

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 UTF8_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 TACAGGCTTGTCACCTCGGGGT 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 adalimumab. 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 Pro112; (2) deletion of Val13; (3) deletion of Val1 and deletion of Val13; (4) deletion of Val1 through and including Pro112 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 delPro12 amino acid sequence

[0043]

MSTESMIRDVELAEEALPKKTGGPQGSRRCLFLSLFSFLIVAGATTFLCLLHFGVIGPQREEFPRDLSLIS
PLAQARSSSRTPSDKVAHVVANPQAEGLQWLNRRANALLANGVELRDNQLVVPSEGLYLIYSQVLFKGQGCPSTHV
LLTHTISRIAVSYQTKVNLLSAIKSPCQRETPEGAEAKPWYEP IYLGGVFQLEKGDRLSAEINRPDYLDFAESGQVY
FGIIAL (SEQ ID NO:1)

[0044] TNF-alpha mutant- delVal1 del Prol12 nucleic acid sequence**[0045]**

atgagcactgaaagcatgatccgggacgtggagctggccgaggaggcgctccccaagaa
gacagggggggccccagggtccaggcggtgcttggtcctcagcctcttctccttcctgatcgtg
gcaggcgccaccacgctcttctgcctgctgcactttggagtgatcgccccccagagggaagagt
tccccagggacctctctctaatacagccctctggcccaggcaagatcatcttctcgaacccccgag
tgacaaggtagcccatgttgtagcaaaccctcaagctgaggggacgctccagtggctgaaccgc
cggggccaatgccctcctggccaatggcgtggagctgagagataaccagctggtggtgccatcag
agggcctgtacctcatctactcccaggctcctcttcaaggggccaaggtgccccctccacccatgt
gtcctcaccacacaccatcagccgcacgcgtctcctaccagaccaaggtcaacctcctctct
gccatcaagagccccctgccagaggggagaccccagagggggctgaggccaagccctggtatgagc
ccatctatctgggaggggtcttccagctggagaagggtgaccgactcagcgctgagatcaatcg
gcccgactatctcgactttgccgagctctgggcaggtctactttgggatcattgccctgtcg
(SEQ ID NO:2)

[0046] TNFa mutant- del Val1 to Val13 amino acid sequence (delete 13aa)**[0047]**

MSTESMIRDVELAEEALPKKTGGPQGSRRCLFLSLFSFLIVAGATTFLCLLHFGVIGPQ
REEFPRDLSLISPLAQAAHVVANPQAEGLQWLNRRANALLANGVELRDNQLVVPSEGLYLIYS
QVLFKGQGCPSTHVLLTHTISRIAVSYQTKVNLLSAIKSPCQRETPEGAEAKPWYEP IYLGGVF
QLEKGDRLSAEINRPDYLDFAESGQVYFGIIAL (SEQ ID NO:3)

[0048] TNFa mutant- del Val1 to Prol12 delVal13 (delete 13 aa) nucleic acid sequence**[0049]**

atgagcactgaaagcatgatccgggacgtggagctggccgaggaggcgctccccaagaagacaggggggccc
ccagggtccaggcggtgcttggtcctcagcctcttctccttcctgatcgtggcaggcgccaccacgctcttctgcc
tgctgcactttggagtgatcgccccccagagggaagagttccccagggacctctctctaatacagccctctggcccag
gcagcccatgtttagcaaaccctcaagctgaggggacgctccagtggctgaaccgcggggccaatgccctcctggc

caatggcggtggagctgagagataaccagctggtggtgccatcagagggcctgtacctcatctactcccaggtcctct
tcaagggccaaggctgcccctccacccatgtgctcctcaccacacccatcagccgcacgcgtctcctaccagacc
aaggtcaacctcctctctgccatcaagagcccctgccagagggagaccccagagggggctgaggccaagccctggta
tgagcccatctatctgggaggggtcttccagctggagaagggtagccgactcagcgctgagatcaatcgcccgact
atctcgactttgccgagctctgggcaggtctactttgggatcattgcctgtcg (SEQ ID NO:4)

[0050] TNF-alpha delVal1 delVal13 amino acid sequence

[0051]

MSTESMIRDVELAEEALPKKTGGPQGSRRCLFLSLFSFLIVAGATTFLCLLHFGVIGPQREEFPRDLSLIS
PLAQARSSSRTPSDKPAHVVANPQAEGLQLQWLNRRANALLANGVELRDNLVVPSEGLYLIYSQVLFKGGCPSTHV
LLTHTISRIAVSYQTKVNLLSAIKSPCQRETPEGAEAKPWYEPYILGGVFQLEKGDRLSAEINRPDYLDFAESGQVY
FGIIAL (SEQ ID NO:5)

[0052] TNF-alpha delVal1 delVal13 nucleic acid sequence

[0053]

atgagcactgaaagcatgatccgggacgtggagctggccgaggaggcgctccccaagaa
gacagggggggcccccagggtccaggcggtgcttgttcctcagcctcttctccttctctgatcgtg
gcaggcgccaccacgctcttctgctgctgcactttggagtgatcgccccccagagggaagagt
tccccagggaacctctctaatcagccctctggcccagggaagatcatcttctcgaacccccgag
tgacaagcctgcccattgtttagcaaaccctcaagctgaggggagcctccagtggctgaaccgc
cgggccaatgccctcctggccaatggcgtagctgagagataaccagctggtggtgccatcag
agggcctgtacctcatctactcccaggtcctcttcaagggccaaggctgcccctccacccatgt
gctcctcaccacacccatcagccgcacgcgtctcctaccagaccaaggtcaacctcctctct
gccatcaagagcccctgccagagggagaccccagagggggctgaggccaagccctggtatgagc
ccatctatctgggaggggtcttccagctggagaagggtagccgactcagcgctgagatcaatcg
gcccgactatctcgactttgccgagctctgggcaggtctactttgggatcattgcctgtcg
(SEQ ID NO:6)

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

[0055]

TCGAGTCGAGATGAGCACTGAAAGCATGATCCGGGACGTGGAGCTGGCCGAGGAGGCGCTCCCCAAGAAGA
CAGGGGGGGCCCCAGGGCTCCAGGCGGTGCTTGTTCCTCAGCCTCTTCTCCTTCCTGATCGTGGCAGGCGCCACCACG
CTCTTCTGCCTGCTGCACTTTGGAGTGATCGCCCCCAGAGGGAAGAGTTCCCCAGGGACCTCTCTTAATCAGCCC
TCTGCAGGCAGCCCATGTGTAGCAAACCCTCAAGCTGAGGGGACGCTCCAGTGGCTGAACCGCCGGGCCAATGCCC
TCCTGGCCAATGGCGTGGAGCTGAGAGATAACCAGCTGGTGGTGCCATCAGAGGGCCTGTACCTCATCTACTCCAG

GTCTCTTCAAGGGCCAAGGCTGCCCCCTCCACCCATGTGCTCCTCACCACACCATCAGCCGCATCGCCGTCTCCTA
CCAGACCAAGGTCAACCTCCTCTCTGCCATCAAGAGCCCCCTGCCAGAGGGAGACCCAGAGGGGGCTGAGGCCAAGC
CCTGGTATGAGCCCATCTATCTGGGAGGGGTCTTCCAGCTGGAGAAGGGTGACCGACTCAGCGCTGAGATCAATCGG
CCCGACTATCTCgACTTTGCCGAGTCTGGGCAGGTCTACTTTGGGATCATTGCCCTGTCTGTCG (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]

MSTESMIRDVELAEALPKKTGGPQGSRRCLFLSLFSFLIVAGATTFLCLLHFGVIGPQREEFPRLSLIS
PLQAAHVVANPQAEGQLQWLNRRANALLANGVELRDNLVVPSEGLYLIYSQVLFGQGCPSTHVLLTHTISRIAVS
YQTKVNLLSAIKSPCQRETPEGAEAKPWYEPYIYLGGVFQLEKGDRLSAEINRPDYLDFAESGQVYFGIIAL (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]

ATGAGCACTGAAATGCATCCCGAAGGGGGTCTGGCACGAGGAGGCGCTCCCCAAGAAGACAGGGGGGCC
CCAGGGCTCCAGGCGGTGCTTGTTCCTCAGCCTCTTCTCCTTCCTGATCGTGGCAGGCGCCACCACGCTCTTCTTCC
TGCTGCACTTTGGAGTGATCGGCCCCCAGAGGGAAGAGTTCCCAGGGACCTCTCTCTAATCAGCCCTCTGGCCCAT
GTTGTAGCAAACCCTCAAGCTGAGGGGCAGCTCCAGTGGCTGAACCGCCGGGCCAATGCCCTCCTGGCCAATGGCGT
GGAGCTGAGAGATAACCAGCTGGTGGTGCCATCAGAGGGCCTGTACCTCATCTACTCCCAGGTCTCTTCAAGGGCC
AAGGCTGCCCTCCACCCATGTGCTCCTCACCACACCATCAGCCGCATCGCCGTCTCCCACCAGACCAAGGTCAAC
CTCCTCTTCGCCATCAAGAGCCCCCTGCCAGAGGGAGACCCAGAGGGGGCTGAGGCCAAGCCCTGGTATGAGCCCAT
CTATCTGGGAGGGGTCTTCCAGCTGGAGAAGGGTGACCGACTCATCGCTGAGATCAATCGGCCCGACTATCTCTACT
TTGCCGAGTATGGGCAGGTCTACTTTGGGATCATTGCCCTGTCTG (SEQ ID NO:40)

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

[0061]

MSTEMHPGRGSWHEEALPKKTGGPQGSRRCLFLSLFSFLIVAGATTFLFLHFGVIGPQREEFPRLSLIS
PLAHVVANPQAEGQLQWLNRRANALLANGVELRDNLVVPSEGLYLIYSQVLFGQGCPSTHVLLTHTISRIAVSHQ
TKVNLLFAIKSPCQRETPEGAEAKPWYEPYIYLGGVFQLEKGDRLIAEINRPDYLYFAEYGVYFGIIALS (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]

MSTESMIRDVELAEEALPKKTGGPQGSRRCLFLSLFSFLIVAGATTFLCLLHFGVIGPQREEFPRDLSLIS
PLAQAVRSSSRTPSDKPVAVHVVANPQAEGQLQWLNRRANALLANGVELRDNLVVPSEGLYLIYSQVLFKGGCPST
HVLTLTHTISRIAVSYQTKVNLLSAIKSPCQRETPEGAEAKPWYEPYIYLGGVFQLEKGDRLSAEINRPDYLDFAESGQ
VYFGIIAL (SEQ ID NO:7)

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

[0066]

VRSSSRTPSDKPVAVHVVANPQAEGQLQWLNRRANALLANGVELRDNLVVPSEGLYLIYSQVLFKGGCPST
THVLLTHTISRIAVSYQTKVNLLSAIKSPCQRETPEGAEAKPWYEPYIYLGGVFQLEKGDRLSAEINRPDYLDFAESG
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 FSFLIVAGATTFLCLLHFGVI (SEQ ID NO:19). The SPPL2b cut site is SL/LI.

The linker comprises GPQREEFPRLDSLISPLAQA (SEQ ID NO:20). The TACE cut 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]

atgagcactgaaaTGCATCCCGGAAGGGGGTCCTGGCACgaggaggcgctccccaagaagacaggggggcccagggctccaggcgggtgcttggttcctcagcctcttctccttctgatcggtggcaggcgccaccacgctcttctgccgtgctgactttggagtgatcggtcccccagagggaagagttccccaggacctctctctaatacagccctctggcccagcagcccatggtgtagcaaacctcaagctgaggggcagctccagtggtgaaccgcgggccaatgccctcctggccaatggcgtggagctgagagataaccagctggtggtgccatcagagggcctgtacctcatctactcccaggtcctcttcaagggccaaggctgccctccaccatgtgctcctcaccacaccatcagccgcatcgccgtctcctaccagaccaggtcaacctcctctctgccatcaagagccctgccagaggagagccccagagggggctgaggccaagccctggtagagcccatctatctgggaggggtcttcagctggagaagggtagccgactcagcgctgagatcaatcgcccgactatctcgactttgccgagctctgggcaggtctactttgggatcattgccctgcg (SEQ ID NO:22)

[0073]

MSTEMHPGRGSWHEEALPKKTGGPQGSRRCLFLSLFSFLIVAGATTFLCLLHFGVIGPQREEFPRLDSLISPLAQAVRSSSRTPSDKPVAVHVVANPQAEQQLQWLNRRANALLANGVELRDNLVVPSEGLYLIYSQVLFKGGCPSTHVLLTHTISRIAVSYQTKVNLLSAIKSPCQRETPEGAEAKPWYEPIYLGGVFQLEKGDRLSAEINRPDYLDFAESQVYFGIIAL (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]

atgagcactgaaagcaAgatccgggacgtggagctggccgaggaggcgctccccaagaagacaggggggcccagggctccaggcgggtgcttggttcctcagcctcttctccttctgatcggtggcaggcgccaccacgctcttctgccgtgctgactttggagtgatcggtcccccagagggaagagttccccaggacctctctctaatacagccctctggcccagcagcccatggtgtagcaaacctcaagctgaggggcagctccagtggtgaaccgcgggccaatgccctcctggccaatggcgtggagctgagagataaccagctggtggtgccatcagagggcctgtacctcatctactcccaggtcctcttcaagggccaaggctgccctccaccatgtgctcctcaccacaccatcagccgcatcgccgtctccCaccagaccaggtcaacctcctctctgccatcaagagccctgccagaggagagccccagagggggctgaggccaagccctggtagagcccatctatctgggaggggtcttcagctggagaagggtagccgactcagcgctgagatcaatcgcccgactatctcgactttgccgagctctgggcaggtctactttgggatcattgccctgctg (SEQ ID NO:24)

[0076]

MSTESKIRDVELAEALPKKTGGPQGSRRCLFLSLFSFLIVAGATTLFCLLHFGVIGPQREEFPRDLSLIS
PLAQAAHVVANPQAEGQLQWLNRRANALLANGVELRDNQLVVPSEGLYLIYSQVLFGQGCPSTHVLLTHTISRIAV
SHQTKVNLLSAIKSPCQRETPEGAEAKPWYEPYIYLGGVFQLEKGDRLSAEINRPDYLDFAESGQVYFGIIAL (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]

atgagcactgaaagcatgatccgggacgtggagctggccgaggaggcgctccccaagaagacaggggggccc
ccagggctccaggcggtgcttggttcctcagcctcttctccttctgatcgtggcaggcgccaccacgctcttctTcc
tgctgcactttggagtgatcgccccccagaggggaagagttccccagggacctctctctaatacagccctctggcccag
gcagcccatggttagcaaacctcaagctgaggggcagctccagtggctgaaccgcggggccaatgccctcctggc
caatggcgtggagctgagagataaccagctggtggtgccatcagagggcctgtacctcatctactcccaggtcctct
tcaagggccaaggctgccctccacccatgtgctcctcaccacacccatcagccgcacgcgtctcctaccagacc
aaggtcaacctcctctTCgcatcaagagccctgccagagggagacccagagggggctgaggccaagccctggta
tgagcccatctatctgggaggggtcttcagctggagaagggtagccgactcagcgctgagatcaatcgcccgact
atctcgactttgccgagctctgggcaggtctactttgggatcattgccctgtcg (SEQ ID NO:26)

[0079]

MSTESMIRDVELAEALPKKTGGPQGSRRCLFLSLFSFLIVAGATTLFFLLHFGVIGPQREEFPRDLSLIS
PLAQAAHVVANPQAEGQLQWLNRRANALLANGVELRDNQLVVPSEGLYLIYSQVLFGQGCPSTHVLLTHTISRIAV
SYQTKVNLLFAIKSPCQRETPEGAEAKPWYEPYIYLGGVFQLEKGDRLSAEINRPDYLDFAESGQVYFGIIAL (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]

atgagcactgaaagcatgatccgggacgtggagctggccgaggaggcgctccccaagaagacaggggggccc
ccagggctccaggcggtgcttggttcctcagcctcttctccttctgatcgtggcaggcgccaccacgctcttctgcc
tgctgcactttggagtgatcgccccccagaggggaagagttccccagggacctctctctaatacagccctctggcccag
gcagcccatggttagcaaacctcaagctgaggggcagctccagtggctgaaccgcggggccaatgccctcctggc
caatggcgtggagctgagagataaccagctggtggtgccatcagagggcctgtacctcatctactcccaggtcctct
tcaagggccaaggctgccctccacccatgtgctcctcaccacacccatcagccgcacgcgtctcctaccagacc
aaggtcaacctcctctctgccatcaagagccctgccagagggagacccagagggggctgaggccaagccctggta
tgagcccatctatctgggaggggtcttcagctggagaagggtagccgactcaTcgtgagatcaatcgcccgact
atctcgactttgccgagtAtgggcaggtctactttgggatcattgccctgtcg (SEQ ID NO:28)

[0082]

MSTESMIRDVELAEEALPKKTGGPQGSRRCLFLSLFSFLIVAGATTFLCLLHFGVIGPQREEFPRDLSLIS
 PLAQA~~AAHV~~VANPQAEGQLQWLNRRANALLANGVELRD~~NQ~~LVVPSEGLYLIYSQVLFKGGCPSTHVL~~LT~~HTISRIAV
 SYQTKVNLLSAIKSPCQRETPEGAEAKPWYEPYIYLG~~GV~~FQLEKGDRLIAEINRPDYLDFAEY~~GQ~~VYFGIIAL (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
 ccagggctccaggcgggtgcttgttccctcagcctcttctccttccctgatcgctggcaggcgccaccacgctcttctgcc
 tgctgcactttggagtgatcgccccccagaggggaagagttccccaggacctctctctaatacagccctctg~~g~~ccag
 gcagcccatgtttagcaaacctcaagctgaggggacgctccagtggctgaaccgcggggccaatgccctcctggc
 caatggcgtggagctgagagataaccagctgggtggcgccatcagagggcctgtacctcatctactcccaggtcctct
 tcaagggccaaggtgccctccaccatgtgctcctcaccacaccatcagccgcacgcctgtcctaccagacc
 aaggtcaacctcctctctgccatcaagagcccctgccagaggagagccccagagggggctgaggccaagccctggta
 tgagcccatctatctgggaggggtcttccagctggagaagggtgaccgactcagcgctgagatcaatcgcccgact
 atctcTactttgccgagctctgggcaggtctactttgggatcattgccctgtcg (SEQ ID NO:30)

[0085]

MSTESMIRDVELAEEALPKKTGGPQGSRRCLFLSLFSFLIVAGATTFLCLLHFGVIGPQREEFPRDLSLIS
 PLAQA~~AAHV~~VANPQAEGQLQWLNRRANALLANGVELRD~~NQ~~LVVPSEGLYLIYSQVLFKGGCPSTHVL~~LT~~HTISRIAV
 SYQTKVNLLSAIKSPCQRETPEGAEAKPWYEPYIYLG~~GV~~FQLEKGDRLSAEINRPDYLYFAESGQVYFGIIAL (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
 ccagggctccaggcgggtgcttgttccctcagcctcttctccttccctgatcgctggcaggcgccaccacgctcttctgcc
 tgctgcactttggagtgatcgccccccagaggggaagagttccccaggacctctctctaatacagccctctgcaggca
 gccatgtttagcaaacctcaagctgaggggacgctccagtggctgaaccgcggggccaatgccctcctggccaa
 tggcgtggagctgagagataaccagctgggtggcgccatcagagggcctgtacctcatctactcccaggtcctcttca
 agggccaaggtgccctccaccatgtgctcctcaccacaccatcagccgcacgcctgtcctaccagaccaag
 gtcaacctcctctctgccatcaagagcccctgccagaggagagccccagagggggctgaggccaagccctggatatga

gcccactctatctgggaggggtcttccagctggagaaggggtgaccgactcagcgctgagatcaatcggccgactatc
tcTactttgccgagctctgggcaggtctactttgggatcattgccctgtcg (SEQ ID NO:32)

[0088]

MSTESMIRDVELAEEALPKKTGGPQGSRRCLFLSLFSLIVAGATTFLCLLHFGVIGPQREEFPRDLSLIS
PLAQAHVVANPQAEGQLQWLNRRANALLANGVELRDNLVVPSEGLYLIYSQVLFKQGQCPSTHVLLTHTISRIAVS
YQTKVNLLSAIKSPCQRETPEGAEAKPWYEPYILGGVFQLEKGDRLSAEINRPDYLYFAESGQVYFGIIAL (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]

ATGCTCGAGTcgagatgagcactgaaaTGCATCCCGGAAGGGGTCCTGGCACgaggaggcgctccccaag
aagacaggggggccccagggctccaggcggtgcttgttctcagcctcttctccttctgatcgtggcaggcgccac
cagctcttctTcctgctgcactttggagtgateggccccagagggaagagttccccagggaacctctctaatca
gccctctggcagcccatgtttagcaaaccctcaagctgaggggcagctccagtggctgaaccgcgggccaatgcc
ctcctggccaatggcgtggagctgagagataaccagctggtggtgccatcagagggcctgtacctcatctactccca
ggctctctcaagggccaaggctgcccctccaccatgtgctcctcaccacaccatcagccgcacgcgtctccC
accagaccaaggtcaacctcctctTCgcatcaagagccctgccagaggagagccccagaggggctgaggccaag
ccctggtatgagcccatctatctgggaggggtcttccagctggagaaggggtgaccgactcaTcgtgagatcaatcg
gcccgactatctcTactttgccgagtAtgggcaggtctactttgggatcattgccctgtcg (SEQ ID NO:34)

[0091]

MSTEMHPGRGSWHEEALPKKTGGPQGSRRCLFLSLFSLIVAGATTFLFLLHFGVIGPQREEFPRDLSLIS
PLAQAHVVANPQAEGQLQWLNRRANALLANGVELRDNLVVPSEGLYLIYSQVLFKQGQCPSTHVLLTHTISRIAVS
HQTKVNLLFAIKSPCQRETPEGAEAKPWYEPYILGGVFQLEKGDRLIAEINRPDYLYFAEYGQVYFGIIAL (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 $\alpha \nu \beta 3$, 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
RGAAEAATRKRITETESPYQELQGQRSDVYSDLNTQRPYYK (SEQ ID NO:9)

[0100] DAP12 nucleic acid sequence

[0101]

ATGGGGGGACTTGAACCTGCAGCAGGCTCCTGCTCCTGCCTCTCCTGCTGGCTGTAAGTGGTCTCCGTCC
TGTCCAGGCCAGGCCAGAGCGATTGCAGTTGCTCTACGGTGAGCCGGGCGTGCTGGCAGGGATCGTGATGGGAG
ACCTGGTGCTGACAGTGCTCATTGCCCTGGCCGTGACTTCCTGGGCCGGCTGGTCCCTCGGGGGCGAGGGGCTGCG
GAGGCAGCGACCCGAAACAGCGTATCACTGAGACCGAGTCGCCTTATCAGGAGCTCCAGGGTCAGAGGTCGGATGT
CTACAGCGACCTCAACACACAGAGCCGTATTACAAATGA (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]

KFWVLVVVGVLACYSLLVTVAFIIFWVRSKRSRLHSDYMNMTPRRPGPTRKHYPYAPPRDFAAYRSRV
 KFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGE
 RRRGKGHDGLYQGLSTATKDTYDALHMQALPPRG (SEQ ID NO:11)

[0105] CD28 nucleic acid sequence**[0106]**

ATTTTGGGTGCTGGTGGTGGTTGGTGGAGTCCTGGCTTGCTATAGCTTGCTAGTAACAG
 TGGCCTTTATTATTTTCTGGGTGAGGAGTAAGAGGAGCAGGCTCCTGCACAGTGACTACATGAA
 CATGACTCCCCGCCGCCCGGGCCACCCGCAAGCATTACCAGCCCTATGCCCCACCACGCGAC
 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
 NWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLR
 PSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFSQSVMH
 EALHNHYTQKSLSLSPGKDKPK (SEQ ID NO:13)

[0111] IgG1 hinge nucleic acid sequence**[0112]**

GTACGTCACTGTCTCTTCACAGGATCCCGCCGAGCCCAAATCTCCTGACAAAACCTCACACATGCCACCGT
GCCCAGCACCTGAACCTCTGGGGGGACCGTCAGTCTTCTCTTCCCCCAAAACCAAGGACACCCCTCATGATCTCC
CGGACCCCTGAGGTACATGCGTGGTGGTGGACGTGAGCCACGAAGACCCTGAGGTCAAGTTCAACTGGTACGTGGA
CGGCGTGGAGGTGCATAATGCCAAGACAAAGCCGCGGGAGGAGCAGTACAACAGCACGTACCGTGTGGTCAGCGTCC
TCACCGTCTGCACCAGGACTGGCTGAATGGCAAGGAGTACAAGTGCAAGGTCTCCAACAAAGCCCTCCCAGCCCCC
ATCGAGAAAACCATCTCCAAAGCCAAAGGGCAGCCCGAGAACCACAGGTGTACACCCTGCCCCCATCCCGGGATGA
GCTGACCAAGAACCAGGTACGCTGACCTGCCTGGTCAAAGGCTTCTATCCCAGCGACATCGCCGTGGAGTGGGAGA
GCAATGGGCAACCGGAGAACAACTACAAGACCACGCCTCCCGTGCTGGACTCCGACGGCTCCTTCTCTCTACAGC
AAGCTCACCGTGGACAAGAGCAGGTGGCAGCGGGAACGTCTTCTCATGCTCCGTGATGCATGAGGCTCTGCACAA
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]

AATGAAAGACCCACCTGTAGGTTTGGCAAGCTAGCTTAAGTAACGCCATTTTGCAAGGCATGGAAAAATA
CATAACTGAGAATAGAAAAGTTCAGATCAAGGTCAGGAACAGATGGAACAGCTGAATATGGGCCAAACAGGATATCT
GTGGTAAGCAGTTCTTCCCCGGCTCAGGGCCAAGAACAGATGGAACAGCTGAATATGGGCCAAACAGGATATCTGT
GGTAAGCAGTTCTTCCCCGGCTCAGGGCCAAGAACAGATGGTCCCCAGATGCGGTCCAGCCCTCAGCAGTTTCTAG
AGAACCATCAGATGTTTTCCAGGGTGGCCCAAGGACCTGAAATGACCCTGTGCCTTATTTGAACTAACCAATCAGTTC
GCTTCTCGCTTCTGTTTCGCGCTTATGCTCCCCGAGCTCAATAAAAGAGCCCAACCCCTCACTCGGGGCGCCAG
TCCTCCGATTGACTGAGTCGCCCCGGTACCCGTGTATCCAATAAACCCCTCTTGCACTTGCATCCGACTTGTGGTCTC
GCTGTTCTTTGGGAGGGTCTCTCTGAGTGATTGACTACCCGTGACGGGGGTCTTTCATTTGGGGGCTCGTCCGGG
ATCGGGAGACCCCTGCCCAGGGACACCGACCCACCACCGGGAGGTAAGCTGGCCAGCAACTTATCTGTGTCTGTCC
GATTGTCTAGTGTCTATGACTGATTTTATGCGCTGCGTCTAGTACTAGCTAACTAGCTCTGTATCTGGCGGAC
CCGTGGTGAACTGACGAGTTCGGAACACCCGGCCGCAACCCTGGGAGACGTCCCAGGGACTTCGGGGGCGCTTTTT
GTGGCCCGACCTGAGTCTAAATCCCGATCGTTTATAGGACTCTTTGGTGCACCCCCCTTAGAGGAGGGATATGTGGT
TCTGGTAGGAGACGAGAACCTAAACAGTTCCCGCTCCGTCTGAATTTTTGCTTTCGGTTTGGGACCGAAGCCGCG
CCGCGCGTCTTGTCTGCTGCAGCATCGTTCTGTGTGTCTCTGTCTGACTGTGTTTCTGTATTGTCTGAAAATATG
GGCCCGGGCTAGCCTGTTACCACTCCCTTAAGTTTGACCTTAGGTCACCTGGAAGATGTCGAGCGGATCGCTCACAA
CCAGTCGGTAGATGTCAAGAAGAGACGTTGGGTTACCTTCTGCTCTGCAGAAATGGCCAACCTTTAACGTCGGATGGC
CGCGAGACGGCACCTTTAACCGAGACCTCATACCCAGGTTAAGATCAAGGTCTTTTACCTGGCCCCGATGGACAC
CCAGACCAGGTGGGTACATCGTGACCTGGGAAGCCTTGGCTTTTGACCCCCCTCCCTGGGTCAAGCCCTTTGTACA
CCCTAAGCCTCCGCTCCTCTTCTCCATCCGCCCCGTCTCTCCCCCTTGAACCTCCTCGTTGACCCCGCCTCGAT
CCTCCCTTTATCCAGCCCTCACTCCTTCTTAGGCGCCCCCATATGGCCATATGAGATCTTATATGGGGCACCCCG
CCCCCTTGTAACCTTCCCTGACCCTGACATGACAAGAGTTACTAACAGCCCCCTCTCTCCAAGCTCACTTACAGGCTCT
CTACTTAGTCCAGCACGAAGTCTGGAGACCTCTGGCGGCAGCCTACCAAGAACAAGTGGACCGACCGGTGGTACCTC
ACCCTTACCGAGTCGGCGACACAGTGTGGGTCCGCGACACCAGACTAAGAACCTAGAACCTCGCTGGAAAGGACCT
TACACAGTCCTGCTGACCACCCCAACCGCCCTCAAAGTAGACGGCATCGCAGCTTGGATACACGCCGCCACGTGAA
GGCTGCCGACCCCGGGGTGGACCATCTCTAGACTGCCATGCTCGAGATGAGCACTGAAAGCATGATCCGGGACGT
GGAGCTGGCCGAGGAGGCGCTCCCCAAGAAGACAGGGGGGCCCCAGGGCTCCAGGCGGTGCTTGTTCCTCAGCCTCT
TCTCCTTCTGATCGTGGCAGGCGCCACCACGCTCTTCTGCCTGCTGCACCTTGGAGTGATCGGCCCCCAGAGGGAA
GAGTTCCCCAGGGACCTCTCTCTAATCAGCCCTCTGGCCCAGGCAAGATCATCTCTCGAACCCCGAGTGACAAGGT
AGCCCATGTTGTAGCAAACCCCTCAAGCTGAGGGGACGCTCCAGTGGCTGAACCGCCGGGCCAATGCCCTCTGGCCA

GTAATCTGCTGCTTGCAAACAAAAAACCACCGCTACCAGCGGTGGTTTGTGTTGCCGGATCAAGAGCTACCAACTCT
TTTTCCGAAGGTAAGTGGCTTCAGCAGAGCGCAGATACCAAATACTGTTCTTCTAGTGTAGCCGTAGTTAGGCCACC
ACTTCAAGAACTCTGTAGCACCGCCTACATACCTCGCTCTGCTAATCCTGTTACCAGTGGCTGCTGCCAGTGGCGAT
AAGTCGTGTCTTACCGGGTTGGACTCAAGACGATAGTTACCGGATAAGGCGCAGCGGTGCGGCTGAACGGGGGGTTC
GTGCACACAGCCCAGCTTGGAGCGAACGACCTACACCGAACTGAGATACCTACAGCGTGAGCTATGAGAAAGCGCCA
CGCTTCCCGAAGGGAGAAAGGCGGACAGGTATCCGGTAAGCGGCAGGGTCGGAACAGGAGAGCGCACGAGGGAGCTT
CCAGGGGGAAACGCCTGGTATCTTTATAGTCCTGTGCGGGTTTCGCCACCTCTGACTTGAGCGTCGATTTTTGTGATG
CTCGTCAGGGGGGCGGAGCCTATGGAACAGCCAGCAACGCGGCTTTTACGGTTTCTGGCCTTTTGTGTCGCTT
TTGCTCACATGTTCTTTCTGCGTTATCCCCTGATTCTGTGGATAACCGTATTACCGCCTTTGAGTGAGCTGATACC
GCTCGCCGAGCCGAACGACCGAGCGCAGCGAGTCAGTGAGCGAGGAAGCGGAAGAGCGCCCAATACGAAACCGCC
TCTCCCCGCGCGTTGGCCGATTCAATTAATGCAGCTGGCAGCAGAGGTTTCCCGACTGGAAGCGGGCAGTGAGCGCA
ACGCAATTAATGTGAGTTAGCTCACTCATTAGGCACCCAGGCTTTACACTTTATGCTTCCGGCTCGTATGTTGTGT
GGAATTGTGAGCGGATAACAATTTACACAGGAAACAGCTATGACCATGATTACGCCAAGCTTTGCTCTTAGGAGTT
TCCTAATACATCCCAAACCTCAAATATATAAAGCATTGACTTGTCTATGCCCTAGGGGGCGGGGGAAGCTAAGCC
AGCTTTTTTTAACATTTAAAAATGTTAATTCCATTTTAAATGCACAGATGTTTTTATTTTATAAGGGTTTCAATGTGC
ATGAATGCTGCAATATTCCTGTTACCAAAGCTAGTATAAAATAAAATAGATAAACGTGGAATTACTTAGAGTTTCT
GTCATTACGTTTCTTCTCAGTTGACAACATAAATGCGCTGCTGAGCAAGCCAGTTTGCATCTGTCAGGATCAAT
TTCCCATATGCCAGTCATATTAATTACTAGTCAATTAGTTGATTTTTATTTTTGACATATACATGTG (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]

MSTESMIRDVELAEALPKKTGGPQGSRRCLFLSLFSFLIVAGATTLFCLLHFGVIGPQREEFPRDLISLIS
PLAQARSSSRTPSDKVAHVVANPQAEGLQWLNRRANALLANGVELRDNLVVPSEGLYLIYSQVLFKGGQCPSTHV
LLTHTISRIVSYQTKVNLLSAIKSPCQRETPEGAEAKPWYEPYILGGVFQLEKGDRLSAEINRPDYLDFAESGQVY
FGIIALSRAEGRGSLTTCGDVEENPGPMEFGLSWLFLVAILKGVQCSDIQMTQTTSSLSASLGDRVTISCRASQDI
SKYLNWYQQKPDGTVKLLIYHTSRLHSGVPSRFSGSGSGTDYSLTISNLEQEDIATYFCQQGNTLPYTFGGGKLEL
KRGGGGSGGGGSGGGGSGGGSEVQLQQSGPGLVAPSSQLSVTCTVSGVSLPDYGVSWIRQPPRKGLEWLGVIWGSE
TTYNSALKSRLTIKDNSKSQVFLKMNSLQTDITAIYYCAKHYGGSYAMDYWGQTTVTVSSYVTVSSQDPAEP
KSPDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREE
QYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKG
FYPDSIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFSQSVMEALHNHYTQKSLSLSPGKK
DPKFWVLVVGVLACYSLLVTVAFIIIFWVRSKRSRLHSDYMNMTFRRPGPTRKHYPYAPPRDFAAYRSRVKFSR
SADAPAYQQGQNQLYNELNLGRREEYDVLDRRGRDPEMGGKPRKPNPQEGLYNELQDKMAEAYSEIGMKGERRRG
KGHDGLYQGLSTATKDTYDALHMQALPPRGPQCTNYALLKLAGDVESNPGPMRISKPHLRSISIQCYLCLLLNSHFL

TEAGIHVFIILGCF SAGLPKTEANWVNVISDLKKIEDLIQSMHIDATLYTESDVHP SCKVTAMKCFLELQVISLESG
DASIHD TVENLIILANN SLSSNGNVTESGCKECEELEEKNIKEFLQSFVHIVQMFIN TS (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]

AATGAAAGACCCACCTGTAGGTTTGGCAAGCTAGCTTAAGTAACGCCATTTTGCAAGGCATGGAAAAATA
CATAACTGAGAATAGAAAAGTTCAGATCAAGGTCAGGAACAGATGGAACAGCTGAATATGGGCCAAACAGGATATCT
GTGGTAAGCAGTTCTGCCCCGGCTCAGGGCCAAGAACAGATGGAACAGCTGAATATGGGCCAAACAGGATATCTGT
GGTAAGCAGTTCTGCCCCGGCTCAGGGCCAAGAACAGATGGTCCCCAGATGCGGTCCAGCCCTCAGCAGTTTCTAG
AGAACCATCAGATGTTTCCAGGGTGCCCCAAGGACCTGAAATGACCCTGTGCCTTATTTGAACTAACCAATCAGTTC
GCTTCTCGCTTCTGTTTCGCGCGCTTATGCTCCCCGAGCTCAATAAAAGAGCCCAACCCCTCACTCGGGGGCCGAG
TCCTCCGATTGACTGAGTCGCCCCGGGTACCCGTGTATCCAATAAACCCCTCTTGCAAGTTGCATCCGACTTGTGGTCTC
GCTGTTCTTGGGAGGGTCTCCTCTGAGTGATTGACTACCCGTCAGCGGGGGTCTTTCATTTGGGGGCTCGTCCGGG
ATCGGGAGACCCCTGCCAGGGACACCGACCCACCCAGGGAGGTAAGCTGGCCAGCAACTATCTGTGTCTGTCC
GATTGTCTAGTGTCTATGACTGATTTTATGCGCTGCGTCTGCTAGTTAGCTAACTAGCTCTGTATCTGGCGGAC
CCGTGGTGGAAGTACGAGTTTCGGAACACCCGGCCGCAACCCTGGGAGACGTCCAGGGACTTCGGGGGGCGCTTTT
GTGGCCCGACCTGAGTCCTAAAATCCCGATCGTTTAGGACTCTTTGGTGACCCCCCTTAGAGGAGGGATATGTGGT
TCTGGTAGGAGACGAGAACCTAAAACAGTTCCCGCTCCGTCTGAATTTTGGCTTTCGGTTTGGGACCGAAGCCGCG
CCGCGCGTCTTGTCTGTCTGAGCATCGTTCTGTGTGTCTCTGTCTGACTGTGTTTCTGTATTTGTCTGAAAATATG
GGCCCGGGCTAGCCTGTTACCACTCCCTTAAGTTTGACCTTAGGTCACTGGAAGATGTCGAGCGGATCGCTCACAA
CCAGTCGGTAGATGTCAAGAAGAGACGTTGGGTTACCTTCTGCTCTGCAGAATGGCCAACCTTTAACGTCGGATGGC
CGCGAGACGGCACCTTTAACCGAGACCTCATCACCAGGTTAAGATCAAGGTCCTTTACCTGGCCCCGCATGGACAC
CCAGACCAGGTGGGTACATCGTGACCTGGGAAGCCTTGGCTTTTGACCCCCCTCCCTGGGTCAAGCCCTTTGTACA
CCCTAAGCTCCGCTCTCTCTCCATCGCCCCGTCTCTCCCCCTTGAACCTCCTCGTTGCACCCCGCTCGAT
CCTCCCTTTATCCAGCCCTCACTCCTTCTCTAGGCGCCCCCATATGGCCATATGAGATCTTATATGGGGACCCCCG
CCCCCTTGTAACCTTCCCTGACCCTGACATGACAAGAGTTACTAACAGCCCCTCTCTCCAAGCTCACTTACAGGCTCT
CTACTTAGTCCAGCACGAAGTCTGGAGACCTCTGGCGGCAGCCTACCAAGAACAACCTGGACCGACCGGTGGTACCTC
ACCCCTTACCGAGTCGGCGACACAGTGTGGGTCCGCCGACACCAGACTAAGAACCTAGAACCTCGCTGGAAGGACCT
TACACAGTCTCTGCTGACCACCCCAACCGCCCTCAAAGTAGACGGCATCGCAGCTTGGATACACGCCGCCACGTGAA
GGCTGCCGACCCCGGGGTGGACCATCCTCTAGACTGCCATGCTCGAGATGAGCACTGAAAGCATGATCCGGGACGT
GGAGCTGGCCGAGGAGGCGCTCCCCAAGAAGACAGGGGGGGCCCCAGGGCTCCAGGCGGTGCTTGTTCTCAGCCTCT
TCTCCTTCTGATCGTGGCAGGCGCCACCACGCTCTTCTGCCTGCTGCACTTTGGAGTGATCGGCCCCCAGAGGGAA
GAGTTCCCCAGGGACCTCTCTCTAATCAGCCCTCTGGCCCAGGCAAGATCATCTTCTCGAACCCCGAGTGACAAGGT
AGCCCATGTTGTAGCAAACCTCAAGCTGAGGGGCGAGCTCCAGTGGCTGAACCGCCGGGCCAATGCCCTCCTGGCCA
ATGGCGTGGAGCTGAGAGATAACCAGCTGGTGGTGCCATCAGAGGGCCTGTACCTCATCTACTCCAGGTCTCTTTC
AAGGGCCAAGGCTGCCCTCCACCCATGTGCTCCTCACCCACACCATCAGCCGCATCGCGCTCTCTACCAGACCAA
GGTCAACCTCCTCTCTGCCATCAAGAGCCCCTGCCAGAGGGAGACCCAGAGGGGGCTGAGGCCAAGCCCTGGTATG

AGCCCATCTATCTGGGAGGGGTCTTCCAGCTGGAGAAGGGTGACCGACTCAGCGCTGAGATCAATCGGCCCCACTAT
CTCGACTTTGCCGAGTCTGGGCAGGTCTACTTTGGGATCATTGCCCTGTGCGGAGCCGAGGGCAGGGGAAGTCTTCT
AACATGCGGGGACGTGGAGGAAAATCCCGGGCCCATGGAGTTTGGGCTGAGCTGGCTTTTTCTTGTGGCTATTTTAA
AAGGTGTCCAGTGCTCTAGAGACATCCAGATGACACAGACTACATCCTCCCTGTCTGCCTCTCTGGGAGACAGAGTC
ACCATCAGTTGCAGGGCAAGTCAGGACATTAGTAAATATTTAAATTTGGTATCAGCAGAAACCAGATGGAACGTGTTAA
ACTCCTGATCTACCATACATCAAGATTACACTCAGGAGTCCCATCAAGGTTCACTGGCAGTGGGTCTGGAACAGATT
ATTCTCTCACCATTAGCAACCTGGAGCAAGAAGATATTGCCACTTACTTTTGCCAACAGGGTAATACGCTTCCGTAC
ACGTTTCGGAGGGGGGACCAAGCTGGAGCTGAAACGTGGTGGTGGTGGTTCCTGGTGGTGGTGGTTCCTGGCGGCGGCGG
CTCCGGTGGTGGTGGATCCGAGGTGCAGCTGCAGCAGTCTGGACCTGGCCTGGTGGCGCCCTCACAGAGCCTGTCCG
TCACATGCACTGTCTCAGGGGTCTCATTACCCGACTATGGTGTAAAGCTGGATTGCGCCAGCCTCCACGAAAGGGTCTG
GAGTGGCTGGGAGTAATATGGGGTAGTGAAACCACATACTATAATTCACTCTCAAATCCAGACTGACCATCATCAA
GGACAACCTCCAAGAGCCAAGTTTTCTTAAAAATGAACAGTCTGCAAACTGATGACACAGCCATTTACTACTGTGCCA
AACATTATTACTACGGTGGTAGCTATGCTATGGACTACTGGGGCCAAGGGACCACGGTCACTGTCTCCTCGTACGTC
ACTGTCTCTTCACAGGATCCCGCCGAGCCCAAATCTCCTGACAAAACCTCACACATGCCACCGTGCCAGCACCTGA
ACTCCTGGGGGGACCGTCAGTCTTCTCTTCCCCCAAAACCAAGGACACCCCTCATGATCTCCCGGACCCCTGAGG
TCACATGCGTGGTGGTGGACGTGAGCCACGAAGACCTGAGGTCAAGTTCAACTGGTACGTGGACGGCGTGGAGGTG
CATAATGCCAAGACAAAGCCGCGGGAGGAGCAGTACAACAGCAGCTACCGTGTGGTCAGCGTCTCACCCTCCTGCA
CCAGGACTGGCTGAATGGCAAGGAGTACAAGTGCAAGGTCTCCAACAAAGCCCTCCAGCCCCCATCGAGAAAACCA
TCTCCAAAGCCAAAGGGCAGCCCCGAGAACCACAGGTGTACACCCTGCCCCCATCCCGGGATGAGCTGACCAAGAAC
CAGGTCAAGCTGACCTGCCTGGTCAAAGGCTTCTATCCAGCGACATCGCCGTGGAGTGGGAGAGCAATGGGCAACC
GGAGAACAACATAAGACCACGCTCCCGTGTGGACTCCGACGGCTCCTTCTCTCTACAGCAAGCTCACCGTGG
ACAAGAGCAGGTGGCAGCAGGGGAACGTCTTCTCATGCTCCGTGATGCATGAGGCTCTGCACAACCACTACACGCAG
AAGAGCCTCTCCCTGTCTCCGGGTAAAAAAGATCCCAAATTTTGGGGCGTGTGGCCGAATCGTGATGGGCGACCT
GGTGCTGACAGTGCTGATCGCCCTGGCTGTGTACTTCTGGGCAGACTGGTGCCAGAGGAAGAGGCGCTGCCGAAG
CCGCCACCCGGAAGCAGAGAATCACCGAGACAGAGAGCCCCCTATCAGGAACGCAGGGCCAGCGGAGCGACGTGTAC
AGCGACCTGAATACCCAGCGGCCCTACTACAAACGCGGACCGCAGTGTACTAATTATGCTCTCTTGAAATTGGCTGG
AGATGTTGAGAGCAATCCCGGGCCCATGCGCATTAGCAAGCCCCACCTGCGGAGCATCAGCATCCAGTGCTACCTGT
GCCTGTGTGAACAGCCACTTCTGACCGAGGCCGGCATCCAGTGTTTCATCCTGGGCTGCTTCAGCGCCGGACTG
CCCAAGACCGAGGCCAACTGGGTGAACGTGATCAGCGACCTGAAGAAGATCGAGGACCTGATCCAGAGCATGCACAT
CGACGCCACCCGTGTACACCGAGAGCGACGTGCACCCAGCTGCAAGGTGACCGCCATGAAGTGCTTTCTGTGGAAC
TGCAGGTGATCAGCCTGGAAGCGGCGACGCCAGCATCCACGACACCGTGGAGAACCTGATCATCCTGGCCAACAAC
AGCCTGAGCAGCAACGGCAACGTGACCGAGAGCGGCTGCAAGAGTGCAGGGAACCTGGAAGAGAAGAACATCAAAGA
GTTTCTGCAGAGCTTCGTGCACATCGTGCAGATGTTTCATCAACACCAGCTGACAATTGCGCGTCATCATCGATCCGG
ATTAGTCCAATTTGTTAAAGACAGGATATCAGTGGTCCAGGCTCTAGTTTTGACTCAACAATATCACCAGCTGAAGC
CTATAGAGTACGAGCCATAGATAAAATAAAAGATTTTATTTAGTCTCCAGAAAAAGGGGGGAATGAAAGACCCACC
TGTAGGTTTGGCAAGCTAGCTTAAGTAACGCCATTTTGCAAGGCATGGAAAAATACATAACTGAGAATAGAGAAGTT
CAGATCAAGGTCAGGAACAGATGGAACAGCTGAATATGGGGCCAAACAGGATATCTGTGGTAAGCAGTTTCTGCCCG
GCTCAGGGCCAAGAACAGATGGAACAGCTGAATATGGGGCCAAACAGGATATCTGTGGTAAGCAGTTTCTGCCCGGC
TCAGGGCCAAGAACAGATGGTCCCCAGATGCGGTCCAGCCCTCAGCAGTTTCTAGAGAACCATCAGATGTTTCCAGG
GTGCCCCAAGGACCTGAAATGACCCTGTGCCTTATTTGAACTAACCAATCAGTTCGCTTCTCGCTTCTGTTCGCGCG

TCAGGGGGGCGGAGCCTATGGAAAAACGCCAGCAACGCGGCCCTTTTACGGTTCCTGGCCTTTTGCTGGCCTTTTGCTG
 TCACATGTTCTTTCTGCGTTATCCCCTGATTCTGTGGATAACCGTATTACCGCCTTTGAGTGAGCTGATACCGCTC
 GCCGCAGCCGAACGACCGAGCGCAGCGAGTCAGTGAGCGAGGAAGCGGAAGAGCGCCCAATACGCAAACCGCCTCTC
 CCCGCGCGTTGGCCGATTCAATTAATGCAGCTGGCAGCAGAGGTTTCCCGACTGGAAAGCGGGCAGTGAGCGCAACGC
 AATTAATGTGAGTTAGCTCACTCATTAGGCACCCAGGCTTTACACTTTATGCTTCCGGCTCGTATGTTGTGTGGAA
 TTGTGAGCGGATAACAATTTACACAGGAAACAGCTATGACCATGATTACGCCAAGCTTTGCTCTTAGGAGTTTCTT
 AATACATCCCAAACCTCAAATATATAAAGCATTGACTTGTTCTATGCCCTAGGGGGCGGGGGAAGCTAAGCCAGCT
 TTTTTTAACATTTAAATGTTAATTCATTTTAAATGCACAGATGTTTTATTTCATAAGGGTTTCAATGTGCATGA
 ATGCTGCAATATTCCTGTTACCAAAGCTAGTATAAATAAAATAGATAAACGTGGAATTACTTAGAGTTTCTGTCA
 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]

MSTESMIRDVELAEALPKKTGGPQGSRRCLFLSLFSLIVAGATTLFCLLHFGVIGPQREEFPRDLSLIS
 PLAQARSSSRTPSDKVAHVVANPQAEQLQWLNRRANALLANGVELRDNLVVPSEGLYLIYSQVLFKQGCPSTHV
 LLTHTISRIVSYQTKVNLLSAIKSPQRETPEGAEAKPWYEPYILGGVFQLEKGDRLSAEINRPDYLDFAESGQVY
 FGIIALSRAEGRGSLLTCDGVEENPGPMEFGLSWLFLVAILKGVCQRDIQMTQTSSLSASLGDRVTISCRASQDI
 SKYLNWYQQKPDGTVKLLIYHTSRLHSGVPSRFSGSGSGTDYSLTISNLEQEDIATYFCQQGNTLPYTFGGGKLEL
 KRGGGSGGGGSGGGGSGGGGSEVQLQQSGPGLVAPSQSLSVTCTVSGVSLPDYGVSWIRQPPRKGLEWLGVWGS
 TTYNSALKSRLTIKDNSKSQVFLKMNSLQTDDTAIYYCAKHYYYGGSYAMDYWGQGTTVTVSSYVTVSSQDPAEP
 KSPDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPRE
 QYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVK
 GYFPDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVSCFVSVMHEALHNHYTQKSLSLSPGK
 DPKFVGVLGAGIVMGDLVLTVLIALAVYFLGRLVPRGRGAEEAATRKQRITETESPYQELQQRSDVYSDLNTQRPYY
 KRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGM
 KGERRRGKGDGLYQGLSTATKDTYDALHMQALPPRGPQCTNYALLKLAGDVESNPGPMRISKPHLRSISIQCYLCL
 LLNSHFLTEAGIHVFIILGCFSGALPKTEANWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCVKVTAMKCFLELQ
 VISLESGLDASIHDTVENLIILANNSLSSNGNVTESGCKECEELEEKNIKEFLQSFVHIVQMFINTS (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-szss, 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 adalimumab. 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 Transduced 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.