glu Glu Gln Pro Met Pro Glu Thr Pro Lys Trp Phe His Leu
Pro Tyr Leu Phe Gln Met Pro Ala Tyr Ala Ser Ser Ser Lys Phe Val
Thr Arg Val Thr Thr Pro Ala Gln Arg Gly Lys Val Pro Pro Val His
His Ser Ser Pro Thr Thr Gln Ile Thr His Arg Pro Arg Val Ser Arg
Ala Ser Ser Val Ala Gly Asp Lys Pro Arg Thr Phe Leu Pro Ser Thr
Thr Ala Ser Lys Ile Ser Ala Leu Glu Gly Leu Leu Lys Pro Gln Thr
Pro Ser Tyr Asn His His Thr Arg Leu His Arg Gln Arg Ala Leu Asp

FIG. 1 (cont.)

Tyr Gly Ser Gln Ser Gly Arg Glu Gly Gln Gly Phe His Ile Leu Ile
Pro Thr Ile Leu Gly Leu Phe Leu Leu Ala Leu Leu Gly Leu Val Val
Lys Arg Ala Val Glu Arg Arg Lys Ala Leu Ser Arg Arg Ala Arg Arg
Leu Ala Val Arg Met Arg Ala Leu Glu Ser Ser Gln Arg Pro Arg Gly
Ser Pro Arg Pro Arg Ser Gln Asn Asn Ile Tyr Ser Ala Cys Pro Arg
Arg Ala Arg Gly Ala Asp Ala Ala Gly Thr Gly Glu Ala Pro Val Pro
Gly Pro Gly Ala Pro Leu Pro Pro Ala Pro Leu Gln Val Ser Glu Ser
Pro Trp Leu His Ala Pro Ser Leu Lys Thr Ser Cys Glu Tyr Val Ser
Leu Tyr His Gln Pro Ala Ala Met Met Glu Asp Ser Asp Ser Asp Asp
Tyr Ile Asn Val Pro Ala

SEQ ID NO: 8

Pro Glu Val Lys Val Glu Gly Glu Leu Gly Ser Val Thr Ile Lys
Cys Pro Leu Pro Glu Met His Val Arg Ile Tyr Leu Cys Arg Glu Met
Ala Gly Ser Gly Thr Cys Gly Thr Val Val Ser Thr Thr Asn Phe Ile
Lys Ala Glu Tyr Lys Gly Arg Val Thr Leu Lys Gln Tyr Pro Arg Lys
Asn Leu Phe Leu Val Glu Val Thr Gln Leu Thr Glu Ser Asp Ser Gly
Val Tyr Ala Cys Gly Ala Gly Met Asn Thr Asp Arg Gly Lys Thr Gln
Lys Val Thr Leu Asn Val His Ser Glu Tyr Glu Pro Ser Trp Glu Glu
Gln Pro Met Pro Glu Thr Pro Lys Trp Phe His Leu Pro Tyr Leu Phe
Gln Met Pro Ala Tyr Ala Ser Ser Lys Phe Val Thr Arg Val Thr
Thr Pro Ala Gln Arg Gly Lys Val Pro Pro Val His His Ser Ser Pro
Thr Thr Gln Ile Thr His Arg Pro Arg Val Ser Arg Ala Ser Ser Val
Ala Gly Asp Lys Pro Arg Thr Phe Leu Pro Ser Thr Thr Ala Ser Lys
Ile Ser Ala Leu Glu Gly Leu Leu Lys Pro Gln Thr Pro Ser Tyr Asn

FIG. 1 (cont.)

His His Thr Arg Leu His Arg Gln Arg Ala Leu Asp Tyr Gly Ser Gln
Ser Gly Arg Glu Gly Gln Gly

SEQ ID NO: 9

Glu Met His Val Arg Ile Tyr Leu Cys Arg Glu Met Ala Gly Ser Gly
Thr Cys Gly Thr Val Val Ser Thr Thr Asn Phe Ile Lys Ala Glu Tyr
Lys Gly Arg Val Thr Leu Lys Gln Tyr Pro Arg Lys Asn Leu Phe Leu
Val Glu Val Thr Gln Leu Thr Glu Ser Asp Ser Gly Val Tyr Ala Cys
Gly Ala Gly Met Asn Thr Asp Arg Gly Lys Thr Gln Lys Val Thr Leu
Asn Val His Ser Glu Tyr Glu Pro Ser Trp Glu Gln Pro Met Pro
Glu Thr Pro Lys Trp Phe His Leu Pro Tyr Leu Phe Gln Met Pro Ala
Tyr Ala Ser Ser Ser Lys Phe Val Thr Arg Val Thr Pro Ala Gln
Arg Gly Lys Val Pro Pro Val His His Ser Ser Pro Thr Thr Gln Ile
Thr His Arg Pro Arg Val Ser Arg Ala Ser Ser Val Ala Gly Asp Lys
Pro Arg Thr Phe Leu Pro Ser Thr Thr Ala Ser Lys Ile Ser Ala Leu
Glu Gly Leu Leu Lys Pro Gln Thr Pro Ser Tyr Asn His His Thr Arg
Leu His Arg Gln Arg Ala Leu Asp Tyr Gly Ser Gln Ser Gly Arg Glu
Gly Gln Gly

SEQ ID NO: 10

Met Tyr Arg Met Gln Leu Leu Ser Cys Ile Ala Leu Ser Leu Ala Leu
Val Thr Asn Ser Ile Ser Ala Met Val Arg Ser Glu Met His Val Arg
Ile Tyr Leu Cys Arg Glu Met Ala Gly Ser Gly Thr Cys Gly Thr Val
Val Ser Thr Thr Asn Phe Ile Lys Ala Glu Tyr Lys Gly Arg Val Thr
Leu Lys Gln Tyr Pro Arg Lys Asn Leu Phe Leu Val Glu Val Thr Gln

Leu Thr Glu Ser Asp Ser Gly Val Tyr Ala Cys Gly Ala Gly Met Asn
Thr Asp Arg Gly Lys Thr Gln Lys Val Thr Leu Asn Val His Ser Glu
Tyr Glu Pro Ser Trp Glu Glu Gln Pro Met Pro Glu Thr Pro Lys Trp
Phe His Leu Pro Tyr Leu Phe Gln Met Pro Ala Tyr Ala Ser Ser Ser
Lys Phe Val Thr Arg Val Thr Pro Ala Gln Arg Gly Lys Val Pro
Pro Val His His Ser Ser Pro Thr Thr Gln Ile Thr His Arg Pro Arg
Val Ser Arg Ala Ser Ser Val Ala Gly Asp Lys Pro Arg Thr Phe Leu
Pro Ser Thr Thr Ala Ser Lys Ile Ser Ala Leu Glu Gly Leu Leu Lys
Pro Gln Thr Pro Ser Tyr Asn His His Thr Arg Leu His Arg Gln Arg
Ala Leu Asp Tyr Gly Ser Gln Ser Gly Arg Glu Gly Gln Gly Phe His
Arg Ser Val Glu Cys Pro Pro Cys Pro Ala Pro Pro Val Ala Gly Pro
Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser
Arg Thr Pro Glu Val Thr Cys Val Val Asp Val Ser His Glu Asp
Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn
Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val
Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu
Tyr Lys Cys Lys Val Ser Asn Lys Gly Leu Pro Ser Ser Ile Glu Lys
Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr
Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr
Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu
Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu
Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys
Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu
Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly

SEQ ID NO: 11

Gly Thr Cys Gly Thr Val Val Ser Thr Thr Asn Phe Ile Lys Ala Glu
Tyr Lys Gly Arg Val Thr Leu Lys Gln Tyr Pro Arg Lys Asn Leu Phe
Leu Val Glu Val Thr Gln Leu Thr Glu Ser Asp Ser Gly Val Tyr Ala
Cys Gly Ala Gly Met Asn Thr Asp Arg Gly Lys Thr Gln Lys Val Thr
Leu Asn Val His Ser Glu Tyr Glu Pro Ser Trp Glu Glu Gln Pro Met
Pro Glu Thr Pro Lys Trp Phe His Leu Pro Tyr Leu Phe Gln Met Pro
Ala Tyr Ala Ser Ser Ser Lys Phe Val Thr Arg Val Thr Thr Pro Ala
Gln Arg Gly Lys Val Pro Pro Val His His Ser Ser Pro Thr Thr Gln
Ile Thr His Arg Pro Arg Val Ser Arg Ala Ser Ser Val Ala Gly Asp
Lys Pro Arg Thr Phe Leu Pro Ser Thr Thr Ala Ser Lys Ile Ser Ala
Leu Glu Gly Leu Leu Lys Pro Gln Thr Pro Ser Tyr Asn His His Thr
Arg Leu His Arg Gln Arg Ala Leu Asp Tyr Gly Ser Gln Ser Gly Arg
Glu Gly Gln Gly

SEQ ID NO: 12

Met Tyr Arg Met Gln Leu Leu Ser Cys Ile Ala Leu Ser Leu Ala Leu
Val Thr Asn Ser Ile Ser Ala Met Val Arg Ser Gly Thr Cys Gly Thr
Val Val Ser Thr Thr Asn Phe Ile Lys Ala Glu Tyr Lys Gly Arg Val
Thr Leu Lys Gln Tyr Pro Arg Lys Asn Leu Phe Leu Val Glu Val Thr
Gln Leu Thr Glu Ser Asp Ser Gly Val Tyr Ala Cys Gly Ala Gly Met
Asn Thr Asp Arg Gly Lys Thr Gln Lys Val Thr Leu Asn Val His Ser
Glu Tyr Glu Pro Ser Trp Glu Glu Gln Pro Met Pro Glu Thr Pro Lys
Trp Phe His Leu Pro Tyr Leu Phe Gln Met Pro Ala Tyr Ala Ser Ser
Ser Lys Phe Val Thr Arg Val Thr Pro Ala Gln Arg Gly Lys Val

FIG. 1 (cont.)

Pro Pro Val His His Ser Ser Pro Thr Thr Gln Ile Thr His Arg Pro
Arg Val Ser Arg Ala Ser Ser Val Ala Gly Asp Lys Pro Arg Thr Phe
Leu Pro Ser Thr Thr Ala Ser Lys Ile Ser Ala Leu Glu Gly Leu Leu
Lys Pro Gln Thr Pro Ser Tyr Asn His His Thr Arg Leu His Arg Gln
Arg Ala Leu Asp Tyr Gly Ser Gln Ser Gly Arg Glu Gly Gln Gly Phe
His Arg Ser Val Glu Cys Pro Pro Cys Pro Ala Pro Pro Val Ala Gly
Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile
Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu
Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His
Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg
Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys
Glu Tyr Lys Cys Lys Val Ser Asn Lys Gly Leu Pro Ser Ser Ile Glu
Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr
Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val Ser Leu
Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp
Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val
Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp
Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His
Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro
Gly Lys

SEQ ID NO: 13

Gly Met Asn Thr Asp Arg Gly Lys Thr Gln Lys Val Thr Leu Asn Val
His Ser Glu Tyr Glu Pro Ser Trp Glu Glu Gln Pro Met Pro Glu Thr
Pro Lys Trp Phe His Leu Pro Tyr Leu Phe Gln Met Pro Ala Tyr Ala

FIG. 1 (cont.)

Ser Ser Ser Lys Phe Val Thr Arg Val Thr Thr Pro Ala Gln Arg Gly
Lys Val Pro Pro Val His His Ser Ser Pro Thr Thr Gln Ile Thr His
Arg Pro Arg Val Ser Arg Ala Ser Ser Val Ala Gly Asp Lys Pro Arg
Thr Phe Leu Pro Ser Thr Thr Ala Ser Lys Ile Ser Ala Leu Glu Gly
Leu Leu Lys Pro Gln Thr Pro Ser Tyr Asn His His Thr Arg Leu His
Arg Gln Arg Ala Leu Asp Tyr Gly Ser Gln Ser Gly Arg Glu Gly Gln

SEQ ID NO: 14

Met Tyr Arg Met Gln Leu Leu Ser Cys Ile Ala Leu Ser Leu Ala Leu
Val Thr Asn Ser Ile Ser Ala Met Val Arg Ser Gly Met Asn Thr Asp
Arg Gly Lys Thr Gln Lys Val Thr Leu Asn Val His Ser Glu Tyr Glu
Pro Ser Trp Glu Glu Gln Pro Met Pro Glu Thr Pro Lys Trp Phe His
Leu Pro Tyr Leu Phe Gln Met Pro Ala Tyr Ala Ser Ser Ser Lys Phe
Val Thr Arg Val Thr Pro Ala Gln Arg Gly Lys Val Pro Pro Val
His His Ser Ser Pro Thr Thr Gln Ile Thr His Arg Pro Arg Val Ser
Arg Ala Ser Ser Val Ala Gly Asp Lys Pro Arg Thr Phe Leu Pro Ser
Thr Thr Ala Ser Lys Ile Ser Ala Leu Glu Gly Leu Leu Lys Pro Gln
Thr Pro Ser Tyr Asn His His Thr Arg Leu His Arg Gln Arg Ala Leu
Asp Tyr Gly Ser Gln Ser Gly Arg Glu Gly Gln Gly Phe His Arg Ser
Val Glu Cys Pro Pro Cys Pro Ala Pro Pro Val Ala Gly Pro Ser Val
Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr
Pro Glu Val Thr Cys Val Val Asp Val Ser His Glu Asp Pro Glu
Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys
Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser
Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys

FIG. 1 (cont.)

Cys Lys Val Ser Asn Lys Gly Leu Pro Ser Ser Ile Glu Lys Thr Ile
Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro
Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu
Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn
Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser
Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg
Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu
His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys

SEQ ID NO: 15

Glu Met His Val Arg Ile Tyr Leu Cys Arg Glu Met Ala Gly Ser Gly
Thr Cys Gly Thr Val Val Ser Thr Thr Asn Phe Ile Lys Ala Glu Tyr
Lys Gly Arg Val Thr Leu Lys Gln Tyr Pro Arg Lys Asn Leu Phe Leu
Val Glu Val Thr Gln Leu Thr Glu Ser Asp Ser Gly Val Tyr Ala Cys
Gly Ala Gly Met Asn Thr Asp Arg Gly Lys Thr Gln Lys Val Thr Leu
Asn Val His Ser Glu Tyr Glu Pro Ser Trp Glu Glu Gln Pro Met Pro
Glu Thr Pro Lys Trp Phe His Leu Pro Tyr Leu Phe Gln Met Pro Ala
Tyr Ala Ser Ser Ser Lys Phe Val Thr Arg Val Thr Thr Pro Ala Gln
Arg Gly Lys Val Pro Pro Val His His Ser Ser Pro Thr Thr Gln Ile
Thr His Arg Pro Arg Val Ser Arg Ala Ser Ser Val Ala Gly Asp Lys
Pro Arg Thr Phe Leu Pro Ser Thr Thr Ala Ser Lys Ile Ser Ala Leu
Glu Gly Leu Leu Lys Pro Gln Thr Pro Ser Tyr Asn His His Thr Arg

SEQ ID NO: 16

Met Tyr Arg Met Gln Leu Leu Ser Cys Ile Ala Leu Ser Leu Ala Leu

FIG. 1 (cont.)

Val Thr Asn Ser Ile Ser Ala Met Val Arg Ser Glu Met His Val Arg
Ile Tyr Leu Cys Arg Glu Met Ala Gly Ser Gly Thr Cys Gly Thr Val
Val Ser Thr Thr Asn Phe Ile Lys Ala Glu Tyr Lys Gly Arg Val Thr
Leu Lys Gln Tyr Pro Arg Lys Asn Leu Phe Leu Val Glu Val Thr Gln
Leu Thr Glu Ser Asp Ser Gly Val Tyr Ala Cys Gly Ala Gly Met Asn
Thr Asp Arg Gly Lys Thr Gln Lys Val Thr Leu Asn Val His Ser Glu
Tyr Glu Pro Ser Trp Glu Glu Gln Pro Met Pro Glu Thr Pro Lys Trp
Phe His Leu Pro Tyr Leu Phe Gln Met Pro Ala Tyr Ala Ser Ser Ser
Lys Phe Val Thr Arg Val Thr Pro Ala Gln Arg Gly Lys Val Pro
Pro Val His His Ser Ser Pro Thr Thr Gln Ile Thr His Arg Pro Arg
Val Ser Arg Ala Ser Ser Val Ala Gly Asp Lys Pro Arg Thr Phe Leu
Pro Ser Thr Thr Ala Ser Lys Ile Ser Ala Leu Glu Gly Leu Leu Lys
Pro Gln Thr Pro Ser Tyr Asn His His Thr Arg Arg Ser Val Glu Cys
Pro Pro Cys Pro Ala Pro Pro Val Ala Gly Pro Ser Val Phe Leu Phe
Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val
Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe
Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro
Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr
Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val
Ser Asn Lys Gly Leu Pro Ser Ser Ile Glu Lys Thr Ile Ser Lys Ala
Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg
Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly
Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro
Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser
Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln

Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His
Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys

SEQ ID NO: 17

Arg Ile Leu Pro Glu Val Lys Val Glu Gly Glu Leu Gly Ser Val
Thr Ile Lys Cys Pro Leu Pro Glu Met His Val Arg Ile Tyr Leu Cys
Arg Glu Met Ala Gly Ser Gly Thr Cys Gly Thr Val Val Ser Thr Thr
Asn Phe Ile Lys Ala Glu Tyr Lys Gly Arg Val Thr Leu Lys Gln Tyr
Pro Arg Lys Asn Leu Phe Leu Val Glu Val Thr Gln Leu Thr Glu Ser
Asp Ser Gly Val Tyr Ala Cys Gly Ala Gly Met Asn Thr Asp Arg Gly
Lys Thr Gln Lys Val Thr Leu Asn Val His Ser Glu Tyr Glu Pro Ser
Trp Glu Glu Gln Pro Met Pro Glu Thr Pro Lys Trp Phe His Leu Pro
Tyr Leu Phe Gln Met Pro Ala Tyr Ala Ser Ser Ser Lys Phe Val Thr
Arg Val Thr Thr Pro Ala Gln Arg Gly Lys Val Pro Pro Val His His
Ser Ser Pro Thr Thr Gln Ile Thr His Arg Pro Arg Val Ser Arg Ala
Ser Ser Val Ala Gly Asp Lys Pro Arg Thr Phe Leu Pro Ser Thr Thr
Ala Ser Lys Ile Ser Ala Leu Glu Gly Leu Leu Lys Pro Gln Thr Pro
Ser Tyr Asn His His Thr Arg

SEQ ID NO: 18

Met Tyr Arg Met Gln Leu Leu Ser Cys Ile Ala Leu Ser Leu Ala Leu
Val Thr Asn Ser Ile Ser Ala Met Val Arg Ser Arg Ile Leu Pro Glu
Val Lys Val Glu Gly Glu Leu Gly Ser Val Thr Ile Lys Cys Pro
Leu Pro Glu Met His Val Arg Ile Tyr Leu Cys Arg Glu Met Ala Gly
Ser Gly Thr Cys Gly Thr Val Val Ser Thr Thr Asn Phe Ile Lys Ala

FIG. 1 (cont.)

Glu Tyr Lys Gly Arg Val Thr Leu Lys Gln Tyr Pro Arg Lys Asn Leu
Phe Leu Val Glu Val Thr Gln Leu Thr Glu Ser Asp Ser Gly Val Tyr
Ala Cys Gly Ala Gly Met Asn Thr Asp Arg Gly Lys Thr Gln Lys Val
Thr Leu Asn Val His Ser Glu Tyr Glu Pro Ser Trp Glu Glu Gln Pro
Met Pro Glu Thr Pro Lys Trp Phe His Leu Pro Tyr Leu Phe Gln Met
Pro Ala Tyr Ala Ser Ser Lys Phe Val Thr Arg Val Thr Thr Pro
Ala Gln Arg Gly Lys Val Pro Pro Val His His Ser Ser Pro Thr Thr
Gln Ile Thr His Arg Pro Arg Val Ser Arg Ala Ser Ser Val Ala Gly
Asp Lys Pro Arg Thr Phe Leu Pro Ser Thr Thr Ala Ser Lys Ile Ser
Ala Leu Glu Gly Leu Leu Lys Pro Gln Thr Pro Ser Tyr Asn His His
Thr Arg Arg Ser Val Glu Cys Pro Pro Cys Pro Ala Pro Pro Val Ala
Gly Pro Ser Val Phe Pro Pro Lys Pro Lys Asp Thr Leu Met
Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Asp Val Ser His
Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val
His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr
Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly
Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Gly Leu Pro Ser Ser Ile
Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val
Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val Ser
Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu
Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro
Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val
Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met
His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser
Pro Gly Lys

SEQ ID NO: 19

Arg Ile Leu Pro Glu Val Lys Val Glu Gly Glu Leu Gly Gly Ser Val
Thr Ile Lys Cys Pro Leu Pro Glu Met His Val Arg Ile Tyr Leu Cys
Arg Glu Met Ala Gly Ser Gly Thr Cys Gly Thr Val Val Ser Thr Thr
Asn Phe Ile Lys Ala Glu Tyr Lys Gly Arg Val Thr Leu Lys Gln Tyr
Pro Arg Lys Asn Leu Phe Leu Val Glu Val Thr Gln Leu Thr Glu Ser
Asp Ser Gly Val Tyr Ala Cys Gly Ala Gly Met Asn Thr Asp Arg Gly
Lys Thr Gln Lys Val Thr Leu Asn Val His Ser Glu Tyr Glu Pro Ser
Trp Glu Glu Gln Pro Met Pro Glu Thr Pro Lys Trp Phe His Leu Pro
Tyr Leu Phe Gln Met Pro Ala Tyr Ala Ser Ser Ser Lys Phe Val Thr
Arg Val Thr Thr Pro Ala Gln Arg Gly Lys Val

SEQ ID NO: 20

Met Tyr Arg Met Gln Leu Leu Ser Cys Ile Ala Leu Ser Leu Ala Leu
Val Thr Asn Ser Ile Ser Ala Met Val Arg Ser Arg Ile Leu Pro Glu
Val Lys Val Glu Gly Glu Leu Gly Gly Ser Val Thr Ile Lys Cys Pro
Leu Pro Glu Met His Val Arg Ile Tyr Leu Cys Arg Glu Met Ala Gly
Ser Gly Thr Cys Gly Thr Val Val Ser Thr Thr Asn Phe Ile Lys Ala
Glu Tyr Lys Gly Arg Val Thr Leu Lys Gln Tyr Pro Arg Lys Asn Leu
Phe Leu Val Glu Val Thr Gln Leu Thr Glu Ser Asp Ser Gly Val Tyr
Ala Cys Gly Ala Gly Met Asn Thr Asp Arg Gly Lys Thr Gln Lys Val
Thr Leu Asn Val His Ser Glu Tyr Glu Pro Ser Trp Glu Glu Gln Pro
Met Pro Glu Thr Pro Lys Trp Phe His Leu Pro Tyr Leu Phe Gln Met
Pro Ala Tyr Ala Ser Ser Lys Phe Val Thr Arg Val Thr Thr Pro
Ala Gln Arg Gly Lys Val Arg Ser Val Glu Cys Pro Pro Cys Pro Ala

FIG. 1 (cont.)

Pro Pro Val Ala Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys
Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val
Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp
Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr
Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp
Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Gly Leu
Pro Ser Ser Ile Glu Lys Thr Ile Ser Lys Ala Lys Gln Pro Arg
Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys
Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp
Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys
Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser
Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser
Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser
Leu Ser Leu Ser Pro Gly Lys

SEQ ID NO: 21

Arg Ile Leu Pro Glu Val Lys Val Glu Gly Glu Leu Gly Gly Ser Val
Thr Ile Lys Cys Pro Leu Pro Glu Met His Val Arg Ile Tyr Leu Cys
Arg Glu Met Ala Gly Ser Gly Thr Cys Gly Thr Val Val Ser Thr Thr
Asn Phe Ile Lys Ala Glu Tyr Lys Gly Arg Val Thr Leu Lys Gln Tyr
Pro Arg Lys Asn Leu Phe Leu Val Glu Val Thr Gln Leu Thr Glu Ser
Asp Ser Gly Val Tyr Ala Cys Gly Ala Gly Met Asn Thr Asp Arg Gly
Lys Thr Gln Lys Val Thr Leu Asn Val His

SEQ ID NO: 22

FIG. 1 (cont.)

Met Tyr Arg Met Gln Leu Leu Ser Cys Ile Ala Leu Ser Leu Ala Leu
Val Thr Asn Ser Ile Ser Ala Met Val Arg Ser Arg Ile Leu Pro Glu
Val Lys Val Glu Gly Glu Leu Gly Gly Ser Val Thr Ile Lys Cys Pro
Leu Pro Glu Met His Val Arg Ile Tyr Leu Cys Arg Glu Met Ala Gly
Ser Gly Thr Cys Gly Thr Val Val Ser Thr Thr Asn Phe Ile Lys Ala
Glu Tyr Lys Gly Arg Val Thr Leu Lys Gln Tyr Pro Arg Lys Asn Leu
Phe Leu Val Glu Val Thr Gln Leu Thr Glu Ser Asp Ser Gly Val Tyr
Ala Cys Gly Ala Gly Met Asn Thr Asp Arg Gly Lys Thr Gln Lys Val
Thr Leu Asn Val His Arg Ser Val Glu Cys Pro Pro Cys Pro Ala Pro
Pro Val Ala Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp
Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp
Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly
Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn
Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp
Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Gly Leu Pro
Ser Ser Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu
Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn
Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile
Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr
Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys
Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys
Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu
Ser Leu Ser Pro Gly Lys

SEQ ID NO: 23

FIG. 1 (cont.)

Arg Ile Leu Pro Glu Val Lys Val Glu Gly Glu Leu Gly Gly Ser Val
Thr Ile Lys Cys Pro Leu Pro Glu Met His Val Arg Ile Tyr Leu Cys
Arg Glu Met Ala Gly Ser Gly Thr Cys Gly Thr Val Val Ser Thr Thr
Asn Phe Ile Lys Ala Glu Tyr Lys Gly Arg Val Thr Leu Lys Gln Tyr
Pro Arg Lys Asn Leu Phe Leu Val Glu Val Thr Gln Leu Thr Glu Ser
Asp Ser Gly Val Tyr Ala Cys Gly Ala Gly Met Asn Thr Asp

SEQ ID NO: 24

Met Tyr Arg Met Gln Leu Leu Ser Cys Ile Ala Leu Ser Leu Ala Leu
Val Thr Asn Ser Ile Ser Ala Met Val Arg Ser Arg Ile Leu Pro Glu
Val Lys Val Glu Gly Glu Leu Gly Gly Ser Val Thr Ile Lys Cys Pro
Leu Pro Glu Met His Val Arg Ile Tyr Leu Cys Arg Glu Met Ala Gly
Ser Gly Thr Cys Gly Thr Val Val Ser Thr Thr Asn Phe Ile Lys Ala
Glu Tyr Lys Gly Arg Val Thr Leu Lys Gln Tyr Pro Arg Lys Asn Leu
Phe Leu Val Glu Val Thr Gln Leu Thr Glu Ser Asp Ser Gly Val Tyr
Ala Cys Gly Ala Gly Met Asn Thr Asp Arg Ser Val Glu Cys Pro Pro
Cys Pro Ala Pro Pro Val Ala Gly Pro Ser Val Phe Leu Phe Pro Pro
Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys
Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp
Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu
Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu
His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn
Lys Gly Leu Pro Ser Ser Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly
Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu
Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr

FIG. 1 (cont.)

Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn
Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe
Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn
Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr
Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys

SEQ ID NO: 25

Ile Ser Ala Met Val Arg Ser

SEQ ID NO: 26

gtgaatacgt gagcttggc tacc

SEQ ID NO: 27

caagtgatgg gggattacag tgaa

SEQ ID NO: 28

tgttaatat gatgtgtcag gctg

SEQ ID NO: 29

agggccagct cattcctccc actcat

SEQ ID NO: 30

aactctgccc ctgctcccttc atttcc

FIG. 1 (cont.)

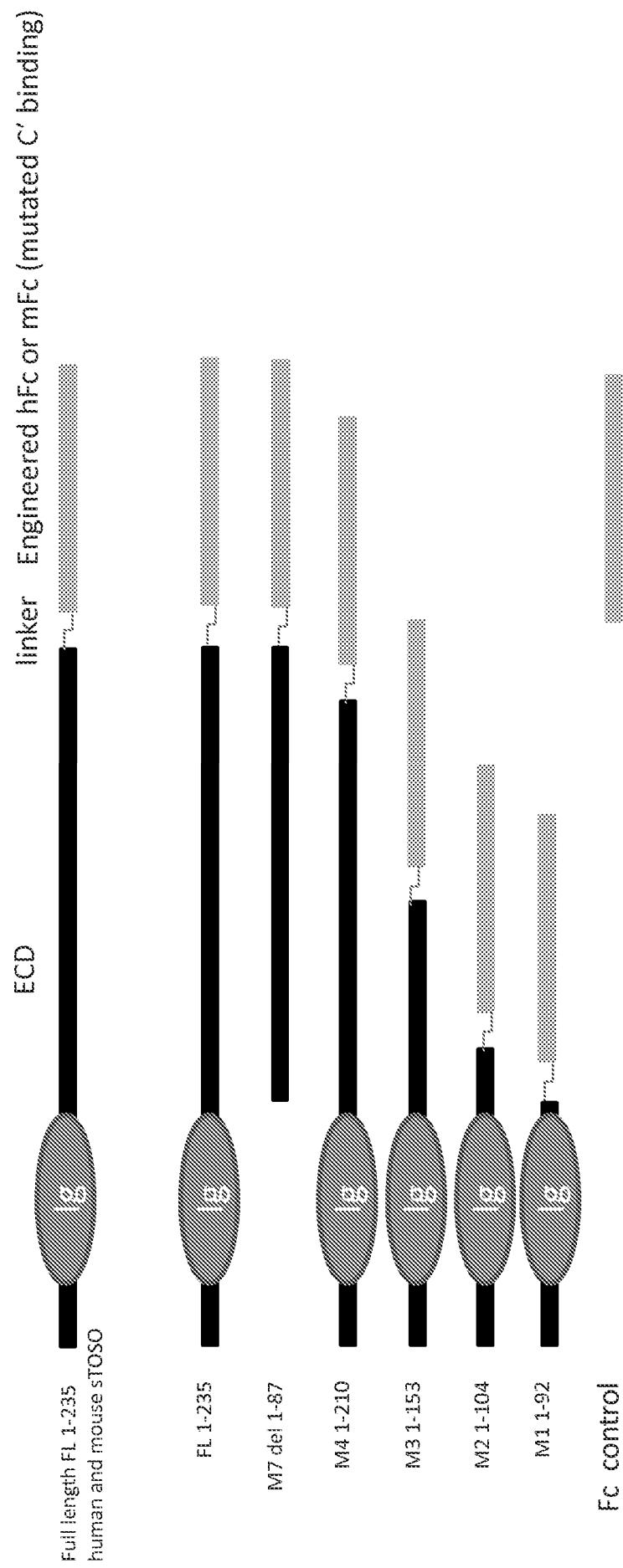
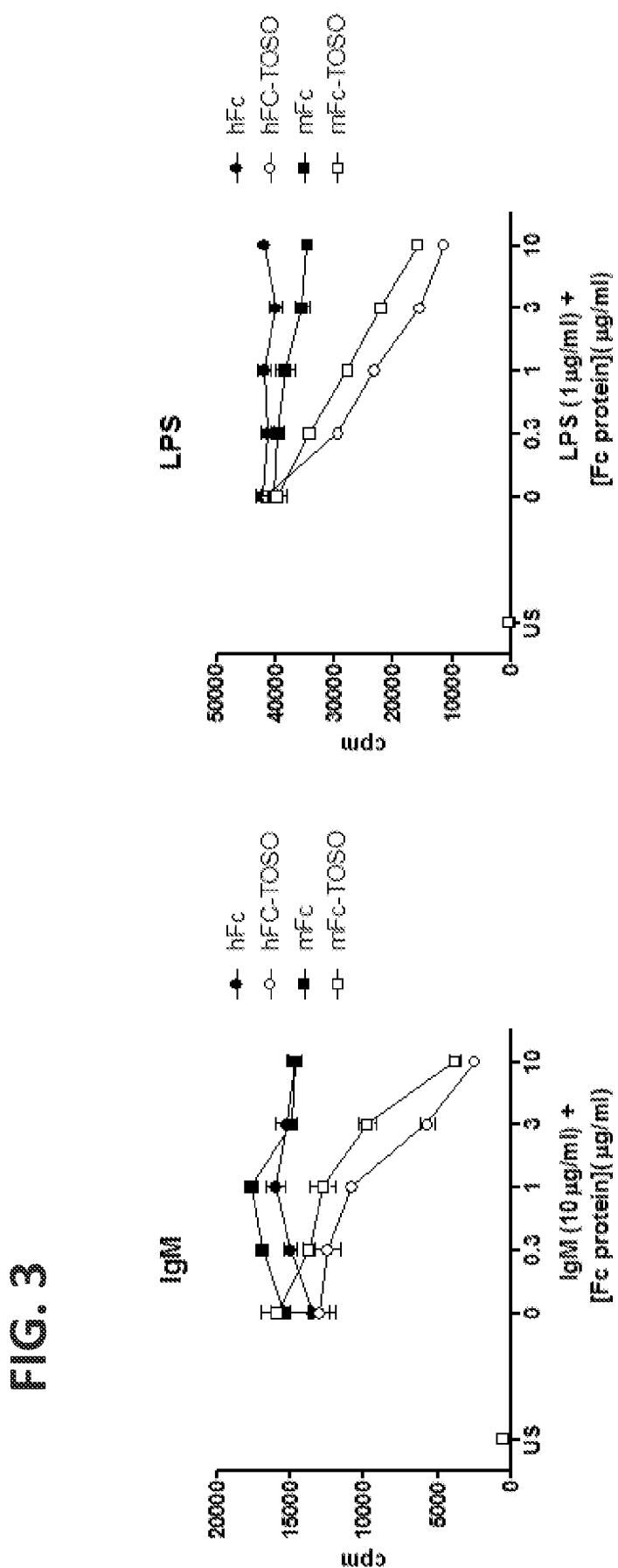


FIG. 2



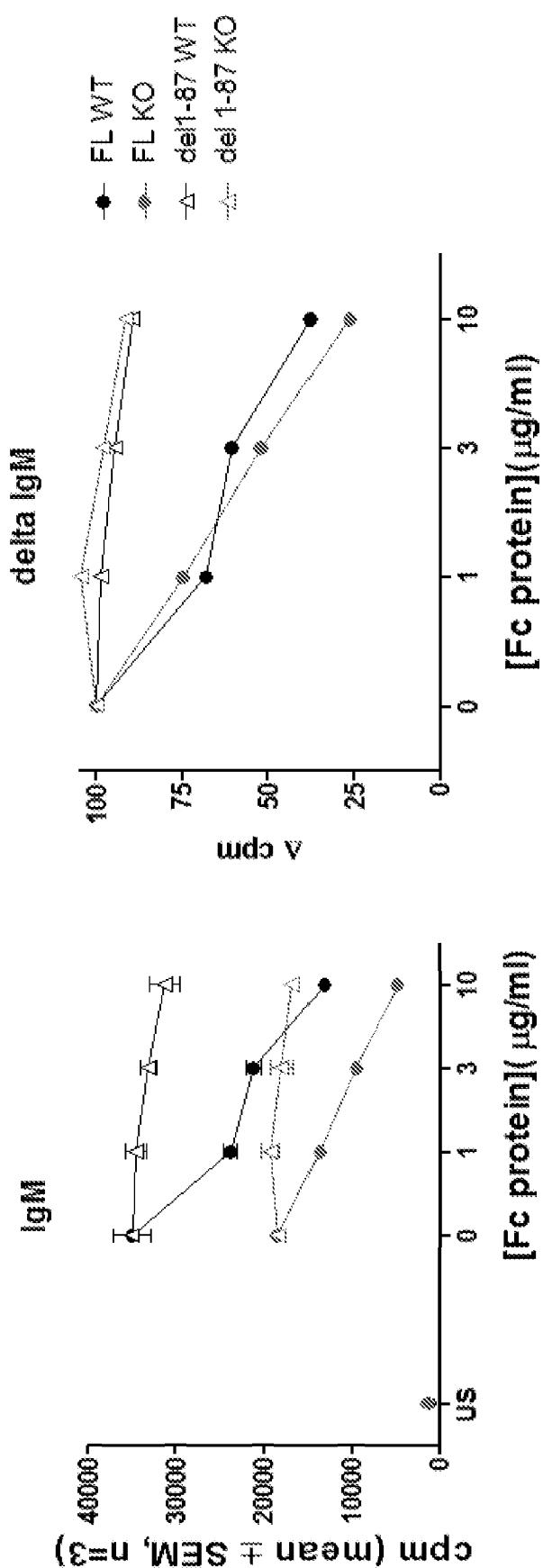


FIG. 4

FIG. 5

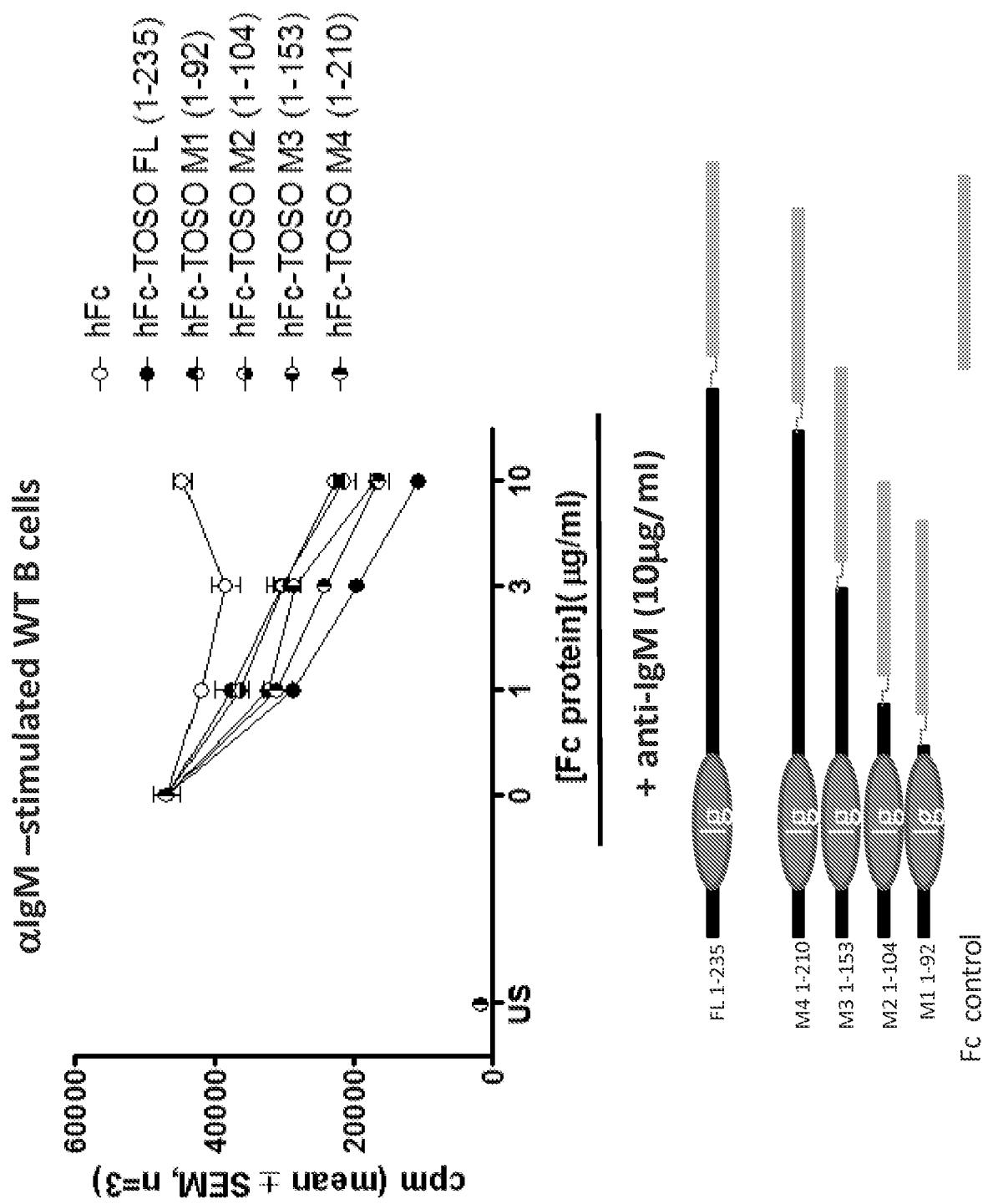


FIG. 6

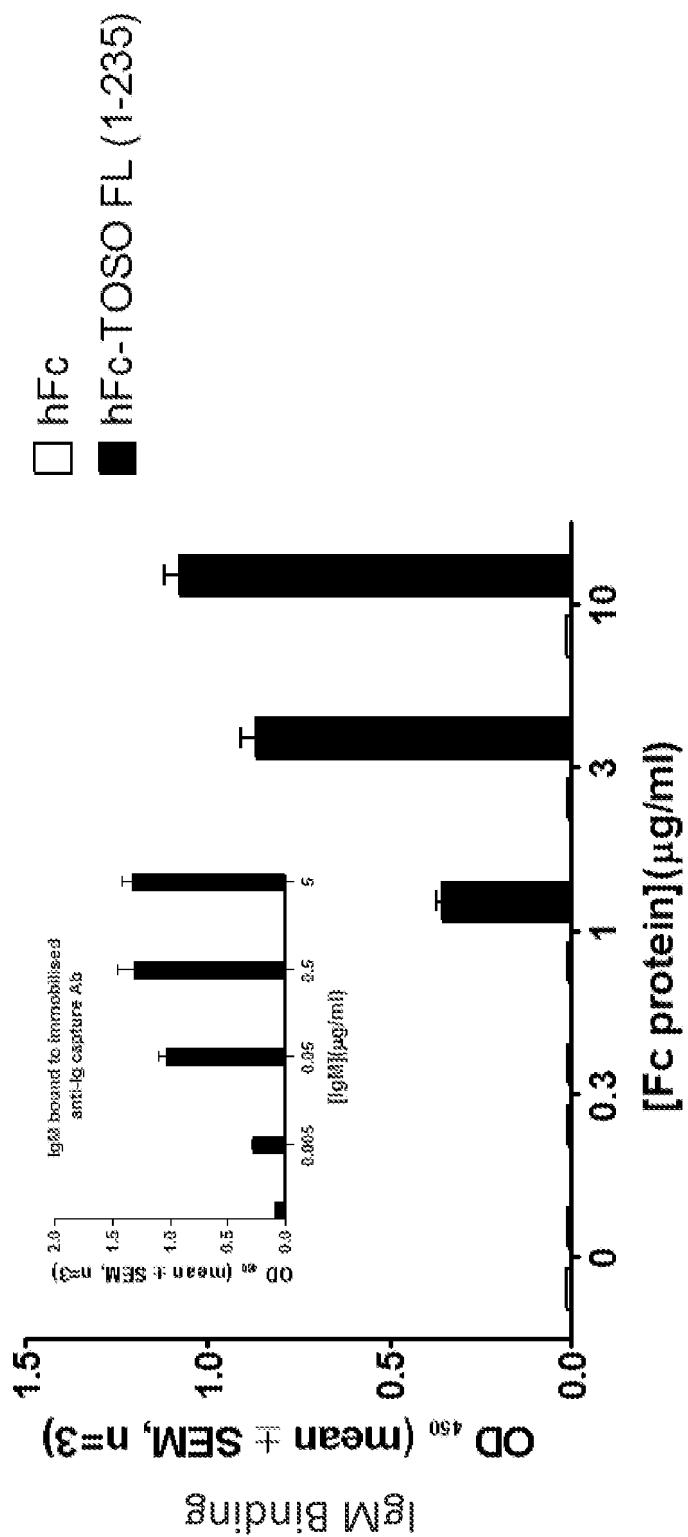
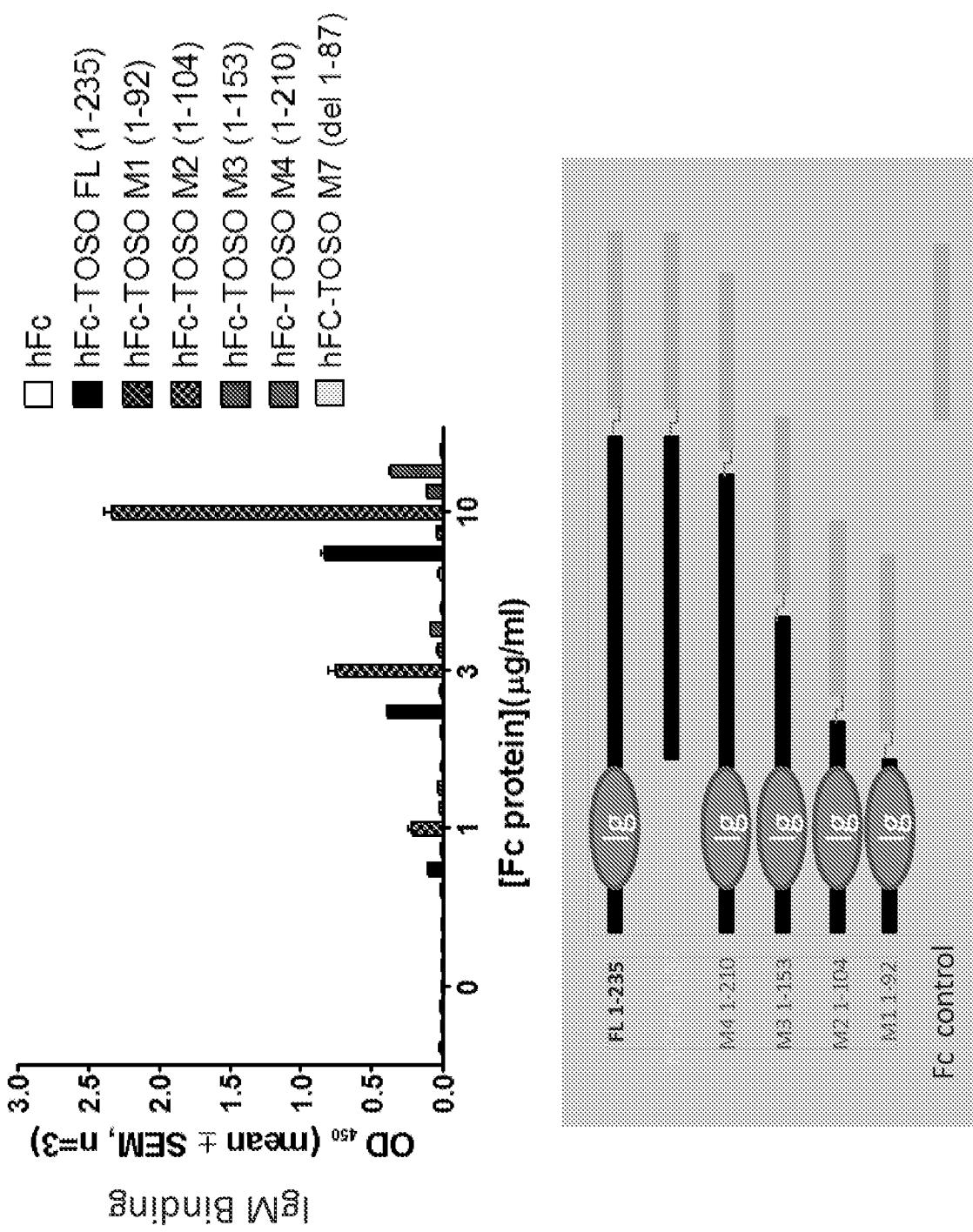


FIG. 7



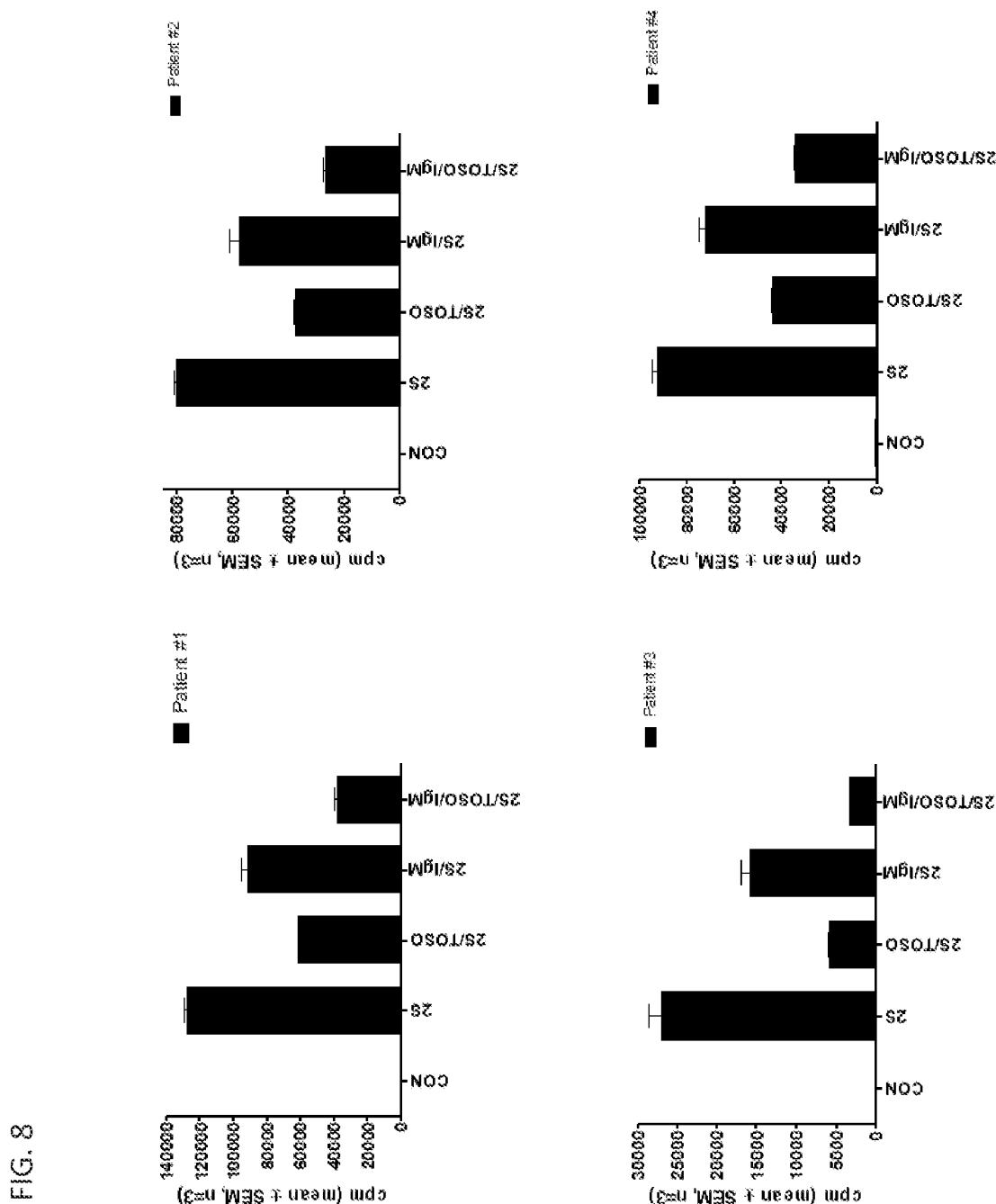


Figure 9

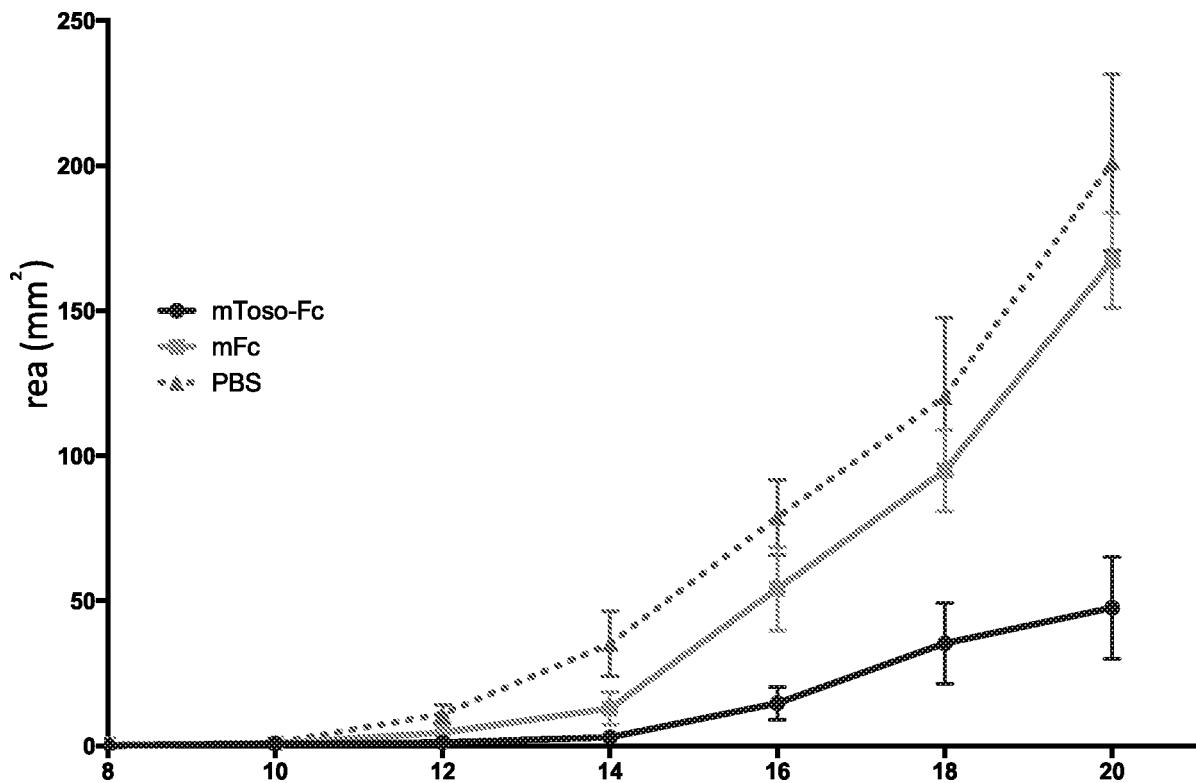


Figure 10

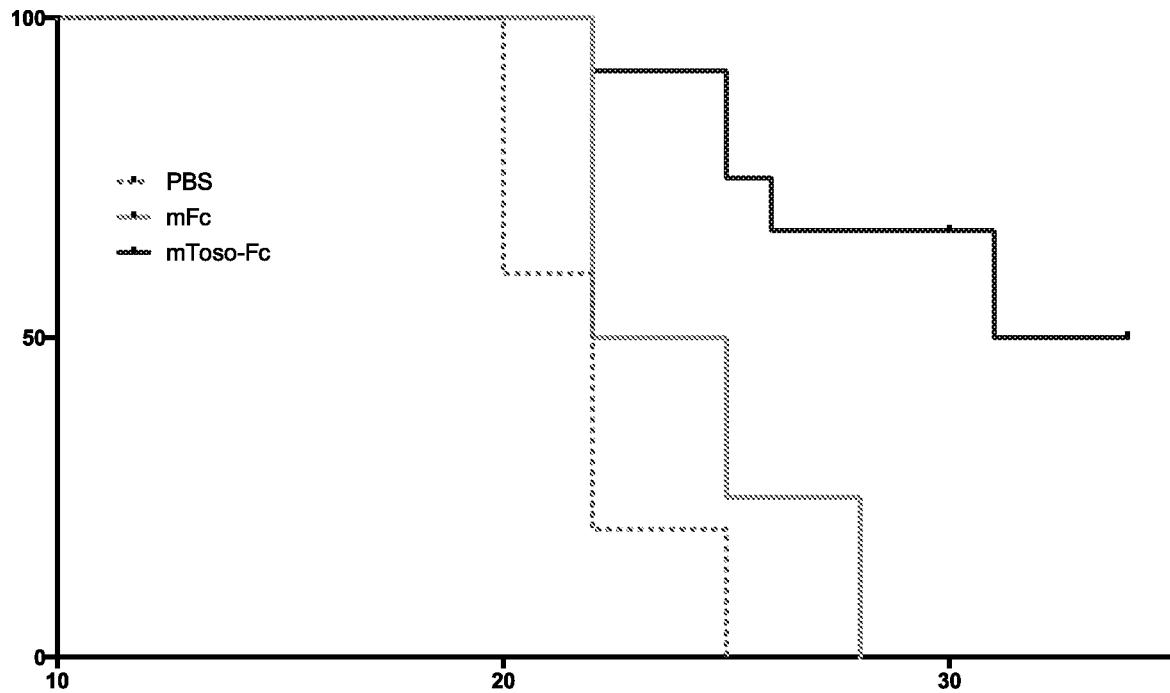


Figure 11

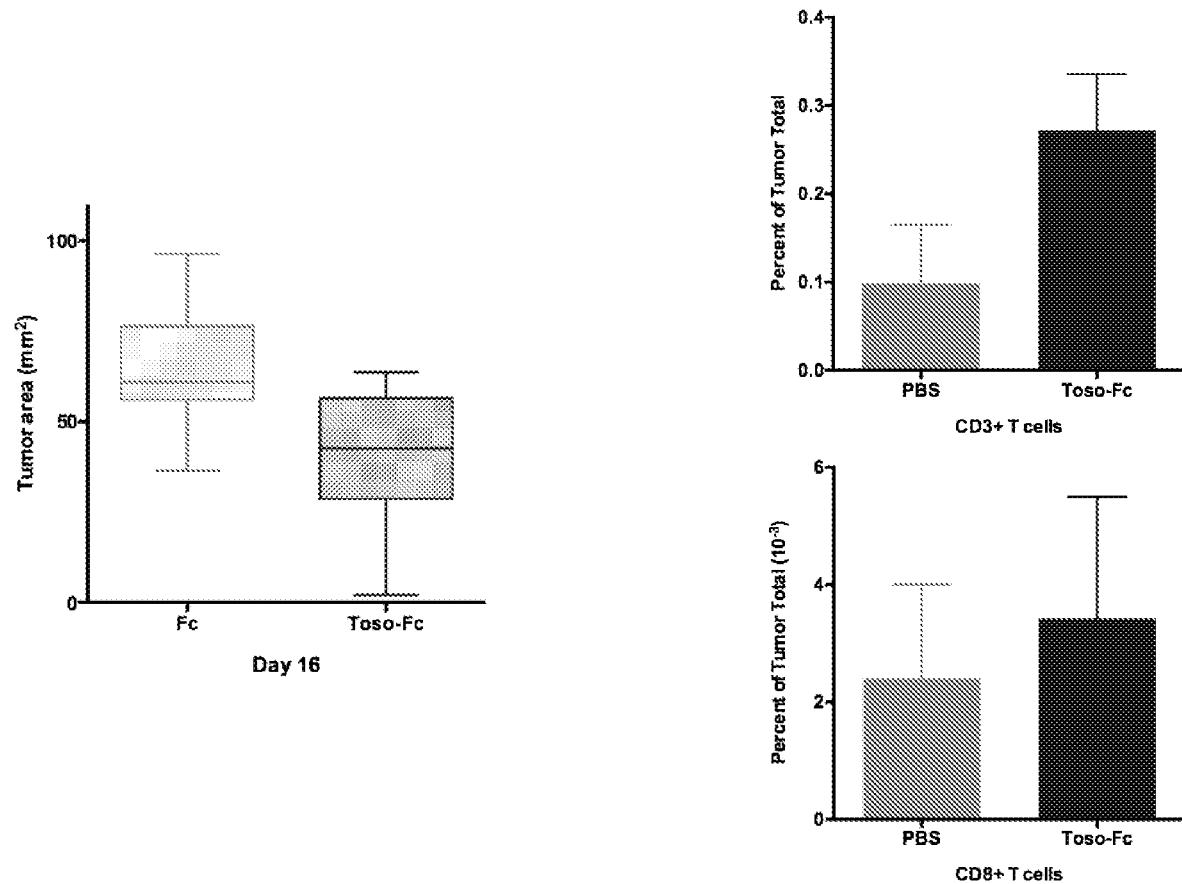


Figure 12

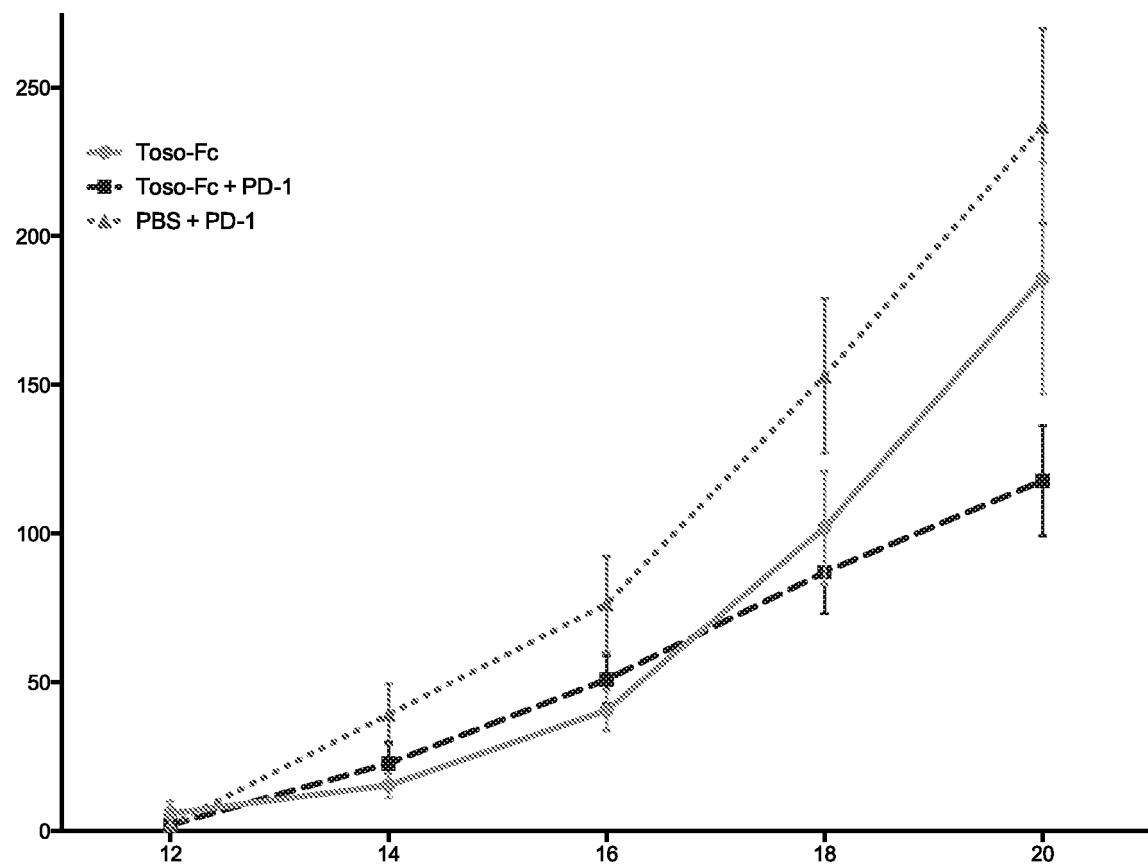


Figure 13

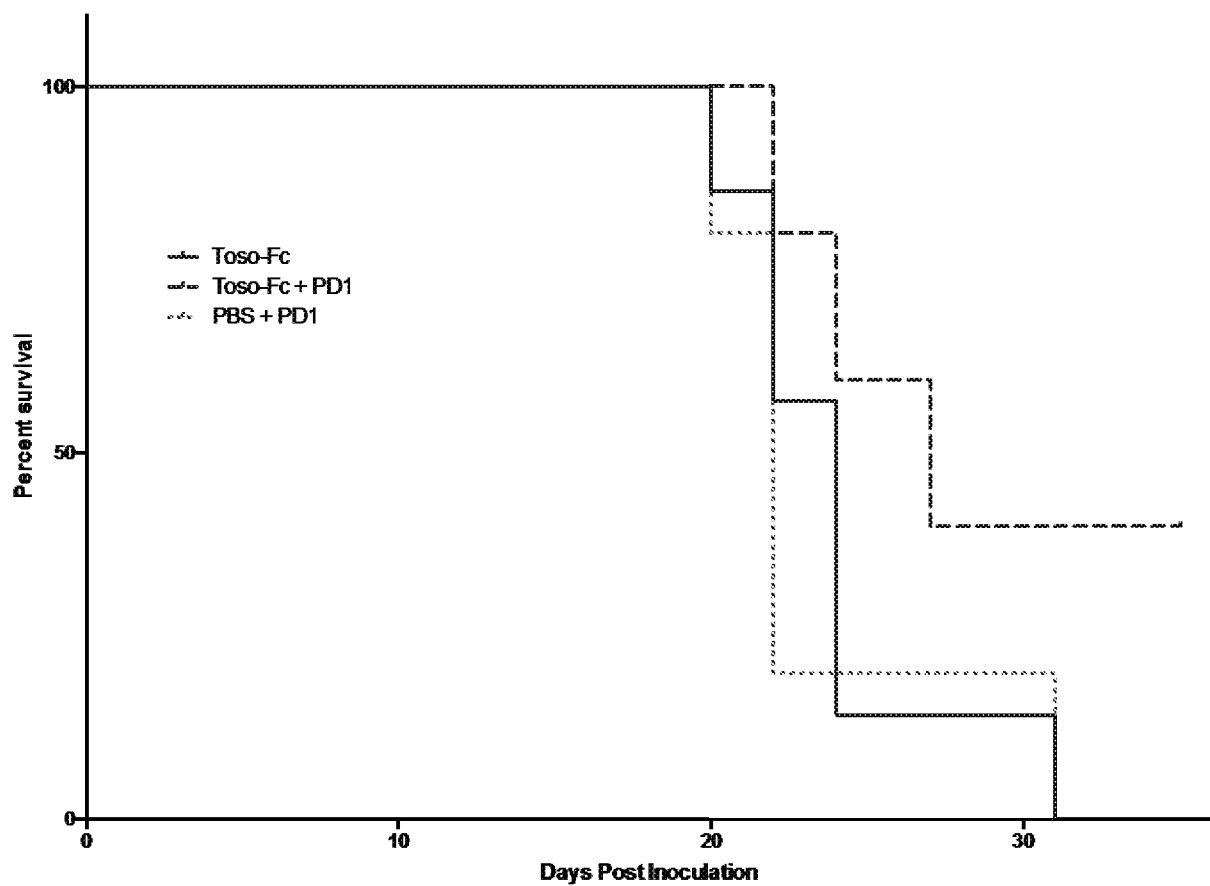


Figure 14

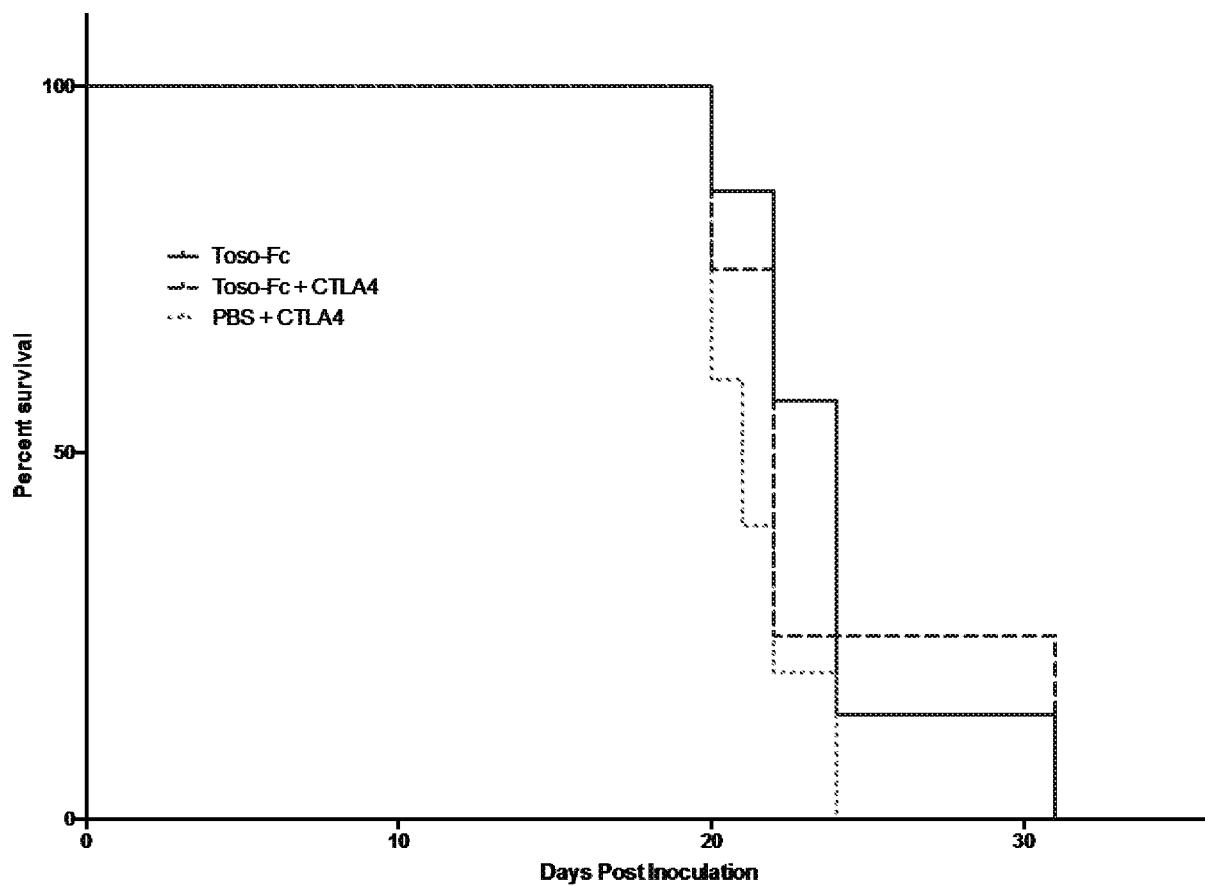


Figure 15

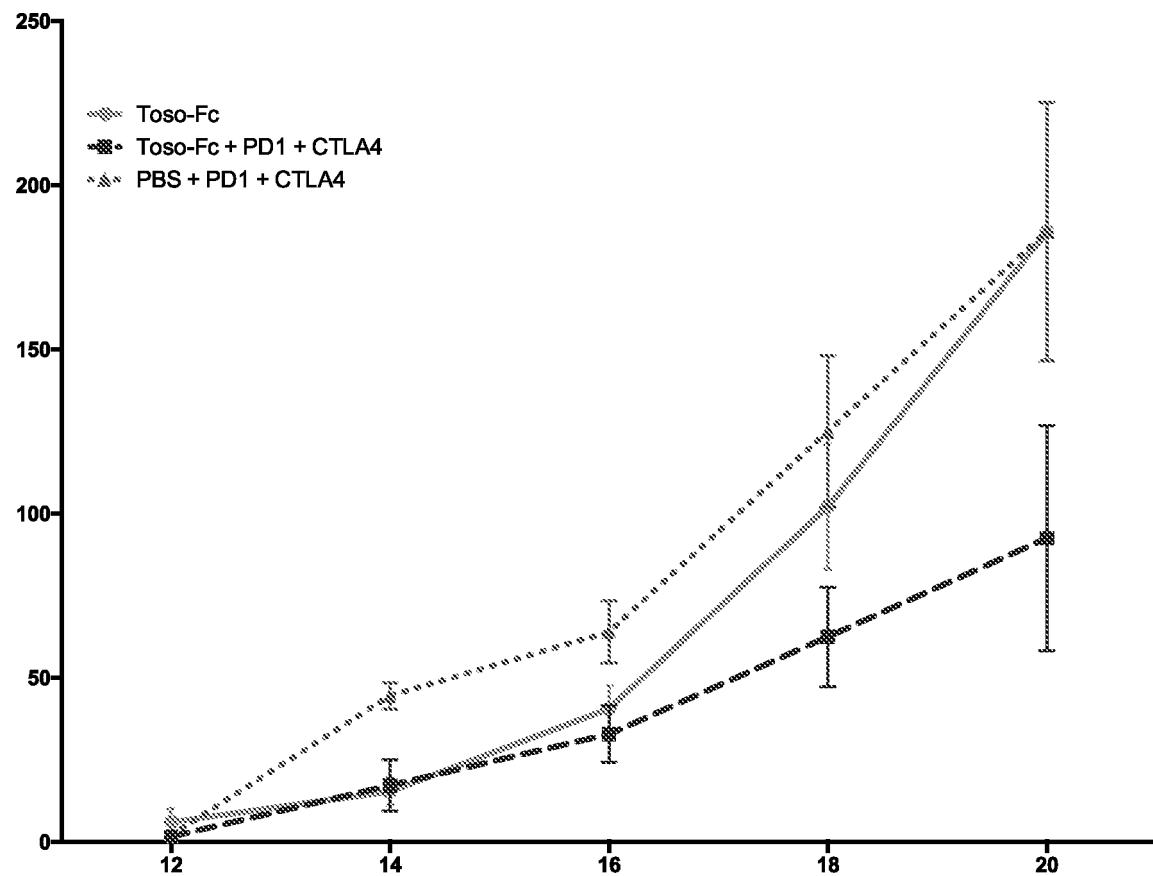


Figure 16

