

On one aspect, the present disclosure refers to a chimeric antigen receptor (CAR) comprising an extracellular antigen-binding domain, a transmembrane domain, and an intracellular signaling domain comprising a modified human CD3 ζ polypeptide, wherein the modified human CD3 ζ polypeptide comprises a deletion of ITAM1 and a deletion of ITAM2.

5 On another aspect, the present disclosure refers to an immunoresponsive cell comprising a CAR as described herein, wherein the cell is selected from the group consisting of a T cell, a Natural Killer (NK) cell, a cytotoxic T lymphocyte (CTL), a regulatory T cell, a Natural Killer T (NKT) cell, and a lymphoid cell differentiated from a myeloid cell, an embryonic stem cell, or a pluripotent stem cell.

10 On another aspect, the present disclosure refers to a pharmaceutical composition comprising an effective amount of an immunoresponsive cell as described herein and a pharmaceutically acceptable excipient.

On another aspect, the present disclosure refers to use of the immunoresponsive cell as described herein or the pharmaceutical composition as described herein in the manufacture of a pharmaceutical composition for reducing tumor burden in a subject, or in treating a neoplasm in
15 a subject.

On another aspect, the present disclosure refers to use of the immunoresponsive cell as described herein or a pharmaceutical composition as described herein in the manufacture of a pharmaceutical composition for treating a subject having a relapse of a neoplasm, wherein the
20 subject has a relapse of a disease, wherein the subject received treatment which leads to residual tumor cells, or the subject received an immunoresponsive cell comprising an antigen recognizing receptor that comprises a 4-1BB costimulatory signal.

On another aspect, the present disclosure refers to a method for producing an antigen-specific immunoresponsive cell, wherein the cell is selected from the group consisting of a T
25 cell, a Natural Killer (NK) cell, a cytotoxic T lymphocyte (CTL), a regulatory T cell, a Natural Killer T (NKT) cell, and a lymphoid cell differentiated from a myeloid cell, an embryonic stem cell, or a pluripotent stem cell, the method comprising introducing into the immunoresponsive cell a nucleic acid sequence encoding a CAR as described herein.

On another aspect, the present disclosure refers to a nucleic acid molecule encoding the
30 CAR as described herein.

On another aspect, the present disclosure refers to a vector comprising the nucleic acid molecule as described herein.

On another aspect, the present disclosure refers to a kit comprising a CAR as described herein, an immunoresponsive cell as described herein, a pharmaceutical composition as
35 described herein, a nucleic acid molecule as described herein, or a vector as described herein.

On another aspect, the present disclosure refers to the immunoresponsive cell as described herein or the pharmaceutical composition as described herein for use in therapy.

What is claimed is:

1. A chimeric antigen receptor (CAR) comprising an extracellular antigen-binding domain, a transmembrane domain, and an intracellular signaling domain comprising a modified human CD3 ζ polypeptide, wherein the modified human CD3 ζ polypeptide comprises a deletion of ITAM1 and a deletion of ITAM2.
2. The CAR of claim 1, wherein the modified human CD3 ζ polypeptide lacks all or part of basic-rich stretch (BRS) regions, wherein the BRS regions are BRS1, BRS2, and BRS3.
3. The CAR of claim 2, wherein the modified human CD3 ζ polypeptide
 - a) lacks BRS2 or a portion thereof;
 - b) lacks BRS1 or a portion thereof; and/or
 - c) lacks BRS3 or a portion thereof.
4. The CAR of claim 2, wherein the modified human CD3 ζ polypeptide
 - i) lacks BRS1 or a portion thereof, lacks BRS2 or a portion thereof, and lacks BRS3 or a portion thereof;
 - ii) lacks BRS2 or a portion thereof and lacks BRS3 or a portion thereof; or
 - iii) lacks BRS1 or a portion thereof and lacks BRS3 or a portion thereof;
 - iv) lacks BRS1 or a portion thereof and lacks BRS2 or a portion thereof.
5. The CAR of claim 1, wherein the modified human CD3 ζ polypeptide lacks ITAM1, ITAM2, BRS1, BRS2, and BRS3.
6. The CAR of claim 1, wherein the modified human CD3 ζ polypeptide comprises
 - a) a deletion of BRS2 or a portion thereof;
 - b) a deletion of BRS1 or a portion thereof; and/or
 - c) a deletion of BRS3 or a portion thereof.
7. The CAR of claim 6, wherein the modified human CD3 ζ polypeptide comprises
 - i) a deletion of BRS1 or a portion thereof, a deletion of BRS2 or a portion thereof, and a deletion of BRS3 or a portion thereof;
 - ii) a deletion of BRS2 or a portion thereof and a deletion of BRS3 or a portion thereof;

- iii) a deletion of BRS1 or a portion thereof, a deletion of BRS3 or a portion thereof; or
 - iv) a deletion of BRS1 or a portion thereof, a deletion of BRS2 or a portion thereof.
8. The CAR of claim 1, wherein the modified human CD3 ζ polypeptide comprises a deletion of ITAM1 or a portion thereof, a deletion of ITAM2 or a portion thereof, a deletion of BRS1 or a portion thereof, a deletion of BRS2 or a portion thereof, and a deletion of BRS3 or a portion thereof.
9. The CAR of claim 1, wherein the modified human CD3 ζ polypeptide consists of a deletion of ITAM1 or a portion thereof, a deletion of ITAM2 or a portion thereof, a deletion of BRS1 or a portion thereof, a deletion of BRS2 or a portion thereof, and a deletion of BRS3 or a portion thereof.
10. The CAR of claim 9, wherein the modified CD3 ζ polypeptide comprises or consists of amino acids 374 to 416 of SEQ ID NO: 45.
11. The CAR of claim 1, wherein the CAR comprises the amino acid sequence set forth in SEQ ID NO: 45 or SEQ ID NO: 47.
12. The CAR of any one of claims 1-11, further comprising a hinge/spacer region, wherein the hinge/spacer region comprises a CD8 polypeptide, a CD28 polypeptide, a CD3 ζ polypeptide, a CD4 polypeptide, a 4-1BB polypeptide, an OX40 polypeptide, a CD166 polypeptide, a CD166 polypeptide, a CD8a polypeptide, a CD8b polypeptide, an ICOS polypeptide, an ICAM-1 polypeptide, a CTLA-4 polypeptide, a CD27 polypeptide, a CD40/My88 peptide, a NKGD2 peptide, or a combination thereof.
13. The CAR of claim 12, wherein the hinge/spacer region comprises a CD166 polypeptide.
14. The CAR of claim 13, wherein the CD166 polypeptide consists of amino acids 489 to 527 of SEQ ID NO: 3.
15. The CAR of claim 12, wherein the hinge/spacer region comprises a CD28 polypeptide.
16. The CAR of claim 15, wherein the CD28 polypeptide consists of the amino acid set forth in SEQ ID NO: 15.

17. The CAR any one of claims 1-16, wherein the transmembrane domain comprises a CD8 polypeptide, a CD28 polypeptide, a CD3 ζ polypeptide, a CD4 polypeptide, a 4-1BB polypeptide, an OX40 polypeptide, a CD166 polypeptide, a CD166 polypeptide, a CD8a polypeptide, a CD8b polypeptide, an ICOS polypeptide, an ICAM-1 polypeptide, a CTLA-4 polypeptide, a CD27 polypeptide, a CD40/My88 peptide, a NKGD2 peptide, or a combination thereof.
18. The CAR of claim 17, wherein the transmembrane domain comprises a CD166 polypeptide.
19. The CAR of claim 18, wherein the CD166 polypeptide comprises amino acid 528 to 553 of SEQ ID NO: 3.
20. The CAR of any one of claims 12-19, wherein the transmembrane domain and the hinge/spacer region are derived from the same molecule.
21. The CAR of any one of claims 12-19, wherein
- a) the hinge/spacer region comprises a CD28 polypeptide and the transmembrane domain comprises a CD28 polypeptide;
 - b) the hinge/spacer region comprises a CD84 polypeptide and the transmembrane domain comprises a CD84 polypeptide; or
 - c) the hinge/spacer region comprises a CD166 polypeptide and the transmembrane domain comprises a CD166 polypeptide;
 - d) the hinge/spacer region comprises a CD8a polypeptide and the transmembrane domain comprises a CD8a polypeptide; or
 - e) the hinge/spacer region comprises a CD8b polypeptide and the transmembrane domain comprises a CD8b polypeptide.
22. The CAR of claim 21, wherein the CAR comprises amino acids 489 to 553 of SEQ ID NO: 3 or the amino acid sequence set forth in SEQ ID NO: 792.
23. The CAR of any one of claims 12-19, wherein the transmembrane domain and the hinge/spacer region are derived from different molecules.

24. The CAR of claim 23, wherein the hinge/spacer region comprises a CD28 polypeptide and the transmembrane domain comprises an ICOS polypeptide.
25. The CAR of any one of claims 1-24, wherein the intracellular signaling domain further comprises a co-stimulatory signaling domain.
26. The CAR of claim 25, wherein the co-stimulatory signaling domain comprises a CD28 polypeptide.
27. An immunoresponsive cell comprising a CAR of any one of claims 1-26, wherein the cell is selected from the group consisting of a T cell, a Natural Killer (NK) cell, a cytotoxic T lymphocyte (CTL), a regulatory T cell, a Natural Killer T (NKT) cell, and a lymphoid cell differentiated from a myeloid cell, an embryonic stem cell, or a pluripotent stem cell.
28. The immunoresponsive cell of claim 27, wherein the CAR is recombinantly expressed, and/or expressed from a vector.
29. The immunoresponsive cell of claim 27 or 28, wherein the CAR is placed at an endogenous gene locus of the immunoresponsive cell.
30. The immunoresponsive cell of claim 29, wherein the endogenous gene locus is a TRAC locus, a TRBC locus or a TRGC locus.
31. The immunoresponsive cell of claim 30, wherein the endogenous gene locus is a TRAC locus.
32. The immunoresponsive cell of any one of claims 29-31, wherein the placement of the CAR disrupts or abolishes the endogenous expression of a TCR.
33. The immunoresponsive cell of any one of claims 26-32, wherein the cell is a T cell.
34. The immunoresponsive cell of any one of claims 27-33, wherein the antigen is a tumor antigen.
35. The immunoresponsive cell of claim 34, wherein the tumor antigen is selected from the group consisting of CD19, MUC16, MUC1, CAIX, CEA, CD8, CD7, CD10, CD20, CD22,

CD30, CLL1, CD33, CD34, CD38, CD41, CD44, CD49f, CD56, CD74, CD133, CD138, EGP-2, EGP-40, EpCAM, erb-B2,3,4, FBP, Fetal acetylcholine receptor, folate receptor-a, GD2, GD3, HER-2, hTERT, IL-13R-a2, K-light chain, KDR, LeY, L1 cell adhesion molecule, MAGE-A1, Mesothelin, ERBB2, MAGEA3, p53, MART1, GP100, Proteinase3 (PR1), Tyrosinase, Survivin, hTERT, EphA2, NKG2D ligands, NY-ESO-1, oncofetal antigen (h5T4), PSCA, PSMA, ROR1, TAG-72, VEGF-R2, WT-1, BCMA, CD123, CD44V6, NKCS1, EGF1R, EGFR-VIII, CD99, CD70, ADGRE2, CCR1, LILRB2, PRAME, CCR4, CD5, CD3, TRBC1, TRBC2, TIM-3, Integrin B7, ICAM-1, CD70, Tim3, CLEC12A and ERBB.

36. The immunoresponsive cell of claim 35, wherein the antigen is CD19.
37. A pharmaceutical composition comprising an effective amount of an immunoresponsive cell of any one of claims 27-36 and a pharmaceutically acceptable excipient.
38. The pharmaceutical composition of claim 37, which is for treating a neoplasm.
39. Use of the immunoresponsive cell of any one of claims 27-36 or the pharmaceutical composition of claim 37 or 38 in the manufacture of a pharmaceutical composition for reducing tumor burden in a subject, or in treating a neoplasm in a subject.
40. The use of claim 39, wherein the neoplasm or tumor is selected from the group consisting of blood cancer, B cell leukemia, multiple myeloma, acute lymphoblastic leukemia (ALL), chronic lymphocytic leukemia, non-Hodgkin's lymphoma, and ovarian cancer.
41. The use of claim 39 or 40, wherein
 - a) the tumor or neoplasm is B cell leukemia, multiple myeloma, acute lymphoblastic leukemia (ALL), chronic lymphocytic leukemia, or non-Hodgkin's lymphoma, and the CAR binds to CD19; or
 - b) the tumor or neoplasm is B cell leukemia, multiple myeloma, acute lymphoblastic leukemia (ALL), chronic lymphocytic leukemia, or non-Hodgkin's lymphoma, and the CAR binds to BCMA, ADGRE2, MSLN, PSMA or combinations thereof.
42. Use of the immunoresponsive cell of any one of claims 27-36 or the pharmaceutical composition of claim 37 or 38 in the manufacture of a pharmaceutical composition for treating a

subject having a relapse of a neoplasm, wherein the subject has a relapse of a disease, wherein the subject received treatment which leads to residual tumor cells, or the subject received an immunoresponsive cell comprising an antigen recognizing receptor that comprises a 4-1BB costimulatory signal.

43. The use of claim 42, wherein the neoplasm is selected from the group consisting of blood cancer, B cell leukemia, multiple myeloma, acute lymphoblastic leukemia (ALL), chronic lymphocytic leukemia and non-Hodgkin's lymphoma.

44. The use of claim 42 or 43, wherein the neoplasm is B cell leukemia, multiple myeloma, acute lymphoblastic leukemia (ALL), chronic lymphocytic leukemia, or non-Hodgkin's lymphoma, and the CAR binds to CD19.

45. The use of any one of claims 42-44, wherein the neoplasm is CD19+ ALL.

46. The use of any one of claims 42-45, wherein the neoplasm comprises a tumor cell having a low density of a tumor specific antigen on the surface of the tumor cell.

47. A method for producing an antigen-specific immunoresponsive cell, wherein the cell is selected from the group consisting of a T cell, a Natural Killer (NK) cell, a cytotoxic T lymphocyte (CTL), a regulatory T cell, a Natural Killer T (NKT) cell, and a lymphoid cell differentiated from a myeloid cell, an embryonic stem cell, or a pluripotent stem cell, the method comprising introducing into the immunoresponsive cell a nucleic acid sequence encoding a CAR of any one of claims 1-26.

48. A nucleic acid molecule encoding the CAR of any one of claims 1-26.

49. The nucleic acid molecule of claim 49, comprising the nucleotide sequence set forth in SEQ ID NO: 45 or SEQ ID NO: 47.

50. A vector comprising the nucleic acid molecule of claim 49 or 50.

51. The vector of claim 51, wherein the vector is a retroviral vector.

52. A kit comprising a CAR of any one of claims 1-26, an immunoresponsive cell of any one of claims 27-36, a pharmaceutical composition of claim 37 or 38, a nucleic acid molecule of claim 48 or 49, or a vector of claim 50 or 51.

53. The immunoresponsive cell of any one of claims 27-36 or the pharmaceutical composition of claim 37 or 38 for use in therapy.