

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 or a portion thereof and a deletion of ITAM2 or a portion thereof~~lacks all or part of immunoreceptor tyrosine-based activation motifs (ITAMs), wherein the ITAMs are ITAM1, ITAM2, and ITAM3.~~

~~On another 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 CD3 ζ polypeptide, wherein the modified CD3 ζ polypeptide lacks all or part of basic-rich stretch (BRS) regions, wherein the BRS regions are BRS1, BRS2, and BRS3.~~

~~On another 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 CD3 ζ polypeptide, wherein the modified CD3 ζ polypeptide comprises a BRS variant selected from a BRS1 variant, a BRS2 variant, and a BRS3 variant, wherein the BRS variant comprises one or more loss-of-function mutations.~~

~~On another aspect, the present disclosure refers to a chimeric antigen receptor (CAR) comprising an extracellular antigen-binding domain, a hinge/spacer region, a transmembrane domain, and an intracellular signaling domain comprising a modified CD3 ζ polypeptide, wherein the modified CD3 ζ polypeptide comprises one or more ITAM variant comprising one or more loss-of-function mutations, wherein each of the one or more ITAM variant is independently selected from the group consisting of an ITAM1 variant, an ITAM2 variant, and an ITAM3 variant, and 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.~~

On another aspect, the present disclosure refers to an immunoresponsive cell comprising a CAR as described herein.

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 and/or preventing a neoplasm in a subject.

~~On another aspect, the present disclosure refers to use of an immunoresponsive cell or a pharmaceutical composition comprising thereof in the manufacture of a pharmaceutical composition for reducing tumor burden in a subject or in treating and/or preventing a neoplasm in a subject, wherein the immunoresponsive cells comprises: a chimeric antigen receptor (CAR) comprising an extracellular antigen binding domain, a transmembrane domain, and an intracellular signaling domain comprising a modified CD3 ζ polypeptide, wherein the modified CD3 ζ polypeptide comprises one or more ITAM variant comprising one or more loss of function mutations, wherein each of the one or more ITAM variant is independently selected from the group consisting of an ITAM1 variant, an ITAM2 variant, and an ITAM3 variant.~~ On another aspect, the present disclosure refers to use of thean

immunoresponsive cell as described herein or a pharmaceutical composition as described herein ~~comprising thereof~~ 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, ~~wherein the immunoresponsive cells comprises:~~

~~—— a chimeric antigen receptor (CAR) comprising an extracellular antigen binding domain, a transmembrane domain, and an intracellular signaling domain comprising a modified CD3 ζ polypeptide, wherein the modified CD3 ζ polypeptide comprises one or more ITAM variant comprising one or more loss of function mutations, wherein each of the one or more ITAM variant is independently selected from the group consisting of an ITAM1 variant, an ITAM2 variant, and an ITAM3 variant.~~

On another aspect, the present disclosure refers to a method for producing an antigen-specific immunoresponsive cell, the method comprising introducing into an 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 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 CARas descrbied herein, an immunoresponsive cell as descrbied herein, a pharmaceutical

composition as described herein, a nucleic acid molecule as described herein, or a vector as described herein.

On another aspect, the present disclosure refers to an immunoresponsive cell comprising a) a first CAR comprising a first extracellular antigen-binding domain that binds to a first antigen, a first transmembrane domain, and a first intracellular signaling domain; and b) a second CAR comprising a second extracellular antigen-binding domain that binds to a second antigen, a second transmembrane domain, and a second intracellular signaling domain, wherein the first CAR is a CAR as described herein or the first intracellular signaling domain comprises a modified CD3 ζ polypeptide that comprises one or more ITAM variant comprising one or more loss-of-function mutations, wherein each of the one or more ITAM variant is independently selected from the group consisting of an ITAM1 variant, an ITAM2 variant, and an ITAM3 variant.

On another aspect, the present disclosure refers to a pharmaceutical composition comprising an effective amount of the immunoresponsive cells 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, treating and/or preventing a neoplasm, and/or treating a subject having a relapse of a neoplasm.

On yet another aspect, the present disclosure refers to use of the immunoresponsive cell of any one of claims 72-77 or the pharmaceutical composition of claim 78 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. 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.

On another aspect, the present disclosure refers to an immunoresponsive cell or a pharmaceutical composition comprising thereof for use in therapy, wherein the immunoresponsive cells comprises: a chimeric antigen receptor (CAR) comprising an extracellular antigen-binding domain, a transmembrane domain, and an intracellular signaling domain comprising a modified CD3 ζ polypeptide, wherein the modified CD3 ζ polypeptide comprises one or more ITAM variant comprising one or more loss-of-function mutations, wherein each of the one or more ITAM variant is independently selected from the group consisting of an ITAM1 variant, an ITAM2 variant, and an ITAM3 variant.

On yet another aspect, the present disclosure refers to an immunoresponsive cell or a pharmaceutical composition comprising thereof for use in therapy, wherein the subject received an immunoresponsive cell comprising an antigen recognizing receptor comprises a 4-1BB costimulatory signal, wherein the immunoresponsive cells comprises: a chimeric antigen receptor (CAR) comprising an extracellular antigen binding domain, a transmembrane domain, and an intracellular signaling domain comprising a modified CD3 ζ polypeptide, wherein the modified CD3 ζ polypeptide comprises one or more ITAM variant comprising one or more loss of function mutations, wherein each of the one or more ITAM variant is independently selected from the group consisting of an ITAM1 variant, an ITAM2 variant, and an ITAM3 variant.

On yet 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 or a portion thereof and a deletion of ITAM2 or a portion thereof~~lacks all or part of immunoreceptor tyrosine-based activation motifs (ITAMs), wherein the ITAMs are ITAM1, ITAM2, and ITAM3.~~

~~2. The CAR of claim 1, wherein the modified CD3 ζ polypeptide~~

~~—— a) lacks ITAM2 or a portion thereof;~~

~~—— b) lacks ITAM1 or a portion thereof; and/or~~

~~—— c) lacks ITAM3 or a portion thereof.~~

~~3. The CAR of claim 2, wherein the modified CD3 ζ polypeptide~~

~~—— i) lacks ITAM1 or a portion thereof and lacks ITAM2 or a portion thereof;~~

~~—— ii) lacks ITAM2 or a portion thereof and lacks ITAM3 or a portion thereof;~~

~~—— iii) lacks ITAM1 or a portion thereof and lacks ITAM3 or a portion thereof.~~

~~4. The CAR of claim 1, wherein the modified CD3 ζ polypeptide comprises~~

~~—— a) a deletion of ITAM2 or a portion thereof;~~

~~—— b) a deletion of ITAM1 or a portion thereof; and/or~~

~~—— c) a deletion of ITAM3 or a portion thereof.~~

~~5. The CAR of claim 4, wherein the modified CD3 ζ polypeptide comprises~~

~~—— i) a deletion of ITAM1 or a portion thereof and a deletion of ITAM2 or a portion thereof;~~

~~—— ii) a deletion of ITAM2 or a portion thereof and a deletion of ITAM3 or a portion thereof;~~

~~—— iii) a deletion of ITAM1 or a portion thereof and a deletion of ITAM3 or a portion thereof.~~

6.2. The CAR of ~~any one of claims 1-5~~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.

~~7.3.~~ The CAR of claim ~~62~~, wherein the modified human CD3ζ polypeptide

- a) lacks BRS2 or a portion thereof;
- b) lacks BRS1 or a portion thereof; and/or
- c) lacks BRS3 or a portion thereof.

~~8.4.~~ The CAR of claim ~~72~~, wherein the modified human CD3ζ 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.

~~9.5.~~ The CAR of claim 1, wherein the modified human CD3ζ polypeptide

- ~~a) lacks ITAM1, ITAM2, BRS1, BRS2, and BRS3; or~~
- ~~b) lacks ITAM2, ITAM3, BRS2, and BRS3.~~

~~10.6.~~ The CAR of claim ~~61~~, wherein the modified human CD3ζ 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.

~~11.7.~~ The CAR of claim ~~106~~, wherein the modified human CD3ζ 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.

~~12.8.~~ The CAR of claim 1, wherein the modified human CD3ζ polypeptide comprises

- ~~a) 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; or~~

~~———b) a deletion of ITAM2 or a portion thereof, a deletion of ITAM3 or a portion thereof, a deletion of BRS2 or a portion thereof, and a deletion of BRS3 or a portion thereof.~~

13.9. The CAR of claim 1, wherein the modified human CD3 ζ polypeptide ~~comprises or~~ 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.

14.10. The CAR of claim ~~139~~, wherein the modified CD3 ζ polypeptide comprises or consists of amino acids 374 to 416 of SEQ ID NO: 45.

~~15.——The CAR of claim 1, wherein the modified CD3 ζ polypeptide comprises or consists of a deletion of ITAM2 or a portion thereof, a deletion of ITAM3 or a portion thereof, a deletion of BRS2 or a portion thereof, and a deletion of BRS3 or a portion thereof.~~

~~16.——The CAR of claim 15, wherein the modified CD3 ζ polypeptide comprises or consists of amino acids 374 to 416 of SEQ ID NO: 47.~~

17.11. 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.

~~18.——A chimeric antigen receptor (CAR) comprising an extracellular antigen-binding domain, a transmembrane domain, and an intracellular signaling domain comprising a modified CD3 ζ polypeptide, wherein the modified CD3 ζ polypeptide lacks all or part of basic-rich stretch (BRS) regions, wherein the BRS regions are BRS1, BRS2, and BRS3.~~

~~19.——The CAR of claim 18, wherein the modified CD3 ζ polypeptide~~

~~———a) lacks BRS2 or a portion thereof;~~

~~———b) lacks BRS1 or a portion thereof, lacks BRS2 or a portion thereof, and lacks BRS3 or a portion thereof;~~

~~———c) lacks BRS2 or a portion thereof, lacks BRS3 or a portion thereof;~~

~~———d) lacks BRS1 or a portion thereof, lacks BRS3 or a portion thereof; or~~

~~———e) lacks BRS1 or a portion thereof, lacks BRS2 or a portion thereof.~~

~~20. —A chimeric antigen receptor (CAR) comprising an extracellular antigen-binding domain, a transmembrane domain, and an intracellular signaling domain comprising a modified CD3ζ polypeptide, wherein the modified CD3ζ polypeptide comprises a BRS variant selected from a BRS1 variant, a BRS2 variant, and a BRS3 variant, wherein the BRS variant comprises one or more loss-of-function mutations.~~

~~21.12.~~ The CAR of any one of claims 1-~~2011~~, further comprising a hinge/spacer region, wherein the hinge/spacer region comprises a CD8 polypeptide, a CD28 polypeptide, a CD3ζ 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.

~~22.13.~~ The CAR of claim ~~2412~~, wherein the hinge/spacer region comprises a CD166 polypeptide.

~~23.14.~~ The CAR of claim ~~2213~~, wherein the CD166 polypeptide consists of amino acids 489 to 527 of SEQ ID NO: 3.

~~24.15.~~ The CAR of claim ~~2412~~, wherein the hinge/spacer region comprises a CD28 polypeptide.

~~25.16.~~ The CAR of claim ~~2415~~, wherein the CD28 polypeptide consists of the amino acid set forth in SEQ ID NO: 15.

~~26. —A chimeric antigen receptor (CAR) comprising an extracellular antigen-binding domain, a hinge/spacer region, a transmembrane domain, and an intracellular signaling domain comprising a modified CD3ζ polypeptide,
—wherein the modified CD3ζ polypeptide comprises one or more ITAM variant comprising one or more loss-of-function mutations, wherein each of the one or more ITAM variant is independently selected from the group consisting of an ITAM1 variant, an ITAM2 variant, and an ITAM3 variant, and
—wherein the hinge/spacer region comprises a CD8 polypeptide, a CD28 polypeptide, a CD3ζ 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.

~~27. The CAR of claim 26, wherein the modified CD3 ζ polypeptide comprises an ITAM2 variant and an ITAM3 variant.~~

~~28. The CAR of claim 27, wherein the ITAM2 variant consists of the amino acid sequence set forth in SEQ ID NO: 29, and/or the ITAM3 variant consists of the amino acid sequence set forth in SEQ ID NO: 33.~~

~~29. The CAR of any one of claims 26-28, wherein the modified CD3 ζ polypeptide comprises or consists of amino acids 374 to 485 of SEQ ID NO: 43.~~

~~30. The CAR of any one of claims 26-29, wherein the CAR comprises the amino acid sequence set forth in SEQ ID NO: 43.~~

~~31. The CAR of any one of claims 26-30, wherein one or both of the ITAM2 variant and the ITAM3 variant comprise two loss of function mutations.~~

~~32. The CAR of claim 31, wherein the one or more loss of function mutation is at a tyrosine amino acid residue.~~

33.17. The CAR any one of claims 1-~~32~~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.

34.18. The CAR of claim 33.17, wherein the transmembrane domain comprises a CD166 polypeptide.

35.19. The CAR of claim 34.18, wherein the CD166 polypeptide comprises amino acid 528 to 553 of SEQ ID NO: 3.

~~36.20.~~ The CAR of any one of claims ~~26-35~~12-19, wherein the transmembrane domain and the hinge/spacer region are derived from the same molecule.

~~37.21.~~ The CAR of any one of claims ~~26-35~~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.

~~38.22.~~ The CAR of claim ~~37~~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.

~~39.23.~~ The CAR of any one of claims ~~26-35~~12-19, wherein the transmembrane domain and the hinge/spacer region are derived from different molecules.

~~40.24.~~ The CAR of claim ~~39~~23, wherein the hinge/spacer region comprises a CD28 polypeptide and the transmembrane domain comprises an ICOS polypeptide.

~~41.25.~~ The CAR of any one of claims 1-~~40~~24, wherein the intracellular signaling domain further comprises a co-stimulatory signaling domain.

~~42.26.~~ The CAR of claim ~~41~~25, wherein the co-stimulatory signaling domain comprises a CD28 polypeptide.

~~43.27.~~ An immunoresponsive cell comprising a CAR of any one of claims 1-~~42~~26.

~~44.28.~~ The immunoresponsive cell of claim ~~43~~27, wherein the CAR is recombinantly expressed, and/or expressed from a vector.

45.29. The immunoresponsive cell of claim 43.27 or 44.28, wherein the CAR is placed at an endogenous gene locus of the immunoresponsive cell.

46.30. The immunoresponsive cell of claim 45.29, wherein the endogenous gene locus is a TRAC locus, a TRBC locus or a TRGC locus.

47.31. The immunoresponsive cell of claim 46.30, wherein the endogenous gene locus is a TRAC locus.

48.32. The immunoresponsive cell of any one of claims 45-47.29-31, wherein the placement of the CAR disrupts or abolishes the endogenous expression of a TCR.

49.33. The immunoresponsive cell of any one of claims 43-48.27-32, 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, a myeloid cell, an embryonic stem cell, and a pluripotent stem cell from which a lymphoid cell may be differentiated.

50.34. The immunoresponsive cell of claim 49.33, wherein the cell is a T cell.

51.35. The immunoresponsive cell of any one of claims 43-50.27-34, wherein the antigen is a tumor antigen.

52.36. The immunoresponsive cell of claim 51.35, 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.

53.37. The immunoresponsive cell of claim 52.36, wherein the antigen is CD19.

~~54.38.~~ A pharmaceutical composition comprising an effective amount of an immunoresponsive cell of any one of claims ~~43-53~~23-37 and a pharmaceutically acceptable excipient.

~~55.39.~~ The pharmaceutical composition of claim ~~54~~38, which is for treating a neoplasm.

~~56.40.~~ Use of the immunoresponsive cell of any one of claims ~~43-53~~23-37 or the pharmaceutical composition of claim ~~54~~38 or ~~55~~39 in the manufacture of a pharmaceutical composition for reducing tumor burden in a subject, or in treating and/or preventing a neoplasm in a subject.

~~57.—Use of an immunoresponsive cell or a pharmaceutical composition comprising thereof in the manufacture of a pharmaceutical composition for reducing tumor burden in a subject or in treating and/or preventing a neoplasm in a subject, wherein the immunoresponsive cells comprises:~~

~~——a chimeric antigen receptor (CAR) comprising an extracellular antigen-binding domain, a transmembrane domain, and an intracellular signaling domain comprising a modified CD3 ζ polypeptide, wherein the modified CD3 ζ polypeptide comprises one or more ITAM variant comprising one or more loss of function mutations, wherein each of the one or more ITAM variant is independently selected from the group consisting of an ITAM1 variant, an ITAM2 variant, and an ITAM3 variant.~~

~~58.41.~~ The use of claim ~~56 or 57~~40, 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.

~~59.42.~~ The use of ~~any one of claims 56-58~~claim 40 or 41, 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.

~~60.43.~~ Use of the immunoresponsive cell of any one of claims ~~43-53~~23-37 or the pharmaceutical composition of claim ~~54~~38 or ~~55~~39 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.

~~61.—Use of an immunoresponsive cell or a pharmaceutical composition comprising thereof 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, wherein the immunoresponsive cells comprises:~~

~~——a chimeric antigen receptor (CAR) comprising an extracellular antigen-binding domain, a transmembrane domain, and an intracellular signaling domain comprising a modified CD3 ζ polypeptide, wherein the modified CD3 ζ polypeptide comprises one or more ITAM variant comprising one or more loss of function mutations, wherein each of the one or more ITAM variant is independently selected from the group consisting of an ITAM1 variant, an ITAM2 variant, and an ITAM3 variant.~~

~~62.44.~~ The use of claim ~~60 or 61~~43, 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.

~~63.45.~~ The use of ~~any one of claims 60-62~~claim 43 or 44, 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.

~~64.46.~~ The use of ~~claim 60-63~~any one of claims 43-45, wherein the neoplasm is CD19+ ALL.

~~65.47.~~ The use of ~~claim 60-64~~any one of claims 43-46, wherein the neoplasm comprises a tumor cell having a low density of a tumor specific antigen on the surface of the tumor cell.

~~66.48.~~ A method for producing an antigen-specific immunoresponsive cell, the method comprising introducing into an immunoresponsive cell a nucleic acid sequence encoding a CAR of any one of claims 1-~~42~~22.

~~67.49.~~ A nucleic acid molecule encoding the CAR of any one of claims 1-~~4222~~.

~~68.50.~~ The nucleic acid molecule of claim ~~6749~~, comprising the nucleotide sequence set forth in SEQ ID NO: 45 or SEQ ID NO: 47.

~~69.51.~~ A vector comprising the nucleic acid molecule of claim ~~6749~~ or ~~6850~~.

~~70.52.~~ The vector of claim ~~6951~~, wherein the vector is a retroviral vector.

~~71.53.~~ A kit comprising a CAR of any one of claims 1-~~4222~~, an immunoresponsive cell of any one of claims ~~43-5323-37~~, a pharmaceutical composition of claim ~~5438~~ or ~~5539~~, a nucleic acid molecule of claim ~~6749~~ or ~~6850~~, or a vector of claim ~~6951~~ or ~~7052~~.

~~72. —An immunoresponsive cell comprising a) a first CAR comprising a first extracellular antigen-binding domain that binds to a first antigen, a first transmembrane domain, and a first intracellular signaling domain; and b) a second CAR comprising a second extracellular antigen-binding domain that binds to a second antigen, a second transmembrane domain, and a second intracellular signaling domain, wherein the first CAR is a CAR of any one of claims 1-42 or the first intracellular signaling domain comprises a modified CD3 ζ polypeptide that comprises one or more ITAM variant comprising one or more loss-of-function mutations, wherein each of the one or more ITAM variant is independently selected from the group consisting of an ITAM1 variant, an ITAM2 variant, and an ITAM3 variant.~~

~~73. —The immunoresponsive cell of claim 72, wherein the second CAR is a CAR of any one of claims 1-42.~~

~~74. —The immunoresponsive cell of claim 72, wherein~~

~~—— a) the second intracellular signaling domain of the second CAR comprises a modified CD3 ζ polypeptide that comprises one or more ITAM variant comprising one or more loss-of-function mutations, wherein each of the one or more ITAM variant is independently selected from the group consisting of an ITAM1 variant, an ITAM2 variant, and an ITAM3 variant;~~

~~—— b) the second intracellular signaling domain of the second CAR comprises a modified CD3 ζ polypeptide that is the same as the modified CD3 ζ polypeptide comprised in the first~~

intracellular signaling domain of the first CAR;

—— c) the second intracellular signaling domain of the second CAR comprises a modified CD3 ζ polypeptide that is different from the modified CD3 ζ polypeptide comprised in the first intracellular signaling domain of the first CAR;

—— d) the second intracellular signaling domain of the second CAR comprises a native CD3 ζ polypeptide;

—— e) the first intracellular signaling domain of the first CAR is the same as or different from the second intracellular signaling domain of the second CAR;

—— f) the first antigen is different from the second antigen;

—— g) the first CAR further comprises a first hinge/spacer region; and/or

—— h) the second CAR further comprises a second hinge/spacer region.

75. — The immunoresponsive cell of any one of claims 72-74, wherein

—— i) the first intracellular signaling domain comprises or consists of an ITAM2 variant and an ITAM3 variant, and the second intracellular signaling domain comprises or consists of an ITAM1 variant and an ITAM2 variant; or

—— ii) the first intracellular signaling domain comprises or consists of an ITAM2 variant and an ITAM3 variant, and the second intracellular signaling domain comprises or consists of a deletion of ITAM2 or a portion thereof and a deletion of ITAM3 or a portion thereof.

76. — The immunoresponsive cell of any one of claims 72-75, further comprising a third CAR comprising a third extracellular antigen-binding domain that binds to a third antigen, a third transmembrane domain, and a third intracellular signaling domain.

77. — The immunoresponsive cell of claim 76, wherein

—— i) the first intracellular signaling domain comprises or consists of an ITAM2 variant and an ITAM3 variant, the second intracellular signaling domain comprises or consists of a deletion of ITAM2 or a portion thereof and a deletion of ITAM3 or a portion thereof, and the third intracellular signaling domain comprises or consists of an ITAM1 variant and an ITAM2 variant;

—— ii) the first intracellular signaling domain comprises or consists of a deletion of ITAM2 or a portion thereof and a deletion of ITAM3 or a portion thereof, the second intracellular signaling domain comprises or consists of a deletion of ITAM2 or a portion thereof and a deletion of

~~ITAM3 or a portion thereof, and the third intracellular signaling domain comprises or has consists of an ITAM1 variant and an ITAM2 variant;~~

~~—— iii) the first intracellular signaling domain comprises or consists of a deletion of ITAM2 or a portion thereof and a deletion of ITAM3 or a portion thereof, the second intracellular signaling domain comprises or consists of an ITAM1 variant and an ITAM2 variant, and the third intracellular signaling domain comprises or consists of an ITAM1 variant and an ITAM2 variant; or~~

~~—— iv) the first intracellular signaling domain comprises or consists of an ITAM1 variant and an ITAM2 variant, the second intracellular signaling domain comprise or consists of an ITAM1 variant and an ITAM2 variant, and the third intracellular signaling domain comprises or has consists of an ITAM1 variant and an ITAM2 variant.~~

78. ~~A pharmaceutical composition comprising an effective amount of the immunoresponsive cells of any one of claims 72-77 and a pharmaceutically acceptable excipient.~~

79. ~~Use of the immunoresponsive cell of any one of claims 72-77 or the pharmaceutical composition of claim 78 in the manufacture of a pharmaceutical composition for reducing tumor burden, treating and/or preventing a neoplasm, and/or treating a subject having a relapse of a neoplasm.~~

80. ~~The use of claim 79, 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.~~

81. ~~The use of claim 79 or 80, 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 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.~~

82. ~~Use of the immunoresponsive cell of any one of claims 72-77 or the pharmaceutical composition of claim 78 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.

83. — The use of claim 82, 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.

84. — The use of claim 82 or 83, 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.

85. — The use of any one of claims 82-84, wherein the neoplasm is CD19+ ALL.

86. — The use of any one of claims 82-85, wherein the neoplasm comprises a tumor cell having a low density of a tumor specific antigen on the surface of the tumor cell.

87.54. The immunoresponsive cell of any one of claims 43-5323-37 or the pharmaceutical composition of claim 54-38 or 55-39 for use in therapy.

88. — An immunoresponsive cell or a pharmaceutical composition comprising thereof for use in therapy, wherein the immunoresponsive cells comprises:

—— a chimeric antigen receptor (CAR) comprising an extracellular antigen-binding domain, a transmembrane domain, and an intracellular signaling domain comprising a modified CD3ζ polypeptide, wherein the modified CD3ζ polypeptide comprises one or more ITAM variant comprising one or more loss-of-function mutations, wherein each of the one or more ITAM variant is independently selected from the group consisting of an ITAM1 variant, an ITAM2 variant, and an ITAM3 variant.

89. — An immunoresponsive cell or a pharmaceutical composition comprising thereof for use in therapy, wherein the subject received an immunoresponsive cell comprising an antigen recognizing receptor comprises a 4-1BB costimulatory signal, wherein the immunoresponsive cells comprises:

~~—— a chimeric antigen receptor (CAR) comprising an extracellular antigen-binding domain, a transmembrane domain, and an intracellular signaling domain comprising a modified CD3 ζ polypeptide, wherein the modified CD3 ζ polypeptide comprises one or more ITAM variant comprising one or more loss of function mutations, wherein each of the one or more ITAM variant is independently selected from the group consisting of an ITAM1 variant, an ITAM2 variant, and an ITAM3 variant.~~

~~90. — The immunoresponsive cell of any one of claims 72-77 or the pharmaceutical composition of claim 78 for use in therapy.~~