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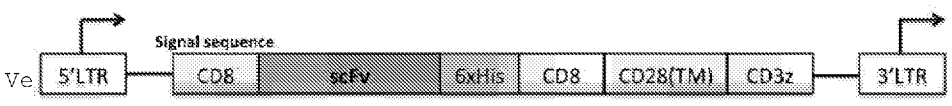
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(54) Title: CD46-SPECIFIC EFFECTOR CELLS AND USES THEREOF

FIG. 1




(57) Abstract: Engineered effector cells, such as chimeric antigen receptors (CARs), are used for enhanced immunogenic response to specific antigen, such as CD46. Disclosed herein are compositions and methods of treatment a cancer overexpressing CD46, which comprises a pharmaceutical composition comprising an engineered effector cell.

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# CD46-SPECIFIC EFFECTOR CELLS AND USES THEREOF

## CROSS-REFERENCE

**[0001]** This application claims the benefit of U.S. Provisional Application No. 62/420,508, filed November 10, 2016, which application is incorporated herein by reference.

## SEQUENCE LISTING

**[0002]** The instant application contains a Sequence Listing which has been submitted electronically in ASCII format and is hereby incorporated by reference in its entirety. Said ASCII copy, created on November 9, 2017, is named 39442-704_601_SL.txt and is 94,317 bytes in size.

## BACKGROUND OF THE DISCLOSURE

**[0003]** Engineered effector cells, such as chimeric antigen receptors (CARs), combine the binding specificity of monoclonal antibodies with the potency of immune cells, such as T cells and natural killer (NK) cells. In some instances, an engineered effector cell is utilized for specific targeting of a cancer marker for treatment.

## SUMMARY OF THE DISCLOSURE

**[0004]** Disclosed herein, in certain embodiments, are engineered effector cells and pharmaceutical compositions comprising one or more engineered effector cells. In some embodiments, also described herein are treatment methods targeting a patient population characterized by an overexpression of CD46 with one or more engineered effector cells.

**[0005]** Disclosed herein, in certain embodiments, is an engineered effector cell that specifically binds CD46, comprising an engineered antigen binding domain that binds to an epitope of CD46, wherein the engineered antigen binding domain is displayed on the surface of the engineered effector cell. In some embodiments, the engineered effector cell is an engineered T cell. In some embodiments, the engineered effector cell is an engineered natural killer T cell. In some embodiments, the engineered effector cell is an engineered natural killer cell. In some embodiments, the engineered antigen binding domain comprises Fab, Fab', $F(ab')_2$, scFv, $sc(Fv)_2$, dsFv, diabody or binding fragments thereof. In some embodiments, the engineered antigen binding domain comprises scFv. In some embodiments, the engineered antigen binding domain binds to an epitope of CD46 bound by one or more antibodies selected from SEQ ID NOs: 1, 2, 9, 10, 17, 18, 25, 26, 33, 34, 41, 42, 49, 50, 57, 58, 65, 66, 73, 74, 81, 82, 89, 90, 97, 98, 105, 106, 113, 114, 121, 122, 129, 130, 137, 138, 145, 146, 153, 154, 161, 162, 169, 170, 177, 178, 185, 186, 193 or 194. In some embodiments, the engineered antigen binding domain comprises a heavy chain variable region comprising three complementarity determining regions

(CDRs) and a light chain variable region comprising three CDRs, wherein the heavy chain variable region comprises (i) a variable heavy (VH) CDR1 that has an amino acid sequence of SEQ ID NO: 3, (ii) a variable heavy (VH) CDR2 that has an amino acid sequence of SEQ ID NO: 4, and (iii) a variable heavy (VH) CDR3 that has an amino acid sequence of SEQ ID NO: 5, and wherein the light chain variable region comprises (iv) a variable light (VL) CDR 1 that has an amino acid sequence of SEQ ID NO: 6, (v) a variable light (VL) CDR 2 that has an amino acid sequence of SEQ ID NO: 7, and (vi) a variable light (VL) CDR 3 that has an amino acid sequence of SEQ ID NO: 8. In some embodiments, the engineered binding domain comprises a heavy chain variable region comprising three complementarity determining regions (CDRs) and a light chain variable region comprising three CDRs, wherein the heavy chain variable region comprises (i) a variable heavy (VH) CDR1 that has an amino acid sequence of SEQ ID NO: 11, (ii) a variable heavy (VH) CDR2 that has an amino acid sequence of SEQ ID NO: 12, and (iii) a variable heavy (VH) CDR3 that has an amino acid sequence of SEQ ID NO: 13, and wherein the light chain variable region comprises (iv) a variable light (VL) CDR 1 that has an amino acid sequence of SEQ ID NO: 14, (v) a variable light (VL) CDR 2 that has an amino acid sequence of SEQ ID NO: 15, and (vi) a variable light (VL) CDR 3 that has an amino acid sequence of SEQ ID NO: 16. In some embodiments, the engineered antigen binding domain comprises a heavy chain variable region comprising three complementarity determining regions (CDRs) and a light chain variable region comprising three CDRs, wherein the heavy chain variable region comprises (i) a variable heavy (VH) CDR1 that has an amino acid sequence of SEQ ID NO: 19, (ii) a variable heavy (VH) CDR2 that has an amino acid sequence of SEQ ID NO: 20, and (iii) a variable heavy (VH) CDR3 that has an amino acid sequence of SEQ ID NO: 21, and wherein the light chain variable region comprises (iv) a variable light (VL) CDR 1 that has an amino acid sequence of SEQ ID NO: 22, (v) a variable light (VL) CDR 2 that has an amino acid sequence of SEQ ID NO: 23, and (vi) a variable light (VL) CDR 3 that has an amino acid sequence of SEQ ID NO: 24. In some embodiments, the engineered antigen binding domain comprises a heavy chain variable region comprising three complementarity determining regions (CDRs) and a light chain variable region comprising three CDRs, wherein the heavy chain variable region comprises (i) a variable heavy (VH) CDR1 that has an amino acid sequence of SEQ ID NO: 27, (ii) a variable heavy (VH) CDR2 that has an amino acid sequence of SEQ ID NO: 28, and (iii) a variable heavy (VH) CDR3 that has an amino acid sequence of SEQ ID NO: 29, and wherein the light chain variable region comprises (iv) a variable light (VL) CDR 1 that has an amino acid sequence of SEQ ID NO: 30, (v) a variable light (VL) CDR 2 that has an amino acid sequence of SEQ ID NO: 31, and (vi) a variable light (VL) CDR 3 that has an amino acid sequence of SEQ ID NO: 32. In some embodiments, the engineered antigen binding domain comprises a

heavy chain variable region having at least 80%, 85%, 90%, 95%, or 99% sequence identity to SEQ ID NOs: 1, 9, 17 and 25; and a light chain variable region having at least 80%, 85%, 90%, 95% or 99% sequence identity to SEQ ID NOs: 2, 10, 18 and 26. In some embodiments, the engineered antigen binding domain comprises a heavy chain variable region comprising an amino acid sequence of SEQ ID NO: 1 and a light chain variable region comprising an amino acid sequence of SEQ ID NO: 2. In some embodiments, the engineered effector cell further comprises a stalk region, a transmembrane domain, at least one costimulatory domains, and a signaling domain. In some embodiments, the at least one costimulatory signaling domain comprises CD8, CD27, CD28z, 4-1BB, ICOS, OX40 or fragment or combination thereof. In some embodiments, the at least one costimulatory signaling domain comprises CD28 and 4-1BB or fragments thereof. In some embodiments, the at least one costimulatory signaling domain comprises CD28 or fragment thereof. In some embodiments, a first costimulatory signaling domain is encoded by a first vector and a second costimulatory signaling domain is encoded by a second vector. In some embodiments, the engineered effector cell is prepared from an autologous effector cell. In some embodiments, the engineered effector cell is prepared from an allogeneic effector cell. In some embodiments, the engineered effector cell is a CAR-T cell. In some embodiments, the engineered effector cell is a CAR-NK cell.

**[0006]** Disclosed herein, in certain embodiments, is a pharmaceutical composition comprising an engineered effector cell described above; and an excipient.

**[0007]** Disclosed herein, in certain embodiments, is a method of treating a subject having a cancer characterized by an overexpression of CD46, comprising administering to the subject a pharmaceutical composition comprising a plurality of engineered effector cells, wherein each engineered effector cell comprises an engineered antigen binding domain that binds to an epitope of CD46, and wherein the engineered antigen binding domain is displayed on the surface of the engineered effector cell. In some embodiments, the engineered effector cell is an engineered T cell. In some embodiments, the engineered effector cell is an engineered natural killer T cell. In some embodiments, the engineered effector cell is an engineered natural killer cell. In some embodiments, the engineered antigen binding domain comprises Fab, Fab', $F(ab')_2$, scFv, $sc(Fv)_2$, dsFv, diabody or binding fragments thereof. In some embodiments, the engineered antigen binding domain comprises scFv. In some embodiments, the engineered antigen binding domain binds to an epitope of CD46 bound by one or more antibodies selected from SEQ ID NOs: 1, 2, 9, 10, 17, 18, 25, 26, 33, 34, 41, 42, 49, 50, 57, 58, 65, 66, 73, 74, 81, 82, 89, 90, 97, 98, 105, 106, 113, 114, 121, 122, 129, 130, 137, 138, 145, 146, 153, 154, 161, 162, 169, 170, 177, 178, 185, 186, 193 or 194. In some embodiments, the engineered antigen binding domain comprises a heavy chain variable region comprising three complementarity

determining regions (CDRs) and a light chain variable region comprising three CDRs, wherein the heavy chain variable region comprises (i) a variable heavy (VH) CDR1 that has an amino acid sequence of SEQ ID NO: 3, (ii) a variable heavy (VH) CDR2 that has an amino acid sequence of SEQ ID NO: 4, and (iii) a variable heavy (VH) CDR3 that has an amino acid sequence of SEQ ID NO: 5, and wherein the light chain variable region comprises (iv) a variable light (VL) CDR 1 that has an amino acid sequence of SEQ ID NO: 6, (v) a variable light (VL) CDR 2 that has an amino acid sequence of SEQ ID NO: 7, and (vi) a variable light (VL) CDR 3 that has an amino acid sequence of SEQ ID NO: 8. In some embodiments, the engineered antigen binding domain comprises a heavy chain variable region comprising three complementarity determining regions (CDRs) and a light chain variable region comprising three CDRs, wherein the heavy chain variable region comprises (i) a variable heavy (VH) CDR1 that has an amino acid sequence of SEQ ID NO: 11, (ii) a variable heavy (VH) CDR2 that has an amino acid sequence of SEQ ID NO: 12, and (iii) a variable heavy (VH) CDR3 that has an amino acid sequence of SEQ ID NO: 13, and wherein the light chain variable region comprises (iv) a variable light (VL) CDR 1 that has an amino acid sequence of SEQ ID NO: 14, (v) a variable light (VL) CDR 2 that has an amino acid sequence of SEQ ID NO: 15, and (vi) a variable light (VL) CDR 3 that has an amino acid sequence of SEQ ID NO: 16. In some embodiments, the engineered antigen binding domain comprises a heavy chain variable region comprising three complementarity determining regions (CDRs) and a light chain variable region comprising three CDRs, wherein the heavy chain variable region comprises (i) a variable heavy (VH) CDR1 that has an amino acid sequence of SEQ ID NO: 19, (ii) a variable heavy (VH) CDR2 that has an amino acid sequence of SEQ ID NO: 20, and (iii) a variable heavy (VH) CDR3 that has an amino acid sequence of SEQ ID NO: 21, and wherein the light chain variable region comprises (iv) a variable light (VL) CDR 1 that has an amino acid sequence of SEQ ID NO: 22, (v) a variable light (VL) CDR 2 that has an amino acid sequence of SEQ ID NO: 23, and (vi) a variable light (VL) CDR 3 that has an amino acid sequence of SEQ ID NO: 24. In some embodiments, the engineered antigen binding domain comprises a heavy chain variable region comprising three complementarity determining regions (CDRs) and a light chain variable region comprising three CDRs, wherein the heavy chain variable region comprises (i) a variable heavy (VH) CDR1 that has an amino acid sequence of SEQ ID NO: 27, (ii) a variable heavy (VH) CDR2 that has an amino acid sequence of SEQ ID NO: 28, and (iii) a variable heavy (VH) CDR3 that has an amino acid sequence of SEQ ID NO: 29, and wherein the light chain variable region comprises (iv) a variable light (VL) CDR 1 that has an amino acid sequence of SEQ ID NO: 30, (v) a variable light (VL) CDR 2 that has an amino acid sequence of SEQ ID NO: 31, and (vi) a variable light (VL) CDR 3 that has an amino acid sequence of SEQ ID NO: 32. In

some embodiments, the engineered antigen binding domain comprises a heavy chain variable region having at least 80%, 85%, 90%, 95%, or 99% sequence identity to SEQ ID NOs: 1, 9, 17 and 25; and a light chain variable region having at least 80%, 85%, 90%, 95% or 99% sequence identity to SEQ ID NOs: 2, 10, 18 and 26. In some embodiments, the engineered antigen binding domain comprises a heavy chain variable region comprising an amino acid sequence of SEQ ID NO: 1 and a light chain variable region comprising an amino acid sequence of SEQ ID NO: 2. In some embodiments, the engineered effector cell further comprises a stalk region, a transmembrane domain, at least one costimulatory domains, and a signaling domain. In some embodiments, the at least one costimulatory signaling domain comprises CD8, CD27, CD28z, 4-1BB, ICOS, OX40 or fragment or combination thereof. In some embodiments, the at least one costimulatory signaling domain comprises CD28 and 4-1BB or fragments thereof. In some embodiments, the at least one costimulatory signaling domain comprises CD28 or fragment thereof. In some embodiments, a first costimulatory signaling domain is encoded by a first vector and a second costimulatory signaling domain is encoded by a second vector. In some embodiments, the engineered effector cell is prepared from an autologous effector cell. In some embodiments, the engineered effector cell is prepared from an allogeneic effector cell. In some embodiments, the CAR effector cell is a CAR-T cell. In some embodiments, the CAR effector cell is a CAR-NK cell. In some embodiments, the cancer comprises breast cancer, cervical cancer, colorectal cancer, kidney cancer, liver cancer, lung cancer, ovarian cancer, pancreatic cancer, prostate cancer or urothelial cancer. In some embodiments, the cancer comprises mesothelioma, lymphoma, or leukemia. In some embodiments, the cancer is prostate cancer. In some embodiments, the cancer is multiple myeloma. In some embodiments, the pharmaceutical composition further comprises an excipient. In some embodiments, the method further comprises an additional therapeutic agent. In some embodiments, the additional therapeutic agent comprises a cytokine. In some embodiments, the cytokine comprises IL2, IL7, IL12, IL15, IL21, IFNγ or TNF-α. In some embodiments, the additional therapeutic agent comprises chemotherapeutic agent. In some embodiments, the chemotherapeutic agent is selected from among bortezomib, CAL-101, chlorambucil, cyclophosphamide, dexamethasone, docetaxel, doxorubicin, endostatin, everolimus, fludarabine, fostamatinib, ifosfamide, ibritumomab, lenalidomide, mesalazine, ofatumumab, paclitaxel, pentostatin, prednisone, rituximab, temsirolimus, thalidomide, tositumomab or a combination thereof. In some embodiments, the engineered effector cell and the additional therapeutic agent is administered sequentially. In some embodiments, the engineered effector cell is administered after administering the additional therapeutic agent. In some embodiments, the additional therapeutic agent comprises cyclophosphamide and optionally further comprising fludarabine. In some embodiments, the

additional therapeutic agent is administered to induce lymphopenia. In some embodiments, the pharmaceutical composition is formulated for parenteral administration. In some embodiments, the pharmaceutical composition is administered to the subject as an injection. In some embodiments, the pharmaceutical composition is administered to the subject as an infusion. In some embodiments, the subject is a human.

**[0008]** Disclosed herein, in certain embodiments, is a method of treating a subject having a cancer characterized by an overexpression of CD46, comprising administering to the subject a pharmaceutical composition comprising a plurality of chimeric antigen receptor (CAR) effector cells, wherein each CAR effector cell recognizes an epitope recognized by an antibody of SEQ ID NOs: 1, 2, 9, 10, 17, 18, 25, 26, 33, 34, 41, 42, 49, 50, 57, 58, 65, 66, 73, 74, 81, 82, 89, 90, 97, 98, 105, 106, 113, 114, 121, 122, 129, 130, 137, 138, 145, 146, 153, 154, 161, 162, 169, 170, 177, 178, 185, 186, 193 or 194.

**[0009]** Disclosed herein, in certain embodiments, is a method of depleting CD46 overexpressed cells, comprising (a) contacting an effector cell *ex vivo* with a vector comprising a polynucleotide encoding an engineered antigen binding domain that recognizes an epitope of CD46 to generate a CD46-specific effector cell, wherein the engineered antigen binding domain is displayed on the surface of the CD46-specific effector cell; and (b) administering the CD46-specific effector cell to a subject having CD46 overexpressed cells, thereby depleting the population of CD46 overexpressed cells. In some embodiments, the CD46 overexpressed cells are cancer cells. In some embodiments, the cancer cells comprise breast cancer cells, cervical cancer cells, colorectal cancer cells, kidney cancer cells, liver cancer cells, lung cancer cells, ovarian cancer cells, pancreatic cancer cells, prostate cancer cells or urothelial cancer cells. In some embodiments, the cancer cells comprise cells from mesothelioma, cells from lymphoma, or cells from leukemia. In some embodiments, the cancer cells comprise prostate cancer cells. In some embodiments, the cancer cells comprise cells from multiple myeloma. In some embodiments, the engineered effector cell is an engineered T cell. In some embodiments, the engineered effector cell is an engineered natural killer T cell. In some embodiments, the engineered effector cell is an engineered natural killer cell. In some embodiments, the engineered antigen binding domain comprises Fab, Fab', $F(ab')_2$, scFv, $sc(Fv)_2$, dsFv, diabody or binding fragments thereof. In some embodiments, the engineered antigen binding domain comprises scFv. In some embodiments, the engineered antigen binding domain comprises a heavy chain variable region comprising three complementarity determining regions (CDRs) and a light chain variable region comprising three CDRs, wherein the heavy chain variable region comprises (i) a variable heavy (VH) CDR1 that has an amino acid sequence of SEQ ID NO: 3, (ii) a variable heavy (VH) CDR2 that has an amino acid sequence of SEQ ID NO: 4, and (iii) a

variable heavy (VH) CDR3 that has an amino acid sequence of SEQ ID NO: 5, and wherein the light chain variable region comprises (iv) a variable light (VL) CDR 1 that has an amino acid sequence of SEQ ID NO: 6, (v) a variable light (VL) CDR 2 that has an amino acid sequence of SEQ ID NO: 7, and (vi) a variable light (VL) CDR 3 that has an amino acid sequence of SEQ ID NO: 8. In some embodiments, the engineered antigen binding domain comprises a heavy chain variable region comprising three complementarity determining regions (CDRs) and a light chain variable region comprising three CDRs, wherein the heavy chain variable region comprises (i) a variable heavy (VH) CDR1 that has an amino acid sequence of SEQ ID NO: 11, (ii) a variable heavy (VH) CDR2 that has an amino acid sequence of SEQ ID NO: 12, and (iii) a variable heavy (VH) CDR3 that has an amino acid sequence of SEQ ID NO: 13, and wherein the light chain variable region comprises (iv) a variable light (VL) CDR 1 that has an amino acid sequence of SEQ ID NO: 14, (v) a variable light (VL) CDR 2 that has an amino acid sequence of SEQ ID NO: 15, and (vi) a variable light (VL) CDR 3 that has an amino acid sequence of SEQ ID NO: 16. In some embodiments, the engineered antigen binding domain comprises a heavy chain variable region comprising three complementarity determining regions (CDRs) and a light chain variable region comprising three CDRs, wherein the heavy chain variable region comprises (i) a variable heavy (VH) CDR1 that has an amino acid sequence of SEQ ID NO: 19, (ii) a variable heavy (VH) CDR2 that has an amino acid sequence of SEQ ID NO: 20, and (iii) a variable heavy (VH) CDR3 that has an amino acid sequence of SEQ ID NO: 21, and wherein the light chain variable region comprises (iv) a variable light (VL) CDR 1 that has an amino acid sequence of SEQ ID NO: 22, (v) a variable light (VL) CDR 2 that has an amino acid sequence of SEQ ID NO: 23, and (vi) a variable light (VL) CDR 3 that has an amino acid sequence of SEQ ID NO: 24. In some embodiments, the engineered antigen binding domain comprises a heavy chain variable region comprising three complementarity determining regions (CDRs) and a light chain variable region comprising three CDRs, wherein the heavy chain variable region comprises (i) a variable heavy (VH) CDR1 that has an amino acid sequence of SEQ ID NO: 27, (ii) a variable heavy (VH) CDR2 that has an amino acid sequence of SEQ ID NO: 28, and (iii) a variable heavy (VH) CDR3 that has an amino acid sequence of SEQ ID NO: 29, and wherein the light chain variable region comprises (iv) a variable light (VL) CDR 1 that has an amino acid sequence of SEQ ID NO: 30, (v) a variable light (VL) CDR 2 that has an amino acid sequence of SEQ ID NO: 31, and (vi) a variable light (VL) CDR 3 that has an amino acid sequence of SEQ ID NO: 32. In some embodiments, the engineered antigen binding domain comprises a heavy chain variable region having at least 80%, 85%, 90%, 95%, or 99% sequence identity to SEQ ID NOs: 1, 9, 17 and 25; and a light chain variable region having at least 80%, 85%, 90%, 95% or 99% sequence identity to SEQ ID NOs: 2, 10, 18 and 26. In some embodiments, the

engineered antigen binding domain comprises a heavy chain variable region comprising an amino acid sequence of SEQ ID NO: 1 and a light chain variable region comprising an amino acid sequence of SEQ ID NO: 2. In some embodiments, the engineered effector cell further comprises a stalk region, a transmembrane domain, at least one costimulatory domains, and a signaling domain. In some embodiments, the at least one costimulatory signaling domain comprises CD8, CD27, CD28z, 4-1BB, ICOS, OX40 or fragment or combination thereof. In some embodiments, the at least one costimulatory signaling domain comprises CD28 and 4-1BB or fragments thereof. In some embodiments, the at least one costimulatory signaling domain comprises CD28 or fragment thereof. In some embodiments, a first costimulatory signaling domain is encoded by a first vector and a second costimulatory signaling domain is encoded by a second vector. In some embodiments, the engineered effector cell is prepared from an autologous effector cell. In some embodiments, the engineered effector cell is prepared from an allogeneic effector cell. In some embodiments, the CAR effector cell is a CAR-T cell. In some embodiments, the CAR effector cell is a CAR-NK cell. In some embodiments, the vector is a viral vector. In some embodiments, the vector is a non-viral vector.

**[0010]** Disclosed herein, in certain embodiments, is a method of depleting CD46 overexpressed cells, comprising (a) contacting a T cell *ex vivo* with a vector comprising a polynucleotide encoding an engineered antigen binding domain that recognizes an epitope of CD46 to generate a CD46-specific CAR-T cell, wherein the engineered antigen binding domain is displayed on the surface of the CD46-specific CAR-T cell; and (b) administering the CD46-specific CAR-T cell to a subject having CD46 overexpressed cells, thereby depleting the population of CD46 overexpressed cells.

**[0011]** Disclosed herein, in certain embodiments, is chimeric polynucleotide comprising a first polynucleotide segment encoding an antigen binding domain that binds to an epitope of CD46; and a second polynucleotide segment encoding a polypeptide comprising a stalk region, a transmembrane domain, at least one costimulatory domains, and a signaling domain; wherein the chimeric polynucleotide, upon transfection to an effector cell, expresses the CD46 antigen binding domain and the polypeptide as a continuous chain, and wherein expression of the CD46 antigen binding domain and the polypeptide triggers the effector cell to activate and/or proliferate.

**[0012]** Disclosed herein, in certain embodiments, is a vector comprising a chimeric polynucleotide comprising a first polynucleotide segment encoding an antigen binding domain that binds to an epitope of CD46; and a second polynucleotide segment encoding a polypeptide comprising a stalk region, a transmembrane domain, at least one costimulatory domains, and a signaling domain; wherein the chimeric polynucleotide, upon transfection to an effector cell,

expresses the CD46 antigen binding domain and the polypeptide as a continuous chain, and wherein expression of the CD46 antigen binding domain and the polypeptide triggers the effector cell to activate and/or proliferate.

**[0013]** Disclosed herein, in certain embodiments, is a polynucleotide encoding a chimeric receptor comprising an antigen binding domain that binds to an epitope of CD46, a stalk region, a transmembrane domain, at least one costimulatory domains, and a signaling domain.

**[0014]** Disclosed herein, in certain embodiments, is a vector comprising a polynucleotide encoding a chimeric receptor comprising an antigen binding domain that binds to an epitope of CD46, a stalk region, a transmembrane domain, at least one costimulatory domains, and a signaling domain.

**[0015]** Disclosed herein, in certain embodiments, is an engineered single-chain polypeptide comprising an antigen binding domain that binds to an epitope of CD46, a stalk region, a transmembrane domain, at least one costimulatory domains, and a signaling domain.

**[0016]** Disclosed herein, in certain embodiments, is a vector comprising a polynucleotide encoding an engineered single-chain polypeptide comprising an antigen binding domain that binds to an epitope of CD46, a stalk region, a transmembrane domain, at least one costimulatory domains, and a signaling domain.

## BRIEF DESCRIPTION OF THE DRAWINGS

**[0017]** The novel features of the invention are set forth with particularity in the appended claims. A better understanding of the features and advantages of the present invention will be obtained by reference to the following detailed description that sets forth illustrative embodiments, in which the principles of the invention are utilized, and the accompanying drawings of which:

**[0018]** **FIG. 1** illustrates a schematic of a CAR cassette. "6xHis" is disclosed as SEQ ID NO: 204.

**[0019]** **FIG. 2** illustrates exemplary configurations of a first, second, third, and fourth generation CAR.

**[0020]** **FIG. 3** illustrates impedance curves of BPH-1 cells, the vertical line at 25 hours indicating administration of the one of three treatments (fresh media alone, non-transduced T cells, or anti-CD46 CAR-T cells) to the BPH-1 cells.

**[0021]** **FIG. 4** illustrates impedance curves of LNCAP-C4-B2 cells, the vertical line at 25 hours indicating administration of the one of three treatments (fresh media alone, non-transduced T cells, or anti-CD46 CAR-T cells) to the LNCAP-C4-B2 cells.

**[0022]** **FIG. 5** illustrates impedance curves of HeLa cells, the vertical line at 25 hours indicating administration of the one of three treatments (fresh media alone, non-transduced T cells, or anti-CD19 CAR-T cells) to the HeLa cells.

**[0023]** **FIG. 6** illustrates impedance curves of CD19 HeLa cells, the vertical line at 25 hours indicating administration of the one of three treatments (fresh media alone, non-transduced T cells, or anti-CD19 CAR-T cells) to the CD19 HeLa cells.

**[0024]** **FIG. 7** illustrates six exemplary lentivirus-based CAR-T constructs.

**[0025]** **FIG. 8A-FIG. 8G** show FACS analysis of CAR expression on T cells on the day of *in vitro* cytotoxicity assay.

**[0026]** **FIG. 9A-FIG. 9D** show *in vitro* cytotoxicity assay on prostate cancer cell lines using six different variants of CAR-T cells targeted to CD46.

**[0027]** **FIG. 10** illustrates 3 different lots of CAR-T cells compared with non-transduced T cells for their ability to kill PC-3 prostate cancer cells.

**[0028]** **FIG. 11** illustrates MiBio PC-3 cells compared to a PC-3 luc cell line.

**[0029]** **FIG. 12** illustrates the *in vitro* cytotoxicity of CAR T cells on MM.1S-luc.

## DETAILED DESCRIPTION OF THE INVENTION

**[0030]** CD46, also known as CD46 complement regulatory protein, cluster of differentiation 46 and membrane cofactor protein, is an inhibitory complement receptor. Overexpression of CD46 has been observed in several cancers, such as breast cancer, colorectal cancer, liver cancer, lung cancer, or prostate cancer. In some cases, overexpression of CD46 has been characterized as a negative prognostic factor. For example, overexpression of CD46 has been correlated with shorter progression-free time and shorter overall survival time in breast cancer patients and ovarian cancer patients.

**[0031]** Chimeric antigen receptor (CAR) therapy comprises reprograming of endogenous effector cells to target specific tumor antigens. In some instance, CAR therapy targeting CD19, a pan-B cell antigen present in most B cell leukemias and lymphomas, has shown efficacy in several clinical trials, for example, targeting acute lymphoblastic leukemia (ALL), chronic lymphocytic leukemia (CLL), or B-cell non-Hodgkin lymphoma (NHL).

**[0032]** In some embodiments, described herein are engineered effector cells and compositions comprising engineered effector cells. In some instances, also described herein include methods of targeting tumor antigen CD46 for treatment of a cancer characterized with an overexpression of CD46 or methods of depleting a cell with an overexpression of CD46.

**[0033]** In some cases, engineered effector cells binds specifically to CD46, and comprises an engineered antigen binding domain that binds to an epitope of CD46, wherein the engineered

antigen binding domain is displayed on the surface of the engineered effector cell. In some embodiments, an engineered effector cell described herein comprises an engineered extracellular antigen recognition domain. In some instances, an engineered effector cell described herein further comprises a trans-membrane domain and at least one signaling region that controls engineered effector cell activation, generating a chimeric antigen receptor (CAR) architecture. In some embodiments, the at least one signaling region further comprises one or more co-stimulatory domains.

**[0034]** Disclosed herein, in certain embodiments, are engineered effector cells comprising an engineered antigen binding domain that specifically binds to CD46. In some embodiments, the engineered effector cell is an engineered T cell, an engineered natural killer T cell, an engineered natural killer cell, or an engineered chimeric antigen receptor (CAR). In some embodiments, the engineered CAR is an engineered CAR-T. In some embodiments, the engineered CAR is an engineered CAR-NK.

**Engineered Antigen Binding Domain**

**[0035]** In some embodiments, an engineered effector cell described herein comprises an engineered extracellular antigen binding domain. In some instances, an antigen binding domain is displayed on the surface of the effector cell. In other instances, an antigen binding domain is displayed near the surface or at a distal point away from the surface of the effector cell (e.g., for enhanced interaction with a target receptor). In some embodiments, an engineered antigen binding domain described herein is joined through a stalk region and a transmembrane domain to an intracellular signaling domain of the engineered effector cell.

**[0036]** In some embodiments, an engineered antigen binding domain described herein comprises a humanized antibody or binding fragment thereof, murine antibody or binding fragment thereof, chimeric antibody or binding fragment thereof, monoclonal antibody or binding fragment thereof, monovalent Fab', divalent Fab2, single-chain variable fragment (scFv), bis-scFv, (scFv)2, diabody, minibody, nanobody, triabody, tetrabody, disulfide stabilized Fv protein (dsFv), single-domain antibody (sdAb), Ig NAR, camelid antibody or binding fragment thereof. In some embodiments, the engineered antigen binding domain comprises Fab, Fab', $F(ab')_2$, scFv, $sc(Fv)_2$, dsFv, diabody or binding fragments thereof. In some embodiments, the engineered antigen binding domain comprises Fab, Fab', $F(ab')_2$, scFv, $sc(Fv)_2$ or binding fragments thereof. In some embodiments, the engineered antigen binding domain comprises a scFv.

**[0037]** In some embodiments, the engineered antigen binding domain binds to an epitope of CD46 bound by one or more antibodies selected from SEQ ID NOs: 1, 2, 9, 10, 17, 18, 25, 26, 33, 34, 41, 42, 49, 50, 57, 58, 65, 66, 73, 74, 81, 82, 89, 90, 97, 98, 105, 106, 113, 114, 121, 122,

129, 130, 137, 138, 145, 146, 153, 154, 161, 162, 169, 170, 177, 178, 185, 186, 193 or 194. In some embodiments, the engineered antigen binding domain binds to an epitope of CD46 bound by one or more antibodies selected from SEQ ID NOs: 1, 2, 9, 10, 17, 18, 25 or 26. In some embodiments, the engineered antigen binding domain binds to an epitope of CD46 bound by one or more antibodies selected from SEQ ID NOs: 33, 34, 41, 42, 49, 50, 57, 58, 65, 66, 73, 74, 81, 82, 89, 90, 97, 98, 105, 106, 113, 114, 121, 122, 129, 130, 137, 138, 145, 146, 153, 154, 161, 162, 169, 170, 177, 178, 185, 186, 193 or 194.

**[0038]** In some cases, the anti-CD46 antibody binds to at least a portion of sushi domain 1 of CD46 comprising the amino acid sequence KPYYEIGERVDYKCKKGYFYIPPLATHTICDR (SEQ ID NO: 201).

*Antibody YS5 and Variants*

**[0039]** In some embodiments, an engineered antigen binding domain described herein comprises a heavy chain variable region comprising three complementarity determining regions (CDRs) and a light chain variable region comprising three CDRs, wherein the heavy chain variable region comprises a variable heavy chain (VH) CDR1 that has an amino acid sequence of SEQ ID NO: 3, a variable heavy chain (VH) CDR2 that has an amino acid sequence of SEQ ID NO: 4, a variable heavy chain (VH) CDR3 that has an amino acid sequence of SEQ ID NO: 5, and wherein the light chain variable region comprises a variable light chain (VL) CDR1 that has an amino acid sequence of SEQ ID NO: 6, a variable light chain (VL) CDR2 that has an amino acid sequence of SEQ ID NO: 7, and a variable light chain (VL) CDR3 that has an amino acid sequence of SEQ ID NO: 8.

**[0040]** In some embodiments, an engineered antigen binding domain described herein comprises a heavy chain variable region having at least 80%, 85%, 90%, 95%, or 99% sequence identity to SEQ ID NO. 1. In some embodiments, an engineered antigen binding domain described herein comprises a heavy chain variable region having at least 80% sequence identity to SEQ ID NO: 1. In some embodiments, an engineered antigen binding domain described herein comprises a heavy chain variable region having at least 85% sequence identity to SEQ ID NO: 1. In some embodiments, an engineered antigen binding domain described herein comprises a heavy chain variable region having at least 90% sequence identity to SEQ ID NO: 1. In some embodiments, an engineered antigen binding domain described herein comprises a heavy chain variable region having at least 95% sequence identity to SEQ ID NO: 1. In some embodiments, an engineered antigen binding domain described herein comprises a heavy chain variable region having at least 99% sequence identity to SEQ ID NO: 1.

**[0041]** In some embodiments, an engineered antigen binding domain described herein comprises a light chain variable region having at least 80%, 85%, 90%, 95%, or 99% sequence

identity to SEQ ID NO. 2. In some embodiments, an engineered antigen binding domain described herein comprises a light chain variable region having at least 80% sequence identity to SEQ ID NO: 2. In some embodiments, an engineered antigen binding domain described herein comprises a light chain variable region having at least 85% sequence identity to SEQ ID NO: 2. In some embodiments, an engineered antigen binding domain described herein comprises a light chain variable region having at least 90% sequence identity to SEQ ID NO: 2. In some embodiments, an engineered antigen binding domain described herein comprises a light chain variable region having at least 95% sequence identity to SEQ ID NO: 2. In some embodiments, an engineered antigen binding domain described herein comprises a light chain variable region having at least 99% sequence identity to SEQ ID NO: 2.

**[0042]** In some embodiments, an engineered antigen binding domain described herein comprises a heavy chain variable region of SEQ ID NO: 1 and a light chain variable region of SEQ ID NO: 2.

**[0043]** In some embodiments, an engineered antigen binding domain described herein comprises an antibody of SEQ ID NO: 203.

*Antibody YS12 and Variants*

**[0044]** In some embodiments, an engineered antigen binding domain described herein comprises a heavy chain variable region comprising three complementarity determining regions (CDRs) and a light chain variable region comprising three CDRs, wherein the heavy chain variable region comprises a variable heavy chain (VH) CDR1 that has an amino acid sequence of SEQ ID NO: 11, a variable heavy chain (VH) CDR2 that has an amino acid sequence of SEQ ID NO: 12, a variable heavy chain (VH) CDR3 that has an amino acid sequence of SEQ ID NO: 13, and wherein the light chain variable region comprises a variable light chain (VL) CDR1 that has an amino acid sequence of SEQ ID NO: 14, a variable light chain (VL) CDR2 that has an amino acid sequence of SEQ ID NO: 15, and a variable light chain (VL) CDR3 that has an amino acid sequence of SEQ ID NO: 16.

**[0045]** In some embodiments, an engineered antigen binding domain described herein comprises a heavy chain variable region having at least 80%, 85%, 90%, 95%, or 99% sequence identity to SEQ ID NO:. 9. In some embodiments, an engineered antigen binding domain described herein comprises a heavy chain variable region having at least 80% sequence identity to SEQ ID NO: 9. In some embodiments, an engineered antigen binding domain described herein comprises a heavy chain variable region having at least 85% sequence identity to SEQ ID NO: 9. In some embodiments, an engineered antigen binding domain described herein comprises a heavy chain variable region having at least 90% sequence identity to SEQ ID NO: 9. In some embodiments, an engineered antigen binding domain described herein comprises a

heavy chain variable region having at least 95% sequence identity to SEQ ID NO: 9. In some embodiments, an engineered antigen binding domain described herein comprises a heavy chain variable region having at least 99% sequence identity to SEQ ID NO: 9.

**[0046]** In some embodiments, an engineered antigen binding domain described herein comprises a light chain variable region having at least 80%, 85%, 90%, 95%, or 99% sequence identity to SEQ ID NO: 10. In some embodiments, an engineered antigen binding domain described herein comprises a light chain variable region having at least 80% sequence identity to SEQ ID NO: 10. In some embodiments, an engineered antigen binding domain described herein comprises a light chain variable region having at least 85% sequence identity to SEQ ID NO: 10. In some embodiments, an engineered antigen binding domain described herein comprises a light chain variable region having at least 90% sequence identity to SEQ ID NO: 10. In some embodiments, an engineered antigen binding domain described herein comprises a light chain variable region having at least 95% sequence identity to SEQ ID NO: 10. In some embodiments, an engineered antigen binding domain described herein comprises a light chain variable region having at least 99% sequence identity to SEQ ID NO: 10.

**[0047]** In some embodiments, an engineered antigen binding domain described herein comprises a heavy chain variable region of SEQ ID NO: 9 and a light chain variable region of SEQ ID NO: 10.

*Antibody YS5iVD and Variants*

**[0048]** In some embodiments, an engineered antigen binding domain described herein comprises a heavy chain variable region comprising three complementarity determining regions (CDRs) and a light chain variable region comprising three CDRs, wherein the heavy chain variable region comprises a variable heavy chain (VH) CDR1 that has an amino acid sequence of SEQ ID NO: 19, a variable heavy chain (VH) CDR2 that has an amino acid sequence of SEQ ID NO: 20, a variable heavy chain (VH) CDR3 that has an amino acid sequence of SEQ ID NO: 21, and wherein the light chain variable region comprises a variable light chain (VL) CDR1 that has an amino acid sequence of SEQ ID NO: 22, a variable light chain (VL) CDR2 that has an amino acid sequence of SEQ ID NO: 23, and a variable light chain (VL) CDR3 that has an amino acid sequence of SEQ ID NO: 24.

**[0049]** In some embodiments, an engineered antigen binding domain described herein comprises a heavy chain variable region having at least 80%, 85%, 90%, 95%, or 99% sequence identity to SEQ ID NO: 17. In some embodiments, an engineered antigen binding domain described herein comprises a heavy chain variable region having at least 80% sequence identity to SEQ ID NO: 17. In some embodiments, an engineered antigen binding domain described herein comprises a heavy chain variable region having at least 85% sequence identity to SEQ

ID NO: 17. In some embodiments, an engineered antigen binding domain described herein comprises a heavy chain variable region having at least 90% sequence identity to SEQ ID NO: 17. In some embodiments, an engineered antigen binding domain described herein comprises a heavy chain variable region having at least 95% sequence identity to SEQ ID NO: 17. In some embodiments, an engineered antigen binding domain described herein comprises a heavy chain variable region having at least 99% sequence identity to SEQ ID NO: 17.

**[0050]** In some embodiments, an engineered antigen binding domain described herein comprises a light chain variable region having at least 80%, 85%, 90%, 95%, or 99% sequence identity to SEQ ID NO: 18. In some embodiments, an engineered antigen binding domain described herein comprises a light chain variable region having at least 80% sequence identity to SEQ ID NO: 18. In some embodiments, an engineered antigen binding domain described herein comprises a light chain variable region having at least 85% sequence identity to SEQ ID NO: 18. In some embodiments, an engineered antigen binding domain described herein comprises a light chain variable region having at least 90% sequence identity to SEQ ID NO: 18. In some embodiments, an engineered antigen binding domain described herein comprises a light chain variable region having at least 95% sequence identity to SEQ ID NO: 18. In some embodiments, an engineered antigen binding domain described herein comprises a light chain variable region having at least 99% sequence identity to SEQ ID NO: 18.

**[0051]** In some embodiments, an engineered antigen binding domain described herein comprises a heavy chain variable region of SEQ ID NO: 17 and a light chain variable region of SEQ ID NO: 18.

*Antibody SB1HGNY and Variants*

**[0052]** In some embodiments, an engineered antigen binding domain described herein comprises a heavy chain variable region comprising three complementarity determining regions (CDRs) and a light chain variable region comprising three CDRs, wherein the heavy chain variable region comprises a variable heavy chain (VH) CDR1 that has an amino acid sequence of SEQ ID NO: 27, a variable heavy chain (VH) CDR2 that has an amino acid sequence of SEQ ID NO: 28, a variable heavy chain (VH) CDR3 that has an amino acid sequence of SEQ ID NO: 29, and wherein the light chain variable region comprises a variable light chain (VL) CDR1 that has an amino acid sequence of SEQ ID NO: 30, a variable light chain (VL) CDR2 that has an amino acid sequence of SEQ ID NO: 31, and a variable light chain (VL) CDR3 that has an amino acid sequence of SEQ ID NO: 32.

**[0053]** In some embodiments, an engineered antigen binding domain described herein comprises a heavy chain variable region having at least 80%, 85%, 90%, 95%, or 99% sequence identity to SEQ ID NO: 25. In some embodiments, an engineered antigen binding domain

described herein comprises a heavy chain variable region having at least 80% sequence identity to SEQ ID NO: 25. In some embodiments, an engineered antigen binding domain described herein comprises a heavy chain variable region having at least 85% sequence identity to SEQ ID NO: 25. In some embodiments, an engineered antigen binding domain described herein comprises a heavy chain variable region having at least 90% sequence identity to SEQ ID NO: 25. In some embodiments, an engineered antigen binding domain described herein comprises a heavy chain variable region having at least 95% sequence identity to SEQ ID NO: 25. In some embodiments, an engineered antigen binding domain described herein comprises a heavy chain variable region having at least 99% sequence identity to SEQ ID NO: 25.

**[0054]** In some embodiments, an engineered antigen binding domain described herein comprises a light chain variable region having at least 80%, 85%, 90%, 95%, or 99% sequence identity to SEQ ID NO: 26. In some embodiments, an engineered antigen binding domain described herein comprises a light chain variable region having at least 80% sequence identity to SEQ ID NO: 26. In some embodiments, an engineered antigen binding domain described herein comprises a light chain variable region having at least 85% sequence identity to SEQ ID NO: 26. In some embodiments, an engineered antigen binding domain described herein comprises a light chain variable region having at least 90% sequence identity to SEQ ID NO: 26. In some embodiments, an engineered antigen binding domain described herein comprises a light chain variable region having at least 95% sequence identity to SEQ ID NO: 26. In some embodiments, an engineered antigen binding domain described herein comprises a light chain variable region having at least 99% sequence identity to SEQ ID NO: 26.

**[0055]** In some embodiments, an engineered antigen binding domain described herein comprises a heavy chain variable region of SEQ ID NO: 25 and a light chain variable region of SEQ ID NO: 26.

**[0056]** In some embodiments, the engineered effector cell described herein comprises a sequence selected from Table 1.

Table 1.

| Identifier | Sequence | SEQ ID NO: |
|---|---|---|
| YS5, VH | QVQLVQSGGGVVQPGRSLRLACAASGLTVNNYAMHWVR QAPGKGLEWVAVISYDGNNKYYADSVKGRFTISRDNSKNT LYLQMNSLRAEDTAVYYCAKGGGYFDLWGRGTLVTVSS | 1 |
| YS5, VL | QSVLTQPPSVSGAPGQRVTISCTGSSSNIGAGYDVHWYQQL PGTAPKLLIYGNNNRPSGVPDRFSGSKSGTSASLAITGLQAE DEADYYCSSYTSGTWLFGGGTKLTVL | 2 |
| YS5, VH CDR1 | GLTVNNYA | 3 |
| YS5, VH CDR2 | ISYDGNNK | 4 |
| YS5, VH CDR3 | AKGGGYFDL | 5 |

| | | |
|---|---|---|
| YS5, VL CDR1 | SSNIGAGYD | 6 |
| YS5, VL CDR2 | GNN | 7 |
| YS5, VL CDR3 | SSYTSGTWL | 8 |
| YS12, VH | QVQLVESGGGVVQPGRSLRLSCAASGFTFSTYGMHWVRQAPGKGLEWLSFISYDGDEKYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYWCAKASGYGMGILDYWGQGTLVTVSS | 9 |
| YS12, VL | SSELTQDPAVSVALGQTVRITCQGDSLRSYYVSWFQQKPGQAPVFVMYGQNNRPSGISERFSGSSSGNTASLIITGAQAEDEADYYCHSRDSSGTHLRVFGGGTKLTVL | 10 |
| YS12, VH CDR1 | GFTFSTYG | 11 |
| YS12, VH CDR2 | FISYDGDEK | 12 |
| YS12, VH CDR3 | AKASGYGMGILDY | 13 |
| YS12, VL CDR1 | SLRSYY | 14 |
| YS12, VL CDR2 | GQN | 15 |
| YS12, VL CDR3 | HSRDSSGTHLRV | 16 |
| YS5vID, VH | QVQLVQSGGGVVQPGRSLRLACAASGFTVNNYAMHWVRQAPGKGLEWVAVISYDGNNKYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAKGGGYFDLWGRGTLVTVSS | 17 |
| YS5vID, VL | QSVLTQPPSVSGAPGQRVTISCTGSSSNIGAGYDVHWYQQLPGTAPKLLIYGDNNRPSGVPDRFSGSKSGTSASLAITGLQAEDEADYYCSSYTSGTWLFGGGTKLTVL | 18 |
| YS5vID, VH CDR1 | GFTVNNYA | 19 |
| YS5vID, VH CDR2 | ISYDGNNK | 20 |
| YS5vID, VH CDR3 | AKGGGYFDL | 21 |
| YS5vID, VL CDR1 | SSNIGAGYD | 22 |
| YS5vID, VL CDR2 | GDN | 23 |
| YS5vID, VL CDR3 | SSYTSGTWL | 24 |
| SB1HGNY, VH | QVQLQQSGGGVVQPGRSLRLSCAASGFTFSSYAMHWVRQAPGKGLEWVAFIRSDGSKKYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARHGNYFDSWGQGTLVTVSS | 25 |
| SB1HGNY, VL | DIQMTQSPSFLSASVGDRVTITCRASQGISSYLAWYQQKPGKAPKLLIYAASTLQSGVPSSFSGSGSGTEFTLTISSLQPEDFATYYCQQLASYPLTFGGGTKVDIK | 26 |
| SB1HGNY, VH CDR1 | GFTFSSYA | 27 |
| SB1HGNY, VH CDR2 | IRSDGSKK | 28 |
| SB1HGNY, VH CDR3 | ARHGNYFDS | 29 |
| SB1HGNY, VL CDR1 | QGISSY | 30 |
| SB1HGNY, VL | AAS | 31 |

| | | |
|---|---|---|
| CDR2 | | |
| SB1HGNY, VL CDR3 | QQLASYPLT | 32 |

**[0057]** In some embodiments, the engineered effector cell described herein comprises a sequence selected from Table 2.

Table 2.

| Identifier | Sequence | Variable chain SEQ ID NO: | CDR1 SEQ ID NO*: | CDR2 SEQ ID NO*: | CDR3 SEQ ID NO*: |
|---|---|---|---|---|---|
| YS5F, VH | QVQLVQSGGGVVQPGRSLRLACAAS GFTVNNYAMHWVRQAPGKGLEWV AVISYDGNNKYYADSVKGRFTISRD NSKNTLYLQMNSLRAEDTAVYYCA KGGGYFDLWGRGTLVTVSS | 33 | 35 | 36 | 37 |
| YS5F, VL | QSVLTQPPSVSGAPGQRVTISCTGSSS NIGAGYDVHWYQQLPGTAPKLLIYG NNNRPSGVPDRFSGSKSGTSASLAIT GLQAEDEADYYCSSYTSGTWLFGGG TKLTVL | 34 | 38 | 39 | 40 |
| 3G7RY, VH | EVQLVESGGGLVQPGGSLRLSCAAS GFTFSDYYMSWIRQAPGKGLEWVSY ISSSGSTIYYADSVKGRFTISRDNSKN TLYLQMNSLRAEDTAVYYCARDYG RIAAAGRRYWGQGTLVTVSS | 41 | 43 | 44 | 45 |
| 3G7RY, VL | QSALTQPPSASATPGQRVTISCSGRTS NIGSNHVYWYQQLPGTAPKLLIYRN NQRPSGVPDRFSGSKSGTSASLAISGL RSEDEADYYCATWDDSLSGEVFGGG TKLTVL | 42 | 46 | 47 | 48 |
| YS6, VH | QVQLQESGGGVVRPGGSLRLSCAAS GFTFSDYYMSWIRQAPGKGLEWVSY ISSSGSTIYYADSVKGRFTISRDNSKN TLYLQMNSLRAEDTAVYYCARDYG RIAAAGRHYWGQGTLVTVSS | 49 | 51 | 52 | 53 |
| YS6, VL | SSELTQDPAVSVALGQTVRITCQGDS LRSYYASWYQQKPGQAPVLVIYGKN NRPSGIPDRFSGSSSGNTASLTITGAQ AEDEADYYCNSRDSSGTHLEVFGGG TKVTVL | 50 | 54 | 55 | 56 |
| YS1, VH | EVQLVESGGGLVQPGGSLRLSCAAS GFTFSDYYMSWIRQAPGKGLEWVSY ISSSGSTIYYADSVKGRFTISRDNSKN TLYLQMNSLRAEDTAVYYCARDYG RIAAAGRHYWGQGTLVTVSS | 57 | 59 | 60 | 61 |
| YS1, VL | SSELTQDPAVSVALGQTVRITCQGDT LSTYYANWYQQKPGQAPVLVIYGKN NRPSGIPDRFSGSSSGNTASLTITGAQ AEDEADYYCHSRDISGNYLFASGTKL TVL | 58 | 62 | 63 | 64 |
| YS3, VH | QVQLQESGGGLVQPGGSLRLSCAAS | 65 | 67 | 68 | 69 |

| | | | | | |
|---|---|---|---|---|---|
| | GFTFSSYWMSWVRQAPGKGLEWVA DIKQDGSEKYYVDSVKGRFTISGDNA KNSLYLQMNSLRAEDTAVYYCAKD VGSTAINYVRAYTWFDPWGQGTLVT VSS | | | | |
| YS3, VL | QSVLTQPPSASGTPGQRVTISCSGSSS NIGSNTVNWSRQLPGTAPKLLIYSNN QRPSGVPDRFSGSKSGTSASLAISGLQ SEDEADYYCAAWDDSLNVYVFGTG TKVTVL | 66 | 70 | 71 | 72 |
| YS4, VH | QVQLQESGGGLVQPGGSLRLSCAAS GFTFSNYAMSWVRQAPGKGLEWVS TISGSGSSTFYVDSVKGRFTISRDNSK NTLYLQMNSLRAEDTAVYYCAQGL YSSGWANWFDPRGQGTLVTVSS | 73 | 75 | 76 | 77 |
| YS4, VL | KIVLTQSPSSLSASVGDTVTIACRASR DIRNDLAWYQQKPGKAPKLLIYGAS SLQSGVPSRFSGSGSGTEFILTISSLQP EDFATYYCHRLNSYPLTFGGGTKVDI K | 74 | 78 | 79 | 80 |
| YS8, VH | QVQLQESGGGVVQPGRSLRLSCAAS GFTFSSYGMHWVRQAPGKGLEWVA VISYDGSNKYYADSVKGRFTISRDNS KNTLYLQMNSLRAEDTAVYYCAKV MGLAAAGLDAFDIWGQGTTVTVSS | 81 | 83 | 84 | 85 |
| YS8, VL | NFMLTQPASLSGSPGQSITISCTGTSS DVGGYNYVSWYQQHPGYAPKLMIY DVSNRPSGVSNRFSGSKSGNTASLTIS GLQAEDEADYYCSSYTSSSTPWVFG GGTKLTVL | 82 | 86 | 87 | 88 |
| YS7, VH | QVQLVQSGGGVVQPGRSLRLSCAAS GFTFSSYAMHWVRQAPGKGLEWVA VISYDGSNKYYADSVKGRFTISRDTS TNTLYLQMNSLRADDTAVYYCGRES SGSPGVWGQGTTVTVSS | 89 | 91 | 92 | 93 |
| YS7, VL | SYVLTQDPAVSVALGQTVRITCQGDS LRSYYASWYQQKPGQAPVLVIYGKN NRPSGIPDRFSGSSSGNTASLTITGAQ AEDEADYYCNSRDSSGNQFGGGTKL TVL | 90 | 94 | 95 | 96 |
| YS9, VH | QVQLVESGGGLIQPGGSLRLSCAASG FTVSSNYMSWVRQAPGKGLEWVSVI YTDGSTYYADSVKGRFTISRDNSKNT LYLQMNSLRAEDTAIYYCARDRGTS GYDWAWFDLWGQGTLVTVSS | 97 | 99 | 100 | 101 |
| YS9, VL | SSELTQDPAVSVALGQTVRITCQGDS LRTYYASWYQQRPGQAPILVLYGKN NRPSGIPDRFSGSSSGNTASLTITGAQ AEDEADYYCNSRDSSGNHVVFGGGT KLTVL | 98 | 102 | 103 | 104 |
| YS10, VH | QVQLQESGGGLVQPGGSLRLSCAAS GFTFSSYAMSWVRQAPGKGLEWVS AISGSGGSTYYADSVKGRFTISRDNS KNTLYMQMNSLRAEDTAVYYCAKD RYYYGSGKDAFDIWGRGTMVTVSS | 105 | 107 | 108 | 109 |
| YS10, VL | QSVLTQPASVSGSPGQSITISCTGTGS | 106 | 110 | 111 | 112 |

| | | | | | |
|---|---|---|---|---|---|
| | DVGSYNYVSWYQQNPGKAPKLMIY EVSNRPSGVSNRFSGSKSGNTASLTIS GLQAEDEADYYCSSYTTSSTLVFGG GTKVTVL | | | | |
| YS11, VH | QVQLVESGGGLVQPGGSLGLSCAAS GFTFSNYWMSWVRQAPGKGLEWVA NVRQDGGQKYYVDSVKGRFTISRDN AKNSLYLQMNSLRTEDTAVYFCVSQ RNSGEHDYWGQGTLVTVSS | 113 | 115 | 116 | 117 |
| YS11, VL | SELTQDPAVSVALGQTVRITCQGDSL RSYYASWYQQKPGQAPVLVIYGENS RPSGIPDRFSGSSSGNTASLTITGAQA EDEADYYCNSWDSSGNHVVFGGGT KLTVL | 114 | 118 | 119 | 120 |
| 3G7HY, VH | EVQLVESGGGLVQPGGSLRLSCAAS GFTFSDYYMSWIRQAPGKGLEWVSY ISSSGSTIYYADSVKGRFTISRDNSKN TLYLQMNSLRAEDTAVYYCARDYG RIAAAGRHYWGQGTLVTVSS | 121 | 123 | 124 | 125 |
| 3G7HY, VL | AIRMTQSPSSLSASVGDRVTITCRASQ SISSYLNWYQQKPGKAPKLLIYAASS LQSGVPSRFSGSGSGTDFTLTISSLQP EDFATYYCQQSYSTPRTFGQGTKLEI K | 122 | 126 | 127 | 128 |
| 3G7NY, VH | EVQLVESGGGLVQPGGSLRLSCAAS GFTFSDYYMSWIRQAPGKGLEWVSY ISSSGSTIYYADSVKGRFTISRDNSKN TLYLQMNSLRAEDTAVYYCARDYG RIAAAGRNYWGQGTLVTVSS | 129 | 131 | 132 | 133 |
| 3G7NY, VL | DIVMTQSPLSLPVTPGEPASISCRSSQ SLLHSNGYDYLDWYLQKPGQSPQLL IYLGSNRASGVPDRFSGSGSGTDFTL KISRVETEDVGIYYCMQGLQTPSFGQ GTKLEIK | 130 | 134 | 135 | 136 |
| 3G7, VH | QVQLQESGGGVVRPGGSLRLSCAAS GFTFSDYYMSWIRQAPGKGLEWVSY ISSSGSTIYYADSVKGRFTISRDNSKN TLYLQMNSLRAEDTAVYYCARDYG RIAAAGRHYWGQGTLVTVSS | 137 | 139 | 140 | 141 |
| 3G7, VL | SSELTQDPAVSVALGQTVRITCQGDS LRSYYASWYQQKPGQAPVPVIYGKN NRPSGIPDRFSGSSSGNTASLTITGAQ AEDEADYYCNSRDSSSTHRGVFGGG TKLTVL | 138 | 142 | 143 | 144 |
| SB2, VH | EVQLVESGGGLVKPGGSLRLSCAAS GFTFSDYYMSWIRQAPGKGLEWVSY ISSSGSSIYYADSVKGRFTISRDNAKN SLYLQMNSLKAEDTAVYYCARDITD VVGVSFDYWGQGTLVTVSS | 145 | 147 | 148 | 149 |
| SB2, VL | DIQLTQSPSSLSASVGDRVTITCRASR SISTYLSWYQQKPGKAPKLLIYDASR LQNGVPSRFSGSGSDTDFTLTISSLQP EDFATYFCQQSYNPPWTFGQGTKLEI K | 146 | 150 | 151 | 152 |
| 2C8, VH | EVQLVESGGGVVQPGRSLRLSCAAS GFTFSSYGMHWVRQAPGKGLEWVA | 153 | 155 | 156 | 157 |

| | | | | | |
|---|---|---|---|---|---|
| | VISYDGSNKYYADSVKGRFTISRDNS KNTLYLQMNSLRAEDTAEYYCAKV MGLAAAGLDAFDIWGQGTLVTVSS | | | | |
| 2C8, VL | QSALTQPASVSGSPGQSITISCTGTSS DVGGYNYVSWYQQHPGKAPKLMIY DVSNRPSGVSNRFSGSKSGNTASLTIS GLQAEDEAYYYCSSYTSSSDPWVFG GGTQLTVL | 154 | 158 | 159 | 160 |
| UA8kappa, VH | EVQLVESGGGVVQPGRSLRLSCAAS GFTFSSFGMHWVRRAPGKGLEWVA VISYDGSNQYYADSVKGRFTISRDNS KNTLYLQMNSLRAEDTAVYYCGSRP GGGYASGSTVAYWGQGTLVTVSS | 161 | 163 | 164 | 165 |
| UA8 kappa, VL | NIQMTQSPSSLSASVGDRVTITCRAG QPISTYVNWYQHKPGKAPKLLIYGAS NLQSGVPSRFSGGGSATDFTLTISSLQ PEDFATYYCQQSYSSLLTFGDGTKVE IK | 162 | 166 | 167 | 168 |
| 2B10, VH | QVQLQEPGGGLVQPGRSLRLSCAAS GFTFDDYAMHWVRQAPGKGLEWVG RIKSKTDEGTTDYAAPVKGRFSISRD DSKNTLYLQMNSLKTEDTGVYYCTA TKGLGGSKLGQGTLVTVSS | 169 | 171 | 172 | 173 |
| 2B10, VL | QSVLTQPPSASGTPGQRVTISCSGSSS NIGSNTVSWSRQLPGTAPKLLIYSND QRPSGVPDRFSGSKSGTSASLAITGLQ PEDEADYYCGTWDSSLSAYVFGTGT KLTVL | 170 | 174 | 175 | 176 |
| UA20, VH | QVQLQESGGGLVKPGGSLRLSCAAS GFTFSNAWMNWVRQAPGKGLEWV GRIKSKTDEGTTDYAAPVKGRFSISR DDSKNTLYLQMNSLKTEDTGVYYCT ATKGLGGSKLGQGTLVTVSS | 177 | 179 | 180 | 181 |
| UA20, VL | QSVLTQPPSASGTPGQRVTISCSGSSS NIGNNTVNWSRQLPGTAPKLLIYSND QRPSGVPDRFSGSKSGTSASLAITGLQ PEDEADYYCGTWDSSLSAYVFGTGT KLTVL | 178 | 182 | 183 | 184 |
| 585II41, VH | QVQLVESGGGLVQPGGSLRLSCAAS GFTFSSYAMGWVRQAPGKGLEWVS AISGSGGSTYYADSVKGRFTISRDNS KDTLYLQMNSLRAEDTAVYYCASRS LLDYWGQGTLVTVSS | 185 | 187 | 188 | 189 |
| 585II41, VL | NFMLTQDPAVSVALGQTVRITCQGD SLRSYYASWYQQKPGQAPLLVIYGK NNRPSGIPDRFSGSSSGNTASLTITGA QAEDEADYYCNSRDSSGNPVFGGGT KVTVL | 186 | 190 | 191 | 192 |
| 585II41.1, VH | QVQLVESGGGLVQPGGSLRLSCAAS GFTFSSYAMSWVRQAPGKGLEWVS AISGSGGSTYYADSVKGRFTISRDNS KDTLYLQMNSLRAEDTAVYYCASRS LLDYWGQGTLVTVSS | 193 | 195 | 196 | 197 |
| 585II41.1, VL | NFMLTQDPAVSVALGQTVRITCQGD SLRSYYASWYQQKPGQAPLLVIYGK NNRPSGIPDRFSGSSSGNTASLTITGA | 194 | 198 | 199 | 200 |

| | | | | | |
|---|---|---|---|---|---|
| | QAEDEADYYCNSRDSSGNPVFGGGT KVTVL | | | | |

** CDR1, CDR2, and CDR3 of each of the VH and VL chains are represented by the first, second, and third underlined regions, respectively*

**[0058]** In some embodiments, an engineered effector cell comprises an antigen binding domain that binds to an epitope bound by antibody 3051.1, G12FC3, M6c42b, 4F3YW, M40pr146, UA8, 585II56, 3076, 3051, M49R, RCI-14, II79.4, II79.3, T5II-4B.1, T5II-4B.2, RCI-11, RCI-20, CI-11A, CI-14A and S95-2. In some embodiments, an engineered effector cell comprises an antibody selected from 3051.1, G12FC3, M6c42b, 4F3YW, M40pr146, UA8, 585II56, 3076, 3051, M49R, RCI-14, II79.4, II79.3, T5II-4B.1, T5II-4B.2, RCI-11, RCI-20, CI-11A, CI-14A and S95-2.

**Stalk region**

**[0059]** In some embodiments, an engineered effector cell described herein further comprises a stalk region, also known as a hinge or a spacer. The stalk region is an extracellular component connecting the engineered antigen binding domain to the transmembrane region. In some embodiments, the stalk region provides flexibility to the engineered antigen binding domain. In some embodiments, the length of the stalk region is adjustable. In some embodiments, the engineered effector cell does not comprise a stalk region.

**[0060]** In some cases, a stalk region described herein is derived from an immunoglobulin molecule. In some embodiments, the immunoglobulin molecule comprises IgG1, IgG4, or IgD. In other instances, a stalk region described herein comprises CD3 or CD8. In some cases, the stalk region comprises the CH2CH3 region of an immunoglobulin and optionally portions of CD3. In some cases, the stalk region comprises CD8 (e.g., CD8α).

**Transmembrane domain**

**[0061]** In some embodiments, an engineered effector cell described herein further comprises a transmembrane domain. In some embodiments, the transmembrane domain anchors the engineered antigen binding domain to a plasma membrane of the engineered effector cell. In some instances, suitable transmembrane domains include the transmembrane region(s) of alpha, beta or zeta chain of the T-cell receptor; or a transmembrane region from CD28, CD3 epsilon, CD3ζ, CD45, CD4, CD5, CD8alpha, CD9, CD16, CD22, CD33, CD37, CD64, CD80, CD86, CD134, CD137 or CD154. In alternative embodiments, a transmembrane domain is a synthetic transmembrane domain.

**[0062]** In some embodiments, a transmembrane domain comprises a native transmembrane portion of a CD3zeta, CD28, a CD4, or a CD8. In some cases, a transmembrane domain described herein comprises a CD3zeta transmembrane domain. In some cases, a transmembrane

domain described herein comprises a CD8 transmembrane domain (e.g., a CD8α transmembrane domain).

**Intracellular signaling domain**

**[0063]** In some embodiments, the engineered effector cell further comprises an intracellular signaling domain. In some embodiments, the intracellular signaling domain is derived from a CD3 zeta chain or an FcεRIγ. In some embodiments, the intracellular signaling domain is derived from a CD3zeta chain.

**[0064]** In some embodiments, the signaling domain comprises signaling motifs which are known as immunoreceptor tyrosine-based activation motifs (ITAMs). Examples of ITAM containing cytoplasmic signaling sequences include those derived from TCR zeta, FcR gamma, FcR beta, CD3 gamma, CD3 delta, CD3 epsilon, CD5, CD22, CD79a, CD79b, and CD66d.

**[0065]** In some embodiments, the intracellular signaling domain further comprises at least one co-stimulatory domain. In some embodiments, the at least one co-stimulatory domain is linked to the intracellular signaling domain. In some embodiments, the at least one co-stimulatory domain is linked to a cytoplasmic domain of the CD3 zeta chain. In some embodiments, the co-stimulatory domain provides greater strength of signaling and greater potency to the engineered effector cell.

**[0066]** In some embodiments, illustrative costimulatory domains comprise CD8, CD27, CD28, 4-1BB (CD137), DAP10, OX40 (CD134), ICOS, CD27, NKG2D, Lck, CD70, CD80 or CD86. In some embodiments, an engineered effector cell described herein comprises one or more costimulatory domains, or two or more costimulatory domains selected from CD8, CD27, CD28, 4-1BB (CD137), DAP10, OX40 (CD134), ICOS, CD27, NKG2D, Lck, CD70, CD80, CD86 or a combination thereof. In some cases, an engineered effector cell described herein comprises one or more costimulatory domains, or two or more costimulatory domains selected from CD28, 4-1BB or a combination thereof. In some cases, an engineered effector cell described herein comprises constimulatory domains CD28 and 4-1BB. In some cases, an engineered effector cell described herein comprises constimulatory domains CD28. In some cases, an engineered effector cell described herein comprises constimulatory domains 4-1BB.

**Chimeric Antigen Receptor Architectures**

**[0067]** In some embodiments, an engineered effector cell described herein comprises a first generation, a second generation, third generation or a fourth generation CAR. In some embodiments, an engineered effector cell described herein comprises a first generation CAR. In some cases, a first generation CAR comprises an antigen binding domain, a stalk region, a

transmembrane domain and a signaling domain. In some cases, a co-stimulatory domain is not present within a chimeric antigen receptor (CAR) architecture.

**[0068]** In some embodiments, an engineered effector cell described herein comprises a second generation CAR. In some cases, a second generation CAR comprises an antigen binding domain, a stalk region, a transmembrane domain, a signaling domain and a costimulatory domain.

**[0069]** In some embodiments, an engineered effector cell described herein comprises a third generation CAR. In some cases, the third generation CAR comprises two costimulatory domains in additional to an antigen binding domain, a stalk region and a transmembrane domain. In some embodiments, the two costimulatory domains are engineered in tandem within the chimeric antigen receptor (CAR) architecture.

**[0070]** In some embodiments, an engineered effector cell described herein comprises a fourth generation CAR. In a fourth generation CAR, for example, CAR comprises at least one costimulatory domains and at least one additional cellular component comprising a cytokine transgene or a co-stimulatory ligand. In some instances, the fourth generation CAR comprises at least one costimulatory domain and at least one additional cellular component comprising a co-stimulatory ligand. In some instances, a co-stimulatory ligand comprises CD8, CD27, CD28, 4-1BB (CD137), DAP10, OX40 (CD134), ICOS, CD27, NKG2D, Lck, CD70, CD80, or CD86.

**[0071]** In some embodiments, an engineered effector cell described herein comprises a fourth generation CAR in which the additional cellular component comprises a co-stimulatory ligand. In some cases, the co-stimulatory ligand comprises CD8, CD27, CD28, 4-1BB (CD137), DAP10, OX40 (CD134), ICOS, CD27, NKG2D, Lck, CD70, CD80, or CD86. In some cases, an engineered effector cell described herein comprises a fourth generation CAR in which the additional cellular component comprises a co-stimulatory ligand comprising CD28 or 4-1BB (CD137). In some cases, an engineered effector cell described herein comprises a fourth generation CAR in which the additional cellular component comprises a co-stimulatory ligand comprising CD28. In some cases, an engineered effector cell described herein comprises a fourth generation CAR in which the additional cellular component comprises a co-stimulatory ligand comprising 4-1BB (CD137).

**[0072]** In some embodiments, the fourth generation CAR comprises at least one costimulatory domain and at least one additional cellular component comprising a cytokine transgene. Exemplary cytokines include, but are not limited to, IL2, IL7, IL12, IL15, IL21, IFNγ or TNF-α. In some cases, an engineered effector cell described herein comprises a fourth generation CAR in which the additional cellular component comprises a cytokine transgene. In some cases, an engineered effector cell described herein comprises a fourth generation CAR in which the

additional cellular component comprises a cytokine transgene comprising IL2, IL7, IL12, IL15, IL21, IFNγ or TNF-α.

**[0073]** In some embodiments, an engineered effector cell described herein comprises a CAR encoded by a vector as illustrated in FIG. 7.

*Engineered Single-chain Polypeptide*

**[0074]** In some embodiments, a chimeric antigen receptor comprises an engineered single-chain polypeptide. In some instances, the engineered single-chain polypeptide comprising an antigen binding domain that binds to an epitope of CD46, a stalk region, a transmembrane domain, at least one costimulatory domains, and a signaling domain.

**[0075]** In some instances, the antigen binding domain comprises Fab, Fab', $F(ab')_2$, scFv, $sc(Fv)_2$, dsFv, diabody or binding fragments thereof. In some cases, the antigen binding domain comprises scFv.

**[0076]** In some embodiments, the antigen binding domain binds to an epitope of CD46 bound by one or more antibodies selected from SEQ ID NOs: 1, 2, 9, 10, 17, 18, 25, 26, 33, 34, 41, 42, 49, 50, 57, 58, 65, 66, 73, 74, 81, 82, 89, 90, 97, 98, 105, 106, 113, 114, 121, 122, 129, 130, 137, 138, 145, 146, 153, 154, 161, 162, 169, 170, 177, 178, 185, 186, 193 or 194.

**[0077]** In some instances, the antigen binding domain comprises a heavy chain variable region comprising three complementarity determining regions (CDRs) and a light chain variable region comprising three CDRs, wherein the heavy chain variable region comprises i) a variable heavy (VH) CDR1 that has an amino acid sequence of SEQ ID NO: 3, ii) a variable heavy (VH) CDR2 that has an amino acid sequence of SEQ ID NO: 4, and iii) a variable heavy (VH) CDR3 that has an amino acid sequence of SEQ ID NO: 5, and wherein the light chain variable region comprises iv) a variable light (VL) CDR 1 that has an amino acid sequence of SEQ ID NO: 6, v) a variable light (VL) CDR 2 that has an amino acid sequence of SEQ ID NO: 7, and vi) a variable light (VL) CDR 3 that has an amino acid sequence of SEQ ID NO: 8.

**[0078]** In some instances, the binding domain comprises a heavy chain variable region comprising three complementarity determining regions (CDRs) and a light chain variable region comprising three CDRs, wherein the heavy chain variable region comprises i) a variable heavy (VH) CDR1 that has an amino acid sequence of SEQ ID NO: 11, ii) a variable heavy (VH) CDR2 that has an amino acid sequence of SEQ ID NO: 12, and iii) a variable heavy (VH) CDR3 that has an amino acid sequence of SEQ ID NO: 13, and wherein the light chain variable region comprises iv) a variable light (VL) CDR 1 that has an amino acid sequence of SEQ ID NO: 14, v) a variable light (VL) CDR 2 that has an amino acid sequence of SEQ ID NO: 15, and vi) a variable light (VL) CDR 3 that has an amino acid sequence of SEQ ID NO: 16.

**[0079]** In some instances, the antigen binding domain comprises a heavy chain variable region comprising three complementarity determining regions (CDRs) and a light chain variable region comprising three CDRs, wherein the heavy chain variable region comprises i) a variable heavy (VH) CDR1 that has an amino acid sequence of SEQ ID NO: 19, ii) a variable heavy (VH) CDR2 that has an amino acid sequence of SEQ ID NO: 20, and iii) a variable heavy (VH) CDR3 that has an amino acid sequence of SEQ ID NO: 21, and wherein the light chain variable region comprises iv) a variable light (VL) CDR 1 that has an amino acid sequence of SEQ ID NO: 22, v) a variable light (VL) CDR 2 that has an amino acid sequence of SEQ ID NO: 23, and vi) a variable light (VL) CDR 3 that has an amino acid sequence of SEQ ID NO: 24.

**[0080]** In some instances, the antigen binding domain comprises a heavy chain variable region comprising three complementarity determining regions (CDRs) and a light chain variable region comprising three CDRs, wherein the heavy chain variable region comprises i) a variable heavy (VH) CDR1 that has an amino acid sequence of SEQ ID NO: 27, ii) a variable heavy (VH) CDR2 that has an amino acid sequence of SEQ ID NO: 28, and iii) a variable heavy (VH) CDR3 that has an amino acid sequence of SEQ ID NO: 29, and wherein the light chain variable region comprises iv) a variable light (VL) CDR 1 that has an amino acid sequence of SEQ ID NO: 30, v) a variable light (VL) CDR 2 that has an amino acid sequence of SEQ ID NO: 31, and vi) a variable light (VL) CDR 3 that has an amino acid sequence of SEQ ID NO: 32.

**[0081]** In some cases, the antigen binding domain comprises a heavy chain variable region having at least 80%, 85%, 90%, 95%, or 99% sequence identity to SEQ ID NOs: 1, 9, 17 and 25; and a light chain variable region having at least 80%, 85%, 90%, 95% or 99% sequence identity to SEQ ID NOs: 2, 10, 18 and 26.

**[0082]** In some cases, the antigen binding domain comprises a heavy chain variable region comprising an amino acid sequence of SEQ ID NO: 1 and a light chain variable region comprising an amino acid sequence of SEQ ID NO: 2.

**[0083]** In some embodiments, the at least one costimulatory signaling domain comprises CD8, CD27, CD28z, 4-1BB, ICOS, OX40, or a fragment or combination thereof. In some instances, the at least one costimulatory signaling domain comprises CD28 and 4-1BB or fragments thereof. In some cases, the at least one costimulatory signaling domain comprises CD28 or fragment thereof.

**[0084]** In some embodiments, the engineered single-chain polypeptide is encoded by a vector and further transfected in an effector cell. In some instances, the transfected effector cell is prepared from an autologous effector cell. In other instances, the transfected effector cell is prepared from an allogeneic effector cell. In some cases, the transfected effector cell is a CAR-T cell. In some cases, the transfected effector cell is a CAR-NK cell.

**[0085]** In some embodiments, the engineered single-chain polypeptide is encoded by a vector as illustrated in FIG. 7.

*Polynucleotide Encoding A Chimeric Antigen Receptor*

**[0086]** In some embodiments, a chimeric antigen receptor is encoded by a chimeric polynucleotide. In such embodiments, the chimeric polynucleotide comprises a first polynucleotide segment encoding an antigen binding domain that binds to an epitope of CD46; and a second polynucleotide segment encoding a polypeptide comprising a stalk region, a transmembrane domain, at least one costimulatory domains, and a signaling domain; in which the chimeric polynucleotide, upon transfection to an effector cell, expresses the CD46 antigen binding domain and the polypeptide as a continuous chain, and wherein expression of the CD46 antigen binding domain and the polypeptide triggers the effector cell to activate and/or proliferate.

**[0087]** In some instances, the effector cell is a T cell, optionally a natural killer T cell; or a natural killer cell.

**[0088]** In some instances, the first polynucleotide segment encodes Fab, Fab', $F(ab')_2$, scFv, $sc(Fv)_2$, dsFv, diabody or binding fragments thereof. In some cases, the first polynucleotide segment encodes scFv.

**[0089]** In some embodiments, the antigen binding domain binds to an epitope of CD46 bound by one or more antibodies selected from SEQ ID NOs: 1, 2, 9, 10, 17, 18, 25, 26, 33, 34, 41, 42, 49, 50, 57, 58, 65, 66, 73, 74, 81, 82, 89, 90, 97, 98, 105, 106, 113, 114, 121, 122, 129, 130, 137, 138, 145, 146, 153, 154, 161, 162, 169, 170, 177, 178, 185, 186, 193 or 194.

**[0090]** In some instances, the first polynucleotide segment encodes a heavy chain variable region comprising three complementarity determining regions (CDRs) and a light chain variable region comprising three CDRs, wherein the heavy chain variable region comprises i) a variable heavy (VH) CDR1 that has an amino acid sequence of SEQ ID NO: 3, ii) a variable heavy (VH) CDR2 that has an amino acid sequence of SEQ ID NO: 4, and iii) a variable heavy (VH) CDR3 that has an amino acid sequence of SEQ ID NO: 5, and wherein the light chain variable region comprises iv) a variable light (VL) CDR 1 that has an amino acid sequence of SEQ ID NO: 6, v) a variable light (VL) CDR 2 that has an amino acid sequence of SEQ ID NO: 7, and vi) a variable light (VL) CDR 3 that has an amino acid sequence of SEQ ID NO: 8.

**[0091]** In some instances, the first polynucleotide segment encodes a heavy chain variable region comprising three complementarity determining regions (CDRs) and a light chain variable region comprising three CDRs, wherein the heavy chain variable region comprises i) a variable heavy (VH) CDR1 that has an amino acid sequence of SEQ ID NO: 11, ii) a variable heavy (VH) CDR2 that has an amino acid sequence of SEQ ID NO: 12, and iii) a variable heavy (VH)

CDR3 that has an amino acid sequence of SEQ ID NO: 13, and wherein the light chain variable region comprises iv) a variable light (VL) CDR 1 that has an amino acid sequence of SEQ ID NO: 14, v) a variable light (VL) CDR 2 that has an amino acid sequence of SEQ ID NO: 15, and vi) a variable light (VL) CDR 3 that has an amino acid sequence of SEQ ID NO: 16.

**[0092]** In some instances, the first polynucleotide segment encodes a heavy chain variable region comprising three complementarity determining regions (CDRs) and a light chain variable region comprising three CDRs, wherein the heavy chain variable region comprises i) a variable heavy (VH) CDR1 that has an amino acid sequence of SEQ ID NO: 19, ii) a variable heavy (VH) CDR2 that has an amino acid sequence of SEQ ID NO: 20, and iii) a variable heavy (VH) CDR3 that has an amino acid sequence of SEQ ID NO: 21, and wherein the light chain variable region comprises iv) a variable light (VL) CDR 1 that has an amino acid sequence of SEQ ID NO: 22, v) a variable light (VL) CDR 2 that has an amino acid sequence of SEQ ID NO: 23, and vi) a variable light (VL) CDR 3 that has an amino acid sequence of SEQ ID NO: 24.

**[0093]** In some instances, the first polynucleotide segment encodes a heavy chain variable region comprising three complementarity determining regions (CDRs) and a light chain variable region comprising three CDRs, wherein the heavy chain variable region comprises i) a variable heavy (VH) CDR1 that has an amino acid sequence of SEQ ID NO: 27, ii) a variable heavy (VH) CDR2 that has an amino acid sequence of SEQ ID NO: 28, and iii) a variable heavy (VH) CDR3 that has an amino acid sequence of SEQ ID NO: 29, and wherein the light chain variable region comprises iv) a variable light (VL) CDR 1 that has an amino acid sequence of SEQ ID NO: 30, v) a variable light (VL) CDR 2 that has an amino acid sequence of SEQ ID NO: 31, and vi) a variable light (VL) CDR 3 that has an amino acid sequence of SEQ ID NO: 32.

**[0094]** In some cases, the first polynucleotide segment encodes a heavy chain variable region having at least 80%, 85%, 90%, 95%, or 99% sequence identity to SEQ ID NOs: 1, 9, 17 and 25; and a light chain variable region having at least 80%, 85%, 90%, 95% or 99% sequence identity to SEQ ID NOs: 2, 10, 18 and 26.

**[0095]** In some cases, the first polynucleotide segment encodes a heavy chain variable region comprising an amino acid sequence of SEQ ID NO: 1 and a light chain variable region comprising an amino acid sequence of SEQ ID NO: 2.

**[0096]** In some embodiments, the at least one costimulatory signaling domain comprises CD8, CD27, CD28z, 4-1BB, ICOS, OX40, or a fragment or combination thereof. In some instances, the at least one costimulatory signaling domain comprises CD28 and 4-1BB or fragments thereof. In some cases, the at least one costimulatory signaling domain comprises CD28 or fragment thereof.

**[0097]** In some embodiments, a vector comprises the chimeric polynucleotide and is further transfected in an effector cell. In some instances, the transfected effector cell is prepared from an autologous effector cell. In other instances, the transfected effector cell is prepared from an allogeneic effector cell. In some cases, the transfected effector cell is a CAR-T cell. In some cases, the transfected effector cell is a CAR-NK cell.

**[0098]** In some embodiments, the transfected effector cell has MHC non-restricted antibody-type specificity.

**[0099]** In some embodiments, a polynucleotide described herein encodes a chimeric receptor comprising an antigen binding domain that binds to an epitope of CD46, a stalk region, a transmembrane domain, at least one costimulatory domains, and a signaling domain.

**[0100]** In such instances, the antigen binding domain comprises Fab, Fab', $F(ab')_2$, scFv, $sc(Fv)_2$, dsFv, diabody or binding fragments thereof. In some cases, the antigen binding domain comprises scFv.

**[0101]** In some embodiments, the antigen binding domain binds to an epitope of CD46 bound by one or more antibodies selected from SEQ ID NOs: 1, 2, 9, 10, 17, 18, 25, 26, 33, 34, 41, 42, 49, 50, 57, 58, 65, 66, 73, 74, 81, 82, 89, 90, 97, 98, 105, 106, 113, 114, 121, 122, 129, 130, 137, 138, 145, 146, 153, 154, 161, 162, 169, 170, 177, 178, 185, 186, 193 or 194.

**[0102]** In some instances, the antigen binding domain comprises a heavy chain variable region comprising three complementarity determining regions (CDRs) and a light chain variable region comprising three CDRs, wherein the heavy chain variable region comprises i) a variable heavy (VH) CDR1 that has an amino acid sequence of SEQ ID NO: 3, ii) a variable heavy (VH) CDR2 that has an amino acid sequence of SEQ ID NO: 4, and iii) a variable heavy (VH) CDR3 that has an amino acid sequence of SEQ ID NO: 5, and wherein the light chain variable region comprises iv) a variable light (VL) CDR 1 that has an amino acid sequence of SEQ ID NO: 6, v) a variable light (VL) CDR 2 that has an amino acid sequence of SEQ ID NO: 7, and vi) a variable light (VL) CDR 3 that has an amino acid sequence of SEQ ID NO: 8.

**[0103]** In some instances, the binding domain comprises a heavy chain variable region comprising three complementarity determining regions (CDRs) and a light chain variable region comprising three CDRs, wherein the heavy chain variable region comprises i) a variable heavy (VH) CDR1 that has an amino acid sequence of SEQ ID NO: 11, ii) a variable heavy (VH) CDR2 that has an amino acid sequence of SEQ ID NO: 12, and iii) a variable heavy (VH) CDR3 that has an amino acid sequence of SEQ ID NO: 13, and wherein the light chain variable region comprises iv) a variable light (VL) CDR 1 that has an amino acid sequence of SEQ ID NO: 14, v) a variable light (VL) CDR 2 that has an amino acid sequence of SEQ ID NO: 15, and vi) a variable light (VL) CDR 3 that has an amino acid sequence of SEQ ID NO: 16.

**[0104]** In some instances, the antigen binding domain comprises a heavy chain variable region comprising three complementarity determining regions (CDRs) and a light chain variable region comprising three CDRs, wherein the heavy chain variable region comprises i) a variable heavy (VH) CDR1 that has an amino acid sequence of SEQ ID NO: 19, ii) a variable heavy (VH) CDR2 that has an amino acid sequence of SEQ ID NO: 20, and iii) a variable heavy (VH) CDR3 that has an amino acid sequence of SEQ ID NO: 21, and wherein the light chain variable region comprises iv) a variable light (VL) CDR 1 that has an amino acid sequence of SEQ ID NO: 22, v) a variable light (VL) CDR 2 that has an amino acid sequence of SEQ ID NO: 23, and vi) a variable light (VL) CDR 3 that has an amino acid sequence of SEQ ID NO: 24.

**[0105]** In some instances, the antigen binding domain comprises a heavy chain variable region comprising three complementarity determining regions (CDRs) and a light chain variable region comprising three CDRs, wherein the heavy chain variable region comprises i) a variable heavy (VH) CDR1 that has an amino acid sequence of SEQ ID NO: 27, ii) a variable heavy (VH) CDR2 that has an amino acid sequence of SEQ ID NO: 28, and iii) a variable heavy (VH) CDR3 that has an amino acid sequence of SEQ ID NO: 29, and wherein the light chain variable region comprises iv) a variable light (VL) CDR 1 that has an amino acid sequence of SEQ ID NO: 30, v) a variable light (VL) CDR 2 that has an amino acid sequence of SEQ ID NO: 31, and vi) a variable light (VL) CDR 3 that has an amino acid sequence of SEQ ID NO: 32.

**[0106]** In some cases, the antigen binding domain comprises a heavy chain variable region having at least 80%, 85%, 90%, 95%, or 99% sequence identity to SEQ ID NOs: 1, 9, 17 and 25; and a light chain variable region having at least 80%, 85%, 90%, 95% or 99% sequence identity to SEQ ID NOs: 2, 10, 18 and 26.

**[0107]** In some cases, the antigen binding domain comprises a heavy chain variable region comprising an amino acid sequence of SEQ ID NO: 1 and a light chain variable region comprising an amino acid sequence of SEQ ID NO: 2.

**[0108]** In some embodiments, the at least one costimulatory signaling domain comprises CD8, CD27, CD28z, 4-1BB, ICOS, OX40, or a fragment or combination thereof. In some instances, the at least one costimulatory signaling domain comprises CD28 and 4-1BB or fragments thereof. In some cases, the at least one costimulatory signaling domain comprises CD28 or fragment thereof.

**[0109]** In some embodiments, a vector comprises the polynucleotide that encodes a chimeric receptor and is further transfected in an effector cell. In some instances, the transfected effector cell is prepared from an autologous effector cell. In other instances, the transfected effector cell is prepared from an allogeneic effector cell. In some cases, the transfected effector cell is a CAR-T cell. In some cases, the transfected effector cell is a CAR-NK cell.

**[0110]** In some embodiments, a vector comprising a polynucleotide described above is as illustrated in FIG. 7.

**Methods of Use**

**[0111]** In certain embodiments, described herein are methods of treating a subject having a cancer characterized by an overexpression of CD46 and methods of depleting CD46 overexpressed population of cells in a subject with an engineered effector cell described *supra*. In some embodiments, disclosed herein are methods of treating a subject having a cancer characterized by an overexpression of CD46, which comprises administering to the subject a pharmaceutical composition comprising a plurality of engineered effector cells, wherein each engineered effector cell comprises an engineered antigen binding domain that binds to an epitope of CD46, and wherein the engineered antigen binding domain is displayed on the surface of the engineered effector cell. In some instances, also described herein are methods of treating a subject having a cancer characterized by overexpression of CD46, which comprises administering to the subject a pharmaceutical composition comprising a plurality of chimeric antigen receptor (CAR) effector cells, wherein each CAR effector cell recognizes an epitope by an antibody of SEQ ID NOs: 1, 2, 9, 10, 17, 18, 25, 26, 33, 34, 41, 42, 49, 50, 57, 58, 65, 66, 73, 74, 81, 82, 89, 90, 97, 98, 105, 106, 113, 114, 121, 122, 129, 130, 137, 138, 145, 146, 153, 154, 161, 162, 169, 170, 177, 178, 185, 186, 193 or 194.

**[0112]** In some embodiments, a cancer characterized by an overexpression of CD46 comprises breast cancer, cervical cancer, colorectal cancer, kidney cancer, liver cancer, lung cancer, ovarian cancer, pancreatic cancer, prostate cancer, or urothelial cancer. In some instances, a cancer characterized by an overexpression of CD46 comprises breast cancer, liver cancer, lung cancer, ovarian cancer or prostate cancer. In some cases, a cancer characterized by an overexpression of CD46 comprises breast cancer, ovarian cancer or prostate cancer. In some cases, the cancer characterized by an overexpression of CD46 is a relapsed or refractory cancer (e.g., a relapsed or refractory breast cancer, cervical cancer, colorectal cancer, kidney cancer, liver cancer, lung cancer, ovarian cancer, pancreatic cancer, prostate cancer, or urothelial cancer). In other cases, the cancer characterized by an overexpression of CD46 is a metastatic cancer (e.g., a metastatic breast cancer, cervical cancer, colorectal cancer, kidney cancer, liver cancer, lung cancer, ovarian cancer, pancreatic cancer, prostate cancer, or urothelial cancer).

**[0113]** In some embodiments, a cancer characterized by an overexpression of CD46 comprises mesothelioma, a lymphoma, or a leukemia. In some instances, a cancer characterized by an overexpression of CD46 comprises mesothelioma. In some cases, a cancer characterized by an overexpression of CD46 comprises a lymphoma. In some cases, a cancer characterized by an

overexpression of CD46 comprises a leukemia. In some cases, mesothelioma, a lymphoma, or a leukemia is a relapsed or refractory.

**[0114]** In some embodiments, a cancer characterized by an overexpression of CD46 is prostate cancer. In some embodiments, the prostate cancer is further classified into adenocarcinoma, a transition cell cancer, a squamous cell cancer, a small cell prostate cancer, a carcinoid, or a sarcoma.

**[0115]** In some embodiments, described herein is a method of treating a subject having prostate cancer, which comprises administering to the subject a pharmaceutical composition comprising a plurality of engineered effector cells, wherein each engineered effector cell comprises an engineered antigen binding domain that binds to an epitope of CD46, and wherein the engineered antigen binding domain is displayed on the surface of the engineered effector cell. In some instances, also described herein is a method of treating a subject having prostate cancer, which comprises administering to the subject a pharmaceutical composition comprising a plurality of chimeric antigen receptor (CAR) effector cells, wherein each CAR effector cell recognizes an epitope by an antibody of SEQ ID NOs: 1, 2, 9, 10, 17, 18, 25, 26, 33, 34, 41, 42, 49, 50, 57, 58, 65, 66, 73, 74, 81, 82, 89, 90, 97, 98, 105, 106, 113, 114, 121, 122, 129, 130, 137, 138, 145, 146, 153, 154, 161, 162, 169, 170, 177, 178, 185, 186, 193 or 194.

**[0116]** In some embodiments, a cancer characterized by an overexpression of CD46 is multiple myeloma. In some instances, multiple myeloma (MM) is further classified as smouldering (indolent) multiple myeloma (also known as asymptomatic myeloma), active (symptomatic) multiple myeloma, solitary plamacytoma of the bone, extramedullary plasmacytoma, light chain myeloma, non-secretory myeloma, immunoglobulin D (IgD) myeloma, or immunoglobulin E (IgE) myeloma. In some cases, multiple myeloma is characterized with a chromosomal aberration, e.g., such as an amplification of chromosome 1q. In some cases, multiple myeloma is characterized with a copy number gain at 1q21. In additional cases, the presence of 1q21 gain correlates with an overexpression of CD46 in multiple myeloma.

**[0117]** In some embodiments, described herein is a method of treating a subject having multiple myeloma, which comprises administering to the subject a pharmaceutical composition comprising a plurality of engineered effector cells, wherein each engineered effector cell comprises an engineered antigen binding domain that binds to an epitope of CD46, and wherein the engineered antigen binding domain is displayed on the surface of the engineered effector cell. In some instances, also described herein is a method of treating a subject having multiple myeloma, which comprises administering to the subject a pharmaceutical composition comprising a plurality of chimeric antigen receptor (CAR) effector cells, wherein each CAR effector cell recognizes an epitope by an antibody of SEQ ID NOs: 1, 2, 9, 10, 17, 18, 25, 26, 33,

34, 41, 42, 49, 50, 57, 58, 65, 66, 73, 74, 81, 82, 89, 90, 97, 98, 105, 106, 113, 114, 121, 122, 129, 130, 137, 138, 145, 146, 153, 154, 161, 162, 169, 170, 177, 178, 185, 186, 193 or 194.

**[0118]** In some embodiments, also described herein are methods of depleting CD46 overexpressed cells, which comprises a) contacting an effector cell *ex vivo* with a vector comprising a polynucleotide encoding an engineered antigen binding domain that recognizes an epitope of CD46 to generate a CD46-specific effector cell; and b) administering the CD46-specific effector cell to a subject having CD46 overexpressed cells, thereby depleting the population of CD46 overexpressed cells. In additional cases, described herein include methods of depleting CD46 overexpressed cells, which comprises a) contacting a T cell *ex vivo* with a vector comprising a polynucleotide encoding an engineered antigen binding domain that recognizes an epitope of CD46 to generate a CD46-specific CAR-T cell, wherein the engineered antigen binding domain is displayed on the surface of the CD46-specific CAR-T cell; and b) administering the CD46-specific CAR-T cell to a subject having CD46 overexpressed cells, thereby depleting the population of CD46 overexpressed cells.

**[0119]** In some cases, CD46 overexpressed cells are cancer cells. In some instances, cancer cells comprise breast cancer cells, cervical cancer cells, colorectal cancer cells, kidney cancer cells, liver cancer cells, lung cancer cells, ovarian cancer cells, pancreatic cancer cells, prostate cancer cells or urothelial cancer cells. In some cases, CD46 overexpressed cells comprise breast cancer cells, liver cancer cells, lung cancer cells, ovarian cancer cells, or prostate cancer cells. In some cases, CD46 overexpressed cells comprise breast cancer cells, ovarian cancer cells, or prostate cancer cells. In some cases, CD46 overexpressed cells comprise prostate cancer cells.

**[0120]** In some instances, CD46 overexpressed cells comprise cells from mesothelioma, cells from lymphoma, or cells from leukemia. In some instances, CD46 overexpressed cells comprise cells from mesothelioma. In some cases, CD46 overexpressed cells comprise cells from lymphoma. In some cases, CD46 overexpressed cells comprise cells from leukemia.

**[0121]** In some instances, CD46 overexpressed cells comprise cells from multiple myeloma.

**[0122]** In some embodiments, also described herein are methods of depleting prostate cancer cells, which comprises a) contacting an effector cell *ex vivo* with a vector comprising a polynucleotide encoding an engineered antigen binding domain that recognizes an epitope of CD46 to generate a CD46-specific effector cell; and b) administering the CD46-specific effector cell to a subject having prostate cancer, thereby depleting the population of prostate cancer cells. In additional cases, described herein include methods of depleting prostate cancer cells, which comprises a) contacting a T cell *ex vivo* with a vector comprising a polynucleotide encoding an engineered antigen binding domain that recognizes an epitope of CD46 to generate a CD46-specific CAR-T cell, wherein the engineered antigen binding domain is displayed on the surface

of the CD46-specific CAR-T cell; and b) administering the CD46-specific CAR-T cell to a subject having prostate cancer, thereby depleting the population of prostate cancer cells.

**[0123]** In some embodiments, also described herein are methods of depleting cells from multiple myeloma, which comprises a) contacting an effector cell *ex vivo* with a vector comprising a polynucleotide encoding an engineered antigen binding domain that recognizes an epitope of CD46 to generate a CD46-specific effector cell; and b) administering the CD46-specific effector cell to a subject having multiple myeloma, thereby depleting the population of cells from multiple myeloma. In additional cases, described herein include methods of depleting cells from multiple myeloma, which comprises a) contacting a T cell *ex vivo* with a vector comprising a polynucleotide encoding an engineered antigen binding domain that recognizes an epitope of CD46 to generate a CD46-specific CAR-T cell, wherein the engineered antigen binding domain is displayed on the surface of the CD46-specific CAR-T cell; and b) administering the CD46-specific CAR-T cell to a subject having multiple myeloma, thereby depleting the population of cells from multiple myeloma.

**Additional Therapeutic Agents**

**[0124]** In some embodiments, one or more methods described herein further comprises administering an additional therapeutic agent. In some embodiments, the additional therapeutic agent comprises a cytokine. Cytokine is a category of small proteins between about 5-20 kDa that are involved in cell signaling. In some instances, cytokines include chemokines, interferons, interleukins, colony-stimulating factors or tumor necrosis factors. In some embodiments, chemokines play a role as a chemoattractant to guide the migration of cells, and is classified into four subfamilies: CXC, CC, CX3C, and XC. Exemplary chemokines include chemokines from the CC subfamily: CCL1, CCL2 (MCP-1), CCL3, CCL4, CCL5 (RANTES), CCL6, CCL7, CCL8, CCL9 (or CCL10), CCL11, CCL12, CCL13, CCL14, CCL15, CCL16, CCL17, CCL18, CCL19, CCL20, CCL21, CCL22, CCL23, CCL24, CCL25, CCL26, CCL27, and CCL28; the CXC subfamily: CXCL1, CXCL2, CXCL3, CXCL4, CXCL5, CXCL6, CXCL7, CXCL8, CXCL9, CXCL10, CXCL11, CXCL12, CXCL13, CXCL14, CXCL15, CXCL16, and CXCL17; the XC subfamily: XCL1 and XCL2; and the CX3C subfamily CX3CL1.

**[0125]** Interferons (IFNs) comprise interferon type I (e.g. IFN-α, IFN-β, IFN-ε, IFN-κ, and IFN-ω), interferon type II (e.g. IFN-γ), and interferon type III. In some embodiments, IFN-α is further classified into about 13 subtypes including IFNA1, IFNA2, IFNA4, IFNA5, IFNA6, IFNA7, IFNA8, IFNA10, IFNA13, IFNA14, IFNA16, IFNA17, and IFNA21.

**[0126]** Interleukins are expressed by leukocytes or white blood cells and they promote the development and differentiation of T and B lymphocytes and hematopoietic cells. Exemplary interleukins include IL-1, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8 (CXCL8), IL-9, IL-10, IL-11,

IL-12, IL-13, IL-14, IL-15, IL-16, IL-17, IL-18, IL-19, IL-20, IL-21, IL-22, IL-23, IL-24, IL-25, IL-26, IL-27, IL-28, IL-29, IL-30, IL-31, IL-32, IL-33, IL-35, and IL-36.

**[0127]** Tumor necrosis factors (TNFs) are a group of cytokines that modulate apoptosis. In some instances, there are about 19 members within the TNF family, including, not limited to, TNFα, lymphotoxin-alpha (LT-alpha), lymphotoxin-beta (LT-beta), T cell antigen gp39 (CD40L), CD27L, CD30L, FASL, 4-1BBL, OX40L, and TNF-related apoptosis inducing ligand (TRAIL).

**[0128]** Colony-stimulating factors (CSFs) are secreted glycoproteins that interact with receptor proteins on the surface of hemopoietic stem cells, which subsequently modulates cell proliferation and differentiation into specific kind of blood cells. In some instances, a CSF comprises macrophage colony-stimulating factor, granulocyte macrophage colony-stimulating factor (GM-CSF), granulocyte colony-stimulating factor (G-CSF) or promegapoietin.

**[0129]** In some embodiments, one or more methods described herein further comprises administration of a cytokine. In some instances, the cytokine comprises a chemokine, an interferon, an interleukin, a colony-stimulating factor or a tumor necrosis factor. In some cases, the cytokine comprises IL2, IL7, IL12, IL15, IL21, IFNγ, TNF-α, or a combination thereof.

**[0130]** In some embodiments, the additional therapeutic agent comprises a chemotherapeutic agent. In some embodiments, the chemotherapeutic agent is selected from among bortezomib, CAL-101, chlorambucil, cyclophosphamide, dexamethasone, docetaxel, doxorubicin, endostatin, everolimus, fludarabine, fostamatinib, ifosfamide, ibritumomab, lenalidomide, mesalazine, ofatumumab, paclitaxel, pentostatin, prednisone, rituximab, temsirolimus, thalidomide, tositumomab or a combination thereof.

**[0131]** In some embodiments, one or more methods described herein further comprises administration of a chemotherapeutic agent. In some cases, the chemotherapeutic agent is selected from among bortezomib, CAL-101, chlorambucil, cyclophosphamide, dexamethasone, docetaxel, doxorubicin, endostatin, everolimus, fludarabine, fostamatinib, ifosfamide, ibritumomab, lenalidomide, mesalazine, ofatumumab, paclitaxel, pentostatin, prednisone, rituximab, temsirolimus, thalidomide, tositumomab or a combination thereof. In some cases, the chemotherapeutic agent comprises cyclophosphamide and optionally with fludarabine.

**[0132]** In some embodiments, the engineered effector cell and the additional therapeutic agent are administered at the same time. In some embodiments, the engineered effector cell and the additional therapeutic agent are administered sequentially. In some embodiments, the engineered effector cell is administered after administering the additional therapeutic agent. In some embodiments, the engineered effector cell is administered before administering the additional therapeutic agent.

**Vector Systems and Delivery Methods**

**[0133]** In some embodiments, a vector comprises a polynucleotide encoding the engineered antigen binding domain, the stalk region, the transmembrane domain, the intracellular signaling domain, or a combination thereof. In some embodiments, the polynucleotide further comprises a promoter and a 3'untranslated region comprising a polyadenylation site (i.e. an expression cassette, as is shown in **FIG. 1**)

**[0134]** In some embodiments, any suitable vector system is used. In some embodiments, the vector system is a viral vector system. In some embodiments, the viral vector system is a retroviral vector, an adenovirus vector, a poxvirus vector, a herpesvirus vector, or an adeno-associated virus vector. In some embodiments, the retroviral vector is a gammaretrovirus vector or a lentivirus vector. In some embodiments, the vector system comprises a transcription factor, a nuclease, a transgene, or a combination thereof. In some embodiments, the vector system is a non-viral vector system. In some embodiments, the non-viral vector system is a plasmid vector, naked nucleic acid, or an mRNA. In some embodiments, the non-viral vector system is Sleeping Beauty (SB) Transposon System. In some embodiments, the naked nucleic acid is complexed with a delivery vehicle. In some embodiments, the delivery vehicle is a liposome or a poloxamer. In some embodiments, the naked nucleic acid is a DNA.

**[0135]** In some embodiments, methods of non-viral delivery of nucleic acids include electroporation, lipofection, nucleofection, gold nanoparticle delivery, microinjection, biolistics, virosomes, liposomes, immunoliposomes, polycation or lipid: nucleic acid conjugates, naked DNA, mRNA, artificial virions, and agent-enhanced uptake of DNA. In some embodiments, sonoporation using, *e.g.*, the Sonitron 2000 system (Rich-Mar) is used for delivery of nucleic acids.

**[0136]** In some embodiments, the gene or portion of the gene is inserted using a restriction enzyme-based technique.

**[0137]** In some embodiments, vectors are delivered to cells *ex vivo*. In some embodiments, the cells are explanted from the subject. In some embodiments, the cells explanted from the subject comprises lymphocytes, T cells, bone marrow aspirates, or tissue biopsy. In some embodiments, cells are reimplanted into the subject, in which reimplanation of the cells into the subject occurs after selection for cells which have incorporated the vector. In some embodiments, prior to or after selection, the cells are expanded. In some embodiments, the vector further comprises a reporter gene so that the incorporation of the vector is detected via activation of the reporter gene. In some embodiments, any suitable reporter gene is used. In some embodiments, the reporter gene is a GFP, a resistance gene, a cell surface marker, or an endogenous tag. In some

embodiments, suitable cells are selected using any suitable technology. In some embodiments, the technology to select suitable cells is flow cytometry or magnetic columns.

**[0138]** In some embodiments, the vector further encodes a suicide gene. In some embodiments, the suicide gene induces elimination of the engineered effector cell. In some embodiments, the suicide gene is any gene that induces apoptosis in the engineered effector cell. In some embodiments, the suicide gene encodes a factor able to convert a non-toxic prodrug into a toxic compound. In some embodiments, administration of the prodrug to the subject results in selective elimination of the engineered effector cell comprising the suicide gene.

**Suitable Cells**

**[0139]** In some embodiments, the engineered effector cells are derived from a source of cells. In some embodiments, the cells are autologous and the source is obtained from the subject. In other embodiments, the cells are allogenic. In some embodiments, the source of cells are obtained from PBMCs, bone marrow, lymph node tissue, cord blood, thymus tissue, tissue from a site of infection, ascites, pleural effusion, spleen tissue, tumors, or a combination thereof. In some embodiments, the source of cells are T cells or NK cells.

**[0140]** In certain embodiments, the source of cells is obtained from a unit of blood collected from the subject. In some embodiments, the blood is collected from the subject using any suitable technique, such as Ficoll™ separation. In some embodiments, cells from the circulating blood of the subject are obtained by apheresis. In some embodiments, the apheresis product contains lymphocytes, including T cells, monocytes, granulocytes, B cells, other nucleated white blood cells, red blood cells, and platelets. In some embodiments, the cells collected by apheresis are washed to remove the plasma fraction and to place the cells in an appropriate buffer or media for subsequent processing steps.

**Pharmaceutical Composition and Formulation**

**[0141]** In some embodiments, pharmaceutical compositions comprise a plurality of engineered effector cells described herein and a pharmaceutically acceptable carrier. In some embodiments, the pharmaceutically acceptable carrier is any suitable pharmaceutically acceptable carrier. The term "pharmaceutically acceptable carrier" as used herein means one or more compatible solid or liquid fillers, diluents, other excipients, or encapsulating substances which are suitable for administration into a human or veterinary patient (e.g., a physiologically acceptable carrier or a pharmacologically acceptable carrier). The term "carrier" denotes an organic or inorganic ingredient, natural or synthetic, with which the engineered effector cell is combined to facilitate the application. In some embodiments, the pharmaceutically acceptable carrier is co-mingled with one or more of the engineered effector cells and with each other, when more than one pharmaceutically acceptable carrier

is present in the composition in a manner so as not to substantially impair the desired pharmaceutical efficacy.

**[0142]** "Pharmaceutically acceptable" materials typically are capable of administration to a subject without the production of significant undesirable physiological effects such as nausea, dizziness, rash, or gastric upset. It is, for example, desirable for a composition comprising a pharmaceutically acceptable carrier not to be immunogenic when administered to a human patient for therapeutic purposes.

**[0143]** In some embodiments, the pharmaceutical formulations include a carrier or carrier materials selected on the basis of compatibility with the composition disclosed herein, and the release profile properties of the desired dosage form. Exemplary carrier materials include, e.g., binders, suspending agents, disintegration agents, filling agents, surfactants, solubilizers, stabilizers, lubricants, wetting agents, diluents, and the like. Pharmaceutically compatible carrier materials include, but are not limited to, acacia, gelatin, colloidal silicon dioxide, calcium glycerophosphate, calcium lactate, maltodextrin, glycerine, magnesium silicate, polyvinylpyrrollidone (PVP), cholesterol, cholesterol esters, sodium caseinate, soy lecithin, taurocholic acid, phosphotidylcholine, sodium chloride, tricalcium phosphate, dipotassium phosphate, cellulose and cellulose conjugates, sugars sodium stearoyl lactylate, carrageenan, monoglyceride, diglyceride, pregelatinized starch, and the like. See, e.g., *Remington: The Science and Practice of Pharmacy*, Nineteenth Ed (Easton, Pa.: Mack Publishing Company, 1995), Hoover, John E., *Remington's Pharmaceutical Sciences*, Mack Publishing Co., Easton, Pennsylvania 1975, Liberman, H.A. and Lachman, L., Eds., *Pharmaceutical Dosage Forms*, Marcel Decker, New York, N.Y., 1980, and *Pharmaceutical Dosage Fo*rms *and* Drug Delivery Systems, Seventh Ed. (Lippincott Williams & Wilkins1999).

**[0144]** In some instances, the pharmaceutical formulations further include pH adjusting agents or buffering agents which include acids such as acetic, boric, citric, lactic, phosphoric and hydrochloric acids, bases such as sodium hydroxide, sodium phosphate, sodium borate, sodium citrate, sodium acetate, sodium lactate and tris-hydroxymethylaminomethane, and buffers such as citrate/dextrose, sodium bicarbonate and ammonium chloride. Such acids, bases and buffers are included in an amount required to maintain pH of the composition in an acceptable range.

**[0145]** In some instances, the pharmaceutical formulation includes one or more salts in an amount required to bring osmolality of the composition into an acceptable range. Such salts include those having sodium, potassium or ammonium cations and chloride, citrate, ascorbate, borate, phosphate, bicarbonate, sulfate, thiosulfate or bisulfite anions, suitable salts include sodium chloride, potassium chloride, sodium thiosulfate, sodium bisulfite and ammonium sulfate.

**[0146]** In some embodiments, the pharmaceutical formulations include, but are not limited to, sugars like trehalose, sucrose, mannitol, maltose, glucose, or salts like potassium phosphate,

sodium citrate, ammonium sulfate and/or other agents such as heparin to increase the solubility and *in vivo* stability of polypeptides.

**[0147]** In some instances, the pharmaceutical formulations further include diluent which are used to stabilize compounds because they can provide a more stable environment. Salts dissolved in buffered solutions (which also can provide pH control or maintenance) are utilized as diluents in the art, including, but not limited to a phosphate buffered saline solution.

**[0148]** In some cases, the pharmaceutical formulations include disintegration agents or disintegrants to facilitate the breakup or disintegration of a substance. The term "disintegrate" include both the dissolution and dispersion of the dosage form when contacted with gastrointestinal fluid. Examples of disintegration agents include a starch, e.g., a natural starch such as corn starch or potato starch, a pregelatinized starch such as National 1551 or Amijel®, or sodium starch glycolate such as Promogel® or Explotab®, a cellulose such as a wood product, methylcrystalline cellulose, e.g., Avicel®, Avicel® PH101, Avicel® PH102, Avicel® PH105, Elcema® P100, Emcocel®, Vivacel®, Ming Tia®, and Solka-Floc®, methylcellulose, croscarmellose, or a cross-linked cellulose, such as cross-linked sodium carboxymethylcellulose (Ac-Di-Sol®), cross-linked carboxymethylcellulose, or cross-linked croscarmellose, a cross-linked starch such as sodium starch glycolate, a cross-linked polymer such as crospovidone, a cross-linked polyvinylpyrrolidone, alginate such as alginic acid or a salt of alginic acid such as sodium alginate, a clay such as Veegum® HV (magnesium aluminum silicate), a gum such as agar, guar, locust bean, Karaya, pectin, or tragacanth, sodium starch glycolate, bentonite, a natural sponge, a surfactant, a resin such as a cation-exchange resin, citrus pulp, sodium lauryl sulfate, sodium lauryl sulfate in combination starch, and the like.

**[0149]** Lubricants and glidants are also optionally included in the pharmaceutical formulations described herein for preventing, reducing or inhibiting adhesion or friction of materials. Exemplary lubricants include, e.g., stearic acid, calcium hydroxide, talc, sodium stearyl fumerate, a hydrocarbon such as mineral oil, or hydrogenated vegetable oil such as hydrogenated soybean oil (Sterotex®), higher fatty acids and their alkali-metal and alkaline earth metal salts, such as aluminum, calcium, magnesium, zinc, stearic acid, sodium stearates, glycerol, talc, waxes, Stearowet®, boric acid, sodium benzoate, sodium acetate, sodium chloride, leucine, a polyethylene glycol (e.g., PEG-4000) or a methoxypolyethylene glycol such as Carbowax™, sodium oleate, sodium benzoate, glyceryl behenate, polyethylene glycol, magnesium or sodium lauryl sulfate, colloidal silica such as Syloid™, Cab-O-Sil®, a starch such as corn starch, silicone oil, a surfactant, and the like.

**[0150]** Solubilizers include compounds such as triacetin, triethylcitrate, ethyl oleate, ethyl caprylate, sodium lauryl sulfate, sodium doccusate, vitamin E TPGS, dimethylacetamide, N-methylpyrrolidone, N-hydroxyethylpyrrolidone, polyvinylpyrrolidone, hydroxypropylmethyl cellulose, hydroxypropyl cyclodextrins, ethanol, n-butanol, isopropyl alcohol, cholesterol, bile

salts, polyethylene glycol 200-600, glycofurol, transcutol, propylene glycol, and dimethyl isosorbide and the like.

**[0151]** Stabilizers include compounds such as any antioxidation agents, buffers, acids, preservatives and the like. Exemplary stabilizers include L-arginine hydrochloride, tromethamine, albumin (human), citric acid, benzyl alcohol, phenol, disodium biphosphate dehydrate, propylene glycol, metacresol or m-cresol, zinc acetate, polysorbate-20 or Tween® 20, or trometamol.

**[0152]** Suspending agents include compounds such as polyvinylpyrrolidone, e.g., polyvinylpyrrolidone K12, polyvinylpyrrolidone K17, polyvinylpyrrolidone K25, or polyvinylpyrrolidone K30, vinyl pyrrolidone/vinyl acetate copolymer (S630), polyethylene glycol, e.g., the polyethylene glycol can have a molecular weight of about 300 to about 6000, or about 3350 to about 4000, or about 7000 to about 5400, sodium carboxymethylcellulose, methylcellulose, hydroxypropylmethylcellulose, hydroxymethylcellulose acetate stearate, polysorbate-80, hydroxyethylcellulose, sodium alginate, gums, such as, e.g., gum tragacanth and gum acacia, guar gum, xanthans, including xanthan gum, sugars, cellulosics, such as, e.g., sodium carboxymethylcellulose, methylcellulose, sodium carboxymethylcellulose, hydroxypropylmethylcellulose, hydroxyethylcellulose, polysorbate-80, sodium alginate, polyethoxylated sorbitan monolaurate, polyethoxylated sorbitan monolaurate, povidone and the like.

**[0153]** Surfactants include compounds such as sodium lauryl sulfate, sodium docusate, Tween 60 or 80, triacetin, vitamin E TPGS, sorbitan monooleate, polyoxyethylene sorbitan monooleate, polysorbates, polaxomers, bile salts, glyceryl monostearate, copolymers of ethylene oxide and propylene oxide, e.g., Pluronic® (BASF), and the like. Additional surfactants include polyoxyethylene fatty acid glycerides and vegetable oils, *e.g.*, polyoxyethylene (60) hydrogenated castor oil, and polyoxyethylene alkylethers and alkylphenyl ethers, *e.g.*, octoxynol 10, octoxynol 40. Sometimes, surfactants is included to enhance physical stability or for other purposes.

**[0154]** Viscosity enhancing agents include, e.g., methyl cellulose, xanthan gum, carboxymethyl cellulose, hydroxypropyl cellulose, hydroxypropylmethyl cellulose, hydroxypropylmethyl cellulose acetate stearate, hydroxypropylmethyl cellulose phthalate, carbomer, polyvinyl alcohol, alginates, acacia, chitosans and combinations thereof.

**[0155]** Wetting agents include compounds such as oleic acid, glyceryl monostearate, sorbitan monooleate, sorbitan monolaurate, triethanolamine oleate, polyoxyethylene sorbitan monooleate, polyoxyethylene sorbitan monolaurate, sodium docusate, sodium oleate, sodium lauryl sulfate, sodium doccusate, triacetin, Tween 80, vitamin E TPGS, ammonium salts and the like.

**Dosage Forms**

**[0156]** In some instances, a pharmaceutical composition described herein is formulated for parenteral administration. In some cases, parenteral administration comprises intravenous, subcutaneous, intramuscular, intra-arterial, intraosseous infusion, intracerebral, intracerebroventricular, or intrathecal administration. In some instances, a pharmaceutical composition described herein is formulated for intravenous, subcutaneous, intramuscular, intra-arterial, intraosseous infusion, intracerebral, intracerebroventricular, or intrathecal administration. In some cases, a pharmaceutical composition described herein is administered to a subject as an injection. In some cases, a pharmaceutical composition described herein is administered to a subject as an infusion.

**[0157]** In some instances, a pharmaceutical composition described herein is presented in any unit dosage form and is prepared by any suitable method, many of which are well known in the art of pharmacy. Such methods include the step of bringing the engineered effector cell into association with a carrier that constitutes one or more accessory ingredients.

**[0158]** In some embodiments, a composition suitable for parenteral administration comprises a sterile aqueous preparation of the inventive composition, which preferably is isotonic with the blood of the recipient. In some embodiments, the aqueous preparation is formulated according to known methods using suitable dispersing or wetting agents and suspending agents. In some embodiments the sterile injectable preparation is a sterile injectable solution or suspension in a non-toxic parenterally-acceptable diluent or solvent, for example, as a solution in 1,3-butane diol. In some embodiments, the vehicle or solvent is water, Ringer's solution, or isotonic sodium chloride solution. In some embodiments, sterile, fixed oils are conventionally employed as a solvent or suspending medium. In some embodiments, the oil is a synthetic mono-or di-glycerides. In some embodiments a fatty acids such as oleic acid is used in the preparation of injectables. In some embodiments, carrier formulations suitable for oral, subcutaneous, intravenous, intramuscular, etc. administrations are found in *Remington's Pharmaceutical Sciences,* Mack Publishing Co., Easton, PA.

**Therapeutic Regimens**

**[0159]** In some embodiments, one or more pharmaceutical compositions described herein are administered for therapeutic applications. In some embodiments, the pharmaceutical composition is administered once per day, twice per day, three times per day or more. The pharmaceutical composition is administered daily, every day, every alternate day, five days a week, once a week, every other week, two weeks per month, three weeks per month, once a month, twice a month, three times per month, or more. The pharmaceutical composition is

administered for at least 1 month, 2 months, 3 months, 4 months, 5 months, 6 months, 7 months, 8 months, 9 months, 10 months, 11 months, 12 months, 18 months, 2 years, 3 years, or more.

**[0160]** In the case wherein the patient's status does improve, upon the doctor's discretion the administration of the composition is given continuously, alternatively, the dose of the composition being administered is temporarily reduced or temporarily suspended for a certain length of time (i.e., a "drug holiday"). In some instances, the length of the drug holiday varies between 2 days and 1 year, including by way of example only, 2 days, 3 days, 4 days, 5 days, 6 days, 7 days, 10 days, 12 days, 15 days, 20 days, 28 days, 35 days, 50 days, 70 days, 100 days, 120 days, 150 days, 180 days, 200 days, 250 days, 280 days, 300 days, 320 days, 350 days, or 365 days. The dose reduction during a drug holiday is from 10%-100%, including, by way of example only, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 100%.

**[0161]** Once improvement of the patient's conditions has occurred, a maintenance dose is administered if necessary. In some embodiments, the dosage or the frequency of administration, or both, is reduced, as a function of the symptoms, to a level at which the improved disease, disorder or condition is retained.

**[0162]** In some embodiments, the amount of a given agent that correspond to such an amount varies depending upon factors such as the particular compound, the severity of the disease, the identity (e.g., weight) of the subject or host in need of treatment, but nevertheless is routinely determined in a manner known in the art according to the particular circumstances surrounding the case, including, e.g., the specific agent being administered, the route of administration, and the subject or host being treated. In some instances, the desired dose is conveniently presented in a single dose or as divided doses administered simultaneously (or over a short period of time) or at appropriate intervals, for example as two, three, four or more sub-doses per day.

**[0163]** The foregoing ranges are merely suggestive, as the number of variables in regard to an individual treatment regime is large, and considerable excursions from these recommended values are not uncommon. Such dosages is altered depending on a number of variables, not limited to the activity of the compound used, the disease or condition to be treated, the mode of administration, the requirements of the subject, the severity of the disease or condition being treated, and the judgment of the practitioner.

**[0164]** In some embodiments, toxicity and therapeutic efficacy of such therapeutic regimens are determined by standard pharmaceutical procedures in cell cultures or experimental animals, including, but not limited to, the determination of the LD50 (the dose lethal to 50% of the population) and the ED50 (the dose therapeutically effective in 50% of the population). The dose ratio between the toxic and therapeutic effects is the therapeutic index and it is expressed as the ratio between LD50 and ED50. Compounds exhibiting high therapeutic indices are preferred. The data obtained from cell culture assays and animal studies are used in formulating

a range of dosage for use in human. The dosage of such compounds lies preferably within a range of circulating concentrations that include the ED50 with minimal toxicity. The dosage varies within this range depending upon the dosage form employed and the route of administration utilized.

**Kits/Article of Manufacture**

**[0165]** Disclosed herein are kits and articles of manufacture suitable for carrying out the methods disclosed herein. In some embodiments, the kit comprises two or more components required for performing a therapeutic method described herein. In some embodiments, kit components include, but are not limited to, one or more engineered effector cells of the invention, appropriate reagents, and/or equipment. In some embodiments, the kit is packaged in a vial, pouch, ampoule, and/or any container suitable for a therapeutic method. Additional examples of pharmaceutical packaging materials include, but are not limited to, blister packs, bottles, tubes, bags, containers, bottles, and any packaging material suitable for a selected formulation and optionally intended mode of administration and treatment. In some embodiments, kit components are provided as concentrates (including lyophilized compositions), which are further diluted prior to use or provided at the concentration of use. In some embodiments, when the engineered effector cell is for use *in vivo,* a single dosage is provided in a sterilized container having the desired amount and concentration of the engineered effector cell.

**[0166]** In some cases, a kit includes labels listing contents and/or instructions for use, and package inserts with instructions for use. A set of instructions will also typically be included.

**[0167]** In some embodiments, a label is on or associated with the container. In one embodiment, a label is on a container when letters, numbers or other characters forming the label are attached, molded or etched into the container itself; a label is associated with a container when it is present within a receptacle or carrier that also holds the container, e.g., as a package insert. In one embodiment, a label is used to indicate that the contents are to be used for a specific therapeutic application. The label also indicates directions for use of the contents, such as in the methods described herein.

**Certain Terminologies**

**[0168]** The terminology used herein is for the purpose of describing particular cases only and is not intended to be limiting. As used herein, the singular forms “a”, “an” and “the” are intended to include the plural forms as well, unless the context clearly indicates otherwise. Furthermore, to the extent that the terms “including”, “includes”, “having”, “has”, “with”, or variants thereof are used in either the detailed description and/or the claims, such terms are intended to be inclusive in a manner similar to the term “comprising.”

**[0169]** The term "about" or "approximately" means within an acceptable error range for the particular value as determined by one of ordinary skill in the art, which will depend in part on how the value is measured or determined, *e.g.*, the limitations of the measurement system. For example, "about" can mean within 1 or more than 1 standard deviation, per the practice in the given value. Where particular values are described in the application and claims, unless otherwise stated the term "about" should be assumed to mean an acceptable error range for the particular value.

**[0170]** The terms "individual," "patient," or "subject" are used interchangeably. None of the terms require or are limited to situation characterized by the supervision (e.g. constant or intermittent) of a health care worker (e.g. a doctor, a registered nurse, a nurse practitioner, a physician's assistant, an orderly, or a hospice worker). Further, these terms refer to human or animal subjects. Animal subjects include, but are not limited to, animal models, such as, mammalian models of conditions or disorders associated with elevated CD46 expression such as breast cancer, cervical cancer, colorectal cancer, kidney cancer, liver cancer, lung cancer, multiple myeloma, ovarian cancer, pancreatic cancer, prostate cancer or urothelial cancer.

**[0171]** "Treating" or "treatment" of a state, disorder or condition (e.g., cancer) includes: (1) preventing or delaying the appearance of clinical or sub-clinical symptoms of the disorder developing in a human that is afflicted with or pre-disposed to the disorder but does not yet experience or display clinical or subclinical symptoms of the disorder; and/or (2) inhibiting the disorder, including arresting, reducing or delaying the clinical manifestation of the disorder or at least one clinical or sub-clinical symptom thereof; and/or (3) relieving the disorder, e.g., causing regression of the disorder or at least one of its clinical or sub-clinical symptoms; and/or (4) causing a decrease in the severity of one or more symptoms of the disorder. The benefit to a subject to be treated is either statistically significant or at least perceptible to the patient or to the physician.

**[0172]** The term "antibody" as used herein refers to immunoglobulin molecules and immunologically active portions of immunoglobulin molecules, i.e., molecules that contain an antigen binding site that immunospecifically binds an antigen. The term also refers to antibodies comprised of two immunoglobulin heavy chains and two immunoglobulin light chains as well as a variety of forms including full length antibodies and portions thereof; including, for example, an immunoglobulin molecule, a polyclonal antibody, a monoclonal antibody, a recombinant antibody, a chimeric antibody, a humanized antibody, a CDR-grafted antibody, $F(ab)_2$, Fv, scFv, $IgG\Delta CH_2$, F(ab')2, $scFv2CH_3$, F(ab), VL, VH, scFv4, scFv3, scFv2, dsFv, Fv, scFv-Fc, (scFv)2, a disulfide linked Fv, a single domain antibody (dAb), a diabody, a multispecific antibody, a dual specific antibody, an anti-idiotypic antibody, a bispecific antibody, any isotype (including, without limitation IgA, IgD, IgE, IgG, or IgM) a modified antibody, and a synthetic

antibody (including, without limitation non-depleting IgG antibodies, T-bodies, or other Fc or Fab variants of antibodies). Each heavy chain is composed of a variable region of said heavy chain (abbreviated here as HCVR or VH) and a constant region of said heavy chain. Each light chain is composed of a variable region of said light chain (abbreviated here as LCVR or VL) and a constant region of said light chain. The VH and VL regions may be further divided into hypervariable regions referred to as complementarity-determining regions (CDRs) and interspersed with conserved regions referred to as framework regions (FR). Each VH and VL region thus consists of three CDRs and four FRs which are arranged from the N terminus to the C terminus in the following order: FR1, CDR1, FR2, CDR2, FR3, CDR3, FR4.

**[0173]** As used herein, the term "CDR" refers to the complementarity determining region within antibody variable sequences. There are three CDRs in each of the variable regions of the heavy chain and of the light chain, which are designated CDR1, CDR2 and CDR3, for each of the variable regions. The exact boundaries of these CDRs have been defined differently according to different systems, including those described by: Kabat et al. (1991), "Sequences of Proteins of Immunological Interest," 5th Ed. Public Health Service, National Institutes of Health, Bethesda, MD ("Kabat" numbering scheme), Al-Lazikani et al., (1997) JMB 273,927-948 ("Chothia" numbering scheme), MacCallum et al., J. Mol. Biol. 262:732-745 (1996), "Antibody-antigen interactions: Contact analysis and binding site topography," J. Mol. Biol. 262, 732-745." ("Contact" numbering scheme), Lefranc MP et al., "IMGT unique numbering for immunoglobulin and T cell receptor variable domains and Ig superfamily V-like domains," Dev Comp Immunol, 2003 Jan;27(1):55-77 ("IMGT" numbering scheme), and Honegger A and Plückthun A, "Yet another numbering scheme for immunoglobulin variable domains: an automatic modeling and analysis tool," J Mol Biol, 2001 Jun 8;309(3):657-70, ("Aho" numbering scheme). The boundaries of a given CDR or FR may vary depending on the scheme used for identification. For example, the Kabat scheme is based on structural alignments, while the Chothia scheme is based on structural information. Numbering for both the Kabat and Chothia schemes is based upon the most common antibody region sequence lengths, with insertions accommodated by insertion letters, for example, "30a," and deletions appearing in some antibodies. The two schemes place certain insertions and deletions ("indels") at different positions, resulting in differential numbering. The Contact scheme is based on analysis of complex crystal structures and is similar in many respects to the Chothia numbering scheme. The EU index or EU index as in Kabat or EU numbering scheme refers to the numbering of the EU antibody (Edelman et al., 1969, Proc Natl Acad Sci USA 63:78-85. In some embodiments, the methods used herein utilize CDRs defined according to any of these systems. In some embodiments, the methods used herein utilize CDRs defined according to the Kabat system.

**[0174]** As used herein, the term "engineered effector cell activation" or "engineered effector cell triggering" refers to the state of an engineered effector cell that has been sufficiently

stimulated to induce detectable cellular proliferation, cytokine production and/or detectable effector function. Engineered effector cell activation can be measured using any suitable assay, such as an ELISA to measure cytokine secretion, an ELISPOT, flow cytometry assays to measure intracellular cytokine expression (CD107), flow cytometry assays to measure proliferation, and cytotoxicity assays (51Cr release assay) to determine target cell elimination. In some embodiments, these assays typically use controls (e.g. non-engineered cells) to compare to engineered effector cells (e.g. CAR-T) to determine relative activation of the engineered effector cell compared to a control. In some embodiments, these assays compare engineered effector cells incubated or put in contact with a target cell not expressing the target antigen.

**[0175]** As used herein, the term "affinity" refers to measures the strength of interaction between an epitope and an antibody's antigen binding site. Affinity is measured by the equilibrium dissociation constant ($K_D$). Lower values of $K_D$ indicate a higher affinity, and vice versa. In some embodiments, the antibody has affinity for CD46 of less than about $1.0 \times 10^{-6}$ M. In some embodiments, the dissociation constant is between about $1.0 \times 10^{-6}$ and $1.0 \times 10^{-7}$ M. In other embodiments, the dissociation constant is between about $1.0 \times 10^{-7}$ and $1.0 \times 10^{-8}$ M. In still other embodiments, the dissociation constant is between about $1.0 \times 10^{-8}$ and $1.0 \times 10^{-9}$ M. In yet other embodiments, the dissociation constant is less than $9.9 \times 10^{-10}$ M. In some embodiments, affinity is measured using art-known techniques, such as ELISA or BIACORE.

**[0176]** As used herein, the term "avidity" refers to measure of the overall strength of an antibody-antigen complex. In some embodiments, the antibody has avidity for CD46 of about 10 μM or less, 5 μM or less, 2 μM or less, 1 μM or less, 500 nM or less, 400 nM or less, 300 nM or less, or 200 nM or less. In some embodiments, the antibody has avidity for CD46 of about 100 nM or less, about 75 nM or less, about 50 nM or less, about 25 nM or less, about 10 nM or less, or about 5 nM or less. In some embodiments, the antibody has avidity for CD46 of about 1 nM or less, about 800 pM or less, about 700 pM or less, about 600 pM or less, about 500 pM or less, about 400 pM or less, about 300 pM or less, about 200 pM or less, or about 100 pM or less. In some embodiments, avidity is measured using art-known techniques, such as ELISA or BIACORE.

## EXAMPLES

**[0177]** These examples are provided for illustrative purposes only and not to limit the scope of the claims provided herein.

### Example 1: Illustrative CAR configurations

**[0178]** VS5FL Fab sequence was converted into an scFv format and the resulting sequences were subsequently inserted into a second generation lentivirus CAR containing CD28 and CD3zeta (**FIG. 1**). High titer lentivirus stocks were used to transduce activated human T cells. CAR-T scFv expression was demonstrated by FACs and the resulting cells were used in T cell

mediated cytotoxicity assays. Table 3 illustrates the nucleic acid sequence and polypeptide sequence of the YS5FL-scFV.

| YS5FL-scFV Sequences | SEQ ID NO: |
|---|---|
| GCTAGCCAGGTGCAGCTGGTGCAGTCTGGGGGAGGCGTGGTCCAGC<br>CTGGGAGGTCCCTGAGACTCGCCTGTGCAGCCTCTGGACTCACCGT<br>CAACAATTATGCTATGCACTGGGTCCGCCAGGCTCCAGGCAAGGGA<br>CTGGAGTGGGTGGCAGTTATATCATATGATGGAAACAATAAATACT<br>ATGCAGACTCCGTGAAGGGCCGATTCACCATCTCCAGAGACAATTC<br>CAAGAACACGCTGTATCTGCAAATGAACAGCCTGAGAGCTGAGGA<br>CACGGCTGTGTATTACTGTGCGAAAGGGGGTGGATACTTCGATCTC<br>TGGGGCCGTGGCACCCTGGTCACCGTCTCCTCAGGTGGAGGCGGTT<br>CAGGCGGAGGTGGCTCTGGCGGTGGCGGATCGCAGTCTGTGTTGAC<br>GCAGCCGCCCTCAGTGTCTGGGGCCCCAGGGCAGAGGGTCACCATC<br>TCCTGCACTGGGAGCAGCTCCAACATCGGGGCAGGTTATGATGTAC<br>ACTGGTACCAGCAGCTTCCAGGAACAGCCCCCAAACTCCTCATCTA<br>TGGTAACAACAATCGGCCCTCAGGGGTCCCTGATCGATTCTCTGGC<br>TCCAAGTCTGGCACCTCAGCCTCCCTGGCCATCACTGGGCTCCAGG<br>CTGAGGATGAGGCTGATTATTACTGCAGCTCATATACAAGCGGCAC<br>TTGGCTGTTCGGCGGGGGGACCAAGCTGACCGTCCTACATCATCAC<br>CATCACCATCTCGAG | 202 |
| QVQLVQSGGGVVQPGRSLRLACAASGLTVNNYAMHWVRQAPGKGLE<br>WVAVISYDGNNKYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAV<br>YYCAKGGGYFDLWGRGTLVTVSSGGGGSGGGGSGGGGSQSVLTQPPS<br>VSGAPGQRVTISCTGSSSNIGAGYDVHWYQQLPGTAPKLLIYGNNNRP<br>SGVPDRFSGSKSGTSASLAITGLQAEDEADYYCSSYTSGTWLFGGGTK<br>LTVLHHHHHH | 203 |

**[0179]** **FIG. 2** shows additional illustrative configurations of CD46-specific CARs. As shown in FIG. 2, a first generation CAR comprises a YS5FL scFv as the engineered antigen binding domain, a CD8 hinge, and a CD3zeta signaling domain. A second generation CAR comprises a YS5FL scFv as the engineered antigen binding domain, a CD8 hinge, a CD28 costimulatory domain, and a CD3zeta signaling domain. A third generation CAR comprises a YS5FL scFv as the engineered antigen binding domain, a CD8 hinge, a CD28 first costimulatory domain, a CD3zeta signaling domain, and a 4-1BB second costimulatory domain. A fourth generation CAR comprises a YS5FL scFv as the engineered antigen binding domain, a CD8 hinge, CD28 costimulatory domain, and a CD3zeta signaling domain, and further comprised a 4-1BB costimulatory ligand.

**[0180]** In some instances, use of two signaling domains (CD28 and CD3zeta) and the 4-1BB ligand provides therapeutic *in vivo* efficiency, showing balanced tumoricidal function (CD28), increased T cell persistence (4-1BB), and elevated CD8/CD4 ratio, and decreased exhaustion.

**Example 2: Real Time Cytotoxicity Assay (RTCA) of anti-CD46 CAR-T cells**

**[0181]** Effector cells were freshly activated and expanded on day 1. Viral transduction occurred on day 5 in CAR-T cells, but not in the non-transduced (Non-T) cells. On day 15, a real time

cytotoxicity assay (RTCA) was performed. Target cells corresponding to each population of effector cells (Tables 4 and 5) were seeded at 10,000 cells/well at hour 0 of the RTCA. Effector cells were then added at the 25 hour mark of the RTCA. The effector cell: target cell ratio was 10:1. In some instances, during transduction, about 10% of the effector cells were transduced, yielding a ratio of transduced effector cell: target cell of 1:1. Data was collected by ACEA Biosciences software v2.0.

Table 4. Experimental group

| | **Effector cells** | **Target cells** |
|---|---|---|
| True | Anti-CD46 CAR-T | LNCAP-C4-B2 |
| Null | Non-T | BPH-1 |

Table 5. Control group

| | **Effector cells** | **Target cells** |
|---|---|---|
| True | Anti-CD19 CAR-T | CD-19 HeLa |
| Null | Non-T | HeLa |

***Real Time Cell Analysis (RTCA) Assay***

**[0182]** Electrical impedance was recorded every 5 minutes to provide a normalized Cell Index (CI) (**FIGS. 3-6**). Results were grouped by the target cell, with each cell line having a characteristic impedance curve over 82 hours. At 25 hours, the target cells were given one of the following: 1) fresh media alone; 2) fresh media plus non-transduced T cells; or 3) fresh media plus transduced T cells. In some instances, the additional of non-T cells initially lowered the Cell Index, but the cells continued to grow and after a period of time reach to a level relative to cells from the "Fresh media alone group."

**[0183]** The effect of anti-CD46 CARs was compared to anti-CD19 CARs for both control non-target cells (**FIGS. 3 and 5**, respectively) and target cells (**FIGS. 4 and 6**, respectively). The control non-target cells (BPH-1) for the anti-CD46 CAR-T cells responded similarly to transduced and non-transduced T cells (**FIG. 3**) LNCAP-C4-B2 were the target cells for the anti-CD46 CAR-T cells. These CARs showed a killing rate of over 99% of LNCAP-C4-B2 cells within the first 2 hours following administration of the anti-CD46CARs at the 25 hour mark (**FIG. 4**). This is compared to the anti-CD19 CARs, which showed a drop in target cell growth after approximately 10 hours following administration of the anti-CD19 CARs at the 25 hour mark (**FIG. 5**).

**Example 3: *In vitro* cytotoxicity of CAR-T cells**

**[0184]** A number of different CAR-T expression vectors were generated and subsequently transduced into human T cells. The resulting CAR-T cells were analyzed for surface CD46 expression and compared for their ability to kill various prostate cancer cells *in vitro.* Various published reports have shown that different vectors have varying degrees of potency *in vivo*, but minimal effects *in vitro*.

**[0185]** **FIG. 7** illustrates six exemplary lentivirus-based CAR-T constructs. The lentivirus vectors illustrate Generations 2, 3, and 4 CARs.

**[0186]** **FIG. 8A-FIG. 8G** show FACS analysis of CAR expression on T cells on the day of *in vitro* cytotoxicity assay. As illustrated in the figure, >85% CAR(BB) (or CARBB) expressions are observed for EGFP and Puro constructs while only 25-32% of CAR(BB) expression is detected for 4-1BBL constructs.

**[0187]** **FIG. 9A-FIG. 9D** show *in vitro* cytotoxicity assay on prostate cancer cell lines using six different variants of CAR-T cells targeted to CD46. Single time point cell viability measurements were done 24 hr after adding CAR-T (T-cells) A CytoTox 96 Non-Radioactive Cytotoxicity Assay (Promega) was used.

**[0188]** As illustrated in **FIG. 9A-FIG. 9D**, effector-to-target (E-T) ratio of 10:1 is effective at killing most prostate cancer cells, relative to the negative control cell line BPH which has minimum expression of CD46. In addition, T cells transduced with the second generation CAR is superior in cell kill effect relative to the remaining third and fourth generation CARs *in vitro*.

**Example 4: *In Vitro* CAR-T Studies On PC-3 Cell Lines**

**[0189]** Prior to initiating *in vivo* xenograft studies at MiBio research (CRO), the MiBio PC-3 cell line was tested *in vitro*. Human T cells were transduced with Generation 3 lentivirus vector expressing a CD46 chimeric antigen receptor. 3 lots of transduced T cells were generated for anti-proliferation studies. To determine which lots, if any, were capable of killing PC-3 prostate cancer cells, a 10:1 effector (CAR-T)/tumor cell (PC-3) (T/C) was used (**FIG. 10**). *R*eal *T*ime *C*ell *A*nalysis (RTCA) was used to measure viable cells. RTCA uses an electrical current that passes through the tumor cell monolayer. As cells die, the impedance id decreased and this is observed in the **FIG. 10** and **FIG. 11**.

**[0190]** In **FIG. 10**, 3 different lots of CAR-T cells were compared with non-transduced T cells for their ability to kill PC-3 prostate cancer cells. Although all 3 lots effectively killed PC-3 cells, lots 2 and 3 appeared to be far more potent, severely reducing the number of tumor cells relative to untreated or non-transduced T cells. Lot 2 was selected for subsequent *in vivo* studies.

**[0191]** In **FIG. 11**, MiBio PC-3 cells were compared to a PC-3 luc cell line obtained from UCSF. PC-3 luc appears to be far more sensitive to killing by all three lots of CAR-T cells. Cells transfected with the firefly luciferase gene historically are more sensitive to anti-tumor agents.

**Example 5: *In vitro* Cytotoxicity of CAR-T Cells on MM.1S-luc (MiBio cell line)**

**[0192]** Cell viability was measured 24 hr after co-culture using luciferin based assays. Table 6 illustrates the E-T ratio of two exemplary CAR constructs. **FIG. 12** illustrates the *in vitro* cytotoxicity of CAR T cells on MM.1S-luc.

**Table 6**

| | **Cell Viability (%)** | |
|---|---|---|
| **E:T ratio** | **CAR-Puro 080317** | **T cells (non-transduced)** |
| 0:1 | 100 | 100 |
| 1:1 | 1.4 | 44 |
| 2.5:1 | 0.76 | 21 |
| 5:1 | 0.56 | 8.7 |
| 10:1 | 0.37 | 8.4 |
| 25:1 | 0.31 | 7.2 |
| 50:1 | 0.45 | 0.47 |

**[0193]** While preferred embodiments of the present invention have been shown and described herein, it will be obvious to those skilled in the art that such embodiments are provided by way of example only. Numerous variations, changes, and substitutions will now occur to those skilled in the art without departing from the invention. It should be understood that various alternatives to the embodiments of the invention described herein may be employed in practicing the invention. It is intended that the following claims define the scope of the invention and that methods and structures within the scope of these claims and their equivalents be covered thereby.

## CLAIMS

WHAT IS CLAIMED IS:

1. An engineered effector cell that specifically binds CD46, comprising:

an engineered antigen binding domain that binds to an epitope of CD46, wherein the engineered antigen binding domain is displayed on the surface of the engineered effector cell.

2. The engineered effector cell of claim 1, wherein the engineered effector cell is

an engineered T cell, optionally an engineered natural killer T cell; or

an engineered natural killer cell.

3. The engineered effector cell of claim 1, wherein the engineered antigen binding domain comprises Fab, Fab', $F(ab')_2$, scFv, $sc(Fv)_2$, dsFv, diabody or binding fragments thereof.

4. The engineered effector cell of claim 1, wherein the engineered antigen binding domain comprises scFv.

5. The engineered effector cell of claim 1, wherein the engineered antigen binding domain binds to an epitope of CD46 bound by one or more antibodies selected from SEQ ID NOs: 1, 2, 9, 10, 17, 18, 25, 26, 33, 34, 41, 42, 49, 50, 57, 58, 65, 66, 73, 74, 81, 82, 89, 90, 97, 98, 105, 106, 113, 114, 121, 122, 129, 130, 137, 138, 145, 146, 153, 154, 161, 162, 169, 170, 177, 178, 185, 186, 193 or 194.

6. The engineered effector cell of claim 1, wherein the engineered antigen binding domain comprises a heavy chain variable region comprising three complementarity determining regions (CDRs) and a light chain variable region comprising three CDRs, wherein the heavy chain variable region comprises

i) a variable heavy (VH) CDR1 that has an amino acid sequence of SEQ ID NO: 3,

ii) a variable heavy (VH) CDR2 that has an amino acid sequence of SEQ ID NO: 4, and

iii) a variable heavy (VH) CDR3 that has an amino acid sequence of SEQ ID NO: 5, and

wherein the light chain variable region comprises

iv) a variable light (VL) CDR 1 that has an amino acid sequence of SEQ ID NO: 6,

v) a variable light (VL) CDR 2 that has an amino acid sequence of SEQ ID NO: 7, and

vi) a variable light (VL) CDR 3 that has an amino acid sequence of SEQ ID NO: 8.

7. The engineered effector cell of claim 1, wherein the engineered binding domain comprises a heavy chain variable region comprising three complementarity determining

regions (CDRs) and a light chain variable region comprising three CDRs, wherein the heavy chain variable region comprises

i) a variable heavy (VH) CDR1 that has an amino acid sequence of SEQ ID NO: 11,

ii) a variable heavy (VH) CDR2 that has an amino acid sequence of SEQ ID NO: 12, and

iii) a variable heavy (VH) CDR3 that has an amino acid sequence of SEQ ID NO: 13, and

wherein the light chain variable region comprises

iv) a variable light (VL) CDR 1 that has an amino acid sequence of SEQ ID NO: 14,

v) a variable light (VL) CDR 2 that has an amino acid sequence of SEQ ID NO: 15, and

vi) a variable light (VL) CDR 3 that has an amino acid sequence of SEQ ID NO: 16.

8. The engineered effector cell of claim 1, wherein the engineered antigen binding domain comprises a heavy chain variable region comprising three complementarity determining regions (CDRs) and a light chain variable region comprising three CDRs, wherein the heavy chain variable region comprises

i) a variable heavy (VH) CDR1 that has an amino acid sequence of SEQ ID NO: 19,

ii) a variable heavy (VH) CDR2 that has an amino acid sequence of SEQ ID NO: 20, and

iii) a variable heavy (VH) CDR3 that has an amino acid sequence of SEQ ID NO: 21, and

wherein the light chain variable region comprises

iv) a variable light (VL) CDR 1 that has an amino acid sequence of SEQ ID NO: 22,

v) a variable light (VL) CDR 2 that has an amino acid sequence of SEQ ID NO: 23, and

vi) a variable light (VL) CDR 3 that has an amino acid sequence of SEQ ID NO: 24.

9. The engineered effector cell of claim 1, wherein the engineered antigen binding domain comprises a heavy chain variable region comprising three complementarity determining regions (CDRs) and a light chain variable region comprising three CDRs, wherein the heavy chain variable region comprises

i) a variable heavy (VH) CDR1 that has an amino acid sequence of SEQ ID NO: 27,

ii) a variable heavy (VH) CDR2 that has an amino acid sequence of SEQ ID NO: 28, and

iii) a variable heavy (VH) CDR3 that has an amino acid sequence of SEQ ID NO: 29, and

wherein the light chain variable region comprises

iv) a variable light (VL) CDR 1 that has an amino acid sequence of SEQ ID NO: 30,

v) a variable light (VL) CDR 2 that has an amino acid sequence of SEQ ID NO: 31, and

vi) a variable light (VL) CDR 3 that has an amino acid sequence of SEQ ID NO: 32.

10. The engineered effector cell of claim 1, wherein the engineered antigen binding domain comprises a heavy chain variable region having at least 80%, 85%, 90%, 95%, or 99% sequence identity to SEQ ID NOs: 1, 9, 17 and 25; and a light chain variable region having at least 80%, 85%, 90%, 95% or 99% sequence identity to SEQ ID NOs: 2, 10, 18 and 26.

11. The engineered effector cell of claim 1, wherein the engineered antigen binding domain comprises a heavy chain variable region comprising an amino acid sequence of SEQ ID NO: 1 and a light chain variable region comprising an amino acid sequence of SEQ ID NO: 2.

12. The engineered effector cell of claim 1, wherein the engineered effector cell further comprises a stalk region, a transmembrane domain, at least one costimulatory domains, and a signaling domain.

13. The engineered effector cell of claim 12, wherein the at least one costimulatory signaling domain comprises CD8, CD27, CD28z, 4-1BB, ICOS, OX40, or a fragment or combination thereof.

14. The engineered effector cell of claim 12, wherein the at least one costimulatory signaling domain comprises CD28 and 4-1BB or fragments thereof.

15. The engineered effector cell of claim 12, wherein the at least one costimulatory signaling domain comprises CD28 or fragment thereof.

16. The engineered effector cell of claim 12, wherein a first costimulatory signaling domain is encoded by a first vector and a second costimulatory signaling domain is encoded by a second vector.

17. The engineered effector cell of any one of the claims 1-16, wherein the engineered effector cell is prepared from an autologous effector cell.

18. The engineered effector cell of any one of the claims 1-16, wherein the engineered effector cell is prepared from an allogeneic effector cell.

19. The engineered effector cell of any one of the claims 1-18, wherein the engineered effector cell is a CAR-T cell.

20. The engineered effector cell of any one of the claims 1-18, wherein the engineered effector cell is a CAR-NK cell.

21. A pharmaceutical composition comprising an engineered effector cell of claims 1-20; and an excipient.

22. A chimeric polynucleotide comprising:

a first polynucleotide segment encoding an antigen binding domain that binds to an epitope of CD46; and

a second polynucleotide segment encoding a polypeptide comprising a stalk region, a transmembrane domain, at least one costimulatory domains, and a signaling domain;

wherein the chimeric polynucleotide, upon transfection to an effector cell, expresses the CD46 antigen binding domain and the polypeptide as a continuous chain, and wherein expression of the CD46 antigen binding domain and the polypeptide triggers the effector cell to activate and/or proliferate.

23. The chimeric polynucleotide of claim 22, wherein the effector cell is

a T cell, optionally a natural killer T cell; or

a natural killer cell.

24. The chimeric polynucleotide of claim 22, wherein the first polynucleotide segment encodes Fab, Fab', $F(ab')_2$, scFv, $sc(Fv)_2$, dsFv, diabody or binding fragments thereof.

25. The chimeric polynucleotide of claim 22, wherein the first polynucleotide segment encodes scFv.

26. The chimeric polynucleotide of claim 22, wherein the antigen binding domain binds to an epitope of CD46 bound by one or more antibodies selected from SEQ ID NOs: 1, 2, 9, 10, 17, 18, 25, 26, 33, 34, 41, 42, 49, 50, 57, 58, 65, 66, 73, 74, 81, 82, 89, 90, 97, 98, 105, 106, 113, 114, 121, 122, 129, 130, 137, 138, 145, 146, 153, 154, 161, 162, 169, 170, 177, 178, 185, 186, 193 or 194.

27. The chimeric polynucleotide of claim 22, wherein the first polynucleotide segment encodes a heavy chain variable region comprising three complementarity determining regions (CDRs) and a light chain variable region comprising three CDRs, wherein the heavy chain variable region comprises

i) a variable heavy (VH) CDR1 that has an amino acid sequence of SEQ ID NO: 3,

ii) a variable heavy (VH) CDR2 that has an amino acid sequence of SEQ ID NO: 4, and

iii) a variable heavy (VH) CDR3 that has an amino acid sequence of SEQ ID NO: 5, and

wherein the light chain variable region comprises

iv) a variable light (VL) CDR 1 that has an amino acid sequence of SEQ ID NO: 6,

v) a variable light (VL) CDR 2 that has an amino acid sequence of SEQ ID NO: 7, and

vi) a variable light (VL) CDR 3 that has an amino acid sequence of SEQ ID NO: 8.

28. The chimeric polynucleotide of claim 22, wherein the first polynucleotide segment encodes a heavy chain variable region comprising three complementarity determining regions (CDRs) and a light chain variable region comprising three CDRs, wherein the heavy chain variable region comprises

i) a variable heavy (VH) CDR1 that has an amino acid sequence of SEQ ID NO: 11,

ii) a variable heavy (VH) CDR2 that has an amino acid sequence of SEQ ID NO: 12, and

iii) a variable heavy (VH) CDR3 that has an amino acid sequence of SEQ ID NO: 13, and

wherein the light chain variable region comprises

iv) a variable light (VL) CDR 1 that has an amino acid sequence of SEQ ID NO: 14,

v) a variable light (VL) CDR 2 that has an amino acid sequence of SEQ ID NO: 15, and

vi) a variable light (VL) CDR 3 that has an amino acid sequence of SEQ ID NO: 16.

29. The chimeric polynucleotide of claim 22, wherein the first polynucleotide segment encodes a heavy chain variable region comprising three complementarity determining regions (CDRs) and a light chain variable region comprising three CDRs, wherein the heavy chain variable region comprises

i) a variable heavy (VH) CDR1 that has an amino acid sequence of SEQ ID NO: 19,

ii) a variable heavy (VH) CDR2 that has an amino acid sequence of SEQ ID NO: 20, and

iii) a variable heavy (VH) CDR3 that has an amino acid sequence of SEQ ID NO: 21, and

wherein the light chain variable region comprises

iv) a variable light (VL) CDR 1 that has an amino acid sequence of SEQ ID NO: 22,

v) a variable light (VL) CDR 2 that has an amino acid sequence of SEQ ID NO: 23, and

vi) a variable light (VL) CDR 3 that has an amino acid sequence of SEQ ID NO: 24.

30. The chimeric polynucleotide of claim 22, wherein the first polynucleotide segment encodes a heavy chain variable region comprising three complementarity determining regions (CDRs) and a light chain variable region comprising three CDRs, wherein the heavy chain variable region comprises

i) a variable heavy (VH) CDR1 that has an amino acid sequence of SEQ ID NO: 27,

ii) a variable heavy (VH) CDR2 that has an amino acid sequence of SEQ ID NO: 28, and

iii) a variable heavy (VH) CDR3 that has an amino acid sequence of SEQ ID NO: 29, and

wherein the light chain variable region comprises

iv) a variable light (VL) CDR 1 that has an amino acid sequence of SEQ ID NO: 30,

v) a variable light (VL) CDR 2 that has an amino acid sequence of SEQ ID NO: 31, and

vi) a variable light (VL) CDR 3 that has an amino acid sequence of SEQ ID NO: 32.

31. The chimeric polynucleotide of claim 22, wherein the first polynucleotide segment encodes a heavy chain variable region having at least 80%, 85%, 90%, 95%, or 99% sequence identity to SEQ ID NOs: 1, 9, 17 and 25; and a light chain variable region having at least 80%, 85%, 90%, 95% or 99% sequence identity to SEQ ID NOs: 2, 10, 18 and 26.

32. The chimeric polynucleotide of claim 22, wherein the first polynucleotide segment encodes a heavy chain variable region comprising an amino acid sequence of SEQ ID NO: 1 and a light chain variable region comprising an amino acid sequence of SEQ ID NO: 2.

33. The chimeric polynucleotide of claim 22, wherein the at least one costimulatory signaling domain comprises:

CD8, CD27, CD28z, 4-1BB, ICOS, OX40, or a fragment or combination thereof;

CD28 and 4-1BB or fragments thereof; or

CD28 or fragment thereof.

34. The chimeric polynucleotide of any one of the claims 22-33, wherein the transfected effector cell is a CAR-T cell.

35. The chimeric polynucleotide of any one of the claims 22-33, wherein the transfected effector cell is a CAR-NK cell.

36. The chimeric polynucleotide of any one of the claims 22-35, wherein the transfected effector cell has MHC non-restricted antibody-type specificity.

37. A vector comprising a chimeric polynucleotide of claims 22-36.

38. A polynucleotide encoding a chimeric receptor comprising an antigen binding domain that binds to an epitope of CD46, a stalk region, a transmembrane domain, at least one costimulatory domains, and a signaling domain.

39. A vector comprising a polynucleotide of claim 38.

40. An engineered single-chain polypeptide comprising an antigen binding domain that binds to an epitope of CD46, a stalk region, a transmembrane domain, at least one costimulatory domains, and a signaling domain.

41. A vector comprising a polynucleotide encoding an engineered single-chain polypeptide of claim 40.

42. A method of treating a subject having a cancer characterized by an overexpression of CD46, comprising:

administering to the subject a pharmaceutical composition comprising a plurality of engineered effector cells, wherein each engineered effector cell comprises an engineered antigen binding domain that binds to an epitope of CD46, and wherein the engineered antigen binding domain is displayed on the surface of the engineered effector cell.

43. The method of claim 42, wherein the engineered effector cell is

an engineered T cell, optionally an engineered natural killer T cell; or

an engineered natural killer cell.

44. The method of claim 42, wherein the engineered antigen binding domain comprises Fab, Fab', $F(ab')_2$, scFv, $sc(Fv)_2$, dsFv, diabody, or binding fragments thereof.

45. The method of claim 42, wherein the engineered antigen binding domain comprises scFv.

46. The method of claim 42, wherein the engineered antigen binding domain binds to an epitope of CD46 bound by one or more antibodies selected from SEQ ID NOs: 1, 2, 9, 10, 17, 18, 25, 26, 33, 34, 41, 42, 49, 50, 57, 58, 65, 66, 73, 74, 81, 82, 89, 90, 97, 98, 105, 106, 113, 114, 121, 122, 129, 130, 137, 138, 145, 146, 153, 154, 161, 162, 169, 170, 177, 178, 185, 186, 193 or 194.

47. The method of claim 42, wherein the engineered antigen binding domain comprises a heavy chain variable region comprising three complementarity determining regions

(CDRs) and a light chain variable region comprising three CDRs, wherein the heavy chain variable region comprises

i) a variable heavy (VH) CDR1 that has an amino acid sequence of SEQ ID NO: 3,

ii) a variable heavy (VH) CDR2 that has an amino acid sequence of SEQ ID NO: 4, and

iii) a variable heavy (VH) CDR3 that has an amino acid sequence of SEQ ID NO: 5, and

wherein the light chain variable region comprises

iv) a variable light (VL) CDR 1 that has an amino acid sequence of SEQ ID NO: 6,

v) a variable light (VL) CDR 2 that has an amino acid sequence of SEQ ID NO: 7, and

vi) a variable light (VL) CDR 3 that has an amino acid sequence of SEQ ID NO: 8.

48. The method of claim 42, wherein the engineered antigen binding domain comprises a heavy chain variable region comprising three complementarity determining regions (CDRs) and a light chain variable region comprising three CDRs, wherein the heavy chain variable region comprises

i) a variable heavy (VH) CDR1 that has an amino acid sequence of SEQ ID NO: 11,

ii) a variable heavy (VH) CDR2 that has an amino acid sequence of SEQ ID NO: 12, and

iii) a variable heavy (VH) CDR3 that has an amino acid sequence of SEQ ID NO: 13, and

wherein the light chain variable region comprises

iv) a variable light (VL) CDR 1 that has an amino acid sequence of SEQ ID NO: 14,

v) a variable light (VL) CDR 2 that has an amino acid sequence of SEQ ID NO: 15, and

vi) a variable light (VL) CDR 3 that has an amino acid sequence of SEQ ID NO: 16.

49. The method of claim 42, wherein the engineered antigen binding domain comprises a heavy chain variable region comprising three complementarity determining regions (CDRs) and a light chain variable region comprising three CDRs, wherein the heavy chain variable region comprises

i) a variable heavy (VH) CDR1 that has an amino acid sequence of SEQ ID NO: 19,

ii) a variable heavy (VH) CDR2 that has an amino acid sequence of SEQ ID NO: 20, and

iii) a variable heavy (VH) CDR3 that has an amino acid sequence of SEQ ID NO: 21, and

wherein the light chain variable region comprises

iv) a variable light (VL) CDR 1 that has an amino acid sequence of SEQ ID NO: 22,

v) a variable light (VL) CDR 2 that has an amino acid sequence of SEQ ID NO: 23, and

vi) a variable light (VL) CDR 3 that has an amino acid sequence of SEQ ID NO: 24.

50. The method of claim 42, wherein the engineered antigen binding domain comprises a heavy chain variable region comprising three complementarity determining regions (CDRs) and a light chain variable region comprising three CDRs, wherein the heavy chain variable region comprises

i) a variable heavy (VH) CDR1 that has an amino acid sequence of SEQ ID NO: 27,

ii) a variable heavy (VH) CDR2 that has an amino acid sequence of SEQ ID NO: 28, and

iii) a variable heavy (VH) CDR3 that has an amino acid sequence of SEQ ID NO: 29, and

wherein the light chain variable region comprises

iv) a variable light (VL) CDR 1 that has an amino acid sequence of SEQ ID NO: 30,

v) a variable light (VL) CDR 2 that has an amino acid sequence of SEQ ID NO: 31, and

vi) a variable light (VL) CDR 3 that has an amino acid sequence of SEQ ID NO: 32.

51. The method of claim 42, wherein the engineered antigen binding domain comprises a heavy chain variable region having at least 80%, 85%, 90%, 95%, or 99% sequence identity to SEQ ID NOs: 1, 9, 17 and 25; and a light chain variable region having at least 80%, 85%, 90%, 95% or 99% sequence identity to SEQ ID NOs: 2, 10, 18 and 26.

52. The method of claim 42, wherein the engineered antigen binding domain comprises a heavy chain variable region comprising an amino acid sequence of SEQ ID NO: 1 and a light chain variable region comprising an amino acid sequence of SEQ ID NO: 2.

53. The method of claim 42, wherein the engineered effector cell further comprises a stalk region, a transmembrane domain, at least one costimulatory domains, and a signaling domain.

54. The method of claim 53, wherein the at least one costimulatory signaling domain comprises:

CD8, CD27, CD28z, 4-1BB, ICOS, OX40, or fragment or combination thereof;

CD28 and 4-1BB or fragments thereof; or

CD28 or fragment thereof.

55. The method of claim 53, wherein a first costimulatory signaling domain is encoded by a first vector and a second costimulatory signaling domain is encoded by a second vector.

56. The method of any one of the claims 42-55, wherein the engineered effector cell is prepared from an autologous effector cell.

57. The method of any one of the claims 42-55, wherein the engineered effector cell is prepared from an allogeneic effector cell.

58. The method of any one of the claims 42-57, wherein the CAR effector cell is a CAR-T cell.

59. The method of any one of the claims 42-57, wherein the CAR effector cell is a CAR-NK cell.

60. The method of claim 42, wherein the cancer comprises breast cancer, cervical cancer, colorectal cancer, kidney cancer, liver cancer, lung cancer, ovarian cancer, pancreatic cancer, prostate cancer or urothelial cancer.

61. The method of claim 42, wherein the cancer comprises mesothelioma, lymphoma, or leukemia.

62. The method of claim 42 or 60, wherein the cancer is prostate cancer.

63. The method of claim 42, wherein the cancer is multiple myeloma.

64. The method of any one of the claims 42-63, wherein the pharmaceutical composition further comprises an excipient.

65. The method of any one of the claims 42-64, further comprising an additional therapeutic agent.

66. The method of claim 65, wherein the additional therapeutic agent comprises a cytokine.

67. The method of claim 66, wherein the cytokine comprises IL2, IL7, IL12, IL15, IL21, IFNγ or TNF-α.

68. The method of claim 65, wherein the additional therapeutic agent comprises chemotherapeutic agent.

69. The method of claim 68, wherein the chemotherapeutic agent is selected from among bortezomib, CAL-101, chlorambucil, cyclophosphamide, dexamethasone, docetaxel, doxorubicin, endostatin, everolimus, fludarabine, fostamatinib, ifosfamide, ibritumomab, lenalidomide, mesalazine, ofatumumab, paclitaxel, pentostatin, prednisone, rituximab, temsirolimus, thalidomide, tositumomab or a combination thereof.

70. The method of any one of the claims 42-69, wherein the engineered effector cell and the additional therapeutic agent is administered sequentially.

71. The method of claim 70, wherein the engineered effector cell is administered after administering the additional therapeutic agent.

72. The method of claim 70 or 71, wherein the additional therapeutic agent comprises cyclophosphamide and optionally further comprising fludarabine.

73. The method of any one of the claims 42-65 or 68-72, wherein the additional therapeutic agent is administered to induce lymphopenia.

74. The method of any one of the claims 42-73, wherein the pharmaceutical composition is formulated for parenteral administration.

75. The method of any one of the claims 42-74, wherein the pharmaceutical composition is administered to the subject as an injection or as an infusion.

76. The method of any one of the claims 42-75, wherein the subject is a human.

77. A method of treating a subject having a cancer characterized by an overexpression of CD46, comprising:

administering to the subject a pharmaceutical composition comprising a plurality of chimeric antigen receptor (CAR) effector cells, wherein each CAR effector cell recognizes an epitope recognized by an antibody of SEQ ID NOs: 1, 2, 9, 10, 17, 18, 25, 26, 33, 34, 41, 42, 49, 50, 57, 58, 65, 66, 73, 74, 81, 82, 89, 90, 97, 98, 105, 106, 113, 114, 121, 122, 129, 130, 137, 138, 145, 146, 153, 154, 161, 162, 169, 170, 177, 178, 185, 186, 193 or 194.

78. A method of depleting CD46 overexpressed cells, comprising:

a) contacting an effector cell *ex vivo* with a vector comprising a polynucleotide encoding an engineered antigen binding domain that recognizes an epitope of CD46 to generate a CD46-specific effector cell, wherein the engineered antigen binding domain is displayed on the surface of the CD46-specific effector cell; and

b) administering the CD46-specific effector cell to a subject having CD46 overexpressed cells, thereby depleting the population of CD46 overexpressed cells.

79. The method of claim 78, wherein the CD46 overexpressed cells are cancer cells.

80. The method of claim 79, wherein the cancer cells comprise

breast cancer cells, cervical cancer cells, colorectal cancer cells, kidney cancer cells, liver cancer cells, lung cancer cells, ovarian cancer cells, pancreatic cancer cells, prostate cancer cells or urothelial cancer cells;

cells from mesothelioma, cells from lymphoma, or cells from leukemia;

prostate cancer cells; or

cells from multiple myeloma.

81. The method of claim 78, wherein the engineered effector cell is

an engineered T cell, optionally an engineered natural killer T cell; or

an engineered natural killer cell.

82. The method of claim 78, wherein the engineered antigen binding domain comprises Fab, Fab', $F(ab')_2$, scFv, $sc(Fv)_2$, dsFv, diabody, or binding fragments thereof.

83. The method of claim 78, wherein the engineered antigen binding domain comprises scFv.

84. The method of any one of the claims 78, 82 or 83, wherein the engineered antigen binding domain comprises a heavy chain variable region comprising three complementarity determining regions (CDRs) and a light chain variable region comprising three CDRs, wherein the heavy chain variable region comprises

i) a variable heavy (VH) CDR1 that has an amino acid sequence of SEQ ID NO: 3,

ii) a variable heavy (VH) CDR2 that has an amino acid sequence of SEQ ID NO: 4, and

iii) a variable heavy (VH) CDR3 that has an amino acid sequence of SEQ ID NO: 5, and

wherein the light chain variable region comprises

iv) a variable light (VL) CDR 1 that has an amino acid sequence of SEQ ID NO: 6,

v) a variable light (VL) CDR 2 that has an amino acid sequence of SEQ ID NO: 7, and

vi) a variable light (VL) CDR 3 that has an amino acid sequence of SEQ ID NO: 8.

85. The method of any one of the claims 78, 82 or 83, wherein the engineered antigen binding domain comprises a heavy chain variable region comprising three complementarity determining regions (CDRs) and a light chain variable region comprising three CDRs, wherein the heavy chain variable region comprises

i) a variable heavy (VH) CDR1 that has an amino acid sequence of SEQ ID NO: 11,

ii) a variable heavy (VH) CDR2 that has an amino acid sequence of SEQ ID NO: 12, and

iii) a variable heavy (VH) CDR3 that has an amino acid sequence of SEQ ID NO: 13, and

wherein the light chain variable region comprises

iv) a variable light (VL) CDR 1 that has an amino acid sequence of SEQ ID NO: 14,

v) a variable light (VL) CDR 2 that has an amino acid sequence of SEQ ID NO: 15, and

vi) a variable light (VL) CDR 3 that has an amino acid sequence of SEQ ID NO: 16.

86. The method of any one of the claims 78, 82 or 83, wherein the engineered antigen binding domain comprises a heavy chain variable region comprising three complementarity determining regions (CDRs) and a light chain variable region comprising three CDRs, wherein the heavy chain variable region comprises

i) a variable heavy (VH) CDR1 that has an amino acid sequence of SEQ ID NO: 19,

ii) a variable heavy (VH) CDR2 that has an amino acid sequence of SEQ ID NO: 20, and

iii) a variable heavy (VH) CDR3 that has an amino acid sequence of SEQ ID NO: 21, and

wherein the light chain variable region comprises

iv) a variable light (VL) CDR 1 that has an amino acid sequence of SEQ ID NO: 22,

v) a variable light (VL) CDR 2 that has an amino acid sequence of SEQ ID NO: 23, and

vi) a variable light (VL) CDR 3 that has an amino acid sequence of SEQ ID NO: 24.

87. The method of any one of the claims 78, 82 or 83, wherein the engineered antigen binding domain comprises a heavy chain variable region comprising three complementarity determining regions (CDRs) and a light chain variable region comprising three CDRs, wherein the heavy chain variable region comprises

i) a variable heavy (VH) CDR1 that has an amino acid sequence of SEQ ID NO: 27,

ii) a variable heavy (VH) CDR2 that has an amino acid sequence of SEQ ID NO: 28, and

iii) a variable heavy (VH) CDR3 that has an amino acid sequence of SEQ ID NO: 29, and

wherein the light chain variable region comprises

iv) a variable light (VL) CDR 1 that has an amino acid sequence of SEQ ID NO: 30,

v) a variable light (VL) CDR 2 that has an amino acid sequence of SEQ ID NO: 31, and

vi) a variable light (VL) CDR 3 that has an amino acid sequence of SEQ ID NO: 32.

88. The method of any one of the claims 78 or 82-87, wherein the engineered antigen binding domain comprises a heavy chain variable region having at least 80%, 85%, 90%,

95%, or 99% sequence identity to SEQ ID NOs: 1, 9, 17 and 25; and a light chain variable region having at least 80%, 85%, 90%, 95% or 99% sequence identity to SEQ ID NOs: 2, 10, 18 and 26.

89. The method of any one of the claims 78 or 82-88, wherein the engineered antigen binding domain comprises a heavy chain variable region comprising an amino acid sequence of SEQ ID NO: 1 and a light chain variable region comprising an amino acid sequence of SEQ ID NO: 2.

90. The method of any one of the claims 78-89, wherein the engineered effector cell further comprises a stalk region, a transmembrane domain, at least one costimulatory domains, and a signaling domain.

91. The method of claim 90, wherein the at least one costimulatory signaling domain comprises:

CD8, CD27, CD28z, 4-1BB, ICOS, OX40, or a fragment or combination thereof;

CD28 and 4-1BB or fragments thereof; or

CD28 or fragment thereof.

92. The method of claim 90 or 91, wherein a first costimulatory signaling domain is encoded by a first vector and a second costimulatory signaling domain is encoded by a second vector.

93. The method of any one of the claims 78-92, wherein the engineered effector cell is prepared from an autologous effector cell.

94. The method of any one of the claims 78-92, wherein the engineered effector cell is prepared from an allogeneic effector cell.

95. The method of any one of the claims 78-94, wherein the CAR effector cell is a CAR-T cell.

96. The method of any one of the claims 78-94, wherein the CAR effector cell is a CAR-NK cell.

97. The method of any one of the claims 78-80, wherein the vector is a viral vector.

98. The method of any one of the claims 78-80, wherein the vector is a non-viral vector.

**FIG. 1**

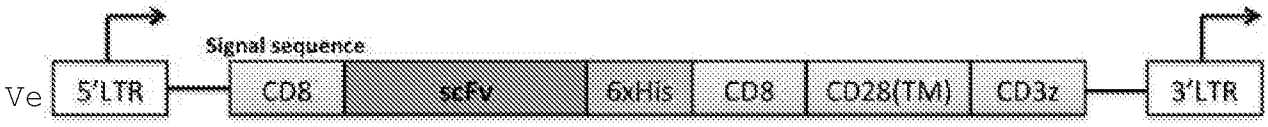

Signal sequence
Ve
5'LTR
CD8
scFv
6xHis
CD8
CD28(TM)
CD3z
3'LTR

**FIG. 2**

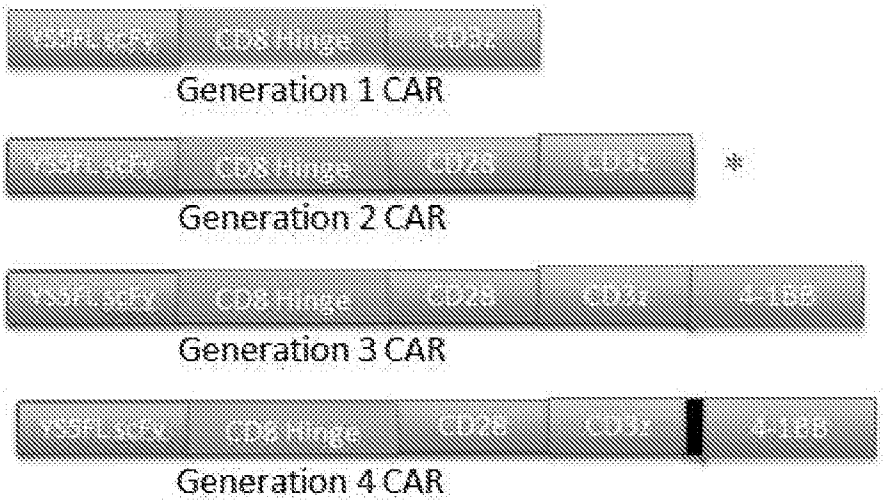

Generation 1 CAR
Generation 2 CAR
Generation 3 CAR
Generation 4 CAR

FIG. 3

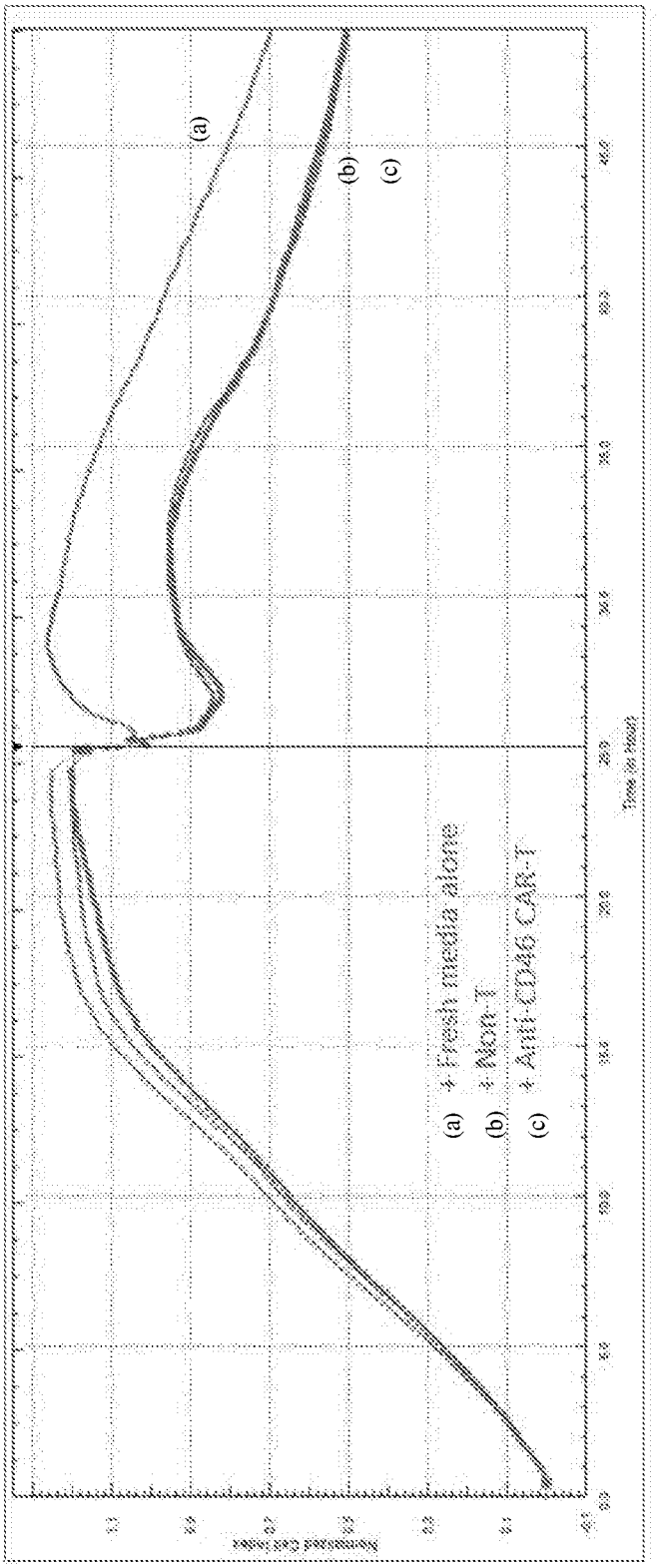


FIG. 4

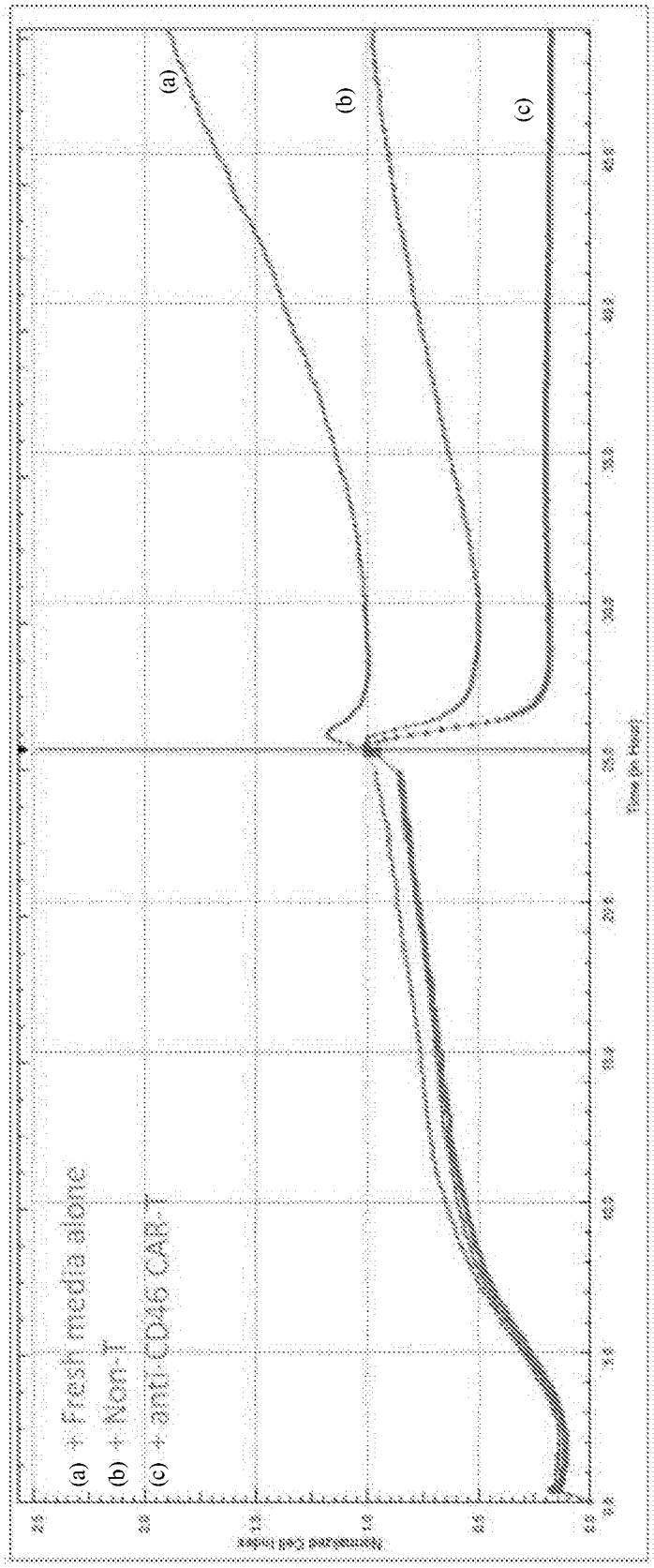


FIG. 5

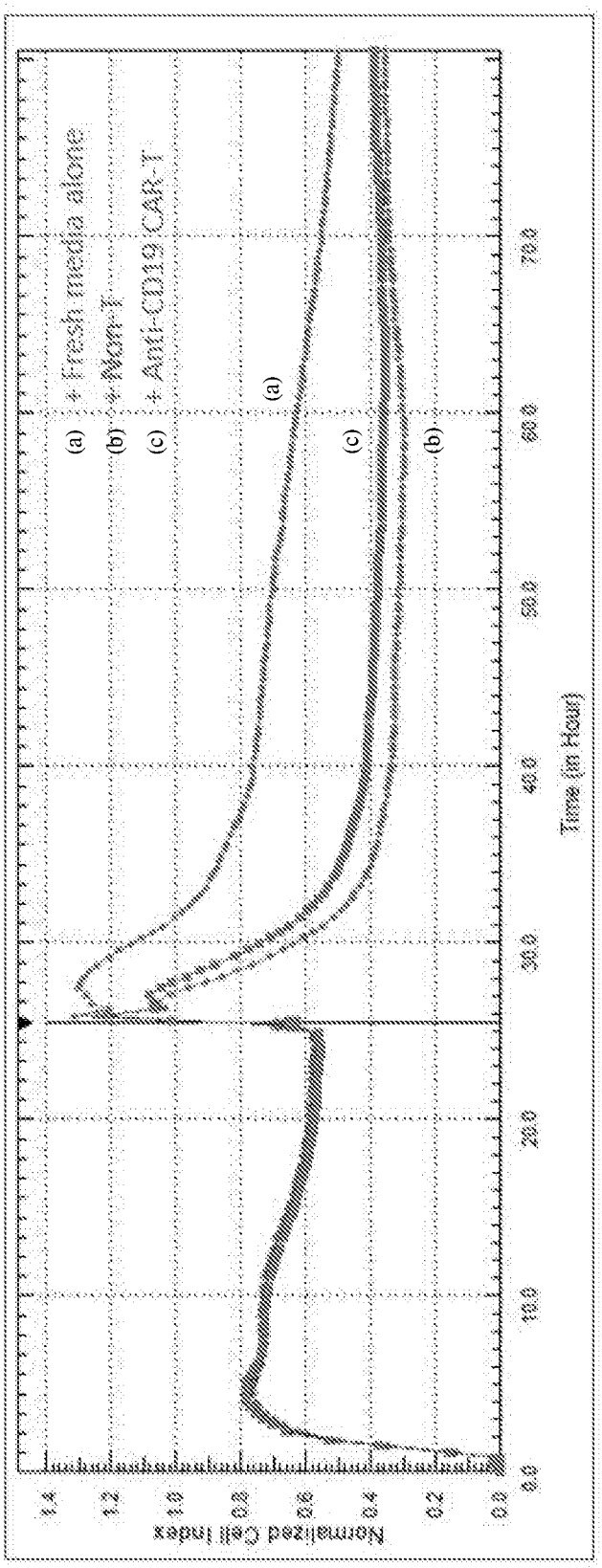

(a) + fresh media alone
(b) + Non-T
(c) + Anti-CD19 CAR-T
Normalized Cell Index
Time (in Hour)
0.0
0.2
0.4
0.6
0.8
1.0
1.2
1.4
0.0
10.0
20.0
30.0
40.0
50.0
60.0
70.0
(a)
(c)
(b)

FIG. 6

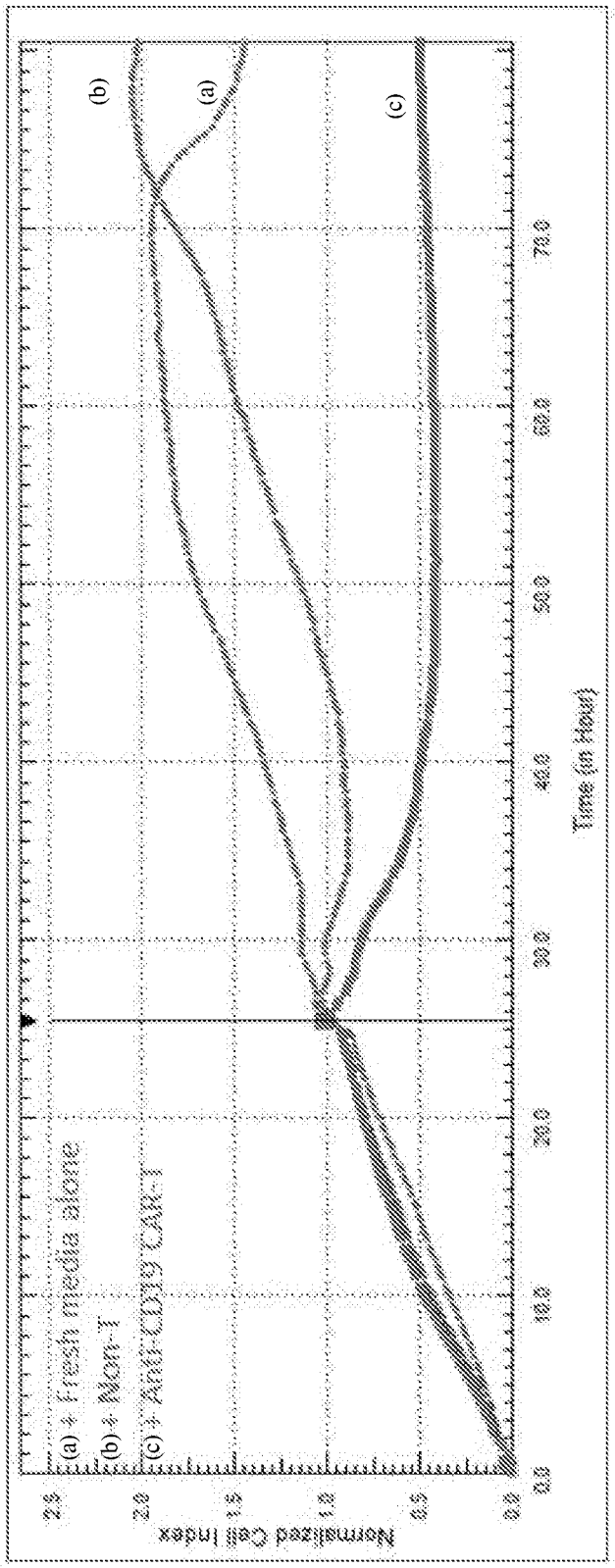
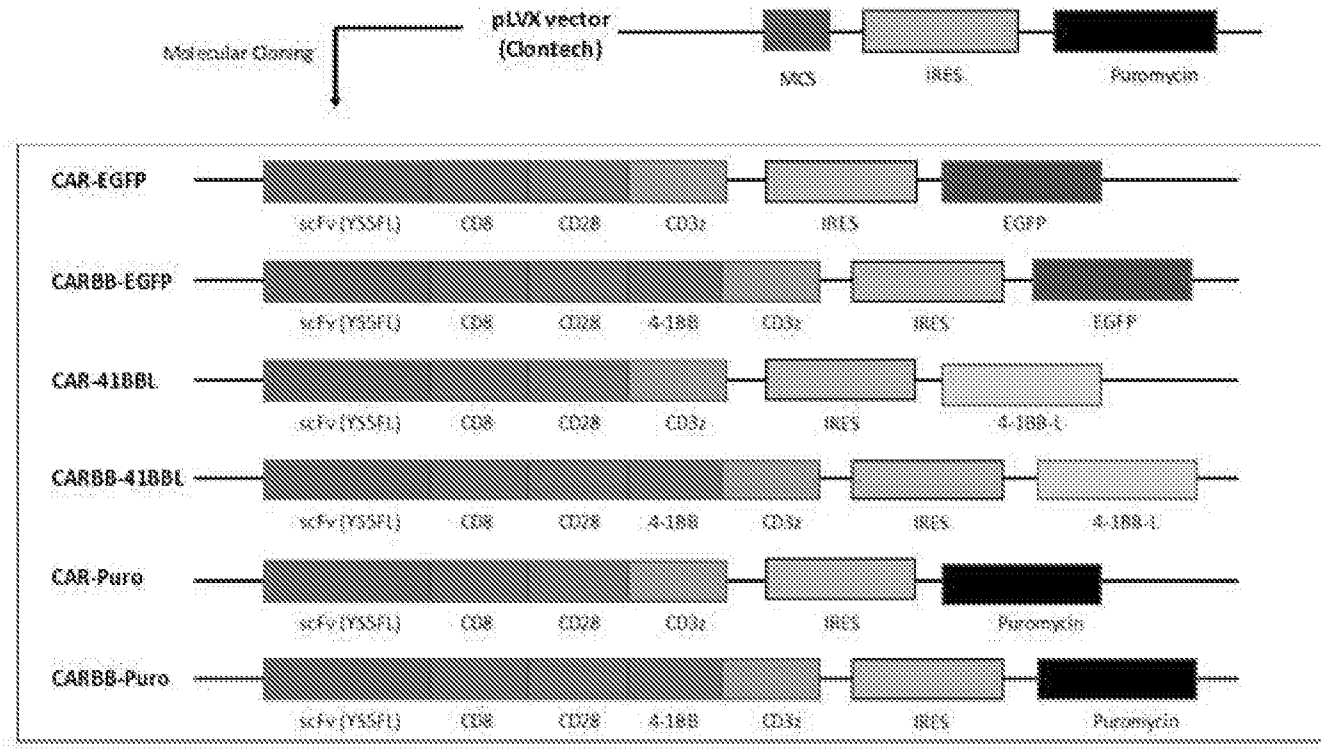

(a) + fresh media alone
(b) + Mon-T
(c) + Anti-CD19 CAR-T
Normalized Cell Index
Time (in Hour)
(a)
(b)
(c)
0.0
0.5
1.0
1.5
2.0
2.5
0.0
10.0
20.0
30.0
40.0
50.0
60.0
70.0

**FIG. 7**




CD8—hinge region
CD28/4-1BB- co-stimulatory domains
CD3z – CD3 Zeta domain (T-cell activating)
IRES – internal ribosome entry
4-1BB-L - 41-BB ligand

FIG. 8A

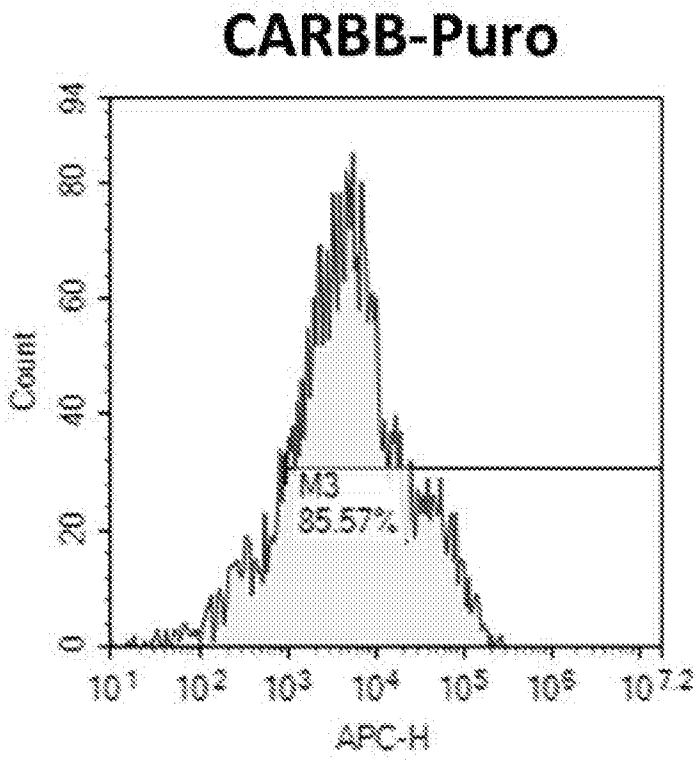

CARBB-Puro
Count
0
20
40
60
80
94
M3
85.57%
$10^1$
$10^2$
$10^3$
$10^4$
$10^5$
$10^6$
$10^{7.2}$
APC-H

FIG. 8B

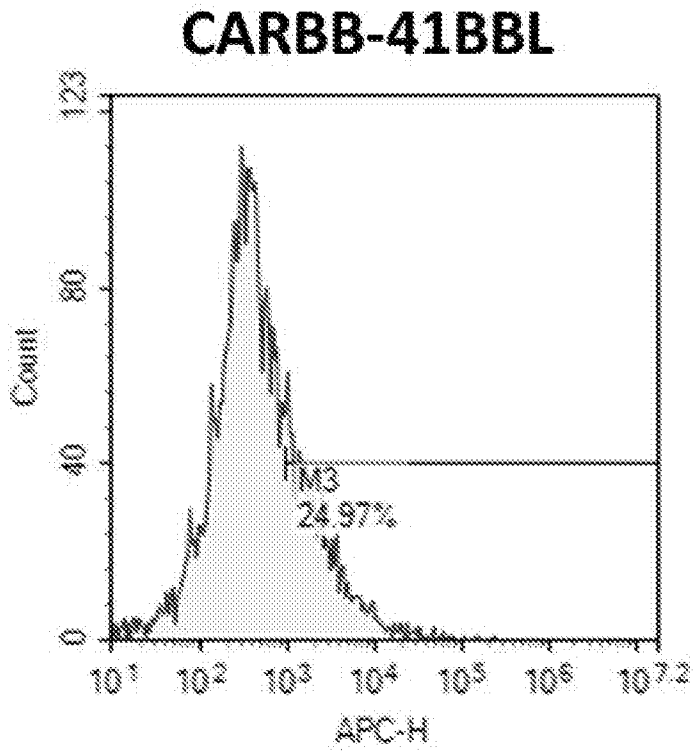

CARBB-41BBL
Count
0
40
80
123
M3
24.97%
$10^1$
$10^2$
$10^3$
$10^4$
$10^5$
$10^6$
$10^{7.2}$
APC-H

FIG. 8C

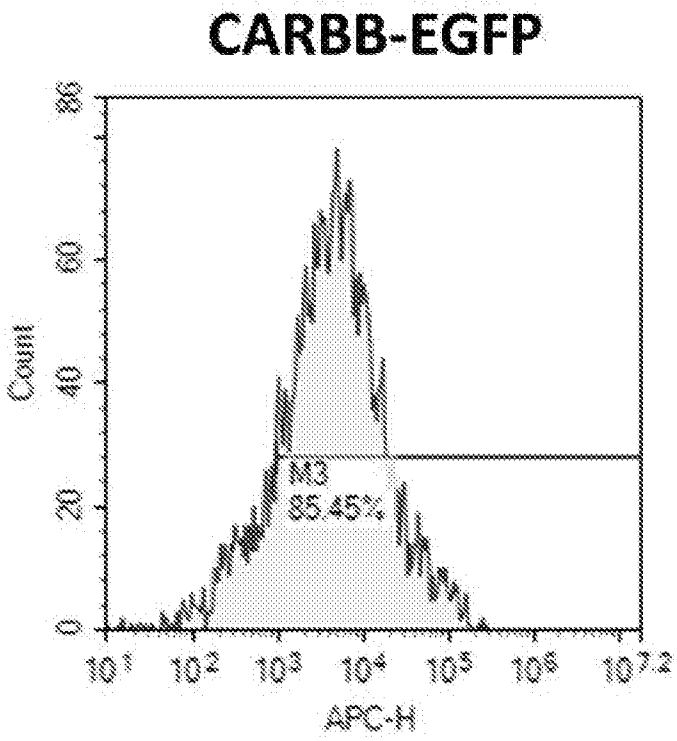



FIG. 8D

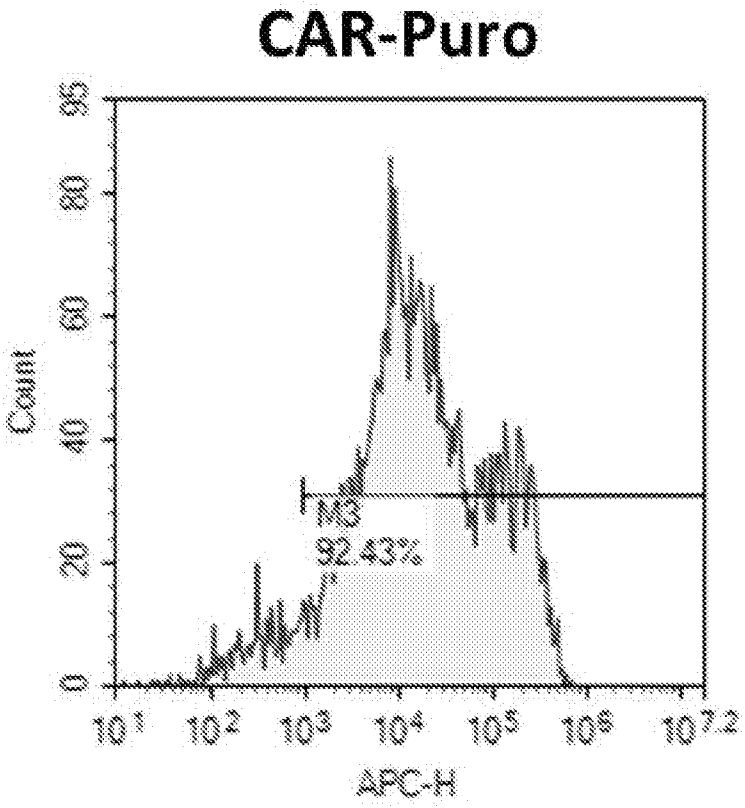


FIG. 8E

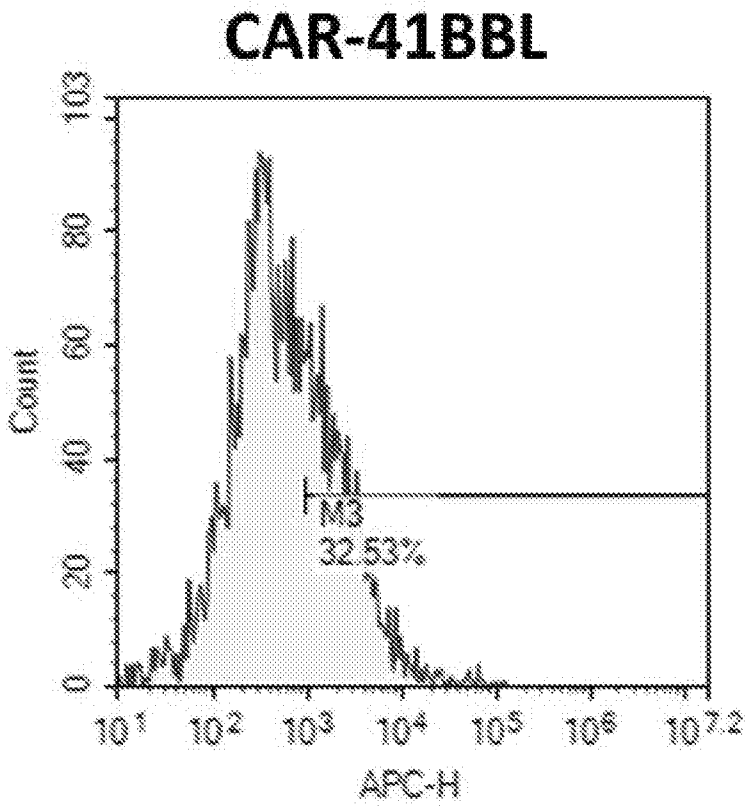

CAR-41BBL
Count
0
20
40
60
80
100
M3
32.53%
$10^1$
$10^2$
$10^3$
$10^4$
$10^5$
$10^6$
$10^{7.2}$
APC-H

FIG. 8F

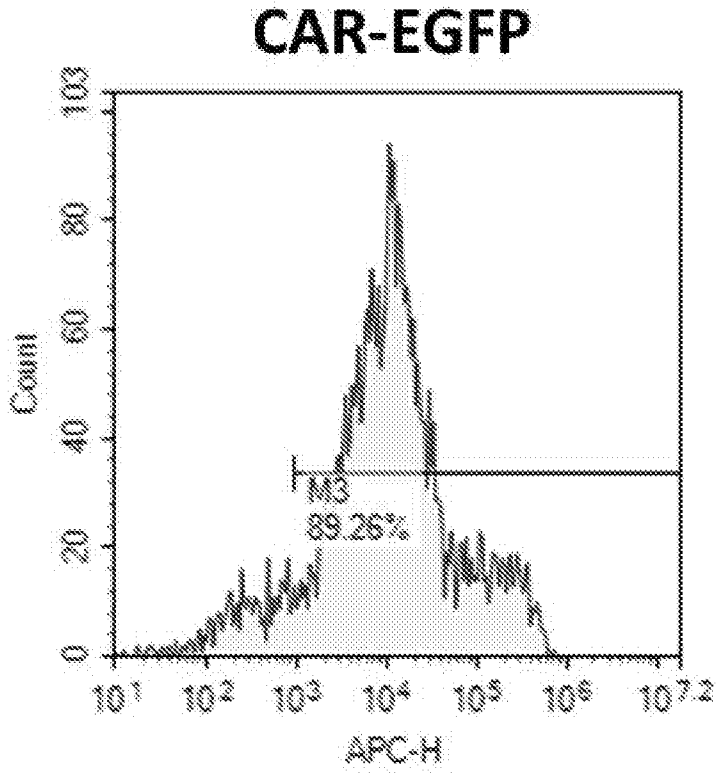

CAR-EGFP
Count
0
20
40
60
80
100
M3
89.26%
$10^1$
$10^2$
$10^3$
$10^4$
$10^5$
$10^6$
$10^{7.2}$
APC-H

FIG. 8G

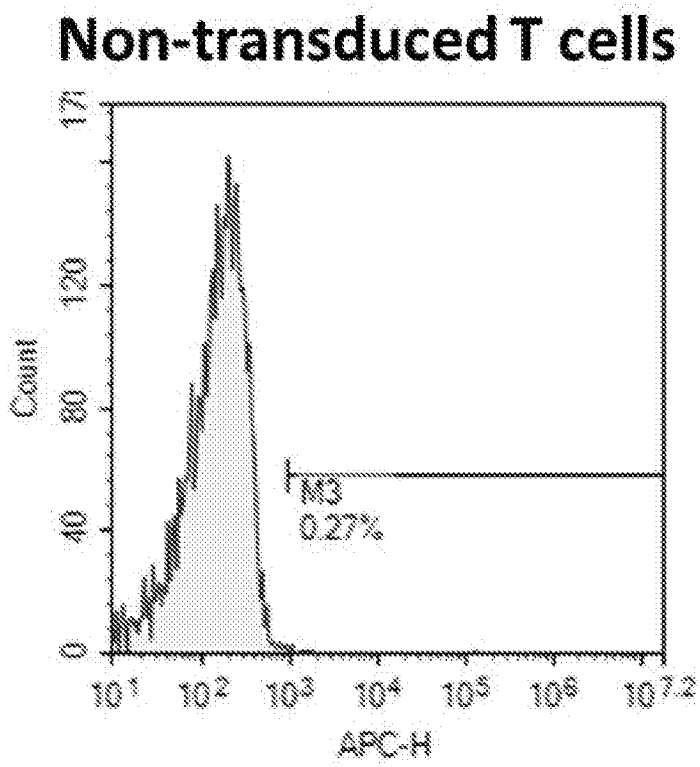

Non-transduced T cells
Count
APC-H
M3
0.27%

FIG. 9A

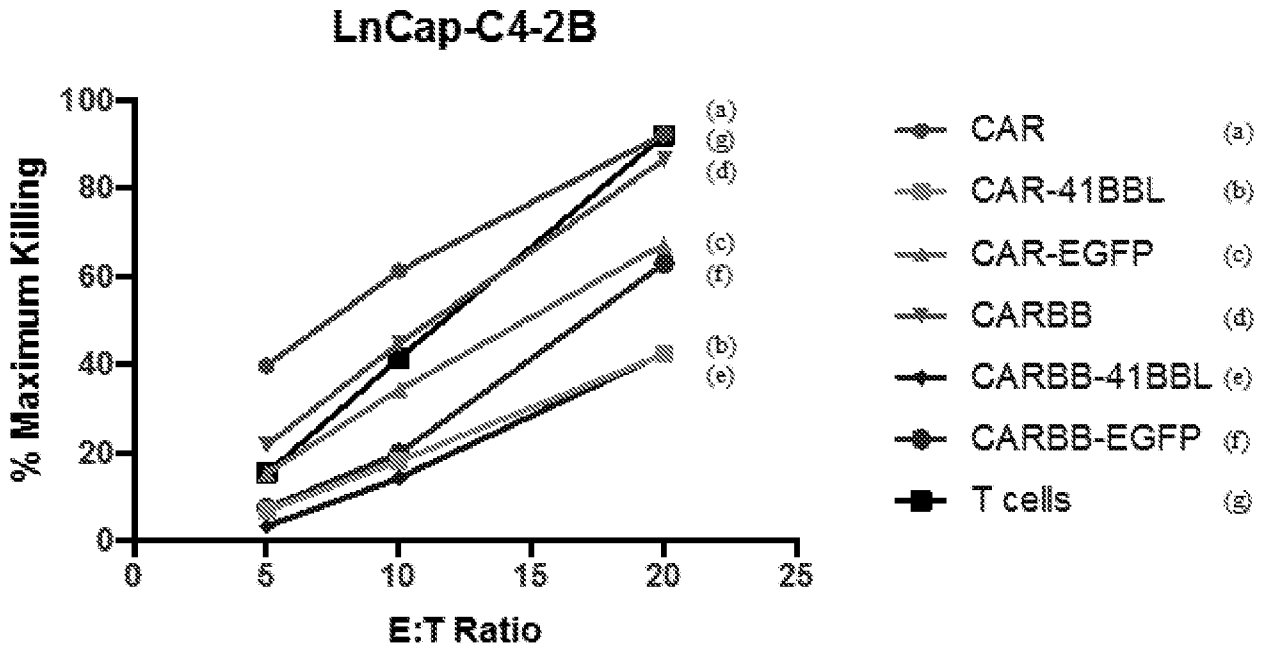

LnCap-C4-2B
% Maximum Killing
E:T Ratio
CAR (a)
CAR-41BBL (b)
CAR-EGFP (c)
CARBB (d)
CARBB-41BBL (e)
CARBB-EGFP (f)
T cells (g)

FIG. 9B

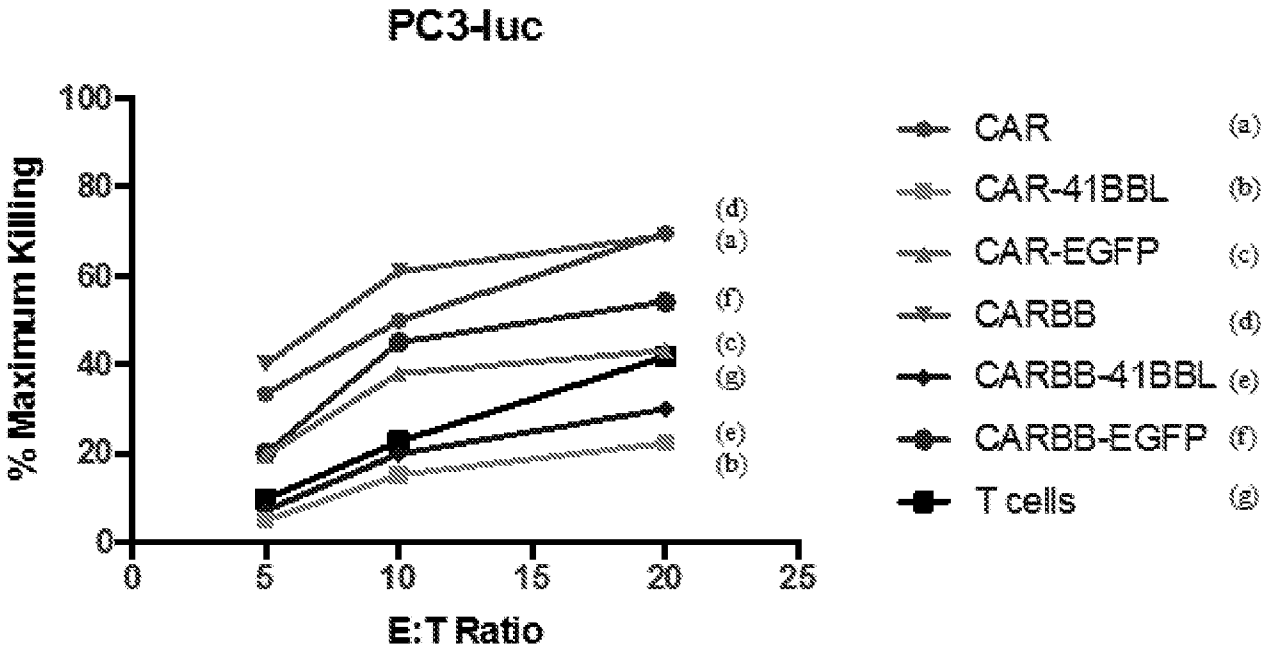

PC3-luc
% Maximum Killing
100
80
60
40
20
0
0
5
10
15
20
25
E:T Ratio
(d)
(a)
(f)
(c)
(g)
(e)
(b)
CAR (a)
CAR-41BBL (b)
CAR-EGFP (c)
CARBB (d)
CARBB-41BBL (e)
CARBB-EGFP (f)
T cells (g)

FIG. 9C

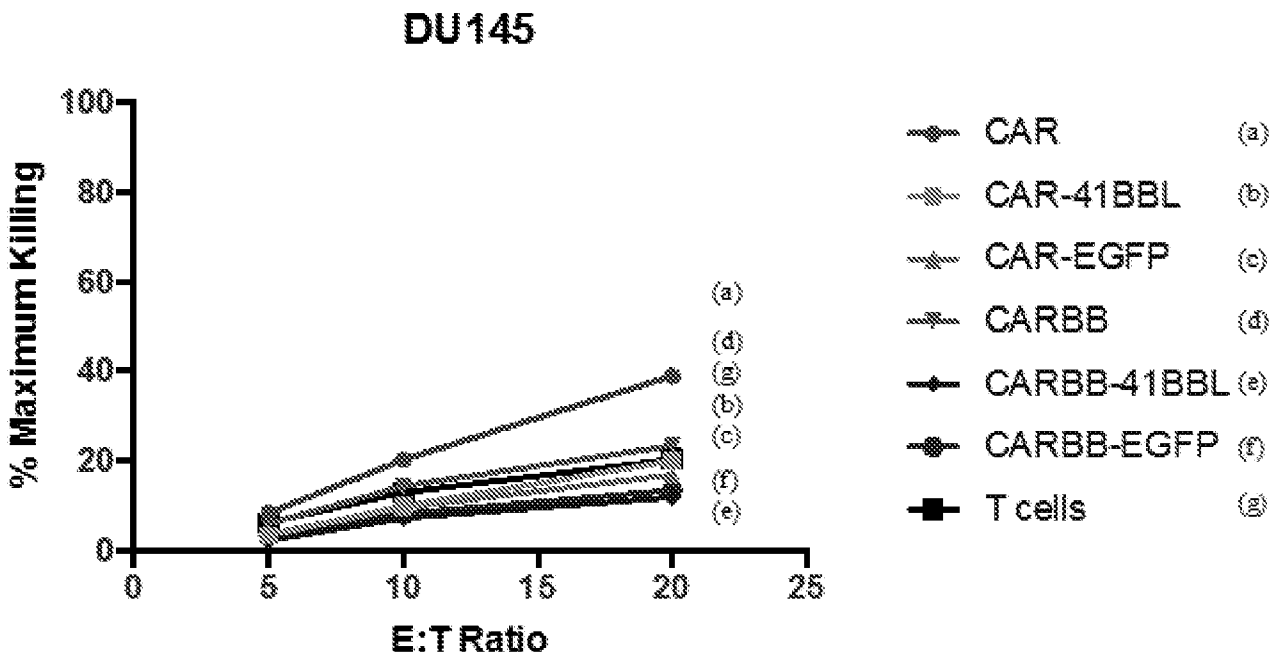

DU145
% Maximum Killing
100
80
60
40
20
0
0
5
10
15
20
25
E:T Ratio
(a)
(d)
(g)
(b)
(c)
(f)
(e)
CAR (a)
CAR-41BBL (b)
CAR-EGFP (c)
CARBB (d)
CARBB-41BBL (e)
CARBB-EGFP (f)
T cells (g)

FIG. 9D

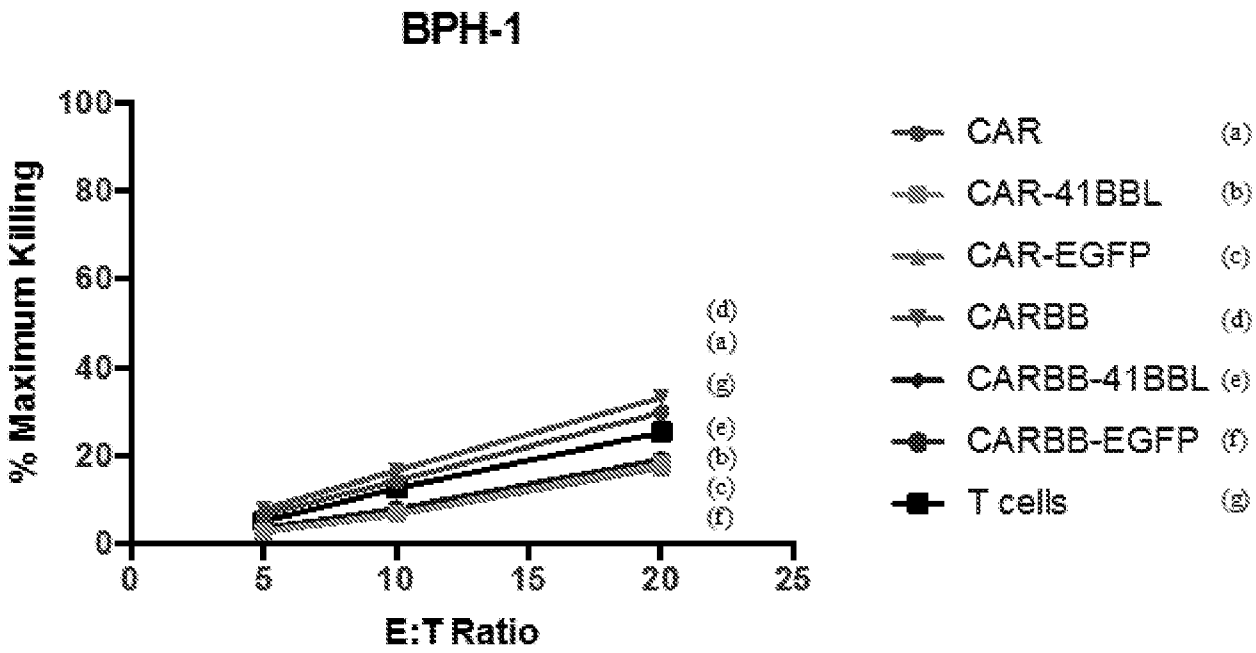
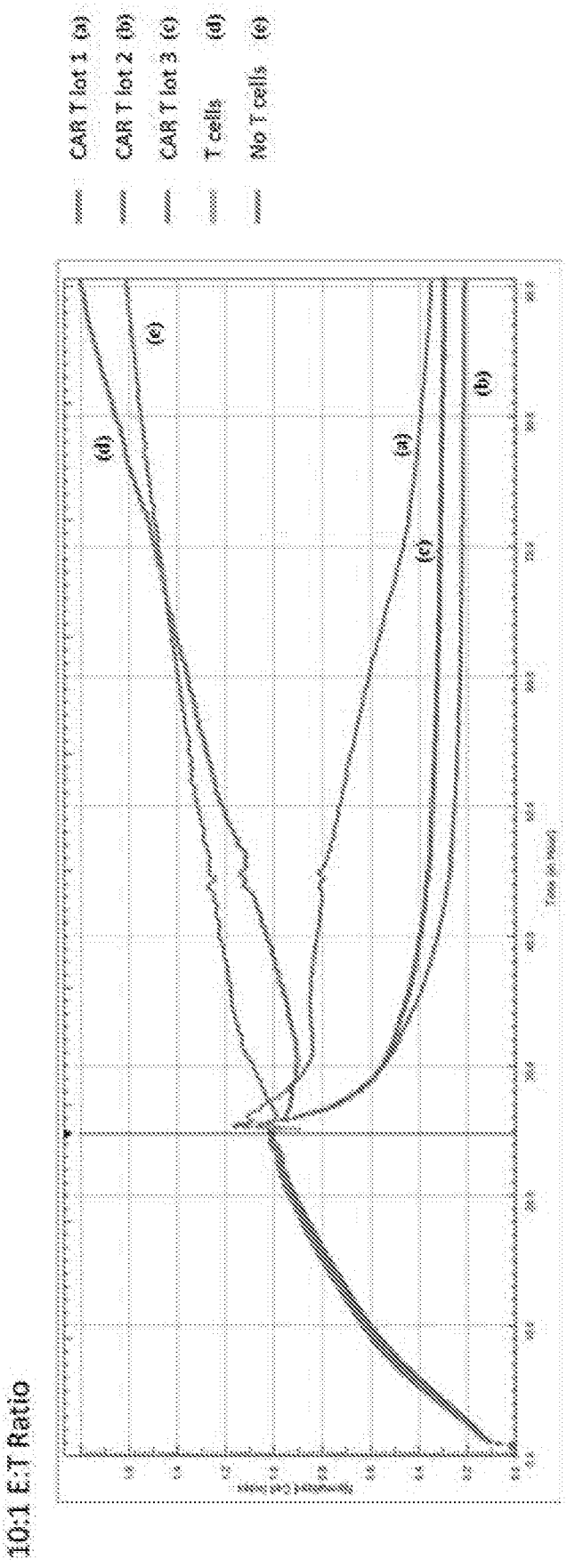

BPH-1
% Maximum Killing
100
80
60
40
20
0
0
5
10
15
20
25
E:T Ratio
(d)
(a)
(g)
(e)
(b)
(c)
(f)
CAR (a)
CAR-41BBL (b)
CAR-EGFP (c)
CARBB (d)
CARBB-41BBL (e)
CARBB-EGFP (f)
T cells (g)

FIG. 10


CAR T lot 1 (a)
CAR T lot 2 (b)
CAR T lot 3 (c)
T cells (d)
No T cells (e)
10:1 E:T Ratio
(a)
(b)
(c)
(d)
(e)

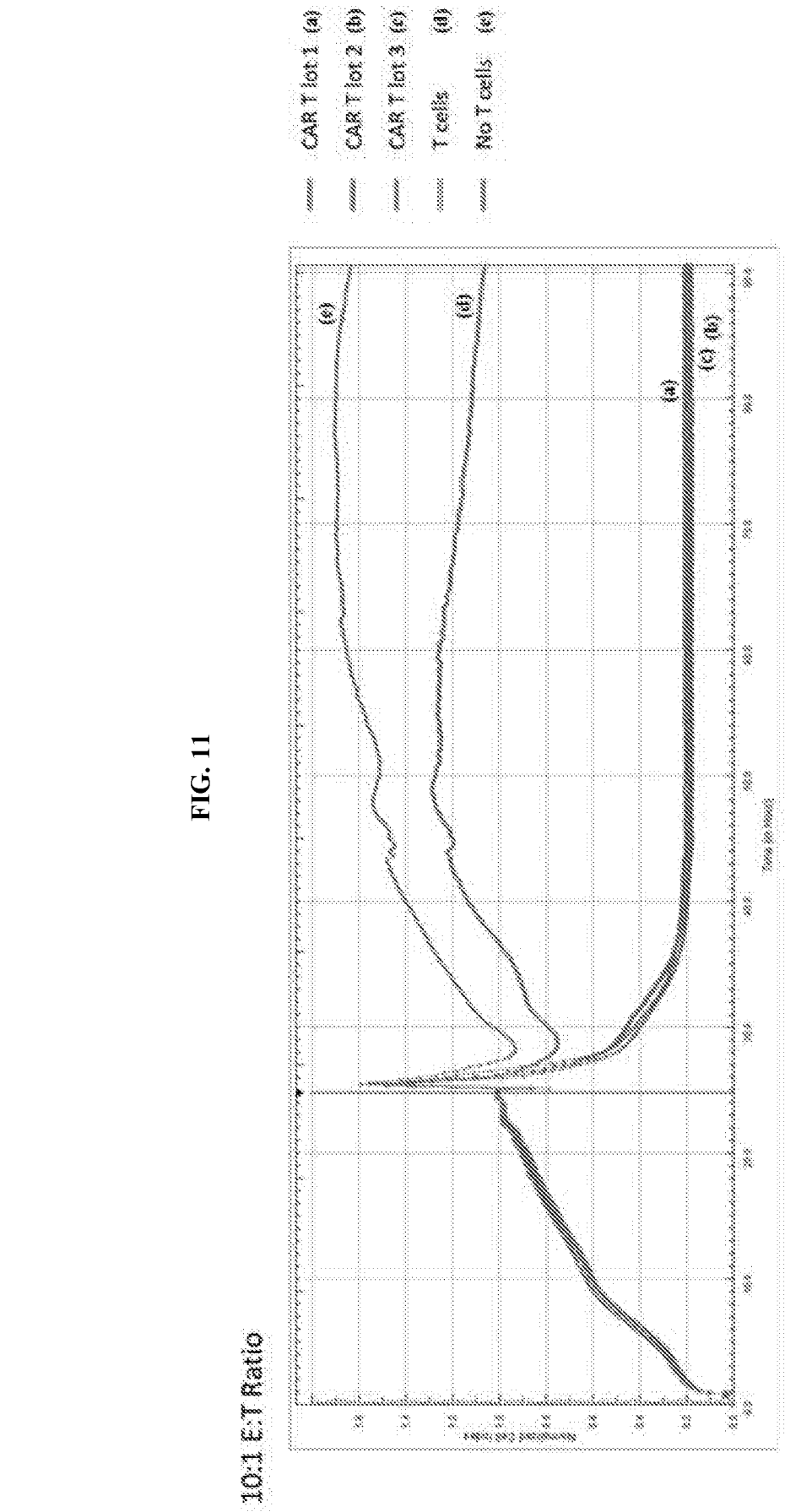
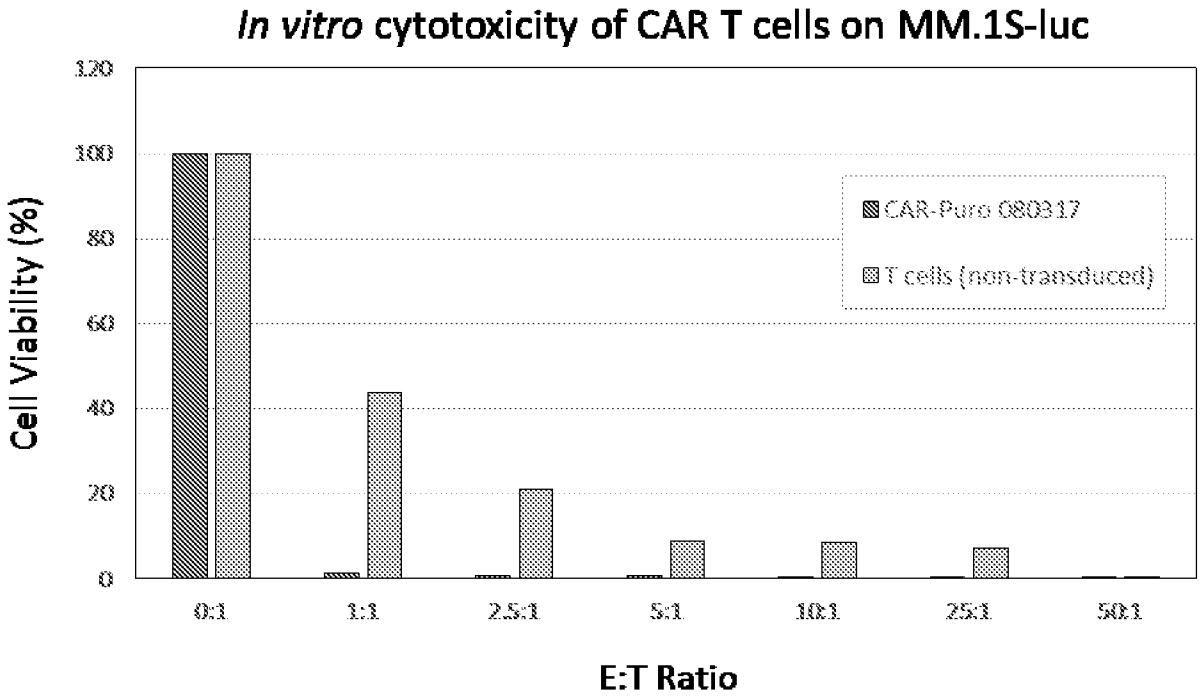

CAR T lot 1 (a)
CAR T lot 2 (b)
CAR T lot 3 (c)
T cells (d)
No T cells (e)
10:1 E:T Ratio
(e)
(d)
(a)
(c) (b)

FIG. 11

FIG. 12


*In vitro* cytotoxicity of CAR T cells on MM.1S-luc
Cell Viability (%)
120
100
80
60
40
20
0
CAR-Puro 080317
T cells (non-transduced)
0:1
1:1
2.5:1
5:1
10:1
25:1
50:1
E:T Ratio

# INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 17/61153

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - C07K 14/725, 16/30, 16/28; C12N 5/0783, 15/64 (2018.01)
CPC - C07K 14/70592, 2317/622, 2319/00, 2319/03, 14/705, 14/4748; A61K 39/0011

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
See Search History Document

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
See Search History Document

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
See Search History Document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| Y | WO 2016/040683 A1 (The Regents of the University of California) 17 March 2016 (17.03.2016). Especially para [0007], [0013], [0022], [0036], [0048], [0049], [0052], sheet 1 fig 1A, sheet 4 fig 1B | 1-6, 10-16, (17,18)/(1-6,10-16), 22-27, 31-33, (34,35)/(22-27,31-33), 38-41 |
| Y | WO 2016/042461 A1 (Molmed Spa) 24 March 2016 (24.03.2016). Especially para 1 para 3-4, pg 2 para 9, pg 3 para 1-3, 7, 10, pg 28 para 5-6, pg 29 para 1. | 1-6, 10-16, (17,18)/(1-6,10-16), 22-27, 31-33, (34,35)/(22-27,31-33), 38-41<br><br>18/(1-6,10-16) |
| Y | WO 2016/019300A1 (Novartis AG et al.) 4 February 2016 (04.02.2016). Especially pg 7 ln 14-19 | |

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

| Date of the actual completion of the international search | Date of mailing of the international search report |
|---|---|
| 12 March 2018 | 27 MAR 2018 |
| Name and mailing address of the ISA/US<br>Mail Stop PCT, Attn: ISA/US, Commissioner for Patents<br>P.O. Box 1450, Alexandria, Virginia 22313-1450<br>Facsimile No. 571-273-8300 | Authorized officer:<br>Lee W. Young<br>PCT Helpdesk: 571-272-4300<br>PCT OSP: 571-272-7774 |

Form PCT/ISA/210 (second sheet) (January 2015)

# INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 17/61153

**Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)**

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:

   a. ☒ forming part of the international application as filed:

      ☒ in the form of an Annex C/ST.25 text file.

      ☐ on paper or in the form of an image file.

   b. ☐ furnished together with the international application under PCT Rule 13*ter*.1(a) for the purposes of international search only in the form of an Annex C/ST.25 text file.

   c. ☐ furnished subsequent to the international filing date for the purposes of international search only:

      ☐ in the form of an Annex C/ST.25 text file (Rule 13*ter*.1(a)).

      ☐ on paper or in the form of an image file (Rule 13*ter*.1(b) and Administrative Instructions, Section 713).

2. ☐ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that forming part of the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:
GenCore ver 6.4.1 SEQ ID NOs. 1, 2

# INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 17/61153

**Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)**

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☒ Claims Nos.: 19-21, 36, 37, 58, 59, 64-76, 88-96
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

**Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)**

This International Searching Authority found multiple inventions in this international application, as follows:
----Go to Extra Sheet for continuation------

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
1-6, 10-16, (17,18)(in part), 22-27, 31-33, (34,35)(in part), 38-41limited to SEQ ID NOs: 1-2

**Remark on Protest**

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

**INTERNATIONAL SEARCH REPORT**

International application No.
PCT/US 17/61153

*Continued from Box III: Observations where Unity of Invention is Lacking*

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

Group I+: Claims 1-18, 22-35, 38-41, drawn to an engineered effector cell comprising an engineered antigen binding domain that binds to an epitope of CD46, a polynucleotide encoding said chimeric antigen receptor.
The engineered effector cell will be searched to the extent that the antigen binding domain is the first named antigen binding domain comprising VH SEQ ID NO: 1 and VL SEQ ID NO: 2 [Note, SEQ ID NO: 1 comprises CDR-H1, -H2, -H3 of SEQ ID NOs: 3, 4, 5, respectively; VL SEQ ID NO: 2 comprises CDR-L1, -L2, -L3 of SEQ ID NOs: 6, 7, 8, respectively.]. It is believed that claims 1-6, 10-16, (17,18)(in part), 22-27, 31-33, (34,35)(in part), 38-41 read on this first named invention and thus these claims will be searched without fee to the extent that they encompass SEQ ID NOs: 1-2. Additional antigen binding domains will be searched upon payment of additional fees. Applicant must specify the claims that encompass any additional antigen binding domains(s). Applicants must further indicate, if applicable, the claims which read on the first named invention if different than what was indicated above for this group. Failure to clearly identify how any paid additional invention fees are to be applied to the "+" group(s) will result in only the first claimed invention to be searched/examined. An exemplary election would be: antigen binding domain polypeptide comprising VH SEQ ID NO: 9 and VL SEQ ID NO: 10 (claims 1-5, 7, 10, 12-16, (17,18)(in part), 22-26, 28, 31, 33, (34,35)(in part), 38-41).

Group II+: Claims 42-57, 60-63, 77-87, 97, 98, drawn to a method of treating a subject having cancer characterized by an overexpression of CD46.
Group II+ will be searched upon payment of additional fee(s). The composition may be searched, for example, to the extent that, the antigen binding domain comprises VH SEQ ID NO: 1 and VL SEQ ID NO: 2. It is believed that claims 42-47,51-55, (56,57)(in part), 60-63, 77-84, read on this exemplary invention. Additional antigen binding domains will be searched upon the payment of additional fees. Applicants must indicate, if applicable, which claims read on this named invention if different than what was indicated above for this group. Failure to clearly identify how any paid additional invention fees are to be applied to the '+' group(s) will result in only the first named invention to be searched/examined. An exemplary election would be antigen binding domain comprising VH SEQ ID NO: 9 and VL SEQ ID NO: 10 (42-46, 48, 51-55, (56,57)(in part), 60-63, 77-83, 85).

The inventions listed as Groups I+ and II+ do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

Technical Features:

Group I+ has the special technical feature of a composition comprising an engineered effector cell comprising an engineered antigen binding domain that binds to an epitope of CD46, not required by Group II+.

Group II+ has the special technical feature of a method involving the step of administrating a plurality of engineered effector cells, wherein each engineered effector cell comprises an engineered antigen binding domain that binds to an epitope of CD46, not required by Group I+.

No technical features are shared among the amino acid sequences of antigen binding domains that bind to CD46 of Groups I+ and II+ and, accordingly, these groups lack unity a priori.

Additionally, even if Groups I+ and II+ were considered to share the technical features of:

1. an engineered antigen binding domain that binds to an epitope of CD46, wherein the engineered antigen binding domain is displayed on the surface of the engineered effector cell.

2. an antigen binding domain comprising a heavy chain variable region (VH) comprising VH-CDRs 1-3 and a light chain variable region (VL) comprising VL-CDRs 1-3.

3. a chimeric polynucleotide comprising: a first polynucleotide segment encoding an antigen binding domain that binds to an epitope; and a second polynucleotide segment encoding a polypeptide comprising a stalk region, a transmembrane domain, at least one costimulatory domains, and a signaling domain; wherein the chimeric polynucleotide, upon transfection to an effector cell, expresses the CD46 antigen binding domain and the polypeptide as a continuous chain, and wherein expression of the CD46 antigen binding domain and the polypeptide triggers the effector cell to activate and/or proliferate.

4. Group II+ inventions share the common technical feature of a method of treating a subject having cancer characterized by an overexpression of CD46.

-----continued on next sheet----

----continued from previous sheet----

these shared technical features are previously disclosed by WO 2016/040683 A1 to The Regents of the University of California (hereinafter "Univ California") [published 17 March 2016], in view of WO 2016/042461 A1 to Molmed Spa (hereinafter "Molmed")[published 24 March 2016]

As to shared technical features #1 and #2, Univ California teaches an engineered antigen binding domain that binds to an epitope of CD46, comprising a heavy chain variable region (VH) comprising VH-CDRs 1-3 and a light chain variable region (VL) comprising VL-CDRs 1-3 (para [0009]; "Embodiment 1 : An isolated human antibody that specifically binds CD46 and is internalized into a cell expressing or overexpressing CD46, wherein: said antibody is an antibody that specifically binds cells that express or overexpress a CD46"; para [0022]; "Embodiment 14: The antibody according to any one of embodiments 1-8, wherein said antibody is a single chain antibody"; para [0048]; "Embodiment 40: The antibody according to any one of embodiments 1-16, wherein said antibody comprises VL CDR1, and/or VL CDR2, and/or VL CDR3 of the YS5 antibody"; para [0049] Embodiment 41 : The antibody according to any one of embodiments 1-16, wherein said antibody comprises VH CDR1, VH CDR2, VH CDR3, VL CDR1, VLCDR2, and VL CDR3 of the YS5 antibody"). Univ California does not teach that the antigen binding domain is incorporated into a chimeric antigen receptor (CAR), or that said CAR is expressed in an engineered effector cell. However, incorporation of an antibody scFv as an antigen binding domain as part of a CAR was well-known in the art, as for example, taught by Molmed (pg 1 para 3; "CARs comprise an extracellular ligand binding domain, most commonly a single chain variable fragment of a monoclonal antibody (scFv) linked to intracellular signaling components"). By way of analogy, it would have been obvious that the anti-CD86 antibody taught by Univ California could have incorporated into a CAR, without undue effort, and a high probability of success).

As to shared technical feature #3, Molmed teaches a chimeric polynucleotide comprising: a first polynucleotide segment encoding an antigen binding domain that binds to an epitope; and a second polynucleotide segment encoding a polypeptide comprising a stalk region, a transmembrane domain, at least one costimulatory domains, and a signaling domain (pg 3 para 1-3; "According to an aspect of the present invention there is provided a chimeric antigen receptor (CAR) comprising (i) an antigen-specific targeting domain; (ii) an extracellular spacer domain as defined herein; (iii) a transmembrane domain; (iv) optionally at least one costimulatory domain; and (v) an intracellular signaling domain"; pg 2 para 9; "In another embodiment, the spacer comprises the serine /threonine-rich stalk of LNGFR"; pg 3 para 10; "In another aspect of the present invention there is provided a polynucleotide encoding a CAR of the invention and as defined herein"; pg 4 para 3; "In another aspect of the present invention there is provided a vector comprising the polynucleotide of the invention"; pg 4 para 5; "In another aspect of the present invention there is provided a cell comprising a CAR, a polynucleotide, or a vector of the present invention. Preferably the cell is a T-cell."). Molmed further teaches expression of the antigen binding domain and the polypeptide triggers the effector cell to activate and/or proliferate (pg 29 para 3; "Stimulation of the T-cells by an antigen under proper conditions results in proliferation (expansion) of the cells and/or production of IL-2. The cells comprising the CAR of the invention will expand in number in response to the binding of one or more antigens to the antigen-specific targeting regions of the CAR. The invention also provides a method of making and expanding cells expressing a CAR").

As to shared technical feature #4, Univ California teaches method of treating a subject having cancer characterized by an overexpression of CD46 (para [0350]; "The methods of "treatment" employ administration to a subject (e.g., a subject in need thereof), an anti-CD46 antibody or antigen binding portion or an immunoconjugate comprising such an antibody or antigen binding portion described herein. In certain embodiments the subject is a subject diagnosed with and/or under treatment for a CD46 positive cancer (e.g., prostate cancer) in order to prevent, cure, delay, reduce the severity of, or ameliorate one or more symptoms of the disease or disorder or recurring disease or disorder, or in order to prolong the survival of a subject beyond that expected in the absence of such treatment"; para [0412]-"the anti-CD46 antibodies described herein were constructed and it was found that they kill CD46-overexpressing cancer cell lines in vitro including but not limited to metastatic castration resistant prostate cancer, multiple myeloma, colorectal cancer, mesothelioma, ovarian cancer, etc").

As the shared technical features were known in the art at the time of the invention, they cannot be considered common shared technical features that would otherwise unify the groups. The inventions lack unity with one another.

Therefore, Groups I+ and II+ inventions each lack unity of invention under PCT Rule 13 because they do not share a same or corresponding special technical feature

Note concerning item 4: Claims 19-21, 36, 37, 58, 59, 64-76, 88-96 are multiple dependent claims and are not drafted according to the second and third sentences of PCT Rule 6.4(a).