

CLAIMS

1. A cell comprising a first chimeric antigen receptor (CAR) and a second CAR, each of which comprises an antigen binding domain, a transmembrane domain, and an intracellular signaling domain, wherein the antigen binding domain of said first CAR binds to a B-Cell antigen and the antigen binding domain of said second CAR binds to a tumor antigen other than a B-Cell antigen, optionally wherein the B-Cell antigen and the tumor antigen other than a B-Cell antigen are not expressed on the same cell, optionally wherein the second CAR binds:

- (a) a solid tumor antigen;
- (b) a myeloid tumor antigen; or
- (c) an antigen of a hematological tumor not of B-cell lineage.

2. The cell of claim 1, wherein said B-Cell antigen is selected from the group consisting of CD5, CD10, CD19, CD20, CD21, CD22, CD23, CD24, CD25, CD27, CD30, CD34, CD37, CD38, CD40, CD53, CD69, CD72, CD73, CD74, CD75, CD77, CD79a, CD79b, CD80, CD81, CD82, CD83, CD84, CD85, CD86, CD123, CD135, CD138, CD179, CD269, Flt3, ROR1, BCMA, FcRn5, FcRn2, CS-1, CXCR4, 5, 7, IL-7/3R, IL7/4/3R, and IL4R, optionally wherein said B-Cell antigen is selected from the group consisting of CD19, CD20, CD22, FcRn5, FcRn2, BCMA, CS-1, and CD138.

3. The cell of claim 2, wherein said B-Cell antigen is BCMA, optionally wherein:

(a) said antigen binding domain of said first CAR comprises a heavy chain complementary determining region 1 (HC CDR1), a heavy chain complementary determining region 2 (HC CDR2), and a heavy chain complementary determining region 3 (HC CDR3) of any heavy chain binding domain amino acid sequence listed in Table 12 or 13;

(b) said antigen binding domain of said first CAR further comprises a light chain complementary determining region 1 (LC CDR1), a light chain complementary determining region 2 (LC CDR2), and a light chain complementary determining region 3 (LC CDR3) of any light chain binding domain amino acid sequence listed in Table 12 or 13;

(c) said antigen binding domain of said first CAR comprises:

(i) the amino acid sequence of any light chain variable region listed in Table 12 or 13;

(ii) an amino acid sequence having at least one, two or three modifications but not more than 20 or 10 modifications of the amino acid sequence of any of the light chain variable regions provided in Table 12 or 13; or

(iii) an amino acid sequence with 95-99% identity to the amino acid sequence of any of the light chain variable regions provided in Table 12 or 13;

(d) said antigen binding domain of said first CAR comprises:

(i) the amino acid sequence of any heavy chain variable region listed in Table 12 or 13;

(ii) an amino acid sequence having at least one, two or three modifications but not more than 20 or 10 modifications of the amino acid sequence of any of the heavy chain variable regions provided in Table 12 or 13; or

(iii) an amino acid sequence with 95-99% identity to the amino acid sequence of any of the heavy chain variable regions provided in Table 12 or 13;

(e) said antigen binding domain of said first CAR comprises a polypeptide having the amino acid sequence of any light chain variable region listed in Table 12 or 13, and the amino acid sequence of any heavy chain variable region listed in Table 12 or 13;

(f) said antigen binding domain of said first CAR comprises a polypeptide having the sequence of SEQ ID NO: 349; SEQ ID NO: 339, SEQ ID NO: 340; SEQ ID NO: 341; SEQ ID NO: 342; SEQ ID NO: 343; SEQ ID NO: 344, SEQ ID NO: 345, SEQ ID NO: 346, SEQ ID NO: 347, SEQ ID NO: 348, SEQ ID NO: 350, SEQ ID NO: 351, SEQ ID NO: 352, SEQ ID NO: 353, SEQ ID NO: 429, SEQ ID NO: 430, SEQ ID NO: 431, SEQ ID NO: 432, SEQ ID NO: 433, SEQ ID NO: 434, SEQ ID NO: 435, SEQ ID NO: 436, SEQ ID NO: 437, SEQ ID NO: 438, SEQ ID NO: 439, SEQ ID NO: 440, SEQ ID NO: 441, SEQ ID NO: 442, SEQ ID NO: 443, SEQ ID NO: 444, SEQ ID NO: 445, SEQ ID NO: 446, SEQ ID NO: 447, SEQ ID NO: 448, SEQ ID NO: 449, SEQ ID NO: 563, SEQ ID NO: 564, SEQ ID NO: 565 or SEQ ID NO: 566; or

(g) the first CAR comprises a polypeptide having an amino acid sequence selected from the group consisting of SEQ ID NO: 949, SEQ ID NO: 950, SEQ ID NO: 951, SEQ ID NO: 952, SEQ ID NO: 953, SEQ ID NO: 954, SEQ ID NO: 955, SEQ ID NO: 956, SEQ ID NO: 957, SEQ ID NO: 958, SEQ ID NO: 959, SEQ ID NO: 960, SEQ ID NO: 961, SEQ ID NO: 962, SEQ ID NO: 963, SEQ ID NO: 979, SEQ ID NO: 980, SEQ ID NO: 981, SEQ ID NO: 982, SEQ ID NO: 983, SEQ ID NO: 984, SEQ ID NO: 985, SEQ ID NO: 986, SEQ ID NO: 987, SEQ ID NO: 988, SEQ ID NO: 989, SEQ ID NO: 990, SEQ ID NO: 991, SEQ ID NO: 992, SEQ ID NO: 993, SEQ ID NO: 994, SEQ ID NO: 995, SEQ ID NO: 996, SEQ ID NO: 997, SEQ ID NO: 998, and SEQ ID NO: 999.

4. The cell of claim 2, wherein said B-Cell antigen is CD19, optionally wherein:

(a) said antigen binding domain of said first CAR comprises a heavy chain complementary determining region 1 (HC CDR1), a heavy chain complementary determining region 2 (HC CDR2), and a heavy chain complementary determining region 3 (HC CDR3) of any heavy chain binding domain amino acid sequence listed in Table 6, Table 7 or Table 9;

(b) said antigen binding domain of said first CAR further comprises a light chain complementary determining region 1 (LC CDR1), a light chain complementary determining region 2 (LC CDR2), and a light chain complementary determining region 3 (LC CDR3) of any light chain binding domain amino acid sequence listed in Table 6, Table 8 or Table 9;

(c) said antigen binding domain of said first CAR comprises:

(i) the amino acid sequence of any light chain variable region listed in Table 6 or Table 9;

(ii) an amino acid sequence having at least one, two or three modifications but not more than 20 or 10 modifications of the amino acid sequence of any of the light chain variable regions provided in Table 6 or Table 9; or

(iii) an amino acid sequence with 95-99% identity to the amino acid sequence of any of the light chain variable regions provided in Table 6 or Table 9;

(d) said antigen binding domain of said first CAR comprises:

(i) the amino acid sequence of any heavy chain variable region listed in Table 6 or Table 9;

(ii) an amino acid sequence having at least one, two or three modifications but not more than 20 or 10 modifications of the amino acid sequence of any of the heavy chain variable regions provided in Table 6 or Table 9; or

(iii) an amino acid sequence with 95-99% identity to the amino acid sequence of any of the heavy chain variable regions provided in Table 6 or Table 9;

(e) said antigen binding domain of said first CAR comprises a polypeptide having the amino acid sequence of any light chain variable region listed in Table 6 or Table 9, and the amino acid sequence of any heavy chain variable region listed in Table 6 or Table 9;

(f) said antigen binding domain of said first CAR comprises a polypeptide having the sequence of SEQ ID NO: 83; SEQ ID NO: 84, SEQ ID NO: 85; SEQ ID NO: 86; SEQ ID NO: 87; SEQ ID NO: 88; SEQ ID NO: 89, SEQ ID NO: 90, SEQ ID NO: 91, SEQ ID NO: 92, SEQ ID NO: 93, SEQ ID NO: 94, SEQ ID NO: 95, or SEQ ID NO: 112; or

(g) the first CAR comprises a polypeptide having an amino acid sequence selected from the group consisting of SEQ ID NO: 269, SEQ ID NO: 270, SEQ ID NO: 271, SEQ ID NO: 272, SEQ ID NO: 273, SEQ ID NO: 274, SEQ ID NO: 275, SEQ ID NO: 276, SEQ ID NO: 277, SEQ ID NO: 278, SEQ ID NO: 279, SEQ ID NO: 280, and SEQ ID NO: 281.

5. The cell of any one of claims 1-4, wherein said second CAR binds a myeloid tumor antigen, and wherein said myeloid tumor antigen is selected from the group consisting of CD123, CD33 and CLL-1.

6. The cell of any one of claims 1-4, wherein said second CAR binds a T cell lymphoma antigen.

7. The cell of any one of claims 1-4, wherein said second CAR binds a solid tumor antigen, and wherein said solid tumor antigen is selected from the group consisting of EGFRvIII, mesothelin, GD2, Tn antigen, sTn antigen, Tn-O-Glycopeptides, sTn-O-Glycopeptides, PSMA, CD97, TAG72, CD44v6, CEA, EPCAM, KIT, IL-13Ra2, leguman, GD3, CD171, IL-11Ra, PSCA, MAD-CT-1, MAD-CT-2, VEGFR2, LewisY, CD24, PDGFR-beta, SSEA-4, folate receptor alpha, ERBBs (e.g., ERBB2), Her2/neu, MUC1, EGFR, NCAM, Ephrin B2,

CAIX, LMP2, sLe, HMWMAA, o-acetyl-GD2, folate receptor beta, TEM1/CD248, TEM7R, FAP, Legumain, HPV E6 or E7, ML-IAP, CLDN6, TSHR, GPRC5D, ALK, Polysialic acid, Fos-related antigen, neutrophil elastase, TRP-2, CYP1B1, sperm protein 17, beta human chorionic gonadotropin, AFP, thyroglobulin, PLAC1, globoH, RAGE1, MN-CA IX, human telomerase reverse transcriptase, intestinal carboxyl esterase, mut hsp 70-2, NA-17, NY-BR-1, UPK2, HAVCR1, ADRB3, PANX3, NY-ESO-1, GPR20, Ly6k, OR51E2, TARP, GFR α 4, and a peptide of any of these antigens presented on MHC, optionally wherein said solid tumor antigen is selected from the group consisting of CLDN6, mesothelin and EGFRvIII.

8. The cell of claim 7, wherein said solid tumor antigen is EGFRvIII, optionally wherein:

(a) said antigen binding domain of said second CAR comprises a heavy chain complementary determining region 1 (HC CDR1), a heavy chain complementary determining region 2 (HC CDR2), and a heavy chain complementary determining region 3 (HC CDR3) of any anti-EGFRvIII heavy chain binding domain amino acid sequence listed in Table 5;

(b) said antigen binding domain of said second CAR further comprises a light chain complementary determining region 1 (LC CDR1), a light chain complementary determining region 2 (LC CDR2), and a light chain complementary determining region 3 (LC CDR3) of any anti-EGFRvIII light chain binding domain amino acid sequence listed in Table 5;

(c) said antigen binding domain of said second CAR comprises:

(i) the amino acid sequence of any anti-EGFRvIII light chain variable region listed in Table 5;

(ii) an amino acid sequence having at least one, two or three modifications but not more than 20 or 10 modifications of the amino acid sequence of any of the anti-EGFRvIII light chain variable regions provided in Table 5; or

(iii) an amino acid sequence with 95-99% identity to the amino acid sequence of any of the anti-EGFRvIII light chain variable regions provided in Table 5;

(d) said antigen binding domain of said second CAR comprises:

(i) the amino acid sequence of any anti-EGFRvIII heavy chain variable region listed in Table 5;

(ii) an amino acid sequence having at least one, two or three modifications but not more than 20 or 10 modifications of the amino acid sequence of any of the anti-EGFRvIII heavy chain variable regions provided in Table 5; or

(iii) an amino acid sequence with 95-99% identity to the amino acid sequence of any of the anti-EGFRvIII heavy chain variable regions provided in Table 5;

(e) said antigen binding domain of said second CAR comprises a polypeptide having the amino acid sequence of any anti-EGFRvIII light chain variable region listed in Table 5, and the amino acid sequence of any anti-EGFRvIII heavy chain variable region listed in Table 5;

(f) said antigen binding domain of said second CAR comprises a polypeptide having a sequence of any of SEQ ID NOS: 71-79; or

(g) the second CAR comprises a polypeptide having an amino acid sequence selected from the group consisting of SEQ ID NO: 1043, SEQ ID NO: 1049, SEQ ID NO: 1055, SEQ ID NO: 1061, SEQ ID NO: 1067, SEQ ID NO: 1073, SEQ ID NO: 1079, SEQ ID NO: 1085, SEQ ID NO: 1090, and SEQ ID NO: 1096.

9. The cell of claim 7, wherein said solid tumor antigen is mesothelin, optionally wherein:

(a) said antigen binding domain of said second CAR comprises a heavy chain complementary determining region 1 (HC CDR1), a heavy chain complementary determining region 2 (HC CDR2), and a heavy chain complementary determining region 3 (HC CDR3) of any heavy chain binding domain amino acid sequence listed in Table 2 or 3;

(b) said antigen binding domain of said second CAR further comprises a light chain complementary determining region 1 (LC CDR1), a light chain complementary determining region 2 (LC CDR2), and a light chain complementary determining region 3 (LC CDR3) of any light chain binding domain amino acid sequence listed in Table 2 or 4;

(c) said antigen binding domain of said second CAR comprises:

(i) the amino acid sequence of any light chain variable region listed in Table 2;

(ii) an amino acid sequence having at least one, two or three modifications but not more than 20 or 10 modifications of the amino acid sequence of any of the light chain variable regions provided in Table 2; or

(iii) an amino acid sequence with 95-99% identity to the amino acid sequence of any of the light chain variable regions provided in Table 2;

(d) said antigen binding domain of said second CAR comprises:

(i) the amino acid sequence of any heavy chain variable region listed in Table 2;

(ii) an amino acid sequence having at least one, two or three modifications but not more than 20 or 10 modifications of the amino acid sequence of any of the heavy chain variable regions provided in Table 2; or

(iii) an amino acid sequence with 95-99% identity to the amino acid sequence of any of the heavy chain variable regions provided in Table 2;

(e) said antigen binding domain of said second CAR comprises a polypeptide having the amino acid sequence of any light chain variable region listed in Table 2, and the amino acid sequence of any heavy chain variable region listed in Table 2;

(f) said antigen binding domain of said second CAR comprises a polypeptide having a sequence of any one of SEQ ID NOS: 46-70; or

(g) the second CAR comprises a polypeptide having an amino acid sequence selected from the group consisting of SEQ ID NO: 282, SEQ ID NO: 283, SEQ ID NO: 284, SEQ ID NO: 285, SEQ ID NO: 286, SEQ ID NO: 287, SEQ ID NO: 288, SEQ ID NO: 289, SEQ ID NO: 290, SEQ ID NO: 291, SEQ ID NO: 292, SEQ ID NO: 293, SEQ ID NO: 294, SEQ ID NO: 295, SEQ ID NO: 296, SEQ ID NO: 297, SEQ ID NO: 298, SEQ ID NO: 299, SEQ ID NO: 300, SEQ ID NO: 301, SEQ ID NO: 302, SEQ ID NO: 303, SEQ ID NO: 304, SEQ ID NO: 305, and SEQ ID NO: 306.

10. The cell of any one of claims 1-9, wherein:

(a) said antigen binding domain of said first CAR is in the format of an scFv; and/or

(b) said antigen binding domain of said second CAR is in the format of an scFv.

11. The cell of any one of claims 1-10, wherein:

(a) said intracellular signaling domain of said first or said second CAR comprises one or more primary signaling domains;

(b) said intracellular signaling domains of said first CAR and said second CAR comprise a primary signaling domain;

(c) said intracellular signaling domain of said first or said second CAR comprises one or more costimulatory signaling domains; or

(d) said intracellular signaling domains of said first CAR and said second CAR comprise one or more costimulatory signaling domains.

12. The cell of claim 11, wherein:

(a) the primary signaling domains comprise a CD3-zeta stimulatory domain;

(b) said costimulatory signaling domain is an intracellular domain of a costimulatory protein selected from the group consisting of CD27, CD28, 4-1BB (CD137), OX40, GITR, CD30, CD40, ICOS, BAFFR, HVEM, ICAM-1, lymphocyte function-associated antigen-1 (LFA-1), CD2, CDS, CD7, CD287, LIGHT, NKG2C, NKG2D, SLAMF7, NKp80, NKp30, NKp44, NKp46, CD160, B7-H3, and a ligand that specifically binds with CD83;

(c) the costimulatory domain of both said first and said second CAR comprise an intracellular domain of 4-1BB;

(d) said one or more of said costimulatory domains comprises an intracellular domain of CD28; or

(e) said first or second CAR comprises two costimulatory domains:

(1) a 4-1BB costimulatory domain and a CD28 costimulatory domain;

(2) a 4-1BB costimulatory domain and an ICOS costimulatory domain; or

(3) a CD28 costimulatory domain and an ICOS costimulatory domain.

13. The cell of any one of claims 1-12, wherein:

(a) said cell is derived from a patient diagnosed with a myeloid tumor, or a hematological tumor not of B-Cell lineage, optionally wherein said patient is diagnosed with a myeloid tumor expressing an antigen selected from the group consisting of CD123, CD33 and CLL-1;

(b) said cell is derived from a patient diagnosed with a solid tumor, optionally wherein said patient is diagnosed with a solid tumor expressing an antigen selected from the group consisting of: EGFRvIII, mesothelin, GD2, Tn Ag, PSMA, TAG72, CD44v6, CEA, EPCAM, KIT, IL-13Ra2, GD3, CD171, IL-11Ra, PSCA, VEGFR2, LewisY, CD24, PDGFR-beta, SSEA-4, folate receptor alpha, ERBB2, Her2/neu, MUC1, EGFR, NCAM, Ephrin B2, CAIX, LMP2, sLe, HMWMAA, o-acetyl-GD2, folate receptor beta, TEM1/CD248, TEM7R, FAP, Legumain, HPV E6 or E7, CLDN6, TSHR, GPRC5D, ALK, Pysialic acid, PLAC1, globoH, NY-BR-1, UPK2, HAVCR1, ADRB3, PAX3, GPR20, Ly6k, OR51E2, TARP, and GFR α 4;

(c) said cell is a human cell and is not derived from a patient diagnosed with a tumor;
or

(d) said cell is a T cell, a natural killer (NK) cell, a cytotoxic T lymphocyte (CTL), a tumor infiltrating lymphocyte (TIL), or a regulatory T cell.

14. Use of the cell of any one of claims 1-4 or 7-13 in the manufacture of a medicament for the treatment of a disease associated with expression of a solid tumor antigen in a mammal, optionally wherein said solid tumor expresses an antigen selected from the group consisting of: EGFRvIII, mesothelin, CS-1, GD2, Tn Ag, PSMA, TAG72, CD44v6, CEA, EPCAM, KIT, IL-13Ra2, GD3, CD171, IL-11Ra, PSCA, VEGFR2, LewisY, CD24, PDGFR-beta, SSEA-4, folate receptor alpha, ERBB2, Her2/neu, MUC1, EGFR, NCAM, Ephrin B2, CAIX, LMP2, sLe, HMWMAA, o-acetyl-GD2, folate receptor beta, TEM1/CD248, TEM7R, FAP, Legumain, HPV E6 or E7, CLDN6, TSHR, GPRC5D, ALK, Pysialic acid, PLAC1, globoH, NY-BR-1, UPK2, HAVCR1, ADRB3, PAX3, GPR20, Ly6k, OR51E2, TARP, and GFR α 4, optionally wherein said mammal has a tumor characterized as glioblastoma, ovarian cancer, lung cancer, prostate cancer, colorectal cancer, pancreatic cancer, breast carcinoma, adenocarcinoma or mesothelioma.

15. Use of the cell of any one of claims 1-5 or 10-13 in the manufacture of a medicament for the treatment of a disease associated with expression of a myeloid tumor antigen in a mammal, optionally wherein said myeloid tumor expresses an antigen selected from the group consisting of CD123, CD33 and CLL-1, optionally wherein said mammal has a tumor characterized as acute myeloid leukemia (AML), acute lymphoblastic B-cell leukemia (B-cell acute lymphoid leukemia, BALL), acute lymphoblastic T-cell leukemia (T cell acute lymphoid leukemia (TALL)), B-cell prolymphocytic leukemia, chronic lymphocytic leukemia, chronic myeloid leukemia (CML), myelodysplastic syndrome, plasma cell myeloma, or a combination thereof.

16. The use of claim 14 or 15, wherein:

- (a) said cells are autologous to the treated mammal;
 - (b) said cells are allogeneic to the treated mammal;
 - (c) said mammal is a human;
 - (d) the administration of said cells results in partial or complete elimination of said tumor cells and, thereafter, continue to persist in said subject at a level greater than, or for a length of time longer than, otherwise identical cells that lack said first CAR;
 - (e) said mammal is to be administered a lymphodepleting therapy prior to, concurrently with, or after administration of said cells; or
 - (f) said mammal is not to be administered a lymphodepleting therapy prior to or concurrently with administration of said cells.
17. A nucleic acid encoding the first CAR and the second CAR of any one of claims 1-13, optionally wherein the sequence of said first CAR and said second CAR are separated by an independent ribosomal entry site, a promoter element, or a sequence encoding a T2A, P2A, E2A, or F2A element.
18. A vector comprising the nucleic acid of claim 17, optionally wherein said vector is a lentiviral vector.
19. A composition comprising a first nucleic acid encoding the first CAR and a second nucleic acid encoding the second CAR of any one of claims 1-13, optionally wherein said first and said second nucleic acids are comprised within separate vectors, optionally wherein said vectors are lentiviral vectors.
20. A method of generating the cell of any one of claims 1-13, comprising introducing into said cell the nucleic acid of claim 17, the vector of claim 18 or the composition of claim 19.
21. A method of generating the cell of any one of claims 1-13, comprising introducing into said cell a first vector comprising a nucleic acid encoding the first CAR of any one of claims 1-13, and introducing into said cell a second vector comprising a nucleic acid encoding the second CAR of any one of claims 1-13, optionally wherein said introduction of said first vector and said second vector is simultaneous or sequential.
22. A cell comprising a nucleic acid encoding the first CAR of any one of claims 1-13 and the second CAR of any one of claims 1-13.
23. The cell of any one of claims 1-4 or 7-13 for use in the treatment of a disease associated with expression of a solid tumor antigen in a mammal.
24. The cell of any one of claims 1-5 or 10-13 for use in the treatment of a disease associated with expression of a myeloid tumor antigen in a mammal.