

3. SUMMARY-OF-THE-INVENTION

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.

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

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 and/or preventing 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.

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 YMN₁M motif.

5 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 YMN₁M motif.

10 In certain embodimentsexamples, 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 YMN₁M motif. In certain examplesembodiments, a p85 subunit of a PI3K does not bind to the mutated YMN₁M motif. In certain examplesembodiments, the mutated YMN₁M motif consists of the amino acid sequence set forth in YxNx (SEQ ID NO: 21), wherein x is not a methionine (M). In certain examplesembodiments, the
15 mutated YMN₁M 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), YHNL (SEQ ID NO: 28), YVNL (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),
20 YKNV (SEQ ID NO: 73), or YANG (SEQ ID NO: 87).. In certain examplesembodiments, the mutated YMN₁M 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 examplesembodiments, the mutated YMN₁M motif consists of the amino acid sequence set forth in YSNV (SEQ ID NO: 23). In certain examplesembodiments,
25 the mutated YMN₁M motif binds to growth factor receptor bound receptor 2 (Grb2) and/or Grb2-related adaptor downstream of Shc (GADS).

In certain examplesembodiments, the mutated YMN₁M motif does not bind to Grb2 and/or GADS. In certain examplesembodiments, the mutated YMN₁M motif consists of the amino acid sequence set forth in YM₁M (SEQ ID NO: 20), wherein x is
30 not an aspartic acid (N). In certain examplesembodiments, the mutated YMN₁M 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 examplesembodiments, the mutated YMN₁M motif consists of the amino acid sequence set forth in YMDM (SEQ ID NO: 32). In certain examplesembodiments, the mutated

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

sequence set forth in YTHM (SEQ ID NO: 34), YVLM (SEQ ID NO: 35), YIAM (SEQ ID NO: 36), YVEM (SEQ ID NO: 83), YVKM (SEQ ID NO: 85), or YVPM (SEQ ID NO: 86).

In certain exampleembodiments, the mutated YMN_M motif consists of the amino acid sequence set forth in YM_xb (SEQ ID NO: 65), wherein x is not an aspartic acid (N), and b is not a methionine (M). In certain exampleembodiments, the mutated YMN_M motif consists of the amino acid sequence set forth in YMAP (SEQ ID NO: 77). In certain exampleembodiments, a p85 subunit of a PI3K signaling binds to the mutated YMN_M motif.

In certain exampleembodiments, the mutated YMN_M motif does not bind to Grb2 and/or GADS or a p85 subunit of a PI3K. In certain exampleembodiments, the mutated YMN_M motif consists of the amino acid sequence set forth in Yb_xb (SEQ ID NO: 43), wherein x is not an aspartic acid (N), and b is not a methionine (M). In certain exampleembodiments, the mutated YMN_M motif consists of the amino acid sequence set forth in YGGG (SEQ ID NO: 44), YAAA (SEQ ID NO: 45), YFFF (SEQ ID NO: 46), YETV (SEQ ID NO: 69), YQQQ (SEQ ID NO: 70), YHAE (SEQ ID NO: 71), YLDL (SEQ ID NO: 74), YLIP (SEQ ID NO: 75), YLRV (SEQ ID NO: 76), YTAV (SEQ ID NO: 82), or YVHV (SEQ ID NO: 84). In certain exampleembodiments, the mutated YMN_M motif consists of the amino acid sequence set forth in YGGG (SEQ ID NO: 44).

In certain exampleembodiments, the mutated YMN_M motif is capable of modulating PI3K signaling by limiting the number of methionine residues that can bind to a p85 subunit of PI3K. In certain exampleembodiments, the mutated YMN_M motif consists of the amino acid sequence set forth in YMN_x (SEQ ID NO: 38) or Y_xNM (SEQ ID NO: 39), wherein x is not a methionine (M). In certain exampleembodiments, the mutated YMN_M motif consists of the amino acid sequence set forth in YMN_V (SEQ ID NO: 40), YENM (SEQ ID NO: 41), and YMN_Q (SEQ ID NO: 42), YMN_L (SEQ ID NO: 78), or YSNM (SEQ ID NO: 81).

In certain exampleembodiments, the extracellular antigen-binding domain binds to an antigen. In certain exampleembodiments, the antigen is a tumor antigen or a pathogen antigen. In certain exampleembodiments, the antigen is a tumor antigen. In certain exampleembodiments, the tumor antigen is selected from the group consisting of CD19, mesothelin, AXL, TIM3, HVEM, MUC16, MUC1, CAIX, 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 (e.g., Eerb-B2, Erb-B3, Erb-B4), 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, ERBB2, 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. In certain [example embodiments](#), the tumor antigen is CD19.

In certain [example embodiments](#), the mutated YMN motif consists of the amino acid sequence set forth in YMDM (SEQ ID NO: 32). In certain [example embodiments](#), the extracellular antigen-binding domain binds to CD19. In certain [example embodiments](#), the CAR comprises the amino acid sequence set forth in SEQ ID NO: 51.

In certain [example embodiments](#), the mutated YMN motif consists of the amino acid sequence set forth in YKNI (SEQ ID NO: 26). In certain [example embodiments](#), the extracellular antigen-binding domain binds to CD19. In certain [example embodiments](#), the CAR comprises the amino acid sequence set forth in SEQ ID NO: 55.

In certain [example embodiments](#), the mutated YMN motif consists of the amino acid sequence set forth in YENV (SEQ ID NO: 22). In certain [example embodiments](#), the extracellular antigen-binding domain binds to CD19. In certain [example embodiments](#), the CAR comprises the amino acid sequence set forth in SEQ ID NO: 53.

In certain [example embodiments](#), the mutated YMN motif consists of the amino acid sequence set forth in YSNV (SEQ ID NO: 64). In certain [example embodiments](#), the extracellular antigen-binding domain binds to CD19. In certain [example embodiments](#), the CAR comprises the amino acid sequence set forth in SEQ ID NO: 57.

In certain [example embodiments](#), the mutated YMN motif consists of the amino acid sequence set forth in YGGG (SEQ ID NO: 63). In certain [example embodiments](#), the extracellular antigen-binding domain binds to CD19. In certain [example embodiments](#), the CAR comprises the amino acid sequence set forth in SEQ ID NO: 61.

The presently disclosed subject matter also provides cells comprising a CAR described herein. In certain [example embodiments](#), the cell is an immunoresponsive cell. In certain [example embodiments](#), the cell is a cell of the lymphoid lineage or a cell of the myeloid lineage. In certain [example embodiments](#), the cell is selected from the group consisting of T cells, Natural Killer (NK) cells, and stem cells from which lymphoid cells may be differentiated. In certain [example embodiments](#), the cell is a T cell. In certain [example embodiments](#), the T cell is selected from the group consisting of a cytotoxic T lymphocyte (CTL), a $\gamma\delta$ T cell, a tumor-infiltrating lymphocyte (TIL), a regulatory T cell, a Natural Killer T (NKT) cell, and a tumor-reactive lymphocyte.

Furthermore, the presently discloses subject matter provides compositions comprising a cell described herein. In certain example embodiments, the composition is a pharmaceutical composition that further comprises a pharmaceutically acceptable excipient. In certain example embodiments, the composition is for treating and/or preventing a neoplasm, and/or a pathogen infection.

The presently discloses subject matter further provides methods of reducing tumor burden in a subject. In certain example embodiments, the method comprises administering to the subject a cell described herein or a composition described herein. In certain example embodiments, the method reduces the number of tumor cells, reduces tumor size, and/or eradicates the tumor in the subject.

The presently discloses subject matter further provides methods of treating and/or preventing a neoplasm. In certain example embodiments, the method comprises administering to the subject a cell described herein or a composition described herein.

The presently discloses subject matter further provides methods of lengthening survival of a subject having a neoplasm. In certain example embodiments, the method comprises administering to the subject a cell described herein or a composition described herein.

In certain example embodiments, 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).

The presently discloses subject matter further provides methods for producing an antigen-specific cell. In certain example embodiments, the method comprises introducing into a cell a nucleic acid sequence encoding a CAR described herein. In certain example embodiments, the nucleic acid sequence is present on a vector. In certain example embodiments, the vector is a retroviral vector.

In addition, the presently discloses subject matter provides nucleic acid molecules encoding CARs described herein. In certain example embodiments, the nucleic acid molecule comprises the nucleotide sequence set forth in SEQ ID NO: 52, SEQ ID NO: 54, SEQ ID NO: 56, or SEQ ID NO: 58. The presently discloses subject matter further provides vectors comprising the nucleic acid molecules described herein. In certain example embodiments, the vector is a γ -retroviral vector.

The presently discloses subject matter further provides host cells expressing the nucleic acid molecule described herein. In certain example embodiments, the host cell is a T cell.

Furthermore, the presently discloses subject matter kits comprising a CAR described herein, a cell described herein, a composition described herein, a nucleic acid molecule described herein, or a vector described herein. In certain

examplesembodiments, the kit further comprises written instructions for treating and/or preventing a neoplasm and/or a pathogen infection.

4. BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 is a schematic showing of physiological CD28 signaling.

Fig. 2 is a schematic showing of CD28 modifications to modulate PI3Kp85 binding to CD28.

Figs. 3A-3D show that CD28-YKNI mutant CAR T cells had potent killing capacity *in vitro*, which was comparable to certain CAR T cells. Human CD19-targeted CAR T cells expressing a truncated EGFR domain (Etah19) were cocultured with CD19+ NALM6 cells expressing GFP-ffLuciferase (NALM6gL) at different effector:tumor ratios. Tumor cell lysis (relative to a non-signaling CAR T cell) was measured by bioluminescence 24 hours later. h28Z: CD28-based + CD3Z signaling domain; hBBZ: 4-1BB-based + CD3Z signaling domain; h28h1XX: CD28-based + CD3Z signaling domain with mutated ITAM 2 and ITAM 3; hYKNIZ: mutated CD28-based (YMNM->YKNI) + CD3Z signaling domain. pStimx# refers to post-stimulation, where the number indicates the number of prior stimulations. Fig. 3A shows tumor cell lysis results of post manufacture. Fig. 3B shows tumor cell lysis results of post 1 stimulation. Fig. 3C shows tumor cell lysis results of post 2 stimulations. Fig. 3D shows tumor cell lysis results of post 4 stimulations.

Figs. 4A-4N show that mutant CD28 CAR T cells demonstrated a divergent pro-inflammatory cytokine secretion profile. Human CD19-targeted CAR T cells were cultured ALONE cocultured with CD19+ NALM6 cells at an effector:tumor ratio of 1:1. 24 hours later, supernatant was collected and cytokines were measured utilizing a bead-based multiplex assay. Figs. 4A-4G show the cytokines profile from donor V, and Figs. 4H-4N show the cytokine profile from donor IV. (Fig. 4A, 4H) GMCSF; (Figs. 4B, 4I) IFN- γ ; (Figs. 4C, 4J) IL-13; (Figs. 4D-4K) IL-17; (Figs. 4E-4L) IL-9; (Figs. 4F-4M) IL-2; (Figs. 4G-4N) TNF- α .

Fig. 5 shows that CD28-YKNI mutant CAR T cells had no quantitative differences in proliferation in response to repeated antigen exposure. Human CD19-targeted CAR T cells were cocultured with NALM6 at an E:T ratio of 1:5 and at a concentration of 50,000 CAR T cells/mL. Roughly every 5 days, CAR T cells were

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.
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 claim 3, wherein 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).
5. The CAR of claim 3 or 4, wherein 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), YHNL (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).
6. The CAR of any one of claims 3-5, 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).
7. The CAR of any one of claims 3-6, wherein the mutated YMNM motif consists of the amino acid sequence set forth in YSNV (SEQ ID NO: 23).
8. The CAR of any one of claims 3-7, wherein the mutated YMNM motif binds to growth factor receptor bound receptor 2 (Grb2) and/or Grb2-related adaptor downstream of Shc (GADS).
9. The CAR of claim 1 or 2, wherein the mutated YMNM motif does not bind to Grb2 and/or GADS.

10. The CAR of claim 9, wherein 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).
11. The CAR of claim 9 or 10, wherein 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).
12. The CAR of any one of claims 9-11, wherein the mutated YMNm motif consists of the amino acid sequence set forth in YMDM (SEQ ID NO: 32).
13. The CAR of claim 9, wherein the mutated YMNm 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).
14. The CAR of claim 13, wherein the mutated YMNm motif consists of the amino acid sequence set forth in YTHM (SEQ ID NO: 34), YVLM (SEQ ID NO: 35), YIAM (SEQ ID NO: 36), YVEM (SEQ ID NO: 83), YVKM (SEQ ID NO: 85), or YVPM (SEQ ID NO: 86).
15. The CAR of claim 9, wherein the mutated YMNm motif consists of the amino acid sequence set forth in YMxb (SEQ ID NO: 65), wherein x is not an aspartic acid (N), and b is not a methionine (M).
16. The CAR of claim 15, wherein the mutated YMNm motif consists of the amino acid sequence set forth in YMAP (SEQ ID NO: 77).
17. The CAR of any one of claims 9-16, wherein a p85 subunit of a PI3K signaling binds to the mutated YMNm motif.
18. The CAR of claim 1 or 2, wherein the mutated YMNm motif does not bind to Grb2 and/or GADS or a p85 subunit of a PI3K.
19. The CAR of claim 18, wherein the mutated YMNm motif consists of the amino acid sequence set forth in Ybxb (SEQ ID NO: 43), wherein x is not an aspartic acid (N), and b is not a methionine (M).
20. The CAR of claim 19, wherein the mutated YMNm motif consists of the amino acid sequence set forth in YGGG (SEQ ID NO: 44), YAAA (SEQ ID NO: 45), YFFF (SEQ ID NO: 46), YETV (SEQ ID NO: 69), YQQQ (SEQ ID NO: 70), YHAE (SEQ ID

NO: 71), YLDL (SEQ ID NO: 74), YLIP (SEQ ID NO: 75), YLRV (SEQ ID NO: 76), YTAV (SEQ ID NO: 82), or YVHV (SEQ ID NO: 84).

21. The CAR of any one of claims 18-20, wherein the mutated YMN_M motif consists of the amino acid sequence set forth in YGGG (SEQ ID NO: 44).

5 22. The CAR of claim 1 or 2, wherein the mutated YMN_M motif is capable of modulating PI3K signaling by limiting the number of methionine residues that can bind to a p85 subunit of PI3K.

23. The CAR of claim 22, wherein the mutated YMN_M motif consists of the amino acid sequence set forth in YMN_x (SEQ ID NO: 38) or Y_xNM (SEQ ID NO: 39), wherein
10 x is not a methionine (M).

24. The CAR of claim 22 or 23, wherein the mutated YMN_M motif consists of the amino acid sequence set forth in YMN_V (SEQ ID NO: 40), YEN_M (SEQ ID NO: 41), and YMN_Q (SEQ ID NO: 42), YMN_L (SEQ ID NO: 78), or YSN_M (SEQ ID NO: 81).

25. The CAR of any one of claims 1-24, wherein the extracellular antigen-binding
15 domain binds to an antigen.

26. The CAR of claim 25, wherein the antigen is a tumor antigen or a pathogen antigen.

27. The CAR of claim 25 or 26, wherein the antigen is a tumor antigen.

28. The CAR of claim 27, wherein the tumor antigen is selected from the group
20 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,
25 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.

30 29. The CAR of claim 28, wherein the tumor antigen is CD19.

30. The CAR of any one of claims 1-29, wherein the mutated YMNm motif consists of the amino acid sequence set forth in YMDM (SEQ ID NO: 32).
31. The CAR of claim 30, wherein the extracellular antigen-binding domain binds to CD19.
- 5 32. The CAR of claim 31, wherein the CAR comprises the amino acid sequence set forth in SEQ ID NO: 51.
33. The CAR of any one of claims 1-29, wherein the mutated YMNm motif consists of the amino acid sequence set forth in YKNI (SEQ ID NO: 26).
34. The CAR of claim 33, wherein the extracellular antigen-binding domain binds to
10 CD19.
35. The CAR of claim 34, wherein the CAR comprises the amino acid sequence set forth in SEQ ID NO: 55.
36. The CAR of any one of claims 1-29, wherein the mutated YMNm motif consists of the amino acid sequence set forth in YENV (SEQ ID NO: 22).
- 15 37. The CAR of claim 36, wherein the extracellular antigen-binding domain binds to CD19.
38. The CAR of claim 37, wherein the CAR comprises the amino acid sequence set forth in SEQ ID NO: 53.
39. The CAR of any one of claims 1-29, wherein the mutated YMNm motif consists
20 of the amino acid sequence set forth in YSNV (SEQ ID NO: 64).
40. The CAR of claim 39 wherein the extracellular antigen-binding domain binds to CD19.
41. The CAR of claim 40, wherein the CAR comprises the amino acid sequence set forth in SEQ ID NO: 57.
- 25 42. The CAR of any one of claims 1-29, wherein the mutated YMNm motif consists of the amino acid sequence set forth in YGGG (SEQ ID NO: 63).
43. The CAR of claim 42, wherein the extracellular antigen-binding domain binds to CD19.

44. The CAR of claim 43, wherein the CAR comprises the amino acid sequence set forth in SEQ ID NO: 61.
45. A cell comprising the CAR of any one of claims 1-44.
46. The cell of claim 45, wherein the cell is an immunoresponsive cell.
- 5 47. The cell of claim 45 or 46, wherein the cell is a cell of the lymphoid lineage or a cell of the myeloid lineage.
48. The cell of any one of claims 45-47, wherein the cell is selected from the group consisting of T cells, Natural Killer (NK) cells, stem cells from which lymphoid cells may be differentiated.
- 10 49. The cell of any one of claims 45-48, wherein the cell is a T cell.
50. The cell of claim 48 or 49, 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.
- 15 51. A composition comprising a cell of any one of claims 45-50.
52. The composition of claim 51, which is a pharmaceutical composition that further comprises a pharmaceutically acceptable excipient.
53. The composition of claim 51 or 52, which is for treating and/or preventing a neoplasm or a tumor, and/or a pathogen infection.
- 20 54. Use of the cell of any one of claims 45-50 or the composition of any one of claims 51-53 in the manufacture of a medicament for~~A method of~~ reducing tumor burden in a subject, ~~wherein the method comprising administering to the subject the~~ a cell of any one of claims 45-50 or the composition ~~of any one of claims 51-53~~ is to be administered to the subject.
- 25 55. The ~~use~~method of claim 54, wherein ~~the method reduces~~ the number of tumor cells is reduced, ~~reduces the~~ tumor size is reduced, and/or ~~eradicates~~ the tumor is eradicated in the subject.
56. Use of the cell of any one of claims 45-50 or the composition of any one of claims 51-53 in the manufacture of a medicament for ~~A method of~~ treating and/or
30 preventing a neoplasm or a tumor, wherein the cell or the composition ~~the method~~

~~comprising is to be administered to the subject a cell of any one of claims 45-50 or a composition of any one of claims 51-53.~~

57. Use of the cell of any one of claims 45-50 or the composition of any one of claims 51-53 in the manufacture of a medicament for ~~A method of~~ lengthening survival of a subject having a neoplasm or a tumor, wherein the cell or the composition is to be administered to ~~the method comprising administering to the subject a cell of any one of claims 45-50 or a composition of any one of claims 51-53.~~

58. The ~~use method~~ of any one of claims 54-57, 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).

59. 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 1-44.

60. The method of claim 59, wherein the nucleic acid molecule is present on a vector.

61. The method of claim 60, wherein the vector is a retroviral vector.

62. A nucleic acid molecule encoding the CAR of any one of claims 1-44.

63. The nucleic acid molecule of claim 62, comprising the nucleotide sequence set forth in SEQ ID NO: 52, SEQ ID NO: 54, SEQ ID NO: 56, or SEQ ID NO: 58.

64. A vector comprising the nucleic acid molecule of claim 62 or 63.

65. The vector of claim 64, wherein the vector is a γ -retroviral rector.

66. A host cell expressing the nucleic acid molecule of claim 64 or 65.

67. The host cell of claim 66, wherein the host cell is a T cell.

68. A kit comprising a CAR of any one of claims 1-44, a cell of any one of claims 35-40, a composition of any one of claims 51-53, a nucleic acid molecule of claim 62 or 63, or a vector of claim 64 or 65.

69. The kit of claim 68, wherein the kit further comprises written instructions for treating and/or preventing a neoplasm, a tumor, and/or a pathogen infection.

70. The cell of any one of claims 45-50 or the composition of any one of claims 51-53 for use in therapy.