



US 20210332101A1

(19) **United States**(12) **Patent Application Publication**
Wang et al.(10) **Pub. No.: US 2021/0332101 A1**(43) **Pub. Date: Oct. 28, 2021**(54) **COMPOSITIONS AND METHODS FOR T
CELL ENGINEERING**Dec. 18, 2018 (CN) 201811549651.0
Jun. 6, 2019 (CN) 201910492882.0(71) Applicants: **Gracell Biotechnologies (Shanghai)
Co., Ltd.**, Shanghai (CN); **Suzhou
Gracell Biotechnologies Co., Ltd.**,
Jiangsu (CN)**Publication Classification**(51) **Int. Cl.****C07K 14/725** (2006.01)**A61K 35/17** (2006.01)**A61P 35/00** (2006.01)**C07K 14/705** (2006.01)(52) **U.S. Cl.**CPC **C07K 14/7051** (2013.01); **A61K 35/17**
(2013.01); **A61K 38/00** (2013.01); **C07K**
14/70596 (2013.01); **C07K 14/70507**
(2013.01); **A61P 35/00** (2018.01)(72) Inventors: **Xinxin Wang**, Shanghai (CN); **Tao
JIN**, Shanghai (CN); **Chunhui YANG**,
Shanghai (CN); **Zhongdong SHI**,
Shanghai (CN); **Liping LIU**, Shanghai
(CN); **Jing SUN**, Shanghai (CN); **Shuyi
QIU**, Shanghai (CN); **Wei CAO**,
Shanghai (CN)(21) Appl. No.: **17/246,394**

(57)

ABSTRACT(22) Filed: **Apr. 30, 2021****Related U.S. Application Data**(63) Continuation of application No. PCT/CN2019/
114939, filed on Nov. 1, 2019, which is a continua-
tion-in-part of application No. PCT/CN2019/101651,
filed on Aug. 20, 2019.The present disclosure relates to an engineered immune cell
and use thereof. The present disclosure provides an engi-
neered immune cell comprising a CAR or engineered TCR,
which CAR or engineered TCR can comprise a first antigen
binding domain and a second antigen binding domain. The
engineered immune cells of the present disclosure, when
administered into a subject, can inhibit the host immune
cells such as T cells and/or NK cells and enhance the
survival and persistence of the engineered immune cells in
vivo, thereby exhibiting more effective tumor killing activ-
ity.**Specification includes a Sequence Listing.**(30) **Foreign Application Priority Data**Nov. 1, 2018 (CN) 201811297174.3
Dec. 14, 2018 (CN) 201811535338.1

IEGFR	2A	SP	CD3ε scFv	Lk	CD19 scFv	H	TM	CD28	CD3z
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Parallel:

IEGFR	2A	CD19 scFv	CD28	CD3z	2A	CD3 scFv	41BB	CD3z
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Tandem:

IEGFR	2A	CD3 scFv	CD19 scFv	41BB	CD3z
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Loop:

CD19 VL	CD3 VL	CD3 VH	CD19 VH	41BB	CD3z
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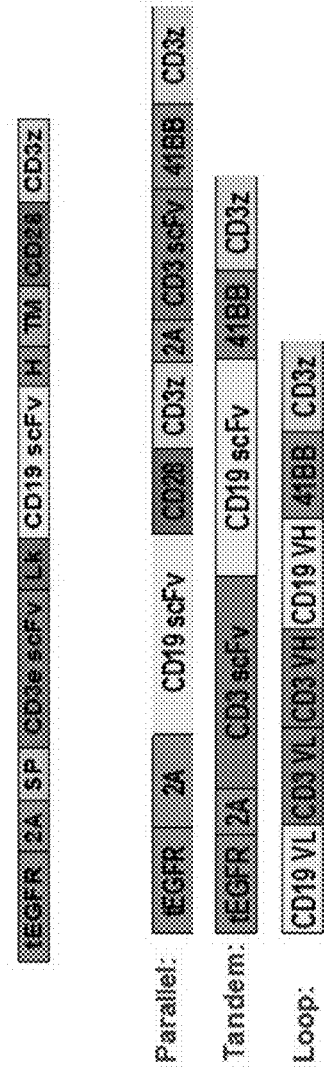


FIG. 1

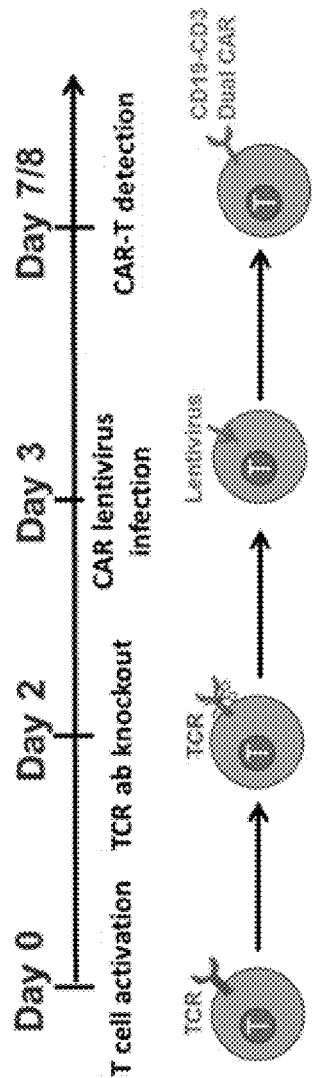


FIG. 2

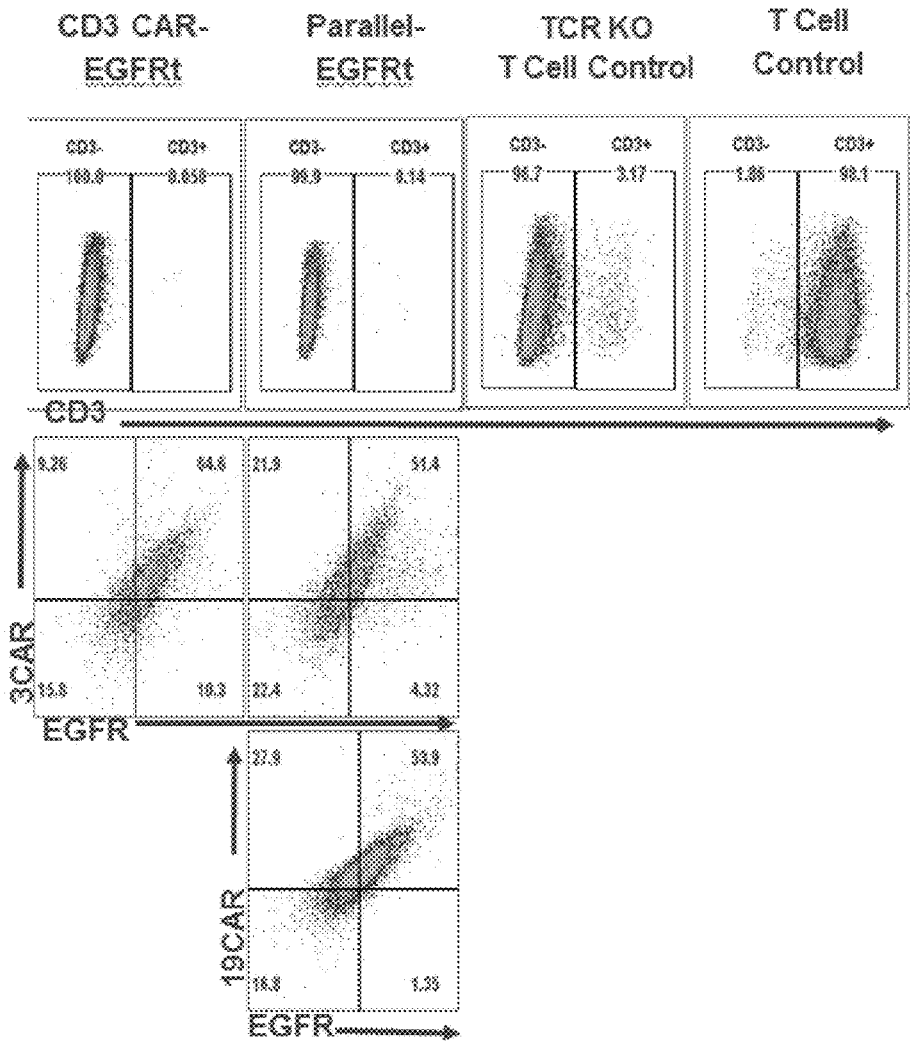


FIG. 3

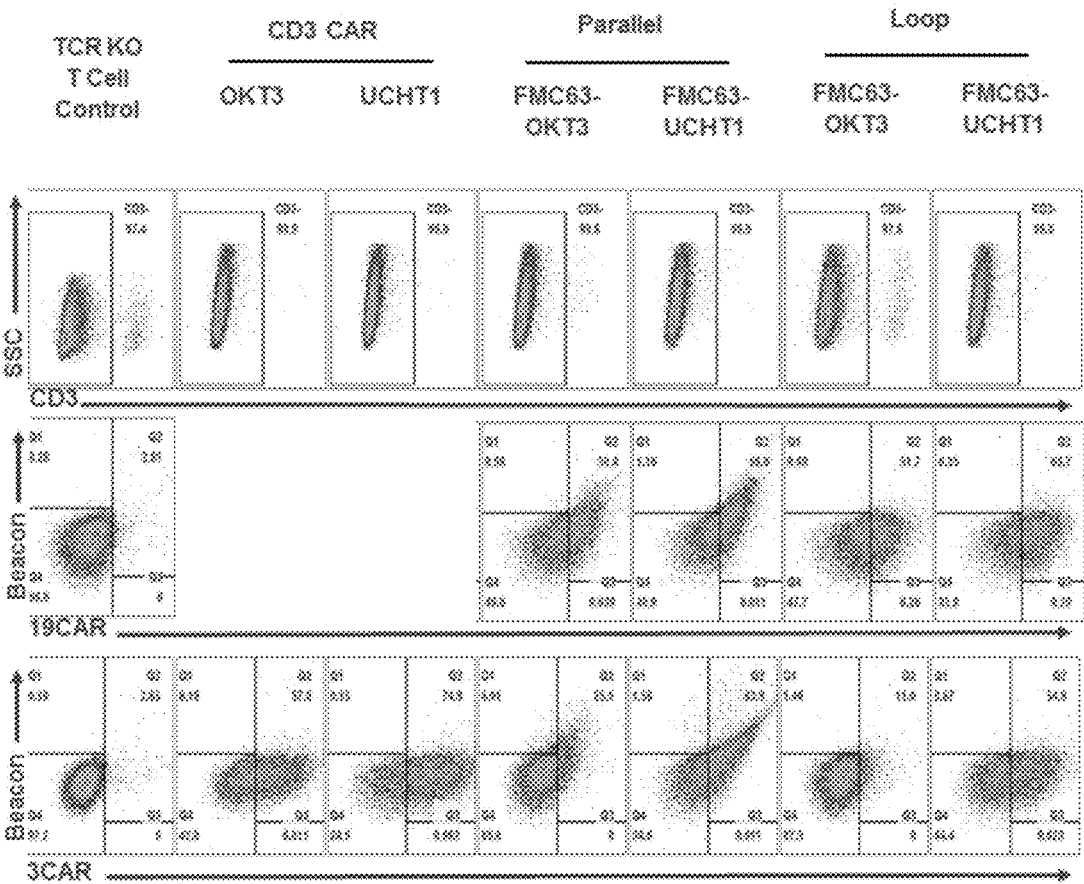


FIG. 4

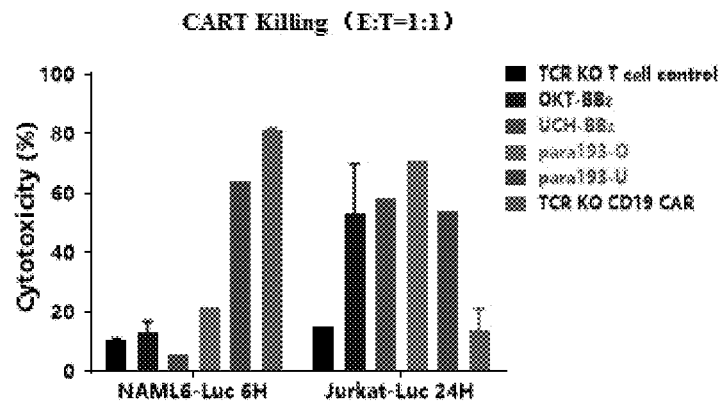


FIG. 5A

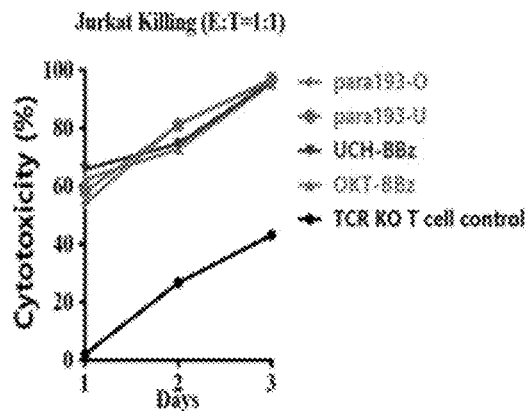


FIG. 5B

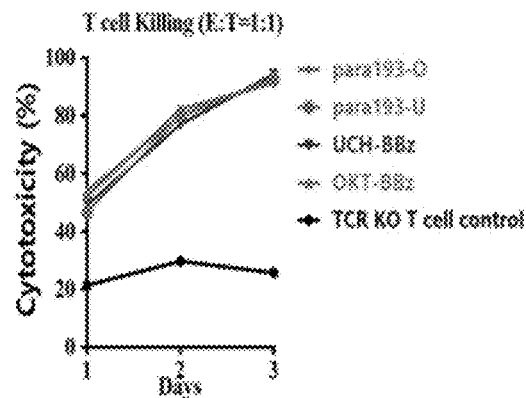


FIG. 5C

NALM6-LucG Killing-6H

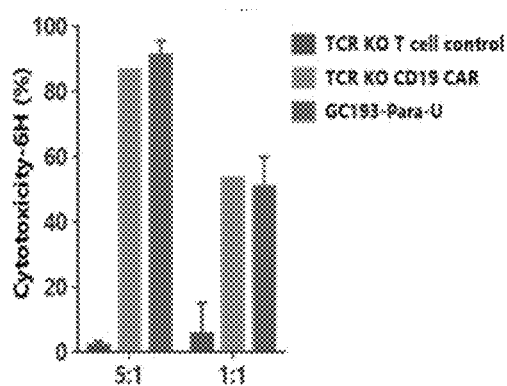


FIG. 6A

Jurkat-LucG Killing-6H

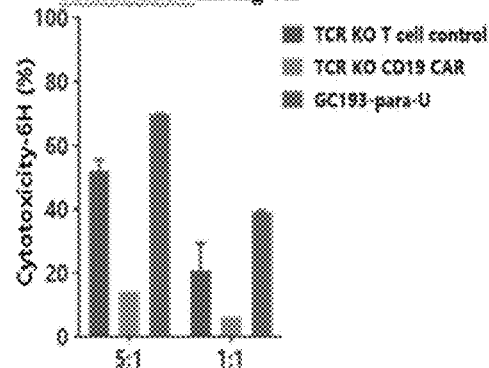


FIG. 6B

CART Killing (E:T=1:1)

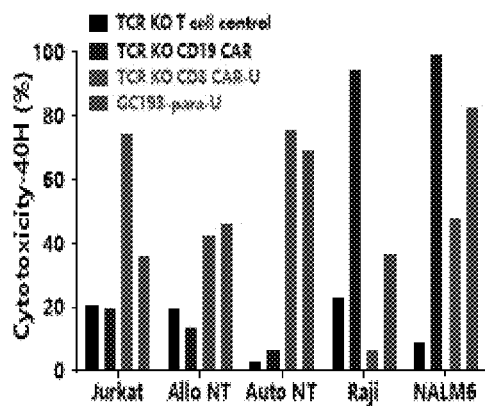


FIG. 6C

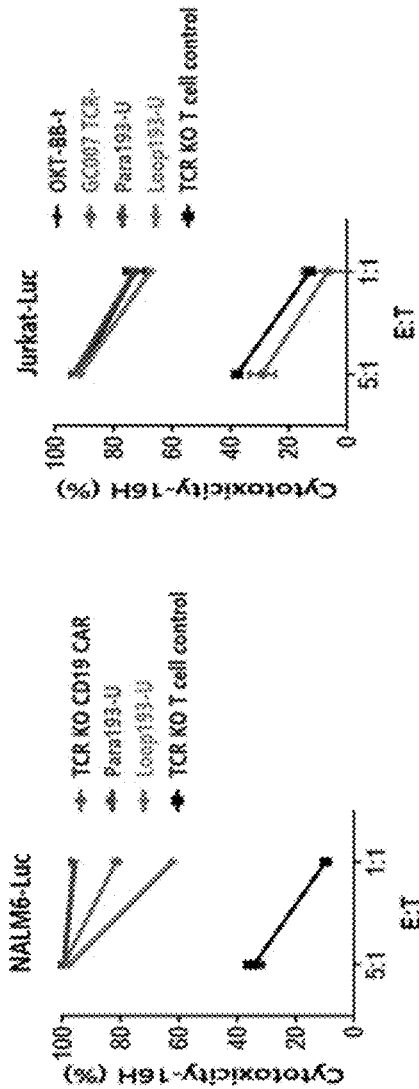


FIG. 7B

FIG. 7A

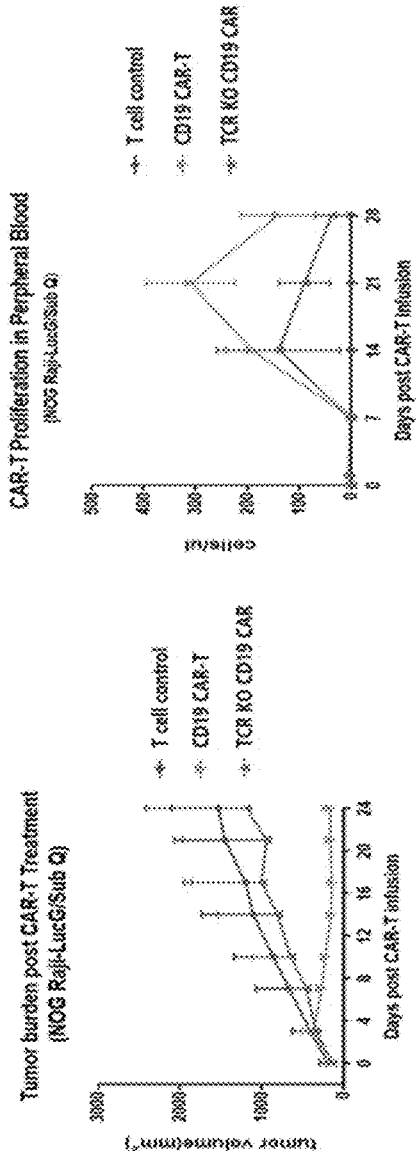


FIG. 8A

FIG. 8B

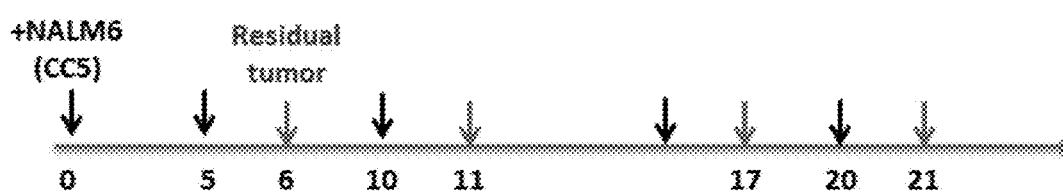


FIG. 9A

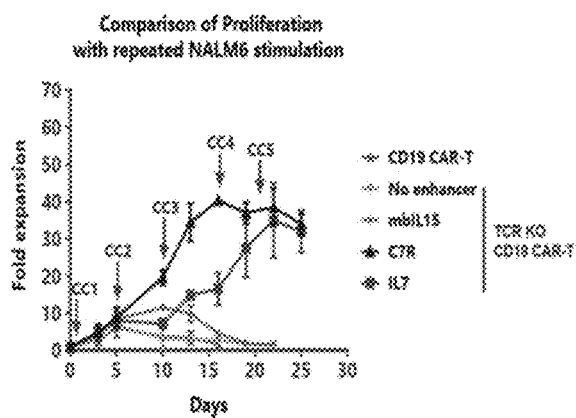


FIG. 9B

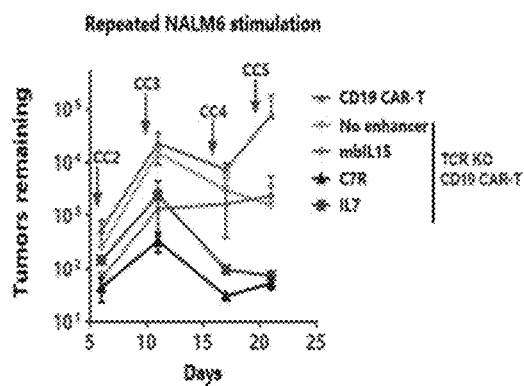


FIG. 9C

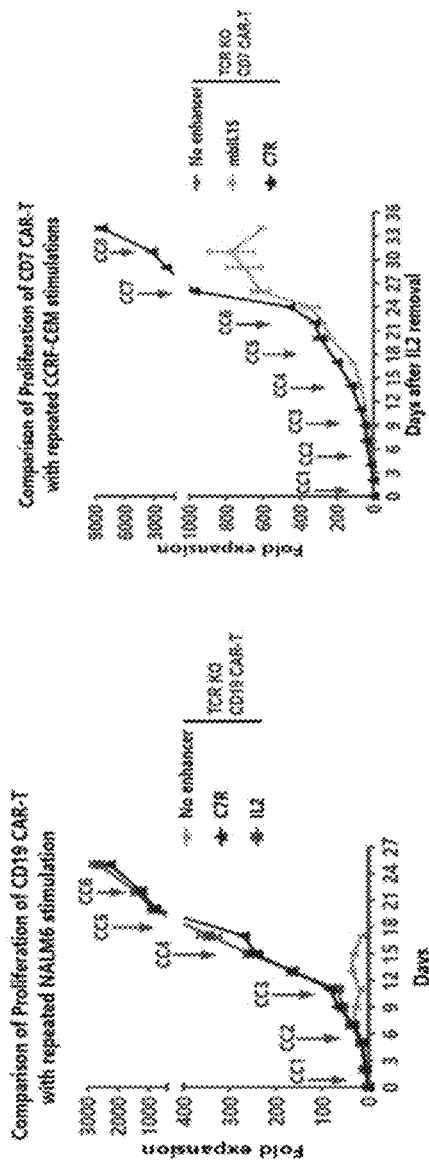


FIG. 10A

FIG. 10B

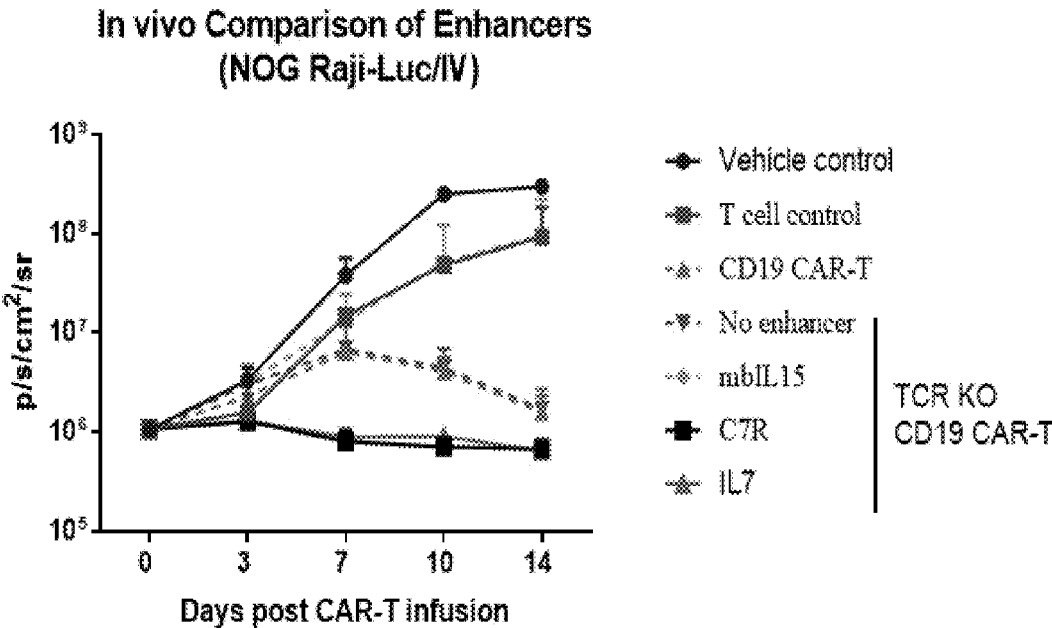


FIG. 11

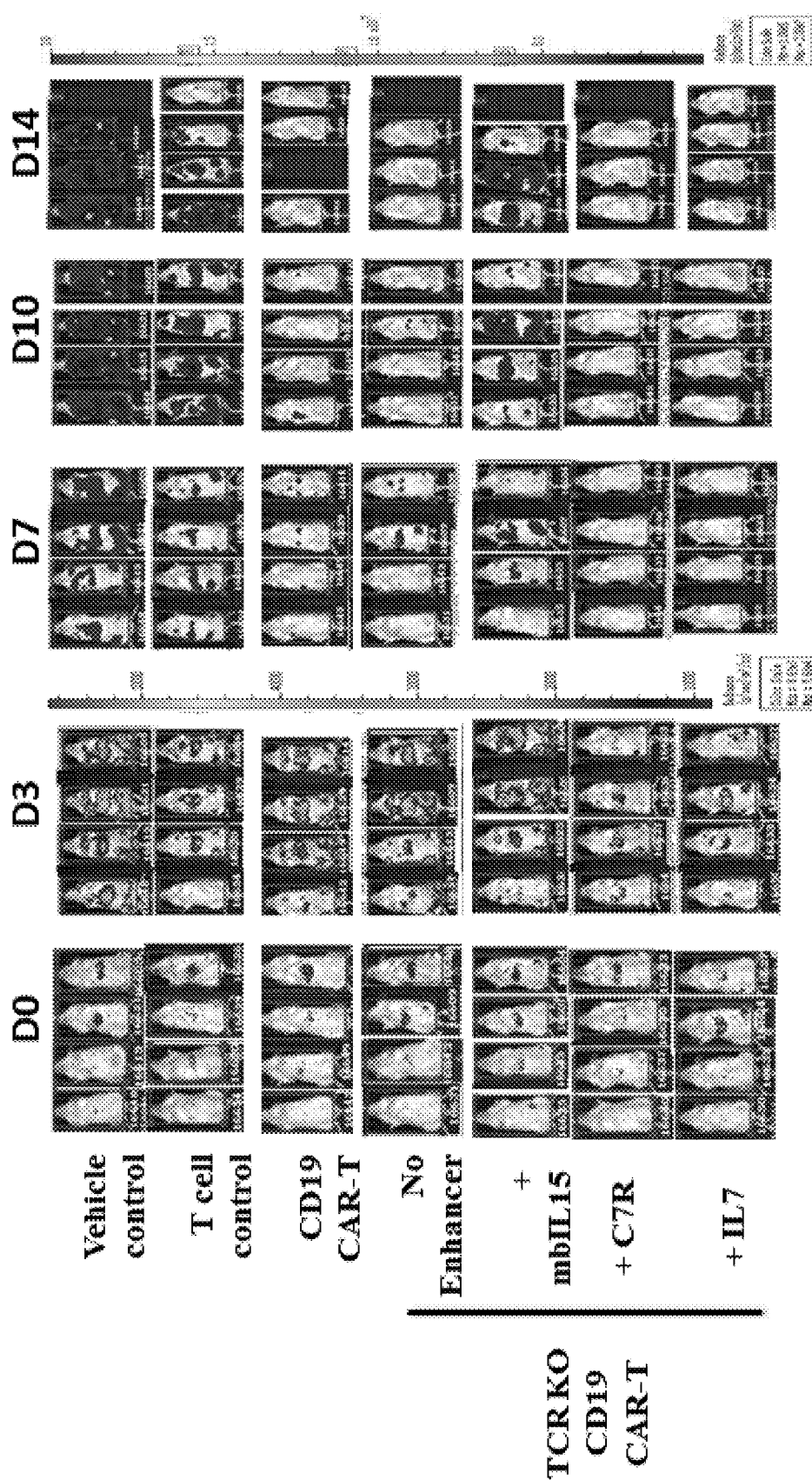


FIG. 12



FIG. 14

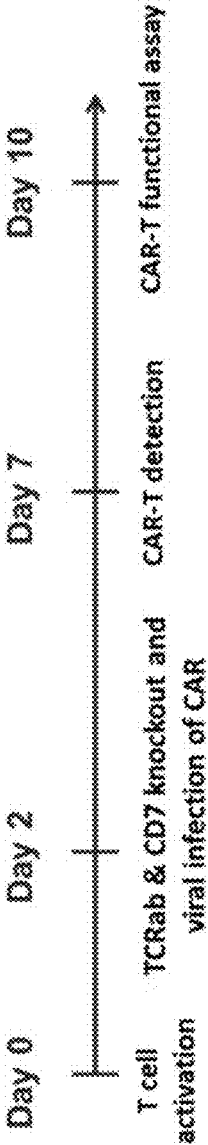


FIG. 15

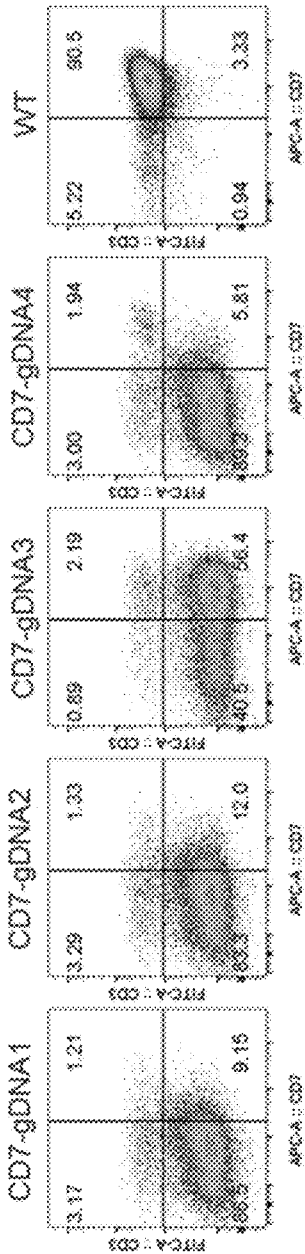


FIG. 16

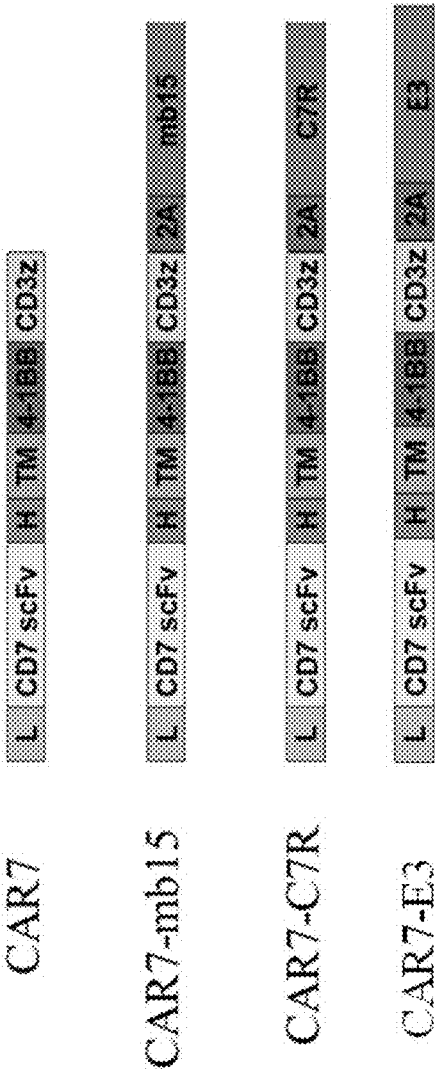


FIG. 17

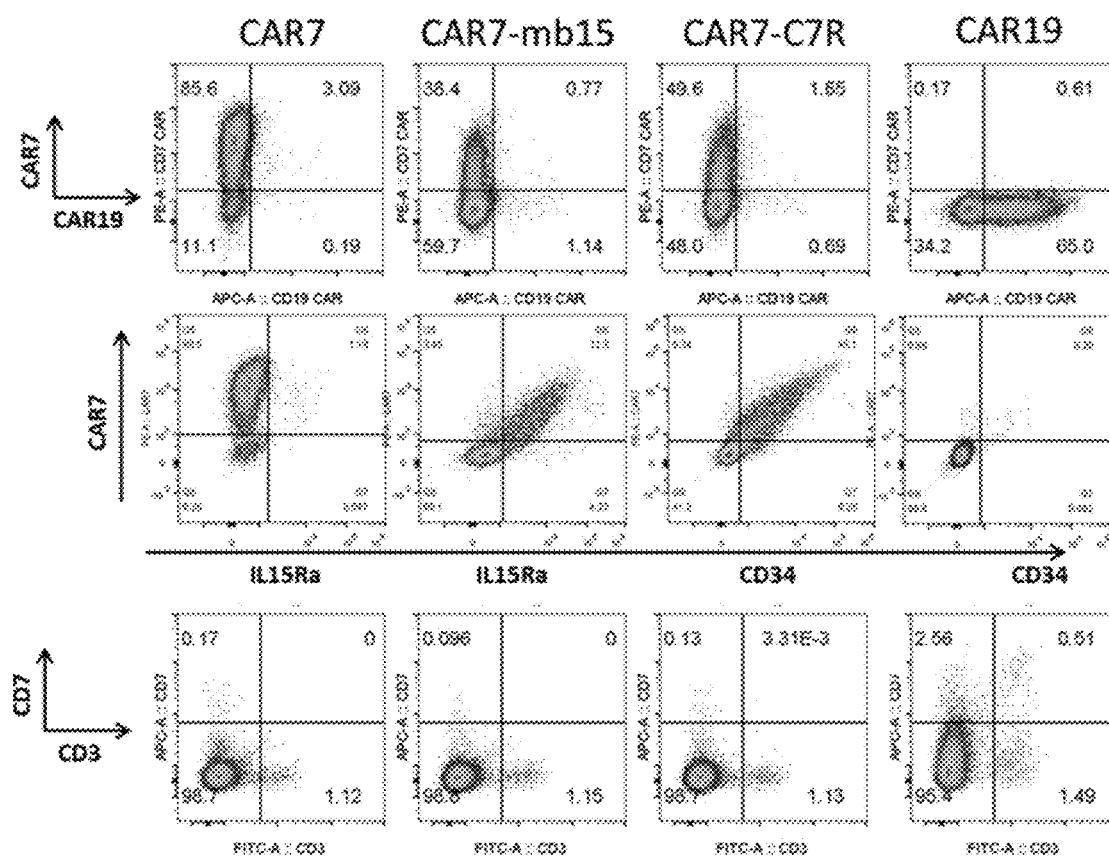


FIG. 18

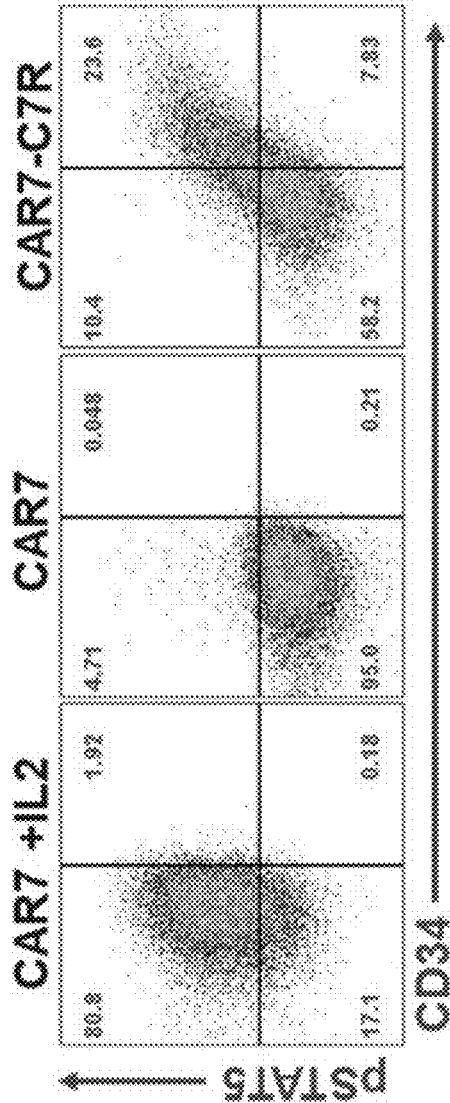


FIG. 19

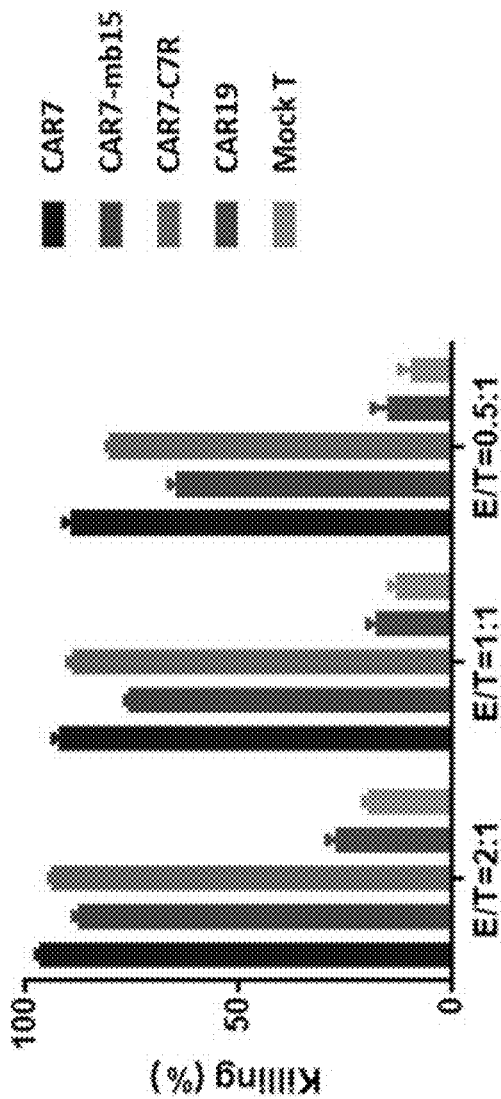


FIG. 20

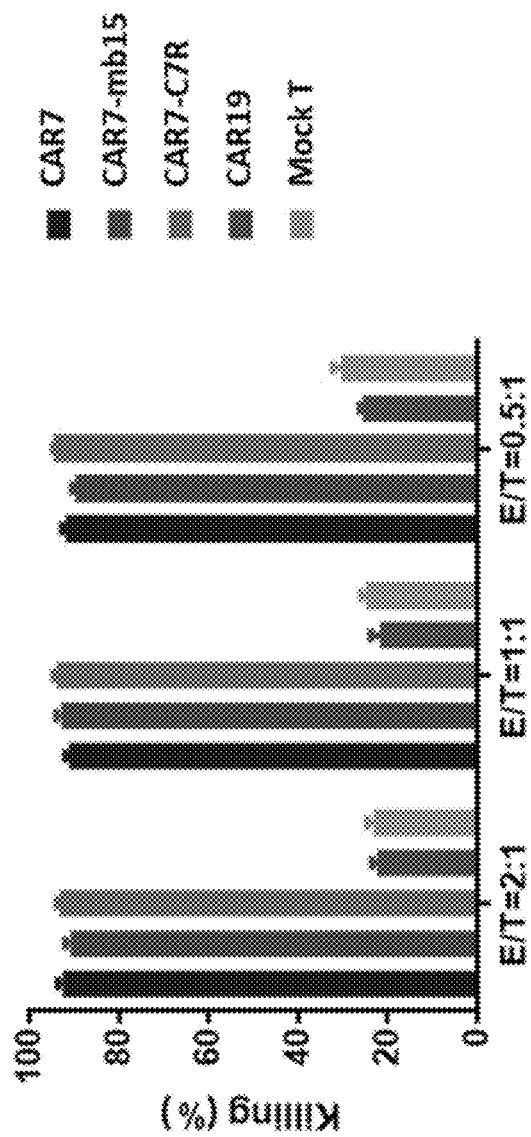


FIG. 21

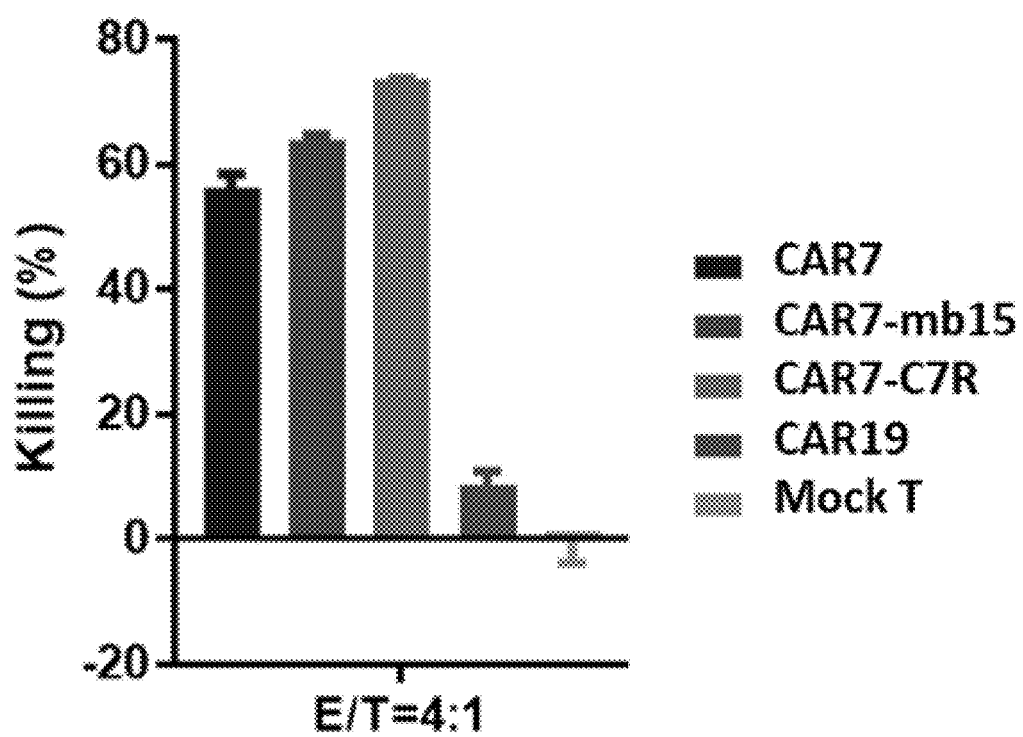


FIG. 22

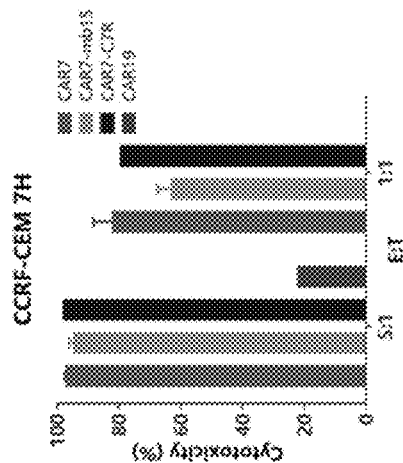


FIG. 23B

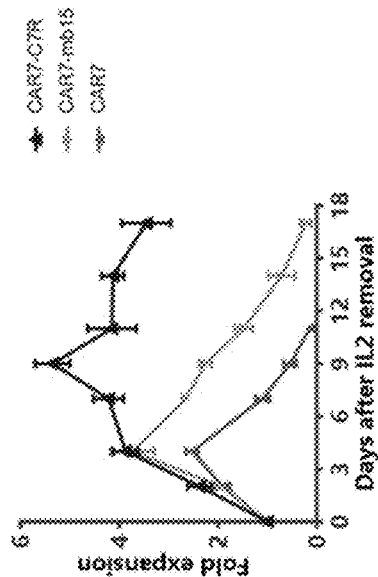


FIG. 23A

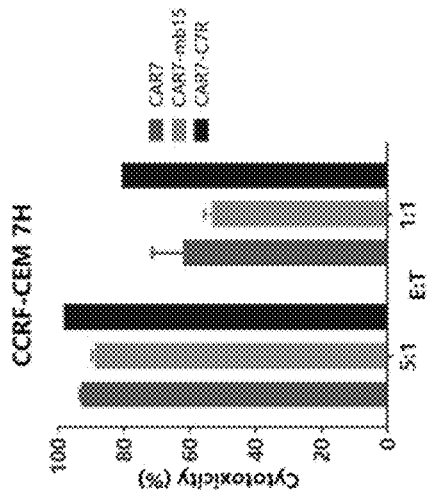


FIG. 24B

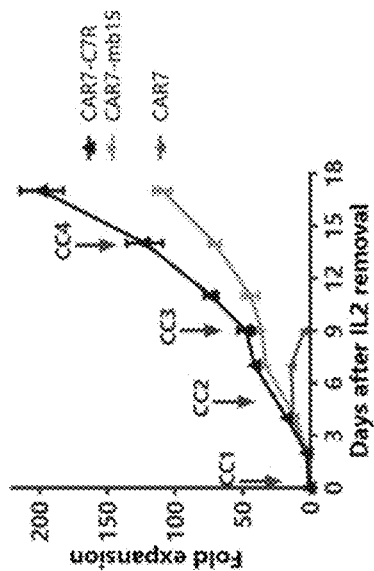


FIG. 24A

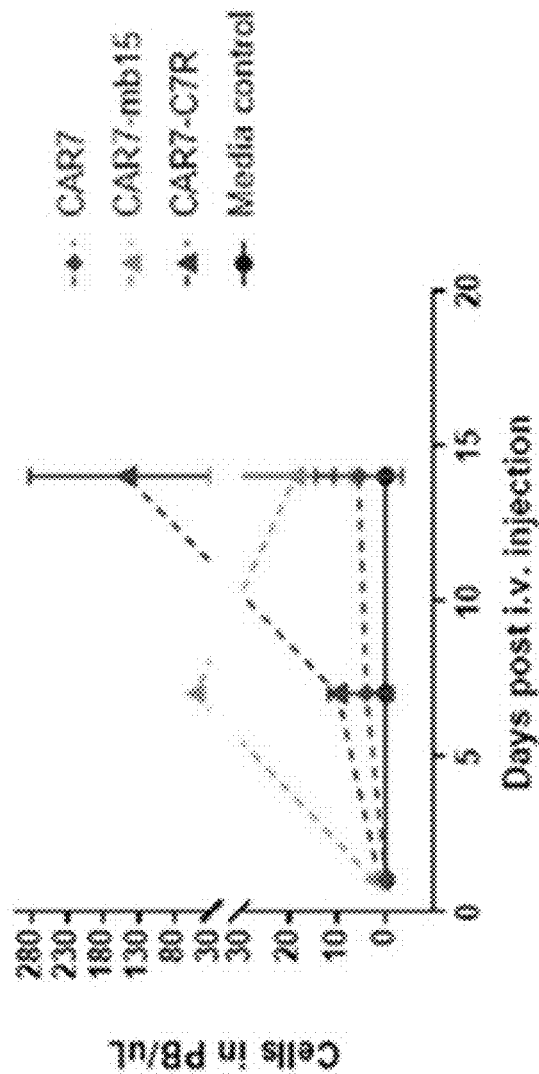


FIG. 25

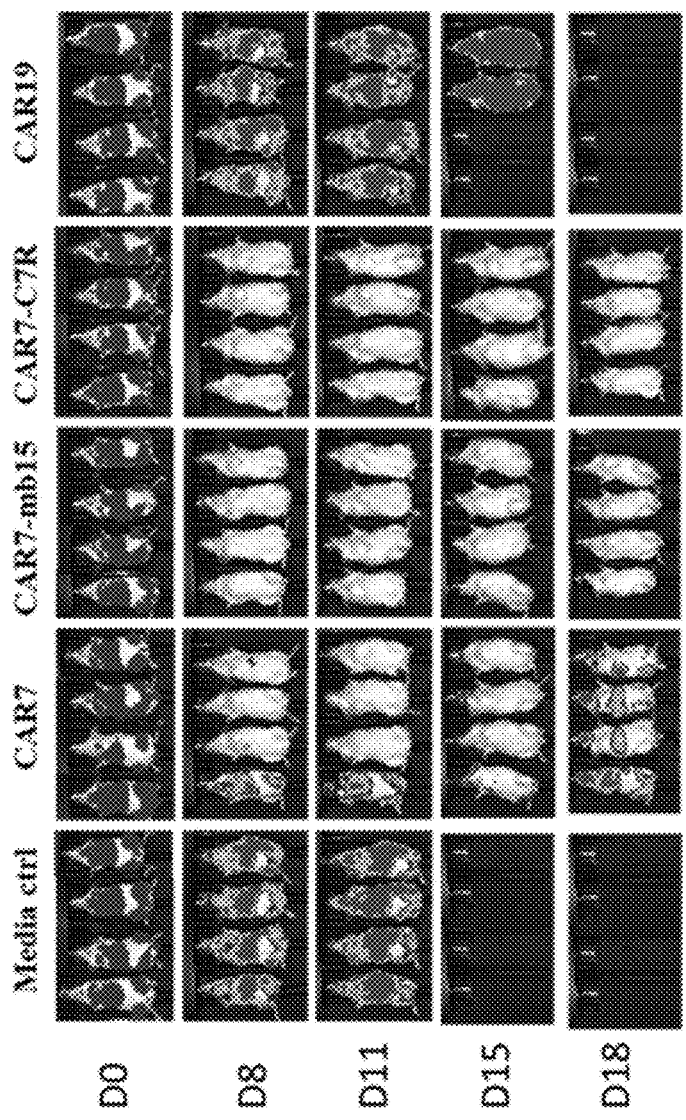


FIG. 26



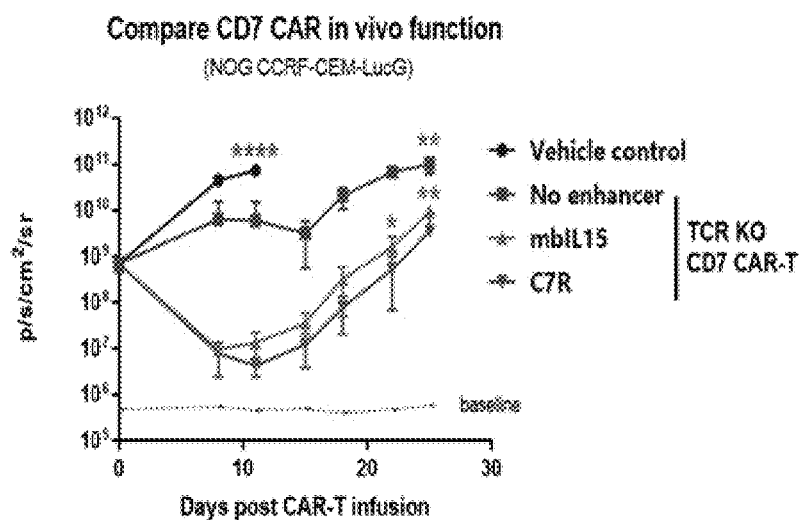


FIG. 28A

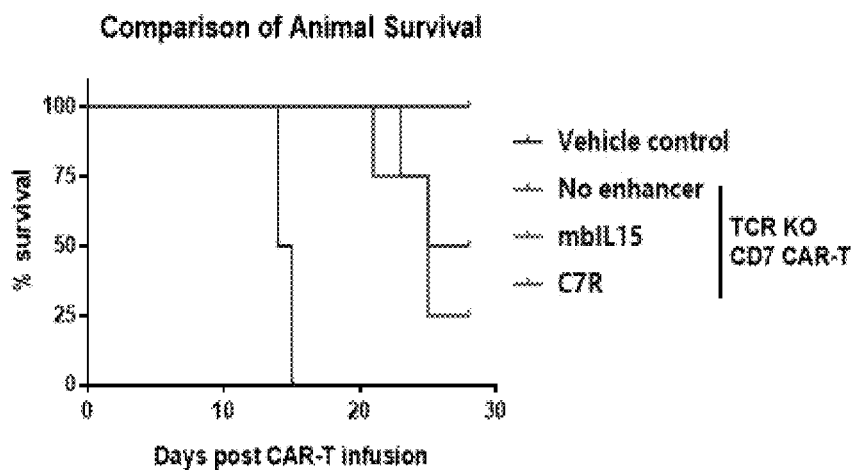


FIG. 28B

RQR8-CD19CAR- mbIL15	RQR8	2A	CD19 scFv	bc	CD28	CD3z	2A	mbIL15		
RQR8-CD19CAR- mbIL15-JL7	RQR8	2A	CD19 scFv	bc	CD28	CD3z	2A	mbIL15	2A	siL7
RQR8-CD19CAR- C7R	RQR8	2A	CD19 scFv	bc	CD28	CD3z	2A	C7R		
RQR8-CD19CAR- mbIL7	RQR8	2A	CD19 scFv	bc	CD28	CD3z	2A	mbIL7		

FIG. 29

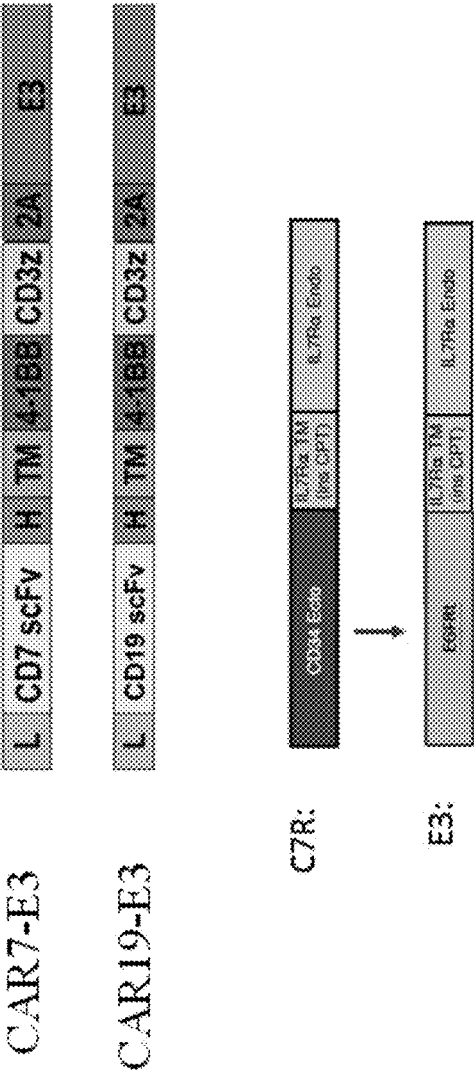


FIG. 30

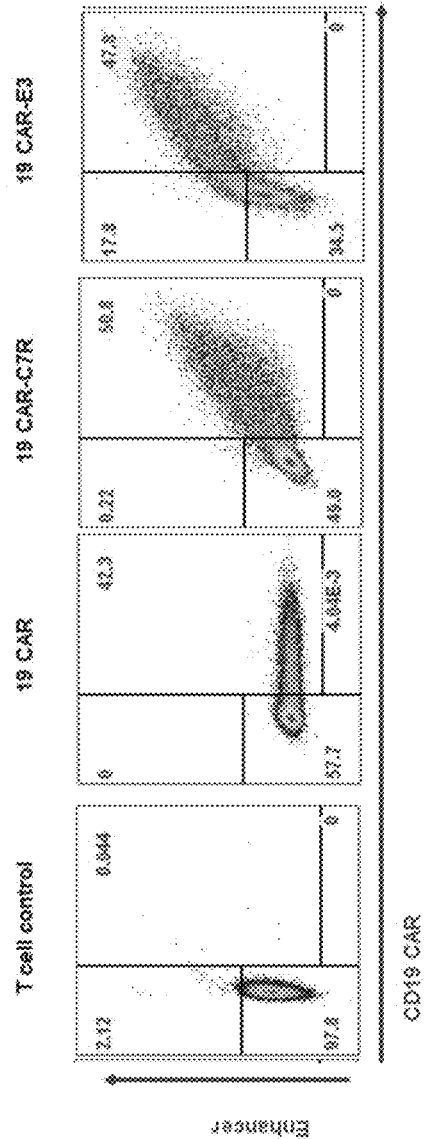


FIG. 31

Killing of Hela-CD19
E:T=5:1

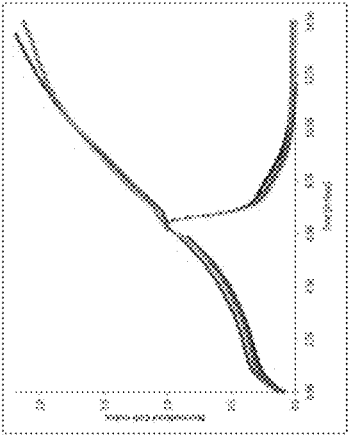


FIG. 32A

Killing of Hela-CD19
E:T=1:1

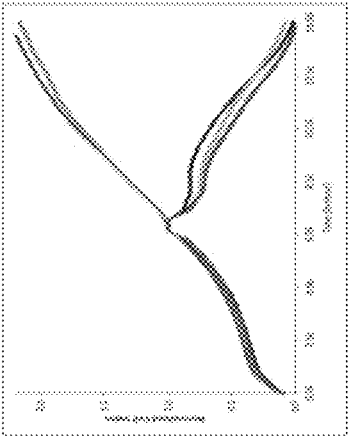


FIG. 32B

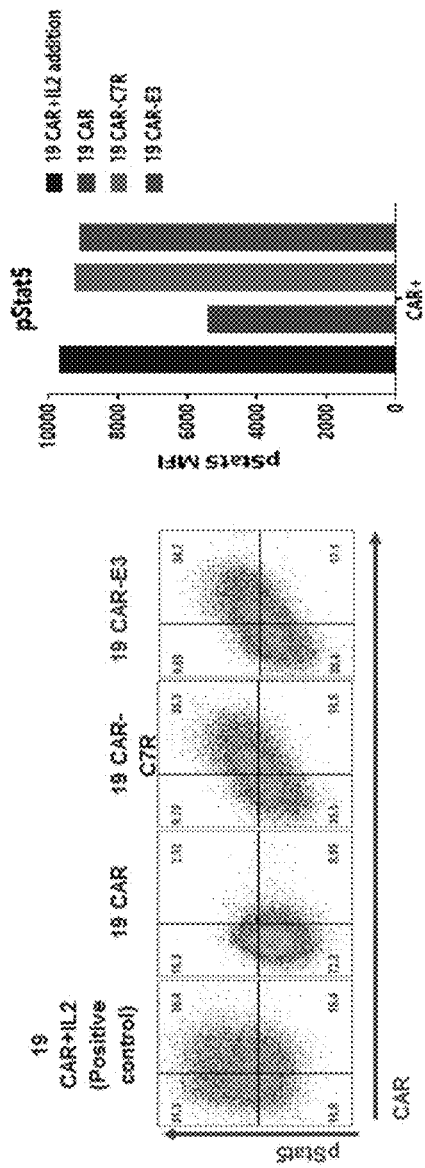


FIG. 33B

FIG. 33A

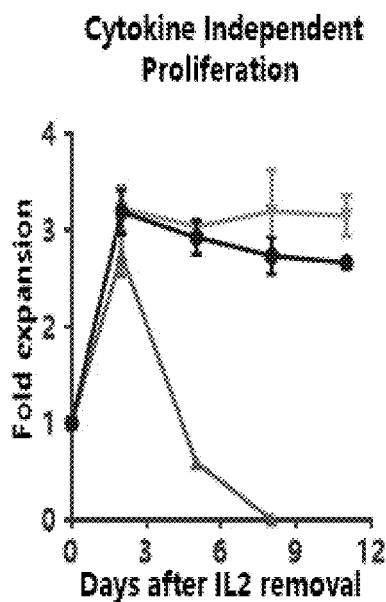


FIG. 34A

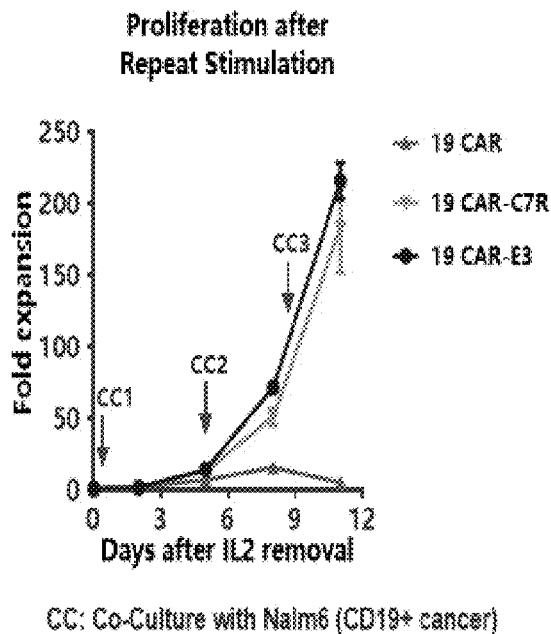


FIG. 34B

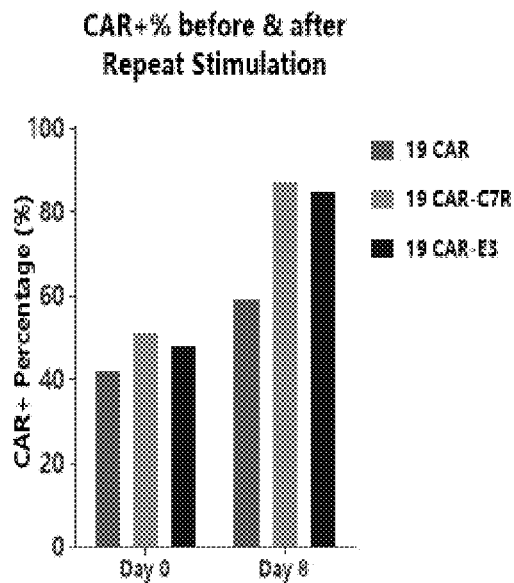


FIG. 34C

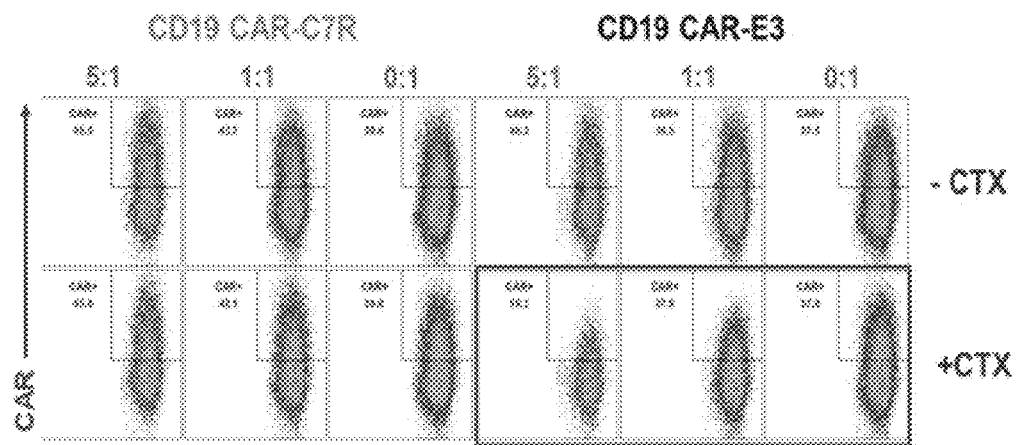


FIG. 35A

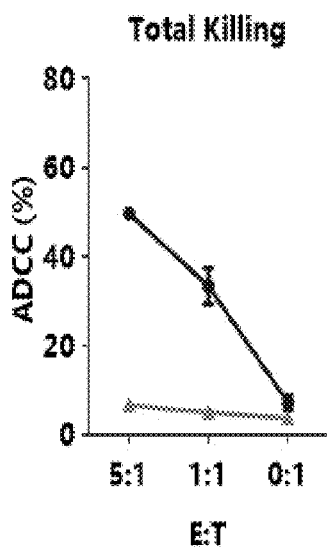


FIG. 35B

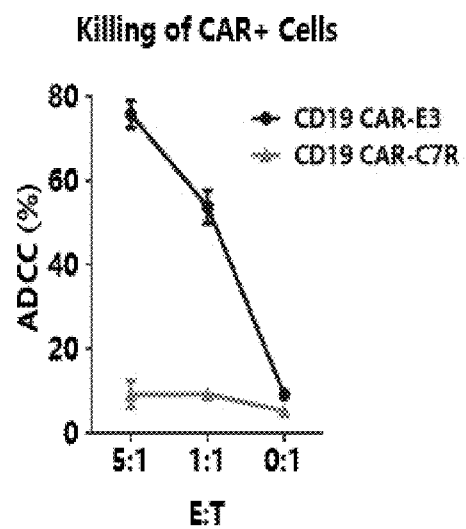


FIG. 35C

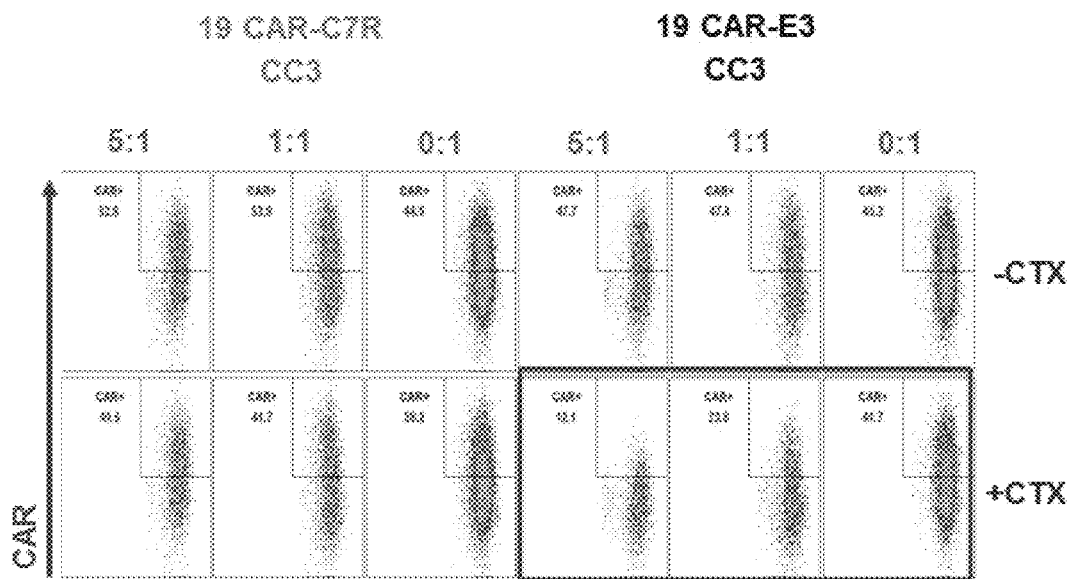


FIG. 36A

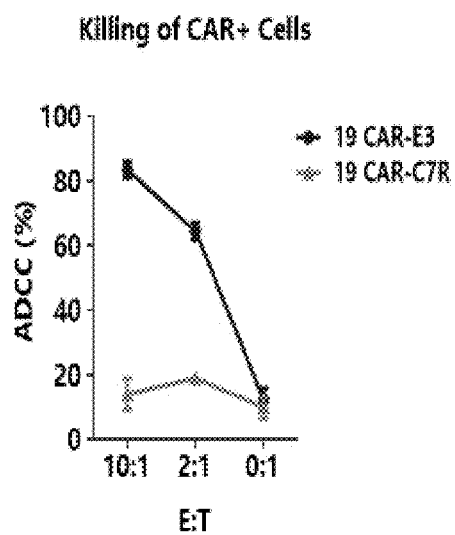
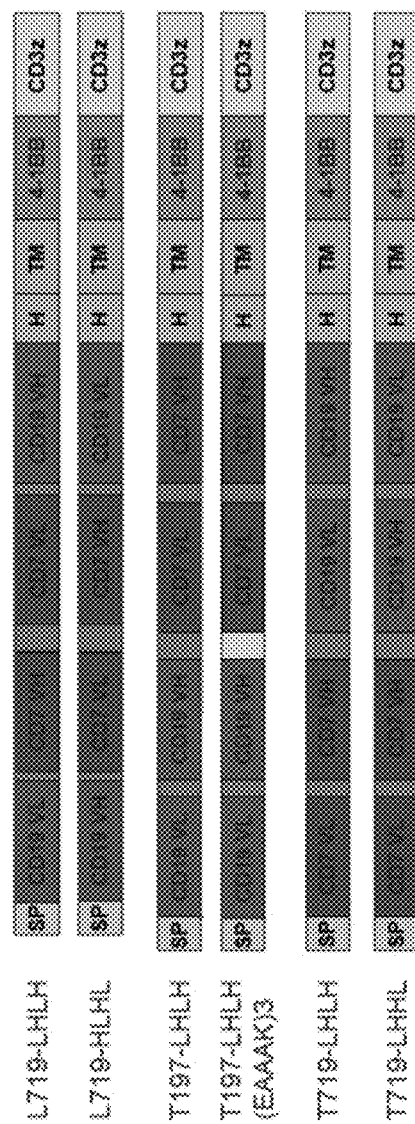


FIG. 36B



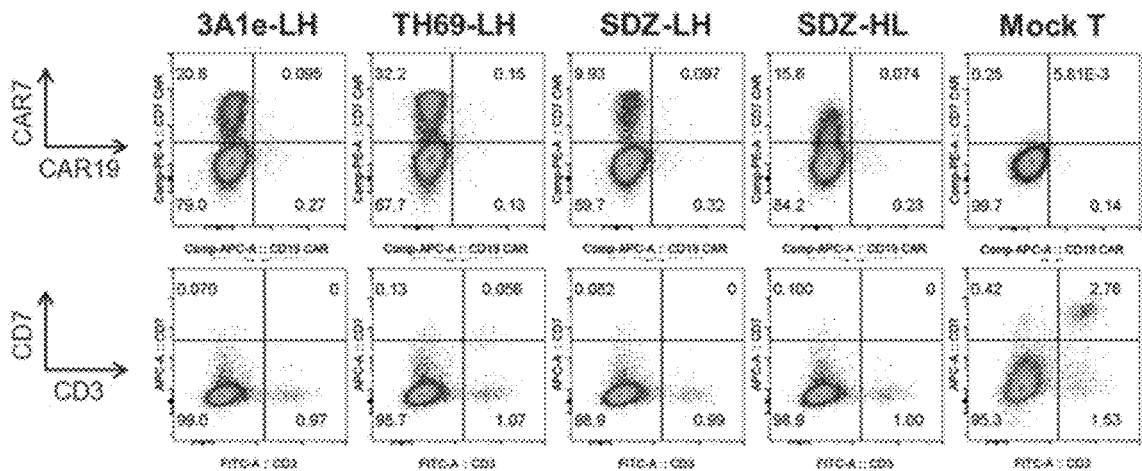


FIG. 38A

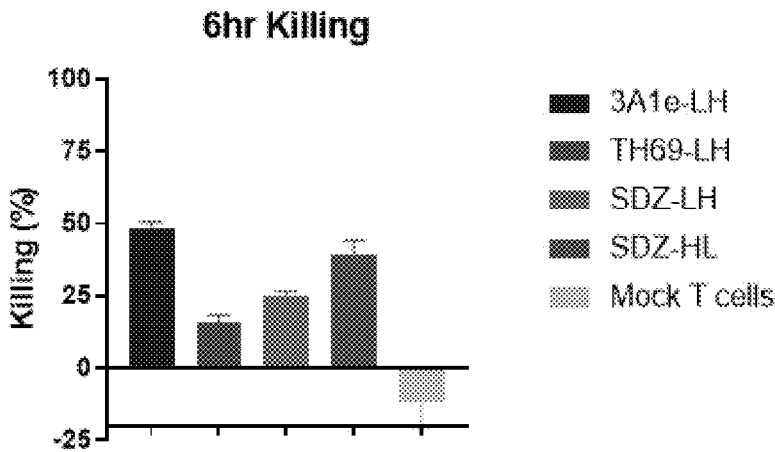


FIG. 38B

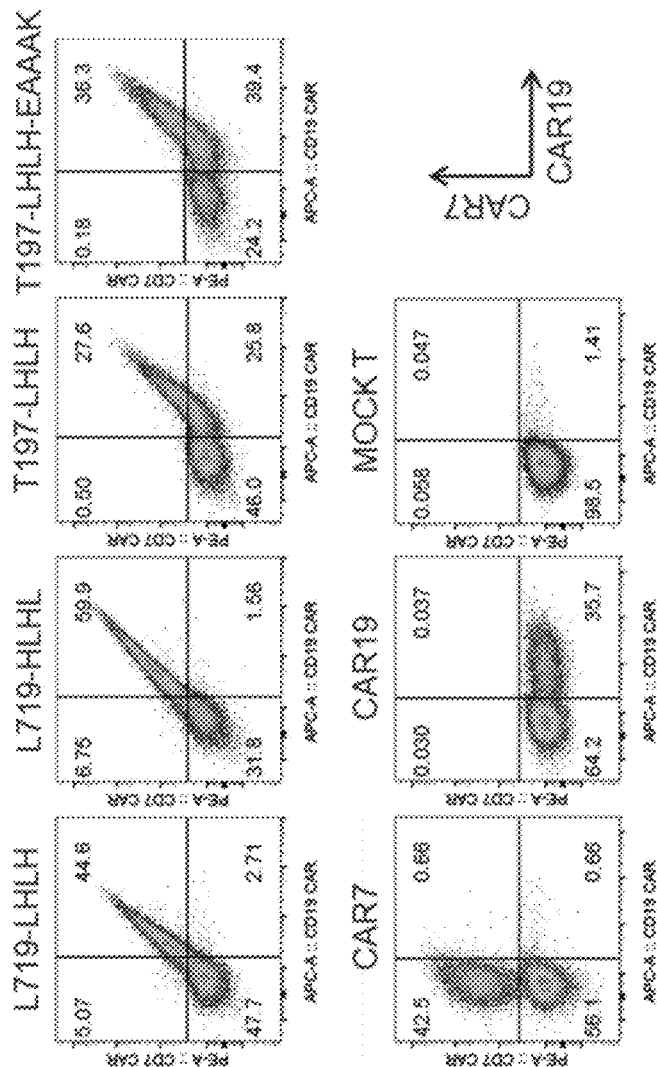


FIG. 39

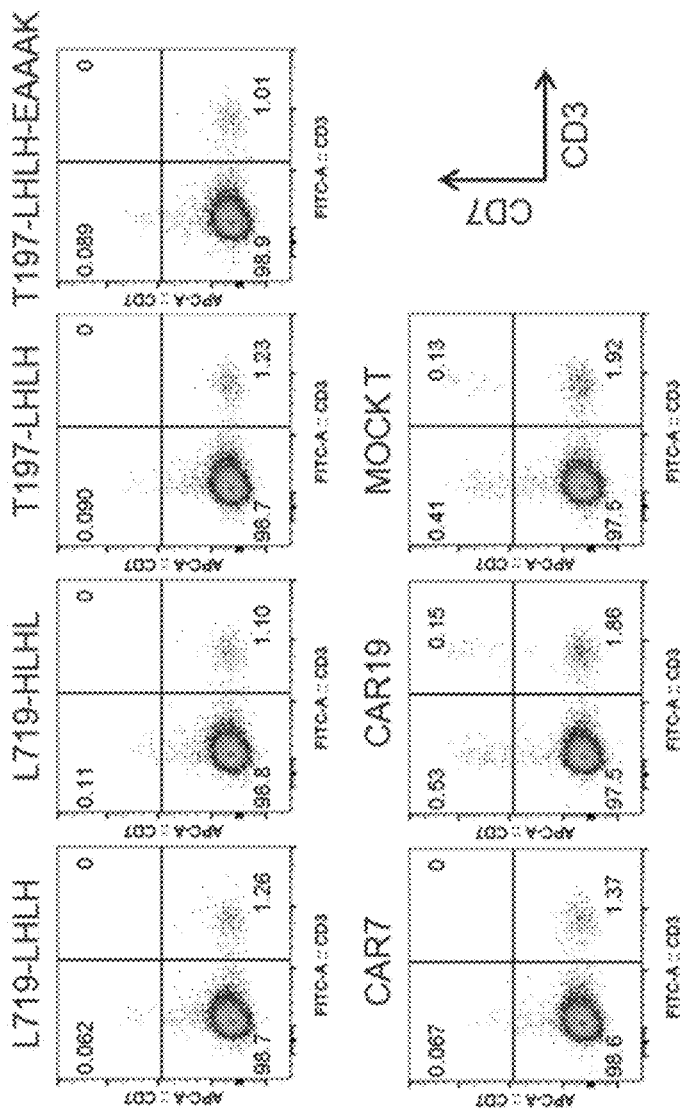


FIG. 40

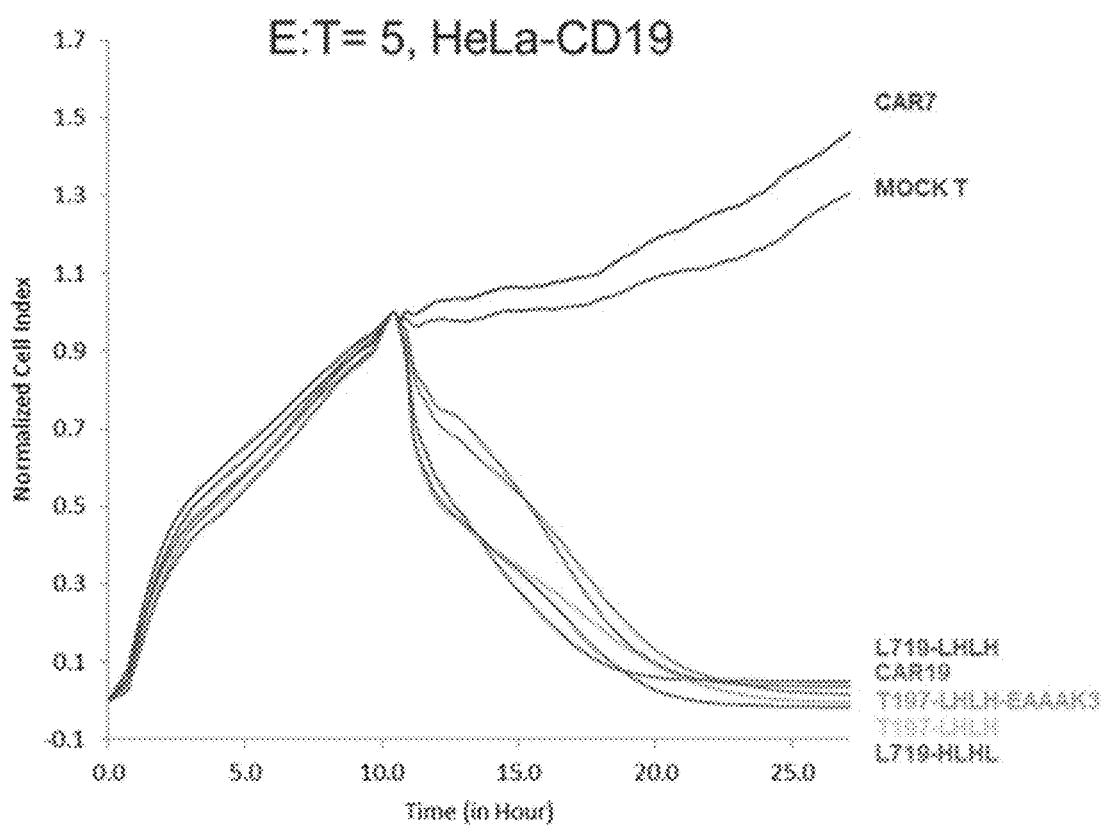


FIG. 41

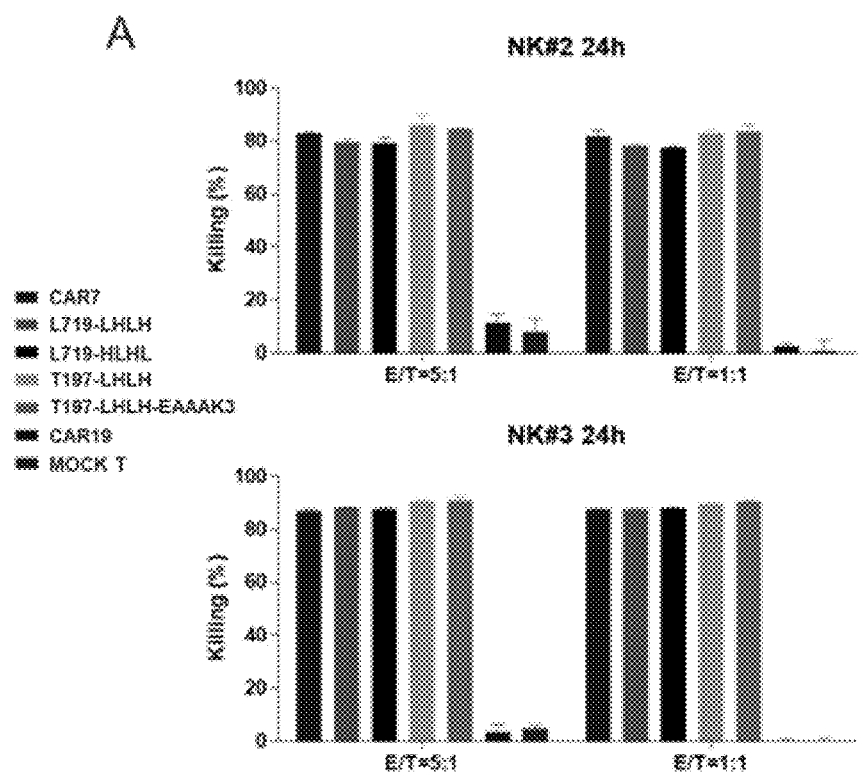


FIG. 42A

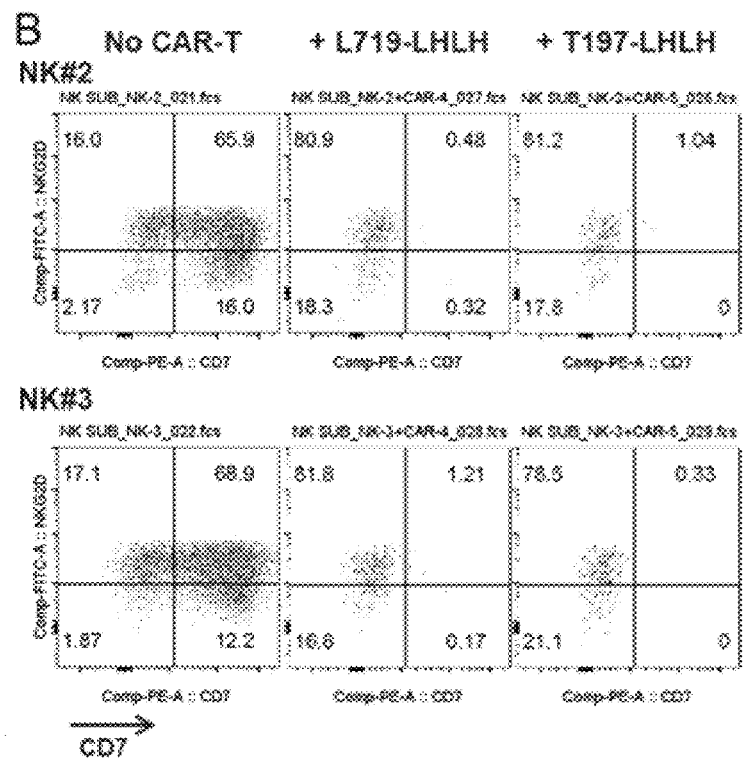


FIG. 42B

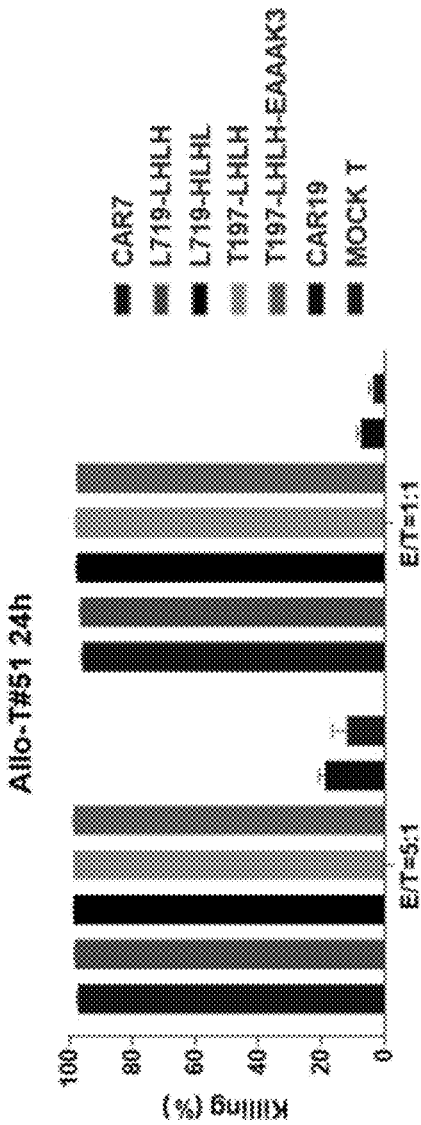


FIG. 43

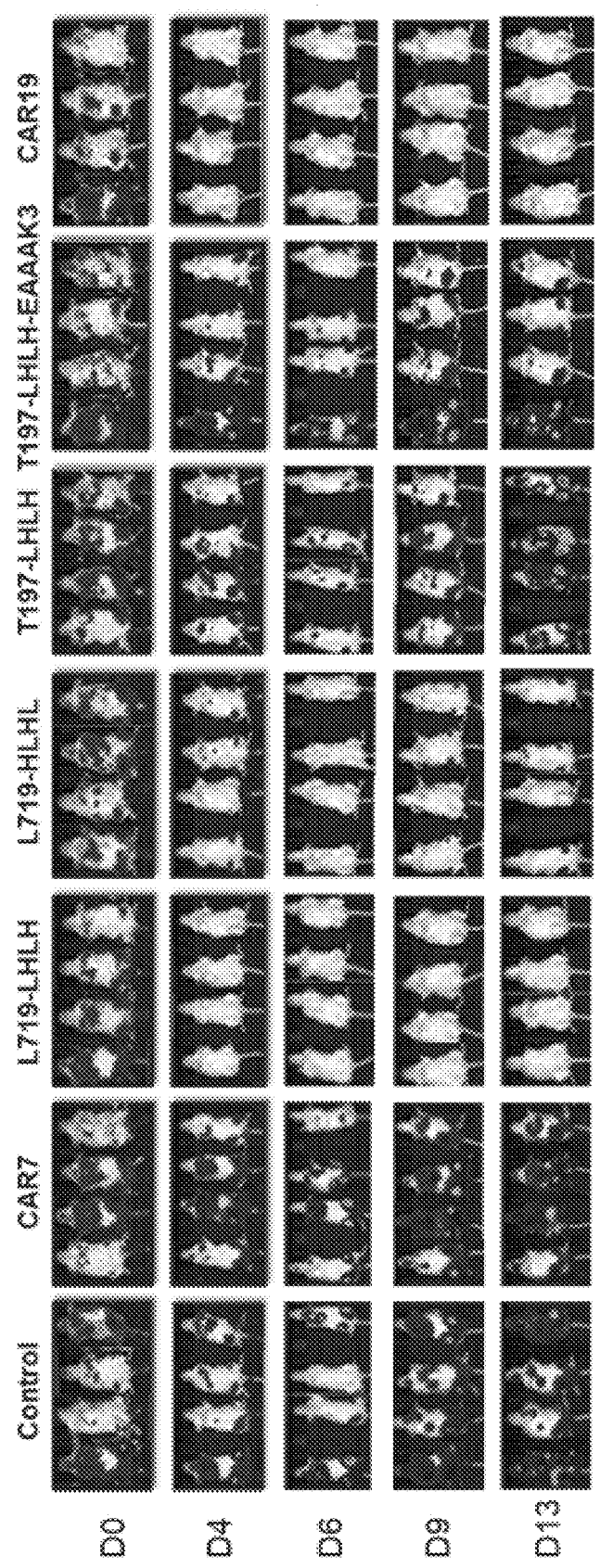


FIG. 44

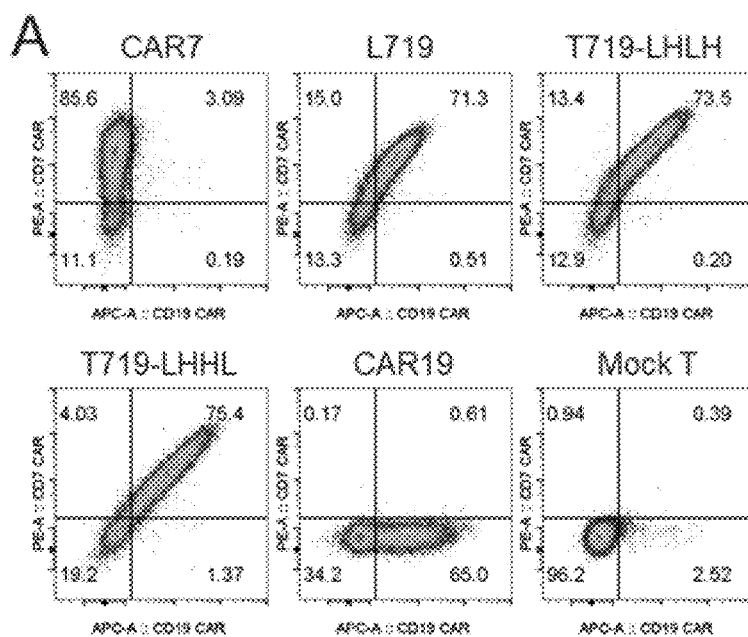


FIG. 45A

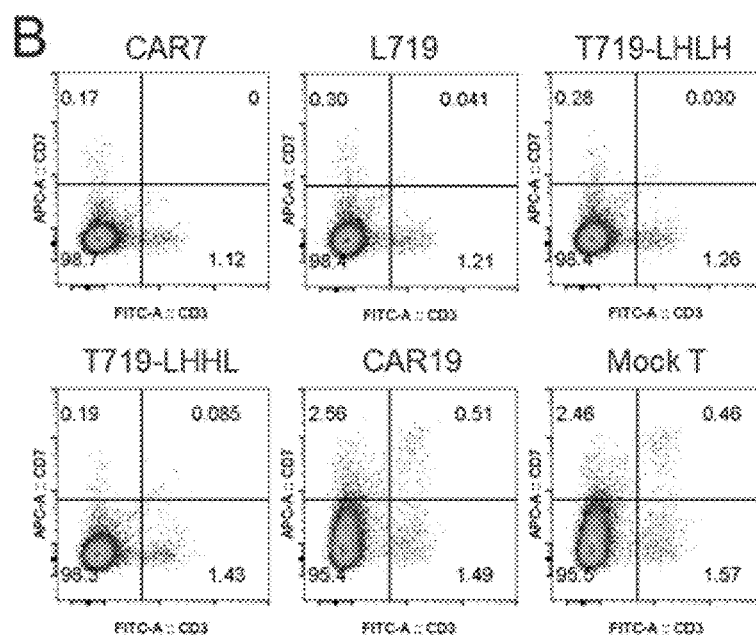


FIG. 45B

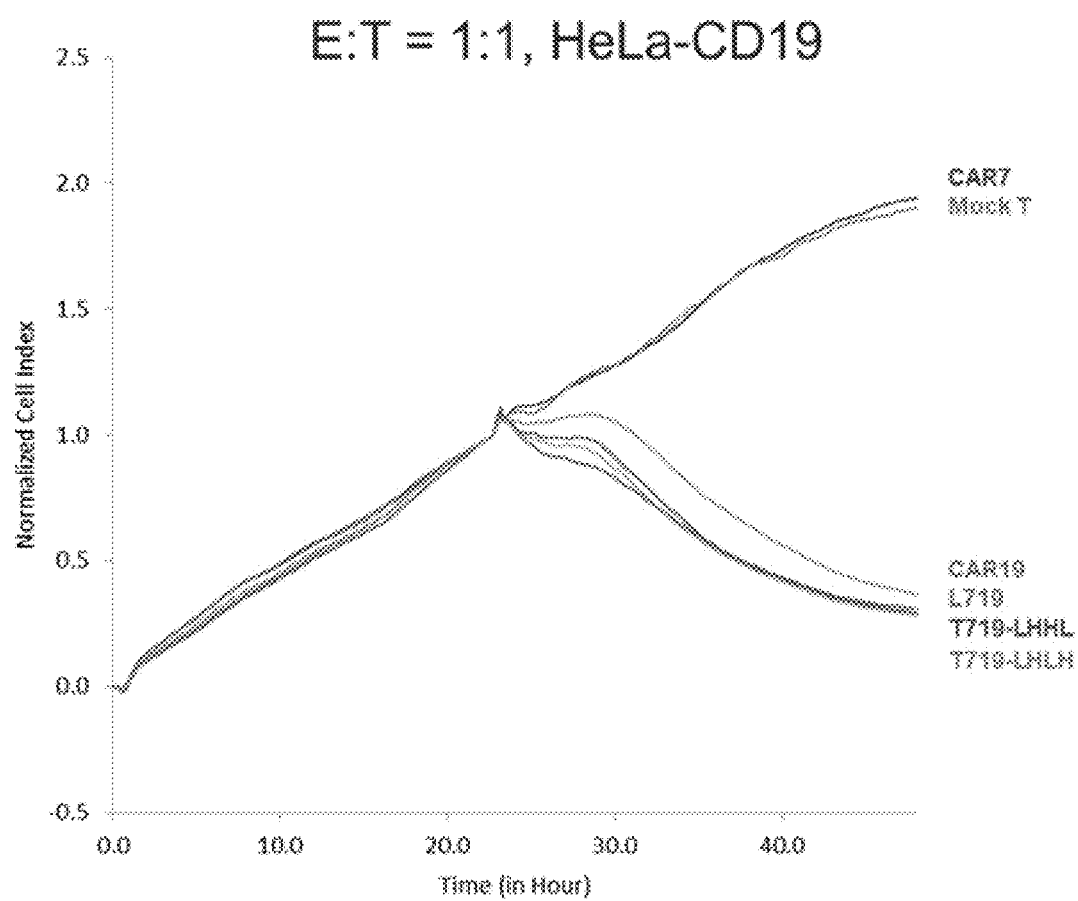


FIG. 46

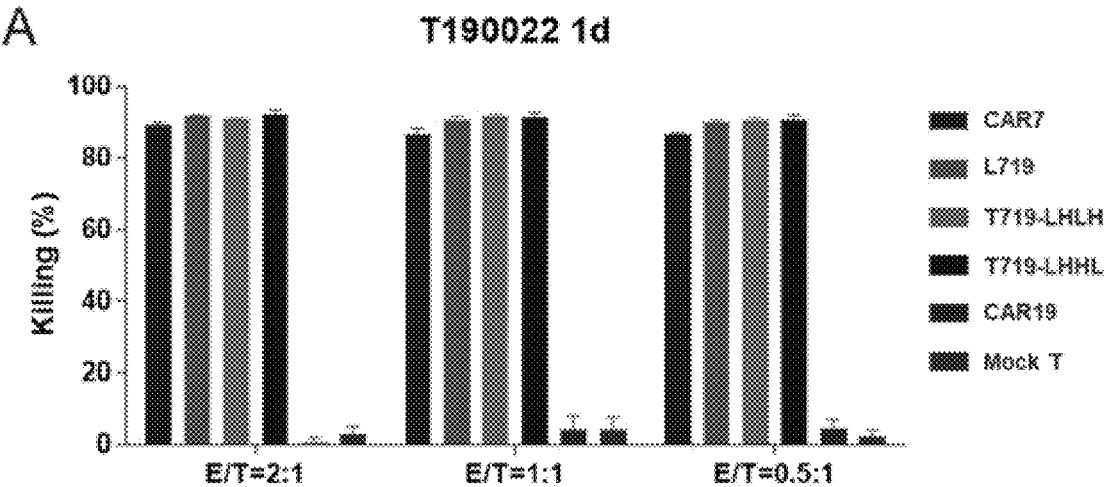


FIG. 47A

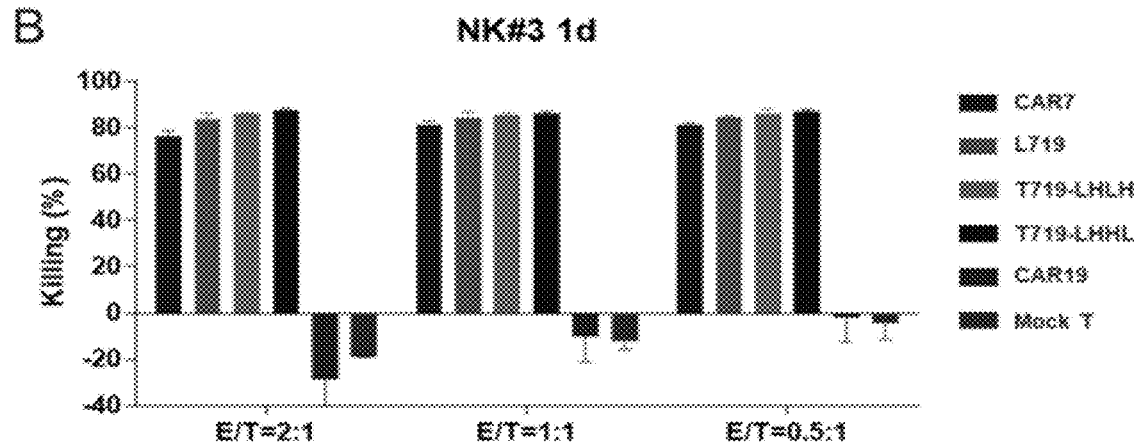


FIG. 47B

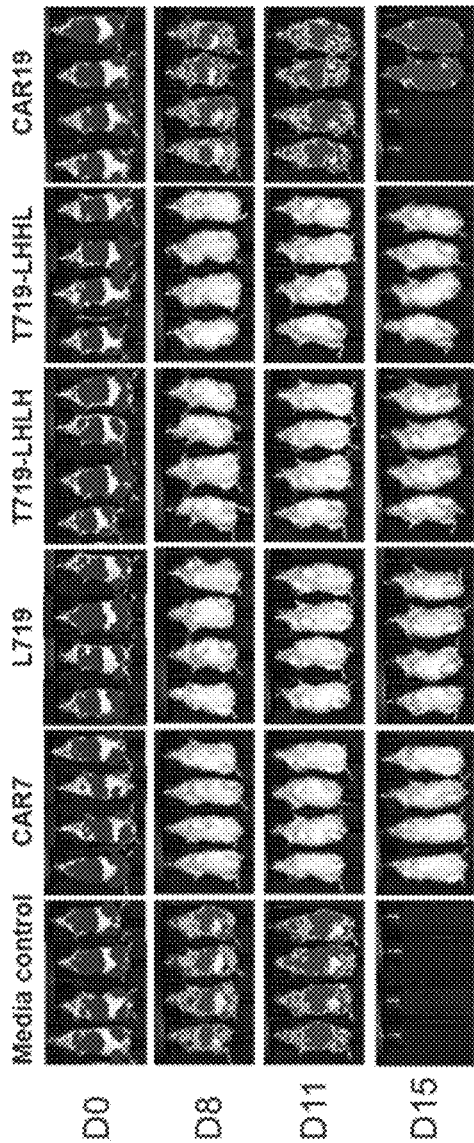


FIG. 48

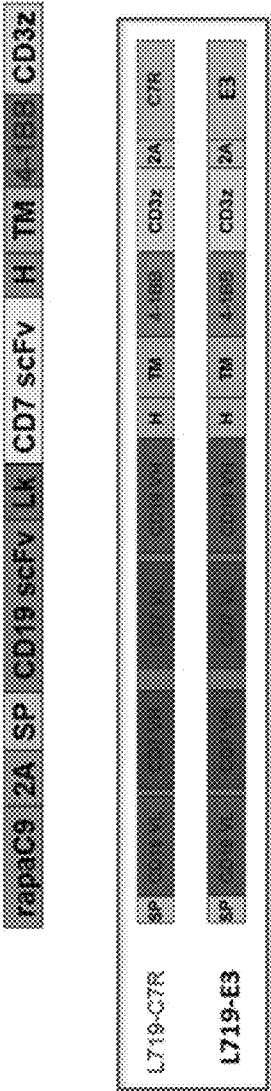


FIG. 49

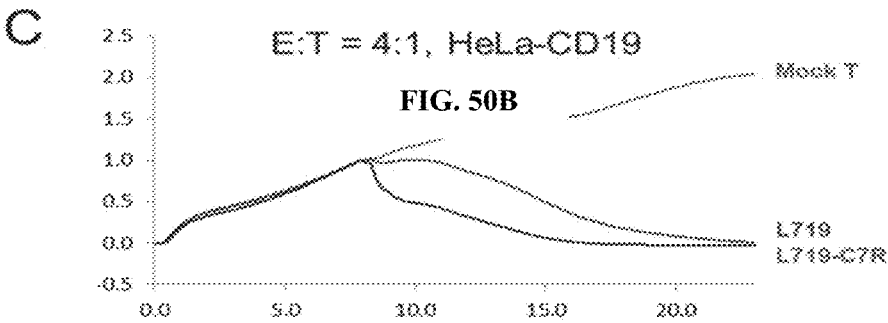
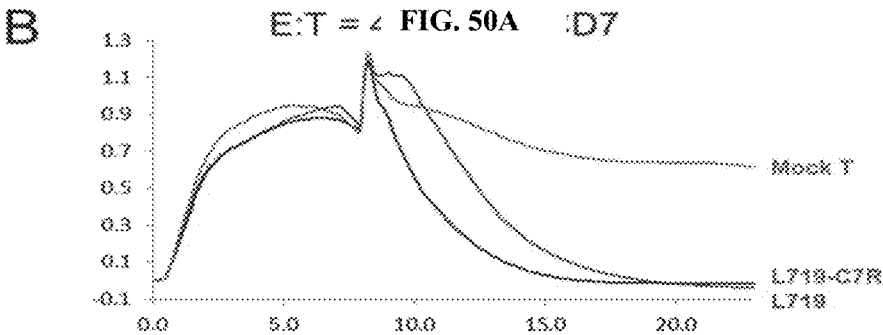
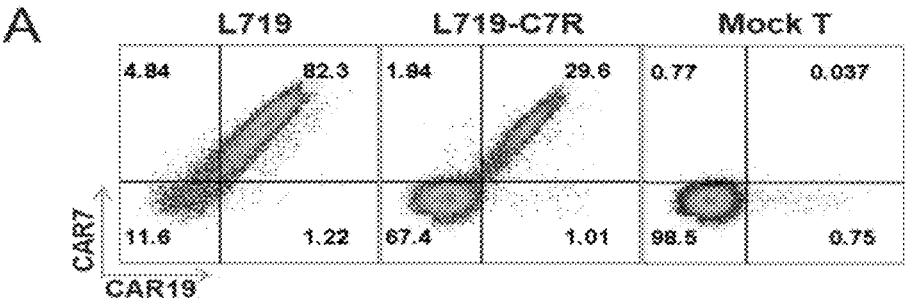


FIG. 50C

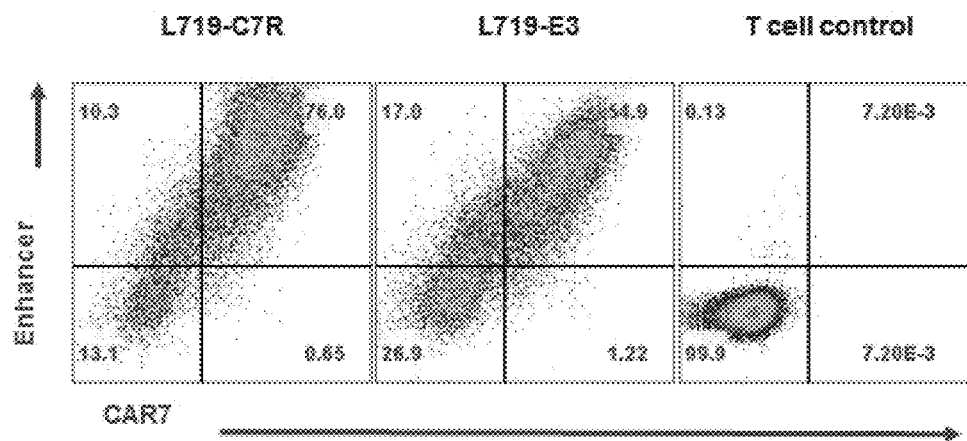


FIG. 50D

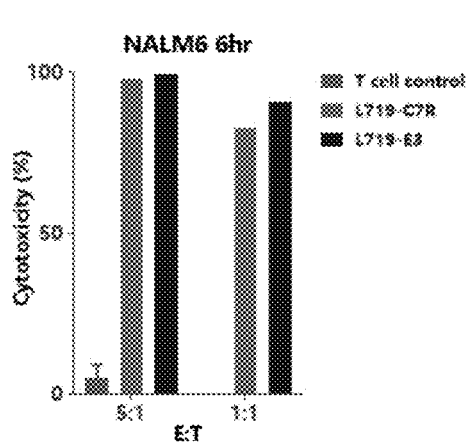


FIG. 50E

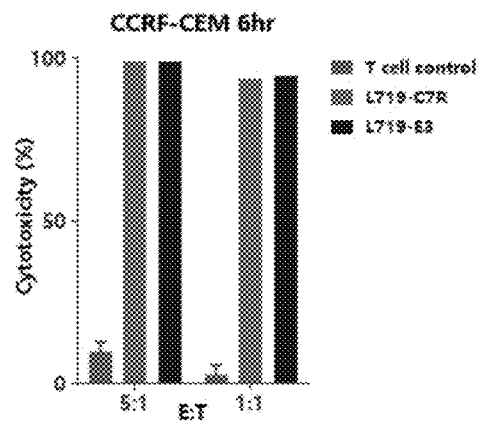


FIG. 50F

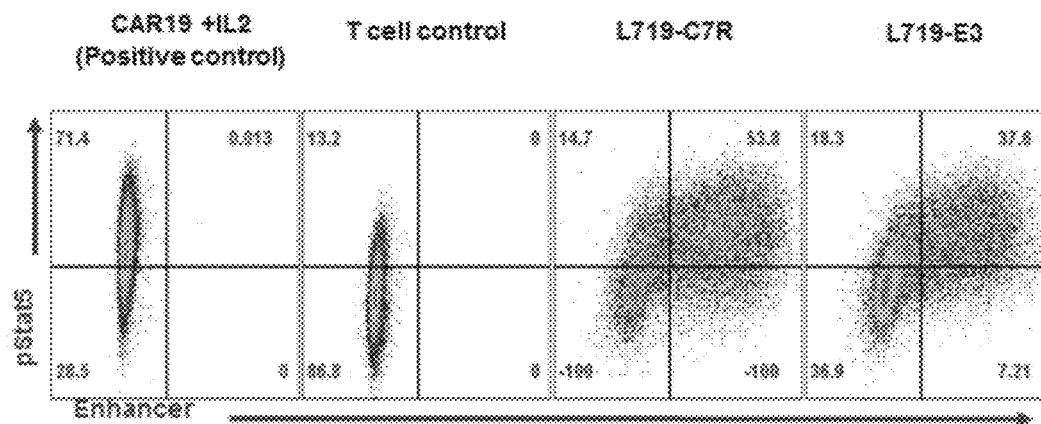


FIG. 50G

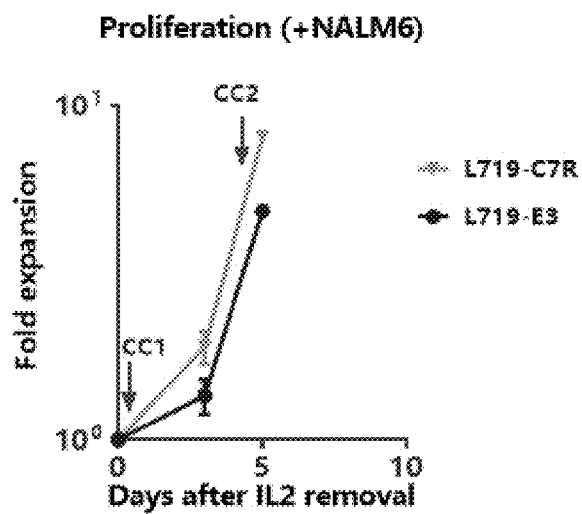


FIG. 50H

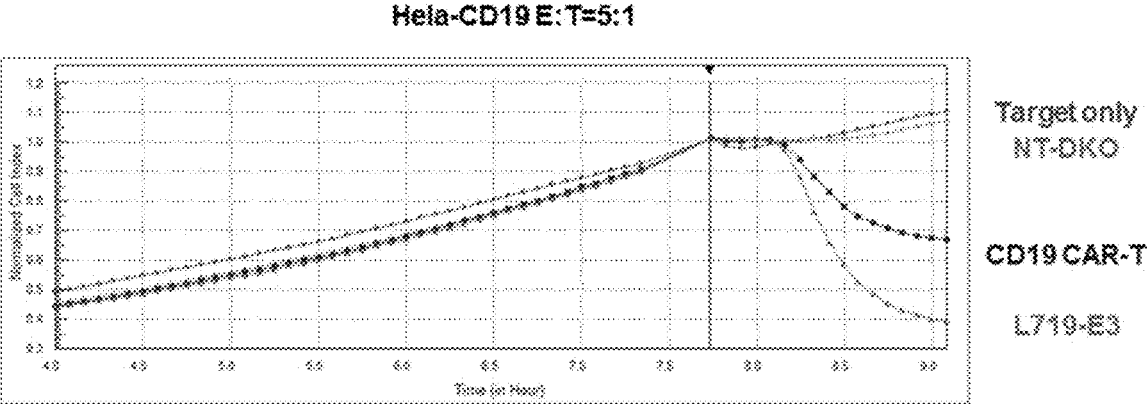


FIG. 50I

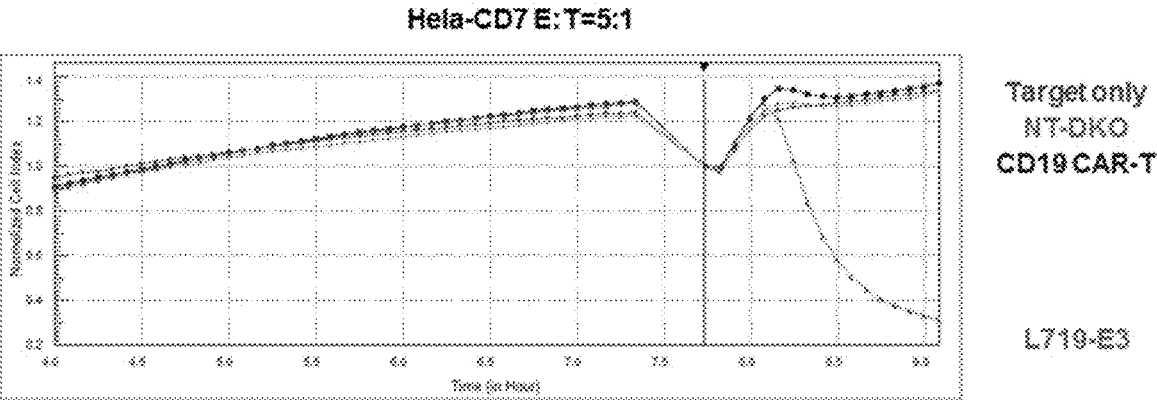


FIG. 50J



FIG. 51



FIG. 52

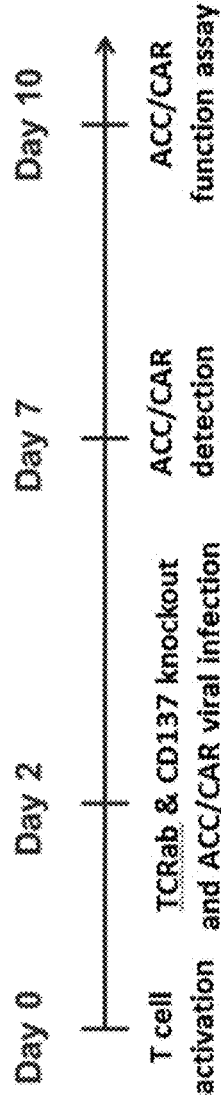


FIG. 53

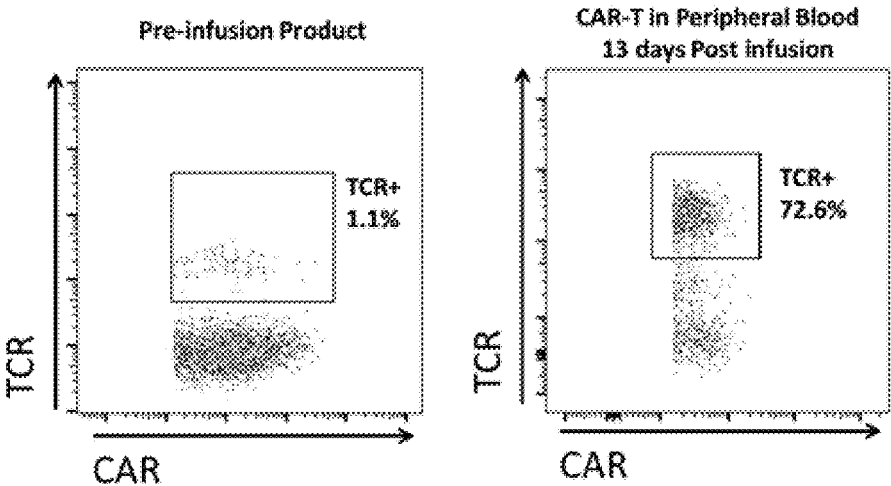


FIG. 54A

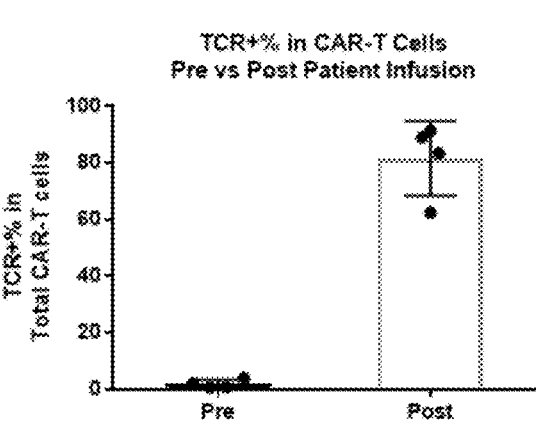


FIG. 54B

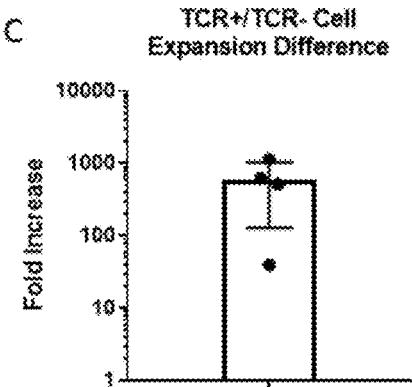
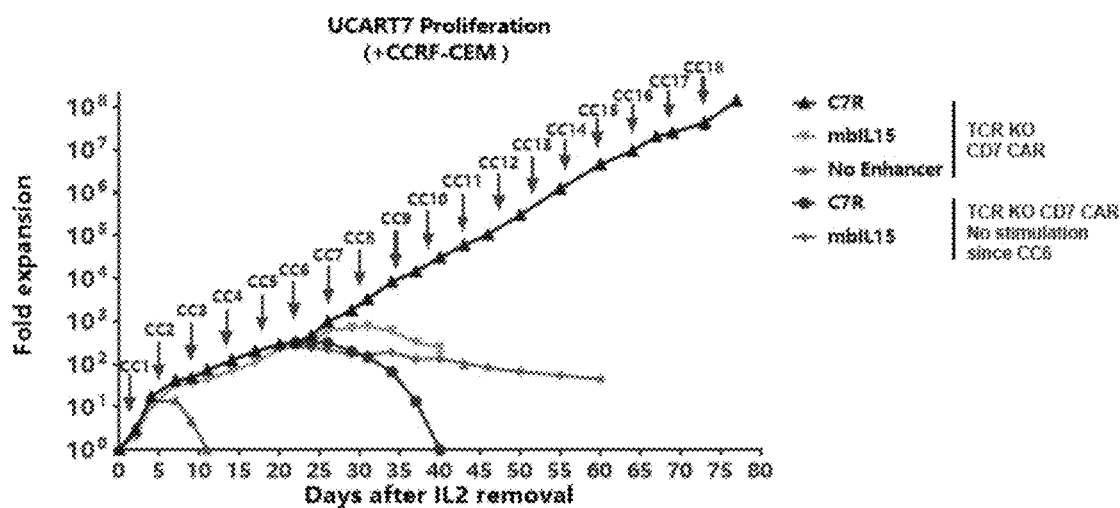
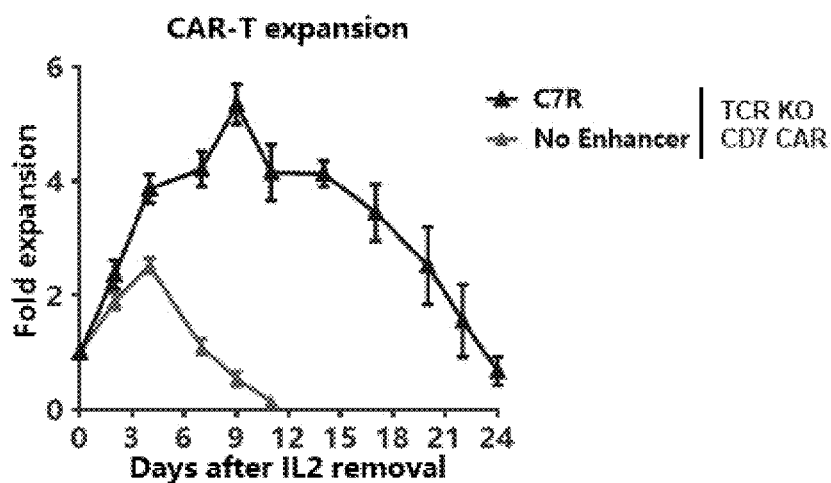


FIG. 54C



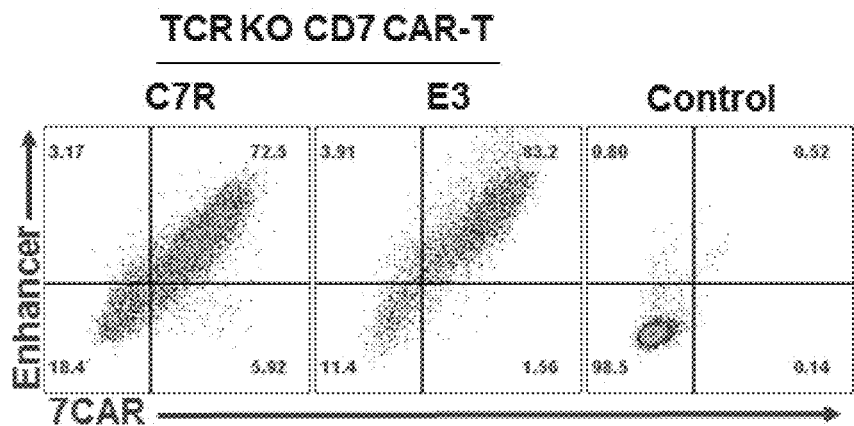


FIG. 56A

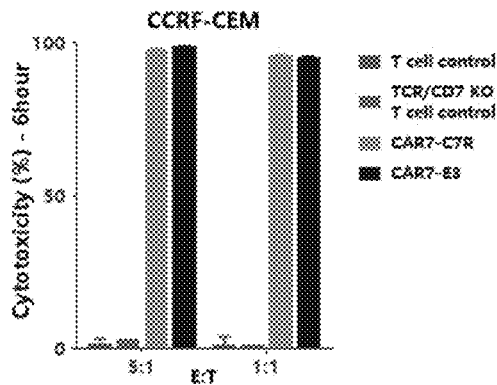


FIG. 56B

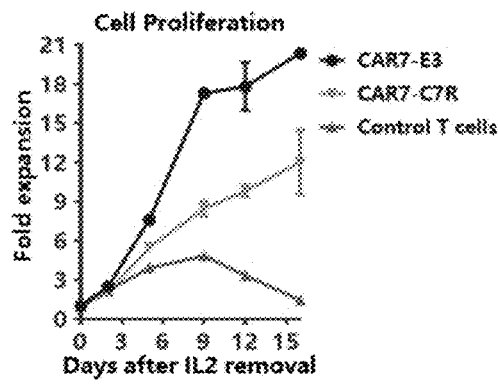


FIG. 56C

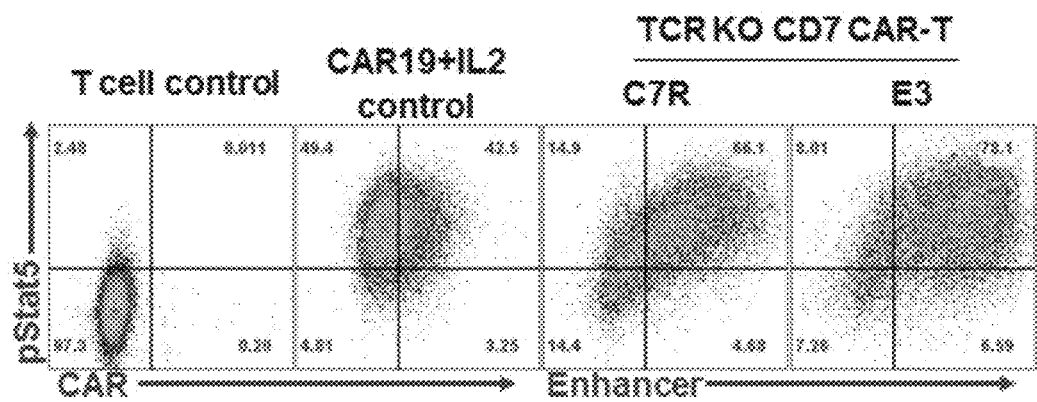


FIG. 56D

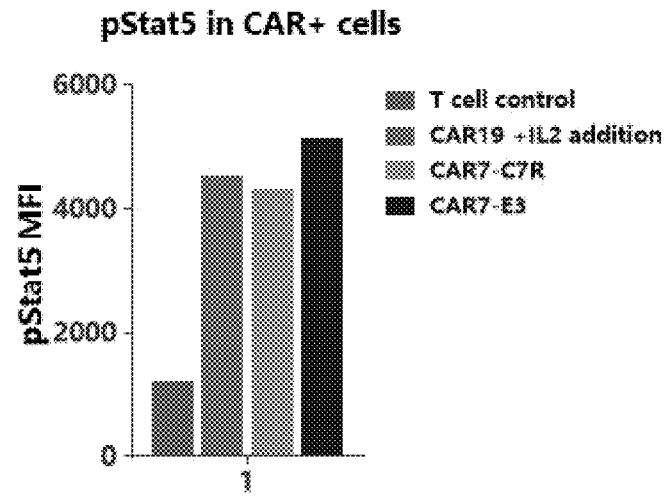


FIG. 56E

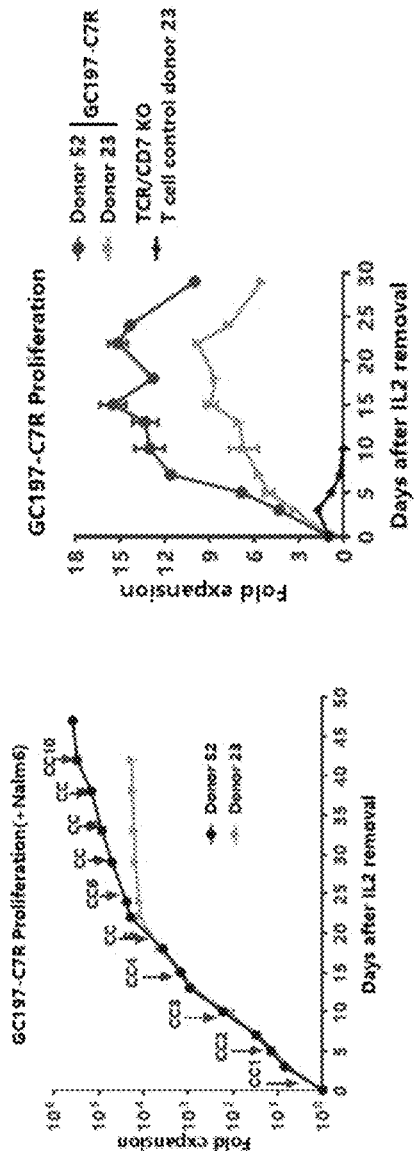


FIG. 57A

FIG. 57B

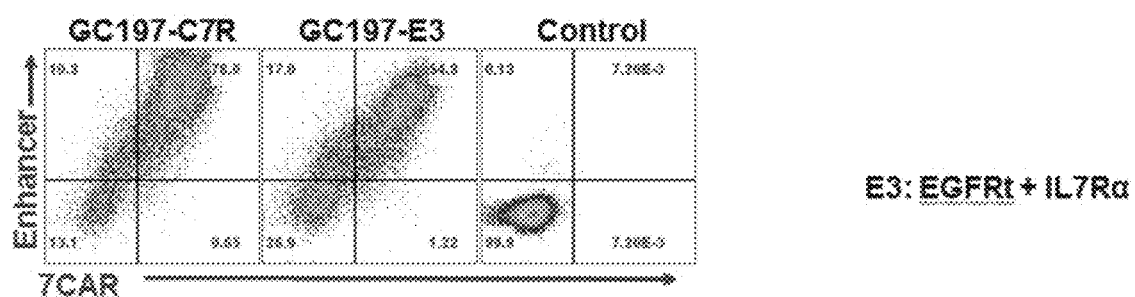


FIG. 58A

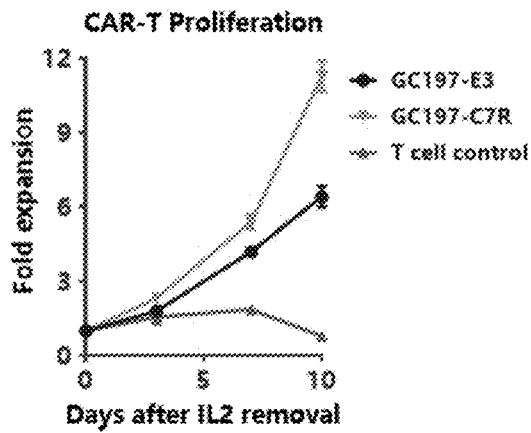


FIG. 58B

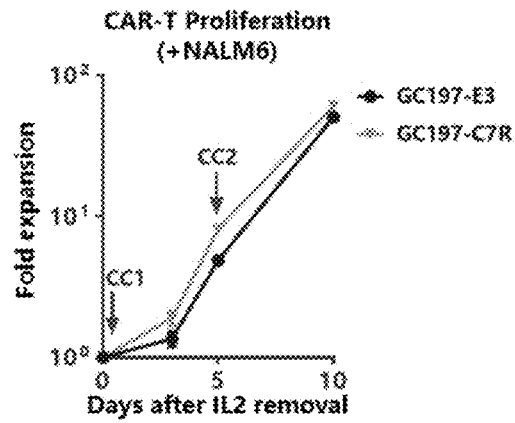


FIG. 58C

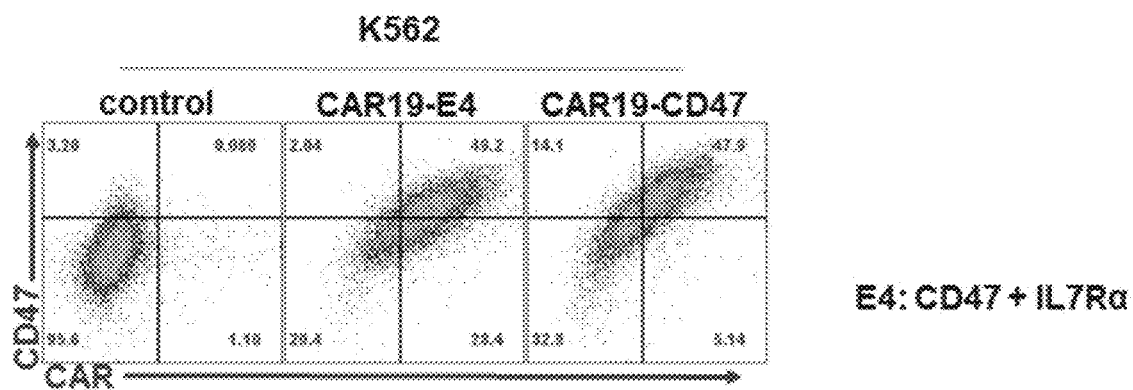


FIG. 59A

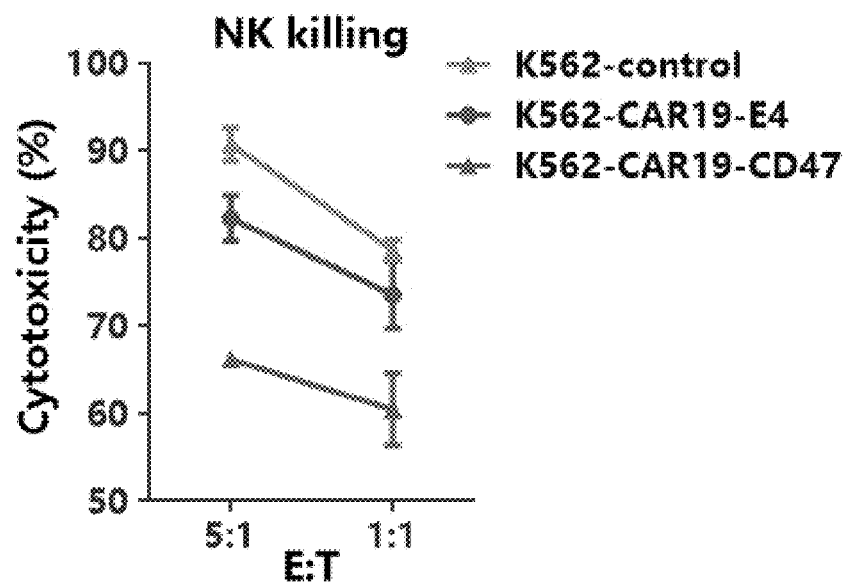


FIG. 59B

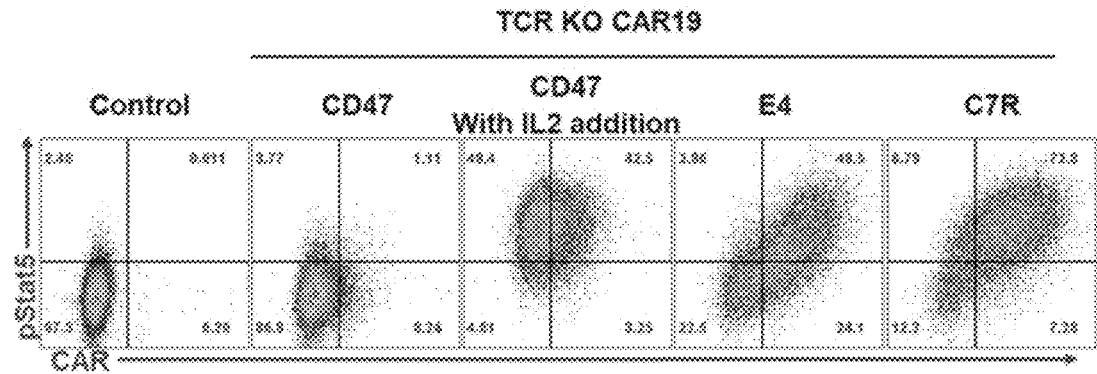


FIG. 60A

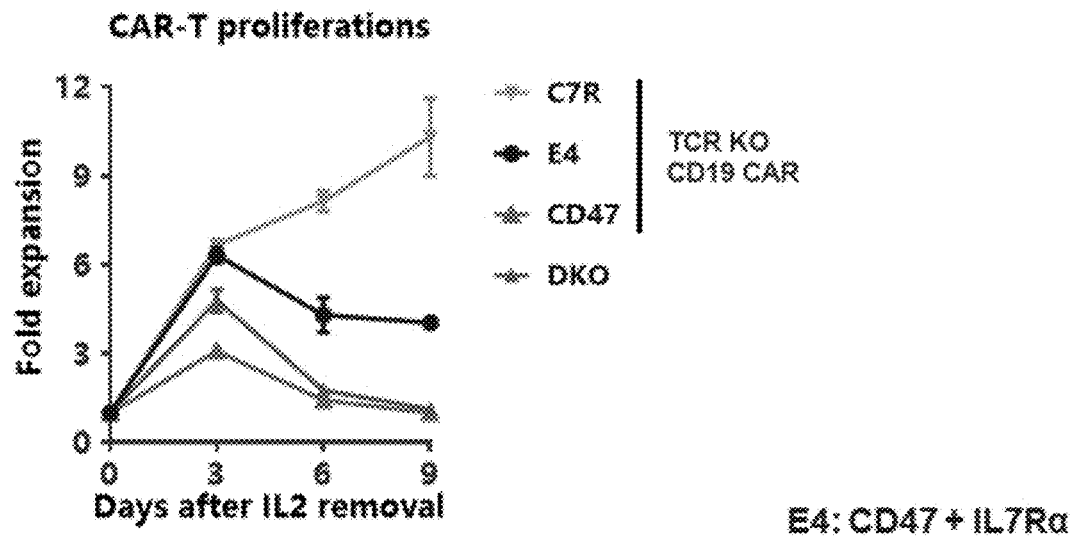


FIG. 60B

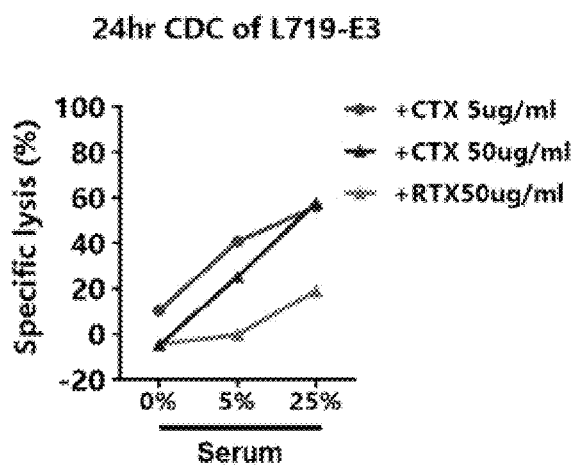


FIG. 61A

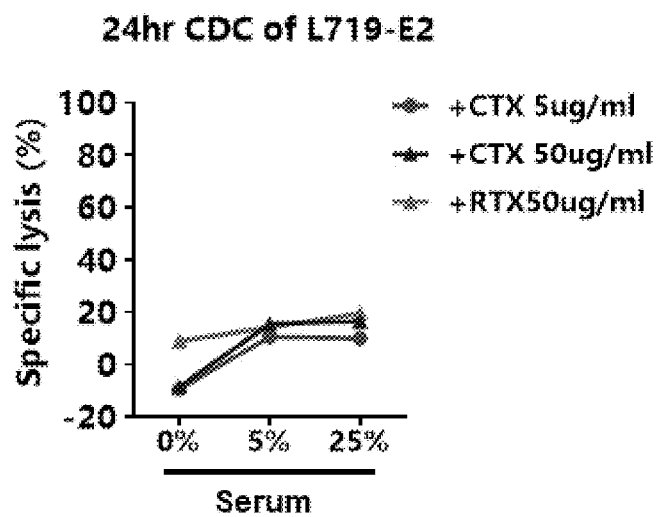


FIG. 61B

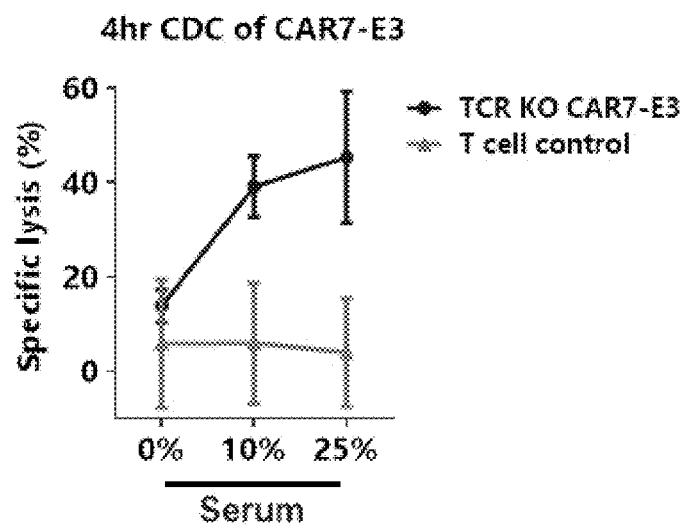


FIG. 62A

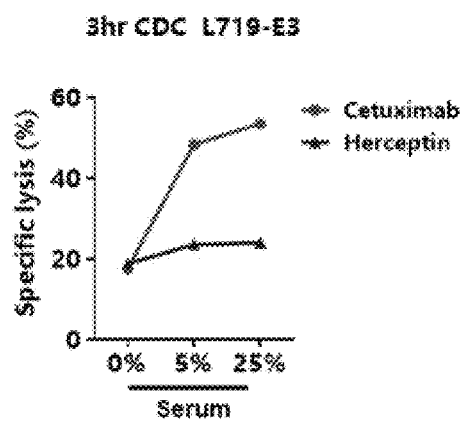


FIG. 62B

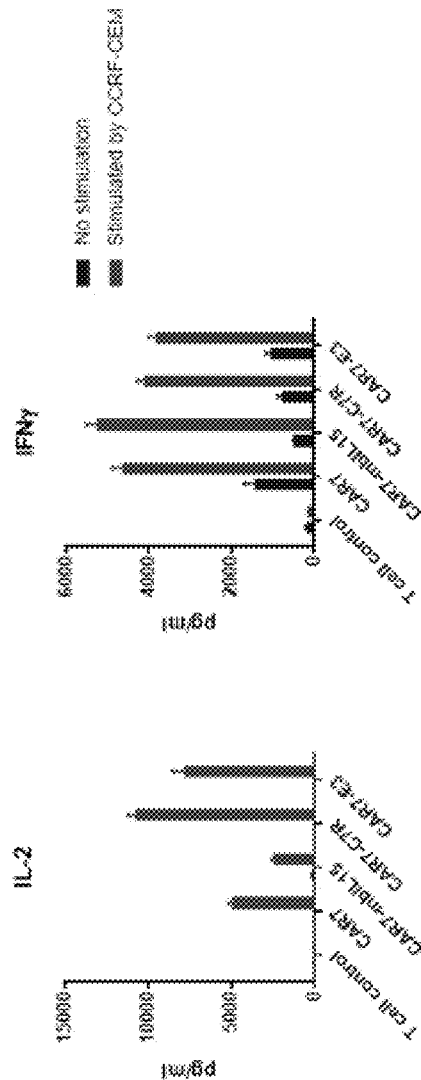


FIG. 63A

FIG. 63B

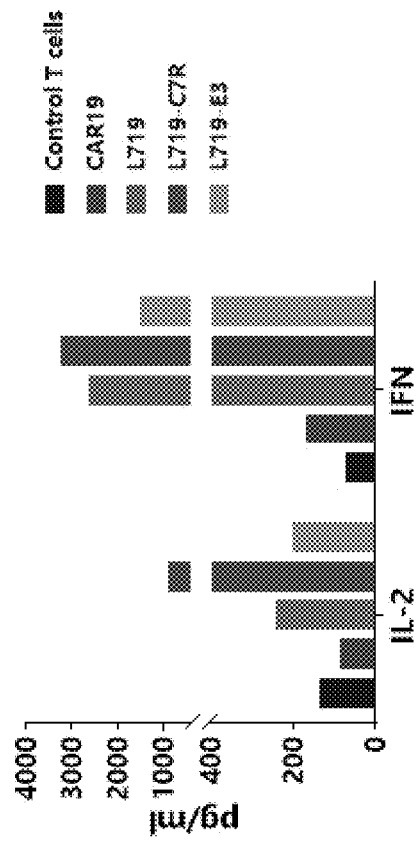


FIG. 64

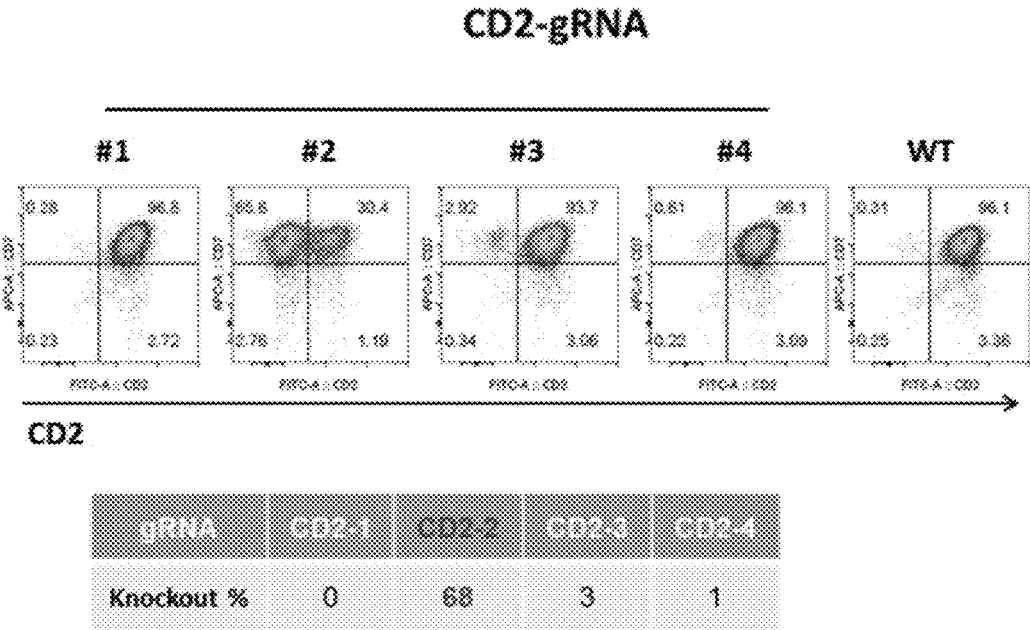


FIG. 65

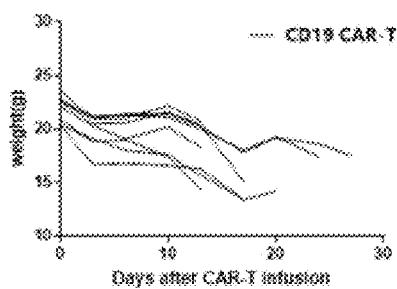


FIG. 66A

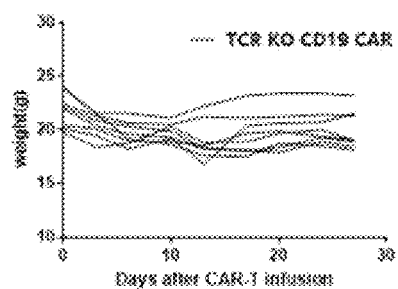


FIG. 66B

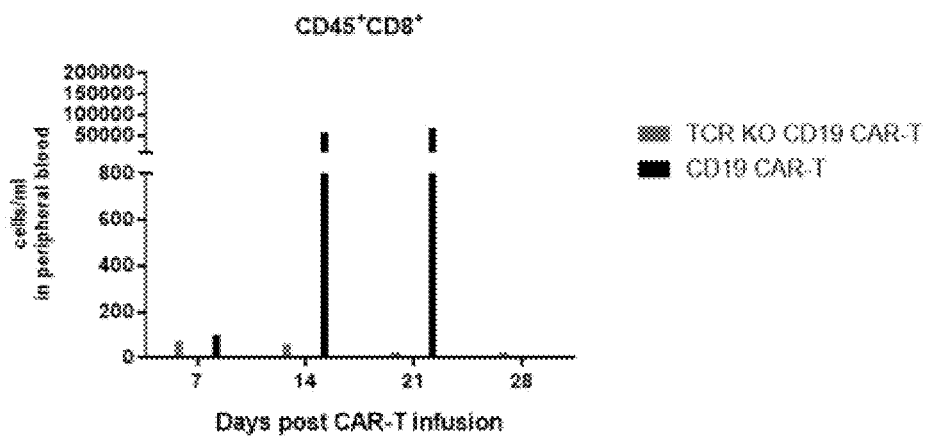


FIG. 66C

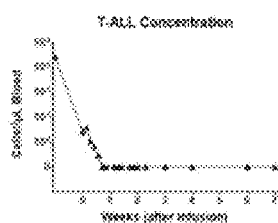


Fig. 67A

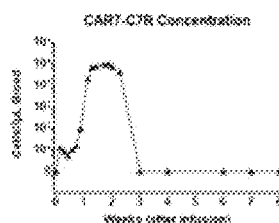


Fig. 67B

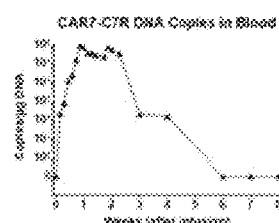


Fig. 67C

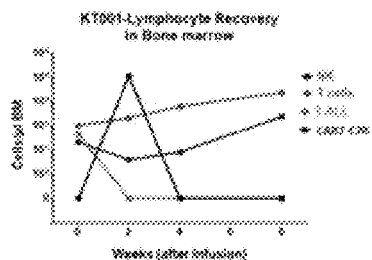


Fig. 67D

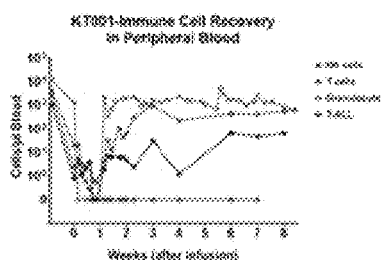


Fig. 67E

FIG. 67

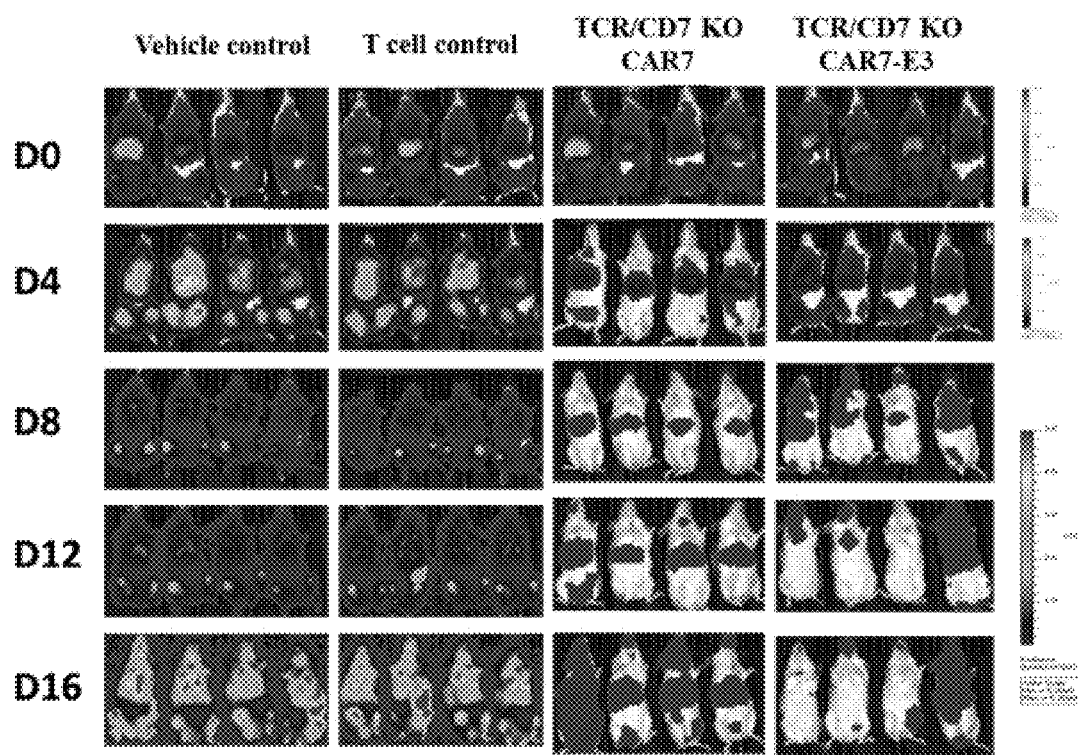


FIG. 68

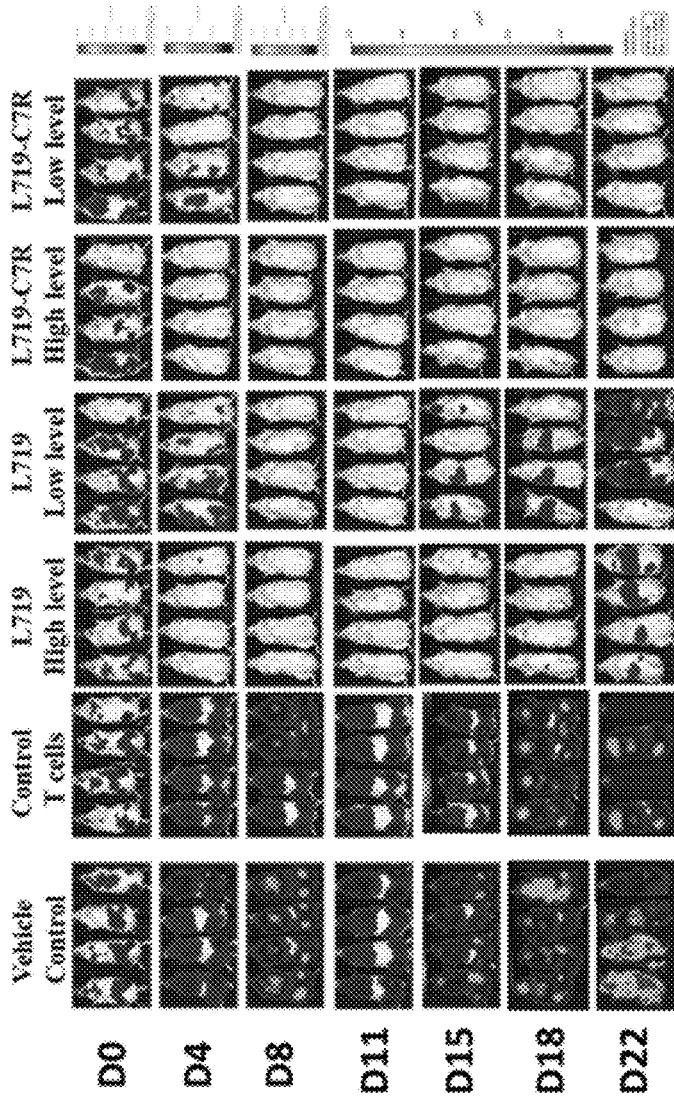


FIG. 69

COMPOSITIONS AND METHODS FOR T CELL ENGINEERING

CROSS-REFERENCE

[0001] This application is continuation application which claims priority to International Application No. PCT/CN2019/114939, filed Nov. 1, 2019, which claims priority to Chinese Patent Application No. 201811297174.3, filed Nov. 1, 2018; Chinese Patent Application No. 201811535338.1, filed Dec. 14, 2018; Chinese Patent Application No. 201811549651.0, filed Dec. 18, 2018; Chinese Patent Application No. 201910492882.0, filed Jun. 6, 2019; and International Application No. PCT/CN2019/101651, filed Aug. 20, 2019, each of which is entirely incorporated herein by reference.

SEQUENCE LISTING

[0002] 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 Apr. 30, 2021, is named 56758-702 (generic)_SL.txt and is 242,531 bytes in size.

BACKGROUND

[0003] The generation of tumor-specific T lymphocytes by genetic modification to express chimeric antigen receptors (CARs) is gaining traction as a form of synthetic biology generating powerful antitumor effects. Because the specificity is conferred by antibody fragments, the CAR-T cells are not MHC restricted and are therefore more practical than approaches based on T-cell receptors that require MHC matching.

[0004] Although early CAR-T cell clinical data obtained in the treatment of cancer have shown promising results, the risk to patients is higher, and some patients' T cells are effective even after TCR or CAR redirection. Treatment is also not sufficiently effective, which promotes the modification of allogeneic donor T cells. However, endogenous $\alpha\beta$ T cell receptors on infused allogeneic T cells can recognize primary and secondary histocompatibility antigens in the recipient, which results in graft versus host disease (GVHD). As a result, most current clinical trials using autologous CAR-T cell infusion rely on immune tolerance to prevent TCR-mediated deleterious recognition of normal tissues following adoptive cell transfer. This approach has achieved early clinical success, but is limited by the time and expense of manufacturing patient-specific T cell products.

SUMMARY

[0005] Recognized herein is a need for compositions and methods for genetically modifying immune cells (e.g., T cells) for allogeneic cell therapy. Also recognized herein is a need for methods of modifying immune cells (e.g., T cells) while circumventing the time and expense of making patient-specific T cell products.

[0006] In an aspect, the present disclosure provides an engineered immune cell, comprising: a chimeric polypeptide comprising (i) an enhancer moiety capable of enhancing one or more activities of the engineered immune cell, and (ii) an inducible cell death moiety capable of effecting death of the engineered immune cell upon contacting the chimeric polypeptide with a cell death activator, wherein the enhancer moiety is linked to the inducible cell death moiety, and one

or more chimeric polypeptide receptors (CPRs) comprising a binding moiety, wherein the binding moiety comprises (i) a first antigen binding domain, which first antigen binding domain suppresses or reduces a subject's immune response toward the engineered immune cell when administered into the subject and (ii) a second antigen binding domain capable of binding to a disease-associated antigen, wherein an individual CPR of the one or more CPRs comprises (i) the first antigen binding domain, (ii) the second antigen binding domain, or (iii) both the first antigen binding domain and the second antigen binding domain, wherein each CPR of the one or more CPRs further comprises a transmembrane domain and an intracellular signaling domain.

[0007] In some embodiments, the one or more CPRs are one or more chimeric antigen receptor (CARs) or engineered T cell receptors (TCRs). In some embodiments, the first antigen binding domain binds to an immune cell antigen. In some embodiments, a gene encoding an endogenous surface marker of the engineered immune cell is inactivated (e.g., silenced or knocked out), wherein the endogenous surface marker is capable of binding to the first antigen binding domain when expressed. In some embodiments, an endogenous T cell receptor (TCR) of the engineered immune cell is inactivated. In some embodiments, a function of the endogenous TCR of the engineered immune cell is inhibited by an inhibitor. In some embodiments, a gene encoding a subunit of the endogenous TCR is inactivated such that the endogenous TCR is inactivated. In some embodiments, the gene encoding the subunit is TCR α , TCR β , CD3 ϵ , CD3 δ , CD3 γ , or CD3 ζ . In some embodiments, the chimeric polypeptide does not comprise any self-cleaving peptide flanked by the enhancer moiety and the inducible cell death moiety. In some embodiments, the enhancer moiety is configured to constitutively enhance the one or more activities of the engineered immune cell. In some embodiments, the enhancer moiety is configured to constitutively upregulate one or more intracellular signaling pathways of the engineered immune cell. In some embodiments, the one or more intracellular signaling pathways are one or more cytokine signaling pathways. In some embodiments, the enhancer moiety is self-activating through self-oligomerizing. In some embodiments, the enhancer moiety is self-activating through self-dimerizing. In some embodiments, the chimeric polypeptide is a secreted protein. In some embodiments, the chimeric polypeptide is an intracellular protein. In some embodiments, the chimeric polypeptide is a transmembrane protein. In some embodiments, the enhancer moiety or the inducible cell death moiety is contained in an ectodomain of the transmembrane protein. In some embodiments, the enhancer moiety or the inducible cell death moiety is contained in an endodomain of the transmembrane protein. In some embodiments, (i) the enhancer moiety is contained in an endodomain of the transmembrane protein and (ii) the inducible cell death moiety are contained in an ectodomain of the transmembrane protein. In some embodiments, (i) the enhancer moiety is contained in an ectodomain of the transmembrane protein, and (ii) the inducible cell death moiety is contained in an endodomain of the transmembrane protein. In some embodiments, the enhancer moiety is a cytokine or a cytokine receptor. In some embodiments, the enhancer moiety is selected from the group consisting of IL-2, IL-3, IL-4, IL-6, IL-7, IL-8, IL-10, IL-11, IL-12, IL-15, IL-17, IL-18, IL-21, IL-23, PD-1, PD-L1, CD122, CSF1R, CTAL-4, TIM-3, CCL21, CCL19, TGFR beta,

receptors for the same, functional fragments thereof, functional variants thereof, and combinations thereof. In some embodiments, the enhancer moiety functions as a trans-activating factor or a cis-activating factor. In some embodiments, the inducible cell death moiety is selected from the group consisting of rapaCasp9, iCasp9, HSV-TK, Δ CD20, mTMPK, Δ CD19, RQR8, Her2t, CD30, BCMA and EGFRt. In some embodiments, the inducible cell death moiety is EGFRt, and the cell death activator is an antibody or an antigen binding fragment thereof that binds EGFRt. In some embodiments, the inducible cell death moiety is HSV-TK, and the cell death activator is GCV. In some embodiments, the inducible cell death moiety is iCasp9, and the cell death activator is AP1903. In some embodiments, the cell death activator comprises a nucleic acid, a polynucleotide, an amino acid, a polypeptide, lipid, a carbohydrate, a small molecule, an enzyme, a ribosome, a proteasome, a variant thereof, or any combination thereof. In some embodiments, the individual CPR of the one or more CPRs comprises both the first antigen binding domain and the second antigen binding domain. In some embodiments, the first antigen binding domain and the second antigen binding domain is linked via a linker. In some embodiments, the linker does not comprise a self-cleaving peptide. In some embodiments, the first antigen binding domain or the second antigen binding is a scFv. In some embodiments, the first antigen binding domain and the second antigen binding domain is arranged, from amino terminus to carboxyl terminus, as: (i) VL2-VH1-VL1-VH2; (ii) VH2-VL1-VH1-VL2; (iii) VL1-VH2-VL2-VH1; (iv) VH1-VL2-VH2-VL1; (v) VL2-VL1-VH1-VH2; (vi) VH2-VH1-VL1-VL2; (vii) VL1-VL2-VH2-VH1; or (viii) VH1-VH2-VL2-VL1; wherein VH1 is heavy chain variable domain of the first antigen binding domain, VL1 is light chain variable light domain of the first antigen binding domain, VH2 is heavy chain variable domain of the second antigen binding domain, and VL2 is light chain variable domain of the second antigen binding domain. In some embodiments, the first antigen binding domain and the second antigen binding domain is arranged, from amino terminus to carboxyl terminus, as: (i) VL2-VH2-VL1-VH1; (ii) VL2-VH2-VH1-VL1; (iii) VL1-VH1-VL2-VH2; (iv) VL1-VH1-VH2-VL2; (v) VH2-VL2-VL1-VH1; (vi) VH2-VL2-VH1-VL1; (vii) VH1-VL1-VL2-VH2; or (viii) VH1-VL1-VH2-VL2, wherein VH1 is heavy chain variable domain of the first antigen binding domain, VL1 is light chain variable light domain of the first antigen binding domain, VH2 is heavy chain variable domain of the second antigen binding domain, and VL2 is light chain variable domain of the second antigen binding domain. In some embodiments, the first antigen binding domain and the second antigen binding domain bind to the immune cell antigen and the disease-associated antigen. In some embodiments, the individual CPR of the one or more CPRs comprises only the first antigen binding domain and an additional individual CPR of the one or more CPRs comprises only the second antigen binding domain. In some embodiments, the immune cell antigen is a surface protein or a secreted protein of an immune cell. In some embodiments, the immune cell is an NK cell, a T cell, a monocyte, an innate lymphocyte, a macrophage or a granulocyte. In some embodiments, the immune cell is obtained from peripheral blood, cord blood, or is derived from a stem cell. In some embodiments, the immune cell antigen is selected from the group consisting of CD2, CD3, CD4, CD5, CD7, CD8,

CD16a, CD16b, CD25, CD27, CD28, CD30, CD38, CD45, CD48, CD50, CD52, CD56, CD57, CD62L, CD69, CD94, CD100, CD102, CD122, CD127, CD132, CD137, CD160, CD161, CD178, CD218, CD226, CD244, CD159a (NKG2A), CD159c (NKG2C), NKG2E, CD279, CD314 (NKG2D), CD305, CD335 (NKP46), CD337, CD319 (CS1), TCR α , TCR β and SLAMF7. In some embodiments, the disease-associated antigen is a tumor-associated antigen. In some embodiments, the tumor-associated antigen is CD19, CD2, CD3, CD4, CD5, CD7, CD8, CD19, CD20, CD22, CD25, CD28, CD30, CD33, CD38, CD40, CD44V6, CD47, CD52, CD56, CD57, CD58, CD79b, CD80, CD86, CD81, CD123, CD133, CD137, CD151, CD171, CD276, CLL1, B7H4, BCMA, VEGFR-2, EGFR, GPC3, PMSA, CEACAM6, c-Met, EGFRvIII, ErbB2/HER2, ErbB3, HER-2, HER3, ErbB4/HER-4, EphA2, IGF1R, GD2, O-acetyl GD2, O-acetyl GD3, GHRHR, GHR, Flt1, KDR, Flt4, Flt3, CEA, CA125, CTLA-4, GITR, BTLA, TGFBR1, TGFBR2, TGFBR1, IL6R, gp130, Lewis, TNFR1, TNFR2, PD1, PD-L1, PD-L2, PSCA, HVEM, MAGE-A, MSLN, NY-ESO-1, PSMA, RANK, ROR1, TNFRSF4, TWEAK-R, LTPR, LIFRP, LRP5, MUC1, MUC16, TCR α , TCR β , TLR7, TLR9, PTCH1, WT-1, Robo1, Frizzled, OX40, Notch-1-4, APRIL, CS1, MAGE3, Claudin 18.2, Folate receptor α , Folate receptor β , GPC2, CD70, BAFF-R or TROP-2. In some embodiments, the first antigen binding domain binds to an immune cell antigen selected from the group consisting of CD2, CD3, CD5, CD7 and CD137, and the second antigen binding domain binds to CD19. In some embodiments, the first antigen binding domain binds to CD3, and the second antigen binding domain binds to CD19. In some embodiments, the first antigen binding domain binds to CD7, and the second antigen binding domain binds to CD19. In some embodiments, the first antigen binding domain binds to CD137, and the second antigen binding domain binds to CD19. In some embodiments, expression of one or more endogenous human leukocyte antigen (HLA) genes of the engineered immune cell remains intact. In some embodiments, expression of endogenous HLA-I and/or HLA-II genes of the engineered immune cell remains intact. In some embodiments, expression of endogenous HLA-E and/or HLA-G and/or HLA-C genes of the engineered immune cell remains intact. In some embodiments, expression of one or more endogenous HLA genes of the engineered immune cell is upregulated. In some embodiments, expression of endogenous HLA-E and/or HLA-G and/or HLA-C genes of the engineered immune cell is upregulated. In some embodiments, expression of an endogenous HLA-I, an endogenous HLA-II, or both of the engineered immune cell is inhibited. In some embodiments, a gene encoding endogenous HLA-I, endogenous HLA-II, or both is inactivated. In some embodiments, HLA-E or HLA-G of the engineered immune cell is overexpressed. In some embodiments, CD24, CD47, FASL and/or PD-1 of the engineered immune cell is overexpressed. In some embodiments, the engineered immune cell is a T cell, an NKT cell or an NK cell. In some embodiments, the engineered immune cell is derived from a stem cell. In some embodiments, the stem cell is a hematopoietic stem cell (HSC) or an induced pluripotent stem cell (iPSC). In some embodiments, the engineered immune cell is an autologous cell or an allogeneic cell. In some embodiments, the engineered immune cell

is obtained from a subject having a condition. In some embodiments, the engineered immune cell is obtained from a healthy donor.

[0008] In another aspect, the present disclosure provides an engineered immune cell, comprising: (a) one or more chimeric antigen receptors (CARs) comprising a binding moiety, wherein the binding moiety comprises a first antigen binding domain capable of binding to an immune cell antigen and a second antigen binding domain capable of binding to a disease-associated antigen, and wherein each CAR of the one or more CARs further comprises a transmembrane domain and an intracellular signaling domain; and (b) an enhancer moiety capable of enhancing one or more activities of the engineered immune cell, wherein an endogenous T cell receptor (TCR) of the engineered immune cell is inactivated, and wherein the engineered immune cell exhibits (i) enhanced degree of persistence by remaining viable in vitro for at least about 20 days while in presence of cells that are heterologous to the engineered immune cell, (ii) enhanced degree of expansion by at least about 10-fold within 15 days, or (iii) enhanced cytotoxicity against a target cell comprising the immune cell antigen or the disease-associated antigen, compared to an additional engineered immune cell comprising the one or more CARs in (a) but not the enhancer moiety in (b).

[0009] In some embodiments, the engineered immune cell is characterized by exhibiting two or more of (i), (ii), and (iii). In some embodiments, (i), (ii), and/or (iii) is measured in absence of any exogenous enhancer moiety. In some embodiments, the immune cell antigen is selected from the group consisting of CD2, CD3, CD4, CD5, CD7, CD8, CD16a, CD16b, CD25, CD27, CD28, CD30, CD38, CD45, CD48, CD50, CD52, CD56, CD57, CD62L, CD69, CD94, CD100, CD102, CD122, CD127, CD132, CD160, CD161, CD178, CD218, CD226, CD244, CD159a (NKG2A), CD159c (NKG2C), NKG2E, CD314 (NKG2D), CD305, CD335 (NKP46), CD337, CS1, TCR α , TCR β , and SLAMF7. In some embodiments, the immune cell antigen is CD7. In some embodiments, the enhancer moiety is configured to constitutively enhance the one or more activities of the engineered immune cell. In some embodiments, the enhancer moiety is configured to constitutively upregulate one or more intracellular signaling pathways of the engineered immune cell. In some embodiments, the one or more intracellular signaling pathways are one or more cytokine signaling pathways. In some embodiments, the enhancer moiety is self-activating through self-oligomerizing. In some embodiments, the enhancer moiety is self-activating through self-dimerizing. In some embodiments, the enhancer moiety is a cytokine or a cytokine receptor. In some embodiments, the enhancer moiety is selected from the group consisting of IL-2, IL-3, IL-4, IL-6, IL-7, IL-8, IL-10, IL-11, IL-12, IL-15, IL-17, IL-18, IL-21, IL-23, PD-1, PD-L1, CD122, CSF1R, CTLA-4, TIM-3, CCL21, CCL19, TGF β beta, receptors for the same, functional fragments thereof, functional variants thereof, and combinations thereof. In some embodiments, a gene encoding a subunit of the endogenous TCR is inactivated such that the endogenous TCR is inactivated. In some embodiments, the gene encoding the subunit is TCR α , TCR β , CD3 ϵ , CD3 δ , CD3 γ , or CD3 ζ . In some embodiments, the engineered immune cell further comprises an inducible cell death moiety, which inducible cell death moiety effects suicide of the engineered immune cell upon contact with a cell death

activator. In some embodiments, the inducible cell death moiety is selected from the group consisting of rapaCasp9, iCasp9, HSV-TK, ACD20, mTMPK, ACD19, RQR8, Her2t, CD30, BCMA, and EGFRt. In some embodiments, the inducible cell death moiety is EGFRt, and the cell death activator is an antibody or an antigen binding fragment thereof that binds EGFRt. In some embodiments, the inducible cell death moiety is HSV-TK, and the cell death activator is GCV. In some embodiments, the inducible cell death moiety is iCasp9, and the cell death activator is AP1903. In some embodiments, the cell death activator comprises a nucleic acid, a polynucleotide, an amino acid, a polypeptide, lipid, a carbohydrate, a small molecule, an enzyme, a ribosome, a proteasome, a variant thereof, or any combination thereof. In some embodiments, expression of one or more endogenous human leukocyte antigen (HLA) genes of the engineered immune cell remains intact. In some embodiments, expression of endogenous HLA-I and/or HLA-II genes of the engineered immune cell remains intact. In some embodiments, expression of endogenous HLA-E and/or HLA-G and/or HLA-C genes of the engineered immune cell remains intact. In some embodiments, expression of one or more endogenous HLA genes of the engineered immune cell is upregulated. In some embodiments, expression of endogenous HLA-E and/or HLA-G and/or HLA-C genes of the engineered immune cell is upregulated. In some embodiments, expression of an endogenous HLA-I, an endogenous HLA-II, or both of the engineered immune cell is inhibited. In some embodiments, a gene encoding endogenous HLA-I, endogenous HLA-II, or both is inactivated. In some embodiments, HLA-E or HLA-G of the engineered immune cell is overexpressed. In some embodiments, CD24, CD47, FASL and/or PD-1 of the engineered immune cell is overexpressed. In some embodiments, the disease-associated antigen is a tumor-associated antigen. In some embodiments, the first antigen binding domain or the second antigen binding domain is a scFv. In some embodiments, a gene encoding an endogenous surface marker of the engineered immune cell is inactivated, wherein the endogenous surface marker is capable of binding to the first antigen binding domain when expressed. In some embodiments, the endogenous surface marker is CD2, CD3, CD4, CD5, CD7, CD8, CD16a, CD16b, CD25, CD27, CD28, CD30, CD38, CD45, CD48, CD50, CD52, CD56, CD57, CD62L, CD69, CD94, CD100, CD102, CD122, CD127, CD132, CD137, CD160, CD161, CD178, CD218, CD226, CD244, CD159a (NKG2A), CD159c (NKG2C), NKG2E, CD279, CD314 (NKG2D), CD305, CD335 (NKP46), CD337, CD319 (CS1), TCR α , TCR β or SLAMF7. In some embodiments, the engineered immune cell is a T cell, an NKT cell or an NK cell. In some embodiments, the engineered immune cell is derived from a stem cell. In some embodiments, the stem cell is a hematopoietic stem cell (HSC) or an induced pluripotent stem cell (iPSC). In some embodiments, the engineered immune cell is an autologous cell or an allogeneic cell. In some embodiments, the engineered immune cell is obtained from a subject having a condition. In some embodiments, the engineered immune cell is obtained from a healthy donor.

[0010] In another aspect, the present disclosure provides a pharmaceutical composition for treating a disease, comprising the engineered immune cell described herein, and a pharmaceutically acceptable carrier. In another aspect, the present disclosure provides a method of treating or diagnos-

ing a disease in a subject, comprising administering the pharmaceutical composition to the subject. In some embodiments, the engineered immune cell in the pharmaceutical composition is derived from an allogeneic immune cell. In some embodiments, the engineered immune cell derived from the allogeneic immune cell does not induce graft versus host disease (GvHD) in the subject. In some embodiments, the engineered immune cell in the pharmaceutical composition is derived from an autologous immune cell. In some embodiments, an endogenous TCR of the engineered immune cell in the pharmaceutical composition is functionally inactive. In some embodiments, the engineered immune cell reduces GvHD in the subject compared to an additional immune cell having a functionally active TCR. In some embodiments, the disease is a cancer. In some embodiments, the cancer is lymphoma or leukemia.

[0011] In another aspect, the present disclosure provides a cell, comprising: a functionally inactive T cell receptor (TCR), and one or more chimeric antigen receptors (CARs), wherein each individual CAR of the one or more CARs comprises a binding moiety, which binding moiety comprises (i) a first antigen binding domain, which first antigen binding domain suppresses or reduces a subject's immune response toward the engineered immune cell when administered into the subject and (ii) a second antigen binding domain that binds to a disease-associated antigen, and wherein each CAR of the one or more CARs further comprises a transmembrane domain and an intracellular signaling domain.

[0012] In some embodiments, the first antigen binding domain and the second antigen binding domain is linked by a linker. In some embodiments, the linker does not comprise a self-cleaving peptide. In some embodiments, the first antigen binding domain and the second antigen binding domain is arranged, from amino terminus to carboxyl terminus, as: (i) VL2-VH1-VL1-VH2; (ii) VH2-VL1-VH1-VL2; (iii) VL1-VH2-VL2-VH1; (iv) VH1-VL2-VL1-VL1; (v) VL2-VL1-VH1-VH2; (vi) VH2-VH1-VL1-VL2; (vii) VL1-VL2-VH2-VH1; or (viii) VH1-VH2-VL2-VL1, wherein VH1 is heavy chain variable domain of the first antigen binding domain, VL1 is light chain variable light domain of the first antigen binding domain, VH2 is heavy chain variable domain of the second antigen binding domain, and VL2 is light chain variable domain of the second antigen binding domain. In some embodiments, the first antigen binding domain and the second antigen binding domain is arranged, from amino terminus to carboxyl terminus, as: (i) VL2-VH2-VL1-VH1; (ii) VL2-VH2-VH1-VL1; (iii) VL1-VH1-VL2-VH2; (iv) VL1-VH1-VH2-VL2; (v) VH2-VL2-VL1-VH1; (vi) VH2-VL2-VH1-VL1; (vii) VH1-VL1-VL2-VH2; or (viii) VH1-VL1-VH2-VL2, wherein VH1 is heavy chain variable domain of the first antigen binding domain, VL1 is light chain variable light domain of the first antigen binding domain, VH2 is heavy chain variable domain of the second antigen binding domain, and VL2 is light chain variable domain of the second antigen binding domain. In some embodiments, a gene encoding a subunit of an endogenous TCR of the cell is inactivated, thereby generating the functionally inactive TCR. In some embodiments, the gene encoding the subunit is TCR α , TCR β , CD3 ϵ , CD3 δ , CD3 γ , or CD3 ζ . In some embodiments, the first antigen binding domain binds to an immune cell antigen. In some embodiments, the immune cell antigen is a surface protein or a secreted protein of an

immune cell. In some embodiments, the immune cell is an NK cell, a T cell, a monocyte, a macrophage, or a granulocyte. In some embodiments, the immune cell antigen is CD2, CD3, CD4, CD5, CD7, CD8, CD16a, CD16b, CD25, CD27, CD28, CD30, CD38, CD45, CD48, CD50, CD52, CD56, CD57, CD62L, CD69, CD94, CD100, CD102, CD122, CD127, CD132, CD137, CD160, CD161, CD178, CD218, CD226, CD244, CD159a (NKG2A), CD159c (NKG2C), NKG2E, CD279, CD314 (NKG2D), CD305, CD335 (NKP46), CD337, CD319 (CS1), TCR α , TCR β or SLAMF7. In some embodiments, the disease-associated antigen is a tumor-associated antigen. In some embodiments, the tumor-associated antigen is CD19, CD2, CD3, CD4, CD5, CD7, CD8, CD19, CD20, CD22, CD25, CD28, CD30, CD33, CD38, CD40, CD44V6, CD47, CD52, CD56, CD57, CD58, CD79b, CD80, CD86, CD81, CD123, CD133, CD137, CD151, CD171, CD276, CLL1, B7H4, BCMA, VEGFR-2, EGFR, GPC3, PMSA, CEACAM6, c-Met, EGFRvIII, ErbB2/HER2, ErbB3, HER-2, HER3, ErbB4/HER-4, EphA2, IGF1R, GD2, O-acetyl GD2, O-acetyl GD3, GHRHR, GHR, Flt1, KDR, Flt4, Flt3, CEA, CA125, CTLA-4, GITR, BTLA, TGFBR1, TGFBR2, TGFBR1, TL6R, gp130, Lewis, TNFR1, TNFR2, PD1, PD-L1, PD-L2, PSCA, HVEM, MAGE-A, MSLN, NY-ESO-1, PSMA, RANK, ROR1, TNFRSF4, TWEAK-R, LTPR, LIFR, LRP5, MUC1, MUC16, TCR α , TCR β , TLR7, TLR9, PTCH1, WT-1, Robo1, Frizzled, OX40, Notch-1-4, APRIL, CS1, MAGE3, Claudin 18.2, Folate receptor α , Folate receptor β , GPC2, CD70, BAFF-R or TROP-2. In some embodiments, the first antigen binding domain binds to an immune cell antigen selected from the group consisting of CD2, CD3, CD4, CD5, CD7, CD8, CD16a, CD16b, CD25, CD27, CD28, CD30, CD38, CD45, CD48, CD50, CD52, CD56, CD57, CD62L, CD69, CD94, CD100, CD102, CD122, CD127, CD132, CD137, CD160, CD161, CD178, CD218, CD226, CD244, CD159a (NKG2A), CD159c (NKG2C), NKG2E, CD279, CD314 (NKG2D), CD305, CD335 (NKP46), CD337, CD319 (CS1), TCR α , TCR β and SLAMF7, and the second antigen binding domain binds to CD19. In some embodiments, the first antigen binding domain binds to CD3, and the second antigen binding domain binds to CD19. In some embodiments, the first antigen binding domain binds to CD7, and the second antigen binding domain binds to CD19. In some embodiments, the first antigen binding domain binds to CD137, and the second antigen binding domain binds to CD19. In some embodiments, the cell further comprises an enhancer moiety, which enhancer moiety enhances one or more activities of the engineered immune cell. In some embodiments, the enhancer moiety is configured to constitutively enhance the one or more activities of the engineered immune cell. In some embodiments, the enhancer moiety is configured to constitutively upregulate one or more intracellular signaling pathways of the engineered immune cell. In some embodiments, the one or more intracellular signaling pathways are one or more cytokine signaling pathways. In some embodiments, the enhancer moiety is a cytokine or a cytokine receptor. In some embodiments, the enhancer moiety is selected from the group consisting of IL-2, IL-3, IL-4, IL-6, IL-7, IL-8, IL-10, IL-11, IL-12, IL-15, IL-17, IL-18, IL-21, IL-23, PD-1, PD-L1, CD122, CSF1R, CTAL-4, TIM-3, CCL21, CCL19, TGF β , receptors for the same, functional fragments thereof, functional variants thereof, and combinations thereof. In some embodiments, the cell

further comprises an inducible cell death moiety capable of effecting death of the cell upon contacting the inducible cell death moiety with a cell death activator. In some embodiments, the inducible cell death moiety is selected from the group consisting of rapaCasp9, iCasp9, HSV-TK, ACD20, mTMPK, ACD19, RQR8, Her2t, CD30, BCMA, and EGFRt. In some embodiments, the inducible cell death moiety is EGFRt, and the cell death activator is an antibody or an antigen binding fragment thereof that binds EGFRt. In some embodiments, the inducible cell death moiety is HSV-TK, and the cell death activator is GCV. In some embodiments, the inducible cell death moiety is iCasp9, and the cell death activator is AP1903.

[0013] In another aspect, the present disclosure provides a nucleic acid molecule comprising a first sequence encoding a chimeric antigen receptor (CAR), wherein the CAR comprises a binding moiety, which binding moiety comprises (i) a first antigen binding domain, which first antigen binding domain suppresses or reduces a subject's immune response toward the engineered immune cell when administered into the subject linked to (ii) a second antigen binding domain capable of binding to a disease-associated antigen, and wherein each CAR of the one or more CARs further comprises a transmembrane domain and an intracellular signaling domain.

[0014] In some embodiments, the first antigen binding domain binds to an antigen selected from the group consisting of CD2, CD3, CD4, CD5, CD7, CD8, CD16a, CD16b, CD25, CD27, CD28, CD30, CD38, CD45, CD48, CD50, CD52, CD56, CD57, CD62L, CD69, CD94, CD100, CD102, CD122, CD127, CD132, CD137, CD160, CD161, CD178, CD218, CD226, CD244, CD159a (NKG2A), CD159c (NKG2C), NKG2E, CD279, CD314 (NKG2D), CD305, CD335 (NKP46), CD337, CD319 (CS1), TCR α , TCR β and SLAMF7. In some embodiments, the second antigen binding domain binds to CD19, CD2, CD3, CD4, CD5, CD7, CD8, CD19, CD20, CD22, CD25, CD28, CD30, CD33, CD38, CD40, CD44V6, CD47, CD52, CD56, CD57, CD58, CD79b, CD80, CD86, CD81, CD123, CD133, CD137, CD151, CD171, CD276, CLL1, B7H4, BCMA, VEGFR-2, EGFR, GPC3, PMSA, CEACAM6, c-Met, EGFRvIII, ErbB2/HER2, ErbB3, HER-2, HER3, ErbB4/HER-4, EphA2, IGF1R, GD2, O-acetyl GD2, O-acetyl GD3, GHRHR, GHR, Flt1, KDR, Flt4, Flt3, CEA, CA125, CTLA-4, GITR, BTLA, TGFBR1, TGFBR2, TGFBR1, IL6R, gp130, Lewis, TNFR1, TNFR2, PD1, PD-L1, PD-L2, PSCA, HVEM, MAGE-A, MSLN, NY-ESO-1, PSMA, RANK, ROR1, TNFRSF4, TWEAK-R, LTPR, LIFRP, LRP5, MUC1, MUC16, TCR α , TCR β , TLR7, TLR9, PTCH1, WT-1, Robo1, Frizzled, OX40, Notch-1-4, APRIL, CS1, MAGE3, Claudin 18.2, Folate receptor α , Folate receptor β , GPC2, CD70, BAFF-R or TROP-2. In some embodiments, the nucleic acid molecule further comprises a second sequence encoding an enhancer moiety, which enhancer moiety enhances one or more activities of the CAR when expressed in a cell. In some embodiments, the enhancer moiety is selected from the group consisting of IL-2, IL-3, IL-4, IL-6, IL-7, IL-8, IL-10, IL-11, IL-12, IL-15, IL-17, IL-18, IL-21, IL-23, PD-1, PD-L1, CD122, CSF1R, CTAL-4, TIM-3, CCL21, CCL19, TGFR beta, receptors for the same, functional fragments thereof, and combinations thereof. In some embodiments, the nucleic acid molecule further comprises a second sequence encoding an inducible cell death moiety,

which inducible cell death moiety, when expressed in a cell, effects death of the cell upon contacting the inducible cell death moiety with a cell death activator. In some embodiments, the inducible cell death moiety is selected from the group consisting of rapaCasp9, iCasp9, HSV-TK, Δ CD20, mTMPK, Δ CD19, RQR8, Her2t, CD30, BCMA, and EGFRt. In some embodiments, the nucleic acid molecule further comprises a third sequence flanked by the first sequence and the second sequence, wherein the third sequence encodes a cleavable linker. In some embodiments, the cleavable linker is a self-cleaving peptide. In some embodiments, the nucleic acid molecule further comprises a regulatory sequence regulating expression of the first sequence and/or the second sequence.

[0015] In another aspect, the present disclosure provides a kit comprising a nucleic acid molecule described herein.

[0016] In another aspect, the present disclosure provides a method of generating an engineered cell, comprising (a) delivering the nucleic acid molecule described herein into a cell; and (b) expressing the nucleic acid molecule in the cell, thereby generating the engineered cell.

[0017] In another aspect, the present disclosure provides an engineered immune cell, comprising: an enhancer moiety capable of enhancing one or more activities of the engineered immune cell; a chimeric antigen receptor (CAR) comprising an antigen binding domain that specifically binds CD7, wherein the CAR further comprises a transmembrane domain and an intracellular signaling domain; wherein an endogenous CD7 in the engineered immune cell is inactivated. In some embodiments, the enhancer moiety is selected from the group consisting of IL-2, IL-7, IL-12, IL-15, IL-18, IL-21, PD-1, PD-L1, CSF1R, CTAL-4, TIM-3, CCL21, CCL19, and TGFR beta, receptors for the same, and a combination thereof. In some embodiments, an endogenous T cell receptor (TCR) of the engineered immune cell is inactivated. In some embodiments, the engineered immune cell further comprises a polypeptide that comprises an inducible cell death moiety capable of effecting death of the engineered immune cell upon contacting the polypeptide with a cell death activator. In some embodiments, the enhancer moiety is part of the polypeptide. In some embodiments, the enhancer moiety is not a part of the CAR. In some embodiments, the enhancer moiety is a part of the CAR. In some embodiments, the engineered immune cell is a T cell, an NKT cell or an NK cell. In some embodiments, the engineered immune cell is derived from a stem cell. In some embodiments, the stem cell is a hematopoietic stem cell (HSC) or an induced pluripotent stem cell (iPSC). In some embodiments, the engineered immune cell is an autologous cell or an allogeneic cell. In some embodiments, the engineered immune cell is obtained from a subject having a condition. In some embodiments, the engineered immune cell is obtained from a healthy donor.

[0018] In another aspect, the present disclosure provides a method of generating the engineered immune cell described herein, comprising (a) delivering one or more nucleic acid molecules encoding (i) the enhancer moiety and (ii) the CAR in to an immune cell; and (b) expressing the one or more nucleic acid molecules in the immune cell, thereby generating the engineered immune cell. In some embodiments, a single nucleic acid molecule encodes (i) the enhancer moiety and (ii) the CAR. In some embodiments, the single nucleic acid molecule comprises a self-cleaving peptide flanked by (i) the enhancer moiety and (ii) the CAR.

In some embodiments, the single nucleic acid molecule does not comprise any self-cleaving peptide flanked by (i) the enhancer moiety and (ii) the CAR. In some embodiments, (i) a first nucleic acid molecule encodes the enhancer moiety and (ii) a second nucleic acid molecule encodes the CAR.

[0019] In another aspect, the present disclosure provides a method of treating or diagnosing a condition in a subject, comprising administering the engineered immune cell described herein to the subject. In some embodiments, the engineered immune cell is derived from an autologous immune cell of the subject. In some embodiments, the engineered immune cell is derived from an allogeneic immune cell. In some embodiments, the condition is cancer (e.g., T cell and/or NK cell malignancies).

[0020] In another aspect, the present disclosure provides an engineered immune cell, comprising: a single chimeric antigen receptor (CAR) comprising (i) a first antigen binding domain that specifically binds CD7 and (ii) a second antigen binding domain capable of binding to a disease-associated antigen, the CAR further comprises a transmembrane domain and an intracellular signaling domain, wherein a gene encoding endogenous CD7 is inactivated in the engineered immune cell. In some embodiments, the engineered immune further comprises an enhancer moiety capable of enhancing one or more activities of the engineered immune cell. In some embodiments, the enhancer moiety is selected from the group consisting of IL-2, IL-7, IL-12, IL-15, IL-18, IL-21, PD-1, PD-L1, CSF1R, CTAL-4, TIM-3, CCL21, CCL19, and TGF β receptors for the same, and a combination thereof. In some embodiments, an endogenous T cell receptor (TCR) of the engineered immune cell is inactivated. In some embodiments, the engineered immune cell further comprises a polypeptide that comprises an inducible cell death moiety capable of effecting death of the engineered immune cell upon contacting the polypeptide with a cell death activator. In some embodiments, the enhancer moiety is part of the polypeptide. In some embodiments, the engineered immune cell is a T cell, an NKT cell or an NK cell. In some embodiments, the engineered immune cell is derived from a stem cell. In some embodiments, the stem cell is a hematopoietic stem cell (HSC) or an induced pluripotent stem cell (iPSC). In some embodiments, the engineered immune cell is an autologous cell or an allogeneic cell. In some embodiments, the engineered immune cell is obtained from a subject having a condition. In some embodiments, the engineered immune cell is obtained from a healthy donor.

[0021] In another aspect, the present disclosure provides a method of generating the engineered immune cell, comprising (a) delivering one or more nucleic acid molecules encoding the single CAR into an immune cell; and (b) expressing the one or more nucleic acid molecules in the immune cell, thereby generating the engineered immune cell. In some embodiments, a single nucleic acid molecule encodes the single CAR.

[0022] In another aspect, the present disclosure provides a method of treating or diagnosing a condition in a subject, comprising administering the engineered immune cell to the subject. In some embodiments, the engineered immune cell is derived from an autologous immune cell of the subject. In some embodiments, the engineered immune cell is derived from an allogeneic immune cell. In some embodiments, the condition is cancer (e.g., T cell and/or NK cell malignancies).

[0023] In another aspect, the present disclosure provides a method of delivering an allogeneic cell therapy comprising: administering to a subject in need thereof a population of engineered immune cells, an individual engineered immune cell of the population comprises: (a) one or more chimeric antigen receptors (CARs) comprising a binding moiety, wherein the binding moiety comprises a first antigen binding domain capable of binding to an immune cell antigen and a second antigen binding domain capable of binding to a disease-associated antigen, which first antigen binding domain suppresses or reduces a subject's immune response toward the engineered immune cell when administered into the subject; (b) an enhancer moiety capable of enhancing one or more activities of the engineered immune cell, wherein an endogenous T cell receptor (TCR) of the engineered immune cell is inactivated, and wherein the engineered immune cell exhibits (i) enhanced degree of persistence by remaining viable in vitro for at least about 20 days while in presence of cells that are heterologous to the engineered immune cell, (ii) enhanced degree of expansion by at least about 10-fold within 15 days, or (iii) enhanced cytotoxicity against a target cell comprising the immune cell antigen or the disease-associated antigen.

[0024] In some embodiments, the administering of the population of the engineered immune cells alone without the use of an additional proliferation agent yields (i) enhanced degree of persistence by remaining viable in vitro for at least about 20 days while in presence of immune cells that are heterologous to the engineered immune cell, (ii) enhanced degree of expansion by at least about 10-fold within 15 days, or (iii) enhanced cytotoxicity against a target cell comprising the immune cell antigen or the disease-associated antigen. In some embodiments, each CAR of the one or more CARs further comprises a transmembrane domain and an intracellular signaling domain, wherein the first antigen binding domain suppresses or reduces a subject's immune response toward the engineered immune cell when administered into the subject. In some embodiments, an endogenous CD7 of the individual engineered immune cell is inactivated. In some embodiments, the immune cell antigen is CD7.

[0025] In some embodiments, the enhancer moiety is configured to constitutively enhance the one or more activities of the individual engineered immune cell. In some embodiments, the enhancer moiety is configured to constitutively upregulate one or more intracellular signaling pathways of the engineered immune cell. In some embodiments, the one or more intracellular signaling pathways are one or more cytokine signaling pathways. In some embodiments, the enhancer moiety is self-activating through self-oligomerizing. In some embodiments, the enhancer moiety is self-activating through self-dimerizing. In some embodiments, the enhancer moiety is a cytokine or a cytokine receptor. In some embodiments, the enhancer moiety is selected from the group consisting of IL-2, IL-3, IL-4, IL-6, IL-7, IL-8, IL-10, IL-11, IL-12, IL-15, IL-17, IL-18, IL-21, IL-23, PD-1, PD-L1, CD122, CSF1R, CTAL-4, TIM-3, CCL21, CCL19, TGF β receptors for the same, functional fragments thereof, functional variants thereof, and combinations thereof. In some embodiments, a gene encoding a subunit of the endogenous TCR is inactivated such that the endogenous TCR is inactivated. In some embodiments, the gene encoding the subunit is TCR α , TCR β , CD3 ϵ , CD3 δ , CD3 γ , or CD3 ζ . In some embodiments, the individual engineered immune cell further comprises an inducible cell

death moiety, which inducible cell death moiety effects suicide of the engineered immune cell upon contact with a cell death activator. In some embodiments, the inducible cell death moiety is selected from the group consisting of rapaCasp9, iCasp9, HSV-TK, ACD20, mTMPK, ACD19, RQR8, Her2t, CD30, BCMA, and EGFRt. In some embodiments, the inducible cell death moiety is EGFRt, and the cell death activator is an antibody or an antigen binding fragment thereof that binds EGFRt. In some embodiments, the inducible cell death moiety is HSV-TK, and the cell death activator is GCV. In some embodiments, the inducible cell death moiety is iCasp9, and the cell death activator is AP1903. In some embodiments, the cell death activator comprises a nucleic acid, a polynucleotide, an amino acid, a polypeptide, lipid, a carbohydrate, a small molecule, an enzyme, a ribosome, a proteasome, a variant thereof, or any combination thereof. In some embodiments, expression of one or more endogenous human leukocyte antigen (HLA) genes of the individual engineered immune cell remains intact. In some embodiments, expression of endogenous HLA-I and/or HLA-II genes of the individual engineered immune cell remains intact. In some embodiments, expression of endogenous HLA-E and/or HLA-G and/or HLA-C genes of the individual engineered immune cell remains intact. In some embodiments, expression of one or more endogenous HLA genes of the individual engineered immune cell is upregulated. In some embodiments, expression of endogenous HLA-E and/or HLA-G and/or HLA-C genes of the individual engineered immune cell is upregulated. In some embodiments, expression of an endogenous HLA-I, an endogenous HLA-II, or both of the individual engineered immune cell is inhibited. In some embodiments, a gene encoding endogenous HLA-I, endogenous HLA-II, or both is inactivated. In some embodiments, HLA-E or HLA-G of the engineered immune cell is overexpressed. In some embodiments, CD24, CD47, FASL and/or PD-1 of the engineered immune cell is overexpressed.

[0026] In some embodiments, the disease-associated antigen is a tumor-associated antigen. In some embodiments, the first antigen binding domain or the second antigen binding domain is a scFv. In some embodiments, the individual engineered immune cell is a T cell, an NKT cell or an NK cell. In some embodiments, the individual engineered immune cell is derived from a stem cell. In some embodiments, the stem cell is a hematopoietic stem cell (HSC) or an induced pluripotent stem cell (iPSC). In some embodiments, the individual engineered immune cell is an allogeneic cell. In some embodiments, the individual engineered immune cell is obtained from a healthy donor.

[0027] In an aspect, the present disclosure provides a cell comprising: (a) one or more chimeric antigen receptors (CARs) comprising a binding moiety, wherein the binding moiety comprises an antigen binding domain capable of binding to an immune cell antigen, and wherein each CAR of the one or more CARs further comprises a transmembrane domain and an intracellular signaling domain; and (b) an enhancer moiety capable of enhancing one or more activities of the cell, wherein an endogenous T cell receptor (TCR) of the cell is inactivated.

[0028] In some embodiments, the enhancer moiety enhances one or more activities of the cell. In some embodiments, the enhancer moiety is configured to constitutively enhance the one or more activities of the cell. In some embodiments, the enhancer moiety is configured to consti-

tutively upregulate one or more intracellular signaling pathways of the cell. In some embodiments, the one or more intracellular signaling pathways are one or more cytokine signaling pathways. In some embodiments, the enhancer moiety is a cytokine or a cytokine receptor. In some embodiments, the enhancer moiety is selected from the group consisting of IL-2, IL-3, IL-4, IL-6, IL-7, IL-8, IL-10, IL-11, IL-12, IL-15, IL-17, IL-18, IL-21, IL-23, PD-1, PD-L1, CD122, CSF1R, CTAL-4, TIM-3, CCL21, CCL19, TGF β , receptors for the same, functional fragments thereof, functional variants thereof, and combinations thereof. In some embodiments, the method further comprises an inducible cell death moiety capable of effecting death of the cell upon contacting the inducible cell death moiety with a cell death activator. In some embodiments, the inducible cell death moiety is selected from the group consisting of rapaCasp9, iCasp9, HSV-TK, ACD20, mTMPK, ACD19, RQR8, Her2t, CD30, BCMA, and EGFRt. In some embodiments, the inducible cell death moiety is EGFRt, and the cell death activator is an antibody or an antigen binding fragment thereof that binds EGFRt. In some embodiments, the inducible cell death moiety is HSV-TK, and the cell death activator is GCV. In some embodiments, the inducible cell death moiety is iCasp9, and the cell death activator is AP1903. In some embodiments, a gene encoding an endogenous surface marker of the cell is inactivated, wherein the endogenous surface marker is capable of binding to the first antigen binding domain when expressed. In some embodiments, the endogenous surface marker is CD2, CD3, CD4, CD5, CD7, CD8, CD16a, CD16b, CD25, CD27, CD28, CD30, CD38, CD45, CD48, CD50, CD52, CD56, CD57, CD62L, CD69, CD94, CD100, CD102, CD122, CD127, CD132, CD137, CD160, CD161, CD178, CD218, CD226, CD244, CD159a (NKG2A), CD159c (NKG2C), NKG2E, CD279, CD314 (NKG2D), CD305, CD335 (NKP46), CD337, CD319 (CS1), TCR α , TCR β or SLAMF7.

[0029] In an aspect, the present disclosure provides a method for treating a lymphoid malignancy, comprising administering to a patient in need thereof a population of engineered immune cells, wherein an individual engineered immune cell of the population comprises: (a) one or more chimeric antigen receptors (CARs) comprising a binding moiety, wherein the binding moiety comprises an antigen binding domain capable of binding to an immune cell antigen, and wherein each CAR of the one or more CARs further comprises a transmembrane domain and an intracellular signaling domain; and (b) an enhancer moiety capable of enhancing one or more activities of the engineered immune cell, wherein an endogenous T cell receptor (TCR) of the engineered immune cell is inactivated, wherein (i) the number of affected cells in peripheral blood or the number of affected cells in bone marrow is reduced by at least 50% within 3 weeks after a last dosing of the engineered immune cells, and (ii) the number of any one or more of autologous T cell, granulocyte, and NK cell in peripheral blood starts to increase within 3 weeks after a last dosing of the engineered immune cells.

[0030] In some embodiments, the enhancer moiety enhances one or more activities of the engineered immune cell. In some embodiments, the enhancer moiety is configured to constitutively enhance the one or more activities of the engineered immune cell. In some embodiments, the enhancer moiety is configured to constitutively upregulate one or more intracellular signaling pathways of the engi-

neered immune cell. In some embodiments, the one or more intracellular signaling pathways are one or more cytokine signaling pathways. In some embodiments, the enhancer moiety is a cytokine or a cytokine receptor. In some embodiments, the enhancer moiety is selected from the group consisting of IL-2, IL-3, IL-4, IL-6, IL-7, IL-8, IL-10, IL-11, IL-12, IL-15, IL-17, IL-18, IL-21, IL-23, PD-1, PD-L1, CD122, CSF1R, CTAL-4, TIM-3, CCL21, CCL19, TGFR beta, receptors for the same, functional fragments thereof, functional variants thereof, and combinations thereof. In some embodiments, the engineered immune cell further comprises an inducible cell death moiety capable of effecting death of the cell upon contacting the inducible cell death moiety with a cell death activator.

[0031] In some embodiments, the inducible cell death moiety is selected from the group consisting of rapaCasp9, iCasp9, HSV-TK, ACD20, mTMPK, ACD19, RQR8, Her2t, CD30, BCMA, and EGFRt. In some embodiments, the inducible cell death moiety is EGFRt, and the cell death activator is an antibody or an antigen binding fragment thereof that binds EGFRt. In some embodiments, the inducible cell death moiety is HSV-TK, and the cell death activator is GCV. In some embodiments, the inducible cell death moiety is iCasp9, and the cell death activator is API903. In some embodiments, a gene encoding an endogenous surface marker of the cell is inactivated, wherein the endogenous surface marker is capable of binding to the first antigen binding domain when expressed. In some embodiments, the endogenous surface marker is CD2, CD3, CD4, CD5, CD7, CD8, CD16a, CD16b, CD25, CD27, CD28, CD30, CD38, CD45, CD48, CD50, CD52, CD56, CD57, CD62L, CD69, CD94, CD100, CD102, CD122, CD127, CD132, CD137, CD160, CD161, CD178, CD218, CD226, CD244, CD159a (NKG2A), CD159c (NKG2C), NKG2E, CD279, CD314 (NKG2D), CD305, CD335 (NKP46), CD337, CD319 (CS1), TCR α , TCR β or SLAMF7. In some embodiments, the number of any one or more of autologous T cell, granulocyte, and NK cell in peripheral blood starts to increase before the number of affected cells in peripheral blood or the number of affected cells in bone marrow is reduced by at least 50%. In some embodiments, the number of any one or more of autologous T cell, granulocyte, and NK cell in peripheral blood starts to increase after the number of affected cells in peripheral blood or the number of affected cells in bone marrow is reduced by at least 50%.

[0032] Additional aspects and advantages of the present disclosure will become readily apparent to those skilled in this art from the following detailed description, wherein only illustrative embodiments of the present disclosure are shown and described. As will be realized, the present disclosure is capable of other and different embodiments, and its several details are capable of modifications in various obvious respects, all without departing from the disclosure. Accordingly, the drawings and description are to be regarded as illustrative in nature, and not as restrictive.

INCORPORATION BY REFERENCE

[0033] All publications, patents, and patent applications mentioned in this specification are herein incorporated by reference to the same extent as if each individual publication, patent, or patent application was specifically and individually indicated to be incorporated by reference. To the extent publications and patents or patent applications incorporated by reference contradict the disclosure contained in

the specification, the specification is intended to supersede and/or take precedence over any such contradictory material.

BRIEF DESCRIPTION OF THE DRAWINGS

[0034] The novel features of the invention are set forth with particularity in the appended claims. A better understanding of the features and advantages of the present invention will be obtained by reference to the following detailed description that sets forth illustrative embodiments, in which the principles of the invention are utilized, and the accompanying drawings (also “Figure” and “FIG.” herein), of which:

[0035] FIG. 1 illustrates an example structure of EGFRt-CD3-CD19 CAR.

[0036] FIG. 2 illustrates an example preparation process of EGFRt-CD3-CD19 universal CAR-T.

[0037] FIG. 3 illustrates expression of Parallel CAR (FMC63-OKT3) and the tEGFR switch.

[0038] FIG. 4 illustrates expression of Parallel and Loop CARs.

[0039] FIGS. 5A-C illustrate experimental data comparing the UCHT1 vs OKT3.

[0040] FIGS. 6A-C illustrate the anti-cancer function of parallel-UCHT1.

[0041] FIGS. 7A and 7B illustrate experimental data comparing the anti-cancer function of parallel CAR vs Loop CAR.

[0042] FIGS. 8A and 8B illustrate that TCR KO CD19 CAR-T cell showed a proliferation defect in subcutaneous tumor model. 5e5 Raji-luciferase cells were grafted into NOG mice by subcutaneous injection. 1e6 WT, TCR KO CD19 CAR-T cells were infused (IV) into Raji grafted mice. Tumor burden was assessed by caliper measurements of the actual tumor sizes. Although both were Raji based tumor bearing models, subcutaneous model generates solid tumors and has much higher requirements on CAR-T cell proliferation for controlling the tumors than intravenous model. TCR KO CAR-T cells showed a proliferation defects compared to WT CAR-T cells, consistent with their tumor control effects.

[0043] FIGS. 9A-C illustrate proliferation and killing efficacy of CAR-T cells with different enhancers after multiple rounds of stimulation by cancer cells.

[0044] FIGS. 10A and 10B illustrate the proliferation of CAR-T cells with different enhancers after multiple rounds of stimulation by cancer cells.

[0045] FIG. 11 illustrates In vivo comparison of enhancers in TCR KO CD19 CAR-T cells. 5e5 Raji-luciferase cells were grafted into NOG mice by intravenous (IV) injection. WT, TCR KO CD19 CAR-T cells with/without enhancers were infused (IV) into Raji grafted mice. Tumor burden was assessed by bioluminescence intensity (BLI).

[0046] FIG. 12 illustrates in vivo comparison of enhancers in TCR KO CD19 CAR-T cells.

[0047] FIG. 13 illustrates an example tissue distribution of CD7 mRNA.

[0048] FIG. 14 illustrates an example tissue distribution of CD2 mRNA.

[0049] FIG. 15 illustrates an example preparation of CD7 CAR-T.

[0050] FIG. 16 illustrates an experimental data of CD7 gRNA screening.

[0051] FIG. 17 illustrates example structures of CAR7, CAR7-mb15 and CAR7-C7R.

[0052] FIG. 18 illustrates experimental data of CAR expression of CAR7, CAR7-mb15, CAR7-C7R and control CAR19, expression of IL15Ra and CD34, and the knockout efficiency of CD7 and CD3.

[0053] FIG. 19 illustrates experimental data of the activation of STAT5 in UCAR-T cells. Adding IL-2 activated the STAT5 of CAR7 T cells and remove of IL-2 significantly decreased pSTAT5 level. In CAR7-C7R, the level of pSTAT5 was at high level with or without exogenous IL-2.

[0054] FIG. 20 illustrates experimental data of the killing effect of UCAR-T cells on CCRF-CEM-luc cell line.

[0055] FIG. 21 illustrates experimental data of the killing effect of UCAR-T cells on alloreactive T (allo-T) cells.

[0056] FIG. 22 illustrates the killing effect of UCAR-T cells on NK92 cell line in 24 hours.

[0057] FIG. 23A illustrates experimental data of in vitro expansion of several UCAR-T cells, where the UCAR-T cells expressing enhancers survived longer. FIG. 23B illustrates experimental data of comparison of their killing effects on CCRF-CEM tumor cells after removing IL-2.

[0058] FIG. 24A illustrates experimental data of in vitro expansion of several UCAR-T cells. FIG. 24B illustrates experimental data of comparison of their killing effects on CCRF-CEM tumor cells after removing IL-2 and co-culturing with CCRF-CEM for multiple rounds, where the UCAR-T cells expressing enhancers showed stronger expansion under the stimulation of tumor antigens.

[0059] FIG. 25 illustrates experimental data of expansion of UCAR-T cells expressing enhancers in immuno-deficient mice carrying CCRF-CEM tumor.

[0060] FIG. 26 illustrates experimental data of the killing effect of UCAR-T cells expressing enhancers on CCRF-CEM tumor in mice.

[0061] FIG. 27 illustrates in vivo comparison of enhancers in TCR KO CD7 CAR-T cells.

[0062] FIGS. 28A and 28B illustrate in vivo comparison of enhancers in TCR KO CD7 CAR-T cells.

[0063] FIG. 29 illustrates an example structure of RQR8-CD19CAR-MBIL15, RQR8-CD19CAR-MBIL15-IL7, RQR8-CD19CAR-C7R and RQR8-CD19CAR-MBIL7.

[0064] FIG. 30 illustrates a design of E3: constitutive active IL7R α based enhancer+safety switch.

[0065] FIG. 31 illustrates expression of CAR and the enhancer.

[0066] FIGS. 32A and 32B illustrate test of CAR-T function.

[0067] FIGS. 33A and 33B illustrate test of enhancer function.

[0068] FIGS. 34A-C illustrate test of enhancer function.

[0069] FIGS. 35A-C illustrate test of safety switch by ADCC.

[0070] FIGS. 36A and 36B illustrate test of safety switch by ADCC after repeat stimulations.

[0071] FIG. 37 illustrates examples of the design of CD7-CD19 dual CARs. L719 represents Loop-CAR, LHLH=VLVH-VLVH, HLHL=VHVL-VHVL; T197 and T719 are Tandem-CARs, EAAK is the linker (SEQ ID NO: 92) between two LH. SP: signaling peptide; H: hinge; TM: transmembrane domain.

[0072] FIGS. 38A and 38B illustrate an experimental data of the expression of CD7 single CARs constructed with scFvs of different antibodies and the knockout efficiencies of

CD7/CD3 (TRAC) (FIG. 38A), as well as the killing effects of CARs on CD7+ cells (FIG. 38B). LH represents VLVH, and HL represents VHVL.

[0073] FIG. 39 illustrates experimental data of the expressions of Loop-CARs and Tandem-CARs. CAR7 and CAR19 are single CAR controls, and Mock T is negative control.

[0074] FIG. 40 illustrates experimental data of the knockout efficiencies of CD7 and CD3 (TRAC) and clearances of CD7+ cells by CARs.

[0075] FIG. 41 illustrates experimental data of the clearance of HeLa-CD19+ cells by Loop-CARs and Tandem CARs.

[0076] FIGS. 42A and 42B illustrate experimental data of the complete killing of NK by CD7 single CAR and CD7-CD19 dual CARs. FIG. 42A shows the experimental data of the clearance efficiencies of in vitro expanded peripheral blood NK by different CARs. FIG. 42B illustrates experimental data of the in vitro expanded NK comprises ~80% CD7+ cells; after killing by L719-LHLH and T197-LHLH, the remaining NK cells are CD7-cells.

[0077] FIG. 43 illustrates experimental data of the rapid killing of allogeneic T cells by CD7 single CAR and CD7-CD19 dual CARs.

[0078] FIG. 44 illustrates experimental data of the in vivo functions of dual CARs. Loop CARs and Tandem CARs both showed potent killing effects on Raji tumor cells.

[0079] FIGS. 45A and 45B illustrate experimental data comparing two Tandem CARs. FIG. 45A shows experimental data using L719: L719-LHLH as a standard control for screening, and CAR7, CAR19 and Mock T as other controls. FIG. 45B shows experimental data of the clearance of the remaining CD7+ cells by different CARs after CD7 knock-out.

[0080] FIG. 46 illustrates experimental data of the two Tandem CARs showing similar clearance effects on HeLa-CD19+ cells as L719. 8 FIGS. 47A and 47 B illustrate experimental data of the two Tandem CARs showing similar clearance effects on allogeneic T cells (FIG. 47A) and NK cells (FIG. 47B) as L719.

[0081] FIG. 48 illustrates experimental data of the in vivo functional screening of dual CARs. Both Loop and Tandem dual CARs showed potent killing effects on CCRF-CEM tumor cells in vivo.

[0082] FIG. 49 illustrates the structure of rapaC, L719-C7R, and L719-E3.

[0083] FIGS. 50A-J illustrate experimental data of the effects of cytokine signaling on the functions of dual CARs. FIG. 50A illustrates experimental data of the expressions of L719 and L719-C7R dual CARs. FIG. 50B illustrates experimental data of the killing effects of dual CARs on HeLa-CD7+ cells. FIG. 50C illustrates experimental data of the killing effects of dual CARs on HeLa-CD19+ cells. FIG. 50D illustrates experimental data of the expressions of enhancer and CD7 in L718 dual CAR-T cells. FIG. 50E illustrates experimental data of cytotoxicity of L719 dual CAR-T cells. FIG. 50F illustrates experimental data of cytotoxicity of L719 dual CAR-T cells. FIG. 50G illustrates experimental data of the expressions of phosphorylated STAT5 and the enhancer in L719 dual CAR-T cells. FIG. 50H illustrates experimental data of L719 dual CAR-T cell proliferation. FIG. 50I illustrates experimental data of the killing effects of dual CARs on HeLa-CD19+ cells. FIG. 50J illustrates experimental data of the killing effects of dual CARs on HeLa-CD7+ cells.

[0084] FIG. 51 illustrates an example tissue distribution of CD137 mRNA.

[0085] FIG. 52 illustrates an example structural diagram of CD137-CD19 AAC/CAR.

[0086] FIG. 53 illustrates an example preparing process of CD137-CD19 universal CAR-T.

[0087] FIGS. 54A-C illustrate experimental data showing defects in cell expansion of TCR knockout autologous CAR-T cells. FIG. 54A illustrates FACS data showing fraction of TCR+ cells pre- and post-infusion. FIG. 54B illustrates bar-graph of the FACS data in FIG. 54A. FIG. 54C illustrates comparison of expansion differences of CAR-T cells with or without TCRs.

[0088] FIGS. 55A and 55B illustrate experimental data showing effects of C7R on TCR knockout (KO) CD7 CAR-T cells (e.g., a CAR-T cell having a CAR targeting CD7, also referred to as UCART7 cells or CAR7 cells) expansion and persistence. FIG. 55A illustrates TCR KO CD7 CAR-T expansion data with or without enhancer C7R. FIG. 55B illustrates proliferation data of TCR KO CD7 CAR-T cells with or without enhancer.

[0089] FIGS. 56A-E illustrate experimental data showing STAT5 phosphorylation, cell persistence and cytotoxicity of TCR KO CD7 CAR-T cells expressing enhancer C7R or E3 (EGFRt+IL7Ra). FIG. 56A illustrates experimental data of fraction of CAR-T cells expressing enhancer. FIG. 56B illustrates cytotoxicity of TCR KO CD7 CAR-T cells. FIG. 56C illustrates proliferation data of CAR-T cells in the absence of IL2. FIG. 56D illustrates experimental data of fractions of CAR-T cells expressing enhancer and having phosphorylated STAT5. FIG. 56E illustrates a bar graph of mean fluorescent index (MFI) of phosphorylated STAT5 of FIG. 56D.

[0090] FIGS. 57A and 57B illustrate experimental data showing expansion and persistence of UCAR-T GC197 cells (e.g., multi-specific CAR-T cells having CARs targeting both CD19 and CD7). FIG. 57A illustrates proliferation data of UCAR-T GC197 cells expressing enhancer C7R from two different donors in the presence of cancer antigen stimulation. FIG. 57B illustrates proliferation data of UCAR-T GC197 cells expressing enhancer C7R in the absence of IL2 or antigen stimulation.

[0091] FIGS. 58A-C illustrate experimental data showing expansion and persistence of UCAR-T GC197 cells. FIG. 58A illustrates experimental data showing fractions of CAR-T cells expressing CD7 and the enhancer C7R or E3 (EGFRt+IL7Ra). FIG. 58B illustrates proliferation data of UCAR-T GC197 cells expressing enhancer C7R or E3 (EGFRt+IL7Ra). FIG. 58C illustrates proliferation data of UCAR-T GC197 cells expressing enhancer C7R or E3 (EGFRt+IL7Ra) in the presence of cancer antigen stimulation.

[0092] FIGS. 59A and 59B illustrate experimental data of K562-CAR19 profiling and natural killer (NK) cell killing assay. FIG. 59A illustrates experimental data of fractions of K562 cells expressing CAR (e.g., CAR19 or CAR targeting CD19) and enhancer CD47 or E4 (CD47+IL7Ra). FIG. 59B illustrates experimental data of cytotoxicity of NK cells toward K562-CAR19 expressing the enhancer CD47 or E4 (CD47+IL7Ra).

[0093] FIGS. 60A and 60B illustrate experimental data showing STAT5 phosphorylation and cell persistence of UCART19 cells (e.g., CAR-T cells having CARs targeting CD19, also referred to as CD19 CAR-T cells or CAR19

cells). FIG. 60A illustrates experimental data of fractions of TCR KO CAR19 cells having phosphorylated STAT5 and CAR19. FIG. 60B illustrates proliferation data of TCR KO CD19 CAR expressing enhancer C7R, CD47 or E4 (CD47+IL7Ra).

[0094] FIGS. 61A and 62B illustrate safety switch function of E2 and E3 in L719 cells. FIG. 61A illustrates safety switch function of E3 in L719-E3 cells. FIG. 61B illustrates safety switch function of E2 in L719-E2 cells.

[0095] FIGS. 62A and 62B illustrate safety switch function of E3 tested in CAR7-E3 and L719-E3 by Complement dependent cytotoxicity assay (CDC assay). FIG. 62A illustrates safety switch function of E3 tested in CAR7-E3 cells. FIG. 62B illustrates safety switch function of E3 tested in L719-E3 cells.

[0096] FIGS. 63A and 63B illustrate expression of IL2 and IFN γ in TCR/CD7 double knockout CAR7 cells with no cytokine signaling enhancement, or with mbIL15, C7R, or E3 expression. FIG. 63A illustrates expression of IL2 in TCR/CD7 double knockout CAR7 cells with no cytokine signaling enhancement, or with mbIL15, C7R, or E3 expression. FIG. 63B illustrates IFN γ in TCR/CD7 double knockout CAR7 cells with no cytokine signaling enhancement, or with mbIL15, C7R, or E3 expression.

[0097] FIG. 64 illustrates expression of IL2 and IFN γ in TCR/CD7 double knockout CD19/CD7 dual CAR-L719 cells with no cytokine signaling enhancement, or with C7R, or E3 expression.

[0098] FIG. 65 illustrates CD2 gRNA screening.

[0099] FIGS. 66A-C illustrate the in vivo GvHD study comparing CD19 CAR-T vs TCR KO CD19 CAR-T cells. FIG. 66A illustrates the in vivo GvHD study of CD19 CAR-T. FIG. 66B illustrates the in vivo GvHD study of TCR KO CD19 CAR-T cells. FIG. 66C illustrates the in vivo GvHD study comparing CD19 CAR-T vs TCR KO CD19 CAR-T cells.

[0100] FIGS. 67A-E illustrate the clinical data of CAR7-C7R for treating T-acute lymphoblastic leukemia (T-ALL) patients. FIG. 67A shows the kinetics of T-ALL elimination in the peripheral blood. FIG. 67B shows the CAR7-C7R cell concentration in the peripheral blood. FIG. 67C shows CAR7-C7R DNA copy/ug DNA of peripheral blood cells. FIG. 67D shows the granulocyte and lymphocyte (T and NK cells) recovery in the bone marrow. FIG. 67E shows the granulocyte and lymphocyte (T and NK cells) recovery in peripheral blood.

[0101] FIG. 68 illustrates BLI imaging of the in vivo efficacy of CAR7 and CAR7-E3 UCAR-T cells.

[0102] FIG. 69 illustrates BLI imaging of the in vivo efficacy of L719 and L719-C7R UCAR-T cells.

DETAILED DESCRIPTION

[0103] While various embodiments of the invention have been shown and described herein, it will be obvious to those skilled in the art that such embodiments are provided by way of example only. Numerous variations, changes, and substitutions may occur to those skilled in the art without departing from the invention. It should be understood that various alternatives to the embodiments of the invention described herein may be employed.

[0104] The term “about,” as used herein, refers to a value or composition within a range of acceptable tolerances for a particular value or composition as determined, which will depend in part on how the value or composition is measured

or measured, i.e., the limitations of the measurement system. For example, “about” can mean within 1 or more than 1 standard deviation, per the practice in the art. Alternatively, “about” can mean a range of up to 20%, up to 10%, up to 5%, or up to 1% of a given value. Alternatively, particularly with respect to biological systems or processes, the term can mean within an order of magnitude, preferably within 5-fold, and more preferably within 2-fold, of a value. Where particular values are described in the application and claims, unless otherwise stated, the term “about” meaning within an acceptable error range for the particular value should be assumed.

[0105] The term “administering,” as used herein, refers to physically introducing a product of the present disclosure into a subject using any of a variety of methods and delivery systems, including intravenous, intramuscular, subcutaneous, intraperitoneal, spinal or other routes of parenteral administration, for example by injection or infusion.

[0106] The term “antigen,” as used herein, refers to a molecule or a fragment thereof capable of being bound by a selective binding agent. As an example, an antigen can be a ligand that can be bound by a selective binding agent such as a receptor. As another example, an antigen can be an antigenic molecule that can be bound by a selective binding agent such as an immunological protein (e.g., an antibody). An antigen can also refer to a molecule or fragment thereof capable of being used in an animal to produce antibodies capable of binding to that antigen. In some cases, an antigen may be bound to a substrate (e.g., a cell membrane). Alternatively, an antigen may not be bound to a substrate (e.g., a secreted molecule, such as a secreted polypeptide).

[0107] The term “antibody (Ab),” as used herein, include, but is not limited to, an immunoglobulin that specifically binds to an antigen and comprises at least two heavy (H) chains and two light (L) chains interconnected by disulfide bonds, or an antigen thereof. Each H chain comprises a heavy chain variable region (abbreviated herein as VH) and a heavy chain constant region. The heavy chain constant region comprises three constant domains CH1, CH2 and CH3. Each light chain comprises a light chain variable region (abbreviated herein as VL) and a light chain constant region. The light chain constant region comprises a constant domain CL. The VH and VL regions can be further subdivided into hypervariable regions called complementarity determining regions (CDRs) interspersed with more conserved regions called framework regions (FR). Each VH and VL contains three CDRs and four FRs, arranged from the amino terminus to the carboxy terminus in the following order: FR1, CDR1, FR2, CDR2, FR3, CDR3, FR4. The variable regions of the heavy and light chains contain a binding domain that interacts with the antigen.

[0108] The term “nucleotide,” as used herein, generally refers to a base-sugar-phosphate combination. A nucleotide can comprise a synthetic nucleotide. A nucleotide can comprise a synthetic nucleotide analog. Nucleotides can be monomeric units of a nucleic acid sequence (e.g. deoxyribonucleic acid (DNA) and ribonucleic acid (RNA)). The term nucleotide can include ribonucleoside triphosphates adenosine triphosphate (ATP), uridine triphosphate (UTP), cytosine triphosphate (CTP), guanosine triphosphate (GTP) and deoxyribonucleoside triphosphates such as dATP, dCTP, dTTP, dUTP, dGTP, dTTP, or derivatives thereof. Such derivatives can include, for example, [aS]dATP, 7-deaza-dGTP and 7-deaza-dATP, and nucleotide derivatives that

confer nuclease resistance on the nucleic acid molecule containing them. The term nucleotide as used herein can refer to dideoxyribonucleoside triphosphates (ddNTPs) and their derivatives. Illustrative examples of dideoxyribonucleoside triphosphates can include, but are not limited to, ddATP, ddCTP, ddGTP, ddTTP, and ddTTP. A nucleotide can be unlabeled or detectably labeled by well-known techniques. Labeling can also be carried out with quantum dots. Detectable labels can include, for example, radioactive isotopes, fluorescent labels, chemiluminescent labels, bioluminescent labels and enzyme labels. Fluorescent labels of nucleotides can include but are not limited fluorescein, 5-carboxyfluorescein (FAM), 2'7'-dimethoxy-4'-dichloro-6-carboxyfluorescein (JOE), rhodamine, 6-carboxyrhodamine (R6G), N,N,N',N'-tetramethyl-6-carboxyrhodamine (TAMRA), 6-carboxy-X-rhodamine (ROX), 4-(4'-dimethylaminophenylazo) benzoic acid (DABCYL), Cascade Blue, Oregon Green, Texas Red, Cyanine and 5-(2'-aminoethyl) aminonaphthalene-1-sulfonic acid (EDANS). Specific examples of fluorescently labeled nucleotides can include [R6G]dUTP, [TAMRA]dUTP, [R110]dCTP, [R6G]dCTP, [TAMRA]dCTP, [JOE]ddATP, [R6G]ddATP, [FAM]ddCTP, [R110]ddCTP, [TAMRA]ddGTP, [ROX]ddTTP, [dR6G]ddATP, [dR110]ddCTP, [dTAMRA]ddGTP, and [dROX]ddTTP available from Perkin Elmer, Foster City, Calif., FluoroLink DeoxyNucleotides, FluoroLink Cy3-dCTP, FluoroLink Cy5-dCTP, FluoroLink Fluor X-dCTP, FluoroLink Cy3-dUTP, and FluoroLink Cy5-dUTP available from Amersham, Arlington Heights, Ill.; Fluorescein-15-dATP, Fluorescein-12-dUTP, Tetramethyl-rhodamine-6-dUTP, IR770-9-dATP, Fluorescein-12-ddUTP, Fluorescein-12-UTP, and Fluorescein-15-2'-dATP available from Boehringer Mannheim, Indianapolis, Ind.; and Chromosome Labeled Nucleotides, BODIPY-FL-14-UTP, BODIPY-FL-4-UTP, BODIPY-TMR-14-UTP, BODIPY-TMR-14-dUTP, BODIPY-TR-14-UTP, BODIPY-TR-14-dUTP, Cascade Blue-7-UTP, Cascade Blue-7-dUTP, fluorescein-12-UTP, fluorescein-12-dUTP, Oregon Green 488-5-dUTP, Rhodamine Green-5-UTP, Rhodamine Green-5-dUTP, tetramethylrhodamine-6-UTP, tetramethylrhodamine-6-dUTP, Texas Red-5-UTP, Texas Red-5-dUTP, and Texas Red-12-dUTP available from Molecular Probes, Eugene, Oreg. Nucleotides can also be labeled or marked by chemical modification. A chemically-modified single nucleotide can be biotin-dNTP. Some non-limiting examples of biotinylated dNTPs can include, biotin-dATP (e.g., bio-N6-ddATP, biotin-14-dATP), biotin-dCTP (e.g., biotin-11-dCTP, biotin-14-dCTP), and biotin-dUTP (e.g. biotin-11-dUTP, biotin-16-dUTP, biotin-20-dUTP).

[0109] The terms “polynucleotide,” “oligonucleotide,” and “nucleic acid” are used interchangeably to refer to a polymeric form of nucleotides of any length, either deoxyribonucleotides or ribonucleotides, or analogs thereof, either in single-, double-, or multi-stranded form. A polynucleotide can be exogenous or endogenous to a cell. A polynucleotide can exist in a cell-free environment. A polynucleotide can be a gene or fragment thereof. A polynucleotide can be DNA. A polynucleotide can be RNA. A polynucleotide can have any three dimensional structure, and can perform any function, known or unknown. A polynucleotide can comprise one or more analogs (e.g. altered backbone, sugar, or nucleobase). If present, modifications to the nucleotide structure can be imparted before or after assembly of the polymer. Some non-limiting examples of analogs include: 5-bromou-

racil, peptide nucleic acid, xeno nucleic acid, morpholinos, locked nucleic acids, glycol nucleic acids, threose nucleic acids, dideoxynucleotides, cordycepin, 7-deaza-GTP, fluorophores (e.g. rhodamine or fluorescein linked to the sugar), thiol containing nucleotides, biotin linked nucleotides, fluorescent base analogs, CpG islands, methyl-7-guanosine, methylated nucleotides, inosine, thiouridine, pseudouridine, dihydrouridine, queuosine, and wyosine. Non-limiting examples of polynucleotides include coding or non-coding regions of a gene or gene fragment, loci (locus) defined from linkage analysis, exons, introns, messenger RNA (mRNA), transfer RNA (tRNA), ribosomal RNA (rRNA), short interfering RNA (siRNA), short-hairpin RNA (shRNA), microRNA (miRNA), ribozymes, cDNA, recombinant polynucleotides, branched polynucleotides, plasmids, vectors, isolated DNA of any sequence, isolated RNA of any sequence, cell-free polynucleotides including cell-free DNA (cfDNA) and cell-free RNA (cfrRNA), nucleic acid probes, and primers. The sequence of nucleotides can be interrupted by non-nucleotide components.

[0110] The term “gene,” as used herein, refers to a nucleic acid (e.g., DNA such as genomic DNA and cDNA) and its corresponding nucleotide sequence that is involved in encoding an RNA transcript. The term as used herein with reference to genomic DNA includes intervening, non-coding regions as well as regulatory regions and can include 5' and 3' ends. In some uses, the term encompasses the transcribed sequences, including 5' and 3' untranslated regions (5'-UTR and 3'-UTR), exons and introns. In some genes, the transcribed region will contain “open reading frames” that encode polypeptides. In some uses of the term, a “gene” comprises only the coding sequences (e.g., an “open reading frame” or “coding region”) necessary for encoding a polypeptide. In some cases, genes do not encode a polypeptide, for example, ribosomal RNA genes (rRNA) and transfer RNA (tRNA) genes. In some cases, the term “gene” includes not only the transcribed sequences, but in addition, also includes non-transcribed regions including upstream and downstream regulatory regions, enhancers and promoters. A gene can refer to an “endogenous gene” or a native gene in its natural location in the genome of an organism. A gene can refer to an “exogenous gene” or a non-native gene. A non-native gene can refer to a gene not normally found in the host organism but which is introduced into the host organism by gene transfer. A non-native gene can also refer to a gene not in its natural location in the genome of an organism. A non-native gene can also refer to a naturally occurring nucleic acid or polypeptide sequence that comprises mutations, insertions and/or deletions (e.g., non-native sequence).

[0111] The term “endogenous,” as used herein, refers to a nucleic acid molecule or polypeptide normally expressed in a cell or tissue.

[0112] The term “exogenous,” as used herein, refers to the nucleic acid molecule or polypeptide is not endogenously present in the cell or is present at a level sufficient to achieve the functional effects obtained upon overexpression. Thus, the term “exogenous” includes any recombinant nucleic acid molecule or polypeptide expressed in a cell, e.g., a foreign, heterologous, and overexpressed nucleic acid molecule and polypeptide.

[0113] The term “T cell and NK cell consensus marker,” as used herein, refers to a marker co-existing on T cells and NK cells, including but not limited to: CD2, CD7, CD38,

CD45, CD48, CD50, CD52, CD56, CD69, CD100, CD122, CD132, CD161, CD159a, CD159c, CD314.

[0114] The term “marker of T cells and/or NK cells,” as used herein, refers to markers present in T cells or NK cells, respectively, or both T cells and NK cells, including but not limited to: CD2, CD3, CD4, CD5, CD7, CD8, CD16a, CD16b, CD25, CD27, CD28, CD38, CD45, CD48, CD50, CD52, CD56, CD57, CD62L, CD69, CD94, CD100, CD102, CD122, CD127, CD132, CD160, CD161 CD178, CD218, CD226, CD244, CD159a (NKG2A), CD159c (NKG2C), NKG2E, CD314 (NKG2D), CD305, CD335 (NKP46), CD337, SLAMF7.

[0115] The term “functionally inactivate” or “functional inactivation” as used herein refers to that a functional gene or the product of the gene such as mRNA or protein is prevented or inhibited. The inactivation may be achieved by deletion, addition or substitution of the gene or the promoter thereof, so that expression does not occur, or mutation of the coding sequence of the gene so that the gene product such as mRNA or protein is inactive. The functional inactivation may be complete or partial. Inactivation of a gene can encompass all degrees of inactivation, including gene silencing, knockout, inhibition and disruption. In some embodiments, the functional inactivation is introduced by CRISPR-Cas9 system.

[0116] The terms “subject,” “individual,” and “patient” are used interchangeably herein to refer to a vertebrate, preferably a mammal such as a human. Mammals include, but are not limited to, murines, simians, humans, farm animals, sport animals, and pets. Tissues, cells and their progeny of a biological entity obtained in vivo or cultured in vitro are also encompassed.

[0117] The terms “treatment” and “treating,” as used herein, refer to an approach for obtaining beneficial or desired results including but not limited to a therapeutic benefit and/or a prophylactic benefit. For example, a treatment can comprise administering a system or cell population disclosed herein. By therapeutic benefit is meant any therapeutically relevant improvement in or effect on one or more conditions (e.g., diseases or symptoms) under treatment. For prophylactic benefit, a composition can be administered to a subject at risk of developing a particular condition, or to a subject reporting one or more of the physiological symptoms of a disease, even though the condition may not have yet been manifested.

Overview

[0118] CARs can comprise an extracellular antigen recognition region, for example, a scFv (single-chain variable fragment), a transmembrane region, and an intracellular costimulatory signal region. The extracellular domain of CARs can recognize a specific antigen and then transduce the signal through the intracellular domain, causing T cell activation and proliferation, cytotoxicity, and secretion of cytokines, thereby eliminating target cells. The patient's autologous T cells (or heterologous donors) can be first isolated, activated and genetically engineered to produce CAR-T cells, which can be then injected into the same patient. In this way, the probability of graft-versus-host disease may be reduced, and the antigen can be recognized by T cells in a non-MHC-restricted manner. In addition, a CAR-T can treat all cancers that express the antigen.

[0119] The present disclosure provides compositions and methods to engineer a cell, e.g., an immune cell, such that

it can target both disease-associated antigen (e.g., tumor-associated antigen or tumor cell marker) and immune cell antigen (e.g., CD3, CD7 or CD137) through bispecific or multivalent CAR(s). For example, the present disclosure provides an engineered immune cell that can target a tumor cell marker and an immune cell antigen such as CD3. The endogenous TCR can be inactivated (e.g., disrupted, inhibited, knocked out or silenced). The CAR-T of the present disclosure which targets the tumor cell marker and the immune cell antigen can eliminate positive tumor cells and clear host immune cell antigen positive T and NK cells, thereby avoiding host rejection (HVG). In the present disclosure, the endogenous TCR of the engineered immune cell can be knocked out, and graft-versus-host disease (GVHD) can be prevented, thereby preparing a general-purpose or universal CAR-T (UCAR-T) cell. The engineered immune cell can be derived from an autologous T cell or an allogeneic T cell.

[0120] Moreover, the engineered immune cell can comprise a cell suicide element (e.g., inducible cell death moiety), and the CAR-T can be inactivated/cleared at any time to reduce side effects. In some cases, the engineered immune cell can further comprise an enhancer moiety. The enhancer moiety can regulate one or more activities of the engineered immune cell when the engineered immune cell is administered to a subject. For example, the enhancer moiety can be a cytokine (e.g., IL-5 or IL-7) or a cytokine receptor (e.g., IL-5R or IL-7R). The enhancer moiety can enhance a signaling pathway within the engineered immune cell, for example, STAT5 signaling pathway. In some embodiments, the engineered immune cell comprises a bispecific CAR targeting both CD19 and CD3. The engineered immune cell show in this example can further comprise an inducible cell death moiety such as a truncated epidermal growth factor receptor (EGFRt or tEGFR, which can be used interchangeably herein; see U.S. Pat. No. 9,447,194B2 and PCT Publication No. WO2018038945).

[0121] The inducible cell death moiety or the enhancer moiety can be introduced in the immune cell via a separate expression vector. In some cases, the inducible cell death moiety and the enhancer moiety may be introduced into the immune cell via an expression vector comprising sequences encoding both moieties. In some cases, the inducible cell death moiety and the enhancer moiety are linked and are expressed as a chimeric polypeptide.

[0122] The application of the engineered immune cells provided herein in cell therapy can treat the disease (e.g., cancer) of a patient, be prepared in large-scale in advance to avoid GVHD and HvG, reduce treatment costs, inactivate CAR-T at any time if necessary, reduce side effects of immunotherapy, and ensure product safety. The engineered cells provided herein can be referred to as universal CAR T cells (UCAR-T cells).

Chimeric Antigen Receptor (CAR)

[0123] The cell (e.g., immune cell or engineered immune cell) provided herein can comprise one or more CARs. The CAR can include an extracellular domain, a transmembrane domain, and an intracellular signaling domain. The extracellular domain can include a target-specific binding element (also known as an antigen binding domain). The intracellular domain can include a costimulatory signaling region and a zeta (ζ) chain portion. A costimulatory signaling region refers to a portion of the CAR comprising the

intracellular domain of a costimulatory molecule. Costimulatory molecules are cell surface molecules other than antigens receptors or their ligands that may be needed for an efficient response of lymphocytes to antigen. Between the extracellular domain and the transmembrane domain of the CAR, or between the cytoplasmic domain and the transmembrane domain of the CAR, there may be incorporated a spacer domain. As used herein, the term “spacer domain” generally means any oligo- or polypeptide that functions to link the transmembrane domain to, either the extracellular domain or, the cytoplasmic domain in the polypeptide chain. A spacer domain may comprise up to 300 amino acids, preferably 10 to 100 amino acids and most preferably 25 to 50 amino acids.

[0124] With respect to the transmembrane domain, the CAR can be designed to comprise a transmembrane domain that is fused to the extracellular domain of the CAR. In one embodiment, the transmembrane domain that naturally is associated with one of the domains in the CAR is used. In some instances, the transmembrane domain can be selected or modified by amino acid substitution to avoid binding of such domains to the transmembrane domains of the same or different surface membrane proteins to minimize interactions with other members of the receptor complex.

[0125] The transmembrane domain may be derived either from a natural or from a synthetic source. Where the source is natural, the domain may be derived from any membrane-bound or transmembrane protein. Transmembrane regions of particular use in the present disclosure may be derived from (e.g., comprise at least the transmembrane region(s) of) the alpha, beta or zeta chain of the T-cell receptor, CD28, CD3 epsilon, CD45, CD4, CD5, CD8, CD9, CD16, CD22, CD33, CD3 γ , CD64, CD80, CD86, CD134, CD137, CD154, or from an immunoglobulin such as IgG4. Alternatively the transmembrane domain may be synthetic, in which case it will comprise predominantly hydrophobic residues such as leucine and valine. Preferably a triplet of phenylalanine, tryptophan and valine will be found at each end of a synthetic transmembrane domain. Optionally, a short oligo- or polypeptide linker, preferably between 2 and 10 amino acids in length may form the linkage between the transmembrane domain and the cytoplasmic signaling domain of the CAR. A glycine-serine doublet provides a particularly suitable linker.

[0126] The cytoplasmic domain or otherwise the intracellular signaling domain of the CAR of the present disclosure can be responsible for activation of at least one of the normal effector functions of the immune cell in which the CAR has been placed in. The term “effector function” refers to a specialized function of a cell. Effector function of a T cell, for example, may be cytolytic activity or helper activity including the secretion of cytokines. Thus the term “intracellular signaling domain” refers to the portion of a protein which transduces the effector function signal and directs the cell to perform a specialized function. While usually the entire intracellular signaling domain can be employed, in many cases it is not necessary to use the entire chain. To the extent that a truncated portion of the intracellular signaling domain is used, such truncated portion may be used in place of the intact chain as long as it transduces the effector function signal. The term intracellular signaling domain is thus meant to include any truncated portion of the intracellular signaling domain sufficient to transduce the effector function signal.

[0127] Examples of intracellular signaling domains for use in the CAR of the present disclosure include the cytoplasmic sequences of the TCR and co-receptors that act in concert to initiate signal transduction following antigen receptor engagement, as well as any derivative or variant of these sequences and any synthetic sequence that has the same functional capability.

[0128] Signals generated through the TCR alone may be insufficient for full activation of the T cell and that a secondary or co-stimulatory signal may be included. Thus, T cell activation can be said to be mediated by two distinct classes of cytoplasmic signaling sequence: those that initiate antigen-dependent primary activation through the TCR (primary cytoplasmic signaling sequences) and those that act in an antigen-independent manner to provide a secondary or co-stimulatory signal (secondary cytoplasmic signaling sequences).

[0129] Primary cytoplasmic signaling sequences can regulate primary activation of the TCR complex either in a stimulatory way, or in an inhibitory way. Primary cytoplasmic signaling sequences that act in a stimulatory manner may contain signaling motifs which are known as immunoreceptor tyrosine-based activation motifs or ITAMs. Examples of ITAM containing primary cytoplasmic signaling sequences that are of particular use in the present disclosure include those derived from TCR zeta, FcR gamma, FcR beta, CD3 gamma, CD3 delta, CD3 epsilon, CD5, CD22, CD79a, CD79b, and CD66d. It is particularly preferred that cytoplasmic signaling molecule in the CAR of the present disclosure comprises a cytoplasmic signaling sequence derived from CD3-zeta.

[0130] In some embodiments, the cytoplasmic domain of the CAR can be designed to comprise the CD3-zeta signaling domain by itself or combined with any other desired cytoplasmic domain(s) useful in the context of the CAR of the present disclosure. For example, the cytoplasmic domain of the CAR can comprise a CD3 zeta chain portion and a costimulatory signaling region. The costimulatory signaling region refers to a portion of the CAR comprising the intracellular domain of a costimulatory molecule. A costimulatory molecule is a cell surface molecule other than an antigen receptor or its ligands that may be needed for an efficient response of lymphocytes to an antigen. Examples of such molecules include CD27, CD28, 4-1BB (CD137), OX40, CD30, CD40, PD-1, ICOS, lymphocyte function-associated antigen-1 (LFA-1), CD2, CD7, LIGHT, NKG2C, B7-H3, and a ligand that specifically binds with CD83, and the like. Thus, while the present disclosure is, in some cases, exemplified with 4-1BB as the co-stimulatory signaling element, other costimulatory elements are within the scope of the present disclosure.

[0131] The cytoplasmic signaling sequences within the cytoplasmic signaling portion of the CAR of the present disclosure may be linked to each other in a random or specified order. Optionally, a short oligo- or polypeptide linker, preferably between 2 and 10 amino acids in length may form the linkage. A glycine-serine doublet provides a particularly suitable linker.

[0132] In some embodiments, the cytoplasmic domain is designed to comprise the signaling domain of CD3-zeta and the signaling domain of CD28. In another embodiment, the cytoplasmic domain is designed to comprise the signaling domain of CD3-zeta and the signaling domain of 4-1BB. In yet another embodiment, the cytoplasmic domain is

designed to comprise the signaling domain of CD3-zeta and the signaling domain of CD28 and 4-1BB.

[0133] A CAR provided herein can comprise one or more antigen binding domains. In some cases, a CAR provided herein comprises an antigen binding domain that can target both an immune cell antigen (e.g., to inhibit killing activity of a T cell or NK cell) and a disease-associated antigen (e.g., a tumor-associated antigen). For example, an antigen binding domain targeting both immune cell antigens and cancer antigens include, but not limited to, CD2, CD3, CD4, CD5, CD7, CD8, CD30, CD38, CD45, CD48, CD50, CD52, CD56, CD69, CD100, CD122, CD132, CD137, CD161, CD159a, CD159c, CD279, CD314, CD319 (CS1) and TCR.

[0134] In some cases, a CAR provided herein comprises two antigen binding domains such that one individual CAR is a bispecific CAR, targeting two different antigens. For bispecific CAR, one antigen binding domain can target immune cell antigen, and the other antigen binding domain can target disease-associated antigen. The two antigen binding domains of a bispecific CAR can have a tandem structure, a parallel structure or a loop structure.

[0135] For example, a CAR can target a tumor cell marker and CD3. The CAR can have a structure as formula I. L-scFv1-I-scFv2-H-TM-C-CD3 (I), wherein each “-” is independently a linker peptide or a peptide bond; L is optionally a signaling peptide sequence; I is a flexible linker; H is optionally a hinge region; TM is a transmembrane domain; C is a costimulatory domain; CD3 ζ is a cytoplasmic signaling sequence derived from CD3 ζ ; one of scFv1 and scFv2 is an antigen binding domain targeting a tumor cell marker, and the other one is an antigen binding domain targeting CD3. The CAR can have a structure as formula II or II': L-VL-scFv-VH-H-TM-C-CD3 ζ (II), L-VH-scFv-VL-H-TM-C-CD3 ζ (II'), wherein each “-” is independently a linker peptide or a peptide bond; the elements L, H, TM, C and CD3 ζ as described above; scFv is an antigen binding domain targeting a tumor cell marker, VH is an anti-CD3 antibody heavy chain variable region, and VL is an anti-CD3 antibody light chain variable region; or scFv is an antigen binding domain targeting CD-3, VH is an anti-tumor cell marker antibody heavy chain variable region, and VL is an anti-tumor cell marker antibody light chain variable region.

[0136] In some cases, a CAR can comprise the structure of EGFRt-CD3 scFv-CD19 scFv-Hinge-TM-CD28/41BB-CD33, wherein EGFRt is a truncated EGFR, as a safety switch (e.g., inducible cell death moiety), CD3 scFv is the svFCv fragment of the heavy and light chain variable regions of the monoclonal antibody OKT3 or UCHT1 linked by a GS linker, and the CD19 scFv fragment is the heavy and light chain variable region of the monoclonal FMC63 antibody linked by a GS linker. The structure of the CAR can further comprise a hinge, transmembrane regions, costimulatory signaling region of CD28 or 41BB, and/or CD3 (intracellular domain). In the present disclosure, the nucleic acid construct of EGFRt-CD3 scFv-CD19 scFv-Hinge-TM-CD28/41BB-CD3 ζ can be inserted into a vector (e.g., a lentiviral vector). The vector can be packaged in 293T cells. T cells can be sorted from PBMC, and after activation, TCR and PD-1 genes can be knocked out by CRISPR/CAS technology. T cells can then be infected with the vectors to express the CARs. The prepared CAR-T cells can be used to detect the infection efficiency and gene editing efficiency of CAR by flowcytometry.

[0137] The immune cell marker, e.g., CD3, of the above examples can be replaced with other immune cell markers such as CD7 and CD137.

[0138] In some cases, a CAR comprising two antigen binding domains arranged in a tandem form. In some embodiments, the first antigen binding domain and the second antigen binding domain is arranged, from amino terminus to carboxyl terminus, as: (i) VL2-VH2-VL1-VH1; (ii) VL2-VH2-VH1-VL1; (iii) VL1-VH1-VL2-VH2; (iv) VL1-VH1-VH2-VL2; (v) VH2-VL2-VL1-VH1; (vi) VH2-VL2-VH1-VL1; (vii) VH1-VL1-VL2-VH2; or (viii) VH1-VL1-VH2-VL2, wherein VH1 is heavy chain variable domain of the first antigen binding domain, VL1 is light chain variable light domain of the first antigen binding domain, VH2 is heavy chain variable domain of the second antigen binding domain, and VL2 is light chain variable domain of the second antigen binding domain. For example, the CAR can have a structure represented by the following formula IV or IV': L3-scFv1-R-scFv2-H3-TM3-C3-CD3 (IV); L3-scFv2-R-scFv1-H3-TM3-C3-CD3 ζ (IV'), wherein each "-" is independently a linker peptide or peptide bond; L3 is an optional signal peptide sequence; scFv1 is an antigen binding domain that targets tumor cell markers; R is a rigid or flexible joint; scFv2 is an antigen binding domain (e.g., an antibody single-chain variable region sequence) that targets T cell and NK cell consensus markers; H3 is an optional hinge region; TM3 is a transmembrane domain; C3 is a costimulatory domain; CD3 ζ is a cytoplasmic signaling sequence derived from CD3 ζ .

[0139] In some cases, a CAR comprising two antigen binding domains arranged in a loop form. In some cases, the first antigen binding domain and the second antigen binding domain is arranged, from amino terminus to carboxyl terminus, as: (i) VL2-VH1-VL1-VH2; (ii) VH2-VL1-VH1-VL2; (iii) VL1-VH2-VL2-VH1; (iv) VH1-VL2-VH2-VL1; (v) VL2-VL1-VH1-VH2; (vi) VH2-VH1-VL1-VL2; (vii) VL1-VL2-VH2-VH1; or (viii) VH1-VH2-VL2-VL1, wherein VH1 is heavy chain variable domain of the first antigen binding domain, VL1 is light chain variable light domain of the first antigen binding domain, VH2 is heavy chain variable domain of the second antigen binding domain, and VL2 is light chain variable domain of the second antigen binding domain. For example, the CAR can have the following formula VI, VI', VI'' or VI''' structure: L8-VL1-VH2-I-VL2-VH1-H8-TM8-C8-CD3 (VI); L8-VH1-VL2-I-VH2-VL1-H8-TM8-C8-CD3 ζ (VI'); L8-VL2-VH1-I-VL1-VH2-H8-TM8-C8-CD3 (VI''); L8-VH2-VL1-I-VH1-VL2-H8-TM8-C8-CD3 ζ (VI'''), wherein each "-" is independently a linker peptide or peptide bond; L8 is an optional signal peptide sequence; VH1 is an anti-tumor cell marker antibody heavy chain variable region, and VL1 is an anti-tumor cell marker antibody light chain variable region; VH2 is an anti-T cell and NK cell consensus marker (such as CD7 or CD2) antibody heavy chain variable region; and VL2 is an anti-T cell and NK cell consensus marker (such as CD7 or CD2) antibody light chain variable region; I is a flexible joint; H8 is an optional hinge region; TM8 is a transmembrane domain; C8 is a costimulatory domain; CD3 ζ is a cytoplasmic signaling sequence derived from CD3 ζ .

[0140] In some cases, a CAR comprising two antigen binding domains are arranged in a parallel form. The parallel form can comprise a full construct of a first CAR having a first antigen binding domain linked to a full construct of a

second CAR having a second antigen binding domain. An example of parallel form can be tEGFR-CD19 scFv-CD28-CD3 ζ -CD3 scFv-41BB-CD3 ζ . The tEGFR shown here can function as a safety switch, which can be replaced by other safety switches as described in the present disclosure. As described herein, CD19 scFv and CD3 scFv are two examples of antigen binding domains, which may be replaced with various antigen binding domains as described in the present disclosure. CD28 can be an example of transmembrane domain and can be replaced with other transmembrane domains described herein. 41BB can be an example of co-stimulatory domain and can be replaced with other co-stimulatory domains described herein. In some cases, a linker is used to link the first CAR and the second CAR. The linker can be a cleavable linker. The cleavable linker can be self-cleaving peptide such as 2A self-cleaving peptide.

[0141] Also contemplated in the present disclosure is a nucleic acid molecule encoding a CAR or a bispecific CAR. The nucleic acid can comprise a first sequence encoding a chimeric antigen receptor (CAR), wherein the CAR can comprise a binding moiety, which binding moiety comprises (i) a first antigen binding domain, which first antigen binding domain suppresses or reduces a subject's immune response toward the engineered immune cell when administered into the subject linked to (ii) a second antigen binding domain capable of binding to a disease-associated antigen, and wherein each CAR of the one or more CARs can further comprise a transmembrane domain and an intracellular signaling domain. The first antigen binding domain can target an immune cell antigen selected from the group consisting of CD2, CD3, CD4, CD5, CD7, CD8, CD16a, CD16b, CD25, CD27, CD28, CD30, CD38, CD45, CD48, CD50, CD52, CD56, CD57, CD62L, CD69, CD94, CD100, CD102, CD122, CD127, CD132, CD137, CD160, CD161, CD178, CD218, CD226, CD244, CD159a (NKG2A), CD159c (NKG2C), NKG2E, CD279, CD314 (NKG2D), CD305, CD335 (NKP46), CD337, CD319 (CS1), TCR α , TCR β and SLAMF7. The second antigen binding domain can target a disease-associated antigen such as CD19. Other non-limiting examples of disease-associated antigen includes BCMA, VEGFR2, CD19, CD20, CD30, CD22, CD25, CD28, CD30, CD33, CD52, CD56, CD80, CD86, CD81, CD123, cd171, CD276, B7H4, CD133, EGFR, GPC3; PMSA, CD 3, CEACAM6, c-Met, EGFRvIII, ErbB2, ErbB3 HER-2, HER3, ErbB4/HER-4, EphA2, IGF1R, GD2, O-acetyl GD2, O-acetyl GD3, GHRHR, GHR, Flt1, KDR, Flt4, CD44V6, CEA, CA125, CD151, CTLA-4, GITR, BTLA, TGFBR2, TGFBR1, IL6R, gp130, Lewis, TNFR1, TNFR2, PD1, PD-L1, PD-L2, HVEM, MAGE-A, Mesothelin, NY-ESO-1, PSMA, RANK, RORI, TNFRSF4, CD40, CD137, TWEAK-R, LTPR, LIFRP, LRP5, MUC1, TCR α , TCR β , TLR7, TLR9, PTCH1, WT-1, Robl, Frizzled, OX40, CD79b, Claudin 18.2, Folate receptor α , Folate receptor β , GPC2, CD70, BAFF-R and Notch-1-4.

[0142] The nucleic acid molecule can further comprise a second sequence encoding an enhancer moiety, which enhancer moiety can enhance one or more activities of the CAR when expressed in a cell. The enhancer moiety can be selected from the group consisting of IL-2, IL-3, IL-4, IL-6, IL-7, IL-8, IL-10, IL-11, IL-12, IL-15, IL-17, IL-18, IL-21, IL-23, PD-1, PD-L1, CD122, CSF1R, CTAL-4, TIM-3, CCL21, CCL19, TGFR beta, receptors for the same, functional fragments thereof, functional variants thereof, and

combinations thereof. The nucleic acid molecule can further comprise a second sequence encoding an inducible cell death moiety, which inducible cell death moiety, when expressed in a cell, can effect death of the cell upon contacting the inducible cell death moiety with a cell death activator. The inducible cell death moiety can be selected from the group consisting of rapaCasp9, iCasp9, HSV-TK, Δ CD20, mTMPK, Δ CD19, RQR8, and EGFRt.

[0143] The nucleic acid molecule can further comprise a third sequence flanked by the first sequence and the second sequence, wherein the third sequence can encode a cleavable linker. The cleavable linker can be a self-cleaving peptide.

[0144] The nucleic acid molecule can further comprise a regulatory sequence regulating expression of the first sequence and/or the second sequence.

[0145] Also contemplated in the present disclosure is a kit comprising the nucleic acid molecule described herein.

[0146] In some cases, the nucleic acid encoding the CAR described herein can be delivered into an immune cell for expression of the CAR to generate an engineered cell.

Source Cell

[0147] The present disclosure provides an engineered cell, such as an engineered immune cell. The engineered immune cell can be prepared from a cell (e.g., an immune cell) isolated from a sample obtained from a subject. The engineered immune cell can be prepared from a cell line cell. The immune cell used to prepare the engineered immune cell can be a T cell, a B cell, a natural killer (NK) cell or a macrophage. The immune cell used to prepare the engineered immune cell can be an innate lymphocyte (ILC).

[0148] The immune cell used to prepare the engineered immune cell can be a stem cell. The stem cell can be a hematopoietic stem cell (HSC) or an induced pluripotent stem cell (iPSC).

[0149] The immune cell may comprise a T-cell receptor (TCR). The TCR can be endogenous TCR of the immune cell. In some cases, the endogenous TCR can be inactivated. For example, a gene encoding a subunit of the TCR can be inactivated. For example, the immune cell can be an alpha beta T cells with impaired TCRs such that the immune cells can avoid GVHD. For another example, the function of the endogenous TCR can be inhibited by an inhibitor such as TCR-derived peptides, peptides derived from amino acid sequences of fusion and other protein regions of various viruses, antibodies and small molecule inhibitors. The viruses from which the TCR inhibiting peptides can be derived from include, but are not limited to, severe acute respiratory syndrome coronavirus (SARS-CoV), herpesvirus saimiri (HVS), human herpesvirus 6 (HHV-6), Lassa virus (LASV), lymphocytic choriomeningitis virus (LCMV), Mopeia virus (MOPV), Tacaribe virus (TACV), Friend murine leukemia virus (MLV); human T lymphotropic virus type 1 (HTLV-1); herpesvirus ateles (HVA); Marburg virus (MARV); Sudan Ebola virus (SEBOV); and Zaire Ebola virus (ZEBOV).

[0150] In some cases, the immune cells can be T cells containing TCRs that may not cause GVHD responses. For example, the immune cell can be an alpha beta T cell with TCRs that can recognize specific antigens such as viral specific antigen, tumor-associated antigens (TAAs) or tumor-specific antigens (TSAs). For another example, the immune cell can be a gamma delta T cell or a natural killer T (NKT) cell. For another example, the immune cell can be

induced pluripotent stem cells produced from antigen-specific T cells (e.g., antigen-specific cytotoxic T cells). The immune cell can be cord-blood T cells.

[0151] The immune cell may comprise a cell surface marker. The cell surface marker can be an immune cell antigen. The gene encoding the immune cell antigen of the immune cell used for preparing the engineered immune cell can be inactivated. Examples of immune cell antigens include, but are not limited to, CD2, CD3, CD4, CD5, CD7, CD8, CD16a, CD16b, CD25, CD27, CD28, CD30, CD38, CD45, CD48, CD50, CD52, CD56, CD57, CD62L, CD69, CD94, CD100, CD102, CD122, CD127, CD132, CD137, CD160, CD161, CD178, CD218, CD226, CD244, CD159a (NKG2A), CD159c (NKG2C), NKG2E, CD279, CD314 (NKG2D), CD305, CD335 (NKP46), CD337, CD319 (CS1), TCR α , TCR β and SLAMF7. For example, in some cases, the gene encoding CD7 of the immune cell is inactivated. In some cases, the gene encoding CD3 of the immune cell is inactivated. In some cases, the gene encoding CD137 of the immune cell is inactivated.

[0152] The immune cells can be isolated from a sample from a subject. The subject can be a healthy donor. The subject can have a condition (e.g., a disease such as cancer). The sample can be a bodily fluid or a tissue, including but not limited to, peripheral blood mononuclear cells, bone marrow, lymph node tissue, cord blood, thymus tissue, tissue from a site of infection, ascites, pleural effusion, spleen tissue, and tumors. In some cases, a sample comprises NK cells, NKT cells, T-cells or T-cell progenitor cells. For example, in some cases, the sample is an umbilical cord blood sample, a peripheral blood sample (e.g., a mononuclear cell fraction) or a sample from the subject comprising pluripotent cells. In some aspects, a sample from the subject can be cultured to generate induced pluripotent stem (iPS) cells and these cells used to produce NK cells, NKT cells or T-cells. Cell samples may be cultured directly from the subject or may be cryopreserved prior to use. In some aspects, obtaining a cell sample comprises collecting a cell sample. In other aspects, the sample is obtained by a third party. In still further aspects, a sample from a subject can be treated to purify or enrich the T-cells or T-cell progenitors in the sample. For example, the sample can be subjected to gradient purification, cell culture selection and/or cell sorting (e.g., via fluorescence-activated cell sorting (FACS)).

[0153] The immune cell can be an NK cell. The NK cells can be obtained from peripheral blood, cord-blood, or other sources described herein. The NK cells can be derived from induced pluripotent stem cells.

[0154] In some embodiments, a cell that can be utilized in a method provided herein can be positive or negative for a given factor. In some embodiments, a cell utilized in a method provided herein can be a CD3+ cell, CD3- cell, a CD5+ cell, CD5- cell, a CD7+ cell, CD7- cell, a CD14+ cell, CD14- cell, CD8+ cell, a CD8- cell, a CD103+ cell, CD103- cell, CD11b+ cell, CD11b- cell, a BDCA1+ cell, a BDCA1- cell, an L-selectin+ cell, an L-selectin- cell, a CD25+ cell, a CD25- cell, a CD27+ cell, a CD27- cell, a CD28+ cell, CD28- cell, a CD44+ cell, a CD44- cell, a CD56+ cell, a CD56- cell, a CD57+ cell, a CD57- cell, a CD62L+ cell, a CD62L- cell, a CD69+ cell, a CD69- cell, a CD45RO+ cell, a CD45RO- cell, a CD127+ cell, a CD127- cell, a CD132+ cell, a CD132- cell, an IL-7+ cell, an IL-7- cell, an IL-15+ cell, an IL-15- cell, a lectin-like receptor G1 positive cell, a lectin-like receptor G1 negative cell, or an differen-

tiated or de-differentiated cell thereof. The examples of factors expressed by cells is not intended to be limiting, and a person having skill in the art will appreciate that a cell may be positive or negative for any factor known in the art. In some embodiments, a cell may be positive for two or more factors. For example, a cell may be CD4+ and CD8+. In some embodiments, a cell may be negative for two or more factors. For example, a cell may be CD25-, CD44-, and CD69-. In some embodiments, a cell may be positive for one or more factors, and negative for one or more factors. For example, a cell may be CD4+ and CD8-. In some aspects, a cellular marker provided herein can be utilized to select, enrich, or deplete a population of cells. In some aspects, enriching comprises selecting a monocyte fraction. In some aspects, enriching comprises sorting a population of immune cells from a monocyte fraction. In some embodiments, the cells may be selected for having or not having one or more given factors (e.g., cells may be separated based on the presence or absence of one or more factors). In some embodiments, the selected cells can also be transduced and/or expanded in vitro. The selected cells can be expanded in vitro prior to infusion. In some embodiments, selected cells can be transduced with a vector provided herein. It should be understood that cells used in any of the methods disclosed herein may be a mixture (e.g., two or more different cells) of any of the cells disclosed herein. For example, a method of the present disclosure may comprise cells, and the cells are a mixture of CD4+ cells and CD8+ cells. In another example, a method of the present disclosure may comprise cells, and the cells are a mixture of CD4+ cells and naïve cells. In some cases, a cell can be a stem memory TSCM cell comprised of CD45RO (-), CCR7(+), CD45RA (+), CD62L+(L-selectin), CD27+, CD28+ and IL-7Ra+, stem memory cells can also express CD95, IL-2RP, CXCR3, and LFA-1, and show numerous functional attributes distinctive of stem memory cells. Cells provided herein can also be central memory TCM cells comprising L-selectin and CCR7, where the central memory cells can secrete, for example, IL-2, but not IFN γ or IL-4. Cells can also be effector memory TEM cells comprising L-selectin or CCR7 and produce, for example, effector cytokines such as IFN γ and IL-4. In some cases, a population of cells can be introduced to a subject. For example, a population of cells can be a combination of T cells and NK cells. In other cases, a population can be a combination of naïve cells and effector cells. A population of cells can be TILs.

[0155] The source immune cells can be T cells. The T cells can be alpha beta T cells or gamma delta T cells. T cells can be obtained from a number of sources, including peripheral blood mononuclear cells, bone marrow, lymph node tissue, cord blood, thymus tissue, tissue from a site of infection, ascites, pleural effusion, spleen tissue, and tumors. In certain embodiments of the present disclosure, various T cell lines may be used. In certain embodiments of the present disclosure, T cells can be obtained from a unit of blood collected from a subject using any number of techniques known to the skilled artisan, such as Ficoll™ separation. In some embodiments, cells from the circulating blood of an individual are obtained by apheresis. The apheresis product typically contains lymphocytes, including T cells, monocytes, granulocytes, B cells, other nucleated white blood cells, red blood cells, and platelets. In some embodiments, the cells collected by apheresis may be washed to remove the plasma fraction and to place the cells in an appropriate buffer or media for

subsequent processing steps. In some embodiments of the present disclosure, the cells are washed with phosphate buffered saline (PBS). In an alternative embodiment, the wash solution lacks calcium and may lack magnesium or may lack many if not all divalent cations. Initial activation steps in the absence of calcium may lead to magnified activation. A washing step may be accomplished by methods such as by using a semi-automated “flow-through” centrifuge (for example, the Cobe 2991 cell processor, the Baxter CytoMate, or the Haemonetics Cell Saver 5) according to the manufacturer’s instructions. After washing, the cells may be resuspended in a variety of biocompatible buffers, such as, for example, Ca2+-free, Mg2+-free PBS, PlasmaLyte A, or other saline solution with or without buffer. Alternatively, the undesirable components of the apheresis sample may be removed and the cells directly resuspended in culture media.

[0156] In another embodiment, T cells are isolated from peripheral blood lymphocytes by lysing the red blood cells and depleting the monocytes, for example, by centrifugation through a PERCOLL™ gradient or by counterflow centrifugal elutriation. A specific subpopulation of T cells, such as CD3+, CD28+, CD4+, CD8+, CD45RA+, and CD45RO+ T cells, can be further isolated by positive or negative selection techniques. For example, in one embodiment, T cells are isolated by incubation with anti-CD3/anti-CD28 (i.e., 3 $\times$ 28)-conjugated beads, such as DYNABEADS® M-450 CD3/CD28 T, for a time period sufficient for positive selection of the desired T cells. In one embodiment, the time period is about 30 minutes. In a further embodiment, the time period ranges from 30 minutes to 36 hours or longer and all integer values there between. In a further embodiment, the time period is at least 1, 2, 3, 4, 5, or 6 hours. In yet another preferred embodiment, the time period is 10 to 24 hours. In some embodiments, the incubation time period is 24 hours. For isolation of T cells from patients with leukemia, use of longer incubation times, such as 24 hours, can increase cell yield. Longer incubation times may be used to isolate T cells in any situation where there are few T cells as compared to other cell types. Further, use of longer incubation times can increase the efficiency of capture of CD8+ T cells. Thus, by simply shortening or lengthening the time T cells are allowed to bind to the CD3/CD28 beads and/or by increasing or decreasing the ratio of beads to T cells (as described further herein), subpopulations of T cells can be preferentially selected for or against at culture initiation or at other time points during the process. Additionally, by increasing or decreasing the ratio of anti-CD3 and/or anti-CD28 antibodies on the beads or other surface, subpopulations of T cells can be preferentially selected for or against at culture initiation or at other desired time points. In some cases, multiple rounds of selection can also be used. In certain embodiments, it may be useful to perform the selection procedure and use the “unselected” cells in the activation and expansion process. “Unselected” cells can also be subjected to further rounds of selection.

[0157] Enrichment of a T cell population by negative selection can be accomplished with a combination of antibodies directed to surface markers unique to the negatively selected cells. An example method can be cell sorting and/or selection via negative magnetic immunoadherence or flow cytometry that uses a cocktail of monoclonal antibodies directed to cell surface markers present on the cells negatively selected. For example, to enrich for CD4+ cells by negative selection, a monoclonal antibody cocktail typically

includes antibodies to CD14, CD20, CD11b, CD16, HLA-DR, and CD8. In certain embodiments, it may be desirable to enrich for or positively select for regulatory T cells which typically express CD4+, CD25+, CD62Lhi, GITR+, and FoxP3+. Alternatively, in certain embodiments, T regulatory cells are depleted by anti-CD25 conjugated beads or other similar method of selection.

[0158] For isolation of a desired population of cells by positive or negative selection, the concentration of cells and surface (e.g., particles such as beads) can be varied. In certain embodiments, it may be desirable to significantly decrease the volume in which beads and cells are mixed together (i.e., increase the concentration of cells), to ensure maximum contact of cells and beads. For example, in one embodiment, a concentration of 2 billion cells/ml is used. In one embodiment, a concentration of 1 billion cells/ml is used. In a further embodiment, greater than 100 million cells/ml is used. In a further embodiment, a concentration of cells of 10, 15, 20, 25, 30, 35, 40, 45, or 50 million cells/ml is used. In yet another embodiment, a concentration of cells from 75, 80, 85, 90, 95, or 100 million cells/ml is used. In further embodiments, concentrations of 125 or 150 million cells/ml can be used. Using high concentrations can result in increased cell yield, cell activation, and cell expansion. Further, use of high cell concentrations allows more efficient capture of cells that may weakly express target antigens of interest, such as CD28-negative T cells, or from samples where there are many tumor cells present (i.e., leukemic blood, tumor tissue, etc.). Such populations of cells may have therapeutic value and would be desirable to obtain. For example, using high concentration of cells allows more efficient selection of CD8+ T cells that normally have weaker CD28 expression.

[0159] In a related embodiment, lower concentrations of cells may be used. By significantly diluting the mixture of T cells and surface (e.g., particles such as beads), interactions between the particles and cells is minimized. This method can select for cells that express high amounts of desired antigens to be bound to the particles. For example, CD4+ T cells express higher levels of CD28 and are more efficiently captured than CD8+ T cells in dilute concentrations. In some embodiments, the concentration of cells used is 5×10^6 /ml. In other embodiments, the concentration used can be from about 1×10^5 /ml to 1×10^6 /ml, and any integer value in between. In other embodiments, the cells may be incubated on a rotator for varying lengths of time at varying speeds at either $2-10^\circ \text{C}$. or at room temperature.

[0160] T cells for stimulation can also be frozen after a washing step. Wishing not to be bound by theory, the freeze and subsequent thaw step provides a more uniform product by removing granulocytes and to some extent monocytes in the cell population. After the washing step that removes plasma and platelets, the cells may be suspended in a freezing solution. While many freezing solutions and parameters are known in the art and will be useful in this context, one method involves using PBS containing 20% DMSO and 8% human serum albumin, or culture media containing 10% Dextran 40 and 5% Dextrose, 20% Human Serum Albumin and 7.5% DMSO, or 31.25% Plasmalyte-A, 31.25% Dextrose 5%, 0.45% NaCl, 10% Dextran 40 and 5% Dextrose, 20% Human Serum Albumin, and 7.5% DMSO or other suitable cell freezing media containing for example, Hespan and Plasmalyte A, the cells then are frozen to -80°C . at a rate of 1° per minute and stored in the vapor phase of a liquid

nitrogen storage tank. Other methods of controlled freezing may be used as well as uncontrolled freezing immediately at -20°C . or in liquid nitrogen.

[0161] In some embodiments, cryopreserved cells are thawed and washed as described herein and allowed to rest for one hour at room temperature prior to activation using the methods of the present disclosure.

[0162] In some embodiments, the cells are isolated from a blood sample or an apheresis from a subject prior to any number of relevant treatment modalities, including but not limited to treatment with agents such as natalizumab, efalizumab, antiviral agents, chemotherapy, radiation, immunosuppressive agents, such as cyclosporin, azathioprine, methotrexate, mycophenolate, and FK506, antibodies, or other immunoablative agents such as CAMPATH, anti-CD3 antibodies, cytoxan, fludarabine, cyclosporin, FK506, rapamycin, mycophenolic acid, steroids, FR901228, and irradiation. These drugs inhibit either the calcium dependent phosphatase calcineurin (cyclosporine and FK506) or inhibit the p70S6 kinase that is important for growth factor induced signaling (rapamycin) (Liu et al., Cell 66:807-815, 1991; Henderson et al., Immun. 73:316-321, 1991; Bierer et al., Curr. Opin. Immun. 5:763-773, 1993). In a further embodiment, the cells are isolated for a patient and frozen for later use in conjunction with (e.g., before, simultaneously or following) bone marrow or stem cell transplantation, T cell ablative therapy using either chemotherapy agents such as, fludarabine, external-beam radiation therapy (XRT), cyclophosphamide, or antibodies such as OKT3 or CAMPATH. In another embodiment, the cells are isolated prior to and can be frozen for later use for treatment following B-cell ablative therapy such as agents that react with CD20, e.g., Rituxan.

Engineered Immune Cell

[0163] The engineered immune cell provided herein can exhibit enhanced activity toward tumor cells, but with reduced side effects such as GVHD. The engineered immune cell can target a disease-associated antigen (e.g., tumor-associated antigen, or tumor cell marker) and at the same time suppress host immune cells. One or more endogenous genes (e.g., a gene encoding a subunit of a TCR, or a gene encoding a cell surface marker) of the engineered immune cell can be inactivated. In some cases, the engineered immune cell comprises a first CAR and a second CAR, each targeting a different antigen. In some cases, the engineered immune cell comprises a CAR having a first antigen binding domain and a second antigen binding domain.

[0164] The engineered immune cell can comprise one or more chimeric antigen receptors (CARs) comprising a binding moiety. The binding moiety can comprise a first antigen binding domain capable of binding to an immune cell antigen and a second antigen binding domain capable of binding to a disease-associated antigen. Each CAR of the one or more CARs may further comprise a transmembrane domain and an intracellular signaling domain. The engineered immune cell can also comprise an enhancer moiety capable of enhancing one or more activities of the engineered immune cell. The endogenous T cell receptor (TCR) of the engineered immune cell can be inactivated. In some cases, the engineered immune cell can exhibit (i) enhanced degree of persistence by remaining viable in vitro for at least about 20 days while in presence of cells that are heterologous to the engineered immune cell, (ii) enhanced degree of

expansion by at least about 10-fold within 15 days, or (iii) enhanced cytotoxicity against a target cell comprising the immune cell antigen or the disease-associated antigen, compared to an additional engineered immune cell comprising the one or more CARs but not the enhancer moiety. In some cases, the engineered immune cell can be characterized by exhibiting two or more of (i) enhanced degree of persistence, (ii) enhanced degree of expansion, and (iii) enhanced cytotoxicity. The (i), (ii), and/or (iii) characteristics can be measured in absence of any exogenous enhancer moiety such as exogenous cytokines.

[0165] The engineered immune cell can comprise a multi-specific CAR. In some cases, the engineered immune cell comprises a bispecific CAR targeting an immune cell antigen and a disease-associated antigen. The two antigen binding domains of the bispecific CAR can be arranged in any form as described in the present disclosure, for example, parallel form, loop form, and tandem form. For example, an engineered immune cell described herein can comprise comprising a single chimeric antigen receptor (CAR) comprising (i) a first antigen binding domain that specifically binds CD7 and (ii) a second antigen binding domain capable of binding to a disease-associated antigen. The CAR can further comprise a transmembrane domain and an intracellular signaling domain. In various cases, a gene encoding endogenous CD7 can be inactivated (e.g., silenced or knocked out) in the engineered immune cell.

[0166] The immune cell antigen can be selected from the group consisting of CD2, CD3, CD4, CD5, CD7, CD8, CD16a, CD16b, CD25, CD27, CD28, CD30, CD38, CD45, CD48, CD50, CD52, CD56, CD57, CD62L, CD69, CD94, CD100, CD102, CD122, CD127, CD132, CD137, CD160, CD161, CD178, CD218, CD226, CD244, CD159a (NKG2A), CD159c (NKG2C), NKG2E, CD279, CD314 (NKG2D), CD305, CD335 (NKP46), CD337, CD319 (CS1), TCR α , TCR β and SLAMF7. In some cases, the immune cell antigen is CD2, CD3, CD4, CD5, CD7, CD8, CD30, CD38, CD45, CD48, CD50, CD52, CD56, CD69, CD100, CD122, CD132, CD137, CD161, CD159a, CD159c, CD279, CD314, CD319 (CS1), TCR α or TCR β . In some cases, the immune cell antigen is CD2, CD3, CD5, CD7, or CD137. In some cases, the immune cell antigen is CD7. The enhancer moiety can be configured to constitutively enhance the one or more activities of the engineered immune cell. The enhancer moiety can be configured to constitutively upregulate one or more intracellular signaling pathways of the engineered immune cell. The one or more intracellular signaling pathways can be one or more cytokine signaling pathways. The enhancer moiety can be self-activating through self-oligomerizing. The enhancer moiety can be self-activating through self-dimerizing.

[0167] The enhancer moiety can be a cytokine or a cytokine receptor. The enhancer moiety can be selected from the group consisting of IL-2, IL-3, IL-4, IL-6, IL-7, IL-8, IL-10, IL-11, IL-12, IL-15, IL-17, IL-18, IL-21, IL-23, PD-1, PD-L1, CD122, CSF1R, CTAL-4, TIM-3, CCL21, CCL19, TGF β receptors for the same, functional fragments thereof, functional variants thereof, and combinations thereof.

[0168] In some cases, a gene encoding a subunit of the endogenous TCR of the engineered immune cell can be inactivated such that the endogenous TCR is inactivated. The gene encoding the subunit can be TCR α , TCR β , CD3 ϵ , CD3 δ , CD3 γ , or CD3 ζ .

[0169] The engineered immune cell can further comprise an inducible cell death moiety, which inducible cell death moiety can effect suicide of the engineered immune cell upon contact with a cell death activator. The inducible cell death moiety can be selected from the group consisting of rapaCasp9, iCasp9, HSV-TK, ACD20, mTMPK, ACD19, RQR8, and EGFRt. In some cases, the inducible cell death moiety is EGFRt, and the cell death activator is an antibody or an antigen binding fragment thereof that binds EGFRt. In some cases, the inducible cell death moiety is HSV-TK, and the cell death activator is GCV. In some cases, the inducible cell death moiety is iCasp9, and the cell death activator is AP1903. The cell death activator can comprise a nucleic acid, a polynucleotide, an amino acid, a polypeptide, lipid, a carbohydrate, a small molecule, an enzyme, a ribosome, a proteasome, a variant thereof, or any combination thereof.

[0170] The expression of one or more endogenous human leukocyte antigen (HLA) genes of the engineered immune cell may remain intact. In some cases, the expression of endogenous HLA-I and/or HLA-II genes of the engineered immune cell may remain intact. The expression of endogenous HLA-E and/or HLA-G and/or HLA-C genes of the engineered immune cell may remain intact.

[0171] The expression of one or more endogenous HLA genes of the engineered immune cell can be upregulated. In some cases, the expressions of endogenous HLA-E and/or HLA-G and/or HLA-C genes of the engineered immune cell are upregulated.

[0172] The expression of one or more endogenous HLA genes of the engineered immune cell may be knocked out or partially knocked out. For example, HLA-I, HLA-II or both can be knocked out. In some cases, HLA-I, HLA-2, or both can be partially knocked out. In some cases, HLA-I/II can be partially knockout. In some cases, an endogenous HLA can be knocked out to reduce T cell killing activity but keep anti-NK killer function. For example, HLA-A/B can be knocked out while keeping HLA-C/E in the engineered immune cell. In some cases, HLA-A, HLA-B, or both is knocked out. In some cases, HLA-C, HLA-E, or both remains intact. In some cases, a killer/phagocyte inhibitor of the engineered immune cell can be overexpressed. In some other cases, an endogenous HLA can be knocked out with co-expression of killer/phagocyte inhibitor(s). For example, HLA-I, HLA-II, or both can be knocked out with co-expression of killer/phagocyte inhibitors. The killer/phagocyte inhibitor may suppress immune response toward the engineered immune cell. The killer/phagocyte inhibitors include, but are not limited to, CD47, CD24, FASL, PDL1, or functional domains thereof.

[0173] The disease-associated antigen described herein can be a tumor-associated antigen or a tumor-specific antigen. The first antigen binding domain or the second antigen binding domain can be an antibody or fragment thereof, for example, a scFv or a single domain antibody.

[0174] A gene encoding an endogenous surface marker of the engineered immune cell can be inactivated, wherein the endogenous surface marker is capable of binding to the first antigen binding domain when expressed. The endogenous surface marker can be the antigen that CAR targets. In various embodiments, when a CAR of the engineered immune cell targets an antigen that is endogenously expressed by the engineered immune cell, the endogenous antigen or the gene encoding such antigen may be inactivated (e.g., disrupted, inhibited, silenced or knocked out).

Various gene editing methods described herein can be used. The endogenous surface marker can be, for example, CD2, CD3, CD4, CD5, CD7, CD8, CD16a, CD16b, CD25, CD27, CD28, CD30, CD38, CD45, CD48, CD50, CD52, CD56, CD57, CD62L, CD69, CD94, CD100, CD102, CD122, CD127, CD132, CD137, CD160, CD161, CD178, CD218, CD226, CD244, CD159a (NKG2A), CD159c (NKG2C), NKG2E, CD279, CD314 (NKG2D), CD305, CD335 (NKP46), CD337, CD319 (CS1), TCR α , TCR β and SLAMF7.

[0175] The engineered immune cell provided herein can comprise a chimeric polypeptide comprising (i) an enhancer moiety capable of enhancing one or more activities of the engineered immune cell, and (ii) an inducible cell death moiety capable of effecting death of the engineered immune cell upon contacting the chimeric polypeptide with a cell death activator, wherein the enhancer moiety is linked to the inducible cell death moiety. In some cases, the enhancer moiety and the inducible moiety may be linked by a linker. The linker can be a cleavable linker, for example, a self-cleaving peptide. The engineered immune cell can further comprise one or more chimeric polypeptide receptors (CPRs) comprising a binding moiety, wherein the binding moiety comprises (i) a first antigen binding domain, which first antigen binding domain suppresses or reduces a subject's immune response toward the engineered immune cell when administered into the subject and (ii) a second antigen binding domain capable of binding to a disease-associated antigen. An individual CPR of the one or more CPRs can comprise (i) the first antigen binding domain, (ii) the second antigen binding domain, or (iii) both the first antigen binding domain and the second antigen binding domain. A CPR of the one or more CPRs can further comprise a transmembrane domain and an intracellular signaling domain. In some cases, the one or more CPRs in the engineered immune cell are one or more chimeric antigen receptors (CARs) or engineered T cell receptors (TCRs). In some cases, the engineered immune cells comprise both CARs and engineered TCRs. The engineered TCR can be a TCR fusion protein. For example, the TCR fusion protein can comprise a heterologous antigen binding domain fused to one or more subunits of a TCR complex. In some cases, the TCR fusion protein can comprise a TCR subunit comprising at least a portion of a TCR extracellular domain and a TCR intracellular domain; and an antibody domain comprising an antigen binding domain, where the TCR subunit and the antibody domain are linked. The TCR fusion protein can incorporate into a TCR complex when expressed in a T cell. In some cases, the TCR fusion protein can further comprise a TCR transmembrane domain. The TCR extracellular domain, the TCR intracellular domain, or the TCR transmembrane domain can be derived from TCR alpha chain, TCR beta chain, TCR gamma chain, TCR delta chain, CD3 epsilon, CD3 gamma, CD3 delta or CD3 zeta. In some cases, an endogenous TCR of the engineered immune cell comprising an engineered TCR is inactivated. In some cases, the engineered immune cell comprising inactivated endogenous TCR may not cause GVHD. For example, a gene encoding an endogenous TCR subunit can be inactivated. For another example, a gene encoding an endogenous TCR subunit may be mutated such that an endogenous TCR may not be formed.

[0176] The engineered immune cell provided herein can comprise a chimeric polypeptide comprising (i) an enhancer

moiety capable of enhancing one or more activities of the engineered immune cell, and (ii) an inducible cell death moiety capable of effecting death of the engineered immune cell upon contacting the chimeric polypeptide with a cell death activator. In some cases, the enhancer moiety is linked to the inducible cell death moiety. The one or more chimeric antigen receptors (CARs) can comprise a binding moiety. The binding moiety can comprise (i) a first antigen binding domain, which first antigen binding domain suppresses or reduces a subject's immune response toward the engineered immune cell when administered into the subject and (ii) a second antigen binding domain capable of binding to a disease-associated antigen. In some cases, an individual CAR of the one or more CARs comprises (i) the first antigen binding domain or (ii) the second antigen binding domain. In some cases, an individual CAR of the one or more CARs comprises both the first antigen binding domain and the second antigen binding domain. In some cases, each CAR of the one or more CARs further comprises a transmembrane domain and an intracellular signaling domain.

[0177] The first antigen binding domain of the engineered immune cell can bind to an immune cell antigen. In some cases, endogenous T cell receptors (TCRs) of the engineered immune cell is inactivated. Various methods can be used to inactivate endogenous TCRs. For example, a gene encoding a subunit of the endogenous TCR can be inactivated such that the endogenous TCR is inactivated. The gene encoding the subunit can be TCR α , TCR β , CD3 ϵ , CD3 δ , CD3 γ , or CD3 ζ .

[0178] The chimeric polypeptide may or may not comprise any self-cleaving peptide flanked by the enhancer moiety and the inducible cell death moiety. The enhancer moiety can be configured to constitutively enhance the one or more activities of the engineered immune cell. The enhancer moiety can be configured to constitutively upregulate one or more intracellular signaling pathways of the engineered immune cell. The one or more intracellular signaling pathways can be one or more cytokine signaling pathways. The enhancer moiety can be self-activating through self-oligomerizing. The enhancer moiety can be self-activating through self-dimerizing.

[0179] The chimeric polypeptide described herein can be a secreted protein. The chimeric polypeptide can be an intracellular protein. The chimeric polypeptide can be a transmembrane protein. The enhancer moiety or the inducible cell death moiety can be contained in an ectodomain of the transmembrane protein. The enhancer moiety or the inducible cell death moiety is contained in an endodomain of the transmembrane protein. The enhancer moiety can be contained in an endodomain of the transmembrane protein and the inducible cell death moiety can be contained in an ectodomain of the transmembrane protein. The enhancer moiety can be contained in an ectodomain of the transmembrane protein, and the inducible cell death moiety can be contained in an endodomain of the transmembrane protein. The enhancer moiety can be a cytokine or a cytokine receptor. For example, the enhancer moiety can be selected from the group consisting of IL-2, IL-3, IL-4, IL-6, IL-7, IL-8, IL-10, IL-11, IL-12, IL-15, IL-17, IL-18, IL-21, IL-23, PD-1, PD-L1, CD122, CSF1R, CTAL-4, TIM-3, CCL21, CCL19, TGF β receptor, receptors for the same, functional fragments thereof, functional variants thereof, and combinations thereof. The inducible cell death moiety can be selected from the group consisting of rapaCasp9, iCasp9,

HSV-TK, ACD20, mTMPK, ACD19, RQR8, and EGFRt. In some cases, the inducible cell death moiety is EGFRt, and the cell death activator is an antibody or an antigen binding fragment thereof that binds EGFRt. In some cases, the inducible cell death moiety is HSV-TK, and the cell death activator is GCV. In some cases, the inducible cell death moiety is iCasp9, and the cell death activator is AP1903. The cell death activator may comprise a nucleic acid, a polynucleotide, an amino acid, a polypeptide, lipid, a carbohydrate, a small molecule, an enzyme, a ribosome, a proteasome, a variant thereof, or any combination thereof.

[0180] The engineered immune cell can be a CAR-T cell. The CAR-T cell can express a CAR targeting a tumor cell marker and CD3. The expression of endogenous CD3 gene can be silenced in the CAR-T cell. The CAR can be a single CAR targeting both a tumor cell marker and CD3. The CAR can comprise a first CAR targeting a tumor cell marker and a second CAR targeting CD3. The CAR-T cell can have one or more of the following characteristics: (a) expression of PD-1 gene is silenced in the CAR-T cell; (b) expression of TCR gene is silenced in the CAR-T cell; (c) the CAR-T cell expresses an exogenous cellular suicide element (e.g., inducible cell death moiety).

[0181] The engineered immune cell can express a CAR and/or an exogenous TCR, and the CAR and/or exogenous TCR target a tumor cell marker. The engineered immune cell can comprise a cytokine-related signaling pathway that is enhanced. The cytokine-related signaling pathway can comprise a related signaling pathway of a cytokine selected from a group consisting of IL-2, IL-3, IL-4, IL-6, IL-7, IL-8, IL-10, IL-11, IL-12, IL-15, IL-17, IL-18, IL-21, or a combination thereof. Enhancing the cytokine-related signaling pathway can comprise introducing a gene encoding a cytokine and/or a receptor thereof, up-regulating a gene encoding a cytokine and/or a receptor thereof, or exogenously adding a cytokine, a receptor of cytokine that is introduced, or a combination thereof. The engineered immune cell can be a CAR-T cell having one or more characteristics selected from a group consisting of (a) gene expression an endogenous TCR being silenced; (b) expressing an element for cellular suicide; (c) normal expression of endogenous HLA-I and HLA-II genes; (d) normal expression or over-expression of endogenous HLA-E and/or HLA-G.

[0182] The engineered immune cell can comprise a CAR or an exogenous TCR targeting a tumor cell marker. The engineered immune cell can comprise a substance targeting a T cell or NK cell. For example, the engineered immune cell can comprise a CAR targeting a T cell and/or NK cell. The engineered immune cell can comprise a bispecific CAR targeting both (i) a tumor cell marker and (ii) a T cell and/or NK cell marker. In some cases, the substance is an antibody. The antibody target both T cell and NK cell can be H-69, 3A1e, 3A1f, T3-3A1, RFT2, SDZ214-380 (SDZCHH380), CD7-6B7, 124-1D1, 4H9, RPA-2.10, TS1/8, OKT11, AB75, 3E11, BH1, or Lo-CD2a.

[0183] The CAR-T cell provided herein can be a universal CAR-T cell. The CAR-T cell can express a chimeric antigen receptor CAR that targets a tumor cell marker and the binding of the T cell receptor to PD-1 is inhibited. The CAR-T cell can target a tumor cell marker and an immune cell marker such as CD2 or CD7. The endogenous TCR expression in the CAR-T cells provided herein can be knocked out by gene editing technology. Upon knocking out the endogenous TCRs of the CAR-T cells, the normal cells

may not be recognized and killed by the CAR-T cells during the allogeneic infusion. The GVHD reaction may be inhibited. Moreover, targeting tumor cells through CD19 while eliminating host T cells and/or NK cells through CD2 or CD7 can avoid host versus graft (HVG) and/or NK killing and improve the survival and anti-tumor effect of the allogeneic CAR-T cells in the recipient. The CAR-T can further comprise a suicide gene switch (e.g., an inducible cell death moiety). The CAR-T cells can be inactivated or removed by turning on the suicide gene switch (e.g., binding of an activator to the inducible cell death moiety) to reduce the side effects of the CAR-T cell therapy. For example, a CAR provided herein can have a structure of CD19 scFv-CD7 scFv-Hinge-TM-CD28/41BB-CD3 ζ , wherein the CD7 scFv fragment is a monoclonal 3A1e antibody, the heavy and light chain variable regions are joined by a GS linker, and the CD19 scFv fragment is the heavy and light chain variable region of the monoclonal FMC63 antibody linked by a GS linker. The CAR can also include a hinge region and a transmembrane region in tandem, human CD28 and/or 41BB intracellular co-stimulatory elements, as well as human CD3 intracellular domain. In some cases of the present disclosure, a gene fragment of a CAR construct CD19 scFv-CD7 scFv-Hinge-TM-CD28/41BB-CD3 ζ can be inserted into a lentiviral vector, and the recombinant vector can be packaged into viral particles in 293T cells. To prepare universal CAR-T cells, T cells can be isolated from peripheral blood mononuclear cells, and after activation, some endogenous genes (e.g., CD7, TCR and PD-1 genes) can be knocked out by gene editing technologies such as CRISPR/CAS technology. Next, T cells can be infected by the viral particles containing the CAR construct describe herein to express the CAR. The prepared CAR-T cells can be used to detect the infection efficiency and gene editing efficiency of CAR by flowcytometry.

[0184] The engineered immune cell may have one or more characteristics described herein: (a) the expression of the endogenous CD7 or CD2 gene of the engineered immune cell is silenced; (b) the PD-1 gene expression of the engineered immune cell is silenced; (c) the TCR gene expression of the engineered immune cell is silenced; (d) the engineered immune cell expresses a cytokine or cytokine receptor complex and the pSTAT5 signaling level is up-regulated; (e) the engineered immune cell expresses an exogenous inducible cell death moiety; (f) the first CAR, and/or the second CAR in the engineered immune cell is co-expressed with the inducible cell death moiety.

[0185] The engineered immune cell may comprise two different CARs, each having a different antigen binding domain target a different antigen. The engineered immune cell may comprise a single CAR, which further comprises two antigen binding domains targeting two different antigens. In some cases, a first CAR, and/or a second CAR is linked to an inducible cell death moiety and/or an enhancer moiety by a self-cleaving element. In some cases, the enhancer moiety is a cytokine or cytokine complex. Examples of cytokines or cytokine complexes include IL2, IL7, IL15, membrane-bound IL15 (mbIL15 or mb15), and a constitutive activating cytokine receptor such as an IL7 receptor (C7R). As used herein, "mbIL" and "mb" are used interchangeably to refer to a membrane-bound interleukin factor, for example, mbIL7 or mb7, and mbIL17 or mb17.

[0186] The engineered immune cell described herein may have the following characteristics: (a) the engineered

immune cell expresses a CAR and/or an exogenous TCR, and the CAR and/or exogenous TCR targets tumor cell markers; and (b) the cytokine-associated signaling pathway is enhanced. The engineered immune cell may be (i) chimeric antigen receptor T cells (CAR-T cells); (ii) chimeric antigen receptor NK cells (CAR-NK cells); or (iii) Exogenous T cell receptor (TCR) T cells (TCR-T cells). The engineered immune cell can be a CAR-T cell, preferably a universal CAR-T cell (UCAR-T cell). The “cytokine-related signaling pathway,” as used herein, refers to a signaling pathway initiated by the cytokine binding to the corresponding receptor, converting the extracellular signal into an intracellular signal, which is then amplified, dispersed, and regulated by a signal cascade. A series of cellular responses can be produced. In some cases, the cytokine-related signaling pathway comprises a related signaling pathway of a cytokine selected from the group consisting of IL-2, IL-3, IL-4, IL-6, IL-7, IL-8, IL-10, IL-11, IL-12, IL-15, IL-17, IL-18, IL-21, 25 or a combination thereof.

[0187] The engineered immune cell can comprise a bispecific CAR (or a dual CAR). For example, the bispecific CAR can comprise both a first antigen binding domain and a second antigen binding domain. The first antigen binding domain and the second antigen binding domain can be linked via a linker. The linker may not comprise a self-cleaving peptide. The first antigen binding domain or the second antigen binding can be a scFv.

[0188] The first antigen binding domain and the second antigen binding domain can be arranged, from amino terminus to carboxyl terminus, as: (i) VL2-VH1-VL1-VH2; (ii) VH2-VL1-VH1-VL2; (iii) VL1-VH2-VL2-VH1; or (iv) VH1-VL2-VH2-VL1, wherein VH1 is heavy chain variable domain of the first antigen binding domain, VL1 is light chain variable light domain of the first antigen binding domain, VH2 is heavy chain variable domain of the second antigen binding domain, and VL2 is light chain variable domain of the second antigen binding domain.

[0189] The first antigen binding domain and the second antigen binding domain can be arranged, from amino terminus to carboxyl terminus, as: (i) VL2-VH2-VL1-VH1; (ii) VL2-VH2-VH1-VL1; (iii) VL1-VH1-VL2-VH2; or (iv) VL1-VH1-VH2-VL1, wherein VH1 is heavy chain variable domain of the first antigen binding domain, VL1 is light chain variable light domain of the first antigen binding domain, VH2 is heavy chain variable domain of the second antigen binding domain, and VL2 is light chain variable domain of the second antigen binding domain. The first antigen binding domain and the second antigen binding domain can bind to the immune cell antigen and the disease-associated antigen.

[0190] In some cases, the engineered immune cell may not comprise a bispecific CAR. For example, an individual CAR of the engineered immune cell can comprise only the first antigen binding domain and an additional individual CAR of the engineered immune cell can comprise only the second antigen binding domain.

[0191] The immune cell antigen can be a surface protein or a secreted protein of an immune cell. The immune cell can be an NK cell, a T cell, a monocyte, a macrophage or a granulocyte. The immune cell antigen can be selected from the group consisting of CD2, CD3, CD4, CD5, CD7, CD8, CD16a, CD16b, CD25, CD27, CD28, CD30, CD38, CD45, CD48, CD50, CD52, CD56, CD57, CD62L, CD69, CD94, CD100, CD102, CD122, CD127, CD132, CD137, CD160,

CD161, CD178, CD218, CD226, CD244, CD159a (NKG2A), CD159c (NKG2C), NKG2E, CD279, CD314 (NKG2D), CD305, CD335 (NKP46), CD337, CD319 (CS1), TCR α , TCR β and SLAMF7.

[0192] The disease-associated antigen can be a tumor-associated antigen. The tumor-associated antigen can be CD19 or other antigens described herein. In some cases, the first antigen binding domain can bind to an immune cell antigen selected from the group consisting of CD2, CD3, CD5, CD7 and CD137, and the second antigen binding domain can bind to CD19. In some cases, the first antigen binding domain can bind to CD3, and the second antigen binding domain can bind to CD19. In some cases, the first antigen binding domain can bind to CD7, and the second antigen binding domain can bind to CD19. In some cases, the first antigen binding domain can bind to CD137, and the second antigen binding domain can bind to CD19. The expression of one or more endogenous human leukocyte antigen (HLA) genes of the engineered immune cell can remain intact. The expression of endogenous HLA-I and/or HLA-II genes of the engineered immune cell can remain intact. The expression of endogenous HLA-E and/or HLA-G and/or HLA-C genes of the engineered immune cell can remain intact. The expression of one or more endogenous HLA genes of the engineered immune cell can be upregulated. The expression of endogenous HLA-E and/or HLA-G and/or HLA-C genes of the engineered immune cell can be upregulated.

[0193] In various embodiments, the engineered immune cell is a T cell, an NKT cell or an NK cell. In some cases, the engineered immune cell is derived from a stem cell. The stem cell can be a hematopoietic stem cell (HSC) or an induced pluripotent stem cell (iPSC).

[0194] A cell (e.g., an engineered immune cell) provided herein can comprise one or more chimeric antigen receptors (CARs) comprising a binding moiety, where the binding moiety can comprise an antigen binding domain capable of binding to an immune cell antigen. Each CAR of the one or more CARs can further comprise a transmembrane domain and an intracellular signaling domain. The cell can further comprise an enhancer moiety capable of enhancing one or more activities of the cell, where an endogenous T cell receptor (TCR) of the cell may be inactivated.

[0195] The enhancer moiety can enhance one or more activities of the cell. The enhancer moiety can be configured to constitutively enhance the one or more activities of the cell. The enhancer moiety can be configured to constitutively upregulate one or more intracellular signaling pathways of the cell. For example, the one or more intracellular signaling pathways can be one or more cytokine signaling pathways. The enhancer moiety can be a cytokine or a cytokine receptor. The enhancer moiety can be selected from the group consisting of IL-2, IL-3, IL-4, IL-6, IL-7, IL-8, IL-10, IL-11, IL-12, IL-15, IL-17, IL-18, IL-21, IL-23, PD-1, PD-L1, CD122, CSF1R, CTAL-4, TIM-3, CCL21, CCL19, TGF β receptors for the same, functional fragments thereof, functional variants thereof, and combinations thereof.

[0196] The cell can further comprise an inducible cell death moiety capable of effecting death of the cell upon contacting the inducible cell death moiety with a cell death activator. The inducible cell death moiety can be selected from the group consisting of rapaCasp9, iCasp9, HSV-TK, ACD20, mTMPK, ACD19, RQR8, Her2t, CD30, BCMA,

and EGFRt. For example, the inducible cell death moiety can be EGFRt, and the cell death activator can be an antibody or an antigen binding fragment thereof that binds EGFRt. For another example, the inducible cell death moiety can be HSV-TK, and the cell death activator can be GCV. For another example, the inducible cell death moiety can be iCasp9, and the cell death activator can be AP1903.

[0197] A gene encoding an endogenous surface marker of the cell can be inactivated, where the endogenous surface marker may be capable of binding to the first antigen binding domain when expressed. The endogenous surface marker can be CD2, CD3, CD4, CD5, CD7, CD8, CD16a, CD16b, CD25, CD27, CD28, CD30, CD38, CD45, CD48, CD50, CD52, CD56, CD57, CD62L, CD69, CD94, CD100, CD102, CD122, CD127, CD132, CD137, CD160, CD161, CD178, CD218, CD226, CD244, CD159a (NKG2A), CD159c (NKG2C), NKG2E, CD279, CD314 (NKG2D), CD305, CD335 (NKP46), CD337, CD319 (CS1), TCR α , TCR β or SLAMF7.

Antigen Binding Domain

[0198] The engineered immune cell can comprise a first antigen binding domain and a second antigen binding domain. In some cases, a single or individual CAR of the engineered immune cell comprises both the first antigen binding domain and the second antigen binding domain. In some cases, two CARs of the engineered immune cell comprise the first antigen binding domain and the second antigen binding domain with each CAR contains only one antigen binding domain. In some cases, two engineered immune cells comprise the first antigen binding domain and the second antigen binding domain with each engineered immune cell comprises only one type of antigen binding domain. In some cases, the first antigen binding domain can target an immune cell antigen and the second antigen binding domain can target a disease-associated antigen. The antigen binding domain can be a Fab, F(ab')₂, single domain antibody, single chain Fv (scFv), centyrin, darpin, or other polypeptides with antigen binding specificities.

[0199] The antigen binding domain can target an immune cell antigen. Examples of immune cell antigen include, but are not limited to, CD2, CD3, CD4, CD5, CD7, CD8, CD16a, CD16b, CD25, CD27, CD28, CD30, CD38, CD45, CD48, CD50, CD52, CD56, CD57, CD62L, CD69, CD94, CD100, CD102, CD122, CD127, CD132, CD137, CD160, CD161, CD178, CD218, CD226, CD244, CD159a (NKG2A), CD159c (NKG2C), NKG2E, CD279, CD314 (NKG2D), CD305, CD335 (NKP46), CD337, CD319 (CS1), TCR α , TCR β and SLAMF7. In some cases, the immune cell antigen is a cell marker expressed on both T cells and NK cells, including, but not limited to, CD2, CD7, CD38, CD45, CD48, CD50, CD52, CD56, CD69, CD100, CD122, CD132, CD134 (OX40), CD137 (4-1BB), CD178 (ICOS), CD161, CD159a, CD159c and CD314.

[0200] CD3 is a marker present on the surface of T lymphocytes. There are three subtypes, CD 3 δ , CD 3 ϵ and CD 3 γ . CD 3 δ and CD 3 ϵ have a molecular weight of 20 kDa, and CD37 has a molecular weight of 26 kDa, which is expressed on the surface of T lymphocytes, thymocytes and NK cell membranes. CD3 can be expressed in 61% to 85% of normal peripheral blood lymphocytes and 60% to 85% in thymocytes. It belongs to the immunoglobulin superfamily. CD3 is a component of the T-lymphocyte receptor (TCR) complex and forms a complex with the α - β and γ / δ T-lym-

phocyte receptors (TCRs), which can be the main membrane protein for conducting TCR signals upon TCR binding to peptide/MHC. TCR can be essential for cell surface expression, antigen recognition, and signaling. CD3 is a surface-specific molecule of T lymphocytes through which T lymphocytes with killing effects can be recruited. Monoclonal antibodies to CD3 can induce or prevent T lymphocyte activation. Anti-CD3 antibodies induce apoptosis of T lymphocytes in the presence of anti-CD28 antibodies or IL-2. CD3 is one of the markers of mature T lymphocytes in peripheral blood. Determination of CD3+ T lymphocytes for evaluation of immunodeficiency (T lymphocyte deficiency), leukemia, lymphoma (T lymphocyte type) classification diagnosis. Anti-CD3 monoclonal antibodies can be used for immunosuppressive therapy in organ transplantation or bone marrow transplantation, and can also be used for immunomodulatory treatment of severe autoimmune diseases to remove T lymphocytes.

[0201] In some cases, the antigen binding domain can target CD7. CD7 is a transmembrane protein and is a member of the immunoglobulin superfamily. CD7 proteins are expressed on the surface of mature T cells and NK cells as well as their precursor cells. CD7 can bind to its ligand K12/SECTM1 and function as a co-stimulatory effector on T cell activation. In mice, CD7 knockout T cell precursors can develop into normal T cells with only a slight effect on T cell effector function. More than 90% of T-cell acute lymphoblastic leukemia (T-ALL) can express CD7, and therefore CD7 can be a marker for T-ALL. Moreover, CD7 can also be expressed in NK lymphoma, T-cell lymphoma/leukemia, chronic myelogenous leukemia, acute myeloid leukemia, and lymphocyte-rich thymoma. An example tissue distribution of CD7 expression is shown in FIG. 13.

[0202] In some cases, the antigen binding domain can target CD2. Similar to CD7, CD2 adhesion molecules may be expressed on all peripheral blood T cells and natural killer cells, but not on B lymphocytes. The CD2 extracellular domain contains an immunoglobulin-like domain that mediates homodimerization. Binding of CD2 to CD58 (LFA-3) or CD48 can help T cells adhere to antigen presenting cells, triggering signal transduction of T cell receptors for antigen binding. The function of CD2 may be similar to other T cell costimulatory receptors (such as CD28). CD2 knockout mice can have normal immune function. CD2 expression in cells of T-ALL, T-cell lymphoma/leukemia, acute promyelocytic leukemia (microparticle variant), systemic mastocytosis, mast cell disease, thymoma, and acute myeloid lymphoma and NK cell leukemia. An example tissue distribution of CD2 expression is shown in FIG. 14.

[0203] The antigen binding domain can target CD137. CD137, also known as 4-1BB, is a member of the TNF receptor superfamily. CD137 protein can be an activation-induced co-stimulatory receptor, which can be widely expressed in activated T cells, NK cells, dendritic cells, granulocytes and other immune cell types and certain tumor cells. CD137 expression was found on activated T and NK cells with little expression in naïve T cells and inactivated T and NK cells. In T cells, CD137 can initiate NF- κ B pathway through TRAF2 and participate in T cell proliferation, cytokine secretion and anti-apoptosis. In clinical applications, CD137 can be used as a marker for reactive T cells. Activation of CD137 signaling pathway by CD137 natural ligand or agonistic antibody can increase cytokine secretion and antitumor activity of cytotoxic lymphocytes. Inhibition

of reactive T cells with specific inhibitory CD137 antibodies can reduce autologous rejection in transplant rejection or reduce GVHD response caused by autoreactive T cells of an allogeneic origin. CD137 expression can be regulated by TCR signaling and downstream signaling of cytokine TL-2/TL-15. Knockout of CD137 molecule may have no effect on the function of mature T cells in a non-activated state. The endogenous CD137 of the engineered immune cell can be knocked out to avoid fratricide. The endogenous TCRs of the engineered immune cell can be inactivated to prevent GVHD. An example tissue distribution of CD137 expression is shown in FIG. 51.

[0204] The antigen binding domain can target a disease-associated antigen, for example, tumor-associated antigen. Examples of the tumor-associated antigens include, but are not limited to, BCMA, VEGFR2, CD19, CD20, CD30, CD22, CD25, CD28, CD30, CD33, CD52, CD56, CD80, CD86, CD81, CD123, cd171, CD276, B7H4, CD133, EGFR, GPC3; PMSA, CD 3, CEACAM6, c-Met, EGFRvIII, ErbB2, ErbB3 HER-2, HER3, ErbB4/HER-4, EphA2, IGF1R, GD2, O-acetyl GD2, O-acetyl GD3, GHRHR, GHR, Flt1, KDR, Flt4, CD44V6, CEA, CA125, CD151, CTLA-4, GITR, BTLA, TGFBR2, TGFBR1, IL6R, gp130, Lewis, TNFR1, TNFR2, PD1, PD-L1, PD-L2, HVEM, MAGE-A, Mesothelin, NY-ESO-1, PSMA, RANK, ROR1, TNFRSF4, CD40, CD137, TWEAK-R, LTPR, LIFRP, LRP5, MUC1, TCR α , TCR β , TLR7, TLR9, PTCH1, WT-1, Robl, Frizzled, OX40, CD79b, Claudin 18.2, Folate receptor α , Folate receptor β , GPC2, CD70, BAFF-R and Notch-1-4. In some cases, the tumor-associated antigens comprise CD19, CD2, CD3, CD4, CD5, CD7, CD8, CD19, CD20, CD22, CD25, CD28, CD30, CD33, CD38, CD40, CD44V6, CD47, CD52, CD56, CD57, CD58, CD79b, CD80, CD86, CD81, CD123, CD133, CD137, CD151, CD171, CD276, CLL1, B7H4, BCMA, VEGFR-2, EGFR, GPC3, PMSA, CEACAM6, c-Met, EGFRvIII, ErbB2/HER2, ErbB3, HER-2, HER3, ErbB4/HER-4, EphA2, IGF1R, GD2, O-acetyl GD2, O-acetyl GD3, GHRHR, GHR, Flt1, KDR, Flt4, Flt3, CEA, CA125, CTLA-4, GITR, BTLA, TGFBR1, TGFBR2, TGFBR1, IL6R, gp130, Lewis, TNFR1, TNFR2, PD1, PD-L1, PD-L2, PSCA, HVEM, MAGE-A, MSLN, NY-ESO-1, PSMA, RANK, ROR1, TNFRSF4, TWEAK-R, LTPR, LIFRP, LRP5, MUC1, MUC16, TCR α , TCR β , TLR7, TLR9, PTCH1, WT-1, Robl, Frizzled, OX40, Notch-1-4, APRIL, CS1, MAGE3, Claudin 18.2, Folate receptor α , Folate receptor β , GPC2, CD70, BAFF-R or TROP-2.

[0205] In some cases, the tumor-associated antigen is CD19. CD19 is a 95 kDa glycoprotein on the surface of B cells that begins to express from the early development of B cells until it differentiates into plasma cells. CD19 is a member of the immunoglobulin (Ig) superfamily and is involved in the regulation of the signal transduction process of B cell receptors as one of the constituent elements of the B cell surface signal transduction complex. In a CD19-deficient mouse model, the number of B cells in peripheral lymphoid tissue can be significantly reduced, and the response to vaccines and mitogens is also reduced, accompanied by a decrease in serum Ig levels. It can be generally believed that the expression of CD19 is restricted to the B-cell lineage and not to the surface of pluripotent hematopoietic stem cells. CD19 can also be expressed on the surface of most B cell lymphomas, mantle cell lymphomas, ALLs, CLLs, hairy cell leukemias, and some acute myeloid

leukemia cells. CD19 can be a target for immunotherapy in the treatment of leukemia/lymphoma. CD19 may not be expressed on most normal cell surfaces other than B cells, including pluripotent hematopoietic stem cells. This feature can make CD19 a safe therapeutic target for autoimmune diseases because the risk of irreversible bone marrow toxicity damage can be minimized.

[0206] The antigen binding domain provided herein can have a structure shown as V_H - V_L or VL-VH, wherein VH is a heavy chain variable region of an antibody; V_L is a light chain variable region of an antibody; "-" is a linker peptide (or flexible linker) or a peptide bond. In some embodiments, the antigen binding domain targets a tumor-associated antigen. In some embodiments, the tumor-associated antigen comprises CD19. In some embodiments, the antigen binding domain that targets CD19 comprises the heavy chain variable region and the light chain variable region of the monoclonal FMC63 antibody. In some embodiments, the sequence of the linker peptide or flexible linker comprises 2-6, preferably 3-4 consecutive (GGGGS) amino acid sequences (SEQ ID NO: 93). In some embodiments, the amino acid sequence of V_{L1} is as shown in SEQ ID NO.: 87, and the amino acid sequence of VH1 is shown in SEQ ID NO.: 88. An example amino acid sequence of CAR targeting CD19 is shown in SEQ ID NO.: 89. In some embodiments, the antigen binding domain targets an immune cell antigen. In some embodiments, the immune cell antigen targets CD7. In some other embodiments, the immune cell antigen targets CD2. In some embodiments, the monoclonal antibody of CD7 is selected from the group consisting of TH-69, 3Ale, 3Alf, T3-3A1, RFT2, CD7-6B7, 124-1D1, 4H9, SDZ214-380, or a combination thereof. In some other embodiments, the monoclonal antibody to CD2 is selected from the group consisting of RPA-2.10, TS1/8, OKT11, AB75, 3E11, BH1, or a combination thereof. In some embodiments, the amino acid sequence of V_L is as shown in SEQ ID NO.: 90, and the amino acid sequence of VH is shown in SEQ ID NO.: 91.

Enhancer Moiety

[0207] The engineered immune cell provided herein can comprise an enhancer moiety. The enhancer moiety can regulate one or more activities of the engineered immune cell, for example, enhance or upregulate one or more signaling pathways to enhance or upregulate effector functions of the engineered immune cell. The signaling pathways can be a cytokine-related signaling pathway. The enhancer moiety can be a cytokine. The enhancer moiety can be a cytokine receptor.

[0208] The cytokine-related signaling pathway can comprise a related signaling pathway of a cytokine. Examples of cytokines include, but are not limited to, IL-2, IL-3, IL-4, IL-6, IL-7, IL-8, IL-10, IL-11, IL-12, IL-15, IL-17, IL-18, IL-21 and IL25. In some cases, the cytokine-related signaling pathway comprises a related signaling pathway of two or more cytokines, wherein the cytokines include: IL-2 and IL-7, IL-2 and IL-15, IL-7 and IL-15, IL15 and IL21. The cellular response can include regulation of downstream gene expression, changes in intracellular enzyme activity, changes in cellular bone architecture, changes in DNA synthesis, promotion of gene transcription, regulation of immune cell differentiation, proliferation, and resistance to cell death. In some cases, the cytokine-related signaling pathway is enhanced comprising: introducing or up-regulating a gene encoding a cytokine and/or a receptor thereof,

exogenously adding a cytokine, being introduced into a cytokine receptor, or a combination thereof. In some cases, up-regulating the gene encoding the cytokine and/or its receptor comprises up-regulating the level of transcription and/or translation of the encoding gene. In some cases, the enhanced cytokine-related signaling pathway can be achieved by one or more of the following methods: expressing a gene encoding the cytokine and/or its receptor in the immune cell, increasing the copy number of the gene encoding the cytokine and/or its receptor in the immune cell, engineering a regulatory sequence (e.g., a promoter) of the encoding gene to enhance transcription speed (e.g., transcriptional initiation rate), modifying a translational regulatory region of a messenger RNA carrying the encoded gene to enhance translational strength, modifying the coding gene itself to enhance mRNA stability, protein stability and to release protein feedback inhibition.

[0209] The cytokine-related signaling pathway can be enhanced by membrane expression of a cytokine and its receptor, secretion of a cytokine, enhancement of transcriptional regulation of a cytokine and/or its receptor, or a combination thereof. The membrane-expressed cytokine and its receptors can include: IL-15 and its receptor (e.g., mbIL15 fusion protein), IL-7 and its receptor (e.g., mbIL7 fusion protein), IL-17 and its receptor (e.g., mbIL17 fusion protein), IL-2 and its receptor (e.g., mbTL2 fusion protein), IL-21 and its receptor (e.g., mbIL21 fusion protein), constitute the activated IL-7 receptor (C7R), or a combination thereof. In some cases, the enhancer moiety comprised in the engineered immune cell is a secretive cytokine. The secretive cytokine can function with various mechanisms, for example, the secretive cytokine can be a trans-activating factor or a cis-activating factor. The secretive cytokines can include IL-2, IL-3, IL-4, IL-6, IL-7, IL-8, IL-10, IL-11, IL-12, IL-15, IL-17, IL-18, IL-21, or a combination thereof. In some cases, the enhancer is a membrane bound protein such as mbIL15, mbIL7, mbIL21 and mbIL2. In some cases, the enhancer moiety is constitutively active cytokine receptor downstream signaling protein such as STAT5 and STAT3. In some cases, the enhancer moiety is a constitutively active cytokine receptor such as constitutively active IL-7 receptor (C7R) or derivatives thereof. For example, the constitutively active cytokine receptor can be an engineered protein (e.g., referred to as “E3” in the present disclosure) where the ecto domain of C7R is replaced by a safety switch, such as EGFRt or truncated form of human epidermal growth factor receptor 2 (Her2t; see U.S. Patent Application Publication No. 20170267742A1) or other peptides described in the present disclosure. For another example, the constitutively active cytokine receptor can be an engineered protein (e.g., referred to as “E4” in the present disclosure) where the ectodomain of C7R is replaced by an immune cell inhibitor, such as CD47, CD24 or other peptides that inhibit killer or phagocytic immune cell function and protect therapeutic cells (e.g., the engineered immune cells described herein). In some cases, the cytokine can be a chemokine such as CCL21 and CCL19. Other non-limiting examples of chemokines that may be used include CCL27, CCL28, CCL20, CXCL9, CXCL10, CXCL11, CXCL16, CXCL13, CXCL5, CXCL6, CXCL8, CXCL12, CCL2, CCL8, CCL13, CCL25, CCL3, CCL4, CCL5, CCL7, CCL14, CCL15, CCL16, CCL23, CX3CL1, XCL1, XCL2, CCL1, CCL17, CCL22, CCL11, CCL24, CCL26, CXCL1, CXCL2, CXCL3 and CXCL7. In some cases, the enhancer moiety is a ligand

of CCR7, which can function to enhance infiltration of T cells, NK cells or dendritic cells. CCR7 ligand includes, but not limited to, CCL21 and CCL19. In some cases, the enhancer moiety comprises co-expression of chemokines CCL21 and CCL19 for therapeutic use to treat lymphomas or other solid tumors.

[0210] In some situations, the engineered immune cell is used as a therapeutic agent to treat liquid tumors, and in such situations, the enhancer moiety can comprise any cytokine in any form as described herein. In some situations, the engineered immune cell is used as a therapeutic agent to treat solid tumors, and in such situations, the enhancer moiety can comprise any cytokine in any form and further comprise one or more chemokines.

[0211] In some cases, two cytokines may be used to enhance the cytokine-related signaling pathway in the engineered immune cell, including IL-2 and IL-7, IL-2 and IL-15, IL-7 and IL-15, and TL15 and IL21. The cytokine-related signaling pathway enhancement can comprise the expression of a polypeptide selected from the group consisting of a mbTL fusion protein, a constitutively active IL-7 receptor (C7R), an interleukin, or a combination thereof.

[0212] The enhancer moiety described herein can be interleukin 15 (IL-15) or IL-15 receptor. IL-15 is a 14-15kDs glycoprotein composed of 114 amino acids and belongs to the family of four helix bundle cytokines. IL-15 is structurally homologous to interleukin 2 (IL-2). IL-15 receptor comprises a high affinity IL-15 receptor alpha chain, an IL2/15 receptor beta chain, and a common gamma chain. Therefore, IL-15 may have some functions similar to IL-2, such as stimulating T cell activation and proliferation, enhancing NK cell killing activity and promoting B cell production of immunoglobulin. Recent studies have found that IL-15 may play a role in the differentiation, proliferation and activation of NK cells, NKT cells and intestinal epithelial cells. IL-15 and IL-17 may play a role in the regulation of CD8+ memory T cells. Studies have also shown that IL-15 can regulate the proliferation of CD8+ memory T cells and the survival cycle of NK cells through a mechanism, in which a cell expressing IL-15a chain receptor can present IL-15 to a cell expressing an IL-150 chain and a common gamma chain. IL-15 may also play a role in the non-immune system, such as regulation of skeletal muscle anabolism. The enhancer moiety described herein can be interleukin 7 (IL-7). IL-7 can promote the growth of pre-B cells, pro-B cells, B cells, and T cells. It can also promote growth and anti-apoptosis of B cells and T cells. IL-7 can play a role in the early differentiation and proliferation of thymus and the development and differentiation of dendritic cells. However, IL-7 may not have an enhanced effect on the killing activity of antigen-specific cytotoxic T lymphocytes. It can first transfer from the thymus to the peripheral blood, then induce thymocytes or peripheral blood lymphocytes to produce lymphokines, activate and enhance lymphokine-activated killer cell activity of LAK cells. CD8+ subpopulation can be the main effector cell of IL-7, and IL-7 can also support memory CD8+ T cell expansion and survival. IL-7 can promote bone marrow tissue production. IL-7 not only can stimulate myeloid precursor cells and megakaryocytes to produce colony forming units and platelets, but also can restore the body from the immunosuppression of cyclophosphamide. At higher concentrations, it can also induce cytotoxicity that enhances macrophages, function as a synergistic factor for the production of CTL cells, NK cells, and

activated monocytes, induce monocyte-macrophages to secrete various cytokines and promote the expression of inflammatory factors such as macrophage inflammatory protein alpha (MIP-alpha), MIP-P, IL-8 and monocyte chemoattractant protein-1 (MCP-1) and the like. By activating a large number of inflammatory factors produced by inflammatory cells, IL-7 not only can regulate the interaction between the components of the inflammatory process, but also enhance the inflammatory cytokine receptors (CCR) such as CCR1, CCR2 and CCR5. In addition, IL-7 can play a role in inducing immune responses. IL-7 can induce type I immune responses and increase the production of IFN- γ and IL2. IL-7 can synergize with IL12 to induce IFN- γ and T cell proliferation. IL-7 and transforming growth factor beta (TGF β) can play a regulatory role and can be part of the immune regulatory mechanism. IL-7 not only can promote immune reconstitution of T cells, but also can induce up-regulation of T cell cycle and BCL-2 expression, which broadens the diversity and persistence of circulating T cell receptor pools and increases the number of CD4+ and CD8+ T cells. Moreover, for HIV antigens, expanded T cells can also secrete IL2 and IFN- γ , and have good antiviral function. Therefore, IL-7 can reverse the defects of HIV-specific T lymphocytes in proliferation, cytokine secretion and cell function.

[0213] The enhance moiety can regulate (e.g., activate) signal transducer and activator of transcription 5 (STAT5)-mediated signaling pathway. STAT5 can be widely present in the cytoplasm. When cytokines (e.g., IL2, IL7, IL15 and IL21) bind to the cytokine receptors, the receptor-coupled JAK is activated, thereby phosphorylating the Tyr residue at the C-terminus of the STAT5 protein. The phosphorylated STAT5 can form homologous or heterologous dimers through its SH2 region. The homologous or heterodimer can be transferred to the nucleus and bind to the target gene, thereby regulating the expression of the target gene including the cell regulatory factor and the anti-apoptotic gene. Activation of STAT5 can play a role in maintaining normal cell function and regulating cell proliferation and differentiation. Therefore, regulating the activity of STAT5 signaling pathway may regulate the survival and persistence of CAR-T cells described herein.

[0214] The enhancer moiety can be introduced into a cell (e.g., an immune cell or an engineered immune cell) by delivering a nucleic acid molecule encoding the enhancer moiety into the cell. The nucleic acid molecule can be a vector. The enhancer moiety can be a part of a fusion construct. A fusion protein or corresponding nucleic acid construct can have a structure as presented by a formula selected from: S-2A-L1-scFv-H-TM-C-CD3 ζ -2A-L2-IL15-IL15Ra (A); S-2A-L1-scFv-H-TM-C-CD3 ζ -2A-L2-IL15-IL15Ra-2A-L3-IL7 (B); S-2A-L1-scFv-H-TM-C-CD3 ζ -2A-L2-C7R (C); S-2A-L1-scFv-H-TM-C-CD3 ζ -2A-L2-IL7-IL7Ra (D); wherein: each “-” is independently a linker peptide or a peptide bond; S is a safety switch; 2A is an optional self-cleaving peptide; each of L1, L2 and L3 is independently null or a signal peptide sequence; C7R is as described above; scFv is an antigen binding domain; H is null or a hinge region; TM is a transmembrane domain; C is a costimulatory signaling molecule; CD3 ζ is a cytoplasmic signaling sequence derived from CD3 ζ ; IL15 is interleukin 15, IL15Ra is IL-15 receptor α ; IL7 is interleukin 7, IL7Ra is IL-7 receptor α ; C7R is a constitutively activated IL-7 receptor.

[0215] The enhancer moiety can be part of a chimeric polypeptide. For example, the enhancer moiety can be linked to an inducible cell death moiety. The enhancer moiety can be linked to the inducible cell death moiety by a linker. The linker may not be cleaved. The linker may not comprise a self-cleaving peptide. In some other cases, the enhancer moiety and the inducible cell death moiety can be expressed in a cell from a same nucleic acid molecule and can be cleaved to form two polypeptides.

Inducible Cell Death Moiety

[0216] The engineered immune cell described herein may comprise an inducible cell death moiety, also referred to as “suicide gene switch,” “suicide switch,” “safety switch,” or “cell suicide element.” The inducible cell death moiety can be used to effectively remove of the engineered immune cells (e.g., CAR-T cells) in vivo under the action of exogenous factors (e.g., drugs). The inducible cell death moiety described herein may be rapaCasp9, iCasp9, CD20 (and its mimotope), RQR8, Her2t, CD30, BCMA, EGFRt, HSV-TK, mTMPK and the like. iCasp9, CD20 (and its mimotope), RQR8, and HSV-TK may have the same ability to clear T cells, but rapaCasp9, iCasp9, RQR8, and CD20 (and their mimotope) may be faster in comparison with HSV-TK.

[0217] In some cases, an inducible cell death moiety is capable of effecting death of said cell upon contacting said inducible cell death moiety with a cell death activator. The inducible cell death moiety can be, for example, rapaCasp9, iCasp9, HSV-TK, ACD20, mTMPK, ACD19, RQR8, or EGFRt. In some cases, the inducible cell death moiety is EGFRt, and said cell death activator is an antibody or an antigen binding fragment thereof that binds EGFRt. In some cases, the inducible cell death moiety is HSV-TK, and said cell death activator is GCV. In some cases, the inducible cell death moiety is iCasp9, and said cell death activator is AP1903.

[0218] The inducible cell death moiety can be linked to an enhancer moiety and can be co-expressed in a cell as a chimeric polypeptide as described above.

Graft Versus Host Disease (GVHD)

[0219] To prepare “off-the-shelf” allogeneic T cells for the treatment of malignant and infectious diseases, cell therapy by infusion of T cells can be designed to re-establish immunity against pathogens and malignancies. The amount of time required to produce the T cells with tumor-targeting properties with a sufficient number of T cells in vitro can be generally incompatible with the patient’s therapeutic window. Furthermore, autologous T cells from patients with advanced disease may have impaired function and are tolerant to the desired antigen.

[0220] To address these issues, patients can be administered with allogeneic T cells but need to be prevented from immune-mediated rejection by host T cells by recognizing different major or minor histocompatibility antigens on the infused cells. Infusion of T cells without the expression of TCR alpha and beta chains and HLA-A molecules may not cause GVHD and HVG. Thus the T cells edited with CRISPR/CAS9 to delete TCR alpha chain and HLA-A molecular can serve as a source of universal effector donor cells.

[0221] Although knockdown of Beta-2-Microglobulin (B2M) may prevent donor CAR-T cells from being attacked

by the host T cells. The donor CAR-T cells may be attacked by host NK cells and affect the survival of CAR-T cells. Therefore, the present disclosure provides engineered immune cells which target tumor cells and host T cells and/or NK cells. The engineered immune cells described herein can scavenge host T cells and/or NK cells, and enhance the survival, persistence and expansion ability of CAR-T cells, thereby being more effective against tumor cells.

Gene Editing

[0222] Various gene editing methods can be used in the present disclosure to make the engineered immune cells, including CRISPR, RNA interference technology, TALENs (transcription activator-like (TAL) effector nucleases) and Zinc finger nucleases (ZFNs).

[0223] In some cases, CRISPR/Cas9 system is used to edit the genes of the immune cells. For example, CRISPR/Cas9 system can be used to knockout endogenous TCRs or cell surface markers (e.g., CD7) of the immune cells to generate the engineered immune cells for T cell therapy. The CRISPR/Cas9 (clustered regular interspaced short palindromic repeats)/Cas (CRISPR-associated) system is a natural immune system unique to prokaryotes that is resistant to viruses or exogenous plasmids. The Type II CRISPR/Cas system has been applied in many eukaryotic and prokaryotic organisms as a direct genome-directed genome editing tool. The development of the CRISPR/Cas9 system has revolutionized the ability of people to edit DNA sequences and regulate the expression levels of target genes, providing a powerful tool for accurate genome editing of organisms. The simplified CRISPR/Cas9 system can comprise Cas9 protein and gRNA. The principle of action is that gRNA forms a Cas9-gRNA complex with Cas9 protein through its own Cas9 handle, and the base complementary pairing sequence of gRNA in the Cas9-gRNA complex is paired with the target sequence of the target gene by the principle of base complementary pairing. Cas9 uses its own endonuclease activity to cleave the target DNA sequence. Compared to traditional genome editing techniques, the CRISPR/Cas9 system has several distinct advantages: ease of use, simplicity, low cost, programmability, and the ability to edit multiple genes simultaneously.

Pharmaceutical Composition

[0224] The present disclosure also provides a pharmaceutical composition comprising an engineered immune cell described herein and a pharmaceutically acceptable carrier, diluent or excipient. In some embodiments, the pharmaceutical composition is a liquid composition. The pharmaceutical composition can be administered into a subject, for example, by injection. The concentration of the engineered immune cells in the preparation can be at least about 10^2 , 10^3 , 10^4 , 10^5 , 10^6 , 10^7 , 10^8 , 10^9 , or more cells/ml. In some case, the concentration of the engineered immune cells in the preparation can be 1×10^3 - 1×10^8 cells/ml, or 1×10^4 - 1×10^7 cells/ml.

[0225] The pharmaceutical compositions of the present disclosure may comprise engineered immune cells as described herein, in combination with one or more pharmaceutically or physiologically acceptable carriers, diluents or excipients. Such compositions may comprise buffers such as neutral buffered saline, phosphate buffered saline and the

like; carbohydrates such as glucose, mannose, sucrose or dextrans, mannitol; proteins; polypeptides or amino acids such as glycine; antioxidants; chelating agents such as EDTA or glutathione; adjuvants (e.g., aluminum hydroxide); and preservatives. Compositions of the present disclosure may be formulated for intravenous administration.

[0226] Pharmaceutical compositions of the present disclosure may be administered in a manner appropriate to the disease to be treated (or prevented). The quantity and frequency of administration will be determined by such factors as the condition of the patient, and the type and severity of the patient's disease, although appropriate dosages may be determined by clinical trials.

[0227] When "an immunologically effective amount", "an anti-tumor effective amount", "an tumor-inhibiting effective amount", or "therapeutic amount" is indicated, the precise amount of the compositions of the present disclosure to be administered can be determined by a physician with consideration of individual differences in age, weight, tumor size, extent of infection or metastasis, and condition of the patient (subject). It can generally be stated that a pharmaceutical composition comprising the engineered immune cells (e.g., CAR-T cells) described herein may be administered at a dosage of 10^4 to 10^9 cells/kg body weight, or in some cases, 10^5 to 10^6 cells/kg body weight, including all integer values within those ranges. T cell compositions may also be administered multiple times at these dosages. The cells can be administered by using infusion techniques. The optimal dosage and treatment regime for a particular patient can readily be determined by monitoring the patient for signs of disease and adjusting the treatment accordingly.

[0228] The administration of the subject compositions may be carried out in any convenient manner, including by aerosol inhalation, injection, ingestion, transfusion, implantation or transplantation. The compositions described herein may be administered to a patient subcutaneously, intradermally, intratumorally, intranodally, intramedullary, intramuscularly, by intravenous (i.v.) injection, or intraperitoneally. In some embodiments, the T cell compositions of the present disclosure are administered to a patient by intradermal or subcutaneous injection. In some other embodiments, the T cell compositions of the present disclosure are preferably administered by i.v. injection. The compositions of T cells may be injected directly into a tumor, lymph node, or site of infection.

Therapeutics

[0229] The present disclosure provides therapeutic applications with engineered immune cells (e.g., T cells or NK cells) transduced with a lentiviral vector (LV) encoding an expression cassette described herein. Transduced T cells or NK cells can target tumor cell markers (such as CD19) and activated T cell and/or NK cell consensus markers (such as CD3, CD7, CD137, etc.). The engineered immune cells can be used for allogeneic tumor treatment and can be prepared on a large scale.

[0230] Accordingly, the present disclosure also provides a method of stimulating a T cell mediated immune response to a target cell population or tissue of a subject (e.g., a mammal) comprising the step of administering to the subject an engineered immune cell (e.g., CAR-T cell) of the disclosure.

[0231] In some embodiments, the present disclosure provides a type of cell therapy comprising directly administer-

ing engineered universal CAR-T cells of the present disclosure to a patient in need thereof. The CAR-T cells of the present disclosure may have the endogenous TCR expression knocked out or silenced in the cells by gene editing technology. Inactivation of the endogenous TCRs can prevent killing of normal cells by the TCRs during the allogeneic infusion. The GVHD reaction may be prevented. The CAR-T cells targeting a tumor cell marker (such as CD19) and a marker for activated T cells and/or NK cells (such as CD137) can remove activated T cells and/or NK cells while scavenging tumor cells. In addition, host versus graft response (HVG) can also be prevented. The cell therapy provided herein can also improve the survival and anti-tumor effect of allogeneic CAR-T cells in the subject.

[0232] In some embodiments, provided herein is a method of treating or diagnosing a disease in a subject, comprising administering the pharmaceutical composition described herein to said subject.

[0233] The engineered immune cell in said pharmaceutical composition can be derived from an allogeneic immune cell. The engineered immune cell derived from said allogeneic immune cell may not induce graft versus host disease (GvHD) in said subject. The engineered immune cell in said pharmaceutical composition can be derived from an autologous immune cell.

[0234] The endogenous TCR of said engineered immune cell in said pharmaceutical composition may be functionally inactive. The engineered immune cell can reduce GVHD in said subject compared to an additional immune cell having a functionally active TCR. The disease can be a cancer. The cancer can be, for example, lymphoma or leukemia.

[0235] The CAR-T cells of the present disclosure can undergo robust *in vivo* cell expansion and can be extended. The CAR-mediated immune response can be part of a step of adoptive immunotherapy in which CAR-modified T cells can induce an immune response specific for the antigen-binding domain in the CAR. For example, anti-CD19 CAR-T cells elicit a specific immune response against cells expressing CD19.

[0236] The engineered immune cells provided herein can be used to treat cancers. Cancers that may be treated include tumors that are not vascularized, or not yet substantially vascularized, as well as vascularized tumors. The cancers may comprise non-solid tumors (such as hematological tumors, for example, leukemias and lymphomas) or may comprise solid tumors. Types of cancers to be treated with the CARs of the present disclosure include, but are not limited to, carcinoma, blastoma, and sarcoma, and certain leukemia or lymphoid malignancies, benign and malignant tumors, and malignancies e.g., sarcomas, carcinomas, and melanomas. Adult tumors/cancers and pediatric tumors/cancers are also included.

[0237] Hematologic cancers are cancers of the blood or bone marrow. Examples of hematological (or hematogenous) cancers include leukemias, including acute leukemias (such as acute lymphocytic leukemia, acute myelocytic leukemia, acute myelogenous leukemia and myeloblastic, promyelocytic, myelomonocytic, monocytic and erythroleukemia), chronic leukemias (such as chronic myelocytic (granulocytic) leukemia, chronic myelogenous leukemia, and chronic lymphocytic leukemia), polycythemia vera, lymphoma, Hodgkin's disease, non-Hodgkin's lymphoma (indolent and high grade forms), multiple myeloma,

Waldenstrom's macroglobulinemia, heavy chain disease, myelodysplastic syndrome, hairy cell leukemia and myelodysplasia.

[0238] Solid tumors are abnormal masses of tissue that usually do not contain cysts or liquid areas. Solid tumors can be benign or malignant. Different types of solid tumors are named for the type of cells that form them (such as sarcomas, carcinomas, and lymphomas). Examples of solid tumors, such as sarcomas and carcinomas, include fibrosarcoma, myxosarcoma, liposarcoma, chondrosarcoma, osteosarcoma, and other sarcomas, synovioma, mesothelioma, Ewing's tumor, leiomyosarcoma, rhabdomyosarcoma, colon carcinoma, lymphoid malignancy, pancreatic cancer, breast cancer, lung cancers, ovarian cancer, prostate cancer, hepatocellular carcinoma, squamous cell carcinoma, basal cell carcinoma, adenocarcinoma, sweat gland carcinoma, medullary thyroid carcinoma, papillary thyroid carcinoma, pheochromocytomas sebaceous gland carcinoma, papillary carcinoma, papillary adenocarcinomas, medullary carcinoma, bronchogenic carcinoma, renal cell carcinoma, hepatoma, bile duct carcinoma, choriocarcinoma, Wilms' tumor, cervical cancer, testicular tumor, seminoma, bladder carcinoma, melanoma, and CNS tumors (such as a glioma (such as brainstem glioma and mixed gliomas), glioblastoma (also known as glioblastoma multiforme) astrocytoma, CNS lymphoma, germinoma, medulloblastoma, Schwannoma craniopharyngioma, ependymoma, pinealoma, hemangioblastoma, acoustic neuroma, oligodendroglioma, meningioma, neuroblastoma, retinoblastoma and brain metastases).

[0239] In some embodiments, the antigen bind moiety portion of the CAR of the present disclosure is designed to treat a particular cancer. For example, the CAR designed to target CD19 can be used to treat cancers and disorders including but are not limited to pre-B ALL (pediatric indication), adult ALL, mantle cell lymphoma, diffuse large B-cell lymphoma, salvage post allogeneic bone marrow transplantation, and the like. In some embodiments, the CAR can be designed to target CD22 to treat diffuse large B-cell lymphoma. In some embodiments, cancers and disorders include but are not limited to pre-B ALL (pediatric indication), adult ALL, mantle cell lymphoma, diffuse large B-cell lymphoma, salvage post allogeneic bone marrow transplantation, and the like can be treated using a combination of CARs that target CD19, CD20, CD22, and ROR1. In some embodiments, the CAR can be designed to target mesothelin to treat mesothelioma, pancreatic cancer, ovarian cancer, and the like. In some embodiments, the CAR can be designed to target CD33/IL3Ra to treat acute myelogenous leukemia and the like. In some embodiments, the CAR can be designed to target c-Met to treat triple negative breast cancer, non-small cell lung cancer, and the like. In some embodiments, the CAR can be designed to target PSMA to treat prostate cancer and the like. In some embodiments, the CAR can be designed to target Glycolipid F77 to treat prostate cancer and the like. In some embodiments, the CAR can be designed to target EGFRvIII to treat glioblastoma and the like. In some embodiments, the CAR can be designed to target GD-2 to treat neuroblastoma, melanoma, and the like. In some embodiments, the CAR can be designed to target NY-ESO-1 TCR to treat myeloma, sarcoma, melanoma, and the like. In some embodiments, the CAR can be designed to target MAGE A3 TCR to treat myeloma, sarcoma, melanoma, and the like.

[0240] The present disclosure should not be construed to be limited to solely to the antigen targets and diseases disclosed herein. Rather, the present disclosure should be construed to include any antigenic target that is associated with a disease where a CAR can be used to treat the disease.

[0241] The cell therapy disclosed herein can be co-formulated with, and/or co-administered with, one or more additional therapeutic agents, e.g., one or more anti-cancer agents, cytotoxic or cytostatic agents, hormone treatment, vaccines, and/or other immunotherapies. In some embodiments, the engineered immune cells are administered in combination with other therapeutic treatment modalities, including surgery, radiation, cryosurgery, and/or chemotherapy. Such combination therapies may advantageously utilize lower dosages of the administered therapeutic agents, thus avoiding possible toxicities or complications associated with the various monotherapies.

[0242] In certain embodiments, the methods and compositions described herein are administered in combination with one or more antibody molecules, chemotherapy, other anti-cancer therapy (e.g., targeted anti-cancer therapies, or oncolytic drugs), cytotoxic agents, immune-based therapies (e.g., cytokines), surgical and/or radiation procedures. Exemplary cytotoxic agents that can be administered in combination with include antimicrotubule agents, topoisomerase inhibitors, anti-metabolites, mitotic inhibitors, alkylating agents, anthracyclines, vinca alkaloids, intercalating agents, agents capable of interfering with a signal transduction pathway, agents that promote apoptosis, proteasome inhibitors, and radiation (e.g., local or whole body irradiation).

[0243] In certain embodiments, the combination therapy, is used in combination with a standard of cancer care chemotherapeutic agent including, but not limited to, anastrozole (Arimidex®), bicalutamide (Casodex®), bleomycin sulfate (Blenoxane®), busulfan (Myleran®), busulfan injection (Busulfex®), capecitabine (Xeloda®), N4-pentoxycarbonyl-5-deoxy-5-fluorocytidine, carboplatin (Paraplatin®), carmustine (BiCNU®), chlorambucil (Leukeran®), cisplatin (Platinol®), cladribine (Leustatin®), cyclophosphamide (Cytosan® or Neosar®), cytarabine, cytosine arabinoside (Cy-tosar-U®), cytarabine liposome injection (DepoCyt®), dacarbazine (DTIC-Dome®), dactinomycin (Actinomycin D, Cosmegen®), daunorubicin hydrochloride (Cerubidine®), daunorubicin citrate liposome injection (DaunoXome®), dexamethasone, docetaxel (Taxotere®), doxorubicin hydrochloride (Adriamycin®, Rubex®), etoposide (Vepesid®), fludarabine phosphate (Fludara®), 5-fluorouracil (Adrucil®, Efudex®), flutamide (Eulexin®), tezacitabine, Gemcitabine (difluorodeoxy-cytidine), hydroxyurea (Hydrea®), Idarubicin (Idamycin®), ifosfamide (IFEX®), irinotecan (Carnotarsar®), L-asparaginase (ELSPAR®), leucovorin calcium, melphalan (Alkeran®), 6-mercaptopurine (Purinethol®), methotrexate (Folex®), mitoxantrone (Novantrone®), mylotarg, paclitaxel (Taxol®), nab-paclitaxel (Abraxane®), phoenix (Yttrium90/MX-DTPA), pentostatin, polifeprosan 20 with carmustine implant (Gliadel®), tamoxifen citrate (Nolvadex®), teniposide (Vumon®), 6-thioguanine, thiopeta, tirapazamine (Tirazone®), topotecan hydrochloride for injection (Hycamptin®), vinblastine (Velban®), vincristine (Oncovin®), and vinorelbine (Navelbine®).

[0244] Examples of alkylating agents include, without limitation, nitrogen mustards, ethylenimine derivatives, alkyl sulfonates, nitrosoureas and triazines): uracil mustard

(Aminouracil Mustard®, Chlorethaminacil®, Demethyldopan®, Desmethyldopan®, Haemanthamine®, Nordopan®, Uracil nitrogen Mustard®, Uracillost®, Uracilmostaza®, Uramustin®, Uramustine®), chlormethine (Mustargen®), cyclophosphamide (Cytosan®, Neosar®, Clafen®, Endoxan®, Procytox®, Revimmune™), ifosfamide (Mitoxana®), melphalan (Alkeran®), Chlorambucil (Leukeran®), pi-pobroman (Amedel®, Vercyte®), triethylenemelamine (Hemel®, Hexalen®, Hexastat®), triethylenethiophosphoramine, Temozolomide (Temodar®), thiotepa (Thioplex®), busulfan (Busilvex®, Myleran®), carmustine (BiCNU®), lomustine (CeeNU®), streptozocin (Zanosar®), and Dacarbazine (DTIC-Dome®). Additional exemplary alkylating agents include, without limitation, Oxaliplatin (Eloxatin®); Temozolomide (Temodar® and Temodal®); Dactinomycin (also known as actinomycin-D, Cosmegen®); Melphalan (also known as L-PAM, L-sarcosylsin, and phenylalanine mustard, Alkeran®); Altretamine (also known as hexamethylmelamine (HMM), Hexalen®); Carmustine (BiCNU®); Bendamustine (Treanda®); Busulfan (Busulfex® and Myleran®); Carboplatin (Paraplatin®); Lomustine (also known as CCNU, CeeNU®); Cisplatin (also known as CDDP, Platinol® and Platinol®-AQ); Chlorambucil (Leukeran®); Cyclophosphamide (Cytosan® and Neosar®); Dacarbazine (also known as DTIC, DIC and imidazole carboxamide, DTIC-Dome®); Altretamine (also known as hexamethylmelamine (HMM), Hexalen®); Ifosfamide (Ifex®); Prednimustine; Procarbazine (Matulane®); Mechlorethamine (also known as nitrogen mustard, mustine and mechlorethamine hydrochloride, Mustargen®); Streptozocin (Zanosar®); Thiotepa (also known as thiophosphoramide, TESP and TSPA, Thioplex®); Cyclophosphamide (Endoxan®, Cytosan®, Neosar®, Procytox®, Revimmune®); and Bendamustine HCl (Treanda®).

[0245] Examples of anthracyclines include, e.g., doxorubicin (Adriamycin® and Rubex®); bleomycin (Blenoxane®); daunorubicin (daunorubicin hydrochloride, daunomycin, and rubidomycin hydrochloride, Cerubidine®); daunorubicin liposomal (daunorubicin citrate liposome, DaunoXome®); mitoxantrone (DHAD, Novantrone®); epirubicin (Ellence™); idarubicin (Idamycin®, Idamycin PFS®); mitomycin C (Mutamycin®); geldanamycin; herbimycin; ravidomycin; and desacetyl-ravidomycin.

[0246] Examples of vinca alkaloids that can be used in combination with the cell therapy described herein, include, but are not limited to, vinorelbine tartrate (Navelbine®), Vincristine (Oncovin®), and Vindesine (Eldisine®); vinblastine (also known as vinblastine sulfate, vincalurekoblamine and VLB, Alkaban-AQ® and Velban®); and vinorelbine (Navelbine®).

[0247] Examples of proteasome inhibitors that can be used in combination with the cell therapy described herein, include, but are not limited to, bortezomib (Velcade®); carfilzomib (PX-171-007, (S)-4-Methyl-N-((S)-1-((S)-4-methyl-1-((R)-2-methyloxiran-2-yl)-1-oxopentan-2-yl)amino)-1-oxo-3-phenylpropan-2-yl)-2-((S)-2-(2-morpholinoacetamido)-4-phenylbutanamido)-pentanamide); marizomib (NPI-0052); ixazomib citrate (MLN-9708); delanzomib (CEP-18770); 0-Methyl-N-[(2-methyl-5-thiazolyl)carbonyl]-L-seryl-O-methyl-N-[(1S)-2-[(2R)-2-methyl-2-oxiranyl]-2-oxo-1-(phenylmethyl)ethyl]-L-serinamide (ONX-0912); danoprevir (RG7227, CAS 850876-88-9); ixazomib (MHLN2238, CAS 1072833-77-2); and (S)

N-[(phenylmethoxy)carbonyl]-L-leucyl-N-(1-formyl-3-methylbutyl)-L-Leucinamide (MG-132, CAS 133407-82-6).

[0248] In some embodiments, the cell therapy may be used in combination with a tyrosine kinase inhibitor (e.g., a receptor tyrosine kinase (RTK) inhibitor). Exemplary tyrosine kinase inhibitor include, but are not limited to, an epidermal growth factor (EGF) pathway inhibitor (e.g., an epidermal growth factor receptor (EGFR) inhibitor), a vascular endothelial growth factor (VEGF) pathway inhibitor (e.g., a vascular endothelial growth factor receptor (VEGFR) inhibitor (e.g., a VEGFR-1 inhibitor, a VEGFR-2 inhibitor, a VEGFR-3 inhibitor)), a platelet derived growth factor (PDGF) pathway inhibitor (e.g., a platelet derived growth factor receptor (PDGFR) inhibitor (e.g., a PDGFR- α inhibitor)), a RAF-1 inhibitor, a KIT inhibitor and a RET inhibitor. In some embodiments, the anti-cancer agent used in combination with the hedgehog inhibitor is selected from the group consisting of: axitinib (AG013736), bosutinib (SKI-606), cediranib (RE-CENTIN, AZD2171), dasatinib (SPRYCEL®, BMS-354825), erlotinib (TARCEVA®), gefitinib (IRESSA®), imatinib (Gleevec®, CGP57148B, STI-571), lapatinib (TYKERB®, TYVERB®), lestaurtinib (CEP-701), neratinib (HKI-272), nilotinib (TASIGNA®), semaxanib (semaxinib, SU5416), sunitinib (SUTENT®, SU11248), toceranib (PALLADIA®), vandetanib (ZAC-TIMA®, ZD6474), vatalanib (PTK787, PTK/ZK), trastuzumab (HERCEPTIN®), bevacizumab (AVASTIN®), rituximab (RITUXAN®), cetuximab (ERBITUX®), panitumumab (VECTIBIX®), ranibizumab (Lucentis®), nilotinib (TASIGNA®), sorafenib (NEXAVAR®), alemtuzumab (CAM-PATH®), gemtuzumab ozogamicin (MYLOTARG®), ENMD-2076, PCI-32765, AC220, dovitinib lactate (TK1258, CHIR-258), BIBW 2992 (TO-VOK™), SGX523, PF-04217903, PF-02341066, PF-299804, BMS-777607, ABT-869, MP470, BIBF 1120 (VARGATEF®), AP24534, JNJ-26483327, MGCD265, DCC-2036, BMS-690154, CEP-11981, tivozanib (AV-951), OSI-930, MM-121, XL-184, XL-647, XL228, AEE788, AG-490, AST-6, BMS-599626, CUDC-101, PD153035, pelitinib (EKB-569), vandetanib (zactima), WZ3146, WZ4002, WZ8040, ABT-869 (linifanib), AEE788, AP24534 (ponatinib), AV-951 (tivozanib), axitinib, BAY 73-4506 (regorafenib), brivanib alaninate (BMS-582664), brivanib (BMS-540215), cediranib (AZD2171), CHIR-258 (dovitinib), CP 673451, CYC116, E7080, Ki8751, masitinib (AB1010), MGCD-265, motesanib diphosphate (AMG-706), MP-470, OSI-930, Pazopanib Hydrochloride, PD173074, Sorafenib Tosylate (Bay 43-9006), SU 5402, TSU-68 (SU6668), vatalanib, XL880 (GSK1363089, EXEL-2880). Further examples of hedgehog inhibitors include, but are not limited to, vismodegib (2-chloro-N-[4-chloro-3-(2-pyridinyl)phenyl]-4-(methylsulfonyl)-benzamide, GDC-0449); 1-(4-Chloro-3-(trifluoromethyl)phenyl)-3-((3-(4-fluorophenyl)-3,4-dihydro-4-oxo-2-quinazolinyl)methyl)urea (CAS 330796-24-2); N-[(2S,3R,3'R,3aS,4'aR,6S,6'aR,6'bS,7aR,12'aS,12'bS)-2', 3', 3a,4,4', 4'a,5,5', 6,6', 6'a,6'b,7,7', 7a,8', 10', 12', 12'a,12'b-Eicosa-hydro-3,6,11', 12'b-tetramethylspiro[furo[3,2-b]pyridine-2(3H),9'(1H)-naphth[2,1-a]azulen]-3'-yl]-methanesulfonamide (IPI926, CAS 1037210-93-7); and 4-Fluoro-N-methyl-N-[1-[4-(1-methyl-1H-pyrazol-5-yl)-1-phthalazinyl]-4-piperidinyl]-2-(trifluoromethyl)-benzamide (LY2940680, CAS 1258861-20-9); and Erismodegib (LDE225). Selected tyrosine kinase inhibitors are chosen from sunitinib, erlo-

tinib, gefitinib, or sorafenib erlotinib hydrochloride (Tarceva®); linifanib (N-[4-(3-amino-1H-indazol-4-yl)phenyl]-N'-(2-fluoro-5-methylphenyl)urea, also known as ABT 869, available from Genentech); sunitinib malate (Sutent®); bosutinib (4-[(2,4-dichloro-5-methoxyphenyl)amino]-6-methoxy-7-[3-(4-methylpiperazin-1-yl)propoxy]quinoline-3-carbonitrile, also known as SKI-606, described in U.S. Pat. No. 6,780,996); dasatinib (Sprycel®); pazopanib (Votrient®); sorafenib (Nexavar®); zactima (ZD6474); and imatinib or imatinib mesylate (Gleevec® and Gleevec®).

[0249] In certain embodiments, the cell therapy can be used in combination with a Vascular Endothelial Growth Factor (VEGF) receptor inhibitors, including but not limited to, Bevacizumab (Avastin®), axitinib (Inlyta®); Brivanib alaninate (BMS-582664, (S)-((R)-1-(4-(4-Fluoro-2-methyl-1H-indol-5-yloxy)-5-methylpyrrolo[2,1-f][1,2,4]triazin-6-yloxy)propan-2-yl)2-aminopropanoate); Sorafenib (Nexavar®); Pazopanib (Votrient®); Sunitinib malate (Sutent®); Cediranib (AZD2171, CAS 288383-20-1); Vargatef (BIBF1120, CAS 928326-83-4); Foretinib (GSK1363089); Telatinib (BAY57-9352, CAS 332012-40-5); Apatinib (YN968D1, CAS 811803-05-1); Imatinib (Gleevec®); Ponatinib (AP24534, CAS 943319-70-8); Tivozanib (AV951, CAS 475108-18-0); Regorafenib (BAY73-4506, CAS 755037-03-7); Vatalanib dihydrochloride (PTK787, CAS 212141-51-0); Brivanib (BMS-540215, CAS 649735-46-6); Vandetanib (Caprelsa® or AZD6474); Motesanib diphosphate (AMG706, CAS 857876-30-3, N-(2,3-dihydro-3,3-dimethyl-1H-indol-6-yl)-2-[(4-pyridinylmethyl)amino]-3-pyridinecarboxamide, described in PCT Publication No. WO 02/066470); Dovitinib dilactate acid (TK1258, CAS 852433-84-2); Linfan-ib (ABT869, CAS 796967-16-3); Cabozantinib (XL184, CAS 849217-68-1); Lestaurtinib (CAS 111358-88-4); N-[5-[[[5-(1,1-Dimethyl-ethyl)-2-oxazolyl]methyl]thio]-2-thiazolyl]-4-piperidinecarboxamide (BMS38703, CAS 345627-80-7); (3R,4R)-4-Amino-1-((4-((3-methoxyphenyl)amino)pyrrolo[2,1-f][1,2,4]triazin-5-yl)methyl)piperidin-3-ol (BMS690514); N-(3,4-Dichloro-2-fluorophenyl)-6-methoxy-7-[[3aa,50,6aa]-octahydro-2-methylcyclopenta[c]pyrrol-5-yl]methoxy]-4-quinazolinamine (XL647, CAS 781613-23-8); 4-Methyl-3-[[1-methyl-6-(3-pyridinyl)-1H-pyrazolo[3,4-d]pyrimidin-4-yl]amino]-N-[3-(trifluoromethyl)phenyl]-benzamide (BHG712, CAS 940310-85-0); and Afibercept (Eylea®).

[0250] In some embodiments, the cell therapy described herein can be used in combination with a PI3K inhibitor. The PI3K inhibitor can be an inhibitor of delta and gamma isoforms of PI3K. Examples of PI3K inhibitors include, but are not limited to, 4-[2-(1H-Indazol-4-yl)-6-[[4-(methylsulfonyl)piperazin-1-yl]methyl]thieno[3,2-d]pyrimidin-4-yl]morpholine; 2-Methyl-2-[4-[3-methyl-2-oxo-8-(quinolin-3-yl)-2,3-dihydroimidazo[4,5-c]quinolin-1-yl]phenyl]propionitrile; 4-(trifluoromethyl)-5-(2,6-dimorpholinopyrimidin-4-yl)pyridin-2-amine; Tozasertib (VX680 or MK-0457, CAS 639089-54-6); (5Z)-5-[[4-(4-Pyridinyl)-6-quinolinyl]methylene]-2,4-thiazolidinedione (GSK1059615, CAS 958852-01-2); (1E,4S,4aR,5R,6aS,9aR)-5-(Acetyloxy)-1-[(di-2-propenylamino)methylene]-4,4a,5,6,6a,8,9,9a-octahydro-11-hydroxy-4-(methoxymethyl)-4a,6a-dimethyl-cyclopenta[5,6]naphtho[1,2-c]pyran-2,7,10(1H)-trione (PX866, CAS 502632-66-8); 8-Phenyl-2-(morpholin-4-yl)-chromen-4-one (LY294002, CAS 154447-36-6); 2-Amino-8-ethyl-4-methyl-6-(1H-pyrazol-5-yl)pyrido[2,3-d]pyrimidin-7(8H)-one (SAR 245409 or XL

765); 1,3-Dihydro-8-(6-methoxy-3-pyridinyl)-3-methyl-1-[4-(1-piperazinyl)-3-(trifluoromethyl)phenyl]-2H-imidazo[4,5-c]quinolin-2-one, (2Z)-2-butenedioate (1:1) (BGT 226); 5-Fluoro-3-phenyl-2-[(1S)-1-(9H-purin-6-ylamino)ethyl]-4(3H)-quinazolinone (CAL101); 2-Amino-N-[3-[N-[3-[(2-chloro-5-methoxyphenyl)amino]quinoxalin-2-yl]sulfamoyl]phenyl]-2-methylpropanamide (SAR 245408 or XL 147); and (S)-Pyrrolidine-1,2-dicarboxylic acid 2-amide 1-({4-methyl-5-[2-(2,2,2-trifluoro-1,1-dimethyl-ethyl)-pyridin-4-yl]-thiazol-2-yl}-amide) (BYL719).

[0251] In some embodiments, the cell therapy described herein can be used in combination with a mTOR inhibitor, e.g., one or more mTOR inhibitors chosen from one or more of rapamycin, temsirolimus (TORISEL®), AZD8055, BEZ235, BGT226, XL765, PF-4691502, GDC0980, SF1126, OSI-027, GSK1059615, KU-0063794, WYE-354, Palomid 529 (P529), PF-04691502, or PKI-587. ridaforolimus (formally known as deferolimus, (1R,2R,4S)-4-[(2R)-2-[(1R,9S,12S,15R,16E,18R,19R,21R, 23S,24E,26E,28Z, 30S,32S,35R)-1,18-dihydroxy-19,30-dimethoxy-15,17,21, 23, 29,35-hexamethyl-2,3,10,14,20-penta-oxo-11,36-dioxo-4-azatricyclo[30.3.1.04,9] hexatriaconta-16,24,26,28-tetraen-12-yl]propyl]-2-methoxycyclohexyl di-methylphosphinate, also known as AP23573 and MK8669, and described in PCT Publication No. WO 03/064383); everolimus (Afinitor® or RAD001); rapamycin (AY22989, Sirolimus®); simapi-mod (CAS 164301-51-3); emsirolimus, (5-{2,4-Bis[(3S)-3-methylmorpholin-4-yl]pyrido[2,3-d]pyrimidin-7-yl}-2-methoxyphenyl)methanol (AZD8055); 2-Amino-8-[trans-4-(2-hydroxyethoxy)cyclohexyl]-6-(6-methoxy-3-pyridinyl)-4-methyl-pyrido[2,3-d]pyrimidin-7(8H)-one (PF04691502, CAS 1013101-36-4); and N2-[1,4-dioxo-4-[[4-(4-oxo-8-phenyl-4H-1-benzopyran-2-yl)morpholinium-4-yl]methoxy]butyl]-L-arginylglycyl-L-aspartyl-L-serine (SEQ ID NO: 94), inner salt (SF1126, CAS 936487-67-1), (1r,4r)-4-(4-amino-5-(7-methoxy-1H-indol-2-yl)imidazo[1,5-f][1,2,4]triazin-7-yl) cyclohexanecarboxylic acid (OSI-027); and XL765.

[0252] In some embodiments, the cell therapy can be used in combination with a BRAF inhibitor, e.g., GSK2118436, RG7204, PLX4032, GDC-0879, PLX4720, and sorafenib tosylate (Bay 43-9006). In further embodiments, a BRAF inhibitor includes, but is not limited to, regorafenib (BAY73-4506, CAS 755037-03-7); tivantinib (AV951, CAS 475108-18-0); vemurafenib (Zel-boraf®, PLX-4032, CAS 918504-65-1); encorafenib (also known as LGX818); 1-Methyl-5-[[2-[5-(trifluoromethyl)-1H-imidazol-2-yl]-4-pyridinyl]oxy]-N-[4-(trifluoromethyl)phenyl]-1H-benzimidazol-2-amine (RAF265, CAS 927880-90-8); 5-[1-(2-Hydroxyethyl)-3-(pyridin-4-yl)-1H-pyrazol-4-yl]-2,3-dihydroinden-1-one oxime (GDC-0879, CAS 905281-76-7); 5-[2-[4-[2-(Dimethylamino)ethoxy]phenyl]-5-(4-pyridinyl)-1H-imidazol-4-yl]-2,3-dihydro-1H-Inden-1-one oxime (GSK2118436 or SB590885); (+/-)-Methyl (5-(2-(5-chloro-2-methylphenyl)-1-hydroxy-3-oxo-2,3-dihydro-1H-isoindol-1-yl)-1H-benzimidazol-2-yl)carbamate (also known as XL-281 and BMS908662) and N-(3-(5-chloro-1H-pyrrolo[2,3-b]pyridine-3-carbonyl)-2,4-difluorophenyl) propane-1-sulfonamide (also known as PLX4720).

[0253] In some embodiments, the cell therapy described herein can be used in combination with a MEK inhibitor. Any MEK inhibitor can be used in combination including, but not limited to, selumetinib (5-[(4-bromo-2-chlorophenyl)amino]-4-fluoro-N-(2-hydroxyethoxy)-1-methyl-1H-

benzimidazole-6-carboxamide, also known as AZD6244 or ARRY 142886); ARRY-142886 trametinib dimethyl sulfoxide (GSK-1120212, CAS 1204531-25-80); G02442104 (also known as GSK1120212), RDEA436; N-[3,4-Difluoro-2-[(2-fluoro-4-iodophenyl)amino]-6-methoxyphenyl]-1-[(2R)-2,3-dihydroxypropyl]-cyclopropanesulfonamide (also known as RDEA119 or BAY869766); RDEA119/BAY 869766, AS703026; G00039805 (also known as AZD-6244 or selumetinib), BIX 02188; BIX 02189; 2-[(2-Chloro-4-iodophenyl)amino]-N-(cyclopropylmethoxy)-3,4-difluoro-benzamide (also known as CI-1040 or PD184352); CI-1040 (PD-184352), N-[(2R)-2,3-Dihydroxypropoxy]-3,4-difluoro-2-[(2-fluoro-4-iodophenyl)amino]-benzamide (also known as PD0325901); PD03259012'-amino-3'-methoxyflavone (also known as PD98059 available from BIAffin GmbH & Co., KG, Germany); PD98059, 2,3-bis[amino[(2-aminophenyl)thio]methylene]-butanedinitrile (also known as U0126); U0126, XL-518 (also known as GDC-0973, Cas No. 1029872-29-4, available from ACC Corp.); GDC-0973 (Methanone, [3,4-difluoro-2-[(2-fluoro-4-iodophenyl)amino]phenyl][3-hydroxy-3-(25)-2-piperidinyl-1-azetidinyl]-), G-38963; and G02443714 (also known as AS703206), or a pharmaceutically acceptable salt or solvate thereof. Further examples of MEK inhibitors include, but are not limited to, benimetinib (6-(4-bromo-2-fluorophenylamino)-7-fluoro-3-methyl-3H-benzimidazole-5-carboxylic acid (2-hydroxyethoxy)-amide, also known as MEK162, CAS 1073666-70-2); 2,3-Bis[amino[(2-aminophenyl)thio]methylene]-butanedinitrile (also known as U0126 and described in U.S. Pat. No. 2,779,780); (3S,4R, 5Z,8S,9S,11E)-14-(Ethylamino)-8,9,16-trihydroxy-3,4-dimethyl-3,4,9, 19-tetrahydro-1H-2-benzoxacyclotetradecine-1,7(8H)-dione] (also known as E6201); vemurafenib (PLX-4032, CAS 918504-65-1); (R)-3-(2,3-Dihydroxypropyl)-6-fluoro-5-(2-fluoro-4-iodophenylamino)-8-methylpyrido[2,3-d]pyrimidine-4,7(3H,8H)-dione (TAK-733, CAS 1035555-63-5); pimasertib (AS-703026, CAS 1204531-26-9); 2-(2-Fluoro-4-iodophenylamino)-N-(2-hydroxyethoxy)-1,5-dimethyl-6-oxo-1,6-dihydropyridine-3-carboxamide (AZD 8330); and 3,4-Difluoro-2-[(2-fluoro-4-iodophenyl)amino]-N-(2-hydroxyethoxy)-5-[(3-oxo-[1,2]oxazinan-2-yl)methyl]benzamide (CH 4987655 or Ro 4987655).

[0254] In some embodiments, the cell therapy described herein can be used in combination with a JAK2 inhibitor, e.g., CEP-701, INCB18424, CP-690550 (tasocitinib). Example JAK inhibitors include, but are not limited to, ruxolitinib (Jakafi®); tofacitinib (CP690550); axitinib (AG013736, CAS 319460-85-0); 5-Chloro-N2-[(1S)-1-(5-fluoro-2-pyrimidinyl)ethyl]-N4-(5-methyl-1H-pyrazol-3-yl)-12,4-pyrimidinediamine (AZD1480, CAS 935666-88-9); (9E)-15-[2-(1-Pyrrolidinyl)ethoxy]-7,12,26-trioxa-19,21, 24-triazatetracyclo[18.3.1.12,5.114,18]-hexacos-1(24),2,4, 9,14,16,18(25),20,22-nonaene (SB-1578, CAS 937273-04-6); momelotinib (CYT 387); baricitinib (INCB-028050 or LY-3009104); pacritinib (SB1518); (16E)-14-Methyl-20-oxa-5,7,14,27-tetraazatetracyclo[19.3.1.12,6.18,12]heptacos-1(25),2,4,6(27),8,10,12(26),16,21,23-decaene (SB 1317); gandotinib (LY 2784544); and N,N-cyclopropyl-4-[(1,5-dimethyl-1H-pyrazol-3-yl)amino]-6-ethyl-1,6-dihydro-1-methyl-imidazo[4,5-d]pyrrolo[2,3-b]pyridine-7-carboxamide (BMS 911543).

[0255] In some embodiments, the combination therapies disclosed herein include paclitaxel or a paclitaxel agent, e.g., TAXOL®, protein-bound paclitaxel (e.g., ABRAXANE®).

Exemplary paclitaxel agents include, but are not limited to, nanoparticle albumin-bound paclitaxel (ABRAX-ANE, marketed by Abraxis Bioscience), docosahexaenoic acid bound-paclitaxel (DHA-paclitaxel, Taxoprexin, marketed by Protarga), polyglutamate bound-paclitaxel (PG-paclitaxel, paclitaxel poliglumex, CT-2103, XYOTAX, marketed by Cell Therapeutic), the tumor-activated prodrug (TAP), ANG105 (Angiopep-2 bound to three molecules of paclitaxel, marketed by Im-munoGen), paclitaxel-EC-1 (paclitaxel bound to the erbB2-recognizing peptide EC-1; see Li et al., *Biopolymers* (2007) 87:225-230), and glucose-conjugated paclitaxel (e.g., 2'-paclitaxel me-thyl 2-glucopyranosyl succinate).

Methods

[0256] The present disclosure provides methods for generating an engineered cell. In some aspects, the method can comprise (a) delivering a nucleic acid molecule expressing a chimeric polypeptide into a cell; and (b) expressing the nucleic acid molecule in the cell, thereby generating the engineered cell. The chimeric polypeptide can be a chimeric antigen receptor as described herein.

[0257] The present disclosure also provides methods for administering an engineered cell as described herein. The engineered cell can be an engineered immune cell. The engineered immune cell can be a T cell. The engineered immune cell can be derived from an autologous T cell. The engineered immune cell can be derived from an allogeneic T cell.

[0258] In some aspects, provided herein is a method for administering an engineered immune cell comprising a chimeric polypeptide comprising (i) an enhancer moiety capable of enhancing one or more activities of the engineered immune cell, and (ii) an inducible cell death moiety capable of effecting death of the engineered immune cell upon contacting the chimeric polypeptide with a cell death activator. The enhancer moiety can be linked to the inducible cell death moiety. The engineered immune cell can further comprise one or more chimeric antigen receptors (CARs) comprising a binding moiety. The binding moiety can comprise a first antigen binding domain, which first antigen binding domain suppresses or reduces a subject's immune response toward the engineered immune cell when administered into the subject. The binding moiety can further comprise a second antigen binding domain capable of binding to a disease-associated antigen. An individual CAR of the one or more CARs can comprise (i) the first antigen binding domain, (ii) the second antigen binding domain, or (iii) both the first antigen binding domain and the second antigen binding domain. Each CAR of the one or more CARs can further comprise a transmembrane domain and an intracellular signaling domain.

[0259] In some aspects, provided herein is a method of administering an engineered immune cell comprising one or more chimeric antigen receptors (CARs) comprising a binding moiety. The binding moiety can comprise a first antigen binding domain capable of binding to an immune cell antigen and a second antigen binding domain capable of binding to a disease-associated antigen. Each CAR of the one or more CARs can further comprise a transmembrane domain and an intracellular signaling domain. The engineered immune cell can further comprise an enhancer moiety capable of enhancing one or more activities of the engineered immune cell. In some cases, an endogenous T

cell receptor (TCR) of the engineered immune cell can be inactivated. The engineered immune cell can exhibit (i) enhanced degree of persistence by remaining viable in vitro for at least about 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 150, 200, 250, 300, 350, 400, 450, 500, or more days while in presence of cells (e.g., cancer cells, immune cells, or both) that are heterologous to the engineered immune cell, (ii) enhanced degree of expansion by at least about 2-fold, 3-fold, 4-fold, 5-fold, 6-fold, 7-fold, 8-fold, 9-fold, 10-fold, 15-fold, 20-fold, 25-fold, 30-fold, 35-fold, 40-fold, 45-fold, 50-fold, 55-fold, 60-fold, 65-fold, 70-fold, 75-fold, 80-fold, 85-fold, 90-fold, 95-fold, 100-fold, 110-fold, 120-fold, 130-fold, 140-fold, 150-fold, 160-fold, 170-fold, 180-fold, 190-fold, 200-fold, 210-fold, 220-fold, 230-fold, 240-fold, 250-fold, 260-fold, 270-fold, 280-fold, 290-fold, 300-fold, 350-fold, 400-fold, 450-fold, 500-fold, or more within 30 days. In some cases, the engineered immune cell can exhibit enhanced degree of expansion by at least about 100-fold, 200-fold, 300-fold, 400-fold, 500-fold, 600-fold, 700-fold, 800-fold, 900-fold, 1,000-fold, 2,000-fold, 3,000-fold, 4,000-fold, 5,000-fold, 6,000-fold, 7,000-fold, 8,000-fold, 9,000-fold, 10,000-fold, 20,000-fold, 30,000-fold, 40,000-fold, 50,000-fold, 60,000-fold, 70,000-fold, 80,000-fold, 90,000-fold, 100,000-fold, 200,000-fold, 300,000-fold, 400,000-fold, 500,000-fold, 600,000-fold, 700,000-fold, 800,000-fold, 900,000-fold, 1,000,000-fold, or more within 60 days. The viability or expansion can be measured in the presence of stimulation, for example, stimulation by a cancer antigen or a cancer cell. The viability or expansion can be measured in the presence of multiple rounds or repeated stimulations.

[0260] In some aspects, provided herein is a method of administering a cell (e.g., an engineered immune cell), comprising a functionally inactive T cell receptor (TCR). The cell can further comprise one or more chimeric antigen receptors (CARs). Each individual CAR of the one or more CARs can comprise a binding moiety. The binding moiety can comprise (i) a first antigen binding domain, which first antigen binding domain suppresses or reduces a subject's immune response toward the engineered immune cell when administered into the subject and (ii) a second antigen binding domain that binds to a disease-associated antigen. Each CAR of the one or more CARs can further comprise a transmembrane domain and an intracellular signaling domain.

[0261] In some aspects, provided herein is a method of administering an engineered immune cell comprising an enhancer moiety capable of enhancing one or more activities of the engineered immune cell. The engineered cell can further comprise a chimeric antigen receptor (CAR) comprising an antigen binding domain that specifically binds CD7. The CAR can further comprise a transmembrane domain and an intracellular signaling domain. The endogenous CD7 in the engineered immune cell can be inactivated. In some embodiments, the engineered cell can com-

prise a CAR comprising an antigen binding domain that specifically binds an immune cell antigen. The immune cell antigen can be any immune cell antigen described herein such as CD2, CD3, CD4, CD5, CD8, CD16a, CD16b, CD25, CD27, CD28, CD30, CD38, CD45, CD48, CD50, CD52, CD56, CD57, CD62L, CD69, CD94, CD100, CD102, CD122, CD127, CD132, CD137, CD160, CD161, CD178, CD218, CD226, CD244, CD159a (NKG2A), CD159c (NKG2C), NKG2E, CD279, CD314 (NKG2D), CD305, CD335 (NKP46), CD337, CD319 (CS1), TCR α , TCR β and SLAMF7. The endogenous immune cell antigen of the engineered cell, which the antigen binding domain binds, can be inactivated in the engineered cell.

[0262] In some aspects, provided herein is a method of administering an engineered immune cell comprising a single chimeric antigen receptor (CAR) comprising (i) a first antigen binding domain that specifically binds CD7 and (ii) a second antigen binding domain capable of binding to a disease-associated antigen. The CAR can further comprise a transmembrane domain and an intracellular signaling domain. A gene encoding endogenous CD7 can be inactivated in the engineered immune cell.

[0263] The present disclosure also provides methods of treating or diagnosing a disease in a subject. In some cases, the method comprises administering a pharmaceutical composition comprising an engineered immune cell into a subject. The engineered immune cell in the pharmaceutical composition can be derived from an allogeneic immune cell. The engineered immune cell derived from the allogeneic immune cell may not induce graft versus host disease (GVHD) in the subject. The engineered immune cell in the pharmaceutical composition can be derived from an autologous immune cell. In some cases, an endogenous TCR of the engineered immune cell in the pharmaceutical composition is functionally inactive. The engineered immune cell can reduce GVHD in the subject compared to an immune cell having a functionally active TCR. The disease can be a cancer. For example, the cancer can be lymphoma or leukemia.

[0264] The present disclosure also provides a method of delivering an allogeneic cell therapy comprising administering to a subject in need thereof a population of engineered immune cells. An individual engineered immune cell of the population can comprise one or more chimeric antigen receptors (CARs) comprising a binding moiety. The binding moiety can comprise a first antigen binding domain capable of binding to an immune cell antigen. The binding moiety can further comprise a second antigen binding domain capable of binding to a disease-associated antigen. The first antigen binding domain can suppress or reduce a subject's immune response toward the engineered immune cell when administered into the subject. The engineered immune cell can further comprise an enhancer moiety capable of enhancing one or more activities of the engineered immune cell. The endogenous T cell receptor (TCR) of the engineered immune cell can be inactivated. For example, a gene encoding a subunit of TCR can be inactivated. Various gene editing methods described herein can be used to inactivate endogenous TCRs of a T cell.

[0265] In some embodiments, a method provided herein can include activation of a population of cells. In some cases, the cell used to prepare the engineered immune cell can be activated before preparing the engineered immune cell. In some cases, the engineered immune cell can be

activated. Activation as used herein can refer to a process whereby a cell transitions from a resting state to an active state. This process can comprise a response to an antigen, migration, and/or a phenotypic or genetic change to a functionally active state. In some aspects, activation can refer to the stepwise process of T cell activation. In some cases, a T cell can require one or more signals to become activated. For example, a T cell can require at least two signals to become fully activated. The first signal can occur after engagement of a TCR by the antigen-MHC complex, and the second signal can occur by engagement of co-stimulatory molecules. Anti-CD3 antibody (or a functional variant thereof) can mimic the first signal and anti-CD28 antibody (or a functional variant thereof) can mimic the second signal in vitro.

[0266] In some aspects, a method provided herein can comprise activation of a population of cells. Activation can be performed by contacting a population of cells with a surface having attached thereto an agent that can stimulate a CD3 TCR complex associated signal and a ligand that can stimulate a co-stimulatory molecule on the surface of the cells. In particular, T cell populations can be stimulated in vitro such as by contact with an anti-CD3 antibody or antigen-binding fragment thereof, or an anti-CD2 antibody immobilized on a surface, or by contact with a protein kinase C activator (e.g., bryostatin) sometimes in conjunction with a calcium ionophore. For co-stimulation of an accessory molecule on the surface of the T cells, a ligand that binds the accessory molecule can be used. For example, a population of cells can be contacted with an anti-CD3 antibody and an anti-CD28 antibody, under conditions that can stimulate proliferation of the T cells. In some cases, 4-1BB can be used to stimulate cells. For example, cells can be stimulated with 4-1BB and IL-21 or another cytokine. For activation of either CD4 T cells or CD8 T cells, an anti-CD3 antibody and an anti-CD28 antibody can be used. For example, the agents providing a signal may be in solution or conjugated to a solid phase surface. The ratio of particles to cells may depend on particle size relative to the target cell. In further embodiments, the cells, such as T cells, can be combined with agent-coated beads, where the beads and the cells can be subsequently separated, and optionally cultured. Each bead can be coated with either anti-CD3 antibody or an anti-CD28 antibody, or in some cases, a combination of the two. In an alternative embodiment, prior to culture, the agent-coated beads and cells are not separated but are cultured together. Cell surface proteins may be conjugated by allowing paramagnetic beads to which anti-CD3 antibody and anti-CD28 antibody can be attached (3 $\times$ 28 beads) to contact the T cells. In one embodiment the cells and beads (for example, DYNABEADS[®] M-450 CD3/CD28 T paramagnetic beads at a ratio of 1:1) are combined in a buffer, for example, phosphate buffered saline (PBS) (e.g., without divalent cations such as, calcium and magnesium). Any cell concentration may be used. The mixture may be cultured for or for about several hours (e.g., about 3 hours) to or to about 14 days or any hourly integer value in between. In another embodiment, the mixture may be cultured for or for about 21 days or for up to or for up to about 21 days. Conditions appropriate for T cell culture can include an appropriate media (e.g., Minimal Essential Media or RPMI Media 1640 or, X-vivo 5, (Lonza)) that may contain factors necessary for proliferation and viability, including serum (e.g., fetal bovine or human serum), interleukin-2 (IL-2), insulin, IFN-

g, TL-4, TL-7, GM-CSF, IL-10, IL-21, IL-15, TGF beta, and TNF alpha or any other additives for the growth of cells. Other additives for the growth of cells include, but are not limited to, surfactant, plasmanate, and reducing agents such as N-acetyl-cysteine and 2-mercaptoethanol. Media can include RPMI 1640, AI M-V, DMEM, MEM, a-MEM, F-12, X-Vivo 1, and X-Vivo 20, Optimizer, with added amino acids, sodium pyruvate, and vitamins, either serum-free or supplemented with an appropriate amount of serum (or plasma) or a defined set of hormones, and/or an amount of cytokine(s) sufficient for the growth and expansion of T cells. Antibiotics, e.g., penicillin and streptomycin, can be included only in experimental cultures, possibly not in cultures of cells that are to be infused into a subject. The target cells can be maintained under conditions necessary to support growth; for example, an appropriate temperature (e.g., 37°C) and atmosphere (e.g., air plus 5% CO₂). In some instances, T cells that have been exposed to varied stimulation times may exhibit different characteristics. In some cases, a soluble monospecific tetrameric antibody against human CD3, CD28, CD2, or any combination thereof may be used. In some embodiments, activation can utilize an activation moiety, a costimulatory agent, and any combination thereof. In some aspects, an activation moiety binds: a CD3/T cell receptor complex and/or provides costimulation. In some aspects, an activation moiety is any one of anti-CD3 antibody and/or anti-CD28 antibody. In some aspects, a solid phase is at least one of a bead, plate, and/or matrix. In some aspects, a solid phase is a bead. Alternatively or in addition to, the activation moiety may be not be conjugated a substrate, e.g., the activation moiety may be free-floating in a medium.

[0267] In some cases, a population of cells can be activated or expanded by co-culturing with tissue or cells. A cell can be an antigen presenting cell. An artificial antigen presenting cells (aAPCs) can express ligands for T cell receptor and costimulatory molecules and can activate and expand T cells for transfer, while improving their potency and function in some cases. An aAPC can be engineered to express any gene for T cell activation. An aAPC can be engineered to express any gene for T cell expansion. An aAPC can be a bead, a cell, a protein, an antibody, a cytokine, or any combination. An aAPC can deliver signals to a cell population that may undergo genomic transplant. For example, an aAPC can deliver a signal 1, signal 2, signal 3 or any combination. A signal 1 can be an antigen recognition signal. For example, signal 1 can be ligation of a TCR by a peptide-MHC complex or binding of agonistic antibodies directed towards CD3 that can lead to activation of the CD3 signal-transduction complex. Signal 2 can be a co-stimulatory signal. For example, a co-stimulatory signal can be anti-CD28, inducible co-stimulator (ICOS), CD27, and 4-1BB (CD137), which bind to ICOS-L, CD70, and 4-1BBL, respectively. Signal 3 can be a cytokine signal. A cytokine can be any cytokine. A cytokine can be IL-2, IL-7, IL-12, IL-15, IL-21, or any combination thereof. In some cases an artificial antigen presenting cell (aAPC) may be used to activate and/or expand a cell population. In some cases, an artificial may not induce allospecificity. An aAPC may not express HLA in some cases. An aAPC may be genetically modified to stably express genes that can be used to activation and/or stimulation. In some cases, a K562 cell may be used for activation. A K562 cell may also be used for expansion. A K562 cell can be a human erythroleukemic cell

line. A K562 cell may be engineered to express genes of interest. K562 cells may not endogenously express HLA class I, II, or CD1d molecules but may express ICAM-1 (CD54) and LFA-3 (CD58). K562 may be engineered to deliver a signal 1 to T cells. For example, K562 cells may be engineered to express HLA class I. In some cases, K562 cells may be engineered to express additional molecules such as B7, CD80, CD83, CD86, CD32, CD64, 4-1BBL, anti-CD3, anti-CD3 mAb, anti-CD28, anti-CD28mAb, CD1d, anti-CD2, membrane-bound IL-15, membrane-bound IL-17, membrane-bound IL-21, membrane-bound IL-2, truncated CD19, or any combination. In some cases, an engineered K562 cell can express a membranous form of anti-CD3 mAb, clone OKT3, in addition to CD80 and CD83. In some cases, an engineered K562 cell can express a membranous form of anti-CD3 mAb, clone OKT3, membranous form of anti-CD28 mAb in addition to CD80 and CD83.

[0268] An aAPC can be a bead. A spherical polystyrene bead can be coated with antibodies against CD3 and CD28 and be used for T cell activation. A bead can be of any size. In some cases, a bead can be or can be about 3 and 6 micrometers. A bead can be or can be about 4.5 micrometers in size. A bead can be utilized at any cell to bead ratio. For example, a 3 to 1 bead to cell ratio at 1 million cells per milliliter can be used. An aAPC can also be a rigid spherical particle, a polystyrene latex microbeads, a magnetic nano- or micro-particles, a nanosized quantum dot, a 4, poly(lactic-co-glycolic acid) (PLGA) microsphere, a nonspherical particle, a 5, carbon nanotube bundle, a 6, ellipsoid PLGA microparticle, a 7, nanoworms, a fluidic lipid bilayer-containing system, an 8, 2D-supported lipid bilayer (2D-SLBs), a 9, liposome, a 10, RAFTsomes/microdomain liposome, an 11, SLB particle, or any combination thereof. In some cases, an aAPC can expand CD4 T cells. For example, an aAPC can be engineered to mimic an antigen processing and presentation pathway of HLA class II-restricted CD4 T cells. A K562 can be engineered to express HLA-D, DP a, DP R chains, Ii, DM α , DM β , CD80, CD83, or any combination thereof. For example, engineered K562 cells can be pulsed with an HLA-restricted peptide in order to expand HLA-restricted antigen-specific CD4 T cells. In some cases, the use of aAPCs can be combined with exogenously introduced cytokines for T cell activation, expansion, or any combination. Cells can also be expanded in vivo, for example in the subject's blood after administration of genomically transplanted cells into a subject.

[0269] In some embodiments, a method provided herein can comprise transduction of a population of cells. In some embodiments, a method comprises introducing a polynucleotide encoding for a cellular receptor such as a chimeric antigen receptor and/or a T cell receptor. In some cases, a transfection of a cell can be performed.

[0270] In some embodiments, a viral supernatant comprising a polynucleotide encoding for a cellular receptor such as a CAR and/or TCR is generated. In some embodiments, a viral vector can be a retroviral vector, a lentiviral vector and/or an adeno-associated viral vector. Packaging cells can be used to form virus particles capable of infecting a host cell. Such cells can include 293 cells, (e.g., for packaging adenovirus), and Psi2 cells or PA317 cells (e.g., for packaging retrovirus). Viral vectors can be generated by producing a cell line that packages a nucleic acid vector into a viral particle. The vectors can contain the minimal viral

sequences required for packaging and subsequent integration into a host. The vectors can contain other viral sequences being replaced by an expression cassette for the polynucleotide(s) to be expressed. The missing viral functions can be supplied in trans by the packaging cell line. For example, AAV vectors can comprise ITR sequences from the AAV genome which are required for packaging and integration into the host genome. Viral DNA can be packaged in a cell line, which can contain a helper plasmid encoding the other AAV genes, namely rep and cap, while lacking ITR sequences. The cell line can also be infected with adenovirus as a helper. The helper virus can promote replication of the AAV vector and expression of AAV genes from the helper plasmid. Contamination with adenovirus can be reduced by, e.g., heat treatment to which adenovirus is more sensitive than AAV. Additional methods for the delivery of nucleic acids to cells can be used, for example, as described in US20030087817, incorporated herein by reference.

[0271] In some embodiments, a host cell can be transiently or non-transiently transfected with one or more vectors described herein. A cell can be transfected as it naturally occurs in a subject. A cell can be taken or derived from a subject and transfected. A cell can be derived from cells taken from a subject, such as a cell line. In some embodiments, a cell transfected with one or more vectors described herein is used to establish a new cell line comprising one or more vector-derived sequences. Non-limiting examples of vectors for eukaryotic host cells include but are not limited to: pBs, pQE-9 (Qiagen), phagescript, PsiX174, pBluescript SK, pBsKS, pNH8a, pNH16a, pNH18a, pNH46a (Stratagene); pTrc99A, pKK223-3, pKK233-3, pDR540, pRIT5 (Pharmacia). Eukaryotic: pWL-neo, pSv2cat, pOG44, pXT1, pSG (Stratagene) pSVK3, pBPv, pMSG, pSVL (Pharmacia). Also, any other plasmids and vectors can be used as long as they are replicable and viable in a selected host. Any vector and those commercially available (and variants or derivatives thereof) can be engineered to include one or more recombination sites for use in the methods. Such vectors can be obtained from, for example, Vector Laboratories Inc., Invitrogen, Promega, Novagen, NEB, Clontech, Boehringer Mannheim, Pharmacia, EpiCenter, OriGenes Technologies Inc., Stratagene, PerkinElmer, Pharmingen, and Research Genetics. Other vectors of interest include eukaryotic expression vectors such as pFastBac, pFastBacHT, pFastBacDUAL, pSFV, and pTet-Splice (Invitrogen), pEUK-C1, pPUR, pMAM, pMAMneo, pBI101, pBI121, pDR2, pCMVEBNA, and pYACneo (Clontech), pSVK3, pSVL, pMSG, pCH110, and pKK232-8 (Pharmacia, Inc.), p3'SS, pXT1, pSG5, pPbac, pMbac, pMClneo, and pOG44 (Stratagene, Inc.), and pYES2, pAC360, pBlueBacHis A, B, and C, pVL1392, pBlueBac111, pCDM8, pcDNA1, pZeoSV, pcDNA3 pREP4, pCEP4, and pEBVHis (Invitrogen, Corp.), and variants or derivatives thereof. Other vectors include pUC18, pUC19, pBlueScript, pSPORT, cosmid, phagemids, YAC's (yeast artificial chromosomes), BAC's (bacterial artificial chromosomes), P1 (*Escherichia coli* phage), pQE70, pQE60, pQE9 (quagan), pBS vectors, PhageScript vectors, BlueScript vectors, pNH8A, pNH16A, pNH18A, pNH46A (Stratagene), pcDNA3 (Invitrogen), pGEX, pTrsfus, pTrc99A, pET-5, pET-9, pKK223-3, pKK233-3, pDR540, pRIT5 (Pharmacia), pSPORT1, pSPORT2, pCMVSPORT2.0 and pSYS-PORT1 (Invitrogen) and variants or derivatives thereof. Additional vectors of interest can also include pTrxFus,

pThioHis, pLEX, pTrcHis, pTrcHis2, pRSET, pBlueBacHis2, pcDNA3.1/His, pcDNA3.1(-)/Myc-His, pSecTag, pEBVHis, pPIC9K, pPIC3.5K, pA081S, pPICZ, pPICZA, pPICZB, pPICZC, pGAPZA, pGAPZB, pGAPZC, pBlueBac4.5, pBlueBacHis2, pMelBac, pSinRep5, pSinHis, pIND, pIND(SP1), pVgRXXR, pcDNA2.1, pYES2, pZer01.1, pZerO-2.1, pCR-Blunt, pSE280, pSE380, pSE420, pVL1392, pVL1393, pCDM8, pcDNA1.1, pcDNA1.1/Amp, pcDNA3.1, pcDNA3.1/Zeo, pSe, SV2, pRc/CMV2, pRc/RSV, pREP4, pREP7, pREP8, pREP9, pREP 10, pCEP4, pEBVHis, pCR3.1, pCR2.1, pCR3.1-Uni, and pCRBac from Invitrogen; X ExCell, X gtl1, pTrc99A, pKK223-3, pGEX-1X T, pGEX-2T, pGEX-2TK, pGEX-4T-1, pGEX-4T-2, pGEX-4T-3, pGEX-3X, pGEX-5X-1, pGEX-5X-2, pGEX-5X-3, pEZZ18, pRIT2T, pMC1871, pSVK3, pSVL, pMSG, pCH110, pKK232-8, pSL1180, pNEO, and pUC4K from Pharmacia; pSCREEN-lb(+), pT7Blue(R), pT7Blue-2, pCITE-4-abc(+), pOCUS-2, pTag, pET-32LIC, pET-30LIC, pBAC-2 cp LIC, pBACgus-2 cp LIC, pT7Blue-2 LIC, pT7Blue-2, X SCREEN-1, XBlueSTAR, pET-3abcd, pET-7abc, pET9abcd, pET 11 abcd, pET12abc, pET-14b, pET-15b, pET-16b, pET-17b-pET-17xb, pET-19b, pET-20b(+), pET-21abcd(+), pET-22b(+), pET-23abcd(+), pET-24abcd(+), pET-25b(+), pET-26b(+), pET-27b(+), pET-28abc(+), pET-29abc(+), pET-30abc(+), pET-31b(+), pET-32abc(+), pET-33b(+), pBAC-1, pBACgus-1, pBAC4x-1, pBACgus4x-1, pBAC-3 cp, pBACgus-2 cp, pBACsurf-1, plg, Signal plg, pYX, Selecta Vecta-Neo, Selecta Vecta-Hyg, and Selecta Vecta-Gpt from Novagen; pLexA, pB42AD, pGBT9, pAS2-1, pGAD424, pACT2, pGAD GL, pGAD GH, pGAD10, pGilda, pEZM3, pEGFP, pEGFP-1, pEGFPN, pEGFP-C, pEBFP, pGFPuv, pGFP, p6xHis-GFP, pSEAP2-Basic, pSEAP2-Contra, pSEAP2-Promoter, pSEAP2-Enhancer, p I3 gal-Basic, p I3 gal-Control, p I3 gal-Promoter, p I3 gal-Enhancer, pCMV, pTet-Off, pTet-On, pTK-Hyg, pRetro-Off, pRetro-On, pIRESneo, pIRESihyg, pLXSN, pLNCX, pLAPSN, pMAMneo, pMAMneo-CAT, pMAMneo-LUC, pPUR, pSV2neo, pYEX4T-1/2/3, pYEX-S1, pBacPAK-His, pBacPAK8/9, pAcUW31, pBacPAK6, pTriplEx, 2Xgt10, Xgt11, pWE15, and X TriplEx from Clontech; Lambda ZAP II, pBK-CMV, pBK-RSV, pBlue-script II KS+/-, pBluescript II SK+/-, pAD-GAL4, pBD-GAL4 Cam, pSurfsript, Lambda FIX II, Lambda DASH, Lambda EMBL3, Lambda EMBL4, SuperCos, pCR-Script Amp, pCR-Script Cam, pCR-Script Direct, pBS+/-, pBC KS+/-, pBC SK+/-, Phag-escrpt, pCAL-n-EK, pCAL-n, pCAL-c, pCAL-kc, pET-3abcd, pET-11abcd, pSPUTK, pESP-1, pCMVLacI, pOPRSVI/MCS, pOPI3 CAT, pXT1, pSG5, pPbac, pMbac, pMClneo, pMClneo Poly A, pOG44, pOG45, pFRTI3GAL, pNEO13GAL, pRS403, pRS404, pRS405, pRS406, pRS413, pRS414, pRS415, and pRS416 from Stratagene, pPC86, pDBLeu, pDBTrp, pPC97, p2.5, pGAD1-3, pGAD10, pAct, pACT2, pGADGL, pGADGH, pAS2-1, pGAD424, pGBT8, pGBT9, pGAD-GAL4, pLexA, pBD-GAL4, pHISi, pHISi-1, placZi, pB42AD, pDG202, pJK202, pJG4-5, pNLexA, pYESTrp, and variants or derivatives thereof. In some embodiments, a vector can be a minicircle vector. A vector provided herein can be used to deliver a polypeptide coding for a CAR and/or TCR.

[0272] Transduction and/or transfection can be performed by any one of: non-viral transfection, biolistics, chemical transfection, electroporation, nucleofection, heat-shock transfection, lipofection, microinjection, or viral transfection. In some embodiments a provided method comprises

viral transduction, and the viral transduction comprises a lentivirus. Viral particles can be used to deliver a viral vector comprising a polypeptide sequence coding for a cellular receptor into a cell *ex vivo* or *in vivo*. In some cases, a viral vector as disclosed herein may be measured as pfu (plaque forming units). In some cases, the pfu of recombinant virus or viral vector of the compositions and methods of the disclosure may be about 10^8 to about 5×10^{10} pfu. In some cases, recombinant viruses of this disclosure are at least about 1×10^8 , 2×10^8 , 3×10^8 , 4×10^8 , 5×10^8 , 6×10^8 , 7×10^8 , 8×10^8 , 9×10^8 , 1×10^9 , 2×10^9 , 3×10^9 , 4×10^9 , 5×10^9 , 6×10^9 , 7×10^9 , 8×10^9 , 9×10^9 , 1×10^{10} , 2×10^{10} , 3×10^{10} , 4×10^{10} , and 5×10^{10} pfu. In some cases, recombinant viruses of this disclosure are at most about 1×10^8 , 2×10^8 , 3×10^8 , 4×10^8 , 5×10^8 , 6×10^8 , 7×10^8 , 8×10^8 , 9×10^8 , 1×10^9 , 2×10^9 , 3×10^9 , 4×10^9 , 5×10^9 , 6×10^9 , 7×10^9 , 8×10^9 , 9×10^9 , 1×10^{10} , 2×10^{10} , 3×10^{10} , 4×10^{10} , and 5×10^{10} pfu. In some aspects, the viral vector of the disclosure may be measured as vector genomes. In some cases, recombinant viruses of this disclosure are 1×10^{10} to 3×10^{12} vector genomes, or 1×10^9 to 3×10^{13} vector genomes, or 1×10^8 to 3×10^{14} vector genomes, or at least about 1×10^1 , 1×10^2 , 1×10^3 , 1×10^4 , 1×10^5 , 1×10^6 , 1×10^7 , 1×10^8 , 1×10^9 , 1×10^{10} , 1×10^{11} , 1×10^{12} , 1×10^{13} , 1×10^{14} , 1×10^{15} , 1×10^{16} , 1×10^{17} , and 1×10^{18} vector genomes, or are 1×10^8 to 3×10^{14} vector genomes, or are at most about 1×10^1 , 1×10^2 , 1×10^3 , 1×10^4 , 1×10^5 , 1×10^6 , 1×10^7 , 1×10^8 , 1×10^9 , 1×10^{10} , 1×10^{11} , 1×10^{12} , 1×10^{13} , 1×10^{14} , 1×10^{15} , 1×10^{16} , 1×10^{17} , and 1×10^{18} vector genomes. In some cases, a viral vector provided herein can be measured using multiplicity of infection (MOI). In some cases, MOI may refer to the ratio, or multiple of vector or viral genomes to the cells to which the nucleic acid may be delivered. In some cases, the MOI may be 1×10^6 . In some cases, the MOI may be 1×10^5 to 1×10^7 . In some cases, the MOI may be 1×10^4 to 1×10^8 . In some cases, recombinant viruses of the disclosure are at least about 1×10^1 , 1×10^2 , 1×10^3 , 1×10^4 , 1×10^5 , 1×10^6 , 1×10^7 , 1×10^8 , 1×10^9 , 1×10^{10} , 1×10^{11} , 1×10^{12} , 1×10^{13} , 1×10^{14} , 1×10^{15} , 1×10^{16} , 1×10^{17} , and 1×10^{18} MOI. In some cases, recombinant viruses of this disclosure are 1×10^8 to 3×10^{14} MOI, or are at most about 1×10^1 , 1×10^2 , 1×10^3 , 1×10^4 , 1×10^5 , 1×10^6 , 1×10^7 , 1×10^8 , 1×10^9 , 1×10^{10} , 1×10^{11} , 1×10^{12} , 1×10^{13} , 1×10^{14} , 1×10^{15} , 1×10^{16} , 1×10^{17} , and 1×10^{18} MOI. In some cases, a viral vector is introduced at a multiplicity of infection (MOI) from about 1×10^5 , 2×10^5 , 3×10^5 , 4×10^5 , 5×10^5 , 6×10^5 , 7×10^5 , 8×10^5 , 9×10^5 , 1×10^6 , 2×10^6 , 3×10^6 , 4×10^6 , 5×10^6 , 6×10^6 , 7×10^6 , 8×10^6 , 9×10^6 , 1×10^7 , 2×10^7 , 3×10^7 , or up to about 9×10^9 genome copies/virus particles per cell.

[0273] The transfection efficiency of cells with any of the nucleic acid delivery platforms described herein, for example, transduction, can be or can be about 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5%, 99.9%, or more than 99.9%. In some embodiments, a method can comprise adding an infective agent to a composition comprising a population of cells. An infective agent can comprise polybrene. In some aspects, an infective agent can enhance efficiency of viral infection. An infective agent can enhance viral infectivity from about 100 to 1,000 fold. Polybrene can be added to a composition at a concentration from about 5 μ g to 10 μ g per ml.

[0274] In some embodiments, a method provided herein can comprise a non-viral approach of introducing a cellular receptor to a cell. Non-viral approaches can include but are

not limited to: CRISPR associated proteins (Cas proteins, e.g., Cas9), Zinc finger nuclease (ZFN), Transcription Activator-Like Effector Nuclease (TALEN), Argonate nucleases, and meganucleases. Nucleases can be naturally existing nucleases, genetically modified, and/or recombinant. Non-viral approaches can also be performed using a transposon-based system (e.g. PiggyBac, Sleeping beauty).

[0275] In some embodiments, a method provided herein can utilize a PiggyBac system to introduce an exogenous polypeptide to a cell. A PiggyBac system comprises two components, a transposon and a transposase. The PiggyBac transposase facilitates the integration of the transposon specifically at 'TTAA' sites randomly dispersed in the genome. The predicted frequency of 'TTAA' in the genome is approximately 1 in every 256 base-pairs of DNA sequence. Unlike other transposons, the PB transposase also enables the excision of the transposon in a completely seamless manner, leaving no sequences or mutations behind. Furthermore, PiggyBac offers a large cargo-carrying capacity (over 200 kb has been demonstrated) with no known upper limit. PB performance levels can be increased by codon-optimization strategies, mutations, deletions, additions, substitutions, and any combination thereof. In some cases, PB can have a larger cargo (approximately 9.1-14.3 kb), a higher transposition activity, and its footprint-free characteristic can make it appealing as a gene editing tool. In some aspects, PB can comprise a few features: high efficiency transposition; large cargo; steady long-term expression; the trans-gene is integrated as a single copy; tracking the target gene *in vivo* by a noninvasive mark instead of traditional method such as PCR; easy to determine the integration site, and combinations thereof.

[0276] In some aspects, a method provided herein can utilize a Sleeping Beauty (SB) System to introduce a polypeptide coding for a cellular receptor to a cell. SB was engineered from ancient Tc1/mariner transposon fossils found within the Salmonid genomes by *in vitro* evolution. The SB ITRs (230 bp) contain imperfect direct repeats (DRs) of 32 bp in length that can serve as recognition signals for the transposase. Binding affinity and spacing between the DR elements within ITR has involved in transpositional activities. The SB transposase can be a 39 kDa protein that possess DNA binding polypeptide, a nuclear localization signal (NLS) and the catalytic domain, featured by a conserved amino acid motif (DDE). Various screens mutagenizing the primary amino acid sequence of the SB transposase resulted in hyperactive transposase versions. In some cases, a modified SB can be utilized. Modified SBs can contain mutations, deletions and additions within ITRs of the original SB transposon. Modified SBs can comprise: pT2, pT3, pT2B, pT4, SB100X, and combinations thereof. Non-limited examples of modified SBs can be selected from: SB10, SB11 (3-fold higher than SB10), SB12 (4-fold higher than SB10), HSB1-HSB5 (up to 10-fold higher than SB10), HSB13-HSB17 (HSB17 is 17-fold higher than SB10), SB100X (100-fold higher than SB10), SB150X (130-fold higher than SB10), and any combination thereof. In some cases, SB100X is 100-fold hyperactive compared to the originally resurrected transposase (SB10). In some aspects, SB transposition excision leaves a footprint (3 bp) at the cargo site. Integration occurs into TA dinucleotides of the genome, and results in target site duplications, generated by the host repair machinery. In some cases, SB appears to possess a nearly unbiased, close-to-random integration pro-

file. Transposon integration can be artificially targeted (~10%) to a predetermined genomic locus in wildtype systems, however in chimeric systems provided herein, SB transposon integration can be directed to a predetermined locus with efficiencies over 10%.

[0277] In some aspects, a non-viral approach may be taken to introduce an exogenous polynucleic acid to a population of cells. In some aspects, a non-viral vector or nucleic acid may be delivered without the use of a virus and may be measured according to the quantity of nucleic acid. Generally, any suitable amount of nucleic acid can be used with the compositions and methods of this disclosure. In some cases, nucleic acid may be at least about 1 pg, 10 pg, 100 pg, 1 pg, 10 pg, 100 pg, 200 pg, 300 pg, 400 pg, 500 pg, 600 pg, 700 pg, 800 pg, 900 pg, 1 μ g, 100 μ g, 200 μ g, 300 μ g, 400 μ g, 500 μ g, 600 μ g, 700 μ g, 800 μ g, 900 μ g, 1 ng, 10 ng, 100 ng, 200 ng, 300 ng, 400 ng, 500 ng, 600 ng, 700 ng, 800 ng, 900 ng, 1 mg, 10 mg, 100 mg, 200 mg, 300 mg, 400 mg, 500 mg, 600 mg, 700 mg, 800 mg, 900 mg, 1 g, 2 g, 3 g, 4 g, or 5 g. In some cases, nucleic acid may be at most about 1 pg, 10 pg, 100 pg, 1 pg, 10 pg, 100 pg, 200 pg, 300 pg, 400 pg, 500 pg, 600 pg, 700 pg, 800 pg, 900 pg, 1 μ g, 10 μ g, 100 μ g, 200 μ g, 300 μ g, 400 μ g, 500 μ g, 600 μ g, 700 μ g, 800 μ g, 900 μ g, 1 ng, 10 ng, 100 ng, 200 ng, 300 ng, 400 ng, 500 ng, 600 ng, 700 ng, 800 ng, 900 ng, 1 mg, 10 mg, 100 mg, 200 mg, 300 mg, 400 mg, 500 mg, 600 mg, 700 mg, 800 mg, 900 mg, 1 g, 2 g, 3 g, 4 g, or 5 g.

[0278] In some embodiments, a non-viral approach of introducing a CAR and/or TCR sequence to a cell can include electroporation. Electroporation can be performed using, for example, the Neon® Transfection System (ThermoFisher Scientific) or the AMAXA® Nucleofector (AMAXA® Biosystems). Electroporation parameters may be adjusted to optimize transfection efficiency and/or cell viability. Electroporation devices can have multiple electrical wave form pulse settings such as exponential decay, time constant and square wave. Every cell type has a unique optimal Field Strength (E) that is dependent on the pulse parameters applied (e.g., voltage, capacitance and resistance). Application of optimal field strength causes electroporation through induction of transmembrane voltage, which allows nucleic acids to pass through the cell membrane. In some cases, the electroporation pulse voltage, the electroporation pulse width, number of pulses, cell density, and tip type may be adjusted to optimize transfection efficiency and/or cell viability.

[0279] In some embodiments, electroporation pulse voltage may be varied to optimize transfection efficiency and/or cell viability. In some cases, the electroporation voltage may be less than about 500 volts. In some cases, the electroporation voltage may be at least about 500 volts, at least about 600 volts, at least about 700 volts, at least about 800 volts, at least about 900 volts, at least about 1000 volts, at least about 1100 volts, at least about 1200 volts, at least about 1300 volts, at least about 1400 volts, at least about 1500 volts, at least about 1600 volts, at least about 1700 volts, at least about 1800 volts, at least about 1900 volts, at least about 2000 volts, at least about 2100 volts, at least about 2200 volts, at least about 2300 volts, at least about 2400 volts, at least about 2500 volts, at least about 2600 volts, at least about 2700 volts, at least about 2800 volts, at least about 2900 volts, or at least about 3000 volts. In some cases, the electroporation pulse voltage required for optimal transfection efficiency and/or cell viability may be specific to the

cell type. For example, an electroporation voltage of 1900 volts may optimal (e.g., provide the highest viability and/or transfection efficiency) for macrophage cells. In another example, an electroporation voltage of about 1350 volts may optimal (e.g., provide the highest viability and/or transfection efficiency) for Jurkat cells or primary human cells such as T cells. In some cases, a range of electroporation voltages may be optimal for a given cell type. For example, an electroporation voltage between about 1000 volts and about 1300 volts may optimal (e.g., provide the highest viability and/or transfection efficiency) for human 578T cells. In some cases, a primary cell can be a primary lymphocyte. In some cases, a population of primary cells can be a population of lymphocytes.

[0280] In some embodiments, electroporation pulse width may be varied to optimize transfection efficiency and/or cell viability. In some cases, the electroporation pulse width may be less than about 5 milliseconds. In some cases, the electroporation width may be at least about 5 milliseconds, at least about 6 milliseconds, at least about 7 milliseconds, at least about 8 milliseconds, at least about 9 milliseconds, at least about 10 milliseconds, at least about 11 milliseconds, at least about 12 milliseconds, at least about 13 milliseconds, at least about 14 milliseconds, at least about 15 milliseconds, at least about 16 milliseconds, at least about 17 milliseconds, at least about 18 milliseconds, at least about 19 milliseconds, at least about 20 milliseconds, at least about 21 milliseconds, at least about 22 milliseconds, at least about 23 milliseconds, at least about 24 milliseconds, at least about 25 milliseconds, at least about 26 milliseconds, at least about 27 milliseconds, at least about 28 milliseconds, at least about 29 milliseconds, at least about 30 milliseconds, at least about 31 milliseconds, at least about 32 milliseconds, at least about 33 milliseconds, at least about 34 milliseconds, at least about 35 milliseconds, at least about 36 milliseconds, at least about 37 milliseconds, at least about 38 milliseconds, at least about 39 milliseconds, at least about 40 milliseconds, at least about 41 milliseconds, at least about 42 milliseconds, at least about 43 milliseconds, at least about 44 milliseconds, at least about 45 milliseconds, at least about 46 milliseconds, at least about 47 milliseconds, at least about 48 milliseconds, at least about 49 milliseconds, or at least about 50 milliseconds. In some cases, the electroporation pulse width required for optimal transfection efficiency and/or cell viability may be specific to the cell type. For example, an electroporation pulse width of 30 milliseconds may optimal (e.g., provide the highest viability and/or transfection efficiency) for macrophage cells. In another example, an electroporation width of about 10 milliseconds may optimal (e.g., provide the highest viability and/or transfection efficiency) for Jurkat cells. In some cases, a range of electroporation widths may be optimal for a given cell type. For example, an electroporation width between about 20 milliseconds and about 30 milliseconds may optimal (e.g., provide the highest viability and/or transfection efficiency) for human 578T cells.

[0281] In some embodiments, the number of electroporation pulses may be varied to optimize transfection efficiency and/or cell viability. In some cases, electroporation may comprise a single pulse. In some cases, electroporation may comprise more than one pulse. In some cases, electroporation may comprise 2 pulses, 3 pulses, 4 pulses, 5 pulses, 6 pulses, 7 pulses, 8 pulses, 9 pulses, or 10 or more pulses. In some cases, the number of electroporation pulses required for optimal transfection efficiency and/or cell viability may

be specific to the cell type. For example, electroporation with a single pulse may be optimal (e.g., provide the highest viability and/or transfection efficiency) for macrophage cells. In another example, electroporation with a 3 pulses may be optimal (e.g., provide the highest viability and/or transfection efficiency) for primary cells. In some cases, a range of electroporation widths may be optimal for a given cell type. For example, electroporation with between about 1 to about 3 pulses may be optimal (e.g., provide the highest viability and/or transfection efficiency) for human cells.

[0282] In some cases, the starting cell density for electroporation may be varied to optimize transfection efficiency and/or cell viability. In some cases, the starting cell density for electroporation may be less than about 1×10^5 cells. In some cases, the starting cell density for electroporation may be at least about 1×10^5 cells, at least about 2×10^5 cells, at least about 3×10^5 cells, at least about 4×10^5 cells, at least about 5×10^5 cells, at least about 6×10^5 cells, at least about 7×10^5 cells, at least about 8×10^5 cells, at least about 9×10^5 cells, at least about 1×10^6 cells, at least about 1.5×10^6 cells, at least about 2×10^6 cells, at least about 2.5×10^6 cells, at least about 3×10^6 cells, at least about 3.5×10^6 cells, at least about 4×10^6 cells, at least about 4.5×10^6 cells, at least about 5×10^6 cells, at least about 5.5×10^6 cells, at least about 6×10^6 cells, at least about 6.5×10^6 cells, at least about 7×10^6 cells, at least about 7.5×10^6 cells, at least about 8×10^6 cells, at least about 8.5×10^6 cells, at least about 9×10^6 cells, at least about 9.5×10^6 cells, at least about 1×10^7 cells, at least about 1.2×10^7 cells, at least about 1.4×10^7 cells, at least about 1.6×10^7 cells, at least about 1.8×10^7 cells, at least about 2×10^7 cells, at least about 2.2×10^7 cells, at least about 2.4×10^7 cells, at least about 2.6×10^7 cells, at least about 2.8×10^7 cells, at least about 3×10^7 cells, at least about 3.2×10^7 cells, at least about 3.4×10^7 cells, at least about 3.6×10^7 cells, at least about 3.8×10^7 cells, at least about 4×10^7 cells, at least about 4.2×10^7 cells, at least about 4.4×10^7 cells, at least about 4.6×10^7 cells, at least about 4.8×10^7 cells, or at least about 5×10^7 cells. In some cases, the starting cell density for electroporation required for optimal transfection efficiency and/or cell viability may be specific to the cell type. For example, a starting cell density for electroporation of 1.5×10^6 cells may optimal (e.g., provide the highest viability and/or transfection efficiency) for macrophage cells. In another example, a starting cell density for electroporation of 5×10^6 cells may optimal (e.g., provide the highest viability and/or transfection efficiency) for human cells. In some cases, a range of starting cell densities for electroporation may be optimal for a given cell type. For example, a starting cell density for electroporation between of 5.6×10^6 and 5×10^7 cells may optimal (e.g., provide the highest viability and/or transfection efficiency) for human cells such as T cells.

[0283] A method for treating a lymphoid malignancy is provided. The method can comprise administering to a patient in need thereof a population of engineered immune cells. An individual engineered immune cell of the population can comprise one or more chimeric antigen receptors (CARs) comprising a binding moiety, where the binding moiety can comprise an antigen binding domain capable of binding to an immune cell antigen, and where each CAR of the one or more CARs can further comprise a transmembrane domain and an intracellular signaling domain. An individual engineered immune cell of the population can further comprise an enhancer moiety capable of enhancing

one or more activities of the engineered immune cell. An endogenous T cell receptor (TCR) of the engineered immune cell may be inactivated. In some cases, the number of affected cells in peripheral blood or the number of affected cells in bone marrow of the patient can be reduced by at least about 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95% or more within a period (e.g., 3 weeks) after a last dosing of the engineered immune cells. In some cases, the period after a last dosing of the engineered immune cell can be about 1 week, 2 weeks, 3 weeks, 4 weeks, 5 weeks or more. The number of any one or more of autologous T cell, granulocyte, and NK cell in peripheral blood of the patient can start to increase within a period (e.g., 3 weeks) after a last dosing of the engineered immune cells. In some cases, the period after a last dosing of the engineered immune cell can be about 1 week, 2 weeks, 3 weeks, 4 weeks, 5 weeks or more.

[0284] The enhancer moiety can enhance one or more activities of the engineered immune cell. The enhancer moiety can be configured to constitutively enhance the one or more activities of the engineered immune cell. The enhancer moiety can be configured to constitutively upregulate one or more intracellular signaling pathways of the engineered immune cell. The one or more intracellular signaling pathways can be one or more cytokine signaling pathways. The enhancer moiety can be a cytokine or a cytokine receptor. The enhancer moiety can be selected from the group consisting of IL-2, IL-3, IL-4, IL-6, IL-7, IL-8, IL-10, IL-11, IL-12, IL-15, IL-17, IL-18, IL-21, IL-23, PD-1, PD-L1, CD122, CSF1R, CTAL-4, TIM-3, CCL21, CCL19, TGF β receptors for the same, functional fragments thereof, functional variants thereof, and combinations thereof.

[0285] The engineered immune cell can further comprise an inducible cell death moiety capable of effecting death of the cell upon contacting the inducible cell death moiety with a cell death activator. The inducible cell death moiety can be selected from the group consisting of rapaCasp9, iCasp9, HSV-TK, ACD20, mTMPK, ACD19, RQR8, Her2t, CD30, BCMA, and EGFRt. For example, the inducible cell death moiety can be EGFRt, and the cell death activator can be an antibody or an antigen binding fragment thereof that binds EGFRt. For another example, the inducible cell death moiety can be HSV-TK, and the cell death activator can be GCV. For another example, the inducible cell death moiety can be iCasp9, and the cell death activator can be AP1903.

[0286] A gene encoding an endogenous surface marker of the cell can be inactivated. The endogenous surface marker can be capable of binding to the first antigen binding domain when expressed. The endogenous surface marker can be CD2, CD3, CD4, CD5, CD7, CD8, CD16a, CD16b, CD25, CD27, CD28, CD30, CD38, CD45, CD48, CD50, CD52, CD56, CD57, CD62L, CD69, CD94, CD100, CD102, CD122, CD127, CD132, CD137, CD160, CD161, CD178, CD218, CD226, CD244, CD159a (NKG2A), CD159c (NKG2C), NKG2E, CD279, CD314 (NKG2D), CD305, CD335 (NKP46), CD337, CD319 (CS1), TCR α , TCR β or SLAMF7.

[0287] The number of any one or more of autologous T cell, granulocyte, and NK cell in peripheral blood of the patient may start to increase before the number of affected cells in peripheral blood or the number of affected cells in bone marrow is reduced by at least about 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%,

75%, 80%, 85%, 90%, 95% or more. The number of any one or more of autologous T cell, granulocyte, and NK cell in peripheral blood may start to increase after the number of affected cells in peripheral blood or the number of affected cells in bone marrow is reduced by at least about 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95% or more.

EXAMPLES

Example 1 Study of the U-CAR T Cells Expressing CD3+ CD19 Dual CAR and EGFRt Switch with TCR Knockout

General Materials and Methods

[0288] Isolation of Peripheral Blood Mononuclear Cells (PBMCs) from Donor Blood and Expansion of T Cells

[0289] Peripheral blood mononuclear cells (PBMCs) were isolated from donor blood by using Histopaque-1077 (Sigma-Aldrich) through density gradient centrifuge. Then T cells were enriched, activated by magnetic beads coupled with anti-CD3/anti-CD28, cultured and expanded.

[0290] Cell Lines and the Culture of PBMCs

[0291] Raji cells (Burkitt's lymphoma cells, ATCC-CCL86);

[0292] K562 cells (Human erythroleukemia cell line, ATCC-CCL243);

[0293] Raji-fluc cell line (obtained by screening Raji cells transfected with lentivirus having firefly luciferase);

[0294] 293T cells (ATCC-CRL3216).

[0295] Raji cells, K562 cells and Raji-fluc cell line were cultured in RPMI1640 medium, and 293T cells were cultured in DMEM medium. Both RPMI1640 and DMEM were

supplemented with 10% (v/v) fetal bovine serum and 100 U/ml penicillin and streptomycin, 2 mM glutamine and 1 mM sodium pyruvate. All of the cells were cultured in an incubator at 37° C., 5% CO₂.

[0296] T cells and the obtained CAR-T cells were cultured in X-vivo15 medium (containing 5% FBS, 2 mM L-glutamine, 1 mM sodium pyruvate and 300 IU/ml rhIL2). The culture medium for CAR-T cells was further supplemented with rhIL-2 (ThermoFisher Scientific) at a final concentration of 300 IU/ml every two days. All of the cells were cultured in an incubator at 37° C., 5% CO₂.

1.1 Design of CAR and Package of the Virus

[0297] The structure of the chimeric antigen receptor is EGFRt-CD3 scFv-CD19 scFv-Hinge-TM-CD28-CD3ζ (tandem), tEGFR-CD19 scFv-CD28-CD3ζ-CD3 scFv-41BB-CD3ζ (Parallel), tEGFR-CD3 scFv-CD19 scFv41BB-CD3ζ (Tandem), and CD19VL-CD3VL-CD3VH-CD19VH-41BB-CD3ζ (Loop), wherein EGFRt (or tEGFR) is a truncated EGFR as a switch, CD3 scFv fragment is the heavy chain and light chain variable region of monoclonal antibody OKT3 or UCHT1 (connected by a GS linker), CD 19 scFv fragment is the heavy chain and light chain variable region of monoclonal antibody FMC63 (connected by a GS linker) containing a beacon sequence for CAR detection, and also included are hinge region, transmembrane region, human CD 28 intracellular co-stimulatory element and human CD3ζ intracellular region in tandem. An example construct of the chimeric antigen receptor is shown in FIG. 1.

[0298] The amino acid sequence of EGFRt-OKT3-FMC63-CD28z (SEQ ID NO.: 1) is as set forth below:

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MLLLVTSLLLCELPHPAFLLIPRKVCNGIGIGEFKDSLSINATNIKHFNKNTSISGDLHILPVAFRGDSF
THTPPLDPQELDILKTVKEITGFLLIQAWPENRTDLHAFENLEIRGRTKHQGFSLAVVSLNITSLGLR
SLKEISDGDVVISGNKNLCYANTINWKKLFGTSGQTKIISNRGENSKATGQVCHALCSPEGCWGPEPR
DCVSCRNVSRGREVDKCNLLEGEPEFVENSECIQCHPECLPQAMNITCTGRGPDNCIQCAHYIDGPHC
VKTCPAGVMGENNTLVWKYADAGHVCHLCHPNCTYGCTGPGLEGCP TNGPKIPSIATGMVGALLLLVVA
LGIGLFMRKRGSGATNFSLLKQAGDVEENPGPMALPVTALLLPLALLLHAARFQVQLQQSGAELARPGA
SVKMSCKASGYTFTRYTMHWVKRQPGQGLEWIGYINPSRGYTNYNQKFKDKATLTDDKSSSTAYMQLSSL
TSEDSAVYYCARYYDDHYCLDYWGQGTTLTVSSGSGTSGGGSGGGSGGGSSQIVLTQSPAIMSASPGKV
TMTCSASSSVSYMNWYQQKSGTSPKRWIYDTSKLASGVP AHFRGSGSGTSYSLTISGMEAEADAATYYCQQ
WSSNPF TFGSGTKLEINRGGGGSGGGSGGGSGGGSDIQMTQTSSLSASLGDRVTISCRASQDISKYLWNYQ
QKPDGTVKLLIYHSTRLHSGVPSRFSGSGSGTDYSLTISNLEQEDIATYFCQQGNTLPYTFGGGKLEIT

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GSTSGSGKPGSGEGSTKGEVQLQESGPGLVAPSQSLSVTCTVSGVSLPDYGVSWIRQPPRKGLEWLGVIV

GSETTYNSALKSRLTI IKDNSKSQVFLKMNSLQTDDTAIYYCAKHYYGGSYAMDYWGQTSVTVSAA

ADYKDDDDK IEVMYPPPYLDNEKSNGTIIHVKGKHLCPSPFPGPSKPFWLVVVGGVLACYSLLVTVAF

IIFWVRSKRSRLHSDYMMTPRRPGPTRKHYQPYAPPRDFAAYRSRVKFSRSADAPAYQQGQNLYNEL

NLGRREEYDVLDRRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGDGLYQGLS

TATKDTYDALHMQALPPR

[0299] In the EGFRt-OKT3-FMC63-CD28z amino acid sequence, the underlined part is the safety switch signal peptide region, the italic part is tEGFR, the italic underlined part is tEGFR TM, the curve underlined part is P2A, the italic curve underlined part is CAR signal peptide, the bold part is OKT3 VH, the dashed underlined part is a linking peptide, the bold underlined part is OKT3 VL, the bold italic part is FMC63 VL, the bold italic underlined part is FMC63 VH, the boxed part is CD28 hinge region transmembrane region and costimulatory domain, and the boxed italic part is CD3z.

[0300] The nucleotide acid sequence of EGFRt-OKT3-FMC63-CD28z is as set forth in SEQ ID NO.: 2.

SEQ ID NO.: 2:

ATGTTGCTCCTTGTGACGAGCTCTGTCTGCGAGCTGCCCATCCAGC
CTTCTCTCATCCCGCGAAGGTGTGCAATGGCATAGGCATTGGCGAGT
TTAAAGATTCTCTGAGCATAAATGCTACGAATATTAAGCATTTCAGAAT
TGACTTCTATTAGTGCGGACCTCCATATTCTTCGGTTGCCTTCAGGGG
TGACTCTTTACCCACACACCTCCATTGGATCCACAAGAAGTTGACATCC
TGAAGACGGTTAAAGAGATTACAGGCTTCTCTTATCCAGCGTGGCCC
GAGAACAGAACGGACTTGCACGCCTTTGAGAACCTCGAAATAATACGGGG
TCGGACGAAGCAACACGGCCAATTTAGCCTTGCGGTTGTTAGTCTGAACA
TTACTTCTCTCGGCCTTCGCTCTTTGAAGAAATCAGCGACGGAGATGTC
ATCATTAGTGAACAAGAAGCTGTGCTACGCGAACACAATCAACTGGAA
GAAGCTCTTCGGTACTTCAGGCCAAAAGACAAAGATTATTAGTAACAGAG
GAGAGAATAGCTGAAGGCTACCGGACAAGTTTGTACGCCTTGTGTAGT
CCAGAGGGTTGCTGGGGACCGGAACCAAGGATTGCGTCAGTTGCCGGAA
CGTGAGTCGCGGACGCGAGTGTGTGGATAAGTGCAATCTTCTGGAAGGGG
AACC CGAGAGATTGTAGAAAAATCCGAATGTATACAGTGCATCCCGAG
TGTCTTCCACAAGCAATGAATATCATGTACAGGAGGGGCTCTGATAA
CTGTATCCAATGTGCACACTACATAGATGGTCTCACTGTGTAAGACGT
GCCCCGCGGAGTAATGGGTGAAAAACAACCTCGTGTGAAGTACGCC

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GATGCCGGGCATGTCTGTCAATTTGTGTATCCCACTGCACATATGGCTG
TACCGGTCTGGATTGGAGGGCTGTCCAACAAACGGGCGGAAAAATACCGA
GTATCGCAACAGGCATGGTGGGAGCACTTTTGCTTCTCTCGTTGTGCGCC
CTGGGCATCGGCTTGTTTCATGCGAGCTAAACGAGGCTCAGGCGCGACGAA
CTTTAGTTTGCTGAAGCAAGCTGGGGATGTAGAGGAAAAATCCGGGTCCCA
TGGCCCTTCCAGTGACAGCCTTGTGTTGCCACTTGCTCTGCTGCTCCAC
GCTGCGCGGCCACAGGTCAGTTGCGAGAGTACAGCGCCGAATTTGGCGCG
ACCAGGGGCAAGCGTAAAGATGAGCTGTAAGGCATCCGGGTACACGTTCA
CTCGCTATACCATGCATTGGGTTAAACAACGGCCTGGGCGGGCCTTGAG
TGGATTGGGTATATCAACCCATCCCGGGGCTACACTAACTATAATCAAAA
GTTTAAAGATAAAGCAACCCCTTACGACCGACAAATCATCTTCTACCGCAT
ACATGCAGCTCAGCTCCCTCACCAGTGAAGATTCTGCCGTTTATTATTGT
GCACGATACTATGACGATCACTATTGCGTGGACTACTGGGTCAAGGCAC
CACACTTACTGTGAGTTCGGGAAGTACCAGTGGGGGAGGTTCTGGCGGTG
GCAGCGGGGTGGGGGTAGCTCACAAATCGTGTGACCCAGAGTCCCGCT
ATCATGAGCGCCTCCCCAGGGGAAAAGGTGACGATGACATGCTCAGCCAG
CTCCAGTGTATCCTACATGAATTGGTATCAACAGAAGAGTGGGACGTACAC
CCAAAAGATGGATTTATGACACAGCAAAATGGCCAGCGGAGTACACGCG
CATTTCAGAGGCAGTGGGAGTGAACATCTTATTCTCTCACCATTAGCGG
CATGGAAGCAGAGGATGACAGCAACGTACTATTGTCAACAATGGAGCTCTA
ATCCCTTTACGTTCCGCGAGCGGCACTAAGCTCGAAATTAATAGGGGTGGC
GGCGGCTCCGGCGGTGGCGGGTCTGGAGGTGGGGGAGTGACATCCAGAT
GACACAGACTACATCCTCCCTGTCTGCTCTCTGGGAGACAGAGTACCA
TCAGTTGCAGGGCAAGTACAGACATTAGTAATAATTTAAATTTGGTATCAG
CAGAAACCAGATGGAACGTGTTAAACTCCTGATCTACCATACATCAAGATT
ACACTCAGGAGTCCCATCAAGGTTCAAGTGGCAGTGGGTCTGGAACAGATT
ATTCTCTCACCATTAGCAACCTGGAGCAAGAAGATATTGCCACTTACTTT

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TGCCAACAGGGTAATACGCTTCCGTACACGTTTCGAGGGGGGACTAAGTT
 GGAAATAACAGGCTCCACCTCTGGATCCGGCAAGCCCGGATCTGGCGAGG
 GATCCACCAAGGGCGAGGTGAAACTGCAGGAGTCAGGACCTGGCCTGGTG
 GCGCCCTCACAGAGCCTGTCCGTACATGCAGTGTCTCAGGGGTCTCAT
 ACCCGACTATGGTGTAACTGGATTTCGCCAGCCTCCACGAAAGGGTCTGG
 AGTGGCTGGGAGTAATATGGGGTAGTGAAACCACATACTATAATTCACT
 CTCAAATCCAGACTGACCATCATCAAGGACAACCTCAAGAGCCAAGTTTT
 CTTAAAAATGAACAGTCTGCAAACTGATGACACAGCCATTTACTACTGTG
 CCAAACATTATTACTACGGTGGTAGCTATGCTATGGACTACTGGGGTCAA
 GGAACCTCAGTCACCGTCTCCTCAGCGCGCCGAGACTACAAGACGATGA
 CGACAAGATTGAAGTTATGTATCTCTCTCTTACCTAGACAATGAGAAGA
 GCAATGGAACCATTTATCCATGTGAAAGGGAACACCTTTGTCCAAGTCCC
 CTATTTCCCGACCTTCTAAGCCCTTTTGGGTGCTGGTGGTGGTGGGGG
 AGTCCTGGCTTGCTATAGCTTGCTAGTAACAGTGGCCTTTATTATTTCT

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GGGTGAGGAGTAAGAGGAGCAGGCTCCTGCACAGTGACTACATGAACATG
 ACTCCCCCGCGCCCCGGGCCCCACCGCAAGCATTACCAGCCCTATGCCCC
 ACCACGCGACTTCGCAGCCTATCGCTCCAGAGTGAAGTTACAGCAGGAGCG
 CAGACGCCCCCGCTACCAGCAGGGCCAGAACCAGCTCTATAACGAGCTC
 AATCTAGGACGAAGAGAGGAGTACGATGTTTTGGACAAGAGACGTGGCCG
 GGACCTGAGATGGGGGAAAGCCGAGAAGGAAGAACCTCAGGAAGGCC
 TGTACAATGAAGTGCAGAAAGATAAGATGGCGGAGGCCTACAGTGAGATT
 GGGATGAAAGGCGAGCGCCGAGGGGCAAGGGGCACGATGGCCTTTACCA
 GGGTCTCAGTACAGCCACCAAGGACACCTACGACGCCCTTCACATGCAGG
 CCCTGCCCCCTCGCTAA

[0301] The amino acid sequence of EGFR-UCHT1-FMC63-CD28z (SEQ ID NO.: 3) is as set forth below:

MLLLVTSLLLCELPHPAPLLIPRKVCNGIGIGEFKDSLSINATNIKHFKNCTSIGDLHLIPVAFRGDSF

THTPPLDPQELDILKTVKEITGFLLIQAWPENRTDLHAFENLEIIRGRTKQHGFSLAVVSLNITSLGLR

SLKEISGDVVISGNKNLCYANTINWKKLFGTSGQKTKIISNRGENSCKATGQVCHALCSPEGCWGPPEPR

DCVSCRNVSRGREVDKCNLLEGEPRFVENSECIQCHPECLPQAMNITCTGRGPDNCIQCAHYIDGPHC

VKTCAPAGVMGENNTLVWKYADAGHVCHLCHPNCTYGTGPGLEGCPNGPKIPSIATGMVGALLLLLVVA

LGIGLFMRKRGSGATNFSLLKOAGDVEENPGPMALPVTALLPLALLHAARPDIQMTQSPSSLSASVG

DRVITICRASQDIRNYLNWYQQKPGKAPKLLIYYTSRLESGVPSRFSGSGSGTDYTLTISSLQPEDFATYY

CQQGNTLPWTFGQGTKEIKGGGSGGGGSGGGGSEVQLVESGGGLVQPGGSLRLSCAASGYSFTGYTMN

WVRQAPGKLEWVALINPYKGVSTYNQKFKDRFTISVDKSKNTAYLQMNSLRAEDTAVYYCARSGYYGDS

DWYFDVWGQTLVTVSSGGGSGGGGSGGGGSEVKLQESGPGLVAPSQSLSVTCTVSGVSLPDYGVSWIR

QPPRKLEWLGVWGETTYNSALKSRLTIKDNKSQVFLKMNSLQTDDTAIYYCAKHHYYGGSYAMD

YWGGQTSVTVSSGSTSGSGKPGSGEGSTKGDIDQMTQTSSLSASLGDRVITICRASQDISKYNWYQQKP

DGTVKLLIYHTSRLEHSGVPSRFSGSGSGTDYSLTISNLEQEDIATYFCQQGNTLPYTFGGGKLEITAAA

DYKDDDDKLEVMYPPPYLDNEKSNGTIIHVKGKHLCPSPLPFGPSKPFVVLVVVGGVLCYSLLVTVAFI

IFWVRSKRSLHSDYMNMTPRRPGPTRKHYPYAPPRDFAAYRSRVKFSRSADAPAYQQGQNQLYNELN

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LGRREYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQDKMAEAYSEIGMKGERRRGKGHDGLYQGLST

ATKDTYDALHMQALPPR

[0302] In the amino acid sequence of EGFRt-UCHT1-FMC63-CD28z, the underlined part is the safety switch signal peptide (GMCSFR-L), the italic part is tEGFR, the italic underlined part is tEGFR TM, the curve underlined part is P2A, the italic curve underlined part is CAR signal peptide (CD8 L), the bold part is UCHT1 VL, the dashed underlined part is the linking peptide, the bold underlined part is UCHT1 VH, the bold italic part is FMC63 VH, the bold italic underlined part is FMC63 VL, the boxed part is CD28 hinge region transmembrane region and costimulatory domain, and the boxed italic part is CD3z.

[0303] The nucleotide acid sequence of EGFRt-UCHT1-FMC63-CD28z is as set forth in SEQ ID NO.: 4.

SEQ ID NO.: 4:

ATGCTGCTGCTCGTGACATCCCTCTTATTATGCGAACTCCCTACCCCGC
TTTTCTGTGATCCCCAGAAAGGTGTGCAACGGCATCGGCATTGGAGAGT
TCAAGGATTCTTTAAGCATCAACGCTACCAACATCAAACATTTCAAGAAC
TGTAACCTCCATTTCCGGCGATTACACATCTCCCGTTGCTTTCTGTGG
CGATAGCTTCACACACACACCCCTCTGGACCCTCAAGAACTGGACATTT
TAAAGACCGTGAAGGAGATCACTGGTTTTTACTGATCCAAGCTTGGCCC
GAAAATAGGACAGATCTCCACGCTTTCGAGAAATTTAGAGATCATTCTGTG
CAGAACCAAGCAGCAGCGACAGTTCTCTTTAGCCGTCTGTCTTTAAATA
TCACCTCTTTAGGTTTAAAGATCTTTAAAGAGATCAGCGATGGCGACGTC
ATCATCTCCGGCAACAAGAACCTCTGTTACGCCAACACCATTAATTGGAA
AAAGCTGTTTGGCACTCCGGACAGAAGACCAAGATCATCAGCAACAGAG
GCGAGAACAGCTGCAAAGCTACCGGCCAAGTTTGCCACGCTCTGTGTAGC
CCCGAAGGCTGCTGGGGACCCGAACCTCGTGACTGTGTGAGCTGTAGGAA
CGTGTCTCGTGGTCTGTGAGTGTGTGGATAAGTGCAATTTACTCGAGGGCG
AGCCAGAGAGTTTGTGGAAATAGCGAGTGCATCCAGTGCCACCCCGAA
TGTCTGCCCCAAGCTATGAACATTACTTGTACTGGTCTGTGCCCCGATAA
CTGTATCCAGTGCGCTCACTACATTGACGGCCCCACTGCGTCAAGACAT
GCCCCGCTGGCGTGATGGGAGAAAACAACACTGGTGTGGAAGTATGCC
GATGCCGGCCACGTCTGTCTGTGTCACCTTAACGTACCTACGGCTG
TACCGGACCCGGACTGGAGGGCTGTCCACCAACGGACCCAAGATCCCTA
GCATCGCCACCGGCATGGTGGGAGCCTTACTGCTGTTACTGGTGGTGGCT
CTGGGCATTGGTTTATTATGAGGGCCAAGAGAGGATCCGGCGCCACCAA
CTTTTCTTTACTGAAACAAGCTGGAGAGCTCGAGGAAACCCCGGACCCA
TGGCTCTCCCGTGACAGCTCTGCTGCTGCCTCTCGCTTTTACTGACAC
GCCGCTAGGCCGATATTAGATGACCCAAAGCCCTAGCTCTTTATCCGC

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CAGCGTCGGAGATAGAGTCACAATCACTTGTAGAGCCAGCCAAGATATTC
GTAATTATCTCAACTGGTACCAGCAGAAGCCCGGTAAAGCCCCAAGCTG
CTCATCTATTACACCTCTCGTCTGGAGAGCGGCGTGCCTTCCAGATTACG
CGGCTCCGGCAGCGGCACCGACTATACACTCACCATTAGCTCTTTACAGC
CCGAAGATTTGCTACCTACTACTGCCAGCAAGGTAATACTTTACCTTGG
ACCTTCGGCCAAGGTACCAAGGTGAAATCAAGGGCGGCGGCGGATCCGG
CGGCGGTGGATCTGGTGGCGCGGCTCCGAAGTCCAGCTGGTGAATCCG
GAGGCGGACTGGTCCAGCCCGGTGGATCCCTCAGACTGAGCTGCCCGCT
AGCGGCTATTCTTACCGGCTACACCATGAAGTGGGTGAGACAAGCTCC
CGGCAAAGGACTGGAATGGGTGGCCCTCATCAACCCCTACAAGGGCGTGT
CCACATATAATCAAAAGTTTAAAGGACAGATTACCATCAGCGTCGACAAG
TCCAAGAACACCGCTTATTTACAGATGAATCTTTAAGAGCTGAGGATAC
CGCCGTGTACTATTGTGCTAGGTCCGGCTACTACGGCGACAGCGACTGGT
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GGCGGTCTTGGCGGCGGAGGCTCCGGAGGAGGCGGAGCGAGGTGAAGCT
GCAAGAAAGCGGACCCGGTCTCGTGGCTCCCTCCCAATCTTTATCCGTGA
CTTGTAACCGTGTCCGGAGTCTCTTACCCGACTACGGCGTGAGCTGGATT
AGACAGCCCCCAGAAAAGGTTTAGAGTGGCTGGCGTGATCTGGGGATC
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GACGACACAGCCATTACTACTGCGCCAAGCATTTACTACGGCGGCAG
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GCAGCACATCCGGAAGCGGAAAGCCCGGACGCGGAGAGGGCAGCAGAAAG
GGAGACATCCAGATGACCCAGACCACCTCTCTTTAAGCGCTCTTTAGG
AGATAGGGTGACCATTAGCTGCAGAGCCTCCCAAGACATCAGCAAGTATC
TCAATTGGTACCAGCAAAAGCCGATGGCACCGCTCAAGCTGCTGATCTAC
CACACCTCTCGTCTCCATTCCGGCGTGCCAGCAGATTTAGCGGAAGCGG
ATCCGGAACAGACTATTCTTTAACAATCAGCAATTTAGAGCAAGAAGACA
TCGCCACATATTTCTGCCACAAGGTAACACTTTACCTACACCTTCGGA
GGCGGCACCAAACCTGGAGATTACAGCGGCGGAGACTACAAAGACGATGA
CGACAAGATTGAAGTTATGTATCCTCCTCTTACCTAGACAATGAGAAGA
GCAATGGAACCATATCCATGTGAAAGGGAACACCTTTGTCCAAGTCCC
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AGTCCTGGCTTGCTATAGCTTGCTAGTAACAGTGGCCTTTATTATTTCT
GGGTGAGGAGTAAGAGGAGCAGGCTCTGCACAGTGACTACATGAACATG
ACTCCCCCGCCCGGGCCACCCGCAAGCATTACCAGCCCTATGCCCC
ACCACGCGACTTCGCGAGCCTATCGCTCCAGAGTGAAGTTCAGCAGGAGCG
CAGACGCCCCCGCTACCAGCAGGGCCAGAACAGCTCTATAACGAGCTC
AATCTAGGACGAAGAGAGGAGTACGATGTTTGGACAAGAGACGTGGCCG
GGACCCCTGAGATGGGGGAAAGCCGAGAAGGAAGAACCTCAGGAAGGCC
TGTACAATGAAGTGCAGAAAGATAAGATGGCGGAGGCCCTACAGTGAGATT
GGGATGAAAGGCGAGCGCCGGAGGGGCAAGGGGCACGATGGCCCTTACCA
GGGTCTCAGTACAGCCACCAAGGACACCTACGACGCCCTTCACATGCAGG
CCCTGCCCCCTCGCTAA

[0304] The CAR gene was cloned into the FUW lentiviral vector backbone under the promoter of EF1 α (EF-1 α) to form Fuw-EF1 α -CAR. Fuw-EF1 α -CAR, lentiviral envelope plasmid pMD2.G (Addgene, Plasmid #12259) and lentiviral packaging plasmid psPAX2 (Addgene, Plasmid #12260) were transfected into 293 T cells by using Lipofectamine3000 to prepare the whole lentiviral expression vector. The supernatant was collected at 48 and 72 h and subject to ultra-centrifuge (Merck Millipore) for concentration. The concentrated virus was ready for the transfection of T cells.

[0305] The result shows that the lentiviral vector was successfully constructed and expression of EGFRt and CD19+CD3 CAR were both detected as expected.

1.2 Design and Construction of CRISPR

[0306] First, gRNA sequences targeting the first exon of the TCR conserved region were designed at <http://crispr.mit.edu>, and two gRNA sequences with scores above 80 were selected for higher knockout efficiency. For the gRNA primer, the forward primer comprises a T7 promoter followed by 20 bp of the target sequence, and the reverse primer has 20 bp of the complementary sequence. pX330 plasmid was used as the PCR template. After purification, T7-PCR product was further used as the template for MEGAshortscript T7 kit to obtain RNA. The RNA was purified with a MEGAclear column and eluted with RNA-free water. Cas9 plasmid was purchased from Addgene.

[0307] The gRNA targeting sequences are as below:

TRAC-gRNA: TGTGCTAGACATGAGGTCTA,; SEQ ID NO: 5

[0308] Meanwhile, the knockout efficiency of the gRNA was analyzed by Surveyor assay, TIDE.

1.3 Preparation of CAR-T Cells

[0309] Cell Isolation and Activation

[0310] After apheresis, monocytes were isolated by using Histopaque-1077 (Sigma-Aldrich) through density gradient centrifugation. Then T cells were enriched, activated by magnetic beads coupled with anti-CD3/anti-CD28, cultured and expanded.

[0311] X-vivo 15 with 5% FBS, 2 mM L-glutamine, 1 mM sodium pyruvate and 300 IU/ml rIL2 was used as CAR-T cell medium. The cells were incubated and cultured at 37° C., 5% CO₂.

[0312] Cell line expressing CD 19: Raji (Burkitt's lymphoma cell line, ATCC-CCL86); K562 cells (Human erythroleukemia cell line, ATCC-CCL243); Raji-fluc cell line was obtained by screening Raji cells transfected with lentivirus having firefly luciferase;

[0313] Cell line expressing CD3: Jurkat cell.

[0314] All of the cells were cultured in RPMI1640 medium and 293T cells (ATCC-CRL3216) were cultured in DMEM medium. Both RPMI1640 and DMEM were supplemented with 10% (v/v) fetal bovine serum and 100 U/ml penicillin and streptomycin, 2 mM glutamine and 1 mM sodium pyruvate.

[0315] Electroporation

[0316] Two days after enriching and activating T cells, the anti-CD3/anti-CD28 magnetic beads and the cells were collected into a tube and subject to centrifuge at 300 g for 5 min, and then washed twice with DPBS and re-suspended in opti-mem at a cell density of 1-3 $\times 10^8$ /ml. The amount of cas9/gRNA required was calculated based on the density of the cells, 30 μ g/ml each. Cas9 and gRNA were 1:1 mixed, and incubated at room temperature for 10 mins and then transferred to electroporation buffer to further mix with the cells for electroporation by 4D-Nucleofector System N (Lonza) system. Then the cells were re-suspended in a pre-warmed medium to a density of 1-2 $\times 10^6$ /ml, transferred to a plate and then cultured in an incubator at 37° C., 5% CO₂.

[0317] Lentiviral Transfection

[0318] 1 day after electroporation, the cells were transfected by lentiviral vector at MOI of 2-8, transferred to a flask and cultured at 37° C., 5% CO₂.

[0319] Cell Proliferation and Detection of CAR Positive Ratio

[0320] 3 days after the transfection, the number of cells and CAR positive cells were detected, and the positive ratio of CAR in T cells was calculated. The positive ratio of CD3 and PD1 was also detected to determine the knockout efficiency. Then the cells were continuously cultured in the incubator and half of the medium was replaced every 2-3 days till day 14, when the cells were ready for cryopreservation. An example workflow is shown in FIG. 2.

[0321] FIG. 3 shows flow cytometry data of fractions of the engineered immune cells expressing dual-CARs or control cells with or without specified markers. For example, CD3 can be a marker indicative of endogenous TCR knockout efficiency, 3CAR (CAR targeting CD3) can be a marker indicative of CAR-T cells targeting CD3, 19CAR (CAR targeting CD19) can be a marker indicative of CAR-T cells targeting CD19, and EGFR can be a marker indicative of CAR-T expressing EGFRt. FIG. 4 shows another flow cytometry data of fractions of the engineered immune cells expressing dual-CARs or control cells with or without specified markers. In FIG. 4, the CAR targeting CD19 was a scFv of monoclonal antibody FMC63, and the CAR targeting CD3 was a scFv of monoclonal antibody OKT3 or UCHT1. The result shows that positive ratio of EGFR and CAR and negative ratio of CD3 were high in the prepared EGFRt-CD3-CD19 CAR-T cells, indicating the gene knock-out dual CAR-T cells (e.g., parallel-EGFRt) with safety switch (e.g., EGFRt) were successfully prepared.

1.4 Release of the Cytokines

[0322] The dual CAR-T cells prepared in Example 1.3 and CD19 positive tumor cells (Raji), CD3 positive tumor cells (Jurkat) or primary allogeneic PBMCs, 100 μ l each, were mixed in RPMI medium at 1:1 ratio to a density of 1×10^6 /ml for each cell, and then cultured overnight in a 96-well plate. The medium was then collected and subject to centrifuge and the released cytokine IFN- γ and IL-2 were detected by Cytokine bead array kit (CBA kit, BD Biosciences).

[0323] The result shows that co-incubation of EGFRt-CD3-CD19 CAR-T cells with Raji, Jurkat or primary allogeneic PBMCs produce a large amount of IFN- γ and IL-2, indicating function of CAR-T against CD19 positive or CD3 positive target cells.

1.5 In Vitro Killing Activity

[0324] A stably transfected cell line was obtained by introducing the luciferase gene into the target cell followed by screening. After adding fluorescein, luciferase could react with the fluorescein to produce fluorescence. By detecting the intensity of the fluorescence, the activity of luciferase can be measured, and the survival of the cells can be detected so that to evaluate the killing effect of CAR-T cells.

[0325] FIG. 5A shows bar-graphs of cytotoxicity of CAR-T cells, including CAR-T cells targeting CD3 with scFv of monoclonal antibody OKT3 or UCHT1, dual-specific CAR-T cells targeting CD19 and CD3 (para193-O and para193-U), CD19 CAR with endogenous TCR knocked out (TCR KO CD19 CAR), and T cells controls with endogenous TCR knocked out (TCR KO T cell control), toward CD19+ NALM6-Luc cells and CD3+ Jurkat-Luc cells at 1:1 E:T (Effector CAR-T cells to Target cancer cells) ratio for 6 hours and 24 hours, respectively. FIG. 5B shows cytotoxicity of CAR-T cells toward Jurkat cells measured from Day 1 to Day3 of co-culture of Jurkat cells and CAR-T cells at 1:1 E:T ratio. FIG. 5C shows cytotoxicity of CAR-T cells toward T cells expressing CD3 measured from Day1 to Day 3 of co-culture of NALM6 cells and CAR-T cells at 1:1 E:T ratio.

[0326] The cytotoxicity of the dual-specific CAR-T cells targeting CD19 and CD3 (e.g., dual-specific CAR-T cells in parallel form with scFv from monoclonal antibody UCHT1: GC193-para-U) were also tested against NALM6-LucG, Jurkat-LucG, Raji, allogeneic NTcells (non-treated T cells), and autologous NT cells. FIG. 6A shows 40 hour cytotoxicity of CAR-T cells toward NALM6 cells at different effector: target ratios. FIG. 6B shows 6 hour cytotoxicity of CAR-T cells toward Jurkat cells at different effector: target ratios. FIG. 6C shows 40 hour cytotoxicity of CAR-T cells toward Jurkat, allogeneic NT cells, autologous NT cells, Raji cells, and NALM6 cells. FIG. 7A shows 16 hour cytotoxicity of dual-specific CAR-T cells in parallel or loop form toward NALM6 cells. CD19-CD3 dual-specific CAR-T cells showed comparable anti-tumor efficacy to single CD19 CAR-T cells. FIG. 7B shows 16 hour cytotoxicity of dual-specific CAR-T cells in parallel or loop form toward Jurkat cells. CD19-CD3 dual-specific CAR-T cells showed comparable anti-tumor efficacy to single CD3 CAR-T cells. The result shows that EGFRt-CD3-CD19 CAR-T cells significantly killed Nalm6, Raji, Jurkat and primary allogeneic PBMCs, indicating the function of CAR-T against CD19-positive or CD3-positive target cells,

and their capabilities of simultaneous targeting both CD19+ cancer cells and CD3+ killer T cells.

1.6 In Vivo Efficacy

[0327] 6-12 weeks NOD-Prkdcscid Il2rgnull (NPG) mice were selected and intraperitoneally injected with 2×10^5 Raji-fluc cells or Jurkat-fluc cells, 50 μ L DPBS and 50 μ L matrigel matrix (Corning). Two days later, the tumor graft burden of the mice was measured, and the mice were divided into 4 groups based on the tumor burden. One day later, 200 μ L DPBS, 5×10^6 NT cells and 5×10^6 EGFRt-CD3-CD19 CAR-T cells were respectively injected to each mouse. The tumor burden of the mice was further evaluated 7 days after the CAR-T treatment. Then each mouse was injected intraperitoneally with 3 mg d-luciferin for a 4-minutes reaction, and subsequently photographed by using a Xenogen IVIS Imaging System with 30 s exposure.

[0328] The result shows that EGFRt-CD3-CD19 CAR-T significantly decreased the tumor burden compared to NT cells for tumors from by both Raji-fluc and Jurkat-fluc cells, indicating EGFRt-CD3-CD19 CAR-T can clear CD19 positive or CD3 positive target cells in vivo.

1.7 In Vivo GVHD Study

[0329] The mice were systemically treated with a sub-lethal dose of irradiation (175 cGy) first. Then CAR-T cells were re-suspended in PBS and injected into the thorax of the treated mice. The mice were observed 2-3 times a week by using GVHD clinical criteria including weight loss, arch-back, activity, fur texture, and skin integrity.

[0330] The result shows that mice without TCR knockout showed GVHD symptoms, however, no GVHD was detected in the EGFRt-CD3-CD19 CAR-T+TCR dual knockout group.

[0331] FIGS. 66A-C show the in vivo GvHD study comparing CD19 CAR-T vs TCR KO CD19 CAR-T cells. 2×10^7 CD19 CAR-T or TCR KO CD19 CAR-T cells were infused into each Raji-tumor bearing NOG mice to compare GvHD response. Mice from CD19 CAR-T group showed significant CAR-T expansion along with severe weight loss (>20%), arch-back, ruffled fur, and early death compared with TCR KO CAR-T cells. No GVHD was detected in any TCR KO CD19 CAR-T infused mice.

1.8 In Vitro HVG Study

[0332] EGFRt-CD3-CD19 CAR-T+TCR knockout and EGFRt-CD3-CD19 CAR-T+TCR/B2M dual knockout cells were 1:1 incubated with NK cells overnight to detect apoptosis and cytokine release (IFN- γ).

[0333] The results show that compared to the control group, EGFRt-CD3-CD19 CAR-T+TCR knockout group had almost no apoptosis, and the cytokine released was significantly less, indicating that the product of the present disclosure will not cause HVG reaction by NK cells. Meanwhile, the TCR/B2M dual knockout cells were significantly killed by NK cell and were not able to survive long.

[0334] HVG can also induce allogeneic T cells against HLA intact CAR-T cells. FIG. 6C shows that dual CAR-T cells can target allogeneic T cells within 40 hours, preventing CAR-T rejection by host allogeneic T cells, which usually takes 72 hours.

1.9 Clearance of EGFRt Gene-Mediated CAR-T Cells

[0335] NK cells were isolated from PBMCs as effector cells, and 1:1 co-cultured with CD19-28z CART or EGFRt-CD3-CD19 CAR-T, with or without Cetuximab at a final concentration of 10 µg/mL. 4 h and 24 h later, the expression of CD3 and CAR was detected by flow cytometry.

ADCC percentage=(1-monoclonal antibody group
CAR positive ratio/no antibody group CAR
positive ratio)*100%

[0336] The results show that, 4 h and 24 h later, in the presence of Cetuximab, NK cells significantly cleared EGFRt-CD3-CD19 CAR-T cells, but failed to clear CD19-28z CART. Without Cetuximab, NK failed to kill either of the cells. This indicates that Cetuximab functions as a safety switch, which binds to EGFRt-CD3-CD19 CAR-T, and allows NK to mediate the ADCC function so that to clear CART cells.

Example 2 Study of U-CAR-T Cells Expressing CAR19 and the Enhancer with TCR Knockout

[0337] In this example, 5×10^5 Raji-luciferase cells were grafted into NOG mice by subcutaneous injection and grew till reach 100 mm³. 1×10^6 wildtype or TCR KO CD19 CAR-T cells were intravenously infused (IV) into Raji grafted mice. Tumor burden was assessed by caliper measurements of the actual tumor sizes. CAR-T cell expansion in the peripheral blood was measured by flow cytometry analysis. FIG. 8A shows tumor burden post CAR-T treatment. TCR intact CD19 CAR-T cells were able to eliminate tumors, but TCR KO CD19 CAR-T wasn't able to control Raji tumor growth. Although both were Raji based tumor bearing models, subcutaneous model generates solid tumors and has much higher requirements on CAR-T cell proliferation for controlling the tumors than intravenous model. FIG. 8B shows CAR-T proliferation in peripheral blood. TCR KO CAR-T cells showed a proliferation defects compared to WT CAR-T cells, the peak of CAR-T cell expansion is much weaker than that of WT cells, which is consistent with their tumor control effects.

[0338] FIGS. 9A-9C show proliferation of CD19 CAR-T cells with TCR knockout. FIG. 9A shows an experimental timeline of repeated stimulations by NALM6 cells and measurements of residual tumor cells. FIG. 9B shows the proliferation data of CD19 CAR-T cells with TCR knockout and expression of different enhancers in comparison to CD19 CAR-T cells without TCR knockout and CD19 CAR-T cells with TCR knockout but without expression of the enhancers. Additions of NALM6 cells are indicated by arrows according to the experimental timeline in FIG. 9A. FIG. 9B shows the clearing activity of various CD19 CAR-T cells as indicated by the amount of residual tumor cells measured at the indicated days according to the experimental timeline in FIG. 9A. Under multiple rounds or repeated stimulations by NALM6 cells, the CD19 CAR-T cells with TCR knockout and C7R or IL7 exhibited persistent expansion and enhanced expansion ability compared with other cells tested in this experiment.

[0339] FIGS. 10A and 10B show additional proliferation data comparing expansion ability of engineered CAR-T cells. FIG. 10A shows proliferation data of CD19 CAR-T cells with TCR knockout and two different enhancers (C7R and IL2) in comparison to cells without the expression of the enhancer. The results showed that under multiple rounds or

repeated stimulation by NALM6 cells, CD19 CAR-T cells with TCR knockout and C7R or IL2 exhibited persistent expansion and enhanced expansion ability compared with the cell without the expression of any enhancer. FIG. 10B shows proliferation data of CD7 CAR-T cells with TCR knockout and two different enhancers (C7R and mbIL15) in comparison to cells without the expression of the enhancer. The results showed that under multiple rounds or repeated stimulation by CCRF-CEM cells, CD7 CAR-T cells with TCR knockout and C7R or mbIL15 exhibited persistent expansion and enhanced expansion ability compared with the cell without the expression of any enhancer. The cells with the expression of C7R exhibited better expansion ability than the cells expressing mbIL15.

[0340] FIG. 11 shows an example of in vivo comparison of enhancers in TCR KO CD19 CAR-T cells. In this example, 5×10^5 Raji-luciferase cells were grafted into NOG mice by intravenous (IV) injection and grew for 5 days. TCR KO CD19 CAR-T cells (CD19 CAR-T cells with TCR knockout) with/without enhancers as well as vehicle and T cell controls were intravenously infused (IV) into Raji grafted mice. Tumor burden was assessed by bioluminescence intensity (BLI). The TCR KO CD19 CAR-T cells expressing enhancer C7R, IL7 exhibited the better tumor control in comparison to the cells without the expression of enhancers or with expression of mbIL15 or other control cells. FIG. 12 shows corresponding images of the NOG mice indicating tumor burden assessed by BLI.

Example 3 Study of U-CAR-T Cells Expressing CAR7 and the Enhancer with TCR and CD 7 Double Knockout, as Well as U-CAR-T Cells Expressing CD2 CAR and the Enhancer with TCR and CD 2 Double Knockout

General Materials and Methods

[0341] Isolation of Peripheral Blood Mononuclear Cells (PBMCs) from Donor Blood and Expansion of T Cells

[0342] Peripheral blood mononuclear cells (PBMCs) were isolated from donor blood by using Histopaque-1077 (Sigma-Aldrich) through density gradient centrifuge. Then T cells were enriched, activated by magnetic beads coupled with anti-CD3/anti-CD28, cultured and expanded.

[0343] Cell Lines and Culture of T Cells

[0344] CCRF-CEM (T lymphoblast, ATCC® CCL-119™);

[0345] CCRF-CEM-luc cells (obtained by screening CCRF-CEM cells transfected with lentivirus having firefly luciferase);

[0346] K562 cells (Human erythroleukemia cell line, ATCC-CCL243);

[0347] 293T cells (ATCC-CRL3216);

[0348] NK92 cells (ATCC-CRL2407).

[0349] The CCRF-CEM-luc cell line was cultured in RPMI1640 medium, and 293T cells were cultured in DMEM medium. Both RPMI1640 and DMEM were supplemented with 10% (v/v) fetal bovine serum and 100 U/ml penicillin and streptomycin, 2 mM glutamine and 1 mM sodium pyruvate. All of the cells were cultured in an incubator at 37° C., 5% CO₂.

[0350] NK92 cells were cultured in RPMI1640 medium supplemented with 10% (v/v) fetal bovine serum, 2 mM glutamine, 1 mM sodium pyruvate, 1% NEAA, 0.1 mM mercaptoethanol and 200 IU/ml rhIL2.

[0351] T cells and the obtained CAR-T cells were cultured in X-vivo15 medium (containing 5% FBS, 2 mM L-glutamine, 1 mM sodium pyruvate and 300 IU/ml rhIL2). The culture medium for CAR-T cells was further supplemented with rhIL-2 (ThermoFisher Scientific) at a final concentration of 300 IU/ml every two days. All of the cells were cultured in an incubator at 37° C., 5% CO₂.

3.1 Design and Construction of CRISPR

[0352] CD7 and TCR gRNAs for gene editing were selected after evaluating gene editing efficiency and off-target risk at website <http://crispr.mit.edu>, www.idtdna.com and <https://www.synthego.com>. gRNAs and HiFi-Cas9 protein used in the present disclosure were purchased from Integrated DNA Technologies, Inc (IDT).

[0353] For gene editing, 3 µg Cas9 protein and 1.5 µg gRNA were mixed in 20 µl and incubated at 37° C. or room temperature for 15 minutes to form ribonucleoprotein (RNP). Then RNP was transfected into T cells by Lonza 4D Nucleofector. Gene knockout efficiency was determined by the ratio of the cells expressing the protein as detected by flow cytometry (FACS).

[0354] Our previous study indicated that TRAC-gRNA1 has a higher knockout efficiency for TCR (the sequence is set forth as SEQ ID NO.: 20). When gRNA of CD7 and TRAC-gRNA were used together to knockout CD7 and TCR

simultaneously, three of four CD7-gRNAs showed high efficiencies (as shown in FIG. 16). CD7-gRNA1 (SEQ ID NO.: 34) was selected as the gRNA to knockout CD7.

TRAC-gRNA1: TTCGGAACCCAATCACTGAC, ; SEQ ID NO 20

CD7-gRNA1: GAGGTCAATGTCTACGGCTC, . SEQ ID NO 25

[0355] The gDNA of CD2 were designed as below, and CD2 gRNA2 showed the highest CD2 knockout efficiencies (as shown in FIG. 65).

CD2-gRNA1: CAAAGAGATTACGAATGCCT (SEQ ID NO: 29)

CD2-gRNA2: GTGCCACAAAGACCATCAAG (SEQ ID NO: 30)

CD2-gRNA3: AGAGGGTCATCACACACAAG (SEQ ID NO: 31)

CD2-gRNA4: CTGTAGATATCTGTATCAT (SEQ ID NO: 32)

3.2 Design of CAR and Cytokine and Package of the Virus

[0356] The structure of the chimeric antigen receptor and the cytokines are: CAR7, CAR7-mb15 and CAR7-C7R (as shown in FIG. 17). The nucleotide sequences and amino acid sequences are set forth as SEQ ID NO.: 35-40.

CAR7 (SEQ ID NO 35):

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atggcactccctgtaactgcacttcttttgccacttgccctgctcctgcacgcagcgcggccggacatc
gagctgacccagagccccgccatcatgagcgcagcctggcgaggagatcacctgacctgcagcgcc
agcagcagcgtgagctacatgcactggtagcagcagaagagcggcaccagccccaaagctgctgactac
agcaccagcaacctggccagcggcggtgccagcaggttcagcggcagcggcagcggcaccttctacagc
ctgaccatcagcagcgtggaggccgaggacgccgcgactactactgccaccagtgaggacgctacacc
ttcggcgggcgccaccaagctggagatcaagagggcgggcgggcagcggcgggcgggcagcggcgggc
ggcgggcagccaggtgaagctgcaggagagcggcgggcggtggaagccggcgggcagcgtgaagctg
agctgcgccgcagcggcttcaccttcagcagctacgccatgagctgggtgaggcagacccccgagaag
aggctggagtggtggccaccatcagcagcggcgggcagctacacactactacccgacagcgtgaagggc
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gacaccgccatgtactactgcgcaggcaggacggctactaccccgctggttcgccaaactggggccag
ggcaccaccgtgaccgtgagcagctccggaacaacgacaccagcaccacggccaccactcctgctccg
acaattgcgtctcagccctttcccttcgacccgaagcttgctcgccctgctgcgggaggagcggccac
acgcgcgggcttgacttcgcttgcgacatctacatttgggcacccttgccgggacatgcggcgtcttg
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ccctttatgaggcccgtaacaaccacacaagaggaggtgggtgctcatgcagatttccctgaagaggaa
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aatcaattgtataatgaacttaatctcgggagggcgagaggtatgatgtgctggacaagcgacggggt
cgagaccagagatggcggttaaccccgccgaagaacccccaggaggactgtataatgagctgcaa
aaggacaaaatggcagaagcctattccgaaataggatgaaggagagcggcgggcaggtgaaggacat
gacggtctttatcaaggtcttagtactgcaactaaggacacctatgacgcgtgcatatgcaggctctc
ccacctagataa
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CAR7 (SEQ ID NO 36):

MALPVTALLPLALLLHAARPDIELTQSPAIMSASLGEEITLTCSASSSVSYMHW

YQQKSGTSPKLLIYSTSNLASGVPSRFSGSGSGTFYSLTISSVEAEDAADYYCHQWSSYT

FGGGTKLEIKRGGGSGGGGSGGGGSGQVKLQESGGGLVKPGGSLKLSCAASGFTFSSY

AMSWVRQTPEKRLEWVATISSGGSYTYYPDSVKGRFTISRDNAKNTLYLQMSSLRSED

TAMYICARQDGYYPGWFWANWGQGTTVTVSSSGTTPAPRPPTPAPTIASQPLSLRPEAC

RPAAGGAVHTRGLDFACDIYIWAPLAGTCGVLLLSLVI TLCKRGRKKLLYIFKQPFMR

PVQTTQEEDGCSRFPEEEEGGCELRVKFSRSADAPAYKQGQNQLYNELNLGRREEYD

VLDKRRGRDPEMGGKPRKNPQEGLYNELQDKMAEAYSEIGMKGERRRGKGHDGL

YQGLSTATKDTYDALHMQALPPR

CAR7-mb15 (SEQ ID NO 37):

atggcactccctgtaacgtcacttcttttggcacttgcttgcctcctgcacgcagcgcggccggacatc
gagctgacccagagccccgccatcatgagcgccagcctgggagagagatcacctgacctgcagcgcc
agcagcagcgtgagctacatgcactggtaccagcagaagagcgccaccagccccagctgctgatctac
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ttcgggcgcgccaccaagctggagatcaagagggcgcgcgcgagcgcgcgcgcgagcgcgcgcg
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cgagaccagagatggcggttaaccccgcggaagaaccccgaggaggactgtataatgagctgcaa
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agtctgcagataaacatgccccccacctatgagtggtgaacatgctgatatctgggttaaattctactcc
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gaatgtgtattgaataaaggacaaatgtcgacactggactacccttccctcaaatgcattcgcgat
cctgccttgggtgcatcagcgaccagcaccgccgtccacggtaactaccgcaggagtaacaccgcagccc
gagagcctttccccctcaggcaagagccggccgcatcctccccatcttccaataataaccgcagctacc
accgcagcaatcgtaccgggacccagctgatgccagcaaaagtcagtagtactggaacgactgaaatc
tccagtacagagtccttctcatggaactccgagtcactacagcaagaatgggagctgactgcttcc
gcttcacaccagccgccaggcgcttatcctcagggacactcagataccacggtgccgattagcacaagc
accgtcctcctgtgtgggtgtagtgagtgctacttctcgctgtactttaagtcagacagacaccc
cctttggcaagcgttgaaatggaagccatggaagccttgctgtcacatgggggacttcatcccgcat
gaagacttgagaaactgctcacaccatctt

CAR7-mb15 (SEQ ID NO 38):

MALPVTALLPLALLHAARPDIELTQSPAIMASLGEETITLCSASSSVSYMHW

YQQSGTSPKLLIYSTSNLASGVPSRFGSGSGTFYSLTISSVEAEDAADYYCHQWSSYT

FGGGTKLEIKRGGGSGGGGSGGGGSGVQLQESGGGLVKPGGSLKLSCAASGFTFSSY

AMSWVRQTPEKRLEWVATISSGGSYTYYPDSVKGRFTISRDNKNTLYLQMSLSLRS

TAMYCARQDGYYPGFANWGGTTVTVSSSGTTTPAPRPPTPAPTIASQPLSLRPEAC

RPAAGGAVHTRGLDFACDIYIWAPLAGTCGVLLLSLVI TLYCKRGRKKLLYIFKQPFMR

PVQTTQEDGCS CRPPEEEGGCEL RVKFSRSADAPAYKQGQNQLYNELNLGRREEYD

VLDKRRGRDPMEGKPRRKNPQEGLYNELQDKMAEAYSEIGMKGERRRGKHDGL

YQGLSTATKDYDALHMQALP PRAKRGSGATNFSLLKQAGDVEENPGPMDWTWILF

LVAATR VHSNWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLELQV

ISLES GDASIHD TVENLII LANNSLSSNGNVTESGCKECEELEEKNIKEFLQSFVHIVQMFI

NTSSGGGSGGGSGGGSGGGSGGGSLQITCPPMSVEHADIWKSYSLYSRERYICN

SGFKRKAGTSSLTECVLNKATNVAHWTTPSLKCIRD PALVHQR PAPPSTVTTAGVTPQP

ESLSPSGKEPAASSPSSNNTAATTAI VPGSQLMPKSPSTGTTEISSHESHGTPSQTTAK

NWELTASASHQPPGVYPQGHSDTTVAISTSTVLLCGLSAVSL LACYLKSRQTPPLASVE

MEAMEALPVTWGTSSRDELENC SHHL

CAR7-C7R (SEQ ID NO 39):

atggcactccctgtaactgcacttcttttggcacttgctctgctcctgcacgcagcgccggacatc
gagctgacccagagccccgccatcatgagcgccagcctgggcgaggagatcacctgacctgcagcgcc
agcagcagcgtgagctacatgcactggtagcagcagaagagcgccaccagccccagctgctgatctac
agcaccagcaacctggccagcgcggtgcccagcagggtcagcggcagcggcagcggcaccttctacagc
ctgacctcagcagcgtggaggccgaggacgcccgcgactactactgccaccagtgaggagcagctacacc
ttcgcgcgcgccaccaagctggagatcaagagggcgcgcgcgcgagcgcgcgcgcgagcgcgcgcc
ggcgcgagccaggtgaagctgcaggagagcgcgcgcgcgctggtagacccggcgcgagcctgaagctg
agctgcgcgcgcagcggttcacctcagcagctacgccatgagctgggtgaggcagacccccgagaag
aggctggagtggtggccaccatcagcagcgcgcgagctacacctactaccccgacagcgtgaagggc
aggttcaccatcagcagggacaacccaagaacacctgtacctgcagatgagcagcctgaggagcgag
gacaccgcatgtactactgcgccaggcaggacggctactaccccgctggttcgccaactggggccag

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ggcaccacccgtgaccgtgagcagctccggaacaacgacaccagcaccacggccaccactcctgctccg
acaattgcgtctcagcccccttcccttcgacccgaagcttgctcgccctgctgctggaggagcggtccac
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aaggacaaaatggcagaagcctattccgaaataggatgaaggagagcgccggcgaggtaaggacat
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ggcagtagcagcttgcatccctcgacgaacacggcaacgaggcaacaactaacatcacagagactacg
gtcaagtttactagtagtctccgtaatcacgtctgtgtacgggaatacaaattcatcagttcagagtc aa
acgtcagttatatctacagttcttactactcccggaatgtatccacccagaaaccacctcaaacct
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ccgtacacttccagcagtcctactgtccgacattaaggcagaaataaaatgttctgggatcaggggag
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gagaaacagcgcttggtggagatgtccagtcctcgaactgtccgagcgaggacgtagtaattacccca
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ctcaatccagtcgcacaagggaacctatcctgacctccttgggttctaaccaggaagaggeatcagtc
actatgtccagcttctatcagaatcag

CAR-C7R (SEQ ID NO 40):

MALPVTALLLLPLALLLHAARPDIELTQSPAIMASLGEETLTCSASSSVSYMH

YQQKSGTSPKLLIYSTSNLASGVPSRFRSGSGSTFYSLTISSVEAEDAADYYCHQWSSYT

FGGGTKLEIKRGGGSGGGGSGGGGSGVQLQESGGLVKPGGSLKLSCAASGFTFSSY

AMSWVRQTPEKRLWVATISSGGSYTYYPDSVKGRFTISRDNKNTLYLQMSSLRSED

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TAMYYCARQDGYYPGWANWGQTTVTVSSSGTTTPAPRPPTPAPTIASQPLSLRPEAC
 RPAAGGAVHTRGLDFACDIYIWAPLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMR
 PVQTTQEEDGCS CRFPEEEEGGCELRVKFSRSADAPAYKQGQNQLYNELNLGRREEYD
 VLDKRRGRDPEMGKPRRKNPQEGLYNELQDKMAEAYSEIGMKGERRRGKGHDGL
 YQGLSTATKDTYDALHMQALPPRGSGATNFSLLKQAGDVEENPGPMLVRRGARAGR
 MPRGWTALCLLSLLPSGFMSLDNNGTATPELPTQGTFSNVSTNVSYQETTTPTSLGSTSL
 HPVSHQHGNEATTNITETTVKFTSTSVITSVYNTNSSVQSQTSTVISTVFTTPANVSTPETT
 LKPSLSPGNVSDLSTTSTSLATSPTKPYTSSSPILSDIKAEIKCSGIREVKLTQGICLEQNKT
 SSCAEFKKDRGEGLARVLCGEEQADADAGAVCSLLLAQSEVRPQCCLLVLANRTEISS
 KLQLMKKHQSDLKKLGILDFTEQDVASHQSYSQKTPILLTCPTISILSFFSVALLVILACV
 LWKKRIKPIVWPSPDPHKKTLLEHLCKKPRKNLNVSNPESFLDCQIHRVDDIQARDEVE
 GFLQDTFPQQLEESEKQRLGGDVQSPNCPSEDVITPESFRDSSLTCLAGNVSAACDAPI
 LSSSRSLDCRESGKNGPHVYQDLLLSLGTNTSLPPPFSLSGILTLNPVAQGGPILTSLG
 SNQEEAYVTMSSFYQNG

[0357] The amino acid sequence of two CD2 scFv constructed to single or a dual CAR are as below:

CD2 scFv1 VH: (SEQ ID NO: 95)
 QLQQPGAELVRPGSSVKLSCKASGYTFTRYWIHWVKQRPIQGLEWIGNID
 PSDSETHYNQKFKDKATLTVDKSSGTAYMQLSSTSEDSAVYYCATEDLY
 YAMEYWGQGTSTVTVSS

CD2 scFv1 VL: (SEQ ID NO: 96)
 DIMMTQSPSSSLAVSAGEKVTMTCKSSQSVLYSSNQKNYLAWYQQKPGQSP
 KLLIYWASTRESGVDRFTGSGSGTDFTLTISVQPEDLAVYYCHQYLSS
 HTFGGGTKLEIKR

CD2 scFv2 VH: (SEQ ID NO: 97)
 QVQLQQPGTELVRPGSSVKLSCKASGYTFTSYWVNVKQRPDQGLEWIGR
 IDPYDSETHYNQKFTDKAISTIDTSNTAYMQLSLTSDASAVYYCSRSP
 RDSSTNLADWGQGTSLVTVSS

CD2 scFv2 VL: (SEQ ID NO: 98)
 DIVMTQSPATLSVTPGDRVSLSCRASQSIIDYLVHWYQQKSHESPRLLIKY
 ASQSISGIPSRFSGSGSGSDFTLSINSVEPEDVGYYCQNGHSFPLTFGA
 GTKLELRR

[0358] The CAR genes were cloned into the FUW lentiviral vector backbone under the promoter of EF α (EF-1 α) to form Fuw-EF1 α -CAR. Fuw-EF1 α -CAR, lentiviral envelope plasmid pMD2.G (Addgene, Plasmid #12259) and lentiviral packaging plasmid psPAX2 (Addgene, Plasmid #12260) were transfected into 293 T cells by using Lipofectamine3000 to prepare the whole lentiviral expression vector. The supernatant was collected at 48 and 72 h and subject to ultra-centrifuge (Merck Millipore) for concentration.

3.3 Preparation of UCAR-T Cells

[0359] The whole preparation process is illustrated in FIG. 15.

[0360] Cell Isolation and Activation

[0361] After apheresis, monocytes were isolated using Histopaque-1077 (Sigma-Aldrich) by density gradient centrifugation. T cells were then enriched, activated by magnetic beads coupled with anti-CD3/anti-CD28, cultured and expanded.

[0362] X-vivo 15 with 5% FBS, 2 mM L-glutamine, 1 mM sodium pyruvate and 300 IU/ml rhIL2 was used as UCAR-T cell medium. The cells were incubated and cultured at 37° C., 5% CO₂.

[0363] Electroporation and Lentivirus Infection

[0364] Two days after enriching and activating the T cells, the anti-CD3/anti-CD28 magnetic beads were removed and the cells were collected into a tube and subject to centrifuge at 300 g for 5 min, and then washed twice with DPBS and re-suspended in opti-mem at a cell density of 50×10⁶/ml. The amount of cas9/gRNA RNP required was calculated based on the density of the cells, and then mixed with cells and transferred for electroporation by 4D-Nucleofector System N (Lonza) system. Then the cells were re-suspended in a pre-warmed medium to a density of 2×10⁶/ml. The cells were further subject to lentiviral transfection at MOI of 2-8, transferred to a flask and cultured in an incubator at 37° C., 5% CO₂.

[0365] Cell Proliferation and Detection of CAR Positive Ratio

[0366] 3 days after the transfection, the number of cells and CAR positive cells were detected, and the positive ratio of CAR in T cells was calculated. The positive ratio of TCR (or CD3) and CD7 was also detected to determine the knockout efficiency. Then cells were continuously cultured in the incubator for 10 days and then the cells were ready for cryopreservation.

[0367] The result shows that (FIG. 18) the expression of CAR, IL15R α or C7R (CD34) was successfully detected by flow cytometry for CAR7, CAR7-mb15 and CAR7-C7R,

and the positive ratio was higher than 30%. The negative ratio of CD3/CD7 was up to about 98%, indicating that the TCR knockout enhanced and universal UCAR-T cells were successfully prepared.

[0368] As shown in FIG. 19, after removing exogenous IL-2, pSTAT5 level of CAR7 went down to basal level. However, the level of pSTAT5 in CAR7-C7R T cells (CD34+ cells) or CAR7-T cells with exogenous IL2 addition were at quite high levels, indicating expression of C7R and exogenously added IL-2 can both enhance the activation of STAT5 signaling pathway.

3.4 In Vitro Killing Effect

[0369] A stably transfected CCRF-CEM-luc cell line was obtained by introducing the luciferase gene into the target cell CCRF-CEM followed by screening. After adding fluorescein, luciferase could react with the fluorescein to produce fluorescence. By detecting the intensity of the fluorescence, the activity of luciferase can be measured, and the survival of the cells can be detected so that to evaluate the killing effect of UCAR-T cells.

[0370] The UCAR-T cells prepared in Example 3.3 were mixed with CD-7 positive T cell CCRF-CEM-luc, 100 ul each, in RPMI medium at 1:1 ratio to a density of 1×10^6 /ml for each cell, and then cultured for a certain period of time in a 96-well plate. The medium was then collected and subject to centrifuge and the cytokine IFN- γ and IL-2 were detected by Cytokine bead array kit (CBA kit, BD Biosciences). In addition, fluorescein substrate was added to the cell culture to detect the killing effect of the UCAR-T cells. The result shows that UCAR-T has a significant killing effect on CCRF-CEM tumor within 24 hours (FIG. 20).

[0371] The killing effect of UCAR-T on allogeneic T cells: allogeneic T cells were labeled with CFSE and incubated with CAR-T for a predetermined period of time. Then changes in the number of CFSE positive cells were analyzed by flow cytometry. The result shows that CAR-T cells efficiently killed allogeneic T cells within 24 hours, which is much shorter than the time for allo-T cell mediated HVG to happen, and thus CAR7 can effectively protect CAR-T cells from allogeneic T cell killing (FIG. 21).

[0372] The 24 hour killing effect of UCAR-T on NK tumor cell line (NK92): NK92 was labeled with carboxyfluorescein succinimidyl ester (CFSE) and incubated with UCAR-T for a predetermined period of time. Then changes in the number of CFSE positive cells were analyzed by flow cytometry, so that to calculate the in vitro killing effect of UCAR-T on NK92 tumor cell line (FIG. 22).

3.5 Proliferation of CAR-T Cells

[0373] IL-2 was removed from CAR-T. CAR-T cells were continuously cultured for 2 weeks and counted twice a week to measure the proliferation.

[0374] The result in FIGS. 23A and 23B shows that CAR7-mb15 and CAR7-C7R CAR-T cells had stronger proliferation rate than CAR7 CAR-T cells, and in absence of IL-2, they still had higher survival ratio within 2 weeks. CAR7-C7R showed better survival than CAR7-mb15. In vitro killing assay indicates that the UCAR-T cells have superior killing effect against T-ALL cell line CCRF-CEM within 7 hours.

3.6 Re-Activation of Cells In Vitro

[0375] IL-2 was removed from UCAR-T. CCRF-CEM was added twice a week at 1:1 effector: target ratio and counted twice a week to measure the proliferation.

[0376] FIGS. 24A and 24B show that after adding CCRF-CEM, CAR7-mb15 and CAR7-C7R UCAR-T cells showed significantly stronger proliferation than CAR7 UCAR-T and CAR7-C7R UCAR-T cells proliferated better than CAR7-mb15 UCAR-T cells. After repeated stimulation, UCAR-T cells still showed significant ability to remove tumor cells, indicating after repeated antigen stimulation, the cytokine-related signaling pathway enhanced UCAR-T cells has a higher proliferation rate, and can survive better.

3.7 Proliferation and Efficacy of UCAR-T Cells in Tumor Bearing Mice

[0377] 6-12 weeks NOD/Shi-scid/IL-2R γ null (NOG) mice were selected and intraperitoneally injected with 2×10^6 CCRF-CEM-luc cells. Two days later, the tumor graft burden of the mice was measured, and the mice were divided into 5 groups based on the tumor burden. 6 days after the tumor implantation, 1×10^6 UCAR-T or control was injected intravenously to each mouse, and the tumor burden was further evaluated every 1-2 weeks. Each mouse was injected intraperitoneally with 3 mg D-luciferin for a 4-minutes reaction, and then photographed using a Xenogen IVIS Imaging System with 30 s exposure (BLI). UCAR-T cell number in peripheral blood was counted every week.

[0378] FIG. 25 shows that CAR7-mb15 and CAR7-C7R UCAR-T had significant expansion, and CAR7 UCAR-T did not show any significant expansion. FIG. 26 shows the result of BLI imaging, and it can be seen that CAR7-mb15 and CAR7-C7R UCAR-T cells had better effect on tumor clearance than CAR7 UCAR-T cells.

[0379] FIG. 27 shows BLI images of the mice treated with vehicle control or CAR7 UCAR-T cells with TCR knockout and with or without expression of enhancers. CAR7 UCAR-T cells with TCR knockout and the expression of C7R or mbIL15 exhibited better efficiency on tumor clearance in comparison to vehicle control and TCR KO CAR7 UCAR-T cells without the expression of any enhancers. C7R expressing CAR7 cells showed better tumor control and mouse survival than that expressing mbIL15. As used herein, "CAR7," "CAR7 UCAR-T," or CD7 CAR-T" can be used interchangeably to indicate an engineered CAR-T cell comprising a CAR targeting CD7.

[0380] FIG. 28A BLI imaging shows in vivo function of CD7 CAR-T cells in clearing tumor cells. TCR KO CD7 CAR-T cells with the expression C7R or mbIL15 exhibited better in vivo efficiency on tumor clearance in comparison to vehicle control and TCR KO CD7 CAR-T cells without the expression of any enhancers. FIG. 28B shows animal survival data of the NOG mice treated with TCR KO CD7 CAR-T with or without the expression of enhancer and vehicle control. Percentage of survived mice were measured against days after CAR-T cell infusion. Mice treated with TCR KO CD7 CAR-T with expression of C7R or mbIL15 had a better survival rate in comparison to mice treated with CAR-T cells without the expression enhancer or vehicle control. C7R expressing CAR7 cells showed better tumor control and mouse survival than that expressing mbIL15.

[0381] FIG. 55A shows expansion data of CAR-T cells. Freshly prepared CD7 CAR-T cells expressing C7R were

cultured in T cell culture media without supplement of IL2 or antigen stimulation, compared to control cells that do not have C7R or any enhancer expression, CD7 CAR-T cells expressing C7R maintained better expansion and persisted in culture. FIG. 55B shows proliferation of CAR-T cells in the presence of stimulation. CD7 CAR-T (or UCART7), CD7 CAR-T expressing mbIL15 or C7R were repetitively stimulated by CCRF-CEM (CD7+ T-ALL cells). The CD7 CAR-T expressing C7R showed the best persistence and proliferation. CD7 CAR-T expressing mbIL15/C7R stopped proliferation when antigen stimulation was removed at round 6 of stimulation (CC6). "CC," as used herein, indicates co-culture with cancer cells.

3.8 In Vivo GVHD Study

[0382] The mice were systemically treated with a sub-lethal dose of irradiation (175 cGy) first. Then CAR-T cells were re-suspended in PBS and injected into the thorax of the treated mice. The mice were observed 2-3 times a week by using GVHD clinical criteria including weight loss, arch-back, activity, fur texture, and skin integrity.

[0383] The result shows that mice in groups without TCR knockout all showed symptoms of GVHD, however, no GVHD was detected in TCR knockout group.

3.9 Effect of TCR Knockout

[0384] FIGS. 54A-C show the effect of TCR knockout in the CAR-T cells. In an investigator initiated clinical trial, TCR expression in Mesothelin CAR-T cells were impaired to avoid TCR related adverse event. However, TCR KO CAR-T cells unexpectedly showed significant expansion defects after infusion into the patients. FIG. 54A shows example of TCR KO efficiency in autologous Mesothelin (MSLN) CAR+ T cells (left), and CAR+ T cells in patient's peripheral blood CAR-T cells at 13 days post infusion (right). FIG. 54B shows TCR disruption efficiencies in autologous MSLN CAR-T products were highly effective, with less than 5% TCR+ cells remaining in the product. However, after infusion into patients, majority of the CAR-T cells detected in the peripheral blood were TCR+ cells. FIG. 54C shows that TCR+ cells showed 40 to ~1000 fold more expansion than TCR-cells in cancer patients. Therefore, even in autologous CAR-T applications, where there is no cell survival and expansion pressure from allogeneic killer cells, TCR disruption dramatically impaired CAR-T cell expansion.

3.10 Sequences of Construct Designs

[0385] CD7CAR-mbIL15 construct design: CD7CAR-mbIL15 amino acid sequence (mbIL15=IgE signal peptide+IL15+IL15R α)

(SEQ ID NO: 99)
MALPVTALLPLALLHAARPDIELTQSPAIMSASLGEEITLTCSASSV
SYMHWYQQKSGTSPKLLIYSTNLASGVPSRFGSGSGTFYSLTISSVEA
EDAADYYCHQWSSYTFGGGKLEIKRGGGSGGGGSGGGGQVKLQESGG
GLVKPGGSLKLSCAASGFTFSSYAMSWVRQTPEKRLEWVATISSGGSYTY
YPDSVKGRFTISRDNAKNTLYLQMSLRS EDTAMYYCARQDGYYPGW FAN

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WGQGTTVTVSSSGTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHT
RGLDFACDIYIWAPLAGTCGVLLLSLVTLYCKRGRKKLLYIFKQPFMRP
VQTTQEEDGCSCRFPEEEEGGCELRVKFSRSADAPAYKQGQNQLYNELNL
GRREYDVLDKRRGRDPEMGKPRRKNPQEGLYNELQDKMAEAYSEIGM
KGERRRGKGGHDGLYQGLSTATKDTYDALHMQALPPRGSGATNFSLLKQAG
DVEENPGPMDWTWILFLVAAATRVHSNWNVVISDLKKIEDLIQSMHIDAT
LYTESDVHPSCKVTAMKCFLELQVISLES GDAS IHD TVENLIILANNLS
SSNGNVTESGCKECELEEKNIKEFLQSFVHIVQMFIN TSSGGSGGGGS
GGGSGGGSGGGSLQITCPPPMSVEHADIWVKSYSLSYRERYICNSGFK
RKAGTSSLTECVLNKATNVAHWTPSLKCI RDPALVHQRPA PPTVTTAG
VTPQPESLSPSGKEPAASSPSSNNTAATTAIVPGSQLMPKSPSTGTTE
ISSHESHGTPSQTTAKNWELTASASHQPPGVYPQGHSDTTVAISTSTVL
LCGLSAVSL LACYLKSRQTPPLASVEMEAMEALPVTWGTSSRDELENC
S
HHL

[0386] CD7CAR-C7R construct design: CD7CAR-C7R amino acid sequence (C7R=CD34 ecto domain+constitutively active IL7R α)

(SEQ ID NO: 40)
MALPVTALLPLALLHAARPDIELTQSPAIMSASLGEEITLTCSASSV
SYMHWYQQKSGTSPKLLIYSTNLASGVPSRFGSGSGTFYSLTISSVEA
EDAADYYCHQWSSYTFGGGKLEIKRGGGSGGGGSGGGGQVKLQESGG
GLVKPGGSLKLSCAASGFTFSSYAMSWVRQTPEKRLEWVATISSGGSYTY
YPDSVKGRFTISRDNAKNTLYLQMSLRS EDTAMYYCARQDGYYPGW FAN
WGQGTTVTVSSSGTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHT
RGLDFACDIYIWAPLAGTCGVLLLSLVTLYCKRGRKKLLYIFKQPFMRP
VQTTQEEDGCSCRFPEEEEGGCELRVKFSRSADAPAYKQGQNQLYNELNL
GRREYDVLDKRRGRDPEMGKPRRKNPQEGLYNELQDKMAEAYSEIGM
KGERRRGKGGHDGLYQGLSTATKDTYDALHMQALPPRGSGATNFSLLKQAG
DVEENPGPMLVRRGARAGPRMPRGWTALCLLSLLPSGFMSLDNNGTATPE
LPTQGTFSNVSTNVSYQETTTPTSLGSTSLHPVSHQNEATNITETTVK
FTSTSVITSVYGNTNSSVQSQTSTVISTVFTTPANVSTPETTLKPSLSPGN
VSDLSTTSTSLATSPTKPYTSSSPILSDIKAEIKCSGIREVKLTQGICLE
QNKTS SCAEFKDRGEGLARVLCGEEQADADAGAQCVSLLLAQSEVRPQC
LLLVLANRTEISSKLQLMKKHQSDLLKLGILDFTEQDVASHQSYQKTP I
LLTCPTISILSFFSVALLVILACVLWKKRIKPIVWPSLPDHHKLTLEHLCK
KPRKNLNVSPNPESFLDCQIHRVDDIQARDEVEGFLQDTFPPQGLESEKQ
RLGGDVQSPNCPSEDVVITPESFGRDSSLTCLAGNV SACDAPILSSSRSL
DCRESGKNGPHVYQDLLSLGTTNSTLPPFSLQSGILTLNPAVAGQPIL
TSLGNSQEEAVVTMSSFYQNG

Example 4 Study of U-CAR T Cells Expressing the Enhancer Together with the Safety-Switch

4.1 Design of CAR and Package of the Virus

[0387] The structure of the chimeric antigen receptor is:

[0388] 1) RQR8-CD19CAR-mbIL15;

[0389] 2) RQR8-CD19CAR-mbIL15-IL7;

[0390] 3) RQR8-CD19CAR-C7R;

[0391] 4) RQR8-CD19CAR-mbIL7;

[0392] wherein RQR8 is a safety switch, and the CD19CAR fragment is the heavy chain and light chain variable region of monoclonal antibody FMC63 (connected by a GS linker), and also included are hinge region and transmembrane region, human CD 28 intracellular co-stimulatory element, and human CD3 ζ intracellular region in tandem; mbIL15 consists of IL15+IL15R α in tandem; C7R is a constitutively activated IL7 receptor consisting of CD34 extracellular domain and IL7 receptor transmembrane and intracellular domains; IL7 consists of IL7 signal peptide and mature peptide; mbIL7 consists of IL7+IL7Ra in tandem. Examples of the constructs described herein are shown in FIG. 29.

[0393] The amino acid sequence of RQR8 is as set forth below (SEQ ID NO.: 7):

MGTSLLCWMALCLLGADHADACPYSNPSLCSGGGGSELPTQGTFSNVSTN
VSPAKPTTTACPYSNPSLCSGGGGSPAPRPPTPAPTIASQPLSLRPEACR
PAAGGAVHTRGLDFACDIYIWAPLAGTCGVLLLSLVITLYCNHRNRRRV
KCPRPVV

[0394] The DNA sequence of RQR8 is as set forth below (SEQ ID NO.: 8):

ATGGGCACCTCTCTGCTGTGCTGGATGGCTCTGTGTCTGCTGGGCGCTGA
CCATGCTGATGCTTGCCCTATTCCAACCCCTCTCTGTGCTCCGAGGAG
GAGGATCCGAAGTCCCTACCAAGGCACCTTCAGCAACGTGTCCACCAAC
GTGAGCCCCGTAAGCTACCAACACCGCTTGCCCTTACTCCAATCCCAG
CCTCTGCTCCGAGGCGGAGGATCCCCGCCGCCAGACCTCTACACCCG
CTCCACAATCGCCAGCCAGCTCTGTCTCTGAGACCCGAAGCTTGCGAGA
CCCGTGCCGAGGAGCTGTGCATACAAGGAGCTGGATTTCGCTTGCGA
CATCTACATCTGGGCCCCCTGGCTGGCACATGTGGCGTGTCTGCTGTG
CTCTGGTCATTACTGTACTGCAACCATAGAAATAGAAGAAGGGTGTGC
AAGTGTCCAGACCCGTGGTGGGCGAGGAGAAGGAAGAGGCTCTCTGCT
GACATGCGGAGACGTGGAAGAGAACCCCGGCCCC

[0395] The amino acid sequence of CD19CAR is as set forth below (SEQ ID NO.: 9):

MLLVTSLLLCCLPHPAELLIPDIQMTQTSSLSASLGDRVTISCRASQD
ISKYLNWYQQKPDGTVKLLIYHSLRHSVPSRFSGSGSGTDSLTIENL
EQEDIATYFCQQGNTLPYTFGGGKLEITGSTSGSGKPGSGEGSTKGEVK
LQESGPGLVAPSLSVTCTVSGVSLPDYGVSWIRQPPRKGLEWLGVIWG

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SETTYNSALKSRLTIIKDNSKSQVFLKMNSLQTDITAIYYCAKHYYVGG
SYAMDYWGQGSVTVSSAAADYKDDDDKIEVMYPPPYLDNEKSNGTIIHV
KGKHLCPSPFPGPSKPFVVLVVVGGVLACYSLLVTVAFIIFWVRSKRSR
LLHSDYMNMTPRRPGPTRKHYQPYAPPRDFAAYRSRVKFSRSADAPAYQQ
GQNQLYNELNLRREYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKD
KMAEAYSEIGMKGERRRKGHDGLYQGLSTATKDTYDALHMQALPPR

[0396] The DNA sequence of CD19CAR is as set forth below (SEQ ID NO.: 10):

ATGCTTCTCCTGGTGACAAGCCTTCTGCTCTGTGAGTTACCACACCCAGC
ATTCCTCCTGATCCCAGACATCCAGATGACACAGACTACATCCTCCCTGT
CTGCTCTCTGGGAGACAGAGTCACCATCAGTTGCAGGGCAAGTCAGGAC
ATTAGTAAATATTTAAATTGGTATCAGCAGAAACAGATGGAACCTGTAA
ACTCCTGATCTACCATACTCAAGATTACACTCAGGAGTCCCATCAAGGT
TCAGTGGCAGTGGGTCTGGAACAGATTATTCTCTACCATTAGCAACCTG
GAGCAAGAAGATATTGCCACTTACTTTTGCCAACAGGGTAATACGCTTCC
GTACACGTTTCGGAGGGGGGACTAAGTTGGAATAACAGGCTCCACCTCTG
GATCCGGCAAGCCCGGATCTGGCGAGGATCCACCAAGGGCGAGGTGAAA
CTGCAGGAGTCAGGACCTGGCCTGGTGGCGCCCTCACAGAGCCTGTCCGT
CACATGCACTGTCTCAGGGGTCTCATTACCCGACTATGGTGAAGCTGGA
TTCCGCAGCCTCCACGAAAGGGTCTGGAGTGGCTGGGAGTAATATGGGGT
AGTGAAACCACATACTATAATTGAGCTCTCAAATCCAGACTGACCATCAT
CAAGGACAACTCCAAGAGCCAAGTTTCTTAAAAATGAACAGTCTGCAAA
CTGATGACACAGCCATTTACTACTGTGCCAAACATTATTACTACGGTGGT
AGCTATGCTATGGACTACTGGGGTCAAGGAACCTCAGTCACCGTCTCTCTC
AGCGGCCGAGACTACAAAGACGATGACGACAAGATTGAAGTTATGTATC
CTCTCTCTTACCTAGACAATGAGAAGAGCAATGGAACCATATCCATGTG
AAAGGGAAACACCTTTGTCCAAGTCCCTATTTCCTGGACCTTCTAAGCC
CTTTTGGGTGCTGGTGGTGGTGGGGAGTCTGGCTTGCTATAGCTTGC
TAGTAACAGTGGCCTTTATTATTTCTGGGTGAGGAGTAAGAGGAGCAGG
CTCTGACAGTGACTACATGAACATGACTCCCGCCGCCCGGGCCAC
CCGCAAGCATTACCAGCCCTATGCCCCACACGCGACTTCGACGCTATC
GCTCCAGAGTGAAGTTCAGCAGGAGCGCAGACGCCCGCGGTACCAGCAG
GGCCAGAACCAGCTCTATAACGAGCTCAATCTAGGACGAAGAGAGGAGTA
CGATGTTTTGGACAAGAGACGTGGCCGGGACCTGAGATGGGGGAAAGC
CGAGAAGGAAGAACCCTCAGGAAGGCTGTACAATGAAGTCAGAAAGAT
AAGATGGCGGAGGCCCTACAGTGAAGTGGGATGAAGGCGAGCGCCGGAG
GGGCAAGGGGACGATGGCCTTTACCAGGGTCTCAGTACAGCCACCAAGG
ACACCTACGACGCCCTTCACATGACGGCCCTGCCCTCTCGC

[0397] The amino acid sequence of mbIL15 is as set forth below (SEQ ID NO.: 11):

MDWTWILFLVAAATRVHSNWNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLELQVLSL
ESGDASIHDVTENLIILANNSSNGNVTESGCKECELEEKNIKEFLQSFVHIVQMFINTSSGGSGGG
 GSGGGSGGGSGGGSLQITCPPPMSEHADIIWKSYSLSYRERYICNSGFKRKAGTSSLTECVLNKATN
 VAHWTTPSLKCIRDPAHVHQRAPPSTVTTAGVTPQPESLSPSGKEPAASSPSSNNTAATTAAIVPGSQL
 MPKSPSTGTTEISSHESHGTPSQTTAKNWELTASASHQPPGVYPQGHSDTTVAISTSTVLLCGLSAVS
 LLACYLKSRQTPPLASVEMEAMEALPVTWGTSSRDELENCSHHL

[0398] In the amino acid sequence of mbIL15, the underlined part is the signal peptide region, the italic part is IL-15, the bold part is the linking region, and the dashed underlined part is IL-15 receptor α .

[0399] The DNA sequence of mbIL15 is as set forth below (SEQ ID NO.: 12):

ATGGATTGGACTTGGAATTTGTTCCCTCGTTGCCGAGCGACTCGCGTCCA
 TAGTAATTGGGTGAACGTAATTAGTGACTTGAAAAAATTGAGGACCTTA
 TACAAAGTATGCATATCGATGCAACACTGTACACGAGTCCGACGTGCAC
 CCAAGCTGCAAGGTACCAGCAATGAAATGCTTTTGTCTCGAATTGCAAGT
 TATCTCACTTGAGTCAGGGACGCTTCAATCCATGATACTGTGGAGAATT
 TGATAATCTCTGGCGAACAATAGCCTTAGTTCAAATGGCAACGTCACTGAG
 TCAGGCTGCAAGGAATGTGAGGAATTGGAAGAAAAAATATCAAGGAATT
 TTTGCAATCTTTGTTCACATAGTTTCAATGTTTCACTAGTTCCG
 GGGCGCGAGTGGAGGTGGCGGTAGCGCGGGGTGGCTCTGGTGGAGGC
 GGCTCTGGGGCGGAAGTCTGCAGATAACATGCCCCACCTATGAGTGT
 TGAACATGCTGATATCTGGGTTAAATCTTACTCCCTTACAGTCGAGAAA
 GGTACATTGCAACTCCGGCTTTAAACGCAAAGCCGGACTAGTTCACTG
 ACTGAATGTGTATTGAATAAAGCGACAAATGTGCACACTGGACTACCCC
 TTCCCTCAAATGCATTTCGCGATCTGCCTTGGTGATCAGCGACCCAGCAC
 CGCCGTCCACGGTAACACCGCAGGAGTAACACCGCAGCCGAGAGCCTT
 TCCCCCTCAGGCAAAGACCGGCGCATCCTCCCATCTTCCAATAATAC
 CGCAGCTACCAACCGCAGCAATCGTACCCGGTCCAGCTGATCCCAGCA
 AAAGTCCGAGTACTGGAACGACTGAAATCTCCAGTCACGAGTCTTCTCAT
 GGAACCTCCGAGTCAAACACTACAGCAAAGAATTGGGAGCTGACTGCTCCGC
 TTCACACCAGCCGCCAGGCGTTTATCCTCAGGGACACTCAGATACCACGG
 TGGCGATTAGCACAAAGCACCCTCTCTGTGTGGGTGAGTGAGTGTCAT
 CTTCTCGCCTGCTACCTTAAGTCCAGACAGACACCCCTTTGGCAAGCGT

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TGAAATGGAAGCCATGGAAGCCTTGCTGTACATGGGGGACTTCATCCC

GCGATGAAGACTTGGAGAACTGCTCACACCATCTTTGA

[0400] The amino acid sequence of IL7 is as set forth below (SEQ ID NO.: 13):

MFHVSFRYIFGLPPLILVLLPVASSDCDIEGKDGKQYESVLMVSIQQLLD
 SMKEIGSNCLNNEFNFFKRHICDANKEGMFLFRAARKLRQFLKMNSTGDF
 DLHLLKVSEGTITLLNCTGQVKGRKPAALGEAQPTKSLEENKSLKEQKKL
 NDLCFLKRLLEIKTCWNKILMGTKHE

[0401] The DNA sequence of IL7 is as set forth below (SEQ ID NO.: 14):

GAATTCGGCAGCGCGCTCAAGCAGACACTGAACTTCGATCTGCTGAAGCT
 GGCCGGAGACGTCGAGAGCAACCCGCGCCCTATGTTCCACGTGAGCTTTA
 GATACATCTTCGACTGCCCCCTCTGATTCTGGTGCTGCTGCCGTGGCC
 AGCAGCGACTGCGATATCGAGGGCAAGGACGGCAAGCAGTATGAGTCCGT
 GCTGATGGTCTCCATCGATCAGCTGCTGGACAGCATGAAGGAGATCGGCT
 CCAACTGCCTCAACAACGAGTTCAACTTTTCAAGAGGCACATCTGCGAC
 GCCAACAAGGAGGGCATGTTCTGTCTAGAGCGCTAGAAAGCTGAGGCA
 GTTCTCTGAAGATGAACAGCACCGCGACTTTGATCTGCATCTGTGAAAG
 TGAGCGAGGGCACCACCATCTGCTGAACTGCACCGCCAAGTGAAAGGA
 AGAAAGCCCGCGCTCTGGGCGAGGCTCAGCCTACCAAGTCTCTGGAAGA
 GAACAAGTCTCTGAAGGAGCAGAAGAAGCTCAACGATCTGTGCTTCCTCA
 AGAGGCTGCTGCAAGAGATCAAGACATGCTGGAACAAGATCCTCATGGGC
 ACCAAGGAACACTG

GGAAATTAGAGAGGTC AAGCTGACCCAAGGAATCTGTCTGGAGCAGAATAA
GACCAGCAGCTGCGCCGAGTTCAAGAAAGACAGAGGCGAAGGACTGGCCCA
GAGTGCTCTGCGGCGAGGAACAAGCCGATGCCGATGCGCGAGCTCAAGTG
TGCAGCCTCCTCCTCGCTCAGAGCGAGGTCAGACCCCAATGTCTGCTGCT
CGTGCTGGCCAATAGGACCGAGATCTCTCCAAACTGCAGCTGATGAAGA
AGCACCAGAGCGACCTCAAGAAGCTCGGCATCTCGACTTTACCGAGCAA
GACGTGGCCTCCCATCAATCCTATAGCCAGAAGACCCCCATTCTGCTGAC
ATGTCCCAATCAGCATCCTCAGCTTCTTCAGCGTCGCTCTGCTCGTCA
TTCTGGCTTGTGTGCTGTGGAAGAAGAGGATCAAGCCTATTGTGTGGCCC
TCTCTGCCCGACCACAAGAAGACCTCGAACACCTCTGCAAGAAACCTAG
AAAGAACCTCAACGTGAGCTTCAACCCCGAGTCTTTCTGGAGCTGCAAA
TCCATAGGGTGGATGACATCCAAGCTAGAGACGAGGTCGAGGGCTTTCTG
CAAGACACCTTCCCTCAGCAGCTGGAAGAAAGCGAGAAGCAAAGACTGGG
CGGAGATGTGCAGTCCCCTAATTGCCCTCCGAGGACGTGGTGATTACCC
CCGAGAGCTTCGGAAGAGACAGCTCTCTGACATGTCTGGCCGAAATGTG
TCCGCTTGCATGCCCCCTATTCTGAGCAGCTCCAGATCTCTGGACTGCAG
AGAGTCCGGCAAGAACGGCCCTCATGTGTACCAAGATCTGCTGTGTCTC
TGGAACCACAAACTCCACACTGCCTCCCCCTTTAGCCTCCAGTCCGGC
ATTCTGACACTGAACCCCGTGGCTCAAGGCCAACCTATCCTCACATCCCT
CGGCTCCAATCAAGAGGAAGCCTACGTGACCATGAGCTCCTTCTATCAGA
ACCAAGTGA

LDDYSFSCYSQLEVNGSQHSLTCAFEDPDVNITNLEFEICGALVEVKCLNFRKLOEIYFIETKKFLLIGK

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SNICVKVGEKSLTCKKIDLTITIVKPEAPFDLSVVYREGANDFVVTFNSTSHLQKYVKVLMHVDVAYRQEKD

ENKWTHVNLSSTKLTLLQKRLQPAAMYEEKVRSIPDHYPKGFWESEWSPSYFRTPEINNSSGEMDPILLT

ISILSFFSVALLVILACVLWKKRIKPIVWPSLPDHKKTLLEHLCKKPRKNLNVSNPESFLDCQIHRVDDI

QARDEVEGFLQDTFPOOLEESEKQRLGGDVQSPNCPSEDVVITPESFGRDSSLTCLAGNVSACDAPILSS

SRLDCRESGKNGPHVYQDLLLSLGTNTSLPPPFSLQSGILTLPVAQGQPIILTSLSGNQEEAYVTMSS

FYQNO

[0406] In the amino acid sequence of mbIL7, the underlined part is the signal peptide region, the italic part is IL-7, the bold part is the linking region and the dashed underlined part is IL-7 receptor α .

[0407] The DNA sequence of mbIL7 is as set forth below (SEQ ID NO.: 18):

ATGGCTCTGCCTGTTACAGCTCTGCTGCTGCCTCTGGCTCTGCTTCTGCA
TGCCGCCAGACCTGACTGTGACATCGAGGGCAAAGACGGCAAGCAGTACG
AGAGCGTGCTGATGGTGTCATCGACCAGCTGCTGGACAGCATGAAGGAA
ATCGGCAGCAACTGCCTGAACAACGAGTTCAACTTCTTCAAGCGGCACAT
CTGCGACGCCAACAAAGAAGGCATGTTCTGTTCTAGAGCCGCCAGAAAGC
TGCGGCAGTTCTGAAGATGAACAGCACCGCGCACTTCGACCTGCATCTG
CTGAAAGTGTCTGAGGGCACCACCATCTGCTGAATTGCACCGGCCAAGT
GAAGGGCAGAAAGCCTGCTGCTCTGGGAGAAGCCAGCCTACCAAGAGCC
TGGAAGAGACAAGTCCCTGAAAGAGCAGAAGAAGCTGAACGACCTCTGC
TTCCTGAAGCGGCTGCTGCAAGAGATCAAGACCTGCTGGAACAAGATCCT
GATGGGCACCAAGAGCACGCGGAGGATCTGGCGGAGGTGAAGCGGCG
GAGGCGGTAGCGGTGGCGGAGGAAGTGGTGGCGGATCTGAATCTGGCTAC
GCCCAGAACCGCGACCTGGAAGATGCCGAGCTGGACGACTACAGCTTCAG
CTGTACAGCCAGCTGGAAGTGAACGGCAGCCAGCACTCTGTGACCTGCG
CCTTTGAAGATCCCGACGTGAACATCACCAACCTTGAGTTCGAGATTTGT
GGCGCCCTGGTGAAGTCAAGTGCCTGAATTTCCGGAAGCTGCAAGAAAT
CTACTTTATCGAGACAAAGAAGTTCTGCTGATCGGCAAGAGCAACATCT
GTGTGAAAGTGGGCGAGAAAAGCCTGACCTGCAAGAAGATCGACCTGACC
ACCATCGTGAAGCCCGAGGCTCCTTTCGATCTGAGCGTGGTGTATAGAGA
GGGCGCCAACGACTTCGTGGTCACCTTCAACACCGACCACTCCAAAAGA
AATACGTGAAGGTGCTGATGCACGACGTGGCCTACCGGCAAGAGAAGGAC
GAGAACAAATGGACCCACGTGAACCTGAGCAGCACCAAGCTGACCTGCT
GCAGAGAAAACCTGCAGCTGCCGCTATGTACGAGATCAAAGTGGCGAGCA

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TCCCCGACCACTACTTTAAAGGCTTTGGAGCGAGTGGTCCCCTAGCTAC
TACTTCAGAACCCCTGAGATCAACAACCTCCAGCGGCGAGATGGACCCCAT
TCTGCTGACAATCAGCATCCTGAGCTTTTTCAGCGTGGCCCTGCTGGTCA
TCCTGGCCTGTGTGCTGTGGAAGAAGCGGATCAAGCCCATCGTGTGGCCC
AGCCTGCCTGACCACAAGAAAACCTGGAACACCTGTGCAAGAAGCCCCG
GAAAAACCTGAACGTGTCTTCAATCCCGAGAGCTTCTGGACTGCCAGA
TCCACAGAGTGGACGACATCCAGGCCAGGACGAAGTGAAGGATTTCTG
CAGGACACATTCCCTCAGCAGCTCGAAGAGAGCGAGAAGCAAAGACTCGG
AGGCGACGTGCAGAGCCCTAATTGCCCTTCTGAGGACGTGGTCATCACCC
CAGAGAGCTTCGGCAGAGATAGCAGCCTGACATGTCTGGCCGGCAATGTG
TCCGCTGTGATGCCCTATCCTGTCTCTAGCAGAAGCCTGGATTGCAG
AGAGAGCGGCAAGAACGGCCCTCACGTGTACCAGGATCTGCTCTGTCTC
TGGGCACCACAAACAGCACACTGCCTCCACCATTAGCCTGCAGAGCGGC
ATCCTGACACTGAACCTGTTGCTCAGGGCCAGCCTATCCTGACAAGCCT
GGGCAGCAATCAAGAAGAGGCTACGTACCATGAGCAGCTTCTACCAGA
ACCAG

[0408] The above CAR genes were cloned into the FUW lentiviral vector backbone under the promoter of EF1 α (EF-1 α) to form Fuw-EF1 α -CAR. Fuw-EF1 α -CAR, lentiviral envelope plasmid pMD2.G (Addgene, Plasmid #12259) and lentiviral packaging plasmid psPAX2 (Addgene, Plasmid #12260) were transfected into 293 T cells by using Lipofectamine3000 to prepare the whole lentiviral expression vector. The supernatant was collected at 48 and 72 h and subject to ultra-centrifuge (Merck Millipore) for concentration. The concentrated virus was ready for the transfection of T cells.

[0409] The result shows that expression of RQR8, CAR, IL15 α or C7R was successfully detected by flow cytometry for RQR8-CD19CAR-MBIL15, RQR8-CD19CAR-MBIL15-IL7, RQR8-CD19CAR-C7R cells; secretion of IL-7 was detected by ELISA. The lentiviral vectors were successfully constructed. All of the structures could be expressed simultaneously without interference with each other.

4.2 Design and Construction of CRISPR

[0410] First, gRNA sequences targeting the exon of the TCR conserved region were designed at <http://crispr.mit.edu>, and two gRNA sequences with scores above 80 were selected for higher knockout efficiency. For the gRNA primer, the forward primer comprises a T7 promotor followed by 20 bp of the target sequence, and the reverse primer has 20 bp of the complementary sequence. Plasmids were used as PCR template. After purification, T7-PCR product was further used as the template for MEGAshortscript T7 kit to obtain RNA. The RNA was purified with a MEGAclear column and eluted with RNA-free water. Cas9 plasmid was purchased from Addgene.

[0411] The gRNA targeting sequences are as below:

TRAC-gRNA:	SEQ ID NO.: 5
TGTGCTAGACATGAGGTCTA,	

[0412] Meanwhile, the knockout efficiency of the gRNA was analyzed by T7E1, TIDE and flow cytometry.

[0413] The result shows that the TCR gene was successfully knocked out with a knockout efficiency of higher than 90%.

4.3 Preparation of CAR-T Cells

[0414] Cell Isolation and Activation

[0415] After apheresis, monocytes were isolated using Histopaque-1077 (Sigma-Aldrich) by density gradient centrifugation. Then T cells were enriched, activated by magnetic beads coupled with anti-CD3/anti-CD28, cultured and expanded.

[0416] X-vivo 15 with 5% FBS, 2 mM L-glutamine, 1 mM sodium pyruvate and 300 IU/ml rhIL2 was used as CAR-T cell medium. The cells were incubated and cultured at 37° C., 5% CO₂.

[0417] Cell line expressing CD 19: Raji (Burkitt's lymphoma cell line, ATCC-CCL86); K562 cells (Human erythroleukemia cell line, ATCC-CCL243); Raji-fluc cell line was obtained by screening Raji cells transfected with lentivirus having firefly luciferase;

[0418] All of the cells were cultured in RPMI1640 medium and 293T cells (ATCC-CRL3216) were cultured in DMEM medium. Both RPMI1640 and DMEM were supplemented with 10% (v/v) fetal bovine serum and 100 U/ml penicillin and streptomycin, 2 mM glutamine and 1 mM sodium pyruvate.

[0419] Electroporation

[0420] Two days after enriching and activating the T cells, the anti-CD3/anti-CD28 magnetic beads were removed and the cells were collected into a tube. Cas9 and gRNA as needed were mixed, incubated at room temperature and then transferred to electroporation buffer to further mix with the cells for electroporation by 4D-Nucleofector System N (Lonza) system. Then the cells were re-suspended in a pre-warmed medium to a density of $1-2 \times 10^6$ /ml, transferred to a plate and cultured in an incubator at 37° C., 5% CO₂.

[0421] Lentiviral Transfection

[0422] 1 day after electroporation, the cells were transfected by lentiviral vector at MOI of 2-8, transferred to a flask and cultured at 37° C., 5% CO₂.

[0423] Cell Proliferation and Detection of CAR Positive Ratio

[0424] 3 days after the transfection, the number of cells and CAR positive cells were detected, and the positive ratio of CAR in T cells was calculated. The positive ratio of CD3 was also detected to determine the knockout efficiency. Then cells were continuously cultured in the incubator and half of the medium was replaced every 2-3 days till day 14 when the cells were ready for cryopreservation.

[0425] The result shows that that expression of RQR8, CAR, IL15R α or C7R was successfully detected by flow cytometry for RQR8-CD19CAR-mbIL15, RQR8-CD19CAR-mbIL15-IL7, RQR8-CD19CAR-C7R CAR-T; secretion of IL-7 was detected by ELISA. The CD3 negative ratio was higher than 90%, indicating that the TCR knockout CAR-T with enhanced cytokine-related signaling pathway and the safety switch was successfully constructed, and each of the elements does not interfere with each other.

4.4 Release of the Cytokines

[0426] The CAR-T cells prepared in Example 3 and CD19 positive tumor cells (Raji, NALM6), 100 μ l each, were mixed in RPMI medium at 1:1 ratio to a density of 1×10^6 /ml for each cell, and then cultured overnight in a 96-well plate. The medium was collected and subject to centrifuge and the released cytokine IFN- γ and IL-2 were detected by Cytokine bead array kit (CBA kit, BD Biosciences).

[0427] The result shows that incubation of RQR8-CD19CAR-MBIL15, RQR8-CD19CAR-MBIL15-IL7, RQR8-CD19CAR-C7R CAR-T cells with Raji, NALM6 produced a large amount of IFN- γ and IL-2, indicating function of the cytokine-related signaling pathway enhanced CART against CD19 positive target cells, and the secretion of cytokines by the cytokine-related signaling pathway enhanced CART was increased compared to conventional CAR-T.

4.5 Expression of CD107a after Co-Culture of CAR-T Cells with the Target Cells

[0428] 2×10^5 CAR-T/NT cells and 2×10^5 target cells (Raji, NALM6)/control cells (K562) were added to a v-bottom 96 well plate, and re-suspended in 200 μ l X-VIVO complete medium without IL-2. 11 BD GolgiStop(containing monensin) was added to each 1 ml medium and 2 μ l CD107a antibody (1:50) was added to each well. The cells were then cultured at 37° C. for 4 hours and further subject to collection.

[0429] The sample was centrifuged to remove the medium, washed once with PBS, and then centrifuged at 400 g, 4° C. for 5 minutes. The supernatant was removed and specific surface antibodies CAR, CD3, CD4, and CD8 were added to each tube. Then the cells were re-suspended in 100 μ l and put on ice for 30 minutes in dark.

[0430] Each tube was washed once with 3 mL PBS, and then the sample was centrifuged at 400 g for 5 minutes. The supernatant was carefully removed and the pellet was re-suspended in PBS. CAR, CD3, CD4, CD8, and CD107a were detected by flow cytometry.

[0431] The result shows that incubation of RQR8-CD19CAR-MBIL15, RQR8-CD19CAR-MBIL15-IL7, RQR8-CD19CAR-C7R CAR-T cells with Raji, NALM6 produced a large amount of CD107a, indicating function of the cytokine-related signaling pathway enhanced CART against CD19 positive target cells in vitro.

4.6 In Vitro Killing Activity

[0432] A stably transfected cell line was obtained by introducing the luciferase gene into the target cell followed by screening. The CAR-T cells prepared in Example 3 were mixed with CD19-positive tumor cells (Raji-luc, NALM6-luc) at different effector: target ratios. After adding fluorescein, luciferase could react with the fluorescein to produce fluorescence. By detecting the intensity of the fluorescence, the activity of luciferase can be measured, and the survival of the cells can be detected so that to evaluate the killing effect of CAR-T cells.

[0433] The result shows that RQR8-CD19CAR-MBIL15, RQR8-CD19CAR-MBIL15-IL7, RQR8-CD19CAR-C7R CAR-T cells significantly killed Raji and NALM6, and the cytokine-related signaling pathway enhanced CART showed a more significant killing effect, which is correlated with the effector: target ratio.

4.7 Proliferation of CAR-T Cells

[0434] IL-2 was removed from CAR-T. CAR-T cells were continuously cultured for 2 weeks and counted twice a week to measure the proliferation.

[0435] The result shows that RQR8-CD19CAR-MBIL15, RQR8-CD19CAR-MBIL15-IL7, RQR8-CD19CAR-C7R CAR-T cells had stronger proliferation rate than CD19 CAR-T. Without IL-2, they still had high survival ratio after 2 weeks. CFSE showed certain proliferation and CD19 CAR-T could not survive after 2 weeks.

4.8 Re-Activation of Cells In Vitro

[0436] IL-2 was removed from CAR-T. K562-CD19 was added twice a week at 2:1 effector: target ratio. The cells were cultured for 2 weeks and counted twice a week to measure cell proliferation

[0437] The result shows that after adding K562-CD19, RQR8-CD19CAR-MBIL15, RQR8-CD19CAR-MBIL15-IL7, RQR8-CD19CAR-C7R CAR-T cells showed significantly stronger proliferation rate than CD19 CAR-T. After the stimulation, the cell survival ratio was higher than CD19 CAR-T, indicating the cytokine-related signaling pathway enhanced CART has a higher proliferation rate, and can survive better.

4.9 In Vivo Efficacy

[0438] 6-12 weeks NOD-Prkdcscid Il2rnull (NOG) mice were selected and intraperitoneally injected with 2×10^5 Raji-fluc cells, 50 μ L DPBS and 50 μ L matrigel matrix (Corning). Two days later, the tumor graft burden of the mice was measured, and the mice were divided into 4 groups based on the tumor burden. One day later, 200 μ L DPBS, 5×10^6 NT cells, 5×10^6 CD19 CAR-T cells, RQR8-CD19CAR-MBIL15, RQR8-CD19CAR-MBIL15-IL7, RQR8-CD19CAR-C7R CAR-T cells were respectively injected to each mouse. The tumor burden of the mice was further evaluated 7 days after the CAR-T treatment. Each mouse was injected intraperitoneally with 3 mg d-luciferin for a 4-minutes reaction, and then photographed by using a Xenogen IVIS Imaging System with 30 s exposure.

[0439] The result shows that after two weeks, RQR8-CD19CAR-MBIL15, RQR8-CD19CAR-MBIL15-IL7 and RQR8-CD19CAR-C7R CAR-T cells significantly decreased the tumor burden compared to NT and CD19 CAR-T cells,

and T cells could still be detected in peripheral blood. Mice in the cytokine-related signaling pathway enhanced CART groups overall had longer survival.

4.10 In Vivo GVHD Study

[0440] The mice were systemically treated with a sub-lethal dose of irradiation (175 cGy) first. Then CAR-T cells were re-suspended in PBS and injected into the thorax of the treated mice. The mice were observed 2-3 times a week by using GVHD clinical criteria including weight loss, arch-back, activity, fur texture, and skin integrity.

[0441] The result shows that mice in the group without TCR knockout all showed symptoms of GVHD reaction, and no GVHD reaction was detected in TCR single knockout CAR-T cell groups.

4.11 Clearance of 11 RQR8 Gene-Mediated CAR-T Cells

[0442] NK cells were isolated from PBMCs as effector cells, and 1:1 co-cultured with CAR-T, with or without rituximab (Rutiximab) at a final concentration of 10 g/mL. 4 h and 24 h later, the expression CAR was detected by flow cytometry.

$$\text{ADCC percentage} = (1 - \frac{\text{monoclonal antibody group CAR positive ratio}}{\text{no antibody group CAR positive ratio}}) * 100\%$$

[0443] The results show that, 4 h and 24 h later, NK cells significantly cleared RQR8-CD19CAR-MBIL15, RQR8-CD19CAR-MBIL15-IL7 and RQR8-CD19CAR-C7R CAR-T cells in the presence of Rutiximab, but failed to clear CD19 CAR-T. Without Rutiximab, NK failed to kill any of the cells. This indicates that Rutiximab can function as a safety switch, which binds to RQR8, and allows NK to mediate the ADCC function so that to clear CART cells.

Example 5 Study of U-CAR T Cells Expressing E3

[0444] FIG. 30 shows an example design of CAR-T cells expressing of enhancer E3. In E3, the ecto domain of C7R is replaced by a safety switch, such as EGFRt or Her2t or other peptides described in the present disclosure.

[0445] FIG. 31 shows flow cytometry data of fractions of CAR-T cells expressing CD19 CAR and the enhancer C7R or E3.

[0446] FIGS. 32A and 32B show in vitro killing activity of CAR-T cells toward HeLa cells expressing a CD19 antigen. FIG. 32A shows in vitro killing activity of CD19 CAR-T cells expressing C7R or E3 in comparison with target only or T cell controls. The effector: target ratio in this experiment is 5:1. FIG. 32B shows in vitro killing activity of CD19 CAR-T cells expressing C7R or E3 in comparison with target only or T cell controls. The effector: target ratio in this experiment is 1:1.

[0447] FIGS. 33A and 33B show expression of phosphorylated STAT5 (pSTAT5) and CD19 CAR in the tested engineered cells. FIG. 33A shows flow cytometry data of fractions of CAR-T cells expressing CD19 CAR and pSTAT5. FIG. 33B shows corresponding mean fluorescent index (MFI) of pSTAT5 of the data in FIG. 33A.

[0448] FIGS. 34A-C show proliferation data of CD19 CAR-T cells expressing C7R or E3 in comparison to CD19 CAR-T without enhancer. FIG. 34A shows cytokine independent proliferation of the three different cells. FIG. 34B shows proliferation under repeated NALM6 stimulations.

FIG. 34C shows percentage of CAR-T cells before and after the stimulation by NALM6. CAR stimulation enriched CAR+ cells.

[0449] FIG. 35A shows flow cytometry data of fractions of CAR-T cells co-cultured with NK cells at different effector: target ratios in the presence or absence of Cetuximab (CTX, anti-EGFR Antibody). The data showed that in the presence of CTX, the CD19 CAR-T expressing E3 can be effectively killed by NK cells via NK Cell-Mediated Antibody-Dependent Cellular Cytotoxicity (ADCC), indicating that E3 functions as an effective safety switch and has been effectively turned off. FIG. 35B shows the total killing activity of NK cells toward other cells. FIG. 35C shows the killing activity of NK cells toward CAR-T cells.

[0450] FIGS. 36A and 36B show test of safety switch by ADCC after repeated stimulation. FIG. 36A shows flow cytometry data of fractions of CAR-T cells co-cultured with NK cells at different effector: target ratios in the presence or absence of CTX. The cells shown in this example were repeated stimulated. FIG. 36B shows killing activity of NK cells toward CAR-T cells.

[0451] FIG. 56A shows flow cytometry data of fractions of TCR KO CD7 CAR-T cells expressing CD7 CAR and enhancer C7R or E3 (EGFRt+IL7R α) at day 12 of cell culture. FIG. 56B shows 6-hour cytotoxicity of TCR KO CD7 CAR-T cells expressing CD7 CAR and enhancer C7R or E3 (EGFRt+IL7R α) toward CCRF-CEM cells. The effector: target ratios tested in this experiment are 5:1 and 1:1. FIG. 56C shows proliferation of TCR KO CD7 CART cells expressing C7R or E3 (EGFRt+IL7R α). The CAR-T cells were cultured in T cell culture media without supplement of IL2 and monitored for fold of cell expansion. The results show that TCR KO CD7 CART cells expressing C7R or E3 displayed better cell expansion and cell stability than control T cells. E3 (EGFRt+IL7R α) has better enhancer expression and thus maintained better cell persistence and expansion than C7R. FIG. 56D shows flow cytometry data of fractions of TCR KO CD7 CAR-T cells expressing phosphorylated STAT5 (pSTAT5) and C7R or E3. pSTAT5 was highly upregulated in TCR KO CD7 CAR-T cells expressing C7R or E3. CAR19 plus IL2 stimulation were used as positive control for pSTAT5. FIG. 56E shows corresponding mean fluorescent index (MFI) of pSTAT5 of the data in FIG. 56D.

[0452] Sequence of construct design: The etcdomain of C7R can be replaced by a safety switch, such as truncated EGFR or truncated Her2.

[0453] CD7CAR-E3 amino acid sequence (E3=EGFRt+ constitutively active IL7R α TM and endo domain)

(SEQ ID NO: 100)
MALPVTALLPLALLLHAARPDIELTQSPAIMSASLGEEITLTCSASSV
SYMHWYQQKSGTSPKLLIYSTNLASGVPSRFSGSGSTFYSLTISSVEA
EDAADYYCHQWSSYTFGGGKLEIKRGGGSGGGGSGGGGQVQLQESGG
GLVKPGGSLKLSCAASGFTFSSYAMSWVRQTPEKRLWVATISSGGSYTY
YPDSVKGRFTISRDNKNTLYLQMSLSRSEDAMYYCARQDGYYPGWAFN
WGQGTTVTVSSSGTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHT
RGLDFACDIYIWAPLAGTCGVLLSLVITLYCKRGRKKLLYIFKQPFMRP
VQTTQEEDGCSCRFPEEEEGGCELRVKFSRSADAPAYKQGQNQLYNELNL

-continued

GRREEDVLDKRRGRDPEMGGKPRKPNQEGLYNELQDKMAEAYSEIGM
KGERRRGKGGHDGLYQGLSTATKDTYDALHMQALPPRGSGATNFSLLKQAG
DVEENPGPMLLLVTSLLLCPLPAPAFLLIPKVCNGIGIGEFKDSLSINA
TNIKHFKNCTSIGDLHILPVAFRGDSFTHTPPLDPQELDILTVKEITGF
LLIQAWPENRTDLHAFENLEIIRGRKQHQGQFSLAVVSLNITSLGLRSLK
EISDGDVVISGNKNLCYANTINWKKLFGTSGQKTKIISNRGENSCKATGQ
VCHALCSPEGCGPEPRDCVSCRNVSRGRCVCDKCNLLEGEPEFVENSE
CIQCHPECLPQAMNITCTGRGPDNCIQCAHYIDGPHCVKCTPAGVMGENN
TLVWKYADAGHVCHLCHPNCTYGTGPGLEGCPNPKIPSPILLTCTPTI
SILSFFSVALLVILACVLWKKRIKPIVWPSLPDHKKTLLEHLCKKPRKNLN
VSFNPESFLDCQIHRVDDIQRARDEVEGFLQDTFPQGLEESEKQRLGGDVQ
SPNCPSEDEVITPESFGRDSSLTCLAGNVSAADAPILSSSRSLDCRESGK
NGPHVYQDLLLLSLGTNTSLPPPFSLQSGILTLNLPVAGQGPILTSLSGNSQ
EEAYVTMSFQYQ

[0454] CD7CAR-E3 (E3=Her2t+constitutively active IL7R α TM and endo domain)

(SEQ ID NO: 101)
MALPVTALLPLALLLHAARPDIELTQSPAIMSASLGEEITLTCSASSV
SYMHWYQQKSGTSPKLLIYSTNLASGVPSRFSGSGSTFYSLTISSVEA
EDAADYYCHQWSSYTFGGGKLEIKRGGGSGGGGSGGGGQVQLQESGG
GLVKPGGSLKLSCAASGFTFSSYAMSWVRQTPEKRLWVATISSGGSYTY
YPDSVKGRFTISRDNKNTLYLQMSLSRSEDAMYYCARQDGYYPGWAFN
WGQGTTVTVSSSGTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHT
RGLDFACDIYIWAPLAGTCGVLLSLVITLYCKRGRKKLLYIFKQPFMRP
VQTTQEEDGCSCRFPEEEEGGCELRVKFSRSADAPAYKQGQNQLYNELNL
GRREEDVLDKRRGRDPEMGGKPRKPNQEGLYNELQDKMAEAYSEIGM
KGERRRGKGGHDGLYQGLSTATKDTYDALHMQALPPRGSGATNFSLLKQAG
DVEENPGPMLLLVTSLLLCPLPAPAFLLIPCHPECPQNGSVTCFGPEAD
QCVAHAHYKDPFVCVARCPGSKVCPDLSPYIWKFPDEEGACQPCPINCTH
SCVDLDDKGCPEQRASPLTGGGSGGGSPILLTCTPTISILSFFSVALLVI
LACVLWKKRIKPIVWPSLPDHKKTLLEHLCKKPRKNLNVSNFNPESFLDCQI
HRVDDIQRARDEVEGFLQDTFPQGLEESEKQRLGGDVQSPNCPSEDEVITP
ESFGRDSSLTCLAGNVSAADAPILSSSRSLDCRESGKNGPHVYQDLLLLSL
GTNTSLPPPFSLQSGILTLNLPVAGQGPILTSLSGNSQEEAYVTMSFQYQ

Q

[0455] FIG. 68 shows BLI imaging of the in vivo efficacy of CAR7 and CAR7-E3 UCAR-T in CCRF-CEM (T-ALL) murine xenograft model. Each mouse was inoculated intravenously with 3×10^5 CCRF-CEM cells. Four days later, CAR-T cells were intravenously infused (1×10^6 cells per mouse). BLI imaging indicates CAR7-E3 UCAR-T cells had better effect on tumor clearance than CAR7 UCAR-T cells.

Example 6 Study of U-CAR T Cells Expressing Dual CAR

General Materials and Methods

[0456] Isolation of Peripheral Blood Mononuclear Cells (PBMCs) from Donor Blood and Expansion of T Cells

[0457] Peripheral blood mononuclear cells (PBMCs) were isolated from donor blood by using Histopaque-1077 (Sigma-Aldrich) through density gradient centrifuge. Then T cells were enriched, activated by magnetic beads coupled with anti-CD3/anti-CD28, cultured and expanded.

[0458] Cell Lines and Culture of PBMCs

[0459] Raji cells (Burkitt's lymphoma cells, ATCC-CCL86);

[0460] K562 cells (Human erythroleukemia cell line, ATCC-CCL243);

[0461] Raji-*fluc* cell line (obtained by screening Raji cells transfected with lentivirus having firefly luciferase);

[0462] 293T cells (ATCC-CRL3216);

[0463] CCRF-CEM cells (ATCC-CCL119);

[0464] PBNK: peripheral blood NK sorted from PBMC by using CD56 microbeads and cultured in NK serum free medium kit II (Cyagen Biosciences)+10% FBS+900 IU/ml IL2 (PeproTech);

[0465] NK92 cells (ATCC-CRL2407);

[0466] NK92-*fluc* cells (obtained by screening NK92 cells transfected with lentivirus having firefly luciferase);

[0467] Raji cells, Raji-*fluc* cell line, and K562 cells were cultured in RPMI1640 medium, and 293T cells were cultured in DMEM medium. Both RPMI1640 and DMEM were supplemented with 10% (v/v) fetal bovine serum and 100 U/ml penicillin and streptomycin, 2 mM glutamine and 1 mM sodium pyruvate. All of the cells were cultured in an incubator at 37° C., 5% CO₂.

[0468] NK92 cells were cultured in RPMI1640 medium supplemented with 10% (v/v) fetal bovine serum, 2 mM glutamine, 1 mM sodium pyruvate, 1% NEAA, 0.1 mM mercaptoethanol and 200 IU/ml rhIL2.

[0469] T cells and the obtained CAR-T cells were cultured in X-vivo15 medium (containing 5% FBS, 2 mM L-glutamine, 1 mM sodium pyruvate and 300 IU/ml rhIL2). The culture medium for CAR-T cells was further supplemented with rhIL-2 (ThermoFisher Scientific) at a final concentration of 300 IU/ml every two days. All of the cells were cultured in an incubator at 37° C., 5% CO₂.

6.1 Design and Construction of CRISPR

[0470] CD7, CD2, TCR gRNAs for gene editing were selected after evaluating gene editing efficiency and off-target risk at website <http://crispr.mit.edu>, www.idtdna.com and <https://www.synthego.com>. gRNAs used in the present disclosure were either synthesized by Integrated DNA Tech-

nologies, Inc (IDT) or prepared by in vitro transcription (See Shi et al., 2017, J Vis Exp. doi: 10.3791/55267). HiFi-Cas9 was purchased from IDT.

[0471] For gene editing, 3 µg Cas9 protein and 1.5 µg gRNA were mixed in 20 µl and incubated at 37° C. or room temperature for 15 minutes to form ribonucleoprotein (RNP). Then RNP was transfected into T cells by Lonza 4D Nucleofector. Gene knockout efficiency was determined by the ratio of the cells expressing the protein as detected by flow cytometry.

[0472] gRNA targeting sequences used are as below:

TRAC-gRNA1:	TTCGGAACCCAATCACTGAC,	SEQ ID NO: 20
TRAC-gRNA2:	TCAGGGTTCTGGATATCTGT,	SEQ ID NO: 21
TRAC-gRNA3:	AAGTTCCTGTGATGTCAAGC,	SEQ ID NO: 22
CD7-gRNA1:	GAGGTCAATGTCTACGGCTC,	SEQ ID NO: 25
CD7-gRNA2:	ATCACGGAGGTCAATGTCTA,	SEQ ID NO: 26
CD7-gRNA3:	GTAGACATTGACCTCCGTGA,	SEQ ID NO: 27
CD7-gRNA4:	GGAGCAGGTGATGTTGACGG,	SEQ ID NO: 28
CD2-gRNA1:	CAAAGAGATTACGAATGCCT,	SEQ ID NO: 29
CD2-gRNA2:	GTGCCACAAAGACCATCAAG,	SEQ ID NO: 30
CD2-gRNA3:	AGAGGGTCATCACACACAAG,	SEQ ID NO: 31
CD2-gRNA4:	CTGTAGATATCTGATCAT,	SEQ ID NO: 32

[0473] After screening, gRNA with high knockout efficiency was found for each gene and high efficiency was also observed when knocking out two genes, TCR/CD7 or TCR/CD2, wherein TRAC-gRNA2 and CD7-gRNA1 are gRNAs with high knockout efficiencies. FIG. 16 shows efficiencies of CD7 knockout by using different CD7-gRNAs with TRAC-gRNA1, and CD7-gRNA1 and CD7-gRNA4 were obviously better, and CD7-gRNA1 was used in subsequent experiments.

6.2 Design of CD7 CAR and Package of the Virus

[0474] The scFv of the CD7 single CAR is derived from TH69, 3A1e and SDZCHH380 antibodies. The scFv in the CAR is designed as 3A1e-LH, TH69-LH, SDZ-LH, SDZ-HL. The amino acid sequence of the CAR is as set forth in SEQ ID NO: 75-78.

3A1e-LH

MALPVTALLLLPLALLHAARPDIELTQSPAIMSASLGEEITLTCSASSSVSYMHW (SEQ ID NO 75)

YQQKSGTSPKLLIYSTNLASGVPSRFGSGSGTFYSLTISSVEADAADYCHQWSSYT

FGGGTKLEIKRGGGSGGGGSGGGGSGVQLQESGGGLVKPGGSLKLSAASGFTFSSY

AMSWVRQTPEKRLIEWATISSGGSYTYYPDSVKGRFTISRDNKNTLYLQMSSLRSED

- continued

TAMYYCARQDGYYPGWFWANWQGTTVTVSSSGTTTPAPRPPTPAPTIASQPLSLRPEAC
RPAAGGAVHTRGLDFACDIYIWAPLAGTCGVLLLSLVIITLYCKRGRKKLLYIFKQPFMR
PVQTTQEEDGCS CRFPEEEEGGCELRVKFSRSADAPAYKQGQNQLYNELNLGRREEYD
VLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKHDGL
YQGLSTATKDTYDALHMQUALPPR*

TH69-LH

(SEQ ID NO 76)

MALPVTALLPLALLHAARPAAYKDIQMTQTSSLSASLGDRVTISCSASQGIS
NYLNWYQQKPDGTVKLLIYYTSSLHSGVPSRFSGSGSDYSLTISNLEPEDIATYYCQQ
YSKLPYTFGGGKLEIKRGGGSGGGSGGGSEVQLVESGGGLVKPGGSLKLSCAAS
GLTFSSYAMSWVRQTPEKRLEWVASISSGGFTYYPDSVKGRFTISRDNARNILYLQMS
LRSEDTAMYYCARDEVRYLDVWGAGTTVTVSSSGTTTPAPRPPTPAPTIASQPLSLRP
EACRPAAGGAVHTRGLDFACDIYIWAPLAGTCGVLLLSLVIITLYCKRGRKKLLYIFKQ
FMRPVQTTQEEDGCS CRFPEEEEGGCELRVKFSRSADAPAYKQGQNQLYNELNLGRRE
EYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKH
DGLYQGLSTATKDTYDALHMQUALPPR*

SDZ-LH

(SEQ ID NO 77)

MALPVTALLPLALLHAARPDQMTQSPASLSASVGETVTITCRASGNIHNYL
AWYQQKQKSPQLLVYNAKTLADGVPSRFSGSGGTQYSLKINSLOPEDFGSYQCQHF
WTPPWTFGGGKLEIKGGGSGGGSGGGGSIQLVQSGPELKKPGETVKISCKASGY
TFTNYGMNVWKQAPGKGLKWMGWINTYNGEPTYADDFKGRFDFSLETSASTAYLQIN
NLKNEDTATYFCARRGYYGSRYGAMDYWGQGTSTVTVSSSGTTTPAPRPPTPAPTIAS
QPLSLRPEACRPAAGGAVHTRGLDFACDIYIWAPLAGTCGVLLLSLVIITLYCKRGRKKL
LYIFKQPFMRPVQTTQEEDGCS CRFPEEEEGGCELRVKFSRSADAPAYKQGQNQLYNEL
NLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERR
RGKHDGLYQGLSTATKDTYDALHMQUALPPR*

SDZ-HL

(SEQ ID NO 78)

MALPVTALLPLALLHAARPQIQLVQSGPELKKPGETVKISCKASGYTFTNYG
MNVWKQAPGKGLKWMGWINTYNGEPTYADDFKGRFDFSLETSASTAYLQINNLKNED
TATYFCARRGYYGSRYGAMDYWGQGTSTVTVSSGGGSGGGGSGGGSDIQMTQSP
ASLSASVGETVTITCRASGNIHNYLAWYQQKQKSPQLLVYNAKTLADGVPSRFSGSGS
GTQYSLKINSLOPEDFGSYQCQHFWTTPPWTFGGGKLEIKSGTTTPAPRPPTPAPTIASQ
PLSLRPEACRPAAGGAVHTRGLDFACDIYIWAPLAGTCGVLLLSLVIITLYCKRGRKKLL
YIFKQPFMRPVQTTQEEDGCS CRFPEEEEGGCELRVKFSRSADAPAYKQGQNQLYNELN
LGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERR
GKHDGLYQGLSTATKDTYDALHMQUALPPR*

[0475] The CAR gene was cloned into the FUW lentiviral vector backbone under the promoter of EF1 α (EF-1 α) to form Fuw-EF1 α -CAR. Fuw-EF1 α -CAR, lentiviral envelope plasmid pMD2.G (Addgene, Plasmid #12259) and lentiviral packaging plasmid psPAX2 (Addgene, Plasmid #12260) were transfected into 293 T cells by using Lipofectamine3000 or PEI to prepare the whole expression vector. The supernatant was collected at 48 and 72 h and subject to ultra-centrifuge (Merck Millipore) for concentration. The concentrated virus was ready for the transfection of T cells.

6.3 Preparation of CAR-T Cells

[0476] Cell Isolation and Activation

[0477] After apheresis, monocytes were isolated using Histopaque-1077 (Sigma-Aldrich) by density gradient centrifugation. Then T cells were enriched, activated by magnetic beads coupled with anti-CD3/anti-CD28, cultured and expanded.

[0478] X-vivo 15 with 5% FBS, 2 mM L-glutamine, 1 mM sodium pyruvate and 300 IU/ml rhIL2 was used as CAR-T cell medium. The cells were incubated and cultured at 37° C., 5% CO₂.

[0479] Cell line expressing CD 19: Raji (Burkitt's lymphoma cell line, ATCC-CCL86); K562 (ATCC-CCL243); Raji-fluc and NK92 cell lines was obtained by screening cells transfected with lentivirus having firefly luciferase;

[0480] Cell line expressing CD7 and CD2: Jurkat cell, CCRF-CEM and NK92 cells.

[0481] All of the cells were cultured in RPMI1640 medium and 293T cells (ATCC-CRL3216) were cultured in DMEM medium. Both RPMI1640 and DMEM were supplemented with 10% (v/v) fetal bovine serum and 100 U/ml penicillin and streptomycin, 2 mM glutamine and 1 mM sodium pyruvate.

[0482] Electroporation and Lentivirus Infection

[0483] Two days after enriching and activating the T cells, the anti-CD3/anti-CD28 magnetic beads were removed and the cells were collected into a tube and subject to centrifuge

at 300 g for 5 min, and then washed twice with DPBS and re-suspended in opti-mem at a cell density of 50×10⁶/ml. The amount of cas9/gRNA RNP required was calculated based on the density of the cells, and then mixed with cells and transferred for electroporation by 4D-Nucleofector System N (Lonza) system. Then the cells were re-suspended in a pre-warmed medium to a density of 1-2×10⁶/ml. The cells were further subject to lentiviral transfection at MOI of 2-8, transferred to a flask and cultured in an incubator at 37° C., 5% CO₂.

[0484] Cell Proliferation and Detection of CAR Positive Ratio

[0485] 3 days after the transfection, the number of cells and CAR positive cells were detected, and the positive ratio of CAR in T cells was calculated. The positive ratio of TCR (or CD3), and CD7 was also detected to determine the knockout efficiency. Then the cells were continuously cultured in the incubator and half of the medium was replaced every 2-3 days till day 10 when the cells were ready for cryopreservation.

6.4 Screening of CD7 CAR Structure

[0486] As shown in FIG. 38A, the expression of anti-CD7 CAR was successfully detected, among which TH69-LH shows the strongest expression, followed by 3Ale-LH, then SDZ-LH, and SDZ-HL. In terms of function, as shown in FIG. 38B, 3Ale-LH, CARs all showed the best killing effects on CD7-positive T cells, while TH69-LH, SDZ-LH and SDZ-HL CARs were less effective. Therefore, the scFv of 3Ale was selected for CD7 CAR in the dual CAR.

6.5 Design of CD19-CD7 Dual CAR Structure

[0487] The structure of CD19-CD7 dual CAR targeting CD19 and CD7 is as illustrated in FIG. 37 and below:

[0488] Loop: L719-LHLH and L719-HLHL, sequence as set forth in SEQ ID NO 79 and 80;

L719-LHLH

(SEQ ID NO. 79)

MALPVTALLPLALLLHAARPDIQMTQTSSLSASLGDRVTISCRASQDISKYLN

WYQQKPDGTVKLLIYHTSRHSGVPSRFSGSGSGTDYSLTISNLEQEDIATYFCQQGNTL

PYTFGGGKLEITGGGGSQVKLQESGGGLVKPGGSLKLSCAASGETFSSYAMSWVRQT

PEKRLEWVATISSGGSYTYPPDSVKGRFTISRDNKNTLYLQMSSLRSEDATAMYCAR

QDGYYPGWANWGQTTVTVSSGSTSGSGKPGSGEGSTKGDIELTQSPAIMSASLGEEI

TLTCSASSSVSYMHYQQKSGTSPKLLIYSTSNLASGVPSRFSGSGSGTFYSLTISSEAE

DAADYYCHQWSSYTFGGGKLEIKRGGGSEVKLQESGPGLVAPSQSLSVTCTVSGVS

LPDYGVSIRQPPRKLEWLGVIWGSETTYNSALKSRLTIKDNSKSQVFLKMNSLQT

DDTAIYYCAKHYGGSYAMDYWGQTSVTVSSSGTTTPAPRPPTPAPTASQPLSLRP

EACRPAAGGAVHTRGLDFACDIYIAPLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPF

FMRPVQTTQEEGDCSCRFPEEEEGGCELRVKFSRSADAPAYKQGQNQLYNELNLGRRE

EYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGH

DGLYQGLSTATKDTYDALHMQALPPR*

- continued

L719-HLHL

(SEQ ID NO. 80)

MALPVTALLLPLALLHAARPEVKLQESGPGLVAPSQSLSVTCTVSGVSLPDYG
 VSWIRQPPRKGLEWLGVIWGSETTYNSALKSRLTIKDNSKSQVFLKMNSLQTDDBAI
 YYCAKHHYYGGSYAMDYWGQGTSTVSSGGGSDIELTQSPAIMSASLGEEITLTCSA
 SSSVSyme1WYQKSGTSPKLLIYSTSNLASGVPSRFGSGSGTFYSLTISSEAEADAADY
 YCHQWSSYTFGGGTKEIKRGSTSGSGKPGSGEGSTKGQVKLQESGGGLVKPGSLKL
 SCAASGFTFSSYAMSWVRQTPEKRLEWVATISSGGSYTYYPDSVKGRFTISRDNKNTL
 YLQMSLSRSEDAMYYCARQDGYYPGWANWGQGTTVTVSSGGGSDIQMTQTTSSL
 SASLGDRVTISCRASQDISKYNWYQKPDGTVKLLIYHTSRLHSGVPSRFGSGSGTDY
 SLTISNLEQEDIATYFCQQGNTLPYTFGGGTKEITSGTTTPAPRPPTPAPTIASQPLSLRPE
 ACRPAAGGAVHTRGLDFACDIYIWAPLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPF
 MRPVQTTQEEDGCSRCFPPEEEGGCELVRKFSRSADAPAYKQGQNLYNELNLGRREE
 YDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQDKMAEAYSEIGMKGERRRGKGHD
 GLYQGLSTATKDTYDALHMQALPPR*

[0489] Tandem: T197-LHLH, T197-LHLH-EAAAK3 (by using a linker with three connected EAAAK (SEQ ID NO: 102)), T719-LHLH, T719-LHHL, sequence as set forth in SEQ ID NO 81-84;

T197-LHLH

(SEQ ID NO. 81)

MALPVTALLLPLALLHAARPDQMTQTTSSLSASLGDRVTISCRASQDISKYN
 WYQKPDGTVKLLIYHTSRLHSGVPSRFGSGSGTDYSLTISNLEQEDIATYFCQQGNTL
 PYTFGGGTKEITGGGSGGGGSGGGSEVKLQESGPGLVAPSQSLSVTCTVSGVSLPD
 YGVSWIRQPPRKGLEWLGVIWGSETTYNSALKSRLTIKDNSKSQVFLKMNSLQTDDBAI
 AIYYCAKHHYYGGSYAMDYWGQGTSTVSSGSGTSGSGKPGSGEGSTKGDIELTQSPAT
 MSASLGEEITLTCSASSSVSYMHYQKSGTSPKLLIYSTSNLASGVPSRFGSGSGTFY
 SLTISSEAEADAADYCHQWSSYTFGGGTKEIKRGGGSGGGGSGGGGQVKLQESG
 GGLVKPGSLKLSCAASGFTFSSYAMSWVRQTPEKRLEWVATISSGGSYTYYPDSVKG
 RFTISRDNKNTLYLQMSLSRSEDAMYYCARQDGYYPGWANWGQGTTVTVSSSGT
 TTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYIWAPLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPVQTTQEEDGCSRCFPPEEEGGCELVRKFSRSADA
 PAYKQGQNLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQDK
 MAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR*

T197-LHLH- (EAAAK) 3

(SEQ ID NO. 82)

MALPVTALLLPLALLHAARPDQMTQTTSSLSASLGDRVTISCRASQDISKYN
 WYQKPDGTVKLLIYHTSRLHSGVPSRFGSGSGTDYSLTISNLEQEDIATYFCQQGNTL
 PYTFGGGTKEITGGGSGGGGSGGGSEVKLQESGPGLVAPSQSLSVTCTVSGVSLPD
 YGVSWIRQPPRKGLEWLGVIWGSETTYNSALKSRLTIKDNSKSQVFLKMNSLQTDDBAI
 AIYYCAKHHYYGGSYAMDYWGQGTSTVTSSEAAAKEAAKEAAKDIELTQSPAIMS
 ASLGEEITLTCSASSSVSYMHYQKSGTSPKLLIYSTSNLASGVPSRFGSGSGTFYSLT

- continued

ISSVEAEDAADYYCHQWSSYTFGGGKLEIKRGGGSGGGGSGGGGSGVQLQESGGGL
VKPGGSLKLSCAASGFTFSSYAMSWVRQTPEKRLEWVATISSGGSYTYYPDSVKGRFTI
SRDNAKNTLYLQMSSLRSEDAMYYCARQDGYYPGWANWGQGTTVTVSSSGTTTPA
PRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYIWAPLAGTCGVLLLSLVIT
LYCKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPPEEEGGCELRVKFSRSADAPAYK
QGQNLQYNELNLGRREEYDVLDKRRGRDPEMGKPRRKNPQEGLYNELQKDKMAEA
YSEIGMKGERRRGKHDGLYQGLSTATKDTYDALHMQALPPR*

T719-LHLH

(SEQ ID NO. 83)

MALPVTALLPLALLHAARPDIELTQSPAIMSASLGEEITLTCSASSSVSYMHW
YQQSGTSPKLLIYSTSNLASGVPSRFGSGSGTFYSLTISSVEAEDAADYYCHQWSSYT
FGGGTKLEIKRGGGSGGGGSGGGGSGVQLQESGGGLVKPGGSLKLSCAASGFTFSSY
AMSWVRQTPEKRLEWVATISSGGSYTYYPDSVKGRFTISRDNKNTLYLQMSSLRSED
TAMYYCARQDGYYPGWANWGQGTTVTVSSGSTSGSGKPGSGEGSTKGD IQMTQTTS
SLSASLGDRVTISCRASQDISKYLWYQQKPDGTVKLLIYHTSRLHSGVPSRFGSGSGT
DYSLTISNLEQEDIATYFCQQNTLPYTEGGGKLEITGGGSGGGGSGGGSEVKLQE
SGPGLVAPSQSLSVTCTVSGVSLPDYGVSWIRQPPRKGLEWLGVIWGSETTYNSALKS
RLTI IKDNSKSVFLKMNSLQTD DTAIYYCAKHYYYGGSYAMDYWGQTSVTVSSSGT
TTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYIWAPLAGTCGVLLL
SLVITLYCKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPPEEEGGCELRVKFSRSADA
PAYKQGQNLQYNELNLGRREEYDVLDKRRGRDPEMGKPRRKNPQEGLYNELQKDK
MAEAYSEIGMKGERRRGKHDGLYQGLSTATKDTYDALHMQALPPR*

T719-LHHL

(SEQ ID NO. 84)

MALPVTALLPLALLHAARPDIELTQSPAIMSASLGEEITLTCSASSSVSYMHW
YQQSGTSPKLLIYSTSNLASGVPSRFGSGSGTFYSLTISSVEAEDAADYYCHQWSSYT
FGGGTKLEIKRGGGSGGGGSGGGGSGVQLQESGGGLVKPGGSLKLSCAASGFTFSSY
AMSWVRQTPEKRLEWVATISSGGSYTYYPDSVKGRFTISRDNKNTLYLQMSSLRSED
TAMYYCARQDGYYPGWANWGQGTTVTVSSGSTSGSGKPGSGEGSTKGEVKLQESGP
GLVAPSQSLSVTCTVSGVSLPDYGVSWIRQPPRKGLEWLGVIWGSETTYNSALKSRLT
I IKDNSKSVFLKMNSLQTD DTAIYYCAKHYYYGGSYAMDYWGQTSVTVSSGGGS
GGGSGGGGSDIQMTQTTSLSASLGDRVTISCRASQDISKYLWYQQKPDGTVKLLIY
HTSRLHSGVPSRFGSGSGTDYSLTISNLEQEDIATYFCQQNTLPYTFGGGKLEITSGT
TTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYIWAPLAGTCGVLLL
SLVITLYCKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPPEEEGGCELRVKFSRSADA
PAYKQGQNLQYNELNLGRREEYDVLDKRRGRDPEMGKPRRKNPQEGLYNELQKDK
MAEAYSEIGMKGERRRGKHDGLYQGLSTATKDTYDALHMQALPPR*

[0490] Loop dual CAR connected with a constitutively activated IL7 receptor C7R: L719-C7R, amino acid sequence as set forth in SEQ ID NO 85.

L719-C7R
(SEQ ID NO 85)
MALPVTALLPLALLHAARPDIMTQTSSLSASLGDRVTISCRASQDISKYLN
WYQQKPDGTVKLLIYHTSRLHSGVPSRFGSGSGTDYSLTISNLEQEDIATYFCQQGNTL
PYTEGGGKLEITGGGGSQVKLQESGGGLVKPGGSLKLSAASGFTESYAMSWVRQT
PEKRLEWVATISSGGSYTYYPDSVKGRETISRDNAKNTLYLQMSLSRSEDAMYYCAR
QDGYYPGWFWANWGQTTVTVSSGSTSGSGKPGSGEGSTKGDIELTQSPAIVISASLGEEI
TLTCSASSSVSYMHWYQQKSGTSPKLLIYSTNLASGVPSRFGSGSGTFYSLTISSVEAE
DAADYYCHQWSSYTEGGGKLEIKRGGGSEVKLQESGPGLVAPSQSLSVTCTVSGVS
LPDYGVSWIRQPPRKGLEWLVGIWGSETTYNSALKSRLTIKDNSKQVFLKMNSLQT
DDTAIYYCAKHHYYGGSYAMDYWGQGTSTVTVSSSGTTTPAPRPPTPAPTIASQPLSLRP
EACRPAGGAVHTRGLDFACDIYIWAPLAGTCGVLLLSLVITLYCKRGRKLLYIFKQP
FMRPVQTTQEEDGCSCRFEEEEGGCELRVKFSRSDAPAYKQGQNQLYNELNLGRRE
EYDVLDKRRGRDPEMGGKPRKPNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGH
DGLYQGLSTATKDTYDALHMQALPPRGSGATNFSLLKQAGDVEENPGPMLVRRGARA
GPRMPRGWTALCLLSLPSGFMSLDNNGTATPELPTQGTFSNVSTNVSYQETTTTPSTLG
STSLHPVSQHGEATTNITETTVKFTSTSVITSVYGNNTSSVQSQTSTVISTVFTTPANVSTP
ETTLKPSLSPGNVSDLSTSTSLATSPTKPYTSSSPILSDIKAEIKCSGIREVKLTQGCICLEQ
NKTSSCAEFKKDRGEGLARVLCGEEQADADAGAVCSLLLAQSEVRPQCCLLVLANRT
EISSKLQLMKKHQSDLKKLGILDFTEQDVASHQSYSQKTPILLTCTPTISILSFFSVALLVIL
ACVLWKKRIKPIVWPSLPDHKKTLEHLCKKPRKNLNVSENPESELDCQIHRVDDIQARD
EVEGFLQDTFPQOLEESEKQRLGGDVQSPNCPSEDVVI TPESEGRDSSLTCLAGNVSACD
APILSSSRSLDCRESGKNQPHVYQDLLSLGTTNSTLPPFSLQSGILTLNPVAQGQPIILTS
LGSNQEEAYVTMSSFYQNQ*

6.6 Test for the Dual CAR Structure-1

[0491] 1) Expression of the dual CARs and the knockout efficiency

[0492] We compared two Loop CARs (L719-LHLH and L719-HLHL) and two Tandem CARs (T197-LHLH and T197-LHLH-(EAAAK3)3), where CAR7 and CAR19 were used as single CAR control, and Mock T was a negative control. As shown in FIG. 39, the expression of the two Loop dual CARs was diagonally distributed, indicating successful expression of the dual CARs. CD19 CAR of the Tandem dual CARs showed strong expression, however, CD7 CAR was weakly detected. FIG. 40 shows the knockout efficiencies of CD7 and CD3 (TRAC), and it can be seen that in Mock T and CAR19, the knockout efficiencies were up to 98%. In the other two groups including CD7 single CAR and CD7/CD19 dual CAR, the ratio of CD7+ cells was significantly lower than those in Mock T and CAR 19 groups, indicating the function of CD7 CAR.

[0493] 2) In vitro killing effects of dual CARs on CD19+ cells and CD7+ cells

[0494] First, we compared the killing effects of different CARs on CD 19 target. xCELLigence Real-Time Cell

Analyzer (RTCA) experiment showed that two Loop CARs and two Tandem CARs both had potent killing effects on HeLa-CD19+ cells, similar as CD19 single CAR (CAR19) (FIG. 41).

[0495] Subsequently, we compared the killing effects of different CARs on CD7+NK cells from peripheral blood (FIG. 42A). In vivo expanded PBNK cells (ratio of CD7+ cell was ~80%) were labelled with carboxyfluorescein succinimidyl ester (CFSE), and incubated with CAR-T cells for 1 day. Then the cells were subject to flow cytometry to analyze the change in ratio of CFSE positive cells. FIG. 42A shows that dual CARs and CD7 single CAR had significant killing effects on NK cells, and CD19 single CAR and Mock T did not show any killing effect. FIG. 42B shows that CD7+ cells in NK almost disappeared after co-culturing with dual CARs (L719-LHLH and T197-LHLH), indicating CAR7 and dual CARs completely killed the CD7+ cells in NK.

[0496] Furthermore, we compared the killing effects of different CARs on allogeneic T cells (CD7+) (FIG. 43). The result shows that the allogeneic T cells were completely killed by CAR7 and dual CARs, while CAR19 did not show any killing effect on allogeneic T cells.

[0497] 3) In vivo clearance of CD19+ Raji tumor by dual CARs

[0498] Each mouse was inoculated intravenously with 3×10^5 tumor cells. 6 days later, CAR-T cells were intravenously infused (3×10^6 cells per mouse). BLI imaging indicates that Loop CARs and Tandem CARs both cleared Raji tumors to certain extent, and L719-LHLH showed the best in vivo tumor control (FIG. 44).

6.7 Test for Dual CAR Structure-2

[0499] Two other Tandem dual CAR structures: T719-LHLH and T719-LHHL were designed and compared with Loop CAR (L719-LHLH, also named as L719).

[0500] FIGS. 45A and 45B show that new Tandem dual CARs were successfully expressed (FIG. 45A), and also had significant killing effects on the remaining CD7+ cells after gene knockout (FIG. 45B).

[0501] Real-time Cell Analysis (RTCA) shows that (FIG. 46), Tandem dual CARs had similar killing effects on HeLa-CD19 cells as L719.

[0502] FIGS. 47A and 47B shows that, Tandem dual CARs also had similar strong killing effects on allogeneic T cells and NK cells as L719 over 24 hours.

[0503] FIG. 48 shows that L719 and Tandem (T719-LHLH and T719-LHHL) dual CARs both greatly cleared the CCRF-CEM tumors in vivo (CCRF-CEM intravenous model in NOG mice).

6.8 Release of the Cytokines

[0504] The dual CAR-T cells prepared in Example 6.3 and CD19 positive tumor cells (Raji), CD7 positive tumor cells (Jurkat) or primary allogeneic PBMCs or NK, 100 ul each, were mixed in RPMI medium at 1: 1 ratio to a density of 1×10^6 /ml for each cell, and then cultured overnight in a 96-well plate. The medium was then collected and subject to centrifuge and the released cytokine IFN- γ and IL-2 were detected by Cytokine bead array kit (CBA kit, BD Biosciences).

[0505] The result shows that incubation of CD19-CD7 CAR-T cells with Raji, Jurka, primary allogeneic PBMCs or

NK cells produces a large amount of IFN- γ and IL-2, indicating function of CAR-T against CD19 positive or CD7 positive target cells.

6.9 In Vivo GVHD Study

[0506] The mice were systemically treated with a sub-lethal dose of irradiation (175 cGy) first. Then CAR-T cells were re-suspended in PBS and injected into the thorax of the treated mice. The mice were observed 2-3 times a week by using GVHD clinical criteria including weight loss, arch-back, activity, fur texture, and skin integrity.

[0507] The result shows that mice without TCR knockout showed GVHD symptoms, however, no GVHD was detected in the CD7-CD19 CAR-T+TCR/CD7double knockout group.

6.10 In Vitro HVG Study

[0508] CD7 CAR can effectively and rapidly clear CD7+ cells, and in vitro experiments showed that allogeneic T cells did not display significant rejection on CAR-T cells expressing CD7 CAR, but displayed strong rejection on CAR-T cells expressing CAR19 only.

[0509] The allogeneic NK cells showed significantly less rejection on CAR-T cells expressing CD7 CAR than CAR-T cells without CD7 CAR.

6.11 Clearance of CAR-T Cells by AP1903 or Rapamycin

[0510] 10 nM AP1903 or Rapamycin was added to the medium of rapaCasp9-L719 CAR-T (sequence as set forth in SEQ ID NO: 86) cells. 1, 6 and 24 hours later, apoptosis and survival of the CAR-T cells were detected by flow cytometry.

[0511] The result shows that AP1903 and Rapamycin rapidly cleared rapaCasp9-L719 CAR-T cells but fails to clear CD19 control CART, indicating the specificity of the safety switch.

rapaC9-L719

(SEQ ID NO. 86)

MASRILWHEMWHEGLEEASRLYFGERNVKGMEVLEPLHAMMERGPQTLKET
 SFNQAYGRDLMEAEQWCRKYMKSGNVKDLLQAWDLYYHVFRISKLEYSGGGSLEG
 VQVETISPGDGRTFPPKRGQTCVVHYTGMLDGKKFDRNRNPKPKMLGKQEVIRGW
 EEGVAQMSVGQRAKLTI SPDYAYGATGHPGIIIPPHATLVFDVELLKLESGGGSGGGGS
 GGGSGVDGFGDVGALSLRGNADLAYILSMPCGHCLIIINNVCRESGLRTRTGSNI
 DCEKLRRRPFSSLHFMVEVKDGLTAKKMLALLELAQQDHGALDCCVVVILSHGCQAS
 HLQFPGAVYGTGCPVSVKEIVNIFNGTSCPSLGGKPKLFFIQACGGEQKDGHFEVASTS
 PEDESPGSNPEPDATPFQEGRLTFDQLDAISSLPTPSDIFVSYSSTFPGFVSWRDPKSGSWY
 VETLDDIFEQWAHSEDLQSLLLRVANAVSVKGIYKQMPGCFNPLRKKLFFKTS SGSGATN
 FSLKQAGDVEENPGPMALPVTALLPLALLHAARPDIQMTQTSSLSASLGDRVTISC
 RASQDISKYLNNWYQQKPDGTVKLLIYHTSRLHSGVPSRFGSGSGTDYSLTISNLEQEDI
 ATYFCQQGNTLPYTFGGGKLEITGGGGSQVKLQESGGGLVKPGGSLKLSCAASGFTFS

- continued

SYAMSWVRQTPEKRLIEWATISSGGSYYTPDSVKGRFTISRDNAKNTLYLQMSSLRS
 EDTAMYICARQDGYYPGWFWANWQGTTTVTVSSGSTSGSGKPGSGEGSTKGDIELTQSP
 AIMSASLGEEITLTCASSSSVSYMHWYQQKSGTSPKLLIYSTNLASGVPSRFSGSGSGTGF
 YSLTISSVEAEDAADYYCHQWSSYTFGGGTKEIKRGGGSEVKLQESGPGLVAPSQSL
 SVTCTVSGVSLPDYGVSWIRQPPRKGLEWLGVIWGETTYNSALKSRLTIIKDNSKSQ
 VFLKMNSLQTDITAIYYCAKHYYYGGSYAMDYWGQGTSTVTVSSSGTTTPAPRPPTAP
 TIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYIWAPLAGTCGVLILLSLVITLYCKRGR
 KLLYIFKQPFMRPVQTTQEEDGCSRCFPPEEEGGCELVRKFSRSDAPAYKQGQNLQY
 NELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKG
 ERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR*

[0512] Safety switch functions of E3 in L719-E3 cells were determined by 24 hr Complement dependent cytotoxicity assay (CDC assay). Briefly, TCR/CD7 double knockout L719-E3 cells or L718-E2 cells were incubated in media containing 0%, 5% or 25% donor serum. 24 hr CDC effects were examined by adding 5 or 50 ug/ml of Cetuximab (CTX). Rituximab (RTX), which targeting CD20, was used as negative control. As shown in FIGS. 61A and 61B, L719-E3 cells were specifically lysed by cetuximab in the presence of serum (FIG. 61A), and no CDC effect was observed in L719-E2 cells without EGFRt or CD20 extracellular domain (FIG. 61B).

[0513] Safety switch functions of E3 in TCR/CD7 double knockout CAR7-E3 cells or L719-E3 cells were further determined by Complement dependent cytotoxicity assay (CDC assay). Cells were incubated in media containing 0%, 5% or 25% Guinea pig serum. Then CDC effects were detected in the presence of 5 ug/ml of Cetuximab. As shown in FIG. 62A, the CDC effects corresponded to the concentrations of the serum (complement), and were specific for E3 expressing CAR7-E3 cells but not control T cells. The CDC effects were also examined on L719-E3 cells in the presence of either 5 ug/ml Cetuximab or Herceptin. As shown in FIG. 62B, the CDC effects were only observed in the presence of Cetuximab.

6.12 Cytokine Receptor C7R or E3 Enhanced the Function of Dual CAR

[0514] To further enhance the survival of the universal CAR-T cells in vivo, a constitutive signal factor IL7 receptor (C7R) or E3 was linked to the L719 dual CAR by 2A to activate the pSTAT5 signaling pathway. FIG. 50A shows the expression of CD19 and CD7 antigen binding domains in L719 and L719-C7R dual CAR-T cells. FIG. 50B shows cytotoxicity of L719 dual CAR-T cells with or without C7R expression toward HeLa cells expressing CD7 (HeLa-CD7). FIG. 50C show cytotoxicity of L719 dual CAR-T cells with or without C7R expression toward HeLa cells expressing CD19 (HeLa-CD19). The effector: target ratio tested in FIGS. 50B and 50C is 4:1. FIGS. 50B and 50C show that both L719 and L719-C7R effectively killed HeLa-CD7 and HeLa-CD19. FIG. 50D shows flow cytometry data of fractions of L719 dual CAR-T cells expressing both an enhancer (C7R or E3) and CD7. FIG. 50E shows 6-hour cytotoxicity data of L719 dual CAR-T cells expressing C7R or E3 toward NALM6 cells. The effector: target ratios tested in this

experiment are 5:1 and 1:1. FIG. 50F shows 6-hour cytotoxicity data of L719 dual CAR-T cells expressing C7R or E3 toward CCRF-CEM cells. The effector: target ratios tested in this experiment are 5:1 and 1:1. FIG. 50G shows flow cytometry data of fractions of L719 dual CAR-T cells expressing phosphorylated STAT5 (pSTAT5) and C7R or E3. pSTAT5 was highly upregulated in L719 dual CAR-T cells expressing C7R or E3. CAR19 plus IL2 stimulation were used as positive control for pSTAT5. Non-engineered T cells were used as negative control for pSTAT5. FIG. 50H shows proliferation of L719 dual CAR-T cells expressing C7R or E3 after IL-2 removal. The CAR-T cells were treated with two rounds of stimulations by NALM6 cells.

[0515] FIG. 50I shows cytotoxicity of L719 dual CAR-T cells expressing E3 (L719-E3) toward CD19 expressing HeLa cells (HeLa-CD19). The L719-E3 cells were compared with control groups including target cells only, non-transduced TCR/CD7 double knockout cells (NT-DKO), and CD19 CAR-T cells, and L719-E3 showed better cytotoxicity toward HeLa-CD19 cells. The effector: target ratio tested in this experiment is 5:1. FIG. 50J shows cytotoxicity of L719 dual CAR-T cells expressing E3 (L719-E3) toward CD7 expressing HeLa cells (HeLa-CD7). The L719-E3 cells were compared with control groups including target cells only, non-transduced TCR/CD7 double knockout cells (NT-DKO), and CD19 CAR-T cells, and L719-E3 showed better cytotoxicity toward HeLa-CD7 cells. The effector: target ratio tested in this experiment is 5:1.

[0516] FIGS. 63A and 63B show the expression of IL2 and IFN- γ in TCR/CD7 double knockout CAR7 cells with no cytokine signaling enhancement, or with mbIL15, C7R, or E3 expression. Cells were stimulated by CCRF-CEM cells at 1:1 effector: target cell ratio for 24 hours. Supernatant were collected and measured for IL12 and IFN γ expression by cytokine bead array. As shown in FIG. 63A, CAR7 cells expressing C7R and E3 showed enhanced IL-2 production.

[0517] FIG. 64 shows the expression of IL2 and IFN- γ in TCR/CD7 double knockout CD19/CD7 dual CAR-L719 cells with no cytokine signaling enhancement, or with C7R, or E3 expression.

[0518] Cells were stimulated by CCRF-CEM cells at 1:2 effector: target cell ratio for 24 hours. Supernatant were collected and measured for IL12 and IFN γ expression by cytokine bead array. As shown in FIG. 64, CAR7 cells expressing C7R showed enhanced IL-2 production.

6.13 Additional Sequences Used to Construct CARs as Described Herein

[0519]

FMC63 scFv V_L (SEQ ID NO 87)
 DIQMTQTSSLSASLGDRVTISCRASQDISKYLNWYQQKPDGTVKLLIYHTSRL
 HSGVPSRFSGSGSGTDYSLTISNLEQEDIATYFCQQGNTLPYTFGGGKLEIT

FMC63 scFv V_H (SEQ ID NO 88)
 EVKLQESGPGLVAPSQSLSVTCTVSGVSLPDYGVSWIRQPPRKGLEWLGVIWGS
 ETTYNSALKSRLTIKDNSKSQVFLKMNSLQTDITAIYYCAKHYYYGGSYAMDYWG
 QGTSVTVSS

FMC63 CD19CAR (SEQ ID NO 89)
 MALPVTALLPLALLHAARPDQMTQTSSLSASLGDRVTISCRASQDISKYL
 WYQQKPDGTVKLLIYHTSRLHSGVPSRFSGSGSGTDYSLTISNLEQEDIATYFCQQGNTL
 PYTFGGGKLEITGSTSGSGKPGSGEGSTKGEVKLQESGPGLVAPSQSLSVTCTVSGVSL
 PDYGVSWIRQPPRKGLEWLGVIWGSSETTYNSALKSRLTIKDNSKSQVFLKMNSLQTD
 DTAIYYCAKHYYYGGSYAMDYWGQGTSVTVSSSGTTTPAPRPPTPAPTASQPLSLRPE
 ACRPAAGGAVHTRGLDFACDIYIWAFLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPF
 MRPVQTTQEEDGCSRFPPEEEGGCELVRVKFSRSADAPAYKQGQNLQYNELNLGRREE
 YDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHD
 GLYQGLSTATKDTYDALHMQALPPR*

3A1e CD7 scFv V_L (SEQ ID NO 90)
 DIELTQSPAIMSASLGEEITLTCSASSSVSYMEIWWYQQKSGTSPKWYSTSNLASG
 VPSRFSGSGSGTFYSLTISSVEAEDAADYYCHQWSSYTFGGGKLEIKR

3A1e CD7 scFv V_H (SEQ ID NO 91)
 QVKLQESGGGLVKPGGSLKLSCAASGFTFSSYAMSWVRQTPEKRLEWVATISS
 GGSYTYYPDSVKGRFTISRDNKNTLYLQMSSLSRSEDAMYYCARQDGYYPGWFWANW
 GGGTTVTVSS

6.14 Proliferation of CD19 and CD7 Dual Specific CAR-T Cells

[0520] FIG. 57A shows proliferation of CD19 and CD7 dual specific CAR-T cells expressing C7R (GC197-C7R). The engineered dual specific CAR-T cells were prepared from two different T cell donors. CAR-T cells from both donors displayed superior persistence and proliferation in response to repetitive cancer antigen stimulation. FIG. 57B shows proliferation of GC197-C7R cells without stimulation. Freshly prepared GC197-C7R were cultured in T cell culture media without supplement of IL2 or antigen stimulation, compared to control T cells, GC197-C7R maintained better expansion, and persisted in culture.

[0521] FIG. 58A shows expression of CD7 CAR and enhancer in GC197-C7R and GC197-E3 at day 12 of cell culture. FIG. 58B shows proliferation of GC197 cells expressing C7R (GC197-C7R) or E3 (GC197-E3) without stimulation. GC197-C7R or GC197-E3 cells were cultured in T cell culture media without supplement of IL2 and

monitored for fold of cell expansion and persistence. FIG. 58C shows proliferation of GC197-C7R and GC197-E3 in the presence of antigen stimulation. The cells were repeated stimulated by Nalm6 (CD19+ B-ALL cell line) and monitored for fold of cell expansion. The data showed that GC197-C7R/E3 displayed comparably superior cell expansion and cell stability either with or without cytokine or antigen stimulation.

6.15 In Vivo Efficacy

[0522] FIG. 69 shows BLI imaging of the in vivo efficacy of L719 and L719-C7R UCAR-T in Nalm6 (B-ALL) murine xenograft model. Each mouse was inoculated intravenously with 1×10^6 Nalm6 cells. Four days later, CAR-T cells were intravenously infused (high dose: 1×10^6 or low dose: 3×10^5 cells per mouse). BLI imaging indicates L719-C7R UCAR-T cells had better effect on tumor clearance than L719 UCAR-T cells.

Example 7 Study of U-CAR T Cells with CD137 and CD19 Dual CAR

General Materials and Methods

[0523] Isolation of Peripheral Blood Mononuclear Cells (PBMCs) from Donor Blood and Expansion of T Cells

[0524] Peripheral blood mononuclear cells (PBMCs) were isolated from donor blood by using Histopaque-1077 (Sigma-Aldrich) through density gradient centrifuge. Then T cells were enriched, activated by magnetic beads coupled with anti-CD3/anti-CD28, cultured and expanded.

[0525] Cell Lines and Culture of PBMC and Peripheral Blood Primary NK (PBNK) Cells

[0526] Raji cells (Burkitt's lymphoma cells, ATCC-CCL86);

[0527] K562 cells (Human erythroleukemia cell line, ATCC-CCL243);

[0528] Raji-fluc cell line (obtained by screening Raji cells transfected with lentivirus having firefly luciferase);

[0529] 293T cells (ATCC-CRL3216);

[0530] NK92 cells (ATCC-CRL2407);

[0531] NK92-fluc cells (obtained by screening NK92 cells transfected with lentivirus having firefly luciferase)

[0532] Raji cells, K562 cells and Raji-fluc cell line were cultured in RPMI1640 medium, and 293T cells were cultured in DMEM medium. Both RPMI1640 and DMEM were supplemented with 10% (v/v) fetal bovine serum and 100

U/ml penicillin and streptomycin, 2 mM glutamine and 1 mM sodium pyruvate. All of the cells were cultured in an incubator at 37° C., 5% CO₂.

[0533] NK92 cells and NK92-fluc cells were cultured in RPMI1640 medium supplemented with 10% (v/v) fetal bovine serum, 2 mM glutamine, 1 mM sodium pyruvate, 1% NEAA, 0.1 mM mercaptoethanol and 200 IU/ml rhIL2. PBNKs was sorted from PBMCs by using CD56 microbeads, and then cultured in above medium and expanded under the stimulation of K562 expressing 4-1BBL-mbIL15.

[0534] T cells and the obtained CAR-T cells were cultured in X-vivo15 medium (containing 5% FBS, 2 mM L-glutamine, 1 mM sodium pyruvate and 300 IU/ml rhIL2). The culture medium for CAR-T cells was supplemented with rhIL-2 (ThermoFisher Scientific) at a final concentration of 300 IU/ml every two days. All of the cells were cultured in an incubator at 37° C., 5% CO₂.

7.1 Design of CD19-CD137 Dual CAR and Package of the Virus

[0535] The structure of CD19 CAR is SP-CD19 scFv VL-Linker-CD19 scFv VH-Hinge-TM-4-1BB-CD3 ζ ; and the loop of CD19 and CD137 targeting dual CAR (CD19-CD7 dual-CAR) is SP-CD19 scFv VL-Linker-CD137 scFv VH-linker-CD137 scFv VL-Linker-CD19 scFv VH-Hinge-TM-4-1BB-CD3 ζ (as shown in FIG. 52). The nucleotide sequence of CD19-CD137-loop-CAR is set forth as SEQ ID NO.: 41.

CD19-CD137-Loop-CAR (SEQ ID NO 41):

```
ATGGCACTCCCTGTAACTGCACCTTCTTTTGCCTTGCTGCTCCTGCACGC
AGCGCGGCCGGATATTCAAATGACACAACTACCAGCTCCCTTTCAGCATCTTTGGG
CGATAGAGTAACATATAAGTTGCCGCGCTCCCAAGACATCTCTAAGTACCTTAAGT
GTATCAACAAAAACCGGACGGGACGGTCAAACGTGTGATCTATCACACATCCAGAT
TGCACTCAGGCGTGCCGAGCAGGTTCACTGGGAGTGGGTGAGGAACGATTACAGC
TTGACGATTAGTAACCTGGAGCAAGAAGACATTGCCACCTACTTCTGCCAGCAAGG
TAACACTCTCCCATATACGTTCCGGGGTGGCACCAGCTGGAAATCACTGGCGGCG
GAGGATCCGAGGTGCAGCTGGTGCAGAGCGGCGCGAGGTGAAGAAGCCCGGCGA
GAGCCTGAGGATCAGCTGCAAGGGCAGCGGTACAGCTTCAGCACCTACTGGATCA
GCTGGGTGAGGCAGATGCCCGGCAAGGGCTGGAGTGGATGGGCAAGATCTACCCC
GGCGACAGCTACACCAACTACAGCCCAGCTTCCAGGGCCAGGTGACCATCAGCGC
CGACAAGAGCATCAGCACCGCTACCTGCAGTGGAGCAGCCTGAAGGCCAGCGAC
ACCGCCATGTACTACTGCGCCAGGGGCTACGGCATCTTCGACTACTGGGCCAGGG
CACCTGGTGACCGTGAGCAGCGGCGAGCAACAAGCGCTCTGGCAAGCCTGGATCTG
GCGAGGGCTCTACCAAGGGCATGAGCTACGAGCTGACCCAGCCCCCAGCGTGAGC
GTGAGCCCCGGCCAGCCGAGCATCACCTGCAGCGGCGACAACATCGGCGACCA
GTACGCCCACTGGTACCAGCAGAAGCCCGGCCAGAGCCCCGTGCTGGTGATCTACC
AGGACAAGAAGAGGCCCGAGCGCATCCCCGAGAGGTTGAGCGGCAGCAACAGCGG
CAACACCGCCACCTGACCATCAGCGGACCCAGGCCATGGACGAGGCCGACTACT
ACTGCGCCACCTACACCGGCTTCGGCAGCCTGGCCGTGTTGCGCGGCGGCACCAAG
CTGACCGTGCTGGGGGGAGCGGCGAGGTTGAACTGCAGGAGTCCGGGCCCCG
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GTCTCGTGGCACCTTCCCAGTCACTGTCCGTGACCTGCACCGTATCTGGGGTAAGTC
 TGCCGGATTATGGGGTTTCATGGATCCGGCAACCTCCGAGGAAAGGGTTGGAATGG
 CTGGGAGTCATCTGGGGAAGCGAGACAACTTATTATAATTCTGCTTTGAAGAGCCG
 CTTGACGATAATCAAGGACAACAGTAAGAGTCAGGTTTCTTGAAGATGAATTCTCT
 TCAGACAGATGACACCGCTATTTATTATTGTGCAAAACATTATTATTACGGAGGATC
 CTACGCGATGGACTATTGGGGACAGGTACCTCTGTTACGGTGTCTCATCCGGAAC
 AACGACACCAGCACCGCCACCCACTCCTGCTCCGACAATTGCGTCTCAGCCCTT
 TTCCCTTCGACCCGAAGCTTGTGCGCTGCTGCGGGAGGAGCGGTCCACACGCGCG
 GGCTTGACTTCGCTTGCGACATCTACATTTGGGCACCTTGGCCGGGACATGCGGCG
 TCTTGCTCCTGAGTCTGGTTATAACGCTGATTGTAAGCGAGGTGGAAGAAGCTTT
 TGTATATCTTTAAACAGCCCTTTATGAGGCCGTACAAACCACACAAGAGGAGGAT
 GGGTGCTCATGCAGATTTCTGAAGAGGAAGAGGGCGGTTGCGAACTTAGAGTCAA
 ATTCAGCCGCTCCGAGATGCACCTGCTTATAAACAGGGTCAGAATCAATTGTATA
 ATGAACCTTAATCTCGGGAGGCGCGAGGAGTATGATGTGCTGGACAAGCGACGGGGT
 CGAGACCCAGAGATGGGCGGTAACCCCGCGAAAGAACCCCGAGGAGGACTGT
 ATAATGAGCTGCAAAAGGACAAAATGGCAGAAGCCTATTCCGAAATAGGGATGAA
 GGGAGAGCGGCGCGAGGTAAGGGACATGACGGTCTTTATCAAGGTCTTAGTACTG
 CAACTAAGGACACCTATGACGCGCTGCATATGCAGGCTCTCCACCTAGATAA

[0536] The CAR gene was cloned into the FUW lentiviral vector backbone under the promoter of EF1 α (EF-1 α) to form Fuw-EF1 α -CAR. Fuw-EF1 α -CAR, lentiviral envelope plasmid pMD2.G (Addgene, Plasmid #12259) and lentiviral packaging plasmid psPAX2 (Addgene, Plasmid #12260) were transfected into 293 T cells by using Lipofectamine3000 to prepare the whole lentiviral expression vector. The supernatant was collected at 48 and 72 h and subject to ultra-centrifuge (Merck Millipore) for concentration. The concentrated virus was ready for the transfection of T cells.

[0537] The result shows that the lentiviral vector was successfully constructed and expressions of anti-CD19 and anti-CD19/CD137 of the dual CAR were detected as expected.

7.2 Design and Construction of CRISPR

[0538] CD137 and TCR gRNAs for gene editing were selected after evaluating gene editing efficiency and off-target risk at website <http://crispr.mit.edu>, www.idtdna.com and <https://www.synthego.com>. gRNAs used in the present disclosure were synthesized by Integrated DNA Technologies, Inc (IDT). HiFi-Cas9 was purchased from IDT.

[0539] For gene editing, 3 μ g Cas9 protein and 1.5 μ g gRNA were mixed in 20 μ l and incubated at 37° C. or room temperature for 15 minutes to form ribonucleoprotein (RNP). Then RNP was transfected into T cells by Lonza 4D Nucleofector. Gene knockout efficiency was determined by the ratio of the cells expressing the protein as detected by flow cytometry.

[0540] gRNA targeting sequences used are as below:

TRAC-gRNA1:	TTCGGAACCAATCACTGAC,	SEQ ID NO: 20
TRAC-gRNA2:	TCAGGGTTCTGGATATCTGT,	SEQ ID NO: 21
TRAC-gRNA3:	AAGTTCCTGTGATGTCAAGC,	SEQ ID NO: 22
CD137-gRNA1:	AACGTGGCATCTGTCGACCC,	SEQ ID NO: 45
CD137-gRNA2:	GCGCTGGAGAACTATTGG,	SEQ ID NO: 46
CD137-gRNA3:	ACATTTAACGATCAGAAACG,	SEQ ID NO: 47
CD137-gRNA4:	GGTCCTTTGTCCACCTGCGC,	SEQ ID NO: 48
CD137-gRNA5:	GAGAACTATTGGAGGACA,	SEQ ID NO: 49
CD137-gRNA6:	GGAGAACTATTGGAGGAC,	SEQ ID NO: 50

[0541] After screening, gRNA with high knockout efficiency was found for each gene and high efficiency was also observed when knocking out two genes, TCR/CD137.

7.3 Preparation of CAR-T Cells

[0542] Cell Isolation and Activation

[0543] After apheresis, monocytes were isolated using Histopaque-1077 (Sigma-Aldrich) by density gradient cen-

trifugation. Then T cells were enriched, activated by magnetic beads coupled with anti-CD3/anti-CD28, cultured and expanded.

[0544] X-vivo 15 with 5% FBS, 2 mM L-glutamine, 1 mM sodium pyruvate and 300 IU/ml rhIL2 was used as CAR-T cell medium. The cells were incubated and cultured at 37° C., 5% CO₂.

[0545] Allogeneic T cells were activated by using anti-CD3/anti-CD28 magnetic beads.

[0546] Allogeneic PBNK cells were activated by co-culturing K562 cells with PBNKs.

[0547] The expression of CD137 on the cell surface before and after the activation was determined by flow cytometry.

[0548] Electroporation and Lentivirus Infection

[0549] Two days after enriching and activating the T cells, the anti-CD3/anti-CD28 magnetic beads were removed and the cells were collected into a tube and subject to centrifuge at 300 g for 5 min, and then washed twice with DPBS and re-suspended in opti-mem at a cell density of 50×10⁶/ml. The amount of cas9/gRNA RNP required was calculated based on the density of the cells, and then mixed with cells and transferred for electroporation by 4D-Nucleofector System N (Lonza) system. Then the cells were re-suspended in a pre-warmed medium to a density of 1-2×10⁶/ml. The cells were further subject to lentiviral transfection at MOI of 2-8, and then transferred to a flask and cultured in an incubator at 37° C., 5% CO₂.

[0550] Cell Proliferation and Detection of CAR Positive Ratio

[0551] 4 days after the transfection, the number of cells and CAR positive cells were detected, and the positive ratio of CAR in T cells was calculated. The positive ratio of TCR (or CD3) and CD137 was also detected to determine the knockout efficiency. Then cells were continuously cultured in the incubator and half of the medium was replaced every 2-3 days till day 10 when the cells were ready for cryopreservation. An example workflow is shown in FIG. 53.

[0552] The result shows that positive ratio of CAR and negative ratio of CD3 were both high in the prepared CD19-CD137 CAR-T cells.

7.4 In Vitro Killing Effect and Release of the Cytokines

[0553] The dual CAR-T cells prepared in Example 7.3 and CFSE labeled CD19 positive tumor cells (Raji) and CD137 positive cells (activated allogeneic T cells or activated allogeneic PBNK or NK92), 100 ul each, were mixed in RPMI medium at 1:1 ratio to a density of 1×10⁶/ml for each cell, and then cultured from overnight to 1 week in a 96-well plate. The number of CAR-T cells and CFSE-labeled target cells was detected by flow cytometry, so that to determine the killing effect between CAR-T cells and target cells during co-culture. Furthermore, the co-culture medium was also collected and subject to centrifuge and the released

cytokine IFN-γ and IL-2 were detected by Cytokine bead array kit (CBA kit, BD Biosciences).

[0554] The result shows that incubation of CD19-CD137 CAR-T cells with Raji, allo-T or allo-NK cells produces a large amount of IFN-γ and IL-2, indicating function of CAR-T against CD19 positive or CD137 positive target cells. The flowcytometry result also indicates that the killing effects of allo-T and allo-NK on CD19-CD137 dual CAR T cells were significantly less than that on CD19 single CAR T cells.

[0555] Additional experiments show that CD19-CD137 dual CAR T cells only clear CD137 positive cells and NK cells, but fails to show any significant killing effect on CD137 negative inactivated T cells or NK cells, indicating the specificity of CD137 CAR.

7.5 In Vivo Efficacy

[0556] 6-12 weeks NOD/Shi-scid/IL-2R^γnull (NOG) mice were selected and intravenously injected with 2×10⁵ Raji-fluc cells and allo-T cells. 5 days later, the tumor graft burden of the mice was measured, and the mice were divided into 4 groups based on the tumor burden. One day later, 200 μL DPBS, 0.5×10⁶ CD19 CAR-T cells and 0.5×10⁶ CD19-CD137 CAR-T cells were respectively injected to each mouse. The tumor burden of the mice was further evaluated every 7 days after the CAR-T treatment. Each mouse was injected intraperitoneally with 3 mg d-luciferin for a 4-minutes reaction, and then photographed using a Xenogen IVIS Imaging System with 30 s exposure.

[0557] The result shows that CD19-CD137 CAR-T significantly decreased the tumor burden compared to CD19 CAR-T cells, and CD19-CD137 CAR-T cells showed significant better in vivo expansion than CD19 CAR-T cells, indicating the CD19-CD137 CAR-T cells survive better and have a stronger ability to clear CD19 positive tumor cells in vivo.

7.6 In Vivo GVHD Study

[0558] The mice were systemically treated with a sub-lethal dose of irradiation (175 cGy) first. Then CAR-T cells were re-suspended in PBS and injected into the thorax of the treated mice. The mice were observed 2-3 times a week by using GVHD clinical criteria including weight loss, arch-back, activity, fur texture, and skin integrity.

[0559] The result shows that mice without TCR knockout all showed GVHD symptoms, however, no GVHD was detected in the TCR/CD137 dual knockout CD19-CD137 CAR-T cell group.

7.7 Additional Sequences Used in Constructing CARs as Described Herein

[0560]

4-1BB-L

(SEQ ID NO: 51)

MEYASDASLDPEAPWPPAPRARACRVLPWALVAGLLLLLLAACAFLACP

WAVSGARASPGSAASPRRLREGPELSPDDPAGLLDLRQGMFAQLVAQNVLIDGPLSWY

SDPGLAGVSLTGGLSYKEDTKELVVAKAGVYVFFQLELRRVVAGEGSGSVSLALHLQ

-continued

PLRSAAGAAALALTVDLPPASSEARNSAFGFQGRLLHLSAGQRLGVHLHTEARARHAW

QLTQGATVLGLFRVTPEIPAGLPSPRSE

4-1BB PF-05082566 scFv VH

(SEQ ID NO: 52)

EVQLVQSGAEVKKPGESLRISCKGSGYSFSTYWISWVRQMPGKGLEWMGKIYP

GDSYTNYSFQGVQVTISADKSISTAYLQWSSLKASDTAMYYCARGYGIFDYWGQGT

LVTVSS

4-1BB PF-05082566 scFv VL

(SEQ ID NO: 53)

MSYELTQPPSVSVSPGQTASITCSGDNIGDQYAHWYQQKPGQSPVLVIYQDKN

RPSGIPERFSGSNSGNTATLTISGTQAMDEADYYCATYTGFGSLAVFGGGTKLTVL

4-1BB BMS-663513 scFv VH

(SEQ ID NO: 54)

QVQLQQWGAGLLKPSETLSLTCAVYGGSFSGYYWSWIRQSPEKGLEWIGE

INHGQYVTYNPSLESRVTISVDTSKNQPSLKLSSVTAADTAVYYCARDYG

PGNYDWYFDLWGRGTLVTVSS

4-1BB BMS-663513 scFv VL

(SEQ ID NO: 55)

EIVLTQSPATLSLSPGERATLSCRASQSVSSYLAWYQQKPGQAPRLLIYD

ASNRATGIPARFSGSGTDFTLTISLLEPEDFAVYYCQQRSNWPPALTF

CGGTKVEIKR

AAC VL-VH

(SEQ ID NO: 56)

MALPVTALLLPLALLLHAARPMSEYELTQPPSVSVSPGQTASITCSGDNIGDQYA

HWYQQKPGQSPVLVIYQDKNRPSPGIPERFSGSNSGNTATLTISGTQAMDEADYYCATYT

GFGSLAVFGGGTKLTVLGSTSGSGKPGSGEGSTKGEVQLVQSGAEVKKPGESLRISCKG

SGYSFSTYWISWVRQMPGKGLEWMGKIYPGDSYTNYSFQGVQVTISADKSISTAYLQ

WSSLKASDTAMYYCARGYGIFDYWGQGTLVTVSSGTTTPAPRPPTPAPTIASQPLSLRP

EACRPAAGGAVHTRGLDFACDIYIWAPLAGTCGVLLLSLVITLYCKGRKKLLYIFKQP

FMRPVQTTQEEDGCGSCRFPEEEEGGCELRVKFSRSDAPAYKQGQNLNLYNELNLGRRE

EYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGH

DGLYQGLSTATKDTYDALHMQALPPR*

AAC DNA sequence VL-VH

(SEQ ID NO: 57)

ATGGCCCTGCCCCGTACCGCCCTGCTGCTGCCCCCTGGCCCTGCTGCTGCACG

CCGCCAGGCCCATGAGCTACGAGCTGACCCAGCCCCCAGCGTGAGCGTGAGCCCC

GGCCAGACCGCCAGCATCACCTGCAGCGCGACAACATCGGCGACAGTACGCCCA

CTGGTACCAGCAGAAGCCCGCCAGAGCCCCGTGCTGGTGATCTACCAGGACAAGA

ACAGGCCAGCGGCATCCCCGAGAGGTTTCAGCGGCAGCAACAGCGGCAACACCGC

CACCTTGACCATCAGCGGCACCCAGGCATGGACAGGCGGACTACTACTGCGCCA

CCTACACCGGCTTCGGCAGCCTGGCCGTGTTTCGGCGGCGGCACCAAGCTGACCGTG

CTGGGCAGCACCGCGCAGCGGCAAGCCCGCAGCGGCGAGGGCAGACCAAGG

GCGAGGTGCAGCTGGTGAGAGCGGCGCGAGGTGAAGAAGCCCGCGAGAGCCT

GAGGATCAGCTGCAAGGGCAGCGGCTACAGCTTCAGCACCTACTGGATCAGCTGGG

TGAGGCAGATGCCCGCAAGGGCCTGGAGTGGATGGGCAAGATCTACCCCGCGCA

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CAGCTACACCAACTACAGCCCCAGCTTCCAGGGCCAGGTGACCATCAGCGCCGACA
AGAGCATCAGCACCCGCTACCTGCAGTGGAGCAGCCTGAAGGCCAGCGACACCGCC
ATGTACTACTGCGCCAGGGGCTACGGCATCTTCGACTACTGGGGCCAGGGCACCCCT
GGTGACCGTGAGCAGCAGCGGCACCAACCCCCGCCCCAGGGCCCCACCCCCG
CCCCACCATCGCCAGCCAGCCCCCTGAGCCTGAGGCCCGAGGCCTGCAGGCCCGCC
GCCGGCGCGCCGTGCACACCAGGGGCTGGACTTCGCCTGCGACATCTACATCTG
GGCCCCCTGGCCGGCACCTGCGCGTGCTGCTGCTGAGCCTGGTGATCACCTGTGA
CTGCAAGAGGGGCGAGGAAGAAGCTGCTGTACATCTTCAAGCAGCCCTTCATGAGGC
CCGTGCAGACCACCAGGAGGAGGACGGCTGCAGCTGCAGGTTCCCCGAGGAGGA
GGAGGGCGGCTGCGAGCTGAGGGTGAAGTTGAGCAGGAGCGCCGACGCCCCGCC
TACAAGCAGGGCCAGAACCAGCTGTACAACGAGCTGAACCTGGGCAGGAGGGAGG
AGTACGACGTGCTGGACAAGAGGAGGGGAGGGACCCCGAGATGGGCGGCAAGCC
CAGGAGGAAGAACCCCCAGGAGGGCCTGTACAACGAGCTGCAGAAGGACAAGATG
GCCGAGGCCCTACAGCGAGATCGGCATGAAGGGCGAGAGGAGGAGGGCAAGGGCC
ACGACGGCCTGTACCAGGGCCTGAGCACCGCCACCAAGGACACCTACGACGCCCTG
CACATGCAGGCCCTGCCCCCAGGTGA

AAC VH-VL

(SEQ ID NO: 58)

S01 ATGGCCCTGCCCCGTGACCGCCCTGCTGCTGCCCCCTGGCCCTGCTGCTGCACG
CCGCCAGGCCCGAGGTGCAGCTGGTGCAGAGCGGCGCCGAGGTGAAGAAGCCCGG
CGAGAGCCTGAGGATCAGCTGCAAGGGCAGCGGCTACAGCTTCAGCACCTACTGGA
TCAGCTGGGTGAGGCAGATGCCCGCAAGGGCCTGGAGTGATGGGCAAGATCTAC
CCCGCGCAGCTACACCAACTACAGCCCCAGCTTCCAGGGCCAGGTGACCATCAG
CGCCGACAAGAGCATCAGCACCGCCTACCTGCAGTGGAGCAGCCTGAAGGCCAGCG
ACACCGCCATGTACTACTGCGCCAGGGGCTACGGCATCTTCGACTACTGGGGCCAG
GGCACCTTGGTGACCGTGAGCAGCGGCAGCACAGCGGCAGCGGCAAGCCCGCA
GCGGCGAGGGCAGCACCAAGGGCATGAGCTACGAGCTGACCCAGCCCCCAGCGT
GAGCGTGAGCCCCGGCCAGACCGCCAGCATCACCTGCAGCGGCGACAACATCGGCG
ACCAGTACGCCCACTGGTACCAGCAGAAGCCCGCCAGAGCCCCGTGCTGGTGATC
TACCAGGACAAGAACAGGCCAGCGGCATCCCCGAGAGGTTACGCGGCAGCAACA
GCGGCAACACCGCCACCTGACCATCAGCGGCACCCAGGCCATGGACGAGGCCGAC
TACTACTGCGCCACCTACACCGGCTTCGGCAGCCTGGCCGTGTTGCGCGGCGGCACC
AAGCTGACCGTGCTGAGCGGCACCACCACCCCGCCCCAGGCCCCCAGCCCCGC
CCCCACCATCGCCAGCCAGCCCTGAGCCTGAGGCCCGAGGCCTGCAGGCCCGCCG
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TGCAAGAGGGGCGAGGAAGAAGCTGCTGTACATCTTCAAGCAGCCCTTCATGAGGCC
CGTGCGAGACCACCAGGAGGAGGACGGCTGCAGCTGCAGGTTCCCCGAGGAGGAG
GAGGGCGGCTGCGAGCTGAGGGTGAAGTTGAGCAGGAGCGCCGACGCCCCGCGCT
ACAAGCAGGGCCAGAACCAGCTGTACAACGAGCTGAACCTGGGCAGGAGGGAGGA

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GTACGACGTGCTGGACAAGAGGAGGGGCAGGGACCCGAGATGGGCGGCAAGCCC
 AGGAGGAAGAACCCAGGAGGGCCTGTACAACGAGCTGCAGAAGGACAAGATGG
 CCGAGGCCCTACAGCGAGATCGGCATGAAGGGCGAGAGGAGGGGCAAGGGCCA
 CGACGGCCTGTACCAGGGCCTGAGCACGCCACCAAGGACACCTACGACGCCCTGC
 ACATGCAGGCCCTGCCCCCAGGTGA

FMC63 VH (SEQ ID NO: 59)
 EVKLQESGPGLVAPSQSLSVTCTVSGVSLPDYGVSWIRQPPRKGLEWLGVIWGS
 ETTYNSALKSRLTIKDNSKQVFLKMNSLQTDITAIYYCAKHHYYGGSYAMDYWG
 QGTSVTVSS

FMC63 VL (SEQ ID NO: 60)
 DIQMTQTSSLSASLGDRVTISCRASQDISKYLNWYQQKPDGTVKLLIYHTSRL
 HSGVPSRPSGSGSGTDYSLTISNLEQEDIATYFCQQGNTLPYTFGGGKLEIT

FMC63 CD19 CAR (SEQ ID NO: 61)
 MALPVTALLLPLALLHAARPDQMTQTSSLSASLGDRVTISCRASQDISKYLN
 WYQQKPDGTVKLLIYHTSRLHSGVPSRPSGSGSGTDYSLTISNLEQEDIATYFCQQGNTL
 PYTFGGGKLEITGTSGSGKPGSGEGSTKGEVKLQESGPGLVAPSQSLSVTCTVSGVSL
 PDYGVSWIRQPPRKGLEWLGVIWGS ETTYNSALKSRLTIKDNSKQVFLKMNSLQTD
 DTAIYYCAKHHYYGGSYAMDYWGQGTSVTVSSGTTTPAPRPPTPAPTIASQPLSLRPE
 ACRPAAGGAVHTRGLDFACDIYIWAPLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPF
 MRPVQTTQEEDGCS CRFP EEEEGGCEL RVKFSRSADAPAYKQGQNQLYNELNLGRREE
 YDVLDKRRGRDPEMGKPRRKNPQEGLYNELQDKMAEAYSEIGMKGERRRGKGHD
 GLYQGLSTATKDTYDALHMQALPPR*

Example 8 Study of U-CAR T Cells Expressing E4

[0561] FIG. 59A shows flow cytometry data of fractions of CD19 CAR-T cells expressing enhancer CD47 (CAR19-CD47) or E4 (CAR19-E4). In the E4 design, the ecto domain of C7R is replaced by the ecto domain from an immune cell inhibitor, such as CD47, CD24 or other peptides that inhibit killer or phagocytic immune cell function and protect therapeutic cells. The CAR19-CD47 or CAR19-E4 was expressed in K562 cells, which lacks MHCII expression and are very sensitive to NK cell killing due to lack of MHC expression. Expression of CAR19 and CD47 were analyzed by flow cytometry. FIG. 59B shows NK cell killing activity toward CAR19-CD47 and CAR19-E4 cells. Both CD47 and E4 were able to reduce the extent of NK cell killing for K562 cells. The data show that E4 may protect MHCII negative cells from killing by NK cells, which may be used in protection of MHCII KO CAR-T cells.

[0562] FIG. 60A shows flow cytometry data of fractions of TCR KO CD19 CAR-T cells expressing CD19 and phosphorylated STAT5 (pSTAT5). The CAR19-CD47 or CAR19-E4 was expressed in TCR knockout T cells and accessed for CAR expression and STAT5 phosphorylation. CAR19-E2 and CAR19-CD47 plus IL2 stimulation were used as positive controls. E4 mediates up-regulation of pSTAT5. FIG. 60B shows proliferation of TCR KO CD19 CAR-T cells expressing enhancer CD47 (CAR19-CD47) or

E4 (CAR19-E4). The CAR19-CD47, CAR19-E4, CAR19-C7R expressing TCR knockout T cells were compared for cell proliferation and cell survival without IL12 supplement. E4 can mediate stronger cell proliferation as well as maintenance of cell number after removal of IL2 in culture media. The E4 maintains better CAR-T cell stability without IL2 addition. E4 may protect therapeutic cells, such as cells that with HLA-I KO, from NK cell killing or killing by phagocytes and T killer cells.

[0563] Sequence of construct design: the ecto domain of C7R can be replaced by an immune cell inhibitor, such as truncated CD47, CD24, that can inhibit a killer or phagocytic immune cell function and protect the CAR-T/NK cells from being attached by patient's immune system.

[0564] CD7CAR-E4 amino acid sequence:

(SEQ ID NO: 103)
 MLLLVTSLLLCELPHPAPLLIPQLLFNKTCSVEFTFCNDTVVIPCFTVNM
 EAQNTTEVYVVKWKFGRDIYTFDGLNKS TVPTDFSSAKIEVSQLLKGDA
 SLKMDKSDAVSHTGNYTCEVTELTREGETIIELYRVVSWFSPNEPIILLT
 CPTISILSFFSVALLVILACVLWKKRIKPIVWPSLPDHKKTLLEHLCKKPR
 KNLNVSNFNPESFLDCQIHRVDDIQARDEVEGFLQDTFPPQOLEESEKQRLG

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GDVQSPNCPSEDDVITPESFGRDSSLTCLAGNVSAADAPILSSSRSLDCR
 ESGKNGPHVYQDLLSLGTTNSTLPPFSLQSGILTLPVQAQGQPILTSL
 GSNQEEAYVTMSSFYQNQ.

Example 9 Clinical Data of CAR7-C7R for
 Treating T-Acute Lymphoblastic Leukemia (T-ALL)
 Patients

[0565] This example provides clinical study designs and clinical data using CAR7-C7R. Patients were enrolled with tumor burden at the enrollment that 38.2% of bone marrow cells were T-ALL cells. Before infusing the CAR7-C7R cells, the patients were treated with pre-conditional regimen. The pre-conditional regimen used chemotherapy drug including fludarabine, cyclophosphamide and etoposide for lymphodepletion. The pre-conditional regimen can provide better CAR-T engraftment and tumor clearance. Two days after completion of lymphodepletion regimen, CAR7-C7R cells were intravenously infused into patients at $1.5 \times 10^7/\text{kg}$. CAR7-C7R rapidly eliminated cancer cells (T-ALL cells) and expanded to the peak around 7-10 days, without affecting innate immune cell subsets such as granulocytes and monocytes. Once cancer cells were completely cleared in both the peripheral blood and in the bone marrow, CAR7-C7R cells very quickly return to minimal level, which allows patients' own immune system to start recovery immediately after cancer cell clearance. T cells and NK cells then gradually recover to normal levels.

[0566] FIGS. 67A-E show the clinical data of CAR7-C7R for treating T-acute lymphoblastic leukemia (T-ALL) patients. The construct of CAR7-C7R used in the clinical

studies is shown in FIG. 17. FIG. 67A shows the kinetics of T-ALL elimination in the peripheral blood. T-ALL cells were quickly eliminated within 7-10 days. T-ALL cell concentration were measured in the peripheral blood by flow cytometry. FIGS. 67B and 67C shows the CAR7-C7R cell expansion kinetics, where FIG. 67B shows the CAR7-C7R cell concentration in the peripheral blood and FIG. 67C shows CAR7-C7R DNA copy/ug DNA of peripheral blood cells. FIGS. 67D and 67E show the granulocyte and lymphocyte (T and NK cells) recovery in the bone marrow and in peripheral blood.

[0567] While preferred embodiments of the present invention have been shown and described herein, it will be obvious to those skilled in the art that such embodiments are provided by way of example only. It is not intended that the invention be limited by the specific examples provided within the specification. While the invention has been described with reference to the aforementioned specification, the descriptions and illustrations of the embodiments herein are not meant to be construed in a limiting sense. Numerous variations, changes, and substitutions will now occur to those skilled in the art without departing from the invention. Furthermore, it shall be understood that all aspects of the invention are not limited to the specific depictions, configurations or relative proportions set forth herein which depend upon a variety of conditions and variables. It should be understood that various alternatives to the embodiments of the invention described herein may be employed in practicing the invention. It is therefore contemplated that the invention shall also cover any such alternatives, modifications, variations or equivalents. It is intended that the following claims define the scope of the invention and that methods and structures within the scope of these claims and their equivalents be covered thereby.

SEQUENCE LISTING

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<210> SEQ ID NO 1

<211> LENGTH: 1138

<212> TYPE: PRT

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic polypeptide

<400> SEQUENCE: 1

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 1 5 10 15

Ala Phe Leu Leu Ile Pro Arg Lys Val Cys Asn Gly Ile Gly Ile Gly
 20 25 30

Glu Phe Lys Asp Ser Leu Ser Ile Asn Ala Thr Asn Ile Lys His Phe
 35 40 45

Lys Asn Cys Thr Ser Ile Ser Gly Asp Leu His Ile Leu Pro Val Ala
 50 55 60

Phe Arg Gly Asp Ser Phe Thr His Thr Pro Pro Leu Asp Pro Gln Glu
 65 70 75 80

Leu Asp Ile Leu Lys Thr Val Lys Glu Ile Thr Gly Phe Leu Leu Ile
 85 90 95

Gln Ala Trp Pro Glu Asn Arg Thr Asp Leu His Ala Phe Glu Asn Leu
 100 105 110

Glu 115	Ile	Arg	Gly	Arg	Thr	Lys 120	Gln	His	Gly	Gln	Phe 125	Ser	Leu	Ala
Val 130	Val	Ser	Leu	Asn	Ile	Thr 135	Ser	Leu	Gly	Leu	Arg 140	Ser	Leu	Lys
Ile 145	Ser	Asp	Gly	Asp	Val	Ile 150	Ile	Ser	Gly	Asn	Lys 155	Asn	Leu	Cys
Ala	Asn	Thr	Ile	Asn	Trp	Lys 165	Lys	Leu	Phe	Gly	Thr 170	Ser	Gly	Gln
Thr	Lys	Ile	Ile	Ser	Asn	Arg 180	Gly	Glu	Asn	Ser	Cys 185	Lys	Ala	Thr
Gln	Val	Cys	His	Ala	Leu	Cys 195	Ser	Pro	Glu	Gly	Cys 200	Trp	Gly	Pro
Pro	Arg	Asp	Cys	Val	Ser	Cys 210	Arg	Asn	Val	Ser	Arg 215	Gly	Arg	Glu
Val 225	Asp	Lys	Cys	Asn	Leu	Leu 230	Glu	Gly	Glu	Pro	Arg 235	Glu	Phe	Val
Asn	Ser	Glu	Cys	Ile	Gln	Cys 245	His	Pro	Glu	Cys	Leu 250	Pro	Gln	Ala
Asn	Ile	Thr	Cys	Thr	Gly	Arg 260	Gly	Pro	Asp	Asn	Cys 265	Ile	Gln	Cys
His	Tyr	Ile	Asp	Gly	Pro	His 275	Cys	Val	Lys	Thr	Cys 280	Pro	Ala	Gly
Met	Gly	Glu	Asn	Asn	Thr	Leu 285	Val	Trp	Lys	Tyr	Ala 290	Asp	Ala	Gly
Val 305	Cys	His	Leu	Cys	His	Pro 310	Asn	Cys	Thr	Tyr	Gly 315	Cys	Thr	Gly
Gly	Leu	Glu	Gly	Cys	Pro	Thr 325	Asn	Gly	Pro	Lys	Ile 330	Pro	Ser	Ile
Thr	Gly	Met	Val	Gly	Ala	Leu 340	Leu	Leu	Leu	Leu	Val 345	Val	Ala	Leu
Ile	Gly	Leu	Phe	Met	Arg	Ala 355	Lys	Arg	Gly	Ser	Gly 360	Ala	Thr	Asn
Ser	Leu	Leu	Lys	Gln	Ala	Gly 365	Asp	Val	Glu	Glu	Asn 370	Pro	Gly	Pro
Ala 385	Leu	Pro	Val	Thr	Ala	Leu 390	Leu	Leu	Pro	Leu	Ala 395	Leu	Leu	Leu
Ala	Ala	Arg	Pro	Gln	Val	Gln 405	Leu	Gln	Gln	Ser	Gly 410	Ala	Glu	Leu
Arg	Pro	Gly	Ala	Ser	Val	Lys 420	Met	Ser	Cys	Lys	Ala 425	Ser	Gly	Tyr
Phe	Thr	Arg	Tyr	Thr	Met	His 435	Trp	Val	Lys	Gln	Arg 440	Pro	Gly	Gln
Leu	Glu	Trp	Ile	Gly	Tyr	Ile 445	Asn	Pro	Ser	Arg	Gly 450	Tyr	Thr	Asn
Asn 465	Gln	Lys	Phe	Lys	Asp	Lys 470	Ala	Thr	Leu	Thr	Thr 475	Asp	Lys	Ser
Ser	Thr	Ala	Tyr	Met	Gln	Leu 485	Ser	Ser	Leu	Thr	Ser 490	Glu	Asp	Ser
Val	Tyr	Tyr	Cys	Ala	Arg	Tyr 500	Tyr	Asp	Asp	His	Tyr 505	Cys	Leu	Asp

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Trp	Gly	Gln	Gly	Thr	Thr	Leu	Thr	Val	Ser	Ser	Gly	Ser	Thr	Ser	Gly
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Gly	Gly	Ser	Gly	Gly	Gly	Ser	Gly	Gly	Gly	Gly	Ser	Ser	Gln	Ile	Val
	530					535					540				
Leu	Thr	Gln	Ser	Pro	Ala	Ile	Met	Ser	Ala	Ser	Pro	Gly	Glu	Lys	Val
545					550					555					560
Thr	Met	Thr	Cys	Ser	Ala	Ser	Ser	Ser	Val	Ser	Tyr	Met	Asn	Trp	Tyr
			565						570					575	
Gln	Gln	Lys	Ser	Gly	Thr	Ser	Pro	Lys	Arg	Trp	Ile	Tyr	Asp	Thr	Ser
			580					585					590		
Lys	Leu	Ala	Ser	Gly	Val	Pro	Ala	His	Phe	Arg	Gly	Ser	Gly	Ser	Gly
	595						600					605			
Thr	Ser	Tyr	Ser	Leu	Thr	Ile	Ser	Gly	Met	Glu	Ala	Glu	Asp	Ala	Ala
	610					615					620				
Thr	Tyr	Tyr	Cys	Gln	Gln	Trp	Ser	Ser	Asn	Pro	Phe	Thr	Phe	Gly	Ser
625					630					635					640
Gly	Thr	Lys	Leu	Glu	Ile	Asn	Arg	Gly	Gly	Gly	Gly	Ser	Gly	Gly	Gly
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Ser	Leu	Ser	Ala	Ser	Leu	Gly	Asp	Arg	Val	Thr	Ile	Ser	Cys	Arg	Ala
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Ser	Gln	Asp	Ile	Ser	Lys	Tyr	Leu	Asn	Trp	Tyr	Gln	Gln	Lys	Pro	Asp
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Val	Pro	Ser	Arg	Phe	Ser	Gly	Ser	Gly	Ser	Gly	Thr	Asp	Tyr	Ser	Leu
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Thr	Ile	Ser	Asn	Leu	Glu	Gln	Glu	Asp	Ile	Ala	Thr	Tyr	Phe	Cys	Gln
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Gln	Gly	Asn	Thr	Leu	Pro	Tyr	Thr	Phe	Gly	Gly	Gly	Thr	Lys	Leu	Glu
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Ile	Thr	Gly	Ser	Thr	Ser	Gly	Ser	Gly	Lys	Pro	Gly	Ser	Gly	Glu	Gly
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Ser	Thr	Lys	Gly	Glu	Val	Lys	Leu	Gln	Glu	Ser	Gly	Pro	Gly	Leu	Val
785					790					795					800
Ala	Pro	Ser	Gln	Ser	Leu	Ser	Val	Thr	Cys	Thr	Val	Ser	Gly	Val	Ser
			805						810					815	
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Leu	Glu	Trp	Leu	Gly	Val	Ile	Trp	Gly	Ser	Glu	Thr	Thr	Tyr	Tyr	Asn
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Ser	Ala	Leu	Lys	Ser	Arg	Leu	Thr	Ile	Ile	Lys	Asp	Asn	Ser	Lys	Ser
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Gln	Val	Phe	Leu	Lys	Met	Asn	Ser	Leu	Gln	Thr	Asp	Asp	Thr	Ala	Ile
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Tyr	Tyr	Cys	Ala	Lys	His	Tyr	Tyr	Tyr	Gly	Gly	Ser	Tyr	Ala	Met	Asp
			885						890					895	
Tyr	Trp	Gly	Gln	Gly	Thr	Ser	Val	Thr	Val	Ser	Ser	Ala	Ala	Ala	Asp
	900							905					910		
Tyr	Lys	Asp	Asp	Asp	Asp	Lys	Ile	Glu	Val	Met	Tyr	Pro	Pro	Pro	Tyr

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915	920	925
Leu Asp Asn Glu Lys Ser Asn Gly Thr Ile Ile His Val Lys Gly Lys 930 935 940		
His Leu Cys Pro Ser Pro Leu Phe Pro Gly Pro Ser Lys Pro Phe Trp 945 950 955 960		
Val Leu Val Val Val Gly Gly Val Leu Ala Cys Tyr Ser Leu Leu Val 965 970 975		
Thr Val Ala Phe Ile Ile Phe Trp Val Arg Ser Lys Arg Ser Arg Leu 980 985 990		
Leu His Ser Asp Tyr Met Asn Met Thr Pro Arg Arg Pro Gly Pro Thr 995 1000 1005		
Arg Lys His Tyr Gln Pro Tyr Ala Pro Pro Arg Asp Phe Ala Ala 1010 1015 1020		
Tyr Arg Ser Arg Val Lys Phe Ser Arg Ser Ala Asp Ala Pro Ala 1025 1030 1035		
Tyr Gln Gln Gly Gln Asn Gln Leu Tyr Asn Glu Leu Asn Leu Gly 1040 1045 1050		
Arg Arg Glu Glu Tyr Asp Val Leu Asp Lys Arg Arg Gly Arg Asp 1055 1060 1065		
Pro Glu Met Gly Gly Lys Pro Arg Arg Lys Asn Pro Gln Glu Gly 1070 1075 1080		
Leu Tyr Asn Glu Leu Gln Lys Asp Lys Met Ala Glu Ala Tyr Ser 1085 1090 1095		
Glu Ile Gly Met Lys Gly Glu Arg Arg Arg Gly Lys Gly His Asp 1100 1105 1110		
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<210> SEQ ID NO 2

<211> LENGTH: 3417

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic polynucleotide

<400> SEQUENCE: 2

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cttgacatcc tgaagacggt taaagagatt acaggettcc tccttatcca agcgtggccc 300
gagaacagaa cggacttgca cgcctttgag aacctcgaaa taatacgggg tcggacgaag 360
caacacggcc aatttagcct tgcggttggt agtctgaaca ttacttctct cggccttcgc 420
tctttgaaag aaatcagcga cggagatgtc atcattagtg gaaacaagaa cctgtgctac 480
gogaacacaa tcaactggaa gaagctcttc ggtacttcag gccaaaagac aaagattatt 540
agtaacagag gagagaatag ctgtaaggct accggacaag ttgtcacgc cttgtgtagt 600
ccagagggtt gctggggacc ggaaccaagg gattgcgtca gttgccgga cgtgagtcgc 660

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<210> SEQ ID NO 3

<211> LENGTH: 1138

<212> TYPE: PRT

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic polypeptide

<400> SEQUENCE: 3

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20          25          30

Glu Phe Lys Asp Ser Leu Ser Ile Asn Ala Thr Asn Ile Lys His Phe
35          40          45

Lys Asn Cys Thr Ser Ile Ser Gly Asp Leu His Ile Leu Pro Val Ala
50          55          60

Phe Arg Gly Asp Ser Phe Thr His Thr Pro Pro Leu Asp Pro Gln Glu
65          70          75          80

Leu Asp Ile Leu Lys Thr Val Lys Glu Ile Thr Gly Phe Leu Leu Ile
85          90          95

Gln Ala Trp Pro Glu Asn Arg Thr Asp Leu His Ala Phe Glu Asn Leu
100         105         110

Glu Ile Ile Arg Gly Arg Thr Lys Gln His Gly Gln Phe Ser Leu Ala
115         120         125

Val Val Ser Leu Asn Ile Thr Ser Leu Gly Leu Arg Ser Leu Lys Glu
130         135         140

Ile Ser Asp Gly Asp Val Ile Ile Ser Gly Asn Lys Asn Leu Cys Tyr
145         150         155         160

Ala Asn Thr Ile Asn Trp Lys Lys Leu Phe Gly Thr Ser Gly Gln Lys
165         170         175

Thr Lys Ile Ile Ser Asn Arg Gly Glu Asn Ser Cys Lys Ala Thr Gly
180         185         190

Gln Val Cys His Ala Leu Cys Ser Pro Glu Gly Cys Trp Gly Pro Glu
195         200         205

Pro Arg Asp Cys Val Ser Cys Arg Asn Val Ser Arg Gly Arg Glu Cys
210         215         220

Val Asp Lys Cys Asn Leu Leu Glu Gly Glu Pro Arg Glu Phe Val Glu
225         230         235         240

Asn Ser Glu Cys Ile Gln Cys His Pro Glu Cys Leu Pro Gln Ala Met
245         250         255

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Met	Gly	Glu	Asn	Asn	Thr	Leu	Val	Trp	Lys	Tyr	Ala	Asp	Ala	Gly	His	290	295	300
Val	Cys	His	Leu	Cys	His	Pro	Asn	Cys	Thr	Tyr	Gly	Cys	Thr	Gly	Pro	305	310	315
Gly	Leu	Glu	Gly	Cys	Pro	Thr	Asn	Gly	Pro	Lys	Ile	Pro	Ser	Ile	Ala	320	325	330
Thr	Gly	Met	Val	Gly	Ala	Leu	Leu	Leu	Leu	Leu	Val	Val	Ala	Leu	Gly	335	340	345
Ile	Gly	Leu	Phe	Met	Arg	Ala	Lys	Arg	Gly	Ser	Gly	Ala	Thr	Asn	Phe	350	355	360
Ser	Leu	Leu	Lys	Gln	Ala	Gly	Asp	Val	Glu	Glu	Asn	Pro	Gly	Pro	Met	365	370	375
Ala	Leu	Pro	Val	Thr	Ala	Leu	Leu	Leu	Pro	Leu	Ala	Leu	Leu	Leu	His	380	385	390
Ala	Ala	Arg	Pro	Asp	Ile	Gln	Met	Thr	Gln	Ser	Pro	Ser	Ser	Leu	Ser	395	400	405
Ala	Ser	Val	Gly	Asp	Arg	Val	Thr	Ile	Thr	Cys	Arg	Ala	Ser	Gln	Asp	410	415	420
Ile	Arg	Asn	Tyr	Leu	Asn	Trp	Tyr	Gln	Gln	Lys	Pro	Gly	Lys	Ala	Pro	425	430	435
Lys	Leu	Leu	Ile	Tyr	Tyr	Thr	Ser	Arg	Leu	Glu	Ser	Gly	Val	Pro	Ser	440	445	450
Arg	Phe	Ser	Gly	Ser	Gly	Ser	Gly	Thr	Asp	Tyr	Thr	Leu	Thr	Ile	Ser	455	460	465
Ser	Leu	Gln	Pro	Glu	Asp	Phe	Ala	Thr	Tyr	Tyr	Cys	Gln	Gln	Gly	Asn	470	475	480
Thr	Leu	Pro	Trp	Thr	Phe	Gly	Gln	Gly	Thr	Lys	Val	Glu	Ile	Lys	Gly	485	490	495
Gly	Gly	Gly	Ser	Gly	Gly	Gly	Gly	Ser	Gly	Gly	Gly	Gly	Ser	Glu	Val	500	505	510
Gln	Leu	Val	Glu	Ser	Gly	Gly	Gly	Leu	Val	Gln	Pro	Gly	Gly	Ser	Leu	515	520	525
Arg	Leu	Ser	Cys	Ala	Ala	Ser	Gly	Tyr	Ser	Phe	Thr	Gly	Tyr	Thr	Met	530	535	540
Asn	Trp	Val	Arg	Gln	Ala	Pro	Gly	Lys	Gly	Leu	Glu	Trp	Val	Ala	Leu	545	550	555
Ile	Asn	Pro	Tyr	Lys	Gly	Val	Ser	Thr	Tyr	Asn	Gln	Lys	Phe	Lys	Asp	560	565	570
Arg	Phe	Thr	Ile	Ser	Val	Asp	Lys	Ser	Lys	Asn	Thr	Ala	Tyr	Leu	Gln	575	580	585
Met	Asn	Ser	Leu	Arg	Ala	Glu	Asp	Thr	Ala	Val	Tyr	Tyr	Cys	Ala	Arg	590	595	600
Ser	Gly	Tyr	Tyr	Gly	Asp	Ser	Asp	Trp	Tyr	Phe	Asp	Val	Trp	Gly	Gln	605	610	615
Gly	Thr	Leu	Val	Thr	Val	Ser	Ser	Gly	Gly	Gly	Gly	Ser	Gly	Gly	Gly	620	625	630
Gly	Ser	Gly	Gly	Gly	Gly	Ser	Glu	Val	Lys	Leu	Gln	Glu	Ser	Gly	Pro	635	640	645

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Gly	Leu	Val	Ala	Pro	Ser	Gln	Ser	Leu	Ser	Val	Thr	Cys	Thr	Val	Ser		
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Gly	Val	Ser	Leu	Pro	Asp	Tyr	Gly	Val	Ser	Trp	Ile	Arg	Gln	Pro	Pro		
	690					695					700						
Arg	Lys	Gly	Leu	Glu	Trp	Leu	Gly	Val	Ile	Trp	Gly	Ser	Glu	Thr	Thr		
705					710					715					720		
Tyr	Tyr	Asn	Ser	Ala	Leu	Lys	Ser	Arg	Leu	Thr	Ile	Ile	Lys	Asp	Asn		
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Ser	Lys	Ser	Gln	Val	Phe	Leu	Lys	Met	Asn	Ser	Leu	Gln	Thr	Asp	Asp		
			740					745					750				
Thr	Ala	Ile	Tyr	Tyr	Cys	Ala	Lys	His	Tyr	Tyr	Tyr	Gly	Gly	Ser	Tyr		
		755					760					765					
Ala	Met	Asp	Tyr	Trp	Gly	Gln	Gly	Thr	Ser	Val	Thr	Val	Ser	Ser	Gly		
	770					775					780						
Ser	Thr	Ser	Gly	Ser	Gly	Lys	Pro	Gly	Ser	Gly	Glu	Gly	Ser	Thr	Lys		
785					790					795					800		
Gly	Asp	Ile	Gln	Met	Thr	Gln	Thr	Thr	Ser	Ser	Leu	Ser	Ala	Ser	Leu		
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Tyr	Leu	Asn	Trp	Tyr	Gln	Gln	Lys	Pro	Asp	Gly	Thr	Val	Lys	Leu	Leu		
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Ile	Tyr	His	Thr	Ser	Arg	Leu	His	Ser	Gly	Val	Pro	Ser	Arg	Phe	Ser		
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Tyr	Thr	Phe	Gly	Gly	Gly	Thr	Lys	Leu	Glu	Ile	Thr	Ala	Ala	Ala	Asp		
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Leu	Asp	Asn	Glu	Lys	Ser	Asn	Gly	Thr	Ile	Ile	His	Val	Lys	Gly	Lys		
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His	Leu	Cys	Pro	Ser	Pro	Leu	Phe	Pro	Gly	Pro	Ser	Lys	Pro	Phe	Trp		
945					950					955					960		
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Thr	Val	Ala	Phe	Ile	Ile	Phe	Trp	Val	Arg	Ser	Lys	Arg	Ser	Arg	Leu		
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Leu	His	Ser	Asp	Tyr	Met	Asn	Met	Thr	Pro	Arg	Arg	Pro	Gly	Pro	Thr		
	995						1000					1005					
Arg	Lys	His	Tyr	Gln	Pro	Tyr	Ala	Pro	Pro	Arg	Asp	Phe	Ala	Ala			
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Arg	Arg	Glu	Glu	Tyr	Asp	Val	Leu	Asp	Lys	Arg	Arg	Gly	Arg	Asp			
	1055					1060						1065					

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Glu	Ile	Gly	Met	Lys	Gly	Glu	Arg	Arg	Arg	Gly	Lys	Gly	His	Asp
1100						1105					1110			
Gly	Leu	Tyr	Gln	Gly	Leu	Ser	Thr	Ala	Thr	Lys	Asp	Thr	Tyr	Asp
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<210> SEQ ID NO 4

<211> LENGTH: 3417

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic polynucleotide

<400> SEQUENCE: 4

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atccccagaa aggtgtgcaa cggtcatcgcc attggagagt tcaaggattc ttttaagcatc      120
aacgtaccac acatcaaaca tttcaagaac tgtacctcca tttccggcga tttacacatc      180
ctccccgttg cctttcgtgg cgatagcttc acacacacac cccctctgga ccctcaagaa      240
ctggacattt taaagaccgt gaaggagatc actggttttt tactgatcca agcttgcccc      300
gaaaatagga cagatctcca cgctttcgag aatttagaga tcattcgtgg cagaaccaag      360
cagcacggac agttctcttt agccgtcgtg tctttaaata tcacctcttt aggtttaaga      420
tctttaaaag agatcagcga tggcgacgtc atcatctccg gcaacaagaa cctctgttac      480
gccaacacca ttaattggaa aaagctgttt ggcacctccg gacagaagac caagatcac      540
agcaacagag gcgagaacag ctgcaaagct accggccaag tttgccagc tctgtgtagc      600
cccgaaggct gctggggacc cgaacctcgt gactgtgtga gctgtaggaa cgtgtctcgt      660
ggctgtgagt gtgtgtgataa gtgcaattta ctcgagggcg agcccagaga gtttgtggaa      720
aatagcgagt gcattccagt ccaccccgaa tgtctgcccc aagctatgaa cattacttgt      780
actggtcgtg gccccgataa ctgtatccag tgcgctcact acattgacgg cccccactgc      840
gtcaagacat gccccgctgg cgtgatggga gaaaacaaca cactgggtgtg gaagtatgcc      900
gatgccggcc acgtctgtca tctgtgccac cctaactgta cctacggctg taccggaacc      960
ggactggagg gctgtcccac caacggaccc aagatcccta gcatcgccac cggcatgggt      1020
ggagccttac tgetgttact ggtggtggct ctgggcattg gtttattcat gagggccaag      1080
agaggatccg gcgccaccaa cttttcttta ctgaaacaag ctggagacgt cgaggaaaac      1140
cccggaacca tggtctctcc cgtgacagct ctgctgctgc ctctcgcttt attactgcac      1200
gccgctaggg ccgatattca gatgacccaa agccctagct ctttatccgc cagcgtcgga      1260
gatagagtca caatcacttg tagagccagc caagatattc gtaattatct caactggtac      1320
cagcagaagc ccggtaaagc ccccaagctg ctcatctatt acacctctcg tctggagagc      1380
ggcgtgcctt ccagattcag cggtccggc agcggcaccg actatacact caccattagc      1440
tctttacagc ccgaagattt cgctacctac tactgccagc aaggtaatatc tttaccttgg      1500

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accttcggcc aaggtaccaa ggtcgaaatc aagggcggcg gcggtaccgg cggcggtgga 1560
tctggtggcg gcggtccga agtcagctg gtcgaatccg gagcggaact ggtccagccc 1620
ggtggatccc tcagactgag ctgcgcgcgt agcggctatt ccttcaccgg ctacaccatg 1680
aactgggtga gacaagctcc cggcaaagga ctggaatggg tggccctcat caaccctac 1740
aagggcggtg ccacatataa tcaaaagttt aaggacagat tcaccatcag cgtcgacaag 1800
tccaagaaca ccgcttattt acagatgaac tctttaagag ctgaggatac cgcggtgtac 1860
tattgtgcta ggtccggtc ctacggcgac agcgactggt attttgacgt ctggggacaa 1920
ggtaccctcg tgacagttag cagcggcggt ggcggttctg gcgcgaggag ctccggagga 1980
ggcggcagcg aggtgaagct gcaagaaagc ggaccggctc tcgtggctcc ctccaatct 2040
ttatccgtga cttgtaccgt gtcgggagtc tctttaccgg actacggcgt gagctggatt 2100
agacagcccc ccagaaaagg ttagagtgg ctgggcgtga tctggggatc cgagaccaca 2160
tactacaaca gcgctttaa gtctcgtctg acaatcatca aagacaattc caaaagccaa 2220
gttttctcga agatgaactc ttacagacc gacgacacag ccatttacta ctgcgccaag 2280
cattattact acggcggcag ctacgctatg gactactggg gccaaagtac aagcgtcaca 2340
gtgagctccg gcagcacatc cggaagcggg aagcccgcca gcgagagggg cagcacaaa 2400
ggagacatcc agatgaccca gaccacctcc tctttaagcg cctctttagg agatagggtg 2460
accattagct gcagagctc ccaagacatc agcaagtatc tcaattggta ccagcaaaag 2520
cccgatggca ccgtcaagct gctgatctac cacacctcgc gtctccattc cggcgtgccc 2580
agcagattta gcggaagcgg atccggaaca gactattctt taacaatcag caatttagag 2640
caagaagaca tcgccacata tttctgcca caaggtataa ctttacccta caccttcgga 2700
ggcggcacca aactggagat tacagcggcc gcagactaca aagacgatga cgacaagatt 2760
gaagttagt atcctctccc ttacctagac aatgagaaga gcaatggaac cattatccat 2820
gtgaaagggg aacacctttg tccaagtccc ctatttcccg gaccttctaa gcccttttgg 2880
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actccccgcc gccccggggc cccccgaag cattaccagc cctatgcccc accacgcgac 3060
ttcgagcct atcgctccag agtgaagttc agcaggagcg cagacgcccc cgcgtaccag 3120
cagggccaga accagctcta taacgagctc aatctaggac gaagagagga gtacgatgtt 3180
ttggacaaga gacgtggccg ggaccctgag atggggggaa agccgagaag gaagaaccct 3240
caggaaggcc tgtacaatga actgcagaaa gataagatgg cggaggccta cagttagatt 3300
gggatgaaag gcgagcgccg gaggggcaag gggcacgatg gcctttacca gggctctcagt 3360
acagccacca aggacaccta cgacgccctt cacatgcagg ccctgcccc tcgctaa 3417

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<210> SEQ ID NO 5

<211> LENGTH: 20

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 5

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tgtgctagac atgaggtcta 20

<210> SEQ ID NO 6

<400> SEQUENCE: 6

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<210> SEQ ID NO 7

<211> LENGTH: 157

<212> TYPE: PRT

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic polypeptide

<400> SEQUENCE: 7

Met Gly Thr Ser Leu Leu Cys Trp Met Ala Leu Cys Leu Leu Gly Ala
1 5 10 15

Asp His Ala Asp Ala Cys Pro Tyr Ser Asn Pro Ser Leu Cys Ser Gly
20 25 30

Gly Gly Gly Ser Glu Leu Pro Thr Gln Gly Thr Phe Ser Asn Val Ser
35 40 45

Thr Asn Val Ser Pro Ala Lys Pro Thr Thr Thr Ala Cys Pro Tyr Ser
50 55 60

Asn Pro Ser Leu Cys Ser Gly Gly Gly Gly Ser Pro Ala Pro Arg Pro
65 70 75 80

Pro Thr Pro Ala Pro Thr Ile Ala Ser Gln Pro Leu Ser Leu Arg Pro
85 90 95

Glu Ala Cys Arg Pro Ala Ala Gly Gly Ala Val His Thr Arg Gly Leu
100 105 110

Asp Phe Ala Cys Asp Ile Tyr Ile Trp Ala Pro Leu Ala Gly Thr Cys
115 120 125

Gly Val Leu Leu Leu Ser Leu Val Ile Thr Leu Tyr Cys Asn His Arg
130 135 140

Asn Arg Arg Arg Val Cys Lys Cys Pro Arg Pro Val Val
145 150 155

<210> SEQ ID NO 8

<211> LENGTH: 534

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic polynucleotide

<400> SEQUENCE: 8

atgggcacct ctctgtgtg ctggatggct ctgtgtctgc tgggcgctga ccatgctgat 60

gcttgccctt attccaaccc ctctctgtgc tccggaggag gaggatccga actgcctacc 120

caaggcacct tcagcaacgt gtccaccaac gtgagccccg ctaagcctac caccacgcgt 180

tgcccttact ccaatcccag cctctgctcc ggaggcggag gatccccgc cccagacct 240

cctacacccg ctcccacaat cgccagccag cctctgtctc tgagaccga agcttgacga 300

cccgtgcccg gaggagctgt gcatacaaga ggactggatt tcgcttgoga catctacatc 360

tgggcccctc tggtggcac atgtggcgtg ctgctgtgtg ctctggtcat tacactgtac 420

tgcaaccata gaaatagaag aagggtgtgc aagtgtccca gaccctgggt gggcagcgga 480

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gaaggaagag gctctctgct gacatgcgga gacgtggaag agaaccocgg cccc

534

<210> SEQ ID NO 9

<211> LENGTH: 497

<212> TYPE: PRT

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic polypeptide

<400> SEQUENCE: 9

Met Leu Leu Leu Val Thr Ser Leu Leu Leu Cys Glu Leu Pro His Pro
1 5 10 15

Ala Phe Leu Leu Ile Pro Asp Ile Gln Met Thr Gln Thr Thr Ser Ser
20 25 30

Leu Ser Ala Ser Leu Gly Asp Arg Val Thr Ile Ser Cys Arg Ala Ser
35 40 45

Gln Asp Ile Ser Lys Tyr Leu Asn Trp Tyr Gln Gln Lys Pro Asp Gly
50 55 60

Thr Val Lys Leu Leu Ile Tyr His Thr Ser Arg Leu His Ser Gly Val
65 70 75 80

Pro Ser Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Tyr Ser Leu Thr
85 90 95

Ile Ser Asn Leu Glu Gln Glu Asp Ile Ala Thr Tyr Phe Cys Gln Gln
100 105 110

Gly Asn Thr Leu Pro Tyr Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile
115 120 125

Thr Gly Ser Thr Ser Gly Ser Gly Lys Pro Gly Ser Gly Glu Gly Ser
130 135 140

Thr Lys Gly Glu Val Lys Leu Gln Glu Ser Gly Pro Gly Leu Val Ala
145 150 155 160

Pro Ser Gln Ser Leu Ser Val Thr Cys Thr Val Ser Gly Val Ser Leu
165 170 175

Pro Asp Tyr Gly Val Ser Trp Ile Arg Gln Pro Pro Arg Lys Gly Leu
180 185 190

Glu Trp Leu Gly Val Ile Trp Gly Ser Glu Thr Thr Tyr Tyr Asn Ser
195 200 205

Ala Leu Lys Ser Arg Leu Thr Ile Ile Lys Asp Asn Ser Lys Ser Gln
210 215 220

Val Phe Leu Lys Met Asn Ser Leu Gln Thr Asp Asp Thr Ala Ile Tyr
225 230 235 240

Tyr Cys Ala Lys His Tyr Tyr Tyr Gly Gly Ser Tyr Ala Met Asp Tyr
245 250 255

Trp Gly Gln Gly Thr Ser Val Thr Val Ser Ser Ala Ala Ala Asp Tyr
260 265 270

Lys Asp Asp Asp Asp Lys Ile Glu Val Met Tyr Pro Pro Pro Tyr Leu
275 280 285

Asp Asn Glu Lys Ser Asn Gly Thr Ile Ile His Val Lys Gly Lys His
290 295 300

Leu Cys Pro Ser Pro Leu Phe Pro Gly Pro Ser Lys Pro Phe Trp Val
305 310 315 320

Leu Val Val Val Gly Gly Val Leu Ala Cys Tyr Ser Leu Leu Val Thr
325 330 335

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Val Ala Phe Ile Ile Phe Trp Val Arg Ser Lys Arg Ser Arg Leu Leu
340 345 350

His Ser Asp Tyr Met Asn Met Thr Pro Arg Arg Pro Gly Pro Thr Arg
355 360 365

Lys His Tyr Gln Pro Tyr Ala Pro Pro Arg Asp Phe Ala Ala Tyr Arg
370 375 380

Ser Arg Val Lys Phe Ser Arg Ser Ala Asp Ala Pro Ala Tyr Gln Gln
385 390 395 400

Gly Gln Asn Gln Leu Tyr Asn Glu Leu Asn Leu Gly Arg Arg Glu Glu
405 410 415

Tyr Asp Val Leu Asp Lys Arg Arg Gly Arg Asp Pro Glu Met Gly Gly
420 425 430

Lys Pro Arg Arg Lys Asn Pro Gln Glu Gly Leu Tyr Asn Glu Leu Gln
435 440 445

Lys Asp Lys Met Ala Glu Ala Tyr Ser Glu Ile Gly Met Lys Gly Glu
450 455 460

Arg Arg Arg Gly Lys Gly His Asp Gly Leu Tyr Gln Gly Leu Ser Thr
465 470 475 480

Ala Thr Lys Asp Thr Tyr Asp Ala Leu His Met Gln Ala Leu Pro Pro
485 490 495

Arg

<210> SEQ ID NO 10
<211> LENGTH: 1491
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
polynucleotide

<400> SEQUENCE: 10

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atgcttctcc tggtgacaag ccttctgctc tgtgagttac cacaccagc attcctcctg      60
atcccagaca tccagatgac acagactaca tctcctctgt ctgcctctct gggagacaga      120
gtcaccatca gttgcagggc aagtcaggac attagtaaat atttaaattg gtatcagcag      180
aaaccagatg gaactgttaa actcctgata taccatacat caagattaca ctcaggagtc      240
ccatcaagggt tcagtggcag tgggtctgga acagattatt ctctcccat tagcaacctg      300
gagcaagaag atattgccac ttacttttgc caacagggta atacgcttcc gtacacgttc      360
ggagggggga ctaagttgga aataacaggc tccacctctg gatccggcaa gcccgatct      420
ggcgagggat ccaccaaggg cgagggtgaaa ctgcaggagt caggacctgg cctgggtggcg      480
ccctcacaga gctgtccgt cacatgcact gtctcagggg tctcattacc cgactatggt      540
gtaagctgga ttcgccagcc tccacgaaag ggtctggagt ggctgggagt aatatggggt      600
agtgaacca catactataa ttcagctctc aaatccagac tgaccatcat caaggacaac      660
tccaagagcc aagttttctt aaaaatgaac agtctgcaaa ctgatgacac agccatttac      720
tactgtgcc aacattatta ctacgggtgt agctatgcta tggactactg gggatcaagga      780
acctcagtea ccgtctcctc agcgcccgca gactacaaag acgatgacga caagattgaa      840
gttatgtatc ctctcctta cctagacaat gagaagagca atggaacat tatccatgtg      900
aaagggaaac acctttgtcc aagtcccta tttccggac cttctaagcc cttttgggtg      960

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ctggtggtgg ttgggggagt cctggcttgc tatagcttgc tagtaacagt ggcctttatt 1020
atthttctggg tgaggagtaa gaggagcagg ctctgcaca gtgactacat gaacatgact 1080
ccccgccgcc cggggccac ccgcaagcat taccagccct atgccccacc acgcgacttc 1140
gcagcctatc gctccagagt gaagtccagc aggagcgcag acgccccgc gtaccagcag 1200
ggccagaacc agctctataa cgagctcaat ctaggacgaa gagaggagta cgatgttttg 1260
gacaagagac gtggccggga cctgagatg gggggaaagc cgagaaggaa gaaccctcag 1320
gaaggcctgt acaatgaact gcagaaagat aagatggcgg aggcctacag tgagattggg 1380
atgaaaggcg agcgccggag gggcaagggg cacgatggcc tttaccaggg tctcagtaca 1440
gccaccaagg acacctacga cgccttcac atgcaggccc tgccccctcg c 1491

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<210> SEQ ID NO 11
<211> LENGTH: 395
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
                        polypeptide

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<400> SEQUENCE: 11

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Met Asp Trp Thr Trp Ile Leu Phe Leu Val Ala Ala Thr Arg Val
1      5      10      15
His Ser Asn Trp Val Asn Val Ile Ser Asp Leu Lys Lys Ile Glu Asp
20     25     30
Leu Ile Gln Ser Met His Ile Asp Ala Thr Leu Tyr Thr Glu Ser Asp
35     40     45
Val His Pro Ser Cys Lys Val Thr Ala Met Lys Cys Phe Leu Leu Glu
50     55     60
Leu Gln Val Ile Ser Leu Glu Ser Gly Asp Ala Ser Ile His Asp Thr
65     70     75     80
Val Glu Asn Leu Ile Ile Leu Ala Asn Asn Ser Leu Ser Ser Asn Gly
85     90     95
Asn Val Thr Glu Ser Gly Cys Lys Glu Cys Glu Glu Leu Glu Glu Lys
100    105    110
Asn Ile Lys Glu Phe Leu Gln Ser Phe Val His Ile Val Gln Met Phe
115    120    125
Ile Asn Thr Ser Ser Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly
130    135    140
Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Ser Leu Gln Ile Thr
145    150    155    160
Cys Pro Pro Pro Met Ser Val Glu His Ala Asp Ile Trp Val Lys Ser
165    170    175
Tyr Ser Leu Tyr Ser Arg Glu Arg Tyr Ile Cys Asn Ser Gly Phe Lys
180    185    190
Arg Lys Ala Gly Thr Ser Ser Leu Thr Glu Cys Val Leu Asn Lys Ala
195    200    205
Thr Asn Val Ala His Trp Thr Thr Pro Ser Leu Lys Cys Ile Arg Asp
210    215    220
Pro Ala Leu Val His Gln Arg Pro Ala Pro Pro Ser Thr Val Thr Thr
225    230    235    240
Ala Gly Val Thr Pro Gln Pro Glu Ser Leu Ser Pro Ser Gly Lys Glu
245    250    255

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Pro Ala Ala Ser Ser Pro Ser Ser Asn Asn Thr Ala Ala Thr Thr Ala
 260 265 270

Ala Ile Val Pro Gly Ser Gln Leu Met Pro Ser Lys Ser Pro Ser Thr
 275 280 285

Gly Thr Thr Glu Ile Ser Ser His Glu Ser Ser His Gly Thr Pro Ser
 290 295 300

Gln Thr Thr Ala Lys Asn Trp Glu Leu Thr Ala Ser Ala Ser His Gln
 305 310 315 320

Pro Pro Gly Val Tyr Pro Gln Gly His Ser Asp Thr Thr Val Ala Ile
 325 330 335

Ser Thr Ser Thr Val Leu Leu Cys Gly Leu Ser Ala Val Ser Leu Leu
 340 345 350

Ala Cys Tyr Leu Lys Ser Arg Gln Thr Pro Pro Leu Ala Ser Val Glu
 355 360 365

Met Glu Ala Met Glu Ala Leu Pro Val Thr Trp Gly Thr Ser Ser Arg
 370 375 380

Asp Glu Asp Leu Glu Asn Cys Ser His His Leu
 385 390 395

<210> SEQ ID NO 12
 <211> LENGTH: 1188
 <212> TYPE: DNA
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
 polynucleotide

<400> SEQUENCE: 12

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atggattgga cttggatttt gttcctcggt gccgcagcga ctgcggtcca tagtaattgg      60
gtgaacgtaa ttagtgactt gaaaaaaatt gaggacctta tacaagtat gcatatcgat      120
gcaacactgt acacggagtc cgactgcac ccaagctgca aggtcacgcg aatgaaatgc      180
tttttgctcg aattgcaagt tatctcactt gagtcagggg acgcttcaat ccatgatact      240
gtggagaatt tgataatcct ggcgaacaat agccttagtt caaatggcaa cgtcactgag      300
tcaggctgca aggaatgtga ggaattggaa gaaaaaata tcaaggaatt ttgcaatct      360
tttgttcaca tagttcagat gttcattaac actagtccg gggcgggcag tggaggtggc      420
ggtagcggcg ggggtggctc tgggtggagg ggcctctggg gcggaagtct gcagataaca      480
tgccccccac ctatgagtgt tgaacatgct gatctctggg ttaaatctta ctccctttac      540
agtcgagaaa ggtacatttg caactccggc tttaaacgca aagccgggac tagttcactg      600
actgaatgtg tattgaataa agcgacaaat gtcgcacact ggactacccc ttccctcaaa      660
tgcatctcgc atcctgcctt ggtgcatcag cgaccagcac cgccgtccac ggtaactacc      720
gcaggagtaa caccgcagcc cgagagcctt tccccctcag gcaaagagcc ggccgcatcc      780
tccccatctt ccaataatac cgcagctacc accgcagcaa tcgtaccggg gtcccagctg      840
atgcccgaca aaagtccgag tactggaacg actgaaatct ccagtcacga gtcttctcat      900
ggaactccga gtcaaaactac agcaaagaat tgggagctga ctgcttcgcg ttcacaccag      960
ccgccaggcg tttatctca gggacactca gataccagg tggcgattag cacaagcacc     1020
gtcctcctgt gtgggctgag tgcagtgtca cttctcgct gctaccttaa gtccagacag     1080
acacccctt tggcaagcgt tgaaatggaa gccatggaag ccttgctgtg cacatggggg     1140

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acttcatccc gcgatgaaga cttggagaac tgctcacacc atctttga 1188

<210> SEQ ID NO 13
 <211> LENGTH: 177
 <212> TYPE: PRT
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic polypeptide

<400> SEQUENCE: 13

Met Phe His Val Ser Phe Arg Tyr Ile Phe Gly Leu Pro Pro Leu Ile
 1 5 10 15
 Leu Val Leu Leu Pro Val Ala Ser Ser Asp Cys Asp Ile Glu Gly Lys
 20 25 30
 Asp Gly Lys Gln Tyr Glu Ser Val Leu Met Val Ser Ile Asp Gln Leu
 35 40 45
 Leu Asp Ser Met Lys Glu Ile Gly Ser Asn Cys Leu Asn Asn Glu Phe
 50 55 60
 Asn Phe Phe Lys Arg His Ile Cys Asp Ala Asn Lys Glu Gly Met Phe
 65 70 75 80
 Leu Phe Arg Ala Ala Arg Lys Leu Arg Gln Phe Leu Lys Met Asn Ser
 85 90 95
 Thr Gly Asp Phe Asp Leu His Leu Leu Lys Val Ser Glu Gly Thr Thr
 100 105 110
 Ile Leu Leu Asn Cys Thr Gly Gln Val Lys Gly Arg Lys Pro Ala Ala
 115 120 125
 Leu Gly Glu Ala Gln Pro Thr Lys Ser Leu Glu Glu Asn Lys Ser Leu
 130 135 140
 Lys Glu Gln Lys Lys Leu Asn Asp Leu Cys Phe Leu Lys Arg Leu Leu
 145 150 155 160
 Gln Glu Ile Lys Thr Cys Trp Asn Lys Ile Leu Met Gly Thr Lys Glu
 165 170 175
 His

<210> SEQ ID NO 14
 <211> LENGTH: 614
 <212> TYPE: DNA
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic polynucleotide

<400> SEQUENCE: 14

gaattcggca gcggcgctcaa gcagacactg aacttcgatac tgctgaagct ggccggagac 60
 gtcgagagca accccggccc tatgttcac gtgagcttta gatacatctt cggactgccc 120
 cctctgattc tgggtgctgct gcccgtagcc agcagcgact gcgatatcga gggcaaggac 180
 ggcaagcagt atgagtcogt gctgatggtc tccatcgatac agctgctgga cagcatgaag 240
 gagatcggt ccaactgect caacaacgag ttcaactttt tcaagaggca catctgcgac 300
 gccacaagg agggcatggt tctgtttaga gccgctagaa agctgaggca gtttctgaag 360
 atgaacagca ccggcgactt tgatctgcat ctgctgaaag tgagcgaggg caccaccatt 420
 ctgctgaact gcaccggcca agtgaaagga agaaagcccg ccgctctggg cgaggctcag 480

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cctaccaagt ctctggaaga gaacaagtct ctgaaggagc agaagaagct caacgatctg 540
tgcttctctca agaggctgct gcaagagatc aagacatgct ggaacaagat cctcatgggc 600
accaaggaac actg 614

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<210> SEQ ID NO 15
<211> LENGTH: 513
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
                        polypeptide

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<400> SEQUENCE: 15

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Met Leu Val Arg Arg Gly Ala Arg Ala Gly Pro Arg Met Pro Arg Gly
1      5      10      15
Trp Thr Ala Leu Cys Leu Leu Ser Leu Leu Pro Ser Gly Phe Met Ser
20     25     30
Leu Asp Asn Asn Gly Thr Ala Thr Pro Glu Leu Pro Thr Gln Gly Thr
35     40     45
Phe Ser Asn Val Ser Thr Asn Val Ser Tyr Gln Glu Thr Thr Thr Pro
50     55     60
Ser Thr Leu Gly Ser Thr Ser Leu His Pro Val Ser Gln His Gly Asn
65     70     75     80
Glu Ala Thr Thr Asn Ile Thr Glu Thr Thr Val Lys Phe Thr Ser Thr
85     90     95
Ser Val Ile Thr Ser Val Tyr Gly Asn Thr Asn Ser Ser Val Gln Ser
100    105    110
Gln Thr Ser Val Ile Ser Thr Val Phe Thr Thr Pro Ala Asn Val Ser
115    120    125
Thr Pro Glu Thr Thr Leu Lys Pro Ser Leu Ser Pro Gly Asn Val Ser
130    135    140
Asp Leu Ser Thr Thr Ser Thr Ser Leu Ala Thr Ser Pro Thr Lys Pro
145    150    155    160
Tyr Thr Ser Ser Ser Pro Ile Leu Ser Asp Ile Lys Ala Glu Ile Lys
165    170    175
Cys Ser Gly Ile Arg Glu Val Lys Leu Thr Gln Gly Ile Cys Leu Glu
180    185    190
Gln Asn Lys Thr Ser Ser Cys Ala Glu Phe Lys Lys Asp Arg Gly Glu
195    200    205
Gly Leu Ala Arg Val Leu Cys Gly Glu Glu Gln Ala Asp Ala Asp Ala
210    215    220
Gly Ala Gln Val Cys Ser Leu Leu Leu Ala Gln Ser Glu Val Arg Pro
225    230    235    240
Gln Cys Leu Leu Leu Val Leu Ala Asn Arg Thr Glu Ile Ser Ser Lys
245    250    255
Leu Gln Leu Met Lys Lys His Gln Ser Asp Leu Lys Lys Leu Gly Ile
260    265    270
Leu Asp Phe Thr Glu Gln Asp Val Ala Ser His Gln Ser Tyr Ser Gln
275    280    285
Lys Thr Pro Ile Leu Leu Thr Cys Pro Thr Ile Ser Ile Leu Ser Phe
290    295    300
Phe Ser Val Ala Leu Leu Val Ile Leu Ala Cys Val Leu Trp Lys Lys
305    310    315    320

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Gln

<400> SEQUENCE: 16

gggaagcgggcg	ccacaaactt	ttctctgctg	aagcaagcgg	gcgacgtcga	agagaacccc	60
ggccctatgc	tgttgaggag	aggcgctagg	gctggacca	gaatgcccg	aggctggacc	120
gctctgtgtc	tgtgtctct	gctgccagc	ggcttcata	gcctcgataa	taacggcacc	180
gctacccccg	agctgcccac	acaaggcacc	ttctccaatg	tgagcaccaa	cgtgtcctac	240
caagagacca	ccacccttc	cacactggga	agcacatctc	tgcattccgt	ctcccagcac	300
ggcaatgaag	ccaccacaaa	catcaccgag	accaccgtga	agttcaccag	cacctccgtc	360
attaccagcg	tgtacggcaa	caccaatagc	tccgtgcaaa	gccagacatc	cgtgatttcc	420
accgtgttta	ccacccccgc	caatgtcagc	acaccggaga	caacactgaa	acctttctctg	480
tcccccgaa	acgtgagcga	tctgagcaca	accagcacca	gcctcgccac	cagccccaca	540
aagccttaca	caagcagcag	ccccattctg	agcgacatca	aggccgaaat	caagtgtctc	600
ggaattagag	aggtaagct	gacccaagga	atctgtctgg	agcagaataa	gaccagcagc	660
tgcccgagtg	tcaagaagaa	cagaggcgaa	ggactggcca	gagtgtctctg	cggcgaggaa	720
caagccgatg	ccgatgcccg	agctcaagtg	tgacgcctcc	tcctcgctca	gagcggagtc	780

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agaccccaat gtctgctgct cgtgctggcc aataggaccg agatctcctc caaactgcag    840
ctgatgaaga agcaccagag cgacctcaag aagctcggca tcctcgactt taccgagcaa    900
gacgtggcct cccatcaatc ctatagccag aagaccccca ttctgctgac atgtcccaca    960
atcagcatec tcagcttctt cagcgteget ctgctcgtca ttctggcttg tgtgctgtgg   1020
aagaagagga tcaagcctat tgtgtggccc tctctgcccg accacaagaa gacctcgaa   1080
cacctctgca agaaacctag aaagaacctc aacgtgagct tcaaccocga gtcctttctg   1140
gactgtcaaa tccatagggt ggatgacatc caagctagag acgaggtcga gggctttctg   1200
caagacacct tccctcagca gctggaagaa agcgagaagc aaagactggg cggagatgtg   1260
cagtecccta attgccctc cgaggacgtg gtgattaccc cggagagctt cggaagagac   1320
agctctctga catgtctggc cggaatgtg tccgcttgcg atgccctat tctgagcagc   1380
tccagatctc tggactgcag agagtccggc aagaacggcc ctcatgtgta ccaagatctg   1440
ctgctgtctc tgggaaccac aaactccaca ctgctccccc cctttagcct ccagtccggc   1500
attctgacac tgaaccccg gtctcaaggc caacctatcc tcacatccct cggtccaat   1560
caagaggaag cctacgtgac catgagctcc ttctatcaga accagtga           1608

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<210> SEQ ID NO 17

<211> LENGTH: 635

<212> TYPE: PRT

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic polypeptide

<400> SEQUENCE: 17

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Met Ala Leu Pro Val Thr Ala Leu Leu Leu Pro Leu Ala Leu Leu Leu
1          5          10         15

His Ala Ala Arg Pro Asp Cys Asp Ile Glu Gly Lys Asp Gly Lys Gln
          20         25         30

Tyr Glu Ser Val Leu Met Val Ser Ile Asp Gln Leu Leu Asp Ser Met
          35         40         45

Lys Glu Ile Gly Ser Asn Cys Leu Asn Asn Glu Phe Asn Phe Phe Lys
          50         55         60

Arg His Ile Cys Asp Ala Asn Lys Glu Gly Met Phe Leu Phe Arg Ala
          65         70         75         80

Ala Arg Lys Leu Arg Gln Phe Leu Lys Met Asn Ser Thr Gly Asp Phe
          85         90         95

Asp Leu His Leu Leu Lys Val Ser Glu Gly Thr Thr Ile Leu Leu Asn
          100        105        110

Cys Thr Gly Gln Val Lys Gly Arg Lys Pro Ala Ala Leu Gly Glu Ala
          115        120        125

Gln Pro Thr Lys Ser Leu Glu Glu Asn Lys Ser Leu Lys Glu Gln Lys
          130        135        140

Lys Leu Asn Asp Leu Cys Phe Leu Lys Arg Leu Leu Gln Glu Ile Lys
          145        150        155        160

Thr Cys Trp Asn Lys Ile Leu Met Gly Thr Lys Glu His Gly Gly Gly
          165        170        175

Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Ser
          180        185        190

Gly Gly Gly Ser Glu Ser Gly Tyr Ala Gln Asn Gly Asp Leu Glu Asp

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195					200					205					
Ala 210	Glu	Leu	Asp	Asp	Tyr	Ser 215	Phe	Ser	Cys	Tyr	Ser 220	Gln	Leu	Glu	Val
Asn 225	Gly	Ser	Gln	His	Ser 230	Leu	Thr	Cys	Ala	Phe 235	Glu	Asp	Pro	Asp	Val 240
Asn	Ile	Thr	Asn	Leu	Glu 245	Phe	Glu	Ile	Cys 250	Gly	Ala	Leu	Val	Glu	Val 255
Lys	Cys	Leu	Asn	Phe	Arg 260	Lys	Leu	Gln	Glu 265	Ile	Tyr	Phe	Ile	Glu	Thr 270
Lys	Lys	Phe 275	Leu	Leu	Ile	Gly 280	Lys	Ser	Asn	Ile	Cys 285	Val	Lys	Val	Gly
Glu 290	Lys	Ser	Leu	Thr	Cys 295	Lys	Lys	Ile	Asp	Leu	Thr 300	Thr	Ile	Val	Lys
Pro 305	Glu	Ala	Pro	Phe	Asp 310	Leu	Ser	Val	Val	Tyr 315	Arg	Glu	Gly	Ala	Asn 320
Asp	Phe	Val	Val	Thr	Phe 325	Asn	Thr	Ser	His 330	Leu	Gln	Lys	Lys	Tyr	Val 335
Lys	Val	Leu	Met	His	Asp 340	Val	Ala	Tyr 345	Arg	Gln	Glu	Lys	Asp	Glu	Asn 350
Lys	Trp	Thr 355	His	Val	Asn	Leu	Ser 360	Ser	Thr	Lys	Leu	Thr 365	Leu	Leu	Gln
Arg 370	Lys	Leu	Gln	Pro	Ala 375	Ala	Met	Tyr	Glu	Ile	Lys 380	Val	Arg	Ser	Ile
Pro 385	Asp	His	Tyr	Phe	Lys 390	Gly	Phe	Trp	Ser	Glu 395	Trp	Ser	Pro	Ser	Tyr 400
Tyr	Phe	Arg	Thr	Pro	Glu 405	Ile	Asn	Asn	Ser 410	Ser	Gly	Glu	Met	Asp	Pro 415
Ile	Leu	Leu	Thr	Ile	Ser 420	Ile	Leu	Ser 425	Phe	Phe	Ser	Val	Ala	Leu	Leu 430
Val	Ile	Leu 435	Ala	Cys	Val	Leu	Trp 440	Lys	Lys	Arg	Ile	Lys 445	Pro	Ile	Val 450
Trp	Pro	Ser 450	Leu	Pro	Asp 455	His	Lys	Lys	Thr	Leu	Glu 460	His	Leu	Cys	Lys 465
Lys 465	Pro	Arg	Lys	Asn	Leu 470	Asn	Val	Ser	Phe	Asn 475	Pro	Glu	Ser	Phe	Leu 480
Asp	Cys	Gln	Ile	His	Arg 485	Val	Asp	Asp	Ile 490	Gln	Ala	Arg	Asp	Glu	Val 495
Glu	Gly	Phe	Leu	Gln	Asp 500	Thr	Phe	Pro	Gln 505	Gln	Leu	Glu	Glu	Ser	Glu 510
Lys	Gln	Arg 515	Leu	Gly	Gly 520	Asp	Val	Gln	Ser	Pro	Asn	Cys 525	Pro	Ser	Glu 530
Asp 530	Val	Val	Ile	Thr	Pro 535	Glu	Ser	Phe	Gly	Arg	Asp 540	Ser	Ser	Leu	Thr 545
Cys 545	Leu	Ala	Gly	Asn	Val 550	Ser	Ala	Cys	Asp	Ala 555	Pro	Ile	Leu	Ser	Ser 560
Ser	Arg	Ser	Leu	Asp	Cys 565	Arg	Glu	Ser	Gly 570	Lys	Asn	Gly	Pro	His	Val 575
Tyr	Gln	Asp 580	Leu	Leu	Leu 585	Ser	Leu	Gly 585	Thr	Thr	Asn	Ser	Thr	Leu	Pro 590
Pro	Pro	Phe 595	Ser	Leu	Gln 600	Ser	Gly	Ile	Leu	Thr	Leu	Asn 605	Pro	Val	Ala 610

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Gln Gly Gln Pro Ile Leu Thr Ser Leu Gly Ser Asn Gln Glu Glu Ala
610 615 620

Tyr Val Thr Met Ser Ser Phe Tyr Gln Asn Gln
625 630 635

<210> SEQ ID NO 18

<211> LENGTH: 1905

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic polynucleotide

<400> SEQUENCE: 18

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atggctctgc ctgttacagc tctgctgctg cctctggctc tgcttctgca tgccgccaga    60
cctgactgtg acatcgaggg caaagacggc aagcagtagc agagcgtgct gatgggtgcc    120
atcgaccagc tgctggacag catgaaggaa atcggcagca actgcctgaa caacgagttc    180
aacttcttca agcggcacat ctgcgacgcc aacaaagaag gcatgttctc gttcagagcc    240
gccagaaaagc tgccggcagtt cctgaagatg aacagcaccc gcgacttcga cctgcatctg    300
ctgaaagtgt ctgagggcac caccatctct ctgaattgca ccggccaagt gaagggcaga    360
aagcctgctg ctctgggaga agcccagcct accaagagcc tggaagagaa caagtccctg    420
aaagagcaga agaagctgaa cgacctctgc ttcctgaagc ggctgctgca agagatcaag    480
acctgctgga acaagatctc gatgggcacc aaagagcacg gcggaggatc tggcggaggt    540
ggaagcggcg gagcggttag cggtgccgga ggaagtggcg gcgcatctga atctggctac    600
gcccagaacg gcgacctgga agatgccgag ctggacgact acagcttcag ctgctacagc    660
cagctggaag tgaacggcag ccagcactct ctgacctgcy cctttgaaga tcccagcgtg    720
aacatcacca accttgagtt cgagatttgt ggccgacctg tggaagtcaa gtgctgaat    780
ttccggaagc tgcaagaaat ctactttatc gagacaaaga agttcctgct gatcggaag    840
agcaacatct gtgtgaaagt gggcgagaaa agcctgacct gcaagaagat cgacctgacc    900
accatcgtga agcccagggc tcctttcgat ctgagcgtgg tgtatagaga gggcgccaac    960
gacttcgtgg tcacctcaa caccagccac ctccaaaaga aatacgtgaa ggtgctgatg   1020
cacgacgtgg cctaccggca agagaaggac gagaacaaat ggacccacgt gaacctgagc   1080
agcaccaagc tgacctgctg gcagagaaaa ctgcagcctg ccgctatgta cgagatcaaa   1140
gtgcggagca tccccgacca ctactttaa ggcttttgga gcgagtggc ccctagctac   1200
tacttcagaa cccctgagat caacaactcc agcggcgaga tggaccccat tctgctgaca   1260
atcagcatcc tgagcttttt cagcgtggcc ctgctggtea tcctggcctg tgtgctgtgg   1320
aagaagcggg tcaagcccat cgtgtggccc agcctgcctg accacaagaa aaccctggaa   1380
cacctgtgca agaagcccg gaaaaacctg aacgtgtcct tcaatccga gagcttctg   1440
gactgccaga tccacagagt ggacgacatc caggccaggg acgaagtgga aggatttctg   1500
caggacacat tccctcagca gctcgaagag agcgagaagc aaagactcgg aggcgacgtg   1560
cagagcccta attgcccttc tgaggacgtg gtcacacccc cagagagctt cggcagagat   1620
agcagcctga catgtctggc cggcaatgtg tccgcctgtg atgcccctat cctgtcctct   1680
agcagaagcc tggattgcag agagagcggc aagaacggcc ctcacgtgta ccaggatctg   1740

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ctcctgtctc tgggcaccac aaacagcaca ctgcctccac cattcagcct gcagagcggc 1800
atcctgacac tgaacctgtg tgctcagggc cagcctatcc tgacaagcct gggcagcaat 1860
caagaagagg cctacgtcac catgagcagc ttctaccaga accag 1905

<210> SEQ ID NO 19

<400> SEQUENCE: 19

000

<210> SEQ ID NO 20

<211> LENGTH: 20

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 20

ttcggaaccc aatcactgac 20

<210> SEQ ID NO 21

<211> LENGTH: 20

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 21

tcagggttct ggatatctgt 20

<210> SEQ ID NO 22

<211> LENGTH: 20

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 22

aagttcctgt gatgtcaagc 20

<210> SEQ ID NO 23

<400> SEQUENCE: 23

000

<210> SEQ ID NO 24

<400> SEQUENCE: 24

000

<210> SEQ ID NO 25

<211> LENGTH: 20

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 25

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gaggtcaatg tctacggctc 20

<210> SEQ ID NO 26
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 26

atcacggagg tcaatgtcta 20

<210> SEQ ID NO 27
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 27

gtagacattg acctccgtga 20

<210> SEQ ID NO 28
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 28

ggagcaggtg atgttgacgg 20

<210> SEQ ID NO 29
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 29

caaagagatt acgaatgcct 20

<210> SEQ ID NO 30
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 30

gtgccacaaa gaccatcaag 20

<210> SEQ ID NO 31
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

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<400> SEQUENCE: 31

agagggtcat cacacacaag 20

<210> SEQ ID NO 32

<211> LENGTH: 20

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 32

ctttagata tcctgatcat 20

<210> SEQ ID NO 33

<400> SEQUENCE: 33

000

<210> SEQ ID NO 34

<211> LENGTH: 20

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 34

gaggtcaatg tctacggctc 20

<210> SEQ ID NO 35

<211> LENGTH: 1461

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
polynucleotide

<400> SEQUENCE: 35

atggcactcc ctgtaactgc acttcttttg ccacttgcc tgcctctgca cgcagcgcg 60

ccggacatcg agctgaccca gagcccgcc atcatgagcg ccagcctggg cgaggagatc 120

accctgacct gcagcgccag cagcagcgtg agctacatgc actggtacca gcagaagagc 180

ggcaccagcc ccaagctgct gatctacagc accagcaacc tggccagcgg cgtgcccagc 240

agggtcagcg gcagcggcag cggcaccttc tacagcctga ccatcagcag cgtggaggcc 300

gaggacgccc ccgactacta ctgccaccag tggagcagct acaccttcgg cggcggcacc 360

aagctggaga tcaagagggg cggcggcggc agcggcgcg ggcgcagcgg cggcggcggc 420

agccagggtga agctgcagga gagcggcggc ggcctggtga agcccgcgcg cagcctgaag 480

ctgagctgcg ccgccagcgg cttcaccttc agcagctacg ccatgagctg ggtgaggcag 540

acccccgaga agaggctgga gtgggtggcc accatcagca gcggcggcag ctacacctac 600

taccgagaca gcgtgaaggg cagggtcacc atcagcaggg acaacgcca gaacaccctg 660

tacctgcaga tgagcagcct gaggagcgag gacaccgcca tgtactactg cgccaggcag 720

gacggctact accccggctg gttcgccaac tggggccagg gcaccaccgt gaccgtgagc 780

agctccgga caacgacacc agcaccacgg ccacccactc ctgctccgac aattgcgtct 840

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cagccccctt cccttcgacc cgaagcttgt cgccctgctg cgggaggagc ggtccacacg    900
cgcggggcttg acttcgcttg cgacatctac atttgggcac ccttggccgg gacatgcggc    960
gtcttgctcc tgagtctggt tataacgctg tattgtaagc gaggtcggaa gaagcttttg    1020
tatatcttta aacagccott tatgagggcc gtacaaacca cacaagagga ggatgggtgc    1080
tcatgcagat ttctgaaga ggaagagggc ggttgcgaaac ttagagtcaa attcagccgc    1140
tccgcagatg cacctgttta taaacagggt cagaatcaat tgtataatga acttaatctc    1200
gggaggcgcg aggagtatga tgtgctggac aagcgacggg gtcgagacc agagatgggc    1260
ggtaaaccac gccgaaagaa ccccaggag ggactgtata atgagctgca aaaggacaaa    1320
atggcagaag cctattccga aatagggatg aaggagagc gccggcgagg taagggacat    1380
gacgtctttt atcaaggtct tagtactgca actaaggaca cctatgacgc gctgcatatg    1440
caggctctcc cacctagata a                                     1461

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<210> SEQ ID NO 36

<211> LENGTH: 486

<212> TYPE: PRT

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic polypeptide

<400> SEQUENCE: 36

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Met Ala Leu Pro Val Thr Ala Leu Leu Leu Pro Leu Ala Leu Leu Leu
 1             5             10            15

His Ala Ala Arg Pro Asp Ile Glu Leu Thr Gln Ser Pro Ala Ile Met
      20            25            30

Ser Ala Ser Leu Gly Glu Glu Ile Thr Leu Thr Cys Ser Ala Ser Ser
      35            40            45

Ser Val Ser Tyr Met His Trp Tyr Gln Gln Lys Ser Gly Thr Ser Pro
      50            55            60

Lys Leu Leu Ile Tyr Ser Thr Ser Asn Leu Ala Ser Gly Val Pro Ser
      65            70            75            80

Arg Phe Ser Gly Ser Gly Ser Gly Thr Phe Tyr Ser Leu Thr Ile Ser
      85            90            95

Ser Val Glu Ala Glu Asp Ala Ala Asp Tyr Tyr Cys His Gln Trp Ser
      100           105           110

Ser Tyr Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys Arg Gly Gly
      115           120           125

Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gln Val Lys
      130           135           140

Leu Gln Glu Ser Gly Gly Gly Leu Val Lys Pro Gly Gly Ser Leu Lys
      145           150           155           160

Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr Ala Met Ser
      165           170           175

Trp Val Arg Gln Thr Pro Glu Lys Arg Leu Glu Trp Val Ala Thr Ile
      180           185           190

Ser Ser Gly Gly Ser Tyr Thr Tyr Tyr Pro Asp Ser Val Lys Gly Arg
      195           200           205

Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Thr Leu Tyr Leu Gln Met
      210           215           220

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Ser	Ser	Leu	Arg	Ser	Glu	Asp	Thr	Ala	Met	Tyr	Tyr	Cys	Ala	Arg	Gln
225					230					235					240
Asp	Gly	Tyr	Tyr	Pro	Gly	Trp	Phe	Ala	Asn	Trp	Gly	Gln	Gly	Thr	Thr
				245					250					255	
Val	Thr	Val	Ser	Ser	Ser	Gly	Thr	Thr	Thr	Pro	Ala	Pro	Arg	Pro	Pro
			260					265					270		
Thr	Pro	Ala	Pro	Thr	Ile	Ala	Ser	Gln	Pro	Leu	Ser	Leu	Arg	Pro	Glu
		275					280					285			
Ala	Cys	Arg	Pro	Ala	Ala	Gly	Gly	Ala	Val	His	Thr	Arg	Gly	Leu	Asp
	290					295					300				
Phe	Ala	Cys	Asp	Ile	Tyr	Ile	Trp	Ala	Pro	Leu	Ala	Gly	Thr	Cys	Gly
305				310						315					320
Val	Leu	Leu	Leu	Ser	Leu	Val	Ile	Thr	Leu	Tyr	Cys	Lys	Arg	Gly	Arg
				325					330					335	
Lys	Lys	Leu	Leu	Tyr	Ile	Phe	Lys	Gln	Pro	Phe	Met	Arg	Pro	Val	Gln
		340						345					350		
Thr	Thr	Gln	Glu	Glu	Asp	Gly	Cys	Ser	Cys	Arg	Phe	Pro	Glu	Glu	Glu
		355					360					365			
Glu	Gly	Gly	Cys	Glu	Leu	Arg	Val	Lys	Phe	Ser	Arg	Ser	Ala	Asp	Ala
	370					375					380				
Pro	Ala	Tyr	Lys	Gln	Gly	Gln	Asn	Gln	Leu	Tyr	Asn	Glu	Leu	Asn	Leu
385				390						395					400
Gly	Arg	Arg	Glu	Glu	Tyr	Asp	Val	Leu	Asp	Lys	Arg	Arg	Gly	Arg	Asp
			405					410						415	
Pro	Glu	Met	Gly	Gly	Lys	Pro	Arg	Arg	Lys	Asn	Pro	Gln	Glu	Gly	Leu
		420					425						430		
Tyr	Asn	Glu	Leu	Gln	Lys	Asp	Lys	Met	Ala	Glu	Ala	Tyr	Ser	Glu	Ile
	435						440					445			
Gly	Met	Lys	Gly	Glu	Arg	Arg	Arg	Gly	Lys	Gly	His	Asp	Gly	Leu	Tyr
	450					455					460				
Gln	Gly	Leu	Ser	Thr	Ala	Thr	Lys	Asp	Thr	Tyr	Asp	Ala	Leu	His	Met
465				470						475					480
Gln	Ala	Leu	Pro	Pro	Arg										
				485											

<210> SEQ ID NO 37

<211> LENGTH: 2721

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic polynucleotide

<400> SEQUENCE: 37

atggcaactcc ctgtaactgc acttcttttg ccaattgcct tgctcctgca cgcagcgcg	60
ccggacatcg agctgaccca gagccccgcc atcatgagcg ccagcctggg cgaggagatc	120
accctgacct gcagcgccag cagcagcgtg agctacatgc actggtacca gcagaagagc	180
ggcaccagcc ccaagctgct gatctacagc accagcaacc tggccagcgg cgtgcccagc	240
aggttcagcg gcagcggcag cggcaccctc tacagcctga ccatcagcag cgtggaggcc	300
gaggacgccc ccgactacta ctgccaccag tggagcagct acaccttcgg cggcggcacc	360
aagctggaga tcaagagggg cggcggcggc agcggcgggc gcggcagcgg cggcggcggc	420

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agccaggtga agctgcagga gagcgcgccg	ggcctggtga agcccgccgg cagcctgaag	480
ctgagctcgc ccgccagcgg cttcaccttc	agcagctacg ccatgagctg ggtgaggcag	540
acccccgaga agaggctgga gtgggtggcc	accatcagca gcggcgccag ctacacctac	600
taccccgaca gcgtgaaggg caggttcacc	atcagcaggg acaacgcca gaacacctg	660
tacctgcaga tgagcagcct gaggagcgag	gacacgccca tgtactactg cgccaggcag	720
gacggctact accccgctg gtctcgccaac	tggggccagg gcaccacgt gaccgtgagc	780
agctccgaa caacgacacc agcaccacgg	cccccactc ctgctccgac aattgcgtct	840
cagccctttt ccttcgacc cgaagcttgt	cgccctgctg cgggaggagc ggtccacacg	900
cgcggtctg acttcgctg cgacatctac	atctgggac ccttgccgg gacatcgcc	960
gtcttgctcc tgagtctggt tataacgctg	tattgtaagc gaggtcgga gaagctttg	1020
tatatcttta aacagcctt tatgagggcc	gtacaaacca cacaagagga ggatgggtgc	1080
tcctgcagat ttctgaaga ggaagagggc	ggttcgcaac ttagagtaaa attcagccgc	1140
tccgcagatg cacctgctta taaacagggt	cagaatcaat tgtataatga acttaatctc	1200
gggaggcgcg aggagtatga tgtgctggac	aagcgacggg gtcgagacc agagatgggc	1260
ggtaaacccc gccgaaagaa ccccaggag	ggactgtata atgagctgca aaaggacaaa	1320
atggcagaag cctattccga aatagggatg	aaggagagc ggcggcgagg taaggacat	1380
gacgtcttt atcaaggtct tagtactgca	actaaggaca cctatgacgc gctgcatatg	1440
caggctctcc cacctagacg agctaaacga	ggctcaggcg cgacgaactt tagtttgctg	1500
aagcaagctg gggatgtaga ggaaaatccg	ggctccatgg attggacttg gattttgttc	1560
ctcggtgcg cagcgactcg cgtccatagt	aattgggtga acgtaattag tgacttgaaa	1620
aaaattgagg acctatatac aagtatgcat	atcgatgcaa cactgtacac ggagtcgcac	1680
gtgcacccaa gctgcaaggc caccgcaatg	aaatgctttt tgctogaatt gcaagttatc	1740
tcacttgagt caggggacgc ttcaatccat	gatactgtgg agaatttgat aatcctggcg	1800
aacaatagcc ttagtttcaa tggcaacgtc	actgagtcag gctgcaagga atgtgaggaa	1860
ttgaagaaa aaaatatcaa ggaatttttg	caatcttttg ttcacatagt tcagatgttc	1920
attaacacta gttccggggg cggcagtgga	ggtggcggtg gcggcggggg tggctctggt	1980
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catgctgata tctgggttaa atcttactcc	ctttacagtc gagaaaggta catttgcaac	2100
tccggtttta aacgcaaagc cgggactagt	tcaactgactg aatgtgtatt gaataaagcg	2160
acaaatgtcg cactctggac tacccttcc	ctcaaatgca ttccgatcc tgccctggtg	2220
catcagcgac cagcaccgcc gtccacggta	actaccgcag gagtaacacc gcagcccgag	2280
agcctttccc cctcaggcaa agagccggcc	gcatcctccc catcttccaa taataaccga	2340
gtaccaccg cagcaatcgt acccgatcc	cagctgatgc ccagcaaaag tccgagtact	2400
ggaaagctg aaatctccag tcacgagtct	tctcatggaa ctccgagtca aactacagca	2460
aagaattggg agctgactgc ttccgcttca	caccagccgc caggcgttta tctcaggga	2520
cactcagata ccacgggtggc gattagcaca	agcaccgtcc tctgtgtgg gctgagtgca	2580
gtgtcacttc tcgctgcta ccttaagtcc	agacagacac ccccttggc aagcgttgaa	2640
atggaagcca tggaagcctt gctgtcaca	tgggggactt catcccgca tgaagacttg	2700

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gagaactgct cacaccatct t

2721

<210> SEQ ID NO 38

<211> LENGTH: 907

<212> TYPE: PRT

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic polypeptide

<400> SEQUENCE: 38

Met Ala Leu Pro Val Thr Ala Leu Leu Leu Pro Leu Ala Leu Leu Leu
1 5 10 15

His Ala Ala Arg Pro Asp Ile Glu Leu Thr Gln Ser Pro Ala Ile Met
20 25 30

Ser Ala Ser Leu Gly Glu Glu Ile Thr Leu Thr Cys Ser Ala Ser Ser
35 40 45

Ser Val Ser Tyr Met His Trp Tyr Gln Gln Lys Ser Gly Thr Ser Pro
50 55 60

Lys Leu Leu Ile Tyr Ser Thr Ser Asn Leu Ala Ser Gly Val Pro Ser
65 70 75 80

Arg Phe Ser Gly Ser Gly Ser Gly Thr Phe Tyr Ser Leu Thr Ile Ser
85 90 95

Ser Val Glu Ala Glu Asp Ala Ala Asp Tyr Tyr Cys His Gln Trp Ser
100 105 110

Ser Tyr Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys Arg Gly Gly
115 120 125

Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gln Val Lys
130 135 140

Leu Gln Glu Ser Gly Gly Gly Leu Val Lys Pro Gly Gly Ser Leu Lys
145 150 155 160

Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr Ala Met Ser
165 170 175

Trp Val Arg Gln Thr Pro Glu Lys Arg Leu Glu Trp Val Ala Thr Ile
180 185 190

Ser Ser Gly Gly Ser Tyr Thr Tyr Tyr Pro Asp Ser Val Lys Gly Arg
195 200 205

Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Thr Leu Tyr Leu Gln Met
210 215 220

Ser Ser Leu Arg Ser Glu Asp Thr Ala Met Tyr Tyr Cys Ala Arg Gln
225 230 235 240

Asp Gly Tyr Tyr Pro Gly Trp Phe Ala Asn Trp Gly Gln Gly Thr Thr
245 250 255

Val Thr Val Ser Ser Ser Gly Thr Thr Thr Pro Ala Pro Arg Pro Pro
260 265 270

Thr Pro Ala Pro Thr Ile Ala Ser Gln Pro Leu Ser Leu Arg Pro Glu
275 280 285

Ala Cys Arg Pro Ala Ala Gly Gly Ala Val His Thr Arg Gly Leu Asp
290 295 300

Phe Ala Cys Asp Ile Tyr Ile Trp Ala Pro Leu Ala Gly Thr Cys Gly
305 310 315 320

Val Leu Leu Leu Ser Leu Val Ile Thr Leu Tyr Cys Lys Arg Gly Arg
325 330 335

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Lys	Lys	Leu	Leu	Tyr	Ile	Phe	Lys	Gln	Pro	Phe	Met	Arg	Pro	Val	Gln
			340					345					350		
Thr	Thr	Gln	Glu	Glu	Asp	Gly	Cys	Ser	Cys	Arg	Phe	Pro	Glu	Glu	Glu
		355					360					365			
Glu	Gly	Gly	Cys	Glu	Leu	Arg	Val	Lys	Phe	Ser	Arg	Ser	Ala	Asp	Ala
	370					375					380				
Pro	Ala	Tyr	Lys	Gln	Gly	Gln	Asn	Gln	Leu	Tyr	Asn	Glu	Leu	Asn	Leu
	385				390					395					400
Gly	Arg	Arg	Glu	Glu	Tyr	Asp	Val	Leu	Asp	Lys	Arg	Arg	Gly	Arg	Asp
			405						410					415	
Pro	Glu	Met	Gly	Gly	Lys	Pro	Arg	Arg	Lys	Asn	Pro	Gln	Glu	Gly	Leu
			420					425					430		
Tyr	Asn	Glu	Leu	Gln	Lys	Asp	Lys	Met	Ala	Glu	Ala	Tyr	Ser	Glu	Ile
		435					440					445			
Gly	Met	Lys	Gly	Glu	Arg	Arg	Arg	Gly	Lys	Gly	His	Asp	Gly	Leu	Tyr
	450					455					460				
Gln	Gly	Leu	Ser	Thr	Ala	Thr	Lys	Asp	Thr	Tyr	Asp	Ala	Leu	His	Met
	465				470					475					480
Gln	Ala	Leu	Pro	Pro	Arg	Arg	Ala	Lys	Arg	Gly	Ser	Gly	Ala	Thr	Asn
			485						490					495	
Phe	Ser	Leu	Leu	Lys	Gln	Ala	Gly	Asp	Val	Glu	Glu	Asn	Pro	Gly	Pro
			500					505					510		
Met	Asp	Trp	Thr	Trp	Ile	Leu	Phe	Leu	Val	Ala	Ala	Ala	Thr	Arg	Val
		515					520					525			
His	Ser	Asn	Trp	Val	Asn	Val	Ile	Ser	Asp	Leu	Lys	Lys	Ile	Glu	Asp
		530			535						540				
Leu	Ile	Gln	Ser	Met	His	Ile	Asp	Ala	Thr	Leu	Tyr	Thr	Glu	Ser	Asp
	545				550					555					560
Val	His	Pro	Ser	Cys	Lys	Val	Thr	Ala	Met	Lys	Cys	Phe	Leu	Leu	Glu
			565						570					575	
Leu	Gln	Val	Ile	Ser	Leu	Glu	Ser	Gly	Asp	Ala	Ser	Ile	His	Asp	Thr
		580						585					590		
Val	Glu	Asn	Leu	Ile	Ile	Leu	Ala	Asn	Asn	Ser	Leu	Ser	Ser	Asn	Gly
		595				600						605			
Asn	Val	Thr	Glu	Ser	Gly	Cys	Lys	Glu	Cys	Glu	Glu	Leu	Glu	Glu	Lys
	610				615						620				
Asn	Ile	Lys	Glu	Phe	Leu	Gln	Ser	Phe	Val	His	Ile	Val	Gln	Met	Phe
	625				630					635					640
Ile	Asn	Thr	Ser	Ser	Gly	Gly	Gly	Ser	Gly	Gly	Gly	Gly	Ser	Gly	Gly
			645					650						655	
Gly	Gly	Ser	Gly	Gly	Gly	Gly	Ser	Gly	Gly	Gly	Ser	Leu	Gln	Ile	Thr
		660						665					670		
Cys	Pro	Pro	Pro	Met	Ser	Val	Glu	His	Ala	Asp	Ile	Trp	Val	Lys	Ser
		675					680					685			
Tyr	Ser	Leu	Tyr	Ser	Arg	Glu	Arg	Tyr	Ile	Cys	Asn	Ser	Gly	Phe	Lys
		690				695					700				
Arg	Lys	Ala	Gly	Thr	Ser	Ser	Leu	Thr	Glu	Cys	Val	Leu	Asn	Lys	Ala
	705				710					715					720
Thr	Asn	Val	Ala	His	Trp	Thr	Thr	Pro	Ser	Leu	Lys	Cys	Ile	Arg	Asp
			725					730					735		
Pro	Ala	Leu	Val	His	Gln	Arg	Pro	Ala	Pro	Pro	Ser	Thr	Val	Thr	Thr

	740						745						750					
Ala Gly Val Thr Pro Gln Pro Glu Ser Leu Ser Pro Ser Gly Lys Glu																		
755							760						765					
Pro Ala Ala Ser Ser Pro Ser Ser Asn Asn Thr Ala Ala Thr Thr Ala																		
770							775						780					
Ala Ile Val Pro Gly Ser Gln Leu Met Pro Ser Lys Ser Pro Ser Thr																		
785	790						795						800					
Gly Thr Thr Glu Ile Ser Ser His Glu Ser Ser His Gly Thr Pro Ser																		
	805						810						815					
Gln Thr Thr Ala Lys Asn Trp Glu Leu Thr Ala Ser Ala Ser His Gln																		
	820						825						830					
Pro Pro Gly Val Tyr Pro Gln Gly His Ser Asp Thr Thr Val Ala Ile																		
	835						840						845					
Ser Thr Ser Thr Val Leu Leu Cys Gly Leu Ser Ala Val Ser Leu Leu																		
	850						855						860					
Ala Cys Tyr Leu Lys Ser Arg Gln Thr Pro Pro Leu Ala Ser Val Glu																		
865	870						875						880					
Met Glu Ala Met Glu Ala Leu Pro Val Thr Trp Gly Thr Ser Ser Arg																		
	885						890						895					
Asp Glu Asp Leu Glu Asn Cys Ser His His Leu																		
	900						905											
<210>	SEQ ID NO 39																	
<211>	LENGTH: 3063																	
<212>	TYPE: DNA																	
<213>	ORGANISM: Artificial Sequence																	
<220>	FEATURE:																	
<223>	OTHER INFORMATION: Description of Artificial Sequence: Synthetic polynucleotide																	
<400>	SEQUENCE: 39																	
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cggacatcg agctgaccga gagccccgcc atcatgagcg ccagcctggg cgaggagatc	120																	
acctgacct gcagcgccag cagcagcgtg agctacatgc actggtacca gcagaagagc	180																	
ggcaccagcc ccaagctgct gatctacagc accagcaacc tggccagcgg cgtgccccagc	240																	
agggttcagcg gcagcggcgag cggcaccttc tacagcctga ccatcagcag cgtggaggcc	300																	
gaggacgcgg cgcactacta ctgccaccag tggagcagct acaccttcgg cggcggcacc	360																	
aagctggaga tcaagagggg cggcgggcggc agcggcgggc gcggcagcgg cggcgggcggc	420																	
agccagggtga agctgcagga gacgcggcggc ggccctggtga agcccggcgg cagccctgaag	480																	
ctgagctgcg ccgccagcgg cttcaccttc agcagctacg ccatgagctg ggtgaggcag	540																	
acccccgaga agaggctgga gtgggtggcc accatcagca gcggcggcag ctacacctac	600																	
taccccgaca gcgtgaaggg cagggttcacc atcagcaggg acaacgccaa gaacacctg	660																	
tacctgcaga tgagcagcct gaggagcgag gacaccgcc tgtactactg cgccaggcag	720																	
gacggctact accccggtg gttcgccaac tggggccagg gcaccacagt gaccgtgagc	780																	
agctccggaa caacgacacc agcaccacgg ccacccacte ctgctccgac aattgctgt	840																	
cagccccctt cccttcgacc cgaagcttgt cgccctgctg cgggaggagc ggtccacag	900																	
cgcgggcttg acttcgcttg gcagatctac atttgggcac ccttggccgg gacatcgggc	960																	
gtcttgcctc tgagctcgtg tataacgctg tattgtaagc gaggtcggaa gaagcttttg	1020																	

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tatatcttta aacagccott tatgagggcc gtacaaacca cacaagagga ggatgggtgc	1080
tcatgcagat ttctgaaga ggaagagggc ggttgcgaaac ttagagtcaa attcagccgc	1140
tccgcagatg cacctgttta taaacagggt cagaatcaat tgtataatga acttaatctc	1200
gggaggcgcg aggagtatga tgtgtggac aagcgacggg gtcgagaccc agagtgggc	1260
ggtaaacccc gccgaaagaa cccccaggag ggactgtata atgagctgca aaaggacaaa	1320
atggcagaag cctattccga aatagggatg aaggagagc ggcggcgagg taaggacat	1380
gacgtcttt atcaaggtct tagtactgca actaaggaca cctatgacgc gctgcatatg	1440
caggctctcc cacctagagg ctccagcgcg acgaacttta gtttctgaa gcaagctggg	1500
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caaacgtcag ttatatctac agtcttcaact actcccgca atgtatccac cccagaaacc	1920
acctcaaac cttctcttag tccaggaaat gttagcgatt tgtcaacgac gagcacgagt	1980
ttggcgacta gtccaacgaa accgtacact tccagcagtc ccatactgtc cgacattaag	2040
gcagaaataa aatgttctgg gatcagggag gtcaagctta cgcagggaat atgtcttgag	2100
caaaataaga cgtctctatg cgcagaattc aagaaagatc gcggcggaagg tctggccaga	2160
gtcctctgtg gagaggagca ggcggatgct gacgcaggag cgcagggttg tagtctctg	2220
ctcgcgaaa gtgaagttag gccccaatgt ctcttggttg tactggctaa ccgaactgaa	2280
attagcagca agcttcaact catgaaaaaa caccagagtg atttgaaaaa acttgggata	2340
cttgacttca cggagcaaga cgttgctgtc caccaatcct actcacagaa aaccccgata	2400
ctggtgacat gcccaccat atcaatcttg tctttcttca gtgtggctct tctgggtgatc	2460
ttggcgtgcg tgtgtgtgaa aaaaaggatt aaaccgatcg tttggcctag tctgcgggat	2520
cacaaaaaga cactggagca cctctgcaag aagccacgaa aaaacctgaa tgtgagcttt	2580
aaccccgagt cttttttgga ctgtcagata caccgagtcg acgatataca ggcaagagat	2640
gaggttgagg gtttctgca agacacattc ccgcaacaac tcgaagaatc cgagaaacag	2700
cgccttggtg gagatgtcca gtctccgaac tgtccgagcg aggacgtagt aattaccca	2760
gaaagcttcg gtcgagatag tagccttacg tgtctcgccg ggaacgtgtc agcgtgtgac	2820
gcgcctatcc tttcaagttc acgcagtttg gactgtcgag aatcagggaa aaacggacct	2880
cacgtgtatc aggatctcct tctcagcctg ggcacgacaa acagtacctt gcctcctccg	2940
ttttccctgc agtcaggtat tctgacgctc aatccagtcg cacaagggca acctatctg	3000
acctccttgg gttctaacca ggaagaggca tacgtcacta tgtccagctt ctatcagaat	3060
cag	3063

<210> SEQ ID NO 40

<211> LENGTH: 1021

<212> TYPE: PRT

<213> ORGANISM: Artificial Sequence

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<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic polypeptide

<400> SEQUENCE: 40

Met Ala Leu Pro Val Thr Ala Leu Leu Leu Pro Leu Ala Leu Leu Leu
1 5 10 15

His Ala Ala Arg Pro Asp Ile Glu Leu Thr Gln Ser Pro Ala Ile Met
20 25 30

Ser Ala Ser Leu Gly Glu Glu Ile Thr Leu Thr Cys Ser Ala Ser Ser
35 40 45

Ser Val Ser Tyr Met His Trp Tyr Gln Gln Lys Ser Gly Thr Ser Pro
50 55 60

Lys Leu Leu Ile Tyr Ser Thr Ser Asn Leu Ala Ser Gly Val Pro Ser
65 70 75 80

Arg Phe Ser Gly Ser Gly Ser Gly Thr Phe Tyr Ser Leu Thr Ile Ser
85 90 95

Ser Val Glu Ala Glu Asp Ala Ala Asp Tyr Tyr Cys His Gln Trp Ser
100 105 110

Ser Tyr Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys Arg Gly Gly
115 120 125

Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gln Val Lys
130 135 140

Leu Gln Glu Ser Gly Gly Gly Leu Val Lys Pro Gly Gly Ser Leu Lys
145 150 155 160

Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr Ala Met Ser
165 170 175

Trp Val Arg Gln Thr Pro Glu Lys Arg Leu Glu Trp Val Ala Thr Ile
180 185 190

Ser Ser Gly Gly Ser Tyr Thr Tyr Tyr Pro Asp Ser Val Lys Gly Arg
195 200 205

Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Thr Leu Tyr Leu Gln Met
210 215 220

Ser Ser Leu Arg Ser Glu Asp Thr Ala Met Tyr Tyr Cys Ala Arg Gln
225 230 235 240

Asp Gly Tyr Tyr Pro Gly Trp Phe Ala Asn Trp Gly Gln Gly Thr Thr
245 250 255

Val Thr Val Ser Ser Ser Gly Thr Thr Thr Pro Ala Pro Arg Pro Pro
260 265 270

Thr Pro Ala Pro Thr Ile Ala Ser Gln Pro Leu Ser Leu Arg Pro Glu
275 280 285

Ala Cys Arg Pro Ala Ala Gly Gly Ala Val His Thr Arg Gly Leu Asp
290 295 300

Phe Ala Cys Asp Ile Tyr Ile Trp Ala Pro Leu Ala Gly Thr Cys Gly
305 310 315 320

Val Leu Leu Leu Ser Leu Val Ile Thr Leu Tyr Cys Lys Arg Gly Arg
325 330 335

Lys Lys Leu Leu Tyr Ile Phe Lys Gln Pro Phe Met Arg Pro Val Gln
340 345 350

Thr Thr Gln Glu Glu Asp Gly Cys Ser Cys Arg Phe Pro Glu Glu Glu
355 360 365

Glu Gly Gly Cys Glu Leu Arg Val Lys Phe Ser Arg Ser Ala Asp Ala

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370	375	380
Pro Ala Tyr Lys Gln Gly	Gln Asn Gln Leu Tyr Asn Glu Leu Asn Leu	
385	390	395 400
Gly Arg Arg Glu Glu Tyr Asp Val Leu Asp Lys Arg Arg Gly Arg Asp		
	405	410 415
Pro Glu Met Gly Gly Lys Pro Arg Arg Lys Asn Pro Gln Glu Gly Leu		
	420	425 430
Tyr Asn Glu Leu Gln Lys Asp Lys Met Ala Glu Ala Tyr Ser Glu Ile		
	435	440 445
Gly Met Lys Gly Glu Arg Arg Arg Gly Lys Gly His Asp Gly Leu Tyr		
	450	455 460
Gln Gly Leu Ser Thr Ala Thr Lys Asp Thr Tyr Asp Ala Leu His Met		
	465	470 475 480
Gln Ala Leu Pro Pro Arg Gly Ser Gly Ala Thr Asn Phe Ser Leu Leu		
	485	490 495
Lys Gln Ala Gly Asp Val Glu Glu Asn Pro Gly Pro Met Leu Val Arg		
	500	505 510
Arg Gly Ala Arg Ala Gly Pro Arg Met Pro Arg Gly Trp Thr Ala Leu		
	515	520 525
Cys Leu Leu Ser Leu Leu Pro Ser Gly Phe Met Ser Leu Asp Asn Asn		
	530	535 540
Gly Thr Ala Thr Pro Glu Leu Pro Thr Gln Gly Thr Phe Ser Asn Val		
	545	550 555 560
Ser Thr Asn Val Ser Tyr Gln Glu Thr Thr Thr Pro Ser Thr Leu Gly		
	565	570 575
Ser Thr Ser Leu His Pro Val Ser Gln His Gly Asn Glu Ala Thr Thr		
	580	585 590
Asn Ile Thr Glu Thr Thr Val Lys Phe Thr Ser Thr Ser Val Ile Thr		
	595	600 605
Ser Val Tyr Gly Asn Thr Asn Ser Ser Val Gln Ser Gln Thr Ser Val		
	610	615 620
Ile Ser Thr Val Phe Thr Thr Pro Ala Asn Val Ser Thr Pro Glu Thr		
	625	630 635 640
Thr Leu Lys Pro Ser Leu Ser Pro Gly Asn Val Ser Asp Leu Ser Thr		
	645	650 655
Thr Ser Thr Ser Leu Ala Thr Ser Pro Thr Lys Pro Tyr Thr Ser Ser		
	660	665 670
Ser Pro Ile Leu Ser Asp Ile Lys Ala Glu Ile Lys Cys Ser Gly Ile		
	675	680 685
Arg Glu Val Lys Leu Thr Gln Gly Ile Cys Leu Glu Gln Asn Lys Thr		
	690	695 700
Ser Ser Cys Ala Glu Phe Lys Lys Asp Arg Gly Glu Gly Leu Ala Arg		
	705	710 715 720
Val Leu Cys Gly Glu Glu Gln Ala Asp Ala Asp Ala Gly Ala Gln Val		
	725	730 735
Cys Ser Leu Leu Leu Ala Gln Ser Glu Val Arg Pro Gln Cys Leu Leu		
	740	745 750
Leu Val Leu Ala Asn Arg Thr Glu Ile Ser Ser Lys Leu Gln Leu Met		
	755	760 765
Lys Lys His Gln Ser Asp Leu Lys Lys Leu Gly Ile Leu Asp Phe Thr		
	770	775 780

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Glu Gln Asp Val Ala Ser His Gln Ser Tyr Ser Gln Lys Thr Pro Ile
 785 790 795 800
 Leu Leu Thr Cys Pro Thr Ile Ser Ile Leu Ser Phe Phe Ser Val Ala
 805 810 815
 Leu Leu Val Ile Leu Ala Cys Val Leu Trp Lys Lys Arg Ile Lys Pro
 820 825 830
 Ile Val Trp Pro Ser Leu Pro Asp His Lys Lys Thr Leu Glu His Leu
 835 840 845
 Cys Lys Lys Pro Arg Lys Asn Leu Asn Val Ser Phe Asn Pro Glu Ser
 850 855 860
 Phe Leu Asp Cys Gln Ile His Arg Val Asp Asp Ile Gln Ala Arg Asp
 865 870 875 880
 Glu Val Glu Gly Phe Leu Gln Asp Thr Phe Pro Gln Gln Leu Glu Glu
 885 890 895
 Ser Glu Lys Gln Arg Leu Gly Gly Asp Val Gln Ser Pro Asn Cys Pro
 900 905 910
 Ser Glu Asp Val Val Ile Thr Pro Glu Ser Phe Gly Arg Asp Ser Ser
 915 920 925
 Leu Thr Cys Leu Ala Gly Asn Val Ser Ala Cys Asp Ala Pro Ile Leu
 930 935 940
 Ser Ser Ser Arg Ser Leu Asp Cys Arg Glu Ser Gly Lys Asn Gly Pro
 945 950 955 960
 His Val Tyr Gln Asp Leu Leu Leu Ser Leu Gly Thr Thr Asn Ser Thr
 965 970 975
 Leu Pro Pro Pro Phe Ser Leu Gln Ser Gly Ile Leu Thr Leu Asn Pro
 980 985 990
 Val Ala Gln Gly Gln Pro Ile Leu Thr Ser Leu Gly Ser Asn Gln Glu
 995 1000 1005
 Glu Ala Tyr Val Thr Met Ser Ser Phe Tyr Gln Asn Gln
 1010 1015 1020

<210> SEQ ID NO 41

<211> LENGTH: 2181

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic polynucleotide

<400> SEQUENCE: 41

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actataagtt gccgcgcgtc ccaagacatc tctaagtacc ttaactggta tcaacaaaaa    180
ccggacggga cgggtcaaaact gttgatctat cacacatcca gattgcactc aggcgtgccg    240
agcagggttca gtgggagtggt gtcaggaacg gattacagct tgacgattag taacctggag    300
caagaagaca ttgccaccta cttctgccag caaggaataa ctctoccata tacgttcggg    360
ggtggcacca agctggaaat cactggcggc ggaggatccg aggtgcagct ggtgcagagc    420
ggcgccgagg tgaagaagcc cggcgagagc ctgaggatca gctgcaaggg cagcggtac    480
agcttcagca cctactggat cagctgggtg aggcagatgc ccggcaaggg cctggagtgg    540
atgggcaaga tctaccccg cgacagctac accaactaca gcccagctt ccagggccag    600

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gtgaccatca ggcgcgacaa gagcatcagc accgcctacc tgcagtggag cagcctgaag	660
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cagggcaccc tggtgaccgt gagcagcggc agcacaagcg gctctggcaa gcctggatct	780
ggcgagggct ctaccaaggg catgagctac gagctgaccc agccccccag cgtgagcgtg	840
agccccggcc agaccgccag catcacctgc agcggcgaca acatcggcga ccagtaagcc	900
cactgggtacc agcagaagcc cggccagagc cccgtgctgg tgatctacca ggacaagaac	960
agggccagcg gcatccccga gaggttcagc ggcagcaaca gcggcaaac cgcaccctg	1020
accatcagcg gcacccaggc catggacgag gccgactact actgcgccac ctacaccggc	1080
ttcggcagcc tggccgtgtt cggcgcgggc accaagctga ccgtgctggg gggaggcggc	1140
agcgagggtg aactgcagga gtccggggcc ggtctcgtgg caccttccca gtcactgtcc	1200
gtgacctgca ccgtatctgg ggtaaagtctg ccggattatg gggtttcatg gatccggcaa	1260
cctccgagga aagggttgga atggctggga gtcactctgg gaagcgagac aacttattat	1320
aattctgctt tgaagagcgg cttagcgata atcaaggaca acagtaagag tcaggttttc	1380
ttgaagatga attctcttca gacagatgac accgctatct attattgtgc aaaacattat	1440
tattacggag gatcctacgc gatggactat tggggacagg gtacctctgt tacgggtgcc	1500
tcatecggaa caacgacacc agcaccacgg ccacccaactc ctgctccgac aattgcgtct	1560
cagccccctt cccttcgacc cgaagcttgt cgccctgctg cgggaggagc ggtccacacg	1620
cgcgggcttg acttcgcttg cgacatctac atttgggcac ccttggccgg gacatgcggc	1680
gtcttgctcc tgagtctggt tataacgctg tattgtaagc gaggtcggaa gaagcttttg	1740
tatatcttta aacagccctt tatgaggccc gtacaaacca cacaagagga ggatgggtgc	1800
tcatgcagat ttcttgaaga ggaagaggc ggttgcgaa ttagagtcaa attcagccgc	1860
tccgcagatg cacctgctta taaacagggt cagaatcaat tgtataatga acttaatctc	1920
gggaggcgcg aggagtatga tgtgctggac aagcgacggg gtcgagaccc agagatgggc	1980
ggtaaacccc gccgaaagaa cccccaggag ggactgtata atgagctgca aaaggacaaa	2040
atggcagaag cctattccga aatagggatg aaggagagc ggcggcgagg taaggacat	2100
gacgtcttt atcaaggtct tagtactgca actaaggaca cctatgacgc gctgcatatg	2160
caggctctcc cacctagata a	2181

<210> SEQ ID NO 42

<400> SEQUENCE: 42

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<210> SEQ ID NO 43

<400> SEQUENCE: 43

000

<210> SEQ ID NO 44

<400> SEQUENCE: 44

000

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<210> SEQ ID NO 45
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 45

aacgtggcat ctgtcgaccc 20

<210> SEQ ID NO 46
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 46

gcgctggaga aactatttgg 20

<210> SEQ ID NO 47
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 47

acatttaacg atcagaaaacg 20

<210> SEQ ID NO 48
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 48

ggtcctttgt ccacctgcgc 20

<210> SEQ ID NO 49
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 49

gagaaactat ttggaggaca 20

<210> SEQ ID NO 50
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligonucleotide

<400> SEQUENCE: 50

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ggagaaacta tttggaggac

20

<210> SEQ ID NO 51
<211> LENGTH: 254
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic polypeptide

<400> SEQUENCE: 51

Met Glu Tyr Ala Ser Asp Ala Ser Leu Asp Pro Glu Ala Pro Trp Pro
1 5 10 15
Pro Ala Pro Arg Ala Arg Ala Cys Arg Val Leu Pro Trp Ala Leu Val
20 25 30
Ala Gly Leu Leu Leu Leu Leu Leu Ala Ala Ala Cys Ala Val Phe
35 40 45
Leu Ala Cys Pro Trp Ala Val Ser Gly Ala Arg Ala Ser Pro Gly Ser
50 55 60
Ala Ala Ser Pro Arg Leu Arg Glu Gly Pro Glu Leu Ser Pro Asp Asp
65 70 75 80
Pro Ala Gly Leu Leu Asp Leu Arg Gln Gly Met Phe Ala Gln Leu Val
85 90 95
Ala Gln Asn Val Leu Leu Ile Asp Gly Pro Leu Ser Trp Tyr Ser Asp
100 105 110
Pro Gly Leu Ala Gly Val Ser Leu Thr Gly Gly Leu Ser Tyr Lys Glu
115 120 125
Asp Thr Lys Glu Leu Val Val Ala Lys Ala Gly Val Tyr Tyr Val Phe
130 135 140
Phe Gln Leu Glu Leu Arg Arg Val Val Ala Gly Glu Gly Ser Gly Ser
145 150 155 160
Val Ser Leu Ala Leu His Leu Gln Pro Leu Arg Ser Ala Ala Gly Ala
165 170 175
Ala Ala Leu Ala Leu Thr Val Asp Leu Pro Pro Ala Ser Ser Glu Ala
180 185 190
Arg Asn Ser Ala Phe Gly Phe Gln Gly Arg Leu Leu His Leu Ser Ala
195 200 205
Gly Gln Arg Leu Gly Val His Leu His Thr Glu Ala Arg Ala Arg His
210 215 220
Ala Trp Gln Leu Thr Gln Gly Ala Thr Val Leu Gly Leu Phe Arg Val
225 230 235 240
Thr Pro Glu Ile Pro Ala Gly Leu Pro Ser Pro Arg Ser Glu
245 250

<210> SEQ ID NO 52
<211> LENGTH: 116
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic polypeptide

<400> SEQUENCE: 52

Glu Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Glu
1 5 10 15
Ser Leu Arg Ile Ser Cys Lys Gly Ser Gly Tyr Ser Phe Ser Thr Tyr

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

<210> SEQ ID NO 53
 <211> LENGTH: 109
 <212> TYPE: PRT
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic polypeptide

<400> SEQUENCE: 53

Met Ser Tyr Glu Leu Thr Gln Pro Pro Ser Val Ser Val Ser Pro Gly		
1	5	10
Gln Thr Ala Ser Ile Thr Cys Ser Gly Asp Asn Ile Gly Asp Gln Tyr		
20	25	30
Ala His Trp Tyr Gln Gln Lys Pro Gly Gln Ser Pro Val Leu Val Ile		
35	40	45
Tyr Gln Asp Lys Asn Arg Pro Ser Gly Ile Pro Glu Arg Phe Ser Gly		
50	55	60
Ser Asn Ser Gly Asn Thr Ala Thr Leu Thr Ile Ser Gly Thr Gln Ala		
65	70	75
Met Asp Glu Ala Asp Tyr Tyr Cys Ala Thr Tyr Thr Gly Phe Gly Ser		
85	90	95
Leu Ala Val Phe Gly Gly Gly Thr Lys Leu Thr Val Leu		
100	105	

<210> SEQ ID NO 54
 <211> LENGTH: 121
 <212> TYPE: PRT
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic polypeptide

<400> SEQUENCE: 54

Gln Val Gln Leu Gln Gln Trp Gly Ala Gly Leu Leu Lys Pro Ser Glu		
1	5	10
Thr Leu Ser Leu Thr Cys Ala Val Tyr Gly Gly Ser Phe Ser Gly Tyr		
20	25	30
Tyr Trp Ser Trp Ile Arg Gln Ser Pro Glu Lys Gly Leu Glu Trp Ile		
35	40	45
Gly Glu Ile Asn His Gly Gly Tyr Val Thr Tyr Asn Pro Ser Leu Glu		
50	55	60
Ser Arg Val Thr Ile Ser Val Asp Thr Ser Lys Asn Gln Phe Ser Leu		

-continued

65	70	75	80
Lys Leu Ser Ser Val Thr Ala Ala Asp Thr Ala Val Tyr Tyr Cys Ala	85	90	95
Arg Asp Tyr Gly Pro Gly Asn Tyr Asp Trp Tyr Phe Asp Leu Trp Gly	100	105	110
Arg Gly Thr Leu Val Thr Val Ser Ser	115	120	

<210> SEQ ID NO 55
 <211> LENGTH: 110
 <212> TYPE: PRT
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic polypeptide

<400> SEQUENCE: 55

Glu Ile Val Leu Thr Gln Ser Pro Ala Thr Leu Ser Leu Ser Pro Gly	1	5	10	15
Glu Arg Ala Thr Leu Ser Cys Arg Ala Ser Gln Ser Val Ser Ser Tyr	20	25	30	
Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Arg Leu Leu Ile	35	40	45	
Tyr Asp Ala Ser Asn Arg Ala Thr Gly Ile Pro Ala Arg Phe Ser Gly	50	55	60	
Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Glu Pro	65	70	75	80
Glu Asp Phe Ala Val Tyr Tyr Cys Gln Gln Arg Ser Asn Trp Pro Pro	85	90	95	
Ala Leu Thr Phe Cys Gly Gly Thr Lys Val Glu Ile Lys Arg	100	105	110	

<210> SEQ ID NO 56
 <211> LENGTH: 489
 <212> TYPE: PRT
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic polypeptide

<400> SEQUENCE: 56

Met Ala Leu Pro Val Thr Ala Leu Leu Leu Pro Leu Ala Leu Leu Leu	1	5	10	15
His Ala Ala Arg Pro Met Ser Tyr Glu Leu Thr Gln Pro Pro Ser Val	20	25	30	
Ser Val Ser Pro Gly Gln Thr Ala Ser Ile Thr Cys Ser Gly Asp Asn	35	40	45	
Ile Gly Asp Gln Tyr Ala His Trp Tyr Gln Gln Lys Pro Gly Gln Ser	50	55	60	
Pro Val Leu Val Ile Tyr Gln Asp Lys Asn Arg Pro Ser Gly Ile Pro	65	70	75	80
Glu Arg Phe Ser Gly Ser Asn Ser Gly Asn Thr Ala Thr Leu Thr Ile	85	90	95	
Ser Gly Thr Gln Ala Met Asp Glu Ala Asp Tyr Tyr Cys Ala Thr Tyr	100	105	110	
Thr Gly Phe Gly Ser Leu Ala Val Phe Gly Gly Gly Thr Lys Leu Thr				

-continued

115					120					125					
Val	Leu	Gly	Ser	Thr	Ser	Gly	Ser	Gly	Lys	Pro	Gly	Ser	Gly	Glu	Gly
130						135					140				
Ser	Thr	Lys	Gly	Glu	Val	Gln	Leu	Val	Gln	Ser	Gly	Ala	Glu	Val	Lys
145					150					155					160
Lys	Pro	Gly	Glu	Ser	Leu	Arg	Ile	Ser	Cys	Lys	Gly	Ser	Gly	Tyr	Ser
				165					170					175	
Phe	Ser	Thr	Tyr	Trp	Ile	Ser	Trp	Val	Arg	Gln	Met	Pro	Gly	Lys	Gly
			180					185					190		
Leu	Glu	Trp	Met	Gly	Lys	Ile	Tyr	Pro	Gly	Asp	Ser	Tyr	Thr	Asn	Tyr
		195					200					205			
Ser	Pro	Ser	Phe	Gln	Gly	Gln	Val	Thr	Ile	Ser	Ala	Asp	Lys	Ser	Ile
		210				215					220				
Ser	Thr	Ala	Tyr	Leu	Gln	Trp	Ser	Ser	Leu	Lys	Ala	Ser	Asp	Thr	Ala
225					230					235					240
Met	Tyr	Tyr	Cys	Ala	Arg	Gly	Tyr	Gly	Ile	Phe	Asp	Tyr	Trp	Gly	Gln
			245						250					255	
Gly	Thr	Leu	Val	Thr	Val	Ser	Ser	Ser	Gly	Thr	Thr	Thr	Pro	Ala	Pro
		260						265					270		
Arg	Pro	Pro	Thr	Pro	Ala	Pro	Thr	Ile	Ala	Ser	Gln	Pro	Leu	Ser	Leu
		275					280					285			
Arg	Pro	Glu	Ala	Cys	Arg	Pro	Ala	Ala	Gly	Gly	Ala	Val	His	Thr	Arg
		290				295					300				
Gly	Leu	Asp	Phe	Ala	Cys	Asp	Ile	Tyr	Ile	Trp	Ala	Pro	Leu	Ala	Gly
305					310					315					320
Thr	Cys	Gly	Val	Leu	Leu	Leu	Ser	Leu	Val	Ile	Thr	Leu	Tyr	Cys	Lys
			325						330					335	
Arg	Gly	Arg	Lys	Lys	Leu	Leu	Tyr	Ile	Phe	Lys	Gln	Pro	Phe	Met	Arg
			340					345					350		
Pro	Val	Gln	Thr	Thr	Gln	Glu	Glu	Asp	Gly	Cys	Ser	Cys	Arg	Phe	Pro
		355					360					365			
Glu	Glu	Glu	Glu	Gly	Gly	Cys	Glu	Leu	Arg	Val	Lys	Phe	Ser	Arg	Ser
		370				375					380				
Ala	Asp	Ala	Pro	Ala	Tyr	Lys	Gln	Gly	Gln	Asn	Gln	Leu	Tyr	Asn	Glu
385					390					395					400
Leu	Asn	Leu	Gly	Arg	Arg	Glu	Glu	Tyr	Asp	Val	Leu	Asp	Lys	Arg	Arg
			405						410					415	
Gly	Arg	Asp	Pro	Glu	Met	Gly	Gly	Lys	Pro	Arg	Arg	Lys	Asn	Pro	Gln
			420					425					430		
Glu	Gly	Leu	Tyr	Asn	Glu	Leu	Gln	Lys	Asp	Lys	Met	Ala	Glu	Ala	Tyr
		435					440					445			
Ser	Glu	Ile	Gly	Met	Lys	Gly	Glu	Arg	Arg	Arg	Gly	Lys	Gly	His	Asp
		450				455					460				
Gly	Leu	Tyr	Gln	Gly	Leu	Ser	Thr	Ala	Thr	Lys	Asp	Thr	Tyr	Asp	Ala
465					470					475					480
Leu	His	Met	Gln	Ala	Leu	Pro	Pro	Arg							
				485											

<210> SEQ ID NO 57

<211> LENGTH: 1470

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

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<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic polynucleotide

<400> SEQUENCE: 57

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atggccctgc cctgaccgc cctgctgctg cccctggccc tgctgctgca cgccgccagg      60
cccatgagct acgagctgac ccagccccc agcgtgagcg tgagccccgg ccagaccgcc      120
agcatcacct gcagcgcgga caacatcggc gaccagtacg cccactggta ccagcagaag      180
cccgcccaga gccccgtgct ggtgatctac caggacaaga acaggcccag cggcateccc      240
gagaggttca gcggcagcaa cagcggcaac accgcccccc tgaccatcag cggcaccag      300
gccatggacg aggcggaact ctactcgcc acctacacgg gcttcggcag cctggccgtg      360
ttcggcgcg gcaccaagct gaccgtgctg ggcagcacca gcggcagcgg caagcccggc      420
agcggcgagg gcagcaccaa gggcgaggcg cagctggcgc agagcggcgc cgaggtgaag      480
aagcccgcg agagcctgag gatcagctgc aagggcagcg gctacagctt cagcacctac      540
tggatcagct gggtgaggca gatgcccggc aagggcctgg agtggatggg caagatctac      600
cccgcgaca gctacaccaa ctacagcccc agcttcacag gccaggtgac catcagcgcc      660
gacaagagca tcagcaccgc ctacctgcag tggagcagcc tgaaggccag cgacaccgcc      720
atgtactact gcgccagggg ctacggcacc ttcgactact ggggccaggg caccctggtg      780
accgtgagca gcagcggcac caccaccccc gccccaggc cccccacccc cgccccacc      840
atcgccagcc agccctgag cctgaggccc gaggcctgca ggcccccgcc cgcgcgcgcc      900
gtgcacacca ggggcctgga cttcgctgc gacatctaca tctgggcccc cctggccggc      960
acctcgcgcg tgctgctgct gaggcctggtg atcacccctg actgcaagag gggcagggaag      1020
aagctgctgt acatcttcaa gcagcccttc atgaggcccg tgcagaccac ccaggaggag      1080
gacggctgca gctgcaggtt ccccgaggag gaggagggcg gctgcgagct gaggggtgaag      1140
ttcagcagga gcgccgacgc ccccgctac aagcagggcc agaaccagct gtacaacgag      1200
ctgaacctgg gcaggaggga ggagtacgac gtgctggaca agaggagggg cagggacccc      1260
gagatggcg gcaagcccag gaggaagaac ccccgaggag gcctgtacaa cgagctgcag      1320
aaggacaaga tggccgaggc ctacagcgag atcggcata agggcgagag gaggaggggc      1380
aagggccacg acggcctgta ccagggcctg agcaccgcca ccaaggacac ctacgacgcc      1440
ctgcacatgc aggccctgcc ccccgaggtga

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<210> SEQ ID NO 58

<211> LENGTH: 1470

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic polynucleotide

<400> SEQUENCE: 58

```

atggccctgc cctgaccgc cctgctgctg cccctggccc tgctgctgca cgccgccagg      60
cccgaggcgc agctgggtgca gacggcgccc gaggtgaaga agcccgcgga gagcctgagg      120
atcagctgca agggcagcgg ctacagcttc agcacctact ggatcagctg ggtgaggcag      180
atgcccgga agggcctgga gtggatgggc aagatctacc ccggcgacag ctacaccaac      240
tacagcccca gcttcagggg ccaggtgacc atcagcgccg acaagagcat cagcaccgcc      300

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tacctgcagt ggagcagcct gaaggccagc gacaccgcca tgtactactg cgccaggggc 360
tacggcatct tcgactactg gggccagggc accctggtga ccgtgagcag cggcagcacc 420
agcggcagcg gcaagcccgg cagcggcgag ggcagcacca agggcatgag ctacgagctg 480
accagcccc ccagcgtgag cgtgagcccc ggcagaccg ccagcatcac ctgcagcggc 540
gacaacatcg gcgaccagta cgcccactgg taccagcaga agcccggcca gagccccgtg 600
ctggtgatct accaggacaa gaacaggccc agcggcatcc ccgagaggtt cagcggcagc 660
aacagcggca acaccgccac cctgaccatc agcggcacc ccgagaggtt cagcggcagc 720
tactactgcg ccacctacac cggtctcggc agcctggccg tggtcgccgg cggcaccaag 780
ctgaccgtgc tgagcggcac caccaccccc gccccaggc cccccacccc cgccccacc 840
atcgccagcc agcccctgag cctgaggccc gaggcctgca ggcccgccgc cgccggcgcc 900
gtgcacacca ggggcctgga ctctgcctgc gacatctaca tctgggcccc cctggccggc 960
acctgcggcg tgctgctgct gaggcctgtg atcacctgt actgcaagag gggcaggaag 1020
aagctgctgt acatcttcaa gcagccctc atgaggcccg tgcagaccac ccaggaggag 1080
gacggctgca gctgcaggtt ccccgaggag gaggaggcg gctgcgagct gagggggaag 1140
ttcagcagga gcgccgacgc ccccgctac aagcagggcc agaaccagct gtacaacgag 1200
ctgaacctgg gcaggaggga ggagtacgac gtgctggaca agaggagggg cagggacccc 1260
gagatgggcg gcaagcccag gaggaagaac ccccgaggag gcctgtacaa cgagctgcag 1320
aaggacaaga tggccgaggc ctacagcgag atcggcata agggcgagag gagggggggc 1380
aagggccacg acggcctgta ccagggcctg agcaccgcca ccaaggacac ctacgacgcc 1440
ctgcacatgc aggccctgcc ccccgaggtga 1470

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<210> SEQ ID NO 59

<211> LENGTH: 120

<212> TYPE: PRT

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic polypeptide

<400> SEQUENCE: 59

```

Glu Val Lys Leu Gln Glu Ser Gly Pro Gly Leu Val Ala Pro Ser Gln
1           5           10          15
Ser Leu Ser Val Thr Cys Thr Val Ser Gly Val Ser Leu Pro Asp Tyr
20          25          30
Gly Val Ser Trp Ile Arg Gln Pro Pro Arg Lys Gly Leu Glu Trp Leu
35          40          45
Gly Val Ile Trp Gly Ser Glu Thr Thr Tyr Tyr Asn Ser Ala Leu Lys
50          55          60
Ser Arg Leu Thr Ile Ile Lys Asp Asn Ser Lys Ser Gln Val Phe Leu
65          70          75          80
Lys Met Asn Ser Leu Gln Thr Asp Asp Thr Ala Ile Tyr Tyr Cys Ala
85          90          95
Lys His Tyr Tyr Tyr Gly Gly Ser Tyr Ala Met Asp Tyr Trp Gly Gln
100         105         110
Gly Thr Ser Val Thr Val Ser Ser
115         120

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<210> SEQ ID NO 60
<211> LENGTH: 107
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic polypeptide

<400> SEQUENCE: 60

Asp Ile Gln Met Thr Gln Thr Thr Ser Ser Leu Ser Ala Ser Leu Gly
1 5 10 15
Asp Arg Val Thr Ile Ser Cys Arg Ala Ser Gln Asp Ile Ser Lys Tyr
20 25 30
Leu Asn Trp Tyr Gln Gln Lys Pro Asp Gly Thr Val Lys Leu Leu Ile
35 40 45
Tyr His Thr Ser Arg Leu His Ser Gly Val Pro Ser Arg Phe Ser Gly
50 55 60
Ser Gly Ser Gly Thr Asp Tyr Ser Leu Thr Ile Ser Asn Leu Glu Gln
65 70 75 80
Glu Asp Ile Ala Thr Tyr Phe Cys Gln Gln Gly Asn Thr Leu Pro Tyr
85 90 95
Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Thr
100 105

<210> SEQ ID NO 61
<211> LENGTH: 491
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic polypeptide

<400> SEQUENCE: 61

Met Ala Leu Pro Val Thr Ala Leu Leu Leu Pro Leu Ala Leu Leu Leu
1 5 10 15
His Ala Ala Arg Pro Asp Ile Gln Met Thr Gln Thr Thr Ser Ser Leu
20 25 30
Ser Ala Ser Leu Gly Asp Arg Val Thr Ile Ser Cys Arg Ala Ser Gln
35 40 45
Asp Ile Ser Lys Tyr Leu Asn Trp Tyr Gln Gln Lys Pro Asp Gly Thr
50 55 60
Val Lys Leu Leu Ile Tyr His Thr Ser Arg Leu His Ser Gly Val Pro
65 70 75 80
Ser Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Tyr Ser Leu Thr Ile
85 90 95
Ser Asn Leu Glu Gln Glu Asp Ile Ala Thr Tyr Phe Cys Gln Gln Gly
100 105 110
Asn Thr Leu Pro Tyr Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Thr
115 120 125
Gly Ser Thr Ser Gly Ser Gly Lys Pro Gly Ser Gly Glu Gly Ser Thr
130 135 140
Lys Gly Glu Val Lys Leu Gln Glu Ser Gly Pro Gly Leu Val Ala Pro
145 150 155 160
Ser Gln Ser Leu Ser Val Thr Cys Thr Val Ser Gly Val Ser Leu Pro
165 170 175

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Asp	Tyr	Gly	Val	Ser	Trp	Ile	Arg	Gln	Pro	Pro	Arg	Lys	Gly	Leu	Glu
		180						185					190		
Trp	Leu	Gly	Val	Ile	Trp	Gly	Ser	Glu	Thr	Thr	Tyr	Tyr	Asn	Ser	Ala
		195					200					205			
Leu	Lys	Ser	Arg	Leu	Thr	Ile	Ile	Lys	Asp	Asn	Ser	Lys	Ser	Gln	Val
	210					215					220				
Phe	Leu	Lys	Met	Asn	Ser	Leu	Gln	Thr	Asp	Asp	Thr	Ala	Ile	Tyr	Tyr
225				230					235					240	
Cys	Ala	Lys	His	Tyr	Tyr	Tyr	Gly	Gly	Ser	Tyr	Ala	Met	Asp	Tyr	Trp
			245						250					255	
Gly	Gln	Gly	Thr	Ser	Val	Thr	Val	Ser	Ser	Ser	Gly	Thr	Thr	Thr	Pro
			260					265					270		
Ala	Pro	Arg	Pro	Pro	Thr	Pro	Ala	Pro	Thr	Ile	Ala	Ser	Gln	Pro	Leu
		275					280					285			
Ser	Leu	Arg	Pro	Glu	Ala	Cys	Arg	Pro	Ala	Ala	Gly	Gly	Ala	Val	His
	290					295					300				
Thr	Arg	Gly	Leu	Asp	Phe	Ala	Cys	Asp	Ile	Tyr	Ile	Trp	Ala	Pro	Leu
305				310						315					320
Ala	Gly	Thr	Cys	Gly	Val	Leu	Leu	Leu	Ser	Leu	Val	Ile	Thr	Leu	Tyr
			325						330					335	
Cys	Lys	Arg	Gly	Arg	Lys	Lys	Leu	Leu	Tyr	Ile	Phe	Lys	Gln	Pro	Phe
			340					345					350		
Met	Arg	Pro	Val	Gln	Thr	Thr	Gln	Glu	Glu	Asp	Gly	Cys	Ser	Cys	Arg
		355					360					365			
Phe	Pro	Glu	Glu	Glu	Glu	Gly	Gly	Cys	Glu	Leu	Arg	Val	Lys	Phe	Ser
	370					375					380				
Arg	Ser	Ala	Asp	Ala	Pro	Ala	Tyr	Lys	Gln	Gly	Gln	Asn	Gln	Leu	Tyr
385				390						395					400
Asn	Glu	Leu	Asn	Leu	Gly	Arg	Arg	Glu	Glu	Tyr	Asp	Val	Leu	Asp	Lys
			405						410					415	
Arg	Arg	Gly	Arg	Asp	Pro	Glu	Met	Gly	Gly	Lys	Pro	Arg	Arg	Lys	Asn
			420					425					430		
Pro	Gln	Glu	Gly	Leu	Tyr	Asn	Glu	Leu	Gln	Lys	Asp	Lys	Met	Ala	Glu
		435					440					445			
Ala	Tyr	Ser	Glu	Ile	Gly	Met	Lys	Gly	Glu	Arg	Arg	Arg	Gly	Lys	Gly
	450					455					460				
His	Asp	Gly	Leu	Tyr	Gln	Gly	Leu	Ser	Thr	Ala	Thr	Lys	Asp	Thr	Tyr
465					470					475					480
Asp	Ala	Leu	His	Met	Gln	Ala	Leu	Pro	Pro	Arg					
			485					490							

<210> SEQ ID NO 62

<400> SEQUENCE: 62

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<210> SEQ ID NO 63

<400> SEQUENCE: 63

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<210> SEQ ID NO 64

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<400> SEQUENCE: 64

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<210> SEQ ID NO 65

<400> SEQUENCE: 65

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<210> SEQ ID NO 66

<400> SEQUENCE: 66

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<210> SEQ ID NO 67

<400> SEQUENCE: 67

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<210> SEQ ID NO 68

<400> SEQUENCE: 68

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<210> SEQ ID NO 69

<400> SEQUENCE: 69

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<210> SEQ ID NO 70

<400> SEQUENCE: 70

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<210> SEQ ID NO 71

<400> SEQUENCE: 71

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<210> SEQ ID NO 72

<400> SEQUENCE: 72

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<210> SEQ ID NO 73

<400> SEQUENCE: 73

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<210> SEQ ID NO 74

<400> SEQUENCE: 74

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<210> SEQ ID NO 75
<211> LENGTH: 486
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic polypeptide

<400> SEQUENCE: 75

Met Ala Leu Pro Val Thr Ala Leu Leu Leu Pro Leu Ala Leu Leu Leu
1 5 10 15

His Ala Ala Arg Pro Asp Ile Glu Leu Thr Gln Ser Pro Ala Ile Met
20 25 30

Ser Ala Ser Leu Gly Glu Glu Ile Thr Leu Thr Cys Ser Ala Ser Ser
35 40 45

Ser Val Ser Tyr Met His Trp Tyr Gln Gln Lys Ser Gly Thr Ser Pro
50 55 60

Lys Leu Leu Ile Tyr Ser Thr Ser Asn Leu Ala Ser Gly Val Pro Ser
65 70 75 80

Arg Phe Ser Gly Ser Gly Ser Gly Thr Phe Tyr Ser Leu Thr Ile Ser
85 90 95

Ser Val Glu Ala Glu Asp Ala Ala Asp Tyr Tyr Cys His Gln Trp Ser
100 105 110

Ser Tyr Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys Arg Gly Gly
115 120 125

Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gln Val Lys
130 135 140

Leu Gln Glu Ser Gly Gly Gly Leu Val Lys Pro Gly Gly Ser Leu Lys
145 150 155 160

Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr Ala Met Ser
165 170 175

Trp Val Arg Gln Thr Pro Glu Lys Arg Leu Glu Trp Val Ala Thr Ile
180 185 190

Ser Ser Gly Gly Ser Tyr Thr Tyr Tyr Pro Asp Ser Val Lys Gly Arg
195 200 205

Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Thr Leu Tyr Leu Gln Met
210 215 220

Ser Ser Leu Arg Ser Glu Asp Thr Ala Met Tyr Tyr Cys Ala Arg Gln
225 230 235 240

Asp Gly Tyr Tyr Pro Gly Trp Phe Ala Asn Trp Gly Gln Gly Thr Thr
245 250 255

Val Thr Val Ser Ser Ser Gly Thr Thr Thr Pro Ala Pro Arg Pro Pro
260 265 270

Thr Pro Ala Pro Thr Ile Ala Ser Gln Pro Leu Ser Leu Arg Pro Glu
275 280 285

Ala Cys Arg Pro Ala Ala Gly Gly Ala Val His Thr Arg Gly Leu Asp
290 295 300

Phe Ala Cys Asp Ile Tyr Ile Trp Ala Pro Leu Ala Gly Thr Cys Gly
305 310 315 320

Val Leu Leu Leu Ser Leu Val Ile Thr Leu Tyr Cys Lys Arg Gly Arg
325 330 335

Lys Lys Leu Leu Tyr Ile Phe Lys Gln Pro Phe Met Arg Pro Val Gln
340 345 350

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Thr Thr Gln Glu Glu Asp Gly Cys Ser Cys Arg Phe Pro Glu Glu Glu
355 360 365

Glu Gly Gly Cys Glu Leu Arg Val Lys Phe Ser Arg Ser Ala Asp Ala
370 375 380

Pro Ala Tyr Lys Gln Gly Gln Asn Gln Leu Tyr Asn Glu Leu Asn Leu
385 390 395 400

Gly Arg Arg Glu Glu Tyr Asp Val Leu Asp Lys Arg Arg Gly Arg Asp
405 410 415

Pro Glu Met Gly Gly Lys Pro Arg Arg Lys Asn Pro Gln Glu Gly Leu
420 425 430

Tyr Asn Glu Leu Gln Lys Asp Lys Met Ala Glu Ala Tyr Ser Glu Ile
435 440 445

Gly Met Lys Gly Glu Arg Arg Arg Gly Lys Gly His Asp Gly Leu Tyr
450 455 460

Gln Gly Leu Ser Thr Ala Thr Lys Asp Thr Tyr Asp Ala Leu His Met
465 470 475 480

Gln Ala Leu Pro Pro Arg
485

<210> SEQ ID NO 76
<211> LENGTH: 490
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
polypeptide

<400> SEQUENCE: 76

Met Ala Leu Pro Val Thr Ala Leu Leu Leu Pro Leu Ala Leu Leu Leu
1 5 10 15

His Ala Ala Arg Pro Ala Ala Tyr Lys Asp Ile Gln Met Thr Gln Thr
20 25 30

Thr Ser Ser Leu Ser Ala Ser Leu Gly Asp Arg Val Thr Ile Ser Cys
35 40 45

Ser Ala Ser Gln Gly Ile Ser Asn Tyr Leu Asn Trp Tyr Gln Gln Lys
50 55 60

Pro Asp Gly Thr Val Lys Leu Leu Ile Tyr Tyr Thr Ser Ser Leu His
65 70 75 80

Ser Gly Val Pro Ser Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Tyr
85 90 95

Ser Leu Thr Ile Ser Asn Leu Glu Pro Glu Asp Ile Ala Thr Tyr Tyr
100 105 110

Cys Gln Gln Tyr Ser Lys Leu Pro Tyr Thr Phe Gly Gly Gly Thr Lys
115 120 125

Leu Glu Ile Lys Arg Gly Gly Gly Gly Ser Gly Gly Gly Ser Gly
130 135 140

Gly Gly Gly Ser Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val
145 150 155 160

Lys Pro Gly Gly Ser Leu Lys Leu Ser Cys Ala Ala Ser Gly Leu Thr
165 170 175

Phe Ser Ser Tyr Ala Met Ser Trp Val Arg Gln Thr Pro Glu Lys Arg
180 185 190

Leu Glu Trp Val Ala Ser Ile Ser Ser Gly Gly Phe Thr Tyr Tyr Pro
195 200 205

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Asp Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Arg Asn
 210                215                220

Ile Leu Tyr Leu Gln Met Ser Ser Leu Arg Ser Glu Asp Thr Ala Met
 225                230                235                240

Tyr Tyr Cys Ala Arg Asp Glu Val Arg Gly Tyr Leu Asp Val Trp Gly
                245                250                255

Ala Gly Thr Thr Val Thr Val Ser Ser Ser Gly Thr Thr Thr Pro Ala
                260                265                270

Pro Arg Pro Pro Thr Pro Ala Pro Thr Ile Ala Ser Gln Pro Leu Ser
                275                280                285

Leu Arg Pro Glu Ala Cys Arg Pro Ala Ala Gly Gly Ala Val His Thr
 290                295                300

Arg Gly Leu Asp Phe Ala Cys Asp Ile Tyr Ile Trp Ala Pro Leu Ala
 305                310                315                320

Gly Thr Cys Gly Val Leu Leu Leu Ser Leu Val Ile Thr Leu Tyr Cys
                325                330                335

Lys Arg Gly Arg Lys Lys Leu Leu Tyr Ile Phe Lys Gln Pro Phe Met
                340                345                350

Arg Pro Val Gln Thr Thr Gln Glu Glu Asp Gly Cys Ser Cys Arg Phe
                355                360                365

Pro Glu Glu Glu Glu Gly Gly Cys Glu Leu Arg Val Lys Phe Ser Arg
 370                375                380

Ser Ala Asp Ala Pro Ala Tyr Lys Gln Gly Gln Asn Gln Leu Tyr Asn
 385                390                395                400

Glu Leu Asn Leu Gly Arg Arg Glu Glu Tyr Asp Val Leu Asp Lys Arg
                405                410                415

Arg Gly Arg Asp Pro Glu Met Gly Gly Lys Pro Arg Arg Lys Asn Pro
                420                425                430

Gln Glu Gly Leu Tyr Asn Glu Leu Gln Lys Asp Lys Met Ala Glu Ala
                435                440                445

Tyr Ser Glu Ile Gly Met Lys Gly Glu Arg Arg Arg Gly Lys Gly His
 450                455                460

Asp Gly Leu Tyr Gln Gly Leu Ser Thr Ala Thr Lys Asp Thr Tyr Asp
 465                470                475                480

Ala Leu His Met Gln Ala Leu Pro Pro Arg
                485                490

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<210> SEQ ID NO 77

<211> LENGTH: 492

<212> TYPE: PRT

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic polypeptide

<400> SEQUENCE: 77

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Met Ala Leu Pro Val Thr Ala Leu Leu Leu Pro Leu Ala Leu Leu Leu
 1                5                10                15

His Ala Ala Arg Pro Asp Ile Gln Met Thr Gln Ser Pro Ala Ser Leu
                20                25                30

Ser Ala Ser Val Gly Glu Thr Val Thr Ile Thr Cys Arg Ala Ser Gly
                35                40                45

Asn Ile His Asn Tyr Leu Ala Trp Tyr Gln Gln Lys Gln Gly Lys Ser

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50											55											60										
Pro 65	Gln	Leu	Leu	Val	Tyr 70	Asn	Ala	Lys	Thr	Leu 75	Ala	Asp	Gly	Val	Pro 80																	
Ser	Arg	Phe	Ser	Gly 85	Ser	Gly	Ser	Gly	Thr 90	Gln	Tyr	Ser	Leu	Lys 95	Ile																	
Asn	Ser	Leu	Gln	Pro	Glu	Asp	Phe	Gly 105	Ser	Tyr	Tyr	Cys	Gln	His	Phe 110																	
Trp	Thr	Thr	Pro	Pro	Trp	Thr	Phe	Gly 120	Gly	Gly	Gly	Thr	Lys	Leu	Glu	Ile 125																
Lys	Gly 130	Gly	Gly	Gly	Ser	Gly 135	Gly	Gly	Gly	Ser	Gly 140	Gly	Gly	Gly	Ser																	
Gln 145	Ile	Gln	Leu	Val	Gln	Ser	Gly	Pro	Glu	Leu	Lys 155	Lys	Pro	Gly	Glu 160																	
Thr	Val	Lys	Ile	Ser 165	Cys	Lys	Ala	Ser	Gly 170	Tyr	Thr	Phe	Thr	Asn	Tyr 175																	
Gly	Met	Asn	Trp	Val	Lys	Gln	Ala	Pro 185	Gly	Lys	Gly	Leu	Lys	Trp	Met 190																	
Gly	Trp	Ile	Asn	Thr	Tyr	Asn	Gly 200	Glu	Pro	Thr	Tyr	Ala	Asp	Asp	Phe 205																	
Lys	Gly 210	Arg	Phe	Asp	Phe	Ser	Leu	Glu	Thr	Ser	Ala 220	Ser	Thr	Ala	Tyr 225																	
Leu 225	Gln	Ile	Asn	Asn	Leu	Lys	Asn	Glu	Asp	Thr	Ala 235	Thr	Tyr	Phe	Cys 240																	
Ala	Arg	Arg	Gly	Tyr 245	Tyr	Tyr	Gly	Ser	Arg 250	Tyr	Gly	Ala	Met	Asp	Tyr 255																	
Trp	Gly	Gln	Gly	Thr	Ser	Val	Thr	Val	Ser	Ser	Ser	Gly	Thr	Thr	Thr 270																	
Pro	Ala	Pro	Arg	Pro	Pro	Thr	Pro	Ala	Pro	Thr	Ile	Ala	Ser	Gln	Pro 285																	
Leu	Ser	Leu	Arg	Pro	Glu	Ala	Cys	Arg	Pro	Ala	Ala	Gly	Gly	Ala	Val 300																	
His 305	Thr	Arg	Gly	Leu	Asp	Phe	Ala	Cys	Asp	Ile	Tyr	Ile	Trp	Ala	Pro 320																	
Leu	Ala	Gly	Thr	Cys	Gly	Val	Leu	Leu	Leu	Ser	Leu	Val	Ile	Thr	Leu 335																	
Tyr	Cys	Lys	Arg	Gly	Arg	Lys	Lys	Leu	Leu	Tyr	Ile	Phe	Lys	Gln	Pro 350																	
Phe	Met	Arg	Pro	Val	Gln	Thr	Thr	Gln	Glu	Glu	Asp	Gly	Cys	Ser	Cys 365																	
Arg	Phe	Pro	Glu	Glu	Glu	Glu	Gly	Gly	Cys	Glu	Leu	Arg	Val	Lys	Phe 380																	
Ser 385	Arg	Ser	Ala	Asp	Ala	Pro	Ala	Tyr	Lys	Gln	Gly	Gln	Asn	Gln	Leu 400																	
Tyr	Asn	Glu	Leu	Asn	Leu	Gly	Arg	Arg	Glu	Glu	Tyr	Asp	Val	Leu	Asp 415																	
Lys	Arg	Arg	Gly	Arg	Asp	Pro	Glu	Met	Gly	Gly	Lys	Pro	Arg	Arg	Lys 430																	
Asn	Pro	Gln	Glu	Gly	Leu	Tyr	Asn	Glu	Leu	Gln	Lys	Asp	Lys	Met	Ala 445																	
Glu	Ala	Tyr	Ser	Glu	Ile	Gly	Met	Lys	Gly	Glu	Arg	Arg	Arg	Gly	Lys 460																	

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Gly His Asp Gly Leu Tyr Gln Gly Leu Ser Thr Ala Thr Lys Asp Thr
465 470 475 480

Tyr Asp Ala Leu His Met Gln Ala Leu Pro Pro Arg
485 490

<210> SEQ ID NO 78

<211> LENGTH: 492

<212> TYPE: PRT

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic polypeptide

<400> SEQUENCE: 78

Met Ala Leu Pro Val Thr Ala Leu Leu Leu Pro Leu Ala Leu Leu Leu
1 5 10 15

His Ala Ala Arg Pro Gln Ile Gln Leu Val Gln Ser Gly Pro Glu Leu
20 25 30

Lys Lys Pro Gly Glu Thr Val Lys Ile Ser Cys Lys Ala Ser Gly Tyr
35 40 45

Thr Phe Thr Asn Tyr Gly Met Asn Trp Val Lys Gln Ala Pro Gly Lys
50 55 60

Gly Leu Lys Trp Met Gly Trp Ile Asn Thr Tyr Asn Gly Glu Pro Thr
65 70 75 80

Tyr Ala Asp Asp Phe Lys Gly Arg Phe Asp Phe Ser Leu Glu Thr Ser
85 90 95

Ala Ser Thr Ala Tyr Leu Gln Ile Asn Asn Leu Lys Asn Glu Asp Thr
100 105 110

Ala Thr Tyr Phe Cys Ala Arg Arg Gly Tyr Tyr Tyr Gly Ser Arg Tyr
115 120 125

Gly Ala Met Asp Tyr Trp Gly Gln Gly Thr Ser Val Thr Val Ser Ser
130 135 140

Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Asp
145 150 155 160

Ile Gln Met Thr Gln Ser Pro Ala Ser Leu Ser Ala Ser Val Gly Glu
165 170 175

Thr Val Thr Ile Thr Cys Arg Ala Ser Gly Asn Ile His Asn Tyr Leu
180 185 190

Ala Trp Tyr Gln Gln Lys Gln Gly Lys Ser Pro Gln Leu Leu Val Tyr
195 200 205

Asn Ala Lys Thr Leu Ala Asp Gly Val Pro Ser Arg Phe Ser Gly Ser
210 215 220

Gly Ser Gly Thr Gln Tyr Ser Leu Lys Ile Asn Ser Leu Gln Pro Glu
225 230 235 240

Asp Phe Gly Ser Tyr Tyr Cys Gln His Phe Trp Thr Thr Pro Pro Trp
245 250 255

Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys Ser Gly Thr Thr Thr
260 265 270

Pro Ala Pro Arg Pro Pro Thr Pro Ala Pro Thr Ile Ala Ser Gln Pro
275 280 285

Leu Ser Leu Arg Pro Glu Ala Cys Arg Pro Ala Ala Gly Gly Ala Val
290 295 300

His Thr Arg Gly Leu Asp Phe Ala Cys Asp Ile Tyr Ile Trp Ala Pro

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305	310	315	320
Leu Ala Gly Thr Cys Gly Val Leu Leu Leu Ser Leu Val Ile Thr Leu	325	330	335
Tyr Cys Lys Arg Gly Arg Lys Lys Leu Leu Tyr Ile Phe Lys Gln Pro	340	345	350
Phe Met Arg Pro Val Gln Thr Thr Gln Glu Glu Asp Gly Cys Ser Cys	355	360	365
Arg Phe Pro Glu Glu Glu Glu Gly Gly Cys Glu Leu Arg Val Lys Phe	370	375	380
Ser Arg Ser Ala Asp Ala Pro Ala Tyr Lys Gln Gly Gln Asn Gln Leu	385	390	395
Tyr Asn Glu Leu Asn Leu Gly Arg Arg Glu Glu Tyr Asp Val Leu Asp	405	410	415
Lys Arg Arg Gly Arg Asp Pro Glu Met Gly Gly Lys Pro Arg Arg Lys	420	425	430
Asn Pro Gln Glu Gly Leu Tyr Asn Glu Leu Gln Lys Asp Lys Met Ala	435	440	445
Glu Ala Tyr Ser Glu Ile Gly Met Lys Gly Glu Arg Arg Arg Gly Lys	450	455	460
Gly His Asp Gly Leu Tyr Gln Gly Leu Ser Thr Ala Thr Lys Asp Thr	465	470	475
Tyr Asp Ala Leu His Met Gln Ala Leu Pro Pro Arg	485	490	

<210> SEQ ID NO 79

<211> LENGTH: 726

<212> TYPE: PRT

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic polypeptide

<400> SEQUENCE: 79

Met Ala Leu Pro Val Thr Ala Leu Leu Leu Pro Leu Ala Leu Leu Leu	1	5	10	15
His Ala Ala Arg Pro Asp Ile Gln Met Thr Gln Thr Thr Ser Ser Leu	20	25	30	
Ser Ala Ser Leu Gly Asp Arg Val Thr Ile Ser Cys Arg Ala Ser Gln	35	40	45	
Asp Ile Ser Lys Tyr Leu Asn Trp Tyr Gln Gln Lys Pro Asp Gly Thr	50	55	60	
Val Lys Leu Leu Ile Tyr His Thr Ser Arg Leu His Ser Gly Val Pro	65	70	75	80
Ser Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Tyr Ser Leu Thr Ile	85	90	95	
Ser Asn Leu Glu Gln Glu Asp Ile Ala Thr Tyr Phe Cys Gln Gln Gly	100	105	110	
Asn Thr Leu Pro Tyr Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Thr	115	120	125	
Gly Gly Gly Gly Ser Gln Val Lys Leu Gln Glu Ser Gly Gly Gly Leu	130	135	140	
Val Lys Pro Gly Gly Ser Leu Lys Leu Ser Cys Ala Ala Ser Gly Phe	145	150	155	160

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Thr	Phe	Ser	Ser	Tyr	Ala	Met	Ser	Trp	Val	Arg	Gln	Thr	Pro	Glu	Lys	
				165					170					175		
Arg	Leu	Glu	Trp	Val	Ala	Thr	Ile	Ser	Ser	Gly	Gly	Ser	Tyr	Thr	Tyr	
		180						185					190			
Tyr	Pro	Asp	Ser	Val	Lys	Gly	Arg	Phe	Thr	Ile	Ser	Arg	Asp	Asn	Ala	
		195					200					205				
Lys	Asn	Thr	Leu	Tyr	Leu	Gln	Met	Ser	Ser	Leu	Arg	Ser	Glu	Asp	Thr	
	210					215					220					
Ala	Met	Tyr	Tyr	Cys	Ala	Arg	Gln	Asp	Gly	Tyr	Tyr	Pro	Gly	Trp	Phe	
225					230					235					240	
Ala	Asn	Trp	Gly	Gln	Gly	Thr	Thr	Val	Thr	Val	Ser	Ser	Gly	Ser	Thr	
			245						250					255		
Ser	Gly	Ser	Gly	Lys	Pro	Gly	Ser	Gly	Glu	Gly	Ser	Thr	Lys	Gly	Asp	
		260						265					270			
Ile	Glu	Leu	Thr	Gln	Ser	Pro	Ala	Ile	Met	Ser	Ala	Ser	Leu	Gly	Glu	
	275						280					285				
Glu	Ile	Thr	Leu	Thr	Cys	Ser	Ala	Ser	Ser	Ser	Val	Ser	Tyr	Met	His	
	290					295					300					
Trp	Tyr	Gln	Gln	Lys	Ser	Gly	Thr	Ser	Pro	Lys	Leu	Leu	Ile	Tyr	Ser	
305					310					315					320	
Thr	Ser	Asn	Leu	Ala	Ser	Gly	Val	Pro	Ser	Arg	Phe	Ser	Gly	Ser	Gly	
			325					330						335		
Ser	Gly	Thr	Phe	Tyr	Ser	Leu	Thr	Ile	Ser	Ser	Val	Glu	Ala	Glu	Asp	
		340						345					350			
Ala	Ala	Asp	Tyr	Tyr	Cys	His	Gln	Trp	Ser	Ser	Tyr	Thr	Phe	Gly	Gly	
		355					360						365			
Gly	Thr	Lys	Leu	Glu	Ile	Lys	Arg	Gly	Gly	Gly	Gly	Ser	Glu	Val	Lys	
	370					375					380					
Leu	Gln	Glu	Ser	Gly	Pro	Gly	Leu	Val	Ala	Pro	Ser	Gln	Ser	Leu	Ser	
385					390					395					400	
Val	Thr	Cys	Thr	Val	Ser	Gly	Val	Ser	Leu	Pro	Asp	Tyr	Gly	Val	Ser	
			405						410					415		
Trp	Ile	Arg	Gln	Pro	Pro	Arg	Lys	Gly	Leu	Glu	Trp	Leu	Gly	Val	Ile	
			420					425						430		
Trp	Gly	Ser	Glu	Thr	Thr	Tyr	Tyr	Asn	Ser	Ala	Leu	Lys	Ser	Arg	Leu	
		435					440					445				
Thr	Ile	Ile	Lys	Asp	Asn	Ser	Lys	Ser	Gln	Val	Phe	Leu	Lys	Met	Asn	
	450					455					460					
Ser	Leu	Gln	Thr	Asp	Asp	Thr	Ala	Ile	Tyr	Tyr	Cys	Ala	Lys	His	Tyr	
465					470					475					480	
Tyr	Tyr	Gly	Gly	Ser	Tyr	Ala	Met	Asp	Tyr	Trp	Gly	Gln	Gly	Thr	Ser	
				485					490					495		
Val	Thr	Val	Ser	Ser	Ser	Gly	Thr	Thr	Thr	Pro	Ala	Pro	Arg	Pro	Pro	
			500					505					510			
Thr	Pro	Ala	Pro	Thr	Ile	Ala	Ser	Gln	Pro	Leu	Ser	Leu	Arg	Pro	Glu	
		515					520					525				
Ala	Cys	Arg	Pro	Ala	Ala	Gly	Gly	Ala	Val	His	Thr	Arg	Gly	Leu	Asp	
	530					535					540					
Phe	Ala	Cys	Asp	Ile	Tyr	Ile	Trp	Ala	Pro	Leu	Ala	Gly	Thr	Cys	Gly	
545					550				555						560	
Val	Leu	Leu	Leu	Ser	Leu	Val	Ile	Thr	Leu	Tyr	Cys	Lys	Arg	Gly	Arg	

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565					570					575					
Lys	Lys	Leu	Leu	Tyr	Ile	Phe	Lys	Gln	Pro	Phe	Met	Arg	Pro	Val	Gln
		580					585					590			
Thr	Thr	Gln	Glu	Glu	Asp	Gly	Cys	Ser	Cys	Arg	Phe	Pro	Glu	Glu	Glu
		595				600					605				
Glu	Gly	Gly	Cys	Glu	Leu	Arg	Val	Lys	Phe	Ser	Arg	Ser	Ala	Asp	Ala
	610					615					620				
Pro	Ala	Tyr	Lys	Gln	Gly	Gln	Asn	Gln	Leu	Tyr	Asn	Glu	Leu	Asn	Leu
	625					630					635				640
Gly	Arg	Arg	Glu	Glu	Tyr	Asp	Val	Leu	Asp	Lys	Arg	Arg	Gly	Arg	Asp
			645						650					655	
Pro	Glu	Met	Gly	Gly	Lys	Pro	Arg	Arg	Lys	Asn	Pro	Gln	Glu	Gly	Leu
		660					665					670			
Tyr	Asn	Glu	Leu	Gln	Lys	Asp	Lys	Met	Ala	Glu	Ala	Tyr	Ser	Glu	Ile
		675					680					685			
Gly	Met	Lys	Gly	Glu	Arg	Arg	Arg	Gly	Lys	Gly	His	Asp	Gly	Leu	Tyr
	690					695					700				
Gln	Gly	Leu	Ser	Thr	Ala	Thr	Lys	Asp	Thr	Tyr	Asp	Ala	Leu	His	Met
	705					710					715				720
Gln	Ala	Leu	Pro	Pro	Arg										
			725												

<210> SEQ ID NO 80
 <211> LENGTH: 726
 <212> TYPE: PRT
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic polypeptide

<400> SEQUENCE: 80

Met	Ala	Leu	Pro	Val	Thr	Ala	Leu	Leu	Leu	Pro	Leu	Ala	Leu	Leu	Leu
1				5						10				15	
His	Ala	Ala	Arg	Pro	Glu	Val	Lys	Leu	Gln	Glu	Ser	Gly	Pro	Gly	Leu
			20					25					30		
Val	Ala	Pro	Ser	Gln	Ser	Leu	Ser	Val	Thr	Cys	Thr	Val	Ser	Gly	Val
		35				40						45			
Ser	Leu	Pro	Asp	Tyr	Gly	Val	Ser	Trp	Ile	Arg	Gln	Pro	Pro	Arg	Lys
	50					55				60					
Gly	Leu	Glu	Trp	Leu	Gly	Val	Ile	Trp	Gly	Ser	Glu	Thr	Thr	Tyr	Tyr
	65				70				75					80	
Asn	Ser	Ala	Leu	Lys	Ser	Arg	Leu	Thr	Ile	Ile	Lys	Asp	Asn	Ser	Lys
			85					90					95		
Ser	Gln	Val	Phe	Leu	Lys	Met	Asn	Ser	Leu	Gln	Thr	Asp	Asp	Thr	Ala
			100					105					110		
Ile	Tyr	Tyr	Cys	Ala	Lys	His	Tyr	Tyr	Tyr	Gly	Gly	Ser	Tyr	Ala	Met
		115					120					125			
Asp	Tyr	Trp	Gly	Gln	Gly	Thr	Ser	Val	Thr	Val	Ser	Ser	Gly	Gly	Gly
	130					135					140				
Gly	Ser	Asp	Ile	Glu	Leu	Thr	Gln	Ser	Pro	Ala	Ile	Met	Ser	Ala	Ser
	145				150					155					160
Leu	Gly	Glu	Glu	Ile	Thr	Leu	Thr	Cys	Ser	Ala	Ser	Ser	Ser	Val	Ser
			165					170						175	

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Tyr	Met	His	Trp	Tyr	Gln	Gln	Lys	Ser	Gly	Thr	Ser	Pro	Lys	Leu	Leu
			180					185					190		
Ile	Tyr	Ser	Thr	Ser	Asn	Leu	Ala	Ser	Gly	Val	Pro	Ser	Arg	Phe	Ser
		195					200					205			
Gly	Ser	Gly	Ser	Gly	Thr	Phe	Tyr	Ser	Leu	Thr	Ile	Ser	Ser	Val	Glu
	210					215					220				
Ala	Glu	Asp	Ala	Ala	Asp	Tyr	Tyr	Cys	His	Gln	Trp	Ser	Ser	Tyr	Thr
225					230					235					240
Phe	Gly	Gly	Gly	Thr	Lys	Leu	Glu	Ile	Lys	Arg	Gly	Ser	Thr	Ser	Gly
				245					250					255	
Ser	Gly	Lys	Pro	Gly	Ser	Gly	Glu	Gly	Ser	Thr	Lys	Gly	Gln	Val	Lys
		260						265					270		
Leu	Gln	Glu	Ser	Gly	Gly	Gly	Leu	Val	Lys	Pro	Gly	Gly	Ser	Leu	Lys
		275					280					285			
Leu	Ser	Cys	Ala	Ala	Ser	Gly	Phe	Thr	Phe	Ser	Ser	Tyr	Ala	Met	Ser
	290					295					300				
Trp	Val	Arg	Gln	Thr	Pro	Glu	Lys	Arg	Leu	Glu	Trp	Val	Ala	Thr	Ile
305					310					315					320
Ser	Ser	Gly	Gly	Ser	Tyr	Thr	Tyr	Tyr	Pro	Asp	Ser	Val	Lys	Gly	Arg
				325					330					335	
Phe	Thr	Ile	Ser	Arg	Asp	Asn	Ala	Lys	Asn	Thr	Leu	Tyr	Leu	Gln	Met
			340					345					350		
Ser	Ser	Leu	Arg	Ser	Glu	Asp	Thr	Ala	Met	Tyr	Tyr	Cys	Ala	Arg	Gln
		355					360					365			
Asp	Gly	Tyr	Tyr	Pro	Gly	Trp	Phe	Ala	Asn	Trp	Gly	Gln	Gly	Thr	Thr
	370					375					380				
Val	Thr	Val	Ser	Ser	Gly	Gly	Gly	Gly	Ser	Asp	Ile	Gln	Met	Thr	Gln
385					390					395					400
Thr	Thr	Ser	Ser	Leu	Ser	Ala	Ser	Leu	Gly	Asp	Arg	Val	Thr	Ile	Ser
				405					410					415	
Cys	Arg	Ala	Ser	Gln	Asp	Ile	Ser	Lys	Tyr	Leu	Asn	Trp	Tyr	Gln	Gln
			420					425					430		
Lys	Pro	Asp	Gly	Thr	Val	Lys	Leu	Leu	Ile	Tyr	His	Thr	Ser	Arg	Leu
		435					440					445			
His	Ser	Gly	Val	Pro	Ser	Arg	Phe	Ser	Gly	Ser	Gly	Ser	Gly	Thr	Asp
	450					455					460				
Tyr	Ser	Leu	Thr	Ile	Ser	Asn	Leu	Glu	Gln	Glu	Asp	Ile	Ala	Thr	Tyr
465					470					475					480
Phe	Cys	Gln	Gln	Gly	Asn	Thr	Leu	Pro	Tyr	Thr	Phe	Gly	Gly	Gly	Thr
				485					490					495	
Lys	Leu	Glu	Ile	Thr	Ser	Gly	Thr	Thr	Thr	Pro	Ala	Pro	Arg	Pro	Pro
			500					505					510		
Thr	Pro	Ala	Pro	Thr	Ile	Ala	Ser	Gln	Pro	Leu	Ser	Leu	Arg	Pro	Glu
			515				520					525			
Ala	Cys	Arg	Pro	Ala	Ala	Gly	Gly	Ala	Val	His	Thr	Arg	Gly	Leu	Asp
	530					535					540				
Phe	Ala	Cys	Asp	Ile	Tyr	Ile	Trp	Ala	Pro	Leu	Ala	Gly	Thr	Cys	Gly
545					550					555					560
Val	Leu	Leu	Leu	Ser	Leu	Val	Ile	Thr	Leu	Tyr	Cys	Lys	Arg	Gly	Arg
				565					570					575	
Lys	Lys	Leu	Leu	Tyr	Ile	Phe	Lys	Gln	Pro	Phe	Met	Arg	Pro	Val	Gln

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580	585	590
Thr Thr Gln Glu Glu Asp Gly Cys Ser Cys Arg Phe Pro Glu Glu Glu		
595	600	605
Glu Gly Gly Cys Glu Leu Arg Val Lys Phe Ser Arg Ser Ala Asp Ala		
610	615	620
Pro Ala Tyr Lys Gln Gly Gln Asn Gln Leu Tyr Asn Glu Leu Asn Leu		
625	630	635
Gly Arg Arg Glu Glu Tyr Asp Val Leu Asp Lys Arg Arg Gly Arg Asp		
645	650	655
Pro Glu Met Gly Gly Lys Pro Arg Arg Lys Asn Pro Gln Glu Gly Leu		
660	665	670
Tyr Asn Glu Leu Gln Lys Asp Lys Met Ala Glu Ala Tyr Ser Glu Ile		
675	680	685
Gly Met Lys Gly Glu Arg Arg Arg Gly Lys Gly His Asp Gly Leu Tyr		
690	695	700
Gln Gly Leu Ser Thr Ala Thr Lys Asp Thr Tyr Asp Ala Leu His Met		
705	710	715
Gln Ala Leu Pro Pro Arg		
725		

<210> SEQ ID NO 81
 <211> LENGTH: 746
 <212> TYPE: PRT
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic polypeptide

<400> SEQUENCE: 81

Met Ala Leu Pro Val Thr Ala Leu Leu Leu Pro Leu Ala Leu Leu Leu		
1	5	10
His Ala Ala Arg Pro Asp Ile Gln Met Thr Gln Thr Thr Ser Ser Leu		
20	25	30
Ser Ala Ser Leu Gly Asp Arg Val Thr Ile Ser Cys Arg Ala Ser Gln		
35	40	45
Asp Ile Ser Lys Tyr Leu Asn Trp Tyr Gln Gln Lys Pro Asp Gly Thr		
50	55	60
Val Lys Leu Leu Ile Tyr His Thr Ser Arg Leu His Ser Gly Val Pro		
65	70	75
Ser Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Tyr Ser Leu Thr Ile		
85	90	95
Ser Asn Leu Glu Gln Glu Asp Ile Ala Thr Tyr Phe Cys Gln Gln Gly		
100	105	110
Asn Thr Leu Pro Tyr Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Thr		
115	120	125
Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Glu		
130	135	140
Val Lys Leu Gln Glu Ser Gly Pro Gly Leu Val Ala Pro Ser Gln Ser		
145	150	155
Leu Ser Val Thr Cys Thr Val Ser Gly Val Ser Leu Pro Asp Tyr Gly		
165	170	175
Val Ser Trp Ile Arg Gln Pro Pro Arg Lys Gly Leu Glu Trp Leu Gly		
180	185	190

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Val	Ile	Trp	Gly	Ser	Glu	Thr	Thr	Tyr	Tyr	Asn	Ser	Ala	Leu	Lys	Ser	195	200	205
Arg	Leu	Thr	Ile	Ile	Lys	Asp	Asn	Ser	Lys	Ser	Gln	Val	Phe	Leu	Lys	210	215	220
Met	Asn	Ser	Leu	Gln	Thr	Asp	Asp	Thr	Ala	Ile	Tyr	Tyr	Cys	Ala	Lys	225	230	235
His	Tyr	Tyr	Tyr	Gly	Gly	Ser	Tyr	Ala	Met	Asp	Tyr	Trp	Gly	Gln	Gly	245	250	255
Thr	Ser	Val	Thr	Val	Ser	Ser	Gly	Ser	Thr	Ser	Gly	Ser	Gly	Lys	Pro	260	265	270
Gly	Ser	Gly	Glu	Gly	Ser	Thr	Lys	Gly	Asp	Ile	Glu	Leu	Thr	Gln	Ser	275	280	285
Pro	Ala	Ile	Met	Ser	Ala	Ser	Leu	Gly	Glu	Glu	Ile	Thr	Leu	Thr	Cys	290	295	300
Ser	Ala	Ser	Ser	Ser	Val	Ser	Tyr	Met	His	Trp	Tyr	Gln	Gln	Lys	Ser	305	310	315
Gly	Thr	Ser	Pro	Lys	Leu	Leu	Ile	Tyr	Ser	Thr	Ser	Asn	Leu	Ala	Ser	325	330	335
Gly	Val	Pro	Ser	Arg	Phe	Ser	Gly	Ser	Gly	Ser	Gly	Thr	Phe	Tyr	Ser	340	345	350
Leu	Thr	Ile	Ser	Ser	Val	Glu	Ala	Glu	Asp	Ala	Ala	Asp	Tyr	Tyr	Cys	355	360	365
His	Gln	Trp	Ser	Ser	Tyr	Thr	Phe	Gly	Gly	Gly	Thr	Lys	Leu	Glu	Ile	370	375	380
Lys	Arg	Gly	Gly	Gly	Gly	Ser	Gly	Gly	Gly	Gly	Ser	Gly	Gly	Gly	Gly	385	390	395
Ser	Gln	Val	Lys	Leu	Gln	Glu	Ser	Gly	Gly	Gly	Leu	Val	Lys	Pro	Gly	405	410	415
Gly	Ser	Leu	Lys	Leu	Ser	Cys	Ala	Ala	Ser	Gly	Phe	Thr	Phe	Ser	Ser	420	425	430
Tyr	Ala	Met	Ser	Trp	Val	Arg	Gln	Thr	Pro	Glu	Lys	Arg	Leu	Glu	Trp	435	440	445
Val	Ala	Thr	Ile	Ser	Ser	Gly	Gly	Ser	Tyr	Thr	Tyr	Tyr	Pro	Asp	Ser	450	455	460
Val	Lys	Gly	Arg	Phe	Thr	Ile	Ser	Arg	Asp	Asn	Ala	Lys	Asn	Thr	Leu	465	470	475
Tyr	Leu	Gln	Met	Ser	Ser	Leu	Arg	Ser	Glu	Asp	Thr	Ala	Met	Tyr	Tyr	485	490	495
Cys	Ala	Arg	Gln	Asp	Gly	Tyr	Tyr	Pro	Gly	Trp	Phe	Ala	Asn	Trp	Gly	500	505	510
Gln	Gly	Thr	Thr	Val	Thr	Val	Ser	Ser	Ser	Gly	Thr	Thr	Thr	Pro	Ala	515	520	525
Pro	Arg	Pro	Pro	Thr	Pro	Ala	Pro	Thr	Ile	Ala	Ser	Gln	Pro	Leu	Ser	530	535	540
Leu	Arg	Pro	Glu	Ala	Cys	Arg	Pro	Ala	Ala	Gly	Gly	Ala	Val	His	Thr	545	550	555
Arg	Gly	Leu	Asp	Phe	Ala	Cys	Asp	Ile	Tyr	Ile	Trp	Ala	Pro	Leu	Ala	565	570	575
Gly	Thr	Cys	Gly	Val	Leu	Leu	Leu	Ser	Leu	Val	Ile	Thr	Leu	Tyr	Cys	580	585	590
Lys	Arg	Gly	Arg	Lys	Lys	Leu	Leu	Tyr	Ile	Phe	Lys	Gln	Pro	Phe	Met			

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595	600	605
Arg Pro Val Gln Thr Thr Gln Glu Glu Asp Gly Cys Ser Cys Arg Phe		
610	615	620
Pro Glu Glu Glu Glu Gly Gly Cys Glu Leu Arg Val Lys Phe Ser Arg		
625	630	635 640
Ser Ala Asp Ala Pro Ala Tyr Lys Gln Gly Gln Asn Gln Leu Tyr Asn		
645	650	655
Glu Leu Asn Leu Gly Arg Arg Glu Glu Tyr Asp Val Leu Asp Lys Arg		
660	665	670
Arg Gly Arg Asp Pro Glu Met Gly Gly Lys Pro Arg Arg Lys Asn Pro		
675	680	685
Gln Glu Gly Leu Tyr Asn Glu Leu Gln Lys Asp Lys Met Ala Glu Ala		
690	695	700
Tyr Ser Glu Ile Gly Met Lys Gly Glu Arg Arg Arg Gly Lys Gly His		
705	710	715 720
Asp Gly Leu Tyr Gln Gly Leu Ser Thr Ala Thr Lys Asp Thr Tyr Asp		
725	730	735
Ala Leu His Met Gln Ala Leu Pro Pro Arg		
740	745	

<210> SEQ ID NO 82
 <211> LENGTH: 743
 <212> TYPE: PRT
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic polypeptide

<400> SEQUENCE: 82

Met Ala Leu Pro Val Thr Ala Leu Leu Leu Pro Leu Ala Leu Leu Leu		
1	5	10 15
His Ala Ala Arg Pro Asp Ile Gln Met Thr Gln Thr Thr Ser Ser Leu		
20	25	30
Ser Ala Ser Leu Gly Asp Arg Val Thr Ile Ser Cys Arg Ala Ser Gln		
35	40	45
Asp Ile Ser Lys Tyr Leu Asn Trp Tyr Gln Gln Lys Pro Asp Gly Thr		
50	55	60
Val Lys Leu Leu Ile Tyr His Thr Ser Arg Leu His Ser Gly Val Pro		
65	70	75 80
Ser Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Tyr Ser Leu Thr Ile		
85	90	95
Ser Asn Leu Glu Gln Glu Asp Ile Ala Thr Tyr Phe Cys Gln Gln Gly		
100	105	110
Asn Thr Leu Pro Tyr Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Thr		
115	120	125
Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Glu		
130	135	140
Val Lys Leu Gln Glu Ser Gly Pro Gly Leu Val Ala Pro Ser Gln Ser		
145	150	155 160
Leu Ser Val Thr Cys Thr Val Ser Gly Val Ser Leu Pro Asp Tyr Gly		
165	170	175
Val Ser Trp Ile Arg Gln Pro Pro Arg Lys Gly Leu Glu Trp Leu Gly		
180	185	190

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Val	Ile	Trp	Gly	Ser	Glu	Thr	Thr	Tyr	Tyr	Asn	Ser	Ala	Leu	Lys	Ser	195	200	205
Arg	Leu	Thr	Ile	Ile	Lys	Asp	Asn	Ser	Lys	Ser	Gln	Val	Phe	Leu	Lys	210	215	220
Met	Asn	Ser	Leu	Gln	Thr	Asp	Asp	Thr	Ala	Ile	Tyr	Tyr	Cys	Ala	Lys	225	230	235
His	Tyr	Tyr	Tyr	Gly	Gly	Ser	Tyr	Ala	Met	Asp	Tyr	Trp	Gly	Gln	Gly	245	250	255
Thr	Ser	Val	Thr	Val	Ser	Ser	Glu	Ala	Ala	Ala	Lys	Glu	Ala	Ala	Ala	260	265	270
Lys	Glu	Ala	Ala	Ala	Lys	Asp	Ile	Glu	Leu	Thr	Gln	Ser	Pro	Ala	Ile	275	280	285
Met	Ser	Ala	Ser	Leu	Gly	Glu	Glu	Ile	Thr	Leu	Thr	Cys	Ser	Ala	Ser	290	295	300
Ser	Ser	Val	Ser	Tyr	Met	His	Trp	Tyr	Gln	Gln	Lys	Ser	Gly	Thr	Ser	305	310	315
Pro	Lys	Leu	Leu	Ile	Tyr	Ser	Thr	Ser	Asn	Leu	Ala	Ser	Gly	Val	Pro	325	330	335
Ser	Arg	Phe	Ser	Gly	Ser	Gly	Ser	Gly	Thr	Phe	Tyr	Ser	Leu	Thr	Ile	340	345	350
Ser	Ser	Val	Glu	Ala	Glu	Asp	Ala	Ala	Asp	Tyr	Tyr	Cys	His	Gln	Trp	355	360	365
Ser	Ser	Tyr	Thr	Phe	Gly	Gly	Gly	Thr	Lys	Leu	Glu	Ile	Lys	Arg	Gly	370	375	380
Gly	Gly	Gly	Ser	Gly	Gly	Gly	Gly	Ser	Gly	Gly	Gly	Gly	Ser	Gln	Val	385	390	395
Lys	Leu	Gln	Glu	Ser	Gly	Gly	Gly	Leu	Val	Lys	Pro	Gly	Gly	Ser	Leu	405	410	415
Lys	Leu	Ser	Cys	Ala	Ala	Ser	Gly	Phe	Thr	Phe	Ser	Ser	Tyr	Ala	Met	420	425	430
Ser	Trp	Val	Arg	Gln	Thr	Pro	Glu	Lys	Arg	Leu	Glu	Trp	Val	Ala	Thr	435	440	445
Ile	Ser	Ser	Gly	Gly	Ser	Tyr	Thr	Tyr	Tyr	Pro	Asp	Ser	Val	Lys	Gly	450	455	460
Arg	Phe	Thr	Ile	Ser	Arg	Asp	Asn	Ala	Lys	Asn	Thr	Leu	Tyr	Leu	Gln	465	470	475
Met	Ser	Ser	Leu	Arg	Ser	Glu	Asp	Thr	Ala	Met	Tyr	Tyr	Cys	Ala	Arg	485	490	495
Gln	Asp	Gly	Tyr	Tyr	Pro	Gly	Trp	Phe	Ala	Asn	Trp	Gly	Gln	Gly	Thr	500	505	510
Thr	Val	Thr	Val	Ser	Ser	Ser	Gly	Thr	Thr	Thr	Pro	Ala	Pro	Arg	Pro	515	520	525
Pro	Thr	Pro	Ala	Pro	Thr	Ile	Ala	Ser	Gln	Pro	Leu	Ser	Leu	Arg	Pro	530	535	540
Glu	Ala	Cys	Arg	Pro	Ala	Ala	Gly	Gly	Ala	Val	His	Thr	Arg	Gly	Leu	545	550	555
Asp	Phe	Ala	Cys	Asp	Ile	Tyr	Ile	Trp	Ala	Pro	Leu	Ala	Gly	Thr	Cys	565	570	575
Gly	Val	Leu	Leu	Leu	Ser	Leu	Val	Ile	Thr	Leu	Tyr	Cys	Lys	Arg	Gly	580	585	590
Arg	Lys	Lys	Leu	Leu	Tyr	Ile	Phe	Lys	Gln	Pro	Phe	Met	Arg	Pro	Val			

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595					600					605					
Gln	Thr	Thr	Gln	Glu	Glu	Asp	Gly	Cys	Ser	Cys	Arg	Phe	Pro	Glu	Glu
610						615					620				
Glu	Glu	Gly	Gly	Cys	Glu	Leu	Arg	Val	Lys	Phe	Ser	Arg	Ser	Ala	Asp
625					630					635					640
Ala	Pro	Ala	Tyr	Lys	Gln	Gly	Gln	Asn	Gln	Leu	Tyr	Asn	Glu	Leu	Asn
				645					650					655	
Leu	Gly	Arg	Arg	Glu	Glu	Tyr	Asp	Val	Leu	Asp	Lys	Arg	Arg	Gly	Arg
		660						665					670		
Asp	Pro	Glu	Met	Gly	Gly	Lys	Pro	Arg	Arg	Lys	Asn	Pro	Gln	Glu	Gly
		675					680					685			
Leu	Tyr	Asn	Glu	Leu	Gln	Lys	Asp	Lys	Met	Ala	Glu	Ala	Tyr	Ser	Glu
690						695					700				
Ile	Gly	Met	Lys	Gly	Glu	Arg	Arg	Arg	Gly	Lys	Gly	His	Asp	Gly	Leu
705					710					715					720
Tyr	Gln	Gly	Leu	Ser	Thr	Ala	Thr	Lys	Asp	Thr	Tyr	Asp	Ala	Leu	His
			725						730					735	
Met	Gln	Ala	Leu	Pro	Pro	Arg									
			740												
<210> SEQ ID NO 83															
<211> LENGTH: 746															
<212> TYPE: PRT															
<213> ORGANISM: Artificial Sequence															
<220> FEATURE:															
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic polypeptide															
<400> SEQUENCE: 83															
Met	Ala	Leu	Pro	Val	Thr	Ala	Leu	Leu	Leu	Pro	Leu	Ala	Leu	Leu	Leu
1				5					10					15	
His	Ala	Ala	Arg	Pro	Asp	Ile	Glu	Leu	Thr	Gln	Ser	Pro	Ala	Ile	Met
			20					25					30		
Ser	Ala	Ser	Leu	Gly	Glu	Glu	Ile	Thr	Leu	Thr	Cys	Ser	Ala	Ser	Ser
		35				40					45				
Ser	Val	Ser	Tyr	Met	His	Trp	Tyr	Gln	Gln	Lys	Ser	Gly	Thr	Ser	Pro
	50					55					60				
Lys	Leu	Leu	Ile	Tyr	Ser	Thr	Ser	Asn	Leu	Ala	Ser	Gly	Val	Pro	Ser
65					70					75				80	
Arg	Phe	Ser	Gly	Ser	Gly	Ser	Gly	Thr	Phe	Tyr	Ser	Leu	Thr	Ile	Ser
			85					90						95	
Ser	Val	Glu	Ala	Glu	Asp	Ala	Ala	Asp	Tyr	Tyr	Cys	His	Gln	Trp	Ser
			100					105					110		
Ser	Tyr	Thr	Phe	Gly	Gly	Gly	Thr	Lys	Leu	Glu	Ile	Lys	Arg	Gly	Gly
		115					120					125			
Gly	Gly	Ser	Gly	Gly	Gly	Gly	Ser	Gly	Gly	Gly	Gly	Ser	Gln	Val	Lys
		130				135						140			
Leu	Gln	Glu	Ser	Gly	Gly	Gly	Leu	Val	Lys	Pro	Gly	Gly	Ser	Leu	Lys
145					150					155				160	
Leu	Ser	Cys	Ala	Ala	Ser	Gly	Phe	Thr	Phe	Ser	Ser	Tyr	Ala	Met	Ser
			165					170						175	
Trp	Val	Arg	Gln	Thr	Pro	Glu	Lys	Arg	Leu	Glu	Trp	Val	Ala	Thr	Ile
			180				185						190		

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Ser	Ser	Gly	Gly	Ser	Tyr	Thr	Tyr	Tyr	Pro	Asp	Ser	Val	Lys	Gly	Arg	195	200	205	
Phe	Thr	Ile	Ser	Arg	Asp	Asn	Ala	Lys	Asn	Thr	Leu	Tyr	Leu	Gln	Met	210	215	220	
Ser	Ser	Leu	Arg	Ser	Glu	Asp	Thr	Ala	Met	Tyr	Tyr	Cys	Ala	Arg	Gln	225	230	235	240
Asp	Gly	Tyr	Tyr	Pro	Gly	Trp	Phe	Ala	Asn	Trp	Gly	Gln	Gly	Thr	Thr	245	250	255	
Val	Thr	Val	Ser	Ser	Gly	Ser	Thr	Ser	Gly	Ser	Gly	Lys	Pro	Gly	Ser	260	265	270	
Gly	Glu	Gly	Ser	Thr	Lys	Gly	Asp	Ile	Gln	Met	Thr	Gln	Thr	Thr	Ser	275	280	285	
Ser	Leu	Ser	Ala	Ser	Leu	Gly	Asp	Arg	Val	Thr	Ile	Ser	Cys	Arg	Ala	290	295	300	
Ser	Gln	Asp	Ile	Ser	Lys	Tyr	Leu	Asn	Trp	Tyr	Gln	Gln	Lys	Pro	Asp	305	310	315	320
Gly	Thr	Val	Lys	Leu	Leu	Ile	Tyr	His	Thr	Ser	Arg	Leu	His	Ser	Gly	325	330	335	
Val	Pro	Ser	Arg	Phe	Ser	Gly	Ser	Gly	Ser	Gly	Thr	Asp	Tyr	Ser	Leu	340	345	350	
Thr	Ile	Ser	Asn	Leu	Glu	Gln	Glu	Asp	Ile	Ala	Thr	Tyr	Phe	Cys	Gln	355	360	365	
Gln	Gly	Asn	Thr	Leu	Pro	Tyr	Thr	Phe	Gly	Gly	Gly	Thr	Lys	Leu	Glu	370	375	380	
Ile	Thr	Gly	Gly	Gly	Gly	Ser	Gly	Gly	Gly	Gly	Ser	Gly	Gly	Gly	Gly	385	390	395	400
Ser	Glu	Val	Lys	Leu	Gln	Glu	Ser	Gly	Pro	Gly	Leu	Val	Ala	Pro	Ser	405	410	415	
Gln	Ser	Leu	Ser	Val	Thr	Cys	Thr	Val	Ser	Gly	Val	Ser	Leu	Pro	Asp	420	425	430	
Tyr	Gly	Val	Ser	Trp	Ile	Arg	Gln	Pro	Pro	Arg	Lys	Gly	Leu	Glu	Trp	435	440	445	
Leu	Gly	Val	Ile	Trp	Gly	Ser	Glu	Thr	Thr	Tyr	Tyr	Asn	Ser	Ala	Leu	450	455	460	
Lys	Ser	Arg	Leu	Thr	Ile	Ile	Lys	Asp	Asn	Ser	Lys	Ser	Gln	Val	Phe	465	470	475	480
Leu	Lys	Met	Asn	Ser	Leu	Gln	Thr	Asp	Asp	Thr	Ala	Ile	Tyr	Tyr	Cys	485	490	495	
Ala	Lys	His	Tyr	Tyr	Tyr	Gly	Gly	Ser	Tyr	Ala	Met	Asp	Tyr	Trp	Gly	500	505	510	
Gln	Gly	Thr	Ser	Val	Thr	Val	Ser	Ser	Ser	Gly	Thr	Thr	Thr	Pro	Ala	515	520	525	
Pro	Arg	Pro	Pro	Thr	Pro	Ala	Pro	Thr	Ile	Ala	Ser	Gln	Pro	Leu	Ser	530	535	540	
Leu	Arg	Pro	Glu	Ala	Cys	Arg	Pro	Ala	Ala	Gly	Gly	Ala	Val	His	Thr	545	550	555	560
Arg	Gly	Leu	Asp	Phe	Ala	Cys	Asp	Ile	Tyr	Ile	Trp	Ala	Pro	Leu	Ala	565	570	575	
Gly	Thr	Cys	Gly	Val	Leu	Leu	Leu	Ser	Leu	Val	Ile	Thr	Leu	Tyr	Cys	580	585	590	
Lys	Arg	Gly	Arg	Lys	Lys	Leu	Leu	Tyr	Ile	Phe	Lys	Gln	Pro	Phe	Met				

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595	600	605
Arg Pro Val Gln Thr Thr Gln Glu Glu Asp Gly Cys Ser Cys Arg Phe		
610	615	620
Pro Glu Glu Glu Glu Gly Gly Cys Glu Leu Arg Val Lys Phe Ser Arg		
625	630	635 640
Ser Ala Asp Ala Pro Ala Tyr Lys Gln Gly Gln Asn Gln Leu Tyr Asn		
645	650	655
Glu Leu Asn Leu Gly Arg Arg Glu Glu Tyr Asp Val Leu Asp Lys Arg		
660	665	670
Arg Gly Arg Asp Pro Glu Met Gly Gly Lys Pro Arg Arg Lys Asn Pro		
675	680	685
Gln Glu Gly Leu Tyr Asn Glu Leu Gln Lys Asp Lys Met Ala Glu Ala		
690	695	700
Tyr Ser Glu Ile Gly Met Lys Gly Glu Arg Arg Arg Gly Lys Gly His		
705	710	715 720
Asp Gly Leu Tyr Gln Gly Leu Ser Thr Ala Thr Lys Asp Thr Tyr Asp		
725	730	735
Ala Leu His Met Gln Ala Leu Pro Pro Arg		
740	745	

<210> SEQ ID NO 84
 <211> LENGTH: 746
 <212> TYPE: PRT
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic polypeptide

<400> SEQUENCE: 84

Met Ala Leu Pro Val Thr Ala Leu Leu Leu Pro Leu Ala Leu Leu Leu		
1	5	10 15
His Ala Ala Arg Pro Asp Ile Glu Leu Thr Gln Ser Pro Ala Ile Met		
20	25	30
Ser Ala Ser Leu Gly Glu Glu Ile Thr Leu Thr Cys Ser Ala Ser Ser		
35	40	45
Ser Val Ser Tyr Met His Trp Tyr Gln Gln Lys Ser Gly Thr Ser Pro		
50	55	60
Lys Leu Leu Ile Tyr Ser Thr Ser Asn Leu Ala Ser Gly Val Pro Ser		
65	70	75 80
Arg Phe Ser Gly Ser Gly Ser Gly Thr Phe Tyr Ser Leu Thr Ile Ser		
85	90	95
Ser Val Glu Ala Glu Asp Ala Ala Asp Tyr Tyr Cys His Gln Trp Ser		
100	105	110
Ser Tyr Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys Arg Gly Gly		
115	120	125
Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gln Val Lys		
130	135	140
Leu Gln Glu Ser Gly Gly Gly Leu Val Lys Pro Gly Gly Ser Leu Lys		
145	150	155 160
Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr Ala Met Ser		
165	170	175
Trp Val Arg Gln Thr Pro Glu Lys Arg Leu Glu Trp Val Ala Thr Ile		
180	185	190

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Ser	Ser	Gly	Gly	Ser	Tyr	Thr	Tyr	Tyr	Pro	Asp	Ser	Val	Lys	Gly	Arg
		195					200					205			
Phe	Thr	Ile	Ser	Arg	Asp	Asn	Ala	Lys	Asn	Thr	Leu	Tyr	Leu	Gln	Met
	210					215					220				
Ser	Ser	Leu	Arg	Ser	Glu	Asp	Thr	Ala	Met	Tyr	Tyr	Cys	Ala	Arg	Gln
225					230					235					240
Asp	Gly	Tyr	Tyr	Pro	Gly	Trp	Phe	Ala	Asn	Trp	Gly	Gln	Gly	Thr	Thr
				245					250					255	
Val	Thr	Val	Ser	Ser	Gly	Ser	Thr	Ser	Gly	Ser	Gly	Lys	Pro	Gly	Ser
			260					265					270		
Gly	Glu	Gly	Ser	Thr	Lys	Gly	Glu	Val	Lys	Leu	Gln	Glu	Ser	Gly	Pro
		275					280					285			
Gly	Leu	Val	Ala	Pro	Ser	Gln	Ser	Leu	Ser	Val	Thr	Cys	Thr	Val	Ser
	290					295					300				
Gly	Val	Ser	Leu	Pro	Asp	Tyr	Gly	Val	Ser	Trp	Ile	Arg	Gln	Pro	Pro
305					310					315					320
Arg	Lys	Gly	Leu	Glu	Trp	Leu	Gly	Val	Ile	Trp	Gly	Ser	Glu	Thr	Thr
			325						330					335	
Tyr	Tyr	Asn	Ser	Ala	Leu	Lys	Ser	Arg	Leu	Thr	Ile	Ile	Lys	Asp	Asn
		340						345					350		
Ser	Lys	Ser	Gln	Val	Phe	Leu	Lys	Met	Asn	Ser	Leu	Gln	Thr	Asp	Asp
		355					360					365			
Thr	Ala	Ile	Tyr	Tyr	Cys	Ala	Lys	His	Tyr	Tyr	Tyr	Gly	Gly	Ser	Tyr
	370					375					380				
Ala	Met	Asp	Tyr	Trp	Gly	Gln	Gly	Thr	Ser	Val	Thr	Val	Ser	Ser	Gly
385					390					395					400
Gly	Gly	Gly	Ser	Gly	Gly	Gly	Gly	Ser	Gly	Gly	Gly	Gly	Ser	Asp	Ile
			405						410					415	
Gln	Met	Thr	Gln	Thr	Thr	Ser	Ser	Leu	Ser	Ala	Ser	Leu	Gly	Asp	Arg
			420					425					430		
Val	Thr	Ile	Ser	Cys	Arg	Ala	Ser	Gln	Asp	Ile	Ser	Lys	Tyr	Leu	Asn
		435					440					445			
Trp	Tyr	Gln	Gln	Lys	Pro	Asp	Gly	Thr	Val	Lys	Leu	Leu	Ile	Tyr	His
	450					455					460				
Thr	Ser	Arg	Leu	His	Ser	Gly	Val	Pro	Ser	Arg	Phe	Ser	Gly	Ser	Gly
465					470					475					480
Ser	Gly	Thr	Asp	Tyr	Ser	Leu	Thr	Ile	Ser	Asn	Leu	Glu	Gln	Glu	Asp
			485						490					495	
Ile	Ala	Thr	Tyr	Phe	Cys	Gln	Gln	Gly	Asn	Thr	Leu	Pro	Tyr	Thr	Phe
		500						505					510		
Gly	Gly	Gly	Thr	Lys	Leu	Glu	Ile	Thr	Ser	Gly	Thr	Thr	Thr	Pro	Ala
		515					520					525			
Pro	Arg	Pro	Pro	Thr	Pro	Ala	Pro	Thr	Ile	Ala	Ser	Gln	Pro	Leu	Ser
	530					535					540				
Leu	Arg	Pro	Glu	Ala	Cys	Arg	Pro	Ala	Ala	Gly	Gly	Ala	Val	His	Thr
545					550					555					560
Arg	Gly	Leu	Asp	Phe	Ala	Cys	Asp	Ile	Tyr	Ile	Trp	Ala	Pro	Leu	Ala
			565					570					575		
Gly	Thr	Cys	Gly	Val	Leu	Leu	Leu	Ser	Leu	Val	Ile	Thr	Leu	Tyr	Cys
		580						585				590			
Lys	Arg	Gly	Arg	Lys	Lys	Leu	Leu	Tyr	Ile	Phe	Lys	Gln	Pro	Phe	Met

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595	600	605
Arg Pro Val Gln Thr Thr Gln Glu Glu Asp Gly Cys Ser Cys Arg Phe		
610	615	620
Pro Glu Glu Glu Glu Gly Gly Cys Glu Leu Arg Val Lys Phe Ser Arg		
625	630	635 640
Ser Ala Asp Ala Pro Ala Tyr Lys Gln Gly Gln Asn Gln Leu Tyr Asn		
645	650	655
Glu Leu Asn Leu Gly Arg Arg Glu Glu Tyr Asp Val Leu Asp Lys Arg		
660	665	670
Arg Gly Arg Asp Pro Glu Met Gly Gly Lys Pro Arg Arg Lys Asn Pro		
675	680	685
Gln Glu Gly Leu Tyr Asn Glu Leu Gln Lys Asp Lys Met Ala Glu Ala		
690	695	700
Tyr Ser Glu Ile Gly Met Lys Gly Glu Arg Arg Arg Gly Lys Gly His		
705	710	715 720
Asp Gly Leu Tyr Gln Gly Leu Ser Thr Ala Thr Lys Asp Thr Tyr Asp		
725	730	735
Ala Leu His Met Gln Ala Leu Pro Pro Arg		
740	745	

<210> SEQ ID NO 85
 <211> LENGTH: 1261
 <212> TYPE: PRT
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic polypeptide

<400> SEQUENCE: 85

Met Ala Leu Pro Val Thr Ala Leu Leu Leu Pro Leu Ala Leu Leu Leu		
1	5	10 15
His Ala Ala Arg Pro Asp Ile Gln Met Thr Gln Thr Thr Ser Ser Leu		
20	25	30
Ser Ala Ser Leu Gly Asp Arg Val Thr Ile Ser Cys Arg Ala Ser Gln		
35	40	45
Asp Ile Ser Lys Tyr Leu Asn Trp Tyr Gln Gln Lys Pro Asp Gly Thr		
50	55	60
Val Lys Leu Leu Ile Tyr His Thr Ser Arg Leu His Ser Gly Val Pro		
65	70	75 80
Ser Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Tyr Ser Leu Thr Ile		
85	90	95
Ser Asn Leu Glu Gln Glu Asp Ile Ala Thr Tyr Phe Cys Gln Gln Gly		
100	105	110
Asn Thr Leu Pro Tyr Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Thr		
115	120	125
Gly Gly Gly Gly Ser Gln Val Lys Leu Gln Glu Ser Gly Gly Gly Leu		
130	135	140
Val Lys Pro Gly Gly Ser Leu Lys Leu Ser Cys Ala Ala Ser Gly Phe		
145	150	155 160
Thr Phe Ser Ser Tyr Ala Met Ser Trp Val Arg Gln Thr Pro Glu Lys		
165	170	175
Arg Leu Glu Trp Val Ala Thr Ile Ser Ser Gly Gly Ser Tyr Thr Tyr		
180	185	190

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Tyr	Pro	Asp	Ser	Val	Lys	Gly	Arg	Phe	Thr	Ile	Ser	Arg	Asp	Asn	Ala
		195					200					205			
Lys	Asn	Thr	Leu	Tyr	Leu	Gln	Met	Ser	Ser	Leu	Arg	Ser	Glu	Asp	Thr
	210					215					220				
Ala	Met	Tyr	Tyr	Cys	Ala	Arg	Gln	Asp	Gly	Tyr	Tyr	Pro	Gly	Trp	Phe
225					230					235					240
Ala	Asn	Trp	Gly	Gln	Gly	Thr	Thr	Val	Thr	Val	Ser	Ser	Gly	Ser	Thr
				245					250					255	
Ser	Gly	Ser	Gly	Lys	Pro	Gly	Ser	Gly	Glu	Gly	Ser	Thr	Lys	Gly	Asp
			260					265					270		
Ile	Glu	Leu	Thr	Gln	Ser	Pro	Ala	Ile	Met	Ser	Ala	Ser	Leu	Gly	Glu
		275					280					285			
Glu	Ile	Thr	Leu	Thr	Cys	Ser	Ala	Ser	Ser	Ser	Val	Ser	Tyr	Met	His
	290					295					300				
Trp	Tyr	Gln	Gln	Lys	Ser	Gly	Thr	Ser	Pro	Lys	Leu	Leu	Ile	Tyr	Ser
305					310					315					320
Thr	Ser	Asn	Leu	Ala	Ser	Gly	Val	Pro	Ser	Arg	Phe	Ser	Gly	Ser	Gly
				325					330					335	
Ser	Gly	Thr	Phe	Tyr	Ser	Leu	Thr	Ile	Ser	Ser	Val	Glu	Ala	Glu	Asp
			340					345					350		
Ala	Ala	Asp	Tyr	Tyr	Cys	His	Gln	Trp	Ser	Ser	Tyr	Thr	Phe	Gly	Gly
		355					360					365			
Gly	Thr	Lys	Leu	Glu	Ile	Lys	Arg	Gly	Gly	Gly	Gly	Ser	Glu	Val	Lys
	370					375					380				
Leu	Gln	Glu	Ser	Gly	Pro	Gly	Leu	Val	Ala	Pro	Ser	Gln	Ser	Leu	Ser
385					390					395					400
Val	Thr	Cys	Thr	Val	Ser	Gly	Val	Ser	Leu	Pro	Asp	Tyr	Gly	Val	Ser
				405					410					415	
Trp	Ile	Arg	Gln	Pro	Pro	Arg	Lys	Gly	Leu	Glu	Trp	Leu	Gly	Val	Ile
			420				425						430		
Trp	Gly	Ser	Glu	Thr	Thr	Tyr	Tyr	Asn	Ser	Ala	Leu	Lys	Ser	Arg	Leu
		435					440					445			
Thr	Ile	Ile	Lys	Asp	Asn	Ser	Lys	Ser	Gln	Val	Phe	Leu	Lys	Met	Asn
	450					455					460				
Ser	Leu	Gln	Thr	Asp	Asp	Thr	Ala	Ile	Tyr	Tyr	Cys	Ala	Lys	His	Tyr
465					470					475					480
Tyr	Tyr	Gly	Gly	Ser	Tyr	Ala	Met	Asp	Tyr	Trp	Gly	Gln	Gly	Thr	Ser
				485					490					495	
Val	Thr	Val	Ser	Ser	Ser	Gly	Thr	Thr	Thr	Pro	Ala	Pro	Arg	Pro	Pro
			500					505					510		
Thr	Pro	Ala	Pro	Thr	Ile	Ala	Ser	Gln	Pro	Leu	Ser	Leu	Arg	Pro	Glu
		515					520					525			
Ala	Cys	Arg	Pro	Ala	Ala	Gly	Gly	Ala	Val	His	Thr	Arg	Gly	Leu	Asp
	530					535					540				
Phe	Ala	Cys	Asp	Ile	Tyr	Ile	Trp	Ala	Pro	Leu	Ala	Gly	Thr	Cys	Gly
545					550					555					560
Val	Leu	Leu	Leu	Ser	Leu	Val	Ile	Thr	Leu	Tyr	Cys	Lys	Arg	Gly	Arg
				565				570						575	
Lys	Lys	Leu	Leu	Tyr	Ile	Phe	Lys	Gln	Pro	Phe	Met	Arg	Pro	Val	Gln
			580					585					590		
Thr	Thr	Gln	Glu	Glu	Asp	Gly	Cys	Ser	Cys	Arg	Phe	Pro	Glu	Glu	Glu

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595					600					605					
Glu	Gly	Gly	Cys	Glu	Leu	Arg	Val	Lys	Phe	Ser	Arg	Ser	Ala	Asp	Ala
	610					615					620				
Pro	Ala	Tyr	Lys	Gln	Gly	Gln	Asn	Gln	Leu	Tyr	Asn	Glu	Leu	Asn	Leu
625					630					635					640
Gly	Arg	Arg	Glu	Glu	Tyr	Asp	Val	Leu	Asp	Lys	Arg	Arg	Gly	Arg	Asp
				645					650					655	
Pro	Glu	Met	Gly	Gly	Lys	Pro	Arg	Arg	Lys	Asn	Pro	Gln	Glu	Gly	Leu
			660					665					670		
Tyr	Asn	Glu	Leu	Gln	Lys	Asp	Lys	Met	Ala	Glu	Ala	Tyr	Ser	Glu	Ile
	675						680					685			
Gly	Met	Lys	Gly	Glu	Arg	Arg	Arg	Gly	Lys	Gly	His	Asp	Gly	Leu	Tyr
690						695					700				
Gln	Gly	Leu	Ser	Thr	Ala	Thr	Lys	Asp	Thr	Tyr	Asp	Ala	Leu	His	Met
705					710					715					720
Gln	Ala	Leu	Pro	Pro	Arg	Gly	Ser	Gly	Ala	Thr	Asn	Phe	Ser	Leu	Leu
				725					730					735	
Lys	Gln	Ala	Gly	Asp	Val	Glu	Glu	Asn	Pro	Gly	Pro	Met	Leu	Val	Arg
			740					745					750		
Arg	Gly	Ala	Arg	Ala	Gly	Pro	Arg	Met	Pro	Arg	Gly	Trp	Thr	Ala	Leu
	755						760					765			
Cys	Leu	Leu	Ser	Leu	Leu	Pro	Ser	Gly	Phe	Met	Ser	Leu	Asp	Asn	Asn
770						775					780				
Gly	Thr	Ala	Thr	Pro	Glu	Leu	Pro	Thr	Gln	Gly	Thr	Phe	Ser	Asn	Val
785					790					795					800
Ser	Thr	Asn	Val	Ser	Tyr	Gln	Glu	Thr	Thr	Thr	Pro	Ser	Thr	Leu	Gly
			805						810					815	
Ser	Thr	Ser	Leu	His	Pro	Val	Ser	Gln	His	Gly	Asn	Glu	Ala	Thr	Thr
			820					825					830		
Asn	Ile	Thr	Glu	Thr	Thr	Val	Lys	Phe	Thr	Ser	Thr	Ser	Val	Ile	Thr
	835						840					845			
Ser	Val	Tyr	Gly	Asn	Thr	Asn	Ser	Ser	Val	Gln	Ser	Gln	Thr	Ser	Val
850						855					860				
Ile	Ser	Thr	Val	Phe	Thr	Thr	Pro	Ala	Asn	Val	Ser	Thr	Pro	Glu	Thr
865					870					875					880
Thr	Leu	Lys	Pro	Ser	Leu	Ser	Pro	Gly	Asn	Val	Ser	Asp	Leu	Ser	Thr
				885					890					895	
Thr	Ser	Thr	Ser	Leu	Ala	Thr	Ser	Pro	Thr	Lys	Pro	Tyr	Thr	Ser	Ser
			900					905					910		
Ser	Pro	Ile	Leu	Ser	Asp	Ile	Lys	Ala	Glu	Ile	Lys	Cys	Ser	Gly	Ile
915							920					925			
Arg	Glu	Val	Lys	Leu	Thr	Gln	Gly	Ile	Cys	Leu	Glu	Gln	Asn	Lys	Thr
	930					935						940			
Ser	Ser	Cys	Ala	Glu	Phe	Lys	Lys	Asp	Arg	Gly	Glu	Gly	Leu	Ala	Arg
945					950					955					960
Val	Leu	Cys	Gly	Glu	Glu	Gln	Ala	Asp	Ala	Asp	Ala	Gly	Ala	Gln	Val
				965					970					975	
Cys	Ser	Leu	Leu	Leu	Ala	Gln	Ser	Glu	Val	Arg	Pro	Gln	Cys	Leu	Leu
			980					985					990		
Leu	Val	Leu	Ala	Asn	Arg	Thr	Glu	Ile	Ser	Ser	Lys	Leu	Gln	Leu	Met
	995						1000					1005			

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Lys	Lys	His	Gln	Ser	Asp	Leu	Lys	Lys	Leu	Gly	Ile	Leu	Asp	Phe
1010						1015					1020			
Thr	Glu	Gln	Asp	Val	Ala	Ser	His	Gln	Ser	Tyr	Ser	Gln	Lys	Thr
1025						1030					1035			
Pro	Ile	Leu	Leu	Thr	Cys	Pro	Thr	Ile	Ser	Ile	Leu	Ser	Phe	Phe
1040						1045					1050			
Ser	Val	Ala	Leu	Leu	Val	Ile	Leu	Ala	Cys	Val	Leu	Trp	Lys	Lys
1055						1060					1065			
Arg	Ile	Lys	Pro	Ile	Val	Trp	Pro	Ser	Leu	Pro	Asp	His	Lys	Lys
1070						1075					1080			
Thr	Leu	Glu	His	Leu	Cys	Lys	Lys	Pro	Arg	Lys	Asn	Leu	Asn	Val
1085						1090					1095			
Ser	Phe	Asn	Pro	Glu	Ser	Phe	Leu	Asp	Cys	Gln	Ile	His	Arg	Val
1100						1105					1110			
Asp	Asp	Ile	Gln	Ala	Arg	Asp	Glu	Val	Glu	Gly	Phe	Leu	Gln	Asp
1115						1120					1125			
Thr	Phe	Pro	Gln	Gln	Leu	Glu	Glu	Ser	Glu	Lys	Gln	Arg	Leu	Gly
1130						1135					1140			
Gly	Asp	Val	Gln	Ser	Pro	Asn	Cys	Pro	Ser	Glu	Asp	Val	Val	Ile
1145						1150					1155			
Thr	Pro	Glu	Ser	Phe	Gly	Arg	Asp	Ser	Ser	Leu	Thr	Cys	Leu	Ala
1160						1165					1170			
Gly	Asn	Val	Ser	Ala	Cys	Asp	Ala	Pro	Ile	Leu	Ser	Ser	Ser	Arg
1175						1180					1185			
Ser	Leu	Asp	Cys	Arg	Glu	Ser	Gly	Lys	Asn	Gly	Pro	His	Val	Tyr
1190						1195					1200			
Gln	Asp	Leu	Leu	Leu	Ser	Leu	Gly	Thr	Thr	Asn	Ser	Thr	Leu	Pro
1205						1210					1215			
Pro	Pro	Phe	Ser	Leu	Gln	Ser	Gly	Ile	Leu	Thr	Leu	Asn	Pro	Val
1220						1225					1230			
Ala	Gln	Gly	Gln	Pro	Ile	Leu	Thr	Ser	Leu	Gly	Ser	Asn	Gln	Glu
1235						1240					1245			
Glu	Ala	Tyr	Val	Thr	Met	Ser	Ser	Phe	Tyr	Gln	Asn	Gln		
1250						1255					1260			

<210> SEQ ID NO 86

<211> LENGTH: 1263

<212> TYPE: PRT

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic polypeptide

<400> SEQUENCE: 86

Met	Ala	Ser	Arg	Ile	Leu	Trp	His	Glu	Met	Trp	His	Glu	Gly	Leu	Glu
1				5					10					15	

Glu	Ala	Ser	Arg	Leu	Tyr	Phe	Gly	Glu	Arg	Asn	Val	Lys	Gly	Met	Phe
	20						25						30		

Glu	Val	Leu	Glu	Pro	Leu	His	Ala	Met	Met	Glu	Arg	Gly	Pro	Gln	Thr
	35					40						45			

Leu	Lys	Glu	Thr	Ser	Phe	Asn	Gln	Ala	Tyr	Gly	Arg	Asp	Leu	Met	Glu
	50					55				60					

Ala	Gln	Glu	Trp	Cys	Arg	Lys	Tyr	Met	Lys	Ser	Gly	Asn	Val	Lys	Asp
-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----

65	70								75								80	
Leu	Leu	Gln	Ala	Trp	Asp	Leu	Tyr	Tyr	His	Val	Phe	Arg	Arg	Ile	Ser			
				85					90					95				
Lys	Leu	Glu	Tyr	Ser	Gly	Gly	Gly	Ser	Leu	Glu	Gly	Val	Gln	Val	Glu			
			100					105					110					
Thr	Ile	Ser	Pro	Gly	Asp	Gly	Arg	Thr	Phe	Pro	Lys	Arg	Gly	Gln	Thr			
		115					120					125						
Cys	Val	Val	His	Tyr	Thr	Gly	Met	Leu	Glu	Asp	Gly	Lys	Lys	Phe	Asp			
	130					135					140							
Ser	Ser	Arg	Asp	Arg	Asn	Lys	Pro	Phe	Lys	Phe	Met	Leu	Gly	Lys	Gln			
145					150					155					160			
Glu	Val	Ile	Arg	Gly	Trp	Glu	Glu	Gly	Val	Ala	Gln	Met	Ser	Val	Gly			
				165					170					175				
Gln	Arg	Ala	Lys	Leu	Thr	Ile	Ser	Pro	Asp	Tyr	Ala	Tyr	Gly	Ala	Thr			
			180					185					190					
Gly	His	Pro	Gly	Ile	Ile	Pro	Pro	His	Ala	Thr	Leu	Val	Phe	Asp	Val			
		195					200					205						
Glu	Leu	Leu	Lys	Leu	Glu	Ser	Gly	Gly	Gly	Gly	Ser	Gly	Gly	Gly	Gly			
	210					215					220							
Ser	Gly	Gly	Gly	Gly	Ser	Gly	Val	Asp	Gly	Phe	Gly	Asp	Val	Gly	Ala			
225					230					235					240			
Leu	Glu	Ser	Leu	Arg	Gly	Asn	Ala	Asp	Leu	Ala	Tyr	Ile	Leu	Ser	Met			
				245				250					255					
Glu	Pro	Cys	Gly	His	Cys	Leu	Ile	Ile	Asn	Asn	Val	Asn	Phe	Cys	Arg			
			260					265					270					
Glu	Ser	Gly	Leu	Arg	Thr	Arg	Thr	Gly	Ser	Asn	Ile	Asp	Cys	Glu	Lys			
		275				280						285						
Leu	Arg	Arg	Arg	Phe	Ser	Ser	Leu	His	Phe	Met	Val	Glu	Val	Lys	Gly			
	290					295					300							
Asp	Leu	Thr	Ala	Lys	Lys	Met	Val	Leu	Ala	Leu	Leu	Glu	Leu	Ala	Gln			
305					310					315					320			
Gln	Asp	His	Gly	Ala	Leu	Asp	Cys	Cys	Val	Val	Val	Ile	Leu	Ser	His			
			325						330				335					
Gly	Cys	Gln	Ala	Ser	His	Leu	Gln	Phe	Pro	Gly	Ala	Val	Tyr	Gly	Thr			
			340					345					350					
Asp	Gly	Cys	Pro	Val	Ser	Val	Glu	Lys	Ile	Val	Asn	Ile	Phe	Asn	Gly			
		355					360					365						
Thr	Ser	Cys	Pro	Ser	Leu	Gly	Gly	Lys	Pro	Lys	Leu	Phe	Phe	Ile	Gln			
		370				375					380							
Ala	Cys	Gly	Gly	Glu	Gln	Lys	Asp	His	Gly	Phe	Glu	Val	Ala	Ser	Thr			
385					390					395					400			
Ser	Pro	Glu	Asp	Glu	Ser	Pro	Gly	Ser	Asn	Pro	Glu	Pro	Asp	Ala	Thr			
			405						410					415				
Pro	Phe	Gln	Glu	Gly	Leu	Arg	Thr	Phe	Asp	Gln	Leu	Asp	Ala	Ile	Ser			
			420					425					430					
Ser	Leu	Pro	Thr	Pro	Ser	Asp	Ile	Phe	Val	Ser	Tyr	Ser	Thr	Phe	Pro			
		435					440					445						
Gly	Phe	Val	Ser	Trp	Arg	Asp	Pro	Lys	Ser	Gly	Ser	Trp	Tyr	Val	Glu			
	450					455					460							
Thr	Leu	Asp	Asp	Ile	Phe	Glu	Gln	Trp	Ala	His	Ser	Glu	Asp	Leu	Gln			
465					470					475					480			

Ser 485	Leu	Leu	Leu	Arg	Val	Ala	Asn	Ala	Val	Ser	Val	Lys	Gly	Ile	Tyr
Lys 500	Gln	Met	Pro	Gly	Cys	Phe	Asn	Phe	Leu	Arg	Lys	Lys	Leu	Phe	Phe
Lys 515	Thr	Ser	Gly	Ser	Gly	Ala	Thr	Asn	Phe	Ser	Leu	Leu	Lys	Gln	Ala
Gly 530	Asp	Val	Glu	Glu	Asn	Pro	Gly	Pro	Met	Ala	Leu	Pro	Val	Thr	Ala
Leu 545	Leu	Leu	Pro	Leu	Ala	Leu	Leu	Leu	His	Ala	Ala	Arg	Pro	Asp	Ile
Gln 560	Met	Thr	Gln	Thr	Thr	Ser	Ser	Leu	Ser	Ala	Ser	Leu	Gly	Asp	Arg
Val 580	Thr	Ile	Ser	Cys	Arg	Ala	Ser	Gln	Asp	Ile	Ser	Lys	Tyr	Leu	Asn
Trp 595	Tyr	Gln	Gln	Lys	Pro	Asp	Gly	Thr	Val	Lys	Leu	Leu	Ile	Tyr	His
Thr 610	Ser	Arg	Leu	His	Ser	Gly	Val	Pro	Ser	Arg	Phe	Ser	Gly	Ser	Gly
Ser 625	Gly	Thr	Asp	Tyr	Ser	Leu	Thr	Ile	Ser	Asn	Leu	Glu	Gln	Glu	Asp
Ile 645	Ala	Thr	Tyr	Phe	Cys	Gln	Gln	Gly	Asn	Thr	Leu	Pro	Tyr	Thr	Phe
Gly 660	Gly	Gly	Thr	Lys	Leu	Glu	Ile	Thr	Gly	Gly	Gly	Gly	Ser	Gln	Val
Lys 675	Leu	Gln	Glu	Ser	Gly	Gly	Gly	Leu	Val	Lys	Pro	Gly	Gly	Ser	Leu
Lys 690	Leu	Ser	Cys	Ala	Ala	Ser	Gly	Phe	Thr	Phe	Ser	Ser	Tyr	Ala	Met
Ser 705	Trp	Val	Arg	Gln	Thr	Pro	Glu	Lys	Arg	Leu	Glu	Trp	Val	Ala	Thr
Ile 725	Ser	Ser	Gly	Gly	Ser	Tyr	Thr	Tyr	Tyr	Pro	Asp	Ser	Val	Lys	Gly
Arg 740	Phe	Thr	Ile	Ser	Arg	Asp	Asn	Ala	Lys	Asn	Thr	Leu	Tyr	Leu	Gln
Met 755	Ser	Ser	Leu	Arg	Ser	Glu	Asp	Thr	Ala	Met	Tyr	Tyr	Cys	Ala	Arg
Gln 770	Asp	Gly	Tyr	Tyr	Pro	Gly	Trp	Phe	Ala	Asn	Trp	Gly	Gln	Gly	Thr
Thr 785	Val	Thr	Val	Ser	Ser	Gly	Ser	Thr	Ser	Gly	Ser	Gly	Lys	Pro	Gly
Ser 805	Gly	Glu	Gly	Ser	Thr	Lys	Gly	Asp	Ile	Glu	Leu	Thr	Gln	Ser	Pro
Ala 820	Ile	Met	Ser	Ala	Ser	Leu	Gly	Glu	Glu	Ile	Thr	Leu	Thr	Cys	Ser
Ala 835	Ser	Ser	Ser	Val	Ser	Tyr	Met	His	Trp	Tyr	Gln	Gln	Lys	Ser	Gly
Thr 850	Ser	Pro	Lys	Leu	Leu	Ile	Tyr	Ser	Thr	Ser	Asn	Leu	Ala	Ser	Gly
Val 865	Pro	Ser	Arg	Phe	Ser	Gly	Ser	Gly	Ser	Gly	Thr	Phe	Tyr	Ser	Leu

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Thr	Ile	Ser	Ser	Val	Glu	Ala	Glu	Asp	Ala	Ala	Asp	Tyr	Tyr	Cys	His	885	890	895	
Gln	Trp	Ser	Ser	Tyr	Thr	Phe	Gly	Gly	Gly	Thr	Lys	Leu	Glu	Ile	Lys	900	905	910	
Arg	Gly	Gly	Gly	Gly	Ser	Glu	Val	Lys	Leu	Gln	Glu	Ser	Gly	Pro	Gly	915	920	925	
Leu	Val	Ala	Pro	Ser	Gln	Ser	Leu	Ser	Val	Thr	Cys	Thr	Val	Ser	Gly	930	935	940	
Val	Ser	Leu	Pro	Asp	Tyr	Gly	Val	Ser	Trp	Ile	Arg	Gln	Pro	Pro	Arg	945	950	955	960
Lys	Gly	Leu	Glu	Trp	Leu	Gly	Val	Ile	Trp	Gly	Ser	Glu	Thr	Thr	Tyr	965	970	975	
Tyr	Asn	Ser	Ala	Leu	Lys	Ser	Arg	Leu	Thr	Ile	Ile	Lys	Asp	Asn	Ser	980	985	990	
Lys	Ser	Gln	Val	Phe	Leu	Lys	Met	Asn	Ser	Leu	Gln	Thr	Asp	Asp	Thr	995	1000	1005	
Ala	Ile	Tyr	Tyr	Cys	Ala	Lys	His	Tyr	Tyr	Tyr	Gly	Gly	Ser	Tyr		1010	1015	1020	
Ala	Met	Asp	Tyr	Trp	Gly	Gln	Gly	Thr	Ser	Val	Thr	Val	Ser	Ser		1025	1030	1035	
Ser	Gly	Thr	Thr	Thr	Pro	Ala	Pro	Arg	Pro	Pro	Thr	Pro	Ala	Pro		1040	1045	1050	
Thr	Ile	Ala	Ser	Gln	Pro	Leu	Ser	Leu	Arg	Pro	Glu	Ala	Cys	Arg		1055	1060	1065	
Pro	Ala	Ala	Gly	Gly	Ala	Val	His	Thr	Arg	Gly	Leu	Asp	Phe	Ala		1070	1075	1080	
Cys	Asp	Ile	Tyr	Ile	Trp	Ala	Pro	Leu	Ala	Gly	Thr	Cys	Gly	Val		1085	1090	1095	
Leu	Leu	Leu	Ser	Leu	Val	Ile	Thr	Leu	Tyr	Cys	Lys	Arg	Gly	Arg		1100	1105	1110	
Lys	Lys	Leu	Leu	Tyr	Ile	Phe	Lys	Gln	Pro	Phe	Met	Arg	Pro	Val		1115	1120	1125	
Gln	Thr	Thr	Gln	Glu	Glu	Asp	Gly	Cys	Ser	Cys	Arg	Phe	Pro	Glu		1130	1135	1140	
Glu	Glu	Glu	Gly	Gly	Cys	Glu	Leu	Arg	Val	Lys	Phe	Ser	Arg	Ser		1145	1150	1155	
Ala	Asp	Ala	Pro	Ala	Tyr	Lys	Gln	Gly	Gln	Asn	Gln	Leu	Tyr	Asn		1160	1165	1170	
Glu	Leu	Asn	Leu	Gly	Arg	Arg	Glu	Glu	Tyr	Asp	Val	Leu	Asp	Lys		1175	1180	1185	
Arg	Arg	Gly	Arg	Asp	Pro	Glu	Met	Gly	Gly	Lys	Pro	Arg	Arg	Lys		1190	1195	1200	
Asn	Pro	Gln	Glu	Gly	Leu	Tyr	Asn	Glu	Leu	Gln	Lys	Asp	Lys	Met		1205	1210	1215	
Ala	Glu	Ala	Tyr	Ser	Glu	Ile	Gly	Met	Lys	Gly	Glu	Arg	Arg	Arg		1220	1225	1230	
Gly	Lys	Gly	His	Asp	Gly	Leu	Tyr	Gln	Gly	Leu	Ser	Thr	Ala	Thr		1235	1240	1245	
Lys	Asp	Thr	Tyr	Asp	Ala	Leu	His	Met	Gln	Ala	Leu	Pro	Pro	Arg		1250	1255	1260	

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<210> SEQ ID NO 87
<211> LENGTH: 107
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic polypeptide

<400> SEQUENCE: 87

Asp Ile Gln Met Thr Gln Thr Thr Ser Ser Leu Ser Ala Ser Leu Gly
1 5 10 15
Asp Arg Val Thr Ile Ser Cys Arg Ala Ser Gln Asp Ile Ser Lys Tyr
20 25 30
Leu Asn Trp Tyr Gln Gln Lys Pro Asp Gly Thr Val Lys Leu Leu Ile
35 40 45
Tyr His Thr Ser Arg Leu His Ser Gly Val Pro Ser Arg Phe Ser Gly
50 55 60
Ser Gly Ser Gly Thr Asp Tyr Ser Leu Thr Ile Ser Asn Leu Glu Gln
65 70 75 80
Glu Asp Ile Ala Thr Tyr Phe Cys Gln Gln Gly Asn Thr Leu Pro Tyr
85 90 95
Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Thr
100 105

<210> SEQ ID NO 88
<211> LENGTH: 120
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic polypeptide

<400> SEQUENCE: 88

Glu Val Lys Leu Gln Glu Ser Gly Pro Gly Leu Val Ala Pro Ser Gln
1 5 10 15
Ser Leu Ser Val Thr Cys Thr Val Ser Gly Val Ser Leu Pro Asp Tyr
20 25 30
Gly Val Ser Trp Ile Arg Gln Pro Pro Arg Lys Gly Leu Glu Trp Leu
35 40 45
Gly Val Ile Trp Gly Ser Glu Thr Thr Tyr Tyr Asn Ser Ala Leu Lys
50 55 60
Ser Arg Leu Thr Ile Ile Lys Asp Asn Ser Lys Ser Gln Val Phe Leu
65 70 75 80
Lys Met Asn Ser Leu Gln Thr Asp Asp Thr Ala Ile Tyr Tyr Cys Ala
85 90 95
Lys His Tyr Tyr Tyr Gly Gly Ser Tyr Ala Met Asp Tyr Trp Gly Gln
100 105 110
Gly Thr Ser Val Thr Val Ser Ser
115 120

<210> SEQ ID NO 89
<211> LENGTH: 491
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic polypeptide

<400> SEQUENCE: 89

Met 1	Ala	Leu	Pro	Val 5	Thr	Ala	Leu	Leu	Pro 10	Leu	Ala	Leu	Leu 15
His	Ala	Ala	Arg 20	Pro	Asp	Ile	Gln	Met 25	Thr	Gln	Thr	Ser 30	Leu
Ser	Ala	Ser	Leu	Gly	Asp	Arg	Val 40	Thr	Ile	Ser	Cys 45	Ala	Gln
Asp	Ile	Ser	Lys	Tyr	Leu	Asn 55	Trp	Tyr	Gln	Gln	Lys 60	Pro	Thr
Val 65	Lys	Leu	Leu	Ile	Tyr 70	His	Thr	Ser	Arg	Leu 75	His	Ser	Pro
Ser	Arg	Phe	Ser	Gly 85	Ser	Gly	Ser	Gly	Thr 90	Asp	Tyr	Leu	Ile
Ser	Asn	Leu	Glu	Gln	Glu	Asp	Ile	Ala 105	Thr	Tyr	Phe	Cys 110	Gly
Asn	Thr	Leu	Pro	Tyr	Thr	Phe	Gly 120	Gly	Gly	Thr	Lys	Leu	Thr
Gly	Ser	Thr	Ser	Gly	Ser	Gly 135	Lys	Pro	Gly	Ser	Gly 140	Glu	Thr
Lys 145	Gly	Glu	Val	Lys	Leu	Gln	Glu	Ser	Gly	Pro 155	Gly	Val	Pro
Ser	Gln	Ser	Leu	Ser	Val	Thr	Cys	Thr	Val 170	Ser	Gly	Ser	Pro
Asp	Tyr	Gly	Val	Ser	Trp	Ile	Arg	Gln 185	Pro	Pro	Arg	Lys 190	Glu
Trp	Leu	Gly	Val	Ile	Trp	Gly	Ser	Glu 200	Thr	Thr	Tyr	Asn	Ala
Leu	Lys	Ser	Arg	Leu	Thr	Ile 215	Ile	Lys	Asp	Asn 220	Ser	Ser	Val
Phe 225	Leu	Lys	Met	Asn	Ser	Leu	Gln	Thr	Asp	Asp 235	Thr	Ile	Tyr
Cys	Ala	Lys	His	Tyr	Tyr	Tyr	Gly	Gly	Ser 250	Tyr	Ala	Met	Trp
Gly	Gln	Gly	Thr	Ser	Val	Thr	Val	Ser 265	Ser	Ser	Gly	Thr	Pro
Ala	Pro	Arg	Pro	Pro	Thr	Pro	Ala	Pro 280	Thr	Ile	Ala	Ser 285	Leu
Ser	Leu	Arg	Pro	Glu	Ala	Cys 295	Arg	Pro	Ala	Ala	Gly 300	Gly	His
Thr 305	Arg	Gly	Leu	Asp	Phe	Ala	Cys	Asp	Ile	Tyr 315	Ile	Trp	Leu
Ala	Gly	Thr	Cys	Gly	Val	Leu	Leu	Leu 330	Ser	Leu	Val	Thr	Tyr
Cys	Lys	Arg	Gly	Arg	Lys	Lys	Leu	Leu 345	Tyr	Ile	Phe	Gln 350	Phe
Met	Arg	Pro	Val	Gln	Thr	Thr	Gln	Glu 360	Glu	Asp	Gly	Ser 365	Arg
Phe 370	Pro	Glu	Glu	Glu	Glu	Gly	Gly	Cys	Glu	Leu	Arg	Lys	Ser
Arg 385	Ser	Ala	Asp	Ala	Pro	Ala	Tyr	Lys	Gln	Gly	Gln	Asn 395	Tyr

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Asn Glu Leu Asn Leu Gly Arg Arg Glu Glu Tyr Asp Val Leu Asp Lys
    405                                410                                415

Arg Arg Gly Arg Asp Pro Glu Met Gly Gly Lys Pro Arg Arg Lys Asn
    420                                425                                430

Pro Gln Glu Gly Leu Tyr Asn Glu Leu Gln Lys Asp Lys Met Ala Glu
    435                                440                                445

Ala Tyr Ser Glu Ile Gly Met Lys Gly Glu Arg Arg Arg Gly Lys Gly
    450                                455                                460

His Asp Gly Leu Tyr Gln Gly Leu Ser Thr Ala Thr Lys Asp Thr Tyr
    465                                470                                475                                480

Asp Ala Leu His Met Gln Ala Leu Pro Pro Arg
    485                                490

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<210> SEQ ID NO 90
<211> LENGTH: 105
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
                             polypeptide

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<400> SEQUENCE: 90

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Asp Ile Glu Leu Thr Gln Ser Pro Ala Ile Met Ser Ala Ser Leu Gly
1      5      10      15

Glu Glu Ile Thr Leu Thr Cys Ser Ala Ser Ser Ser Val Ser Tyr Met
20     25     30

His Trp Tyr Gln Gln Lys Ser Gly Thr Ser Pro Lys Leu Leu Ile Tyr
35     40     45

Ser Thr Ser Asn Leu Ala Ser Gly Val Pro Ser Arg Phe Ser Gly Ser
50     55     60

Gly Ser Gly Thr Phe Tyr Ser Leu Thr Ile Ser Ser Val Glu Ala Glu
65     70     75     80

Asp Ala Ala Asp Tyr Tyr Cys His Gln Trp Ser Ser Tyr Thr Phe Gly
85     90     95

Gly Gly Thr Lys Leu Glu Ile Lys Arg
100    105

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<210> SEQ ID NO 91
<211> LENGTH: 120
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
                             polypeptide

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<400> SEQUENCE: 91

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Gln Val Lys Leu Gln Glu Ser Gly Gly Gly Leu Val Lys Pro Gly Gly
1      5      10      15

Ser Leu Lys Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
20     25     30

Ala Met Ser Trp Val Arg Gln Thr Pro Glu Lys Arg Leu Glu Trp Val
35     40     45

Ala Thr Ile Ser Ser Gly Gly Ser Tyr Thr Tyr Tyr Pro Asp Ser Val
50     55     60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Thr Leu Tyr
65     70     75     80

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-continued

Leu Gln Met Ser Ser Leu Arg Ser Glu Asp Thr Ala Met Tyr Tyr Cys
85 90 95

Ala Arg Gln Asp Gly Tyr Tyr Pro Gly Trp Phe Ala Asn Trp Gly Gln
100 105 110

Gly Thr Thr Val Thr Val Ser Ser
115 120

<210> SEQ ID NO 92
<211> LENGTH: 5
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic peptide

<400> SEQUENCE: 92

Glu Ala Ala Ala Lys
1 5

<210> SEQ ID NO 93
<211> LENGTH: 30
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic polypeptide
<220> FEATURE:
<221> NAME/KEY: SITE
<222> LOCATION: (1) .. (30)
<223> OTHER INFORMATION: This sequence may encompass 2-6 "Gly Gly Gly Gly Ser" repeating units

<400> SEQUENCE: 93

Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly
1 5 10 15

Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser
20 25 30

<210> SEQ ID NO 94
<211> LENGTH: 4
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic peptide

<400> SEQUENCE: 94

Arg Gly Asp Ser
1

<210> SEQ ID NO 95
<211> LENGTH: 116
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic polypeptide

<400> SEQUENCE: 95

Gln Leu Gln Gln Pro Gly Ala Glu Leu Val Arg Pro Gly Ser Ser Val
1 5 10 15

Lys Leu Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Arg Tyr Trp Ile
20 25 30

-continued

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His Trp Val Lys Gln Arg Pro Ile Gln Gly Leu Glu Trp Ile Gly Asn
   35                               40                               45

Ile Asp Pro Ser Asp Ser Glu Thr His Tyr Asn Gln Lys Phe Lys Asp
   50                               55                               60

Lys Ala Thr Leu Thr Val Asp Lys Ser Ser Gly Thr Ala Tyr Met Gln
   65                               70                               75                               80

Leu Ser Ser Leu Thr Ser Glu Asp Ser Ala Val Tyr Tyr Cys Ala Thr
   85                               90                               95

Glu Asp Leu Tyr Tyr Ala Met Glu Tyr Trp Gly Gln Gly Thr Ser Val
   100                              105                              110

Thr Val Ser Ser
   115

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<210> SEQ ID NO 96
<211> LENGTH: 113
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
                             polypeptide

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<400> SEQUENCE: 96

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Asp Ile Met Met Thr Gln Ser Pro Ser Ser Leu Ala Val Ser Ala Gly
1       5              10              15

Glu Lys Val Thr Met Thr Cys Lys Ser Ser Gln Ser Val Leu Tyr Ser
20      25              30

Ser Asn Gln Lys Asn Tyr Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln
35      40              45

Ser Pro Lys Leu Leu Ile Tyr Trp Ala Ser Thr Arg Glu Ser Gly Val
50      55              60

Pro Asp Arg Phe Thr Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr
65      70              75              80

Ile Ser Ser Val Gln Pro Glu Asp Leu Ala Val Tyr Tyr Cys His Gln
85      90              95

Tyr Leu Ser Ser His Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys
100     105            110

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Arg

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<210> SEQ ID NO 97
<211> LENGTH: 120
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
                             polypeptide

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<400> SEQUENCE: 97

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Gln Val Gln Leu Gln Gln Pro Gly Thr Glu Leu Val Arg Pro Gly Ser
1       5              10              15

Ser Val Lys Leu Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Ser Tyr
20      25              30

Trp Val Asn Trp Val Lys Gln Arg Pro Asp Gln Gly Leu Glu Trp Ile
35      40              45

Gly Arg Ile Asp Pro Tyr Asp Ser Glu Thr His Tyr Asn Gln Lys Phe
50      55              60

Thr Asp Lys Ala Ile Ser Thr Ile Asp Thr Ser Ser Asn Thr Ala Tyr

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65	70	75	80
Met Gln Leu Ser Thr Leu Thr Ser Asp Ala Ser Ala Val Tyr Tyr Cys			
	85	90	95
Ser Arg Ser Pro Arg Asp Ser Ser Thr Asn Leu Ala Asp Trp Gly Gln			
	100	105	110
Gly Thr Leu Val Thr Val Ser Ser			
	115	120	

<210> SEQ ID NO 98
 <211> LENGTH: 108
 <212> TYPE: PRT
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic polypeptide

<400> SEQUENCE: 98

Asp Ile Val Met Thr Gln Ser Pro Ala Thr Leu Ser Val Thr Pro Gly			
1	5	10	15
Asp Arg Val Ser Leu Ser Cys Arg Ala Ser Gln Ser Ile Ser Asp Tyr			
	20	25	30
Leu His Trp Tyr Gln Gln Lys Ser His Glu Ser Pro Arg Leu Leu Ile			
	35	40	45
Lys Tyr Ala Ser Gln Ser Ile Ser Gly Ile Pro Ser Arg Phe Ser Gly			
	50	55	60
Ser Gly Ser Gly Ser Asp Phe Thr Leu Ser Ile Asn Ser Val Glu Pro			
65	70	75	80
Glu Asp Val Gly Val Tyr Tyr Cys Gln Asn Gly His Ser Phe Pro Leu			
	85	90	95
Thr Phe Gly Ala Gly Thr Lys Leu Glu Leu Arg Arg			
	100	105	

<210> SEQ ID NO 99
 <211> LENGTH: 903
 <212> TYPE: PRT
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic polypeptide

<400> SEQUENCE: 99

Met Ala Leu Pro Val Thr Ala Leu Leu Leu Pro Leu Ala Leu Leu Leu			
1	5	10	15
His Ala Ala Arg Pro Asp Ile Glu Leu Thr Gln Ser Pro Ala Ile Met			
	20	25	30
Ser Ala Ser Leu Gly Glu Glu Ile Thr Leu Thr Cys Ser Ala Ser Ser			
	35	40	45
Ser Val Ser Tyr Met His Trp Tyr Gln Gln Lys Ser Gly Thr Ser Pro			
	50	55	60
Lys Leu Leu Ile Tyr Ser Thr Ser Asn Leu Ala Ser Gly Val Pro Ser			
65	70	75	80
Arg Phe Ser Gly Ser Gly Ser Gly Thr Phe Tyr Ser Leu Thr Ile Ser			
	85	90	95
Ser Val Glu Ala Glu Asp Ala Ala Asp Tyr Tyr Cys His Gln Trp Ser			
	100	105	110
Ser Tyr Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys Arg Gly Gly			

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115					120					125					
Gly	Gly	Ser	Gly	Gly	Gly	Gly	Ser	Gly	Gly	Gly	Gly	Ser	Gln	Val	Lys
130						135					140				
Leu	Gln	Glu	Ser	Gly	Gly	Gly	Leu	Val	Lys	Pro	Gly	Gly	Ser	Leu	Lys
145					150					155					160
Leu	Ser	Cys	Ala	Ala	Ser	Gly	Phe	Thr	Phe	Ser	Ser	Tyr	Ala	Met	Ser
				165					170					175	
Trp	Val	Arg	Gln	Thr	Pro	Glu	Lys	Arg	Leu	Glu	Trp	Val	Ala	Thr	Ile
			180					185					190		
Ser	Ser	Gly	Gly	Ser	Tyr	Thr	Tyr	Tyr	Pro	Asp	Ser	Val	Lys	Gly	Arg
		195					200					205			
Phe	Thr	Ile	Ser	Arg	Asp	Asn	Ala	Lys	Asn	Thr	Leu	Tyr	Leu	Gln	Met
210						215					220				
Ser	Ser	Leu	Arg	Ser	Glu	Asp	Thr	Ala	Met	Tyr	Tyr	Cys	Ala	Arg	Gln
225					230					235					240
Asp	Gly	Tyr	Tyr	Pro	Gly	Trp	Phe	Ala	Asn	Trp	Gly	Gln	Gly	Thr	Thr
				245					250					255	
Val	Thr	Val	Ser	Ser	Ser	Gly	Thr	Thr	Thr	Pro	Ala	Pro	Arg	Pro	Pro
			260					265					270		
Thr	Pro	Ala	Pro	Thr	Ile	Ala	Ser	Gln	Pro	Leu	Ser	Leu	Arg	Pro	Glu
		275					280					285			
Ala	Cys	Arg	Pro	Ala	Ala	Gly	Gly	Ala	Val	His	Thr	Arg	Gly	Leu	Asp
290						295					300				
Phe	Ala	Cys	Asp	Ile	Tyr	Ile	Trp	Ala	Pro	Leu	Ala	Gly	Thr	Cys	Gly
305					310					315					320
Val	Leu	Leu	Leu	Ser	Leu	Val	Ile	Thr	Leu	Tyr	Cys	Lys	Arg	Gly	Arg
				325					330					335	
Lys	Lys	Leu	Leu	Tyr	Ile	Phe	Lys	Gln	Pro	Phe	Met	Arg	Pro	Val	Gln
			340					345					350		
Thr	Thr	Gln	Glu	Glu	Asp	Gly	Cys	Ser	Cys	Arg	Phe	Pro	Glu	Glu	Glu
		355				360						365			
Glu	Gly	Gly	Cys	Glu	Leu	Arg	Val	Lys	Phe	Ser	Arg	Ser	Ala	Asp	Ala
370						375					380				
Pro	Ala	Tyr	Lys	Gln	Gly	Gln	Asn	Gln	Leu	Tyr	Asn	Glu	Leu	Asn	Leu
385					390					395					400
Gly	Arg	Arg	Glu	Glu	Tyr	Asp	Val	Leu	Asp	Lys	Arg	Arg	Gly	Arg	Asp
			405					410						415	
Pro	Glu	Met	Gly	Gly	Lys	Pro	Arg	Arg	Lys	Asn	Pro	Gln	Glu	Gly	Leu
			420					425					430		
Tyr	Asn	Glu	Leu	Gln	Lys	Asp	Lys	Met	Ala	Glu	Ala	Tyr	Ser	Glu	Ile
		435				440						445			
Gly	Met	Lys	Gly	Glu	Arg	Arg	Arg	Gly	Lys	Gly	His	Asp	Gly	Leu	Tyr
450						455					460				
Gln	Gly	Leu	Ser	Thr	Ala	Thr	Lys	Asp	Thr	Tyr	Asp	Ala	Leu	His	Met
465					470					475					480
Gln	Ala	Leu	Pro	Pro	Arg	Gly	Ser	Gly	Ala	Thr	Asn	Phe	Ser	Leu	Leu
				485					490					495	
Lys	Gln	Ala	Gly	Asp	Val	Glu	Glu	Asn	Pro	Gly	Pro	Met	Asp	Trp	Thr
			500					505					510		
Trp	Ile	Leu	Phe	Leu	Val	Ala	Ala	Ala	Thr	Arg	Val	His	Ser	Asn	Trp
		515					520					525			

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Val	Asn	Val	Ile	Ser	Asp	Leu	Lys	Lys	Ile	Glu	Asp	Leu	Ile	Gln	Ser
530						535					540				
Met	His	Ile	Asp	Ala	Thr	Leu	Tyr	Thr	Glu	Ser	Asp	Val	His	Pro	Ser
545					550					555					560
Cys	Lys	Val	Thr	Ala	Met	Lys	Cys	Phe	Leu	Leu	Glu	Leu	Gln	Val	Ile
				565					570					575	
Ser	Leu	Glu	Ser	Gly	Asp	Ala	Ser	Ile	His	Asp	Thr	Val	Glu	Asn	Leu
			580					585					590		
Ile	Ile	Leu	Ala	Asn	Asn	Ser	Leu	Ser	Ser	Asn	Gly	Asn	Val	Thr	Glu
		595					600					605			
Ser	Gly	Cys	Lys	Glu	Cys	Glu	Glu	Leu	Glu	Glu	Lys	Asn	Ile	Lys	Glu
	610					615					620				
Phe	Leu	Gln	Ser	Phe	Val	His	Ile	Val	Gln	Met	Phe	Ile	Asn	Thr	Ser
625					630					635					640
Ser	Gly	Gly	Gly	Ser	Gly	Gly	Gly	Gly	Ser	Gly	Gly	Gly	Gly	Ser	Gly
				645					650					655	
Gly	Gly	Gly	Ser	Gly	Gly	Gly	Ser	Leu	Gln	Ile	Thr	Cys	Pro	Pro	Pro
			660					665					670		
Met	Ser	Val	Glu	His	Ala	Asp	Ile	Trp	Val	Lys	Ser	Tyr	Ser	Leu	Tyr
		675					680					685			
Ser	Arg	Glu	Arg	Tyr	Ile	Cys	Asn	Ser	Gly	Phe	Lys	Arg	Lys	Ala	Gly
	690					695					700				
Thr	Ser	Ser	Leu	Thr	Glu	Cys	Val	Leu	Asn	Lys	Ala	Thr	Asn	Val	Ala
705					710					715					720
His	Trp	Thr	Thr	Pro	Ser	Leu	Lys	Cys	Ile	Arg	Asp	Pro	Ala	Leu	Val
				725					730					735	
His	Gln	Arg	Pro	Ala	Pro	Pro	Ser	Thr	Val	Thr	Thr	Ala	Gly	Val	Thr
			740					745					750		
Pro	Gln	Pro	Glu	Ser	Leu	Ser	Pro	Ser	Gly	Lys	Glu	Pro	Ala	Ala	Ser
		755					760					765			
Ser	Pro	Ser	Ser	Asn	Asn	Thr	Ala	Ala	Thr	Thr	Ala	Ala	Ile	Val	Pro
	770				775						780				
Gly	Ser	Gln	Leu	Met	Pro	Ser	Lys	Ser	Pro	Ser	Thr	Gly	Thr	Thr	Glu
785					790					795					800
Ile	Ser	Ser	His	Glu	Ser	Ser	His	Gly	Thr	Pro	Ser	Gln	Thr	Thr	Ala
			805					810						815	
Lys	Asn	Trp	Glu	Leu	Thr	Ala	Ser	Ala	Ser	His	Gln	Pro	Pro	Gly	Val
			820					825					830		
Tyr	Pro	Gln	Gly	His	Ser	Asp	Thr	Thr	Val	Ala	Ile	Ser	Thr	Ser	Thr
		835					840					845			
Val	Leu	Leu	Cys	Gly	Leu	Ser	Ala	Val	Ser	Leu	Leu	Ala	Cys	Tyr	Leu
	850					855					860				
Lys	Ser	Arg	Gln	Thr	Pro	Pro	Leu	Ala	Ser	Val	Glu	Met	Glu	Ala	Met
865					870					875					880
Glu	Ala	Leu	Pro	Val	Thr	Trp	Gly	Thr	Ser	Ser	Arg	Asp	Glu	Asp	Leu
			885					890						895	
Glu	Asn	Cys	Ser	His	His	Leu									
			900												

<210> SEQ ID NO 100

<211> LENGTH: 1065

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<212> TYPE: PRT
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic polypeptide

<400> SEQUENCE: 100

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Met Ala Leu Pro Val Thr Ala Leu Leu Leu Pro Leu Ala Leu Leu Leu
1      5      10      15

His Ala Ala Arg Pro Asp Ile Glu Leu Thr Gln Ser Pro Ala Ile Met
      20      25      30

Ser Ala Ser Leu Gly Glu Glu Ile Thr Leu Thr Cys Ser Ala Ser Ser
      35      40      45

Ser Val Ser Tyr Met His Trp Tyr Gln Gln Lys Ser Gly Thr Ser Pro
      50      55      60

Lys Leu Leu Ile Tyr Ser Thr Ser Asn Leu Ala Ser Gly Val Pro Ser
      65      70      75      80

Arg Phe Ser Gly Ser Gly Ser Gly Thr Phe Tyr Ser Leu Thr Ile Ser
      85      90      95

Ser Val Glu Ala Glu Asp Ala Ala Asp Tyr Tyr Cys His Gln Trp Ser
      100     105     110

Ser Tyr Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys Arg Gly Gly
      115     120     125

Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gln Val Lys
      130     135     140

Leu Gln Glu Ser Gly Gly Gly Leu Val Lys Pro Gly Gly Ser Leu Lys
      145     150     155     160

Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr Ala Met Ser
      165     170     175

Trp Val Arg Gln Thr Pro Glu Lys Arg Leu Glu Trp Val Ala Thr Ile
      180     185     190

Ser Ser Gly Gly Ser Tyr Thr Tyr Tyr Pro Asp Ser Val Lys Gly Arg
      195     200     205

Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Thr Leu Tyr Leu Gln Met
      210     215     220

Ser Ser Leu Arg Ser Glu Asp Thr Ala Met Tyr Tyr Cys Ala Arg Gln
      225     230     235     240

Asp Gly Tyr Tyr Pro Gly Trp Phe Ala Asn Trp Gly Gln Gly Thr Thr
      245     250     255

Val Thr Val Ser Ser Ser Gly Thr Thr Thr Pro Ala Pro Arg Pro Pro
      260     265     270

Thr Pro Ala Pro Thr Ile Ala Ser Gln Pro Leu Ser Leu Arg Pro Glu
      275     280     285

Ala Cys Arg Pro Ala Ala Gly Gly Ala Val His Thr Arg Gly Leu Asp
      290     295     300

Phe Ala Cys Asp Ile Tyr Ile Trp Ala Pro Leu Ala Gly Thr Cys Gly
      305     310     315     320

Val Leu Leu Leu Ser Leu Val Ile Thr Leu Tyr Cys Lys Arg Gly Arg
      325     330     335

Lys Lys Leu Leu Tyr Ile Phe Lys Gln Pro Phe Met Arg Pro Val Gln
      340     345     350

Thr Thr Gln Glu Glu Asp Gly Cys Ser Cys Arg Phe Pro Glu Glu Glu
      355     360     365

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Glu	Gly	Gly	Cys	Glu	Leu	Arg	Val	Lys	Phe	Ser	Arg	Ser	Ala	Asp	Ala
370						375					380				
Pro	Ala	Tyr	Lys	Gln	Gly	Gln	Asn	Gln	Leu	Tyr	Asn	Glu	Leu	Asn	Leu
385					390					395					400
Gly	Arg	Arg	Glu	Glu	Tyr	Asp	Val	Leu	Asp	Lys	Arg	Arg	Gly	Arg	Asp
			405						410					415	
Pro	Glu	Met	Gly	Gly	Lys	Pro	Arg	Arg	Lys	Asn	Pro	Gln	Glu	Gly	Leu
			420					425					430		
Tyr	Asn	Glu	Leu	Gln	Lys	Asp	Lys	Met	Ala	Glu	Ala	Tyr	Ser	Glu	Ile
		435					440					445			
Gly	Met	Lys	Gly	Glu	Arg	Arg	Arg	Gly	Lys	Gly	His	Asp	Gly	Leu	Tyr
	450					455					460				
Gln	Gly	Leu	Ser	Thr	Ala	Thr	Lys	Asp	Thr	Tyr	Asp	Ala	Leu	His	Met
465					470					475					480
Gln	Ala	Leu	Pro	Pro	Arg	Gly	Ser	Gly	Ala	Thr	Asn	Phe	Ser	Leu	Leu
				485					490						495
Lys	Gln	Ala	Gly	Asp	Val	Glu	Glu	Asn	Pro	Gly	Pro	Met	Leu	Leu	Leu
			500					505					510		
Val	Thr	Ser	Leu	Leu	Leu	Cys	Glu	Leu	Pro	His	Pro	Ala	Phe	Leu	Leu
			515					520				525			
Ile	Pro	Arg	Lys	Val	Cys	Asn	Gly	Ile	Gly	Ile	Gly	Glu	Phe	Lys	Asp
	530					535					540				
Ser	Leu	Ser	Ile	Asn	Ala	Thr	Asn	Ile	Lys	His	Phe	Lys	Asn	Cys	Thr
545					550					555					560
Ser	Ile	Ser	Gly	Asp	Leu	His	Ile	Leu	Pro	Val	Ala	Phe	Arg	Gly	Asp
			565						570					575	
Ser	Phe	Thr	His	Thr	Pro	Pro	Leu	Asp	Pro	Gln	Glu	Leu	Asp	Ile	Leu
			580					585					590		
Lys	Thr	Val	Lys	Glu	Ile	Thr	Gly	Phe	Leu	Leu	Ile	Gln	Ala	Trp	Pro
			595				600					605			
Glu	Asn	Arg	Thr	Asp	Leu	His	Ala	Phe	Glu	Asn	Leu	Glu	Ile	Ile	Arg
	610					615					620				
Gly	Arg	Thr	Lys	Gln	His	Gly	Gln	Phe	Ser	Leu	Ala	Val	Val	Ser	Leu
	625				630					635					640
Asn	Ile	Thr	Ser	Leu	Gly	Leu	Arg	Ser	Leu	Lys	Glu	Ile	Ser	Asp	Gly
				645					650					655	
Asp	Val	Ile	Ile	Ser	Gly	Asn	Lys	Asn	Leu	Cys	Tyr	Ala	Asn	Thr	Ile
			660					665					670		
Asn	Trp	Lys	Lys	Leu	Phe	Gly	Thr	Ser	Gly	Gln	Lys	Thr	Lys	Ile	Ile
		675					680					685			
Ser	Asn	Arg	Gly	Glu	Asn	Ser	Cys	Lys	Ala	Thr	Gly	Gln	Val	Cys	His
	690					695					700				
Ala	Leu	Cys	Ser	Pro	Glu	Gly	Cys	Trp	Gly	Pro	Glu	Pro	Arg	Asp	Cys
	705				710					715					720
Val	Ser	Cys	Arg	Asn	Val	Ser	Arg	Gly	Arg	Glu	Cys	Val	Asp	Lys	Cys
				725					730					735	
Asn	Leu	Leu	Glu	Gly	Glu	Pro	Arg	Glu	Phe	Val	Glu	Asn	Ser	Glu	Cys
			740					745					750		
Ile	Gln	Cys	His	Pro	Glu	Cys	Leu	Pro	Gln	Ala	Met	Asn	Ile	Thr	Cys
			755				760						765		

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Thr	Gly	Arg	Gly	Pro	Asp	Asn	Cys	Ile	Gln	Cys	Ala	His	Tyr	Ile	Asp
770						775					780				
Gly	Pro	His	Cys	Val	Lys	Thr	Cys	Pro	Ala	Gly	Val	Met	Gly	Glu	Asn
785					790					795					800
Asn	Thr	Leu	Val	Trp	Lys	Tyr	Ala	Asp	Ala	Gly	His	Val	Cys	His	Leu
			805						810					815	
Cys	His	Pro	Asn	Cys	Thr	Tyr	Gly	Cys	Thr	Gly	Pro	Gly	Leu	Glu	Gly
			820					825					830		
Cys	Pro	Thr	Asn	Gly	Pro	Lys	Ile	Pro	Ser	Pro	Ile	Leu	Leu	Thr	Cys
		835					840					845			
Pro	Thr	Ile	Ser	Ile	Leu	Ser	Phe	Phe	Ser	Val	Ala	Leu	Leu	Val	Ile
	850					855					860				
Leu	Ala	Cys	Val	Leu	Trp	Lys	Lys	Arg	Ile	Lys	Pro	Ile	Val	Trp	Pro
865					870					875					880
Ser	Leu	Pro	Asp	His	Lys	Lys	Thr	Leu	Glu	His	Leu	Cys	Lys	Lys	Pro
				885					890					895	
Arg	Lys	Asn	Leu	Asn	Val	Ser	Phe	Asn	Pro	Glu	Ser	Phe	Leu	Asp	Cys
			900					905					910		
Gln	Ile	His	Arg	Val	Asp	Asp	Ile	Gln	Ala	Arg	Asp	Glu	Val	Glu	Gly
		915					920					925			
Phe	Leu	Gln	Asp	Thr	Phe	Pro	Gln	Gln	Leu	Glu	Glu	Ser	Glu	Lys	Gln
	930					935					940				
Arg	Leu	Gly	Gly	Asp	Val	Gln	Ser	Pro	Asn	Cys	Pro	Ser	Glu	Asp	Val
945					950					955					960
Val	Ile	Thr	Pro	Glu	Ser	Phe	Gly	Arg	Asp	Ser	Ser	Leu	Thr	Cys	Leu
				965					970					975	
Ala	Gly	Asn	Val	Ser	Ala	Cys	Asp	Ala	Pro	Ile	Leu	Ser	Ser	Ser	Arg
			980					985					990		
Ser	Leu	Asp	Cys	Arg	Glu	Ser	Gly	Lys	Asn	Gly	Pro	His	Val	Tyr	Gln
		995					1000					1005			
Asp	Leu	Leu	Leu	Ser	Leu	Gly	Thr	Thr	Asn	Ser	Thr	Leu	Pro	Pro	
	1010					1015						1020			
Pro	Phe	Ser	Leu	Gln	Ser	Gly	Ile	Leu	Thr	Leu	Asn	Pro	Val	Ala	
	1025					1030						1035			
Gln	Gly	Gln	Pro	Ile	Leu	Thr	Ser	Leu	Gly	Ser	Asn	Gln	Glu	Glu	
	1040					1045						1050			
Ala	Tyr	Val	Thr	Met	Ser	Ser	Phe	Tyr	Gln	Asn	Gln				
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<210> SEQ ID NO 101

<211> LENGTH: 851

<212> TYPE: PRT

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic polypeptide

<400> SEQUENCE: 101

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His	Ala	Ala	Arg	Pro	Asp	Ile	Glu	Leu	Thr	Gln	Ser	Pro	Ala	Ile	Met
			20						25				30		

Ser	Ala	Ser	Leu	Gly	Glu	Glu	Ile	Thr	Leu	Thr	Cys	Ser	Ala	Ser	Ser
		35					40					45			

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Ser	Val	Ser	Tyr	Met	His	Trp	Tyr	Gln	Gln	Lys	Ser	Gly	Thr	Ser	Pro	50	55	60
Lys	Leu	Leu	Ile	Tyr	Ser	Thr	Ser	Asn	Leu	Ala	Ser	Gly	Val	Pro	Ser	65	70	75
Arg	Phe	Ser	Gly	Ser	Gly	Ser	Gly	Thr	Phe	Tyr	Ser	Leu	Thr	Ile	Ser	85	90	95
Ser	Val	Glu	Ala	Glu	Asp	Ala	Ala	Asp	Tyr	Tyr	Cys	His	Gln	Trp	Ser	100	105	110
Ser	Tyr	Thr	Phe	Gly	Gly	Gly	Thr	Lys	Leu	Glu	Ile	Lys	Arg	Gly	Gly	115	120	125
Gly	Gly	Ser	Gly	Gly	Gly	Gly	Ser	Gly	Gly	Gly	Gly	Ser	Gln	Val	Lys	130	135	140
Leu	Gln	Glu	Ser	Gly	Gly	Gly	Leu	Val	Lys	Pro	Gly	Gly	Ser	Leu	Lys	145	150	155
Leu	Ser	Cys	Ala	Ala	Ser	Gly	Phe	Thr	Phe	Ser	Ser	Tyr	Ala	Met	Ser	165	170	175
Trp	Val	Arg	Gln	Thr	Pro	Glu	Lys	Arg	Leu	Glu	Trp	Val	Ala	Thr	Ile	180	185	190
Ser	Ser	Gly	Gly	Ser	Tyr	Thr	Tyr	Tyr	Pro	Asp	Ser	Val	Lys	Gly	Arg	195	200	205
Phe	Thr	Ile	Ser	Arg	Asp	Asn	Ala	Lys	Asn	Thr	Leu	Tyr	Leu	Gln	Met	210	215	220
Ser	Ser	Leu	Arg	Ser	Glu	Asp	Thr	Ala	Met	Tyr	Tyr	Cys	Ala	Arg	Gln	225	230	235
Asp	Gly	Tyr	Tyr	Pro	Gly	Trp	Phe	Ala	Asn	Trp	Gly	Gln	Gly	Thr	Thr	245	250	255
Val	Thr	Val	Ser	Ser	Ser	Gly	Thr	Thr	Thr	Pro	Ala	Pro	Arg	Pro	Pro	260	265	270
Thr	Pro	Ala	Pro	Thr	Ile	Ala	Ser	Gln	Pro	Leu	Ser	Leu	Arg	Pro	Glu	275	280	285
Ala	Cys	Arg	Pro	Ala	Ala	Gly	Gly	Ala	Val	His	Thr	Arg	Gly	Leu	Asp	290	295	300
Phe	Ala	Cys	Asp	Ile	Tyr	Ile	Trp	Ala	Pro	Leu	Ala	Gly	Thr	Cys	Gly	305	310	315
Val	Leu	Leu	Leu	Ser	Leu	Val	Ile	Thr	Leu	Tyr	Cys	Lys	Arg	Gly	Arg	325	330	335
Lys	Lys	Leu	Leu	Tyr	Ile	Phe	Lys	Gln	Pro	Phe	Met	Arg	Pro	Val	Gln	340	345	350
Thr	Thr	Gln	Glu	Glu	Asp	Gly	Cys	Ser	Cys	Arg	Phe	Pro	Glu	Glu	Glu	355	360	365
Glu	Gly	Gly	Cys	Glu	Leu	Arg	Val	Lys	Phe	Ser	Arg	Ser	Ala	Asp	Ala	370	375	380
Pro	Ala	Tyr	Lys	Gln	Gly	Gln	Asn	Gln	Leu	Tyr	Asn	Glu	Leu	Asn	Leu	385	390	395
Gly	Arg	Arg	Glu	Glu	Tyr	Asp	Val	Leu	Asp	Lys	Arg	Arg	Gly	Arg	Asp	405	410	415
Pro	Glu	Met	Gly	Gly	Lys	Pro	Arg	Arg	Lys	Asn	Pro	Gln	Glu	Gly	Leu	420	425	430
Tyr	Asn	Glu	Leu	Gln	Lys	Asp	Lys	Met	Ala	Glu	Ala	Tyr	Ser	Glu	Ile	435	440	445

[illegible]

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850

<210> SEQ ID NO 102
<211> LENGTH: 15
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic peptide

<400> SEQUENCE: 102

Glu Ala Ala Ala Lys Glu Ala Ala Ala Lys Glu Ala Ala Ala Lys
1 5 10 15

<210> SEQ ID NO 103
<211> LENGTH: 368
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic polypeptide

<400> SEQUENCE: 103

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Ala Phe Leu Leu Ile Pro Gln Leu Leu Phe Asn Lys Thr Lys Ser Val
20 25 30

Glu Phe Thr Phe Cys Asn Asp Thr Val Val Ile Pro Cys Phe Val Thr
35 40 45

Asn Met Glu Ala Gln Asn Thr Thr Glu Val Tyr Val Lys Trp Lys Phe
50 55 60

Lys Gly Arg Asp Ile Tyr Thr Phe Asp Gly Ala Leu Asn Lys Ser Thr
65 70 75 80

Val Pro Thr Asp Phe Ser Ser Ala Lys Ile Glu Val Ser Gln Leu Leu
85 90 95

Lys Gly Asp Ala Ser Leu Lys Met Asp Lys Ser Asp Ala Val Ser His
100 105 110

Thr Gly Asn Tyr Thr Cys Glu Val Thr Glu Leu Thr Arg Glu Gly Glu
115 120 125

Thr Ile Ile Glu Leu Lys Tyr Arg Val Val Ser Trp Phe Ser Pro Asn
130 135 140

Glu Pro Ile Leu Leu Thr Cys Pro Thr Ile Ser Ile Leu Ser Phe Phe
145 150 155 160

Ser Val Ala Leu Leu Val Ile Leu Ala Cys Val Leu Trp Lys Lys Arg
165 170 175

Ile Lys Pro Ile Val Trp Pro Ser Leu Pro Asp His Lys Lys Thr Leu
180 185 190

Glu His Leu Cys Lys Lys Pro Arg Lys Asn Leu Asn Val Ser Phe Asn
195 200 205

Pro Glu Ser Phe Leu Asp Cys Gln Ile His Arg Val Asp Asp Ile Gln
210 215 220

Ala Arg Asp Glu Val Glu Gly Phe Leu Gln Asp Thr Phe Pro Gln Gln
225 230 235 240

Leu Glu Glu Ser Glu Lys Gln Arg Leu Gly Gly Asp Val Gln Ser Pro
245 250 255

Asn Cys Pro Ser Glu Asp Val Val Ile Thr Pro Glu Ser Phe Gly Arg

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260							265					270				
Asp	Ser	Ser	Leu	Thr	Cys	Leu	Ala	Gly	Asn	Val	Ser	Ala	Cys	Asp	Ala	
	275						280					285				
Pro	Ile	Leu	Ser	Ser	Ser	Arg	Ser	Leu	Asp	Cys	Arg	Glu	Ser	Gly	Lys	
	290					295				300						
Asn	Gly	Pro	His	Val	Tyr	Gln	Asp	Leu	Leu	Leu	Ser	Leu	Gly	Thr	Thr	
305					310					315					320	
Asn	Ser	Thr	Leu	Pro	Pro	Pro	Phe	Ser	Leu	Gln	Ser	Gly	Ile	Leu	Thr	
			325						330					335		
Leu	Asn	Pro	Val	Ala	Gln	Gly	Gln	Pro	Ile	Leu	Thr	Ser	Leu	Gly	Ser	
		340						345					350			
Asn	Gln	Glu	Glu	Ala	Tyr	Val	Thr	Met	Ser	Ser	Phe	Tyr	Gln	Asn	Gln	
	355						360					365				

What is claimed is:

1. An engineered immune cell, comprising:

(i) one or more chimeric polypeptide receptors (CPRs) comprising a binding moiety, wherein the binding moiety comprises:

a first antigen binding domain exhibiting specific binding to an immune cell antigen, wherein, upon engaging the immune cell antigen with the first antigen binding domain, a subject's immune response toward the engineered immune cell is reduced; and a second antigen binding domain exhibiting specific binding to a disease-associated antigen, wherein the disease-associated antigen and the immune cell antigen are different,

wherein each CPR of the one or more CPRs further comprises a transmembrane domain and an intracellular signaling domain; and

(ii) a heterologous enhancer moiety capable of enhancing one or more activities of the engineered immune cell, wherein an endogenous T cell receptor (TCR) of the engineered immune cell is inactivated, and

wherein the engineered immune cell exhibits:

- (1) enhanced degree of persistence by remaining viable for at least about 20 days;
- (2) enhanced degree of expansion by at least about 10-fold within 15 days; and/or
- (3) enhanced cytotoxicity against a target cell comprising the disease-associated antigen, as compared to that of a control immune cell that comprises the one or more CPRs and does not comprise the heterologous enhancer moiety.

2. The engineered immune cell of claim 1, wherein the engineered immune cell exhibits two or more of (1) the enhanced degree of persistence, (2) the enhanced degree of expansion, and (3) the enhanced cytotoxicity against the target cell.

3. The engineered immune cell of claim 1, wherein (1), (2), and/or (3) is measured in absence of any exogenous enhancer moiety.

4. The engineered immune cell of claim 1, wherein the engineered immune cell exhibits (1) the enhanced degree of persistence and/or (2) the enhanced degree of expansion, in a presence of the disease-associated antigen.

5. The engineered immune cell of claim 1, wherein the engineered immune cell exhibits (1) the enhanced degree of

persistence and/or (2) the enhanced degree of expansion, as compared to that of the control immune cell without the heterologous enhancer moiety.

6. The engineered immune cell of claim 1, wherein the enhanced cytotoxicity against the target cell is measured in vivo.

7. The engineered immune cell of claim 1, wherein the heterologous enhancer moiety enhances a cytokine-associated signaling pathway in the engineered immune cell.

8. The engineered immune cell of claim 7, wherein the cytokine is selected from the group consisting of IL-2, IL-3, IL-4, IL-6, IL-7, IL-8, IL-10, IL-11, IL-12, IL-15, IL-17, IL-18, IL-21, IL-23, PD-1, PD-L1, CD122, CSF1R, CTAL-4, TIM-3, CCL21, CCL19, and TGF β .

9. The engineered immune cell of claim 7, wherein heterologous enhancer moiety comprises at least a portion of a receptor of the cytokine.

10. The engineered immune cell of claim 1, wherein the one or more CPRs are (a) one or more chimeric antigen receptors (CARs) or (b) one or more engineered T cell receptors (TCRs).

11. The engineered immune cell of claim 1, wherein an endogenous gene encoding the immune cell antigen is inactivated in the engineered immune cell.

12. The engineered immune cell of claim 1, wherein the engineered immune cell further exhibits enhanced expression of endogenous IL-2 and/or endogenous IFN- γ , as compared to that of the control immune cell without the heterologous enhancer moiety.

13. The engineered immune cell of claim 1, wherein a CPR of the one or more CPRs comprises both the first antigen binding domain and the second antigen binding domain.

14. The engineered immune cell of claim 1, wherein (a) a CPR of the one or more CPRs comprises the first antigen binding domain and does not comprise the second antigen binding domain and (b) an additional CPR of the one or more CPRs comprises the second antigen binding domain and does not comprise the first antigen binding domain.

15. The engineered immune cell of claim 1, wherein the immune cell is an NK cell, a T cell, a monocyte, a macrophage, or a granulocyte.

16. The engineered immune cell of claim 1, wherein the engineered immune cell is allogeneic to the subject.

17. The engineered immune cell of claim 1, wherein the immune cell antigen is selected from the group consisting of CD2, CD3, CD4, CD5, CD7, CD8, CD16a, CD16b, CD25, CD27, CD28, CD30, CD38, CD45, CD48, CD50, CD52, CD56, CD57, CD62L, CD69, CD94, CD100, CD102, CD122, CD127, CD132, CD137, CD160, CD161, CD178, CD218, CD226, CD244, CD159a (NKG2A), CD159c (NKG2C), NKG2E, CD279, CD314 (NKG2D), CD305, CD335 (NKP46), CD337, CD319 (CS1), TCR α , TCR β , and SLAMF7.

18. The engineered immune cell of claim 1, wherein the disease associated antigen is selected from the group consisting of CD2, CD3, CD4, CD5, CD7, CD8, CD19, CD20, CD22, CD25, CD28, CD30, CD33, CD38, CD40, CD44V6, CD47, CD52, CD56, CD57, CD58, CD79b, CD80, CD86, CD81, CD123, CD133, CD137, CD151, CD171, CD276, CLL1, B7H4, BCMA, VEGFR-2, EGFR, GPC3, PMSA, CEACAM6, c-Met, EGFRvIII, ErbB2/HER2, ErbB3, HER-2, HER3, ErbB4/HER-4, EphA2, IGF1R, GD2, O-acetyl GD2, O-acetyl GD3, GHRHR, GHR, Flt1, KDR, Flt4, Flt3, CEA, CA125, CTLA-4, GITR, BTLA, TGFBR1, TGFBR2, TGFBR1, IL6R, gp130, Lewis, TNFR1, TNFR2, PD1, PD-L1, PD-L2, PSMA, HVEM, MAGE-A, MSLN, NY-ESO-1, PSMA, RANK, ROR1, TNFRSF4, TWEAK-R, LTPR, LIFRP, LRP5, MUC1, MUC16, TCR α , TCR β , TLR7, TLR9, PTCH1, WT-1, Robo1, Frizzled, OX40, Notch-I-4, APRIL, CS1, MAGE3, Claudin 18.2, Folate receptor α , Folate receptor β , GPC2, CD70, BAFF-R, and TROP-2.

19. The engineered immune cell of claim 1, wherein the immune cell antigen and the disease-associated antigen are, respectively, (a) CD7 and CD19, (b) CD2 and CD19, or (c) TCR and CD19.

20. The engineered immune cell of claim 1, wherein an endogenous gene encoding a subunit of the endogenous TCR is modified, and wherein the gene is selected from the group consisting of TCR α , TCR β , CD3 ϵ , CD3 δ , CD3 γ , and CD3 ζ .

21. The engineered immune cell of claim 1, wherein the engineered immune cell is derived from an immune cell that is (a) obtained from peripheral blood or cord blood or (b) derived from a stem cell.

22. A method of treatment of a subject in need thereof, comprising administering to the subject a composition comprising an engineered immune cell, wherein the engineered immune cell comprises:

(i) one or more chimeric polypeptide receptors (CPRs) comprising a binding moiety, wherein the binding moiety comprises:

a first antigen binding domain exhibiting specific binding to an immune cell antigen, wherein, upon engaging the immune cell antigen with the first antigen binding domain, a subject's immune response toward the engineered immune cell is reduced, and a second antigen binding domain exhibiting specific binding to a disease-associated antigen, wherein the disease-associated antigen and the immune cell antigen are different,

wherein each CPR of the one or more CPRs further comprises a transmembrane domain and an intracellular signaling domain,

(ii) a heterologous enhancer moiety capable of enhancing one or more activities of the engineered immune cell, wherein an endogenous T cell receptor (TCR) of the engineered immune cell is inactivated, and

wherein the engineered immune cell exhibits

(1) enhanced degree of persistence by remaining viable for at least about 20 days;

(2) enhanced degree of expansion by at least about 10-fold within 15 days; and/or

(3) enhanced cytotoxicity against a target cell comprising the disease-associated antigen as compared to that of a control immune cell that comprises the one or more CPRs and does not comprise the heterologous enhancer moiety.

23. The method of claim 22, wherein the engineered immune cell exhibits two or more of (1) the enhanced degree of persistence, (2) the enhanced degree of expansion, and (3) the enhanced cytotoxicity against the target cell.

24. The method of claim 22, wherein (1), (2), and/or (3) is measured in absence of any exogenous enhancer moiety.

25. The method of claim 22, wherein the heterologous enhancer moiety enhances a cytokine-associated signaling pathway in the engineered immune cell.

26. The method of claim 22, wherein the immune cell antigen and the disease associated antigen are, respectively, (a) CD7 and CD19, (b) CD2 and CD19, or (c) TCR and CD19.

* * * * *