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(71) Applicant: **BOARD OF REGENTS, THE UNIVERSITY OF TEXAS SYSTEM** [US/US]; 210 West 7th Street, Austin, Texas 78701 (US).

(72) Inventors: **REZVANI, Katy**; c/o U.T.M.D. Anderson Cancer Center, Stem Cell Transplantation & Cell Therapy, 1515 Holcombe Blvd., Unit # 0065, Houston, Texas 77030 (US). **SHPALL, Elizabeth**; c/o U.T.M.D. Anderson Cancer Center, Stem Cell Transplantation & Cell Therapy, 1515 Holcombe Blvd., Unit # 0065, Houston, Texas 77030 (US).

(74) Agent: **SISTRUNK, Melissa**; Norton Rose Fulbright US LLP, 1301 McKinney, Suite 5100, Houston, 77010 (US).

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(54) Title: SUICIDE GENE

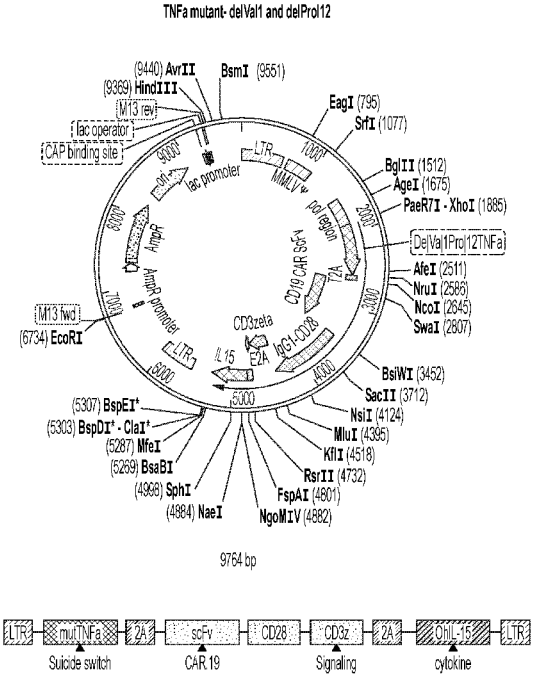

FIG. 2A

(57) Abstract: Embodiments of the disclosure encompass particular TNF-alpha mutants that are nonsecretable and membrane bound, thereby providing a target for inhibition in cells that express the mutants. In specific embodiments, the TNF-alpha mutants are utilized as a suicide gene in cells employed for adoptive cell therapy for an individual, wherein at a desired time the individual is provided one or more anti-TNF-alpha antibodies that bind the membrane bound TNF-alpha and elicit complement-dependent cytotoxicity for the cells. The TNF-alpha mutant can also be used as a way of tracking the transduced cells *in vivo*.

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[Continued on next page]

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SUICIDE GENE

[0001] The present application claims priority to U.S. Provisional Patent Application Serial No. 62/769,405, filed November 19, 2018; U.S. Provisional Patent Application Serial No. 62/773,372, filed November 30, 2018; and U.S. Provisional Patent Application Serial No. 62/791,464, filed January 11, 2019, all of which applications are incorporated by reference herein in their entirety.

SEQUENCE LISTING

[0001.1] The instant application contains a Sequence Listing which has been submitted electronically in ASCII format and is hereby incorporated by reference in its entirety. Said ASCII copy, created on November 13, 2019, is named UTFC_P1151WO_SL.txt and is 108,130 bytes in size.

TECHNICAL FIELD

[0002] Embodiments of the disclosure encompass at least the fields of cell biology, molecular biology, immunology, cell therapy, and medicine.

BACKGROUND

[0003] Adoptive cell therapy with chimeric antigen receptor (CAR)-engineered and T-cell receptor (TCR)-transduced T cells has been associated with reports of serious adverse events such as cytokine release syndrome and neurotoxicity, as well as on-target/off tumor toxicities. As increasing numbers of patients are treated with adoptive cell therapy, the incorporation of a safety mechanism to allow selective deletion of the adoptively infused cells in the face of toxicity is useful.

[0004] The present disclosure provides a solution for a long-felt need in the art of safety mechanisms for cell therapies.

BRIEF SUMMARY

[0005] Embodiments of the present disclosure are directed to systems, methods, and compositions related to cell therapy, including safety mechanisms to control cell therapy. In particular embodiments, a unique suicide gene is utilized in conjunction with cell therapy of any

kind to control its use and allow for controllable termination of the cell therapy at a desired event and/or time. The suicide gene is employed in transduced cells for the purpose of eliciting death for the transduced cells when needed. In specific embodiments, the suicide/depletion gene is a tumor necrosis factor (TNF)-alpha mutant that is uncleavable by standard enzymes that cleave TNF in nature, such as TNF-alpha-converting enzyme (also referred to as TACE). As such, the TNF-alpha mutant is membrane-bound, inactive, and nonsecretable, in particular embodiments. The TNF-alpha mutant of the disclosure is targetable by one or more agents that bind the mutant, including at least an antibody, such that following binding of the agent(s) to the TNF-alpha mutant on the surface of the cell, the cell dies. Embodiments of the disclosure allow the TNF-alpha mutant to be utilized as a marker for cells that express it.

[0006] Embodiments of the disclosure include compositions comprising a transduced cell comprising a nucleic acid that encodes one or more engineered nonsecretable tumor necrosis factor (TNF)-alpha mutant polypeptides and a nucleic acid that encodes one or more therapeutic gene products. In specific embodiments, the TNF-alpha mutant polypeptide comprises a deletion with respect to SEQ ID NO:8 of the following: amino acid residue 1 and amino acid residue 12; amino acid residue 1 and amino acid residue 13; amino acid residues 1-12; amino acid residues 1-13; or amino acid residues 1-14. The therapeutic gene product of the composition may or may not be an engineered receptor, such as a T-cell receptor, a chimeric antigen receptor (CAR), cytokine receptor, homing receptor, or chemokine receptor. Any of the engineered receptors may or may not target an antigen, such as a cancer antigen. When the engineered receptor is a CAR, the CAR may or may not comprises one or more costimulatory domains, such as CD28, DAP12, or both.

[0007] In particular embodiments, the nucleic acid that encodes the TNF-alpha mutant polypeptide and the nucleic acid that encodes the therapeutic gene product are the same nucleic acid molecule, although the nucleic acid that encodes the TNF-alpha mutant polypeptide and the nucleic acid that encodes the therapeutic gene product may be different nucleic acid molecules. In any case, the nucleic acid molecule may be a vector, including a viral vector (retroviral vector, lentiviral vector, adenoviral vector, or adeno-associated viral vector) or a non-viral vector, such one that comprises a plasmid, lipid, transposon, or combination thereof.

[0008] The transduced cells of the composition of the disclosure may be an immune cell or a stem cell, for example. Examples of an immune cell includes a T cell, a NK cell, NKT cell,

iNKT cell, B cell, regulatory T cell, monocyte, macrophage, dendritic cell, or mesenchymal stromal cell. The cell may or may not express one or more exogenously provided cytokines, such as IL-15, IL-12, IL-18, IL-21 or combination thereof. The cytokine may or may not be encoded from the same vector as the TNF-alpha mutant gene. In specific cases, the cytokine is expressed as a separate polypeptide molecule as the TNF-alpha mutant and as an engineered receptor of the cell.

[0009] In particular embodiments of the disclosure, the TNF-alpha mutant polypeptide comprises SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, or SEQ ID NO:39. The TNF-alpha mutant polypeptide may be encoded by a sequence that comprises SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, or SEQ ID NO:38. In certain aspects, the TNF-alpha mutant polypeptide lacks one or more further mutations that prevent binding of the TNF-alpha mutant polypeptide to a TNF receptor.

[0010] Embodiments of the disclosure include methods of inducing death for a transduced cell expressing at least an engineered nonsecretable TNF-alpha mutant polypeptide and optionally expressing a therapeutic gene, such as an engineered receptor, the methods comprising the step of providing an effective amount of at least one agent that binds the TNF-alpha mutant on the transduced cell. An agent that binds TNF-alpha may be an antibody, small molecule, polypeptide, nucleic acid, or combination thereof, for example. When the agent is an antibody, the antibody may be of any kind, including at least a monoclonal antibody. In the methods, the cell may further express an engineered receptor, including a T-cell receptor, a chimeric antigen receptor (CAR), cytokine receptor, homing receptor, or chemokine receptor. Any of the engineered receptors may bind a cancer or other antigen. In specific cases, the method occurs *in vivo* in an individual with a medical condition and when the individual has been provided a therapy for the medical condition that comprises a plurality of the transduced cells. Although the medical condition may be of any kind, in specific cases the medical condition is cancer. The agent may be provided to the individual upon onset of one or more adverse events from the therapy or when an adverse event is suspected of occurring. The individual may exhibit one or more symptoms of cytokine release syndrome, neurotoxicity, anaphylaxis/allergy, and/or on-target/off tumor toxicity. In some cases, the individual has been provided, is provided, and/or will be provided an additional therapy for the medical condition. In particular aspects of the disclosure, the TNF-alpha mutant polypeptide lacks or comprises one or more further mutations

that prevent binding of the TNF-alpha mutant polypeptide to a TNF receptor or prevents reverse signaling.

[0011] Embodiments of the disclosure include methods of reducing the effects of cytokine release syndrome in an individual that has received and/or who is receiving cell therapy with cells that express a nonsecretable TNF-alpha mutant, comprising the step of providing an effective amount of one or more agents that bind the mutant to cause in the individual (a) elimination of at least some of the cells of the cell therapy; and (b) reduction in the level of soluble TNF-alpha.

[0012] Embodiments of the disclosure include methods of reducing the risk of toxicity of a cell therapy for an individual, comprising the step of modifying the cells of the cell therapy to express a nonsecretable TNF-alpha mutant. The cell therapy may be for cancer, for example. The cell therapy may comprise an engineered receptor that targets an antigen.

[0013] Specific embodiments include vectors comprising a sequence that encodes a nonsecretable TNF-alpha mutant and that encodes an engineered receptor. The nonsecretable TNF-alpha mutant and the engineered receptor may or may not be encoded from the vector as separate polypeptides. In specific cases, sequence of the vector that encodes the nonsecretable TNF-alpha mutant and sequence of the vector that encodes the engineered receptor are separated on the vector by a 2A element or an IRES element. The vector may or may not further encode a cytokine, such as IL-15, IL-12, IL-18, IL-2, IL-7, or IL-21. The cytokine may be expressed from the vector as a separate polypeptide as the TNF-alpha mutant and the engineered receptor.

[0014] Embodiments of the disclosure include compositions of matter including a nucleic acid sequence comprising SEQ ID NO:15 or SEQ ID NO:16.

[0015] It is specifically contemplated that any limitation discussed with respect to one embodiment of the invention may apply to any other embodiment of the invention. Furthermore, any composition of the invention may be used in any method of the invention, and any method of the invention may be used to produce or to utilize any composition of the invention. Aspects of an embodiment set forth in the Examples are also embodiments that may be implemented in the context of embodiments discussed elsewhere in a different Example or elsewhere in the application, such as in the Brief Summary, Detailed Description, Claims, and Brief Description of Drawings.

[0016] The foregoing has outlined rather broadly the features and technical advantages of the present disclosure in order that the detailed description that follows may be better understood. Additional features and advantages will be described hereinafter which form the subject of the claims herein. It should be appreciated by those skilled in the art that the conception and specific embodiments disclosed may be readily utilized as a basis for modifying or designing other structures for carrying out the same purposes of the present designs. It should also be realized by those skilled in the art that such equivalent constructions do not depart from the spirit and scope as set forth in the appended claims. The novel features which are believed to be characteristic of the designs disclosed herein, both as to the organization and method of operation, together with further objects and advantages will be better understood from the following description when considered in connection with the accompanying figures. It is to be expressly understood, however, that each of the figures is provided for the purpose of illustration and description only and is not intended as a definition of the limits of the present disclosure.

BRIEF DESCRIPTION OF THE DRAWINGS

[0017] For a more complete understanding of the present disclosure, reference is now made to the following descriptions taken in conjunction with the accompanying drawings.

[0018] FIG. 1 is one example of an experimental plan to mutagenize TNF-alpha in order to ablate membrane cutting sites. Perez *et al.* (1990) reported that deletion in Valine at positions 1 and Proline at position 12 of the extracellular portion of TNF-alpha results in biologically active but non-cleavable TNF-alpha. The underlined nucleotides in the left panel show the deleted nucleotides during mutagenesis corresponding to positions 229-279 of nucleotide sequence. The wild type primer TCGAGAAGATGATCTGACTGCCTGGGCCAGAGG is SEQ ID NO:42, the Del VAL1 mutant primer TCG AGA AGA TGA TCT TGC CTG GGC CAG AGG-3 is SEQ ID NO:43, and the CP496 oligonucleotide TGA TCT TGC CTG is SEQ ID NO:44. The wild type primer TAC AAC ATG GGC TACAGGCTTGTCACTCGGGGT is SEQ ID NO:45, the Del PRO 12 mutant primer TAC AAC ATG GGC TAC CTT GTC ACT CGG GGT is SEQ ID NO:46, and the CP498 oligonucleotide GGC TAC CTT GTC is SEQ ID NO:47. The Perez *et al.* (1990) sequence CAGGCAGTCAGATCATCTTCTCGAACCCCGAGTGACAAGCCTGTAGCC is SEQ ID NO:48, and the sequence QAVRSSSRTPSDKPVA is SEQ ID NO:49.

[0019] FIG. 2A illustrates one example of a vector that separately encodes a TNF-alpha mutant (delVal1 and delProl12) and an example of a CD19-specific chimeric antigen receptor (CAR) (left panel). The right panel illustrates an example of a vector configuration in which the mutant TNF-alpha is encoded as a separate polypeptide from both the CAR molecule and a cytokine. FIG. 2B illustrates one example of a vector that separately encodes a TNF-alpha mutant (delVal13), an example of a CAR, and a cytokine. FIG. 2C illustrates one example of a vector that separately encodes a TNF-alpha mutant (delVal1 and delVal13) and an example of a CAR. FIG. 2D illustrates one example of a vector that separately encodes a TNF-alpha mutant (where 13 aa spanning Val 1 to Val 13 have been deleted) and an example of a CAR. FIG. 2E illustrates one example of a vector that separately encodes a TNF-alpha mutant (delAla-1 to delVal13 where 14 aa spanning from Ala-1 to Val13 have been deleted) and an example of a CAR.

[0020] FIG. 3 shows that NK cells transduced with a vector having a construct separately encoding both a TNF-alpha mutant and a CAR express both the CAR and TNF-alpha on their surface.

[0021] FIG. 4A illustrates examples of TNF-alpha inhibitors.

[0022] FIG. 4B demonstrates that NK cells transduced with a vector having a construct separately encoding both a TNF-alpha mutant and a CAR are targeted by TNF-alpha antagonists and eliminated by complement-dependent cytotoxicity (CDC). Greater than 70% of NK cells expressing mutant TNF-alpha are eliminated by CDC within 90 minutes of treatment with infliximab.

[0023] FIG. 5A shows that NK cells transduced with a vector having a construct separately encoding both a TNF-alpha mutant and a CAR produce more effector cytokines and degranulate more efficiently than CAR19-NK cells in response to Raji targets. FIG. 5B shows NK cells transduced with a vector having a construct separately encoding both a TNF-alpha mutant and a CAR construct kill Raji targets efficiently.

[0024] FIG. 6 demonstrates that NK cells transduced with a vector that separately expresses a CD19-specific CAR and a TNF-alpha mutant do not exhibit off-target activity.

[0025] FIG. 7 demonstrates that NK cells transduced with a vector that separately expresses a CD19-specific CAR and a TNF-alpha mutant do not exhibit off-target activity and do not secrete TNF-alpha non-specifically.

[0026] FIG. 8 illustrates that TNF-alpha receptor binding sites for TNF receptors 1 and 2 *vs.* TNF-alpha antibodies infliximab and adalimumab are different. The sequence in the figure is SEQ ID NO:50.

[0027] FIG. 9 provides a structure of TNF-alpha with noted domains. The sequences in the figure are SEQ ID NOS 17, 54-59, 51, 18, and 18-21, respectively, in order of appearance.

[0028] FIG. 10 illustrates a TNFalpha mutation that combines a mutation in the casein kinase I (CKI) consensus sequence in the cytoplasmic domain (underlined) with deletion of Ala-3 and Gln -2 (in addition to deletion of Ala -1 through and including deletion of Val13 that is not depicted) in addition to six examples of additional mutations that interfere with binding to TNF Receptor 1 and TNF Receptor 2 (such mutated sequences are double underlined). The nucleotide sequence in the figure is SEQ ID NO:52, and the polypeptide sequence in the figure is SEQ ID NO:53.

[0029] FIGS. 11A-11B demonstrate that antitumor activity of NK cells transduced with a TNF-alpha mutant-CAR19-IL15 construct is superior to the iC9-CAR19-IL15 construct. In FIG. 11A, NSG mice with Raji tumor received 3 x 10e6 CAR cord blood NK cells transduced with TNF-alpha mut-CAR19-IL15 construct or iC9-CAR19-IL15 construct. FIG. 11B demonstrates percent survival over time.

DETAILED DESCRIPTION

[0030] The present disclosure incorporates by reference herein U.S. Provisional Patent Application Serial No. 62/769,414, filed November 19, 2018; U.S. Provisional Patent Application Serial No. 62/773,394, filed November 30, 2018; and U.S. Provisional Patent Application Serial No. 62/791491, filed January 11, 2019.

[0031] As used herein the specification, "a" or "an" may mean one or more. As used herein in the claim(s), when used in conjunction with the word "comprising", the words "a" or "an" may mean one or more than one. As used herein “another” may mean at least a second or more. In specific embodiments, aspects of the disclosure may “consist essentially of” or “consist

of" one or more sequences of the disclosure, for example. Some embodiments of the invention may consist of or consist essentially of one or more elements, method steps, and/or methods of the disclosure. It is contemplated that any method or composition described herein can be implemented with respect to any other method or composition described herein. The scope of the present application is not intended to be limited to the particular embodiments of the process, machine, manufacture, composition of matter, means, methods and steps described in the specification. As used herein, the terms "or" and "and/or" are utilized to describe multiple components in combination or exclusive of one another. For example, "x, y, and/or z" can refer to "x" alone, "y" alone, "z" alone, "x, y, and z," "(x and y) or z," "x or (y and z)," or "x or y or z." It is specifically contemplated that x, y, or z may be specifically excluded from an embodiment.

[0032] Throughout this application, the term "about" is used according to its plain and ordinary meaning in the area of cell and molecular biology to indicate that a value includes the standard deviation of error for the device or method being employed to determine the value.

[0033] The term "engineered" as used herein refers to an entity that is generated by the hand of man, including a cell, nucleic acid, polypeptide, vector, and so forth. In at least some cases, an engineered entity is synthetic and comprises elements that are not naturally present or configured in the manner in which it is utilized in the disclosure.

[0034] Reference throughout this specification to "one embodiment," "an embodiment," "a particular embodiment," "a related embodiment," "a certain embodiment," "an additional embodiment," or "a further embodiment" or combinations thereof means that a particular feature, structure or characteristic described in connection with the embodiment is included in at least one embodiment of the present invention. Thus, the appearances of the foregoing phrases in various places throughout this specification are not necessarily all referring to the same embodiment. Furthermore, the particular features, structures, or characteristics may be combined in any suitable manner in one or more embodiments.

I. [0035] General Embodiments

[0036] Embodiments of the present disclosure concern methods and compositions that provide for a cell therapy to be terminated. The present disclosure provides both a marker moiety and a suicide/depletion moiety for cell therapy, based on uncleavable mutants of the 26 kd TNF-alpha. The TNF-alpha mutants are uncleavable that leaves them membrane bound and

nonsecretable. Cells expressing the uncleavable TNF-alpha mutants can be targeted for selective deletion including, for example, using FDA-approved TNF-α antibodies currently in the clinic, such as etanercept, infliximab, or adalilumab. The mutated TNF-alpha polypeptide may be co-expressed with one or more therapeutic transgenes, such as a gene encoding a TCR or CAR. In addition, the TNF-alpha mutant expressing cells have superior activity against the tumor target, mediated by the biological activity of the membrane-bound TNF-alpha protein.

II. [0037] TNF-alpha Mutants

[0038] The present disclosure encompasses mutants of TNF-alpha whose expression in particular cells allows the mutant TNF to be targeted by an agent that binds the mutant, thereby causing death for the particular TNF-alpha mutant-bearing cells. In particular embodiments, the mutant TNF-alpha polypeptides are uncleavable and nonsecretable because of one or more mutations, and such uncleavable and nonsecretable polypeptides render the mutant TNF-alpha to be membrane bound. The association of the membrane bound TNF-alpha in the cell allows the cell to be killed when the membrane bound TNF-alpha is targeted by an agent that binds it directly or indirectly, including an inhibitor. In embodiments wherein the TNF-alpha inhibitor is an antibody, the cell may die by complement-dependent cytotoxicity, and in embodiments wherein the TNF-alpha inhibitor is not an antibody, the cell may die by another mechanism, such as apoptosis.

[0039] Therefore, in specific embodiments of the mutant, the membrane cutting site(s) are ablated, thereby retaining surface expression on the cell and endowing the ability of the cell to be targeted for destruction. Thus, the disclosure contemplates mutant membrane-bound TNF-alpha as a suicide gene for the selective deletion of transduced cells.

[0040] TNF-alpha has a 26kD transmembrane form and a 17 kD secretory component. FIG. 1 herein (right panel from Perez *et al.* (1990)) illustrates some mutants encompassed by the disclosure. In specific embodiments, examples of TNF-alpha mutants of the disclosure include at least the following with respect to the 17 kD TNF: (1) deletion of Val1 and deletion of Prol12; (2) deletion of Val13; (3) deletion of Val1 and deletion of Val13; (4) deletion of Val1 through and including Prol12 and also deletion of Val13 (delete 13aa); (5) deletion of Ala -3 through to and including Val 13 (delete 16 aa); (6) deletion of Ala-1 through to and include Val13 (delete 14aa). In specific embodiments, a TNF-alpha mutant comprises deletion of the respective amino acid at position -3, -2, -1, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, or a

combination thereof. Specific combinations include deletions at positions -3 through and including 13; -3 through and including 12; -3 through and including 11; -3 through and including 10; -3 through and including 9; -3 through and including 8; -3 through and including 7; -3 through and including 6; -3 through and including 5; -3 through and including 4; -3 through and including 3; -3 through and including 2; -3 through and including 1; -3 through and including -1; -3 through and including -2; -2 through and including 13; -2 through and including 12; -2 through and including 11; -2 through and including 10; -2 through and including 9; -2 through and including 8; -2 through and including 7; -2 through and including 6; -2 through and including 5; -2 through and including 4; -2 through and including 3; -2 through and including 2; -2 through and including 1; -2 through and including -1; -1 through and including 13; -1 through and including 12; -1 through and including 11; -1 through and including 10; -1 through and including 9; -1 through and including 8; -1 through and including 7; -1 through and including 6; -1 through and including 5; -1 through and including 4; -1 through and including 3; -1 through and including 2; -1 through and including 1; 1 through and including 13; 1 through and including 12; 1 through and including 11; 1 through and including 10; 1 through and including 9; 1 through and including 8; 1 through and including 7; 1 through and including 6; 1 through and including 5; 1 through and including 4; 1 through and including 3; 1 through and including 2; and so forth.

[0041] The TNF-alpha mutants may be generated by any suitable method, but in specific embodiments they are generated by site-directed mutagenesis. In some cases, the TNF-alpha mutants may have mutations other than those that render the protein uncleavable. In specific cases, the TNF-alpha mutants may have 1, 2, 3, or more mutations other than the deletions at Val1, Pro12, and/or Val13 or the region there between. The mutations other than those that render the mutants nonsecretable may be one or more of an amino acid substitution, deletion, addition, inversion, and so forth. In cases wherein the additional mutation is an amino acid substitution, the substitution may or may not be to a conservative amino acid, for example. In some cases, 1, 2, 3, 4, 5, or more additional amino acids may be present on the N-terminal and/or C-terminal ends of the protein. In some cases, a TNF-alpha mutant has (1) one or more mutations that render the mutant nonsecretable; (2) one or more mutations that prevents outside-in signaling for the mutant; and/or (3) one or more mutations that interfere with binding of the mutant to TNF Receptor 1 and/or TNF Receptor 2 and render them inactive.

[0042] TNF-alpha mutant delVal1 delProl12 amino acid sequence

[0043]

MSTESMIRDVELAEEALPKKTGGPQGSRRCLFLSLFSFLIVAGATTLFCLLHFGVIGPQREEFPRDLSLIS
PLAQARSSSRTPSDKVAHVVANPQAEGQLQWLNRRANALLANGVELRDNQLVVPSEGLYLIYSQVLFKGQGCPSTHV
LLTHTISRIAVSYQTKVNLLSAIKSPCQRETPEGAEAKPWYEPIYLGGVFQLEKGDRLSAEINRPDYLDFAESGQVY
FGIIAL (SEQ ID NO:1)

[0044] TNF-alpha mutant- delVal1 del Prol12 nucleic acid sequence

[0045]

atgagcactgaaagcatgatccgggacgtggagctggccgaggaggcgctccccaagaa
gacaggggggccccagggctccaggcggtgcttgttcctcagcctcttctccttcctgatcgtg
gcaggcgccaccacgctcttctgcctgctgcactttggagtgatcggcccccagagggaagagt
tccccagggacctctctctaatcagccctctggcccaggcaagatcatcttctcgaaccccgag
tgacaaggtagcccatgttgtagcaaaccctcaagctgaggggcagctccagtggctgaaccgc
cgggccaatgccctcctggccaatggcgtggagctgagagataaccagctggtggtgccatcag
agggcctgtacctcatctactcccaggtcctcttcaagggccaaggctgcccctccacccatgt
gctcctcacccacaccatcagccgcatcgccgtctcctaccagaccaaggtcaacctcctctct
gccatcaagagcccctgccagagggagaccccagaggggctgaggccaagccctggtatgagc
ccatctatctgggaggggtcttccagctggagaagggtgaccgactcagcgctgagatcaatcg
gcccgactatctcgactttgccgagtctgggcaggtctactttgggatcattgccctgtcg
(SEQ ID NO:2)

[0046] TNFa mutant- del Val1 to Val13 amino acid sequence (delete 13aa)

[0047]

MSTESMIRDVELAEEALPKKTGGPQGSRRCLFLSLFSFLIVAGATTLFCLLHFGVIGPQ
REEFPRDLSLISPLAQAAHVVANPQAEGQLQWLNRRANALLANGVELRDNQLVVPSEGLYLIYS
QVLFKGQGCPSTHVLLTHTISRIAVSYQTKVNLLSAIKSPCQRETPEGAEAKPWYEPIYLGGVF
QLEKGDRLSAEINRPDYLDFAESGQVYFGIIAL (SEQ ID NO:3)

[0048] TNFa mutant- del Val1 to Prol12 delVal13 (delete 13 aa) nucleic acid sequence

[0049]

atgagcactgaaagcatgatccgggacgtggagctggccgaggaggcgctccccaagaagacaggggggcc
ccagggctccaggcggtgcttgttcctcagcctcttctccttcctgatcgtggcaggcgccaccacgctcttctgcc
tgctgcactttggagtgatcggcccccagagggaagagttccccagggacctctctctaatcagccctctggcccag
gcagcccatgttgtagcaaaccctcaagctgaggggcagctccagtggctgaaccgccgggccaatgccctcctggc

caatggcgtggagctgagagataaccagctggtggtgccatcagagggcctgtacctcatctactcccaggtcctct
tcaagggccaaggctgcccctccacccatgtgctcctcacccacaccatcagccgcatcgccgtctcctaccagacc
aaggtcaacctcctctctgccatcaagagcccctgccagagggagaccccagaggggctgaggccaagccctggta
tgagcccatctatctgggaggggtcttccagctggagaagggtgaccgactcagcgctgagatcaatcggcccgact
atctcgactttgccgagtctgggcaggtctactttgggatcattgccctgtcg (SEQ ID NO:4)

[0050] TNF-alpha delVal1 delVal13 amino acid sequence

[0051]

MSTESMIRDVELAEEALPKKTGGPQGSRRCLFLSLFSFLIVAGATTLFCLLHFGVIGPQREEFPRDLSLIS
PLAQARSSSRTPSDKPAHVVANPQAEGQLQWLNRRANALLANGVELRDNQLVVPSEGLYLIYSQVLFKGQGCPSTHV
LLTHTISRIAVSYQTKVNLLSAIKSPCQRETPEGAEAKPWYEPIYLGGVFQLEKGDRLSAEINRPDYLDFAESGQVY
FGIIAL (SEQ ID NO:5)

[0052] TNF-alpha delVal1 delVal13 nucleic acid sequence

[0053]

atgagcactgaaagcatgatccgggacgtggagctggccgaggaggcgctccccaagaa
gacaggggggccccagggctccaggcggtgcttgttcctcagcctcttctccttcctgatcgtg
gcaggcgccaccacgctcttctgcctgctgcactttggagtgatcggcccccagagggaagagt
tccccagggacctctctctaatcagccctctggcccaggcaagatcatcttctcgaaccccgag
tgacaagcctgcccatgttgtagcaaaccctcaagctgaggggcagctccagtggctgaaccgc
cgggccaatgccctcctggccaatggcgtggagctgagagataaccagctggtggtgccatcag
agggcctgtacctcatctactcccaggtcctcttcaagggccaaggctgcccctccacccatgt
gctcctcacccacaccatcagccgcatcgccgtctcctaccagaccaaggtcaacctcctctct
gccatcaagagcccctgccagagggagaccccagaggggctgaggccaagccctggtatgagc
ccatctatctgggaggggtcttccagctggagaagggtgaccgactcagcgctgagatcaatcg
gcccgactatctcgactttgccgagtctgggcaggtctactttgggatcattgccctgtcg
(SEQ ID NO:6)

[0054] TNF-alpha delAla -3 to Val 13 nucleic acid sequence

[0055]

TCGAGTCGAGATGAGCACTGAAAGCATGATCCGGGACGTGGAGCTGGCCGAGGAGGCGCTCCCCAAGAAGA
CAGGGGGGCCCCAGGGCTCCAGGCGGTGCTTGTTCCTCAGCCTCTTCTCCTTCCTGATCGTGGCAGGCGCCACCACG
CTCTTCTGCCTGCTGCACTTTGGAGTGATCGGCCCCCAGAGGGAAGAGTTCCCCAGGGACCTCTCTCTAATCAGCCC
TCTGCAGGCAGCCCATGTTGTAGCAAACCCTCAAGCTGAGGGGCAGCTCCAGTGGCTGAACCGCCGGGCCAATGCCC
TCCTGGCCAATGGCGTGGAGCTGAGAGATAACCAGCTGGTGGTGCCATCAGAGGGCCTGTACCTCATCTACTCCCAG

GTCCTCTTCAAGGGCCAAGGCTGCCCCTCCACCCATGTGCTCCTCACCCACACCATCAGCCGCATCGCCGTCTCCTA
CCAGACCAAGGTCAACCTCCTCTCTGCCATCAAGAGCCCCTGCCAGAGGGAGACCCCAGAGGGGGCTGAGGCCAAGC
CCTGGTATGAGCCCATCTATCTGGGAGGGGTCTTCCAGCTGGAGAAGGGTGACCGACTCAGCGCTGAGATCAATCGG
CCCGACTATCTCgACTTTGCCGAGTCTGGGCAGGTCTACTTTGGGATCATTGCCCTGTCGTCG (SEQ ID NO:38)

[0056] TNF-alpha del Ala -3 and del of Val 1 through to and including Val 13 amino acid sequence (del -3 and del of 1-13 (but not deletion of -2 and -1)):

[0057]

MSTESMIRDVELAEEALPKKTGGPQGSRRCLFLSLFSFLIVAGATTLFCLLHFGVIGPQREEFPRDLSLIS
PLQAAHVVANPQAEGQLQWLNRRANALLANGVELRDNQLVVPSEGLYLIYSQVLFKGQGCPSTHVLLTHTISRIAVS
YQTKVNLLSAIKSPCQRETPEGAEAKPWYEPIYLGGVFQLEKGDRLSAEINRPDYLDFAESGQVYFGIIAL (SEQ ID NO:39)

[0058] TNF-alpha mutant with del Ala-3 to Val13 nucleic acid sequence in addition to an example of a CIK motif mutation that prevents outside-in signaling and other mutations that interfere with TNFalpha binding to TNF Receptor 1 and TNF Receptor 2 (see FIG. 10)

[0059]

ATGAGCACTGAAATGCATCCCGGAAGGGGGTCCTGGCACGAGGAGGCGCTCCCCAAGAAGACAGGGGGGCC
CCAGGGCTCCAGGCGGTGCTTGTTCCTCAGCCTCTTCTCCTTCCTGATCGTGGCAGGCGCCACCACGCTCTTCTTCC
TGCTGCACTTTGGAGTGATCGGCCCCCAGAGGGAAGAGTTCCCCAGGGACCTCTCTCTAATCAGCCCTCTGGCCCAT
GTTGTAGCAAACCCTCAAGCTGAGGGGCAGCTCCAGTGGCTGAACCGCCGGGCCAATGCCCTCCTGGCCAATGGCGT
GGAGCTGAGAGATAACCAGCTGGTGGTGCCATCAGAGGGCCTGTACCTCATCTACTCCCAGGTCCTCTTCAAGGGCC
AAGGCTGCCCCTCCACCCATGTGCTCCTCACCCACACCATCAGCCGCATCGCCGTCTCCCACCAGACCAAGGTCAAC
CTCCTCTTCGCCATCAAGAGCCCCTGCCAGAGGGAGACCCCAGAGGGGGCTGAGGCCAAGCCCTGGTATGAGCCCAT
CTATCTGGGAGGGGTCTTCCAGCTGGAGAAGGGTGACCGACTCATCGCTGAGATCAATCGGCCCGACTATCTCTACT
TTGCCGAGTATGGGCAGGTCTACTTTGGGATCATTGCCCTGTCG (SEQ ID NO:40)

[0060] TNF-alpha mutant with del Ala-3 to Val13 amino acid sequence encoded by SEQ ID NO:40

[0061]

MSTEMHPGRGSWHEEALPKKTGGPQGSRRCLFLSLFSFLIVAGATTLFFLLHFGVIGPQREEFPRDLSLIS
PLAHVVANPQAEGQLQWLNRRANALLANGVELRDNQLVVPSEGLYLIYSQVLFKGQGCPSTHVLLTHTISRIAVSHQ
TKVNLLFAIKSPCQRETPEGAEAKPWYEPIYLGGVFQLEKGDRLIAEINRPDYLYFAEYGQVYFGIIALS (SEQ ID NO:41)

[0062] In specific embodiments, a TNF-alpha mutant may comprise deletion of Ala-3 to Val13 but not also comprise a CIK motif mutation and a mutation that interferes with binding to TNF Receptor 1 and/or TNF Receptor 2.

[0063] For reference, TNF Wild type, 26 kD, version amino acid sequence

[0064]

MSTESMIRDVELAEEALPKKTGGPQGSRRCLFLSLFSFLIVAGATTLFCLLHFGVIGPQREEFPRDLSLIS PLAQAVRSSSRTPSDKPVAHVVANPQAEGQLQWLNRRANALLANGVELRDNQLVVPSEGLYLIYSQVLFKGQGCPST HVLLTHTISRIAVSYQTKVNLLSAIKSPCQRETPEGAEAKPWYEPIYLGGVFQLEKGDRLSAEINRPDYLDFAESGQ VYFGIIAL (SEQ ID NO:7)

[0065] For reference, TNF Wild type, 17 kD version, amino acid sequence

[0066]

VRSSSRTPSDKPVAHVVANPQAEGQLQWLNRRANALLANGVELRDNQLVVPSEGLYLIYSQVLFKGQGCPS THVLLTHTISRIAVSYQTKVNLLSAIKSPCQRETPEGAEAKPWYEPIYLGGVFQLEKGDRLSAEINRPDYLDFAESG QVYFGIIAL (SEQ ID NO:8)

[0067] TNF-alpha mutants lacking intracellular TNF signaling or TNF-receptor binding capability

[0068] These mutants have mutations in the cytoplasmic signaling domain and/or in the TNF-receptor binding regions and therefore do not exert any biological activity as they lack reverse signaling capability and/or the ability to bind TNF-receptors, respectively. This allows for the TNF-alpha in the construct to be a target for TNF inhibitors, while exerting no biological activity.

[0069] In some embodiments of the disclosure, TNF-alpha mutants lack part or all of the intracytoplasmic domain of TNF-alpha such that the TNF-alpha mutant is unable to exert intracellular signaling (reverse signaling). The nonsecretable TNF-alpha mutants may or may not also be mutated to lack part or all of the intracytoplasmic domain.

[0070] FIG. 9 provides some structure of TNF-alpha. As illustrated in FIG. 9, the intracytoplasmic domain comprises MSTESMIRDVELAEEALPKKTGGPQGSRRCLFL (SEQ ID NO:17). The casein kinase I (CKI) site is STES (SEQ ID NO:18). The transmembrane domain is FSFLIVAGATTLFCLLHFGVI (SEQ ID NO:19). The SPPL2b cut site is SL/LI.

The linker comprises GPQREEFPRDLSLISPLAQA (SEQ ID NO:20). The TACE cute site is VRSSSRTPSDKPV (SEQ ID NO:21). P01375 refers to the UniProt number of the protein. The sequence in FIG. 9 refers to only part of the TNF protein.

[0071] Specific examples of TNF-alpha mutant for the CKI motif (mutated sequence underlined) for nucleic acid and amino acid, respectively, is as follows:

[0072]

atgagcactgaaaTGCATCCCGGAAGGGGGTCCTGGCACgaggaggcgctccccaagaagacagggggcc
ccagggctccaggcggtgcttgttcctcagcctcttctccttcctgatcgtggcaggcgccaccacgctcttctgcc
tgctgcactttggagtgatcggcccccagagggaagagttccccagggacctctctctaatcagccctctggcccag
gcagcccatgttgtagcaaaccctcaagctgaggggcagctccagtggctgaaccgccgggccaatgccctcctggc
caatggcgtggagctgagagataaccagctggtggtgccatcagagggcctgtacctcatctactcccaggtcctct
tcaagggccaaggctgcccctccacccatgtgctcctcacccacaccatcagccgcatcgccgtctcctaccagacc
aaggtcaacctcctctctgccatcaagagcccctgccagagggagaccccagagggggctgaggccaagccctggta
tgagcccatctatctgggaggggtcttccagctggagaagggtgaccgactcagcgctgagatcaatcggcccgact
atctcgactttgccgagtctgggcaggtctactttgggatcattgccctgcg (SEQ ID NO:22)

[0073]

MSTEMHPGRGSWHEEALPKKTGGPQGSRRCLFLSLFSFLIVAGATTLFCLLHFGVIGPQREEFPRDLSLIS
PLAQAVRSSSRTPSDKPVAHVVANPQAEGQLQWLNRRANALLANGVELRDNQLVVPSEGLYLIYSQVLFKGQGCPST
HVLLTHTISRIAVSYQTKVNLLSAIKSPCQRETPEGAEAKPWYEPIYLGGVFQLEKGDRLSAEINRPDYLDFAESGQ
VYFGIIAL (SEQ ID NO:23)

[0074] One example of a TNF-alpha mutant having a mutation at M-71K in the intracytoplasmic sequence and another mutation at Y87H (mutated sequences underlined) for nucleic acid and amino acid, respectively, is as follows:

[0075]

atgagcactgaaagcaAgatccgggacgtggagctggccgaggaggcgctccccaagaagacagggggcc
ccagggctccaggcggtgcttgttcctcagcctcttctccttcctgatcgtggcaggcgccaccacgctcttctgcc
tgctgcactttggagtgatcggcccccagagggaagagttccccagggacctctctctaatcagccctctggcccag
gcagcccatgttgtagcaaaccctcaagctgaggggcagctccagtggctgaaccgccgggccaatgccctcctggc
caatggcgtggagctgagagataaccagctggtggtgccatcagagggcctgtacctcatctactcccaggtcctct
tcaagggccaaggctgcccctccacccatgtgctcctcacccacaccatcagccgcatcgccgtctccCaccagacc
aaggtcaacctcctctctgccatcaagagcccctgccagagggagaccccagagggggctgaggccaagccctggta
tgagcccatctatctgggaggggtcttccagctggagaagggtgaccgactcagcgctgagatcaatcggcccgact
atctcgactttgccgagtctgggcaggtctactttgggatcattgccctgtcg (SEQ ID NO:24)

[0076]

MSTESKIRDVELAEEALPKKTGGPQGSRRCLFLSLFSFLIVAGATTLFCLLHFGVIGPQREEFPRDLSLIS PLAQAAHVVANPQAEGQLQWLNRRANALLANGVELRDNQLVVPSEGLYLIYSQVLFKGQGCPSTHVLLTHTISRIAV SHQTKVNLLSAIKSPCQRETPEGAEAKPWYEPIYLGGVFQLEKGDRLSAEINRPDYLDFAESGQVYFGIIAL (SEQ ID NO:25)

[0077] One example of a TNF-alpha mutant having a mutation at S95F and C-28F (mutated sequences underlined) for nucleic acid and amino acid, respectively, is as follows:

[0078]

atgagcactgaaagcatgatccgggacgtggagctggccgaggaggcgctccccaagaagacaggggggcc ccagggctccaggcggtgcttgttcctcagcctcttctccttcctgatcgtggcaggcgccaccacgctcttctTcc tgctgcactttggagtgatcggcccccagagggaagagttccccagggacctctctctaatcagccctctggcccag gcagcccatgttgtagcaaaccctcaagctgaggggcagctccagtggctgaaccgccgggccaatgccctcctggc caatggcgtggagctgagagataaccagctggtggtgccatcagagggcctgtacctcatctactcccaggtcctct tcaagggccaaggctgcccctccacccatgtgctcctcacccacaccatcagccgcatcgccgtctcctaccagacc aaggtcaacctcctctTCgccatcaagagcccctgccagagggagaccccagagggggctgaggccaagccctggta tgagcccatctatctgggaggggtcttccagctggagaagggtgaccgactcagcgctgagatcaatcggcccgact atctcgactttgccgagtctgggcaggtctactttgggatcattgccctgtcg (SEQ ID NO:26)

[0079]

MSTESMIRDVELAEEALPKKTGGPQGSRRCLFLSLFSFLIVAGATTLFFLLHFGVIGPQREEFPRDLSLIS PLAQAAHVVANPQAEGQLQWLNRRANALLANGVELRDNQLVVPSEGLYLIYSQVLFKGQGCPSTHVLLTHTISRIAV SYQTKVNLLFAIKSPCQRETPEGAEAKPWYEPIYLGGVFQLEKGDRLSAEINRPDYLDFAESGQVYFGIIAL (SEQ ID NO:27)

[0080] One example of a TNF-alpha mutant having a mutation at S133I and S147Y (mutated sequences underlined) for nucleic acid and amino acid, respectively, is as follows:

[0081]

atgagcactgaaagcatgatccgggacgtggagctggccgaggaggcgctccccaagaagacaggggggcc ccagggctccaggcggtgcttgttcctcagcctcttctccttcctgatcgtggcaggcgccaccacgctcttctgcc tgctgcactttggagtgatcggcccccagagggaagagttccccagggacctctctctaatcagccctctggcccag gcagcccatgttgtagcaaaccctcaagctgaggggcagctccagtggctgaaccgccgggccaatgccctcctggc caatggcgtggagctgagagataaccagctggtggtgccatcagagggcctgtacctcatctactcccaggtcctct tcaagggccaaggctgcccctccacccatgtgctcctcacccacaccatcagccgcatcgccgtctcctaccagacc aaggtcaacctcctctctgccatcaagagcccctgccagagggagaccccagagggggctgaggccaagccctggta tgagcccatctatctgggaggggtcttccagctggagaagggtgaccgactcaTcgctgagatcaatcggcccgact atctcgactttgccgagtAtgggcaggtctactttgggatcattgccctgtcg (SEQ ID NO:28)

[0082]

MSTESMIRDVELAEEALPKKTGGPQGSRRCLFLSLFSFLIVAGATTLFCLLHFGVIGPQREEFPRDLSLIS
PLAQAAHVVANPQAEGQLQWLNRRANALLANGVELRDNQLVVPSEGLYLIYSQVLFKGQGCPSTHVLLTHTISRIAV
SYQTKVNLLSAIKSPCQRETPEGAEAKPWYEPIYLGGVFQLEKGDRLIAEINRPDYLDFAEYGQVYFGIIAL (SEQ ID NO:29)

[0083] One example of a TNF-alpha mutant having a mutation at Asp143Tyr and a deletion of Ala at position -1 (mutated sequence underlined and deleted sequence shown by strikethrough) for nucleic acid and amino acid, respectively, is as follows:

[0084]

atgagcactgaaagcatgatccgggacgtggagctggccgaggaggcgctccccaagaagacaggggggcc
ccagggctccaggcggtgcttgttcctcagcctcttctccttcctgatcgtggcaggcgccaccacgctcttctgcc
tgctgcactttggagtgatcggcccccagagggaagagttccccagggacctctctctaatcagccctctg~~gcc~~cag
gcagcccatgttgtagcaaaccctcaagctgaggggcagctccagtggctgaaccgccgggccaatgccctcctggc
caatggcgtggagctgagagataaccagctggtggtgccatcagagggcctgtacctcatctactcccaggtcctct
tcaagggccaaggctgcccctccacccatgtgctcctcacccacaccatcagccgcatcgccgtctcctaccagacc
aaggtcaacctcctctctgccatcaagagcccctgccagagggagaccccagaggggggctgaggccaagccctggta
tgagcccatctatctgggaggggtcttccagctggagaagggtgaccgactcagcgctgagatcaatcggcccgact
atctcTactttgccgagtctgggcaggtctactttgggatcattgccctgtcg (SEQ ID NO:30)

[0085]

MSTESMIRDVELAEEALPKKTGGPQGSRRCLFLSLFSFLIVAGATTLFCLLHFGVIGPQREEFPRDLSLIS
PLAQ~~A~~AHVVANPQAEGQLQWLNRRANALLANGVELRDNQLVVPSEGLYLIYSQVLFKGQGCPSTHVLLTHTISRIAV
SYQTKVNLLSAIKSPCQRETPEGAEAKPWYEPIYLGGVFQLEKGDRLSAEINRPDYLYFAESGQVYFGIIAL (SEQ ID NO:31)

[0086] Versions of SEQ ID NO:30 and SEQ ID NO:31 that lack the deleted sequences are as follows, respectively (with the mutated sequence still underlined).

[0087]

atgagcactgaaagcatgatccgggacgtggagctggccgaggaggcgctccccaagaagacaggggggcc
ccagggctccaggcggtgcttgttcctcagcctcttctccttcctgatcgtggcaggcgccaccacgctcttctgcc
tgctgcactttggagtgatcggcccccagagggaagagttccccagggacctctctctaatcagccctctgcaggca
gcccatgttgtagcaaaccctcaagctgaggggcagctccagtggctgaaccgccgggccaatgccctcctggccaa
tggcgtggagctgagagataaccagctggtggtgccatcagagggcctgtacctcatctactcccaggtcctcttca
agggccaaggctgcccctccacccatgtgctcctcacccacaccatcagccgcatcgccgtctcctaccagaccaag
gtcaacctcctctctgccatcaagagcccctgccagagggagaccccagaggggggctgaggccaagccctggtatga

gcccatctatctgggaggggtcttccagctggagaagggtgaccgactcagcgctgagatcaatcggcccgactatc
tcTactttgccgagtctgggcaggtctactttgggatcattgccctgtcg (SEQ ID NO:32)

[0088]

MSTESMIRDVELAEEALPKKTGGPQGSRRCLFLSLFSFLIVAGATTLFCLLHFGVIGPQREEFPRDLSLIS
PLAQAHVVANPQAEGQLQWLNRRANALLANGVELRDNQLVVPSEGLYLIYSQVLFKGQGCPSTHVLLTHTISRIAVS
YQTKVNLLSAIKSPCQRETPEGAEAKPWYEPIYLGGVFQLEKGDRLSAEINRPDYLYFAESGQVYFGIIAL (SEQ
ID NO:33)

[0089] One example of a TNF-alpha mutant having a combination of the CIK motif mutation and the above-referenced mutations are as follows, with the mutations underlined:

[0090]

ATGCTCGAGtcgagatgagcactgaaaTGCATCCCGGAAGGGGGTCCTGGCACgaggaggcgctccccaag
aagacaggggggccccagggctccaggcggtgcttgttcctcagcctcttctccttcctgatcgtggcaggcgccac
cacgctcttctTcctgctgcactttggagtgatcggcccccagagggaagagttccccagggacctctctctaatca
gccctctggcagcccatgttgtagcaaaccctcaagctgaggggcagctccagtggctgaaccgccgggccaatgcc
ctcctggccaatggcgtggagctgagagataaccagctggtggtgccatcagagggcctgtacctcatctactccca
ggtcctcttcaagggccaaggctgcccctccacccatgtgctcctcacccacaccatcagccgcatcgccgtctccC
accagaccaaggtcaacctcctctTCgccatcaagagcccctgccagagggagaccccagaggggctgaggccaag
ccctggtatgagcccatctatctgggaggggtcttccagctggagaagggtgaccgactcaTcgctgagatcaatcg
gcccgactatctcTactttgccgagtAtgggcaggtctactttgggatcattgccctgtcg (SEQ ID NO:34)

[0091]

MSTEMHPGRGSWHEEALPKKTGGPQGSRRCLFLSLFSFLIVAGATTLFFLLHFGVIGPQREEFPRDLSLIS
PLAQAHVVANPQAEGQLQWLNRRANALLANGVELRDNQLVVPSEGLYLIYSQVLFKGQGCPSTHVLLTHTISRIAVS
HQTKVNLLFAIKSPCQRETPEGAEAKPWYEPIYLGGVFQLEKGDRLIAEINRPDYLYFAEYGQVYFGIIAL (SEQ
ID NO:35)

III. [0092] Therapeutic Gene(s)

[0093] In some cases, cells expressing the TNF-alpha mutant(s) may also express one or more therapeutic genes. In cases where more than one therapeutic gene is employed, the therapeutic genes may or may not be of the same type of molecule. For example, in addition to the TNF-alpha mutant, a single cell may also express an engineered receptor, a cytokine, cytokine receptor, homing receptor, chemokine receptor, or a combination thereof. Encompassed herein are therapeutic gene nucleic acids; therapeutic gene products, including

polypeptides; vectors comprising the therapeutic gene nucleic acid; and cells harboring any thereof.

[0094] In particular embodiments, the mutant is co-expressed with at least one therapeutic gene, including a therapeutic transgene. The therapeutic transgene may be of any kind, but in specific embodiments it encodes an engineered receptor. Examples of engineered receptors include at least a T-cell receptor, chimeric antigen receptor (CAR), chemokine receptor, cytokine receptor, homing receptor, or a combination thereof. Any engineered receptor may target any particular ligand, such as an antigen, including a cancer antigen (such as a tumor antigen). The cancer antigens may be of any kind, including those associated with a particular cancer to be treated and that is desired to be targeted for specific elimination of the cancer.

[0095] In cases wherein the therapeutic gene product is an engineered receptor, the receptor comprises an antigen binding domain that may target any antigen, such as a tumor antigen. The antigen binding domain may comprise an scFv, for example. Antigenic molecules may come from infectious agents, auto-/self-antigens, tumor-/cancer-associated antigens, or tumor neoantigens, for example. Examples of antigens that may be targeted include but are not limited to antigens expressed on B-cells; antigens expressed on carcinomas, sarcomas, lymphomas, leukemia, germ cell tumors, and blastomas; antigens expressed on various immune cells; and antigens expressed on cells associated with various hematologic diseases, autoimmune diseases, and/or inflammatory diseases. Examples of specific antigens to target include CD19, CD5, CD99, CD33, CLL1, CD123, 4-1BB, 5T4, adenocarcinoma antigen, alpha-fetoprotein, BAFF, B-lymphoma cell, C242 antigen, CA-125, carbonic anhydrase 9 (CA-IX), C-MET, CCR4, CD152, CD20, CD200, CD22, CD221, CD23 (IgE receptor), CD28, CD30 (TNFRSF8), CD33, CD4, CD40, CD44 v6, CD51, CD52, CD56, CD74, CD80, CEA, CNTO888, CTLA-4, DRS, EGFR, EpCAM, CD3, FAP, fibronectin extra domain-B, folate receptor 1, GD2, GD3 ganglioside, glycoprotein 75, GPNMB, HER2/neu, HGF, human scatter factor receptor kinase, IGF-1 receptor, IGF-I, IgG1, L1-CAM, IL-13, IL-6, insulin-like growth factor I receptor, integrin-$\alpha 5\beta 1$, integrin$\alpha v\beta 3$, MORAb-009, MS4A1, MUC1, mucin CanAg, N-glycolylneuraminic acid, NPC-1C, PDGF-R .alpha., PDL192, phosphatidylserine, prostatic carcinoma cells, RANKL, RON, ROR1, SCH 900105, SDC1, SLAMF7, TAG-72, tenascin C, TGF $\beta 2$, TGF-β, TRAIL-R1, TRAIL-R2, tumor antigen CTAA16.88, VEGF-A, VEGFR-1, VEGFR2, vimentin, and combinations thereof. Any antigen receptor that may be utilized in methods and compositions of the disclosure may target any one of the above-referenced antigens,

or one or more others, and such an antigen receptor may be a CAR or a TCR. The same cells for therapy may utilize both a CAR and a TCR, in specific embodiments.

[0096] In cases wherein the therapeutic gene encodes a CAR, the CAR may be first generation, second generation, or third or subsequent generation, for example. The CAR may or may not be bispecific to two or more different antigens. The CAR may comprise one or more co-stimulatory domains. Each co-stimulatory domain may comprise the costimulatory domain of any one or more of, for example, members of the TNFR superfamily, CD28, CD137 (4-1BB), CD134 (OX40), Dap10, DAP12, CD27, CD2, CD5, ICAM-1, LFA-1 (CD11a/CD18), Lck, TNFR-I, TNFR-II, Fas, CD30, CD40 or combinations thereof, for example. In specific embodiments, the CAR comprises CD3zeta. In certain embodiments, the CAR lacks one or more specific costimulatory domains; for example, the CAR may lack 4-1BB.

[0097] In a specific embodiment, the CAR comprises at least DAP12 as a costimulatory domain, and in certain aspects the CAR polypeptide comprises a particular DAP12 amino acid sequence or is encoded by a particular DAP12 nucleic acid sequence. Examples are as follows:

[0098] DAP12 amino acid sequence

[0099]

MGGLEPCSRLLLLPLLLAVSGLRPVQAQAQSDCSCSTVSPGVLAGIVMGDLVLTVLIALAVYFLGRLVPRG
RGAAEAATRKQRITETESPYQELQGQRSDVYSDLNTQRPYYK (SEQ ID NO:9)

[0100] DAP12 nucleic acid sequence

[0101]

ATGGGGGGACTTGAACCCTGCAGCAGGCTCCTGCTCCTGCCTCTCCTGCTGGCTGTAAGTGGTCTCCGTCC
TGTCCAGGCCCAGGCCCAGAGCGATTGCAGTTGCTCTACGGTGAGCCCGGGCGTGCTGGCAGGGATCGTGATGGGAG
ACCTGGTGCTGACAGTGCTCATTGCCCTGGCCGTGTACTTCCTGGGCCGGCTGGTCCCTCGGGGGCGAGGGGCTGCG
GAGGCAGCGACCCGGAAACAGCGTATCACTGAGACCGAGTCGCCTTATCAGGAGCTCCAGGGTCAGAGGTCGGATGT
CTACAGCGACCTCAACACACAGAGGCCGTATTACAAATGA (SEQ ID NO:10)

[0102] In a specific embodiment, the CAR comprises at least CD28 as a costimulatory domain, and in certain aspects the CAR polypeptide comprises a particular CD28 amino acid sequence or is encoded by a particular CD28 nucleic acid sequence. Examples are as follows:

[0103] CD28 amino acid sequence

[0104]

KFWVLVVVGGVLACYSLLVTVAFIIFWVRSKRSRLLHSDYMNMTPRRPGPTRKHYQPYAPPRDFAAYRSRV KFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGE RRRGKGHDGLYQGLSTATKDTYDALHMQALPPRG (SEQ ID NO:11)

[0105] CD28 nucleic acid sequence

[0106]

ATTTTGGGTGCTGGTGGTGGTTGGTGGAGTCCTGGCTTGCTATAGCTTGCTAGTAACAG TGGCCTTTATTATTTTCTGGGTGAGGAGTAAGAGGAGCAGGCTCCTGCACAGTGACTACATGAA CATGACTCCCCGCCGCCCCGGGCCCACCCGCAAGCATTACCAGCCCTATGCCCCACCACGCGAC TTCGCAGCCTATCGCTCACGCG (SEQ ID NO:12)

[0107] In particular embodiments, the CAR polypeptide comprises an extracellular spacer domain that links the antigen binding domain and the transmembrane domain. Extracellular spacer domains may include, but are not limited to, Fc fragments of antibodies or fragments or derivatives thereof, hinge regions of antibodies or fragments or derivatives thereof, CH2 regions of antibodies, CH3 regions antibodies, artificial spacer sequences or combinations thereof. Examples of extracellular spacer domains include but are not limited to CD8-alpha hinge, artificial spacers made of polypeptides such as Gly3, or CH1, CH3 domains of IgGs (such as human IgG1 or IgG4). In specific cases, the extracellular spacer domain may comprise (i) a hinge, CH2 and CH3 regions of IgG4, (ii) a hinge region of IgG4, (iii) a hinge and CH2 of IgG4, (iv) a hinge region of CD8-alpha, (v) a hinge, CH2 and CH3 regions of IgG1, (vi) a hinge region of IgG1 or (vi) a hinge and CH2 of IgG1 or a combination thereof.

[0108] In specific embodiments, the hinge is from IgG1 and in certain aspects the CAR polypeptide comprises a particular IgG1 hinge amino acid sequence or is encoded by a particular IgG1 hinge nucleic acid sequence. Examples are as follows:

[0109] IgG1 hinge amino acid sequence

[0110]

SYVTVSSQDPAEPKSPDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKF NWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLP PSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMH EALHNHYTQKSLSLSPGKKDPK (SEQ ID NO:13)

[0111] IgG1 hinge nucleic acid sequence

[0112]

GTACGTCACTGTCTCTTCACAGGATCCCGCCGAGCCCAAATCTCCTGACAAAACTCACACATGCCCACCGT
GCCCAGCACCTGAACTCCTGGGGGGACCGTCAGTCTTCCTCTTCCCCCCAAAACCCAAGGACACCCTCATGATCTCC
CGGACCCCTGAGGTCACATGCGTGGTGGTGGACGTGAGCCACGAAGACCCTGAGGTCAAGTTCAACTGGTACGTGGA
CGGCGTGGAGGTGCATAATGCCAAGACAAAGCCGCGGGAGGAGCAGTACAACAGCACGTACCGTGTGGTCAGCGTCC
TCACCGTCCTGCACCAGGACTGGCTGAATGGCAAGGAGTACAAGTGCAAGGTCTCCAACAAAGCCCTCCCAGCCCCC
ATCGAGAAAACCATCTCCAAAGCCAAAGGGCAGCCCCGAGAACCACAGGTGTACACCCTGCCCCCATCCCGGGATGA
GCTGACCAAGAACCAGGTCAGCCTGACCTGCCTGGTCAAAGGCTTCTATCCCAGCGACATCGCCGTGGAGTGGGAGA
GCAATGGGCAACCGGAGAACAACTACAAGACCACGCCTCCCGTGCTGGACTCCGACGGCTCCTTCTTCCTCTACAGC
AAGCTCACCGTGGACAAGAGCAGGTGGCAGCAGGGGAACGTCTTCTCATGCTCCGTGATGCATGAGGCTCTGCACAA
CCACTACACGCAGAAGAGCCTCTCCCTGTCTCCGGGTAAAAAAGATC (SEQ ID NO:14)

IV. [0113] Vectors

[0114] The TNF-alpha mutant(s) may be delivered to the recipient cell by any suitable vector, including by a viral vector or by a non-viral vector. Examples of viral vectors include at least retroviral, lentiviral, adenoviral, or adeno-associated viral vectors. Examples of non-viral vectors include at least plasmids, transposons, lipids, nanoparticles, and so forth.

[0115] In cases wherein the cell is transduced with a vector encoding the TNF-alpha mutant and also requires transduction of another gene into the cell, such as a therapeutic gene product, the TNF-alpha mutant gene and therapeutic gene may or may not be comprised on or with the same vector. In some cases, the TNF-alpha mutant gene and the therapeutic gene are expressed from the same vector molecule, such as the same viral vector molecule. In such cases, the expression of the TNF-alpha mutant gene and the therapeutic gene may or may not be regulated by the same regulatory element(s). When the TNF-alpha mutant gene and the therapeutic gene are on the same vector, they may or may not be expressed as separate polypeptides. In cases wherein they are expressed as separate polypeptides, they may be separated on the vector by a 2A element or IRES element, for example. In some embodiments the TNF-alpha mutant and the therapeutic gene product are produced as a fusion protein.

[0116] In particular embodiments, the TNF-alpha mutant gene is expressed from a multicistronic vector. The multicistronic vector may encode at least one therapeutic gene in addition to the TNF-alpha mutant gene. In specific embodiments, the multicistronic vector encodes the TNF-alpha mutant and at least one engineered receptor, such as a T-cell receptor

and/or a CAR. In some cases, the multicistronic vector encodes at least one TNF-alpha mutant, at least one engineered receptor, and at least one cytokine. The cytokine may be of a particular type of cytokine, such as human or mouse or any species. In specific cases, the cytokine is interleukin (IL)15, IL12, IL2, IL18, and/or IL21.

[0117] One example of nucleic acid sequence for a vector that encodes a TNF-alpha mutant del Val1 del Pro12 and that separately encodes a CD19-specific CAR with an IgG1 hinge, CD28, and CD3zeta and that separately encodes IL15 is as follows:

[0118]

AATGAAAGACCCCACCTGTAGGTTTGGCAAGCTAGCTTAAGTAACGCCATTTTGCAAGGCATGGAAAAATA
CATAACTGAGAATAGAAAAGTTCAGATCAAGGTCAGGAACAGATGGAACAGCTGAATATGGGCCAAACAGGATATCT
GTGGTAAGCAGTTCCTGCCCCGGCTCAGGGCCAAGAACAGATGGAACAGCTGAATATGGGCCAAACAGGATATCTGT
GGTAAGCAGTTCCTGCCCCGGCTCAGGGCCAAGAACAGATGGTCCCCAGATGCGGTCCAGCCCTCAGCAGTTTCTAG
AGAACCATCAGATGTTTCCAGGGTGCCCCAAGGACCTGAAATGACCCTGTGCCTTATTTGAACTAACCAATCAGTTC
GCTTCTCGCTTCTGTTCGCGCGCTTATGCTCCCCGAGCTCAATAAAAGAGCCCACAACCCCTCACTCGGGGCGCCAG
TCCTCCGATTGACTGAGTCGCCCGGGTACCCGTGTATCCAATAAACCCTCTTGCAGTTGCATCCGACTTGTGGTCTC
GCTGTTCCTTGGGAGGGTCTCCTCTGAGTGATTGACTACCCGTCAGCGGGGGTCTTTCATTTGGGGGCTCGTCCGGG
ATCGGGAGACCCCTGCCCAGGGACCACCGACCCACCACCGGGAGGTAAGCTGGCCAGCAACTTATCTGTGTCTGTCC
GATTGTCTAGTGTCTATGACTGATTTTATGCGCCTGCGTCGGTACTAGTTAGCTAACTAGCTCTGTATCTGGCGGAC
CCGTGGTGGAACTGACGAGTTCGGAACACCCGGCCGCAACCCTGGGAGACGTCCCAGGGACTTCGGGGGCCGTTTTT
GTGGCCCGACCTGAGTCCTAAAATCCCGATCGTTTAGGACTCTTTGGTGCACCCCCCTTAGAGGAGGGATATGTGGT
TCTGGTAGGAGACGAGAACCTAAAACAGTTCCCGCCTCCGTCTGAATTTTTGCTTTCGGTTTGGGACCGAAGCCGCG
CCGCGCGTCTTGTCTGCTGCAGCATCGTTCTGTGTTGTCTCTGTCTGACTGTGTTTCTGTATTTGTCTGAAAATATG
GGCCCGGGCTAGCCTGTTACCACTCCCTTAAGTTTGACCTTAGGTCACTGGAAAGATGTCGAGCGGATCGCTCACAA
CCAGTCGGTAGATGTCAAGAAGAGACGTTGGGTTACCTTCTGCTCTGCAGAATGGCCAACCTTTAACGTCGGATGGC
CGCGAGACGGCACCTTTAACCGAGACCTCATCACCCAGGTTAAGATCAAGGTCTTTTCACCTGGCCCGCATGGACAC
CCAGACCAGGTGGGGTACATCGTGACCTGGGAAGCCTTGGCTTTTGACCCCCCTCCCTGGGTCAAGCCCTTTGTACA
CCCTAAGCCTCCGCCTCCTCTTCCTCCATCCGCCCCGTCTCTCCCCCTTGAACCTCCTCGTTCGACCCCGCCTCGAT
CCTCCCTTTATCCAGCCCTCACTCCTTCTCTAGGCGCCCCCATATGGCCATATGAGATCTTATATGGGGCACCCCCG
CCCCTTGTAAACTTCCCTGACCCTGACATGACAAGAGTTACTAACAGCCCCTCTCTCCAAGCTCACTTACAGGCTCT
CTACTTAGTCCAGCACGAAGTCTGGAGACCTCTGGCGGCAGCCTACCAAGAACAACTGGACCGACCGGTGGTACCTC
ACCCTTACCGAGTCGGCGACACAGTGTGGGTCCGCCGACACCAGACTAAGAACCTAGAACCTCGCTGGAAAGGACCT
TACACAGTCCTGCTGACCACCCCCACCGCCCTCAAAGTAGACGGCATCGCAGCTTGGATACACGCCGCCCACGTGAA
GGCTGCCGACCCCGGGGGTGGACCATCCTCTAGACTGCCATGCTCGAGATGAGCACTGAAAGCATGATCCGGGACGT
GGAGCTGGCCGAGGAGGCGCTCCCCAAGAAGACAGGGGGGCCCCAGGGCTCCAGGCGGTGCTTGTTCCTCAGCCTCT
TCTCCTTCCTGATCGTGGCAGGCGCCACCACGCTCTTCTGCCTGCTGCACTTTGGAGTGATCGGCCCCCAGAGGGAA
GAGTTCCCCAGGGACCTCTCTCTAATCAGCCCTCTGGCCCAGGCAAGATCATCTTCTCGAACCCCGAGTGACAAGGT
AGCCCATGTTGTAGCAAACCCTCAAGCTGAGGGGCAGCTCCAGTGGCTGAACCGCCGGGCCAATGCCCTCCTGGCCA

ATGGCGTGGAGCTGAGAGATAACCAGCTGGTGGTGCCATCAGAGGGCCTGTACCTCATCTACTCCCAGGTCCTCTTC
AAGGGCCAAGGCTGCCCCTCCACCCATGTGCTCCTCACCCACACCATCAGCCGCATCGCCGTCTCCTACCAGACCAA
GGTCAACCTCCTCTCTGCCATCAAGAGCCCCTGCCAGAGGGAGACCCCAGAGGGGGCTGAGGCCAAGCCCTGGTATG
AGCCCATCTATCTGGGAGGGGTCTTCCAGCTGGAGAAGGGTGACCGACTCAGCGCTGAGATCAATCGGCCCGACTAT
CTCGACTTTGCCGAGTCTGGGCAGGTCTACTTTGGGATCATTGCCCTGTCGCGAGCCGAGGGCAGGGGAAGTCTTCT
AACATGCGGGGACGTGGAGGAAAATCCCGGGCCCATGGAGTTTGGGCTGAGCTGGCTTTTTCTTGTGGCTATTTTAA
AAGGTGTCCAGTGCTCTAGAGACATCCAGATGACACAGACTACATCCTCCCTGTCTGCCTCTCTGGGAGACAGAGTC
ACCATCAGTTGCAGGGCAAGTCAGGACATTAGTAAATATTTAAATTGGTATCAGCAGAAACCAGATGGAACTGTTAA
ACTCCTGATCTACCATACATCAAGATTACACTCAGGAGTCCCATCAAGGTTCAGTGGCAGTGGGTCTGGAACAGATT
ATTCTCTCACCATTAGCAACCTGGAGCAAGAAGATATTGCCACTTACTTTTGCCAACAGGGTAATACGCTTCCGTAC
ACGTTCGGAGGGGGGACCAAGCTGGAGCTGAAACGTGGTGGTGGTGGTTCTGGTGGTGGTGGTTCTGGCGGCGGCGG
CTCCGGTGGTGGTGGATCCGAGGTGCAGCTGCAGCAGTCTGGACCTGGCCTGGTGGCGCCCTCACAGAGCCTGTCCG
TCACATGCACTGTCTCAGGGGTCTCATTACCCGACTATGGTGTAAGCTGGATTCGCCAGCCTCCACGAAAGGGTCTG
GAGTGGCTGGGAGTAATATGGGGTAGTGAAACCACATACTATAATTCAGCTCTCAAATCCAGACTGACCATCATCAA
GGACAACTCCAAGAGCCAAGTTTTCTTAAAAATGAACAGTCTGCAAACTGATGACACAGCCATTTACTACTGTGCCA
AACATTATTACTACGGTGGTAGCTATGCTATGGACTACTGGGGCCAAGGGACCACGGTCACTGTCTCCTCGTACGTC
ACTGTCTCTTCACAGGATCCCGCCGAGCCCAAATCTCCTGACAAAACTCACACATGCCCACCGTGCCCAGCACCTGA
ACTCCTGGGGGGGACCGTCAGTCTTCCTCTTCCCCCCAAAACCCAAGGACACCCTCATGATCTCCCGGACCCCTGAGG
TCACATGCGTGGTGGTGGACGTGAGCCACGAAGACCCTGAGGTCAAGTTCAACTGGTACGTGGACGGCGTGGAGGTG
CATAATGCCAAGACAAAGCCGCGGGAGGAGCAGTACAACAGCACGTACCGTGTGGTCAGCGTCCTCACCGTCCTGCA
CCAGGACTGGCTGAATGGCAAGGAGTACAAGTGCAAGGTCTCCAACAAAGCCCTCCCAGCCCCCATCGAGAAAACCA
TCTCCAAAGCCAAAGGGCAGCCCCGAGAACCACAGGTGTACACCCTGCCCCCATCCCGGGATGAGCTGACCAAGAAC
CAGGTCAGCCTGACCTGCCTGGTCAAAGGCTTCTATCCCAGCGACATCGCCGTGGAGTGGGAGAGCAATGGGCAACC
GGAGAACAACTACAAGACCACGCCTCCCGTGCTGGACTCCGACGGCTCCTTCTTCCTCTACAGCAAGCTCACCGTGG
ACAAGAGCAGGTGGCAGCAGGGGAACGTCTTCTCATGCTCCGTGATGCATGAGGCTCTGCACAACCACTACACGCAG
AAGAGCCTCTCCCTGTCTCCGGGTAAAAAAGATCCCAAATTTTGGGTGCTGGTGGTGGTTGGTGGAGTCCTGGCTTG
CTATAGCTTGCTAGTAACAGTGGCCTTTATTATTTTCTGGGTGAGGAGTAAGAGGAGCAGGCTCCTGCACAGTGACT
ACATGAACATGACTCCCCGCCGCCCCGGGCCCACCCGCAAGCATTACCAGCCCTATGCCCCACCACGCGACTTCGCA
GCCTATCGCTCACGCGTGAAGTTCAGCAGGAGCGCAGACGCCCCCGCGTACCAGCAGGGCCAGAACCAGCTCTATAA
CGAGCTCAATCTAGGACGAAGAGAGGAGTACGATGTTTTGGACAAAAGACGTGGCCGGGACCCTGAGATGGGGGGAA
AGCCGAGAAGGAAGAACCCTCAGGAAGGCCTGTACAATGAACTGCAGAAAGATAAGATGGCGGAGGCCTACAGTGAG
ATTGGGATGAAAGGCGAGCGCCGGAGGGGCAAGGGGCACGATGGCCTTTACCAGGGTCTCAGTACAGCCACCAAGGA
CACCTACGACGCCCTTCACATGCAGGCCCTGCCCCCTCGCGGACCGCAGTGTACTAATTATGCTCTCTTGAAATTGG
CTGGAGATGTTGAGAGCAATCCCGGGCCCATGCGCATTAGCAAGCCCCACCTGCGGAGCATCAGCATCCAGTGCTAC
CTGTGCCTGCTGCTGAACAGCCACTTCCTGACCGAGGCCGGCATCCACGTGTTCATCCTGGGCTGCTTCAGCGCCGG
ACTGCCCAAGACCGAGGCCAACTGGGTGAACGTGATCAGCGACCTGAAGAAGATCGAGGACCTGATCCAGAGCATGC
ACATCGACGCCACCCTGTACACCGAGAGCGACGTGCACCCCAGCTGCAAGGTGACCGCCATGAAGTGCTTTCTGCTG
GAACTGCAGGTGATCAGCCTGGAAAGCGGCGACGCCAGCATCCACGACACCGTGGAGAACCTGATCATCCTGGCCAA
CAACAGCCTGAGCAGCAACGGCAACGTGACCGAGAGCGGCTGCAAAGAGTGCGAGGAACTGGAAGAGAAGAACATCA
AAGAGTTTCTGCAGAGCTTCGTGCACATCGTGCAGATGTTCATCAACACCAGCTGACAATTGCGCGTCATCATCGAT

CCGGATTAGTCCAATTTGTTAAAGACAGGATATCAGTGGTCCAGGCTCTAGTTTTGACTCAACAATATCACCAGCTG
AAGCCTATAGAGTACGAGCCATAGATAAAATAAAAGATTTTATTTAGTCTCCAGAAAAAGGGGGGAATGAAAGACCC
CACCTGTAGGTTTGGCAAGCTAGCTTAAGTAACGCCATTTTGCAAGGCATGGAAAAATACATAACTGAGAATAGAGA
AGTTCAGATCAAGGTCAGGAACAGATGGAACAGCTGAATATGGGCCAAACAGGATATCTGTGGTAAGCAGTTCCTGC
CCCGGCTCAGGGCCAAGAACAGATGGAACAGCTGAATATGGGCCAAACAGGATATCTGTGGTAAGCAGTTCCTGCCC
CGGCTCAGGGCCAAGAACAGATGGTCCCCAGATGCGGTCCAGCCCTCAGCAGTTTCTAGAGAACCATCAGATGTTTC
CAGGGTGCCCCAAGGACCTGAAATGACCCTGTGCCTTATTTGAACTAACCAATCAGTTCGCTTCTCGCTTCTGTTCG
CGCGCTTCTGCTCCCCGAGCTCAATAAAAGAGCCCACAACCCCTCACTCGGGGCGCCAGTCCTCCGATTGACTGAGT
CGCCCGGGTACCCGTGTATCCAATAAACCCTCTTGCAGTTGCATCCGACTTGTGGTCTCGCTGTTCCTTGGGAGGGT
CTCCTCTGAGTGATTGACTACCCGTCAGCGGGGGTCTTTCACACATGCAGCATGTATCAAAATTAATTTGGTTTTTT
TTCTTAAGTATTTACATTAAATGGCCATAGTACTTAAAGTTACATTGGCTTCCTTGAAATAAACATGGAGTATTCAG
AATGTGTCATAAATATTTCTAATTTTAAGATAGTATCTCCATTGGCTTTCTACTTTTTCTTTTATTTTTTTTTGTCC
TCTGTCTTCCATTTGTTGTTGTTGTTTGTTTGTTTGTTGGTTGGTTGGTTAATTTTTTTTTAAAGATCCT
ACACTATAGTTCAAGCTAGACTATTAGCTACTCTGTAACCCAGGGTGACCTTGAAGTCATGGGTAGCCTGCTGTTTT
AGCCTTCCCACATCTAAGATTACAGGTATGAGCTATCATTTTTGGTATATTGATTGATTGATTGATTGATGTGTGTG
TGTGTGATTGTGTTTGTGTGTGTGACTGTGAAAATGTGTGTATGGGTGTGTGTGAATGTGTGTATGTATGTGTGTGT
GTGAGTGTGTGTGTGTGTGTGTGCATGTGTGTGTGTGTGACTGTGTCTATGTGTATGACTGTGTGTGTGTGTGTGTG
TGTGTGTGTGTGTGTGTGTGTGTGTGTTGTGAAAAAATATTCTATGGTAGTGAGAGCCAACGCTCCGGCTCAGGT
GTCAGGTTGGTTTTTGAGACAGAGTCTTTCACTTAGCTTGGAATTCACTGGCCGTCGTTTTACAACGTCGTGACTGG
GAAAACCCTGGCGTTACCCAACTTAATCGCCTTGCAGCACATCCCCCTTTCGCCAGCTGGCGTAATAGCGAAGAGGC
CCGCACCGATCGCCCTTCCCAACAGTTGCGCAGCCTGAATGGCGAATGGCGCCTGATGCGGTATTTTCTCCTTACGC
ATCTGTGCGGTATTTCACACCGCATATGGTGCACTCTCAGTACAATCTGCTCTGATGCCGCATAGTTAAGCCAGCCC
CGACACCCGCCAACACCCGCTGACGCGCCCTGACGGGCTTGTCTGCTCCCGGCATCCGCTTACAGACAAGCTGTGAC
CGTCTCCGGGAGCTGCATGTGTCAGAGGTTTTCACCGTCATCACCGAAACGCGCGAGACGAAAGGGCCTCGTGATAC
GCCTATTTTTATAGGTTAATGTCATGATAATAATGGTTTCTTAGACGTCAGGTGGCACTTTTCGGGGAAATGTGCGC
GGAACCCCTATTTGTTTATTTTTCTAAATACATTCAAATATGTATCCGCTCATGAGACAATAACCCTGATAAATGCT
TCAATAATATTGAAAAAGGAAGAGTATGAGTATTCAACATTTCCGTGTCGCCCTTATTCCCTTTTTTGCGGCATTTT
GCCTTCCTGTTTTTGCTCACCCAGAAACGCTGGTGAAAGTAAAAGATGCTGAAGATCAGTTGGGTGCACGAGTGGGT
TACATCGAACTGGATCTCAACAGCGGTAAGATCCTTGAGAGTTTTCGCCCCGAAGAACGTTTTCCAATGATGAGCAC
TTTTAAAGTTCTGCTATGTGGCGCGGTATTATCCCGTATTGACGCCGGGCAAGAGCAACTCGGTCGCCGCATACACT
ATTCTCAGAATGACTTGGTTGAGTACTCACCAGTCACAGAAAAGCATCTTACGGATGGCATGACAGTAAGAGAATTA
TGCAGTGCTGCCATAACCATGAGTGATAACACTGCGGCCAACTTACTTCTGACAACGATCGGAGGACCGAAGGAGCT
AACCGCTTTTTTGCACAACATGGGGGATCATGTAACTCGCCTTGATCGTTGGGAACCGGAGCTGAATGAAGCCATAC
CAAACGACGAGCGTGACACCACGATGCCTGTAGCAATGGCAACAACGTTGCGCAAACTATTAACTGGCGAACTACTT
ACTCTAGCTTCCCGGCAACAATTAATAGACTGGATGGAGGCGGATAAAGTTGCAGGACCACTTCTGCGCTCGGCCCT
TCCGGCTGGCTGGTTTATTGCTGATAAATCTGGAGCCGGTGAGCGTGGGTCTCGCGGTATCATTGCAGCACTGGGGC
CAGATGGTAAGCCCTCCCGTATCGTAGTTATCTACACGACGGGGAGTCAGGCAACTATGGATGAACGAAATAGACAG
ATCGCTGAGATAGGTGCCTCACTGATTAAGCATTGGTAACTGTCAGACCAAGTTTACTCATATATACTTTAGATTGA
TTTAAAACTTCATTTTTAATTTAAAAGGATCTAGGTGAAGATCCTTTTTGATAATCTCATGACCAAAATCCCTTAAC
GTGAGTTTTCGTTCCACTGAGCGTCAGACCCCGTAGAAAAGATCAAAGGATCTTCTTGAGATCCTTTTTTTCTGCGC

GTAATCTGCTGCTTGCAAACAAAAAAACCACCGCTACCAGCGGTGGTTTGTTTGCCGGATCAAGAGCTACCAACTCT
TTTTCCGAAGGTAACTGGCTTCAGCAGAGCGCAGATACCAAATACTGTTCTTCTAGTGTAGCCGTAGTTAGGCCACC
ACTTCAAGAACTCTGTAGCACCGCCTACATACCTCGCTCTGCTAATCCTGTTACCAGTGGCTGCTGCCAGTGGCGAT
AAGTCGTGTCTTACCGGGTTGGACTCAAGACGATAGTTACCGGATAAGGCGCAGCGGTCGGGCTGAACGGGGGGTTC
GTGCACACAGCCCAGCTTGGAGCGAACGACCTACACCGAACTGAGATACCTACAGCGTGAGCTATGAGAAAGCGCCA
CGCTTCCCGAAGGGAGAAAGGCGGACAGGTATCCGGTAAGCGGCAGGGTCGGAACAGGAGAGCGCACGAGGGAGCTT
CCAGGGGGAAACGCCTGGTATCTTTATAGTCCTGTCGGGTTTCGCCACCTCTGACTTGAGCGTCGATTTTTGTGATG
CTCGTCAGGGGGGCGGAGCCTATGGAAAAACGCCAGCAACGCGGCCTTTTTACGGTTCCTGGCCTTTTGCTGGCCTT
TTGCTCACATGTTCTTTCCTGCGTTATCCCCTGATTCTGTGGATAACCGTATTACCGCCTTTGAGTGAGCTGATACC
GCTCGCCGCAGCCGAACGACCGAGCGCAGCGAGTCAGTGAGCGAGGAAGCGGAAGAGCGCCCAATACGCAAACCGCC
TCTCCCCGCGCGTTGGCCGATTCATTAATGCAGCTGGCACGACAGGTTTCCCGACTGGAAAGCGGGCAGTGAGCGCA
ACGCAATTAATGTGAGTTAGCTCACTCATTAGGCACCCCAGGCTTTACACTTTATGCTTCCGGCTCGTATGTTGTGT
GGAATTGTGAGCGGATAACAATTTCACACAGGAAACAGCTATGACCATGATTACGCCAAGCTTTGCTCTTAGGAGTT
TCCTAATACATCCCAAACTCAAATATATAAAGCATTTGACTTGTTCTATGCCCTAGGGGGCGGGGGGAAGCTAAGCC
AGCTTTTTTTAACATTTAAAATGTTAATTCCATTTTAAATGCACAGATGTTTTTATTTCATAAGGGTTTCAATGTGC
ATGAATGCTGCAATATTCCTGTTACCAAAGCTAGTATAAATAAAAATAGATAAACGTGGAAATTACTTAGAGTTTCT
GTCATTAACGTTTCCTTCCTCAGTTGACAACATAAATGCGCTGCTGAGCAAGCCAGTTTGCATCTGTCAGGATCAAT
TTCCCATTATGCCAGTCATATTAATTACTAGTCAATTAGTTGATTTTTATTTTTGACATATACATGTG (SEQ ID NO:15)

[0119] One example of amino acid sequence for a vector that encodes a TNF-alpha mutant del Val1 del Pro12 and that separately encodes a CD19-specific CAR with an IgG1 hinge, CD28, and CD3zeta and that separately encodes IL15 is as follows:

[0120]

MSTESMIRDVELAEEALPKKTGGPQGSRRCLFLSLFSFLIVAGATTLFCLLHFGVIGPQREEFPRDLSLIS
PLAQARSSSRTPSDKVAHVVANPQAEGQLQWLNRRANALLANGVELRDNQLVVPSEGLYLIYSQVLFKGQGCPSTHV
LLTHTISRIAVSYQTKVNLLSAIKSPCQRETPEGAEAKPWYEPIYLGGVFQLEKGDRLSAEINRPDYLDFAESGQVY
FGIIALSRAEGRGSLLTCGDVEENPGPMEFGLSWLFLVAILKGVQCSRDIQMTQTTSSLSASLGDRVTISCRASQDI
SKYLNWYQQKPDGTVKLLIYHTSRLHSGVPSRFSGSGSGTDYSLTISNLEQEDIATYFCQQGNTLPYTFGGGTKLEL
KRGGGGSGGGGSGGGGSGGGGSEVQLQQSGPGLVAPSQSLSVTCTVSGVSLPDYGVSWIRQPPRKGLEWLGVIWGSE
TTYYNSALKSRLTIIKDNSKSQVFLKMNSLQTDDTAIYYCAKHYYYGGSYAMDYWGQGTTVTVSSYVTVSSQDPAEP
KSPDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREE
QYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKG
FYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGKK
DPKFWVLVVVGGVLACYSLLVTVAFIIFWVRSKRSRLLHSDYMNMTPRRPGPTRKHYQPYAPPRDFAAYRSRVKFSR
SADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRG
KGHDGLYQGLSTATKDTYDALHMQALPPRGPQCTNYALLKLAGDVESNPGPMRISKPHLRSISIQCYLCLLLNSHFL

TEAGIHVFILGCFSAGLPKTEANWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLLELQVISLESG
DASIHDTVENLIILANNSLSSNGNVTESGCKECEELEEKNIKEFLQSFVHIVQMFINTS (SEQ ID NO:36)

[0121] One example of nucleic acid sequence for a vector that encodes a TNF-alpha mutant del Val1 del Pro12 and that separately encodes a CD19-specific CAR with an IgG1 hinge, DAP12, and CD3zeta and that separately encodes IL15 is as follows:

[0122]

AATGAAAGACCCCACCTGTAGGTTTGGCAAGCTAGCTTAAGTAACGCCATTTTGCAAGGCATGGAAAAATA
CATAACTGAGAATAGAAAAGTTCAGATCAAGGTCAGGAACAGATGGAACAGCTGAATATGGGCCAAACAGGATATCT
GTGGTAAGCAGTTCCTGCCCCGGCTCAGGGCCAAGAACAGATGGAACAGCTGAATATGGGCCAAACAGGATATCTGT
GGTAAGCAGTTCCTGCCCCGGCTCAGGGCCAAGAACAGATGGTCCCCAGATGCGGTCCAGCCCTCAGCAGTTTCTAG
AGAACCATCAGATGTTTCCAGGGTGCCCCAAGGACCTGAAATGACCCTGTGCCTTATTTGAACTAACCAATCAGTTC
GCTTCTCGCTTCTGTTCGCGCGCTTATGCTCCCCGAGCTCAATAAAAGAGCCCACAACCCCTCACTCGGGGCGCCAG
TCCTCCGATTGACTGAGTCGCCCGGGTACCCGTGTATCCAATAAACCCTCTTGCAGTTGCATCCGACTTGTGGTCTC
GCTGTTCCTTGGGAGGGTCTCCTCTGAGTGATTGACTACCCGTCAGCGGGGGTCTTTCATTTGGGGGCTCGTCCGGG
ATCGGGAGACCCCTGCCCAGGGACCACCGACCCACCACCGGGAGGTAAGCTGGCCAGCAACTTATCTGTGTCTGTCC
GATTGTCTAGTGTCTATGACTGATTTTATGCGCCTGCGTCGGTACTAGTTAGCTAACTAGCTCTGTATCTGGCGGAC
CCGTGGTGGAACTGACGAGTTCGGAACACCCGGCCGCAACCCTGGGAGACGTCCCAGGGACTTCGGGGGCCGTTTTT
GTGGCCCGACCTGAGTCCTAAAATCCCGATCGTTTAGGACTCTTTGGTGCACCCCCCTTAGAGGAGGGATATGTGGT
TCTGGTAGGAGACGAGAACCTAAAACAGTTCCCGCCTCCGTCTGAATTTTTGCTTTCGGTTTGGGACCGAAGCCGCG
CCGCGCGTCTTGTCTGCTGCAGCATCGTTCTGTGTTGTCTCTGTCTGACTGTGTTTCTGTATTTGTCTGAAAATATG
GGCCCGGGCTAGCCTGTTACCACTCCCTTAAGTTTGACCTTAGGTCACTGGAAAGATGTCGAGCGGATCGCTCACAA
CCAGTCGGTAGATGTCAAGAAGAGACGTTGGGTTACCTTCTGCTCTGCAGAATGGCCAACCTTTAACGTCGGATGGC
CGCGAGACGGCACCTTTAACCGAGACCTCATCACCCAGGTTAAGATCAAGGTCTTTTCACCTGGCCCGCATGGACAC
CCAGACCAGGTGGGGTACATCGTGACCTGGGAAGCCTTGGCTTTTGACCCCCCTCCCTGGGTCAAGCCCTTTGTACA
CCCTAAGCCTCCGCCTCCTCTTCCTCCATCCGCCCCGTCTCTCCCCCTTGAACCTCCTCGTTCGACCCCGCCTCGAT
CCTCCCTTTATCCAGCCCTCACTCCTTCTCTAGGCGCCCCCATATGGCCATATGAGATCTTATATGGGGCACCCCCG
CCCCTTGTAAACTTCCCTGACCCTGACATGACAAGAGTTACTAACAGCCCCTCTCTCCAAGCTCACTTACAGGCTCT
CTACTTAGTCCAGCACGAAGTCTGGAGACCTCTGGCGGCAGCCTACCAAGAACAACTGGACCGACCGGTGGTACCTC
ACCCTTACCGAGTCGGCGACACAGTGTGGGTCCGCCGACACCAGACTAAGAACCTAGAACCTCGCTGGAAAGGACCT
TACACAGTCCTGCTGACCACCCCCACCGCCCTCAAAGTAGACGGCATCGCAGCTTGGATACACGCCGCCCACGTGAA
GGCTGCCGACCCCGGGGGTGGACCATCCTCTAGACTGCCATGCTCGAGATGAGCACTGAAAGCATGATCCGGGACGT
GGAGCTGGCCGAGGAGGCGCTCCCCAAGAAGACAGGGGGGCCCCAGGGCTCCAGGCGGTGCTTGTTCCTCAGCCTCT
TCTCCTTCCTGATCGTGGCAGGCGCCACCACGCTCTTCTGCCTGCTGCACTTTGGAGTGATCGGCCCCCAGAGGGAA
GAGTTCCCCAGGGACCTCTCTCTAATCAGCCCTCTGGCCCAGGCAAGATCATCTTCTCGAACCCCGAGTGACAAGGT
AGCCCATGTTGTAGCAAACCCTCAAGCTGAGGGGCAGCTCCAGTGGCTGAACCGCCGGGCCAATGCCCTCCTGGCCA
ATGGCGTGGAGCTGAGAGATAACCAGCTGGTGGTGCCATCAGAGGGCCTGTACCTCATCTACTCCCAGGTCCTCTTC
AAGGGCCAAGGCTGCCCCTCCACCCATGTGCTCCTCACCCACACCATCAGCCGCATCGCCGTCTCCTACCAGACCAA
GGTCAACCTCCTCTCTGCCATCAAGAGCCCCTGCCAGAGGGAGACCCCAGAGGGGGCTGAGGCCAAGCCCTGGTATG

AGCCCATCTATCTGGGAGGGGTCTTCCAGCTGGAGAAGGGTGACCGACTCAGCGCTGAGATCAATCGGCCCGACTAT
CTCGACTTTGCCGAGTCTGGGCAGGTCTACTTTGGGATCATTGCCCTGTCGCGAGCCGAGGGCAGGGGAAGTCTTCT
AACATGCGGGGACGTGGAGGAAAATCCCGGGCCCATGGAGTTTGGGCTGAGCTGGCTTTTTCTTGTGGCTATTTTAA
AAGGTGTCCAGTGCTCTAGAGACATCCAGATGACACAGACTACATCCTCCCTGTCTGCCTCTCTGGGAGACAGAGTC
ACCATCAGTTGCAGGGCAAGTCAGGACATTAGTAAATATTTAAATTGGTATCAGCAGAAACCAGATGGAACTGTTAA
ACTCCTGATCTACCATACATCAAGATTACACTCAGGAGTCCCATCAAGGTTCAGTGGCAGTGGGTCTGGAACAGATT
ATTCTCTCACCATTAGCAACCTGGAGCAAGAAGATATTGCCACTTACTTTTGCCAACAGGGTAATACGCTTCCGTAC
ACGTTCGGAGGGGGGACCAAGCTGGAGCTGAAACGTGGTGGTGGTGGTTCTGGTGGTGGTGGTTCTGGCGGCGGCGG
CTCCGGTGGTGGTGGATCCGAGGTGCAGCTGCAGCAGTCTGGACCTGGCCTGGTGGCGCCCTCACAGAGCCTGTCCG
TCACATGCACTGTCTCAGGGGTCTCATTACCCGACTATGGTGTAAGCTGGATTCGCCAGCCTCCACGAAAGGGTCTG
GAGTGGCTGGGAGTAATATGGGGTAGTGAAACCACATACTATAATTCAGCTCTCAAATCCAGACTGACCATCATCAA
GGACAACTCCAAGAGCCAAGTTTTCTTAAAAATGAACAGTCTGCAAACTGATGACACAGCCATTTACTACTGTGCCA
AACATTATTACTACGGTGGTAGCTATGCTATGGACTACTGGGGCCAAGGGACCACGGTCACTGTCTCCTCGTACGTC
ACTGTCTCTTCACAGGATCCCGCCGAGCCCAAATCTCCTGACAAAACTCACACATGCCCACCGTGCCCAGCACCTGA
ACTCCTGGGGGGACCGTCAGTCTTCCTCTTCCCCCCAAAACCCAAGGACACCCTCATGATCTCCCGGACCCCTGAGG
TCACATGCGTGGTGGTGGACGTGAGCCACGAAGACCCTGAGGTCAAGTTCAACTGGTACGTGGACGGCGTGGAGGTG
CATAATGCCAAGACAAAGCCGCGGGAGGAGCAGTACAACAGCACGTACCGTGTGGTCAGCGTCCTCACCGTCCTGCA
CCAGGACTGGCTGAATGGCAAGGAGTACAAGTGCAAGGTCTCCAACAAAGCCCTCCCAGCCCCCATCGAGAAAACCA
TCTCCAAAGCCAAAGGGCAGCCCCGAGAACCACAGGTGTACACCCTGCCCCCATCCCGGGATGAGCTGACCAAGAAC
CAGGTCAGCCTGACCTGCCTGGTCAAAGGCTTCTATCCCAGCGACATCGCCGTGGAGTGGGAGAGCAATGGGCAACC
GGAGAACAACTACAAGACCACGCCTCCCGTGCTGGACTCCGACGGCTCCTTCTTCCTCTACAGCAAGCTCACCGTGG
ACAAGAGCAGGTGGCAGCAGGGGAACGTCTTCTCATGCTCCGTGATGCATGAGGCTCTGCACAACCACTACACGCAG
AAGAGCCTCTCCCTGTCTCCGGGTAAAAAAGATCCCAAATTTTGGGGCGTGCTGGCCGGAATCGTGATGGGCGACCT
GGTGCTGACAGTGCTGATCGCCCTGGCTGTGTACTTCCTGGGCAGACTGGTGCCCAGAGGAAGAGGCGCTGCCGAAG
CCGCCACCCGGAAGCAGAGAATCACCGAGACAGAGAGCCCCTATCAGGAACTGCAGGGCCAGCGGAGCGACGTGTAC
AGCGACCTGAATACCCAGCGGCCCTACTACAAACGCGGACCGCAGTGTACTAATTATGCTCTCTTGAAATTGGCTGG
AGATGTTGAGAGCAATCCCGGGCCCATGCGCATTAGCAAGCCCCACCTGCGGAGCATCAGCATCCAGTGCTACCTGT
GCCTGCTGCTGAACAGCCACTTCCTGACCGAGGCCGGCATCCACGTGTTCATCCTGGGCTGCTTCAGCGCCGGACTG
CCCAAGACCGAGGCCAACTGGGTGAACGTGATCAGCGACCTGAAGAAGATCGAGGACCTGATCCAGAGCATGCACAT
CGACGCCACCCTGTACACCGAGAGCGACGTGCACCCCAGCTGCAAGGTGACCGCCATGAAGTGCTTTCTGCTGGAAC
TGCAGGTGATCAGCCTGGAAAGCGGCGACGCCAGCATCCACGACACCGTGGAGAACCTGATCATCCTGGCCAACAAC
AGCCTGAGCAGCAACGGCAACGTGACCGAGAGCGGCTGCAAAGAGTGCGAGGAACTGGAAGAGAAGAACATCAAAGA
GTTTCTGCAGAGCTTCGTGCACATCGTGCAGATGTTCATCAACACCAGCTGACAATTGCGCGTCATCATCGATCCGG
ATTAGTCCAATTTGTTAAAGACAGGATATCAGTGGTCCAGGCTCTAGTTTTGACTCAACAATATCACCAGCTGAAGC
CTATAGAGTACGAGCCATAGATAAAATAAAAGATTTTATTTAGTCTCCAGAAAAAGGGGGGAATGAAAGACCCCACC
TGTAGGTTTGGCAAGCTAGCTTAAGTAACGCCATTTTGCAAGGCATGGAAAAATACATAACTGAGAATAGAGAAGTT
CAGATCAAGGTCAGGAACAGATGGAACAGCTGAATATGGGCCAAACAGGATATCTGTGGTAAGCAGTTCCTGCCCCG
GCTCAGGGCCAAGAACAGATGGAACAGCTGAATATGGGCCAAACAGGATATCTGTGGTAAGCAGTTCCTGCCCCGGC
TCAGGGCCAAGAACAGATGGTCCCCAGATGCGGTCCAGCCCTCAGCAGTTTCTAGAGAACCATCAGATGTTTCCAGG
GTGCCCCAAGGACCTGAAATGACCCTGTGCCTTATTTGAACTAACCAATCAGTTCGCTTCTCGCTTCTGTTCGCGCG

CTTCTGCTCCCCGAGCTCAATAAAAGAGCCCACAACCCCTCACTCGGGGCGCCAGTCCTCCGATTGACTGAGTCGCC
CGGGTACCCGTGTATCCAATAAACCCTCTTGCAGTTGCATCCGACTTGTGGTCTCGCTGTTCCTTGGGAGGGTCTCC
TCTGAGTGATTGACTACCCGTCAGCGGGGGTCTTTCACACATGCAGCATGTATCAAAATTAATTTGGTTTTTTTTCT
TAAGTATTTACATTAAATGGCCATAGTACTTAAAGTTACATTGGCTTCCTTGAAATAAACATGGAGTATTCAGAATG
TGTCATAAATATTTCTAATTTTAAGATAGTATCTCCATTGGCTTTCTACTTTTTCTTTTATTTTTTTTGTCCTCTG
TCTTCCATTTGTTGTTGTTGTTTGTTTGTTTGTTTGTTGGTTGGTTGGTTAATTTTTTTTTAAAGATCCTACAC
TATAGTTCAAGCTAGACTATTAGCTACTCTGTAACCCAGGGTGACCTTGAAGTCATGGGTAGCCTGCTGTTTTAGCC
TTCCCACATCTAAGATTACAGGTATGAGCTATCATTTTTGGTATATTGATTGATTGATTGATTGATGTGTGTGTGTG
TGATTGTGTTTGTGTGTGTGACTGTGAAAATGTGTGTATGGGTGTGTGTGAATGTGTGTATGTATGTGTGTGTGA
GTGTGTGTGTGTGTGTGTGCATGTGTGTGTGTGTGACTGTGTCTATGTGTATGACTGTGTGTGTGTGTGTGTGTG
TGTGTGTGTGTGTGTGTGTGTGTTGTGAAAAAATATTCTATGGTAGTGAGAGCCAACGCTCCGGCTCAGGTGTCA
GGTTGGTTTTTGAGACAGAGTCTTTCACTTAGCTTGGAATTCACTGGCCGTCGTTTTACAACGTCGTGACTGGGAAA
ACCCTGGCGTTACCCAACTTAATCGCCTTGCAGCACATCCCCCTTTCGCCAGCTGGCGTAATAGCGAAGAGGCCCGC
ACCGATCGCCCTTCCCAACAGTTGCGCAGCCTGAATGGCGAATGGCGCCTGATGCGGTATTTTCTCCTTACGCATCT
GTGCGGTATTTCACACCGCATATGGTGCACTCTCAGTACAATCTGCTCTGATGCCGCATAGTTAAGCCAGCCCCGAC
ACCCGCCAACACCCGCTGACGCGCCCTGACGGGCTTGTCTGCTCCCGGCATCCGCTTACAGACAAGCTGTGACCGTC
TCCGGGAGCTGCATGTGTCAGAGGTTTTCACCGTCATCACCGAAACGCGCGAGACGAAAGGGCCTCGTGATACGCCT
ATTTTTATAGGTTAATGTCATGATAATAATGGTTTCTTAGACGTCAGGTGGCACTTTTCGGGGAAATGTGCGCGGAA
CCCCTATTTGTTTATTTTTCTAAATACATTCAAATATGTATCCGCTCATGAGACAATAACCCTGATAAATGCTTCAA
TAATATTGAAAAAGGAAGAGTATGAGTATTCAACATTTCCGTGTCGCCCTTATTCCCTTTTTTGCGGCATTTTGCCT
TCCTGTTTTTGCTCACCCAGAAACGCTGGTGAAAGTAAAAGATGCTGAAGATCAGTTGGGTGCACGAGTGGGTTACA
TCGAACTGGATCTCAACAGCGGTAAGATCCTTGAGAGTTTTCGCCCCGAAGAACGTTTTCCAATGATGAGCACTTTT
AAAGTTCTGCTATGTGGCGCGGTATTATCCCGTATTGACGCCGGGCAAGAGCAACTCGGTCGCCGCATACACTATTC
TCAGAATGACTTGGTTGAGTACTCACCAGTCACAGAAAAGCATCTTACGGATGGCATGACAGTAAGAGAATTATGCA
GTGCTGCCATAACCATGAGTGATAACACTGCGGCCAACTTACTTCTGACAACGATCGGAGGACCGAAGGAGCTAACC
GCTTTTTTGCACAACATGGGGGATCATGTAACTCGCCTTGATCGTTGGGAACCGGAGCTGAATGAAGCCATACCAAA
CGACGAGCGTGACACCACGATGCCTGTAGCAATGGCAACAACGTTGCGCAAACTATTAACTGGCGAACTACTTACTC
TAGCTTCCCGGCAACAATTAATAGACTGGATGGAGGCGGATAAAGTTGCAGGACCACTTCTGCGCTCGGCCCTTCCG
GCTGGCTGGTTTATTGCTGATAAATCTGGAGCCGGTGAGCGTGGGTCTCGCGGTATCATTGCAGCACTGGGGCCAGA
TGGTAAGCCCTCCCGTATCGTAGTTATCTACACGACGGGGAGTCAGGCAACTATGGATGAACGAAATAGACAGATCG
CTGAGATAGGTGCCTCACTGATTAAGCATTGGTAACTGTCAGACCAAGTTTACTCATATATACTTTAGATTGATTTA
AAACTTCATTTTTAATTTAAAAGGATCTAGGTGAAGATCCTTTTTGATAATCTCATGACCAAAATCCCTTAACGTGA
GTTTTCGTTCCACTGAGCGTCAGACCCCGTAGAAAAGATCAAAGGATCTTCTTGAGATCCTTTTTTTCTGCGCGTAA
TCTGCTGCTTGCAAACAAAAAAACCACCGCTACCAGCGGTGGTTTGTTTGCCGGATCAAGAGCTACCAACTCTTTTT
CCGAAGGTAACTGGCTTCAGCAGAGCGCAGATACCAAATACTGTTCTTCTAGTGTAGCCGTAGTTAGGCCACCACTT
CAAGAACTCTGTAGCACCGCCTACATACCTCGCTCTGCTAATCCTGTTACCAGTGGCTGCTGCCAGTGGCGATAAGT
CGTGTCTTACCGGGTTGGACTCAAGACGATAGTTACCGGATAAGGCGCAGCGGTCGGGCTGAACGGGGGGTTCGTGC
ACACAGCCCAGCTTGGAGCGAACGACCTACACCGAACTGAGATACCTACAGCGTGAGCTATGAGAAAGCGCCACGCT
TCCCGAAGGGAGAAAGGCGGACAGGTATCCGGTAAGCGGCAGGGTCGGAACAGGAGAGCGCACGAGGGAGCTTCCAG
GGGGAAACGCCTGGTATCTTTATAGTCCTGTCGGGTTTCGCCACCTCTGACTTGAGCGTCGATTTTTGTGATGCTCG

TCAGGGGGGCGGAGCCTATGGAAAAACGCCAGCAACGCGGCCTTTTTACGGTTCCTGGCCTTTTGCTGGCCTTTTGC
TCACATGTTCTTTCCTGCGTTATCCCCTGATTCTGTGGATAACCGTATTACCGCCTTTGAGTGAGCTGATACCGCTC
GCCGCAGCCGAACGACCGAGCGCAGCGAGTCAGTGAGCGAGGAAGCGGAAGAGCGCCCAATACGCAAACCGCCTCTC
CCCGCGCGTTGGCCGATTCATTAATGCAGCTGGCACGACAGGTTTCCCGACTGGAAAGCGGGCAGTGAGCGCAACGC
AATTAATGTGAGTTAGCTCACTCATTAGGCACCCCAGGCTTTACACTTTATGCTTCCGGCTCGTATGTTGTGTGGAA
TTGTGAGCGGATAACAATTTCACACAGGAAACAGCTATGACCATGATTACGCCAAGCTTTGCTCTTAGGAGTTTCCT
AATACATCCCAAACTCAAATATATAAAGCATTTGACTTGTTCTATGCCCTAGGGGGCGGGGGGAAGCTAAGCCAGCT
TTTTTTAACATTTAAAATGTTAATTCCATTTTAAATGCACAGATGTTTTTATTTCATAAGGGTTTCAATGTGCATGA
ATGCTGCAATATTCCTGTTACCAAAGCTAGTATAAATAAAAATAGATAAACGTGGAAATTACTTAGAGTTTCTGTCA
TTAACGTTTCCTTCCTCAGTTGACAACATAAATGCGCTGCTGAGCAAGCCAGTTTGCATCTGTCAGGATCAATTTCC
CATTATGCCAGTCATATTAATTACTAGTCAATTAGTTGATTTTTATTTTTGACATATACATGTG (SEQ ID NO:16).

[0123] One example of amino acid sequence for a vector that encodes a TNF-alpha mutant del Val1 del Pro12 and that separately encodes a CD19-specific CAR with an IgG1 hinge, DAP12, and CD3zeta and that separately encodes IL15 is as follows:

[0124]

MSTESMIRDVELAEEALPKKTGGPQGSRRCLFLSLFSFLIVAGATTLFCLLHFGVIGPQREEFPRDLSLIS
PLAQARSSSRTPSDKVAHVVANPQAEGQLQWLNRRANALLANGVELRDNQLVVPSEGLYLIYSQVLFKGQGCPSTHV
LLTHTISRIAVSYQTKVNLLSAIKSPCQRETPEGAEAKPWYEPIYLGGVFQLEKGDRLSAEINRPDYLDFAESGQVY
FGIIALSRAEGRGSLLTCGDVEENPGPMEFGLSWLFLVAILKGVQCSRDIQMTQTTSSLSASLGDRVTISCRASQDI
SKYLNWYQQKPDGTVKLLIYHTSRLHSGVPSRFSGSGSGTDYSLTISNLEQEDIATYFCQQGNTLPYTFGGGTKLEL
KRGGGGSGGGGSGGGGSGGGGSEVQLQQSGPGLVAPSQSLSVTCTVSGVSLPDYGVSWIRQPPRKGLEWLGVIWGSE
TTYYNSALKSRLTIIKDNSKSQVFLKMNSLQTDDTAIYYCAKHYYYGGSYAMDYWGQGTTVTVSSYVTVSSQDPAEP
KSPDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREE
QYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKG
FYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGKK
DPKFWGVLAGIVMGDLVLTVLIALAVYFLGRLVPRGRGAAEAATRKQRITETESPYQELQGQRSDVYSDLNTQRPYY
KRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGM
KGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPRGPQCTNYALLKLAGDVESNPGPMRISKPHLRSISIQCYLCL
LLNSHFLTEAGIHVFILGCFSAGLPKTEANWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLLELQ
VISLESGDASIHDTVENLIILANNSLSSNGNVTESGCKECEELEEKNIKEFLQSFVHIVQMFINTS (SEQ ID NO:37)

V. [0125] Cells

[0126] Embodiments of the disclosure encompass cells that express one or more TNF-alpha mutants as encompassed herein. The cell comprises a recombinant nucleic acid that encodes one or more engineered nonsecretable, membrane bound TNF-alpha mutant

polypeptides, in specific embodiments. In specific embodiments, in addition to expressing one or more TNF-alpha mutant polypeptides, the cell also comprises a nucleic acid that encodes one or more therapeutic gene products. The nucleic acids may be vectors of any kind. The nucleic acid that encodes the one or more TNF-alpha mutant polypeptides may or may not be the same nucleic acid molecule that encodes the one or more therapeutic gene products.

[0127] The cells of the disclosure may be of any kind, including at least T-cells, NK cells, NKT cells, iNKT cells, macrophages, B cells, MSCs, or stem cells of any kind, including at least hematopoietic stem cells, pluripotent embryonic stem cells or embryonic stem cells.

[0128] The cells may be obtained from an individual directly or may be obtained from a depository or other storage facility. The cells as therapy may be autologous or allogeneic with respect to the individual to which the cells are provided as therapy.

[0129] The cells may be from an individual in need of therapy for a medical condition, and following their manipulation to express the TNF-alpha mutant and therapeutic gene product (using standard techniques for transduction and expansion for adoptive cell therapy, for example), they may be provided back to the individual from which they were originally sourced. In some cases, the cells are stored for later use for the individual or another individual.

[0130] The cells that harbor the one or more engineered receptors and that may be needed to be eliminated by the resident TNF-alpha suicide gene may be of any kind. In specific embodiments the cells are immune cells or stem cells, including those that are being utilized for adoptive cell therapy, for example. The immune cells may be T-cells, NK cells, NKT cells, iNKT cells, B cells, and so forth. The cells may be comprised in a population of cells, and that population may have a majority that are transduced with one or more TNF-alpha mutant suicide genes or both of one or more engineered receptors and one or more TNF-alpha mutant suicide genes. A cell population may comprise 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, or 100% of cells that are transduced with one or more TNF-alpha mutant suicide genes and, optionally, one or more engineered receptors. The TNF-alpha mutant(s) and the engineered receptor(s) are separate polypeptides.

[0131] The cells may be produced with the TNF-alpha mutant suicide gene for the intent of being modular with respect to a specific purpose. For example, cells may be generated,

including for commercial distribution, expressing a TNF-alpha mutant (or distributed with a nucleic acid that encodes the mutant for subsequent transduction), and a user may modify them to express one or more therapeutic genes of interest dependent upon their intended purpose(s). As only one example, an individual interested in treating CD5-positive cancer may obtain or generate the TNF-alpha mutant-expressing cells and modify them to express a CAR comprising a CD5-specific scFv. Alternatively, an individual interested in treating CD5-positive cancer may obtain cells to be transduced, obtain a vector that encodes the TNF-alpha mutant, and modify the vector also to encode a CD5-specific CAR, followed by subsequent transduction of the cells. Either of those embodiments may be applied to any other cancer antigen than CD5.

[0132] In particular embodiments, the genome of the transduced cells expressing the TNF-alpha mutant may be modified. The genome may be modified in any manner, but in specific embodiments the genome is modified by CRISPR gene editing, for example. The genome of the cells may be modified to enhance effectiveness of the TNF-alpha mutant as a suicide gene, to enhance effectiveness of use of the therapeutic gene product, or for another purpose. Specific examples of genes that may be modified in the cells includes the following: knockout of ADAM13/TACE, increase resistance of TNF-alpha mutant expressing cells to the tumor microenvironment such as TGF-beta receptor 1 or 2, IDO, checkpoint molecules such as PD1, TIGIT, KLRG1, TIM3, *etc.*

VI. [0133] Use of TNF-alpha Mutants as a Suicide Gene

[0134] In particular embodiments, the cells for which the TNF-alpha mutant suicide gene are employed are cells that have the potential to be deleterious, for example for an individual exposed to the cells *in vivo*. The cells may be toxic to an individual upon delivery or thereafter, and therefore a need to be able to eliminate the cells may be consistently present for the cells. For instance, any type of cell therapy for use in an individual *in vivo* would be able to employ the disclosed TNF-alpha mutants in the cells, allowing the cell therapy to be terminated when desired. The cell therapy may be subject to utilization of the TNF-alpha mutant suicide gene when an individual receiving the cell therapy and/or having received the cell therapy shows one or more symptoms of one or more adverse events, such as cytokine release syndrome, neurotoxicity, anaphylaxis/allergy, and/or on-target/off tumor toxicities (as examples) or is considered at risk for having the one or more symptoms, including imminently. The use of the TNF-alpha mutant as a suicide gene may be part of a planned protocol for a therapy or may be

used only upon a recognized need for its use. In some cases the cell therapy is terminated by use of agent(s) that targets the TNF-alpha suicide gene because the therapy is no longer required.

[0135] The cells for which the TNF-alpha suicide gene is utilized may be cells engineered for cell therapy for mammals, in particular embodiments. In such cases, the cell therapy may be of any kind and the cells may be of any kind. In specific embodiments, the cells are immune cells or stem cells that have been engineered to express one or more therapeutic gene products. In specific embodiments, the cells are cells that are transduced with one or more engineered receptors for the cells. The engineered receptors may impart a therapeutic characteristic for the cells upon targeting, such as by binding to, a ligand for the receptor. In specific embodiments, the engineered receptor is non-native and made by the hand of man. The engineered receptor may be of any kind including a T-cell receptor, a chimeric antigen receptor (CAR), chemokine receptor, cytokine receptor, homing receptor, gene-edited cells, or a combination thereof. The engineered receptors may be engineered to be able to bind, such as target, a specific antigen, including at least a tumor antigen, as an example. The engineered receptors may be bi-specific or multi-specific for more than one antigen, in some cases, allowing the transduced cells to bind through the engineered receptor to cells that express the multiple antigens.

[0136] In particular embodiments, upon delivering an effective amount of one or more agents to bind to the TNF-alpha mutant-expressing cells, the majority of TNF-alpha mutant-expressing cells are eliminated. In specific embodiments, greater than 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% of cells expressing the TNF-alpha mutants are eliminated in an individual. Following recognition of a need to eliminate the cells, the delivery of the agent(s) to the individual may continue until one or more symptoms are no longer present or until a sufficient number of cells have been eliminated. The cell numbers in the individual may be monitored using the TNF-alpha mutants as markers.

[0137] Embodiments of methods of the disclosure may comprise a first step of providing an effective amount of cell therapy to an individual in need thereof, wherein the cells comprise one or more nonsecretable TNF-alpha mutants; and, a second step of eliminating the cells using the TNF-alpha mutant(s) as suicide genes (directly or indirectly through cell death by any mechanism). The second step may be instigated upon onset of at least one adverse event for the individual, and that adverse event may be recognized by any means, including upon routine

monitoring that may or may not be continuous from the beginning of the cell therapy. The adverse event(s) may be detected upon examination and/or testing. In cases wherein the individual has cytokine release syndrome (which may also be referred to as cytokine storm), the individual may have elevated inflammatory cytokine(s) (merely as examples: interferon-gamma, granulocyte macrophage colony-stimulating factor, IL-10, IL-6 and TNF-alpha); fever; fatigue; hypotension; hypoxia, tachycardia; nausea; capillary leak; cardiac/renal/hepatic dysfunction; or a combination thereof, for example. In cases wherein the individual has neurotoxicity, the individual may have confusion, delirium, aplasia, and/or seizures. In some cases, the individual is tested for a marker associated with onset and/or severity of cytokine release syndrome, such as C-reactive protein, IL-6, TNF-alpha, and/or ferritin

[0138] In additional embodiments, administration of one or more agents that bind the nonsecretable TNF-α during cytokine release syndrome or neurotoxicity, for example, have the added benefit of neutralizing the high levels of soluble TNF-alpha that contribute to the toxicity of the therapy. Soluble TNF-alpha is released at high levels during cytokine release syndrome and is a mediator of toxicity with CAR T-cell therapies. In such cases, the administration of TNF-alpha antibodies encompassed herein have a dual beneficial effect- *i.e.* selective deletion of the TNF-alpha mutant-expressing cells as well as neutralizing soluble TNF-alpha causing toxicity. Thus, embodiments of the disclosure encompass methods of eliminating or reducing the severity of cytokine release syndrome in an individual receiving, or who has received, adoptive cell therapy in which the cells express a nonsecretable TNF-alpha mutant, comprising the step of providing an effective amount of an agent that binds the nonsecretable TNF-alpha mutant, said agent causing in the individual (a) elimination of at least some of the cells of the cell therapy; and (b) reduction in levels of soluble TNF-alpha.

[0139] Embodiments of the disclosure include methods of reducing the effects of cytokine release syndrome in an individual that has received or who is receiving cell therapy with cells that express a nonsecretable TNF-alpha mutant, comprising the step of providing an effective amount of one or more agents that bind the mutant to cause in the individual (a) elimination of at least some of the cells of the cell therapy; and (b) reduction in the level of soluble TNF-alpha.

[0140] When the need arises for the TNF-alpha suicide gene to be utilized, the individual is provided an effective amount of one or more inhibitors that are able to inhibit, such as by

binding directly, the TNF-alpha mutant on the surface of the cells. The inhibitor(s) may be provided to the individual systemically and/or locally in some embodiments. The inhibitor may be a polypeptide (such as an antibody), a nucleic acid, a small molecule (for example, a xanthine derivative), a peptide, or a combination thereof. In specific embodiments, the antibodies are FDA-approved. When the inhibitor is an antibody, the inhibitor may be a monoclonal antibody in at least some cases. When mixtures of antibodies are employed, one or more antibodies in the mixture may be a monoclonal antibody. Examples of small molecule TNF-alpha inhibitors include small molecules such as are described in U.S. Patent No. 5,118,500, which is incorporated by reference herein in its entirety. Examples of polypeptide TNF-alpha inhibitors include polypeptides, such as those described in U.S. Patent No. 6,143,866, which is incorporated by reference herein in its entirety.

[0141] In particular embodiments, at least one antibody is utilized to target the TNF-alpha mutant to trigger its activity as a suicide gene. Examples of antibodies includes at least Adalimumab, Adalimumab-atto, Certolizumab pegol, Etanercept, Etanercept-szzs, Golimumab, Infliximab, Infliximab-dyyb, or a mixture thereof, for example.

[0142] Embodiments of the disclosure include methods of reducing the risk of toxicity of a cell therapy for an individual by modifying cells of a cell therapy to express a nonsecretable TNF-alpha mutant. The cell therapy is for cancer, in specific embodiments, and it may comprise an engineered receptor that targets an antigen, including a cancer antigen.

[0143] In particular embodiments, in addition to the inventive cell therapy of the disclosure, the individual may have been provided, may be provided, and/or may will be provided an additional therapy for the medical condition. In cases wherein the medical condition is cancer, the individual may be provided one or more of surgery, radiation, immunotherapy (other than the cell therapy of the present disclosure), hormone therapy, gene therapy, chemotherapy, and so forth.

[0144] In cases wherein the individual being treated with the cell therapy of the disclosure has cancer, the individual may have any type of cancer. The individual may have leukemia, lymphoma, myeloma, brain cancer, lung cancer, breast cancer, colon cancer, endometrium cancer, cervical cancer, ovarian cancer, testicular cancer, bone cancer, skin cancer, kidney cancer, liver cancer, stomach cancer, spleen cancer, thyroid cancer, head and neck cancer, gall bladder cancer, and so forth.

VII. [0145] Kits of the Disclosure

[0146] Any of the compositions described herein may be comprised in a kit. In a non-limiting example, cells, reagents to produce cells, vectors, and reagents to produce vectors and components thereof may be comprised in a kit. In certain embodiments, alpha-beta T-cells, gamma-delta T cells, NK cells, NKT cells, iNKT cells, B cells, or stem cells may be comprised in a kit. Such a kit may or may not have one or more reagents for manipulation of cells. Such reagents include small molecules, proteins, nucleic acids, antibodies, buffers, primers, nucleotides, salts, and/or a combination thereof, for example. Nucleotides that encode one or more TNF-alpha mutants, engineered receptors, or cytokines may be included in the kit. Proteins, such as cytokines or antibodies, including monoclonal antibodies, may be included in the kit. Nucleotides that encode components of engineered receptors, such as chimeric antigen receptors or T-cell receptors may be included in the kit, including reagents to generate same.

[0147] In particular aspects, the kit comprises the cell therapy of the disclosure and also another cancer therapy. In some cases, the kit, in addition to the cell therapy embodiments, also includes a second cancer therapy, such as chemotherapy, hormone therapy, and/or immunotherapy, for example. The kit(s) may be tailored to a particular cancer for an individual and comprise respective second cancer therapies for the individual.

[0148] The kits may comprise suitably aliquoted compositions of the present disclosure. The components of the kits may be packaged either in aqueous media or in lyophilized form. The container means of the kits will generally include at least one vial, test tube, flask, bottle, syringe or other container means, into which a component may be placed, and preferably, suitably aliquoted. Where there are more than one component in the kit, the kit also may generally contain a second, third or other additional container into which the additional components may be separately placed. However, various combinations of components may be comprised in a vial. The kits of the present invention also will typically include a means for containing the composition and any other reagent containers in close confinement for commercial sale. Such containers may include injection or blow-molded plastic containers into which the desired vials are retained.

EXAMPLES

[0149] The following examples are included to demonstrate preferred embodiments of the disclosure. It should be appreciated by those of skill in the art that the techniques disclosed in

the examples which follow represent techniques discovered by the inventor to function well in the practice of the disclosure, and thus can be considered to constitute preferred modes for its practice. However, those of skill in the art should, in light of the present disclosure, appreciate that many changes can be made in the specific embodiments which are disclosed and still obtain a like or similar result without departing from the spirit and scope of the disclosure.

EXAMPLE 1

TNF-ALPHA SUICIDE GENE

[0150] The present disclosure provides a marker moiety and a suicide moiety for cell therapy, based on uncleavable mutants of the 26 kd tumor necrosis factor alpha (TNF-α) that is normally processed to a 17 kD component. There are a number of advantages to using this approach. FIG. 1 shows an example of an experimental plan to mutagenize TNF-alpha to ablate membrane cutting sites. As described by Perez *et al.* (1990), the right panel of FIG. 1 illustrates three exemplary TNF-alpha mutants that render the TNF-alpha mutant to be uncleavable: (1) deletion of amino acid residues 1-12 of the 17 kD TNF; (2) deletion of amino acid residues 1 and 12 of the 17 kD TNF; and (3) deletion of amino acid residues 1 and 13 of the 17 kD TNF. The left panel of FIG. 1 provides examples of primers for site-directed mutagenesis as an example to generate the mutants.

[0151] FIGS. 2A, 2B, 2C, 2D, and 2E provide examples of vectors that may encode the TNF-alpha mutants. FIG. 2A illustrates a vector map example of a TNF-alpha mutant having deletions of amino acids Val1 and Pro12, and the mutant is co-expressed with a CD19-specific CAR and is also co-expressed with IL-15, all as separate polypeptides, as an example. FIG. 2B illustrates a vector map example of a TNF-alpha mutant having a deletion at Valine 13, and the mutant is separately co-expressed with a CD19-specific CAR and separately co-expressed with IL-15, as an example. FIG. 2C illustrates a vector map example of a TNF-alpha mutant having deletions of amino acids Val1 and Val 13, and the mutant is separately co-expressed with a CD19-specific CAR and IL-15, as an example. FIG. 2D illustrates a vector map example of a TNF-alpha mutant having deletions of amino acids Val1 through to Val 13 (13 aa deletions), and the mutant is separately co-expressed with a CD19-specific CAR and IL-15, as an example. FIG. 2E illustrates a vector map example of a TNF-alpha mutant having deletions of amino acids Ala-1 through to Val 13 (14 aa deletion), and the mutant is separately co-expressed with a CD19-specific CAR and IL-15, as an example.

[0152] The mutated uncleavable TNF-alpha (in cells transduced with a vector encoding both TNF-alpha mutant with deletions at Val1 and Pro12 and a CD19-specific CAR, as an example) is stably expressed on the cell surface after, for example, viral transduction or electroporation of its encoding sequence (FIG. 3).

[0153] Cells expressing the uncleavable TNF-α mutants can be targeted for selective deletion using FDA-approved TNF-α antibodies (for example), such as etanercept, infliximab or adalilumab. FIG. 4A illustrates examples of anti-TNF antibodies. FIG. 4B demonstrates that greater than 70% of NK cells expressing mutant TNF-alpha are eliminated by complement dependent cytotoxicity (CDC) within 90 minutes of treatment with infliximab.

[0154] FIG. 5A demonstrates that in response to Raji targets, the NK cells transduced with a vector that co-expresses TNF-alpha mutant and an CD19-specific CAR produce more effector cytokines and degranulate more efficiently when compared to NK cells that express the anti-CD19 CAR alone. In FIG. 5B, Raji targets are efficiently killed by NK cells transduced with a vector that separately co-expresses a TNF-alpha mutant (deletion of Val1 and Pro12, as an example) and a CD19-specific CAR. The TNF-alpha mutant protein with deletions of Valine at position 1 Proline at position 12 is biologically active and mediates a strong anti-tumor response upon direct cell-cell contact, further contributing to the antitumor activity of the transduced cells.

[0155] The transduced NK cells harboring a vector that separately expresses a CD19-specific CAR and a TNF-alpha mutant do not exhibit off-target activity (FIG. 6). FIG. 7 demonstrates that NK cells transduced with a vector that separately expresses a CD19-specific CAR and a TNF-alpha mutant do not exhibit off-target activity and do not secrete TNF-alpha non-specifically. FIG. 8 illustrates that TNF-alpha receptor binding sites for TNF receptors 1 and 2 *vs.* TNF-alpha antibodies infliximab and adalimumab are different. This demonstrates that the mutations in the TNFalpha gene will not negatively impact the ability of TNFalpha antibodies in recognizing the TNFa mutant protein; *i.e.* the TNFalpha mutant can still be used as a suicide gene and be targeted by the antibodies.

[0156] Additional safety studies may be employed. For example, *in vivo* murine toxicity studies with CD19-specific CAR NK cells may be performed. For example, in an established Raji NSG mouse model one can compare TNF-alpha WT *vs.* TNF-alpha mutant, CD19-specific CAR NK cells also expressing IL15. However, these mutants were previously tested in mice and their safety was demonstrated (Karp *et al.*, 1992).

[0157] One may employ synapse and signaling studies to characterize interaction of TNF-alpha mutant *vs.* TNF-alpha wild type *vs.* exogenous TNF-alpha with TNF-alpha receptor 1 (TNF-R1) and TNF-alpha receptor 2 (TNF-R2). Such studies may incorporate measurement of apoptosis induction and caspase (downstream of TNF-R1) in Ramos cells (which express TNF R1 but not TNFR2). In addition or alternatively, one can measure NFkappaB in Jurkat cells that express both TNFR2 and TNFR1.

EXAMPLE 2

COMPARISON OF ANTITUMOR ACTIVITY OF CAR-NK CELLS TRANSDUCED WITH TNFAMUT-CAR19-IL15 VS. IC9-CAR19-IL15 CONSTRUCT

[0158] FIG. 11 provides a comparison of antitumor activity of CAR-NK cells from cord blood transduced with either TNF-alpha mut-CAR19-IL15 construct or inducible caspase 9 (iC9)-CAR19-IL15 constructs. In FIG. 11A, NSG mice with Raji tumor received 3 x 10e6 CAR cord blood NK cells transduced with TNF-alpha mut-CAR19-IL15 construct or transduced with iC9-CAR19-IL15 construct. FIG. 11B demonstrates percent survival over time. Mice transduced with TNF-alpha mut-CAR19-IL15 construct outlived control mice and mice transduced with iC9-CAR19-IL15 construct.

REFERENCES

[0159] All patents and publications mentioned in the specification are indicative of the level of those skilled in the art to which the embodiments of the disclosure pertain. All patents and publications are herein incorporated by reference in their entirety to the same extent as if each individual publication was specifically and individually indicated to be incorporated by reference.

Patents

[0160] U.S. Patent No. 5,118,500

[0161] U.S. Patent No. 6,143,866

Publications

[0162] Karp, Stephen E., Hwu, Patrick, et al. (1992) In vivo Activity of Tumor Necrosis Factor (TNF) Mutants: Secretory but non Membrane-Bound TNF Mediates the Regression of Retrovirally Tranduced Murine Tumor. *J. Immunol.*, vol. 149(6): 2076-2081.

[0163] Perez, C., Albert, I. *et al.* (1990) A Nonsecretable Cell Surface Mutant of Tumor Necrosis Factor (TNF) Kills by Cell-to-Cell Contact. *Cell*, vol. 63, 251-258.

[0164] Although the present disclosure and its advantages have been described in detail, it should be understood that various changes, substitutions and alterations can be made herein without departing from the spirit and scope of the design as defined by the appended claims. Moreover, the scope of the present application is not intended to be limited to the particular embodiments of the process, machine, manufacture, composition of matter, means, methods and steps described in the specification. As one of ordinary skill in the art will readily appreciate from the present disclosure, processes, machines, manufacture, compositions of matter, means, methods, or steps, presently existing or later to be developed that perform substantially the same function or achieve substantially the same result as the corresponding embodiments described herein may be utilized according to the present disclosure. Accordingly, the appended claims are intended to include within their scope such processes, machines, manufacture, compositions of matter, means, methods, or steps.

CLAIMS

What is claimed is:

1. A composition comprising a transduced cell comprising a nucleic acid that encodes one or more engineered nonsecretable tumor necrosis factor (TNF)-alpha mutant polypeptides and a nucleic acid that encodes one or more therapeutic gene products.

2. The composition of claim 1, wherein the TNF-alpha mutant polypeptide comprises a deletion with respect to SEQ ID NO:8 of the following:

amino acid residue 1 and amino acid residue 12;

amino acid residue 1 and amino acid residue 13;

amino acid residues 1-12;

amino acid residues 1-13; or

amino acid residues -1 to 13.

3. The composition of claim 1 or 2, wherein the therapeutic gene product is an engineered receptor.

4. The composition of any one of claims 1, 2, or 3, wherein the engineered receptor is a T-cell receptor, chimeric antigen receptor (CAR), cytokine receptor, homing receptor, or chemokine receptor.

5. The composition of claim 3 or 4, wherein the engineered receptor targets a cancer antigen.

6. The composition of any one of claims 3-5, wherein the engineered receptor is a CAR that comprises one or more costimulatory domains.

7. The composition of claim 6, wherein the one or more costimulatory domains comprises the costimulatory domain of CD28, DAP12, CD137 (4-1BB), CD134 (OX40), Dap10, CD27, CD2, CD5, ICAM-1, LFA-1 (CD11a/CD18), Lck, TNFR-I, TNFR-II, Fas, CD30, CD40 or a combination thereof.

8. The composition of any one of claims 1-7, wherein the nucleic acid that encodes the TNF-alpha mutant polypeptide and the nucleic acid that encodes the therapeutic gene product are the same nucleic acid molecule.

9. The composition of any one of claims 1-7, wherein the nucleic acid that encodes the TNF-alpha mutant polypeptide and the nucleic acid that encodes the therapeutic gene product are different nucleic acid molecules.

10. The composition of claim 8 or 9, wherein the nucleic acid molecule is a vector.

11. The composition of claim 10, wherein the vector is a viral vector or a non-viral vector.

12. The composition of claim 11, wherein the viral vector is a retroviral vector, lentiviral vector, adenoviral vector, or adeno-associated viral vector.

13. The composition of claim 11, wherein the non-viral vector is a plasmid, lipid, or transposon.

14. The composition of any one of claims 1-13, wherein the cell is an immune cell or a stem cell.

15. The composition of claim 14, wherein the immune cell is a T cell, a NK cell, NKT cell, iNKT cell, B cell, regulatory T cell, monocyte, macrophage, dendritic cell, or mesenchymal stromal cell..

16. The composition of any one of claims 1-15, wherein the TNF-alpha mutant polypeptide comprises SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:39 or SEQ ID NO:41.

17. The composition of any one of claims 1-16, wherein the TNF-alpha mutant polypeptide is encoded by a sequence that comprises SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:38, or SEQ ID NO:40.

18. The composition of any one of claims 1-17, wherein the cell expresses an exogenously provided cytokine.

19. The composition of claim 18, wherein the cytokine is IL-7, IL-2, IL-15, IL-12, IL-18, IL-21 or a combination thereof.

20. The composition of claim 18 or 19, wherein the cytokine is encoded from the same vector as the TNF-alpha mutant gene.

21. The composition of any one of claims 18-20, wherein the cytokine is expressed as a separate polypeptide molecule as the TNF-alpha mutant and as a separate polypeptide molecule as an engineered receptor of the cell.

22. The composition of any one of claims 1-21, wherein the TNF-alpha mutant polypeptide lacks one or more further mutations that prevent binding of the TNF-alpha mutant polypeptide to a TNF receptor.

23. A method of inducing death for a transduced cell expressing an engineered nonsecretable TNF-alpha mutant polypeptide, comprising the step of providing an effective amount of at least one agent that binds the TNF-alpha mutant on the transduced cell.

24. The method of claim 23, wherein the agent that binds TNF-alpha is an antibody, small molecule, polypeptide, nucleic acid, or combination thereof.

25. The method of claim 24, wherein the antibody is a monoclonal antibody.

26. The method of any one of claims 23-25, wherein the cell further expresses an engineered receptor.

27. The method of claim 26, wherein the engineered receptor is a T-cell receptor or a CAR.

28. The method of claim 26 or 27, wherein the engineered receptor targets a cancer antigen.

29. The method of any one of claims 23-28, wherein the method occurs *in vivo* in an individual with a medical condition and the individual has been provided a therapy for the medical condition that comprises a plurality of the transduced cells.

30. The method of claim 29, wherein the medical condition is cancer.

31. The method of claim 29 or 30, wherein the agent is provided to the individual upon onset of one or more adverse events from the therapy.

32. The method of claim 31, wherein the individual exhibits one or more symptoms of cytokine release syndrome, neurotoxicity, anaphylaxis/allergy, and/or on-target/off tumor toxicity.

33. The method of any one of claims 29-32, wherein the individual has been provided, is provided, and/or will be provided an additional therapy for the medical condition.

34. The method of any one of claims 23-33, wherein the TNF-alpha mutant polypeptide lacks one or more further mutations that prevent binding of the TNF-alpha mutant polypeptide to a TNF receptor or prevents reverse signaling.

35. A method of reducing the effects of cytokine release syndrome in an individual that has received and/or who is receiving cell therapy with cells that express a nonsecretable TNF-alpha mutant, comprising the step of providing an effective amount of one or more agents that bind the mutant to cause in the individual (a) elimination of at least some of the cells of the cell therapy; and (b) reduction in the level of soluble TNF-alpha.

36. A method of reducing the risk of toxicity of a cell therapy for an individual, comprising the step of modifying the cells of the cell therapy to express a nonsecretable TNF-alpha mutant.

37. The method of claim 36, wherein the cell therapy is for cancer.

38. The method of claim 36 or 37, wherein the cell therapy comprises an engineered receptor that targets an antigen.

39. A vector, comprising a sequence that encodes a nonsecretable TNF-alpha mutant and that encodes an engineered receptor.

40. The vector of claim 39, wherein the nonsecretable TNF-alpha mutant and the engineered receptor are encoded from the vector as separate polypeptides.

41. The vector of claim 39 or 40, wherein sequence of the vector that encodes the nonsecretable TNF-alpha mutant and sequence of the vector that encodes the engineered receptor are separated on the vector by a 2A element or an IRES element.

42. The vector of any one of claims 39-41, wherein the engineered receptor is a CAR.

43. The vector of any one of claims 39-42, wherein the vector further encodes a cytokine.

44. The vector of claim 43, wherein the cytokine is IL-7, IL-2, IL-15, IL-12, IL-18, or IL-21.

45. The vector of claim 43 or 44, wherein the cytokine is expressed from the vector as a separate polypeptide as the TNF-alpha mutant and the engineered receptor.

46. As a composition of matter, a nucleic acid sequence comprising SEQ ID NO:15.

47. As a composition of matter, a nucleic acid sequence comprising SEQ ID NO:16.

Wild type primer

TCGAGAAGATGATCTGACTGCCTGGGCCAGAGG

CP 495: Del VAL 1

Mutant primer

5′-TCG AGA AGA TGA TCT TGC CTG GGC CAG AGG-3′

CP496: screens Del VAL 1

5′-TGA TCT TGC CTG-3′

Wild type primer

TAC AAC ATG GGC TACAGGCTTGTCACTCGGGGT

CP 497: Del PRO 12

Mutant primer

5′-TAC AAC ATG GGC TAC CTT GTC ACT CGG GGT-3′

CP498: screens Del PRO 12

5′-GGC TAC CTT GTC-3′

FIG. 1

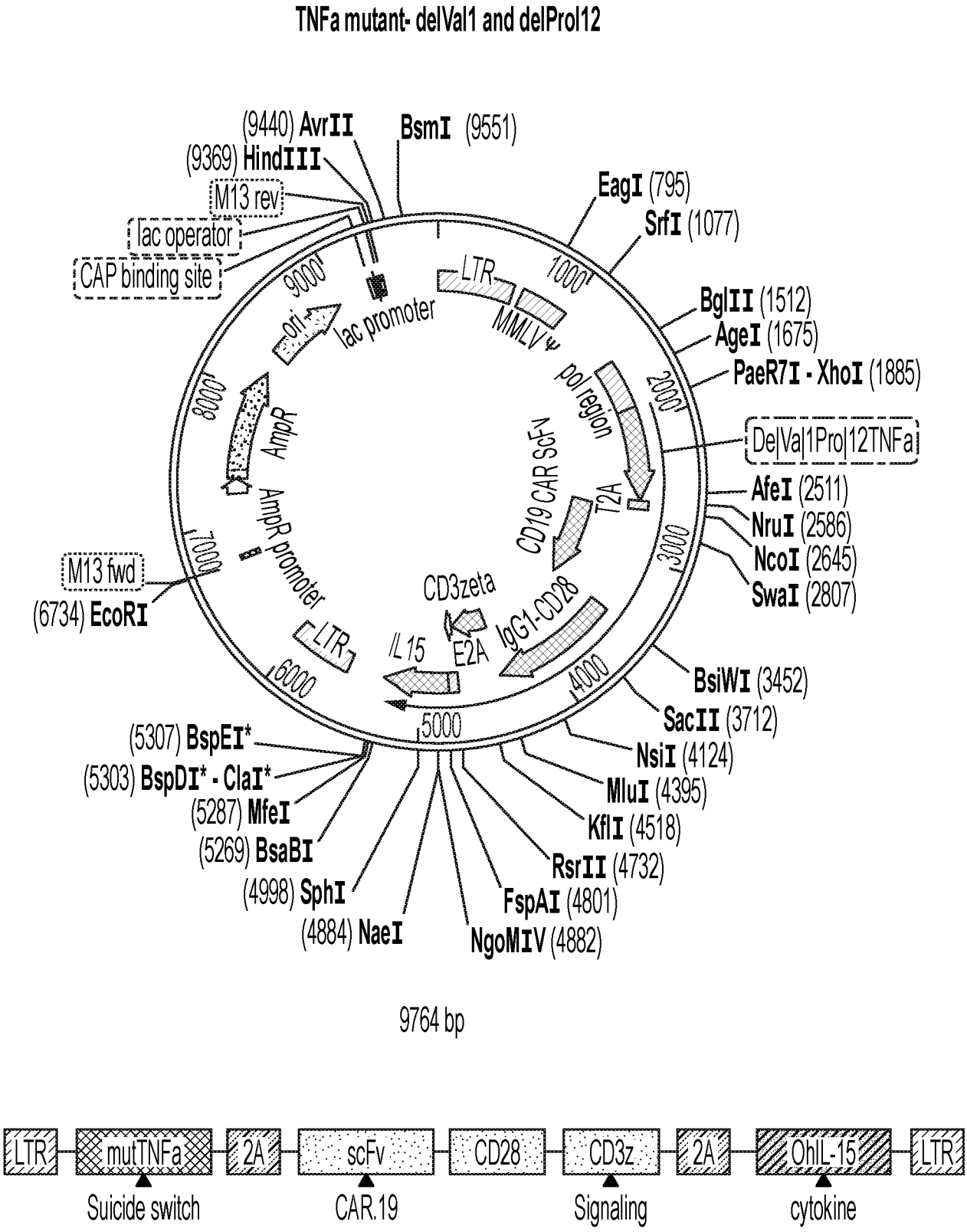
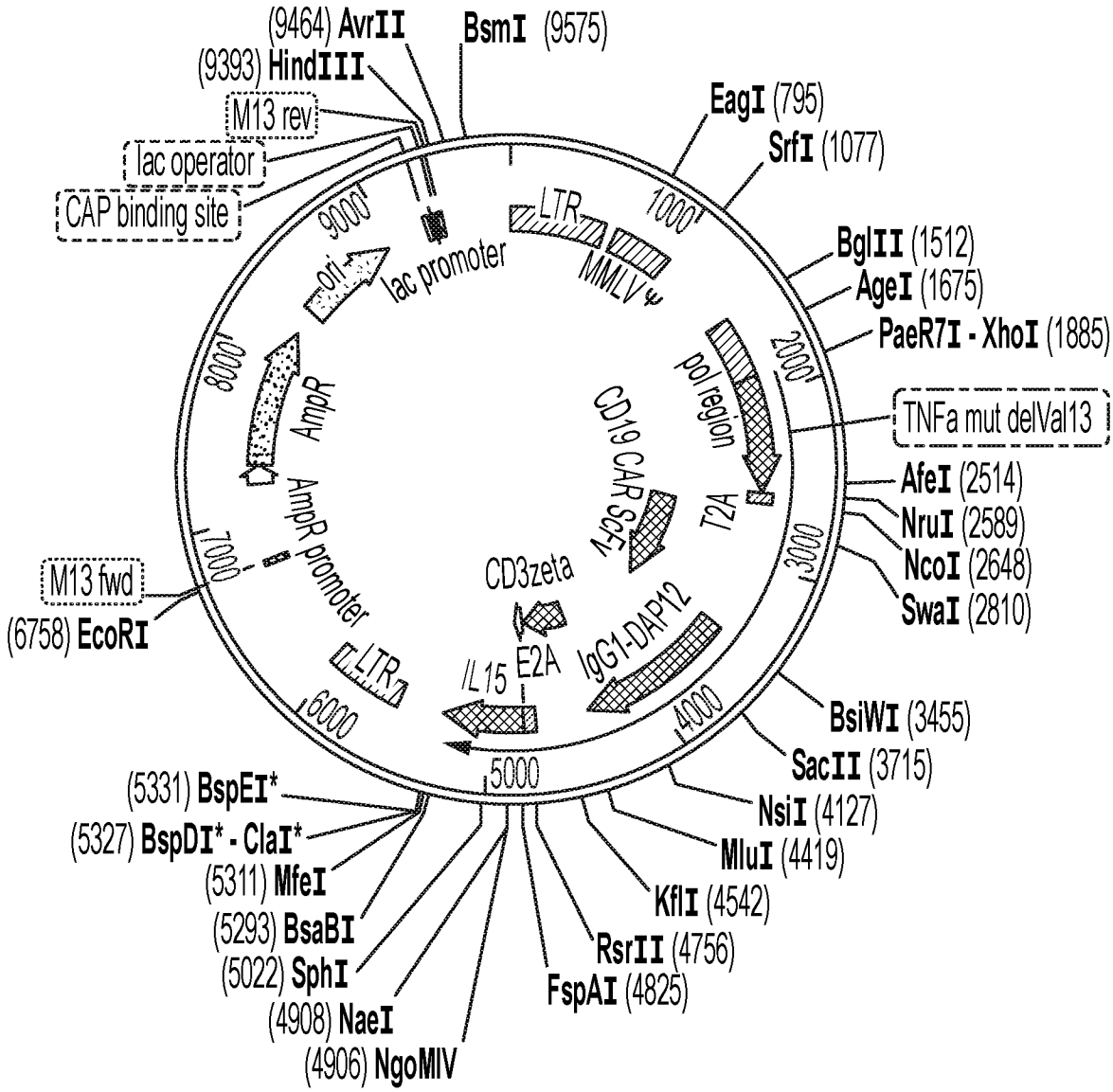

TNFa mutant- delVal1 and delPro12
(9440) AvrII
BsmI (9551)
(9369) HindIII
M13 rev
lac operator
CAP binding site
EagI (795)
SrfI (1077)
BglII (1512)
AgeI (1675)
PaeR7I - XhoI (1885)
DelVal1Pro12TNFa
AfeI (2511)
NruI (2586)
NcoI (2645)
SwaI (2807)
BsiWI (3452)
SacII (3712)
NsiI (4124)
MluI (4395)
KflI (4518)
RsrII (4732)
FspAI (4801)
NgoMIV (4882)
(4884) NaeI
(4998) SphI
(5269) BsaBI
(5287) MfeI
(5303) BspDI* - ClaI*
(5307) BspEI*
M13 fwd
(6734) EcoRI
ori
lac promoter
LTR
MMLV ψ
pol region
T2A
CD19 CAR ScFv
CD3zeta
E2A
IgG1-CD28
IL15
LTR
AmpR promoter
AmpR
1000
2000
3000
4000
5000
6000
7000
8000
9000
9764 bp
LTR
mutTNFa
2A
scFv
CD28
CD3z
2A
OhIL-15
LTR
Suicide switch
CAR.19
Signaling
cytokine

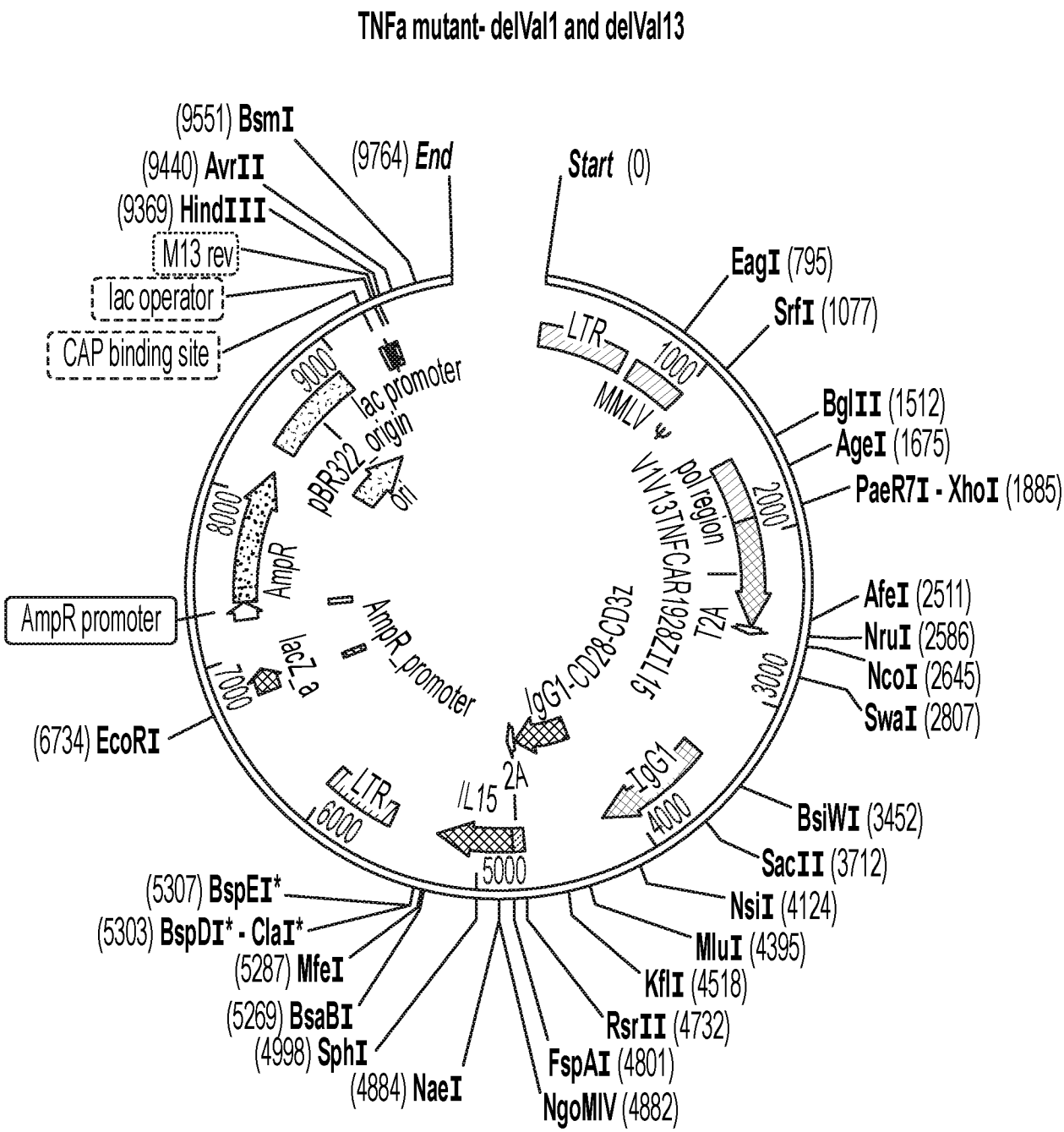
FIG. 2A

TNFa mutant- delVal13



Construct ID 18ACIQEP- TNFa mut delVal13 CAR.CD19.IgG1.DAP12.CD3z.IL15
9788bp

FIG. 2B



Cloned V1V13TNFCAR1928ZIL15 (18AC4AZP)
9764 bp

FIG. 2C

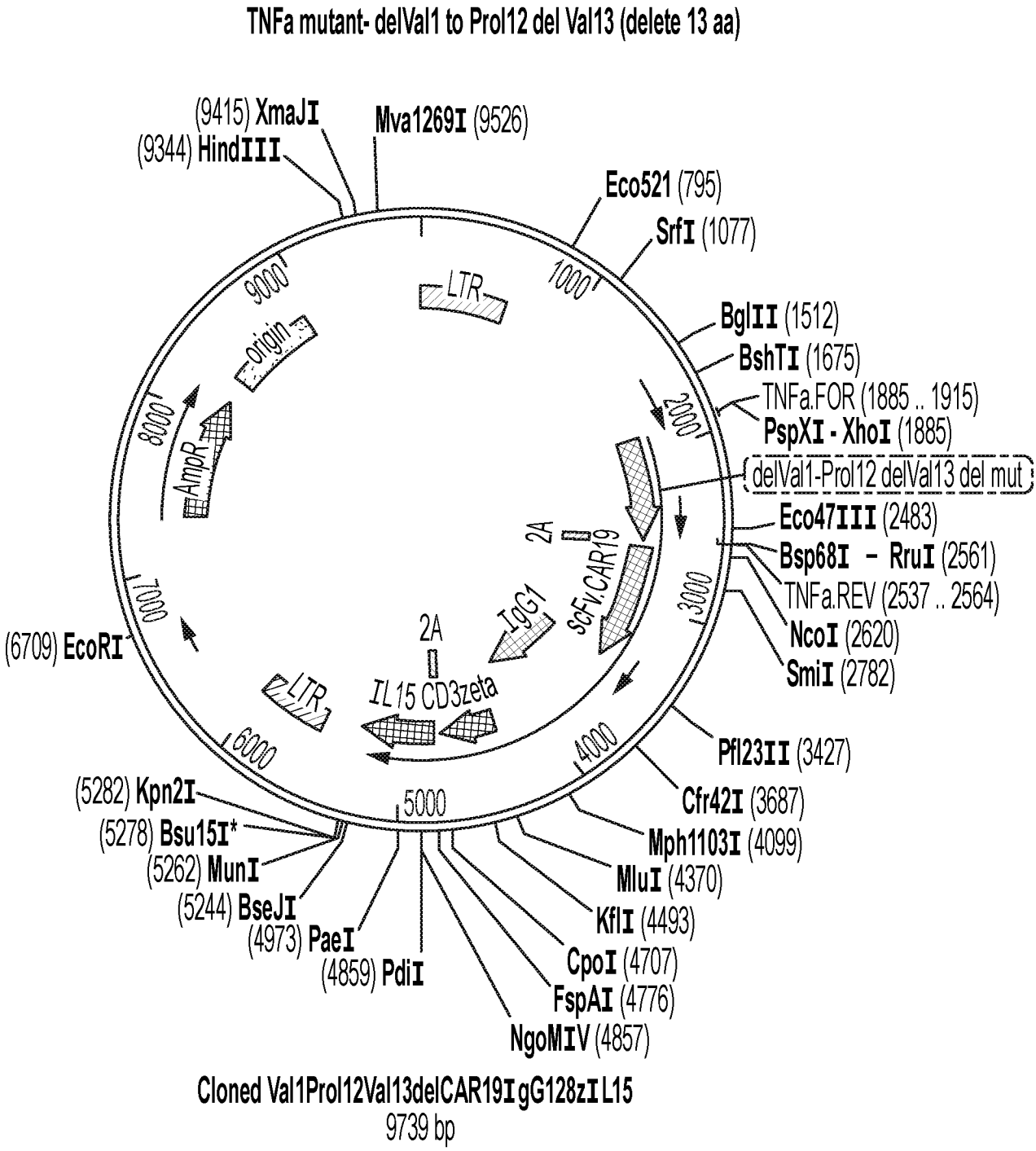

TNFa mutant- delVal1 to Prol12 del Val13 (delete 13 aa)
(9415) XmaJI
Mva1269I (9526)
(9344) HindIII
Eco521 (795)
SrfI (1077)
BglII (1512)
BshTI (1675)
TNFa.FOR (1885 .. 1915)
PspXI - XhoI (1885)
delVal1-Prol12 delVal13 del mut
Eco47III (2483)
Bsp68I - RruI (2561)
TNFa.REV (2537 .. 2564)
NcoI (2620)
SmiI (2782)
Pfl23II (3427)
Cfr42I (3687)
Mph1103I (4099)
MluI (4370)
KflI (4493)
CpoI (4707)
FspAI (4776)
NgoMIV (4857)
(4859) PdiI
(4973) PaeI
(5244) BseJI
(5262) MunI
(5278) Bsu15I*
(5282) Kpn2I
(6709) EcoRI
LTR
origin
AmpR
2A
IgG1
scFvCAR19
2A
IL15 CD3zeta
LTR
1000
2000
3000
4000
5000
6000
7000
8000
9000
Cloned Val1Prol12Val13delCAR19IgG128zIL15
9739 bp

FIG. 2D

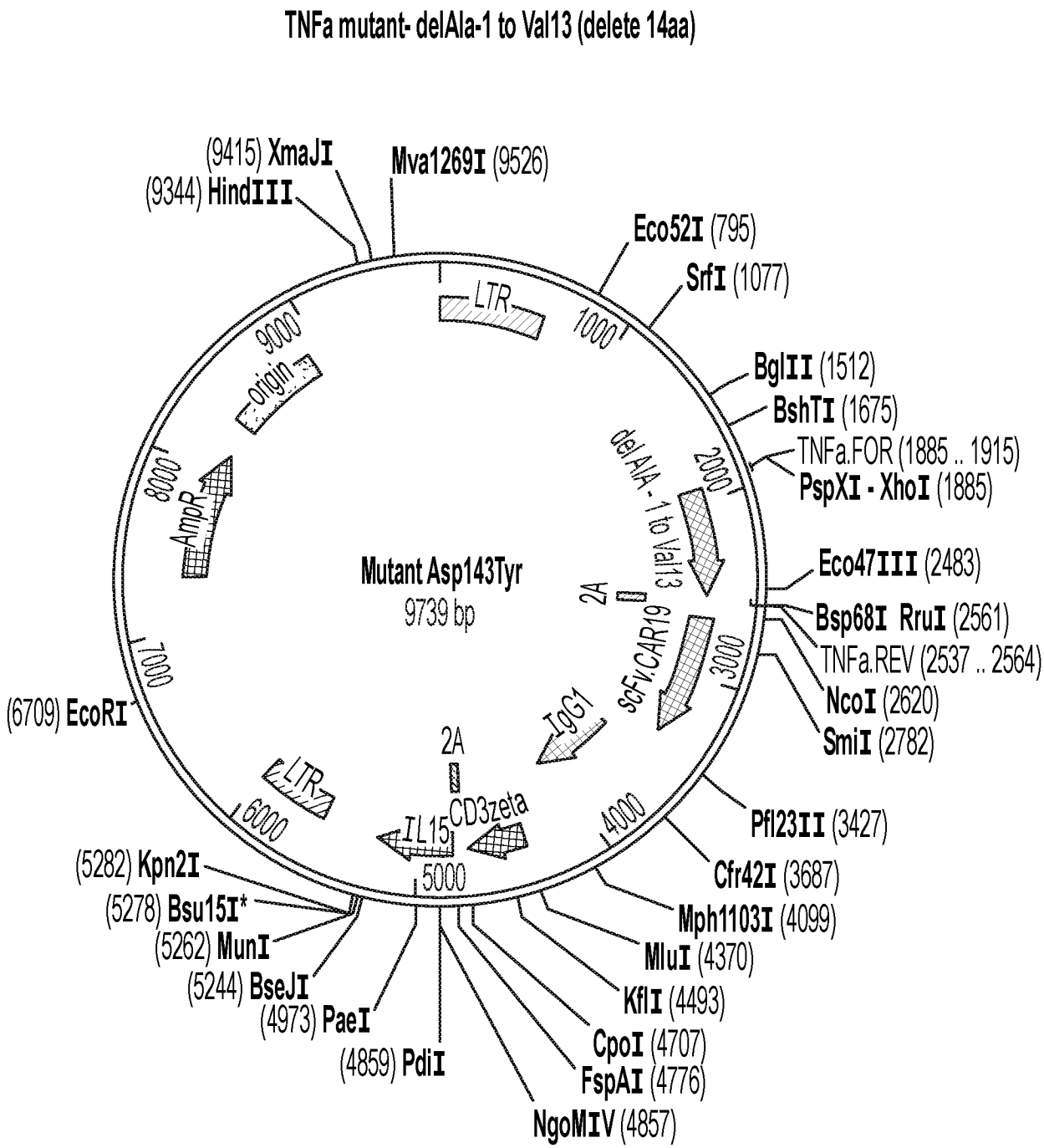

TNFa mutant- delAla-1 to Val13 (delete 14aa)
Mutant Asp143Tyr
9739 bp
(9415) XmaJI
(9344) HindIII
Mva1269I (9526)
Eco52I (795)
SrfI (1077)
BglII (1512)
BshTI (1675)
TNFa.FOR (1885 .. 1915)
PspXI - XhoI (1885)
Eco47III (2483)
Bsp68I RruI (2561)
TNFa.REV (2537 .. 2564)
NcoI (2620)
SmiI (2782)
Pfl23II (3427)
Cfr42I (3687)
Mph1103I (4099)
MluI (4370)
KflI (4493)
CpoI (4707)
FspAI (4776)
NgoMIV (4857)
(4859) PdiI
(4973) PaeI
(5244) BseJI
(5262) MunI
(5278) Bsu15I*
(5282) Kpn2I
(6709) EcoRI
LTR
origin
AmpR
delA1A - 1 to Val13
2A
scFv.CAR19
IgG1
2A
CD3zeta
IL15
LTR
1000
2000
3000
4000
5000
6000
7000
8000
9000

FIG. 2E

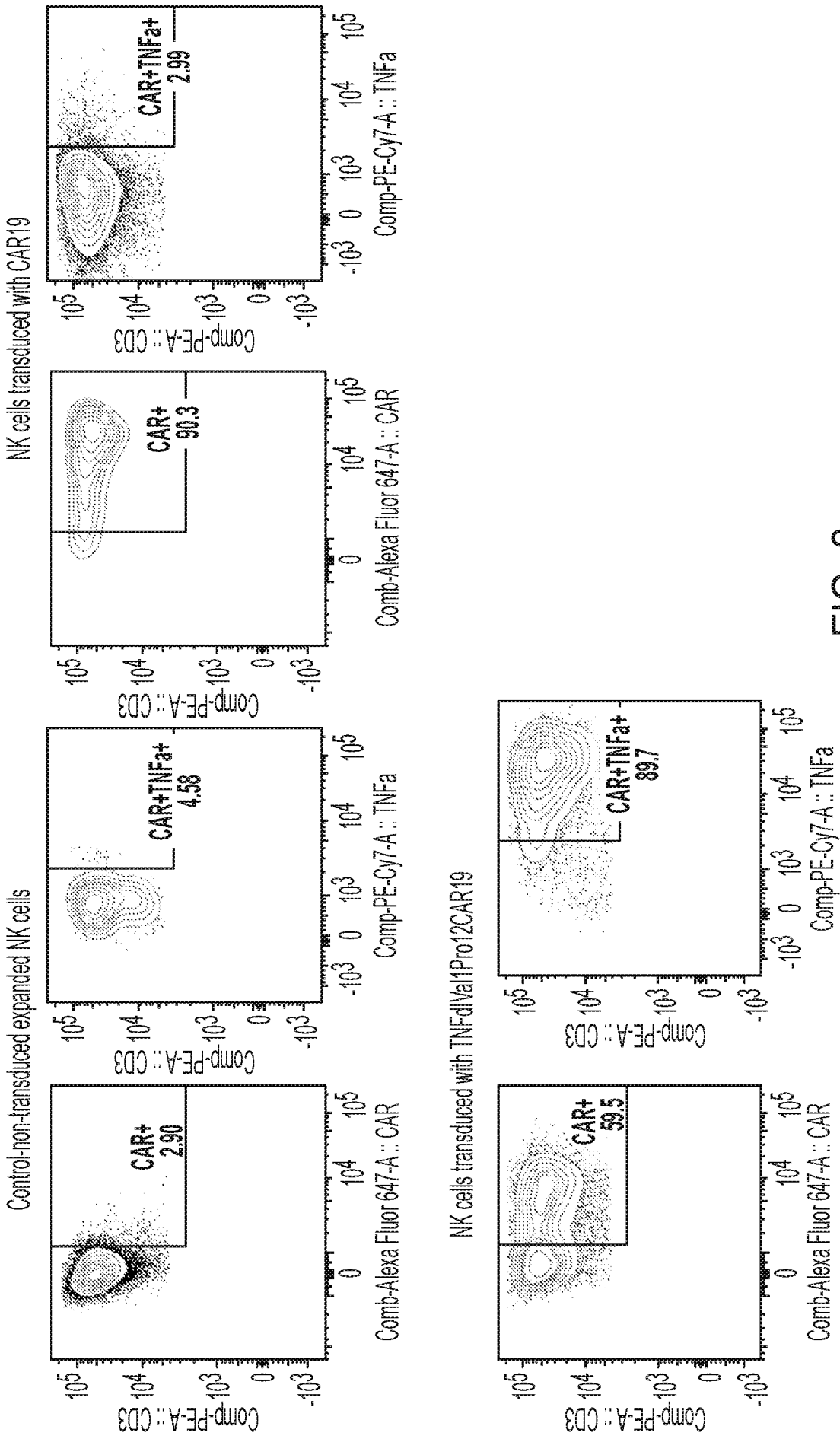

Control-non-transduced expanded NK cells
NK cells transduced with CAR19
NK cells transduced with TNFdiVal1Pro12CAR19
CAR+ 2.90
CAR+ 90.3
CAR+ 59.5
CAR+TNFa+ 4.58
CAR+TNFa+ 2.99
CAR+TNFa+ 89.7
Comb-Alexa Fluor 647-A :: CAR
Comp-PE-A :: CD3
Comp-PE-Cy7-A :: TNFa

FIG. 3

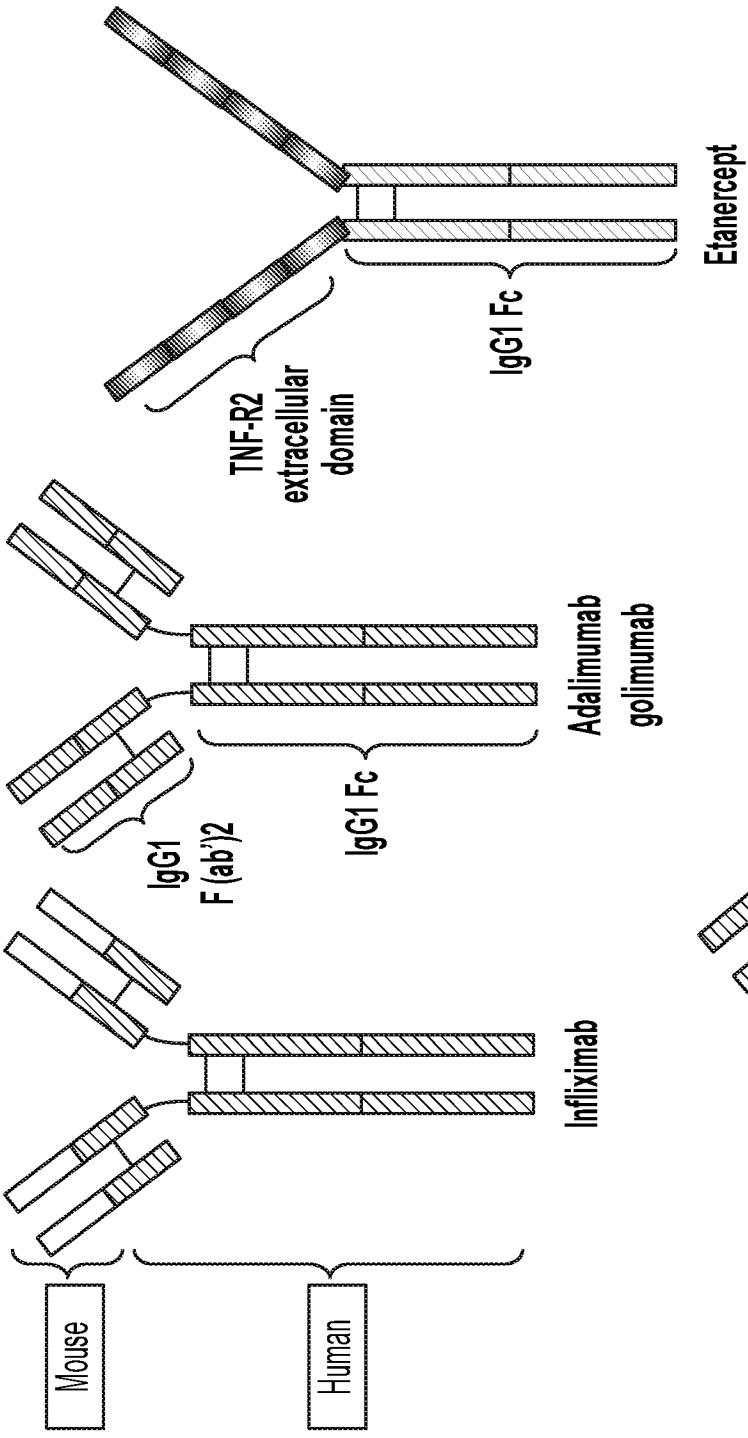

Mouse
Human
Infliximab
IgG1
F (ab')2
IgG1 Fc
Adalimumab
golimumab
TNF-R2
extracellular
domain
IgG1 Fc
Etanercept
Fusion of the
extracellular domain of
TNF-R2 and the Fc
region of IgG1.
PEG
PEG
Certolizumab pegol
PEGylated Fab' fragment of humanized
monoclonal anti-TNF antibody.

FIG. 4A

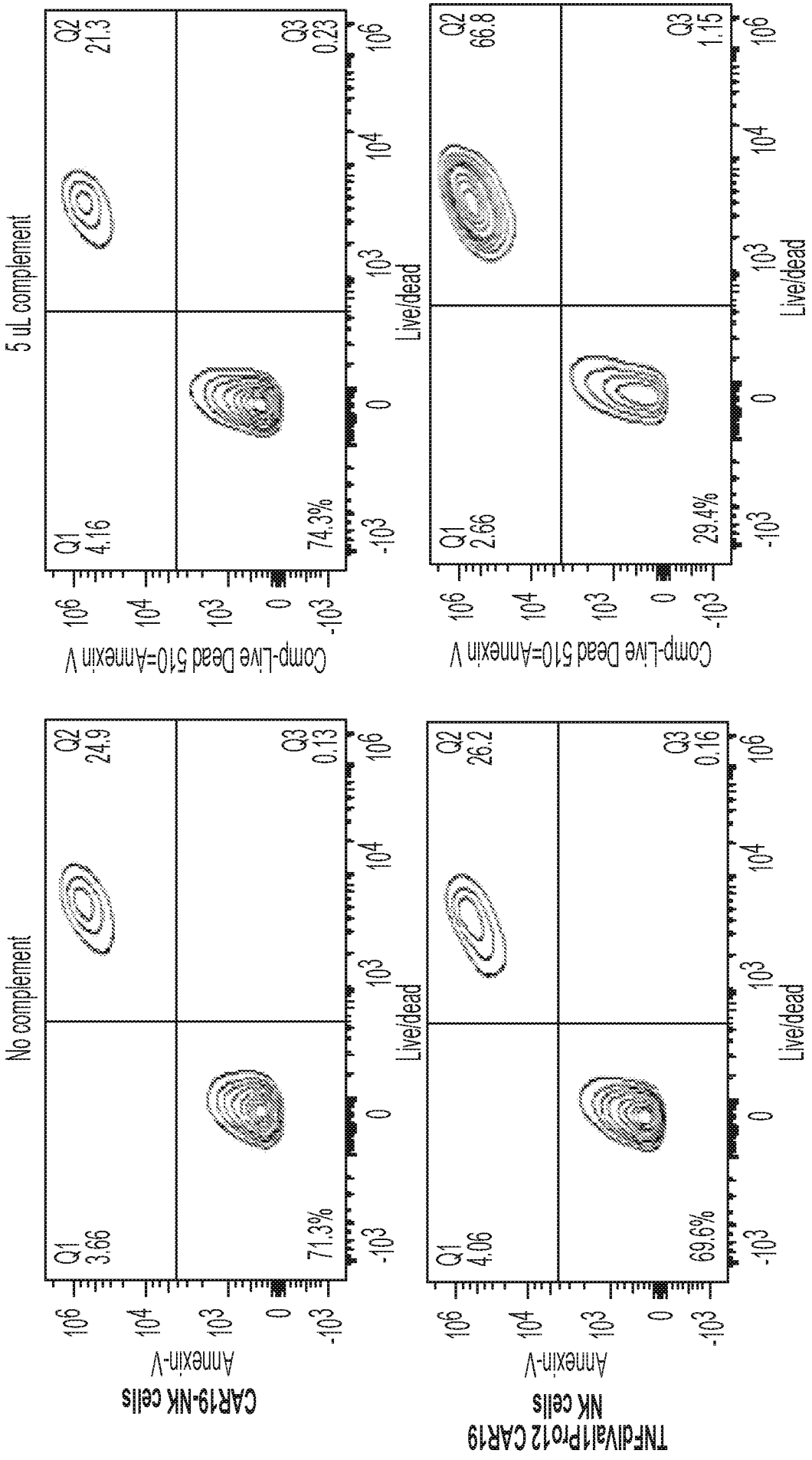

No complement
5 uL complement
CAR19-NK cells
TNFdiVal1Pro12 CAR19 NK cells
Annexin-V
Live/dead
Comp-Live Dead 510=Annexin V
Q1 3.66
Q2 24.9
Q3 0.13
71.3%
Q1 4.16
Q2 21.3
Q3 0.23
74.3%
Q1 4.06
Q2 26.2
Q3 0.16
69.6%
Q1 2.66
Q2 66.8
Q3 1.15
29.4%

FIG. 4B

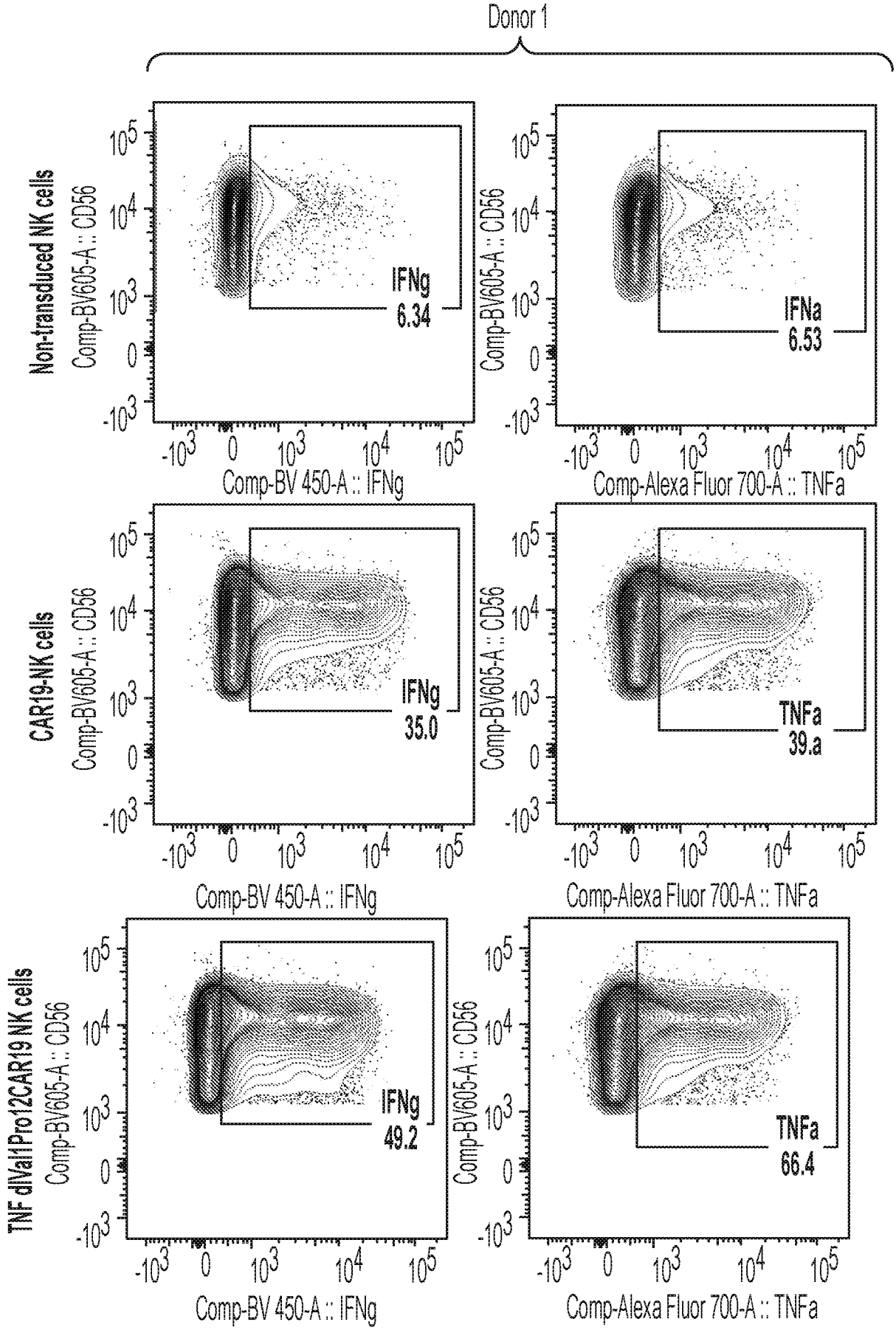

Donor 1
Non-transduced NK cells
CAR19-NK cells
TNF dVal1Pro12CAR19 NK cells
Comp-BV605-A :: CD56
Comp-BV 450-A :: IFNg
Comp-Alexa Fluor 700-A :: TNFa
IFNg
6.34
IFNa
6.53
IFNg
35.0
TNFa
39.a
IFNg
49.2
TNFa
66.4

FIG. 5A

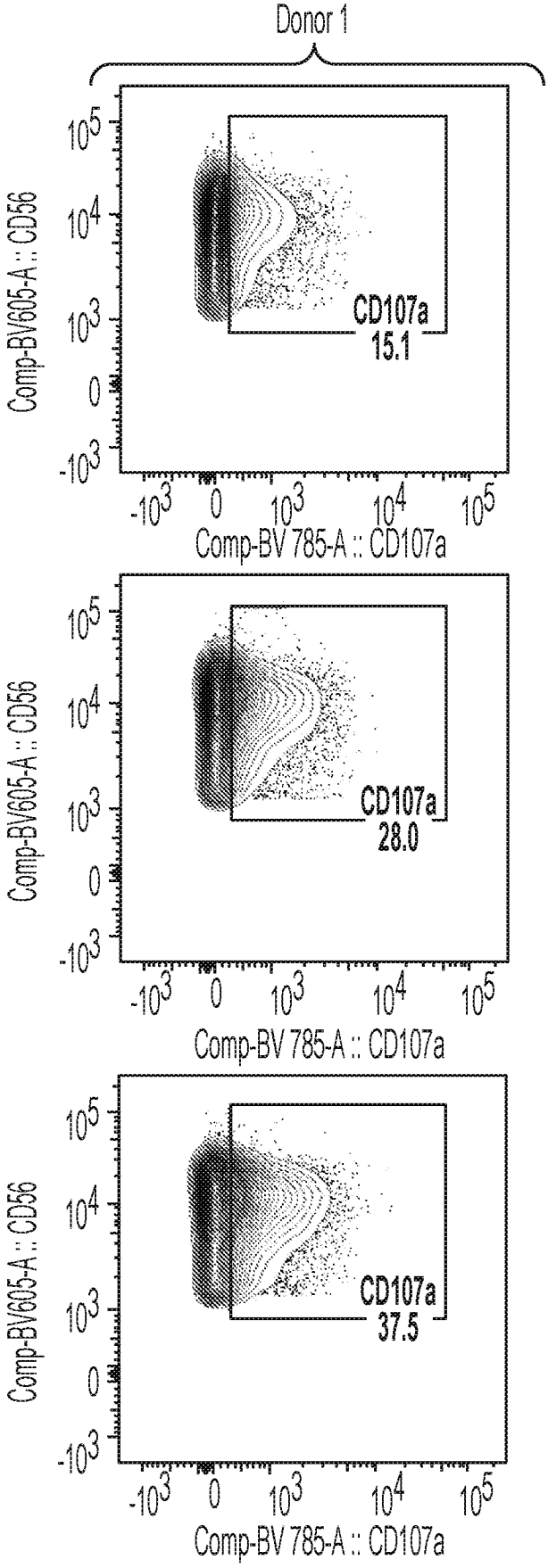


FIG. 5A
CONTINUED

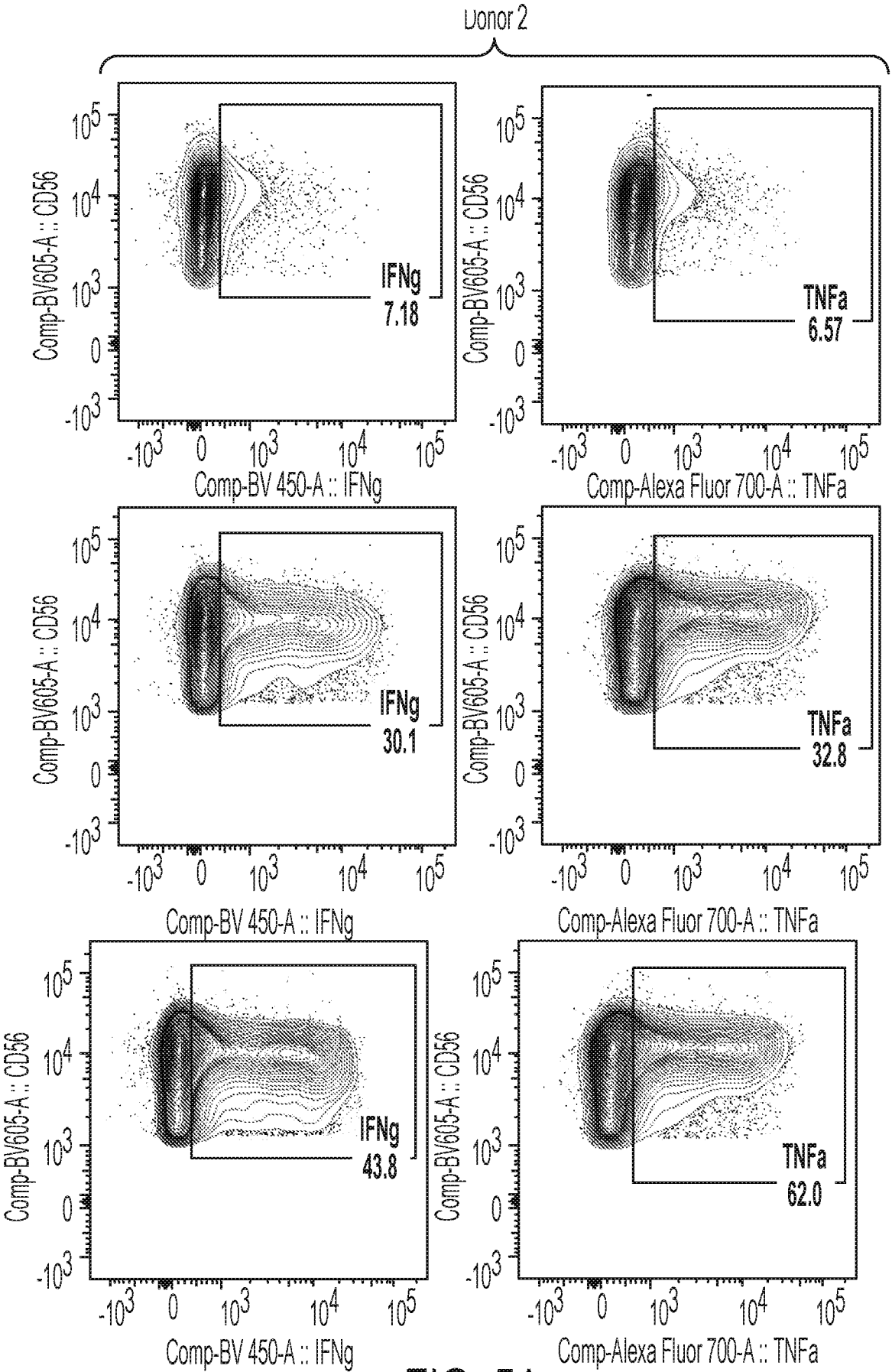


FIG. 5A
CONTINUED

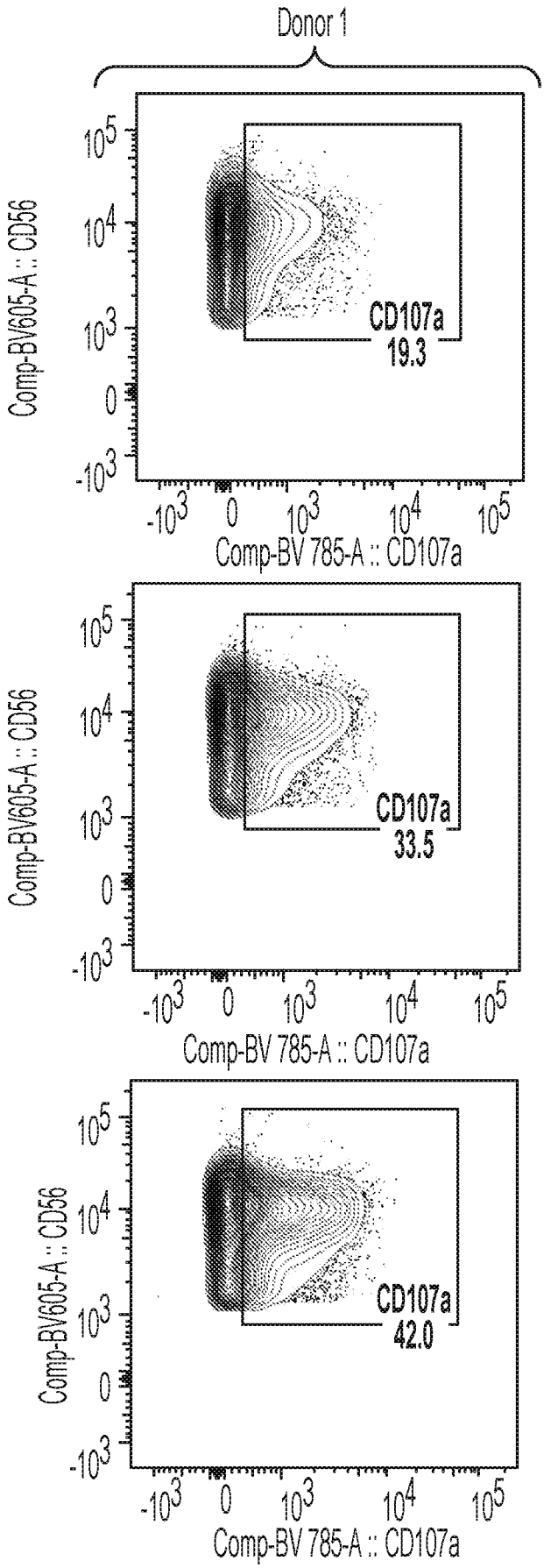


FIG. 5A
CONTINUED

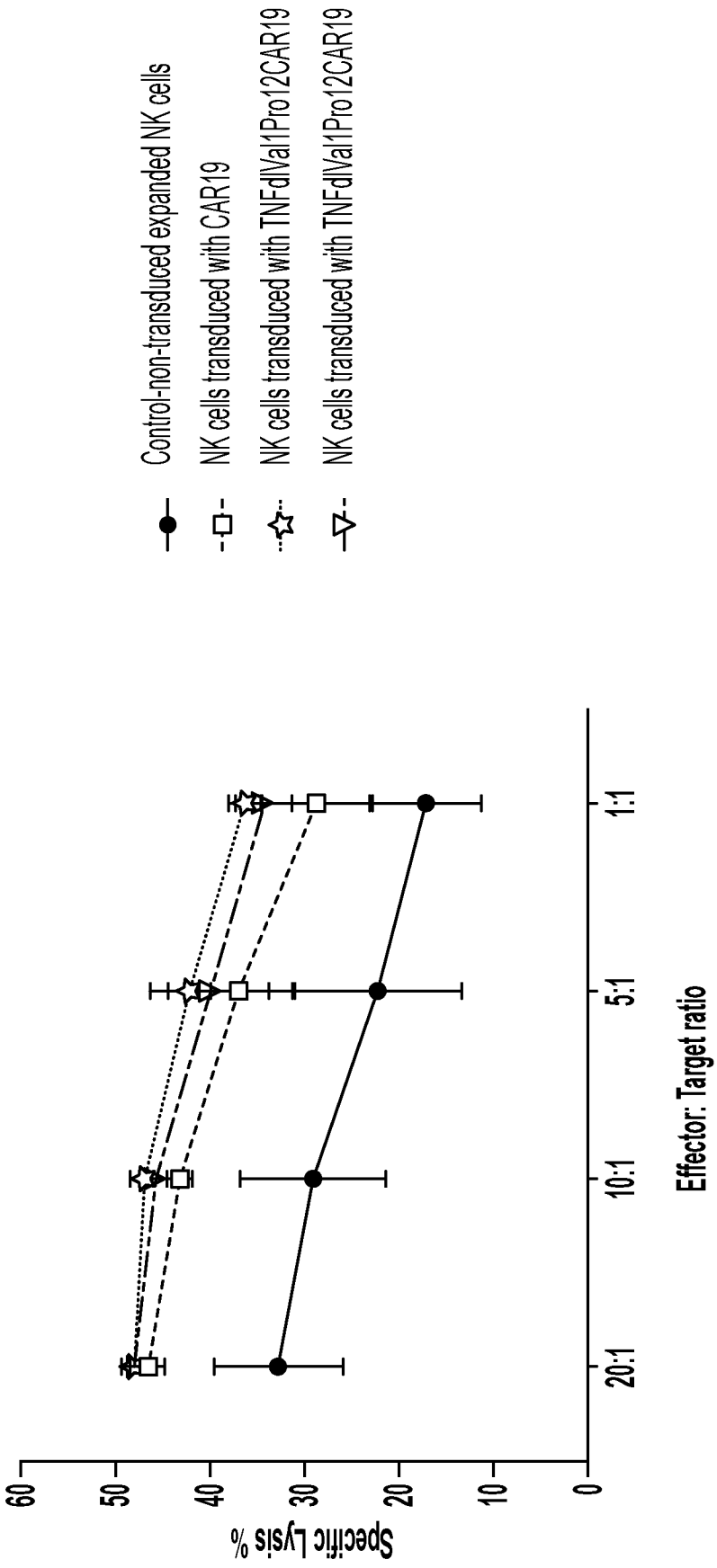

Control-non-transduced expanded NK cells
NK cells transduced with CAR19
NK cells transduced with TNFdlVal1Pro12CAR19
NK cells transduced with TNFdlVal1Pro12CAR19
Specific Lysis %
0
10
20
30
40
50
60
20:1
10:1
5:1
1:1
Effector: Target ratio

FIG. 5B

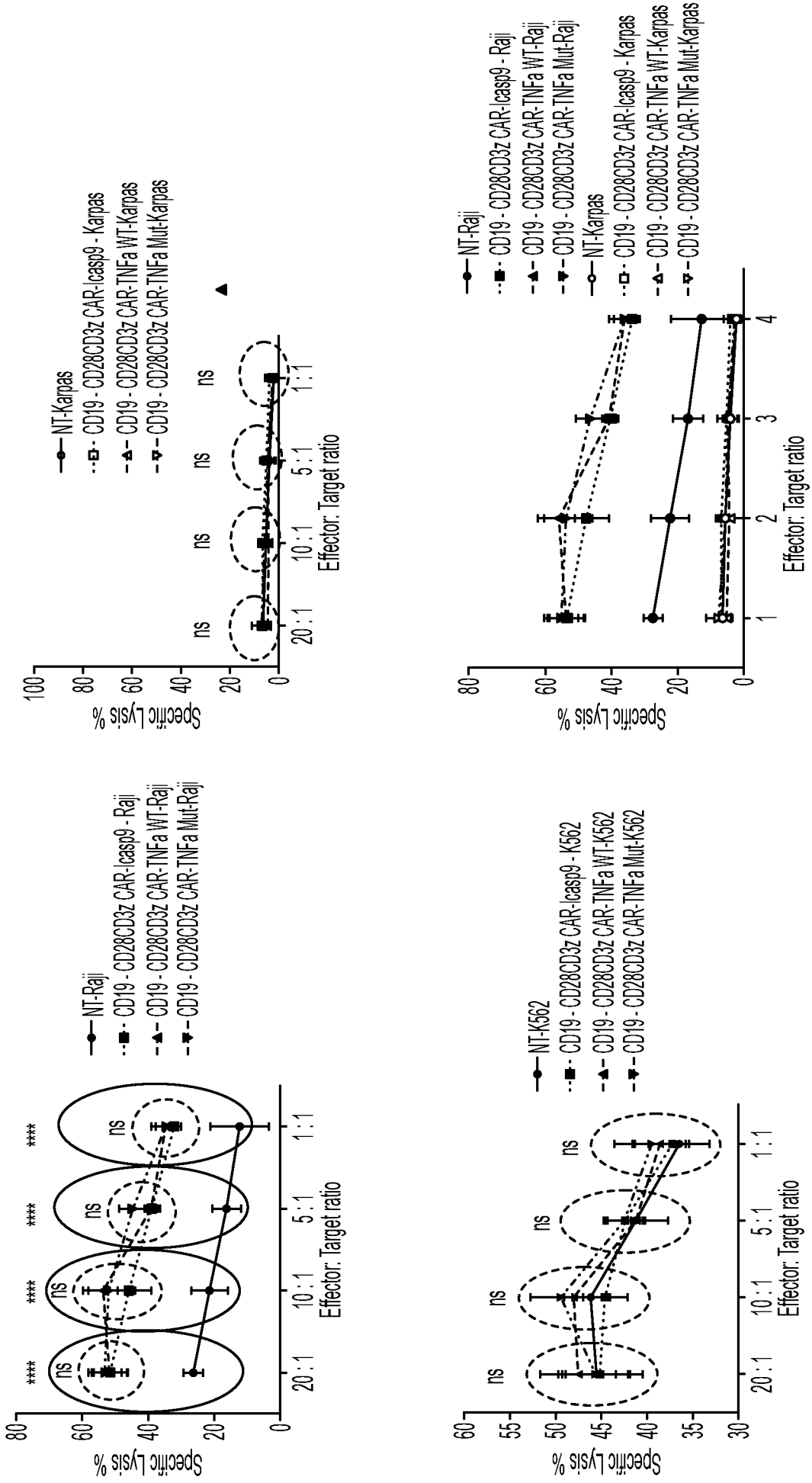

NT-Karpas
CD19 - CD28CD3z CAR-Icasp9 - Karpas
CD19 - CD28CD3z CAR-TNFa WT-Karpas
CD19 - CD28CD3z CAR-TNFa Mut-Karpas
Specific Lysis %
Effector: Target ratio
20 : 1
10 : 1
5 : 1
1 : 1
ns
NT-Raji
CD19 - CD28CD3z CAR-Icasp9 - Raji
CD19 - CD28CD3z CAR-TNFa WT-Raji
CD19 - CD28CD3z CAR-TNFa Mut-Raji
****
NT-K562
CD19 - CD28CD3z CAR-Icasp9 - K562
CD19 - CD28CD3z CAR-TNFa WT-K562
CD19 - CD28CD3z CAR-TNFa Mut-K562

FIG. 6

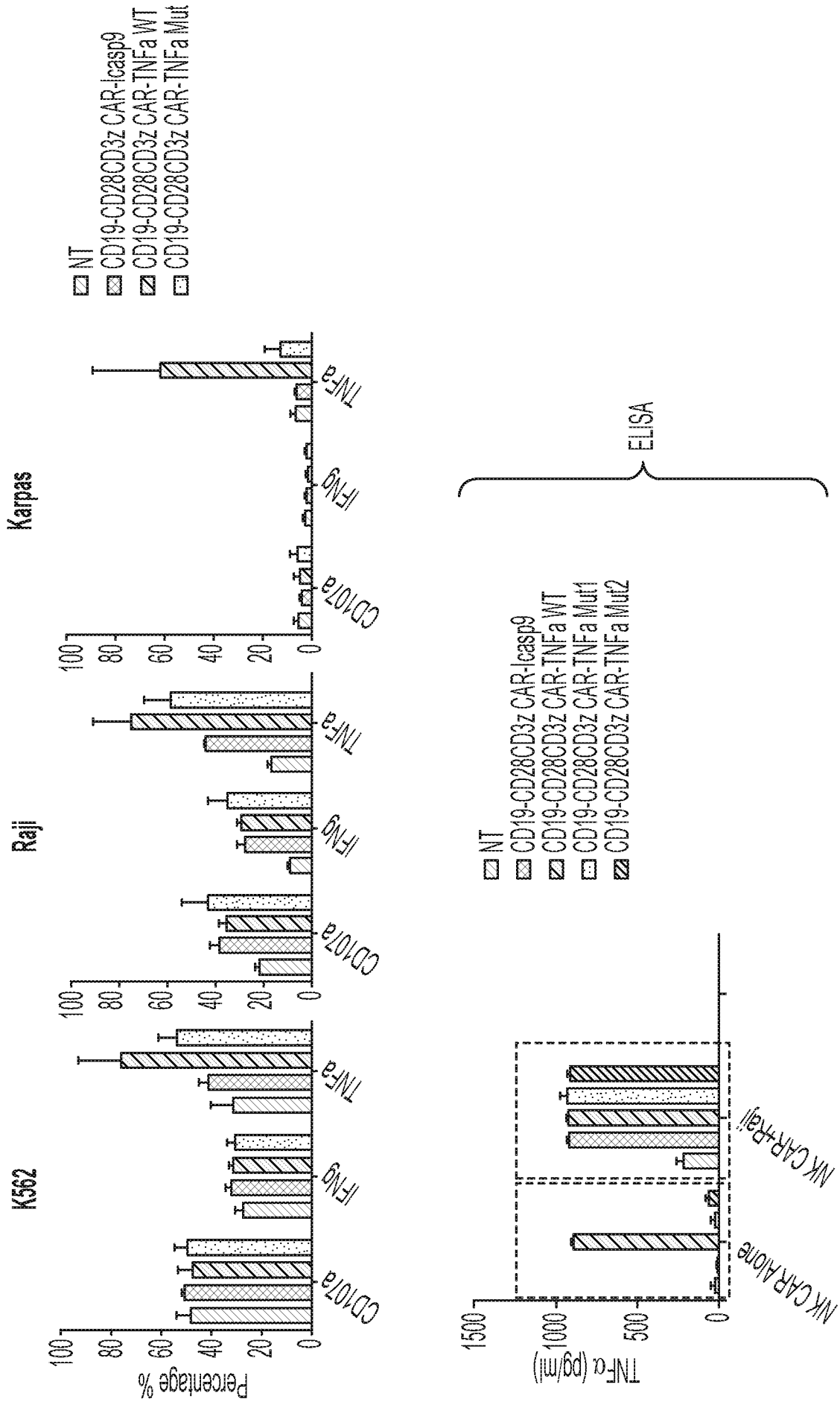

NT
CD19-CD28CD3z CAR-Icasp9
CD19-CD28CD3z CAR-TNFa WT
CD19-CD28CD3z CAR-TNFa Mut
K562
Raji
Karpas
Percentage %
CD107a
IFNg
TNFa
ELISA
NT
CD19-CD28CD3z CAR-Icasp9
CD19-CD28CD3z CAR-TNFa WT
CD19-CD28CD3z CAR-TNFa Mut1
CD19-CD28CD3z CAR-TNFa Mut2
TNFα (pg/ml)
NK CAR Alone
NK CAR+Raji

FIG. 7

MSTESMIRDVELAEEALPKKTGGPQGSRRCLFLSLFSFLIVAGATTLFCLLHFGVIGPQREEFPRD
LSLISPLAQAAHVVANPQAEGQLQWLNRRANALLANGVELRDNQLVVPSEGLYLIYSQVLFKG
QGCPSTHVLLTHTISRIAVSYQTKVNLLSAIKSPCQRETPEGAEAKPWYEPIYLGGVFQLEKGDRL
SAEINRPDYLDFAESGQVYFGIIAL

Single underline represents the receptor binding sites for TNF receptor 1 and 2 – these sites can be mutated to impair interaction of TNFalpha with its receptors.

Double underline E-F LOOP-target for interaction with the adalimumab and infliximab Fab fragments. In contrast, the E-F loops are not involved in the complex interactions of TNFα with TNFR1 or TNFR2

FIG. 8

P01375

MSTESMIRDVELAEEALPKKTGGPQGSRRCLFLSLFSFLIVAGATTLFCLLHFGVIGPQREEFPRDLSLISPLAQAVRSSSRTPSDKP

VAHVVANPQAEGQLQWLNRRANALLANGVELRDNQLVVPSEGLYLIYSQVLFKGQGCPSTHVLLTHTISRIAVSYQTKVNLLSAI

KSPCQRETPEGAEAKPWYEPIYLGGVFQLEKGDRLSAEINRPDYLDFAESGQVYFGIIAL

Intracytoplasmic domain (single underline)

STES The region containing the four amino acids of the casein kinase I (CKI) site (-STES-)

Transmembrane domain is FSFLIVAGATTLFCLLHFGVI

SPPL2b cut site (double underlines)

Linker is GPQREEFPRDLSLISPLAQA

TACE cut site is VRSSSRTPSDKPV

FIG. 9

atgagcactgaaaTGCATCCCGGAAGGGGGTCCTGGCACgaggaggcgctccccaagaagacagggggccccagggct
ccaggcggtgcttgttcctcagcctcttctccttcctgatcgtggcaggcgccaccacgctcttctTcctgctgcactttggagtgatcggccc
ccagagggaagagttccccagggacctctctctaatcagc~~cctctg~~gccgcagcccatgttgtagcaaaccctcaagctgaggggcagct
ccagtggctgaaccgccgggccaatgccctcctggccaatggcgtggagctgagagataaccagctggtggtgccatcagagggcctgt
acctcatctactcccaggtcctcttcaagggccaaggctgcccctccacccatgtgctcctcacccacaccatcagccgcatcgccgtctcc
CaccagaccaaggtcaacctcctctTCgccatcaagagcccctgccagagggagaccccagagggggctgaggccaagccctggtat
gagcccatctatctgggaggggtcttccagctggagaagggtgaccgactcaTcgctgagatcaatcggcccgactatctcTactttgccg
agtAtgggcaggtctactttgggatcattgccctgtcg

MSTEMHPGRGSWHEEALPKKTGGPQGSRRCLFLSLFSFLIVAGATTLFFLLHFGVIGPQREEFPRDLSLISPL~~AQ~~AHVVAN
PQAEGQLQWLNRRANALLANGVELRDNQLVVPSEGLYLIYSQVLFKGQGCPSTHVLLTHTISRIAVSHQTKVNLLFAIKSP
CQRETPEGAEAKPWYEPIYLGGVFQLEKGDRLIAEINRPDYLYFAEYGQVYFGIIALS

FIG. 10

FIG. 11A

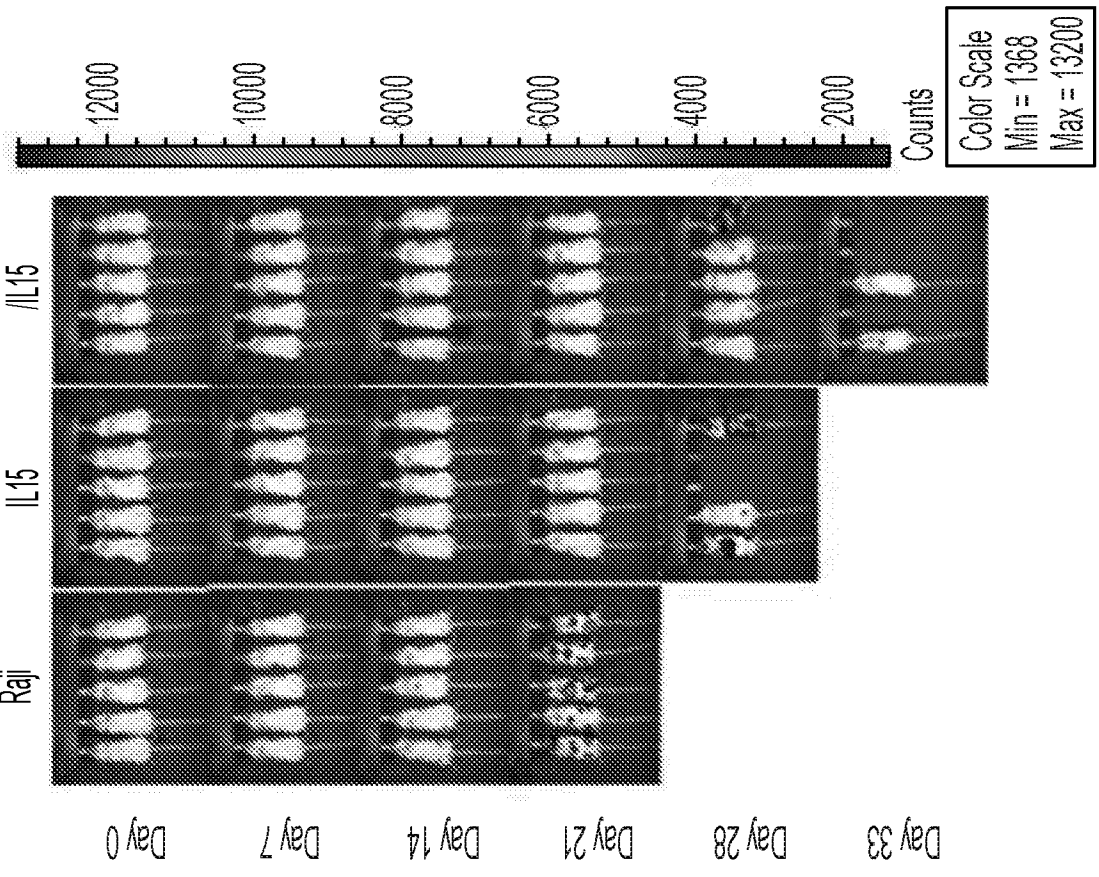
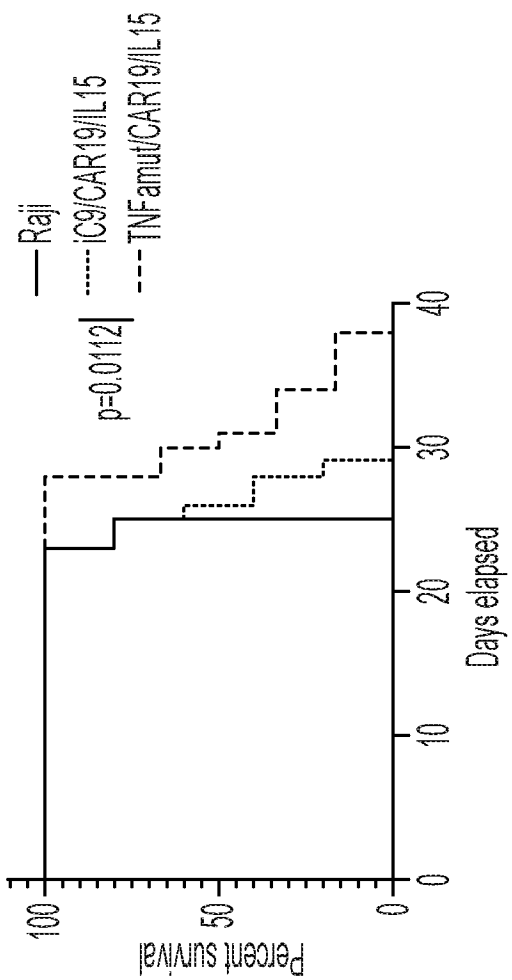


FIG. 11B



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<150> 62/769,405
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<160> 59

<170> PatentIn version 3.5

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<211> 231
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1 5 10 15

Leu Pro Lys Lys Thr Gly Gly Pro Gln Gly Ser Arg Arg Cys Leu Phe
20 25 30

Leu Ser Leu Phe Ser Phe Leu Ile Val Ala Gly Ala Thr Thr Leu Phe
35 40 45

Cys Leu Leu His Phe Gly Val Ile Gly Pro Gln Arg Glu Glu Phe Pro
50 55 60

Arg Asp Leu Ser Leu Ile Ser Pro Leu Ala Gln Ala Arg Ser Ser Ser
65 70 75 80

Arg Thr Pro Ser Asp Lys Val Ala His Val Val Ala Asn Pro Gln Ala
85 90 95

Glu Gly Gln Leu Gln Trp Leu Asn Arg Arg Ala Asn Ala Leu Leu Ala
100 105 110

Asn Gly Val Glu Leu Arg Asp Asn Gln Leu Val Val Pro Ser Glu Gly
115 120 125

Leu Tyr Leu Ile Tyr Ser Gln Val Leu Phe Lys Gly Gln Gly Cys Pro

```
    130                 135                 140

Ser Thr His Val Leu Leu Thr His Thr Ile Ser Arg Ile Ala Val Ser
145                 150                 155                 160

Tyr Gln Thr Lys Val Asn Leu Leu Ser Ala Ile Lys Ser Pro Cys Gln
                165                 170                 175

Arg Glu Thr Pro Glu Gly Ala Glu Ala Lys Pro Trp Tyr Glu Pro Ile
            180                 185                 190

Tyr Leu Gly Gly Val Phe Gln Leu Glu Lys Gly Asp Arg Leu Ser Ala
        195                 200                 205

Glu Ile Asn Arg Pro Asp Tyr Leu Asp Phe Ala Glu Ser Gly Gln Val
    210                 215                 220

Tyr Phe Gly Ile Ile Ala Leu
225                 230
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<210> 2
<211> 696
<212> DNA
<213> Artificial Sequence

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acaggggggc cccagggctc caggcggtgc ttgttcctca gcctcttctc cttcctgatc 120

gtggcaggcg ccaccacgct cttctgcctg ctgcactttg gagtgatcgg cccccagagg 180

gaagagttcc ccagggacct ctctctaatc agccctctgg cccaggcaag atcatcttct 240

cgaaccccga gtgacaaggt agcccatgtt gtagcaaacc ctcaagctga ggggcagctc 300

cagtggctga accgccgggc caatgccctc ctggccaatg gcgtggagct gagagataac 360

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caaggctgcc cctccaccca tgtgctcctc acccacacca tcagccgcat cgccgtctcc 480

taccagacca aggtcaacct cctctctgcc atcaagagcc cctgccagag ggagacccca 540

gagggggctg aggccaagcc ctggtatgag cccatctatc tgggaggggt cttccagctg 600

gagaagggtg accgactcag cgctgagatc aatcggcccg actatctcga ctttgccgag 660

tctgggcagg tctactttgg gatcattgcc ctgtcg 696

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<213> Artificial Sequence

<220>

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polypeptide"

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1 5 10 15

Leu Pro Lys Lys Thr Gly Gly Pro Gln Gly Ser Arg Arg Cys Leu Phe
20 25 30

Leu Ser Leu Phe Ser Phe Leu Ile Val Ala Gly Ala Thr Thr Leu Phe
35 40 45

Cys Leu Leu His Phe Gly Val Ile Gly Pro Gln Arg Glu Glu Phe Pro
50 55 60

Arg Asp Leu Ser Leu Ile Ser Pro Leu Ala Gln Ala Ala His Val Val
65 70 75 80

Ala Asn Pro Gln Ala Glu Gly Gln Leu Gln Trp Leu Asn Arg Arg Ala
85 90 95

Asn Ala Leu Leu Ala Asn Gly Val Glu Leu Arg Asp Asn Gln Leu Val
100 105 110

Val Pro Ser Glu Gly Leu Tyr Leu Ile Tyr Ser Gln Val Leu Phe Lys
115 120 125

Gly Gln Gly Cys Pro Ser Thr His Val Leu Leu Thr His Thr Ile Ser
130 135 140

Arg Ile Ala Val Ser Tyr Gln Thr Lys Val Asn Leu Leu Ser Ala Ile
145 150 155 160

Lys Ser Pro Cys Gln Arg Glu Thr Pro Glu Gly Ala Glu Ala Lys Pro
165 170 175

Trp Tyr Glu Pro Ile Tyr Leu Gly Gly Val Phe Gln Leu Glu Lys Gly
180 185 190

Asp Arg Leu Ser Ala Glu Ile Asn Arg Pro Asp Tyr Leu Asp Phe Ala
195 200 205

Glu Ser Gly Gln Val Tyr Phe Gly Ile Ile Ala Leu
210 215 220

<210> 4
<211> 663
<212> DNA
<213> Artificial Sequence

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gtggcaggcg ccaccacgct cttctgcctg ctgcactttg gagtgatcgg cccccagagg 180

gaagagttcc ccagggacct ctctctaatc agccctctgg cccaggcagc ccatgttgta 240

gcaaaccctc aagctgaggg gcagctccag tggctgaacc gccgggccaa tgccctcctg 300

gccaatggcg tggagctgag agataaccag ctggtggtgc catcagaggg cctgtacctc 360

atctactccc aggtcctctt caagggccaa ggctgcccct ccacccatgt gctcctcacc 420

cacaccatca gccgcatcgc cgtctcctac cagaccaagg tcaacctcct ctctgccatc 480

aagagcccct gccagaggga gaccccagag ggggctgagg ccaagccctg gtatgagccc 540

atctatctgg gaggggtctt ccagctggag aagggtgacc gactcagcgc tgagatcaat 600

cggcccgact atctcgactt tgccgagtct gggcaggtct actttgggat cattgccctg 660

tcg 663

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<212> PRT
<213> Artificial Sequence

<220>
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<223> /note="Description of Artificial Sequence: Synthetic
polypeptide"

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Met Ser Thr Glu Ser Met Ile Arg Asp Val Glu Leu Ala Glu Glu Ala
1 5 10 15

Leu Pro Lys Lys Thr Gly Gly Pro Gln Gly Ser Arg Arg Cys Leu Phe
20 25 30

Leu Ser Leu Phe Ser Phe Leu Ile Val Ala Gly Ala Thr Thr Leu Phe
35 40 45

Cys Leu Leu His Phe Gly Val Ile Gly Pro Gln Arg Glu Glu Phe Pro
50 55 60

Arg Asp Leu Ser Leu Ile Ser Pro Leu Ala Gln Ala Arg Ser Ser Ser
65 70 75 80

Arg Thr Pro Ser Asp Lys Pro Ala His Val Val Ala Asn Pro Gln Ala
85 90 95

Glu Gly Gln Leu Gln Trp Leu Asn Arg Arg Ala Asn Ala Leu Leu Ala
100 105 110

Asn Gly Val Glu Leu Arg Asp Asn Gln Leu Val Val Pro Ser Glu Gly
115 120 125

```
Leu Tyr Leu Ile Tyr Ser Gln Val Leu Phe Lys Gly Gln Gly Cys Pro
    130                 135                 140

Ser Thr His Val Leu Leu Thr His Thr Ile Ser Arg Ile Ala Val Ser
145                 150                 155                 160

Tyr Gln Thr Lys Val Asn Leu Leu Ser Ala Ile Lys Ser Pro Cys Gln
                165                 170                 175

Arg Glu Thr Pro Glu Gly Ala Glu Ala Lys Pro Trp Tyr Glu Pro Ile
            180                 185                 190

Tyr Leu Gly Gly Val Phe Gln Leu Glu Lys Gly Asp Arg Leu Ser Ala
        195                 200                 205

Glu Ile Asn Arg Pro Asp Tyr Leu Asp Phe Ala Glu Ser Gly Gln Val
    210                 215                 220

Tyr Phe Gly Ile Ile Ala Leu
225                 230
```

<210> 6
<211> 696
<212> DNA
<213> Artificial Sequence

<220>
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<223> /note="Description of Artificial Sequence: Synthetic polynucleotide"

<400> 6

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gtggcaggcg ccaccacgct cttctgcctg ctgcactttg gagtgatcgg cccccagagg      180

gaagagttcc ccagggacct ctctctaatc agccctctgg cccaggcaag atcatcttct      240

cgaaccccga gtgacaagcc tgcccatgtt gtagcaaacc ctcaagctga ggggcagctc      300

cagtggctga accgccgggc caatgccctc ctggccaatg gcgtggagct gagagataac      360

cagctggtgg tgccatcaga gggcctgtac ctcatctact cccaggtcct cttcaagggc      420

caaggctgcc cctccaccca tgtgctcctc acccacacca tcagccgcat cgccgtctcc      480

taccagacca aggtcaacct cctctctgcc atcaagagcc cctgccagag ggagacccca      540

gagggggctg aggccaagcc ctggtatgag cccatctatc tgggaggggt cttccagctg      600

gagaagggtg accgactcag cgctgagatc aatcggcccg actatctcga ctttgccgag      660

tctgggcagg tctactttgg gatcattgcc ctgtcg                                 696
```

<210> 7
<211> 233
<212> PRT

<213> Homo sapiens

<400> 7
Met Ser Thr Glu Ser Met Ile Arg Asp Val Glu Leu Ala Glu Glu Ala
1   5   10   15

Leu Pro Lys Lys Thr Gly Gly Pro Gln Gly Ser Arg Arg Cys Leu Phe
            20   25   30

Leu Ser Leu Phe Ser Phe Leu Ile Val Ala Gly Ala Thr Thr Leu Phe
        35   40   45

Cys Leu Leu His Phe Gly Val Ile Gly Pro Gln Arg Glu Glu Phe Pro
    50   55   60

Arg Asp Leu Ser Leu Ile Ser Pro Leu Ala Gln Ala Val Arg Ser Ser
65   70   75   80

Ser Arg Thr Pro Ser Asp Lys Pro Val Ala His Val Val Ala Asn Pro
                85   90   95

Gln Ala Glu Gly Gln Leu Gln Trp Leu Asn Arg Arg Ala Asn Ala Leu
            100   105   110

Leu Ala Asn Gly Val Glu Leu Arg Asp Asn Gln Leu Val Val Pro Ser
        115   120   125

Glu Gly Leu Tyr Leu Ile Tyr Ser Gln Val Leu Phe Lys Gly Gln Gly
    130   135   140

Cys Pro Ser Thr His Val Leu Leu Thr His Thr Ile Ser Arg Ile Ala
145   150   155   160

Val Ser Tyr Gln Thr Lys Val Asn Leu Leu Ser Ala Ile Lys Ser Pro
                165   170   175

Cys Gln Arg Glu Thr Pro Glu Gly Ala Glu Ala Lys Pro Trp Tyr Glu
            180   185   190

Pro Ile Tyr Leu Gly Gly Val Phe Gln Leu Glu Lys Gly Asp Arg Leu
        195   200   205

Ser Ala Glu Ile Asn Arg Pro Asp Tyr Leu Asp Phe Ala Glu Ser Gly
    210   215   220

Gln Val Tyr Phe Gly Ile Ile Ala Leu
225   230

<210> 8
<211> 157
<212> PRT
<213> Homo sapiens

<400> 8

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Val Arg Ser Ser Ser Arg Thr Pro Ser Asp Lys Pro Val Ala His Val
1               5                   10                  15


Val Ala Asn Pro Gln Ala Glu Gly Gln Leu Gln Trp Leu Asn Arg Arg
            20                  25                  30


Ala Asn Ala Leu Leu Ala Asn Gly Val Glu Leu Arg Asp Asn Gln Leu
        35                  40                  45


Val Val Pro Ser Glu Gly Leu Tyr Leu Ile Tyr Ser Gln Val Leu Phe
    50                  55                  60


Lys Gly Gln Gly Cys Pro Ser Thr His Val Leu Leu Thr His Thr Ile
65                  70                  75                  80


Ser Arg Ile Ala Val Ser Tyr Gln Thr Lys Val Asn Leu Leu Ser Ala
                85                  90                  95


Ile Lys Ser Pro Cys Gln Arg Glu Thr Pro Glu Gly Ala Glu Ala Lys
            100                 105                 110


Pro Trp Tyr Glu Pro Ile Tyr Leu Gly Gly Val Phe Gln Leu Glu Lys
        115                 120                 125


Gly Asp Arg Leu Ser Ala Glu Ile Asn Arg Pro Asp Tyr Leu Asp Phe
    130                 135                 140


Ala Glu Ser Gly Gln Val Tyr Phe Gly Ile Ile Ala Leu
145                 150                 155


<210> 9
<211> 113
<212> PRT
<213> Unknown

<220>
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<223> /note="Description of Unknown:
      DAP12 sequence"

<400> 9
Met Gly Gly Leu Glu Pro Cys Ser Arg Leu Leu Leu Leu Pro Leu Leu
1               5                   10                  15


Leu Ala Val Ser Gly Leu Arg Pro Val Gln Ala Gln Ala Gln Ser Asp
            20                  25                  30


Cys Ser Cys Ser Thr Val Ser Pro Gly Val Leu Ala Gly Ile Val Met
        35                  40                  45


Gly Asp Leu Val Leu Thr Val Leu Ile Ala Leu Ala Val Tyr Phe Leu
    50                  55                  60


Gly Arg Leu Val Pro Arg Gly Arg Gly Ala Ala Glu Ala Ala Thr Arg
65                  70                  75                  80
```

```
Lys Gln Arg Ile Thr Glu Thr Glu Ser Pro Tyr Gln Glu Leu Gln Gly
                85                  90                  95

Gln Arg Ser Asp Val Tyr Ser Asp Leu Asn Thr Gln Arg Pro Tyr Tyr
            100                 105                 110

Lys
```

<210> 10
<211> 342
<212> DNA
<213> Unknown

<220>
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DAP12 sequence"

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ggtctccgtc ctgtccaggc ccaggcccag agcgattgca gttgctctac ggtgagcccg 120

ggcgtgctgg cagggatcgt gatgggagac ctggtgctga cagtgctcat tgccctggcc 180

gtgtacttcc tgggccggct ggtccctcgg gggcgagggg ctgcggaggc agcgacccgg 240

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gtctacagcg acctcaacac acagaggccg tattacaaat ga 342

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<211> 182
<212> PRT
<213> Unknown

<220>
<221> source
<223> /note="Description of Unknown:
CD28 sequence"

<400> 11
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Lys Phe Trp Val Leu Val Val Val Gly Gly Val Leu Ala Cys Tyr Ser
1               5                   10                  15

Leu Leu Val Thr Val Ala Phe Ile Ile Phe Trp Val Arg Ser Lys Arg
            20                  25                  30

Ser Arg Leu Leu His Ser Asp Tyr Met Asn Met Thr Pro Arg Arg Pro
        35                  40                  45

Gly Pro Thr Arg Lys His Tyr Gln Pro Tyr Ala Pro Pro Arg Asp Phe
    50                  55                  60

Ala Ala Tyr Arg Ser Arg Val Lys Phe Ser Arg Ser Ala Asp Ala Pro
65                  70                  75                  80
```

```
Ala Tyr Gln Gln Gly Gln Asn Gln Leu Tyr Asn Glu Leu Asn Leu Gly
                85                  90                  95

Arg Arg Glu Glu Tyr Asp Val Leu Asp Lys Arg Arg Gly Arg Asp Pro
            100                 105                 110

Glu Met Gly Gly Lys Pro Arg Arg Lys Asn Pro Gln Glu Gly Leu Tyr
        115                 120                 125

Asn Glu Leu Gln Lys Asp Lys Met Ala Glu Ala Tyr Ser Glu Ile Gly
    130                 135                 140

Met Lys Gly Glu Arg Arg Arg Gly Lys Gly His Asp Gly Leu Tyr Gln
145                 150                 155                 160

Gly Leu Ser Thr Ala Thr Lys Asp Thr Tyr Asp Ala Leu His Met Gln
                165                 170                 175

Ala Leu Pro Pro Arg Gly
            180
```

<210> 12
<211> 209
<212> DNA
<213> Unknown

<220>
<221> source
<223> /note="Description of Unknown:
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ggcctttatt attttctggg tgaggagtaa gaggagcagg ctcctgcaca gtgactacat 120

gaacatgact ccccgccgcc ccgggcccac ccgcaagcat taccagccct atgccccacc 180

acgcgacttc gcagcctatc gctcacgcg 209

<210> 13
<211> 247
<212> PRT
<213> Unknown

<220>
<221> source
<223> /note="Description of Unknown:
IgG1 hinge sequence"

<400> 13
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Ser Tyr Val Thr Val Ser Ser Gln Asp Pro Ala Glu Pro Lys Ser Pro
1               5                   10                  15

Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly
            20                  25                  30

Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met
        35                  40                  45
```

```
Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His
    50                  55                  60

Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val
65                  70                  75                  80

His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr
                85                  90                  95

Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly
            100                 105                 110

Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile
        115                 120                 125

Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val
    130                 135                 140

Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser
145                 150                 155                 160

Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu
                165                 170                 175

Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro
            180                 185                 190

Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val
        195                 200                 205

Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met
    210                 215                 220

His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser
225                 230                 235                 240

Pro Gly Lys Lys Asp Pro Lys
                245
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<210> 14
<211> 734
<212> DNA
<213> Unknown

<220>
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IgG1 hinge sequence"

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atgcccaccg tgcccagcac ctgaactcct gggggggaccg tcagtcttcc tcttcccccc 120

aaaacccaag gacaccctca tgatctcccg gacccctgag gtcacatgcg tggtggtgga 180

cgtgagccac gaagaccctg aggtcaagtt caactggtac gtggacggcg tggaggtgca 240

taatgccaag acaaagccgc gggaggagca gtacaacagc acgtaccgtg tggtcagcgt 300

cctcaccgtc ctgcaccagg actggctgaa tggcaaggag tacaagtgca aggtctccaa 360

caaagccctc ccagccccca tcgagaaaac catctccaaa gccaaagggc agccccgaga 420

accacaggtg tacaccctgc ccccatcccg ggatgagctg accaagaacc aggtcagcct 480

gacctgcctg gtcaaaggct tctatcccag cgacatcgcc gtggagtggg agagcaatgg 540

gcaaccggag aacaactaca agaccacgcc tcccgtgctg gactccgacg gctccttctt 600

cctctacagc aagctcaccg tggacaagag caggtggcag caggggaacg tcttctcatg 660

ctccgtgatg catgaggctc tgcacaacca ctacacgcag aagagcctct ccctgtctcc 720

gggtaaaaaa gatc 734

<210> 15
<211> 9764
<212> DNA
<213> Artificial Sequence

<220>
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polynucleotide"

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atggaaaaat acataactga gaatagaaaa gttcagatca aggtcaggaa cagatggaac 120

agctgaatat gggccaaaca ggatatctgt ggtaagcagt tcctgccccg gctcagggcc 180

aagaacagat ggaacagctg aatatgggcc aaacaggata tctgtggtaa gcagttcctg 240

ccccggctca gggccaagaa cagatggtcc ccagatgcgg tccagccctc agcagtttct 300

agagaaccat cagatgtttc cagggtgccc caaggacctg aaatgaccct gtgccttatt 360

tgaactaacc aatcagttcg cttctcgctt ctgttcgcgc gcttatgctc cccgagctca 420

ataaaagagc ccacaacccc tcactcgggg cgccagtcct ccgattgact gagtcgcccg 480

ggtacccgtg tatccaataa accctcttgc agttgcatcc gacttgtggt ctcgctgttc 540

cttgggaggg tctcctctga gtgattgact acccgtcagc gggggtcttt catttggggg 600

ctcgtccggg atcgggagac ccctgcccag ggaccaccga cccaccaccg ggaggtaagc 660

tggccagcaa cttatctgtg tctgtccgat tgtctagtgt ctatgactga ttttatgcgc 720

ctgcgtcggt actagttagc taactagctc tgtatctggc ggacccgtgg tggaactgac 780

gagttcggaa cacccggccg caaccctggg agacgtccca gggacttcgg gggccgtttt 840

tgtggcccga cctgagtcct aaaatcccga tcgtttagga ctctttggtg cacccccctt 900

agaggaggga tatgtggttc tggtaggaga cgagaaccta aaacagttcc cgcctccgtc 960

tgaatttttg ctttcggttt gggaccgaag ccgcgccgcg cgtcttgtct gctgcagcat 1020

cgttctgtgt tgtctctgtc tgactgtgtt tctgtatttg tctgaaaata tgggcccggg 1080

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cgctcacaac cagtcggtag atgtcaagaa gagacgttgg gttaccttct gctctgcaga 1200

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ccaggttaag atcaaggtct tttcacctgg cccgcatgga cacccagacc aggtggggta 1320

catcgtgacc tgggaagcct tggcttttga ccccccctccc tgggtcaagc cctttgtaca 1380

ccctaagcct ccgcctcctc ttcctccatc cgccccgtct ctccccccttg aacctcctcg 1440

ttcgaccccg cctcgatcct ccctttatcc agccctcact ccttctctag gcgcccccat 1500

atggccatat gagatcttat atggggcacc cccgcccctt gtaaacttcc ctgaccctga 1560

catgacaaga gttactaaca gcccctctct ccaagctcac ttacaggctc tctacttagt 1620

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aagagcgccc aatacgcaaa ccgcctctcc ccgcgcgttg gccgattcat taatgcagct 8880

ggcacgacag gtttcccgac tggaaagcgg gcagtgagcg caacgcaatt aatgtgagtt 8940

agctcactca ttaggcaccc caggctttac actttatgct tccggctcgt atgttgtgtg 9000

gaattgtgag cggataacaa tttcacacag gaaacagcta tgaccatgat tacgccaagc 9060

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ttccatttta aatgcacaga tgtttttatt tcataagggt ttcaatgtgc atgaatgctg 9240

caatattcct gttaccaaag ctagtataaa taaaaataga taaacgtgga aattacttag 9300

agtttctgtc attaacgttt ccttcctcag ttgacaacat aaatgcgctg ctgagcaagc 9360

cagtttgcat ctgtcaggat caatttccca ttatgccagt catattaatt actagtcaat 9420

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<210> 17

<211> 33
<212> PRT
<213> Homo sapiens

<400> 17
Met Ser Thr Glu Ser Met Ile Arg Asp Val Glu Leu Ala Glu Glu Ala
1   5   10   15

Leu Pro Lys Lys Thr Gly Gly Pro Gln Gly Ser Arg Arg Cys Leu Phe
            20   25   30

Leu

<210> 18
<211> 4
<212> PRT
<213> Homo sapiens

<400> 18
Ser Thr Glu Ser
1

<210> 19
<211> 21
<212> PRT
<213> Homo sapiens

<400> 19
Phe Ser Phe Leu Ile Val Ala Gly Ala Thr Thr Leu Phe Cys Leu Leu
1   5   10   15

His Phe Gly Val Ile
            20

<210> 20
<211> 20
<212> PRT
<213> Homo sapiens

<400> 20
Gly Pro Gln Arg Glu Glu Phe Pro Arg Asp Leu Ser Leu Ile Ser Pro
1   5   10   15

Leu Ala Gln Ala
            20

<210> 21
<211> 13
<212> PRT
<213> Homo sapiens

<400> 21
Val Arg Ser Ser Ser Arg Thr Pro Ser Asp Lys Pro Val
1   5   10

<210> 22
<211> 662
<212> DNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
polynucleotide"

<400> 22
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acaggggggc cccagggctc caggcggtgc ttgttcctca gcctcttctc cttcctgatc 120

gtggcaggcg ccaccacgct cttctgcctg ctgcactttg gagtgatcgg cccccagagg 180

gaagagttcc ccagggacct ctctctaatc agccctctgg cccaggcagc ccatgttgta 240

gcaaaccctc aagctgaggg gcagctccag tggctgaacc gccgggccaa tgccctcctg 300

gccaatggcg tggagctgag agataaccag ctggtggtgc catcagaggg cctgtacctc 360

atctactccc aggtcctctt caagggccaa ggctgcccct ccacccatgt gctcctcacc 420

cacaccatca gccgcatcgc cgtctcctac cagaccaagg tcaacctcct ctctgccatc 480

aagagcccct gccagaggga gaccccagag ggggctgagg ccaagccctg gtatgagccc 540

atctatctgg gaggggtctt ccagctggag aagggtgacc gactcagcgc tgagatcaat 600

cggcccgact atctcgactt tgccgagtct gggcaggtct actttgggat cattgccctg 660

cg 662

<210> 23
<211> 233
<212> PRT
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
polypeptide"

<400> 23
Met Ser Thr Glu Met His Pro Gly Arg Gly Ser Trp His Glu Glu Ala
1 5 10 15

Leu Pro Lys Lys Thr Gly Gly Pro Gln Gly Ser Arg Arg Cys Leu Phe
20 25 30

Leu Ser Leu Phe Ser Phe Leu Ile Val Ala Gly Ala Thr Thr Leu Phe
35 40 45

Cys Leu Leu His Phe Gly Val Ile Gly Pro Gln Arg Glu Glu Phe Pro
50 55 60

Arg Asp Leu Ser Leu Ile Ser Pro Leu Ala Gln Ala Val Arg Ser Ser
65 70 75 80

Ser Arg Thr Pro Ser Asp Lys Pro Val Ala His Val Val Ala Asn Pro
85 90 95

Gln Ala Glu Gly Gln Leu Gln Trp Leu Asn Arg Arg Ala Asn Ala Leu
100 105 110

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Leu Ala Asn Gly Val Glu Leu Arg Asp Asn Gln Leu Val Val Pro Ser
        115                 120                 125

Glu Gly Leu Tyr Leu Ile Tyr Ser Gln Val Leu Phe Lys Gly Gln Gly
    130                 135                 140

Cys Pro Ser Thr His Val Leu Leu Thr His Thr Ile Ser Arg Ile Ala
145                 150                 155                 160

Val Ser Tyr Gln Thr Lys Val Asn Leu Leu Ser Ala Ile Lys Ser Pro
                165                 170                 175

Cys Gln Arg Glu Thr Pro Glu Gly Ala Glu Ala Lys Pro Trp Tyr Glu
            180                 185                 190

Pro Ile Tyr Leu Gly Gly Val Phe Gln Leu Glu Lys Gly Asp Arg Leu
        195                 200                 205

Ser Ala Glu Ile Asn Arg Pro Asp Tyr Leu Asp Phe Ala Glu Ser Gly
    210                 215                 220

Gln Val Tyr Phe Gly Ile Ile Ala Leu
225                 230
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<210> 24
<211> 663
<212> DNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic polynucleotide"

<400> 24
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gtggcaggcg ccaccacgct cttctgcctg ctgcactttg gagtgatcgg cccccagagg 180

gaagagttcc ccagggacct ctctctaatc agccctctgg cccaggcagc ccatgttgta 240

gcaaaccctc aagctgaggg gcagctccag tggctgaacc gccgggccaa tgccctcctg 300

gccaatggcg tggagctgag agataaccag ctggtggtgc catcagaggg cctgtacctc 360

atctactccc aggtcctctt caagggccaa ggctgcccct ccacccatgt gctcctcacc 420

cacaccatca gccgcatcgc cgtctcccac cagaccaagg tcaacctcct ctctgccatc 480

aagagcccct gccagaggga gaccccagag ggggctgagg ccaagccctg gtatgagccc 540

atctatctgg gaggggtctt ccagctggag aagggtgacc gactcagcgc tgagatcaat 600

cggcccgact atctcgactt tgccgagtct gggcaggtct actttgggat cattgccctg 660

tcg 663

<210> 25
<211> 220
<212> PRT
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
polypeptide"

<400> 25
Met Ser Thr Glu Ser Lys Ile Arg Asp Val Glu Leu Ala Glu Glu Ala
1 5 10 15

Leu Pro Lys Lys Thr Gly Gly Pro Gln Gly Ser Arg Arg Cys Leu Phe
20 25 30

Leu Ser Leu Phe Ser Phe Leu Ile Val Ala Gly Ala Thr Thr Leu Phe
35 40 45

Cys Leu Leu His Phe Gly Val Ile Gly Pro Gln Arg Glu Glu Phe Pro
50 55 60

Arg Asp Leu Ser Leu Ile Ser Pro Leu Ala Gln Ala Ala His Val Val
65 70 75 80

Ala Asn Pro Gln Ala Glu Gly Gln Leu Gln Trp Leu Asn Arg Arg Ala
85 90 95

Asn Ala Leu Leu Ala Asn Gly Val Glu Leu Arg Asp Asn Gln Leu Val
100 105 110

Val Pro Ser Glu Gly Leu Tyr Leu Ile Tyr Ser Gln Val Leu Phe Lys
115 120 125

Gly Gln Gly Cys Pro Ser Thr His Val Leu Leu Thr His Thr Ile Ser
130 135 140

Arg Ile Ala Val Ser His Gln Thr Lys Val Asn Leu Leu Ser Ala Ile
145 150 155 160

Lys Ser Pro Cys Gln Arg Glu Thr Pro Glu Gly Ala Glu Ala Lys Pro
165 170 175

Trp Tyr Glu Pro Ile Tyr Leu Gly Gly Val Phe Gln Leu Glu Lys Gly
180 185 190

Asp Arg Leu Ser Ala Glu Ile Asn Arg Pro Asp Tyr Leu Asp Phe Ala
195 200 205

Glu Ser Gly Gln Val Tyr Phe Gly Ile Ile Ala Leu
210 215 220

<210> 26

<211> 663
<212> DNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
polynucleotide"

<400> 26
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gtggcaggcg ccaccacgct cttcttcctg ctgcactttg gagtgatcgg cccccagagg 180

gaagagttcc ccagggacct ctctctaatc agccctctgg cccaggcagc ccatgttgta 240

gcaaaccctc aagctgaggg gcagctccag tggctgaacc gccgggccaa tgccctcctg 300

gccaatggcg tggagctgag agataaccag ctggtggtgc catcagaggg cctgtacctc 360

atctactccc aggtcctctt caagggccaa ggctgcccct ccacccatgt gctcctcacc 420

cacaccatca gccgcatcgc cgtctcctac cagaccaagg tcaacctcct cttcgccatc 480

aagagcccct gccagaggga gaccccagag ggggctgagg ccaagccctg gtatgagccc 540

atctatctgg gaggggtctt ccagctggag aagggtgacc gactcagcgc tgagatcaat 600

cggcccgact atctcgactt tgccgagtct gggcaggtct actttgggat cattgccctg 660

tcg 663

<210> 27
<211> 220
<212> PRT
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
polypeptide"

<400> 27
Met Ser Thr Glu Ser Met Ile Arg Asp Val Glu Leu Ala Glu Glu Ala
1 5 10 15

Leu Pro Lys Lys Thr Gly Gly Pro Gln Gly Ser Arg Arg Cys Leu Phe
20 25 30

Leu Ser Leu Phe Ser Phe Leu Ile Val Ala Gly Ala Thr Thr Leu Phe
35 40 45

Phe Leu Leu His Phe Gly Val Ile Gly Pro Gln Arg Glu Glu Phe Pro
50 55 60

Arg Asp Leu Ser Leu Ile Ser Pro Leu Ala Gln Ala Ala His Val Val
65 70 75 80

Ala Asn Pro Gln Ala Glu Gly Gln Leu Gln Trp Leu Asn Arg Arg Ala
85 90 95

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Asn Ala Leu Leu Ala Asn Gly Val Glu Leu Arg Asp Asn Gln Leu Val
            100                 105                 110

Val Pro Ser Glu Gly Leu Tyr Leu Ile Tyr Ser Gln Val Leu Phe Lys
        115                 120                 125

Gly Gln Gly Cys Pro Ser Thr His Val Leu Leu Thr His Thr Ile Ser
    130                 135                 140

Arg Ile Ala Val Ser Tyr Gln Thr Lys Val Asn Leu Leu Phe Ala Ile
145                 150                 155                 160

Lys Ser Pro Cys Gln Arg Glu Thr Pro Glu Gly Ala Glu Ala Lys Pro
                165                 170                 175

Trp Tyr Glu Pro Ile Tyr Leu Gly Gly Val Phe Gln Leu Glu Lys Gly
            180                 185                 190

Asp Arg Leu Ser Ala Glu Ile Asn Arg Pro Asp Tyr Leu Asp Phe Ala
        195                 200                 205

Glu Ser Gly Gln Val Tyr Phe Gly Ile Ile Ala Leu
    210                 215                 220
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<210> 28
<211> 663
<212> DNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
polynucleotide"

<400> 28
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acaggggggc cccagggctc caggcggtgc ttgttcctca gcctcttctc cttcctgatc 120

gtggcaggcg ccaccacgct cttctgcctg ctgcactttg gagtgatcgg cccccagagg 180

gaagagttcc ccagggacct ctctctaatc agccctctgg cccaggcagc ccatgttgta 240

gcaaaccctc aagctgaggg gcagctccag tggctgaacc gccgggccaa tgccctcctg 300

gccaatggcg tggagctgag agataaccag ctggtggtgc catcagaggg cctgtacctc 360

atctactccc aggtcctctt caagggccaa ggctgcccct ccacccatgt gctcctcacc 420

cacaccatca gccgcatcgc cgtctcctac cagaccaagg tcaacctcct ctctgccatc 480

aagagcccct gccagaggga gaccccagag ggggctgagg ccaagccctg gtatgagccc 540

atctatctgg gaggggtctt ccagctggag aagggtgacc gactcatcgc tgagatcaat 600

cggcccgact atctcgactt tgccgagtat gggcaggtct actttgggat cattgccctg 660

tcg 663

<210> 29
<211> 220
<212> PRT
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
polypeptide"

<400> 29
Met Ser Thr Glu Ser Met Ile Arg Asp Val Glu Leu Ala Glu Glu Ala
1 5 10 15

Leu Pro Lys Lys Thr Gly Gly Pro Gln Gly Ser Arg Arg Cys Leu Phe
20 25 30

Leu Ser Leu Phe Ser Phe Leu Ile Val Ala Gly Ala Thr Thr Leu Phe
35 40 45

Cys Leu Leu His Phe Gly Val Ile Gly Pro Gln Arg Glu Glu Phe Pro
50 55 60

Arg Asp Leu Ser Leu Ile Ser Pro Leu Ala Gln Ala Ala His Val Val
65 70 75 80

Ala Asn Pro Gln Ala Glu Gly Gln Leu Gln Trp Leu Asn Arg Arg Ala
85 90 95

Asn Ala Leu Leu Ala Asn Gly Val Glu Leu Arg Asp Asn Gln Leu Val
100 105 110

Val Pro Ser Glu Gly Leu Tyr Leu Ile Tyr Ser Gln Val Leu Phe Lys
115 120 125

Gly Gln Gly Cys Pro Ser Thr His Val Leu Leu Thr His Thr Ile Ser
130 135 140

Arg Ile Ala Val Ser Tyr Gln Thr Lys Val Asn Leu Leu Ser Ala Ile
145 150 155 160

Lys Ser Pro Cys Gln Arg Glu Thr Pro Glu Gly Ala Glu Ala Lys Pro
165 170 175

Trp Tyr Glu Pro Ile Tyr Leu Gly Gly Val Phe Gln Leu Glu Lys Gly
180 185 190

Asp Arg Leu Ile Ala Glu Ile Asn Arg Pro Asp Tyr Leu Asp Phe Ala
195 200 205

Glu Tyr Gly Gln Val Tyr Phe Gly Ile Ile Ala Leu
210 215 220

<210> 30
<211> 663

<212> DNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
polynucleotide"

<400> 30
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acaggggggc cccagggctc caggcggtgc ttgttcctca gcctcttctc cttcctgatc 120

gtggcaggcg ccaccacgct cttctgcctg ctgcactttg gagtgatcgg cccccagagg 180

gaagagttcc ccagggacct ctctctaatc agccctctgg cccaggcagc ccatgttgta 240

gcaaaccctc aagctgaggg gcagctccag tggctgaacc gccgggccaa tgccctcctg 300

gccaatggcg tggagctgag agataaccag ctggtggtgc catcagaggg cctgtacctc 360

atctactccc aggtcctctt caagggccaa ggctgcccct ccacccatgt gctcctcacc 420

cacaccatca gccgcatcgc cgtctcctac cagaccaagg tcaacctcct ctctgccatc 480

aagagcccct gccagaggga gaccccagag ggggctgagg ccaagccctg gtatgagccc 540

atctatctgg gaggggtctt ccagctggag aagggtgacc gactcagcgc tgagatcaat 600

cggcccgact atctctactt tgccgagtct gggcaggtct actttgggat cattgccctg 660

tcg 663

<210> 31
<211> 220
<212> PRT
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
polypeptide"

<400> 31
Met Ser Thr Glu Ser Met Ile Arg Asp Val Glu Leu Ala Glu Glu Ala
1 5 10 15

Leu Pro Lys Lys Thr Gly Gly Pro Gln Gly Ser Arg Arg Cys Leu Phe
20 25 30

Leu Ser Leu Phe Ser Phe Leu Ile Val Ala Gly Ala Thr Thr Leu Phe
35 40 45

Cys Leu Leu His Phe Gly Val Ile Gly Pro Gln Arg Glu Glu Phe Pro
50 55 60

Arg Asp Leu Ser Leu Ile Ser Pro Leu Ala Gln Ala Ala His Val Val
65 70 75 80

Ala Asn Pro Gln Ala Glu Gly Gln Leu Gln Trp Leu Asn Arg Arg Ala
85 90 95

```
Asn Ala Leu Leu Ala Asn Gly Val Glu Leu Arg Asp Asn Gln Leu Val
            100                 105                 110

Val Pro Ser Glu Gly Leu Tyr Leu Ile Tyr Ser Gln Val Leu Phe Lys
        115                 120                 125

Gly Gln Gly Cys Pro Ser Thr His Val Leu Leu Thr His Thr Ile Ser
    130                 135                 140

Arg Ile Ala Val Ser Tyr Gln Thr Lys Val Asn Leu Leu Ser Ala Ile
145                 150                 155                 160

Lys Ser Pro Cys Gln Arg Glu Thr Pro Glu Gly Ala Glu Ala Lys Pro
                165                 170                 175

Trp Tyr Glu Pro Ile Tyr Leu Gly Gly Val Phe Gln Leu Glu Lys Gly
            180                 185                 190

Asp Arg Leu Ser Ala Glu Ile Asn Arg Pro Asp Tyr Leu Tyr Phe Ala
        195                 200                 205

Glu Ser Gly Gln Val Tyr Phe Gly Ile Ile Ala Leu
    210                 215                 220
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<210> 32
<211> 660
<212> DNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic polynucleotide"

<400> 32
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gtggcaggcg ccaccacgct cttctgcctg ctgcactttg gagtgatcgg cccccagagg 180

gaagagttcc ccagggacct ctctctaatc agccctctgc aggcagccca tgttgtagca 240

aaccctcaag ctgaggggca gctccagtgg ctgaaccgcc gggccaatgc cctcctggcc 300

aatggcgtgg agctgagaga taaccagctg gtggtgccat cagagggcct gtacctcatc 360

tactcccagg tcctcttcaa gggccaaggc tgcccctcca cccatgtgct cctcacccac 420

accatcagcc gcatcgccgt ctcctaccag accaaggtca acctcctctc tgccatcaag 480

agcccctgcc agagggagac cccagagggg gctgaggcca agccctggta tgagcccatc 540

tatctgggag gggtcttcca gctggagaag ggtgaccgac tcagcgctga gatcaatcgg 600

cccgactatc tctactttgc cgagtctggg caggtctact ttgggatcat tgccctgtcg 660

<210> 33
<211> 219
<212> PRT

<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
polypeptide"

<400> 33
Met Ser Thr Glu Ser Met Ile Arg Asp Val Glu Leu Ala Glu Glu Ala
1 5 10 15

Leu Pro Lys Lys Thr Gly Gly Pro Gln Gly Ser Arg Arg Cys Leu Phe
20 25 30

Leu Ser Leu Phe Ser Phe Leu Ile Val Ala Gly Ala Thr Thr Leu Phe
35 40 45

Cys Leu Leu His Phe Gly Val Ile Gly Pro Gln Arg Glu Glu Phe Pro
50 55 60

Arg Asp Leu Ser Leu Ile Ser Pro Leu Ala Gln Ala His Val Val Ala
65 70 75 80

Asn Pro Gln Ala Glu Gly Gln Leu Gln Trp Leu Asn Arg Arg Ala Asn
85 90 95

Ala Leu Leu Ala Asn Gly Val Glu Leu Arg Asp Asn Gln Leu Val Val
100 105 110

Pro Ser Glu Gly Leu Tyr Leu Ile Tyr Ser Gln Val Leu Phe Lys Gly
115 120 125

Gln Gly Cys Pro Ser Thr His Val Leu Leu Thr His Thr Ile Ser Arg
130 135 140

Ile Ala Val Ser Tyr Gln Thr Lys Val Asn Leu Leu Ser Ala Ile Lys
145 150 155 160

Ser Pro Cys Gln Arg Glu Thr Pro Glu Gly Ala Glu Ala Lys Pro Trp
165 170 175

Tyr Glu Pro Ile Tyr Leu Gly Gly Val Phe Gln Leu Glu Lys Gly Asp
180 185 190

Arg Leu Ser Ala Glu Ile Asn Arg Pro Asp Tyr Leu Tyr Phe Ala Glu
195 200 205

Ser Gly Gln Val Tyr Phe Gly Ile Ile Ala Leu
210 215

<210> 34
<211> 671
<212> DNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
polynucleotide"

<400> 34
atgctcgagt cgagatgagc actgaaatgc atcccggaag ggggtcctgg cacgaggagg 60

cgctccccaa gaagacaggg gggccccagg gctccaggcg gtgcttgttc ctcagcctct 120

tctccttcct gatcgtggca ggcgccacca cgctcttctt cctgctgcac tttggagtga 180

tcggccccca gagggaagag ttccccaggg acctctctct aatcagccct ctggcagccc 240

atgttgtagc aaaccctcaa gctgaggggc agctccagtg gctgaaccgc cgggccaatg 300

ccctcctggc caatggcgtg gagctgagag ataaccagct ggtggtgcca tcagagggcc 360

tgtacctcat ctactcccag gtcctcttca agggccaagg ctgcccctcc acccatgtgc 420

tcctcaccca caccatcagc cgcatcgccg tctcccacca gaccaaggtc aacctcctct 480

tcgccatcaa gagcccctgc cagagggaga ccccagaggg ggctgaggcc aagccctggt 540

atgagcccat ctatctggga ggggtcttcc agctggagaa gggtgaccga ctcatcgctg 600

agatcaatcg gcccgactat ctctactttg ccgagtatgg gcaggtctac tttgggatca 660

ttgccctgtc g 671

<210> 35
<211> 219
<212> PRT
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
polypeptide"

<400> 35
Met Ser Thr Glu Met His Pro Gly Arg Gly Ser Trp His Glu Glu Ala
1 5 10 15

Leu Pro Lys Lys Thr Gly Gly Pro Gln Gly Ser Arg Arg Cys Leu Phe
20 25 30

Leu Ser Leu Phe Ser Phe Leu Ile Val Ala Gly Ala Thr Thr Leu Phe
35 40 45

Phe Leu Leu His Phe Gly Val Ile Gly Pro Gln Arg Glu Glu Phe Pro
50 55 60

Arg Asp Leu Ser Leu Ile Ser Pro Leu Ala Gln Ala His Val Val Ala
65 70 75 80

Asn Pro Gln Ala Glu Gly Gln Leu Gln Trp Leu Asn Arg Arg Ala Asn
85 90 95

Ala Leu Leu Ala Asn Gly Val Glu Leu Arg Asp Asn Gln Leu Val Val
100 105 110

Pro Ser Glu Gly Leu Tyr Leu Ile Tyr Ser Gln Val Leu Phe Lys Gly
        115   120   125

Gln Gly Cys Pro Ser Thr His Val Leu Leu Thr His Thr Ile Ser Arg
    130   135   140

Ile Ala Val Ser His Gln Thr Lys Val Asn Leu Leu Phe Ala Ile Lys
145   150   155   160

Ser Pro Cys Gln Arg Glu Thr Pro Glu Gly Ala Glu Ala Lys Pro Trp
                165   170   175

Tyr Glu Pro Ile Tyr Leu Gly Gly Val Phe Gln Leu Glu Lys Gly Asp
            180   185   190

Arg Leu Ile Ala Glu Ile Asn Arg Pro Asp Tyr Leu Tyr Phe Ala Glu
        195   200   205

Tyr Gly Gln Val Tyr Phe Gly Ile Ile Ala Leu
    210   215

<210> 36
<211> 1131
<212> PRT
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
      polypeptide"

<400> 36
Met Ser Thr Glu Ser Met Ile Arg Asp Val Glu Leu Ala Glu Glu Ala
1   5   10   15

Leu Pro Lys Lys Thr Gly Gly Pro Gln Gly Ser Arg Arg Cys Leu Phe
            20   25   30

Leu Ser Leu Phe Ser Phe Leu Ile Val Ala Gly Ala Thr Thr Leu Phe
        35   40   45

Cys Leu Leu His Phe Gly Val Ile Gly Pro Gln Arg Glu Glu Phe Pro
    50   55   60

Arg Asp Leu Ser Leu Ile Ser Pro Leu Ala Gln Ala Arg Ser Ser Ser
65   70   75   80

Arg Thr Pro Ser Asp Lys Val Ala His Val Val Ala Asn Pro Gln Ala
                85   90   95

Glu Gly Gln Leu Gln Trp Leu Asn Arg Arg Ala Asn Ala Leu Leu Ala
            100   105   110

Asn Gly Val Glu Leu Arg Asp Asn Gln Leu Val Val Pro Ser Glu Gly

```
        115                 120                 125

Leu Tyr Leu Ile Tyr Ser Gln Val Leu Phe Lys Gly Gln Gly Cys Pro
    130                 135                 140

Ser Thr His Val Leu Leu Thr His Thr Ile Ser Arg Ile Ala Val Ser
145                 150                 155                 160

Tyr Gln Thr Lys Val Asn Leu Leu Ser Ala Ile Lys Ser Pro Cys Gln
                165                 170                 175

Arg Glu Thr Pro Glu Gly Ala Glu Ala Lys Pro Trp Tyr Glu Pro Ile
            180                 185                 190

Tyr Leu Gly Gly Val Phe Gln Leu Glu Lys Gly Asp Arg Leu Ser Ala
        195                 200                 205

Glu Ile Asn Arg Pro Asp Tyr Leu Asp Phe Ala Glu Ser Gly Gln Val
    210                 215                 220

Tyr Phe Gly Ile Ile Ala Leu Ser Arg Ala Glu Gly Arg Gly Ser Leu
225                 230                 235                 240

Leu Thr Cys Gly Asp Val Glu Glu Asn Pro Gly Pro Met Glu Phe Gly
                245                 250                 255

Leu Ser Trp Leu Phe Leu Val Ala Ile Leu Lys Gly Val Gln Cys Ser
            260                 265                 270

Arg Asp Ile Gln Met Thr Gln Thr Thr Ser Ser Leu Ser Ala Ser Leu
        275                 280                 285

Gly Asp Arg Val Thr Ile Ser Cys Arg Ala Ser Gln Asp Ile Ser Lys
    290                 295                 300

Tyr Leu Asn Trp Tyr Gln Gln Lys Pro Asp Gly Thr Val Lys Leu Leu
305                 310                 315                 320

Ile Tyr His Thr Ser Arg Leu His Ser Gly Val Pro Ser Arg Phe Ser
                325                 330                 335

Gly Ser Gly Ser Gly Thr Asp Tyr Ser Leu Thr Ile Ser Asn Leu Glu
            340                 345                 350

Gln Glu Asp Ile Ala Thr Tyr Phe Cys Gln Gln Gly Asn Thr Leu Pro
        355                 360                 365

Tyr Thr Phe Gly Gly Gly Thr Lys Leu Glu Leu Lys Arg Gly Gly Gly
    370                 375                 380

Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly
385                 390                 395                 400
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Ser Glu Val Gln Leu Gln Gln Ser Gly Pro Gly Leu Val Ala Pro Ser
                405                 410                 415

Gln Ser Leu Ser Val Thr Cys Thr Val Ser Gly Val Ser Leu Pro Asp
            420                 425                 430

Tyr Gly Val Ser Trp Ile Arg Gln Pro Pro Arg Lys Gly Leu Glu Trp
        435                 440                 445

Leu Gly Val Ile Trp Gly Ser Glu Thr Thr Tyr Tyr Asn Ser Ala Leu
    450                 455                 460

Lys Ser Arg Leu Thr Ile Ile Lys Asp Asn Ser Lys Ser Gln Val Phe
465                 470                 475                 480

Leu Lys Met Asn Ser Leu Gln Thr Asp Asp Thr Ala Ile Tyr Tyr Cys
                485                 490                 495

Ala Lys His Tyr Tyr Tyr Gly Gly Ser Tyr Ala Met Asp Tyr Trp Gly
            500                 505                 510

Gln Gly Thr Thr Val Thr Val Ser Ser Tyr Val Thr Val Ser Ser Gln
        515                 520                 525

Asp Pro Ala Glu Pro Lys Ser Pro Asp Lys Thr His Thr Cys Pro Pro
    530                 535                 540

Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro
545                 550                 555                 560

Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr
                565                 570                 575

Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn
            580                 585                 590

Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg
        595                 600                 605

Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val
    610                 615                 620

Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser
625                 630                 635                 640

Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys
                645                 650                 655

Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp
            660                 665                 670
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Glu Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe
        675                 680                 685

Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu
    690                 695                 700

Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe
705                 710                 715                 720

Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly
                725                 730                 735

Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr
            740                 745                 750

Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys Lys Asp Pro Lys Phe
        755                 760                 765

Trp Val Leu Val Val Val Gly Gly Val Leu Ala Cys Tyr Ser Leu Leu
    770                 775                 780

Val Thr Val Ala Phe Ile Ile Phe Trp Val Arg Ser Lys Arg Ser Arg
785                 790                 795                 800

Leu Leu His Ser Asp Tyr Met Asn Met Thr Pro Arg Arg Pro Gly Pro
                805                 810                 815

Thr Arg Lys His Tyr Gln Pro Tyr Ala Pro Pro Arg Asp Phe Ala Ala
            820                 825                 830

Tyr Arg Ser Arg Val Lys Phe Ser Arg Ser Ala Asp Ala Pro Ala Tyr
        835                 840                 845

Gln Gln Gly Gln Asn Gln Leu Tyr Asn Glu Leu Asn Leu Gly Arg Arg
    850                 855                 860

Glu Glu Tyr Asp Val Leu Asp Lys Arg Arg Gly Arg Asp Pro Glu Met
865                 870                 875                 880

Gly Gly Lys Pro Arg Arg Lys Asn Pro Gln Glu Gly Leu Tyr Asn Glu
                885                 890                 895

Leu Gln Lys Asp Lys Met Ala Glu Ala Tyr Ser Glu Ile Gly Met Lys
            900                 905                 910

Gly Glu Arg Arg Arg Gly Lys Gly His Asp Gly Leu Tyr Gln Gly Leu
        915                 920                 925

Ser Thr Ala Thr Lys Asp Thr Tyr Asp Ala Leu His Met Gln Ala Leu
    930                 935                 940
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Pro Pro Arg Gly Pro Gln Cys Thr Asn Tyr Ala Leu Leu Lys Leu Ala
945                 950                 955                 960


Gly Asp Val Glu Ser Asn Pro Gly Pro Met Arg Ile Ser Lys Pro His
                965                 970                 975


Leu Arg Ser Ile Ser Ile Gln Cys Tyr Leu Cys Leu Leu Leu Asn Ser
            980                 985                 990


His Phe Leu Thr Glu Ala Gly Ile  His Val Phe Ile Leu  Gly Cys Phe
        995                 1000                1005


Ser Ala  Gly Leu Pro Lys Thr  Glu Ala Asn Trp Val  Asn Val Ile
    1010                1015                1020


Ser Asp  Leu Lys Lys Ile Glu  Asp Leu Ile Gln Ser  Met His Ile
    1025                1030                1035


Asp Ala  Thr Leu Tyr Thr Glu  Ser Asp Val His Pro  Ser Cys Lys
    1040                1045                1050


Val Thr  Ala Met Lys Cys Phe  Leu Leu Glu Leu Gln  Val Ile Ser
    1055                1060                1065


Leu Glu  Ser Gly Asp Ala Ser  Ile His Asp Thr Val  Glu Asn Leu
    1070                1075                1080


Ile Ile  Leu Ala Asn Asn Ser  Leu Ser Ser Asn Gly  Asn Val Thr
    1085                1090                1095


Glu Ser  Gly Cys Lys Glu Cys  Glu Glu Leu Glu Glu  Lys Asn Ile
    1100                1105                1110


Lys Glu  Phe Leu Gln Ser Phe  Val His Ile Val Gln  Met Phe Ile
    1115                1120                1125


Asn Thr  Ser
    1130


<210> 37
<211> 1138
<212> PRT
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
      polypeptide"

<400> 37
Met Ser Thr Glu Ser Met Ile Arg Asp Val Glu Leu Ala Glu Glu Ala
1               5                   10                  15


Leu Pro Lys Lys Thr Gly Gly Pro Gln Gly Ser Arg Arg Cys Leu Phe
            20                  25                  30
```

```
Leu Ser Leu Phe Ser Phe Leu Ile Val Ala Gly Ala Thr Thr Leu Phe
        35                  40                  45

Cys Leu Leu His Phe Gly Val Ile Gly Pro Gln Arg Glu Glu Phe Pro
    50                  55                  60

Arg Asp Leu Ser Leu Ile Ser Pro Leu Ala Gln Ala Arg Ser Ser Ser
65                  70                  75                  80

Arg Thr Pro Ser Asp Lys Val Ala His Val Val Ala Asn Pro Gln Ala
                85                  90                  95

Glu Gly Gln Leu Gln Trp Leu Asn Arg Arg Ala Asn Ala Leu Leu Ala
            100                 105                 110

Asn Gly Val Glu Leu Arg Asp Asn Gln Leu Val Val Pro Ser Glu Gly
        115                 120                 125

Leu Tyr Leu Ile Tyr Ser Gln Val Leu Phe Lys Gly Gln Gly Cys Pro
    130                 135                 140

Ser Thr His Val Leu Leu Thr His Thr Ile Ser Arg Ile Ala Val Ser
145                 150                 155                 160

Tyr Gln Thr Lys Val Asn Leu Leu Ser Ala Ile Lys Ser Pro Cys Gln
                165                 170                 175

Arg Glu Thr Pro Glu Gly Ala Glu Ala Lys Pro Trp Tyr Glu Pro Ile
            180                 185                 190

Tyr Leu Gly Gly Val Phe Gln Leu Glu Lys Gly Asp Arg Leu Ser Ala
        195                 200                 205

Glu Ile Asn Arg Pro Asp Tyr Leu Asp Phe Ala Glu Ser Gly Gln Val
    210                 215                 220

Tyr Phe Gly Ile Ile Ala Leu Ser Arg Ala Glu Gly Arg Gly Ser Leu
225                 230                 235                 240

Leu Thr Cys Gly Asp Val Glu Glu Asn Pro Gly Pro Met Glu Phe Gly
                245                 250                 255

Leu Ser Trp Leu Phe Leu Val Ala Ile Leu Lys Gly Val Gln Cys Ser
            260                 265                 270

Arg Asp Ile Gln Met Thr Gln Thr Thr Ser Ser Leu Ser Ala Ser Leu
        275                 280                 285

Gly Asp Arg Val Thr Ile Ser Cys Arg Ala Ser Gln Asp Ile Ser Lys
    290                 295                 300
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```
Tyr Leu Asn Trp Tyr Gln Gln Lys Pro Asp Gly Thr Val Lys Leu Leu
305                 310                 315                 320

Ile Tyr His Thr Ser Arg Leu His Ser Gly Val Pro Ser Arg Phe Ser
                325                 330                 335

Gly Ser Gly Ser Gly Thr Asp Tyr Ser Leu Thr Ile Ser Asn Leu Glu
            340                 345                 350

Gln Glu Asp Ile Ala Thr Tyr Phe Cys Gln Gln Gly Asn Thr Leu Pro
        355                 360                 365

Tyr Thr Phe Gly Gly Gly Thr Lys Leu Glu Leu Lys Arg Gly Gly Gly
    370                 375                 380

Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly
385                 390                 395                 400

Ser Glu Val Gln Leu Gln Gln Ser Gly Pro Gly Leu Val Ala Pro Ser
                405                 410                 415

Gln Ser Leu Ser Val Thr Cys Thr Val Ser Gly Val Ser Leu Pro Asp
            420                 425                 430

Tyr Gly Val Ser Trp Ile Arg Gln Pro Pro Arg Lys Gly Leu Glu Trp
        435                 440                 445

Leu Gly Val Ile Trp Gly Ser Glu Thr Thr Tyr Tyr Asn Ser Ala Leu
    450                 455                 460

Lys Ser Arg Leu Thr Ile Ile Lys Asp Asn Ser Lys Ser Gln Val Phe
465                 470                 475                 480

Leu Lys Met Asn Ser Leu Gln Thr Asp Asp Thr Ala Ile Tyr Tyr Cys
                485                 490                 495

Ala Lys His Tyr Tyr Tyr Gly Gly Ser Tyr Ala Met Asp Tyr Trp Gly
            500                 505                 510

Gln Gly Thr Thr Val Thr Val Ser Ser Tyr Val Thr Val Ser Ser Gln
        515                 520                 525

Asp Pro Ala Glu Pro Lys Ser Pro Asp Lys Thr His Thr Cys Pro Pro
    530                 535                 540

Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro
545                 550                 555                 560

Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr
                565                 570                 575
```

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Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn
            580                 585                 590

Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg
        595                 600                 605

Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val
    610                 615                 620

Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser
625                 630                 635                 640

Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys
                645                 650                 655

Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp
            660                 665                 670

Glu Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe
        675                 680                 685

Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu
    690                 695                 700

Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe
705                 710                 715                 720

Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly
                725                 730                 735

Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr
            740                 745                 750

Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys Lys Asp Pro Lys Phe
        755                 760                 765

Trp Gly Val Leu Ala Gly Ile Val Met Gly Asp Leu Val Leu Thr Val
    770                 775                 780

Leu Ile Ala Leu Ala Val Tyr Phe Leu Gly Arg Leu Val Pro Arg Gly
785                 790                 795                 800

Arg Gly Ala Ala Glu Ala Ala Thr Arg Lys Gln Arg Ile Thr Glu Thr
                805                 810                 815

Glu Ser Pro Tyr Gln Glu Leu Gln Gly Gln Arg Ser Asp Val Tyr Ser
            820                 825                 830

Asp Leu Asn Thr Gln Arg Pro Tyr Tyr Lys Arg Val Lys Phe Ser Arg
        835                 840                 845

Ser Ala Asp Ala Pro Ala Tyr Gln Gln Gly Gln Asn Gln Leu Tyr Asn
```

```
    850                 855                 860

Glu Leu Asn Leu Gly Arg Arg Glu Glu Tyr Asp Val Leu Asp Lys Arg
865                 870                 875                 880

Arg Gly Arg Asp Pro Glu Met Gly Gly Lys Pro Arg Arg Lys Asn Pro
                885                 890                 895

Gln Glu Gly Leu Tyr Asn Glu Leu Gln Lys Asp Lys Met Ala Glu Ala
            900                 905                 910

Tyr Ser Glu Ile Gly Met Lys Gly Glu Arg Arg Arg Gly Lys Gly His
        915                 920                 925

Asp Gly Leu Tyr Gln Gly Leu Ser Thr Ala Thr Lys Asp Thr Tyr Asp
    930                 935                 940

Ala Leu His Met Gln Ala Leu Pro Pro Arg Gly Pro Gln Cys Thr Asn
945                 950                 955                 960

Tyr Ala Leu Leu Lys Leu Ala Gly Asp Val Glu Ser Asn Pro Gly Pro
                965                 970                 975

Met Arg Ile Ser Lys Pro His Leu Arg Ser Ile Ser Ile Gln Cys Tyr
            980                 985                 990

Leu Cys Leu Leu Leu Asn Ser His  Phe Leu Thr Glu Ala  Gly Ile His
        995                 1000                1005

Val Phe  Ile Leu Gly Cys Phe  Ser Ala Gly Leu Pro  Lys Thr Glu
    1010                1015                1020

Ala Asn  Trp Val Asn Val Ile  Ser Asp Leu Lys Lys  Ile Glu Asp
    1025                1030                1035

Leu Ile  Gln Ser Met His Ile  Asp Ala Thr Leu Tyr  Thr Glu Ser
    1040                1045                1050

Asp Val  His Pro Ser Cys Lys  Val Thr Ala Met Lys  Cys Phe Leu
    1055                1060                1065

Leu Glu  Leu Gln Val Ile Ser  Leu Glu Ser Gly Asp  Ala Ser Ile
    1070                1075                1080

His Asp  Thr Val Glu Asn Leu  Ile Ile Leu Ala Asn  Asn Ser Leu
    1085                1090                1095

Ser Ser  Asn Gly Asn Val Thr  Glu Ser Gly Cys Lys  Glu Cys Glu
    1100                1105                1110

Glu Leu  Glu Glu Lys Asn Ile  Lys Glu Phe Leu Gln  Ser Phe Val
    1115                1120                1125
```

```
His Ile  Val Gln Met Phe Ile  Asn Thr Ser
    1130                1135


<210> 38
<211> 673
<212> DNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
      polynucleotide"

<400> 38
tcgagtcgag atgagcactg aaagcatgat ccgggacgtg gagctggccg aggaggcgct       60

ccccaagaag acaggggggc cccagggctc caggcggtgc ttgttcctca gcctcttctc      120

cttcctgatc gtggcaggcg ccaccacgct cttctgcctg ctgcactttg gagtgatcgg      180

cccccagagg gaagagttcc ccagggacct ctctctaatc agccctctgc aggcagccca      240

tgttgtagca aaccctcaag ctgaggggca gctccagtgg ctgaaccgcc gggccaatgc      300

cctcctggcc aatggcgtgg agctgagaga taaccagctg gtggtgccat cagagggcct      360

gtacctcatc tactcccagg tcctcttcaa gggccaaggc tgcccctcca cccatgtgct      420

cctcacccac accatcagcc gcatcgccgt ctcctaccag accaaggtca acctcctctc      480

tgccatcaag agcccctgcc agagggagac cccagagggg gctgaggcca agccctggta      540

tgagcccatc tatctgggag gggtcttcca gctggagaag ggtgaccgac tcagcgctga      600

gatcaatcgg cccgactatc tcgactttgc cgagtctggg caggtctact ttgggatcat      660

tgccctgtcg tcg                                                         673


<210> 39
<211> 219
<212> PRT
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
      polypeptide"

<400> 39
Met Ser Thr Glu Ser Met Ile Arg Asp Val Glu Leu Ala Glu Glu Ala
1               5                   10                  15


Leu Pro Lys Lys Thr Gly Gly Pro Gln Gly Ser Arg Arg Cys Leu Phe
            20                  25                  30


Leu Ser Leu Phe Ser Phe Leu Ile Val Ala Gly Ala Thr Thr Leu Phe
        35                  40                  45


Cys Leu Leu His Phe Gly Val Ile Gly Pro Gln Arg Glu Glu Phe Pro
    50                  55                  60
```

```
Arg Asp Leu Ser Leu Ile Ser Pro Leu Gln Ala Ala His Val Val Ala
65                  70                  75                  80


Asn Pro Gln Ala Glu Gly Gln Leu Gln Trp Leu Asn Arg Arg Ala Asn
                85                  90                  95


Ala Leu Leu Ala Asn Gly Val Glu Leu Arg Asp Asn Gln Leu Val Val
            100                 105                 110


Pro Ser Glu Gly Leu Tyr Leu Ile Tyr Ser Gln Val Leu Phe Lys Gly
        115                 120                 125


Gln Gly Cys Pro Ser Thr His Val Leu Leu Thr His Thr Ile Ser Arg
    130                 135                 140


Ile Ala Val Ser Tyr Gln Thr Lys Val Asn Leu Leu Ser Ala Ile Lys
145                 150                 155                 160


Ser Pro Cys Gln Arg Glu Thr Pro Glu Gly Ala Glu Ala Lys Pro Trp
                165                 170                 175


Tyr Glu Pro Ile Tyr Leu Gly Gly Val Phe Gln Leu Glu Lys Gly Asp
            180                 185                 190


Arg Leu Ser Ala Glu Ile Asn Arg Pro Asp Tyr Leu Asp Phe Ala Glu
        195                 200                 205


Ser Gly Gln Val Tyr Phe Gly Ile Ile Ala Leu
    210                 215


<210> 40
<211> 654
<212> DNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
      polynucleotide"

<400> 40
atgagcactg aaatgcatcc cggaaggggg tcctggcacg aggaggcgct ccccaagaag       60

acaggggggc cccagggctc caggcggtgc ttgttcctca gcctcttctc cttcctgatc      120

gtggcaggcg ccaccacgct cttcttcctg ctgcactttg gagtgatcgg cccccagagg      180

gaagagttcc ccagggacct ctctctaatc agccctctgg cccatgttgt agcaaaccct      240

caagctgagg ggcagctcca gtggctgaac cgccgggcca atgccctcct ggccaatggc      300

gtggagctga gagataacca gctggtggtg ccatcagagg gcctgtacct catctactcc      360

caggtcctct tcaagggcca aggctgcccc tccacccatg tgctcctcac ccacaccatc      420

agccgcatcg ccgtctccca ccagaccaag gtcaacctcc tcttcgccat caagagcccc      480

tgccagaggg agaccccaga gggggctgag gccaagccct ggtatgagcc catctatctg      540
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ggaggggtct tccagctgga gaagggtgac cgactcatcg ctgagatcaa tcggcccgac 600

tatctctact ttgccgagta tgggcaggtc tactttggga tcattgccct gtcg 654

<210> 41
<211> 218
<212> PRT
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic polypeptide"

<400> 41
Met Ser Thr Glu Met His Pro Gly Arg Gly Ser Trp His Glu Glu Ala
1 5 10 15

Leu Pro Lys Lys Thr Gly Gly Pro Gln Gly Ser Arg Arg Cys Leu Phe
20 25 30

Leu Ser Leu Phe Ser Phe Leu Ile Val Ala Gly Ala Thr Thr Leu Phe
35 40 45

Phe Leu Leu His Phe Gly Val Ile Gly Pro Gln Arg Glu Glu Phe Pro
50 55 60

Arg Asp Leu Ser Leu Ile Ser Pro Leu Ala His Val Val Ala Asn Pro
65 70 75 80

Gln Ala Glu Gly Gln Leu Gln Trp Leu Asn Arg Arg Ala Asn Ala Leu
85 90 95

Leu Ala Asn Gly Val Glu Leu Arg Asp Asn Gln Leu Val Val Pro Ser
100 105 110

Glu Gly Leu Tyr Leu Ile Tyr Ser Gln Val Leu Phe Lys Gly Gln Gly
115 120 125

Cys Pro Ser Thr His Val Leu Leu Thr His Thr Ile Ser Arg Ile Ala
130 135 140

Val Ser His Gln Thr Lys Val Asn Leu Leu Phe Ala Ile Lys Ser Pro
145 150 155 160

Cys Gln Arg Glu Thr Pro Glu Gly Ala Glu Ala Lys Pro Trp Tyr Glu
165 170 175

Pro Ile Tyr Leu Gly Gly Val Phe Gln Leu Glu Lys Gly Asp Arg Leu
180 185 190

Ile Ala Glu Ile Asn Arg Pro Asp Tyr Leu Tyr Phe Ala Glu Tyr Gly
195 200 205

Gln Val Tyr Phe Gly Ile Ile Ala Leu Ser

210 215

<210> 42
<211> 33
<212> DNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic primer"

<400> 42
tcgagaagat gatctgactg cctgggccag agg 33

<210> 43
<211> 30
<212> DNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic primer"

<400> 43
tcgagaagat gatcttgcct gggccagagg 30

<210> 44
<211> 12
<212> DNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 44
tgatcttgcc tg 12

<210> 45
<211> 33
<212> DNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic primer"

<400> 45
tacaacatgg gctacaggct tgtcactcgg ggt 33

<210> 46
<211> 30
<212> DNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic primer"

<400> 46
tacaacatgg gctaccttgt cactcggggt 30

<210> 47
<211> 12
<212> DNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
oligonucleotide"

<400> 47
ggctaccttg tc 12

<210> 48
<211> 48
<212> DNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
oligonucleotide"

<400> 48
caggcagtca gatcatcttc tcgaaccccg agtgacaagc ctgtagcc 48

<210> 49
<211> 16
<212> PRT
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
peptide"

<400> 49
Gln Ala Val Arg Ser Ser Ser Arg Thr Pro Ser Asp Lys Pro Val Ala
1 5 10 15

<210> 50
<211> 220
<212> PRT
<213> Unknown

<220>
<221> source
<223> /note="Description of Unknown:
TNF alpha sequence"

<400> 50
Met Ser Thr Glu Ser Met Ile Arg Asp Val Glu Leu Ala Glu Glu Ala
1 5 10 15

Leu Pro Lys Lys Thr Gly Gly Pro Gln Gly Ser Arg Arg Cys Leu Phe
20 25 30

Leu Ser Leu Phe Ser Phe Leu Ile Val Ala Gly Ala Thr Thr Leu Phe
35 40 45

```
Cys Leu Leu His Phe Gly Val Ile Gly Pro Gln Arg Glu Glu Phe Pro
    50                  55                  60

Arg Asp Leu Ser Leu Ile Ser Pro Leu Ala Gln Ala Ala His Val Val
65                  70                  75                  80

Ala Asn Pro Gln Ala Glu Gly Gln Leu Gln Trp Leu Asn Arg Arg Ala
                85                  90                  95

Asn Ala Leu Leu Ala Asn Gly Val Glu Leu Arg Asp Asn Gln Leu Val
            100                 105                 110

Val Pro Ser Glu Gly Leu Tyr Leu Ile Tyr Ser Gln Val Leu Phe Lys
        115                 120                 125

Gly Gln Gly Cys Pro Ser Thr His Val Leu Leu Thr His Thr Ile Ser
    130                 135                 140

Arg Ile Ala Val Ser Tyr Gln Thr Lys Val Asn Leu Leu Ser Ala Ile
145                 150                 155                 160

Lys Ser Pro Cys Gln Arg Glu Thr Pro Glu Gly Ala Glu Ala Lys Pro
                165                 170                 175

Trp Tyr Glu Pro Ile Tyr Leu Gly Gly Val Phe Gln Leu Glu Lys Gly
            180                 185                 190

Asp Arg Leu Ser Ala Glu Ile Asn Arg Pro Asp Tyr Leu Asp Phe Ala
        195                 200                 205

Glu Ser Gly Gln Val Tyr Phe Gly Ile Ile Ala Leu
    210                 215                 220


<210> 51
<211> 233
<212> PRT
<213> Homo sapiens

<400> 51
Met Ser Thr Glu Ser Met Ile Arg Asp Val Glu Leu Ala Glu Glu Ala
1               5                   10                  15

Leu Pro Lys Lys Thr Gly Gly Pro Gln Gly Ser Arg Arg Cys Leu Phe
            20                  25                  30

Leu Ser Leu Phe Ser Phe Leu Ile Val Ala Gly Ala Thr Thr Leu Phe
        35                  40                  45

Cys Leu Leu His Phe Gly Val Ile Gly Pro Gln Arg Glu Glu Phe Pro
    50                  55                  60

Arg Asp Leu Ser Leu Ile Ser Pro Leu Ala Gln Ala Val Arg Ser Ser
65                  70                  75                  80
```

```
Ser Arg Thr Pro Ser Asp Lys Pro Val Ala His Val Val Ala Asn Pro
                85                  90                  95

Gln Ala Glu Gly Gln Leu Gln Trp Leu Asn Arg Arg Ala Asn Ala Leu
            100                 105                 110

Leu Ala Asn Gly Val Glu Leu Arg Asp Asn Gln Leu Val Val Pro Ser
        115                 120                 125

Glu Gly Leu Tyr Leu Ile Tyr Ser Gln Val Leu Phe Lys Gly Gln Gly
    130                 135                 140

Cys Pro Ser Thr His Val Leu Leu Thr His Thr Ile Ser Arg Ile Ala
145                 150                 155                 160

Val Ser Tyr Gln Thr Lys Val Asn Leu Leu Ser Ala Ile Lys Ser Pro
                165                 170                 175

Cys Gln Arg Glu Thr Pro Glu Gly Ala Glu Ala Lys Pro Trp Tyr Glu
            180                 185                 190

Pro Ile Tyr Leu Gly Gly Val Phe Gln Leu Glu Lys Gly Asp Arg Leu
        195                 200                 205

Ser Ala Glu Ile Asn Arg Pro Asp Tyr Leu Asp Phe Ala Glu Ser Gly
    210                 215                 220

Gln Val Tyr Phe Gly Ile Ile Ala Leu
225                 230
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<210> 52
<211> 660
<212> DNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic polynucleotide"

<220>
<221> misc_feature
<222> (214)..(219)
<223> /note="This region may or may not be present"

<400> 52
atgagcactg aaatgcatcc cggaaggggg tcctggcacg aggaggcgct ccccaagaag 60

acaggggggc cccagggctc caggcggtgc ttgttcctca gcctcttctc cttcctgatc 120

gtggcaggcg ccaccacgct cttcttcctg ctgcactttg gagtgatcgg cccccagagg 180

gaagagttcc ccagggacct ctctctaatc agccctctgg ccgcagccca tgttgtagca 240

aaccctcaag ctgaggggca gctccagtgg ctgaaccgcc gggccaatgc cctcctggcc 300

aatggcgtgg agctgagaga taaccagctg gtggtgccat cagagggcct gtacctcatc 360

tactcccagg tcctcttcaa gggccaaggc tgcccctcca cccatgtgct cctcacccac 420

accatcagcc gcatcgccgt ctcccaccag accaaggtca acctcctctt cgccatcaag 480

agcccctgcc agagggagac cccagagggg gctgaggcca agccctggta tgagcccatc 540

tatctgggag gggtcttcca gctggagaag ggtgaccgac tcatcgctga gatcaatcgg 600

cccgactatc tctactttgc cgagtatggg caggtctact ttgggatcat tgccctgtcg 660

<210> 53
<211> 220
<212> PRT
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
polypeptide"

<220>
<221> SITE
<222> (74)..(75)
<223> /note="This region may or may not be present"

<400> 53
Met Ser Thr Glu Met His Pro Gly Arg Gly Ser Trp His Glu Glu Ala
1 5 10 15

Leu Pro Lys Lys Thr Gly Gly Pro Gln Gly Ser Arg Arg Cys Leu Phe
20 25 30

Leu Ser Leu Phe Ser Phe Leu Ile Val Ala Gly Ala Thr Thr Leu Phe
35 40 45

Phe Leu Leu His Phe Gly Val Ile Gly Pro Gln Arg Glu Glu Phe Pro
50 55 60

Arg Asp Leu Ser Leu Ile Ser Pro Leu Ala Gln Ala His Val Val Ala
65 70 75 80

Asn Pro Gln Ala Glu Gly Gln Leu Gln Trp Leu Asn Arg Arg Ala Asn
85 90 95

Ala Leu Leu Ala Asn Gly Val Glu Leu Arg Asp Asn Gln Leu Val Val
100 105 110

Pro Ser Glu Gly Leu Tyr Leu Ile Tyr Ser Gln Val Leu Phe Lys Gly
115 120 125

Gln Gly Cys Pro Ser Thr His Val Leu Leu Thr His Thr Ile Ser Arg
130 135 140

Ile Ala Val Ser His Gln Thr Lys Val Asn Leu Leu Phe Ala Ile Lys
145 150 155 160

```
Ser Pro Cys Gln Arg Glu Thr Pro Glu Gly Ala Glu Ala Lys Pro Trp
                165                 170                 175


Tyr Glu Pro Ile Tyr Leu Gly Gly Val Phe Gln Leu Glu Lys Gly Asp
            180                 185                 190


Arg Leu Ile Ala Glu Ile Asn Arg Pro Asp Tyr Leu Tyr Phe Ala Glu
        195                 200                 205


Tyr Gly Gln Val Tyr Phe Gly Ile Ile Ala Leu Ser
    210                 215                 220


<210> 54
<211> 56
<212> PRT
<213> Unknown

<220>
<221> source
<223> /note="Description of Unknown:
      monkey TNF alpha sequence"

<400> 54
Met Ser Thr Glu Ser Met Ile Arg Asp Val Glu Leu Ala Glu Glu Ala
1               5                   10                  15


Leu Pro Arg Lys Thr Ala Gly Pro Gln Gly Ser Arg Arg Cys Trp Phe
            20                  25                  30


Leu Ser Leu Phe Ser Phe Leu Leu Val Ala Gly Ala Thr Thr Leu Phe
        35                  40                  45


Cys Leu Leu His Phe Gly Val Ile
    50                  55


<210> 55
<211> 56
<212> PRT
<213> Sus sp.

<400> 55
Met Ser Thr Glu Ser Met Ile Arg Asp Val Glu Leu Ala Glu Glu Ala
1               5                   10                  15


Leu Ala Lys Lys Ala Gly Gly Pro Gln Gly Ser Arg Arg Cys Leu Cys
            20                  25                  30


Leu Ser Leu Phe Ser Phe Leu Leu Val Ala Gly Ala Thr Thr Leu Phe
        35                  40                  45


Cys Leu Leu His Phe Glu Val Ile
    50                  55


<210> 56
<211> 56
<212> PRT
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<213> Mus sp.

<400> 56
Met Ser Thr Glu Ser Met Ile Arg Asp Val Glu Leu Ala Glu Glu Ala
1   5   10   15

Leu Pro Gln Lys Met Gly Gly Phe Gln Asn Ser Arg Arg Cys Leu Cys
            20   25   30

Leu Ser Leu Phe Ser Phe Leu Leu Val Ala Gly Ala Thr Thr Leu Phe
        35   40   45

Cys Leu Leu Asn Phe Gly Val Ile
    50   55

<210> 57
<211> 56
<212> PRT
<213> Oryctolagus sp.

<400> 57
Met Ser Thr Glu Ser Met Ile Arg Asp Val Glu Leu Ala Glu Gly Pro
1   5   10   15

Leu Pro Lys Lys Ala Gly Gly Pro Gln Gly Ser Lys Arg Cys Leu Cys
            20   25   30

Leu Ser Leu Phe Ser Phe Leu Leu Val Ala Gly Ala Thr Thr Leu Phe
        35   40   45

Cys Leu Leu His Phe Arg Val Ile
    50   55

<210> 58
<211> 56
<212> PRT
<213> Capra sp.

<400> 58
Met Ser Thr Lys Ser Met Ile Arg Asp Val Glu Leu Ala Glu Glu Val
1   5   10   15

Leu Ser Lys Lys Ala Gly Gly Pro Gln Gly Ser Arg Ser Cys Trp Cys
            20   25   30

Leu Ser Leu Phe Ser Phe Leu Leu Val Ala Gly Ala Thr Thr Leu Phe
        35   40   45

Cys Leu Leu His Phe Gly Val Ile
    50   55

<210> 59
<211> 56
<212> PRT
<213> Ovis sp.

<400> 59

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Met Ser Thr Lys Ser Met Ile Arg Asp Val Glu Leu Ala Glu Glu Val
1               5                   10                  15


Leu Ser Asn Lys Ala Gly Gly Pro Gln Gly Ser Arg Ser Cys Trp Cys
            20                  25                  30


Leu Ser Leu Phe Ser Phe Leu Leu Val Ala Gly Ala Thr Thr Leu Phe
        35                  40                  45


Cys Leu Leu His Phe Gly Val Ile
    50                  55
```