

Claims

WHAT IS CLAIMED:

1. A method of transducing T cells, the method comprising:
contacting a non-activated T cell with a lentiviral vector comprising a CD8 binding agent,
wherein the lentiviral vector transduces the non-activated T cell, wherein the T cell is a CD8+ T cell.
2. The method of claim 1, wherein the non-activated T cell is surface negative for one or more T cell activation markers selected from the group consisting of CD25, CD44 and CD69.
3. A method of transducing a population of T cells, the method comprising:
contacting a population of non-activated T cells with a composition comprising a lentiviral vector comprising a CD8 binding agent, wherein the population of non-activated T cells is transduced at an efficiency of at least 1%, at least 5%, at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, or at least 35%.
4. Use of a composition comprising a lentiviral vector comprising a CD8 binding agent in the manufacture of a medicament for treating a subject having a disease or condition, optionally a cancer, wherein the subject is not administered a T cell activating treatment (e.g. before, after or concurrently) with administration of the composition, wherein the lentiviral vector comprises a transgene encoding an engineered receptor that binds to or recognizes a protein or antigen expressed by or on cells associated with the disease or condition (e.g. tumor cells), optionally wherein the engineered receptor is a chimeric antigen receptor (CAR) or an engineered T cell receptor (TCR).
5. Use of a composition comprising a lentiviral vector comprising a CD8 binding agent in the manufacture of a medicament for expanding T cells capable of recognizing and killing tumor cells in a subject in need thereof, wherein the subject is not administered a T cell activating treatment (e.g. before, after, or concurrently) with administration of the composition, wherein the lentiviral vector comprises a transgene encoding an engineered receptor that binds to or recognizes a protein expressed on the tumor cells, optionally wherein the engineered receptor is a chimeric antigen receptor (CAR) or an engineered T cell receptor (TCR).

6. The method of any of claims 1-3 or the use of claim 4 or claim 5, wherein the lentiviral vector comprises a transgene encoding an engineered receptor that binds to or recognizes a protein or antigen expressed by or on cells associated with a disease or condition (e.g. tumor cells), wherein the engineered receptor is a chimeric antigen receptor (CAR).

7. The method or use of claim 6, wherein the CAR comprises an antigen-binding domain, a transmembrane domain, and an intracellular signaling domain comprising intracellular components of a CD3zeta signaling domain and a costimulatory signaling domain, and optionally, a hinge domain.

8. The method or use of claim 7, wherein the costimulatory signaling domain is a CD28 costimulatory domain, optionally wherein the CD28 costimulatory signaling domain comprises the amino acid sequence set forth in SEQ ID NO:98; the costimulatory signaling domain is a 4-1BB signaling domain, optionally wherein the 4-1BB signaling domain comprises the amino acid sequence set forth in SEQ ID NO:97; the CD3zeta signaling domain comprises the sequence set forth in SEQ ID NO:99 or SEQ ID NO:100; the transmembrane domain comprises the sequence set forth in any one of SEQ ID NOS:94, 95, and 96; and, optionally, the hinge domain comprises the sequence set forth in any one of SEQ ID NOS:88, 89, 90, 91, 92, 93, and 180.

9. The method or use of claim 7 or claim 8, wherein the antigen binding domain binds to an antigen selected from the group consisting of CD19, CD20, CD22, and BCMA.

10. The method or use of any of claims 7-9, wherein the antigen binding domain binds to CD19, wherein the antigen binding domain comprises:

(a) a CDR-H1, a CDRH-2, and a CDR-H3 comprising the amino acid sequence set forth in SEQ ID NO: 108, 109, and 110, respectively, and a CDR-L1, a CDR-L2, and a CDR-L3 comprising the amino acid sequence set forth in SEQ ID NO: 103, 104, and 105, respectively;

(b) a VH region comprising the amino acid sequence set forth in SEQ ID NO:107, and a VL region comprising the amino acid sequence set forth in SEQ ID NO:102; and/or

(c) the amino acid sequence set forth in SEQ ID NO:101 or 111.

11. The method or use of any one of claims 7-10, wherein the CAR comprises the amino acid sequence set forth in SEQ ID NO:113, 115, 117, or 119 and/or an amino acid sequence encoded by the polynucleotide sequence set forth in SEQ ID NO:112, 114, 116, or 118.

12. The method of any of claims 1-3 and 6-11, wherein the non-activated T cell is in vitro or the non-activated T cell is ex vivo from a subject.

13. The method of any of claims 1-3 and 6-12, wherein, prior to contacting, the non-activated T cell or the population of non-activated T cells has not been treated with: an anti-CD3 antibody (e.g., OKT3), an anti-CD28 antibody (e.g., CD28.2); a bead coupled to an anti-CD3 antibody (e.g. OKT3) and an anti-CD28 antibody (e.g. CD28.2), optionally wherein the bead is a superparamagnetic bead; a T cell activating cytokine (e.g., recombinant IL-2, IL-7, IL-15, IL-21, or combinations thereof), optionally wherein the T cell activating cytokine is a human cytokine; and/or a soluble T cell costimulatory molecule (e.g. anti-CD28 antibody or soluble CD80, soluble CD86, soluble CD137L or soluble ICOS-L).

14. The method of any one of claims 1-3 and 6-13, wherein the non-activated T cell or the population of non-activated T cells is ex vivo from a subject, the population of non-activated T cells comprise peripheral blood mononuclear cells (PBMCs) or a subset thereof comprising CD8+ T cells, the population of non-activated cells is an enriched population of T cells selected from a biological sample from a subject, optionally wherein the T cells are selected for T cells surface positive for a T cell marker (e.g., CD3 or CD8).

15. The method of any of claims 3 and 6-14, further comprising one or more of:
expanding the population of transduced T cells, wherein the expanding comprises incubation of the transduced cells with one or more T cell activating cytokine (e.g., recombinant IL-2, IL-7, IL-15, IL-21, or combinations thereof), optionally wherein the T cell activating cytokine is a human cytokine, and
incubating the transduced T cells with one or more T cell activating cytokine (e.g., recombinant IL-2, IL-7, IL-15, IL-21, or combinations thereof), optionally wherein the T cell activating cytokine is a human cytokine.

16. The use of any of claims 4-11, wherein the T cell activating treatment comprises administration of an anti-CD3 antibody (e.g., OKT3, a soluble T cell costimulatory molecule (e.g., anti-CD28 antibody, or a recombinant CD80, CD86, CD137L, ICOS-L, a T cell activating cytokine (e.g., recombinant IL-2, IL-7, IL-15, IL-21), optionally wherein the T cell activating cytokine is a human cytokine, recombinant IL-7, optionally human IL-7 and/or a lymphodepleting therapy, optionally administration of cyclophosphamide and/or fludarabine.

17. A composition comprising a lentiviral vector comprising a CD8 binding agent for use in expanding T cells capable of recognizing and killing tumor cells in a subject in need thereof.

18. The method of any of claims 1-3 and 6-15, the use of any of claims 4-11 and 16, or the composition of claim 17, wherein the CD8 binding agent is an anti-CD8 antibody or an antigen-binding fragment, wherein the anti-CD8 antibody or antigen-binding fragment is characterized by one or more of the following: the anti-CD8 antibody or antigen-binding fragment is mouse, rabbit, human, or humanized, the anti-CD8 antibody or antigen-binding fragment comprises a single chain variable fragment (scFv), the anti-CD8 antibody or antigen-binding fragment is a single domain antibody, the anti-CD8 antibody or antigen-binding fragment is a camelid (e.g. llama, alpaca, camel) anti-CD8 antibody or antigen-binding fragment (e.g. a VHH).

19. The method, the use, or the composition of claim 18, wherein the anti-CD8 antibody or antigen-binding fragment comprises one or more of the following: is capable of binding to a CD8 alpha chain and/or a CD8 beta chain; is exposed on the surface of the lentiviral vector; is fused to a transmembrane domain incorporated in the viral envelope.

20. The method of any of claims 1-3, 6-15, 18, and 19, the use of any of claims 4-11, 16, 18, and 19, or the composition of any one of claims 17-19, wherein the lentiviral vector is pseudotyped with a viral fusion protein, wherein the viral fusion protein is a Paramyxoviridae fusion protein (e.g., a Morbillivirus or a Henipavirus) or a functional variant thereof; a Morbillivirus fusion protein (e.g., measles virus (MeV), canine distemper virus, Cetacean morbillivirus, Peste-des-petits-ruminants virus, Phocine distemper virus, Rinderpest virus), or a functional variant thereof; or a Henipavirus fusion protein (e.g., Nipah virus, Hendra virus, Cedar virus, Kumasi virus, Mòjiāng virus) or a functional variant thereof.

21. The method, the use, or the composition of claim 20, wherein the viral fusion protein comprises a Nipah virus F glycoprotein (NiV-F) or a biologically active portion thereof and a Nipah virus G glycoprotein (NiV-G) or a biologically active portion thereof, and wherein the CD8 binding agent is fused to the NiV-G or the biologically active portion thereof, optionally wherein the CD8 binding agent is fused to the C-terminus of the Nipah virus G glycoprotein or the biologically active portion thereof, wherein the CD8 binding protein is fused to the viral fusion protein directly or via a peptide linker.

22. The method, the use, or the composition of claim 21, wherein the NiV-G or the biologically active portion thereof is a wild-type NiV-G protein or a functionally active variant or biologically active portion thereof, wherein the NiV-G protein or the biologically active portion is truncated and lacks up to 40 contiguous amino acid residues at or near the N-terminus of the wild-type NiV-G protein, optionally wherein the NiV-G protein or the biologically active portion has: a 5 amino acid truncation at or near the N-terminus of the wild-type NiV-G protein, optionally wherein the NiV-G protein or the biologically active portion thereof has the amino acid sequence set forth in SEQ ID NO:12, or a sequence of amino acids that exhibits at least at or about 80%, 85%, 90% or 95% sequence identity to the sequence set forth in SEQ ID NO:12; a 10 amino acid truncation at or near the N-terminus of the wild-type NiV-G protein, optionally wherein the NiV-G protein or the biologically active portion thereof has the amino acid sequence set forth in SEQ ID NO:44, or a sequence of amino acids that exhibits at least at or about 80%, 85%, 90% or 95% sequence identity to the sequence set forth in SEQ ID NO:44; a 15 amino acid truncation at or near the N-terminus of the wild-type NiV-G protein, optionally wherein the NiV-G protein or the biologically active portion thereof has the amino acid sequence set forth in SEQ ID NO:45, or a sequence of amino acids that exhibits at least at or about 80%, 85%, 90% or 95% sequence identity to the sequence set forth in SEQ ID NO:45; a 20 amino acid truncation at or near the N-terminus of the wild-type NiV-G protein, optionally wherein the NiV-G protein or the biologically active portion thereof has the amino acid sequence set forth in SEQ ID NO:13, or a sequence of amino acids that exhibits at least at or about 80%, 85%, 90% or 95% sequence identity to the sequence set forth in SEQ ID NO:13; a 25 amino acid truncation at or near the N-terminus of the wild-type NiV-G protein, optionally wherein the NiV-G protein or the biologically active portion thereof has the amino acid sequence set forth in SEQ ID NO:14, or a sequence of amino acids that exhibits at least at or about 80%, 85%, 90% or 95% sequence identity to the sequence set forth in SEQ ID NO: 14; a 30 amino acid truncation at or near the N-terminus of the wild-type NiV-G protein, optionally wherein the NiV-G protein or the biologically active portion thereof has the amino acid sequence set forth in SEQ ID NO:43, or a sequence of amino acids that exhibits at least at or about 80%, 85%, 90% or 95% sequence identity to the sequence set forth in SEQ ID NO:43; or a 34 amino acid truncation at or near the N-terminus of the wild-type NiV-G protein, optionally wherein the NiV-G protein or the biologically active portion thereof has the amino acid sequence set forth in SEQ ID NO:42, or a sequence of amino acids that exhibits at least at or about 80%, 85%, 90% or 95% sequence identity to the sequence set forth in SEQ ID NO:42.

23. The method, the use, or the composition of claim 21 or claim 22, wherein the NiV-G-protein or the biologically active portion thereof is a mutant NiV-G protein that exhibits reduced

binding to Ephrin B2 or Ephrin B3, optionally wherein the mutant NiV-G protein or the biologically active portion comprises one or more amino acid substitutions corresponding to amino acid substitutions selected from the group consisting of E501A, W504A, Q530A and E533A with reference to numbering set forth in SEQ ID NO:4.

24. The method, the use, or the composition of any of claims 21-23, wherein (i) the NiV-F protein or the biologically active portion thereof is a wild-type NiV-F protein or is a functionally active variant or a biologically active portion thereof, (ii) the NiV-F protein or the biologically active portion thereof has a 20 amino acid truncation at or near the C-terminus of the wild-type NiV-F protein, optionally wherein the NiV-F protein or the biologically active portion thereof has the sequence set forth in SEQ ID NO: 20 or a sequence of amino acids that exhibits at least at or about 80%, 85%, 90% or 95% sequence identity to the sequence set forth in SEQ ID NO: 20, or (iii) the NiV-F protein or the biologically active portion thereof comprises:

- i) a 20 amino acid truncation at or near the C-terminus of the wild-type NiV-F protein; and
- ii) a point mutation on an N-linked glycosylation site,

optionally wherein the NiV-F protein or the biologically active portion thereof has the sequence set forth in SEQ ID NO: 15, or a sequence of amino acids that exhibits at least at or about 80%, 85%, 90% or 95% sequence identity to the sequence set forth in SEQ ID NO: 15.

25. The method of any of claims 1-3, 6-15, and 18-24, the use of any of claims 4-11, 16, and 18-24, or the composition of any one of claims 17-24, wherein the lentiviral vector does not comprise or encode a T cell activating agent, optionally wherein the T cell activating agent is a lymphoproliferative agent, optionally wherein the T cell activating agent is:

- a polypeptide capable of binding CD3 and/or CD28;
- a CD3 antibody (e.g. anti-CD3 scFv); a T cell activating cytokine (e.g. IL-2, IL-7, IL-15 or IL-21); or a T cell costimulatory molecule (e.g. anti-CD28 antibody, CD80, CD86, CD137L or ICOS-L);
- a cytokine or a cytokine receptor or a signaling domain thereof that activates a STAT3 pathway, a STAT4 pathway, and/or a Jak/STAT5 pathway;
- a T cell survival motif, optionally an IL-7 receptor, an IL-15 receptor, or CD28, or a functional portion thereof; and/or
- a microRNA (miRNA) or short hairpin RNA (shrRNA), wherein the miRNA or the shrRNA stimulates the STAT5 pathway and/or inhibits the SOCS pathway.

26. The use of any of claims 4-11, 16, and 18-25, wherein the subject is not administered a T cell activating treatment concurrently with the lentiviral vector, the subject is not administered a T cell activating treatment within 1 month before the contacting with the lentiviral vector or before the administration of the composition comprising the lentiviral vectors, the subject is not administered a T cell activating treatment within or at or about 1 week, 2 weeks, 3 weeks or 4 weeks, optionally at or about 1, 2, 3, 4, 5, 6 or 7 days, before the contacting with the lentiviral vector or before the administration of the composition comprising the lentiviral vectors, the subject is not administered a T cell activating treatment within 1 month after the contacting with the lentiviral vector or after the administration of the composition comprising the lentiviral vectors, and/or the subject is not administered a T cell activating treatment within or at or about 1 week, 2 weeks, 3 weeks or 4 weeks, optionally at or about 1, 2, 3, 4, 5, 6 or 7 days, after the contacting with the lentiviral vector or after the administration of the composition comprising the lentiviral vectors.

27. The method of any of claims 1-3, 6-15, and 18-25, further comprising editing the T cell or the population of T cells to inactivate one or more of *B2M*, *CIITA*, *TRAC*, and *TRB* genes.

28. The method of claim 27, further comprising inserting a gene encoding CD47 into the T cell or the population of T cells at a defined locus, wherein the defined locus is selected from the group consisting of a *B2M* locus, a *CIITA* locus, a *TRAC* locus, a *TRB* locus, or a safe harbor locus, wherein the safe harbor locus is selected from the group consisting of an *AAVS1* locus, a *CCR5* locus, and a *ROSA26* locus.

29. The method of any of claims 1-3, 6-15, 18-25, 27, and 28, or the use of any one of claims 4-11, 16, and 18-26, wherein the contacting or the administration is carried out by ex vivo administration of the lentiviral vector to a subject using a closed fluid circuit, wherein the ex vivo administration comprises:

- (a) obtaining whole blood from a subject;
- (b) collecting the fraction of blood containing leukocyte components comprising T cells (e.g. CD8+ T cells);
- (c) contacting the leukocyte components comprising T cells (e.g. CD8+ T cells) with a composition comprising the lentiviral vector; and
- (d) reinfusing the contacted leukocyte components comprising T cells (e.g. CD8+ T cells) into the subject, wherein steps (a)-(d) are performed in-line in a closed fluid circuit.

30. The method or use of claim 29, wherein the contacting in step (c) is for no more than 24 hours, no more than 18 hours, no more than 12 hours, or no more than 6 hours.

31. A composition comprising a population of transduced T cells produced by the method of any of claims 3, 6-15, 18-25, and 27-30, optionally wherein the composition is a pharmaceutical composition.