

amino acids (YxxL/I-X₆₋₈-YxxL/I) (PMID: 2927501). ITAMs are usually found in receptors expressed in hematopoietic cells and especially well studied in the context of TCR signaling. TCR binding to peptide-MHC leads to the activation of a Src family kinase Lck, which phosphorylates two tyrosine residues in each of the three ITAMs in CD3ζ (PMID:

5 25861978). Each biphosphorylated ITAM then gains the ability to bind to the two tandem SH2 domains of a Syk family kinase, ZAP-70. This interaction brings ZAP-70 in close proximity to Lck, resulting in the phosphorylation and activation of ZAP-70 by Lck. Activated ZAP-70 further phosphorylates its downstream targets, such as adaptor protein LAT and SLP-76. Phosphorylated LAT and SLP-76 provide scaffolds for many other
10 proteins, such as PLC-γ, Grb2/Sos, Gads and Itk, Vav and Nck, eventually leading to calcium mobilization, Ras/Erk activation and actin cytoskeletal rearrangement and ultimately activation of gene expression (PMID: 20516133). Therefore, the three ITAMs in CD3ζ are the major if not only signaling moieties in TCR signaling.

CD28, on the other hand, doesn't contain any ITAM. Instead, its cytoplasmic domain
15 contains an YNM motif that once phosphorylated upon CD28 binding to its ligand CD80/CD86, can bind to the p85 subunit of PI3K and Grb2/Gads (PMID: 20534709). Additionally, proline-rich regions of CD28 can interact with Itk, Tec, Lck, Grb2/Vav and filamin A (PMID: 20534709). Therefore, antigen-binding initiated TCR signaling through CD3ζ and CD80/86-binding initiated CD28 signaling share many common players, such as
20 Grb2, Vav, Gads, Lck, Itk, which makes crosstalk between these two pathways possible. In addition, the activation of both pathways occurs in the signaling complexes assembled near the plasma membrane at the immunological synapse, physically bringing signaling molecules from the two pathways together in space. Last but not least, CD28 induced calcium signaling occurs seconds after TCR-initiated intracellular calcium increase if not
25 sooner, reflecting the temporal proximity/closeness of two pathways (PMID: 18848472).

SUMMARY

The present disclosure provides chimeric antigen receptors (CARs) that bind to an antigen of interest. The CAR can bind to a tumor antigen or a pathogen antigen. In certain non-limiting examples, the CAR comprises an extracellular antigen-binding domain, a
30 transmembrane domain, and an intracellular signaling domain comprising a modified CD3ζ polypeptide.

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 CD3 ζ polypeptide, wherein the modified CD3 ζ polypeptide 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 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.

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 CARs described herein, an immunoresponsive cell as described 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
5 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
10 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.

In certain examples, the modified CD3 ζ polypeptide lacks all or part of immunoreceptor tyrosine-based activation motifs (ITAMs), wherein the ITAMs are ITAM1, ITAM2, and ITAM3. In certain examples, the modified CD3 ζ polypeptide lacks ITAM2 or a portion thereof. In certain examples, the modified CD3 ζ polypeptide further lacks ITAM3 or a portion thereof. In certain examples, the modified CD3 ζ polypeptide further lacks ITAM1 or a portion thereof. In certain examples, the modified CD3 ζ polypeptide lacks ITAM1 or a portion thereof. In certain examples, the modified CD3 ζ polypeptide further lacks ITAM3 or a portion thereof. In certain examples, the modified CD3 ζ polypeptide lacks ITAM3 or a portion thereof. In certain examples, the modified CD3 ζ polypeptide comprises a deletion of ITAM2 or a portion thereof. In certain examples, the modified CD3 ζ polypeptide further comprises a deletion of ITAM3 or a portion thereof. In certain examples, the modified CD3 ζ polypeptide further comprises a deletion of ITAM1 or a portion thereof. In certain examples, the modified CD3 ζ polypeptide comprises a deletion of ITAM1 or a portion thereof. In certain examples, the modified CD3 ζ polypeptide further comprises a deletion of ITAM3 or a portion thereof. In certain examples, the modified CD3 ζ polypeptide comprises a deletion of ITAM3 or a portion thereof.

In certain examples, the modified CD3 ζ polypeptide further lacks all or part of basic-rich stretch (BRS) regions, wherein the BRS regions are BRS1, BRS2, and BRS3. In certain examples, the modified CD3 ζ polypeptide lacks BRS2 or a portion thereof. In certain examples, the modified CD3 ζ polypeptide further lacks BRS3 or a portion thereof. In certain examples, the modified CD3 ζ polypeptide further lacks BRS1 or a portion thereof. In certain examples, the modified CD3 ζ polypeptide lacks BRS1 or a portion thereof. In certain examples, the modified CD3 ζ polypeptide further lacks BRS3 or a portion thereof. In certain examples, the modified CD3 ζ polypeptide lacks BRS3 or a portion thereof. In certain examples, the modified CD3 ζ polypeptide lacks BRS1 or portion thereof, BRS2 or portion thereof, and BRS3 or a portion thereof. In certain examples, the modified CD3 ζ polypeptide comprises a deletion of BRS2 or a portion thereof. In certain examples, the modified CD3 ζ polypeptide further comprises a deletion of BRS3 or a portion thereof. In certain examples, the modified CD3 ζ polypeptide further comprises a deletion of BRS1 or a portion thereof. In certain examples, the modified CD3 ζ polypeptide comprises a deletion of BRS1 or a portion thereof. In certain examples, the modified CD3 ζ

polypeptide further comprises a deletion of BRS3 or a portion thereof. In certain examples, the modified CD3 ζ polypeptide comprises a deletion of BRS3 or a portion thereof. In certain examples, the modified CD3 ζ polypeptide comprises a deletion of BRS1 or portion thereof, BRS2 or portion thereof, and BRS3 or a portion thereof. In certain examples, the modified
5 CD3 ζ polypeptide comprises a deletion of ITAM2, ITAM3, BRS2, and BRS3. In certain examples, the modified CD3 ζ polypeptide lacks ITAM2, ITAM3, BRS2, and BRS3. In certain examples, the CAR comprises the amino acid sequence set forth in SEQ ID NO: 45 or SEQ ID NO: 47.

In certain examples, the modified CD3 ζ polypeptide lacks all or part of basic-rich
10 stretch (BRS) regions, wherein the BRS regions are BRS1, BRS2, and BRS3. In certain examples, the modified CD3 ζ polypeptide lacks BRS2 or a portion thereof. In certain examples, the modified CD3 ζ polypeptide further lacks BRS3 or a portion thereof. In certain examples, the modified CD3 ζ polypeptide further lacks BRS1 or a portion thereof. In certain examples, the modified CD3 ζ polypeptide lacks BRS1 or a portion thereof. In certain
15 examples, the modified CD3 ζ polypeptide further lacks BRS3 or a portion thereof. In certain examples, the modified CD3 ζ polypeptide lacks BRS3 or a portion thereof. In certain examples, the modified CD3 ζ polypeptide lacks BRS1 or portion thereof, BRS2 or portion thereof, and BRS3 or a portion thereof.

In certain examples, the modified CD3 ζ polypeptide comprises a BRS variant
20 selected from a BRS1 variant, a BRS2 variant, and a BRS3 variant, wherein the BRS variant comprises one or more loss-of-function mutations.

In certain examples, any of the various CARs disclosed above further comprises a hinge/spacer region, wherein the hinge/spacer region comprises a CD8 polypeptide, a CD28 polypeptide, a CD3 ζ polypeptide, a CD40 polypeptide, a 4-1BB polypeptide, an OX40
25 polypeptide, a CD84 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. In certain examples, the hinge/spacer region comprises CD166 polypeptide. In certain examples, the hinge/spacer region comprises a CD166 polypeptide that has amino acids 489 to 527 of SEQ
30 ID NO: 3.

In certain examples, the transmembrane domain any of the various CARs disclosed above comprises a CD8 polypeptide, a CD28 polypeptide, a CD3 ζ polypeptide, a CD40 polypeptide, a 4-1BB polypeptide, an OX40 polypeptide, a CD84 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. In certain examples, the transmembrane domain comprises CD166 polypeptide. In certain examples, the transmembrane domain comprises
5 CD166 polypeptide that has amino acids 528 to 527 of SEQ ID NO: 3.

In certain examples, the transmembrane domain and the hinge/spacer region are derived from the same molecule. In certain examples, the hinge/spacer region comprises a CD28 polypeptide and the transmembrane domain comprises a CD28 polypeptide. In certain examples, the hinge/spacer region comprises a CD84 polypeptide and the
10 transmembrane domain comprises a CD84 polypeptide. In certain examples, the hinge/spacer region comprises a CD166 polypeptide and the transmembrane domain comprises a CD166 polypeptide. In certain examples, the CAR comprises amino acids 489 to 553 of SEQ ID NO: 3.

In certain examples, the hinge/spacer region comprises a CD8a polypeptide and the
15 transmembrane domain comprises a CD8a polypeptide. In certain examples, the hinge/spacer region comprises a CD8b polypeptide and the transmembrane domain comprises a CD8b polypeptide.

In certain examples, the transmembrane domain and the hinge/spacer region are derived from different molecules. In certain examples, the hinge/spacer region comprises a
20 CD28 polypeptide and the transmembrane domain comprises an ICOS polypeptide.

In certain examples, the intracellular signaling domain any of the various CARs disclosed above further comprises a co-stimulatory signaling domain. In certain examples, the co-stimulatory signaling domain comprises a CD28 polypeptide.

The presently disclosed subject matter also provides chimeric antigen receptors
25 (CARs) 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 an ITAM variant comprising one or more loss-of-function mutations, wherein the ITAM variant is selected from the group consisting of an ITAM1 variant, an ITAM2 variant, and an ITAM3 variant.
30 In certain examples, the hinge/spacer region comprises a CD8 polypeptide, a CD28 polypeptide, a CD3 ζ polypeptide, a CD40 polypeptide, a 4-1BB polypeptide, an OX40 polypeptide, a CD84 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. In certain examples, the ITAM2 variant has the amino acid sequence set forth in SEQ ID NO: 29. In certain examples, the ITAM3 variant has the amino acid sequence set forth in SEQ ID NO: 33. In certain examples, the CAR comprises a hinge/spacer region of a CD166 polypeptide and a transmembrane domain of a CD166 polypeptide. In certain examples, the CAR comprises the amino acids 489 to 553 of SEQ ID NO: 3. In certain examples, the modified CD3 ζ polypeptide has amino acids 374 to 485 of SEQ ID NO: 43. In certain examples, the CAR comprises the amino acid sequence set forth in SEQ ID NO: 43.

The presently disclosed subject matter also provides an immunoresponsive cell comprising a CAR disclosed herein. In certain examples, the CAR is recombinantly expressed. In certain examples, the CAR is expressed from a vector. In certain examples, the CAR is placed at an endogenous gene locus of the immunoresponsive cell. In certain examples, the endogenous gene locus is a TRAC locus, a TRBC locus or a TRGC locus. In certain examples, the endogenous gene locus is a TRAC locus. In certain examples, the placement of the CAR disrupts or abolishes the endogenous expression of a TCR.

The presently disclosed subject matter also provides immunoresponsive cells comprising two or more CARs. In certain examples, the immunoresponsive cell comprises 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 disclosed above 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. In certain examples, the first CAR further comprises a first hinge/spacer region. In certain examples, the second CAR further comprises a second hinge/spacer region. In certain examples, each of the first and second hinge/spacer regions can be independently selected from any of the hinge/spacer regions disclosed herein.

In certain examples, the second CAR is a CAR disclosed above. In certain examples, 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 the ITAM variant is selected from the group
5 consisting of an ITAM1 variant, an ITAM2 variant, and an ITAM3 variant. In certain examples, 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. In certain examples, the second intracellular signaling domain of the second CAR comprises a modified CD3 ζ polypeptide
10 that is different from the modified CD3 ζ polypeptide comprised in the first intracellular signaling domain of the first CAR. In certain examples, the second intracellular signaling domain of the second CAR comprises a native CD3 ζ polypeptide.

In certain examples, the first intracellular signaling domain of the first CAR is the same as the second intracellular signaling domain of the second CAR. In certain examples,
15 the first intracellular signaling domain of the first CAR is different from the second intracellular signaling domain of the second CAR.

In certain examples, the first antigen is different from the second antigen.

In certain examples, the first intracellular signaling domain comprises or has an ITAM2 variant and an ITAM3 variant, and the second intracellular signaling domain
20 comprises or has a deletion of ITAM2 or a portion thereof and a deletion of ITAM3 or a portion thereof.

In certain examples, the first intracellular signaling domain comprises or has an ITAM2 variant and an ITAM3 variant, and the second intracellular signaling domain comprises or has an ITAM1 variant and an ITAM2 variant.

25 In certain examples, the cell further comprises 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.

In certain examples, the first intracellular signaling domain comprises or has an ITAM2 variant and an ITAM3 variant, the second intracellular signaling domain comprises
30 or has 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 an ITAM1 variant and an ITAM2 variant.

In certain examples, the first intracellular signaling domain comprises or has a deletion of ITAM2 or a portion thereof and a deletion of ITAM3 or a portion thereof, the second intracellular signaling domain comprises or has 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 an ITAM1 variant and an ITAM2 variant.

In certain examples, the first intracellular signaling domain comprises or has a deletion of ITAM2 or a portion thereof and a deletion of ITAM3 or a portion thereof, the second intracellular signaling domain comprises or has an ITAM1 variant and an ITAM2 variant, and the third intracellular signaling domain comprises or has an ITAM1 variant and an ITAM2 variant.

In certain examples, the first intracellular signaling domain comprises or has an ITAM1 variant and an ITAM2 variant, the second intracellular signaling domain comprises or has an ITAM1 variant and an ITAM2 variant, and the third intracellular signaling domain comprises or has an ITAM1 variant and an ITAM2 variant.

In certain examples, the cell is selected from the group consisting of a T cell, a Natural Killer (NK) cell, a human embryonic stem cell, and a pluripotent stem cell from which lymphoid cells may be differentiated. In certain examples, the cell is a T cell. In certain examples, the T cell is selected from the group consisting of a cytotoxic T lymphocyte (CTL), a regulatory T cell, and a Natural Killer T (NKT) cell. In certain examples, the immunoresponsive cell is a myeloid cell such as macrophage. In certain examples, said immunoresponsive cell is autologous. In certain examples, said antigen is a tumor antigen. In certain examples, 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-ES0-1, oncofetal antigen (h5T4), PSCA, PSMA, ROR1, TAG-72, VEGF-R2, WT-1, BCMA, CD123, CD44V6, NKCS1, EGF1R, EGFR-VIII, and CD99, CD70, ADGRE2, CCR1, LILRB2, PRAME, CCR4, CD5, CD3, TRBC1, TRBC2, TIM-3, Integrin B7, ICAM-1, CD70, Tim3, CLEC12A and ERBB. In certain examples, said antigen is CD19.

The presently disclosed subject matter further provides a pharmaceutical composition comprising an effective amount of an immunoresponsive cell disclosed herein and a pharmaceutically acceptable excipient. In certain examples, the pharmaceutical composition is for treating a neoplasm.

5 The presently disclosed subject matter further provides a method of reducing tumor burden in a subject, the method comprising administering to the subject an effective amount of the immunoresponsive cells or the pharmaceutical composition disclosed herein. The presently disclosed subject matter also provides a method, the method comprising administering to the subject an effective amount of immunoresponsive cells or a
10 pharmaceutical composition comprising thereof, wherein the immunoresponsive cells comprises 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,
15 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. In certain examples, the modified CD3 ζ polypeptide comprises an ITAM2 variant and an ITAM3 variant. In certain examples, one or both of the ITAM2 variant and the ITAM3 variant comprise two loss-of-function mutations. In certain examples, the ITAM2 variant has the
20 amino acid sequence set forth in SEQ ID NO: 29. In certain examples, the ITAM3 variant has the amino acid sequence set forth in SEQ ID NO: 33. In certain examples, the one or more loss-of-function mutation is at a tyrosine amino acid residue. In certain examples, the intracellular signaling domain further comprises a co-stimulatory signaling domain. In certain examples, the co-stimulatory signaling domain comprises a CD28 polypeptide.

25 In certain examples, the method reduces the number of tumor cells. In certain examples, the method reduces tumor size. In certain examples, the method eradicates the tumor in the subject.

 The presently disclosed subject matter further provides methods of treating or preventing a neoplasm. In certain examples, the method comprises administering to the
30 subject an effective amount of the immunoresponsive cells or the pharmaceutical composition disclosed herein. In certain examples, the method comprises administering to the subject an effective amount of immunoresponsive cells or a

pharmaceutical composition comprising thereof, wherein the immunoresponsive cell comprises 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.

The presently disclosed subject matter further provides a method of treating a subject having a relapse of a neoplasm, wherein the subject received an immunoresponsive cell comprising an antigen recognizing receptor, wherein the antigen recognizing receptor comprises a 4-1BB costimulatory signal. In certain examples, the method comprises administering to the subject an effective amount of the immunoresponsive cells or the pharmaceutical composition disclosed herein. In certain examples, the method comprises administering to the subject an effective amount of immunoresponsive cells or a pharmaceutical composition comprising thereof, wherein the immunoresponsive cells comprises 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. In certain examples, the intracellular signaling domain further comprises a co-stimulatory signaling domain. In certain examples, the co-stimulatory signaling domain comprises a CD28 polypeptide.

In certain examples, 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. In certain examples, 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. In certain examples, the neoplasm is CD19+ ALL. In certain examples, the neoplasm comprises a tumor cell having a low expression level of a tumor specific antigen.

The presently disclosed subject matter further provides a method for producing an antigen-specific immunoresponsive cell, the method comprising introducing into an immunoresponsive cell a nucleic acid sequence encoding a CAR disclosed herein. In certain examples, the nucleic acid sequence is comprised in a vector. In certain examples, the vector is a retroviral vector.

The presently disclosed subject matter further provides an isolated nucleotide acid encoding the CAR disclosed herein. In certain examples, the isolated nucleotide acid further comprises the nucleotide sequence set forth in SEQ ID NO: 45 and SEQ ID NO: 47.

The presently disclosed subject matter further provides a nucleic acid composition comprising a CAR disclosed herein. In certain examples, the nucleic acid sequences are comprised in a vector. In certain examples, the vector is a retroviral vector.

The presently disclosed subject matter further provides a vector comprising the nucleic acid composition disclosed herein.

The presently disclosed subject matter further provides a kit comprising a CAR, an immunoresponsive cell, a pharmaceutical composition, a nucleic acid composition or a vector disclosed herein. In certain examples, the kit further comprises written instructions for treating and/or preventing a neoplasm, a pathogen infection, an autoimmune disorder, or an allogeneic transplant.

BRIEF DESCRIPTION OF THE FIGURES

The following Detailed Description, given by way of example, but not intended to limit the presently disclosed subject matter to specific embodiments described, may be understood in conjunction with the accompanying drawings.

Figure 1 depicts cytoplasmic regions of wild-type, mutated and truncated 1928 ζ (1928z) CARs. A) Cytoplasmic regions of different 1928 ζ (1928z) CARs. The ζ chain of the original 1928 ζ CAR is mutated to construct 1928 ζ CAR T cells with distinctive ζ signaling domains. The three ITAM motifs in the ζ domain are named ITAM1, ITAM2 and ITAM3 from membrane proximal to distal direction. In 1XX, X2X, XX3 and X23 CARs, the two tyrosine residues (Y) in the respective ITAM are point mutated to two phenylalanine residues (F) for the indicated ITAMs. In D12 and D23, deletion mutations are made to remove ITAM1 and ITAM2 (D12) or ITAM2 and ITAM3 (D23). B) cytoplasmic regions of 1928z CARs with improved antitumor efficacy compared to the original 1928z structure. In 1XX, the ζ chain of the original 1928 ζ CAR

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 CD3 ζ polypeptide, wherein the modified CD3 ζ polypeptide 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. The CAR of any one of claims 1-5, 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.
7. The CAR of claim 6, wherein the modified 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.
8. The CAR of claim 7, wherein the modified 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.
9. The CAR of claim 1, wherein the modified CD3 ζ polypeptide
- a) lacks ITAM1, ITAM2, BRS1, BRS2, and BRS3; or
 - b) lacks ITAM2, ITAM3, BRS2, and BRS3.
10. The CAR of claim 6, wherein the modified 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.
11. The CAR of claim 10, wherein the modified 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.
12. The CAR of claim 1, wherein the modified CD3 ζ 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. The CAR of claim 1, wherein the modified 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. The CAR of claim 13, 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. 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 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.

21. The CAR of any one of claims 1-20, 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.

22. The CAR of claim 21, wherein the hinge/spacer region comprises a CD166 polypeptide.

23. The CAR of claim 22, wherein the CD166 polypeptide consists of amino acids 489 to 527 of SEQ ID NO: 3.

24. The CAR of claim 21, wherein the hinge/spacer region comprises a CD28 polypeptide.

25. The CAR of claim 24, 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 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.

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. The CAR any one of claims 1-32, 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. The CAR of claim 33, wherein the transmembrane domain comprises a CD166 polypeptide.
35. The CAR of claim 34, wherein the CD166 polypeptide comprises amino acid 528 to 553 of SEQ ID NO: 3.
36. The CAR of any one of claims 26-35, wherein the transmembrane domain and the hinge/spacer region are derived from the same molecule.

37. The CAR of any one of claims 26-35, 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. The CAR of claim 37, 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. The CAR of any one of claims 26-35, wherein the transmembrane domain and the hinge/spacer region are derived from different molecules.
40. The CAR of claim 39, wherein the hinge/spacer region comprises a CD28 polypeptide and the transmembrane domain comprises an ICOS polypeptide.
41. The CAR of any one of claims 1-40, wherein the intracellular signaling domain further comprises a co-stimulatory signaling domain.
42. The CAR of claim 41, wherein the co-stimulatory signaling domain comprises a CD28 polypeptide.
43. An immunoresponsive cell comprising a CAR of any one of claims 1-42.
44. The immunoresponsive cell of claim 43, wherein the CAR is recombinantly expressed, and/or expressed from a vector.
45. The immunoresponsive cell of claim 43 or 44, wherein the CAR is placed at an endogenous gene locus of the immunoresponsive cell.

46. The immunoresponsive cell of claim 45, wherein the endogenous gene locus is a TRAC locus, a TRBC locus or a TRGC locus.
47. The immunoresponsive cell of claim 46, wherein the endogenous gene locus is a TRAC locus.
48. The immunoresponsive cell of any one of claims 45-47, wherein the placement of the CAR disrupts or abolishes the endogenous expression of a TCR.
49. The immunoresponsive cell of any one of claims 43-48, 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. The immunoresponsive cell of claim 49, wherein the cell is a T cell.
51. The immunoresponsive cell of any one of claims 43-50, wherein the antigen is a tumor antigen.
52. The immunoresponsive cell of claim 51, 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. The immunoresponsive cell of claim 52, wherein the antigen is CD19.
54. A pharmaceutical composition comprising an effective amount of an immunoresponsive cell of any one of claims 43-53 and a pharmaceutically acceptable excipient.

55. The pharmaceutical composition of claim 54, which is for treating a neoplasm.
56. Use of the immunoresponsive cell of any one of claims 43-53 or the pharmaceutical composition of claim 54 or 55 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. The use of claim 56 or 57, 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. The use of any one of claims 56-58, 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. Use of the immunoresponsive cell of any one of claims 43-53 or the pharmaceutical composition of claim 54 or 55 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. The use of claim 60 or 61, 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. The use of any one of claims 60-62, 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. The use of claim 60-63, wherein the neoplasm is CD19⁺ ALL.

65. The use of claim 60-64, wherein the neoplasm comprises a tumor cell having a low density of a tumor specific antigen on the surface of the tumor cell.

66. 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.

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67. A nucleic acid molecule encoding the CAR of any one of claims 1-42.
68. The nucleic acid molecule of claim 67, comprising the nucleotide sequence set forth in SEQ ID NO: 45 or SEQ ID NO: 47.
69. A vector comprising the nucleic acid molecule of claim 67 or 68.
70. The vector of claim 69, wherein the vector is a retroviral vector.
71. A kit comprising a CAR of any one of claims 1-42, an immunoresponsive cell of any one of claims 43-53, a pharmaceutical composition of claim 54 or 55, a nucleic acid molecule of claim 67 or 68, or a vector of claim 69 or 70.
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. The immunoresponsive cell of any one of claims 43-53 or the pharmaceutical composition of claim 54 or 55 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 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.

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.