

3. SUMMARY

In 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 at least one co-stimulatory signaling domain that comprises a CD28 polypeptide comprising a mutated YMNM motif, wherein the mutated YMNM motif consists of the amino acid sequence set forth in YSNV (SEQ ID NO: 23), YENV (SEQ ID NO: 22), or YKNI (SEQ ID NO: 26).

In another aspect, the present disclosure refers to a cell comprising the CAR as described herein, wherein the cell is a T cell or an NK cell.

In another aspect, the present disclosure refers to a composition comprising a cell as described herein.

In another aspect, the present disclosure refers to a use of the cell as described herein or the composition as described herein in the manufacture of a medicament for reducing tumor burden in a subject, wherein the cell or the composition is to be administered to the subject.

In another aspect, the present disclosure refers to a use of the cell as described herein or the composition as described herein in the manufacture of a medicament for treating a neoplasm or a tumor, wherein the cell or the composition is to be administered to the subject.

In another aspect, the present disclosure refers to a use of the cell as described herein or the composition as described herein in the manufacture of a medicament for lengthening survival of a subject having a neoplasm or a tumor, wherein the cell or the composition is to be administered to the subject.

In yet another aspect, the present disclosure refers to a method for producing an antigen-specific cell, the method comprising introducing into a cell a nucleic acid molecule encoding a CAR as described herein.

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

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

In yet another aspect, the present disclosure refers to a host cell expressing the nucleic acid molecule as described herein.

In yet another aspect, the present disclosure refers to a kit comprising a CAR as described herein, a cell as described herein, a composition as described herein, a nucleic acid molecule as described herein, or a vector as described herein.

5 In yet another aspect, the present disclosure refers to the cell as described herein or the composition as described herein for use in therapy.

The presently disclosed subject matter provides chimeric antigen receptors (CARs) comprising a mutated CD28 intracellular motif, *i.e.*, a mutated YMNm motif.

10 The present disclosure provides chimeric antigen receptor (CAR) comprising an extracellular antigen-binding domain, a transmembrane domain, and an intracellular signaling domain comprising at least one co-stimulatory signaling domain that comprises a CD28 polypeptide comprising a mutated YMNm motif.

In certain examples, the CD28 polypeptide has reduced recruitment of a p85 subunit of a phosphoinositide 3-kinase (PI3K) as compared to a CD28 molecule comprising a native YMNm motif. In certain examples, a p85 subunit of a PI3K does not bind to the mutated YMNm motif. In certain examples, the mutated YMNm motif consists of the amino acid sequence set forth in YxNx (SEQ ID NO: 21), wherein x is not a methionine (M). In certain examples, the mutated YMNm motif consists of the amino acid sequence set forth in YENV (SEQ ID NO: 22), YSNV (SEQ ID NO: 23), YKNL (SEQ ID NO: 24), YENQ (SEQ ID NO: 25), YKNI (SEQ ID NO: 26), YINQ (SEQ ID NO: 27), YHnk (SEQ ID NO: 28), YVNQ (SEQ ID NO: 29), YLNP (SEQ ID NO: 30), YLNT (SEQ ID NO: 31), YDND (SEQ ID NO: 66), YENI (SEQ ID NO: 67), YENL (SEQ ID NO: 68), YKNQ (SEQ ID NO: 72), YKNV (SEQ ID NO: 73), or YANG (SEQ ID NO: 87).. In certain examples, the mutated YMNm motif consists of the amino acid sequence set forth in YSNV (SEQ ID NO: 23), YENV (SEQ ID NO: 22), or YKNI (SEQ ID NO: 26). In certain examples, the mutated YMNm motif consists of the amino acid sequence set forth in YSNV (SEQ ID NO: 23). In certain examples, the mutated YMNm motif binds to growth factor receptor bound receptor 2 (Grb2) and/or Grb2-related adaptor downstream of Shc (GADS).

30 In certain examples, the mutated YMNm motif does not bind to Grb2 and/or GADS. In certain examples, the mutated YMNm motif consists of the amino acid sequence set forth in YMxM (SEQ ID NO: 20), wherein x is not an aspartic acid (N). In certain examples, the mutated YMNm motif consists of the amino acid sequence set forth in YMDM (SEQ ID NO: 32), YMPM (SEQ ID NO: 79), YMRM (SEQ ID NO: 37), or YMSM (SEQ ID NO: 80). In certain examples, the mutated YMNm motif

consists of the amino acid sequence set forth in YMDM (SEQ ID NO: 32). In certain examples, the mutated YMN_M motif consists of the amino acid sequence set forth in YbxM (SEQ ID NO: 33), wherein x is not an aspartic acid (N), and b is not a methionine (M). In certain examples, the mutated YMN_M motif consists of the amino acid

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 at least one co-stimulatory signaling domain that comprises a CD28 polypeptide comprising a mutated YMNM motif, wherein the mutated YMNM motif consists of the amino acid sequence set forth in YSNV (SEQ ID NO: 23), YENV (SEQ ID NO: 22), or YKNI (SEQ ID NO: 26).
2. The CAR of claim 1, wherein the CD28 polypeptide has reduced recruitment of a p85 subunit of a phosphoinositide 3-kinase (PI3K) as compared to a CD28 molecule comprising a native YMNM motif.
3. The CAR of claim 1 or 2, wherein a p85 subunit of a PI3K does not bind to the mutated YMNM motif.
4. The CAR of any one of claims 1-3, wherein the mutated YMNM motif consists of the amino acid sequence set forth in YSNV (SEQ ID NO: 23).
5. The CAR of any one of claims 1-4, wherein the mutated YMNM motif binds to growth factor receptor bound receptor 2 (Grb2) and/or Grb2-related adaptor downstream of Shc (GADS).
6. The CAR of any one of claims 1-5, wherein the extracellular antigen-binding domain binds to an antigen.
7. The CAR of claim 6, wherein the antigen is a tumor antigen or a pathogen antigen.
8. The CAR of claim 6 or 7, wherein the antigen is a tumor antigen.
9. The CAR of claim 8, wherein the tumor antigen is selected from the group consisting of CD19, mesothelin, AXL, TIM3, HVEM, MUC16, MUC1, CA1X, CEA, CD8, CD7, CD10, CD20, CD22, CD30, CLL1, CD33, CD34, CD38, CD41, CD44, CD49f, CD56, CD70, CD74, CD99, CD123, CD133, CD138, EGP-2, EGP-40, EpCAM, Erb-B, FBP, Fetal acetylcholine receptor, folate receptor- α , GD2, GD3, HER-2, hTERT, IL-13R- α 2, κ -light chain, KDR, LeY, L1 cell adhesion molecule, MAGE-A1, MAGEA3, CT83 (also known as KK-LC-1), 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, CD44V6, NKCS1, EGF1R, EGFR-VIII, ADGRE2, CCR1, LILRB2, PRAME, HPV E6 oncoprotein, and HPV E7 oncoprotein.
10. The CAR of claim 9, wherein the tumor antigen is CD19.

11. The CAR of any one of claims 1-10, wherein the mutated YMNM motif consists of the amino acid sequence set forth in YKNI (SEQ ID NO: 26).
12. The CAR of claim 11, wherein the extracellular antigen-binding domain binds to CD19.
13. The CAR of claim 12, wherein the CAR comprises the amino acid sequence set forth in SEQ ID NO: 55.
14. The CAR of any one of claims 1-10, wherein the mutated YMNM motif consists of the amino acid sequence set forth in YENV (SEQ ID NO: 22).
15. The CAR of claim 14, wherein the extracellular antigen-binding domain binds to CD19.
16. The CAR of claim 15, wherein the CAR comprises the amino acid sequence set forth in SEQ ID NO: 53.
17. The CAR of any one of claims 1-10, wherein the mutated YMNM motif consists of the amino acid sequence set forth in YSNV (SEQ ID NO: 64).
18. The CAR of claim 17, wherein the extracellular antigen-binding domain binds to CD19.
19. The CAR of claim 18, wherein the CAR comprises the amino acid sequence set forth in SEQ ID NO: 57.
20. A cell comprising the CAR of any one of claims 1-19, wherein the cell is a T cell or an NK cell.
21. The cell of claim 20, wherein the cell is a T cell.
22. The cell of claim 20 or 21, wherein the T cell is selected from the group consisting of a cytotoxic T lymphocyte (CTL), a $\gamma\delta$ T cell, a tumor-reactive lymphocyte, a tumor-infiltrating lymphocyte (TIL), a regulatory T cell, and a Natural Killer T (NKT) cell.
23. A composition comprising a cell of any one of claims 20-22.
24. The composition of claim 23, which is a pharmaceutical composition that further comprises a pharmaceutically acceptable excipient.
25. Use of the cell of any one of claims 20-22 or the composition of any one of claims 23-24 in the manufacture of a medicament for reducing tumor burden in a subject, wherein the cell or the composition is to be administered to the subject.
26. The use of claim 25, wherein the number of tumor cells is reduced, the tumor size is reduced, and/or the tumor is eradicated in the subject.

27. Use of the cell of any one of claims 20-22 or the composition of any one of claims 23-24 in the manufacture of a medicament for treating a neoplasm or a tumor, wherein the cell or the composition is to be administered to the subject.
28. Use of the cell of any one of claims 20-22 or the composition of any one of claims 23-24 in the manufacture of a medicament for lengthening survival of a subject having a neoplasm or a tumor, wherein the cell or the composition is to be administered to the subject.
29. The use of any one of claims 25-28, wherein the neoplasm and/or tumor is selected from the group consisting of B cell leukemia, B cell lymphoma, acute lymphoblastic leukemia (ALL), chronic lymphocytic leukemia (CLL), non-Hodgkin's lymphoma, Burkitt lymphoma, acute myeloid leukemia (AML) and Mixed-phenotype acute leukemia (MPAL).
30. A method for producing an antigen-specific cell, the method comprising introducing into a cell a nucleic acid molecule encoding a CAR of any one of claims 1-19.
31. The method of claim 30, wherein the nucleic acid molecule is present on a vector.
32. The method of claim 31, wherein the vector is a retroviral vector.
33. A nucleic acid molecule encoding the CAR of any one of claims 1-19.
34. The nucleic acid molecule of claim 33, comprising the nucleotide sequence set forth in SEQ ID NO: 54, SEQ ID NO: 56, or SEQ ID NO: 58.
35. A vector comprising the nucleic acid molecule of claim 33 or 34.
36. The vector of claim 35, wherein the vector is a γ -retroviral vector.
37. A host cell expressing the nucleic acid molecule of claim 33 or 34.
38. The host cell of claim 37, wherein the host cell is a T cell.
39. A kit comprising a CAR of any one of claims 1-19, a cell of any one of claims 20-22, a composition of any one of claims 23-24, a nucleic acid molecule of claim 33 or 34, or a vector of claim 35 or 36.
40. The kit of claim 39, wherein the kit further comprises written instructions for treating a neoplasm, a tumor, and/or a pathogen infection.
41. The cell of any one of claims 20-22 or the composition of any one of claims 23-24 for use in therapy.