

CLAIMS

1. An isolated T cell or NK cell or a population of T cells or NK cells comprising a chimeric antigen receptor (CAR), wherein said CAR comprises:

(a) a first CAR comprising: a first antigen-binding domain comprising a heavy chain variable domain (VH) and a light chain variable domain (VL) which binds to BCMA (a BCMA CAR); a first transmembrane domain; and a first intracellular signaling domain comprising a first costimulatory signaling domain and a first primary signaling domain;

and

(b) a second CAR comprising: a second antigen-binding domain comprising a VH and a VL which binds to CD19 (a CD19 CAR); a second transmembrane domain; and a second intracellular signaling domain comprising a second costimulatory signaling domain and a second primary signaling domain;

wherein the first CAR and the second CAR each comprise an HCDR1, HCDR2, HCDR3, LCDR1, LCDR2, and LCDR3 as recited in one of SEQ ID NO: 214, 216, 218, 220, or 222; and

wherein the CAR comprises the amino acid sequence of SEQ ID NO: 214, 216, 218, 220, 222, or an amino acid sequence having at least 95%, 96%, 97%, 98%, or 99% identity thereto.

2. The isolated T cell or NK cell or population of T cells or NK cells of claim 1, wherein the first CAR is encoded by a first nucleic acid sequence and the second CAR is encoded by a second nucleic acid sequence, wherein the first and second nucleic acid sequences are disposed on a single nucleic acid molecule.

3. The isolated T cell or NK cell or population of T cells or NK cells of claim 2, wherein:

(a) the single nucleic acid molecule comprises the following configuration in a 5' to 3' orientation:

(i) a nucleic acid sequence encoding the first antigen-binding domain, a nucleic acid sequence encoding the first transmembrane domain, a nucleic acid sequence encoding the first intracellular signaling domain, a nucleic acid sequence encoding a linker comprising a P2A site, a nucleic acid sequence encoding the second antigen-binding domain, a nucleic acid sequence encoding the second transmembrane domain, a nucleic acid sequence encoding the second intracellular signaling domain; or

(ii) a nucleic acid sequence encoding the second antigen-binding domain, a nucleic acid sequence encoding the second transmembrane domain, a nucleic acid sequence encoding the second intracellular signaling domain, a nucleic acid sequence encoding a linker comprising a P2A site, a nucleic acid sequence encoding the first antigen-binding domain, a nucleic acid sequence encoding the first transmembrane domain, a nucleic acid sequence encoding the first intracellular signaling domain;

(b) the single nucleic acid molecule comprises the nucleic acid sequence of SEQ ID NO: 215, 217, 219, 221, or 223, or a nucleic acid sequence having at least 90%, 95%, or 99% sequence identity thereto; or

(c) the single nucleic acid molecule encodes the amino acid sequence of SEQ ID NO: 214, 216, 218, 220, or 222, or an amino acid sequence having at least 95% or 99% sequence identity thereto.

4. The isolated T cell or NK cell or population of T cells or NK cells of claim 3, wherein the linker comprising the P2A site:

(I) is encoded by the nucleic acid sequence of SEQ ID NO: 209, or a nucleic acid sequence having at least 90%, 95%, or 99% sequence identity thereto; or

(II) comprises the amino acid sequence of SEQ ID NO: 208, or an amino acid sequence having at least 90%, 95%, or 99% sequence identity thereto.

5. The isolated T cell or NK cell or population of T cells or NK cells of claim 1, wherein the VH and VL are connected by a linker, and wherein the linker comprises the amino acid sequence of

SEQ ID NO: 5, or an amino acid sequence having at least 90%, 95%, or 99% sequence identity thereto.

6. The isolated T cell or NK cell or population of T cells or NK cells of claim 2, wherein:

(i) the first transmembrane domain or second transmembrane domain comprises the amino acid sequence of SEQ ID NO: 6, or an amino acid sequence having at least 90%, 95%, or 99% sequence identity thereto;

(ii) the first transmembrane domain or second transmembrane domain is encoded by the nucleic acid sequence of SEQ ID NO: 17, or a nucleic acid sequence having at least 90%, 95%, or 99% sequence identity thereto;

(iii) the first antigen-binding domain is connected to the first transmembrane domain by a first hinge region, or the second antigen-binding domain is connected to the first transmembrane domain by a second hinge region;

(iv) the primary signaling domain comprises a functional signaling domain derived from CD3 zeta, wherein:

(a) the primary signaling domain comprises the amino acid sequence of SEQ ID NO: 9 or 10, or an amino acid sequence having at least 90%, 95%, or 99% sequence identity thereto; or

(b) the primary signaling domain is encoded by the nucleic acid sequence of SEQ ID NO: 20, 21, or 205, or a nucleic acid sequence having at least 90%, 95%, or 99% sequence identity thereto;

(v) the costimulatory signaling domain comprises a functional signaling domain derived from 4-1BB (CD137); wherein:

(a) the costimulatory signaling domain comprises the amino acid sequence of SEQ ID NO: 7, or an amino acid sequence having at least 90%, 95%, or 99% sequence identity thereto; or

(b) the costimulatory signaling domain is encoded by the nucleic acid sequence of SEQ ID NO: 18 or 204, or a nucleic acid sequence having at least 90%, 95%, or 99% sequence identity thereto;

(vi) the first intracellular signaling domain or second intracellular signaling domain comprises a functional signaling domain derived from 4-1BB and a functional signaling domain derived from CD3 zeta, wherein:

(a) the first intracellular signaling domain or second intracellular signaling domain comprises the amino acid sequence of SEQ ID NO: 7 or an amino acid sequence having at least 90%, 95%, or 99% sequence identity thereto, and the amino acid sequence of SEQ ID NO: 9 or 10 or an amino acid sequence having at least 90%, 95%, or 99% sequence identity thereto, or

(b) the first intracellular signaling domain or second intracellular signaling domain comprises the amino acid sequence of SEQ ID NO: 7 and the amino acid sequence of SEQ ID NO: 9 or 10; or

(vii) the first CAR further comprises a first leader sequence or second CAR further comprises a second leader sequence, wherein:

(a) the first or second leader sequence comprises the amino acid sequence of SEQ ID NO: 1, or an amino acid sequence having at least 90%, 95%, or 99% sequence identity thereto; or

(b) the first or second leader sequence is encoded by the nucleic acid sequence of SEQ ID NO: 199 or 210, or a nucleic acid sequence having at least 90%, 95%, or 99% sequence identity thereto.

7. The isolated T cell or NK cell or population of T cells or NK cells of claim 6, wherein:

(a) the first or second hinge region comprises the amino acid sequence of SEQ ID NO: 2, or an amino acid sequence having at least 90%, 95%, or 99% sequence identity thereto;

(b) the first or second hinge region is encoded by the nucleic acid sequence of SEQ ID NO: 13, or a nucleic acid sequence having at least 90%, 95%, or 99% sequence identity thereto;

(c) the first or second hinge region and the first or second transmembrane domain comprise the amino acid sequence of SEQ ID NO: 202, or an amino acid sequence having at least 90%, 95%, or 99% sequence identity thereto; or

(d) the first or second hinge region and the first or second transmembrane domain are encoded by the nucleic acid sequence of SEQ ID NO: 203 or 213, or a nucleic acid sequence having at least 90%, 95%, or 99% sequence identity thereto.

8. The isolated T cell or NK cell or population of T cells or NK cells of claim 6, wherein:

(a):

(i) the first leader sequence and the second leader sequence are encoded by different nucleic acid sequences;

(ii) the first hinge region and the second hinge region are encoded by different nucleic acid sequences;

(iii) the first transmembrane domain and the second transmembrane domain are encoded by different nucleic acid sequences; and/or

(iv) the first intracellular signaling domain and the second intracellular signaling domain are encoded by different nucleic acid sequences;

(b):

(i) the first leader sequence and the second leader sequence comprise the same amino acid sequence, or comprise different amino acid sequences;

(ii) the first hinge region and the second hinge region comprise the same amino acid sequence, or comprise different amino acid sequences;

(iii) the first transmembrane domain and the second transmembrane domain comprise the same amino acid sequence, or comprise different amino acid sequences; and/or

(iv) the first intracellular signaling domain and the second intracellular signaling domain comprise the same amino acid sequence, or comprise different amino acid sequences; and/or

(c):

(i) the first leader sequence and the second leader sequence are encoded by nucleic acid sequences comprising SEQ ID NOs: 199 and 210, respectively, or a nucleic acid sequence having at least 90%, 95%, or 99% sequence identity thereto, or SEQ ID NOs: 210 and 199, respectively or a nucleic acid sequence having at least 90%, 95%, or 99% sequence identity thereto;

(ii) the first hinge region and the second hinge region are encoded by nucleic acid sequences comprising SEQ ID NOs: 337 and 13, respectively or a nucleic acid sequence having at least 90%, 95%, or 99% sequence identity thereto; or SEQ ID NOs: 13 and 337, respectively or a nucleic acid sequence having at least 90%, 95%, or 99% sequence identity thereto;

(iii) the first transmembrane domain and the second transmembrane domain are encoded by nucleic acid sequences comprising SEQ ID NOs: 338 and 17, respectively or a nucleic acid sequence having at least 90%, 95%, or 99% sequence identity thereto; or SEQ ID NOs: 17 and 338, respectively or a nucleic acid sequence having at least 90%, 95%, or 99% sequence identity thereto;

(iv) the first costimulatory signaling domain and the second costimulatory signaling domain are encoded by nucleic acid sequences comprising SEQ ID NOs: 204 and 18, respectively or a nucleic acid sequence having at least 90%, 95%, or 99% sequence identity thereto; or SEQ ID NOs: 18 and 204, respectively or a nucleic acid sequence having at least 90%, 95%, or 99% sequence identity thereto; and/or

(v) the first primary signaling domain and the second primary signaling domain are encoded by nucleic acid sequences comprising SEQ ID NOs: 205 and 21, respectively or a nucleic acid sequence having at least 90%, 95%, or 99% sequence identity thereto; or SEQ ID NOs: 21 and 205, respectively or a nucleic acid sequence having at least 90%, 95%, or 99% sequence identity thereto; or

(d) the first CAR or second CAR is encoded by a nucleic acid molecule comprising a woodchuck hepatitis post-transcriptional regulatory element (WPRE).

9. The isolated T cell or NK cell or population of T cells or NK cells of claim 8, wherein:

(a) the first primary signaling domain and the second primary signaling domain are encoded by different nucleic acid sequences;

(b) the first costimulatory signaling domain and the second costimulatory signaling domain are encoded by different nucleic acid sequences;

(c) the first leader sequence and the second leader sequence comprise the amino acid sequence of SEQ ID NO: 1, or an amino acid sequence having at least 90%, 95%, or 99% sequence identity thereto;

(d) the first hinge region and the second hinge region comprise the amino acid sequence of SEQ ID NO: 2, or an amino acid sequence having at least 90%, 95%, or 99% sequence identity thereto;

(e) the first transmembrane domain and the second transmembrane domain comprise the amino acid sequence of SEQ ID NO: 6, or an amino acid sequence having at least 90%, 95%, or 99% sequence identity thereto;

(f) the first primary signaling domain and the second primary signaling domain comprise the amino acid sequence of SEQ ID NO: 10, or an amino acid sequence having at least 90%, 95%, or 99% sequence identity thereto; or

(g) the first costimulatory signaling domain and the second costimulatory signaling domain comprise the amino acid sequence of SEQ ID NO: 7, or an amino acid sequence having at least 90%, 95%, or 99% sequence identity thereto.

10. An isolated nucleic acid molecule encoding a chimeric antigen receptor (CAR), said nucleic acid molecule comprising:

(a) a first nucleic acid sequence encoding a first CAR comprising: a first antigen-binding domain comprising a heavy chain variable domain (VH) and a light chain variable

domain (VL) which binds to BCMA (a BCMA CAR); a first transmembrane domain; and a first intracellular signaling domain comprising a costimulatory signaling domain and a first primary signaling domain; and

(b) a second nucleic acid sequence encoding a second CAR comprising: a second antigen-binding domain comprising a VH and a VL which binds to CD19 (a CD19 CAR); a second transmembrane domain; and a second intracellular signaling domain comprising a second costimulatory signaling domain and a second primary signaling domain;

wherein the first CAR and the second CAR each comprise an HCDR1, HCDR2, HCDR3, LCDR1, LCDR2, and LCDR3 as recited in one of SEQ ID NO: 214, 216, 218, 220, or 222; and

wherein the encoded CAR comprises the amino acid sequence of SEQ ID NO: 214, 216, 218, 220, 222, or an amino acid sequence having at least 95%, 96%, 97%, 98%, or 99% identity thereto; and wherein the first nucleic acid sequence and the second nucleic acid sequence are disposed on a single nucleic acid molecule.

11. An isolated CAR, wherein the CAR comprises:

(a) a first CAR comprising: a first antigen-binding domain comprising a heavy chain variable domain (VH) and a light chain variable domain (VL) which binds to BCMA (a BCMA CAR); a first transmembrane domain; and a first intracellular signaling domain comprising a costimulatory signaling domain and a first primary signaling domain; and

(b) a second CAR comprising a second antigen-binding domain comprising a VH and a VL which binds to CD19 (a CD19 CAR); a second transmembrane domain; and a second intracellular signaling domain comprising a second costimulatory signaling domain and a second primary signaling domain;

wherein the first CAR and the second CAR each comprise an HCDR1, HCDR2, HCDR3, LCDR1, LCDR2, and LCDR3 as recited in one of SEQ ID NO: 214, 216, 218, 220, or 222; and

wherein the CAR comprises the amino acid sequence of SEQ ID NO: 214, 216, 218, 220, 222, or an amino acid sequence having at least 95%, 96%, 97%, 98%, or 99% identity thereto.

12. A vector comprising the nucleic acid molecule of claim 10.

13. The vector of claim 12, wherein the vector is chosen from a DNA vector, an RNA vector, a plasmid, a lentivirus vector, an adenoviral vector, or a retrovirus vector.

14. An isolated T cell or NK cell or a population of T cells or NK cells comprising the nucleic acid molecule of claim 10.

15. A method of making a T cell or NK cell comprising transducing a cell with the vector of claim 12 or 13.

16. A method of making an RNA-engineered cell comprising introducing an in vitro transcribed RNA or synthetic RNA into a cell, wherein the RNA comprises the nucleic acid molecule of claim 10, wherein the cell is a T cell or NK cell.

17. A pharmaceutical composition comprising the T cell or NK cell or population of T cells or NK cells of any one of claims 1-9 or 14, and a pharmaceutically acceptable carrier.

18. Use of an effective amount of:

(a) a T cell or NK cell or population of T cells or NK cells comprising: (i) the nucleic acid molecule of claim 10, (ii) the CAR molecule of claim 11, or (iii) the vector of claim 12 or 13;

(b) the T cell or NK cell or population of T cells or NK cells of any one of claims 1-9 or 14; or

(c) the pharmaceutical composition of claim 17
in the manufacture of a medicament for providing an anti-tumor immunity in a subject or for treating a disease associated with expression of BCMA.

19. The use of claim 18, wherein the disease associated with BCMA expression is:

- (i) a cancer or malignancy, or a precancerous condition chosen from one or more of a myelodysplasia, a myelodysplastic syndrome, or a preleukemia,
- (ii) a non-cancer related indication associated with expression of BCMA;
- (iii) a hematologic cancer or a solid cancer;
- (iv) chosen from: acute leukemia, B-cell acute lymphoblastic leukemia (“BALL”), T-cell acute lymphoblastic leukemia (“TALL”), acute lymphoblastic leukemia (ALL), chronic myelogenous leukemia (CML), chronic lymphocytic leukemia (CLL), B cell prolymphocytic leukemia, blastic plasmacytoid dendritic cell neoplasm, Burkitt's lymphoma, diffuse large B cell lymphoma, follicular lymphoma, hairy cell leukemia, small cell- or large cell-follicular lymphoma, malignant lymphoproliferative conditions, MALT lymphoma, mantle cell lymphoma, Marginal zone lymphoma, multiple myeloma, myelodysplasia and myelodysplastic syndrome, non-Hodgkin's lymphoma, plasmablastic lymphoma, plasmacytoid dendritic cell neoplasm, Waldenstrom macroglobulinemia, prostate cancer, pancreatic cancer, lung cancer, a plasma cell proliferative disorder, monoclonal gammopathy of undetermined significance (MGUS), plasmacytoma, systemic amyloid light chain amyloidosis, or POEMS syndrome (also known as Crow-Fukase syndrome, Takatsuki disease, and PEP syndrome)), or a combination thereof; or
- (v) multiple myeloma.

20. The use of claim 18 or 19, wherein the T cell or NK cell or population of T cells or NK cells is formulated for administration in combination with a second therapeutic agent.

21. The use of claim 20, wherein the second therapeutic agent is chosen from:

- (i) a PD-1 inhibitor;

- (ii) a PD-L1 inhibitor;
- (iii) a LAG-3 inhibitor;
- (iv) a TIM-3 inhibitor;
- (v) a CTLA-4 inhibitor;
- (vi) an interleukin-15 (IL-15) polypeptide, an interleukin-15 receptor alpha (IL-15Ra) polypeptide, or a combination of both an IL-15 polypeptide and an IL-15Ra polypeptide;
- (vii) an interleukin-12 (IL-12) polypeptide; or
- (viii) an mTOR inhibitor.

22. The use of claim 21, wherein:

- (i) the PD-1 inhibitor is selected from the group consisting of PDR001, Nivolumab, Pembrolizumab, Pidilizumab, MEDI0680, REGN2810, TSR-042, PF-06801591, and AMP-224;
- (ii) the PD-L1 inhibitor is selected from the group consisting of FAZ053, Atezolizumab, Avelumab, Durvalumab, and BMS-936559;
- (iii) the LAG-3 inhibitor is selected from the group consisting of LAG525, BMS-986016, TSR-033, MK-4280 and REGN3767;
- (iv) the TIM-3 inhibitor is selected from the group consisting of MBG453, TSR-022, and LY3321367;
- (v) the CTLA-4 inhibitor is Ipilimumab or Tremelimumab; or
- (vi) the mTOR inhibitor is RAD001 or rapamycin.