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(54) Title: CELLS EXPRESSING A BCMA-TARGETING CHIMERIC ANTIGEN RECEPTOR, AND COMBINATION THERAPY WITH A GAMMA SECRETASE INHIBITOR

(57) Abstract: The invention relates to the treatment of diseases associated with expression of BCMA, in particular myelomas. The invention relates to combination therapies of a BCMA CAR-expressing cell and a gamma secretase inhibitor.

BCMA-TARGETING CHIMERIC ANTIGEN RECEPTOR, AND COMBINATION THERAPY WITH A GAMMA SECRETASE INHIBITOR

RELATED APPLICATIONS

This application claims priority to U.S. Serial No. 62/491,598 filed April 28, 2017, and U.S. Serial No. 62/593,655 filed Dec 1, 2017, the contents of each of which are incorporated herein by reference in their entirety.

SEQUENCE LISTING

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 April 23, 2018, is named N2067-7132WO_SL.txt and is 791,851 bytes in size.

FIELD OF THE INVENTION

The present invention relates generally to the use of cells engineered to express a chimeric antigen receptor targeting B-cell maturation antigen protein (BCMA), optionally in combination with a gamma secretase inhibitor, to treat a disease associated with the expression of BCMA.

BACKGROUND OF THE INVENTION

Gamma secretase is a multi-subunit protease complex that cleaves single-pass transmembrane proteins at residues within the transmembrane domain. The gamma secretase complex comprises four subunits: presenilin, nicastrin, gamma-secretase subunit aph-1, and gamma-secretase subunit PEN-2. Another protein, CD147, has been reported as a non-essential regulator of the gamma secretase complex. Exemplary gamma secretase substrates include amyloid precursor protein, Notch, ErbB4, E-cadherin, N-cadherin, and CD44 (Haapasalo et al., J Alzheimers Dis. 2011;25(1):3-28). Recently, B-cell maturation antigen (BCMA) was identified as another substrate of gamma secretase (Laurent et al., Nat Commun. 2015 Jun 11;6:7333).

BCMA is a tumor necrosis family receptor (TNFR) member expressed on cells of the B-cell lineage. BCMA expression is the highest on terminally differentiated B cells that assume the long lived plasma cell fate, including plasma cells, plasmablasts and a subpopulation of activated B cells and memory B cells. BCMA is involved in mediating the survival of plasma cells for maintaining long-term humoral immunity. The expression of BCMA has been recently linked to a number of cancers, autoimmune disorders, and infectious diseases. Cancers with increased expression of BCMA include

some hematological cancers, such as multiple myeloma, Hodgkin's and non-Hodgkin's lymphoma, various leukemias, and glioblastoma.

Given the ongoing need for improved strategies for targeting diseases such as cancer, new compositions and methods for improving therapeutic agents that target BCMA, e.g., anti-BCMA chimeric antigen receptor (CAR) therapies, are highly desirable.

SUMMARY OF THE INVENTION

The disclosure features, at least in part, a method of treating a disease or disorder associated with expression of B-cell maturation antigen (BCMA, also known as TNFRSF17, BCM, or CD269). In certain embodiments, the disorder is a cancer, e.g., a hematological cancer. In some embodiments, the method comprises administering to a subject a BCMA-targeting CAR therapy in combination with a gamma secretase inhibitor (GSI). In some embodiments, the BCMA-targeting CAR therapy is a cell (e.g., a population of cells) that expresses a CAR molecule that binds BCMA. In some embodiments, the combination maintains or has better clinical effectiveness as compared to either therapy alone. The disclosure additionally features a BCMA-targeting CAR therapy, e.g., as a monotherapy or in a combination therapy.

In one aspect, disclosed herein is a method of treating a subject having a disease associated with expression of B-cell maturation antigen (BCMA) comprising administering to the subject an effective amount of a cell (e.g., a population of cells) that expresses a chimeric antigen receptor (CAR) molecule that binds BCMA (a "BCMA CAR-expressing cell"), in combination with a gamma secretase inhibitor (GSI).

In certain embodiments, the GSI is an agent that reduces the expression and/or function of BCMA.

In one aspect, disclosed herein is a method of treating a subject having a disease associated with expression of B-cell maturation antigen (BCMA) comprising administering to the subject an effective amount of a cell (e.g., a population of cells) that expresses a chimeric antigen receptor (CAR) molecule that binds BCMA (a "BCMA CAR-expressing cell"), in combination with a gamma secretase inhibitor (GSI), wherein:

the CAR molecule comprises an anti-BCMA binding domain, a transmembrane domain, and an intracellular signaling domain, and wherein:

the GSI has one or more (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, or all) of the following properties:

(i) the GSI reduces gamma secretase-mediated cleavage of BCMA;

(ii) the GSI, when incubated with BCMA-expressing cells, increases cell surface expression of BCMA, e.g., by at least 2, 4, 6, 8, 10, 15, or 20-fold, e.g., as measured by a method described herein, e.g., a flow cytometry assay, e.g., as measured using methods described in Example 1 with respect to FIG. 1;

(iii) the GSI, when incubated with BCMA-expressing cells, changes conformation and/or posttranslational modification of the extracellular domain of cell surface-expressed BCMA;

(iv) the GSI, when incubated with BCMA-expressing cells, decreases the level of soluble BCMA in the cell supernatant, e.g., by at least 80, 85, 90, 95, 99, or 99.5%, e.g., as measured by a method described herein, e.g., an ELISA assay, e.g., as measured using methods described in Example 1 with respect to Table 28;

(v) the GSI, when administered in vivo, increases cell surface expression of BCMA, e.g., as measured by a method described herein, e.g., a flow cytometry assay;

(vi) the GSI, when administered in vivo, changes conformation and/or posttranslational modification of the extracellular domain of cell surface-expressed BCMA;

(vii) the GSI, when administered in vivo, decreases the level of soluble BCMA in the serum and/or bone marrow, e.g., as measured by a method described herein, e.g., an ELISA assay;

(viii) the GSI is capable of increasing the activity of the BCMA CAR-expressing cell, e.g., increasing the cytotoxicity of the BCMA CAR-expressing cell, e.g., as measured by a method described herein, e.g., as measured using methods described in Example 3 with respect to FIGs. 7B and 7C;

(ix) the GSI is capable of increasing the activity of the BCMA CAR-expressing cell, e.g., increasing the anti-tumor activity of the BCMA CAR-expressing cell, e.g., as measured by a method described herein, e.g., as measured using methods described in Example 3 with respect to FIG. 9D;

(x) the GSI does not reduce gamma secretase-mediated cleavage of Notch, or reduces gamma secretase-mediated cleavage of Notch less efficiently, e.g., at least 2-fold, 5-fold, 10-fold, 50-fold, or 100-fold less efficiently, than the GSI reduces gamma secretase-mediated cleavage of BCMA;

(xi) the GSI reduces gamma secretase-mediated cleavage of BCMA more efficiently, e.g., at least 2-fold, 5-fold, 10-fold, 50-fold, or 100-fold more efficiently, than the GSI reduces gamma secretase-mediated cleavage of another substrate of gamma secretase, e.g., Cadherins, ErbB, or CD44;

(xii) the GSI specifically binds to Presenilin-1, e.g., the GSI binds to Presenilin-1 with higher affinity, e.g., at least 2-fold, 5-fold, 10-fold, 50-fold, or 100-fold higher affinity, than the GSI binds to another subunit of gamma secretase, e.g., nicastrin, anterior pharynx-defective 1, or presenilin enhancer 2; or

(xiii) the GSI exhibits low gastrointestinal toxicity.

In one aspect, disclosed herein is a method of treating a subject having a disease associated with expression of B-cell maturation antigen (BCMA) comprising administering to the subject an effective

amount of a cell (e.g., a population of cells) that expresses a chimeric antigen receptor (CAR) molecule that binds BCMA (a "BCMA CAR-expressing cell"), in combination with a gamma secretase inhibitor (GSI), wherein:

the CAR molecule comprises an anti-BCMA binding domain comprising:

a heavy chain variable region (VH) comprising a heavy chain complementarity determining region 1 (VHCDR1), a VHCDR2, and a VHCDR3 of any anti-BCMA heavy chain binding domain amino acid sequence listed in Tables 1, 20, 22, 24, and 26 (or a sequence at least about 85%, 90%, 95%, 99% or more identical thereto, and/or having one, two, three or more substitutions, insertions or deletions, *e.g.*, conserved substitutions), and/or

a light chain variable region (VL) comprising a light chain complementarity determining region 1 (VLCDR1), a VLCDR2, and a VLCDR3 of any anti-BCMA light chain binding domain amino acid sequence listed in Tables 1, 21, 23, 25, and 26 (or a sequence at least about 85%, 90%, 95%, 99% or more identical thereto, and/or having one, two, three or more substitutions, insertions or deletions, *e.g.*, conserved substitutions).

In one aspect, disclosed herein is a method of treating a subject having a disease associated with expression of B-cell maturation antigen (BCMA) comprising administering to the subject an effective amount of a cell (e.g., a population of cells) that expresses a chimeric antigen receptor (CAR) molecule that binds BCMA (a "BCMA CAR-expressing cell"), in combination with a gamma secretase inhibitor (GSI), wherein:

(i) the GSI is an antibody molecule that reduces the expression and/or function of gamma secretase, optionally wherein the GSI is an antibody molecule that specifically binds to a subunit of gamma secretase (e.g., presenilin, nicastrin, APH-1, or PEN-2);

(ii) the GSI is (1) a gene editing system targeted to one or more sites within a gene encoding a subunit of gamma secretase (e.g., presenilin, nicastrin, APH-1, or PEN-2) or a regulatory element thereof; (2) a nucleic acid encoding one or more components of the gene editing system; or (3) a combination thereof; or

(iii) the GSI is an agent that mediates RNA interference, e.g., an siRNA or shRNA specific for a gene encoding a subunit of gamma secretase (e.g., presenilin, nicastrin, APH-1, or PEN-2), or a nucleic acid encoding the siRNA or shRNA.

In one embodiment, the GSI has one or more (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, or all) of the following properties:

(i) the GSI reduces gamma secretase-mediated cleavage of BCMA or prevents BCMA from shedding;

(ii) the GSI, when incubated with BCMA-expressing cells, increases cell surface expression of BCMA, e.g., by at least 2, 4, 6, 8, 10, 15, or 20-fold, e.g., as measured by a method described herein, e.g., a flow cytometry assay, e.g., as measured using methods described in Example 1 with respect to FIG. 1;

(iii) the GSI, when incubated with BCMA-expressing cells, changes conformation and/or posttranslational modification of the extracellular domain of cell surface-expressed BCMA;

(iv) the GSI, when incubated with BCMA-expressing cells, decreases the level of soluble BCMA in the cell supernatant, e.g., by at least 80, 85, 90, 95, 99, or 99.5%, e.g., as measured by a method described herein, e.g., an ELISA assay, e.g., as measured using methods described in Example 1 with respect to Table 28;

(v) the GSI, when administered in vivo, increases cell surface expression of BCMA, e.g., as measured by a method described herein, e.g., a flow cytometry assay;

(vi) the GSI, when administered in vivo, changes conformation and/or posttranslational modification of the extracellular domain of cell surface-expressed BCMA;

(vii) the GSI, when administered in vivo, decreases the level of soluble BCMA in the serum and/or bone marrow, e.g., as measured by a method described herein, e.g., an ELISA assay;

(viii) the GSI is capable of increasing the activity of the BCMA CAR-expressing cell, e.g., increasing the cytotoxicity of the BCMA CAR-expressing cell, e.g., as measured by a method described herein, e.g., as measured using methods described in Example 3 with respect to FIGs. 7B and 7C;

(ix) the GSI is capable of increasing the activity of the BCMA CAR-expressing cell, e.g., increasing the anti-tumor activity of the BCMA CAR-expressing cell, e.g., as measured by a method described herein, e.g., as measured using methods described in Example 3 with respect to FIG. 9D;

(x) the GSI does not reduce gamma secretase-mediated cleavage of Notch, or reduces gamma secretase-mediated cleavage of Notch less efficiently, e.g., at least 2-fold, 5-fold, 10-fold, 50-fold, or 100-fold less efficiently, than the GSI reduces gamma secretase-mediated cleavage of BCMA;

(xi) the GSI reduces gamma secretase-mediated cleavage of BCMA more efficiently, e.g., at least 2-fold, 5-fold, 10-fold, 50-fold, or 100-fold more efficiently, than the GSI reduces gamma secretase-mediated cleavage of another substrate of gamma secretase, e.g., Cadherins, ErbB, or CD44;

(xii) the GSI specifically binds to Presenilin-1, e.g., the GSI binds to Presenilin-1 with higher affinity, e.g., at least 2-fold, 5-fold, 10-fold, 50-fold, or 100-fold higher affinity, than the GSI binds to another subunit of gamma secretase, e.g., nicastrin, anterior pharynx-defective 1, or presenilin enhancer 2; or

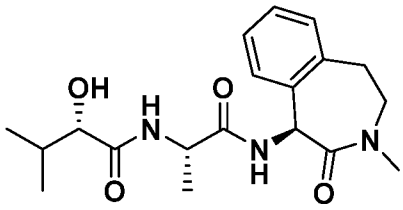
(xiii) the GSI exhibits low gastrointestinal toxicity.

In some embodiments, the GSI is chosen from a small molecule, an antibody molecule, an agent that mediates gene editing, or an agent that mediates RNA interference.

In one embodiment, the GSI is a small molecule that reduces the expression and/or function of gamma secretase, e.g., a small-molecule GSI disclosed herein. In one embodiment, the GSI is chosen from LY-450139, PF-5212362, BMS-708163, MK-0752, ELN-318463, BMS-299897, LY-411575, DAPT, BMS-906024, PF-3084014, RO4929097, or LY3039478. In one embodiment, the GSI is chosen from PF-5212362, ELN-318463, BMS-906024, or LY3039478. Exemplary GSIs are disclosed in Takebe et al., Pharmacol Ther. 2014 Feb;141(2):140-9; and Ran et al., EMBO Mol Med. 2017 Jul;9(7):950-966, both of which are incorporated herein by reference in their entirety.

In some embodiments, MK-0752 is administered in combination with docetaxel. In some embodiments, MK-0752 is administered in combination with gemcitabine. In some embodiments, BMS-906024 is administered in combination with chemotherapy.

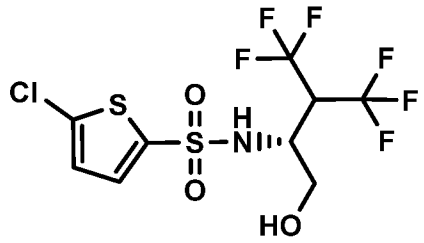
In one embodiment, the GSI is:



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or a pharmaceutically acceptable salt thereof.

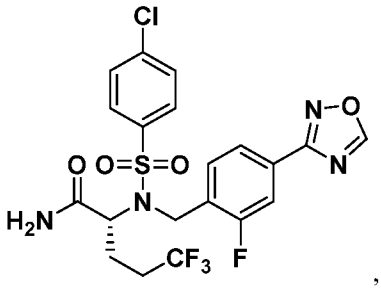
In one embodiment, the GSI is:



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or a pharmaceutically acceptable salt thereof.

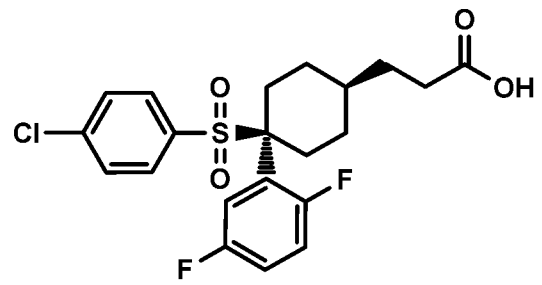
In one embodiment, the GSI is:



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or a pharmaceutically acceptable salt thereof.

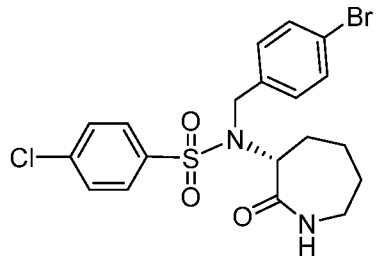
In one embodiment, the GSI is



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or a pharmaceutically acceptable salt thereof.

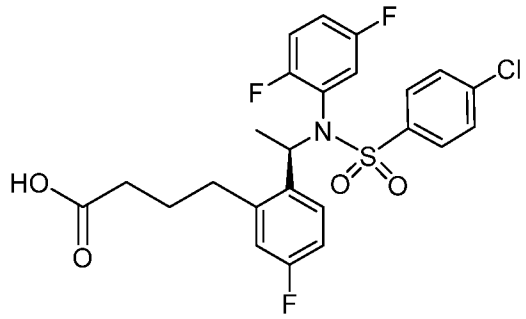
In one embodiment, the GSI is:



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or a pharmaceutically acceptable salt thereof.

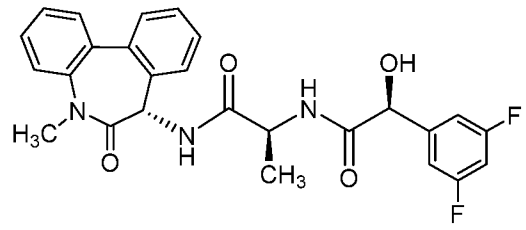
In one embodiment, the GSI is:



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or a pharmaceutically acceptable salt thereof.

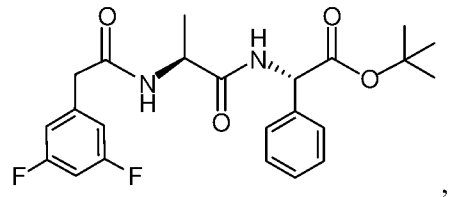
In one embodiment, the GSI is:



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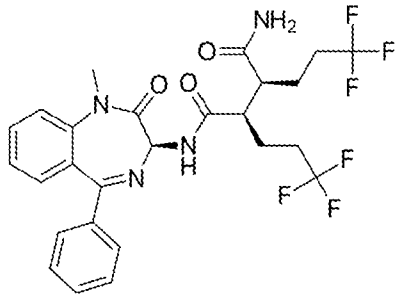
or a pharmaceutically acceptable salt thereof.

In one embodiment, the GSI is:



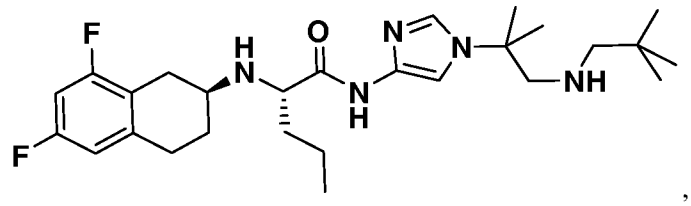
or a pharmaceutically acceptable salt thereof.

In one embodiment, the GSI is:



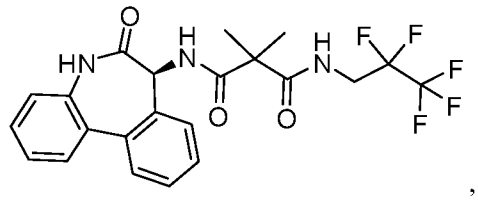
or a pharmaceutically acceptable salt thereof.

In one embodiment, the GSI is:



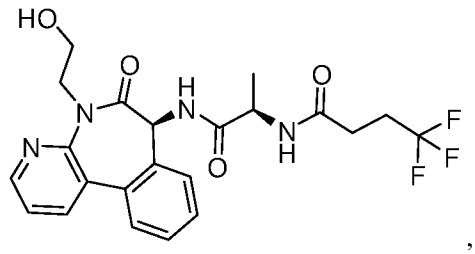
or a pharmaceutically acceptable salt thereof.

In one embodiment, the GSI is:



or a pharmaceutically acceptable salt thereof.

In one embodiment, the GSI is:



or a pharmaceutically acceptable salt thereof.

In one embodiment, the GSI is an antibody molecule that reduces the expression and/or function of gamma secretase, e.g., an antibody-molecule GSI disclosed herein. In one embodiment, the GSI is an antibody molecule that specifically binds to a subunit of gamma secretase (e.g., presenilin, nicastrin, APH-1, or PEN-2).

In one embodiment, the GSI is an agent that mediates gene editing, e.g., a gene editing system disclosed herein. In one embodiment, the GSI is (1) a gene editing system targeted to one or more sites within a gene encoding a subunit of gamma secretase (e.g., presenilin, nicastrin, APH-1, or PEN-2) or a regulatory element thereof; (2) a nucleic acid encoding one or more components of the gene editing system; or (3) a combination thereof. In one embodiment, the gene editing system is chosen from a CRISPR/Cas9 system, a zinc finger nuclease system, a TALEN system, or a meganuclease system.

In one embodiment, the GSI is an agent that mediates RNA interference, e.g., an siRNA or shRNA disclosed herein. In one embodiment, the GSI is an siRNA or shRNA specific for a gene encoding a subunit of gamma secretase (e.g., presenilin, nicastrin, APH-1, or PEN-2), or a nucleic acid encoding the siRNA or shRNA. In one embodiment, the siRNA or shRNA comprises a sequence complementary to a sequence of an mRNA of the gene encoding a subunit of gamma secretase (e.g., presenilin, nicastrin, APH-1, or PEN-2).

In one embodiment, the BCMA CAR-expressing cell comprises a nucleic acid encoding a CAR molecule, wherein the CAR molecule comprises an anti-BCMA binding domain, a transmembrane domain, and an intracellular signaling domain.

In one embodiment, the encoded anti-BCMA binding domain comprises:

a heavy chain variable region (VH) comprising a heavy chain complementarity determining region 1 (VHCDR1), a VHCDR2, and a VHCDR3 of any anti-BCMA heavy chain binding domain amino acid sequence listed in Tables 1, 16, 20, 22, 24, and 26 (or a sequence at least about 85%, 90%, 95%, 99% or more identical thereto, and/or having one, two, three or more substitutions, insertions or deletions, *e.g.*, conserved substitutions), and/or

a light chain variable region (VL) comprising a light chain complementarity determining region 1 (VLCDR1), a VLCDR2, and a VLCDR3 of any anti-BCMA light chain binding domain amino acid sequence listed in Tables 1, 16, 21, 23, 25, and 26 (or a sequence at least about 85%, 90%, 95%, 99% or more identical thereto, and/or having one, two, three or more substitutions, insertions or deletions, *e.g.*, conserved substitutions).

In one embodiment, the encoded anti-BCMA binding domain comprises:

a VH comprising a VH of any anti-BCMA heavy chain binding domain amino acid sequence listed in Tables 1, 16, and 26 (or a sequence at least about 85%, 90%, 95%, 99% or more identical

thereto, and/or having one, two, three or more substitutions, insertions or deletions, *e.g.*, conserved substitutions), and/or

a VL comprising a VL of any anti-BCMA light chain binding domain amino acid sequence listed in Tables 1, 16, and 26 (or a sequence at least about 85%, 90%, 95%, 99% or more identical thereto, and/or having one, two, three or more substitutions, insertions or deletions, *e.g.*, conserved substitutions).

In one embodiment, the encoded anti-BCMA binding domain comprises an scFv comprising an scFv amino acid sequence listed in Tables 1 and 16 (or a sequence at least about 85%, 90%, 95%, 99% or more identical thereto, and/or having one, two, three or more substitutions, insertions or deletions, *e.g.*, conserved substitutions).

In one embodiment, the encoded anti-BCMA binding domain comprises an scFv comprising a VH, a VL, and a linker, wherein the linker comprises the amino acid sequence of GGGGSGGGGSGGGGSGGGGS (SEQ ID NO: 27).

In one embodiment, the encoded anti-BCMA binding domain comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 39, SEQ ID NO: 40, SEQ ID NO: 41, SEQ ID NO: 42, SEQ ID NO: 43, SEQ ID NO: 44, SEQ ID NO: 45, SEQ ID NO: 46, SEQ ID NO: 47, SEQ ID NO: 48, SEQ ID NO: 49, SEQ ID NO: 50, SEQ ID NO: 51, SEQ ID NO: 52, SEQ ID NO: 53, SEQ ID NO: 129, SEQ ID NO: 130, SEQ ID NO: 131, SEQ ID NO: 132, SEQ ID NO: 133, SEQ ID NO: 134, SEQ ID NO: 135, SEQ ID NO: 136, SEQ ID NO: 137, SEQ ID NO: 138, SEQ ID NO: 139, SEQ ID NO: 140, SEQ ID NO: 141, SEQ ID NO: 142, SEQ ID NO: 143, SEQ ID NO: 144, SEQ ID NO: 145, SEQ ID NO: 146, SEQ ID NO: 147, SEQ ID NO: 148, and SEQ ID NO: 149, or a sequence with 95-99% identify thereof.

In one embodiment, the nucleic acid encoding the CAR molecule comprises a nucleotide sequence selected from the group consisting of SEQ ID NO: 54, SEQ ID NO: 55, SEQ ID NO: 56, SEQ ID NO: 57, SEQ ID NO: 58, SEQ ID NO: 59, SEQ ID NO: 60, SEQ ID NO: 61, SEQ ID NO: 62, SEQ ID NO: 63, SEQ ID NO: 64, SEQ ID NO: 65, SEQ ID NO: 66, SEQ ID NO: 67, SEQ ID NO: 68, SEQ ID NO: 150, SEQ ID NO: 151, SEQ ID NO: 152, SEQ ID NO: 153, SEQ ID NO: 154, SEQ ID NO: 155, SEQ ID NO: 156, SEQ ID NO: 157, SEQ ID NO: 158, SEQ ID NO: 159, SEQ ID NO: 160, SEQ ID NO: 161, SEQ ID NO: 162, SEQ ID NO: 163, SEQ ID NO: 164, SEQ ID NO: 165, SEQ ID NO: 166, SEQ ID NO: 167, SEQ ID NO: 168, SEQ ID NO: 169, SEQ ID NO: 170, or a sequence with 95-99% identify thereof.

In one embodiment, the encoded CAR molecule comprises a full CAR amino acid sequence listed in Tables 1 and 16 (or a sequence at least about 85%, 90%, 95%, 99% or more identical thereto, and/or having one, two, three or more substitutions, insertions or deletions, *e.g.*, conserved substitutions).

In one embodiment, the encoded CAR molecule comprises an amino acid sequence selected from the group consisting of residues 22-483 of SEQ ID NO: 109, residues 22-490 of SEQ ID NO: 99, residues 22-488 of SEQ ID NO: 100, residues 22-487 of SEQ ID NO: 101, residues 22-493 of SEQ ID NO: 102, residues 22-490 of SEQ ID NO: 103, residues 22-491 of SEQ ID NO: 104, residues 22-482 of SEQ ID NO: 105, residues 22-483 of SEQ ID NO: 106, residues 22-485 of SEQ ID NO: 107, residues 22-483 of SEQ ID NO: 108, residues 22-490 of SEQ ID NO: 110, residues 22-483 of SEQ ID NO: 111, residues 22-484 of SEQ ID NO: 112, residues 22-485 of SEQ ID NO: 113, residues 22-487 of SEQ ID NO: 213, residues 23-489 of SEQ ID NO: 214, residues 22-490 of SEQ ID NO: 215, residues 22-484 of SEQ ID NO: 216, residues 22-485 of SEQ ID NO: 217, residues 22-489 of SEQ ID NO: 218, residues 22-497 of SEQ ID NO: 219, residues 22-492 of SEQ ID NO: 220, residues 22-490 of SEQ ID NO: 221, residues 22-485 of SEQ ID NO: 222, residues 22-492 of SEQ ID NO: 223, residues 22-492 of SEQ ID NO: 224, residues 22-483 of SEQ ID NO: 225, residues 22-490 of SEQ ID NO: 226, residues 22-485 of SEQ ID NO: 227, residues 22-486 of SEQ ID NO: 228, residues 22-492 of SEQ ID NO: 229, residues 22-488 of SEQ ID NO: 230, residues 22-488 of SEQ ID NO: 231, residues 22-495 of SEQ ID NO: 232, residues 22-490 of SEQ ID NO: 233, or a sequence with 95-99% identify thereof.

In one embodiment, the nucleic acid encoding the CAR molecule comprises a nucleotide sequence listed in Table 1, or a sequence with 95-99% identify thereof.

In one embodiment, the nucleic acid encoding the CAR molecule comprises a nucleotide sequence selected from the group consisting of SEQ ID NO: 114, SEQ ID NO: 115, SEQ ID NO: 116, SEQ ID NO: 117, SEQ ID NO: 118, SEQ ID NO: 119, SEQ ID NO: 120, SEQ ID NO: 121, SEQ ID NO: 122, SEQ ID NO: 123, SEQ ID NO: 124, SEQ ID NO: 125, SEQ ID NO: 126, SEQ ID NO: 127, SEQ ID NO: 128, SEQ ID NO: 234, SEQ ID NO: 235, SEQ ID NO: 236, SEQ ID NO: 237, SEQ ID NO: 238, SEQ ID NO: 239, SEQ ID NO: 240, SEQ ID NO: 241, SEQ ID NO: 242, SEQ ID NO: 243, SEQ ID NO: 244, SEQ ID NO: 245, SEQ ID NO: 246, SEQ ID NO: 247, SEQ ID NO: 248, SEQ ID NO: 249, SEQ ID NO: 250, SEQ ID NO: 251, SEQ ID NO: 252, SEQ ID NO: 253, and SEQ ID NO: 254, or a sequence with 95-99% identify thereof.

In one embodiment, the encoded transmembrane domain comprises a transmembrane domain of a protein selected from the group consisting of the alpha, beta or zeta chain of a T-cell receptor, CD28, CD3 epsilon, CD45, CD4, CD5, CD8, CD9, CD16, CD22, CD33, CD37, CD64, CD80, CD86, CD134, CD137 and CD154.

In one embodiment, the encoded transmembrane domain comprises the amino acid sequence of SEQ ID NO: 6 (or a sequence at least about 85%, 90%, 95%, 99% or more identical thereto, and/or having one, two, three or more substitutions, insertions or deletions, *e.g.*, conserved substitutions).

In one embodiment, the nucleic acid encoding the CAR molecule comprises the nucleotide sequence of SEQ ID NO: 17, or a sequence with 95-99% identify thereof.

In one embodiment, the encoded anti-BCMA binding domain is connected to the transmembrane domain by a hinge region.

In one embodiment, the encoded hinge region comprises the amino acid sequence of SEQ ID NO: 2 or 36 (or a sequence at least about 85%, 90%, 95%, 99% or more identical thereto, and/or having one, two, three or more substitutions, insertions or deletions, *e.g.*, conserved substitutions).

In one embodiment, the nucleic acid encoding the CAR molecule comprises the nucleotide sequence of SEQ ID NO: 13 or 37, or a sequence with 95-99% identify thereof.

In one embodiment, the encoded intracellular signaling domain is a functional signaling domain obtained from a protein chosen from an MHC class I molecule, a TNF receptor, an immunoglobulin-like protein, a cytokine receptor, integrin, signaling lymphocytic activation molecule (SLAM), an activating NK cell receptor, BTLA, a Toll ligand receptor, CD3, OX40, CD2, CD7, CD27, CD28, CD30, CD40, CDS, ICAM-1, LFA-1 (CD11a/CD18), 4-1BB (CD137), B7-H3, CDS, ICAM-1, ICOS (CD278), GITR, BAFFR, LIGHT, HVEM (LIGHTR), KIRDS2, SLAMF7, NKp80 (KLRF1), NKp44, NKp30, NKp46, CD19, CD4, CD8alpha, CD8beta, IL2R beta, IL2R gamma, IL7R alpha, ITGA4, VLA1, CD49a, ITGA4, IA4, CD49D, ITGA6, VLA-6, CD49f, ITGAD, CD11d, ITGAE, CD103, ITGAL, CD11a, LFA-1, ITGAM, CD11b, ITGAX, CD11c, ITGB1, CD29, ITGB2, CD18, LFA-1, ITGB7, NKG2D, NKG2C, TNFR2, TRANCE/RANKL, DNAM1 (CD226), SLAMF4 (CD244, 2B4), CD84, CD96 (Tactile), CEACAM1, CRTAM, Ly9 (CD229), CD160 (BY55), PSGL1, CD100 (SEMA4D), CD69, SLAMF6 (NTB-A, Ly108), SLAM (SLAMF1, CD150, IPO-3), BLAME (SLAMF8), SELPLG (CD162), LTBR, LAT, GADS, SLP-76, PAG/Cbp, CD19a, or a ligand that specifically binds with CD83.

In one embodiment, the encoded intracellular signaling domain is a functional signaling domain of a protein chosen from 4-1BB, CD3 zeta, CD28, or ICOS.

In one embodiment, the encoded intracellular signaling domain comprises a 4-1BB costimulatory domain (or a sequence at least about 85%, 90%, 95%, 99% or more identical thereto, and/or having one, two, three or more substitutions, insertions or deletions, *e.g.*, conserved substitutions). In one embodiment, the encoded intracellular signaling domain comprises a CD28 costimulatory domain (or a sequence at least about 85%, 90%, 95%, 99% or more identical thereto, and/or having one, two, three or more substitutions, insertions or deletions, *e.g.*, conserved substitutions). In one embodiment, the encoded intracellular signaling domain comprises an ICOS costimulatory domain (or a sequence at least about 85%, 90%, 95%, 99% or more identical thereto, and/or having one, two, three or more substitutions, insertions or deletions, *e.g.*, conserved substitutions).

In one embodiment, the encoded intracellular signaling domain comprises a CD3-zeta stimulatory domain (or a sequence at least about 85%, 90%, 95%, 99% or more identical thereto, and/or having one, two, three or more substitutions, insertions or deletions, *e.g.*, conserved substitutions).

In one embodiment, the encoded intracellular signaling domain comprises the amino acid sequence of SEQ ID NO: 7, 9, 10, 1104, or 1106 (or a sequence at least about 85%, 90%, 95%, 99% or more identical thereto, and/or having one, two, three or more substitutions, insertions or deletions, *e.g.*, conserved substitutions).

In one embodiment, the nucleic acid molecule encoding the CAR molecule comprises the nucleotide sequence of SEQ ID NO: 18, 20, 21, 1105, or 1107, or a sequence with 95-99% identify thereof.

In one embodiment, the encoded intracellular signaling domain comprises the amino acid sequence of SEQ ID NO: 7 and the amino acid sequence of SEQ ID NO: 9 or 10, or an amino acid sequence at least about 85%, 90%, 95%, 99% or more identical to SEQ ID NO: 7 and an amino acid sequence at least about 85%, 90%, 95%, 99% or more identical to SEQ ID NO: 9 or 10.

In one embodiment, the nucleic acid encoding the CAR molecule comprises:

(i) a leader sequence encoding the amino acid sequence of SEQ ID NO: 1 (or a sequence at least about 85%, 90%, 95%, 99% or more identical thereto, and/or having one, two, three or more substitutions, insertions or deletions, e.g., conserved substitutions); or

(ii) the nucleotide sequence of SEQ ID NO: 12, or a sequence with 95-99% identify thereof.

In one embodiment, the nucleic acid encoding the CAR molecule is a DNA molecule, optionally wherein the DNA molecule is transcribed under an EF-1 promoter comprising the sequence of SEQ ID NO: 11.

In one embodiment, the nucleic acid encoding the CAR molecule is an RNA molecule.

In one embodiment, the cell is an autologous cell or an allogeneic cell.

In one embodiment, the cell is a T cell or a natural killer (NK) cell.

In one embodiment, the disease associated with expression of BCMA is:

(i) a cancer or malignancy, or a precancerous condition chosen from one or more of a myelodysplasia, a myelodysplastic syndrome or a preleukemia, or

(ii) a non-cancer related indication associated with expression of BCMA.

In one embodiment, the disease is chosen from acute leukemia, B-cell acute lymphoid leukemia (BALL), T-cell acute lymphoid leukemia (TALL), acute lymphoid leukemia (ALL), chronic myelogenous leukemia (CML), chronic lymphocytic leukemia (CLL), B cell prolymphocytic leukemia, blastic plasmacytoid dendritic cell neoplasm, Burkitt's lymphoma, diffuse large B cell lymphoma, follicular lymphoma, hairy cell leukemia, small cell- or large cell-follicular lymphoma, a malignant

lymphoproliferative condition, mucosa associated lymphoid tissue (MALT) lymphoma, mantle cell lymphoma, marginal zone lymphoma, multiple myeloma, myelodysplasia and myelodysplastic syndrome, non-Hodgkin's lymphoma, plasmablastic lymphoma, plasmacytoid dendritic cell neoplasm, Waldenstrom macroglobulinemia, a plasma cell proliferative disorder (e.g., asymptomatic myeloma (smoldering multiple myeloma or indolent myeloma), monoclonal gammapathy of undetermined significance (MGUS), Waldenstrom's macroglobulinemia, plasmacytomas (e.g., plasma cell dyscrasia, solitary myeloma, solitary plasmacytoma, extramedullary plasmacytoma, and multiple plasmacytoma), systemic amyloid light chain amyloidosis, and POEMS syndrome (also known as Crow-Fukase syndrome, Takatsuki disease, and PEP syndrome)), prostate cancer (e.g., castrate-resistant or therapy-resistant prostate cancer, or metastatic prostate cancer), pancreatic cancer, or lung cancer.

In one embodiment, the disease is a hematologic cancer. In one embodiment, the disease is multiple myeloma. In one embodiment, the disease is CD19-negative multiple myeloma.

In one embodiment, the BCMA CAR-expressing cell and the GSI are administered simultaneously or sequentially. In one embodiment, the BCMA CAR-expressing cell is administered prior to the administration of the GSI. In one embodiment, the GSI is administered prior to the administration of the BCMA CAR-expressing cell. In one embodiment, the BCMA CAR-expressing cell and the GSI are administered simultaneously.

In one embodiment, the GSI is administered prior to the administration of the BCMA CAR-expressing cell (e.g., GSI is administered 1, 2, 3, 4, or 5 days prior to the administration of the BCMA CAR-expressing cell), optionally wherein after the administration of the GSI and prior to the administration of the BCMA CAR-expressing cell, the subject shows an increase in cell surface BCMA expression levels and/or a decrease in soluble BCMA levels.

In one embodiment, the method comprises a first treatment regimen and a second treatment regimen, wherein the first treatment regimen is performed prior to the second treatment regimen, wherein:

(i) the first treatment regimen comprises administering a first dose of the BCMA CAR-expressing cell, and

(ii) the second treatment regimen comprises administering a dose of GSI followed by a second dose of the BCMA CAR-expressing cell,

optionally wherein after the administration of the dose of GSI and prior to the administration of the second dose of the BCMA CAR-expressing cell, the subject shows an increase in cell surface BCMA expression levels and/or a decrease in soluble BCMA levels.

In some embodiments, the GSI is administered at a dose that does not reduce or does not substantially reduce T cell proliferation (e.g., reduces T cell proliferation by no more than 2, 4, 6, 8, 10,

or 15%), e.g., as measured using a method described herein, e.g., as measured using methods described in Example 3 with respect to FIGs. 6A and 6B. In some embodiments, the GSI is administered at a sub-optimal dose. In some embodiments, the GSI is administered at a dose that is lower than the dose of GSI used when the GSI is administered as a monotherapy. In some embodiments, the GSI is administered at a dose that is lower than 100, 150, 200, 250, 300, 350, 400, 450, or 500 mg per day.

In one embodiment, the BCMA CAR-expressing cell and the GSI are administered in combination with a third therapeutic agent or procedure, optionally wherein the third therapeutic agent or procedure is chosen from one or more of chemotherapy, a targeted anti-cancer therapy, an oncolytic drug, a cytotoxic agent, an immune-based therapy, a cytokine, surgical procedure, a radiation procedure, an activator of a costimulatory molecule, an inhibitor of an inhibitory molecule (e.g., an inhibitor of a checkpoint inhibitor), a vaccine, or a cellular immunotherapy.

In one embodiment, the third therapeutic agent or procedure is chosen from:

(i) Dexamethasone;

(ii) a PD-1 inhibitor, optionally wherein the PD-1 inhibitor is selected from the group consisting of PDR001, Nivolumab, Pembrolizumab, Pidilizumab, MEDI0680, REGN2810, TSR-042, PF-06801591, and AMP-224;

(iii) a PD-L1 inhibitor, optionally wherein the PD-L1 inhibitor is selected from the group consisting of FAZ053, Atezolizumab, Avelumab, Durvalumab, and BMS-936559;

(iv) a CTLA-4 inhibitor, optionally wherein the CTLA-4 inhibitor is Ipilimumab or Tremelimumab;

(v) a TIM-3 inhibitor, optionally wherein the TIM-3 inhibitor is MGB453 or TSR-022;

(vi) a LAG-3 inhibitor, optionally wherein the LAG-3 inhibitor is selected from the group consisting of LAG525, BMS-986016, and TSR-033;

(vii) an mTOR inhibitor, optionally wherein the mTOR inhibitor is RAD001 or rapamycin; or

(viii) an agent chosen from HetIL-15, an anti-TGFβ antibody, an anti-CD47 antibody, an IDO inhibitor, a STING agonist, a TLR agonist, an immunomodulatory drug (IMiD) (e.g., Thalidomide, Lenalidomide, or Pomalidomide), a proteasome inhibitor (e.g., Bortezomib), or an ADCC-competent antibody (e.g., Daratumumab or Elotuzumab).

In one aspect, provided herein is a composition comprising a cell (e.g., a population of cells) that expresses a CAR molecule that binds BCMA (a "BCMA CAR-expressing cell") and a GSI.

In one embodiment, the BCMA CAR-expressing cell and the GSI are present in a single dose form, or as two or more dose forms.

In one embodiment, provided herein is a composition comprising a cell (e.g., a population of cells) that expresses a CAR molecule that binds BCMA (a "BCMA CAR-expressing cell") and a GSI for use as a medicament.

In one embodiment, provided herein is a composition comprising a cell (e.g., a population of cells) that expresses a CAR molecule that binds BCMA (a "BCMA CAR-expressing cell") and a GSI for use in the treatment of a disease associated with expression of BCMA.

In one embodiment, provided herein is a composition comprising a cell (e.g., a population of cells) that expresses a CAR molecule that binds BCMA (a "BCMA CAR-expressing cell") and a GSI, optionally wherein:

the CAR molecule comprises an anti-BCMA binding domain, a transmembrane domain, and an intracellular signaling domain, and optionally wherein:

the GSI has one or more (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, or all) of the following properties:

(i) the GSI reduces gamma secretase-mediated cleavage of BCMA;

(ii) the GSI, when incubated with BCMA-expressing cells, increases cell surface expression of BCMA, e.g., by at least 2, 4, 6, 8, 10, 15, or 20-fold, e.g., as measured by a method described herein, e.g., a flow cytometry assay, e.g., as measured using methods described in Example 1 with respect to FIG. 1;

(iii) the GSI, when incubated with BCMA-expressing cells, changes conformation and/or posttranslational modification of the extracellular domain of cell surface-expressed BCMA;

(iv) the GSI, when incubated with BCMA-expressing cells, decreases the level of soluble BCMA in the cell supernatant, e.g., by at least 80, 85, 90, 95, 99, or 99.5%, e.g., as measured by a method described herein, e.g., an ELISA assay, e.g., as measured using methods described in Example 1 with respect to Table 28;

(v) the GSI, when administered in vivo, increases cell surface expression of BCMA, e.g., as measured by a method described herein, e.g., a flow cytometry assay;

(vi) the GSI, when administered in vivo, changes conformation and/or posttranslational modification of the extracellular domain of cell surface-expressed BCMA;

(vii) the GSI, when administered in vivo, decreases the level of soluble BCMA in the serum and/or bone marrow, e.g., as measured by a method described herein, e.g., an ELISA assay;

(viii) the GSI is capable of increasing the activity of the BCMA CAR-expressing cell, e.g., increasing the cytotoxicity of the BCMA CAR-expressing cell, e.g., as measured by a method described herein, e.g., as measured using methods described in Example 3 with respect to FIGs. 7B and 7C;

(ix) the GSI is capable of increasing the activity of the BCMA CAR-expressing cell, e.g., increasing the anti-tumor activity of the BCMA CAR-expressing cell, e.g., as measured by a method described herein, e.g., as measured using methods described in Example 3 with respect to FIG. 9D;

(x) the GSI does not reduce gamma secretase-mediated cleavage of Notch, or reduces gamma secretase-mediated cleavage of Notch less efficiently, e.g., at least 2-fold, 5-fold, 10-fold, 50-fold, or 100-fold less efficiently, than the GSI reduces gamma secretase-mediated cleavage of BCMA;

(xi) the GSI reduces gamma secretase-mediated cleavage of BCMA more efficiently, e.g., at least 2-fold, 5-fold, 10-fold, 50-fold, or 100-fold more efficiently, than the GSI reduces gamma secretase-mediated cleavage of another substrate of gamma secretase, e.g., Cadherins, ErbB, or CD44;

(xii) the GSI specifically binds to Presenilin-1, e.g., the GSI binds to Presenilin-1 with higher affinity, e.g., at least 2-fold, 5-fold, 10-fold, 50-fold, or 100-fold higher affinity, than the GSI binds to another subunit of gamma secretase, e.g., nicastrin, anterior pharynx-defective 1, or presenilin enhancer 2; or

(xiii) the GSI exhibits low gastrointestinal toxicity.

In one aspect, provided herein is a kit comprising a cell (e.g., a population of cells) that expresses a CAR molecule that binds BCMA (a "BCMA CAR-expressing cell") and a GSI.

In one aspect, provided herein is a kit comprising a cell (e.g., a population of cells) that expresses a CAR molecule that binds BCMA (a "BCMA CAR-expressing cell") and a GSI, optionally wherein:

the CAR molecule comprises an anti-BCMA binding domain, a transmembrane domain, and an intracellular signaling domain, and optionally wherein:

the GSI has one or more (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, or all) of the following properties:

(i) the GSI reduces gamma secretase-mediated cleavage of BCMA;

(ii) the GSI, when incubated with BCMA-expressing cells, increases cell surface expression of BCMA, e.g., by at least 2, 4, 6, 8, 10, 15, or 20-fold, e.g., as measured by a method described herein, e.g., a flow cytometry assay, e.g., as measured using methods described in Example 1 with respect to FIG. 1;

(iii) the GSI, when incubated with BCMA-expressing cells, changes conformation and/or posttranslational modification of the extracellular domain of cell surface-expressed BCMA;

(iv) the GSI, when incubated with BCMA-expressing cells, decreases the level of soluble BCMA in the cell supernatant, e.g., by at least 80, 85, 90, 95, 99, or 99.5%, e.g., as measured by a method described herein, e.g., an ELISA assay, e.g., as measured using methods described in Example 1 with respect to Table 28;

(v) the GSI, when administered in vivo, increases cell surface expression of BCMA, e.g., as measured by a method described herein, e.g., a flow cytometry assay;

(vi) the GSI, when administered in vivo, changes conformation and/or posttranslational modification of the extracellular domain of cell surface-expressed BCMA;

(vii) the GSI, when administered in vivo, decreases the level of soluble BCMA in the serum and/or bone marrow, e.g., as measured by a method described herein, e.g., an ELISA assay;

(viii) the GSI is capable of increasing the activity of the BCMA CAR-expressing cell, e.g., increasing the cytotoxicity of the BCMA CAR-expressing cell, e.g., as measured by a method described herein, e.g., as measured using methods described in Example 3 with respect to FIGs. 7B and 7C;

(ix) the GSI is capable of increasing the activity of the BCMA CAR-expressing cell, e.g., increasing the anti-tumor activity of the BCMA CAR-expressing cell, e.g., as measured by a method described herein, e.g., as measured using methods described in Example 3 with respect to FIG. 9D;

(x) the GSI does not reduce gamma secretase-mediated cleavage of Notch, or reduces gamma secretase-mediated cleavage of Notch less efficiently, e.g., at least 2-fold, 5-fold, 10-fold, 50-fold, or 100-fold less efficiently, than the GSI reduces gamma secretase-mediated cleavage of BCMA;

(xi) the GSI reduces gamma secretase-mediated cleavage of BCMA more efficiently, e.g., at least 2-fold, 5-fold, 10-fold, 50-fold, or 100-fold more efficiently, than the GSI reduces gamma secretase-mediated cleavage of another substrate of gamma secretase, e.g., Cadherins, ErbB, or CD44;

(xii) the GSI specifically binds to Presenilin-1, e.g., the GSI binds to Presenilin-1 with higher affinity, e.g., at least 2-fold, 5-fold, 10-fold, 50-fold, or 100-fold higher affinity, than the GSI binds to another subunit of gamma secretase, e.g., nicastrin, anterior pharynx-defective 1, or presenilin enhancer 2; or

(xiii) the GSI exhibits low gastrointestinal toxicity.

The materials, methods, and examples are illustrative only and not intended to be limiting.

Headings, sub-headings or numbered or lettered elements, e.g., (a), (b), (i) etc, are presented merely for ease of reading. The use of headings or numbered or lettered elements in this document does not require the steps or elements be performed in alphabetical order or that the steps or elements are necessarily discrete from one another.

All publications, patent applications, patents, and other references mentioned herein are incorporated by reference in their entirety.

Other features, objects, and advantages of the invention will be apparent from the description and drawings, and from the claims.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 is a set of histogram plots showing the staining of BCMA-expressing human cancer cell lines, NCI-H929, MM1S, and U266B1, using a PE conjugated anti-BCMA antibody. The cell lines were untreated, or treated with 1.0μM DAPT or 0.1μM LY-411,575 before subjected to a flow cytometry analysis.

FIG. 2 is a panel of graphs showing the viability of BCMA-expressing cells (NCI-H929$^{[High]}$, MM1S $^{[Med]}$, U266B1 $^{[low]}$) following 72-hour treatment with various gamma secretase inhibitors.

FIG. 3 is a panel of graphs comparing soluble/shed BCMA (sBCMA) levels to membrane-bound BCMA (mBCMA) levels in MM1S cell cultures treated with various gamma secretase inhibitors for 72 hours.

FIG.4A is a panel of histograms showing that a gamma secretase inhibitor increases the expression of BCMA on the surface of multiple myeloma cell lines. FIG. 4B is a panel of flow cytometry plots showing that a gamma secretase inhibitor increases BCMA expression on the surface of primary multiple myeloma samples.

FIGs. 5A and 5B are a pair of graphs showing that a gamma secretase inhibitor reduces soluble BCMA released by MM cell lines. In FIGs. 5A and 5B, the amount of soluble BCMA (pg/ml) as measured by ELISA is plotted for each GSI concentration tested.

FIGs. 6A and 6B are a pair of graphs showing that a gamma secretase inhibitor has minimal impact on T cell proliferation except at high concentrations. In FIGs. 6A and 6B, cellular volume (fL) and cell number (10^6), respectively, are plotted against culture days for each GSI concentration tested.

FIGs. 7A-7C are a panel of graphs showing a gamma secretase inhibitor treatment enhances in vitro cytotoxicity of CART-BCMA cells towards MM cells. % lysis is plotted over different E:T ratios tested. Target cells were cultured in the presence ("BCMA3NP LY 10μM") or absence ("BCMA3NP") of the gamma secretase inhibitor.

FIG. 8 shows a study design testing the combination of BCMA3NP CAR T cells with a gamma-secretase inhibitor.

FIGs. 9A-9D are a panel of graphs showing that a gamma secretase inhibitor treatment enhances in vivo BCMA CART activity. BLI is plotted over days post tumor injection for each condition tested.

DETAILED DESCRIPTION

Membrane-bound BCMA is cleaved by gamma secretase. Without wishing to be bound by theory, BCMA shedding may create challenges for BCMA-targeting CAR therapies. Some of the challenges include the following. First, BCMA shedding may decrease surface BCMA expression on

tumor cells, reducing target binding sites for BCMA-targeting CAR therapies. Second, BCMA shedding may generate a soluble BCMA sink that binds to BCMA-targeting CAR therapies. Third, soluble BCMA molecules may also sequester circulating BCMA ligands, e.g., B-cell activating factor (BAFF) and a proliferation-inducing ligand (APRIL), and prevent them from stimulating BCMA expressed on the surface of B cells and plasma cells, thereby leading to deficient humoral immune responses in patients.

Accordingly, the present invention provides, at least in part, a method of treating a subject having a disease associated with BCMA expression, comprising administering to the subject an effective amount of a cell (e.g., a population of cells) that expresses a CAR molecule that binds BCMA (a "BCMA CAR-expressing cell") and a gamma secretase inhibitor (GSI). In one embodiment, the disease associated with expression of BCMA is a hematologic cancer, e.g., multiple myeloma. In one embodiment, the BCMA CAR-expressing cell and the GSI are administered simultaneously or sequentially. The present invention also provides a composition or kit comprising a BCMA CAR-expressing cell and a GSI.

As used herein, the term "BCMA" refers to B-cell maturation antigen. BCMA (also known as TNFRSF17, BCM or CD269) is a member of the tumor necrosis receptor (TNFR) family and is predominantly expressed on terminally differentiated B cells, e.g., memory B cells and plasma cells. Its ligands include B-cell activating factor (BAFF) and a proliferation-inducing ligand (APRIL). The protein BCMA is encoded by the gene TNFRSF17. Exemplary BCMA sequences are available at the Uniprot database under accession number Q02223.

As used herein, the term "BAFF" refers to B-cell activating factor, also known as Tumor necrosis factor ligand superfamily member 13B, B lymphocyte stimulator (BLyS), dendritic cell-derived TNF-like molecule, TNF- and APOL-related leukocyte expressed ligand 1 (TALL-1), and CD257. The protein BAFF is encoded by the gene TNFSF13B. Exemplary BAFF sequences are available at the Uniprot database under accession number Q9Y275.

As used herein, the term "APRIL" refers to a proliferation-inducing ligand, also known as Tumor necrosis factor ligand superfamily member 13, TNF- and APOL-related leukocyte expressed ligand 2 (TALL-2), TNF-related death ligand 1 (TRDL-1), and CD256. The protein APRIL is encoded by the gene TNFSF13. Exemplary APRIL sequences are available at the Uniprot database under accession number O75888.

As used herein, the term "gamma secretase" refers to any protein or protein complex that exhibits gamma secretase activities including binding to a substrate having a gamma secretase cleavage sequence, and catalyzing the cleavage of the gamma secretase cleavage sequence, at a gamma secretase cleavage site, to produce substrate cleavage products. In one embodiment, gamma secretase is a protein

complex comprising one or more of the following subunits: presenilin, nicastrin, gamma-secretase subunit APH-1, and gamma-secretase subunit PEN-2.

As used herein, the term "gamma secretase inhibitor" or "GSI" refers to any molecule capable of inhibiting or reducing expression and/or function of gamma secretase. In certain embodiment, the GSI reduces expression and/or function of a subunit of gamma secretase (e.g., presenilin, nicastrin, APH-1, or PEN-2). Any form of a "gamma secretase inhibitor" such as a salt, a co-crystal, a crystalline form, a pro-drug, etc., is included within this term.

Additional terms are defined below and throughout the application.

The term "a" and "an" refers to one or to more than one (i.e., to at least one) of the grammatical object of the article. By way of example, "an element" means one element or more than one element.

The term "about" when referring to a measurable value such as an amount, a temporal duration, and the like, is meant to encompass variations of ±20% or in some instances ±10%, or in some instances ±5%, or in some instances ±1%, or in some instances ±0.1% from the specified value, as such variations are appropriate to perform the disclosed methods.

The term "Chimeric Antigen Receptor" or alternatively a "CAR" refers to a recombinant polypeptide construct comprising at least an extracellular antigen binding domain, a transmembrane domain and a cytoplasmic signaling domain (also referred to herein as "an intracellular signaling domain") comprising a functional signaling domain derived from a stimulatory molecule as defined below. In some embodiments, the domains in the CAR polypeptide construct are in the same polypeptide chain, e.g., comprise a chimeric fusion protein. In some embodiments, the domains in the CAR polypeptide construct are not contiguous with each other, e.g., are in different polypeptide chains, e.g., as provided in an RCAR as described herein.

In one aspect, the stimulatory molecule of the CAR is the zeta chain associated with the T cell receptor complex. In one aspect, the cytoplasmic signaling domain comprises a primary signaling domain (e.g., a primary signaling domain of CD3-zeta). In one aspect, the cytoplasmic signaling domain further comprises one or more functional signaling domains derived from at least one costimulatory molecule as defined below. In one aspect, the costimulatory molecule is chosen from 4 1BB (i.e., CD137), CD27, ICOS, and/or CD28. In one aspect, the CAR comprises a chimeric fusion protein comprising an extracellular antigen recognition domain, a transmembrane domain and an intracellular signaling domain comprising a functional signaling domain derived from a stimulatory molecule. In one aspect, the CAR comprises a chimeric fusion protein comprising an extracellular antigen recognition domain, a transmembrane domain and an intracellular signaling domain comprising a functional signaling domain derived from a co-stimulatory molecule and a functional signaling domain derived from a stimulatory molecule. In one aspect, the CAR comprises a chimeric fusion protein comprising an extracellular antigen recognition domain, a transmembrane domain and an

intracellular signaling domain comprising two functional signaling domains derived from one or more co-stimulatory molecule(s) and a functional signaling domain derived from a stimulatory molecule. In one aspect, the CAR comprises a chimeric fusion protein comprising an extracellular antigen recognition domain, a transmembrane domain and an intracellular signaling domain comprising at least two functional signaling domains derived from one or more co-stimulatory molecule(s) and a functional signaling domain derived from a stimulatory molecule. In one aspect the CAR comprises an optional leader sequence at the amino-terminus (N-ter) of the CAR fusion protein. In one aspect, the CAR further comprises a leader sequence at the N-terminus of the extracellular antigen recognition domain, wherein the leader sequence is optionally cleaved from the antigen recognition domain (e.g., an scFv) during cellular processing and localization of the CAR to the cellular membrane.

A CAR that comprises an antigen binding domain (e.g., an scFv, a single domain antibody, or TCR (e.g., a TCR alpha binding domain or TCR beta binding domain)) that targets a specific tumor marker X, wherein X can be a tumor marker as described herein, is also referred to as XCAR. For example, a CAR that comprises an antigen binding domain that targets BCMA is referred to as BCMACAR. The CAR can be expressed in any cell, e.g., an immune effector cell as described herein (e.g., a T cell or an NK cell).

The term "signaling domain" refers to the functional portion of a protein which acts by transmitting information within the cell to regulate cellular activity via defined signaling pathways by generating second messengers or functioning as effectors by responding to such messengers.

The term "antibody," as used herein, refers to a protein, or polypeptide sequence derived from an immunoglobulin molecule, which specifically binds with an antigen. Antibodies can be polyclonal or monoclonal, multiple or single chain, or intact immunoglobulins, and may be derived from natural sources or from recombinant sources. Antibodies can be tetramers of immunoglobulin molecules.

The term "antibody fragment" refers to at least one portion of an intact antibody, or recombinant variants thereof, and refers to the antigen binding domain, e.g., an antigenic determining variable region of an intact antibody, that is sufficient to confer recognition and specific binding of the antibody fragment to a target, such as an antigen. Examples of antibody fragments include, but are not limited to, Fab, Fab', F(ab')2, and Fv fragments, scFv antibody fragments, linear antibodies, single domain antibodies such as sdAb (either VL or VH), camelid VHH domains, and multi-specific molecules formed from antibody fragments such as a bivalent fragment comprising two or more, e.g., two, Fab fragments linked by a disulfide brudge at the hinge region, or two or more, e.g., two isolated CDR or other epitope binding fragments of an antibody linked. An antibody fragment can also be incorporated into single domain antibodies, maxibodies, minibodies, nanobodies, intrabodies, diabodies, triabodies, tetrabodies, v-NAR and bis-scFv (see, e.g., Hollinger and Hudson, Nature Biotechnology 23:1126-1136, 2005). Antibody fragments can also be grafted into scaffolds based on polypeptides such

as a fibronectin type III (Fn3) (see U.S. Patent No.: 6,703,199, which describes fibronectin polypeptide minibodies).

The term "scFv" refers to a fusion protein comprising at least one antibody fragment comprising a variable region of a light chain and at least one antibody fragment comprising a variable region of a heavy chain, wherein the light and heavy chain variable regions are contiguously linked via a short flexible polypeptide linker, and capable of being expressed as a single chain polypeptide, and wherein the scFv retains the specificity of the intact antibody from which it is derived. Unless specified, as used herein an scFv may have the VL and VH variable regions in either order, e.g., with respect to the N-terminal and C-terminal ends of the polypeptide, the scFv may comprise VL-linker-VH or may comprise VH-linker-VL.

The terms "complementarity determining region" or "CDR," as used herein, refer to the sequences of amino acids within antibody variable regions which confer antigen specificity and binding affinity. For example, in general, there are three CDRs in each heavy chain variable region (e.g., HCDR1, HCDR2, and HCDR3) and three CDRs in each light chain variable region (LCDR1, LCDR2, and LCDR3). The precise amino acid sequence boundaries of a given CDR can be determined using any of a number of well-known schemes, 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), or a combination thereof. Under the Kabat numbering scheme, in some embodiments, the CDR amino acid residues in the heavy chain variable domain (VH) are numbered 31-35 (HCDR1), 50-65 (HCDR2), and 95-102 (HCDR3); and the CDR amino acid residues in the light chain variable domain (VL) are numbered 24-34 (LCDR1), 50-56 (LCDR2), and 89-97 (LCDR3). Under the Chothia numbering scheme, in some embodiments, the CDR amino acids in the VH are numbered 26-32 (HCDR1), 52-56 (HCDR2), and 95-102 (HCDR3); and the CDR amino acid residues in the VL are numbered 26-32 (LCDR1), 50-52 (LCDR2), and 91-96 (LCDR3). In a combined Kabat and Chothia numbering scheme, in some embodiments, the CDRs correspond to the amino acid residues that are part of a Kabat CDR, a Chothia CDR, or both. For instance, in some embodiments, the CDRs correspond to amino acid residues 26-35 (HCDR1), 50-65 (HCDR2), and 95-102 (HCDR3) in a VH, e.g., a mammalian VH, e.g., a human VH; and amino acid residues 24-34 (LCDR1), 50-56 (LCDR2), and 89-97 (LCDR3) in a VL, e.g., a mammalian VL, e.g., a human VL.

The portion of the CAR composition of the invention comprising an antibody or antibody fragment thereof may exist in a variety of forms, for example, where the antigen binding domain is expressed as part of a polypeptide chain including, for example, a single domain antibody fragment (sdAb), a single chain antibody (scFv), or e.g., a humanized antibody (Harlow et al., 1999, In: Using Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory Press, NY; Harlow et al., 1989, In:

Antibodies: A Laboratory Manual, Cold Spring Harbor, New York; Houston et al., 1988, Proc. Natl. Acad. Sci. USA 85:5879-5883; Bird et al., 1988, Science 242:423-426). In one aspect, the antigen binding domain of a CAR composition of the invention comprises an antibody fragment. In a further aspect, the CAR comprises an antibody fragment that comprises an scFv.

As used herein, the term "binding domain" or "antibody molecule" (also referred to herein as "anti-target (e.g., BCMA) binding domain") refers to a protein, e.g., an immunoglobulin chain or fragment thereof, comprising at least one immunoglobulin variable domain sequence. The term "binding domain" or "antibody molecule" encompasses antibodies and antibody fragments. In an embodiment, an antibody molecule is a multispecific antibody molecule, e.g., it comprises a plurality of immunoglobulin variable domain sequences, wherein a first immunoglobulin variable domain sequence of the plurality has binding specificity for a first epitope and a second immunoglobulin variable domain sequence of the plurality has binding specificity for a second epitope. In an embodiment, a multispecific antibody molecule is a bispecific antibody molecule. A bispecific antibody has specificity for no more than two antigens. A bispecific antibody molecule is characterized by a first immunoglobulin variable domain sequence which has binding specificity for a first epitope and a second immunoglobulin variable domain sequence that has binding specificity for a second epitope. The term "antibody heavy chain," refers to the larger of the two types of polypeptide chains present in antibody molecules in their naturally occurring conformations, and which normally determines the class to which the antibody belongs.

The term "antibody light chain," refers to the smaller of the two types of polypeptide chains present in antibody molecules in their naturally occurring conformations. Kappa (κ) and lambda (λ) light chains refer to the two major antibody light chain isotypes.

The term "recombinant antibody" refers to an antibody which is generated using recombinant DNA technology, such as, for example, an antibody expressed by a bacteriophage or yeast expression system. The term should also be construed to mean an antibody which has been generated by the synthesis of a DNA molecule encoding the antibody and which DNA molecule expresses an antibody protein, or an amino acid sequence specifying the antibody, wherein the DNA or amino acid sequence has been obtained using recombinant DNA or amino acid sequence technology which is available and well known in the art.

The term "antigen" or "Ag" refers to a molecule that provokes an immune response. This immune response may involve either antibody production, or the activation of specific immunologically-competent cells, or both. The skilled artisan will understand that any macromolecule, including virtually all proteins or peptides, can serve as an antigen. Furthermore, antigens can be derived from recombinant or genomic DNA. A skilled artisan will understand that any DNA, which comprises a nucleotide sequences or a partial nucleotide sequence encoding a protein that elicits an

immune response therefore encodes an "antigen" as that term is used herein. Furthermore, one skilled in the art will understand that an antigen need not be encoded solely by a full length nucleotide sequence of a gene. It is readily apparent that the present invention includes, but is not limited to, the use of partial nucleotide sequences of more than one gene and that these nucleotide sequences are arranged in various combinations to encode polypeptides that elicit the desired immune response. Moreover, a skilled artisan will understand that an antigen need not be encoded by a "gene" at all. It is readily apparent that an antigen can be generated synthesized or can be derived from a biological sample, or might be macromolecule besides a polypeptide. Such a biological sample can include, but is not limited to a tissue sample, a tumor sample, a cell or a fluid with other biological components.

The term "anti-tumor effect" refers to a biological effect which can be manifested by various means, including but not limited to, e.g., a decrease in tumor volume, a decrease in the number of tumor cells, a decrease in the number of metastases, an increase in life expectancy, decrease in tumor cell proliferation, decrease in tumor cell survival, or amelioration of various physiological symptoms associated with the cancerous condition. An "anti-tumor effect" can also be manifested by the ability of the peptides, polynucleotides, cells and antibodies of the invention in prevention of the occurrence of tumor in the first place.

The term "anti-cancer effect" refers to a biological effect which can be manifested by various means, including but not limited to, e.g., a decrease in tumor volume, a decrease in the number of cancer cells, a decrease in the number of metastases, an increase in life expectancy, decrease in cancer cell proliferation, decrease in cancer cell survival, or amelioration of various physiological symptoms associated with the cancerous condition. An "anti-cancer effect" can also be manifested by the ability of the peptides, polynucleotides, cells and antibodies in prevention of the occurrence of cancer in the first place. The term "anti-tumor effect" refers to a biological effect which can be manifested by various means, including but not limited to, e.g., a decrease in tumor volume, a decrease in the number of tumor cells, a decrease in tumor cell proliferation, or a decrease in tumor cell survival. The term "autologous" refers to any material derived from the same individual to whom it is later to be re-introduced into the individual.

The term "allogeneic" refers to any material derived from a different animal of the same species as the individual to whom the material is introduced. Two or more individuals are said to be allogeneic to one another when the genes at one or more loci are not identical. In some aspects, allogeneic material from individuals of the same species may be sufficiently unlike genetically to interact antigenically.

The term "xenogeneic" refers to a graft derived from an animal of a different species.

The term "apheresis" as used herein refers to the art-recognized extracorporeal process by which the blood of a donor or patient is removed from the donor or patient and passed through an apparatus that separates out selected particular constituent(s) and returns the remainder to the circulation

of the donor or patient, e.g., by retransfusion. Thus, in the context of "an apheresis sample" refers to a sample obtained using apheresis.

The term "combination" refers to either a fixed combination in one dosage unit form, or a combined administration where a compound of the present invention and a combination partner (e.g. another drug as explained below, also referred to as "therapeutic agent" or "co-agent") may be administered independently at the same time or separately within time intervals, especially where these time intervals allow that the combination partners show a cooperative, e.g. synergistic effect. The single components may be packaged in a kit or separately. One or both of the components (e.g., powders or liquids) may be reconstituted or diluted to a desired dose prior to administration. The terms "co-administration" or "combined administration" or the like as utilized herein are meant to encompass administration of the selected combination partner to a single subject in need thereof (e.g. a patient), and are intended to include treatment regimens in which the agents are not necessarily administered by the same route of administration or at the same time. The term "pharmaceutical combination" as used herein means a product that results from the mixing or combining of more than one active ingredient and includes both fixed and non-fixed combinations of the active ingredients. The term "fixed combination" means that the active ingredients, e.g. a compound of the present invention and a combination partner, are both administered to a patient simultaneously in the form of a single entity or dosage. The term "non-fixed combination" means that the active ingredients, e.g. a compound of the present invention and a combination partner, are both administered to a patient as separate entities either simultaneously, concurrently or sequentially with no specific time limits, wherein such administration provides therapeutically effective levels of the two compounds in the body of the patient. The latter also applies to cocktail therapy, e.g. the administration of three or more active ingredients.

The term "cancer" refers to a disease characterized by the rapid and uncontrolled growth of aberrant cells. Cancer cells can spread locally or through the bloodstream and lymphatic system to other parts of the body. Examples of various cancers are described herein and include but are not limited to, breast cancer, prostate cancer, ovarian cancer, cervical cancer, skin cancer, pancreatic cancer, colorectal cancer, renal cancer, liver cancer, brain cancer, lymphoma, leukemia, lung cancer and the like. Preferred cancers treated by the methods described herein include multiple myeloma, Hodgkin's lymphoma or non-Hodgkin's lymphoma.

The terms "tumor" and "cancer" are used interchangeably herein, e.g., both terms encompass solid and liquid, e.g., diffuse or circulating, tumors. As used herein, the term "cancer" or "tumor" includes premalignant, as well as malignant cancers and tumors.

"Derived from" as that term is used herein, indicates a relationship between a first and a second molecule. It generally refers to structural similarity between the first molecule and a second molecule and does not connotate or include a process or source limitation on a first molecule that is derived from

a second molecule. For example, in the case of an intracellular signaling domain that is derived from a CD3zeta molecule, the intracellular signaling domain retains sufficient CD3zeta structure such that is has the required function, namely, the ability to generate a signal under the appropriate conditions. It does not connotate or include a limitation to a particular process of producing the intracellular signaling domain, e.g., it does not mean that, to provide the intracellular signaling domain, one must start with a CD3zeta sequence and delete unwanted sequence, or impose mutations, to arrive at the intracellular signaling domain.

The phrase "disease associated with expression of BCMA" includes, but is not limited to, a disease associated with a cell which expresses BCMA (e.g., wild-type or mutant BCMA) or condition associated with a cell which expresses BCMA (e.g., wild-type or mutant BCMA) including, e.g., proliferative diseases such as a cancer or malignancy or a precancerous condition such as a myelodysplasia, a myelodysplastic syndrome or a preleukemia; or a noncancer related indication associated with a cell which expresses BCMA (e.g., wild-type or mutant BCMA). For the avoidance of doubt, a disease associated with expression of BCMA may include a condition associated with a cell which does not presently express BCMA, e.g., because BCMA expression has been downregulated, e.g., due to treatment with a molecule targeting BCMA, e.g., a BCMA inhibitor described herein, but which at one time expressed BCMA. In one aspect, a cancer associated with expression of BCMA (e.g., wild-type or mutant BCMA) is a hematological cancer. In one aspect, the hematological cancer is a leukemia or a lymphoma. In one aspect, a cancer associated with expression of BCMA (e.g., wild-type or mutant BCMA) is a malignancy of differentiated plasma B cells. In one aspect, a cancer associated with expression of BCMA(e.g., wild-type or mutant BCMA) includes cancers and malignancies including, but not limited to, e.g., one or more acute leukemias including but not limited to, e.g., B-cell acute Lymphoid Leukemia ("BALL"), T-cell acute Lymphoid Leukemia ("TALL"), acute lymphoid leukemia (ALL); one or more chronic leukemias including but not limited to, e.g., chronic myelogenous leukemia (CML), Chronic Lymphoid Leukemia (CLL). Additional cancers or hematologic conditions associated with expression of BMCA (e.g., wild-type or mutant BCMA) comprise, but are not limited to, e.g., B cell prolymphocytic leukemia, blastic plasmacytoid dendritic cell neoplasm, Burkitt's lymphoma, diffuse large B cell lymphoma, Follicular lymphoma, Hairy cell leukemia, small cell- or a large cell-follicular lymphoma, malignant lymphoproliferative conditions, MALT lymphoma, mantle cell lymphoma, Marginal zone lymphoma, multiple myeloma, myelodysplasia and myelodysplastic syndrome, non-Hodgkin's lymphoma, plasmablastic lymphoma, plasmacytoid dendritic cell neoplasm, Waldenstrom macroglobulinemia, and "preleukemia" which are a diverse collection of hematological conditions united by ineffective production (or dysplasia) of myeloid blood cells, and the like. In some embodiments, the cancer is multiple myeloma, Hodgkin's lymphoma, non-Hodgkin's lymphoma, or glioblastoma. In embodiments, a disease associated with expression of BCMA includes a plasma cell

proliferative disorder, e.g., asymptomatic myeloma (smoldering multiple myeloma or indolent myeloma), monoclonal gammapathy of undetermined significance (MGUS), Waldenstrom's macroglobulinemia, plasmacytomas (e.g., plasma cell dyscrasia, solitary myeloma, solitary plasmacytoma, extramedullary plasmacytoma, and multiple plasmacytoma), systemic amyloid light chain amyloidosis, and POEMS syndrome (also known as Crow-Fukase syndrome, Takatsuki disease, and PEP syndrome). Further diseases associated with expression of BCMA (e.g., wild-type or mutant BCMA) expression include, but not limited to, e.g., atypical and/or non-classical cancers, malignancies, precancerous conditions or proliferative diseases associated with expression of BCMA (e.g., wild-type or mutant BCMA), e.g., a cancer described herein, e.g., a prostate cancer (e.g., castrate-resistant or therapy-resistant prostate cancer, or metastatic prostate cancer), pancreatic cancer, or lung cancer.

Non-cancer related conditions that are associated with BCMA (e.g., wild-type or mutant BCMA) include viral infections; e.g., HIV, fungal infections, e.g., C. neoformans; autoimmune disease; e.g. rheumatoid arthritis, system lupus erythematosus (SLE or lupus), pemphigus vulgaris, and Sjogren's syndrome; inflammatory bowel disease, ulcerative colitis; transplant-related allospecific immunity disorders related to mucosal immunity; and unwanted immune responses towards biologics (e.g., Factor VIII) where humoral immunity is important. In embodiments, a non-cancer related indication associated with expression of BCMA includes but is not limited to, e.g., autoimmune disease, (e.g., lupus), inflammatory disorders (allergy and asthma) and transplantation. In some embodiments, the tumor antigen-expressing cell expresses, or at any time expressed, mRNA encoding the tumor antigen. In an embodiment, the tumor antigen -expressing cell produces the tumor antigen protein (e.g., wild-type or mutant), and the tumor antigen protein may be present at normal levels or reduced levels. In an embodiment, the tumor antigen -expressing cell produced detectable levels of a tumor antigen protein at one point, and subsequently produced substantially no detectable tumor antigen protein.

The term "conservative sequence modifications" refers to amino acid modifications that do not significantly affect or alter the binding characteristics of the antibody or antibody fragment containing the amino acid sequence. Such conservative modifications include amino acid substitutions, additions and deletions. Modifications can be introduced into an antibody or antibody fragment of the invention by standard techniques known in the art, such as site-directed mutagenesis and PCR-mediated mutagenesis. Conservative substitutions are ones in which the amino acid residue is replaced with an amino acid residue having a similar side chain. Families of amino acid residues having similar side chains have been defined in the art. These families include amino acids with basic side chains (e.g., lysine, arginine, histidine), acidic side chains (e.g., aspartic acid, glutamic acid), uncharged polar side chains (e.g., glycine, asparagine, glutamine, serine, threonine, tyrosine, cysteine, tryptophan), nonpolar side chains (e.g., alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine), beta-branched side chains (e.g., threonine, valine, isoleucine) and aromatic side chains (e.g., tyrosine, phenylalanine,

tryptophan, histidine). Thus, one or more amino acid residues within a CAR of the invention can be replaced with other amino acid residues from the same side chain family and the altered CAR can be tested using the functional assays described herein.

The term "stimulation," refers to a primary response induced by binding of a stimulatory molecule (e.g., a TCR/CD3 complex) with its cognate ligand thereby mediating a signal transduction event, such as, but not limited to, signal transduction via the TCR/CD3 complex. Stimulation can mediate altered expression of certain molecules, such as downregulation of TGF-β, and/or reorganization of cytoskeletal structures, and the like.

The term "stimulatory molecule," refers to a molecule expressed by a T cell that provides the primary cytoplasmic signaling sequence(s) that regulate primary activation of the TCR complex in a stimulatory way for at least some aspect of the T cell signaling pathway. In some embodiments, the ITAM-containing domain within the CAR recapitulates the signaling of the primary TCR independently of endogenous TCR complexes. In one aspect, the primary signal is initiated by, for instance, binding of a TCR/CD3 complex with an MHC molecule loaded with peptide, and which leads to mediation of a T cell response, including, but not limited to, proliferation, activation, differentiation, and the like. A primary cytoplasmic signaling sequence (also referred to as a "primary signaling domain") that acts in a stimulatory manner may contain a signaling motif which is known as immunoreceptor tyrosine-based activation motif or ITAM. Examples of an ITAM containing primary cytoplasmic signaling sequence that is of particular use in the invention includes, but is not limited to, those derived from TCR zeta, FcR gamma, FcR beta, CD3 gamma, CD3 delta , CD3 epsilon, CD5, CD22, CD79a, CD79b, CD278 (also known as "ICOS") , FcεRI and CD66d, DAP10 and DAP12. In a specific CAR of the invention, the intracellular signaling domain in any one or more CARS of the invention comprises an intracellular signaling sequence, e.g., a primary signaling sequence of CD3-zeta. In a specific CAR of the invention, the primary signaling sequence of CD3-zeta is the sequence provided as SEQ ID NO:9, or the equivalent residues from a non-human species, e.g., mouse, rodent, monkey, ape and the like. In a specific CAR of the invention, the primary signaling sequence of CD3-zeta is the sequence as provided in SEQ ID NO:10, or the equivalent residues from a non-human species, e.g., mouse, rodent, monkey, ape and the like. The term "antigen presenting cell" or "APC" refers to an immune system cell such as an accessory cell (e.g., a B-cell, a dendritic cell, and the like) that displays a foreign antigen complexed with major histocompatibility complexes (MHC's) on its surface. T-cells may recognize these complexes using their T-cell receptors (TCRs). APCs process antigens and present them to T-cells.

An "intracellular signaling domain," as the term is used herein, refers to an intracellular portion of a molecule. In embodiments, the intracellular signal domain transduces the effector function signal and directs the cell to perform a specialized function. While the entire intracellular signaling domain can be employed, in many cases it is not necessary to use the entire chain. To the extent that a truncated

portion of the intracellular signaling domain is used, such truncated portion may be used in place of the intact chain as long as it transduces the effector function signal. The term intracellular signaling domain is thus meant to include any truncated portion of the intracellular signaling domain sufficient to transduce the effector function signal.

The intracellular signaling domain generates a signal that promotes an immune effector function of the CAR containing cell, e.g., a CART cell. Examples of immune effector function, e.g., in a CART cell, include cytolytic activity and helper activity, including the secretion of cytokines.

In an embodiment, the intracellular signaling domain can comprise a primary intracellular signaling domain. Exemplary primary intracellular signaling domains include those derived from the molecules responsible for primary stimulation, or antigen dependent simulation. In an embodiment, the intracellular signaling domain can comprise a costimulatory intracellular domain. Exemplary costimulatory intracellular signaling domains include those derived from molecules responsible for costimulatory signals, or antigen independent stimulation. For example, in the case of a CART, a primary intracellular signaling domain can comprise a cytoplasmic sequence of a T cell receptor, and a costimulatory intracellular signaling domain can comprise cytoplasmic sequence from co-receptor or costimulatory molecule.

A primary intracellular signaling domain can comprise a signaling motif which is known as an immunoreceptor tyrosine-based activation motif or ITAM. Examples of ITAM containing primary cytoplasmic signaling sequences include, but are not limited to, those derived from CD3 zeta, FcR gamma, FcR beta, CD3 gamma, CD3 delta, CD3 epsilon, CD5, CD22, CD79a, CD79b, CD278 (also known as "ICOS"), FcεRI, CD66d, DAP10 and DAP12.

The term "zeta" or alternatively "zeta chain", "CD3-zeta" or "TCR-zeta" is defined as the protein provided as GenBan Acc. No. BAG36664.1, or the equivalent residues from a non-human species, e.g., mouse, rodent, monkey, ape and the like, and a "zeta stimulatory domain" or alternatively a "CD3-zeta stimulatory domain" or a "TCR-zeta stimulatory domain" is defined as the amino acid residues from the cytoplasmic domain of the zeta chain that are sufficient to functionally transmit an initial signal necessary for T cell activation. In one aspect the cytoplasmic domain of zeta comprises residues 52 through 164 of GenBank Acc. No. BAG36664.1 or the equivalent residues from a non-human species, e.g., mouse, rodent, monkey, ape and the like, that are functional orthologs thereof. In one aspect, the "zeta stimulatory domain" or a "CD3-zeta stimulatory domain" is the sequence provided as SEQ ID NO:9. In one aspect, the "zeta stimulatory domain" or a "CD3-zeta stimulatory domain" is the sequence provided as SEQ ID NO:10.

The term "costimulatory molecule" refers to the cognate binding partner on a T cell that specifically binds with a costimulatory ligand, thereby mediating a costimulatory response by the T cell, such as, but not limited to, proliferation. Costimulatory molecules are cell surface molecules other than

antigen receptors or their ligands that are required for an efficient immune response. Costimulatory molecules include, but are not limited to an MHC class I molecule, TNF receptor proteins, Immunoglobulin-like proteins, cytokine receptors, integrins, signaling lymphocytic activation molecules (SLAM proteins), activating NK cell receptors, BTLA, Toll ligand receptor, OX40, CD2, CD7, CD27, CD28, CD30, CD40, CDS, ICAM-1, LFA-1 (CD11a/CD18), 4-1BB (CD137), B7-H3, CDS, ICAM-1, ICOS (CD278), GITR, BAFFR, LIGHT, HVEM (LIGHTR), KIRDS2, SLAMF7, NKp80 (KLRF1), NKp44, NKp30, NKp46, CD19, CD4, CD8alpha, CD8beta, IL2R beta, IL2R gamma, IL7R alpha, ITGA4, VLA1, CD49a, ITGA4, IA4, CD49D, ITGA6, VLA-6, CD49f, ITGAD, CD11d, ITGAE, CD103, ITGAL, CD11a, LFA-1, ITGAM, CD11b, ITGAX, CD11c, ITGB1, CD29, ITGB2, CD18, LFA-1, ITGB7, NKG2D, NKG2C, TNFR2, TRANCE/RANKL, DNAM1 (CD226), SLAMF4 (CD244, 2B4), CD84, CD96 (Tactile), CEACAM1, CRTAM, Ly9 (CD229), CD160 (BY55), PSGL1, CD100 (SEMA4D), CD69, SLAMF6 (NTB-A, Ly108), SLAM (SLAMF1, CD150, IPO-3), BLAME (SLAMF8), SELPLG (CD162), LTBR, LAT, GADS, SLP-76, PAG/Cbp, CD19a, and a ligand that specifically binds with CD83.

A costimulatory intracellular signaling domain refers to the intracellular portion of a costimulatory molecule.

The intracellular signaling domain can comprise the entire intracellular portion, or the entire native intracellular signaling domain, of the molecule from which it is derived, or a functional fragment thereof.

The term "4-1BB" refers to a member of the TNFR superfamily with an amino acid sequence provided as GenBank Acc. No. AAA62478.2, or the equivalent residues from a non-human species, e.g., mouse, rodent, monkey, ape and the like; and a "4-1BB costimulatory domain" is defined as amino acid residues 214-255 of GenBank Acc. No. AAA62478.2, or the equivalent residues from a non-human species, e.g., mouse, rodent, monkey, ape and the like. In one aspect, the "4-1BB costimulatory domain" is the sequence provided as SEQ ID NO:7 or the equivalent residues from a non-human species, e.g., mouse, rodent, monkey, ape and the like.

"Immune effector cell," as that term is used herein, refers to a cell that is involved in an immune response, e.g., in the promotion of an immune effector response. Examples of immune effector cells include T cells, e.g., alpha/beta T cells and gamma/delta T cells, B cells, natural killer (NK) cells, natural killer T (NKT) cells, mast cells, and myeloic-derived phagocytes.

"Immune effector function or immune effector response," as that term is used herein, refers to function or response, e.g., of an immune effector cell, that enhances or promotes an immune attack of a target cell. E.g., an immune effector function or response refers a property of a T or NK cell that promotes killing or the inhibition of growth or proliferation, of a target cell. In the case of a T cell, primary stimulation and co-stimulation are examples of immune effector function or response.

The term "effector function" refers to a specialized function of a cell. Effector function of a T cell, for example, may be cytolytic activity or helper activity including the secretion of cytokines.

The term "encoding" refers to the inherent property of specific sequences of nucleotides in a polynucleotide, such as a gene, a cDNA, or an mRNA, to serve as templates for synthesis of other polymers and macromolecules in biological processes having either a defined sequence of nucleotides (e.g., rRNA, tRNA and mRNA) or a defined sequence of amino acids and the biological properties resulting therefrom. Thus, a gene, cDNA, or RNA, encodes a protein if transcription and translation of mRNA corresponding to that gene produces the protein in a cell or other biological system. Both the coding strand, the nucleotide sequence of which is identical to the mRNA sequence and is usually provided in sequence listings, and the non-coding strand, used as the template for transcription of a gene or cDNA, can be referred to as encoding the protein or other product of that gene or cDNA.

Unless otherwise specified, a "nucleotide sequence encoding an amino acid sequence" includes all nucleotide sequences that are degenerate versions of each other and that encode the same amino acid sequence. The phrase nucleotide sequence that encodes a protein or a RNA may also include introns to the extent that the nucleotide sequence encoding the protein may in some version contain an intron(s).

The term "effective amount" or "therapeutically effective amount" are used interchangeably herein, and refer to an amount of a compound, formulation, material, or composition, as described herein effective to achieve a particular biological result.

The term "endogenous" refers to any material from or produced inside an organism, cell, tissue or system.

The term "exogenous" refers to any material introduced from or produced outside an organism, cell, tissue or system.

The term "expression" refers to the transcription and/or translation of a particular nucleotide sequence driven by a promoter.

The term "transfer vector" refers to a composition of matter which comprises an isolated nucleic acid and which can be used to deliver the isolated nucleic acid to the interior of a cell. Numerous vectors are known in the art including, but not limited to, linear polynucleotides, polynucleotides associated with ionic or amphiphilic compounds, plasmids, and viruses. Thus, the term "transfer vector" includes an autonomously replicating plasmid or a virus. The term should also be construed to further include non-plasmid and non-viral compounds which facilitate transfer of nucleic acid into cells, such as, for example, a polylysine compound, liposome, and the like. Examples of viral transfer vectors include, but are not limited to, adenoviral vectors, adeno-associated virus vectors, retroviral vectors, lentiviral vectors, and the like.

The term "expression vector" refers to a vector comprising a recombinant polynucleotide comprising expression control sequences operatively linked to a nucleotide sequence to be expressed.

An expression vector comprises sufficient cis-acting elements for expression; other elements for expression can be supplied by the host cell or in an in vitro expression system. Expression vectors include all those known in the art, including cosmids, plasmids (e.g., naked or contained in liposomes) and viruses (e.g., lentiviruses, retroviruses, adenoviruses, and adeno-associated viruses) that incorporate the recombinant polynucleotide.

The term "lentivirus" refers to a genus of the Retroviridae family. Lentiviruses are unique among the retroviruses in being able to infect non-dividing cells; they can deliver a significant amount of genetic information into the DNA of the host cell, so they are one of the most efficient methods of a gene delivery vector. HIV, SIV, and FIV are all examples of lentiviruses.

The term "lentiviral vector" refers to a vector derived from at least a portion of a lentivirus genome, including especially a self-inactivating lentiviral vector as provided in Milone et al., Mol. Ther. 17(8): 1453–1464 (2009). Other examples of lentivirus vectors that may be used in the clinic, include but are not limited to, e.g., the LENTIVECTOR® gene delivery technology from Oxford BioMedica, the LENTIMAX™ vector system from Lentigen and the like. Nonclinical types of lentiviral vectors are also available and would be known to one skilled in the art.

The term "homologous" or "identity" refers to the subunit sequence identity between two polymeric molecules, e.g., between two nucleic acid molecules, such as, two DNA molecules or two RNA molecules, or between two polypeptide molecules. When a subunit position in both of the two molecules is occupied by the same monomeric subunit; e.g., if a position in each of two DNA molecules is occupied by adenine, then they are homologous or identical at that position. The homology between two sequences is a direct function of the number of matching or homologous positions; e.g., if half (e.g., five positions in a polymer ten subunits in length) of the positions in two sequences are homologous, the two sequences are 50% homologous; if 90% of the positions (e.g., 9 of 10), are matched or homologous, the two sequences are 90% homologous.

"Humanized" forms of non-human (e.g., murine) antibodies are chimeric immunoglobulins, immunoglobulin chains or fragments thereof (such as Fv, Fab, Fab', F(ab')2 or other antigen-binding subsequences of antibodies) which contain minimal sequence derived from non-human immunoglobulin. For the most part, humanized antibodies and antibody fragments thereof are human immunoglobulins (recipient antibody or antibody fragment) in which residues from a complementary-determining region (CDR) of the recipient are replaced by residues from a CDR of a non-human species (donor antibody) such as mouse, rat or rabbit having the desired specificity, affinity, and capacity. In some instances, Fv framework region (FR) residues of the human immunoglobulin are replaced by corresponding non-human residues. Furthermore, a humanized antibody/antibody fragment can comprise residues which are found neither in the recipient antibody nor in the imported CDR or framework sequences. These modifications can further refine and optimize antibody or antibody

fragment performance. In general, the humanized antibody or antibody fragment thereof will comprise substantially all of at least one, and typically two, variable domains, in which all or substantially all of the CDR regions correspond to those of a non-human immunoglobulin and all or a significant portion of the FR regions are those of a human immunoglobulin sequence. The humanized antibody or antibody fragment can also comprise at least a portion of an immunoglobulin constant region (Fc), typically that of a human immunoglobulin. For further details, see Jones et al., Nature, 321: 522-525, 1986; Reichmann et al., Nature, 332: 323-329, 1988; Presta, Curr. Op. Struct. Biol., 2: 593-596, 1992.

"Fully human" refers to an immunoglobulin, such as an antibody or antibody fragment, where the whole molecule is of human origin or consists of an amino acid sequence identical to a human form of the antibody or immunoglobulin.

The term "isolated" means altered or removed from the natural state. For example, a nucleic acid or a peptide naturally present in a living animal is not "isolated," but the same nucleic acid or peptide partially or completely separated from the coexisting materials of its natural state is "isolated." An isolated nucleic acid or protein can exist in substantially purified form, or can exist in a non-native environment such as, for example, a host cell.

In the context of the present invention, the following abbreviations for the commonly occurring nucleic acid bases are used. "A" refers to adenosine, "C" refers to cytosine, "G" refers to guanosine, "T" refers to thymidine, and "U" refers to uridine.

The term "operably linked" or "transcriptional control" refers to functional linkage between a regulatory sequence and a heterologous nucleic acid sequence resulting in expression of the latter. For example, a first nucleic acid sequence is operably linked with a second nucleic acid sequence when the first nucleic acid sequence is placed in a functional relationship with the second nucleic acid sequence. For instance, a promoter is operably linked to a coding sequence if the promoter affects the transcription or expression of the coding sequence. Operably linked DNA sequences can be contiguous with each other and, e.g., where necessary to join two protein coding regions, are in the same reading frame.

The term "parenteral" administration of an immunogenic composition includes, e.g., subcutaneous (s.c.), intravenous (i.v.), intramuscular (i.m.), or intrasternal injection, intratumoral, or infusion techniques.

The term "nucleic acid" or "polynucleotide" refers to deoxyribonucleic acids (DNA) or ribonucleic acids (RNA) and polymers thereof in either single- or double-stranded form. Unless specifically limited, the term encompasses nucleic acids containing known analogues of natural nucleotides that have similar binding properties as the reference nucleic acid and are metabolized in a manner similar to naturally occurring nucleotides. Unless otherwise indicated, a particular nucleic acid sequence also implicitly encompasses conservatively modified variants thereof (e.g., degenerate codon substitutions, e.g., conservative substitutions), alleles, orthologs, SNPs, and complementary sequences

as well as the sequence explicitly indicated. Specifically, degenerate codon substitutions, e.g., conservative substitutions may be achieved by generating sequences in which the third position of one or more selected (or all) codons is substituted with mixed-base and/or deoxyinosine residues (Batzer et al., Nucleic Acid Res. 19:5081 (1991); Ohtsuka et al., J. Biol. Chem. 260:2605-2608 (1985); and Rossolini et al., Mol. Cell. Probes 8:91-98 (1994)).

The terms "peptide," "polypeptide," and "protein" are used interchangeably, and refer to a compound comprised of amino acid residues covalently linked by peptide bonds. A protein or peptide must contain at least two amino acids, and no limitation is placed on the maximum number of amino acids that can comprise a protein's or peptide's sequence. Polypeptides include any peptide or protein comprising two or more amino acids joined to each other by peptide bonds. As used herein, the term refers to both short chains, which also commonly are referred to in the art as peptides, oligopeptides and oligomers, for example, and to longer chains, which generally are referred to in the art as proteins, of which there are many types. "Polypeptides" include, for example, biologically active fragments, substantially homologous polypeptides, oligopeptides, homodimers, heterodimers, variants of polypeptides, modified polypeptides, derivatives, analogs, fusion proteins, among others. A polypeptide includes a natural peptide, a recombinant peptide, or a combination thereof.

The term "promoter" refers to a DNA sequence recognized by the synthetic machinery of the cell, or introduced synthetic machinery, required to initiate the specific transcription of a polynucleotide sequence.

The term "promoter/regulatory sequence" refers to a nucleic acid sequence which is required for expression of a gene product operably linked to the promoter/regulatory sequence. In some instances, this sequence may be the core promoter sequence and in other instances, this sequence may also include an enhancer sequence and other regulatory elements which are required for expression of the gene product. The promoter/regulatory sequence may, for example, be one which expresses the gene product in a tissue specific manner.

The term "constitutive" promoter refers to a nucleotide sequence which, when operably linked with a polynucleotide which encodes or specifies a gene product, causes the gene product to be produced in a cell under most or all physiological conditions of the cell.

The term "inducible" promoter refers to a nucleotide sequence which, when operably linked with a polynucleotide which encodes or specifies a gene product, causes the gene product to be produced in a cell substantially only when an inducer which corresponds to the promoter is present in the cell.

The term "tissue-specific" promoter refers to a nucleotide sequence which, when operably linked with a polynucleotide encodes or specified by a gene, causes the gene product to be produced in a cell substantially only if the cell is a cell of the tissue type corresponding to the promoter.

The terms "cancer associated antigen" or "tumor antigen" interchangeably refers to a molecule (typically a protein, carbohydrate or lipid) that is expressed on the surface of a cancer cell, either entirely or as a fragment (e.g., MHC/peptide), and which is useful for the preferential targeting of a pharmacological agent to the cancer cell. In some embodiments, a tumor antigen is a marker expressed by both normal cells and cancer cells, e.g., a lineage marker, e.g., CD19 on B cells. In some embodiments, a tumor antigen is a cell surface molecule that is overexpressed in a cancer cell in comparison to a normal cell, for instance, 1-fold over expression, 2-fold overexpression, 3-fold overexpression or more in comparison to a normal cell. In some embodiments, a tumor antigen is a cell surface molecule that is inappropriately synthesized in the cancer cell, for instance, a molecule that contains deletions, additions or mutations in comparison to the molecule expressed on a normal cell. In some embodiments, a tumor antigen will be expressed exclusively on the cell surface of a cancer cell, entirely or as a fragment (e.g., MHC/peptide), and not synthesized or expressed on the surface of a normal cell. In some embodiments, the CARs of the present invention includes CARs comprising an antigen binding domain (e.g., antibody or antibody fragment) that binds to a MHC presented peptide. Normally, peptides derived from endogenous proteins fill the pockets of Major histocompatibility complex (MHC) class I molecules, and are recognized by T cell receptors (TCRs) on CD8 + T lymphocytes. The MHC class I complexes are constitutively expressed by all nucleated cells. In cancer, virus-specific and/or tumor-specific peptide/MHC complexes represent a unique class of cell surface targets for immunotherapy. TCR-like antibodies targeting peptides derived from viral or tumor antigens in the context of human leukocyte antigen (HLA)-A1 or HLA-A2 have been described (see, e.g., Sastry et al., J Virol. 2011 85(5):1935-1942; Sergeeva et al., Blood, 2011 117(16):4262-4272; Verma et al., J Immunol 2010 184(4):2156-2165; Willemsen et al., Gene Ther 2001 8(21) :1601-1608 ; Dao et al., Sci Transl Med 2013 5(176) :176ra33 ; Tassev et al., Cancer Gene Ther 2012 19(2):84-100). For example, TCR-like antibody can be identified from screening a library, such as a human scFv phage displayed library.

The term "tumor-supporting antigen" or "cancer-supporting antigen" interchangeably refer to a molecule (typically a protein, carbohydrate or lipid) that is expressed on the surface of a cell that is, itself, not cancerous, but supports the cancer cells, e.g., by promoting their growth or survival e.g., resistance to immune cells. Exemplary cells of this type include stromal cells and myeloid-derived suppressor cells (MDSCs). The tumor-supporting antigen itself need not play a role in supporting the tumor cells so long as the antigen is present on a cell that supports cancer cells.

The term "flexible polypeptide linker" or "linker" as used in the context of an scFv refers to a peptide linker that consists of amino acids such as glycine and/or serine residues used alone or in combination, to link variable heavy and variable light chain regions together. In one embodiment, the flexible polypeptide linker is a Gly/Ser linker and comprises the amino acid sequence (Gly-Gly-Gly-

Ser)n (SEQ ID NO: 38)., where n is a positive integer equal to or greater than 1. For example, n=1, n=2, n=3. n=4, n=5 and n=6, n=7, n=8, n=9 and n=10 In one embodiment, the flexible polypeptide linkers include, but are not limited to, (Gly4 Ser)4 (SEQ ID NO:27) or (Gly4 Ser)3 (SEQ ID NO:28). In another embodiment, the linkers include multiple repeats of (Gly2Ser), (GlySer) or (Gly3Ser) (SEQ ID NO:29). Also included within the scope of the invention are linkers described in WO2012/138475, incorporated herein by reference).

As used herein, a 5' cap (also termed an RNA cap, an RNA 7-methylguanosine cap or an RNA m7G cap) is a modified guanine nucleotide that has been added to the "front" or 5' end of a eukaryotic messenger RNA shortly after the start of transcription. The 5' cap consists of a terminal group which is linked to the first transcribed nucleotide. Its presence is critical for recognition by the ribosome and protection from RNases. Cap addition is coupled to transcription, and occurs co-transcriptionally, such that each influences the other. Shortly after the start of transcription, the 5' end of the mRNA being synthesized is bound by a cap-synthesizing complex associated with RNA polymerase. This enzymatic complex catalyzes the chemical reactions that are required for mRNA capping. Synthesis proceeds as a multi-step biochemical reaction. The capping moiety can be modified to modulate functionality of mRNA such as its stability or efficiency of translation.

As used herein, "in vitro transcribed RNA" refers to RNA, preferably mRNA, that has been synthesized in vitro. Generally, the in vitro transcribed RNA is generated from an in vitro transcription vector. The in vitro transcription vector comprises a template that is used to generate the in vitro transcribed RNA.

As used herein, a "poly(A)" is a series of adenosines attached by polyadenylation to the mRNA. In the preferred embodiment of a construct for transient expression, the polyA is between 50 and 5000 (SEQ ID NO: 30), preferably greater than 64, more preferably greater than 100, most preferably greater than 300 or 400. poly(A) sequences can be modified chemically or enzymatically to modulate mRNA functionality such as localization, stability or efficiency of translation.

As used herein, "polyadenylation" refers to the covalent linkage of a polyadenylyl moiety, or its modified variant, to a messenger RNA molecule. In eukaryotic organisms, most messenger RNA (mRNA) molecules are polyadenylated at the 3' end. The 3' poly(A) tail is a long sequence of adenine nucleotides (often several hundred) added to the pre-mRNA through the action of an enzyme, polyadenylate polymerase. In higher eukaryotes, the poly(A) tail is added onto transcripts that contain a specific sequence, the polyadenylation signal. The poly(A) tail and the protein bound to it aid in protecting mRNA from degradation by exonucleases. Polyadenylation is also important for transcription termination, export of the mRNA from the nucleus, and translation. Polyadenylation occurs in the nucleus immediately after transcription of DNA into RNA, but additionally can also occur later in the cytoplasm. After transcription has been terminated, the mRNA chain is cleaved through the action of an

endonuclease complex associated with RNA polymerase. The cleavage site is usually characterized by the presence of the base sequence AAUAAA near the cleavage site. After the mRNA has been cleaved, adenosine residues are added to the free 3' end at the cleavage site.

As used herein, "transient" refers to expression of a non-integrated transgene for a period of hours, days or weeks, wherein the period of time of expression is less than the period of time for expression of the gene if integrated into the genome or contained within a stable plasmid replicon in the host cell.

As used herein, the terms "treat", "treatment" and "treating" refer to the reduction or amelioration of the progression, severity and/or duration of a proliferative disorder, or the amelioration of one or more symptoms (preferably, one or more discernible symptoms) of a proliferative disorder resulting from the administration of one or more therapies (e.g., one or more therapeutic agents such as a CAR of the invention). In specific embodiments, the terms "treat", "treatment" and "treating" refer to the amelioration of at least one measurable physical parameter of a proliferative disorder, such as growth of a tumor, not necessarily discernible by the patient. In other embodiments the terms "treat", "treatment" and "treating" -refer to the inhibition of the progression of a proliferative disorder, either physically by, e.g., stabilization of a discernible symptom, physiologically by, e.g., stabilization of a physical parameter, or both. In other embodiments the terms "treat", "treatment" and "treating" refer to the reduction or stabilization of tumor size or cancerous cell count.

The term "signal transduction pathway" refers to the biochemical relationship between a variety of signal transduction molecules that play a role in the transmission of a signal from one portion of a cell to another portion of a cell. The phrase "cell surface receptor" includes molecules and complexes of molecules capable of receiving a signal and transmitting signal across the membrane of a cell.

The term "subject" is intended to include living organisms in which an immune response can be elicited (e.g., mammals, human).

The term, a "substantially purified" cell refers to a cell that is essentially free of other cell types. A substantially purified cell also refers to a cell which has been separated from other cell types with which it is normally associated in its naturally occurring state. In some instances, a population of substantially purified cells refers to a homogenous population of cells. In other instances, this term refers simply to cell that have been separated from the cells with which they are naturally associated in their natural state. In some aspects, the cells are cultured in vitro. In other aspects, the cells are not cultured in vitro.

The term "therapeutic" as used herein means a treatment. A therapeutic effect is obtained by reduction, suppression, remission, or eradication of a disease state.

The term "prophylaxis" as used herein means the prevention of or protective treatment for a disease or disease state.

In the context of the present invention, "tumor antigen" or "hyperproliferative disorder antigen" or "antigen associated with a hyperproliferative disorder" refers to antigens that are common to specific hyperproliferative disorders. In certain aspects, the hyperproliferative disorder antigens of the present invention are derived from, cancers including but not limited to primary or metastatic melanoma, thymoma, lymphoma, sarcoma, lung cancer, liver cancer, non-Hodgkin lymphoma, Hodgkin lymphoma, leukemias, uterine cancer, cervical cancer, bladder cancer, kidney cancer and adenocarcinomas such as breast cancer, prostate cancer (e.g., castrate-resistant or therapy-resistant prostate cancer, or metastatic prostate cancer), ovarian cancer, pancreatic cancer, and the like, or a plasma cell proliferative disorder, e.g., asymptomatic myeloma (smoldering multiple myeloma or indolent myeloma), monoclonal gammapathy of undetermined significance (MGUS), Waldenstrom's macroglobulinemia, plasmacytomas (e.g., plasma cell dyscrasia, solitary myeloma, solitary plasmacytoma, extramedullary plasmacytoma, and multiple plasmacytoma), systemic amyloid light chain amyloidosis, and POEMS syndrome (also known as Crow-Fukase syndrome, Takatsuki disease, and PEP syndrome).

The term "transfected" or "transformed" or "transduced" refers to a process by which exogenous nucleic acid is transferred or introduced into the host cell. A "transfected" or "transformed" or "transduced" cell is one which has been transfected, transformed or transduced with exogenous nucleic acid. The cell includes the primary subject cell and its progeny.

The term "specifically binds," refers to an antibody, or a ligand, which recognizes and binds with a cognate binding partner (e.g., a stimulatory and/or costimulatory molecule present on a T cell) protein present in a sample, but which antibody or ligand does not substantially recognize or bind other molecules in the sample.

"Regulatable chimeric antigen receptor (RCAR)," as used herein, refers to a set of polypeptides, typically two in the simplest embodiments, which when in an immune effector cell, provides the cell with specificity for a target cell, typically a cancer cell, and with intracellular signal generation. In some embodiments, an RCAR comprises at least an extracellular antigen binding domain, a transmembrane domain and a cytoplasmic signaling domain (also referred to herein as "an intracellular signaling domain") comprising a functional signaling domain derived from a stimulatory molecule and/or costimulatory molecule as defined herein in the context of a CAR molecule. In some embodiments, the set of polypeptides in the RCAR are not contiguous with each other, e.g., are in different polypeptide chains. In some embodiments, the RCAR includes a dimerization switch that, upon the presence of a dimerization molecule, can couple the polypeptides to one another, e.g., can couple an antigen binding domain to an intracellular signaling domain. In some embodiments, the RCAR is expressed in a cell (e.g., an immune effector cell) as described herein, e.g., an RCAR-expressing cell (also referred to herein as "RCARX cell"). In an embodiment the RCARX cell is a T cell, and is referred to as a RCART cell. In an embodiment the RCARX cell is an NK cell, and is referred to as a RCARN cell. The

RCAR can provide the RCAR-expressing cell with specificity for a target cell, typically a cancer cell, and with regulatable intracellular signal generation or proliferation, which can optimize an immune effector property of the RCAR-expressing cell. In embodiments, an RCAR cell relies at least in part, on an antigen binding domain to provide specificity to a target cell that comprises the antigen bound by the antigen binding domain.

"Membrane anchor" or "membrane tethering domain", as that term is used herein, refers to a polypeptide or moiety, e.g., a myristoyl group, sufficient to anchor an extracellular or intracellular domain to the plasma membrane.

"Switch domain," as that term is used herein, e.g., when referring to an RCAR, refers to an entity, typically a polypeptide-based entity, that, in the presence of a dimerization molecule, associates with another switch domain. The association results in a functional coupling of a first entity linked to, e.g., fused to, a first switch domain, and a second entity linked to, e.g., fused to, a second switch domain. A first and second switch domain are collectively referred to as a dimerization switch. In embodiments, the first and second switch domains are the same as one another, e.g., they are polypeptides having the same primary amino acid sequence, and are referred to collectively as a homodimerization switch. In embodiments, the first and second switch domains are different from one another, e.g., they are polypeptides having different primary amino acid sequences, and are referred to collectively as a heterodimerization switch. In embodiments, the switch is intracellular. In embodiments, the switch is extracellular. In embodiments, the switch domain is a polypeptide-based entity, e.g., FKBP or FRB-based, and the dimerization molecule is small molecule, e.g., a rapalogue. In embodiments, the switch domain is a polypeptide-based entity, e.g., an scFv that binds a myc peptide, and the dimerization molecule is a polypeptide, a fragment thereof, or a multimer of a polypeptide, e.g., a myc ligand or multimers of a myc ligand that bind to one or more myc scFvs. In embodiments, the switch domain is a polypeptide-based entity, e.g., myc receptor, and the dimerization molecule is an antibody or fragments thereof, e.g., myc antibody.

"Dimerization molecule," as that term is used herein, e.g., when referring to an RCAR, refers to a molecule that promotes the association of a first switch domain with a second switch domain. In embodiments, the dimerization molecule does not naturally occur in the subject, or does not occur in concentrations that would result in significant dimerization. In embodiments, the dimerization molecule is a small molecule, e.g., rapamycin or a rapalogue, e.g, RAD001.

The term "bioequivalent" refers to an amount of an agent other than the reference compound (e.g., RAD001), required to produce an effect equivalent to the effect produced by the reference dose or reference amount of the reference compound (e.g., RAD001). In an embodiment the effect is the level of mTOR inhibition, e.g., as measured by P70 S6 kinase inhibition, e.g., as evaluated in an in vivo or in vitro assay, e.g., as measured by an assay described herein, e.g., the Boulay assay, or measurement of

phosphorylated S6 levels by western blot. In an embodiment, the effect is alteration of the ratio of PD-1 positive/PD-1 negative T cells, as measured by cell sorting. In an embodiment a bioequivalent amount or dose of an mTOR inhibitor is the amount or dose that achieves the same level of P70 S6 kinase inhibition as does the reference dose or reference amount of a reference compound. In an embodiment, a bioequivalent amount or dose of an mTOR inhibitor is the amount or dose that achieves the same level of alteration in the ratio of PD-1 positive/PD-1 negative T cells as does the reference dose or reference amount of a reference compound.

The term "low, immune enhancing, dose" when used in conjuction with an mTOR inhibitor, e.g., an allosteric mTOR inhibitor, e.g., RAD001 or rapamycin, or a catalytic mTOR inhibitor, refers to a dose of mTOR inhibitor that partially, but not fully, inhibits mTOR activity, e.g., as measured by the inhibition of P70 S6 kinase activity. Methods for evaluating mTOR activity, e.g., by inhibition of P70 S6 kinase, are discussed herein. The dose is insufficient to result in complete immune suppression but is sufficient to enhance the immune response. In an embodiment, the low, immune enhancing, dose of mTOR inhibitor results in a decrease in the number of PD-1 positive immune effector cells, e.g., T cells or NK cells, and/or an increase in the number of PD-1 negative immune effector cells, e.g., T cells or NK cells, or an increase in the ratio of PD-1 negative immune effector cells (e.g., T cells or NK cells) /PD-1 positive immune effector cells (e.g., T cells or NK cells).

In an embodiment, the low, immune enhancing, dose of mTOR inhibitor results in an increase in the number of naive T cells. In an embodiment, the low, immune enhancing, dose of mTOR inhibitor results in one or more of the following:

an increase in the expression of one or more of the following markers: CD62Lhigh, CD127high, CD27+, and BCL2, e.g., on memory T cells, e.g., memory T cell precursors;

a decrease in the expression of KLRG1, e.g., on memory T cells, e.g., memory T cell precursors; and

an increase in the number of memory T cell precursors, e.g., cells with any one or combination of the following characteristics: increased CD62Lhigh, increased CD127high, increased CD27+, decreased KLRG1, and increased BCL2;

wherein any of the changes described above occurs, e.g., at least transiently, e.g., as compared to a non-treated subject.

"Refractory" as used herein refers to a disease, e.g., cancer, that does not respond to a treatment. In embodiments, a refractory cancer can be resistant to a treatment before or at the beginning of the treatment. In other embodiments, the refractory cancer can become resistant during a treatment. A refractory cancer is also called a resistant cancer.

"Relapsed" or a "relapse" as used herein refers to the reappearance of a disease (e.g., cancer) or the signs and symptoms of a disease such as cancer after a period of improvement or responsiveness,

e.g., after prior treatment of a therapy, e.g., cancer therapy. For example, the period of responsiveness may involve the level of cancer cells falling below a certain threshold, e.g., below 20%, 15%, 10%, 5%, 4%, 3%, 2%, or 1%. The reappearance may involve the level of cancer cells rising above a certain threshold, e.g., above 20%, 15%, 10%, 5%, 4%, 3%, 2%, or 1%.

Ranges: throughout this disclosure, various aspects of the invention can be presented in a range format. It should be understood that the description in range format is merely for convenience and brevity and should not be construed as an inflexible limitation on the scope of the invention. Accordingly, the description of a range should be considered to have specifically disclosed all the possible subranges as well as individual numerical values within that range. For example, description of a range such as from 1 to 6 should be considered to have specifically disclosed subranges such as from 1 to 3, from 1 to 4, from 1 to 5, from 2 to 4, from 2 to 6, from 3 to 6 etc., as well as individual numbers within that range, for example, 1, 2, 2.7, 3, 4, 5, 5.3, and 6. As another example, a range such as 95-99% identity, includes something with 95%, 96%, 97%, 98% or 99% identity, and includes subranges such as 96-99%, 96-98%, 96-97%, 97-99%, 97-98% and 98-99% identity. This applies regardless of the breadth of the range.

A "gene editing system" as the term is used herein, refers to a system, e.g., one or more molecules, that direct and effect an alteration, e.g., a deletion, of one or more nucleic acids at or near a site of genomic DNA targeted by said system. Gene editing systems are known in the art, and are described more fully below.

Certain chemical definitions are provided below.

The term "halo" or "halogen" refers to any radical of fluorine, chlorine, bromine or iodine.

The term "alkyl" refers to a hydrocarbon chain that may be a straight chain or branched chain, containing the indicated number of carbon atoms. For example, C_1-C_{12} alkyl indicates that the group may have from 1 to 12 (inclusive) carbon atoms in it. The term "haloalkyl" refers to an alkyl in which one or more hydrogen atoms are replaced by halo, and includes alkyl moieties in which all hydrogens have been replaced by halo (e.g., perfluoroalkyl). Alkyl may be optionally substituted. Suitable substituents on an alkyl include, without limitation, halo, alkoxy, haloalkoxy (e.g., perfluoroalkoxy such as OCF_3), hydroxy, carboxy, carboxylate, cyano, nitro, amino, alkyl amino, SO_3H, sulfate, phosphate, oxo, thioxo (e.g., C=S), imino (alkyl, aryl, aralkyl), $S(O)_n$alkyl (where n is 0-2), $S(O)_n$aryl (where n is 0-2), $S(O)_n$heteroaryl (where n is 0-2), $S(O)_n$ heterocyclyl (where n is 0-2), amine (mono-, di-, alkyl, cycloalkyl, aralkyl, heteroaralkyl, aryl, heteroaryl, and combinations thereof), ester (alkyl, aralkyl, heteroaralkyl, aryl, heteroaryl), amide (mono-, di-, alkyl, aralkyl, heteroaralkyl, aryl, heteroaryl, and combinations thereof), sulfonamide (mono-, di-, alkyl, aralkyl, heteroaralkyl, and combinations thereof). As used herein, the term "lower alkyl" denotes a saturated straight- or branched-chain alkyl group

containing from 1 to 7 carbon atoms, for example, methyl, ethyl, propyl, isopropyl, n-butyl, i-butyl, 2-butyl, t-butyl and the like. Preferred lower alkyl groups are groups with 1-4 carbon atoms. As used herein, the term "lower alkinyl" denotes a unsaturated straight- or branched-carbon chain containing from 2 to 7 carbon atoms and containing at least one triple bond.

The term "alkoxy" refers to an -O-alkyl radical. The term "lower alkoxy" denotes a group wherein the alkyl residues is as defined above, and which is attached via an oxygen atom.

The terms "arylalkyl" or "aralkyl" refer to an alkyl moiety in which an alkyl hydrogen atom is replaced by an aryl group. Aralkyl includes groups in which more than one hydrogen atom has been replaced by an aryl group. Examples of "arylalkyl" or "aralkyl" include benzyl, 2-phenylethyl, 3-phenylpropyl, 9-fluorenyl, benzhydryl, and trityl groups.

The term "alkylene" refers to a divalent alkyl, e.g., $-CH_2-$, $-CH_2CH_2-$, and $-CH_2CH_2CH_2-$.

The term "alkenyl" refers to a straight or branched hydrocarbon chain containing 2-12 carbon atoms and having one or more double bonds. Examples of alkenyl groups include, but are not limited to, allyl, propenyl, 2-butenyl, 3-hexenyl and 3-octenyl groups. One of the double bond carbons may optionally be the point of attachment of the alkenyl substituent. The term "alkynyl" refers to a straight or branched hydrocarbon chain containing 2-12 carbon atoms and characterized in having one or more triple bonds. Examples of alkynyl groups include, but are not limited to, ethynyl, propargyl, and 3-hexynyl. One of the triple bond carbons may optionally be the point of attachment of the alkynyl substituent.

Unless otherwise indicated, as used herein, the term "alkenoxy", means "alkenyl-O-", wherein "alkenyl" is as defined above. Unless otherwise indicated, as used herein, the term "alkynoxy", means "alkynyl-O-", wherein "alkynyl" is as defined above.

The terms "alkylamino" and "dialkylamino" refer to –NH(alkyl) and $–NH(alkyl)_2$ radicals respectively. The term "aralkylamino" refers to a –NH(aralkyl) radical. The term alkylaminoalkyl refers to a (alkyl)NH-alkyl- radical; the term dialkylaminoalkyl refers to a $(alkyl)_2N$-alkyl- radical The term "alkoxy" refers to an -O-alkyl radical. The term "mercapto" refers to an SH radical. The term "thioalkoxy" refers to an -S-alkyl radical. The term thioaryloxy refers to an –S-aryl radical. "Hydroxy" refers to the radical –OH.

The term "aralkyl", as used herein, refers to an alkyl group substituted with an aryl group (e.g., an aromatic or heteroaromatic group).

The term "aryl" refers to an aromatic monocyclic, bicyclic, or tricyclic hydrocarbon ring system, wherein any ring atom capable of substitution can be substituted (e.g., by one or more substituents). Examples of aryl moieties include, but are not limited to, phenyl, naphthyl, and anthracenyl.

The term "cycloalkyl" as employed herein includes saturated cyclic, bicyclic, tricyclic,or polycyclic hydrocarbon groups having 3 to 12 carbons. Any ring atom can be substituted (e.g., by one or more substituents). The cycloalkyl groups can contain fused rings. Fused rings are rings that share a common carbon atom. Examples of cycloalkyl moieties include, but are not limited to, cyclopropyl, cyclohexyl, methylcyclohexyl, adamantyl, and norbornyl.

"Bicycloalkyl" and "tricycloalkyl" groups are non-aromatic saturated carbocyclic groups consisting of two or three rings respectively, wherein said rings share at least one carbon atom. Unless otherwise indicated, for purposes of the present invention, bicycloalkyl groups include spiro groups and fused ring groups. Examples ofbicycloalkyl groups include, but are not limited to, bicyclo-[3.1.0]-hexyl, bicyclo-2.2.1]-hept-1-yl, norbomyl, spiro[4.5]decyl, spiro[4.4]nonyl, spiro[4.3]octyl, and spiro[4.2]heptyl. An example of a tricycloalkyl group is adamantanyl. Other cycloalkyl, bicycloalkyl, and tricycloalkyl groups are known in the art, and such groups are encompassed by the definitions "cycloalkyl", "bicycloalkyl" and "tricycloalkyl" herein.

"Cycloalkenyl", "bicycloalkenyl", and "tricycloalkenyl" refer to non-aromatic carbocyclic cycloalkyl, bicycloalkyl, and tricycloalkyl moieties as defined above, except comprising one or more carbon-carbon double bonds connecting carbon ring members (an "endocyclic" double bond) and/or one or more carbon-carbon double bonds connecting a carbon ring member and an adjacent non-ring carbon (an "exocyclic"double bond). Examples of cycloalkenyl groups include, but are not limited to, cyclopentenyl, cyclobutenyl, and cyclohexenyl, and a non-limiting example of a bicycloalkenyl group is norbornenyl. Other cycloalkenyl, bicycloalkenyl, and tricycloalkenyl groups are known in the art, and such groups are included within the definitions "cycloalkenyl", "bicycloalkenyl" and "tricycloalkenyl" herein. Cycloalkyl, cycloalkenyl, bicycloalkyl, and bicycloalkenyl groups also include groups that are substituted with one or more oxo moieties. Examples of such groups with oxo moieties are oxocyclopentyl, oxocyclobutyl, oxocyclopentenyl, and norcamphoryl.

The term "heteroaryl" refers to a fully aromatic 5-8 membered monocyclic, 8-12 membered bicyclic, or 11-14 membered tricyclic ring system having 1-3 heteroatoms if monocyclic, 1-6 heteroatoms if bicyclic, or 1-9 heteroatoms if tricyclic, said heteroatoms selected from O, N, or S (e.g., carbon atoms and 1-3, 1-6, or 1-9 heteroatoms selected independently from N, O, or S if monocyclic, bicyclic, or tricyclic, respectively). Any ring atom can be substituted (e.g., by one or more substituents). The point of attachment of a heteroaryl is on the ring containing said heteroatom(s).

The term "heterocyclyl" or "heterocyloalkyl" refers to a nonaromatic 3-10 membered monocyclic, 8-12 membered bicyclic, or 11-14 membered tricyclic ring system having 1-3 heteroatoms if monocyclic, 1-6 heteroatoms if bicyclic, or 1-9 heteroatoms if tricyclic, said heteroatoms selected from O, N, or S (e.g., carbon atoms and 1-3, 1-6, or 1-9 heteroatoms of N, O, or S if monocyclic, bicyclic, or tricyclic, respectively). The point of attachment of a heterocyclyl is on the ring containing

said heteroatom(s). The heteroatom may optionally be the point of attachment of the heterocyclyl substituent. Any ring atom can be substituted (e.g., by one or more substituents). The heterocyclyl groups can contain fused rings. Fused rings are rings that share a common carbon atom. Examples of heterocyclyl include, but are not limited to, tetrahydrofuranyl, tetrahydropyranyl, piperidinyl, morpholino, pyrrolinyl, pyrimidinyl, and pyrrolidinyl.

Bicyclic and tricyclic ring systems containing one or more heteroatoms and both aromatic and non-aromatic rings are considered to be heterocyclyl groups according to the present definition.

The term "saturated or partially saturated heterocyclyl" refers to a non-aromatic cylic structure that includes at least one heteroatom. Heterocyclyl groups include, for example, thiophene, thianthrene, furan, pyran, isobenzofuran, chromene, xanthene, phenoxathiin, pyrrole, imidazole, pyrazole, isothiazole, isoxazole, pyridine, pyrazine, pyrimidine, pyridazine, indolizine, isoindole, indole, indazole, purine, quinolizine, isoquinoline, quinoline, phthalazine, naphthyridine, quinoxaline, quinazoline, cinnoline, pteridine, carbazole, carboline, phenanthridine, acridine, pyrimidine, phenanthroline, phenazine, phenarsazine, phenothiazine, furazan, phenoxazine, pyrrolidine, oxolane, thiolane, oxazole, piperidine, piperazine, morpholine, lactones, lactams such as azetidinones and pyrrolidinones, sultams, sultones, and the like. The heterocyclic ring can be substituted at one or more positions with such substituents as described above, as for example, halogen, alkyl, aralkyl, alkenyl, alkynyl, cycloalkyl, hydroxyl, amino, nitro, sulfhydryl, imino, amido, phosphate, phosphonate, phosphinate, carbonyl, carboxyl, silyl, ether, alkylthio, sulfonyl, ketone, aldehyde, ester, a heterocyclyl, an aromatic or heteroaromatic moiety, $-CF_3$, -CN, or the like.

The term "heterocyclylalkyl", as used herein, refers to an alkyl group substituted with a heterocycle group. The term "cycloalkylalkyl", as used herein, refers to an alkyl group substituted with a cycloalkyl group.

The term "cycloalkenyl" refers to partially unsaturated, nonaromatic, cyclic, bicyclic, tricyclic, or polycyclic hydrocarbon groups having 5 to 12 carbons, preferably 5 to 8 carbons. The unsaturated carbon may optionally be the point of attachment of the cycloalkenyl substituent. Any ring atom can be substituted (e.g., by one or more substituents). The cycloalkenyl groups can contain fused rings. Fused rings are rings that share a common carbon atom. Examples of cycloalkenyl moieties include, but are not limited to, cyclohexenyl, cyclohexadienyl, or norbornenyl.

The term "heterocycloalkenyl" refers to a partially saturated, nonaromatic 5-10 membered monocyclic, 8-12 membered bicyclic, or 11-14 membered tricyclic ring system having 1-3 heteroatoms if monocyclic, 1-6 heteroatoms if bicyclic, or 1-9 heteroatoms if tricyclic, said heteroatoms selected from O, N, or S (e.g., carbon atoms and 1-3, 1-6, or 1-9 heteroatoms of N, O, or S if monocyclic, bicyclic, or tricyclic, respectively). The unsaturated carbon or the heteroatom may optionally be the

point of attachment of the heterocycloalkenyl substituent. Any ring atom can be substituted (e.g., by one or more substituents). The heterocycloalkenyl groups can contain fused rings. Fused rings are rings that share a common carbon atom. Examples of heterocycloalkenyl include but are not limited to tetrahydropyridyl and dihydropyranyl.

The terms "hetaralkyl" and "heteroaralkyl", as used herein, refers to an alkyl group substituted with a heteroaryl group.

The term "oxo" refers to an oxygen atom, which forms a carbonyl when attached to carbon, an N-oxide when attached to nitrogen, and a sulfoxide or sulfone when attached to sulfur.

The term "acyl" refers to an alkylcarbonyl, cycloalkylcarbonyl, arylcarbonyl, heterocyclylcarbonyl, or heteroarylcarbonyl substituent, any of which may be further substituted (e.g., by one or more substituents).

The term "substituents" refers to a group "substituted" on an alkyl, alkoxy, cycloalkyl, cycloalkylalkyl, alkenyl, alkynyl, heterocyclyl, heterocyclylalkyl, heterocycloalkenyl, cycloalkenyl, aryl, aralkyl, heteroaryl or heteroaralkyl group at any atom of that group. Any atom can be substituted. Suitable substituents include, without limitation, alkyl (e.g., C1, C2, C3, C4, C5, C6, C7, C8, C9, C10, C11, C12 straight or branched chain alkyl), cycloalkyl, haloalkyl (e.g., perfluoroalkyl such as CF_3), aryl, heteroaryl, aralkyl, heteroaralkyl, heterocyclyl, alkenyl, alkynyl, cycloalkenyl, heterocycloalkenyl, alkoxy, haloalkoxy (e.g., perfluoroalkoxy such as OCF_3), halo, hydroxy, carboxy, carboxylate, cyano, nitro, amino, alkyl amino, SO_3H, sulfate, phosphate, methylenedioxy (-O-CH_2-O- wherein oxygens are attached to vicinal atoms), ethylenedioxy, oxo, thioxo (e.g., C=S), imino (alkyl, aryl, aralkyl), $S(O)_n$alkyl (where n is 0-2), $S(O)_n$ aryl (where n is 0-2), $S(O)_n$ heteroaryl (where n is 0-2), $S(O)_n$ heterocyclyl (where n is 0-2), amine (mono-, di-, alkyl, cycloalkyl, aralkyl, heteroaralkyl, aryl, heteroaryl, and combinations thereof), ester (alkyl, aralkyl, heteroaralkyl, aryl, heteroaryl), amide (mono-, di-, alkyl, aralkyl, heteroaralkyl, aryl, heteroaryl, and combinations thereof), sulfonamide (mono-, di-, alkyl, aralkyl, heteroaralkyl, and combinations thereof). In one aspect, the substituents on a group are independently any one single, or any subset of the aforementioned substituents. In another aspect, a substituent may itself be substituted with any one of the above substituents.

"Pharmaceutically acceptable" means approved or approvable by a regulatory agency of the Federal or a state government or the corresponding agency in countries other than the United States, or that is listed in the U.S. Pharmacopoeia or other generally recognized pharmacopoeia for use in animals, and more particularly, in humans.

"Pharmaceutically acceptable salt" refers to a salt of a compound of the invention that is pharmaceutically acceptable and that possesses the desired pharmacological activity of the parent compound. In particular, such salts are non-toxic may be inorganic or organic acid addition salts and base addition salts. Specifically, such salts include: (1) acid addition salts, formed with inorganic acids

such as hydrochloric acid, hydrobromic acid, sulfuric acid, nitric acid, phosphoric acid, and the like; or formed with organic acids such as acetic acid, propionic acid, hexanoic acid, cyclopentanepropionic acid, glycolic acid, pyruvic acid, lactic acid, malonic acid, succinic acid, malic acid, maleic acid, fumaric acid, tartaric acid, citric acid, benzoic acid, 3-(4-hydroxybenzoyl) benzoic acid, cinnamic acid, mandelic acid, methanesulfonic acid, ethanesulfonic acid, 1,2-ethane-disulfonic acid, 2-hydroxyethanesulfonic acid, benzenesulfonic acid, 4-chlorobenzenesulfonic acid, 2-naphthalenesulfonic acid, 4-toluenesulfonic acid, camphorsulfonic acid, 4-methylbicyclo[2.2.2]-oct-2-ene-1-carboxylic acid, glucoheptonic acid, 3-phenylpropionic acid, trimethylacetic acid, tertiary butylacetic acid, lauryl sulfuric acid, gluconic acid, glutamic acid, hydroxynaphthoic acid, salicylic acid, stearic acid, muconic acid, and the like; or (2) salts formed when an acidic proton present in the parent compound either is replaced by a metal ion, *e.g.*, an alkali metal ion, an alkaline earth ion, or an aluminum ion; or coordinates with an organic base such as ethanolamine, diethanolamine, triethanolamine, N-methylglucamine and the like. Salts further include, by way of example only, sodium, potassium, calcium, magnesium, ammonium, tetraalkylammonium, and the like; and when the compound contains a basic functionality, salts of non toxic organic or inorganic acids, such as hydrochloride, hydrobromide, tartrate, mesylate, acetate, maleate, oxalate and the like. The term "pharmaceutically acceptable cation" refers to an acceptable cationic counter-ion of an acidic functional group. Such cations are exemplified by sodium, potassium, calcium, magnesium, ammonium, tetraalkylammonium cations, and the like (see, *e.g.,* Berge, *et al., J. Pharm. Sci.* 66(1): 1-79 (Jan."77).

Various aspects of the compositions and methods herein are described in further detail below. Additional definitions are set out throughout the specification.

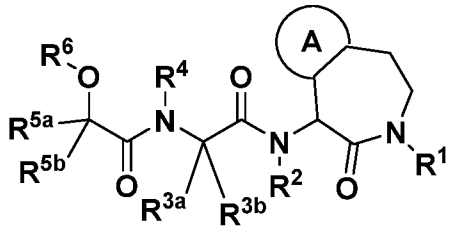
Gamma secretase inhibitor (GSI)

The present invention provides compositions comprising, e.g., a gamma secretase inhibitor (GSI), and methods for enhancing the function of a BCMA CAR-expressing cell, by using such compositions and/or other means as described herein. Any inhibitor of gamma secretase (e.g., a subunit of gamma secretase, e.g., presenilin, nicastrin, APH-1, or PEN-2), and any modulator of a gene encoding gamma secretase (e.g., a subunit of gamma secretase, e.g., presenilin, nicastrin, APH-1, or PEN-2), known in the art, can be used according to the present invention. Examples of GSIs are described below.

Small molecules targeting gamma secretase

The compositions, methods and uses described herein comprise a gamma secretase inhibitor (GSI). In some embodiments, the GSI is a small molecule that reduces the expression and/or function of gamma secretase.

In some embodiments, the compound is a compound of formula (I) or a pharmaceutically acceptable salt thereof;

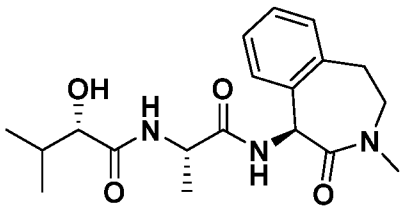


(I)

wherein ring A is aryl or heteroaryl; each of R^1, R^2, and R^4 is independently hydrogen, C_1-C_6 alkyl, cycloalkyl, heterocyclyl, cycloalkylalkyl, heterocyclylalkyl, aryl, heteroaryl, aralkyl, or heteroaralkyl, wherein each C_1-C_6 alkyl, cycloalkyl, heterocyclyl, cycloalkylalkyl, heterocyclylalkyl, aryl, heteroaryl, aralkyl, or heteroaralkyl is substituted with 0-6 independent occurrences of halogen,–OR^A, –SR^A, -$C(O)OR^A$, -$C(O)N(R^A)(R^B)$, -$N(R^A)(R^B)$,or -$C(NR^C)N(R^A)(R^B)$; each R^{3a}, R^{3b}, R^{5a}, and R^{5b} is independently hydrogen, halogen, -OH, C_1-C_6 alkyl, C_1-C_6 alkoxy, cycloalkyl, heterocyclyl, cycloalkylalkyl, heterocyclylalkyl, aryl, heteroaryl, aralkyl, or heteroaralkyl, wherein each C_1-C_6 alkyl, C_1-C_6 alkoxy, cycloalkyl, heterocyclyl, cycloalkylalkyl, heterocyclylalkyl, aryl, heteroaryl, aralkyl, or heteroaralkyl is substituted with 0-6 independent occurrences of halogen, -OH, –OR^A, –SR^A, -$C(O)OR^A$, -$C(O)N(R^A)(R^B)$, -$N(R^A)(R^B)$,or -$C(NR^C)N(R^A)(R^B)$; R^6 is hydrogen, C_1-C_6 alkyl, cycloalkyl, heterocyclyl, cycloalkylalkyl, heterocyclylalkyl, aryl, heteroaryl, aralkyl, or heteroaralkyl, wherein each C_1-C_6 alkyl, cycloalkyl, heterocyclyl, cycloalkylalkyl, heterocyclylalkyl, aryl, heteroaryl, aralkyl, or heteroaralkyl is substituted with 0-6 independent occurrences of halogen, –OH, or C_1-C_6 alkoxy; and each R^A, R^B, and R^C is independently hydrogen, C_1-C_6 alkyl, cycloalkyl, heterocyclyl, cycloalkylalkyl, heterocyclylalkyl, aryl, heteroaryl, aralkyl, or heteroaralkyl, wherein each C_1-C_6 alkyl, cycloalkyl, heterocyclyl, cycloalkylalkyl, heterocyclylalkyl, aryl, heteroaryl, aralkyl, or heteroaralkyl is substituted with 0-6 independent occurrences of halogen, –OH, or C_1-C_6 alkoxy.

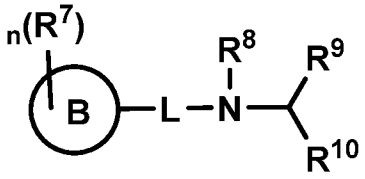
In some embodiments, ring A is aryl (e.g., phenyl). In some embodiments, R^1 is $–CH_3$. In some embodiments, each of R^2 and R^4 is independently hydrogen. In some embodiments, R^{3a} is $–CH_3$ and R^{3b} is hydrogen. In some embodiments, R^{5a} is hydrogen and R^{5b} is $–CH(CH_3)_2$. In some embodiments, R^6 is hydrogen.

In some embodiments, the compound of formula (I) is a compound described in U.S. Patent No. 7,468,365, which is herein incorporated by reference in its entirety. In one embodiment, the compound of formula (I) is LY-450139, *i.e.*, semagacestat, (S)-2-hydroxy-3-methyl-N-((S)-1-(((S)-3-methyl-2-oxo-2,3,4,5-tetrahydro-1H-benzo[d]azepin-1-yl)amino)-1-oxopropan-2-yl)butanamide, or a pharmaceutically acceptable salt thereof. In one embodiment, the compound is



or a pharmaceutically acceptable salt thereof.

In some embodiments, the compound is a compound of formula (II) or a pharmaceutically acceptable salt thereof;

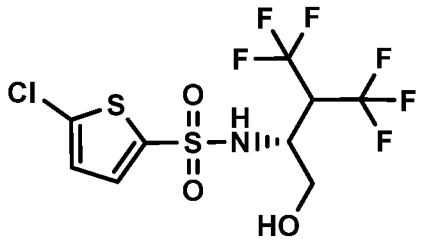


(II)

wherein ring B is aryl or heteroaryl; L is a bond, C_1-C_6 alkylene, -$S(O)_2$-, -C(O)-, -$N(R^E)$(O)C-, or –OC(O)-; each R^7 is independently halogen, -OH, C_1-C_6 alkyl, C_1-C_6 alkoxy, cycloalkyl, heterocyclyl, cycloalkylalkyl, heterocyclylalkyl, aryl, heteroaryl, aralkyl, or heteroaralkyl, wherein each C_1-C_6 alkyl, C_1-C_6 alkoxy, cycloalkyl, heterocyclyl, cycloalkylalkyl, heterocyclylalkyl, aryl, heteroaryl, aralkyl, or heteroaralkyl is independently substituted with 0-6 occurrences of halogen, –OR^D, –SR^D, -C(O)OR^D, -C(O)N(R^D)(R^E), -N(R^D)(R^E),or -C(NR^F)N(R^D)(R^E); R^8 is hydrogen, C_1-C_6 alkyl, C_1-C_6 alkoxy, cycloalkyl, heterocyclyl, cycloalkylalkyl, heterocyclylalkyl, aryl, heteroaryl, aralkyl, or heteroaralkyl, wherein each C_1-C_6 alkyl, C_1-C_6 alkoxy, cycloalkyl, heterocyclyl, cycloalkylalkyl, heterocyclylalkyl, aryl, heteroaryl, aralkyl, or heteroaralkyl is substituted with 0-6 independent occurrences of halogen,–OR^D, –SR^D, -C(O)OR^D, -C(O)N(R^D)(R^E), -N(R^D)(R^E),or -C(NR^F)N(R^D)(R^E); each of R^9 and R^{10} is independently hydrogen, halogen, -OH, C_1-C_6 alkyl, C_1-C_6 alkoxy, cycloalkyl, heterocyclyl, cycloalkylalkyl, heterocyclylalkyl, aryl, heteroaryl, aralkyl, or heteroaralkyl, wherein each C_1-C_6 alkyl, C_1-C_6 alkoxy, cycloalkyl, heterocyclyl, aryl, heteroaryl, aralkyl, or heteroaralkyl is substituted with 0-6 independent occurrences of halogen,–OR^D, –SR^D, -C(O)OR^D, -C(O)N(R^D)(R^E), -N(R^D)(R^E),or -C(NR^I)N(R^G)(R^H); each R^D, R^E, and R^F is independently hydrogen, C_1-C_6 alkyl, cycloalkyl, heterocyclyl, cycloalkylalkyl, heterocyclylalkyl, aryl, heteroaryl, aralkyl, or heteroaralkyl, wherein each C_1-C_6 alkyl, cycloalkyl, heterocyclyl, cycloalkylalkyl, heterocyclylalkyl, aryl, heteroaryl, aralkyl, or heteroaralkyl is substituted with 0-6 independent occurrences of halogen, –OH, or C_1-C_6 alkoxy; and n is 0, 1, 2, 3, 4, or 5.

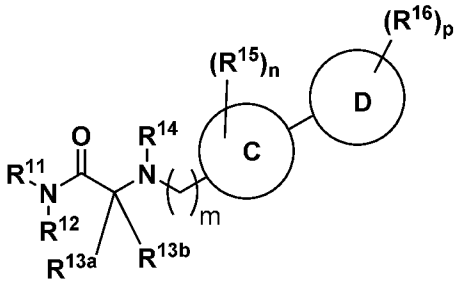
In some embodiments, ring B is heteroaryl (e.g., thiofuranyl). In some embodiments, L is -$S(O)_2$. In some embodiments, R^7 is chloro and n is 1. In some embodiments, R^8 is $–CH_2OH$. In some embodiments, each of R^9 and R^{10} is independently $–CF_3$.

In some embodiments, the compound of formula (II) is a compound described in U.S. Patent No. 7,687,666, which is herein incorporated by reference in its entirety. In one embodiment, the compound of formula (II) is PF-5212362, *i.e.*, begacestat, GSI-953, or (R)-5-chloro-N-(4,4,4-trifluoro-1-hydroxy-3-(trifluoromethyl)butan-2-yl)thiophene-2-sulfonamide, or a pharmaceutically acceptable salt thereof. In one embodiment, the compound is



or a pharmaceutically acceptable salt thereof.

In some embodiments, the compound is a compound is a compound of formula (III) or a pharmaceutically acceptable salt thereof;



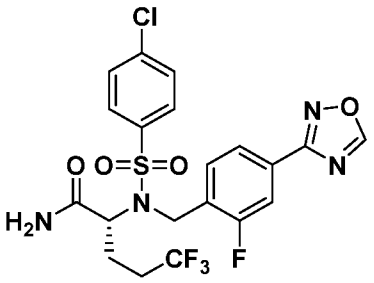
(III)

wherein each of rings C and D is independently aryl or heteroaryl; each of R^{11}, R^{12}, and R^{14} is independently hydrogen, C_1-C_6 alkyl, C_1-C_6 alkoxy, -$S(O)R^G$-, -$S(O)_2R^G$-, cycloalkyl, heterocyclyl, cycloalkylalkyl, heterocyclylalkyl, aryl, heteroaryl, aralkyl, or heteroaralkyl, wherein each C_1-C_6 alkyl, C_1-C_6 alkoxy, cycloalkyl, heterocyclyl, cycloalkylalkyl, heterocyclylalkyl, aryl, heteroaryl, aralkyl, or heteroaralkyl is substituted with 0-6 independent occurrences of halogen, $–OR^G$, $–SR^G$, -$C(O)OR^G$, -$C(O)N(R^G)(R^H)$, -$N(R^G)(R^H)$,or -$C(NR^I)N(R^G)(R^H)$; each of R^{13a} and R^{13b} is hydrogen, halogen, -OH, C_1-C_6 alkyl, C_1-C_6 alkoxy, cycloalkyl, heterocyclyl, cycloalkylalkyl, heterocyclylalkyl, aryl, heteroaryl, aralkyl, or heteroaralkyl, wherein each C_1-C_6 alkyl, C_1-C_6 alkoxy, cycloalkyl, heterocyclyl, cycloalkylalkyl, heterocyclylalkyl, aryl, heteroaryl, aralkyl, or heteroaralkyl is substituted with 0-6 independent occurrences of halogen, $–OR^G$, $–SR^G$, -$C(O)OR^G$, -$C(O)N(R^G)(R^H)$, -$N(R^G)(R^H)$,or -

$C(NR^I)N(R^G)(R^H)$; each R^{15} and R^{16} is independently halogen, -OH, C_1-C_6 alkyl, C_1-C_6 alkoxy, cycloalkyl, heterocyclyl, cycloalkylalkyl, heterocyclylalkyl, aryl, heteroaryl, aralkyl, or heteroaralkyl, wherein each C_1-C_6 alkyl, C_1-C_6 alkoxy, cycloalkyl, heterocyclyl, cycloalkylalkyl, heterocyclylalkyl, aryl, heteroaryl, aralkyl, or heteroaralkyl is substituted with 0-6 independent occurrences of halogen, $-OR^G$, $-SR^G$, $-C(O)OR^G$, $-C(O)N(R^G)(R^H)$, $-N(R^G)(R^H)$,or $-C(NR^I)N(R^G)(R^H)$; each R^G, R^H, and R^I is independently hydrogen, C_1-C_6 alkyl, cycloalkyl, heterocyclyl, cycloalkylalkyl, heterocyclylalkyl, aryl, heteroaryl, aralkyl, or heteroaralkyl, wherein each C_1-C_6 alkyl, cycloalkyl, heterocyclyl, cycloalkylalkyl, heterocyclylalkyl, aryl, heteroaryl, aralkyl, or heteroaralkyl is substituted with 0-6 independent occurrences of halogen, –OH, or C_1-C_6 alkoxy; and each of m, n, and p is independently 0, 1, 2, 3, 4, or 5.

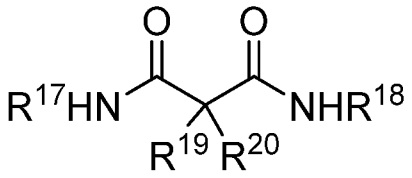
In some embodiments, ring C is aryl (e.g., phenyl). In some embodiments, ring D is heteroaryl (e.g., 1,2,4-oxadiazole). In some embodiments, R^{15} is fluoro and n is 1. In some embodiments, p is 0. In some embodiments, m is 1. In some embodiments, R^{14} is $-S(O)_2R^G$ and R^G is chlorophenyl. In some embodiments, R^{13a} is $-CH_2CH_2CF_3$ and R^{13b} is hydrogen. In some embodiments, each R^{11} and R^{12} is independently hydrogen.

In some embodiments, the compound of formula (III) is a compound described in U.S. Patent No. 8,084,477, which is herein incorporated by reference in its entirety. In one embodiment, the compound of formula (III) is BMS-708163, *i.e.*, avagacestat, or (R)-2-((4-chloro-N-(2-fluoro-4-(1,2,4-oxadiazol-3-yl)benzyl)phenyl)sulfonamido)-5,5,5-trifluoropentanamide, or a pharmaceutically acceptable salt thereof. In one embodiment, the compound is



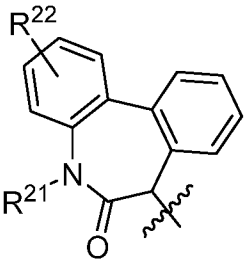
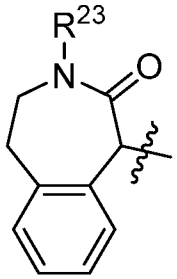
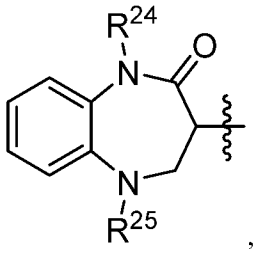
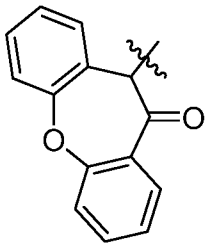
or a pharmaceutically acceptable salt thereof.

In some embodiments, the compound is a compound of formula (IV);



(IV)

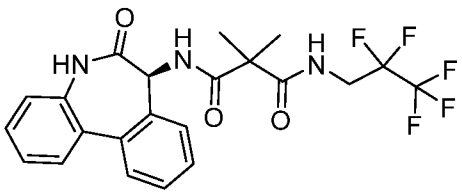
wherein R^{17} is selected from

a.  , b.  , c.  , and d. 

R^{18} is lower alkyl, lower alkinyl, -$(CH_2)_n$-O-lower alkyl, -$(CH_2)_n$-S-lower alkyl, -$(CH_2)_n$-CN, -(CR'R")$_n$-CF_3, -(CR'R")$_n$-CHF_2, -(CR'R")$_n$-CH_2F, -$(CH_2)_n$, -C(O)O-lower alkyl, -$(CH_2)_n$-halogen, or is -(CH2)$_n$-cycloalkyl optionally substituted by one or more substituents selected from the group consisting of phenyl, halogen and CF_3; R',R" are each independently hydrogen, lower alkyl, lower alkoxy, halogen or hydroxy; R^{19}, R^{20} are each independently hydrogen, lower alkyl, lower alkoxy, phenyl or halogen; R^{21} is hydrogen, lower alkyl, -$(CH2)_n$-CF_3 or -$(CH_2)_n$-cycloalkyl; R^{22} is hydrogen or halogen; R^{23} is hydrogen or lower alkyl; R^{24} is hydrogen, lower alkyl, lower alkinyl, -$(CH2)_n$-CF_3, -$(CH_2)_n$-cycloalkyl or -$(CH2)_n$-phenyl optionally substituted by halogen; R^{25} is hydrogen, lower alkyl, -C(O)H, -C(O)-lower alkyl, -C(O)-CF_3, -C(O)-CH_2F, -C(O)-CHF_2, -C(O)-cycloalkyl, -C(O)-$(CH_2)_n$ -O-lower alkyl, -C(O)O-$(CH_2)_n$–cycloalkyl, -C(O)-phenyl optionally substituted by one or more substituents selected from the group consisting of halogen and -C(O)O-lower alkyl, or is -$S(O)_2$-lower alkyl, -$S(O)_2$-CF_3, -$(CH2)_n$-cycloalkyl or is -$(CH_2)_n$-phenyl optionally substituted by halogen; n is 0, 1, 2, 3 or 4.

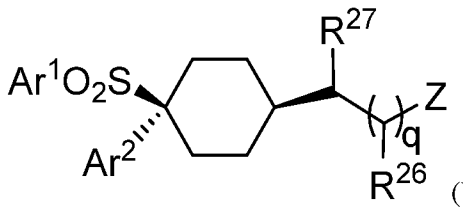
In some embodiments, R^{17} is 5,7-dihydro-6H-dibenzo[b,d]azepin-6-onyl. In some embodiments, each R^{19} and R^{20} is independently –CH_3. In some embodiments, R^{18} is $CH_2CF_2CF_3$.

In some embodiments, the compound of formula (IV) is described in a compound described in U.S. Patent No. 7,160,875, which is herein incorporated by reference in its entirety. In one embodiment, the compound is RO4929097, *i.e.*, (S)-2,2-dimethyl-N1-(6-oxo-6,7-dihydro-5H-dibenzo[b,d]azepin-7-yl)-N3-(2,2,3,3,3-pentafluoropropyl)malonamide, or a pharmaceutically acceptable salt thereof. In one embodiment, the compound is



or a pharmaceutically acceptable salt thereof.

In some embodiments, the compound is a compound of formula (V) or a pharmaceutically acceptable salt thereof;

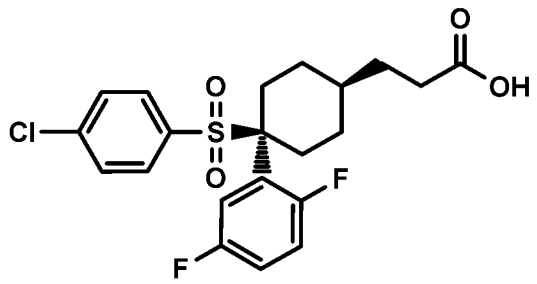


(V)

wherein q is 0 or 1; Z represents halogen, -CN, -NO_2, -N_3, -CF_3, -OR^{2a}, -$N(R^{2a})_2$, -CO_2R^{2a}, -$OCOR^{2a}$, -COR^{2a}, -$CON(R^{2a})_2$, -$OCON(R^{2a})_2$, -$CONR^{2a}(OR^{2a})$, -$CON(R^{2a})N(R^{2a})_2$, -$CONHC(=NOH)R^{2a}$, heterocyclyl, phenyl or heteroaryl, said heterocyclyl, phenyl or heteroaryl bearing 0-3 substituents selected from halogen, -CN, -NO_2, -CF_3, -OR^{2a}, -$N(R^{2a})_2$, -CO_2R^{2a}, -COR^{2a}, -$CON(R^{2a})_2$ and C_{1-4} alkyl; R^{27} represents H, C_{1-4} alkyl, or OH; R^{26} represents H or C_{1-4} alkyl; with the proviso that when m is 1, R^{26} and R^{27} do not both represent C_{1-4} alkyl; Ar^1 represents C_{6-10} aryl or heteroaryl, either of which bears 0-3 substituents independently selected from halogen, -CN, -NO_2, -CF_3, -OH, -OCF_3, C_{1-4} alkoxy or C_{1-4} alkyl which optionally bears a substituent selected from halogen, CN, NO_2, CF_3, OH and C_{1-4} alkoxy; Ar^2 represents C_{6-10} aryl or heteroaryl, either of which bears 0-3 substituents independently selected from halogen, -CN, -NO_2, -CF_3, -OH, -OCF_3, C_{1-4} alkoxy or C_{1-4} alkyl which optionally bears a substituent selected from halogen, -CN, -NO_2, -CF_3, -OH and C_{1-4} alkoxy; R^{2a} represents H, C_{1-6} alkyl, C_{3-6} cycloalkyl, C3 _6cycloalkyl, C_{1-6} alkyl, C_{2-6} alkenyl, any of which optionally bears a substituent selected from halogen, -CN, -NO_2, -CF_3, -OR^{2b}, -CO_2R^{2b}, -$N(R^{2b})_2$, -$CON(R^{2b})_2$, Ar and COAr; or R^{2a} represents Ar; or two R^{2a} groups together with a nitrogen atom to which they are mutually attached may complete an N-heterocyclyl group bearing 0-4 substituents independently selected from =O, =S, halogen, C_{1-4} alkyl, -CN, -NO_2, -CF_3, -OH, C_{1-4} alkoxy, C_{1-4} alkoxycarbonyl, CO_2H, amino, C_{1-4} alkylamino, di(C_{1-4} alkyl)amino, carbamoyl, Ar and COAr; R^{2b} represents H, C_{1-6} alkyl, C_{3-6} cycloalkyl, C_{3-6} cycloalkylC_{1-6} alkyl, C_{2-6} alkenyl, any of which optionally bears a substituent selected from halogen, -CN, -NO_2, -CF_3, -OH, C_{1-4} alkoxy, C_{1-4} alkoxycarbonyl, -CO_2H, amino, C_{1-4} alkylamino, di(C_{1-4} alkyl)amino, carbamoyl, Ar and COAr; or R^{2b} represents Ar; or two R^{2b} groups together with a nitrogen atom to which they are mutually attached may complete an N-heterocyclyl group bearing 0-4 substituents independently selected from =O, =S, halogen, C_{1-4} alkyl, -CN, -NO_2, CF3 , -OH, C_{1-4} alkoxy, C_{1-4} alkoxycarbonyl, -CO_2H, amino, C_{1-4} alkylamino, di(C_{1-4} alkyl)amino, carbamoyl, Ar and COAr; Ar represents phenyl or heteroaryl bearing 0-3 substituents selected from halogen, C_{1-4} alkyl, -CN, -NO_2, -CF_3, -OH, C_{1-4} alkoxy, C_{1-4} alkoxycarbonyl, amino, C_{1-4} alkylamino, di(C_{1-4} alkyl)amino, carbamoyl, C_{1-4} alkylcarbamoyl and di(C_{1-4} alkyl)carbamoyl.

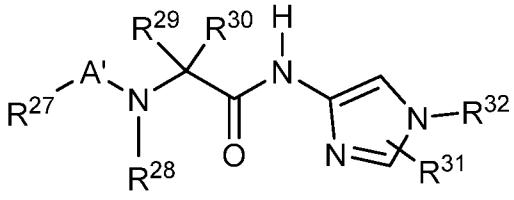
In some embodiments, q is 1. In some embodiments, Z is CO_2H. In some embodiments, each of R^{27} and R^{26} is independently hydrogen. In some embodiments, Ar^1 is chlorophenyl. In some embodiments, Ar^2 is difluorophenyl.

In some embodiments, the compound of formula (V) is described in U.S. Patent No. 6,984,663, which is herein incorporated by reference in its entirety. In one embodiment, the compound of formula (V) is MK-0752, *i.e.*, 3-((1S,4R)-4-((4-chlorophenyl)sulfonyl)-4-(2,5-difluorophenyl)cyclohexyl)propanoic acid, or a pharmaceutically acceptable salt thereof. In one embodiment, the compound is



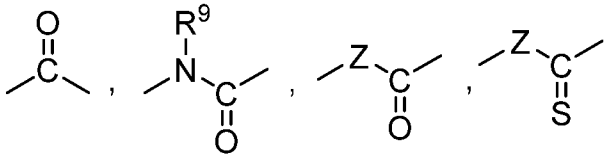
or a pharmaceutically acceptable salt thereof.

In some embodiments, the compound is a compound of formula (VI):



(VI)

or a pharmaceutically acceptable salt thereof, wherein A' is absent or selected from



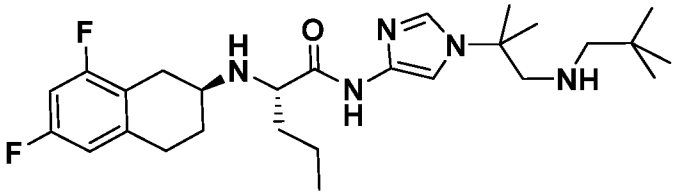
, and $-S(O)_2-$; Z is selected from $-CH_2$, -CH(OH), -$CH(C_1-C_6$ alkyl), -$CH(C_1-C_6$ alkoxy), -$CH(NR^{33}R^{34})$, -$CH(CH_2(OH))$, -$CH(CH(C_1-C_4$ alkyl)(OH)) and -$CH(C(C_1-C_4$ alkyl)$(C_1-C_4$ alkyl)(OH)), for example -$CH(C(CH_3)(CH_3)(OH))$ or -$CH(C(CH_3)(CH_2CH_3)(OH))$; R^{27} is selected from C_1-C_{20} alkyl, C_2-C_{20} alkenyl, C_2-C_{20} alkynyl, C_1-C_{20} alkoxy, C_2-C_{20} alkenoxy, C_1-C_{20} hydroxyalkyl, C_3-C_8 cycloalkyl, benzo(C_3-C_8 cycloalkyl), benzo(C_3-C_8 heterocycloalkyl), C_4-C_8 cycloalkenyl, (C_5-C_{11})bi- or tricycloalkyl, benzo(C_5-C_{11})bi- or tricycloalkyl, C_7-C_{11}tricycloalkenyl, (3-8 membered) heterocycloalkyl, C_6-C_{14} aryl and (5-14 membered) heteroaryl, wherein each hydrogen atom of said alkyl, alkenyl, alkynyl, alkoxy and alkenoxy is optionally independently replaced with halo, and wherein said cycloalkyl, benzo(C_3-C_8 cycloalkyl), cycloalkenyl, (3-8 membered) heterocycloalkyl, C_6-C_{14} aryl and (5-14 membered) heteroaryl is optionally independently substituted with from one to four substituents independently selected from C_1-C_{10} alkyl

optionally substituted with from one to three halo atoms, C_1-C_{10} alkoxy optionally substituted with from one to three halo atoms, C_1-C_{10} hydroxyalkyl, halo, preferably fluorine, -OH, -CN, - $NR^{33}R^{34}$, -$C(=O)NR^{33}R^{34}$, -$C(=O)R^{35}$, C_3-C_8 cycloalkyl and (3-8 membered) heterocycloalkyl; R^{28} is selected from hydrogen, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_3-C_8 cycloalkyl and C_5-C_8 cycloalkenyl, wherein R^{28} is optionally independently substituted with from one to three substituents independently selected from C_1-C_4 alkyl optionally substituted with from one to three halo atoms, C_1-C_4 alkoxy optionally substituted with from one to three halo atoms, halo and -OH; or R^{27} and R^{28} together with the A' group when present and the nitrogen atom to which R^{28} is attached, or R^{27} and R^{28} together with the nitrogen atom to which R^{27} and $R^{28}$are attached when A' is absent, may optionally form a four to eight membered ring; R^{29} is selected from hydrogen, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_3-C_6 cycloalkyl, C_5-C_6 cycloalkenyl and (3-8 membered) heterocycloalkyl, wherein said alkyl, alkenyl, alkynyl, cycloalkyl, cycloalkenyl and heterocycloalkyl are each optionally independently substituted with from one to three substituents independently selected from C_1-C_4alkoxy, halo, -OH-S(C_1-C_4)alkyl and (3-8 membered) heterocycloalkyl; R^{30} is hydrogen, C_1-C_6 alkyl or halo; or R^{29} and R^{30} may together with the carbon atom to which they are attached optionally form a moiety selected from cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, morpholino, piperidino, pyrrolidino, tetrahydrofuranyl and perhydro-2H-pyran, wherein said moiety formed by R^{29} and R^{30} is optionally substituted with from one to three substituents independently selected from C_1-C_6 alkyl optionally substituted with from one to three halo atoms, C_1-C_6 alkoxy optionally substituted with from one to three halo atoms, halo, -OH, -CN and allyl; R^{31} is selected from hydrogen, C_1-C_6 alkyl, C_2-C_6 alkylene, C_1-C_6 alkoxy, halo, -CN, C_3-C_{12} cycloalkyl, C_4-C_{12} cycloalkenyl and C_6-C_{10} aryl, (5-10 membered) heteroaryl, wherein said alkyl, alkylene and alkoxy of $R^{31}$are each optionally independently substituted with from one to three substituents independently selected from halo and -CN, and wherein said cycloalkyl, cycloalkenyl and aryl and heteroaryl of $R^{31}$are each optionally independently substituted with from one to three substituents independently selected from C_1-C_4 alkyl optionally substituted with from one to three halo atoms, C_1-C_4 alkoxy optionally substituted with from one to three halo atoms, halo and -CN; R^{32} is selected from hydrogen, C_1-C_{20} alkyl, C_1-C_{20} alkoxy, C_1-C_{20} hydroxyalkyl, C_3-C_{12} cycloalkyl, C_4-C_{12} cycloalkenyl, (C_5-C_{20}) bi- or tricycloalkyl, (C_7-C_{20})bi- or tricycloalkenyl, (3-12 membered) heterocycloalkyl, (7-20 membered) hetero bi- or heterotricycloalkyl, C_6-C_{14} aryl and (5-15 membered) heteroaryl, wherein R^{32} is optionally independently substituted with from one to four substituents independently selected from C_1-C_{20} alkyl optionally substituted with from one to three halo atoms, C_1-C_{20} alkoxy, -OH, -CN, -NO_2, -$NR^{33}R^{34}$, -$C(=O)NR^{33}R^{34}$, -$C(=O)R^{35}$, -$C(=O)OR^{35}$, -$S(O)_nNR^{33}R^{34}$, -$S(O)_nR^{35}$, C_3-C_{12} cycloalkyl, (4-12 membered) heterocycloalkyl optionally substituted with from one to three OH or halo groups, (4-12 membered) heterocycloalkoxy, C_6-C_{14} aryl, (5-15 membered) heteroaryl, C_6-C_{12} aryloxy and (5-12 membered) heteroaryloxy; or R^{33} and R^{34} may together with the carbon and nitrogen atoms to which

they are respectively attached optionally form a (5-8 membered) heterocycloalkyl ring, a (5-8 membered) heterocycloalkenyl ring or a (6-10 membered) heteroaryl ring, wherein said heterocycloalkyl, heterocycloalkenyl and heteroaryl rings are each optionally independently substituted with from one to three substituents independently selected from halo, C_1-C_6 alkyl, optionally substituted with from one to three halo atoms, C_1-C_6 alkoxy optionally substituted with from one to three halo atoms, C_1-C_6 hydroxyalkyl, -OH, $-(CH_2)_{zero\text{-}10}NR^{33}R^{34}$, $-(CH_2)_{zero\text{-}10}C(=O)NR^{33}R^{34}$, $-S(O)_2NR^{33}R^{34}$ and C_3-C_{12} cycloalkyl; R^{33} and R^{34} are each independently selected from hydrogen, C_1-C_{10} alkyl wherein each hydrogen atom of said C_1-C_{10} alkyl is optionally independently replaced with a halo atom, preferably a fluorine atom, C_2-C_{10} alkenyl, C_2-C_{10} alkynyl, C_1-C_6 alkoxy wherein each hydrogen atom of said C_1-C_6 alkoxy is optionally independently replaced with a halo atom, C_2-C_6 alkenoxy, C_2-C_6 alkynoxy, -C(=O)R11, $-S(O)_nR11$, C_3-C_8 cycloalkyl, C_4-C_8 cycloalkenyl, (C_5-C_{11})bi- or tricycloalkyl, (C_7-C_{11})bi- or tricycloalkenyl, (3-8 membered) heterocycloalkyl, C_6-C_{14} aryl and (5-14 membered) heteroaryl, wherein said alkyl and alkoxy are each optionally independently substituted with from one to three substituents independently selected from halo and -OH, and wherein said cycloalkyl, cycloalkenyl, bi- or tricycloalkyl, bi- or tricycloalkenyl, heterocycloalkyl, aryl and heteroaryl are each optionally independently substituted with from one to three substituents independently selected from halo, -OH, C_1-C_6 alkyl optionally independently substituted with from one to six halo atoms, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_1-C_6 alkoxy, C_2-C_6 alkenoxy, C_2-C_6 alkynoxy and C_1-C_6 hydroxyalkyl; or $NR^{33}R^{34}$ may form a (4-7 membered) heterocycloalkyl, wherein said heterocycloalkyl optionally comprises from one to two further heteroatoms independently selected from N, O, and S, and wherein said heterocycloalkyl optionally contains from one to three double bonds, and wherein said heterocycloalkyl is optionally independently substituted with from one to three substituents independently selected from C_1-C_6 alkyl optionally substituted with from one to six halo atoms, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_1-C_6 alkoxy, C_2-C_6 alkenoxy, C_2-C_6 alkynoxy, C_1-C_6 hydroxyalkyl, C_2-C_6hydroxyalkenyl, C_2-C_6hydroxyalkynyl, halo, -OH, -CN, $-NO_2$, $-C(=O)R^{35}$, $-C(=O)OR^{35}$, $-S(O)_nR^{35}$ and $-S(O)_nNR^{33}R^{34}$; R^{35} is selected from H, C_1-C_8 alkyl, C_3-C_8 cycloalkyl, C_4-C_8 cycloalkenyl, (C_5-C_{11})bi- or tricycloalkyl, -(C_7-C_{11})bi- or tricycloalkenyl, (3-8 membered) heterocycloalkyl, C_6-C_{10} aryl and (5-14 membered) heteroaryl, wherein said alkyl of R^{35} is optionally independently substituted with from one to three substituents independently selected from -OH, -CN and C_3-C_8 cycloalkyl, and wherein each hydrogen atom of said alkyl is optionally independently replaced with a halo atom, preferably a fluorine atom, and wherein said cylcoalkyl, cycloalkenyl, heterocycloalkyl, aryl and hetereoaryl of R^{35} are each optionally independently substituted with from one to three substituents independently selected from halo, C_1-C_8 alkyl optionally substituted with from one to three halo atoms, -OH, -CN and C_3-C_8cycloalkyl; n is in each instance an integer independently selected from zero, 1, 2 and 3; and the pharmaceutically acceptable salts of such compounds.

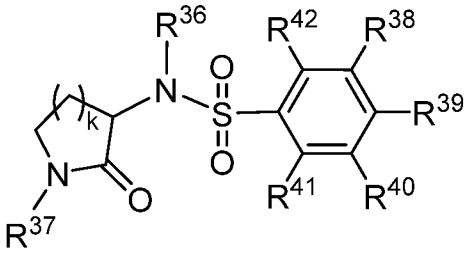
In some embodiments, the compound of formula (VI) is described in U.S. Patent No. 7,795,447, which is herein incorporated by reference in its entirety. In one embodiment, the compound of formula (VI) is PF-3084014, *i.e.*, nirogacestat or (S)-2-(((S)-6,8-difluoro-1,2,3,4-tetrahydronaphthalen-2-yl)amino)-N-(1-(2-methyl-1-(neopentylamino)propan-2-yl)-1H-imidazol-4-yl)pentanamide, or a pharmaceutically acceptable salt thereof.

In some embodiments, the compound is



or a pharmaceutically acceptable salt thereof.

In some embodiments, the compound is a compound of formula (VII):



(VII)

or a pharmaceutically acceptable salt thereof wherein k is 1, 2, or 3; R^{36} is aryl C_1-C_8 alkyl, aryl C_2-C_6 alkenyl, or arylalkynyl, wherein the aryl group is substituted with 0-5 occurrences of C_1-C_6 alkyl, C_1-C_6 alkoxy, halogen, haloalkyl, haloalkoxy, heteroaryl, heteroaryl(C_1-C_6)alkoxy, arylalkoxy, aryloxy, C_1-C_6 alkoxycarbonyl, -OCH_2CH_2O-, -OCH_2O-, -$C(O)NR^{43}R^{44}$, -NHR′, -NR′R″, -$N(R^{16})C(O)R^{17}$, heterocycloalkyl, phenyl, aryl C_1-C_6 alkanoyl, phenylalkoxy, phenyloxy, CN, -SO_2-aryl, -$S(O)_nR^{25}$, -(C_1-C_4 alkyl)-$S(O)_xR^{25}$, -(C_1-C_4 alkyl)-SO_2-aryl, OH, C_1-C_6 thioalkoxy, C_2-C_6 alkenyl, -OSO_2-aryl, or CO_2H, wherein each heteroaryl is independently substituted with 0-3 occurrences of C_1-C_6 alkyl, heteroaryl substituted with 0-2 occurrences of halogen, alkyl, alkoxy, haloalkyl, haloalkoxy, alkoxyalkyl or CN, C_1-C_6 alkoxy, C_1-C_4 alkoxy C_1-C_4 alkyl, C_3-C_6 cycloalkyl, halogen, or phenyl substituted with 0-5 occurrences of halogen, OH, C_1-C_6 alkyl, C_1-C_4 alkoxy, CF_3, OCF_3, CN, or C_1-C_6 thioalkoxy, wherein each heterocycloalkyl and aryl are independently substituted with 0-2 occurrences of halogen, alkyl, alkoxy, haloalkyl, haloalkoxy, alkoxyalkyl or CN, C_1-C_6 alkyl, C_1-C_6 alkoxy, C_1-C_4 alkoxy C_1-C_4 alkyl, C_3-C_6 cycloalkyl, halogen, or phenyl substituted with 0-5 occurrences of halogen, OH, C_1-C_6 alkyl, C_1-C_4 alkoxy, CF_3, OCF_3, CN, or C_1-C_6 thioalkoxy; R^{16} is hydrogen or C_1-C_6 alkyl; R^{17} is C_1-C_6 alkyl, aryl, heteroaryl, C_1-C_6 alkoxy, OH, aryloxy, heteroaryloxy, aryl(C_1-C_6)alkoxy, -$NR^{18}R^{19}$,

cycloalkyl, or arylalkyl, wherein the cyclic portions of each are independently substituted with 0-5 occurrences of alkyl, alkoxy, halo, haloalkyl, haloalkoxy, CN, NH_2, NH(alkyl), N(alkyl) (alkyl), CO_2H, or C_1-C_6 alkoxycarbonyl; R^{18} and R^{19} are independently hydrogen, C_1-C_6 alkyl, aryl, heteroaryl, heterocycloalkyl or aryl(C_1-C_6)alkyl, wherein the cyclic portions of each are substituted with 0-3 occurrences of alkyl, alkoxy, halogen, hydroxyl, CF_3, or OCF_3; each R′ is independently hydrogen, C_1-C_6 alkyl, aryl, aryl(C_1-C_4)alkyl, C_1-C_6 alkanoyl, C_3-C_8 cycloalkyl, aryl(C_1-C_6)alkanoyl, heterocycloalkyl, heteroaryl(C_1-C_4)alkyl, -SO_2-alkyl, -SO_2-aryl, -SO_2-heteroaryl, heterocycloalkyl(C_1-C_6)alkanoyl, or heteroaryl(C_1-C_6)alkanoyl, wherein the alkyl portion of the alkyl and alkanoyl groups are optionally substituted with halogen or C_1-C_6 alkoxy and the aryl and heteroaryl groups are optionally substituted with alkyl, alkoxy, halogen, haloalkyl, haloalkoxy; each R″ is independently hydrogen or C_1-C_6 alkyl, wherein the alkyl group is optionally substituted with halogen;

R^{36} is C_3-C_7 cycloalkyl(C_1-C_6 alkyl) wherein the cyclic portion is substituted with 0-5 occurrences of halogen, C_1-C_6 alkyl, OH, alkoxycarbonyl, or C_1-C_6 alkoxy; or R^{36} is C_1-C_{14} alkyl, C_2-C_{16} alkenyl, or C_2-C_8 alkynyl, each of which is substituted 0-5 occurrences of OH, halogen, C_1-C_6 alkoxy, aryl, arylalkoxy, aryloxy, heteroaryl, heterocycloalkyl, aryl(C_1-C_6)alkyl, -CO_2(C_1-C_6 alkyl), -NR′R″, C_1-C_6 thioalkoxy, -$NHS(O)_xR^{25}$, -N(C_1-C_6 alkyl)-$S(O)_nR^{25}$, -$S(O)_xR^{25}$, -$C(O)NR^{43}R^{44}$, -$N(R^{16})C(O)NR^{16}R^{17}$, or -$N(R_{16})C(O)R^{17}$; wherein the above aryl groups are substituted with 0-3 occurrences of OH, C_1-C_6 alkoxy, C_1-C_6 alkyl, or halogen; R^{43} and R^{44} are independently hydrogen, C_1-C_6 alkyl, aryl, arylalkyl, heteroaryl, heteroarylalkyl, heterocycloalkyl, heterocycloalkylalkyl, arylalkanoyl, alkenyl, cycloalkyl, alkynyl, cycloalkenyl, pyridyl, imidazolyl, thiazolyl, oxazolyl, or indolyl, wherein each alkyl is substituted with 0-3 occurrences of NH_2, NH(C_1-C_6 alkyl), N(C_1-C_6 alkyl) (C_1-C_6 alkyl), OH, C_1-C_6 thioalkoxy, heterocycloalkyl, aryl, heteroaryl, CN, halogen, or alkoxy optionally substituted with OH or phenyl, wherein the aryl, heteroaryl and heterocycloalkyl groups are substituted with 0-3 occurrences of C_1-C_4 alkyl, C_1-C_4 alkoxy, CF_3, OCF_3, OH, halogen, thioalkoxy, phenyl or heteroaryl; or R^{43}, R^{44}, and the nitrogen to which they are attached form a heterocycloalkyl ring containing from 3 to 7 ring members, wherein the cyclic portions of R^{43} and R^{44} or the heterocyclic ring formed from R^{43}, R^{44}, and the nitrogen to which they are attached are substituted with 0-3 occurrences of alkyl, alkoxy, halo, OH, thioalkoxy, NH_2, NH(C_1-C_6 alkyl), N(C_1-C_6 alkyl) (C_1-C_6 alkyl), CF_3, OCF_3, phenyl optionally substituted with a halogen, -(C_1-C_4 alkyl)-N(H or C_1-C_4 alkyl)-phenyl, C_1-C_4 hydroxyalkyl, arylalkoxy, arylalkyl, arylalkanoyl, $C(O)NH_2$, C(O)NH(C_1-C_6 alkyl), C(O)N(C_1-C_6 alkyl) (C_1-C_6 alkyl), heterocycloalkylalkyl, C_1-C_6 alkoxycarbonyl, C_2-C_6 alkanoyl, heteroaryl, or -SO_2(C_1-C_6 alkyl); x is 0, 1, or 2; R^{25} is C_1-C_6 alkyl, OH, $NR^{26}R^{27}$; R^{26} and R^{27} are independently hydrogen, C_1-C_6 alkyl, phenyl(C_1-C_4 alkyl), aryl, or heteroaryl; or R^{26}, R^{27} and the nitrogen to which they are attached form a heterocycloalkyl ring;

R^{36} is heteroaryl(C_1-C_6)alkyl wherein the cyclic portion is substituted 0-5 occurrences of halogen, C_1-C_6 alkyl, C_1-C_6 alkoxy, C_1-C_4 haloalkyl, C_1-C_4 haloalkoxy, aryl, arylalkyl, aryloxy, heteroaryl, -SO_2-aryl, -$S(O)_xR_{25}$, (C_1-C_4 alkyl)-$S(O)_xR_{25}$, CN, C_1-C_6 thioalkoxy, C_1-C_6 alkoxycarbonyl, -NR′R″, -C(O)NR′R″, heterocycloalkyl, wherein the above aryl groups are substituted with 0-4 occurrences of halogen, C_1-C_6 alkyl, C_1-C_6 alkoxy, C_1-C_4 haloalkyl, C_1-C_4 haloalkoxy, or CN; wherein the above heteroaryl and heterocycloalkyl groups are substituted with 0-3 occurrences of halogen, CF_3, (C_1-C_4)alkyl, C_1-C_6 thioalkoxy, OH, C_1-C_4 hydroxyalkyl, or C_1-C_4 alkoxy; or

R^{36} is heterocycloalkyl(C_1-C_6 alkyl) wherein the cyclic portion is substituted with 0-3 occurrences of halogen, C_1-C_6 alkyl, C_1-C_6 alkoxy, C_1-C_4 haloalkyl, C_1-C_4 haloalkoxy, aryl, arylalkyl, aryloxy, heteroaryl, -SO_2-aryl, -$S(O)_xR^{25}$, (C_1-C_4 alkyl)-$S(O)_xR^{25}$, CN, C_1-C_6 thioalkoxy, C_1-C_6 alkoxycarbonyl, -NR′R″, -C(O)NR′R″, heterocycloalkyl;

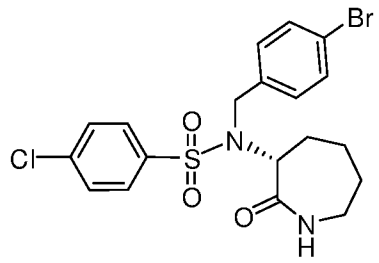
R^{37} is hydrogen, C_1-C_6 alkyl, or phenyl(C_1-C_4)alkyl; R^{38} is hydrogen, halogen, C_1-C_6 alkyl, C_1-C_6 alkoxy, C_1-C_6 haloalkyl, CN; R^{39} is hydrogen, halogen, C_1-C_6 alkyl optionally substituted with -CO_2-(C_1-C_6 alkyl), C_1-C_6 alkoxy, C_1-C_6 haloalkyl, C_1-C_6 haloalkoxy, CN, aryloxy, isocyanato, -SO_2(C_1-C_6 alkyl), -NHR′, -NR′R″, C_1-C_6 alkanoyl, heteroaryl, aryl; or

R^{38} and R^{39} and the carbons to which they are attached form a heterocycloalkyl ring which is substituted with 0-3 occurrences of C_1-C_4 alkyl, C_1-C_4 alkoxy, halogen, or C_1-C_4 alkanoyl wherein the alkanoyl group is substituted with 0-3 halogen atoms; R^{40} is hydrogen, -SO_2NR′R″, halogen; or R^{39} and R^{40} and the carbons to which they are attached form a benzo ring; or R^{39} and R^{40} and the carbons to which they are attached form a 1-oxa-2,3-diazacyclopentyl ring;

R^{40} and R^{41} are independently hydrogen or F; or R^{40}, R^{41}, and the carbons to which they are attached for a 1,2,5-oxadiazolyl ring; or R^{40}, R^{41}, and the carbons to which they are attached form a naphthyl ring.

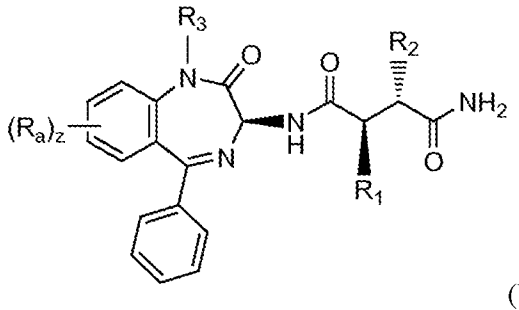
In some embodiments, R^{36} is 4-bromobenzyl. In some embodiments, R^{37} is hydrogen. In some embodiments, k is 2. In some embodiments, each of R^{38}, R^{40}, R^{41}, and R^{42} is independently hydrogen. In some embodiments, R^{39} is chloro.

In some embodiments, the compound of formula (VI) is described in U.S. Patent No. 7,939,657, which is herein incorporated by reference in its entirety. In one embodiment, the compound of formula (VI) is ELN-318463, *i.e.*, HY-50882 or (R)-N-(4-bromobenzyl)-4-chloro-N-(2-oxoazepan-3-yl)benzenesulfonamide, or a pharmaceutically acceptable salt thereof. In some embodiments, the compound is



or a pharmaceutically acceptable salt thereof.

In some embodiments, the compound is a compound of formula (VII):

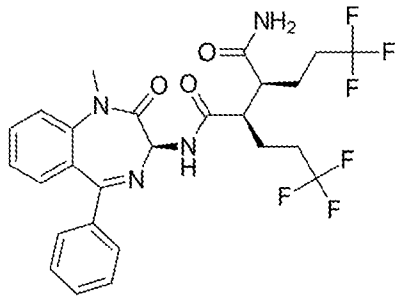


(VII)

or a pharmaceutically acceptable salt thereof, wherein R_1 is $-CH_2CF_3$ or $-CH_2CH_2CF_3$; R_2 is $-CH_2CF_3$, $-CH_2CH_2CF_3$, or $-CH_2CH_2CH_2CF_3$; R_3 is hydrogen or $-CH_3$; each R_a is independently F, CI, -CN, $-OCH_3$, and/or $-NHCH_2CH_2OCH_3$; and z is 0, 1, or 2.

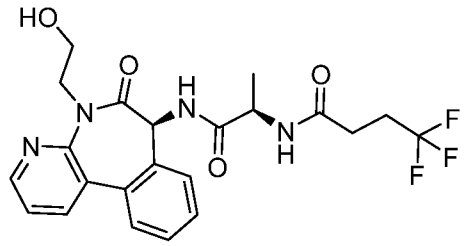
In some embodiments, R_1 is $-CH_2CH_2CF_3CH_2CH_2CF_3$. In some embodiments, R_2 $-CH_2CH_2CF_3$. In some embodiments, R_3 is $-CH_3$. In some embodiments, z is 0.

In some embodiments, the compound of formula (VII) is described in U.S. Patent No. 8,629,136, which is herein incorporated by reference in its entirety. In one embodiment, the compound of formula (VII) is BMS-906024, *i.e.*, (2R,3S)-*N*-[(3S)-1-methyl-2-oxo-5-phenyl-2,3-dihydro-1*H*-1,4-benzodiazepin-3-yl]-2,3-bis(3,3,3-trifluoropropyl)succinamide, or a pharmaceutically acceptable salt thereof. In one embodiment, the compound is



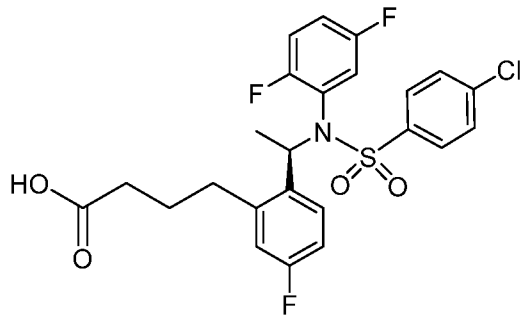
or a pharmaceutically acceptable salt thereof.

In some embodiments, the compound is described in U.S. Patent No. 8,629,136, which is herein incorporated by reference in its entirety. In one embodiment, the compound is LY3039478, *i.e.*, crenigacestat or 4,4,4-trifluoro-N-((R)-1-(((S)-5-(2-hydroxyethyl)-6-oxo-6,7-dihydro-5H-benzo[d]pyrido[2,3-b]azepin-7-yl)amino)-1-oxopropan-2-yl)butanamide, or a pharmaceutically acceptable salt thereof. In some embodiments, the compound is:



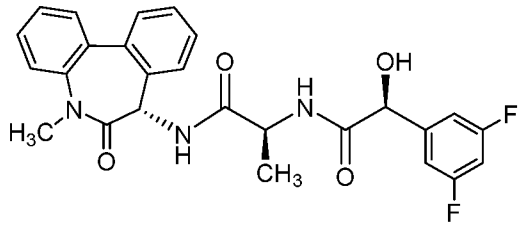
or a pharmaceutically acceptable salt thereof.

In some embodiments, the compound is BMS-299897, *i.e.*, 2-[(1*R*)-1-[[(4-chlorophenyl)sulfonyl](2,5-difluorophenyl)amino]ethyl-5-fluorobenzenebutanoic acid or a pharmaceutically acceptable salt thereof. In some embodiments, the compound is



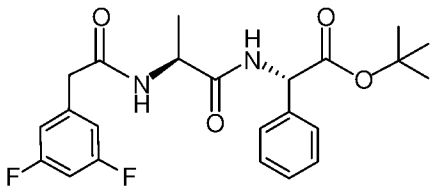
or a pharmaceutically acceptable salt thereof.

In some embodiments, the compound is LY-411575, i.e., LSN-411575, (S)-2-((S)-2-(3,5-difluorophenyl)-2-hydroxyacetamido)-N-((S)-5-methyl-6-oxo-6,7-dihydro-5H-dibenzo[b,d]azepin-7-yl)propanamide, or a pharmaceutically acceptable salt thereof. In some embodiments, the compound is



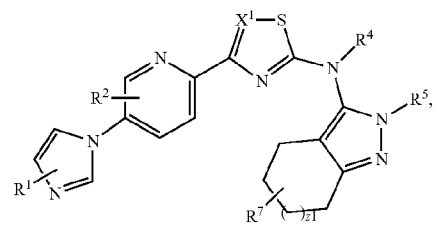
or a pharmaceutically acceptable salt thereof.

In some embodiments, the compound is DAPT, *i.e.*, N-[(3,5-difluorophenyl)acetyl]-L-alanyl-2-phenyl]glycine-1,1-dimethylethyl ester or a pharmaceutically acceptable salt thereof. In some embodiments, the compound is

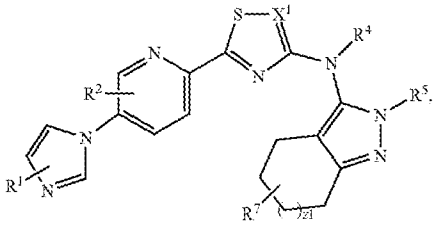


or a pharmaceutically acceptable salt thereof.

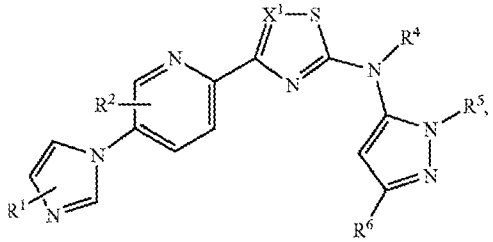
In some embodiments, the compound is a compound of the following formulae:



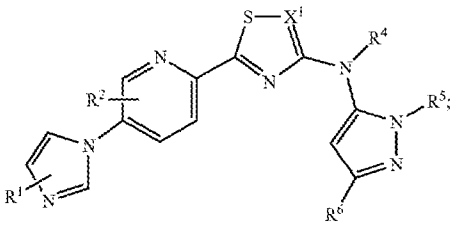
(VIII-a),



(VIII-b),



(VIII-c),

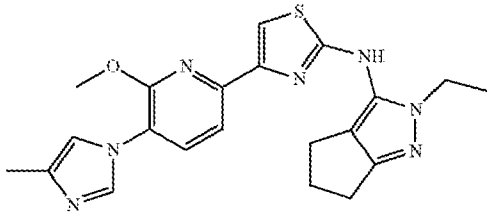


(VIII-d)

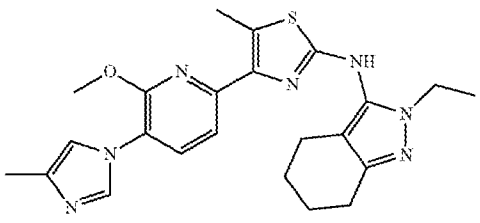
wherein, z1 is 0, 1 or 2; X^1 is $C(R^3)$ or N; R^1 is hydrogen, halogen, —N_3, —CF_3, —CCl_3, —CBr_3, —CI_3, —CN, —CHO, —OR^{1A}, —$NR^{1A}R^{1B}$, —$COOR^{1A}$, —$C(O)NR^{1A}R^{1B}$, —NO_2, —SR^{1A}, —$S(O)_{n1}OR^{1A}$, —$S(O)_{n1}NR^{1A}R^{1B}$, —$NHNR^{1A}R^{1B}$, —$ONR^{1A}R^{1B}$, —$NHC(O)NHNR^{1A}R^{1B}$, substituted or unsubstituted alkyl, substituted or unsubstituted heteroalkyl, substituted or unsubstituted cycloalkyl, substituted or unsubstituted heterocycloalkyl, substituted or unsubstituted aryl, or substituted or unsubstituted heteroaryl; R^2 is hydrogen, halogen, —N_3, —CF_3, —CCl_3, —CBr_3, —CI_3, —CN, —CHO, —OR^{2A}, —$NR^{2A}R^{2B}$, —$COOR^{2A}$, —$C(O)NR^{2A}R^{2B}$, —NO_2, —SR^{2A}, —$S(O)_{n2}R^{2A}$, —$S(O)_{n2}OR^{2A}$, —$S(O)_{n2}NR^{2A}R^{2B}$, —$NHNR^{2A}R^{2B}$, —$ONR^{2A}R^{2B}$, —$NHC(O)NHNR^{2A}R^{2B}$, substituted or unsubstituted alkyl, substituted or unsubstituted heteroalkyl, substituted or unsubstituted cycloalkyl, substituted or unsubstituted heterocycloalkyl, substituted or unsubstituted aryl, or substituted or unsubstituted heteroaryl; R^3 is hydrogen, halogen, —N_3, —CF_3, —CCl_3, —CBr_3, —CI_3, —CN, —CHO, —OR^{3A}, —$NR^{3A}R^{3B}$, —$COOR^{3A}$, —$C(O)NR^{3A}R^{3B}$, —NO_2, —SR^{3A}, —$S(O)_{n3}R^{3A}$, —$S(O)_{n3}OR^{3A}$, —$S(O)_{n3}ONR^{3A}R^{3B}$, —$NHNR^{3A}R^{3B}$, —$ONR^{3A}R^{3B}$, —$NHC(O)NHNR^{3A}R^{3B}$, substituted or unsubstituted alkyl, substituted or unsubstituted heteroalkyl, substituted or unsubstituted cycloalkyl, substituted or unsubstituted heterocycloalkyl, substituted or unsubstituted aryl, or substituted or unsubstituted heteroaryl; R^4 is hydrogen, halogen, —N_3, —CF_3, —CCl_3, —CBr_3, —CI_3, —CN, —CHO, —OR^{4A}, —$NR^{4A}R^{4B}$, —$COOR^{4A}$, —$C(O)NR^{4A}R^{4B}$, —NO_2, —SR^{4A}, —$S(O)_{n4}R^{4A}$, —$S(O)_{n4}OR^{4A}$, —

$S(O)_{n4}NR^{4A}R^{4B}$, —$NHNR^{4A}R^{4B}$, —$ONR^{4A}R^{4B}$, —$NHC(O)NHNR^{4A}R^{4B}$, substituted or unsubstituted alkyl, substituted or unsubstituted heteroalkyl, substituted or unsubstituted cycloalkyl, substituted or unsubstituted heterocycloalkyl, substituted or unsubstituted aryl, or substituted or unsubstituted heteroaryl; R^5 is hydrogen, halogen, —N_3, —CF_3, —CCl_3, —CBr_3, —CI_3, —CN, —CHO, —OR^{5A}, —$NR^{5A}R^{5B}$, —$COOR^{5A}$, —$C(O)NR^{5A}R^{5B}$, —NO_2, —SR^{5A}, —$S(O)_{n5}R^{5A}$, —$S(O)_{n5}OR^{5A}$, —$S(O)_{n5}NR^{5A}R^{5B}$, —$NHNR^{5A}R^{5B}$, —$ONR^{5A}R^{5B}$, —$NHC(O)NHNR^{5A}R^{5B}$, substituted or unsubstituted alkyl, substituted or unsubstituted heteroalkyl, substituted or unsubstituted cycloalkyl, substituted or unsubstituted heterocycloalkyl, substituted or unsubstituted aryl, or substituted or unsubstituted heteroaryl, wherein R^4 and R^5 are optionally joined together to form a substituted or unsubstituted heterocycloalkyl, or substituted or unsubstituted heteroaryl; R^6 is —CF_3, substituted or unsubstituted cyclopropyl, or substituted or unsubstituted cyclobutyl; R^7 is independently hydrogen, halogen, —N_3, —CF_3, —CCl_3, —CBr_3, —CI_3, —CN, —CHO, —OR^{7A}, —$NR^{7A}R^{7B}$, —$COOR^{7A}$, —$C(O)NR^{7A}R^{7B}$, —NO_2, —SR^{7A}, —$S(O)_{n7}R^{7A}$, —$S(O)_{n7}OR^{7A}$, —$S(O)_{n7}NR^{7A}R^{7B}$, —$NHNR^{7A}R^{7B}$, —$ONR^{7A}R^{7B}$, —$NHC(O)NHNR^{7A}R^{7B}$, substituted or unsubstituted alkyl, substituted or unsubstituted heteroalkyl, substituted or unsubstituted cycloalkyl, substituted or unsubstituted heterocycloalkyl, substituted or unsubstituted aryl, or substituted or unsubstituted heteroaryl;R^{1A}, R^{1B}, R^{2A}, R^{2B}, R^{3A}, R^{3B}, R^{4A}, R^{4B}, R^{5A}, R^{5B}, R^{7A} and R^{7B} are independently hydrogen, substituted or unsubstituted alkyl, substituted or unsubstituted heteroalkyl, substituted or unsubstituted cycloalkyl, substituted or unsubstituted heterocycloalkyl, substituted or unsubstituted aryl, or substituted or unsubstituted heteroaryl; and n1, n2, n3, n4, n5 and n7 are independently 1 or 2.

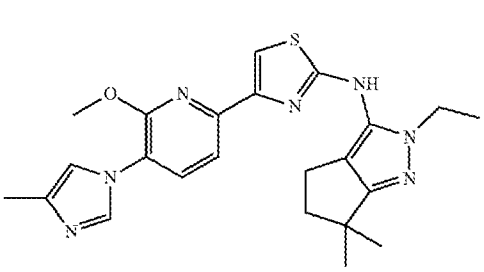
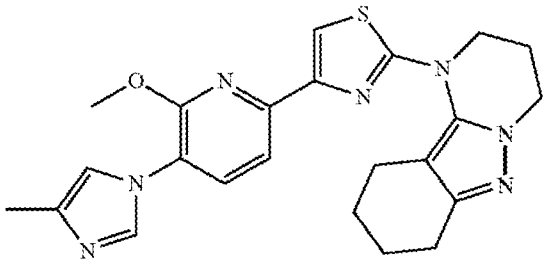
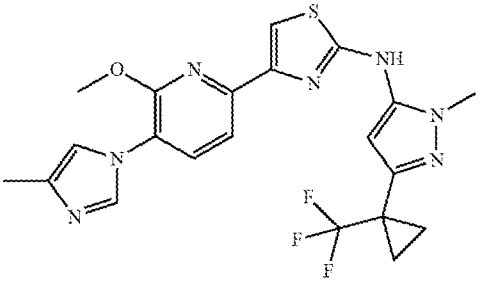
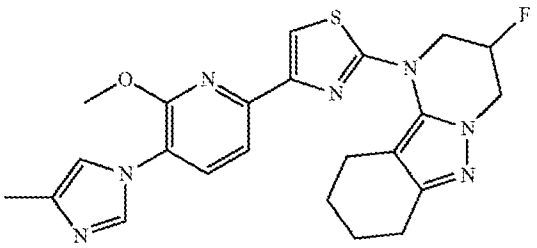
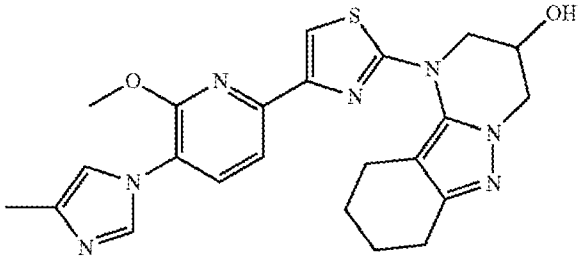
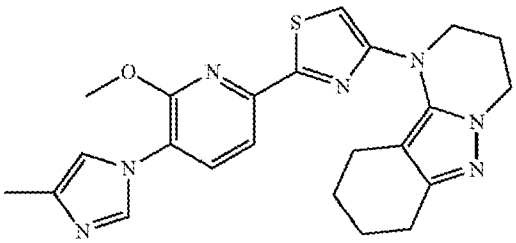
In some embodiments, the compound of formulae (VIII-a), (VIII-b), (VIII-c), or (VIII-d) is described in International Patent Publication No. WO 2014/165263 (e.g., in embodiments P1-P12), which is herein incorporated by reference in its entirety. In some embodiments, the compound of formulae (VIII-a), (VIII-b), (VIII-c), or (VIII-d) is selected from:



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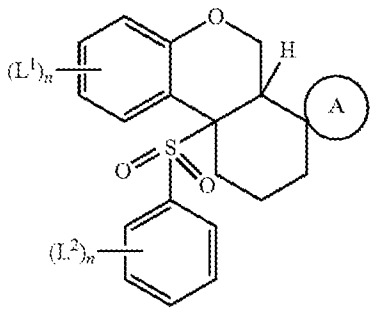


,


,

,

,

,

,and

,

or a pharmaceutically acceptable salt thereof.

In some embodiments, the compound is a compound of formula (IX):



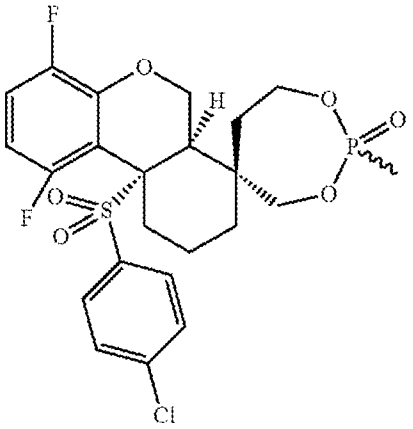
(XIV)

or a pharmaceutically acceptable salt thereof, wherein A is a 4 to 7 membered spirocyclic ring comprising at least one heteroatom selected from the group consisting of N, O, S, $S(O)_2$, $P(O)R^1$, and $N—S(O)_2—R^1$, wherein the spirocyclic ring is optionally substituted with 1 to 3 substituents selected from the group consisting of C1-3alkyl and =O; R^1 is C1-6alkyl optionally substituted with halo; each L^1 is independently selected from the group consisting of 1) C1-3alkyl optionally substituted with halo, and

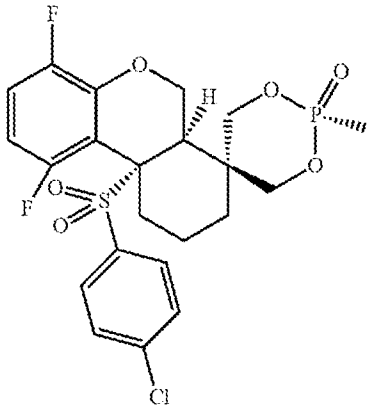
2) halo; each L^2 is independently selected from the group consisting of 1) C1-3alkyl optionally substituted with halo, and 2) halo; and

n is 0 to 3.

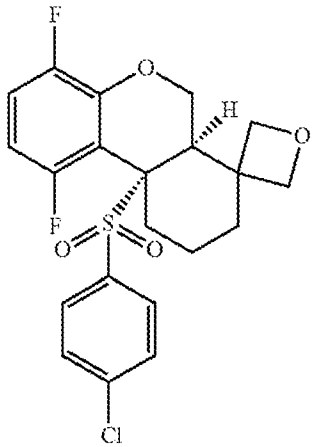
In some embodiments, the compound of formulae (IX) is described in U.S. Patent Publication No. US-2015-307533 (e.g., in the Table on pages 13-16), which is herein incorporated by reference in its entirety. In some embodiments, the compound of formula (IX) is selected from:



,

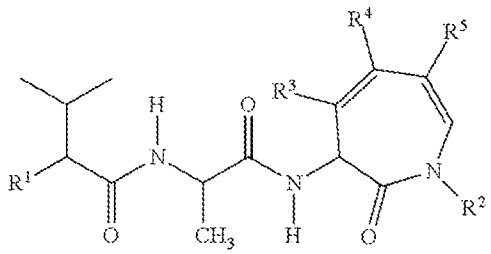


, and



, or a pharmaceutically acceptable salt thereof.

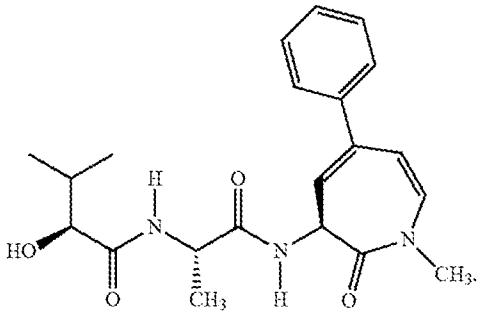
In some embodiments, the compound is a compound of formula (X):



(X)

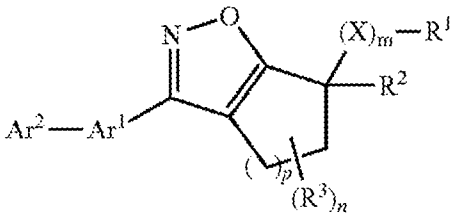
or a pharmaceutically acceptable salt thereof, wherein R^1 is hydroxy or fluoro; R^2 is C_1-C_4 alkyl; R^3 is hydrogen or phenyl; R^4 is hydrogen, phenyl, or C_1-C_4 alkyl; R^5 is hydrogen or phenyl; provided that one of R^3, R^4, and R^5 is other than hydrogen and the other two are hydrogen.

In some embodiments, the compound of formula (X) is described in U.S. Patent No. 8,188,069, which is herein incorporated by reference in its entirety. In one embodiment, the compound is



, or a pharmaceutically acceptable salt thereof.

In some embodiments, the compound is a compound of formula (XI):

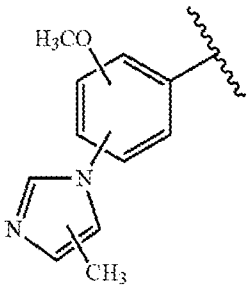
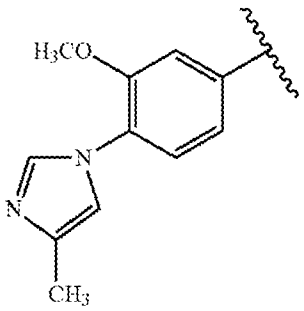
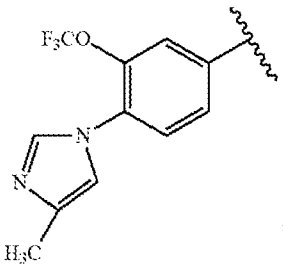
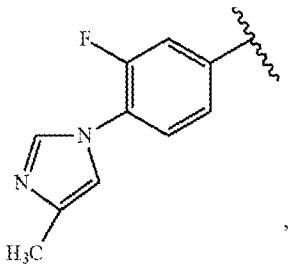
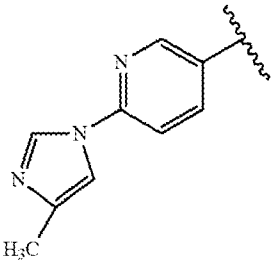
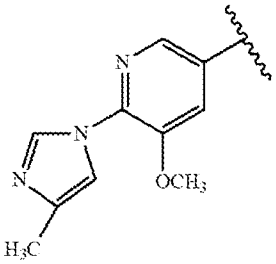
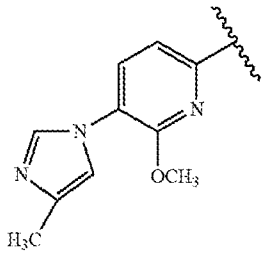
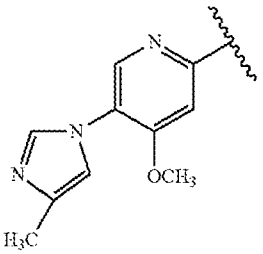
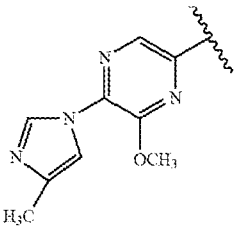


(XI)

or a pharmaceutically acceptable salt thereof, wherein: R^1 is 1) hydrogen, 2) (C1-C6)alkyl optionally substituted with 1 to 5 halogens or phenyl, wherein the phenyl is optionally substituted with 1 to 3 halogens, 3) phenyl optionally substituted with 1 to 3 (C1-C6)alkyls or 1 to 5 halogens, or 4) (C4-C6)cycloalkyl optionally substituted with 1 to 3 (C1-C6)alkyls or 1 to 5 halogens; R^2 is 1) hydrogen, 2) (C1-C6)alkyl optionally substituted with 1 to 5 halogens or phenyl, wherein the phenyl is optionally substituted with 1 to 3 halogens, or 3) phenyl optionally substituted with 1 to 3 halogens; R^3 is (C1-C6)alkyl, —OH or halogen;

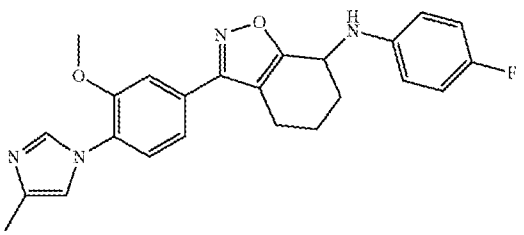
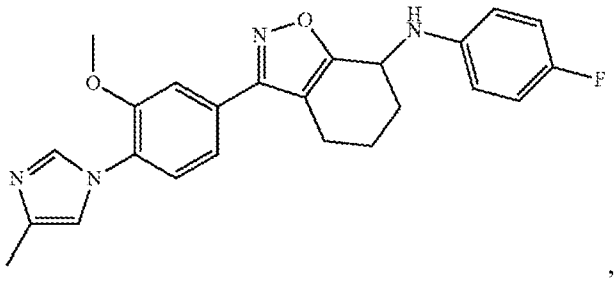
X is —NR^4—, —O—, —S—, or —SO_2—; R^4 is hydrogen or (C1-C3)alkyl;

p is 1 to 3; m is 0 or 1; n is 0 to 3; and Ar^2—Ar^1 is selected from the group consisting of:

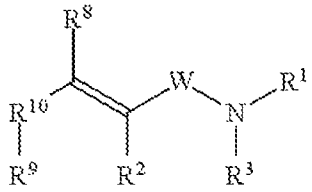
and , or a pharmaceutically acceptable salt thereof.

In some embodiments, the compound of formula (XI) is described in U.S. Patent No. 9,096,582 (e.g., in the Table on pages 13-17), which is herein incorporated by reference in its entirety. In some embodiments, the compound of formula (XI) is selected from:


and ,

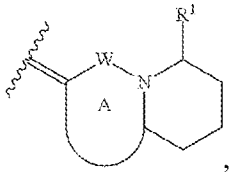
or a pharmaceutically acceptable salt thereof.

In some embodiments, the compound is a compound of formula (XII):



(XII)

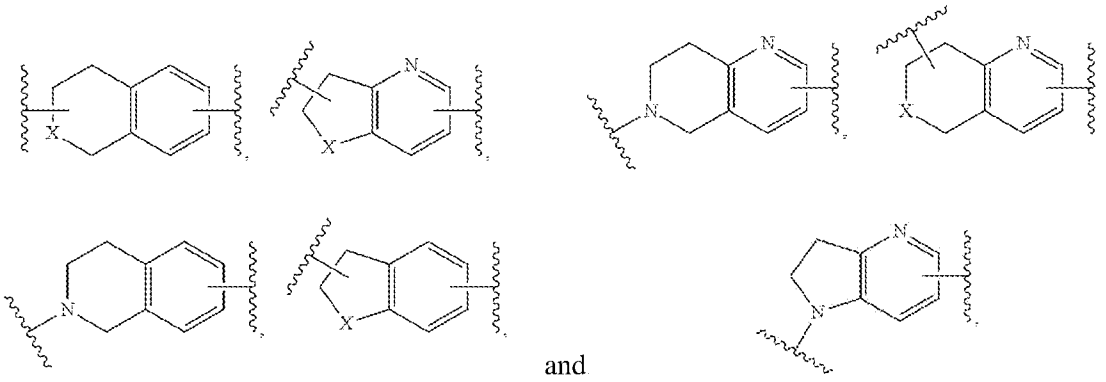
or a pharmaceutically acceptable salt thereof, wherein or the pharmaceutically acceptable salts thereof, wherein: R^1, R^2, R^3, R^8, R^9, R^{10}, and W are independently selected; W is selected from the group consisting of; —S(O)—, and —$S(O)_2$—; R^1 is selected from the group consisting of H, alkyl-, alkenyl-, alkynyl-, aryl-, arylalkyl-, alkylaryl-, cycloalkyl-, cycloalkenyl, cycloalkylalkyl-, fused benzocycloalkyl (i.e., benzofusedcycloalkyl), fused benzoheterocycloalkyl (i.e., benzofusedheterocycloalkyl), fused heteroarylcycloalkyl (i.e., heteroarylfusedcycloalkyl), fused heteroarylheterocycloalkyl (i.e., heteroarylfused-heterocycloalkyl), heteroaryl-, heteroarylalkyl-, heterocyclyl-, heterocyclenyl, -and heterocyclyalkyl-; wherein each of said alkyl-, alkenyl- and alkynyl-, aryl-, arylalkyl-, alkylaryl-, cycloalkyl-, cycloalkenyl-, cycloalkylalkyl-, fused benzocycloalkyl, fused benzoheterocycloalkyl, fused heteroarylcycloalkyl, fused heteroarylheterocycloalkyl, heteroaryl-, heteroarylalkyl-, heterocyclyl-, heterocyclenyl and heterocyclyalkyl-R^1 groups is optionally substituted with 1-5 independently selected R^{21} groups;R^2 and R^3 are each independently selected from the group consisting of H, alkyl-, alkenyl-, alkynyl-, aryl-, arylalkyl-, alkylaryl-, cycloalkyl-, cycloalkenyl-, cycloalkylalkyl-, heteroaryl-, heteroarylalkyl-, heterocyclyl-, heterocyclenyl-, and heterocyclyalkyl-; wherein each of said alkyl-, alkenyl- and alkynyl-, aryl-, arylalkyl-, alkylaryl-, cycloalkyl-, cycloalkenyl, cycloalkylalkyl-, cycloalkenyl-, heteroaryl-, heteroarylalkyl-, heterocyclyl-, heterocyclenyl- and heterocyclyalkyl- R^1 groups is optionally substituted with 1-5 independently selected R^{21} groups; or R^2 and R^3 taken together, along with the atoms to which they are bound, form a ring selected from the group consisting of: (a) a 5 to 6 membered heterocycloalkyl ring, said heterocycloalkyl ring optionally comprising, in addition to W and in addition to the N adjacent to W, at least one other heteroatom independently selected from the group consisting of: —O—, —S(O)—, —$S(O)_2$, and —C(O)—, and (b) a 5 to 6 membered heterocycloalkenyl ring, said heterocycloalkenyl ring optionally comprising, in addition to W and in addition to the N adjacent to W, at least one other heteroatom independently selected from the group consisting of: —O—, —S(O)—, —$S(O)_2$, and —C(O)—; wherein said ring is optionally substituted with 1-5 independently selected R^{21} groups; or R^2 and R^3 taken together along with the atoms to which they are bound, and R^1 and R^3 are taken together along with the atoms to which they are bound, form the fused ring moiety:



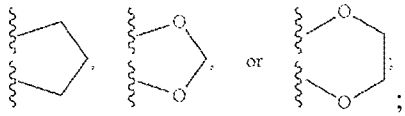
, wherein Ring A is a ring selected from the group consisting of:

(a) a 5 to 6 membered heterocycloalkyl ring, said heterocycloalkyl ring optionally comprising, in addition to W and in addition to the N adjacent to W, at least one other heteroatom independently selected from the group consisting of: —O—, —NR^{14}—, —S(O)—, —$S(O)_2$, and —C(O)—, and (b) a 5 to 6 membered heterocycloalkenyl ring, said heterocycloalkenyl ring optionally comprising, in addition to W and in addition to the N adjacent to W, at least one other heteroatom independently selected from the group consisting of: —O—, —NR^{14}—, —S(O)—, —$S(O)_2$, and —C(O)—, and wherein said fused ring moiety is optionally substituted with 1-5 independently selected R^{21} groups; or R^1 and R^3 taken together with the atoms to which they are bound form a fused benzoheterocycloalkyl ring, and wherein said fused ring is optionally substituted with 1-5 independently selected R^{21} groups, R^8 is selected from the group consisting of H, alkyl-, alkenyl-, alkynyl-, aryl-, arylalkyl-, alkylaryl-, cycloalkyl-, cycloalkenyl, cycloalkylalkyl-, heteroaryl-, heteroarylalkyl-, heterocyclyl-, heterocyclenyl- and heterocyclyalkyl-; wherein each of said R^8 alkyl-, alkenyl- and alkynyl-, aryl-, arylalkyl-, alkylaryl-, cycloalkyl-, cycloalkenyl, cycloalkylalkyl-, heteroaryl-, heteroarylalkyl-, heterocyclyl, heterocyclenyl- and heterocyclyalkyl- is optionally substituted with 1-3 independently selected R^{21} groups; R^9 is selected from the group consisting of: alkyl-, alkenyl-, alkynyl-, aryl-, arylalkyl-, alkylaryl-, cycloalkyl-, cycloalkenyl, cycloalkylalkyl, heteroaryl-, heteroarylalkyl-, heterocyclyl-, heterocyclenyl-, and heterocyclyalkyl-, wherein each of said R^9 alkyl-, alkenyl- and alkynyl-, aryl-, arylalkyl-, alkylaryl-, cycloalkyl-, cycloalkenyl, cycloalkyl alkyl-, heteroaryl-, heteroarylalkyl-, heterocyclyl-, heterocyclenyl-, heterocyclyalkyl- and heterocyclyalkyl- is optionally substituted with 1-3 independently selected R^{21} groups;

R^{10} is selected from the group consisting of: a bond, alkyl-, alkenyl-, alkynyl-, aryl-, arylalkyl-, alkylaryl-, cycloalkyl-, cycloalkenyl, cycloalkylalkyl-, heteroaryl-, heteroarylalkyl-, heterocyclyl-, heterocyclenyl-, heterocyclyalkyl-, heterocyclyalkenyl-,

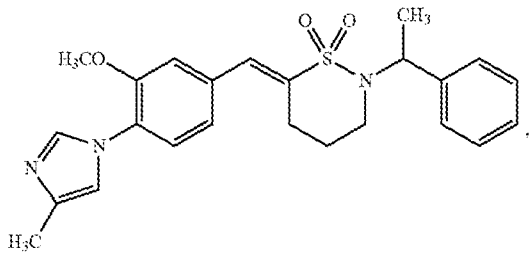
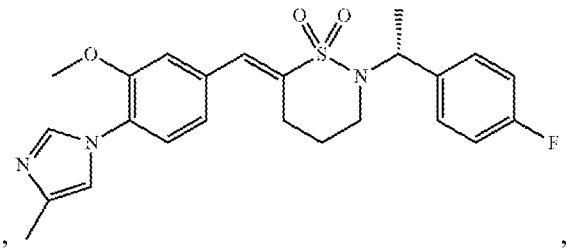
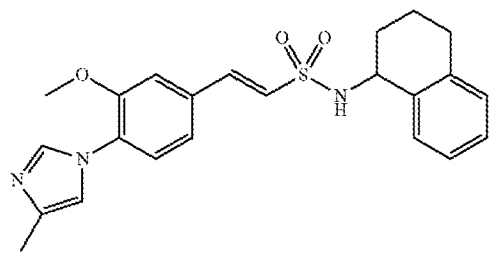
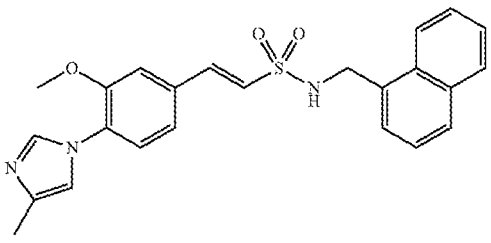
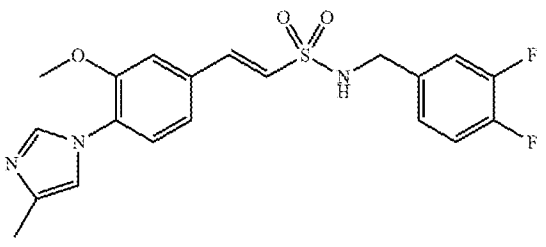
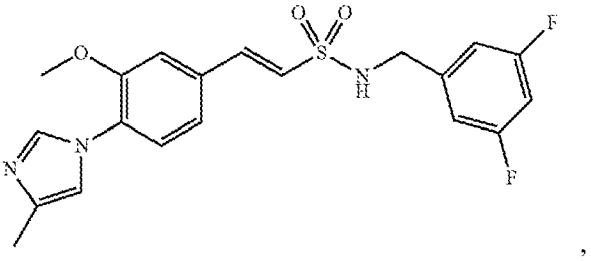


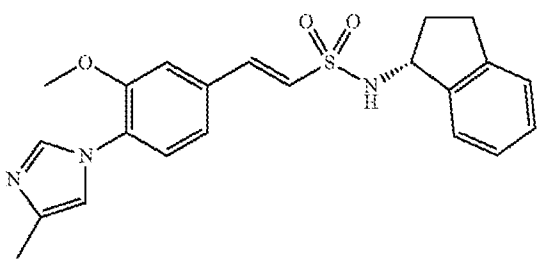
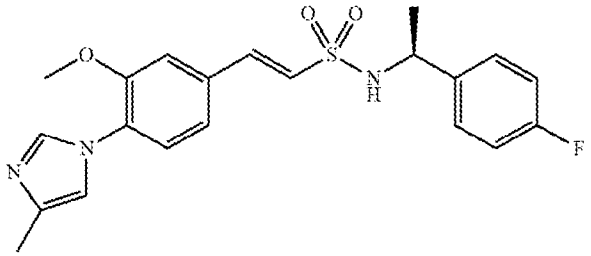
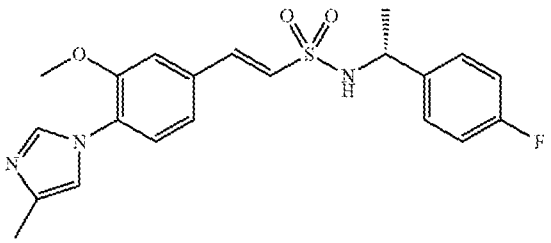
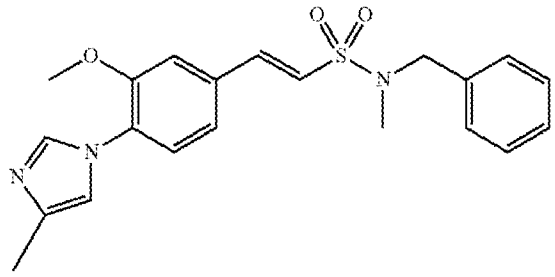
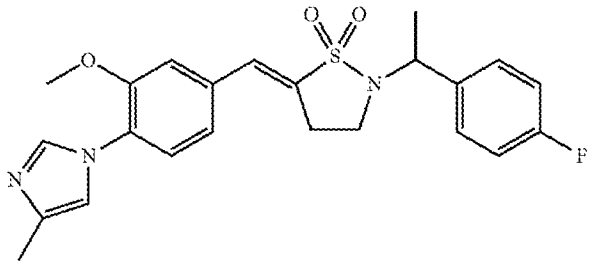
and

wherein X is selected from the group consisting of: O, —N(R^{14})— or —S—; and wherein each of said R^{10} moieties is optionally substituted with 1-3 independently selected R^{21} groups; R^{14} is selected from the group consisting of H, alkyl, alkenyl, alkynyl, cycloalkyl, cycloalkylalkyl, cycloalkenyl, heterocyclyl, heterocyclenyl, heterocyclylalkyl, heterocyclyalkenyl-, aryl, arylalkyl, heteroaryl, heteroarylalkyl, —ON, —C(O)R^{15}, —C(O)OR^{15}, —C(O)N(R^{15})(R^{16}), —S(O)N(R^{15})(R^{16}), —$S(O)_2$N(R^{15})(R^{16}), —C(=NOR^{15})R^{16}, and —P(O)(OR^{15})(OR^{16}); R^{15}, R^{16} and R^{17} are independently selected from the group consisting of H, alkyl, alkenyl, alkynyl, cycloalkyl, cycloalkylalkyl, heterocyclyl, heterocyclylalkyl, aryl, arylalkyl, heteroaryl, heteroarylalkyl, arylcycloalkyl, arylheterocyclyl, $(R^{18})_n$-alkyl, $(R^{18})_n$-cycloalkyl, $(R^{18})_n$-cycloalkylalkyl, $(R^{18})_n$-heterocyclyl, $(R^{18})_n$-heterocyclylalkyl, $(R^{18})_n$-aryl, $(R^{18})_n$-arylalkyl, $(R^{18})_n$-heteroaryl and $(R^{18})_n$-heteroarylalkyl; each R^{18} is independently selected from the group consisting of alkyl, alkenyl, alkynyl, aryl, arylalkyl, arylalkenyl, arylalkynyl, —NO_2, halo, heteroaryl, HO-alkyoxyalkyl, —CF_3, —CN, alkyl-CN, —C(O)R^{19}, —C(O)OH, —C(O)OR^{19}, —C(O)NHR^{20}, —$C(O)NH_2$, —$C(O)NH_2$—C(O)N(alkyl)$_2$, —C(O)N(alkyl)(aryl), —C(O)N(alkyl)(heteroaryl), —SR^{19}, —$S(O)_2R^{20}$, —$S(O)NH_2$, —S(O)NH(alkyl), —S(O)N(alkyl)(alkyl), —S(O)NH(aryl), —$S(O)_2NH_2$, —$S(O)_2$NHR^{19}, —$S(O)_2$NH(heterocyclyl), —$S(O)_2$N(alkyl)$_2$, —$S(O)_2$N(alkyl)(aryl), —OCF_3, —OH, —OR^{20}, —O-heterocyclyl, —O-cycloalkylalkyl, —O-heterocyclylalkyl, —NH_2, —NHR^{20}, —N(alkyl)$_2$, —N(arylalkyl)$_2$, —N(arylalkyl)-(heteroarylalkyl), —NHC(O)R^{20}, —$NHC(O)NH_2$, —NHC(O)NH(alkyl), —NHC(O)N(alkyl)(alkyl), —N(alkyl)C(O)NH(alkyl), —N(alkyl)C(O)N(alkyl)(alkyl), —$NHS(O)_2R^{20}$, —$NHS(O)_2$NH(alkyl), —$NHS(O)_2$N(alkyl)(alkyl), —N(alkyl)$S(O)_2$NH(alkyl) and —N(alkyl)$S(O)_2$N(alkyl)(alkyl); or two R^{18} moieties on adjacent carbons can be linked together to form a  ; R^{19} is selected from the group consisting of: alkyl, cycloalkyl, aryl, arylalkyl and heteroarylalkyl; R^{20} is selected from the group consisting of: alkyl, cycloalkyl, aryl, halo substituted aryl, arylalkyl, heteroaryl and heteroarylalkyl; each R^{21} is independently selected from the group consisting of: alkyl, alkenyl, alkynyl, cycloalkyl, cycloalkylalkyl, cycloalkenyl, heterocycloalkyl, heterocycloalkylalkyl, aryl, arylalkyl, heteroaryl, heteroarylalkyl, halo, —ON, —OR^{15}, —C(O)R^{15}, —C(O)OR^{15}, —C(O)N(R^{15})(R^{16}), —SR^{15}, —S(O)N(R^{16})(R^{16}), —CH(R^{15})(R^{16}), —$S(O)_2$N(R^{16})(R^{16}), —C(=NOR^{16})R^{16}, —P(O)(OR^{15})(OR^{16}), —N(R^{15})(R^{16}), -alkyl-N(R^{15})(R^{16}), —N(R^{15})C(O)R^{16}, —CH_2—N(R^{15})C(O)R^{16}, —CH_2—N(R^{15})C(O)N(R^{16})(R^{17}), —OH_2—R^{15}; —CH_2N(R^{15})(R^{16}), —N(R^{15})S(O)R^{16}, —N(R^{15})$S(O)_2R^{16}$, —CH_2—N(R^{15})$S(O)_2R^{16}$, —N(R^{15})$S(O)_2$N(R^{16})(R^{17}), —N(R^{15})S(O)N(R^{16})(R^{17}), —N(R^{15})C(O)N(R^{16})(R^{17}), —CH_2—N(R^{15})C(O)N(R^{16})(R^{17}), —N(R^{15})C(O)OR^{16}, —CH_2—N(R^{15})C(O)OR^{16}, —S(O)R^{15}, =NOR^{15}, —N_3, —NO_2 and —$S(O)_2R^{15}$; wherein each of said alkyl, alkenyl, alkynyl, cycloalkyl, cycloalkylalkyl, cycloalkenyl, heterocycloalkyl, heterocycloalkylalkyl,

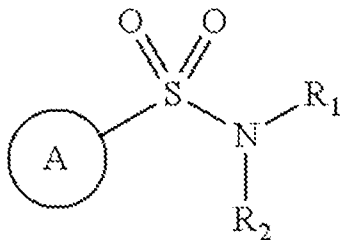
aryl, arylalkyl, heteroaryl, and heteroarylalkyl R^{21} groups is optionally substituted with 1 to 5 independently selected R^{22} groups; and each R^{22} group is independently selected from the group consisting of alkyl, cycloalkyl, cycloalkenyl, heterocycloalkyl, aryl, heteroaryl, halo, —CF_3, —CN, —OR^{15}, —$C(O)R^{15}$, —$C(O)OR^{15}$, -alkyl-$C(O)OR^{15}$, $C(O)N(R^{15})(R^{16})$, —SR^{15}, —$S(O)N(R^{15})(R^{16})$, —$S(O)_2N(R^{15})(R^{16})$, —$C(=NOR^{15})R^{16}$, —$P(O)(OR^{15})(OR^{16})$, —$N(R^{15})(R^{16})$, -alkyl-$N(R^{15})(R^{16})$, —$N(R^{15})C(O)R^{16}$, —CH_2—$N(R^{15})C(O)R^{16}$, —$N(R^{15})S(O)R^{16}$, —$N(R^{15})S(O)_2R^{16}$, —CH_2—$N(R^{15})S(O)_2R^{16}$, —$N(R^{15})S(O)_2N(R^{16})(R^{17})$, —$N(R^{15})S(O)N(R^{16})(R^{17})$, —$N(R^{15})C(O)N(R^{16})(R^{17})$, —$CH_2$—$N(R^{15})C(O)N(R^{16})(R^{17})$; —$N(R^{15})C(O)OR^{16}$, —$CH_2$—$N(R^{15})C(O)OR^{16}$, —$N_3$, =$NOR^{15}$, —$NO_2$, —$S(O)R^{15}$ and —$S(O)_2R^{15}$.

In some embodiments, the compound of formula (XII) is described in U.S. Patent Publication No. US-2011-0257163 (e.g., in paragraphs [0506] to [0553]), which is herein incorporated by reference in its entirety. In some embodiments, the compound of formula (XII) is a pharmaceutically acceptable ester. In some embodiments, the compound of formula (XII) is selected from:


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and , or a pharmaceutically acceptable salt thereof.

In some embodiments, the compound is a compound of formula (XIII):

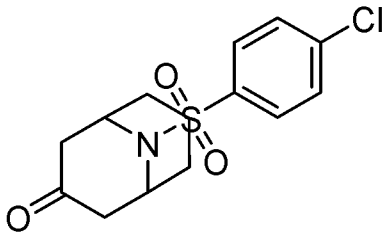

(XIII),

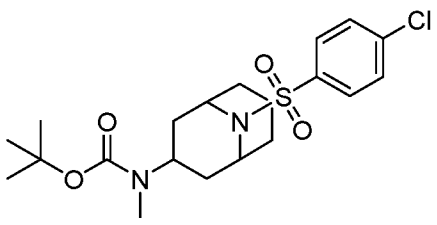
or a pharmaceutically acceptable salt thereof, wherein the A-ring is aryl, cycloalkyl, heteroaryl or heterocycloalkyl, where each ring is optionally substituted at a substitutable position with halogen, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_1-C_6 alkoxy, haloalkyl, haloalkoxy, hydroxyl, hydroxyalkyl, CN, phenoxy, —$S(O)_{0-2}$—(C_1-C_6 alkyl), —$NR_{10}R_{11}$, C_1-C_6 alkanoyl, C_0-C_3alkylCO_2R', heteroaryl, heterocycloalkyl, aryl, aralkyl, or —$SO_2NR_{10}R_{11}$; R_1 and R_2 combine to form a [3.3.1] or a [3.2.1] ring system, where 0 or 1 of the carbons in the ring system is optionally replaced with an —O—, —$S(O)_x$—, or —NR_{15}— group; and where the [3.3.1] or [3.2.1] ring system is optionally substituted with 1, 2, 3, or 4 groups that are independently oxo, halogen, C_1-C_6 alkyl, —O(C_1-C_2 alkyl)O—, —S(C_1-C_2 alkyl)S—,

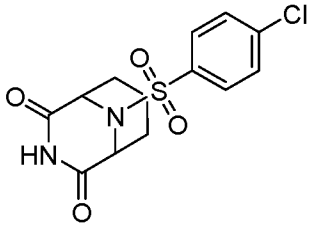
C_2-C_6 alkenyl, C_1-C_6 haloalkyl, C_2-C_6 alkynyl, hydroxy, hydroxyalkyl, C_1-C_6 alkoxy, haloalkoxy, —C(O)OR_{13}, —(C_1-C_4 alkyl)-C(O)OR_{16}, —C$ONR_{10}R_{11}$, —OC(O)$NR_{10}R_{11}$, —NR′C(O)OR″, —NR′S(O)$_2$R″, —OS(O)$_2$R′, —NR′COR″, CN, =N—NR_{12}, or =N—O—R_{13}; where x is 0, 1, or 2; R_{10} and R_{11} at each occurrence are independently hydrogen or C_1-C_6 alkyl, where the alkyl is optionally substituted with an aryl, where the aryl is optionally substituted with 1 to 5 groups that are independently halogen, hydroxyl, alkyl, alkoxy, haloalkyl, haloalkoxy, CN or NO_2; or R_{10} and R_{11} together may form a 3-8 membered ring optionally including an additional heteroatom such as N, O or S; R_{12} is hydrogen, C_1-C_6 alkyl or —SO_2-aryl, where the aryl is optionally substituted with 1 to 5 groups that are independently halogen, hydroxyl, alkyl, alkoxy, haloalkyl, haloalkoxy, CN or NO_2; R_{13} is hydrogen or C_1-C_6 alkyl optionally substituted with aryl, hydroxyl, or halogen, where the aryl is optionally substituted with 1 to 5 groups that are independently halogen, hydroxyl, alkyl, alkoxy, haloalkyl, haloalkoxy, CN or NO_2;

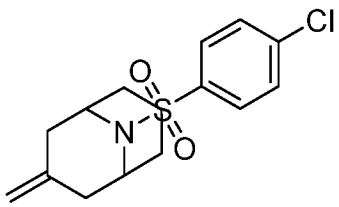
R_{15} is hydrogen, aryl, heteroaryl, —SO_2R′, —C(O)R′, —C(O)OR′, or C_1-C_6 alkyl optionally substituted with aryl, hydroxyl, or halogen, where the aryl groups are optionally substituted with 1 to 5 groups that are independently halogen, hydroxyl, alkyl, alkoxy, haloalkyl, haloalkoxy, CN or NO_2; and R′ and R″ are independently hydrogen, C_1-C_6 alkyl, haloalkyl, C_2-C_6 alkenyl or phenyl optionally substituted with 1 to 5 groups that are independently halogen, C_1-C_6 alkyl, —C(O)OR′, C_1-C_6 alkoxy, haloalkyl, haloalkoxy, hydroxyl, CN, phenoxy, —SO_2—(C_1-C_6 alkyl), —$NR_{10}R_{11}$, C_1-C_6 alkanoyl, pyridyl, phenyl, NO_2, or —$SO_2NR_{10}R_{11}$.

In some embodiments, the compound of formula (XIII) is described in U.S. Patent Publication No. US-2011-178199 (e.g., in paragraphs [0798] to [0799] and Tables 1-4), which is herein incorporated by reference in its entirety. In some embodiments, the compound of formula (XIII) comprises a bridged n-bicyclic sulfonamide or a pharmaceutically acceptable salt thereof. In some embodiments, the compound of formula (XIII) is selected from:



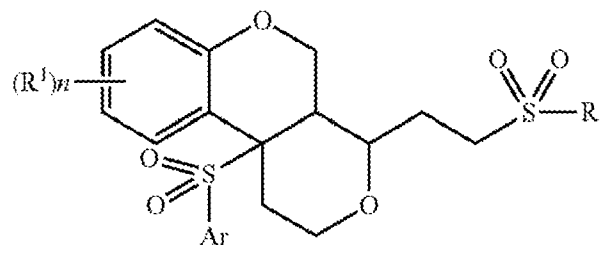
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, and


, or a pharmaceutically acceptable salt thereof.

In some embodiments, the compound is a compound of formula (XIV):



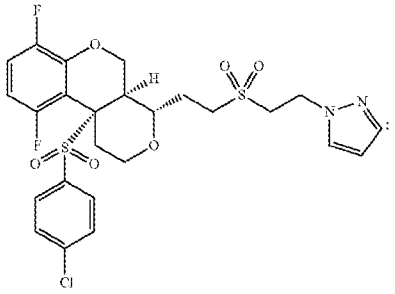
(XIV)

or a pharmaceutically acceptable salt thereof, wherein R is selected from the group consisting of: (1) -pyridinyl, (2) -pyrazolinyl, (3) -1,2,4-oxadiazolyl, (4) -(C1-C2)alkyl-pyridinyl, (5) -(C1-C2)alkyl-pyrazolinyl, and (6) -(C1-C2)alkyl-1,2,4-oxadiazolyl, wherein the pyridinyl, pyrazolinyl, and -1,2,4-oxadiazolyl, is unsubstituted or substited with one L^1 group; R^1 is independently selected from the group consisting halogen, (C1-C6)alkyl, —CN, —CF_3, —O—(C1-C6)alkyl, —O-(halo(C1-C6)alkyl), —C(O)—O—(C1-C6)—OH-substituted (C1-C4)alkyl, halo(C1-C6)alkyl, —(C1-C4)alkoxy-OH, —(C1-C4)alkoxy(C1-C4)alkoxy and —$S(O)_2$(C1-C6)alkyl; n is 0, 1, 2, or 3; Ar is selected from the group consisting of phenyl optionally substituted with 1 or 2 L^2 groups, and pyridyl optionally substituted with 1 or 2 L^2 groups;

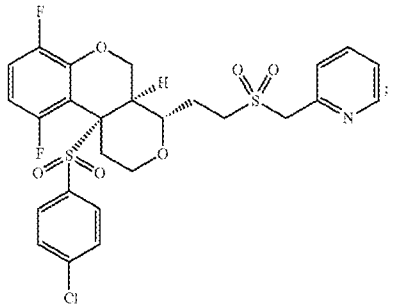
L^1 is independently selected from the group consisting of —OCH_3, —NH_2, =O, and (C1-C5)alkyl; and

L^2 is independently selected from the group consisting of halogen, (C1-C6)alkyl , —CN, —CF_3, —O—(C1-C6)alkyl, —O-(halo(C1-C6)alkyl), —C(O)—O—(C1-C6)alkyl, —OH-substituted(C1-C6)alkyl, halo(C1-C6)alkyl, —OH-substituted (C1-C4)alkoxy, —(C1-C4)alkoxy(C1-C4)alkoxy and —$S(O)_2$(C1-C6)alkyl.

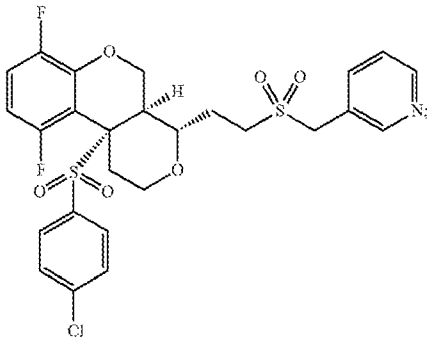
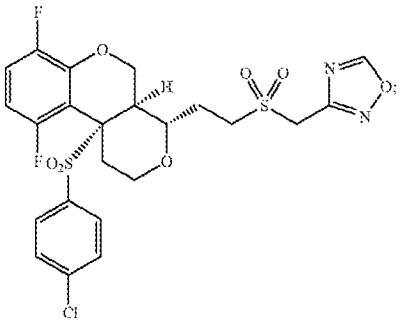
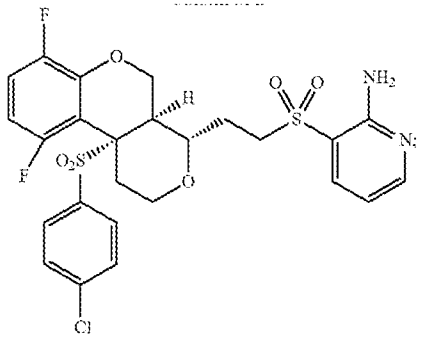
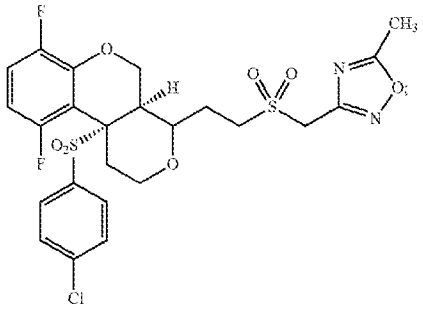
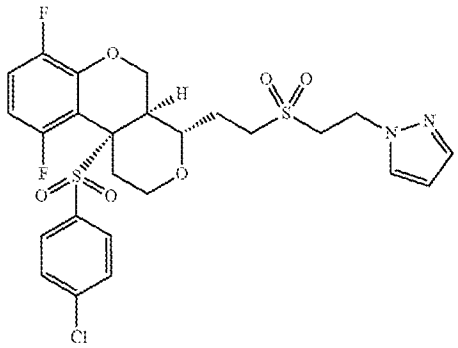
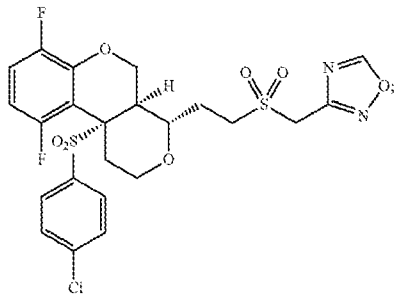
In some embodiments, the compound of formula (XIV) is described in U.S. Patent No. 9,226,927 (e.g., compound 4, 8a, 8b, 11, 14, 25a, 25b, 25c, 25d, 25e, 25f, 25g, 25h, 27a, or 27b), which is herein incorporated by reference in its entirety. In some embodiments, the compound of formula (XIV) comprises a bridged n-bicyclic sulfonamide or a pharmaceutically acceptable salt thereof. In some embodiments, the compound of formula (XIV) is selected from:



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and , or a pharmaceutically acceptable salt thereof.

Antibody molecules targeting gamma secretase

The compositions, methods and uses described herein comprise a gamma secretase inhibitor (GSI). In some embodiments, the GSI is an antibody molecule that reduces the expression and/or function of gamma secretase. In some embodiments, the GSI is an antibody molecule targeting a subunit of gamma secretase. In some embodiments, the GSI is chosen from an anti-presenilin antibody molecule, an anti-nicastrin antibody molecule, an anti-APH-1 antibody molecule, or an anti-PEN-2 antibody molecule.

Exemplary antibody molecules that target a subunit of gamma secretase (e.g., e.g., presenilin, nicastrin, APH-1, or PEN-2) are described in US 8,394,376, US 8,637,274, and US 5,942,400, incorporated by reference herein in their entirety.

Gene editing systems targeting gamma secretase

According to the present invention, gene editing systems can be used as inhibitors of gamma secretase (e.g., a subunit of gamma secretase, e.g., presenilin, nicastrin, APH-1, or PEN-2). Also contemplated by the present invention are the uses of a nucleic acid encoding one or more components of a gene editing system targeting gamma secretase (e.g., a subunit of gamma secretase, e.g., presenilin, nicastrin, APH-1, or PEN-2).

CRISPR/Cas9 gene editing systems

Naturally-occurring CRISPR/Cas systems are found in approximately 40% of sequenced eubacteria genomes and 90% of sequenced archaea. Grissa et al. (2007) BMC Bioinformatics 8: 172. This system is a type of prokaryotic immune system that confers resistance to foreign genetic elements such as plasmids and phages and provides a form of acquired immunity. Barrangou et al. (2007) Science 315: 1709-1712; Marragini et al. (2008) Science 322: 1843-1845.

The CRISPR/Cas system has been modified for use in gene editing (silencing, enhancing or changing specific genes) in eukaryotes such as mice or primates. Wiedenheft et al. (2012) Nature 482: 331-8. This is accomplished by, for example, introducing into the eukaryotic cell a plasmid containing a specifically designed CRISPR and one or more appropriate Cas.

The CRISPR sequence, sometimes called a CRISPR locus, comprises alternating repeats and spacers. In a naturally-occurring CRISPR, the spacers usually comprise sequences foreign to the bacterium such as a plasmid or phage sequence. In an exemplary CRISPR/Cas system targeting gamma secretase (e.g., a subunit of gamma secretase, e.g., presenilin, nicastrin, APH-1, or PEN-2), the spacers are derived from the gene sequence of gamma secretase (e.g., a subunit of gamma secretase, e.g., presenilin, nicastrin, APH-1, or PEN-2), or a sequence of its regulatory elements.

RNA from the CRISPR locus is constitutively expressed and processed into small RNAs. These comprise a spacer flanked by a repeat sequence. The RNAs guide other Cas proteins to silence exogenous genetic elements at the RNA or DNA level. Horvath et al. (2010) Science 327: 167-170; Makarova et al. (2006) Biology Direct 1: 7. The spacers thus serve as templates for RNA molecules, analogously to siRNAs. Pennisi (2013) Science 341: 833-836.

As these naturally occur in many different types of bacteria, the exact arrangements of the CRISPR and structure, function and number of Cas genes and their product differ somewhat from species to species. Haft et al. (2005) PLoS Comput. Biol. 1: e60; Kunin et al. (2007) Genome Biol. 8: R61; Mojica et al. (2005) J. Mol. Evol. 60: 174-182; Bolotin et al. (2005) Microbiol. 151: 2551-2561; Pourcel et al. (2005) Microbiol. 151: 653-663; and Stern et al. (2010) Trends. Genet. 28: 335-340. For example, the Cse (Cas subtype, *E. coli*) proteins (e.g., CasA) form a functional complex, Cascade, that processes CRISPR RNA transcripts into spacer-repeat units that Cascade retains. Brouns et al. (2008)

Science 321: 960-964. In other prokaryotes, Cas6 processes the CRISPR transcript. The CRISPR-based phage inactivation in E. coli requires Cascade and Cas3, but not Cas1 or Cas2. The Cmr (Cas RAMP module) proteins in Pyrococcus furiosus and other prokaryotes form a functional complex with small CRISPR RNAs that recognizes and cleaves complementary target RNAs. A simpler CRISPR system relies on the protein Cas9, which is a nuclease with two active cutting sites, one for each strand of the double helix. Combining Cas9 and modified CRISPR locus RNA can be used in a system for gene editing. Pennisi (2013) Science 341: 833-836.

The CRISPR/Cas system can thus be used to modify, e.g., delete one or more nucleic acids, e.g., a gene encoding gamma secretase (e.g., a subunit of gamma secretase, e.g., presenilin, nicastrin, APH-1, or PEN-2), or a regulatory element of a gene encoding gamma secretase (e.g., a subunit of gamma secretase, e.g., presenilin, nicastrin, APH-1, or PEN-2), or introduce a premature stop which thus decreases expression of a functional gamma secretase (e.g., a subunit of gamma secretase, e.g., presenilin, nicastrin, APH-1, or PEN-2). The CRISPR/Cas system can alternatively be used like RNA interference, turning off a gene encoding gamma secretase (e.g., a subunit of gamma secretase, e.g., presenilin, nicastrin, APH-1, or PEN-2) in a reversible fashion. In a mammalian cell, for example, the RNA can guide the Cas protein to a promoter of a gene encoding gamma secretase (e.g., a subunit of gamma secretase, e.g., presenilin, nicastrin, APH-1, or PEN-2), sterically blocking RNA polymerases.

CRISPR/Cas systems for gene editing in eukaryotic cells typically involve (1) a guide RNA molecule (gRNA) comprising a targeting sequence (which is capable of hybridizing to the genomic DNA target sequence), and sequence which is capable of binding to a Cas, e.g., Cas9 enzyme, and (2) a Cas, e.g., Cas9, protein. The targeting sequence and the sequence which is capable of binding to a Cas, e.g., Cas9 enzyme, may be disposed on the same or different molecules. If disposed on different molecules, each includes a hybridization domain which allows the molecules to associate, e.g., through hybridization.

Artificial CRISPR/Cas systems can be generated which inhibit a gene encoding gamma secretase (e.g., a subunit of gamma secretase, e.g., presenilin, nicastrin, APH-1, or PEN-2), using technology known in the art, e.g., that are described in U.S. Publication No.20140068797, WO2015/048577, and Cong (2013) Science 339: 819-823. Other artificial CRISPR/Cas systems that are known in the art may also be generated which inhibit a gene encoding gamma secretase (e.g., a subunit of gamma secretase, e.g., presenilin, nicastrin, APH-1, or PEN-2), e.g., that described in Tsai (2014) Nature Biotechnol., 32:6 569-576, U.S. Patent No.: 8,871,445; 8,865,406; 8,795,965; 8,771,945; and 8,697,359, the contents of which are hereby incorporated by reference in their entirety. Such systems can be generated which inhibit a gene encoding gamma secretase (e.g., a subunit of gamma secretase, e.g., presenilin, nicastrin, APH-1, or PEN-2), by, for example, engineering a CRISPR/Cas system to include a gRNA molecule comprising a targeting sequence that hybridizes to a sequence of a

target gene, e.g., a gene encoding gamma secretase (e.g., a subunit of gamma secretase, e.g., presenilin, nicastrin, APH-1, or PEN-2). In embodiments, the gRNA comprises a targeting sequence which is fully complementarity to 15-25 nucleotides, e.g., 20 nucleotides, of a target gene, e.g., a gene encoding gamma secretase (e.g., a subunit of gamma secretase, e.g., presenilin, nicastrin, APH-1, or PEN-2). In embodiments, the 15-25 nucleotides, e.g., 20 nucleotides, of a target gene, e.g., a gene encoding gamma secretase (e.g., a subunit of gamma secretase, e.g., presenilin, nicastrin, APH-1, or PEN-2), are disposed immediately 5' to a protospacer adjacent motif (PAM) sequence recognized by the Cas protein of the CRISPR/Cas system (e.g., where the system comprises a *S. pyogenes* Cas9 protein, the PAM sequence comprises NGG, where N can be any of A, T, G or C).

In an embodiment, the CRISPR/Cas system of the present invention comprises Cas9, e.g., S. pyogenes Cas9, and a gRNA comprising a targeting sequence which hybridizes to a sequence of a gene encoding gamma secretase (e.g., a subunit of gamma secretase, e.g., presenilin, nicastrin, APH-1, or PEN-2). In an embodiment, the CRISPR/Cas system comprises nucleic acid encoding a gRNA specific for a gene encoding gamma secretase (e.g., a subunit of gamma secretase, e.g., presenilin, nicastrin, APH-1, or PEN-2), and a nucleic acid encoding a Cas protein, e.g., Cas9, e.g., S. pyogenes Cas9. In an embodiment, the CRISPR/Cas system comprises a gRNA specific for a gene encoding gamma secretase (e.g., a subunit of gamma secretase, e.g., presenilin, nicastrin, APH-1, or PEN-2), and a nucleic acid encoding a Cas protein, e.g., Cas9, e.g., S. pyogenes Cas9.

TALEN Gene Editing Systems

TALENs are produced artificially by fusing a TAL effector DNA binding domain to a DNA cleavage domain. Transcription activator-like effects (TALEs) can be engineered to bind any desired DNA sequence, including a portion of the HLA or TCR gene. By combining an engineered TALE with a DNA cleavage domain, a restriction enzyme can be produced which is specific to any desired DNA sequence, including a HLA or TCR sequence. These can then be introduced into a cell, wherein they can be used for genome editing. Boch (2011) Nature Biotech. 29: 135-6; and Boch et al. (2009) Science 326: 1509-12; Moscou et al. (2009) Science 326: 3501.

TALEs are proteins secreted by Xanthomonas bacteria. The DNA binding domain contains a repeated, highly conserved 33-34 amino acid sequence, with the exception of the 12th and 13th amino acids. These two positions are highly variable, showing a strong correlation with specific nucleotide recognition. They can thus be engineered to bind to a desired DNA sequence.

To produce a TALEN, a TALE protein is fused to a nuclease (N), which is, for example, a wild-type or mutated FokI endonuclease. Several mutations to FokI have been made for its use in TALENs; these, for example, improve cleavage specificity or activity. Cermak et al. (2011) Nucl. Acids Res. 39: e82; Miller et al. (2011) Nature Biotech. 29: 143-8; Hockemeyer et al. (2011) Nature Biotech. 29: 731-

734; Wood et al. (2011) Science 333: 307; Doyon et al. (2010) Nature Methods 8: 74-79; Szczepek et al. (2007) Nature Biotech. 25: 786-793; and Guo et al. (2010) J. Mol. Biol. 200: 96.

The FokI domain functions as a dimer, requiring two constructs with unique DNA binding domains for sites in the target genome with proper orientation and spacing. Both the number of amino acid residues between the TALE DNA binding domain and the FokI cleavage domain and the number of bases between the two individual TALEN binding sites appear to be important parameters for achieving high levels of activity. Miller et al. (2011) Nature Biotech. 29: 143-8.

A TALEN specific for a gene encoding gamma secretase (e.g., a subunit of gamma secretase, e.g., presenilin, nicastrin, APH-1, or PEN-2), can be used inside a cell to produce a double-stranded break (DSB). A mutation can be introduced at the break site if the repair mechanisms improperly repair the break via non-homologous end joining. For example, improper repair may introduce a frame shift mutation.

TALENs specific to sequences in a gene encoding gamma secretase (e.g., a subunit of gamma secretase, e.g., presenilin, nicastrin, APH-1, or PEN-2), can be constructed using any method known in the art, including various schemes using modular components. Zhang et al. (2011) Nature Biotech. 29: 149-53; Geibler et al. (2011) PLoS ONE 6: e19509; US 8,420,782 ; US 8,470,973, the contents of which are hereby incorproated by reference in their entirety.

Zinc Finger Nucleases

"ZFN" or "Zinc Finger Nuclease" refers to a zinc finger nuclease, an artificial nuclease which can be used to modify, e.g., delete one or more nucleic acids of, a desired nucleic acid sequence, e.g., a gene encoding gamma secretase (e.g., a subunit of gamma secretase, e.g., presenilin, nicastrin, APH-1, or PEN-2).

Like a TALEN, a ZFN comprises a FokI nuclease domain (or derivative thereof) fused to a DNA-binding domain. In the case of a ZFN, the DNA-binding domain comprises one or more zinc fingers. Carroll et al. (2011) Genetics Society of America 188: 773-782; and Kim et al. (1996) Proc. Natl. Acad. Sci. USA 93: 1156-1160.

A zinc finger is a small protein structural motif stabilized by one or more zinc ions. A zinc finger can comprise, for example, Cys2His2, and can recognize an approximately 3-bp sequence. Various zinc fingers of known specificity can be combined to produce multi-finger polypeptides which recognize about 6, 9, 12, 15 or 18-bp sequences. Various selection and modular assembly techniques are available to generate zinc fingers (and combinations thereof) recognizing specific sequences, including phage display, yeast one-hybrid systems, bacterial one-hybrid and two-hybrid systems, and mammalian cells.

Like a TALEN, a ZFN must dimerize to cleave DNA. Thus, a pair of ZFNs are required to target non-palindromic DNA sites. The two individual ZFNs must bind opposite strands of the DNA with their nucleases properly spaced apart. Bitinaite et al. (1998) Proc. Natl. Acad. Sci. USA 95: 10570-5.

Also like a TALEN, a ZFN can create a double-stranded break in the DNA, which can create a frame-shift mutation if improperly repaired, leading to a decrease in the expression of a gene encoding gamma secretase (e.g., a subunit of gamma secretase, e.g., presenilin, nicastrin, APH-1, or PEN-2), in a cell. ZFNs can also be used with homologous recombination to mutate a gene encoding gamma secretase (e.g., a subunit of gamma secretase, e.g., presenilin, nicastrin, APH-1, or PEN-2).

ZFNs specific to sequences in a gene encoding gamma secretase (e.g., a subunit of gamma secretase, e.g., presenilin, nicastrin, APH-1, or PEN-2), can be constructed using any method known in the art. See, e.g., Provasi (2011) Nature Med. 18: 807-815; Torikai (2013) Blood 122: 1341-1349; Cathomen et al. (2008) Mol. Ther. 16: 1200-7; and Guo et al. (2010) J. Mol. Biol. 400: 96; U.S. Patent Publication 2011/0158957; and U.S. Patent Publication 2012/0060230, the contents of which are hereby incorporated by reference in their entirety. In embodiments, The ZFN gene editing system may also comprise nucleic acid encoding one or more components of the ZFN gene editing system, e.g., a ZFN gene editing system targeted to a gene encoding gamma secretase (e.g., a subunit of gamma secretase, e.g., presenilin, nicastrin, APH-1, or PEN-2).

Double-stranded RNA, e.g., siRNA or shRNA, targeting gamma secretase

According to the present invention, double stranded RNA ("dsRNA"), e.g., siRNA or shRNA can be used to inhibit a gene encoding gamma secretase (e.g., a subunit of gamma secretase, e.g., presenilin, nicastrin, APH-1, or PEN-2). Also contemplated by the present invention are the uses of a nucleic acid encoding said dsRNA inhibitors of a gene encoding gamma secretase (e.g., a subunit of gamma secretase, e.g., presenilin, nicastrin, APH-1, or PEN-2).

In an embodiment, the GSI is a nucleic acid, e.g., a dsRNA, e.g., a siRNA or shRNA specific for a nucleic acid encoding gamma secretase (e.g., a subunit of gamma secretase, e.g., presenilin, nicastrin, APH-1, or PEN-2).

An aspect of the invention provides a composition comprising a dsRNA, e.g., a siRNA or shRNA, comprising at least 15 contiguous nucleotides, e.g., 15, 16, 17, 18, 19, 20, 21, 22, 23, 24 or 25 contiguous nucleotides, e.g., 21 contiguous nucleotides. It is understood that some of the target sequences and/or shRNA molecules are presented as DNA, but the dsRNA agents targeting these sequences or comprising these sequences can be RNA, or any nucleotide, modified nucleotide or substitute disclosed herein and/or known in the art, provided that the molecule can still mediate RNA interference.

In embodiments, the GSI is a nucleic acid, e.g., DNA, encoding a dsRNA inhibitor, e.g., shRNA or siRNA, of any of the above embodiments. In embodiments, the nucleic acid, e.g., DNA, is disposed on a vector, e.g., any conventional expression system, e.g., as described herein, e.g., a lentiviral vector.

BCMA-targeting Chimeric antigen receptor (CAR)

Provided herein are compositions of matter and methods of use for the treatment of a disease associated with expression of BCMA a cell (e.g., a population of cells) that expresses a chimeric antigen receptor (CAR) molecule that binds BCMA (a "BCMA CAR-expressing cell"), in combination with a gamma secretase inhibitor (GSI).

In one aspect, the present invention provides compositions and their use in medicaments or methods for treating, among other diseases, cancer or any malignancy or autoimmune diseases involving cells or tissues that express BCMA.

In one aspect, the compositions of the invention can be used to eradicate BCMA-expressing normal cells, thereby applicable for use as a conditioning therapy prior to cell transplantation. In one aspect, the BCMA-expressing normal cell is a BCMA-expressing normal stem cell and the cell transplantation is a stem cell transplantation.

In one aspect, the invention provides a number of chimeric antigen receptors (CAR) comprising an antibody or antibody fragment engineered for enhanced binding to a BCMA protein. In one aspect, the invention provides a cell (e.g., an immune effector cell, e.g., T cell or NK cell) engineered to express a CAR, wherein the CAR T cell ("CART") or CAR NK cell exhibits an antitumor property. In one aspect a cell is transformed with the CAR and the CAR is expressed on the cell surface. In some embodiments, the cell (e.g., an immune effector cell, e.g., T cell or NK cell) is transduced with a viral vector encoding a CAR. In some embodiments, the viral vector is a retroviral vector. In some embodiments, the viral vector is a lentiviral vector. In some such embodiments, the cell may stably express the CAR. In another embodiment, the cell (e.g., an immune effector cell, e.g., T cell or NK cell) is transfected with a nucleic acid, e.g., mRNA, cDNA, DNA, encoding a CAR. In some such embodiments, the cell may transiently express the CAR.

In one aspect, the anti-BCMA antigen binding portion of the CAR is a scFv antibody fragment. In one aspect such antibody fragments are functional in that they retain the equivalent binding affinity, e.g., they bind the same antigen with comparable efficacy, as the IgG antibody from which it is derived. In other embodiments, the antibody fragment has a lower binding affinity, e.g., it binds the same antigen with a lower binding affinity than the antibody from which it is derived, but is functional in that it provides a biological response described herein. In one embodiment, the CAR molecule comprises an antibody fragment that has a binding affinity KD of 10-4 M to 10-8 M, e.g., 10-5 M to 10-7 M, e.g., 10-

6 M or 10-7 M, for the target antigen. In one embodiment, the antibody fragment has a binding affinity that is at least five-fold, 10-fold, 20-fold, 30-fold, 50-fold, 100-fold or 1,000-fold less than a reference antibody, e.g., an antibody described herein.

In one aspect such antibody fragments are functional in that they provide a biological response that can include, but is not limited to, activation of an immune response, inhibition of signal-transduction origination from its target antigen, inhibition of kinase activity, and the like, as will be understood by a skilled artisan. In one aspect, the anti-BCMA antigen binding domain of the CAR is a scFv antibody fragment that is humanized compared to the murine sequence of the scFv from which it is derived. In one embodiment, the anti-BCMA antigen binding domain is a human anti-BCMA antigen binding domain. In one embodiment, the anti-BCMA antigen binding domain is a humanized anti-BCMA antigen binding domain.

In some aspects, the antibodies of the invention are incorporated into a chimeric antigen receptor (CAR). In one aspect, the CAR comprises a BCMA binding domain comprising a sequence of SEQ ID NO: 39, SEQ ID NO: 40, SEQ ID NO: 41, SEQ ID NO: 42, SEQ ID NO: 43, SEQ ID NO: 44, SEQ ID NO: 45, SEQ ID NO: 46, SEQ ID NO: 47, SEQ ID NO: 48, SEQ ID NO: 49, SEQ ID NO: 50, SEQ ID NO: 51, SEQ ID NO: 52, SEQ ID NO: 53, SEQ ID NO:129, SEQ ID NO:130, SEQ ID NO:131, SEQ ID NO:132, SEQ ID NO:133, SEQ ID NO:134, SEQ ID NO: 135, SEQ ID NO:136, SEQ ID NO: 137, SEQ ID NO:138, SEQ ID NO: 139, SEQ ID NO: 140, SEQ ID NO: 141, SEQ ID NO: 142, SEQ ID NO: 143, SEQ ID NO: 144, SEQ ID NO: 145, SEQ ID NO: 146, SEQ ID NO: 147, SEQ ID NO: 148, SEQ ID NO: 149, SEQ ID NO: 263, SEQ ID NO: 264, SEQ ID NO: 265, or SEQ ID NO: 266. In one aspect, the scFv domains are human. In another aspect, the scFv domains are humanized variants of the scFv domain of the antibodies or antibody fragments described in PCT Publication No. WO 2012/163805, US Patent No. 7,083,785, EP Patent No. 1975231B1, or PCT Publication No. WO 13/154760 (the contents of each are hereby incorporated by reference in their entireties), which disclose antibodies or scFv fragments of murine origin that specifically binds to human BCMA. Humanization of these mouse antibodies and/or scFvs may be desired for the clinical setting, where the mouse-specific residues may induce a human-anti-mouse antigen (HAMA) response in patients who receive CART-BCMA treatment, e.g., treatment with immune effector cells, e.g., T cells or NK cells, transduced with the anti-BCMA CAR construct.

In one aspect, the anti-BCMA binding domain, e.g., human or humanized scFv, portion of a CAR of the invention is encoded by a transgene whose sequence has been codon optimized for expression in a mammalian cell. In one aspect, entire CAR construct of the invention is encoded by a transgene whose entire sequence has been codon optimized for expression in a mammalian cell. Codon optimization refers to the discovery that the frequency of occurrence of synonymous codons (i.e., codons that code for the same amino acid) in coding DNA is biased in different species. Such codon

degeneracy allows an identical polypeptide to be encoded by a variety of nucleotide sequences. A variety of codon optimization methods is known in the art, and include, e.g., methods disclosed in at least US Patent Numbers 5,786,464 and 6,114,148.

In one aspect, the human anti-BCMA binding domain comprises the scFv portion provided in SEQ ID NO: 39. In one aspect, the human anti-BCMA CAR comprises the scFv portion provided in SEQ ID NO: 40. In one aspect, the human anti-BCMA binding domain comprises the scFv portion provided in SEQ ID NO: 41. In one aspect, the human anti-BCMA binding domain comprises the scFv portion provided in SEQ ID NO: 42. In one aspect, the human anti-BCMA binding domain comprises the scFv portion provided in SEQ ID NO: 43. In one aspect, the human anti-BCMA binding domain comprises the scFv portion provided in SEQ ID NO: 44. In one aspect, the human anti-BCMA binding domain comprises the scFv portion provided in SEQ ID NO: 45. In one aspect, the human anti-BCMA binding domain comprises the scFv portion provided in SEQ ID NO: 46. In one aspect, the human anti-BCMA binding domain comprises the scFv portion provided in SEQ ID NO: 47. In one aspect, the human anti-BCMA binding domain comprises the scFv portion provided in SEQ ID NO: 48. In one aspect, the human anti-BCMA binding domain comprises the scFv portion provided in SEQ ID NO: 49. In one aspect, the human anti-BCMA binding domain comprises the scFv portion provided in SEQ ID NO: 50. In one aspect, the human anti-BCMA binding domain comprises the scFv portion provided in SEQ ID NO: 51. In one aspect, the human anti-BCMA binding domain comprises the scFv portion provided in SEQ ID NO: 52. In one aspect, the human anti-BCMA binding domain comprises the scFv portion provided in SEQ ID NO: 53. In one aspect, the human anti-BCMA binding domain comprises the scFv portion provided in SEQ ID NO: 129. In one aspect, the human anti-BCMA binding domain comprises the scFv portion provided in SEQ ID NO: 130. In one aspect, the human anti-BCMA CAR comprises the scFv portion provided in SEQ ID NO: 131. In one aspect, the human anti-BCMA binding domain comprises the scFv portion provided in SEQ ID NO: 132. In one aspect, the human anti-BCMA binding domain comprises the scFv portion provided in SEQ ID NO: 133. In one aspect, the human anti-BCMA binding domain comprises the scFv portion provided in SEQ ID NO: 134. In one aspect, the human anti-BCMA binding domain comprises the scFv portion provided in SEQ ID NO: 135. In one aspect, the human anti-BCMA binding domain comprises the scFv portion provided in SEQ ID NO: 136. In one aspect, the human anti-BCMA binding domain comprises the scFv portion provided in SEQ ID NO: 137. In one aspect, the human anti-BCMA binding domain comprises the scFv portion provided in SEQ ID NO: 138. In one aspect, the human anti-BCMA binding domaincomprises the scFv portion provided in SEQ ID NO: 139. In one aspect, the human anti-BCMA binding domain comprises the scFv portion provided in SEQ ID NO: 140. In one aspect, the human anti-BCMA binding domain comprises the scFv portion provided in SEQ ID NO: 141. In one aspect, the human anti-BCMA CAR comprises the scFv portion provided in SEQ ID NO: 142. In one aspect, the human anti-BCMA CAR comprises

the scFv portion provided in SEQ ID NO: 143. In one aspect, the human anti-BCMA CAR comprises the scFv portion provided in SEQ ID NO: 144. In one aspect, the human anti-BCMA CAR comprises the scFv portion provided in SEQ ID NO: 145. In one aspect, the human anti-BCMA CAR comprises the scFv portion provided in SEQ ID NO: 146. In one aspect, the human anti-BCMA CAR comprises the scFv portion provided in SEQ ID NO: 147. In one aspect, the human anti-BCMA CAR comprises the scFv portion provided in SEQ ID NO: 148. In one aspect, the human anti-BCMA CAR comprises the scFv portion provided in SEQ ID NO: 149. In one aspect, the humanized anti-BCMA binding domain comprises the scFv portion provided in SEQ ID NO: 255. In one aspect, the humanized anti-BCMA CAR comprises the scFv portion provided in SEQ ID NO: 257.

In one aspect, the human anti-BCMA CAR comprises the scFv portion provided in SEQ ID NO: 263. In one aspect, the human anti-BCMA CAR comprises the scFv portion provided in SEQ ID NO: 264. In one aspect, the human anti-BCMA CAR comprises the scFv portion provided in SEQ ID NO: 265. In one aspect, the human anti-BCMA CAR comprises the scFv portion provided in SEQ ID NO: 266.

In one aspect, the CARs of the invention combine an antigen binding domain of a specific antibody with an intracellular signaling molecule. For example, in some aspects, the intracellular signaling molecule includes, but is not limited to, CD3-zeta chain, 4-1BB and CD28 signaling modules and combinations thereof. In one aspect, the antigen binding domain binds to BCMA. In one aspect, the BCMA CAR comprises a CAR selected from the sequence provided in one or more of residues 22-483 of SEQ ID NO: 109, residues 22-490 of SEQ ID NO: 99, residues 22-488 of SEQ ID NO: 100, residues 22-487 of SEQ ID NO: 101, residues 22-493 of SEQ ID NO: 102, residues 22-490 of SEQ ID NO: 103, residues 22-491 of SEQ ID NO: 104, residues 22-482 of SEQ ID NO: 105, residues 22-483 of SEQ ID NO: 106, residues 22-485 of SEQ ID NO: 107, residues 22-483 of SEQ ID NO: 108, residues 22-490 of SEQ ID NO: 110, residues 22-483 of SEQ ID NO: 111, residues 22-484 of SEQ ID NO: 112, residues 22-485 of SEQ ID NO: 113, residues 22-487 of SEQ ID NO: 213, residues 23-489 of SEQ ID NO: 214, residues 22-490 of SEQ ID NO: 215, residues 22-484 of SEQ ID NO: 216, residues 22-485 of SEQ ID NO: 217, residues 22-489 of SEQ ID NO: 218, residues 22-497 of SEQ ID NO: 219, residues 22-492 of SEQ ID NO: 220, residues 22-490 of SEQ ID NO: 221, residues 22-485 of SEQ ID NO: 222, residues 22-492 of SEQ ID NO: 223, residues 22-492 of SEQ ID NO: 224, residues 22-483 of SEQ ID NO: 225, residues 22-490 of SEQ ID NO: 226, residues 22-485 of SEQ ID NO: 227, residues 22-486 of SEQ ID NO: 228, residues 22-492 of SEQ ID NO: 229, residues 22-488 of SEQ ID NO: 230, residues 22-488 of SEQ ID NO: 231, residues 22-495 of SEQ ID NO: 232, residues 22-490 of SEQ ID NO: 233.

Furthermore, the present invention provides BCMA CAR compositions and their use in medicaments or methods for treating, among other diseases, cancer or any malignancy or autoimmune diseases involving cells or tissues which express BCMA.

In one aspect, the CAR of the invention can be used to eradicate BCMA-expressing normal cells, thereby applicable for use as a cellular conditioning therapy prior to cell transplantation. In one aspect, the BCMA-expressing normal cell is a BCMA-expressing normal stem cell and the cell transplantation is a stem cell transplantation.

In one aspect, the invention provides a cell (e.g., T cell or NK cell) engineered to express a chimeric antigen receptor (CAR), wherein the CAR T cell ("CART") or the CAR NK cell exhibits an antitumor property. A preferred antigen is BCMA. In one aspect, the antigen binding domain of the CAR comprises a human anti-BCMA antibody fragment or a partially humanized anti-BCMA antibody fragment. In one aspect, the antigen binding domain of the CAR comprises human anti-BCMA antibody fragment or a partially humanized anti-BCMA antibody fragment comprising an scFv. Accordingly, the invention provides a BCMA-CAR that comprises a humanized anti-BCMA binding domain and is engineered into a cell, e.g., a T cell or NK cell, and methods of their use for adoptive therapy.

In one aspect, the BCMA-CAR comprises at least one intracellular domain selected from the group of a CD137 (4-1BB) signaling domain, a CD28 signaling domain, a CD3zeta signal domain, and any combination thereof. In one aspect, the BCMA-CAR comprises at least one intracellular signaling domain is from one or more co-stimulatory molecule(s) other than a CD137 (4-1BB) or CD28.

Chimeric Antigen Receptor (CAR)

The present invention provides a CAR (e.g., a CAR polypeptide) that comprises an anti-BCMA binding domain (e.g., human or humanized BCMA binding domain as described herein), a transmembrane domain, and an intracellular signaling domain, and wherein said anti-BCMA binding domain comprises a heavy chain complementary determining region 1 (HC CDR1), a heavy chain complementary determining region 2 (HC CDR2), and a heavy chain complementary determining region 3 (HC CDR3) of any anti-BMCA heavy chain binding domain amino acid sequences listed in Table 1 or 16. The anti-BCMA binding domain of the CAR can further comprise a light chain complementary determining region 1 (LC CDR1), a light chain complementary determining region 2 (LC CDR2), and a light chain complementary determining region 3 (LC CDR3) of any anti-BMCA heavy chain binding domain amino acid sequences listed in Table 1 or 16.

The present invention also provides nucleic acid molecules encoding the CAR as described herein, e.g., encoding a CAR that comprises an anti-BCMA binding domain (e.g., human or humanized BCMA binding domain as described herein), a transmembrane domain, and an intracellular signaling domain, and wherein said anti-BCMA binding domain comprises a heavy chain complementary determining region 1 (HC CDR1), a heavy chain complementary determining region 2 (HC CDR2), and a heavy chain complementary determining region 3 (HC CDR3) of any anti-BMCA heavy chain binding domain amino acid sequences listed in Table 1 or 16 . In one embodiment, the encoded anti-BCMA

binding domain of the CAR can further comprise a light chain complementary determining region 1 (LC CDR1), a light chain complementary determining region 2 (LC CDR2), and a light chain complementary determining region 3 (LC CDR3) of any anti-BMCA heavy chain binding domain amino acid sequences listed in Table 1 or 16.

In specific aspects, a CAR construct of the invention comprises a scFv domain selected from the group consisting of SEQ ID NO: 39, SEQ ID NO: 40, SEQ ID NO: 41, SEQ ID NO: 42, SEQ ID NO: 43, SEQ ID NO: 44, SEQ ID NO: 45, SEQ ID NO: 46, SEQ ID NO: 47, SEQ ID NO: 48, SEQ ID NO: 49, SEQ ID NO: 50, SEQ ID NO: 51, SEQ ID NO: 52, SEQ ID NO: 53, SEQ ID NO: 129, SEQ ID NO: 130, SEQ ID NO: 131, SEQ ID NO: 132, SEQ ID NO: 133, SEQ ID NO: 134, SEQ ID NO: 135, SEQ ID NO: 136, SEQ ID NO: 137, SEQ ID NO: 138, SEQ ID NO: 139, SEQ ID NO: 140, SEQ ID NO: 141, SEQ ID NO: 142, SEQ ID NO: 143, SEQ ID NO: 144, SEQ ID NO: 145, SEQ ID NO: 146, SEQ ID NO: 147, SEQ ID NO: 148, SEQ ID NO: 149, SEQ ID NO: 263, SEQ ID NO: 264, SEQ ID NO: 265, and SEQ ID NO: 266, wherein the scFv may be preceded by an optional leader sequence such as provided in SEQ ID NO: 1, and followed by an optional hinge sequence such as provided in SEQ ID NO:2 or SEQ ID NO:3 or SEQ ID NO:4 or SEQ ID NO:5, a transmembrane region such as provided in SEQ ID NO:6, an intracellular signalling domain that includes SEQ ID NO:7 or SEQ ID NO:8 and a CD3 zeta sequence that includes SEQ ID NO:9 or SEQ ID NO:10, wherein the domains are contiguous with and in the same reading frame to form a single fusion protein. Also included in the invention is a nucleotide sequence that encodes the polypeptide of each of the scFv fragments selected from the group consisting of SEQ ID NO: 39, SEQ ID NO: 40, SEQ ID NO: 41, SEQ ID NO: 42, SEQ ID NO: 43, SEQ ID NO: 44, SEQ ID NO: 45, SEQ ID NO: 46, SEQ ID NO: 47, SEQ ID NO: 48, SEQ ID NO: 49, SEQ ID NO: 50, SEQ ID NO: 51, SEQ ID NO: 52, SEQ ID NO: 53, SEQ ID NO: 129, SEQ ID NO: 130, SEQ ID NO: 131, SEQ ID NO: 132, SEQ ID NO: 133, SEQ ID NO: 134, SEQ ID NO: 135, SEQ ID NO: 136, SEQ ID NO: 137, SEQ ID NO: 138, SEQ ID NO: 139, SEQ ID NO: 140, SEQ ID NO: 141, SEQ ID NO: 142, SEQ ID NO: 143, SEQ ID NO: 144, SEQ ID NO: 145, SEQ ID NO: 146, SEQ ID NO: 147, SEQ ID NO: 148, SEQ ID NO: 149, SEQ ID NO: 263, SEQ ID NO: 264, SEQ ID NO: 265, and SEQ ID NO: 266.

Also included in the invention is a nucleotide sequence that encodes the polypeptide of each of the scFv fragments selected from the group consisting of SEQ ID NO: 39, SEQ ID NO: 40, SEQ ID NO: 41, SEQ ID NO: 42, SEQ ID NO: 43, SEQ ID NO: 44, SEQ ID NO: 45, SEQ ID NO: 46, SEQ ID NO: 47, SEQ ID NO: 48, SEQ ID NO: 49, SEQ ID NO: 50, SEQ ID NO: 51, SEQ ID NO: 52, SEQ ID NO: 53, SEQ ID NO: 129, SEQ ID NO: 130, SEQ ID NO: 131, SEQ ID NO: 132, SEQ ID NO: 133, SEQ ID NO: 134, SEQ ID NO: 135, SEQ ID NO: 136, SEQ ID NO: 137, SEQ ID NO: 138, SEQ ID NO: 139, SEQ ID NO: 140, SEQ ID NO: 141, SEQ ID NO: 142, SEQ ID NO: 143, SEQ ID NO: 144, SEQ ID NO: 145, SEQ ID NO: 146, SEQ ID NO: 147, SEQ ID NO: 148, SEQ ID NO: 149, SEQ ID

NO: 263, SEQ ID NO: 264, SEQ ID NO: 265, and SEQ ID NO: 266, and each of the domains of SEQ ID NOS: 1,2, and 6-9, plus the encoded BCMA CAR fusion protein of the invention.

In one aspect, an exemplary BCMA CAR constructs comprise an optional leader sequence, an extracellular antigen binding domain, a hinge, a transmembrane domain, and an intracellular stimulatory domain. In one aspect an exemplary BCMA CAR construct comprises an optional leader sequence, an extracellular antigen binding domain, a hinge, a transmembrane domain, an intracellular costimulatory domain and an intracellular stimulatory domain. Specific BCMA CAR constructs containing human scFv domains of the invention are provided as SEQ ID NO: 39, SEQ ID NO: 40, SEQ ID NO: 41, SEQ ID NO: 42, SEQ ID NO: 43, SEQ ID NO: 44, SEQ ID NO: 45, SEQ ID NO: 46, SEQ ID NO: 47, SEQ ID NO: 48, SEQ ID NO: 49, SEQ ID NO: 50, SEQ ID NO: 51, SEQ ID NO: 52, SEQ ID NO: 53, SEQ ID NO: 129, SEQ ID NO: 130, SEQ ID NO: 131, SEQ ID NO: 132, SEQ ID NO: 133, SEQ ID NO: 134, SEQ ID NO: 135, SEQ ID NO: 136, SEQ ID NO: 137, SEQ ID NO: 138, SEQ ID NO: 139, SEQ ID NO: 140, SEQ ID NO: 141, SEQ ID NO: 142, SEQ ID NO: 143, SEQ ID NO: 144, SEQ ID NO: 145, SEQ ID NO: 146, SEQ ID NO: 147, SEQ ID NO: 148, and SEQ ID NO: 149. Full-length CAR sequences are also provided herein as SEQ ID NO: 39, SEQ ID NO: 40, SEQ ID NO: 41, SEQ ID NO: 42, SEQ ID NO: 43, SEQ ID NO: 44, SEQ ID NO: 45, SEQ ID NO: 46, SEQ ID NO: 47, SEQ ID NO: 48, SEQ ID NO: 49, SEQ ID NO: 50, SEQ ID NO: 51, SEQ ID NO: 52, SEQ ID NO: 53, SEQ ID NO: 129, SEQ ID NO: 130, SEQ ID NO: 131, SEQ ID NO: 132, SEQ ID NO: 133, SEQ ID NO: 134, SEQ ID NO: 135, SEQ ID NO: 136, SEQ ID NO: 137, SEQ ID NO: 138, SEQ ID NO: 139, SEQ ID NO: 140, SEQ ID NO: 141, SEQ ID NO: 142, SEQ ID NO: 143, SEQ ID NO: 144, SEQ ID NO: 145, SEQ ID NO: 146, SEQ ID NO: 147, SEQ ID NO: 148, and SEQ ID NO: 149 as shown in Table 1.

An exemplary leader sequence is provided as SEQ ID NO: 1. An exemplary hinge/spacer sequence is provided as SEQ ID NO:2 or SEQ ID NO:3 or SEQ ID NO:4 or SEQ ID NO:5. An exemplary transmembrane domain sequence is provided as SEQ ID NO:6. An exemplary sequence of the intracellular signaling domain of the 4-1BB protein is provided as SEQ ID NO: 7. An exemplary sequence of the intracellular signaling domain of CD27 is provided as SEQ ID NO:8. An exemplary CD3zeta domain sequence is provided as SEQ ID NO: 9 or SEQ ID NO:10.

In one aspect, the present invention encompasses a recombinant nucleic acid construct comprising a nucleic acid molecule encoding a CAR, wherein the nucleic acid molecule comprises the nucleic acid sequence encoding an anti-BCMA binding domain, e.g., described herein, that is contiguous with and in the same reading frame as a nucleic acid sequence encoding an intracellular signaling domain. In one aspect, the anti-BCMA binding domain is selected from one or more of SEQ ID NO: 54, SEQ ID NO: 55, SEQ ID NO: 56, SEQ ID NO: 57, SEQ ID NO: 58, SEQ ID NO: 59, SEQ ID NO: 60, SEQ ID NO: 61, SEQ ID NO: 62, SEQ ID NO: 63, SEQ ID NO: 64, SEQ ID NO: 65, SEQ ID NO: 66, SEQ ID NO: 67, SEQ ID NO: 68, SEQ ID NO: 150, SEQ ID NO: 151, SEQ ID NO: 152,

SEQ ID NO: 153, SEQ ID NO: 154, SEQ ID NO: 155, SEQ ID NO: 156, SEQ ID NO: 157, SEQ ID NO: 158, SEQ ID NO: 159, SEQ ID NO: 160, SEQ ID NO: 161, SEQ ID NO: 162, SEQ ID NO: 163, SEQ ID NO: 164, SEQ ID NO: 165, SEQ ID NO: 166, SEQ ID NO: 167, SEQ ID NO: 168, SEQ ID NO: 169, and SEQ ID NO: 170. In one aspect, the anti-BCMA binding domain comprises SEQ ID NO: 54.In one aspect, the anti-BCMA binding domain comprises SEQ ID NO: 55. In one aspect, the anti-BCMA binding domain comprises SEQ ID NO: 56. In one aspect, the anti-BCMA binding domain comprises SEQ ID NO: 57. In one aspect, the anti-BCMA binding domain comprises SEQ ID NO: 58. In one aspect, the anti-BCMA binding domain comprises SEQ ID NO: 59. In one aspect, the anti-BCMA binding domain comprises SEQ ID NO: 60. In one aspect, the anti-BCMA binding domain comprises SEQ ID NO: 61. In one aspect, the anti-BCMA binding domain comprises SEQ ID NO: 62. In one aspect, the anti-BCMA binding domain comprises SEQ ID NO: 63. In one aspect, the anti-BCMA binding domain comprises SEQ ID NO: 64. In one aspect, the anti-BCMA binding domain comprises SEQ ID NO: 65. In one aspect, the anti-BCMA binding domain comprises SEQ ID NO: 66. In one aspect, the anti-BCMA binding domain comprises SEQ ID NO: 67. In one aspect, the anti-BCMA binding domain comprises SEQ ID NO: 68. In one aspect, the anti-BCMA binding domain comprises SEQ ID NO: 150. In one aspect, the anti-BCMA binding domain comprises SEQ ID NO: 151. In one aspect, the anti-BCMA binding domain comprises SEQ ID NO: 152. In one aspect, the anti-BCMA binding domain comprises SEQ ID NO: 153. In one aspect, the anti-BCMA binding domain comprises SEQ ID NO: 154. In one aspect, the anti-BCMA binding domain comprises SEQ ID NO: 155. In one aspect, the anti-BCMA binding domain comprises SEQ ID NO: 156. In one aspect, the anti-BCMA binding domain comprises SEQ ID NO: 157. In one aspect, the anti-BCMA binding domain comprises SEQ ID NO: 158. In one aspect, the anti-BCMA binding domain comprises SEQ ID NO: 159. In one aspect, the anti-BCMA binding domain comprises SEQ ID NO: 160. In one aspect, the anti-BCMA binding domain comprises SEQ ID NO: 161. In one aspect, the anti-BCMA binding domain comprises SEQ ID NO: 162. In one aspect, the anti-BCMA binding domain comprises SEQ ID NO: 163. In one aspect, the anti-BCMA binding domain comprises SEQ ID NO: 164. In one aspect, the anti-BCMA binding domain comprises SEQ ID NO: 165. In one aspect, the anti-BCMA binding domain comprises SEQ ID NO: 166. In one aspect, the anti-BCMA binding domain comprises SEQ ID NO: 167. In one aspect, the anti-BCMA binding domain comprises SEQ ID NO: 168. In one aspect, the anti-BCMA binding domain comprises SEQ ID NO: 169. In one aspect, the anti-BCMA binding domain comprises SEQ ID NO: 170.

In one aspect, the present invention encompasses a recombinant nucleic acid construct comprising a nucleic acid molecule encoding a CAR, wherein the nucleic acid molecule comprises a nucleic acid sequence encoding an anti- BCMA binding domain selected from one or more of SEQ ID

NO: 54, SEQ ID NO: 55, SEQ ID NO: 56, SEQ ID NO: 57, SEQ ID NO: 58, SEQ ID NO: 59, SEQ ID NO: 60, SEQ ID NO: 61, SEQ ID NO: 62, SEQ ID NO: 63, SEQ ID NO: 64, SEQ ID NO: 65, SEQ ID NO: 66, SEQ ID NO: 67, SEQ ID NO: 68, SEQ ID NO: 150, SEQ ID NO: 151, SEQ ID NO: 152, SEQ ID NO: 153, SEQ ID NO: 154, SEQ ID NO: 155, SEQ ID NO: 156, SEQ ID NO: 157, SEQ ID NO: 158, SEQ ID NO: 159, SEQ ID NO: 160, SEQ ID NO: 161, SEQ ID NO: 162, SEQ ID NO: 163, SEQ ID NO: 164, SEQ ID NO: 165, SEQ ID NO: 166, SEQ ID NO: 167, SEQ ID NO: 168, SEQ ID NO: 169, and SEQ ID NO: 170, e.g., wherein the sequence is contiguous with and in the same reading frame as the nucleic acid sequence encoding an intracellular signaling domain. An exemplary intracellular signaling domain that can be used in the CAR includes, but is not limited to, one or more intracellular signaling domains of, e.g., CD3-zeta, CD28, 4-1BB, and the like. In some instances, the CAR can comprise any combination of CD3-zeta, CD28, 4-1BB, and the like. In one aspect the nucleic acid sequence of a CAR construct of the invention is selected from one or more of SEQ ID NO: 114, SEQ ID NO: 115, SEQ ID NO: 116, SEQ ID NO: 117, SEQ ID NO: 118, SEQ ID NO: 119, SEQ ID NO: 120, SEQ ID NO: 121, SEQ ID NO: 122, SEQ ID NO: 123, SEQ ID NO: 124, SEQ ID NO: 125, SEQ ID NO: 126, SEQ ID NO: 127, SEQ ID NO: 128, SEQ ID NO: 234, SEQ ID NO: 235, SEQ ID NO: 236, SEQ ID NO: 237, SEQ ID NO: 238, SEQ ID NO: 239, SEQ ID NO: 240, SEQ ID NO: 241, SEQ ID NO: 242, SEQ ID NO: 243, SEQ ID NO: 244, SEQ ID NO: 245, SEQ ID NO: 246, SEQ ID NO: 247, SEQ ID NO: 248, SEQ ID NO: 249, SEQ ID NO: 250, SEQ ID NO: 251, SEQ ID NO: 252, SEQ ID NO: 253, or SEQ ID NO: 254. In one aspect the nucleic acid sequence of a CAR construct is SEQ ID NO: 114. In one aspect the nucleic acid sequence of a CAR construct is SEQ ID NO: 115. In one aspect the nucleic acid sequence of a CAR construct is SEQ ID NO: 116. In one aspect the nucleic acid sequence of a CAR construct is SEQ ID NO: 117. In one aspect the nucleic acid sequence of a CAR construct is SEQ ID NO: 118. In one aspect the nucleic acid sequence of a CAR construct is SEQ ID NO: 119. In one aspect the nucleic acid sequence of a CAR construct is SEQ ID NO: 120. In one aspect the nucleic acid sequence of a CAR construct is SEQ ID NO: 121. In one aspect the nucleic acid sequence of a CAR construct is SEQ ID NO: 122. In one aspect the nucleic acid sequence of a CAR construct is SEQ ID NO: 123. In one aspect the nucleic acid sequence of a CAR construct is SEQ ID NO: 124. In one aspect the nucleic acid sequence of a CAR construct is SEQ ID NO: 125. In one aspect the nucleic acid sequence of a CAR construct is SEQ ID NO: 126. In one aspect the nucleic acid sequence of a CAR construct is SEQ ID NO: 127. In one aspect the nucleic acid sequence of a CAR construct is SEQ ID NO: 128. In one aspect the nucleic acid sequence of a CAR construct is SEQ ID NO: 234. In one aspect the nucleic acid sequence of a CAR construct is SEQ ID NO: 235. In one aspect the nucleic acid sequence of a CAR construct is SEQ ID NO: 236. In one aspect the nucleic acid sequence of a CAR construct is SEQ ID NO: 237. In one aspect the nucleic acid sequence of a CAR construct is SEQ ID NO: 238. In one aspect the nucleic acid sequence of a CAR construct is SEQ ID

NO: 239. In one aspect the nucleic acid sequence of a CAR construct is SEQ ID NO: 240. In one aspect the nucleic acid sequence of a CAR construct is SEQ ID NO: 241. In one aspect the nucleic acid sequence of a CAR construct is SEQ ID NO: 242. In one aspect the nucleic acid sequence of a CAR construct is SEQ ID NO: 243. In one aspect the nucleic acid sequence of a CAR construct is SEQ ID NO: 244. In one aspect the nucleic acid sequence of a CAR construct is SEQ ID NO: 245. In one aspect the nucleic acid sequence of a CAR construct is SEQ ID NO: 246. In one aspect the nucleic acid sequence of a CAR construct is SEQ ID NO: 247. In one aspect the nucleic acid sequence of a CAR construct is SEQ ID NO: 248. In one aspect the nucleic acid sequence of a CAR construct is SEQ ID NO: 249. In one aspect the nucleic acid sequence of a CAR construct is SEQ ID NO: 250. In one aspect the nucleic acid sequence of a CAR construct is SEQ ID NO: 251. In one aspect the nucleic acid sequence of a CAR construct is SEQ ID NO: 252. In one aspect the nucleic acid sequence of a CAR construct is SEQ ID NO: 253. In one aspect the nucleic acid sequence of a CAR construct is SEQ ID NO: 254.

The nucleic acid sequences coding for the desired molecules can be obtained using recombinant methods known in the art, such as, for example by screening libraries from cells expressing the gene, by deriving the gene from a vector known to include the same, or by isolating directly from cells and tissues containing the same, using standard techniques. Alternatively, the nucleic acid of interest can be produced synthetically, rather than cloned.

The present invention includes retroviral and lentiviral vector constructs expressing a CAR that can be directly transduced into a cell.

The present invention also includes an RNA construct that can be directly transfected into a cell. A method for generating mRNA for use in transfection involves in vitro transcription (IVT) of a template with specially designed primers, followed by polyA addition, to produce a construct containing 3' and 5' untranslated sequence ("UTR"), a 5' cap and/or Internal Ribosome Entry Site (IRES), the nucleic acid to be expressed, and a polyA tail, typically 50-2000 bases in length (SEQ ID NO:35). RNA so produced can efficiently transfect different kinds of cells. In one embodiment, the template includes sequences for the CAR. In an embodiment, an RNA CAR vector is transduced into a cell, e.g., T cell or NK cell, by electroporation.

Antigen binding domain

The CARs of the present invention comprise a target-specific binding domain. The choice of moiety depends upon the type and number of ligands that define the surface of a target cell. For example, the antigen binding domain may be chosen to recognize an antigen that acts as a cell surface marker on target cells associated with a particular disease state.

In one aspect, the CAR-mediated T-cell response can be directed to an antigen of interest by way of engineering an antigen binding domain that specifically binds a desired antigen into the CAR.

In one aspect, the CAR of the present invention comprises a binding domain that specifically binds BCMA. In one aspect, the CAR of the present invention comprises an antigen binding domain that specifically binds human BCMA.

The antigen binding domain can be any protein that binds to the antigen including but not limited to a monoclonal antibody, a polyclonal antibody, a recombinant antibody, a human antibody, a humanized antibody, and a functional fragment thereof, including but not limited to a single-domain antibody such as a heavy chain variable domain (VH), a light chain variable domain (VL) and a variable domain (VHH) of camelid derived nanobody, and to an alternative scaffold known in the art to function as antigen binding domain, such as a recombinant fibronectin domain, and the like. In some instances, it is beneficial for the antigen binding domain to be derived from the same species in which the CAR will ultimately be used in. For example, for use in humans, it may be beneficial for the antigen binding domain of the CAR to comprise human or humanized residues for the antigen binding domain of an antibody or antibody fragment.

Thus, in one aspect, the antigen binding domain comprises a human or a humanized antibody or an antibody fragment. In one embodiment, the human anti-BCMA binding domain comprises one or more (e.g., all three) light chain complementary determining region 1 (LC CDR1), light chain complementary determining region 2 (LC CDR2), and light chain complementary determining region 3 (LC CDR3) of a human anti-BCMA binding domain described herein, and/or one or more (e.g., all three) heavy chain complementary determining region 1 (HC CDR1), heavy chain complementary determining region 2 (HC CDR2), and heavy chain complementary determining region 3 (HC CDR3) of a human anti-BCMA binding domain described herein, e.g., a human anti-BCMA binding domain comprising one or more, e.g., all three, LC CDRs and one or more, e.g., all three, HC CDRs. In one embodiment, the human anti-BCMA binding domain comprises one or more (e.g., all three) heavy chain complementary determining region 1 (HC CDR1), heavy chain complementary determining region 2 (HC CDR2), and heavy chain complementary determining region 3 (HC CDR3) of a human anti-BCMA binding domain described herein, e.g., the human anti-BCMA binding domain has two variable heavy chain regions, each comprising a HC CDR1, a HC CDR2 and a HC CDR3 described herein. In one embodiment, the human anti-BCMA binding domain comprises a human light chain variable region described herein (e.g., in Table 1) and/or a human heavy chain variable region described herein (e.g., in Table 1). In one embodiment, the human anti-BCMA binding domain comprises a human heavy chain variable region described herein (e.g., in Table 1), e.g., at least two human heavy chain variable regions described herein (e.g., in Table 1). In one embodiment, the anti-BCMA binding domain is a scFv comprising a light chain and a heavy chain of an amino acid sequence of Table 1. In an embodiment,

the anti-BCMA binding domain (e.g., an scFv) comprises: a light chain variable region comprising an amino acid sequence having at least one, two or three modifications (e.g., substitutions, e.g., conservative substitutions) but not more than 30, 20 or 10 modifications (e.g., substitutions, e.g., conservative substitutions) of an amino acid sequence of a light chain variable region provided in Table 1, or a sequence with 95-99% identity with an amino acid sequence of Table 11; and/or a heavy chain variable region comprising an amino acid sequence having at least one, two or three modifications (e.g., substitutions, e.g., conservative substitutions) but not more than 30, 20 or 10 modifications (e.g., substitutions, e.g., conservative substitutions) of an amino acid sequence of a heavy chain variable region provided in Table 1, or a sequence with 95-99% identity to an amino acid sequence of Table 1.

In one embodiment, the human anti-BCMA binding domain comprises a sequence selected from a group consisting of SEQ ID NO: 39, SEQ ID NO: 40, SEQ ID NO: 41, SEQ ID NO: 42, SEQ ID NO: 43, SEQ ID NO: 44, SEQ ID NO: 45, SEQ ID NO: 46, SEQ ID NO: 47, SEQ ID NO: 48, SEQ ID NO: 49, SEQ ID NO: 50, SEQ ID NO: 51, SEQ ID NO: 52, SEQ ID NO: 53, SEQ ID NO: 129, SEQ ID NO: 130, SEQ ID NO: 131, SEQ ID NO: 132, SEQ ID NO: 133, SEQ ID NO: 134, SEQ ID NO: 135, SEQ ID NO: 136, SEQ ID NO: 137, SEQ ID NO: 138, SEQ ID NO: 139, SEQ ID NO: 140, SEQ ID NO: 141, SEQ ID NO: 142, SEQ ID NO: 143, SEQ ID NO: 144, SEQ ID NO: 145, SEQ ID NO: 146, SEQ ID NO: 147, SEQ ID NO: 148, and SEQ ID NO: 149, or a sequence with 95-99% identify thereof. In one embodiment, the nucleic acid sequence encoding the human anti-BCMA binding domain comprises a sequence selected from a group consisting of SEQ ID NO: 54, SEQ ID NO: 55, SEQ ID NO: 56, SEQ ID NO: 57, SEQ ID NO: 58, SEQ ID NO: 59, SEQ ID NO: 60, SEQ ID NO: 61, SEQ ID NO: 62, SEQ ID NO: 63, SEQ ID NO: 64, SEQ ID NO: 65, SEQ ID NO: 66, SEQ ID NO: 67, SEQ ID NO: 68, SEQ ID NO: 150, SEQ ID NO: 151, SEQ ID NO: 152, SEQ ID NO: 153, SEQ ID NO: 154, SEQ ID NO: 155, SEQ ID NO: 156, SEQ ID NO: 157, SEQ ID NO: 158, SEQ ID NO: 159, SEQ ID NO: 160, SEQ ID NO: 161, SEQ ID NO: 162, SEQ ID NO: 163, SEQ ID NO: 164, SEQ ID NO: 165, SEQ ID NO: 166, SEQ ID NO: 167, SEQ ID NO: 168, SEQ ID NO: 169, and SEQ ID NO: 170, or a sequence with 95-99% identify thereof. In one embodiment, the human anti-BCMA binding domain is a scFv, and a light chain variable region comprising an amino acid sequence described herein, e.g., in Table 1, is attached to a heavy chain variable region comprising an amino acid sequence described herein, e.g., in Table 1, via a linker, e.g., a linker described herein. In one embodiment, the human anti-BCMA binding domain includes a (Gly4-Ser)n linker, wherein n is 1, 2, 3, 4, 5, or 6, preferably 3 or 4 (SEQ ID NO:26). The light chain variable region and heavy chain variable region of a scFv can be, e.g., in any of the following orientations: light chain variable region-linker-heavy chain variable region or heavy chain variable region-linker-light chain variable region. In one aspect, the antigen binding domain portion comprises one or more sequence selected from SEQ ID NO: 39, SEQ ID NO: 40, SEQ ID NO: 41, SEQ ID NO: 42, SEQ ID NO: 43, SEQ ID NO: 44, SEQ ID NO:

45, SEQ ID NO: 46, SEQ ID NO: 47, SEQ ID NO: 48, SEQ ID NO: 49, SEQ ID NO: 50, SEQ ID NO: 51, SEQ ID NO: 52, SEQ ID NO: 53, SEQ ID NO: 129, SEQ ID NO: 130, SEQ ID NO: 131, SEQ ID NO: 132, SEQ ID NO: 133, SEQ ID NO: 134, SEQ ID NO: 135, SEQ ID NO: 136, SEQ ID NO: 137, SEQ ID NO: 138, SEQ ID NO: 139, SEQ ID NO: 140, SEQ ID NO: 141, SEQ ID NO: 142, SEQ ID NO: 143, SEQ ID NO: 144, SEQ ID NO: 145, SEQ ID NO: 146, SEQ ID NO: 147, SEQ ID NO: 148, and SEQ ID NO: 149. In one aspect the CAR is selected from one or more sequence selected from residues 22-483 of SEQ ID NO: 109, residues 22-490 of SEQ ID NO: 99, residues 22-488 of SEQ ID NO: 100, residues 22-487 of SEQ ID NO: 101, residues 22-493 of SEQ ID NO: 102, residues 22-490 of SEQ ID NO: 103, residues 22-491 of SEQ ID NO: 104, residues 22-482 of SEQ ID NO: 105, residues 22-483 of SEQ ID NO: 106, residues 22-485 of SEQ ID NO: 107, residues 22-483 of SEQ ID NO: 108, residues 22-490 of SEQ ID NO: 110, residues 22-483 of SEQ ID NO: 111, residues 22-484 of SEQ ID NO: 112, residues 22-485 of SEQ ID NO: 113, residues 22-487 of SEQ ID NO: 213, residues 23-489 of SEQ ID NO: 214, residues 22-490 of SEQ ID NO: 215, residues 22-484 of SEQ ID NO: 216, residues 22-485 of SEQ ID NO: 217, residues 22-489 of SEQ ID NO: 218, residues 22-497 of SEQ ID NO: 219, residues 22-492 of SEQ ID NO: 220, residues 22-490 of SEQ ID NO: 221, residues 22-485 of SEQ ID NO: 222, residues 22-492 of SEQ ID NO: 223, residues 22-492 of SEQ ID NO: 224, residues 22-483 of SEQ ID NO: 225, residues 22-490 of SEQ ID NO: 226, residues 22-485 of SEQ ID NO: 227, residues 22-486 of SEQ ID NO: 228, residues 22-492 of SEQ ID NO: 229, residues 22-488 of SEQ ID NO: 230, residues 22-488 of SEQ ID NO: 231, residues 22-495 of SEQ ID NO: 232, residues 22-490 of SEQ ID NO: 233.

In one embodiment, the anti- BCMA binding domain comprises a light chain variable region described herein (e.g., in Table 16) and/or a heavy chain variable region described herein (e.g., in Table 16). In one embodiment, the encoded humanized anti-BCMA binding domain comprises a light chain variable region provided in SEQ ID NO: 259, SEQ ID NO: 260, SEQ ID NO: 261, SEQ ID NO: 262, and/or a heavy chain variable region provided in SEQ ID NO: 255, SEQ ID NO: 256, SEQ ID NO: 257, SEQ ID NO: 258. In one embodiment, the encoded anti- BCMA binding domain is a scFv comprising a light chain and a heavy chain of an amino acid sequence of Table 16. In an embodiment, the human or humanized anti-BCMA binding domain (e.g., an scFv) comprises: a light chain variable region comprising an amino acid sequence having at least one, two or three modifications (e.g., substitutions, e.g., conservative substitutions) but not more than 30, 20 or 10 modifications (e.g., substitutions, e.g., conservative substitutions) of an amino acid sequence of a light chain variable region provided in SEQ ID NO: 259, SEQ ID NO: 260, SEQ ID NO: 261, SEQ ID NO: 262, or a sequence with 95-99% identity thereof; and/or a heavy chain variable region comprising an amino acid sequence having at least one, two or three modifications (e.g., substitutions, e.g., conservative substitutions) but not more than 30, 20 or 10 modifications (e.g., substitutions, e.g., conservative substitutions) of an

amino acid sequence of a heavy chain variable region provided in SEQ ID NO: 255, SEQ ID NO: 256, SEQ ID NO: 257, SEQ ID NO: 258, or a sequence with 95-99% identity thereof. In one embodiment, the encoded anti-BCMA binding domain includes a (Gly4-Ser)n linker, wherein n is 1, 2, 3, 4, 5, or 6, preferably 3 or 4 (SEQ ID NO:26). The light chain variable region and heavy chain variable region of a scFv can be, e.g., in any of the following orientations: light chain variable region-linker-heavy chain variable region or heavy chain variable region-linker-light chain variable region.

In one embodiment, the human anti- BCMA binding domain comprises a sequence selected from a group consisting of SEQ ID NO: 263, SEQ ID NO: 264, SEQ ID NO: 265, and SEQ ID NO: 266, or a sequence with 95-99% identity thereof.

In some aspects, a non-human antibody is humanized, where specific sequences or regions of the antibody are modified to increase similarity to an antibody naturally produced in a human or fragment thereof. In one aspect, the antigen binding domain is humanized.

A humanized antibody can be produced using a variety of techniques known in the art, including but not limited to, CDR-grafting (see, e.g., European Patent No. EP 239,400; International Publication No. WO 91/09967; and U.S. Pat. Nos. 5,225,539, 5,530,101, and 5,585,089, each of which is incorporated herein in its entirety by reference), veneering or resurfacing (see, e.g., European Patent Nos. EP 592,106 and EP 519,596; Padlan, 1991, Molecular Immunology, 28(4/5):489-498; Studnicka et al., 1994, Protein Engineering, 7(6):805-814; and Roguska et al., 1994, PNAS, 91:969-973, each of which is incorporated herein by its entirety by reference), chain shuffling (see, e.g., U.S. Pat. No. 5,565,332, which is incorporated herein in its entirety by reference), and techniques disclosed in, e.g., U.S. Patent Application Publication No. US2005/0042664, U.S. Patent Application Publication No. US2005/0048617, U.S. Pat. No. 6,407,213, U.S. Pat. No. 5,766,886, International Publication No. WO 9317105, Tan et al., J. Immunol., 169:1119-25 (2002), Caldas et al., Protein Eng., 13(5):353-60 (2000), Morea et al., Methods, 20(3):267-79 (2000), Baca et al., J. Biol. Chem., 272(16):10678-84 (1997), Roguska et al., Protein Eng., 9(10):895-904 (1996), Couto et al., Cancer Res., 55 (23 Supp):5973s-5977s (1995), Couto et al., Cancer Res., 55(8):1717-22 (1995), Sandhu J S, Gene, 150(2):409-10 (1994), and Pedersen et al., J. Mol. Biol., 235(3):959-73 (1994), each of which is incorporated herein in its entirety by reference. Often, framework residues in the framework regions will be substituted with the corresponding residue from the CDR donor antibody to alter, for example improve, antigen binding. These framework substitutions, e.g., conservative substitutions are identified by methods well-known in the art, e.g., by modeling of the interactions of the CDR and framework residues to identify framework residues important for antigen binding and sequence comparison to identify unusual framework residues at particular positions. (See, e.g., Queen et al., U.S. Pat. No. 5,585,089; and Riechmann et al., 1988, Nature, 332:323, which are incorporated herein by reference in their entireties.)

A humanized antibody or antibody fragment has one or more amino acid residues remaining in it from a source which is nonhuman. These nonhuman amino acid residues are often referred to as "import" residues, which are typically taken from an "import" variable domain. As provided herein, humanized antibodies or antibody fragments comprise one or more CDRs from nonhuman immunoglobulin molecules and framework regions wherein the amino acid residues comprising the framework are derived completely or mostly from human germline. Multiple techniques for humanization of antibodies or antibody fragments are well-known in the art and can essentially be performed following the method of Winter and co-workers (Jones et al., Nature, 321:522-525 (1986); Riechmann et al., Nature, 332:323-327 (1988); Verhoeyen et al., Science, 239:1534-1536 (1988)), by substituting rodent CDRs or CDR sequences for the corresponding sequences of a human antibody, i.e., CDR-grafting (EP 239,400; PCT Publication No. WO 91/09967; and U.S. Pat. Nos. 4,816,567; 6,331,415; 5,225,539; 5,530,101; 5,585,089; 6,548,640, the contents of which are incorporated herein by reference herein in their entirety). In such humanized antibodies and antibody fragments, substantially less than an intact human variable domain has been substituted by the corresponding sequence from a nonhuman species. Humanized antibodies are often human antibodies in which some CDR residues and possibly some framework (FR) residues are substituted by residues from analogous sites in rodent antibodies. Humanization of antibodies and antibody fragments can also be achieved by veneering or resurfacing (EP 592,106; EP 519,596; Padlan, 1991, Molecular Immunology, 28(4/5):489-498; Studnicka et al., Protein Engineering, 7(6):805-814 (1994); and Roguska et al., PNAS, 91:969-973 (1994)) or chain shuffling (U.S. Pat. No. 5,565,332), the contents of which are incorporated herein by reference herein in their entirety.

The choice of human variable domains, both light and heavy, to be used in making the humanized antibodies is to reduce antigenicity. According to the so-called "best-fit" method, the sequence of the variable domain of a rodent antibody is screened against the entire library of known human variable-domain sequences. The human sequence which is closest to that of the rodent is then accepted as the human framework (FR) for the humanized antibody (Sims et al., J. Immunol., 151:2296 (1993); Chothia et al., J. Mol. Biol., 196:901 (1987), the contents of which are incorporated herein by reference herein in their entirety). Another method uses a particular framework derived from the consensus sequence of all human antibodies of a particular subgroup of light or heavy chains. The same framework may be used for several different humanized antibodies (see, e.g., Nicholson et al. Mol. Immun. 34 (16-17): 1157-1165 (1997); Carter et al., Proc. Natl. Acad. Sci. USA, 89:4285 (1992); Presta et al., J. Immunol., 151:2623 (1993), the contents of which are incorporated herein by reference herein in their entirety). In some embodiments, the framework region, e.g., all four framework regions, of the heavy chain variable region are derived from a VH4_4-59 germline sequence. In one embodiment, the framework region can comprise, one, two, three, four or five modifications, e.g., substitutions, e.g.,

conservative substitutions, e.g., from the amino acid at the corresponding murine sequence. In one embodiment, the framework region, e.g., all four framework regions of the light chain variable region are derived from a VK3_1.25 germline sequence. In one embodiment, the framework region can comprise, one, two, three, four or five modifications, e.g., substitutions, e.g., conservative substitutions, e.g., from the amino acid at the corresponding murine sequence.

In some aspects, the portion of a CAR composition of the invention that comprises an antibody fragment is humanized with retention of high affinity for the target antigen and other favorable biological properties. According to one aspect of the invention, humanized antibodies and antibody fragments are prepared by a process of analysis of the parental sequences and various conceptual humanized products using three-dimensional models of the parental and humanized sequences. Three-dimensional immunoglobulin models are commonly available and are familiar to those skilled in the art. Computer programs are available which illustrate and display probable three-dimensional conformational structures of selected candidate immunoglobulin sequences. Inspection of these displays permits analysis of the likely role of the residues in the functioning of the candidate immunoglobulin sequence, e.g., the analysis of residues that influence the ability of the candidate immunoglobulin to bind the target antigen. In this way, FR residues can be selected and combined from the recipient and import sequences so that the desired antibody or antibody fragment characteristic, such as increased affinity for the target antigen, is achieved. In general, the CDR residues are directly and most substantially involved in influencing antigen binding.

A humanized antibody or antibody fragment may retain a similar antigenic specificity as the original antibody, e.g., in the present invention, the ability to bind human BCMA In some embodiments, a humanized antibody or antibody fragment may have improved affinity and/or specificity of binding to human BCMA.

In one embodiment, the humanized anti-BCMA binding domain of the CAR, comprises one or more (e.g., all three) light chain complementary determining region 1 (LC CDR1), light chain complementary determining region 2 (LC CDR2), and light chain complementary determining region 3 (LC CDR3) of a humanized anti-BCMA binding domain described herein, and/or one or more (e.g., all three) heavy chain complementary determining region 1 (HC CDR1), heavy chain complementary determining region 2 (HC CDR2), and heavy chain complementary determining region 3 (HC CDR3) of a humanized anti-BCMA binding domain described herein, e.g., a humanized anti-BCMA binding domain comprising one or more, e.g., all three, LC CDRs and one or more, e.g., all three, HC CDRs. In one embodiment, the humanized anti-BCMA binding domain comprises one or more (e.g., all three) heavy chain complementary determining region 1 (HC CDR1), heavy chain complementary determining region 2 (HC CDR2), and heavy chain complementary determining region 3 (HC CDR3) of a humanized anti-BCMA binding domain described herein, e.g., the humanized anti-BCMA binding

domain has two variable heavy chain regions, each comprising a HC CDR1, a HC CDR2 and a HC CDR3 described herein. In one embodiment, the humanized anti-BCMA binding domain comprises a humanized light chain variable region described herein (e.g., SEQ ID NO:255 or 257) and/or a human heavy chain variable region described herein (e.g., SEQ ID NO:255 or 257).

In one aspect, the anti-BCMA binding domain is characterized by particular functional features or properties of an antibody or antibody fragment. For example, in one aspect, the portion of a CAR composition of the invention that comprises an antigen binding domain specifically binds human BCMA

In one aspect, the antigen binding domain has the same or a similar binding specificity to human BCMA as mouse BCMA. In one aspect, the invention relates to an antigen binding domain comprising an antibody or antibody fragment, wherein the antibody binding domain specifically binds to a BCMA protein or fragment thereof, wherein the antibody or antibody fragment comprises a variable light chain and/or a variable heavy chain that includes an amino acid sequence of SEQ ID NO: 39, SEQ ID NO: 40, SEQ ID NO: 41, SEQ ID NO: 42, SEQ ID NO: 43, SEQ ID NO: 44, SEQ ID NO: 45, SEQ ID NO: 46, SEQ ID NO: 47, SEQ ID NO: 48, SEQ ID NO: 49, SEQ ID NO: 50, SEQ ID NO: 51, SEQ ID NO: 52, SEQ ID NO: 53, SEQ ID NO: 129, SEQ ID NO: 130, SEQ ID NO: 131, SEQ ID NO: 132, SEQ ID NO: 133, SEQ ID NO: 134, SEQ ID NO: 135, SEQ ID NO: 136, SEQ ID NO: 137, SEQ ID NO: 138, SEQ ID NO: 139, SEQ ID NO: 140, SEQ ID NO: 141, SEQ ID NO: 142, SEQ ID NO: 143, SEQ ID NO: 144, SEQ ID NO: 145, SEQ ID NO: 146, SEQ ID NO: 147, SEQ ID NO: 148, or SEQ ID NO: 149. In one aspect, the antigen binding domain comprises an amino acid sequence of an scFv selected from SEQ ID NO: 39, SEQ ID NO: 40, SEQ ID NO: 41, SEQ ID NO: 42, SEQ ID NO: 43, SEQ ID NO: 44, SEQ ID NO: 45, SEQ ID NO: 46, SEQ ID NO: 47, SEQ ID NO: 48, SEQ ID NO: 49, SEQ ID NO: 50, SEQ ID NO: 51, SEQ ID NO: 52, SEQ ID NO: 53, SEQ ID NO: 129, SEQ ID NO: 130, SEQ ID NO: 131, SEQ ID NO: 132, SEQ ID NO: 133, SEQ ID NO: 134, SEQ ID NO: 135, SEQ ID NO: 136, SEQ ID NO: 137, SEQ ID NO: 138, SEQ ID NO: 139, SEQ ID NO: 140, SEQ ID NO: 141, SEQ ID NO: 142, SEQ ID NO: 143, SEQ ID NO: 144, SEQ ID NO: 145, SEQ ID NO: 146, SEQ ID NO: 147, SEQ ID NO: 148, or SEQ ID NO: 149. In certain aspects, the scFv is contiguous with and in the same reading frame as a leader sequence. In one aspect the leader sequence is the polypeptide sequence provided as SEQ ID NO:1.

In one aspect, the anti-BCMA binding domain is a fragment, e.g., a single chain variable fragment (scFv). In one aspect, the anti-BCMA binding domain is a Fv, a Fab, a (Fab')2, or a bi-functional (e.g. bi-specific) hybrid antibody (e.g., Lanzavecchia et al., Eur. J. Immunol. 17, 105 (1987)). In one aspect, the antibodies and fragments thereof of the invention binds a BCMA protein with wild-type or enhanced affinity.

In some instances, scFvs can be prepared according to method known in the art (see, for example, Bird et al., (1988) Science 242:423-426 and Huston et al., (1988) Proc. Natl. Acad. Sci. USA 85:5879-5883). ScFv molecules can be produced by linking VH and VL regions together using flexible polypeptide linkers. The scFv molecules comprise a linker (e.g., a Ser-Gly linker) with an optimized length and/or amino acid composition. The linker length can greatly affect how the variable regions of a scFv fold and interact. In fact, if a short polypeptide linker is employed (e.g., between 5-10 amino acids) intrachain folding is prevented. Interchain folding is also required to bring the two variable regions together to form a functional epitope binding site. For examples of linker orientation and size see, e.g., Hollinger et al. 1993 Proc Natl Acad. Sci. U.S.A. 90:6444-6448, U.S. Patent Application Publication Nos. 2005/0100543, 2005/0175606, 2007/0014794, and PCT publication Nos. WO2006/020258 and WO2007/024715, is incorporated herein by reference.

An scFv can comprise a linker of at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 35, 40, 45, 50, or more amino acid residues between its VL and VH regions. The linker sequence may comprise any naturally occurring amino acid. In some embodiments, the linker sequence comprises amino acids glycine and serine. In another embodiment, the linker sequence comprises sets of glycine and serine repeats such as (Gly4Ser)n, where n is a positive integer equal to or greater than 1 (SEQ ID NO:25). In one embodiment, the linker can be (Gly4Ser)4 (SEQ ID NO:27) or (Gly4Ser)3(SEQ ID NO:28). Variation in the linker length may retain or enhance activity, giving rise to superior efficacy in activity studies.

Exemplary Human BCMA CAR Constructs and Antigen Binding Domains

Exemplary BCMA CAR constructs disclose herein comprise an scFv (e.g., a scFv as disclosed in Tables 1 or 16, optionally preceded with an optional leader sequence (e.g., SEQ ID NO:1 and SEQ ID NO:12 for exemplary leader amino acid and nucleotide sequences, respectively). The sequences of the scFv fragments (SEQ ID NOs: 39-53, 129-149, or 263-266, not including the optional leader sequence) are provided herein in Tables 1 or 16. The BCMA CAR construct can further include an optional hinge domain, e.g., a CD8 hinge domain (e.g., including the amino acid sequence of SEQ ID NO: 2 or encoded by a nucleic acid sequence of SEQ ID NO:13); a transmembrane domain, e.g., a CD8 transmembrane domain (e.g., including the amino acid sequence of SEQ ID NO: 6 or encoded by the nucleotide sequence of SEQ ID NO: 17); an intracellular domain, e.g., a 4-1BB intracellular domain (e.g., including the amino acid sequence of SEQ ID NO: 7 or encoded by the nucleotide sequence of SEQ ID NO: 18; and a functional signaling domain, e.g., a CD3 zeta domain (e.g., including amino acid sequence of SEQ ID NO: 9 or 10, or encoded by the nucleotide sequence of SEQ ID NO: 20 or 21). In certain embodiments, the domains are contiguous with and in the same reading frame to form a single fusion protein. In other embodiments, the domain are in separate polypeptides, e.g., as in an RCAR molecule as described herein.

In certain embodiments, the full length BCMA CAR molecule includes the amino acid sequence of, or is encoded by the nucleotide sequence of, BCMA-1, BCMA-2, BCMA-3, BCMA-4, BCMA-5, BCMA-6, BCMA-7, BCMA-8, BCMA-9, BCMA-10, BCMA-11, BCMA-12, BCMA-13, BCMA-14, BCMA-15, 149362, 149363, 149364, 149365, 149366, 149367, 149368, 149369, BCMA_EBB-C1978-A4, BCMA_EBB-C1978-G1, BCMA_EBB-C1979-C1, BCMA_EBB-C1978-C7, BCMA_EBB-C1978-D10, BCMA_EBB-C1979-C12, BCMA_EBB-C1980-G4, BCMA_EBB-C1980-D2, BCMA_EBB-C1978-A10, BCMA_EBB-C1978-D4, BCMA_EBB-C1980-A2, BCMA_EBB-C1981-C3, BCMA_EBB-C1978-G4, A7D12.2, C11D5.3, C12A3.2, or C13F12.1 provided in Table 1 or 16, or a sequence substantially (e.g., 95-99%) identical thereto.

In certain embodiments, the BCMA CAR molecule, or the anti-BCMA antigen binding domain, includes the scFv amino acid sequence of BCMA-1, BCMA-2, BCMA-3, BCMA-4, BCMA-5, BCMA-6, BCMA-7, BCMA-8, BCMA-9, BCMA-10, BCMA-11, BCMA-12, BCMA-13, BCMA-14, BCMA-15, 149362, 149363, 149364, 149365, 149366, 149367, 149368, 149369, BCMA_EBB-C1978-A4, BCMA_EBB-C1978-G1, BCMA_EBB-C1979-C1, BCMA_EBB-C1978-C7, BCMA_EBB-C1978-D10, BCMA_EBB-C1979-C12, BCMA_EBB-C1980-G4, BCMA_EBB-C1980-D2, BCMA_EBB-C1978-A10, BCMA_EBB-C1978-D4, BCMA_EBB-C1980-A2, BCMA_EBB-C1981-C3, BCMA_EBB-C1978-G4, A7D12.2, C11D5.3, C12A3.2, or C13F12.1 provided in Table 1 or 16 (with or without the leader sequence), or a sequence substantially identical (e.g., 95-99% identical, or up to 20, 15, 10, 8, 6, 5, 4, 3, 2, or 1 amino acid changes, e.g., substitutions (e.g., conservative substitutions)) to any of the aforesaid sequences.

In certain embodiments, the BCMA CAR molecule, or the anti-BCMA antigen binding domain, includes the heavy chain variable region and/or the light chain variable region of BCMA-1, BCMA-2, BCMA-3, BCMA-4, BCMA-5, BCMA-6, BCMA-7, BCMA-8, BCMA-9, BCMA-10, BCMA-11, BCMA-12, BCMA-13, BCMA-14, BCMA-15, 149362, 149363, 149364, 149365, 149366, 149367, 149368, 149369, BCMA_EBB-C1978-A4, BCMA_EBB-C1978-G1, BCMA_EBB-C1979-C1, BCMA_EBB-C1978-C7, BCMA_EBB-C1978-D10, BCMA_EBB-C1979-C12, BCMA_EBB-C1980-G4, BCMA_EBB-C1980-D2, BCMA_EBB-C1978-A10, BCMA_EBB-C1978-D4, BCMA_EBB-C1980-A2, BCMA_EBB-C1981-C3, BCMA_EBB-C1978-G4, A7D12.2, C11D5.3, C12A3.2, or C13F12.1 provided in Table 1 or 16, or a sequence substantially identical (e.g., 95-99% identical, or up to 20, 15, 10, 8, 6, 5, 4, 3, 2, or 1 amino acid changes, e.g., substitutions (e.g., conservative substitutions)) to any of the aforesaid sequences.

In certain embodiments, the BCMA CAR molecule, or the anti-BCMA antigen binding domain, includes one, two or three CDRs from the heavy chain variable region (e.g., HCDR1, HCDR2 and/or HCDR3), provided in Table 20; and/or one, two or three CDRs from the light chain variable region (e.g., LCDR1, LCDR2 and/or LCDR3) of BCMA-1, BCMA-2, BCMA-3, BCMA-4, BCMA-5, BCMA-

6, BCMA-7, BCMA-8, BCMA-9, BCMA-10, BCMA-11, BCMA-12, BCMA-13, BCMA-14, BCMA-15, 149362, 149363, 149364, 149365, 149366, 149367, 149368, 149369, BCMA_EBB-C1978-A4, BCMA_EBB-C1978-G1, BCMA_EBB-C1979-C1, BCMA_EBB-C1978-C7, BCMA_EBB-C1978-D10, BCMA_EBB-C1979-C12, BCMA_EBB-C1980-G4, BCMA_EBB-C1980-D2, BCMA_EBB-C1978-A10, BCMA_EBB-C1978-D4, BCMA_EBB-C1980-A2, BCMA_EBB-C1981-C3, BCMA_EBB-C1978-G4, A7D12.2, C11D5.3, C12A3.2, or C13F12.1, provided in Table 21; or a sequence substantially identical (e.g., 95-99% identical, or up to 20, 15, 10, 8, 6, 5, 4, 3, 2, or 1 amino acid changes, e.g., substitutions (e.g., conservative substitutions)) to any of the aforesaid sequences.

In certain embodiments, the BCMA CAR molecule, or the anti-BCMA antigen binding domain, includes one, two or three CDRs from the heavy chain variable region (e.g., HCDR1, HCDR2 and/or HCDR3), provided in Table 22; and/or one, two or three CDRs from the light chain variable region (e.g., LCDR1, LCDR2 and/or LCDR3) of BCMA-1, BCMA-2, BCMA-3, BCMA-4, BCMA-5, BCMA-6, BCMA-7, BCMA-8, BCMA-9, BCMA-10, BCMA-11, BCMA-12, BCMA-13, BCMA-14, BCMA-15, 149362, 149363, 149364, 149365, 149366, 149367, 149368, 149369, BCMA_EBB-C1978-A4, BCMA_EBB-C1978-G1, BCMA_EBB-C1979-C1, BCMA_EBB-C1978-C7, BCMA_EBB-C1978-D10, BCMA_EBB-C1979-C12, BCMA_EBB-C1980-G4, BCMA_EBB-C1980-D2, BCMA_EBB-C1978-A10, BCMA_EBB-C1978-D4, BCMA_EBB-C1980-A2, BCMA_EBB-C1981-C3, BCMA_EBB-C1978-G4, A7D12.2, C11D5.3, C12A3.2, or C13F12.1, provided in Table 23; or a sequence substantially identical (e.g., 95-99% identical, or up to 20, 15, 10, 8, 6, 5, 4, 3, 2, or 1 amino acid changes, e.g., substitutions (e.g., conservative substitutions)) to any of the aforesaid sequences.

In certain embodiments, the BCMA CAR molecule, or the anti-BCMA antigen binding domain, includes one, two or three CDRs from the heavy chain variable region (e.g., HCDR1, HCDR2 and/or HCDR3), provided in Table 24; and/or one, two or three CDRs from the light chain variable region (e.g., LCDR1, LCDR2 and/or LCDR3) of BCMA-1, BCMA-2, BCMA-3, BCMA-4, BCMA-5, BCMA-6, BCMA-7, BCMA-8, BCMA-9, BCMA-10, BCMA-11, BCMA-12, BCMA-13, BCMA-14, BCMA-15, 149362, 149363, 149364, 149365, 149366, 149367, 149368, 149369, BCMA_EBB-C1978-A4, BCMA_EBB-C1978-G1, BCMA_EBB-C1979-C1, BCMA_EBB-C1978-C7, BCMA_EBB-C1978-D10, BCMA_EBB-C1979-C12, BCMA_EBB-C1980-G4, BCMA_EBB-C1980-D2, BCMA_EBB-C1978-A10, BCMA_EBB-C1978-D4, BCMA_EBB-C1980-A2, BCMA_EBB-C1981-C3, BCMA_EBB-C1978-G4, A7D12.2, C11D5.3, C12A3.2, or C13F12.1, provided in Table 25; or a sequence substantially identical (e.g., 95-99% identical, or up to 20, 15, 10, 8, 6, 5, 4, 3, 2, or 1 amino acid changes, e.g., substitutions (e.g., conservative substitutions)) to any of the aforesaid sequences.

The sequences of human CDR sequences of the scFv domains are shown in Tables 20, 22, and 24 for the heavy chain variable domains and in Tables 21, 23, and 25 for the light chain variable domains. "ID" stands for the respective SEQ ID NO for each CDR.

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Table 20. Heavy Chain Variable Domain CDRs according to the Kabat numbering scheme (Kabat et al. (1991), "Sequences of Proteins of Immunological Interest," 5th Ed. Public Health Service, National Institutes of Health, Bethesda, MD)

Candidate	HCDR1	ID	HCDR2	ID	HCDR3	ID
139109	NHGMS	394	GIVYSGSTYYAASVKG	434	HGGESDV	474
139103	NYAMS	384	GISRSGENTYYADSVKG	424	SPAHYYGGMDV	464
139105	DYAMH	385	GISWNSGSIGYADSVKG	425	HSFLAY	465
139111	NHGMS	386	GIVYSGSTYYAASVKG	426	HGGESDV	466
139100	NFGIN	387	WINPKNNNTNYAQKFQG	427	GPYYYQSYMDV	467
139101	SDAMT	388	VISGSGGTTYYADSVKG	428	LDSSGYYYARGPRY	468
139102	NYGIT	389	WISAYNGNTNYAQKFQG	429	GPYYYYMDV	469
139104	NHGMS	390	GIVYSGSTYYAASVKG	430	HGGESDV	470
139106	NHGMS	391	GIVYSGSTYYAASVKG	431	HGGESDV	471
139107	NHGMS	392	GIVYSGSTYYAASVKG	432	HGGESDV	472
139108	DYYMS	393	YISSSGSTIYYADSVKG	433	ESGDGMDV	473
139110	DYYMS	395	YISSSGNTIYYADSVKG	435	STMVREDY	475
139112	NHGMS	396	GIVYSGSTYYAASVKG	436	HGGESDV	476
139113	NHGMS	397	GIVYSGSTYYAASVKG	437	HGGESDV	477
139114	NHGMS	398	GIVYSGSTYYAASVKG	438	HGGESDV	478
149362	SSYYYWG	399	SIYYSGSAYYNPSLKS	439	HWQEWPDAFDI	479
149363	TSGMCVS	400	RIDWDEDKFYSTSLKT	440	SGAGGTSATAFDI	480
149364	SYSMN	401	SISSSSSYIYYADSVKG	441	TIAAVYAFDI	481
149365	DYYMS	402	YISSSGSTIYYADSVKG	442	DLRGAFDI	482
149366	SHYIH	403	MINPSGGVTAYSQTLQG	443	EGSGSGWYFDF	483
149367	SGGYYWS	404	YIYYSGSTYYNPSLKS	444	AGIAARLRGAFDI	484
149368	SYAIS	405	GIIPIFGTANYAQK	445	RGGYQLLRWDVG	485

			FQG		LLRSAFDI	
149369	SNSAAWN	406	RTYYRSKWYSFYAISLKS	446	SSPEGLFLYWFDP	486
BCMA_EBB-C1978-A4	SYAMS	407	AISGSGGSTYYADSVKG	447	VEGSGSLDY	487
BCMA_EBB-C1978-G1	RYPMS	408	GISDSGVSTYYADSAKG	448	RAGSEASDI	488
BCMA_EBB-C1979-C1	SYAMS	409	AISGSGGSTYYADSVKG	449	ATYKRELRYYYGMDV	489
BCMA_EBB-C1978-C7	SYAMS	410	AISGSGGSTYYADSVKG	450	ATYKRELRYYYGMDV	490
BCMA_EBB-C1978-D10	DYAMH	411	GISWNSGSIGYADSVKG	451	VGKAVPDV	491
BCMA_EBB-C1979-C12	DYAMH	412	SINWKGNSLAYGDSVKG	452	HQGVAYYNYAMDV	492
BCMA_EBB-C1980-G4	SYAMS	413	AISGSGGSTYYADSVKG	453	VVRDGMDV	493
BCMA_EBB-C1980-D2	SYAMS	414	AISGSGGSTYYADSVKG	454	IPQTGTFDY	494
BCMA_EBB-C1978-A10	SYAMS	415	AISGSGGSTYYADSVKG	455	ANYKRELRYYYGMDV	495
BCMA_EBB-C1978-D4	SYAMS	416	AISGSGGSTYYADSVKG	456	ALVGATGAFDI	496
BCMA_EBB-C1980-A2	SYAMS	417	AISGSGGSTYYADSVKG	457	WFGEGFDP	497
BCMA_EBB-C1981-C3	SYAMS	418	AISGSGGSTYYADSVKG	458	VGYDSSGYYRDYYGMDV	498
BCMA_EBB-C1978-G4	SYAMS	419	AISGSGGSTYYADSVKG	459	MGWSSGYLGAFDI	499
A7D12.2	NFGMN	420	WINTYTGESYFADDFKG	460	GEIYYGYDGGFAY	500
C11D5.3	DYSIN	421	WINTETREPAYAYDFRG	461	DYSYAMDY	501
C12A3.2	HYSMN	422	RINTESGVPIYADDFKG	462	DYLYSLDF	502
C13F12.1	HYSMN	423	RINTETGEPLYADDFKG	463	DYLYSCDY	503

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Table 21. Light Chain Variable Domain CDRs according to the Kabat numbering scheme (Kabat et al. (1991), "Sequences of Proteins of Immunological Interest," 5th Ed. Public Health Service, National Institutes of Health, Bethesda, MD)

Candidate	LCDR1	ID	LCDR2	ID	LCDR3	ID
139109	RASQSISSYLN	514	AASSLQS	554	QQSYSTPYT	594
139103	RASQSISSSFLA	504	GASRRAT	544	QQYHSSPSWT	584
139105	RSSQSLLHSNGYNYLD	505	LGSNRAS	545	MQALQTPYT	585
139111	KSSQSLLRNDGKTPLY	506	EVSNRFS	546	MQNIQFPS	586
139100	RSSQSLLHSNGYNYLN	507	LGSKRAS	547	MQALQTPYT	587
139101	RASQSISSYLN	508	GASTLAS	548	QQSYKRAS	588
139102	RSSQSLLYSNGYNYVD	509	LGSNRAS	549	MQGRQFPYS	589
139104	RASQSVSSNLA	510	GASTRAS	550	QQYGSSLT	590
139106	RASQSVSSKLA	511	GASIRAT	551	QQYGSSSWT	591
139107	RASQSVGSTNLA	512	DASNRAT	552	QQYGSSPPWT	592
139108	RASQSISSYLN	513	AASSLQS	553	QQSYTLA	593
139110	KSSESLVHNSGKTYLN	515	EVSNRDS	555	MQGTHWPGT	595
139112	QASEDINKFLN	516	DASTLQT	556	QQYESLPLT	596
139113	RASQSVGSNLA	517	GASTRAT	557	QQYNDWLPVT	597
139114	RASQSIGSSSLA	518	GASSRAS	558	QQYAGSPPFT	598
149362	KASQDIDDAMN	519	SATSPVP	559	LQHDNFPLT	599
149363	RASQDIYNNLA	520	AANKSQS	560	QHYYRFPYS	600
149364	RSSQSLLHSNGYNYLD	521	LGSNRAS	561	MQALQTPYT	601
149365	GGNNIGTKSVH	522	DDSVRPS	562	QVWDSDSEHVV	602
149366	SGDGLSKKYVS	523	RDKERPS	563	QAWDDTTVV	603
149367	RASQGIRNWLA	524	AASNLQS	564	QKYNSAPFT	604
149368	GGNNIGSKSVH	525	GKNNRPS	565	SSRDSSGDHLRV	605
149369	QGDSLGNYYAT	526	GTNNRPS	566	NSRDSSGHHLL	606
BCMA_EBB-C1978-A4	RASQSVSSAYLA	527	GASTRAT	567	QHYGSSFNGSSLFT	607
BCMA_EBB-C1978-G1	RASQSVSNSLA	528	DASSRAT	568	QQFGTSSGLT	608
BCMA_EBB-C1979-C1	RASQSVSSSFLA	529	GASSRAT	569	QQYHSSPSWT	609
BCMA_EBB-C1978-C7	RASQSVSTTFLA	530	GSSNRAT	570	QQYHSSPSWT	610
BCMA_EBB-	RASQSISSYLN	531	AASSLQS	571	QQSYSTPYS	611

C1978-D10						
BCMA_EBB-C1979-C12	RATQSIGSSFLA	532	GASQRAT	572	QHYESSPSWT	612
BCMA_EBB-C1980-G4	RASQSVSSSYLA	533	GASSRAT	573	QQYGSPPRFT	613
BCMA_EBB-C1980-D2	RASQSVSSSYLA	534	GASSRAT	574	QHYGSSPSWT	614
BCMA_EBB-C1978-A10	RASQRVASNYLA	535	GASSRAT	575	QHYDSSPSWT	615
BCMA_EBB-C1978-D4	RASQSLSSNFLA	536	GASNWAT	576	QYYGTSPMYT	616
BCMA_EBB-C1980-A2	RSSQSLLHSNGYNYLD	537	LGSNRAS	577	MQALQTPLT	617
BCMA_EBB-C1981-C3	RASQSVSSSYLA	538	GTSSRAT	578	QHYGNSPPKFT	618
BCMA_EBB-C1978-G4	RASQSVASSFLA	539	GASGRAT	579	QHYGGSPRLT	619
A7D12.2	RASQDVNTAVS	540	SASYRYT	580	QQHYSTPWT	620
C11D5.3	RASESVSVIGAHLIH	541	LASNLET	581	LQSRIFPRT	621
C12A3.2	RASESVTILGSHLIY	542	LASNVQT	582	LQSRTIPRT	622
C13F12.1	RASESVTILGSHLIY	543	LASNVQT	583	LQSRTIPRT	623

Table 22. Heavy Chain Variable Domain CDRs according to the Chothia numbering scheme (Al-Lazikani et al., (1997) JMB 273,927-948)

Candidate	**HCDR1**	**ID**	**HCDR2**	**ID**	**HCDR3**	**ID**
139109	GFALSNH	634	VYSGS	674	HGGESDV	714
139103	GFTFSNY	624	SRSGEN	664	SPAHYYGGMDV	704
139105	GFTFDDY	625	SWNSGS	665	HSFLAY	705
139111	GFALSNH	626	VYSGS	666	HGGESDV	706
139100	GYIFDNF	627	NPKNNN	667	GPYYYQSYMDV	707
139101	GFTFSSD	628	SGSGGT	668	LDSSGYYYARGPRY	708
139102	GYTFSNY	629	SAYNGN	669	GPYYYYMDV	709
139104	GFALSNH	630	VYSGS	670	HGGESDV	710
139106	GFALSNH	631	VYSGS	671	HGGESDV	711
139107	GFALSNH	632	VYSGS	672	HGGESDV	712
139108	GFTFSDY	633	SSSGST	673	ESGDGMDV	713
139110	GFTFSDY	635	SSSGNT	675	STMVREDY	715
139112	GFALSNH	636	VYSGS	676	HGGESDV	716
139113	GFALSNH	637	VYSGS	677	HGGESDV	717
139114	GFALSNH	638	VYSGS	678	HGGESDV	718
149362	GGSISSSYY	639	YYSGS	679	HWQEWPDAFDI	719
149363	GFSLRTSGM	640	DWDED	680	SGAGGTSATAFDI	720
149364	GFTFSSY	641	SSSSSY	681	TIAAVYAFDI	721

149365	GFTFSDY	642	SSSGST	682	DLRGAFDI	722
149366	GYTVTSH	643	NPSGGV	683	EGSGSGWYFDF	723
149367	GGSISSGGY	644	YYSGS	684	AGIAARLRGAFDI	724
149368	GGTFSSY	645	IPIFGT	685	RGGYQLLRWDVGLLRSAFDI	725
149369	GDSVSSNSA	646	YYRSKWY	686	SSPEGLFLYWFDP	726
BCMA_EBB-C1978-A4	GFTFSSY	647	SGSGGS	687	VEGSGSLDY	727
BCMA_EBB-C1978-G1	GITFSRY	648	SDSGVS	688	RAGSEASDI	728
BCMA_EBB-C1979-C1	GFTFSSY	649	SGSGGS	689	ATYKRELRYYYGMDV	729
BCMA_EBB-C1978-C7	GFTFSSY	650	SGSGGS	690	ATYKRELRYYYGMDV	730
BCMA_EBB-C1978-D10	GFTFDDY	651	SWNSGS	691	VGKAVPDV	731
BCMA_EBB-C1979-C12	GFTFDDY	652	NWKGNS	692	HQGVAYYNYAMDV	732
BCMA_EBB-C1980-G4	GFTFSSY	653	SGSGGS	693	VVRDGMDV	733
BCMA_EBB-C1980-D2	GFTFSSY	654	SGSGGS	694	IPQTGTFDY	734
BCMA_EBB-C1978-A10	GFTFSSY	655	SGSGGS	695	ANYKRELRYYYGMDV	735
BCMA_EBB-C1978-D4	GFSFSSY	656	SGSGGS	696	ALVGATGAFDI	736
BCMA_EBB-C1980-A2	GFTFSSY	657	SGSGGS	697	WFGEGFDP	737
BCMA_EBB-C1981-C3	GFTFSSY	658	SGSGGS	698	VGYDSSGYYRDYYGMDV	738
BCMA_EBB-C1978-G4	GFTFSSY	659	SGSGGS	699	MGWSSGYLGAFDI	739
A7D12.2	GYTFTNF	660	NTYTGE	700	GEIYYGYDGGFAY	740
C11D5.3	GYTFTDY	661	NTETRE	701	DYSYAMDY	741
C12A3.2	GYTFRHY	662	NTESGV	702	DYLYSLDF	742
C13F12.1	GYTFTHY	663	NTETGE	703	DYLYSCDY	743

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Table 23. Light Chain Variable Domain CDRs according to the Chothia numbering scheme (Al-Lazikani et al., (1997) JMB 273,927-948)

Candidate	**LCDR1**	**ID**	**LCDR2**	**ID**	**LCDR3**	**ID**
139109	SQSISSY	754	AAS	794	SYSTPY	834
139103	SQSISSSF	744	GAS	784	YHSSPSW	824
139105	SQSLLHSNGYNY	745	LGS	785	ALQTPY	825
139111	SQSLLRNDGKTP	746	EVS	786	NIQFP	826
139100	SQSLLHSNGYN	747	LGS	787	ALQTPY	827

	Y					
139101	SQSISSY	748	GAS	788	SYKRA	828
139102	SQSLLYSNGYNY	749	LGS	789	GRQFPY	829
139104	SQSVSSN	750	GAS	790	YGSSL	830
139106	SQSVSSK	751	GAS	791	YGSSSW	831
139107	SQSVGSTN	752	DAS	792	YGSSPPW	832
139108	SQSISSY	753	AAS	793	SYTL	833
139110	SESLVHNSGKTY	755	EVS	795	GTHWPG	835
139112	SEDINKF	756	DAS	796	YESLPL	836
139113	SQSVGSN	757	GAS	797	YNDWLPV	837
139114	SQSIGSSS	758	GAS	798	YAGSPPF	838
149362	SQDIDDA	759	SAT	799	HDNFPL	839
149363	SQDIYNN	760	AAN	800	YYRFPY	840
149364	SQSLLHSNGYNY	761	LGS	801	ALQTPY	841
149365	NNIGTKS	762	DDS	802	WDSDSEHV	842
149366	DGLSKKY	763	RDK	803	WDDTTV	843
149367	SQGIRNW	764	AAS	804	YNSAPF	844
149368	NNIGSKS	765	GKN	805	RDSSGDHLR	845
149369	DSLGNYY	766	GTN	806	RDSSGHHL	846
BCMA_EBB-C1978-A4	SQSVSSAY	767	GAS	807	YGSSFNGSSLF	847
BCMA_EBB-C1978-G1	SQSVSNS	768	DAS	808	FGTSSGL	848
BCMA_EBB-C1979-C1	SQSVSSSF	769	GAS	809	YHSSPSW	849
BCMA_EBB-C1978-C7	SQSVSTTF	770	GSS	810	YHSSPSW	850
BCMA_EBB-C1978-D10	SQSISSY	771	AAS	811	SYSTPY	851
BCMA_EBB-C1979-C12	TQSIGSSF	772	GAS	812	YESSPSW	852
BCMA_EBB-C1980-G4	SQSVSSSY	773	GAS	813	YGSPPRF	853
BCMA_EBB-C1980-D2	SQSVSSSY	774	GAS	814	YGSSPSW	854
BCMA_EBB-C1978-A10	SQRVASNY	775	GAS	815	YDSSPSW	855
BCMA_EBB-C1978-D4	SQSLSSNF	776	GAS	816	YGTSPMY	856
BCMA_EBB-C1980-A2	SQSLLHSNGYNY	777	LGS	817	ALQTPL	857
BCMA_EBB-C1981-C3	SQSVSSSY	778	GTS	818	YGNSPPKF	858
BCMA_EBB-	SQSVASSF	779	GAS	819	YGGSPRL	859

C1978-G4						
A7D12.2	SQDVNTA	780	SAS	820	HYSTPW	860
C11D5.3	SESVSVIGAHL	781	LAS	821	SRIFPR	861
C12A3.2	SESVTILGSHL	782	LAS	822	SRTIPR	862
C13F12.1	SESVTILGSHL	783	LAS	823	SRTIPR	863

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Table 24. Heavy Chain Variable Domain CDRs according to a combination of the Kabat numbering scheme (Kabat et al. (1991), "Sequences of Proteins of Immunological Interest," 5th Ed. Public Health Service, National Institutes of Health, Bethesda, MD) and the Chothia numbering scheme (Al-Lazikani et al., (1997) JMB 273,927-948).

Candidate	**HCDR1**	**ID**	**HCDR2**	**ID**	**HCDR3**	**ID**
139109	GFALSNHGMS	874	GIVYSGSTYYAASVKG	914	HGGESDV	954
139103	GFTFSNYAMS	864	GISRSGENTYYADSVKG	904	SPAHYYGGMDV	944
139105	GFTFDDYAMH	865	GISWNSGSIGYADSVKG	905	HSFLAY	945
139111	GFALSNHGMS	866	GIVYSGSTYYAASVKG	906	HGGESDV	946
139100	GYIFDNFGIN	867	WINPKNNNTNYAQKFQG	907	GPYYYQSYMDV	947
139101	GFTFSSDAMT	868	VISGSGGTTYYADSVKG	908	LDSSGYYYARGPRY	948
139102	GYTFSNYGIT	869	WISAYNGNTNYAQKFQG	909	GPYYYYMDV	949
139104	GFALSNHGMS	870	GIVYSGSTYYAASVKG	910	HGGESDV	950
139106	GFALSNHGMS	871	GIVYSGSTYYAASVKG	911	HGGESDV	951
139107	GFALSNHGMS	872	GIVYSGSTYYAASVKG	912	HGGESDV	952
139108	GFTFSDYYMS	873	YISSSGSTIYYADSVKG	913	ESGDGMDV	953
139110	GFTFSDYYMS	875	YISSSGNTIYYADSVKG	915	STMVREDY	955
139112	GFALSNHGMS	876	GIVYSGSTYYAASVKG	916	HGGESDV	956
139113	GFALSNHGMS	877	GIVYSGSTYYAASVKG	917	HGGESDV	957
139114	GFALSNHGMS	878	GIVYSGSTYYAASVKG	918	HGGESDV	958
149362	GGSISSSYYYWG	879	SIYYSGSAYYNPSLKS	919	HWQEWPDAFDI	959
149363	GFSLRTSGMCVS	880	RIDWDEDKFYSTSLKT	920	SGAGGTSATAFDI	960
149364	GFTFSSYSMN	881	SISSSSSYIYYADSVKG	921	TIAAVYAFDI	961

149365	GFTFSDYYMS	882	YISSSGSTIYYADSVKG	922	DLRGAFDI	962
149366	GYTVTSHYIH	883	MINPSGGVTAYSQTLQG	923	EGSGSGWYFDF	963
149367	GGSISSGGYYWS	884	YIYYSGSTYYNPSLKS	924	AGIAARLRGAFDI	964
149368	GGTFSSYAIS	885	GIIPIFGTANYAQKFQG	925	RGGYQLLRWDVGLLRSAFDI	965
149369	GDSVSSNSAAWN	886	RTYYRSKWYSFYAISLKS	926	SSPEGLFLYWFDP	966
BCMA_EBB-C1978-A4	GFTFSSYAMS	887	AISGSGGSTYYADSVKG	927	VEGSGSLDY	967
BCMA_EBB-C1978-G1	GITFSRYPMS	888	GISDSGVSTYYADSAKG	928	RAGSEASDI	968
BCMA_EBB-C1979-C1	GFTFSSYAMS	889	AISGSGGSTYYADSVKG	929	ATYKRELRYYYGMDV	969
BCMA_EBB-C1978-C7	GFTFSSYAMS	890	AISGSGGSTYYADSVKG	930	ATYKRELRYYYGMDV	970
BCMA_EBB-C1978-D10	GFTFDDYAMH	891	GISWNSGSIGYADSVKG	931	VGKAVPDV	971
BCMA_EBB-C1979-C12	GFTFDDYAMH	892	SINWKGNSLAYGDSVKG	932	HQGVAYYNYAMDV	972
BCMA_EBB-C1980-G4	GFTFSSYAMS	893	AISGSGGSTYYADSVKG	933	VVRDGMDV	973
BCMA_EBB-C1980-D2	GFTFSSYAMS	894	AISGSGGSTYYADSVKG	934	IPQTGTFDY	974
BCMA_EBB-C1978-A10	GFTFSSYAMS	895	AISGSGGSTYYADSVKG	935	ANYKRELRYYYGMDV	975
BCMA_EBB-C1978-D4	GFSFSSYAMS	896	AISGSGGSTYYADSVKG	936	ALVGATGAFDI	976
BCMA_EBB-C1980-A2	GFTFSSYAMS	897	AISGSGGSTYYADSVKG	937	WFGEGFDP	977
BCMA_EBB-C1981-C3	GFTFSSYAMS	898	AISGSGGSTYYADSVKG	938	VGYDSSGYYRDYYGMDV	978
BCMA_EBB-C1978-G4	GFTFSSYAMS	899	AISGSGGSTYYADSVKG	939	MGWSSGYLGAFDI	979
A7D12.2	GYTFTNFGMN	900	WINTYTGESYFADDFKG	940	GEIYYGYDGGFAY	980

C11D5.3	GYTFTDYSIN	901	WINTETREPAY AYDFRG	941	DYSYAMDY	981
C12A3.2	GYTFRHYSM N	902	RINTESGVPIYA DDFKG	942	DYLYSLDF	982
C13F12.1	GYTFTHYSM N	903	RINTETGEPLYA DDFKG	943	DYLYSCDY	983

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Table 25. Light Chain Variable Domain CDRs according to a combination of the Kabat numbering scheme (Kabat et al. (1991), "Sequences of Proteins of Immunological Interest," 5th Ed. Public Health Service, National Institutes of Health, Bethesda, MD) and the Chothia numbering scheme (Al-Lazikani et al., (1997) JMB 273,927-948).

Candidate	LCDR1	ID	LCDR2	ID	LCDR3	ID
139109	RASQSISSYLN	994	AASSLQS	1034	QQSYSTPYT	1074
139103	RASQSISSSFLA	984	GASRRAT	1024	QQYHSSPSW T	1064
139105	RSSQSLLHSNGYNYLD	985	LGSNRAS	1025	MQALQTPYT	1065
139111	KSSQSLLRNDGKTPLY	986	EVSNRFS	1026	MQNIQFPS	1066
139100	RSSQSLLHSNGYNYLN	987	LGSKRAS	1027	MQALQTPYT	1067
139101	RASQSISSYLN	988	GASTLAS	1028	QQSYKRAS	1068
139102	RSSQSLLYSNGYNYV D	989	LGSNRAS	1029	MQGRQFPYS	1069
139104	RASQSVSSNLA	990	GASTRAS	1030	QQYGSSLT	1070
139106	RASQSVSSKLA	991	GASIRAT	1031	QQYGSSSWT	1071
139107	RASQSVGSTNLA	992	DASNRAT	1032	QQYGSSPPW T	1072
139108	RASQSISSYLN	993	AASSLQS	1033	QQSYTLA	1073
139110	KSSESLVHNSGKTYLN	995	EVSNRDS	1035	MQGTHWPG T	1075
139112	QASEDINKFLN	996	DASTLQT	1036	QQYESLPLT	1076
139113	RASQSVGSNLA	997	GASTRAT	1037	QQYNDWLP VT	1077
139114	RASQSIGSSSLA	998	GASSRAS	1038	QQYAGSPPF T	1078
149362	KASQDIDDAMN	999	SATSPVP	1039	LQHDNFPLT	1079
149363	RASQDIYNNLA	1000	AANKSQS	1040	QHYYRFPYS	1080
149364	RSSQSLLHSNGYNYLD	1001	LGSNRAS	1041	MQALQTPYT	1081
149365	GGNNIGTKSVH	1002	DDSVRPS	1042	QVWDSDSEH VV	1082
149366	SGDGLSKKYVS	1003	RDKERPS	1043	QAWDDTTV V	1083
149367	RASQGIRNWLA	1004	AASNLQS	1044	QKYNSAPFT	1084
149368	GGNNIGSKSVH	1005	GKNNRPS	1045	SSRDSSGDH LRV	1085
149369	QGDSLGNYYAT	1006	GTNNRPS	1046	NSRDSSGHH LL	1086
BCMA_EB	RASQSVSSAYLA	1007	GASTRAT	1047	QHYGSSFNG	1087

B-C1978-A4					SSLFT	
BCMA_EB B-C1978-G1	RASQSVSNSLA	1008	DASSRAT	1048	QQFGTSSGLT	1088
BCMA_EB B-C1979-C1	RASQSVSSSFLA	1009	GASSRAT	1049	QQYHSSPSWT	1089
BCMA_EB B-C1978-C7	RASQSVSTTFLA	1010	GSSNRAT	1050	QQYHSSPSWT	1090
BCMA_EB B-C1978-D10	RASQSISSYLN	1011	AASSLQS	1051	QQSYSTPYS	1091
BCMA_EB B-C1979-C12	RATQSIGSSFLA	1012	GASQRAT	1052	QHYESSPSWT	1092
BCMA_EB B-C1980-G4	RASQSVSSSYLA	1013	GASSRAT	1053	QQYGSPPRFT	1093
BCMA_EB B-C1980-D2	RASQSVSSSYLA	1014	GASSRAT	1054	QHYGSSPSWT	1094
BCMA_EB B-C1978-A10	RASQRVASNYLA	1015	GASSRAT	1055	QHYDSSPSWT	1095
BCMA_EB B-C1978-D4	RASQSLSSNFLA	1016	GASNWAT	1056	QYYGTSPMYT	1096
BCMA_EB B-C1980-A2	RSSQSLLHSNGYNYLD	1017	LGSNRAS	1057	MQALQTPLT	1097
BCMA_EB B-C1981-C3	RASQSVSSSYLA	1018	GTSSRAT	1058	QHYGNSPPKFT	1098
BCMA_EB B-C1978-G4	RASQSVASSFLA	1019	GASGRAT	1059	QHYGGSPRLT	1099
A7D12.2	RASQDVNTAVS	1020	SASYRYT	1060	QQHYSTPWT	1100
C11D5.3	RASESVSVIGAHLIH	1021	LASNLET	1061	LQSRIFPRT	1101
C12A3.2	RASESVTILGSHLIY	1022	LASNVQT	1062	LQSRTIPRT	1102
C13F12.1	RASESVTILGSHLIY	1023	LASNVQT	1063	LQSRTIPRT	1103

In certain embodiments, the CAR molecule described herein (e.g., the CAR nucleic acid or the CAR polypeptide) or a BCMA binding domain includes:

(1) one, two, or three light chain (LC) CDRs chosen from one of the following:

(i) a LC CDR1 of SEQ ID NO: 504, LC CDR2 of SEQ ID NO: 544 and LC CDR3 of SEQ ID NO: 584 of BCMA-4 CAR (139103);

(ii) a LC CDR1 of SEQ ID NO: 514, LC CDR2 of SEQ ID NO: 554 and LC CDR3 of SEQ ID NO: 594 of BCMA-10 CAR (139109);

(iii) a LC CDR1 of SEQ ID NO: 516, LC CDR2 of SEQ ID NO: 556 and LC CDR3 of SEQ ID NO: 596 of BCMA-13 CAR (139112); or

(iv) a LC CDR1 of SEQ ID NO: 518, LC CDR2 of SEQ ID NO: 558 and LC CDR3 of SEQ ID NO: 598 of BCMA-15 CAR (139114), and/or

(2) one, two, or three heavy chain (HC) CDRs from one of the following:

(i) a HC CDR1 of SEQ ID NO: 384, HC CDR2 of SEQ ID NO: 424 and HC CDR3 of SEQ ID NO: 464 of BCMA-4 CAR (139103);

(ii) a HC CDR1 of SEQ ID NO: 394, HC CDR2 of SEQ ID NO: 434 and HC CDR3 of SEQ ID NO: 474 of BCMA-10 CAR (139109);

(iii) a HC CDR1 of SEQ ID NO: 396, HC CDR2 of SEQ ID NO: 436 and HC CDR3 of SEQ ID NO: 476 of BCMA-13 CAR (139112); or

(iv) a HC CDR1 of SEQ ID NO: 398, HC CDR2 of SEQ ID NO: 438 and HC CDR3 of SEQ ID NO: 478 of BCMA-15 (139114).

In certain embodiments, the CAR molecule described herein (e.g., the CAR nucleic acid or the CAR polypeptide) includes:

(1) one, two, or three light chain (LC) CDRs chosen from one of the following:

(i) a LC CDR1 of SEQ ID NO: 744, LC CDR2 of SEQ ID NO: 784 and LC CDR3 of SEQ ID NO: 824 of BCMA-4 CAR (139103);

(ii) a LC CDR1 of SEQ ID NO: 754, LC CDR2 of SEQ ID NO: 794 and LC CDR3 of SEQ ID NO: 834 of BCMA-10 CAR (139109);

(iii) a LC CDR1 of SEQ ID NO: 756, LC CDR2 of SEQ ID NO: 796 and LC CDR3 of SEQ ID NO: 836 of BCMA-13 CAR (139112); or

(iv) a LC CDR1 of SEQ ID NO: 758, LC CDR2 of SEQ ID NO: 798 and LC CDR3 of SEQ ID NO: 838 of BCMA-15 CAR (139114); and/or

(2) one, two, or three heavy chain (HC) CDRs chosen from one of the following:

(i) a HC CDR1 of SEQ ID NO: 624, HC CDR2 of SEQ ID NO: 664 and HC CDR3 of SEQ ID NO: 704 of BCMA-4 CAR (139103);

(ii) a HC CDR1 of SEQ ID NO: 634, HC CDR2 of SEQ ID NO: 674 and HC CDR3 of SEQ ID NO: 714 of BCMA-10 CAR (139109);

(iii) a HC CDR1 of SEQ ID NO: 636, HC CDR2 of SEQ ID NO: 676 and HC CDR3 of SEQ ID NO: 716 of BCMA-13 CAR (139112); or

(iv) a HC CDR1 of SEQ ID NO: 638, HC CDR2 of SEQ ID NO: 678 and HC CDR3 of SEQ ID NO: 718 of BCMA-15 CAR (139114).

In certain embodiments, the CAR molecule described herein (e.g., the CAR nucleic acid or the CAR polypeptide) includes:

(1) one, two, or three light chain (LC) CDRs chosen from one of the following:

(i) a LC CDR1 of SEQ ID NO: 984 LC CDR2 of SEQ ID NO: 1024 and LC CDR3 of SEQ ID NO: 1064 of BCMA-4 CAR (139103);

(ii) a LC CDR1 of SEQ ID NO: 994, LC CDR2 of SEQ ID NO: 1034 and LC CDR3 of SEQ ID NO: 1074 of BCMA-10 CAR (139109);

(iii) a LC CDR1 of SEQ ID NO: 996, LC CDR2 of SEQ ID NO: 1036 and LC CDR3 of SEQ ID NO: 1076 of BCMA-13 CAR (139112); or

(iv) a LC CDR1 of SEQ ID NO: 998, LC CDR2 of SEQ ID NO: 1038 and LC CDR3 of SEQ ID NO: 1078 of BCMA-15 CAR (139114); and/or

(2) one, two, or three heavy chain (HC) CDRs chosen from one of the following:

(i) a HC CDR1 of SEQ ID NO: 864, HC CDR2 of SEQ ID NO: 904 and HC CDR3 of SEQ ID NO: 944 of BCMA-4 CAR (139103);

(ii) a HC CDR1 of SEQ ID NO: 874, HC CDR2 of SEQ ID NO: 914 and HC CDR3 of SEQ ID NO: 954 of BCMA-10 CAR (139109);

(iii) a HC CDR1 of SEQ ID NO: 876, HC CDR2 of SEQ ID NO: 916 and HC CDR3 of SEQ ID NO: 956 of BCMA-13 CAR (139112);

(iv) a HC CDR1 of SEQ ID NO: 878, HC CDR2 of SEQ ID NO: 918 and HC CDR3 of SEQ ID NO: 958 of BCMA-15 CAR (139114).

In embodiments, anti-BCMA CAR constructs, e.g., human or humanized anti-BCMA CAR constructs, are generated using a method described herein. Exemplary anti-BCMA scFvs include but are not limited to BCMA-1, BCMA-2, BCMA-3, BCMA-4, BCMA-5, BCMA-6, BCMA-7, BCMA-8, BCMA-9, BCMA-10, BCMA-11, BCMA-12, BCMA-13, BCMA-14, and BCMA-15. The sequences of human anti-BCMA scFv fragments (SEQ ID NOS: 39-52), are provided in Table 1 (and the name designations are provided in Table 2).

In embodiments, full BCMA CAR constructs (e.g., SEQ ID NOs: 99-113) are generated using scFv fragments, e.g., the human scFv fragments (e.g., SEQ ID NOs: 39-52), in combination with additional sequences, such as those shown below.

It is noted that the scFv fragments described herein, e.g., in Tables 1 and 16 or in SEQ ID NOS: 39-53, 129-149, 263-266, 271 or 273, without a leader sequence (e.g., without the amino acid sequence of SEQ ID NO: 1 or the nucleotide sequence of SEQ ID NO:12), are encompassed by the present invention. In other embodiments, scFv fragments described herein, e.g., in Tables 1 and 16 or in SEQ ID NOS: 39-53, 129-149, 263-266, 271 or 273 with a leader sequence (e.g., without the amino acid sequence of SEQ ID NO: 1 or the nucleotide sequence of SEQ ID NO:12), are also encompassed by the present invention.

leader (amino acid sequence) (SEQ ID NO: 1)

MALPVTALLLPLALLLHAARP

leader (nucleic acid sequence) (SEQ ID NO: 12)

ATGGCCCTGCCTGTGACAGCCCTGCTGCTGCCTCTGGCTCTGCTGCTGCATGCCGCT AGACCC

CD8 hinge (amino acid sequence) (SEQ ID NO: 2)

TTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACD

CD8 hinge (nucleic acid sequence) (SEQ ID NO: 13)

ACCACGACGCCAGCGCCGCGACCACCAACACCGGCGCCCACCATCGCGTCGCAGC CCCTGTCCCTGCGCCCAGAGGCGTGCCGGCCAGCGGCGGGGGGCGCAGTGCACACGAGGG GGCTGGACTTCGCCTGTGAT

CD8 transmembrane (amino acid sequence) (SEQ ID NO: 6)

IYIWAPLAGTCGVLLLSLVITLYC

CD8 transmembrane (nucleic acid sequence) (SEQ ID NO: 17)

ATCTACATCTGGGCGCCCTTGGCCGGGACTTGTGGGGTCCTTCTCCTGTCACTGGTT ATCACCCTTTACTGC

4-1BB Intracellular domain (amino acid sequence) (SEQ ID NO: 7)

KRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCEL

4-1BB Intracellular domain (nucleic acid sequence) (SEQ ID NO: 18)

AAACGGGGCAGAAAGAAACTCCTGTATATATTCAAACAACCATTTATGAGACCAGT ACAAACTACTCAAGAGGAAGATGGCTGTAGCTGCCGATTTCCAGAAGAAGAAGAAGGAGG ATGTGAACTG

CD28 Intracellular domain (amino acid sequence) (SEQ ID NO: 1104)

RSKRSRLLHSDYMNMTPRRPGPTRKHYQPYAPPRDFAAYRS (SEQ ID NO: 1104)

CD28 Intracellular domain (nucleotide sequence) (SEQ ID NO: 1105)

AGGAGTAAGAGGAGCAGGCTCCTGCACAGTGACTACATGAACATGACTCCCCGCC GCCCCGGGCCCACCCGCAAGCATTACCAGCCCTATGCCCCACCACGCGACTTCGCAGCCTA TCGCTCC (SEQ ID NO: 1105)

ICOS Intracellular domain (amino acid sequence) (SEQ ID NO: 1106)
TKKKYSSSVHDPNGEYMFMRAVNTAKKSRLTDVTL (SEQ ID NO: 1106)

ICOS Intracellular domain (nucleotide sequence) (SEQ ID NO: 1107)
ACAAAAAAGAAGTATTCATCCAGTGTGCACGACCCTAACGGTGAATACATGTTCAT GAGAGCAGTGAACACAGCCAAAAAATCCAGACTCACAGATGTGACCCTA (SEQ ID NO: 1107)

CD3 zeta domain (amino acid sequence) (SEQ ID NO: 9)
RVKFSRSADAPAYKQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQE GLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR
CD3 zeta (nucleic acid sequence) (SEQ ID NO: 20)
AGAGTGAAGTTCAGCAGGAGCGCAGACGCCCCCGCGTACAAGCAGGGCCAGAACC AGCTCTATAACGAGCTCAATCTAGGACGAAGAGAGGAGTACGATGTTTTGGACAAGAGAC GTGGCCGGGACCCTGAGATGGGGGGAAAGCCGAGAAGGAAGAACCCTCAGGAAGGCCTG TACAATGAACTGCAGAAAGATAAGATGGCGGAGGCCTACAGTGAGATTGGGATGAAAGGC GAGCGCCGGAGGGGCAAGGGGCACGATGGCCTTTACCAGGGTCTCAGTACAGCCACCAAG GACACCTACGACGCCCTTCACATGCAGGCCCTGCCCCCTCGC

CD3 zeta domain (amino acid sequence; NCBI Reference Sequence NM_000734.3) (SEQ ID NO:10)
RVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQE GLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR
CD3 zeta (nucleic acid sequence; NCBI Reference Sequence NM_000734.3); (SEQ ID NO:21)
AGAGTGAAGTTCAGCAGGAGCGCAGACGCCCCCGCGTACCAGCAGGGCCAGAACC AGCTCTATAACGAGCTCAATCTAGGACGAAGAGAGGAGTACGATGTTTTGGACAAGAGAC GTGGCCGGGACCCTGAGATGGGGGGAAAGCCGAGAAGGAAGAACCCTCAGGAAGGCCTG TACAATGAACTGCAGAAAGATAAGATGGCGGAGGCCTACAGTGAGATTGGGATGAAAGGC GAGCGCCGGAGGGGCAAGGGGCACGATGGCCTTTACCAGGGTCTCAGTACAGCCACCAAG GACACCTACGACGCCCTTCACATGCAGGCCCTGCCCCCTCGC

IgG4 Hinge (amino acid sequence) (SEQ ID NO:36)
ESKYGPPCPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWY VDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKG

QPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFF
LYSRLTVDKSRWQEGNVFSCSVMHEALHNHYTQKSLSLSLGKM

IgG4 Hinge (nucleotide sequence) (SEQ ID NO:37)

GAGAGCAAGTACGGCCCTCCCTGCCCCCCTTGCCCTGCCCCCGAGTTCCTGGGCGG
ACCCAGCGTGTTCCTGTTCCCCCCCAAGCCCAAGGACACCCTGATGATCAGCCGGACCCCC
GAGGTGACCTGTGTGGTGGTGGACGTGTCCCAGGAGGACCCCGAGGTCCAGTTCAACTGGT
ACGTGGACGGCGTGGAGGTGCACAACGCCAAGACCAAGCCCCGGGAGGAGCAGTTCAATA
GCACCTACCGGGTGGTGTCCGTGCTGACCGTGCTGCACCAGGACTGGCTGAACGGCAAGG
AATACAAGTGTAAGGTGTCCAACAAGGGCCTGCCCAGCAGCATCGAGAAAACCATCAGCA
AGGCCAAGGGCCAGCCTCGGGAGCCCCAGGTGTACACCCTGCCCCCTAGCCAAGAGGAGA
TGACCAAGAACCAGGTGTCCCTGACCTGCCTGGTGAAGGGCTTCTACCCCAGCGACATCGC
CGTGGAGTGGGAGAGCAACGGCCAGCCCGAGAACAACTACAAGACCACCCCCCCTGTGCT
GGACAGCGACGGCAGCTTCTTCCTGTACAGCCGGCTGACCGTGGACAAGAGCCGGTGGCA
GGAGGGCAACGTCTTTAGCTGCTCCGTGATGCACGAGGCCCTGCACAACCACTACACCCAG
AAGAGCCTGAGCCTGTCCCTGGGCAAGATG

In embodiments, the CAR scFv fragments are cloned into lentiviral vectors to create a full length CAR construct in a single coding frame, and using a promoter, e.g., EF1 alpha promoter, for expression (SEQ ID NO: 11).

EF1 alpha promoter

CGTGAGGCTCCGGTGCCCGTCAGTGGGCAGAGCGCACATCGCCCACAGTCCCCGAG
AAGTTGGGGGGAGGGGTCGGCAATTGAACCGGTGCCTAGAGAAGGTGGCGCGGGGTAAAC
TGGGAAAGTGATGTCGTGTACTGGCTCCGCCTTTTTCCCGAGGGTGGGGGAGAACCGTATA
TAAGTGCAGTAGTCGCCGTGAACGTTCTTTTTCGCAACGGGTTTGCCGCCAGAACACAGGT
AAGTGCCGTGTGTGGTTCCCGCGGGCCTGGCCTCTTTACGGGTTATGGCCCTTGCGTGCCTT
GAATTACTTCCACCTGGCTGCAGTACGTGATTCTTGATCCCGAGCTTCGGGTTGGAAGTGG
GTGGGAGAGTTCGAGGCCTTGCGCTTAAGGAGCCCCTTCGCCTCGTGCTTGAGTTGAGGCC
TGGCCTGGGCGCTGGGGCCGCCGCGTGCGAATCTGGTGGCACCTTCGCGCCTGTCTCGCTG
CTTTCGATAAGTCTCTAGCCATTTAAAATTTTTGATGACCTGCTGCGACGCTTTTTTTCTGGC
AAGATAGTCTTGTAAATGCGGGCCAAGATCTGCACACTGGTATTTCGGTTTTTGGGGCCGC
GGGCGGCGACGGGGCCCGTGCGTCCCAGCGCACATGTTCGGCGAGGCGGGGCCTGCGAGC
GCGGCCACCGAGAATCGGACGGGGGTAGTCTCAAGCTGGCCGGCCTGCTCTGGTGCCTGGC
CTCGCGCCGCCGTGTATCGCCCCGCCCTGGGCGGCAAGGCTGGCCCGGTCGGCACCAGTTG
CGTGAGCGGAAAGATGGCCGCTTCCCGGCCCTGCTGCAGGGAGCTCAAAATGGAGGACGC

GGCGCTCGGGAGAGCGGGCGGGTGAGTCACCCACACAAAGGAAAAGGGCCTTTCCGTCCT
CAGCCGTCGCTTCATGTGACTCCACGGAGTACCGGGCGCCGTCCAGGCACCTCGATTAGTT
CTCGAGCTTTTGGAGTACGTCGTCTTTAGGTTGGGGGGAGGGGTTTTATGCGATGGAGTTTC
CCCACACTGAGTGGGTGGAGACTGAAGTTAGGCCAGCTTGGCACTTGATGTAATTCTCCTT
GGAATTTGCCCTTTTTGAGTTTGGATCTTGGTTCATTCTCAAGCCTCAGACAGTGGTTCAAA
GTTTTTTTCTTCCATTTCAGGTGTCGTGA (SEQ ID NO: 11).

Gly/Ser (SEQ ID NO:25)
GGGGS

Gly/Ser (SEQ ID NO:26): This sequence may encompass 1-6 "Gly Gly Gly Gly Ser" repeating units
GGGGSGGGGS GGGGSGGGGS GGGGSGGGGS

Gly/Ser (SEQ ID NO:27)
GGGGSGGGGSGGGGSGGGGS

Gly/Ser (SEQ ID NO:28)
GGGGSGGGGSGGGGS

Gly/Ser (SEQ ID NO:29)
GGGS

PolyA: (A)5000 (SEQ ID NO:30)

PolyA: (T)100 (SEQ ID NO:31)

PolyA: (T)5000 (SEQ ID NO:32)

PolyA: (A)5000 (SEQ ID NO:33)

PolyA: (A)400 (SEQ ID NO:34)

PolyA: (A)2000 (SEQ ID NO:35)

Gly/Ser (SEQ ID NO:38): This sequence may encompass 1-10 "Gly Gly Gly Ser" repeating units

GGGSGGGSGG GSGGGSGGGS GGGSGGGSGG GSGGGSGGGS

The amino acid and nucleic acid sequences of exemplary BCMA scFv domains and exemplary BCMA CAR molecules are provided in Table 1.

Table 2 below designates the nicknames for the BCMA CAR constructs with respect to the DNA ID number, also listed in Table 1.

Table 2. CAR construct IDs

Nickname	Novartis ID	DNA2.0 ID
BCMA-1	ER95-03VA	139100
BCMA-2	UR96-08PA	139101
BCMA-3	KR98-03KA	139102
BCMA-4	JF32-78IB	139103
BCMA-5	AR99-08FA	139104
BCMA-6	ZF34-73CB	139105
BCMA-7	QR91-12ZA	139106
BCMA-8	GR92-17UA	139107
BCMA-9	OG62-93QB	139108
BCMA-10	EG63-98LB	139109
BCMA-11	UG65-93FB	139110
BCMA-12	HU13-58ZB	139111
BCMA-13	KG66-98AB	139112
BCMA-14	HJ64-62PB	139113
BCMA-15	PY43-48LB	139114

Table 1. Amino Acid and Nucleic Acid Sequences of exemplary anti-BCMA scFv domains and BCMA CAR molecules

The amino acid sequences variable heavy chain and variable light chain sequences for each scFv is also provided. Table 2 lists names and CAR construct IDs for several BCMA CARs.

The signal peptide MALPVTALLLPLALLLHAARP (SEQ ID NO: 1) is underlined in each full-length CAR sequence.

Name/ Description	**SEQ ID NO:**	**Sequence**
139109		
139109- aa ScFv domain	49	EVQLVESGGGLVQPGGSLRLSCAVSGFALSNHGMSWVRRAPGKGLEWVSGIVY SGSTYYAASVKGRFTISRDNSRNTLYLQMNSLRPEDTAIYYCSAHGGESDVWG QGTTVTVSSASGGGGSGGRASGGGGSDIQLTQSPSSLSASVGDRVTITCRASQ SISSYLNWYQQKPGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLTISSLQP

		EDFATYYCQQSYSTPYTFGQGTKVEIK
139109- nt ScFv domain	64	GAAGTGCAATTGGTGGAATCAGGGGGAGGACTTGTGCAGCCTGGAGGATCGCT GAGACTGTCATGTGCCGTGTCCGGCTTTGCCCTGTCCAACCACGGGATGTCCT GGGTCCGCCGCGCGCCTGGAAAGGGCCTCGAATGGGTGTCGGGTATTGTGTAC AGCGGTAGCACCTACTATGCCGCATCCGTGAAGGGGAGATTCACCATCAGCCG GGACAACTCCAGGAACACTCTGTACCTCCAAATGAATTCGCTGAGGCCAGAGG ACACTGCCATCTACTACTGCTCCGCGCATGGCGGAGAGTCCGACGTCTGGGGA CAGGGGACCACCGTGACCGTGTCTAGCGCGTCCGGCGGAGGCGGCAGCGGGGG TCGGGCATCAGGGGGCGGCGGATCGGACATCCAGCTCACCCAGTCCCCGAGCT CGCTGTCCGCCTCCGTGGGAGATCGGGTCACCATCACGTGCCGCGCCAGCCAG TCGATTTCCTCCTACCTGAACTGGTACCAACAGAAGCCCGGAAAAGCCCCGAA GCTTCTCATCTACGCCGCCTCGAGCCTGCAGTCAGGAGTGCCCTCACGGTTCT CCGGCTCCGGTTCCGGTACTGATTTCACCCTGACCATTTCCTCCCTGCAACCG GAGGACTTCGCTACTTACTACTGCCAGCAGTCGTACTCCACCCCCTACACTTT CGGACAAGGCACCAAGGTCGAAATCAAG
139109- aa VH	79	EVQLVESGGGLVQPGGSLRLSCAVSGFALSNHGMSWVRRAPGKGLEWVSGIVY SGSTYYAASVKGRFTISRDNSRNTLYLQMNSLRPEDTAIYYCSAHGGESDVWG QGTTVTVSS
139109- aa VL	94	DIQLTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKLLIYAASS LQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQSYSTPYTFGQGTKVEI K
139109- aa Full CAR	109	MALPVTALLLPLALLLHAARPEVQLVESGGGLVQPGGSLRLSCAVSGFALSNH GMSWVRRAPGKGLEWVSGIVYSGSTYYAASVKGRFTISRDNSRNTLYLQMNSL RPEDTAIYYCSAHGGESDVWGQGTTVTVSSASGGGGSGGRASGGGGSDIQLTQ SPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKLLIYAASSLQSGVP SRFSGSGSGTDFTLTISSLQPEDFATYYCQQSYSTPYTFGQGTKVEIKTTTPA PRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYIWAPLAGTCGV LLLSLVITLYCKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCEL RVKFSRSADAPAYKQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKN PQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHM QALPPR
139109- nt Full CAR	124	ATGGCCCTCCCTGTCACCGCCCTGCTGCTTCCGCTGGCTCTTCTGCTCCACGC CGCTCGGCCCGAAGTGCAATTGGTGGAATCAGGGGGAGGACTTGTGCAGCCTG GAGGATCGCTGAGACTGTCATGTGCCGTGTCCGGCTTTGCCCTGTCCAACCAC GGGATGTCCTGGGTCCGCCGCGCGCCTGGAAAGGGCCTCGAATGGGTGTCGGG TATTGTGTACAGCGGTAGCACCTACTATGCCGCATCCGTGAAGGGGAGATTCA CCATCAGCCGGGACAACTCCAGGAACACTCTGTACCTCCAAATGAATTCGCTG AGGCCAGAGGACACTGCCATCTACTACTGCTCCGCGCATGGCGGAGAGTCCGA CGTCTGGGGACAGGGGACCACCGTGACCGTGTCTAGCGCGTCCGGCGGAGGCG GCAGCGGGGGTCGGGCATCAGGGGGCGGCGGATCGGACATCCAGCTCACCCAG TCCCCGAGCTCGCTGTCCGCCTCCGTGGGAGATCGGGTCACCATCACGTGCCG CGCCAGCCAGTCGATTTCCTCCTACCTGAACTGGTACCAACAGAAGCCCGGAA AAGCCCCGAAGCTTCTCATCTACGCCGCCTCGAGCCTGCAGTCAGGAGTGCCC TCACGGTTCTCCGGCTCCGGTTCCGGTACTGATTTCACCCTGACCATTTCCTC CCTGCAACCGGAGGACTTCGCTACTTACTACTGCCAGCAGTCGTACTCCACCC CCTACACTTTCGGACAAGGCACCAAGGTCGAAATCAAGACCACTACCCCAGCA CCGAGGCCACCCACCCCGGCTCCTACCATCGCCTCCCAGCCTCTGTCCCTGCG TCCGGAGGCATGTAGACCCGCAGCTGGTGGGGCCGTGCATACCCGGGGTCTTG ACTTCGCCTGCGATATCTACATTTGGGCCCCTCTGGCTGGTACTTGCGGGGTC CTGCTGCTTTCACTCGTGATCACTCTTTACTGTAAGCGCGGTCGGAAGAAGCT GCTGTACATCTTTAAGCAACCCTTCATGAGGCCTGTGCAGACTACTCAAGAGG AGGACGGCTGTTCATGCCGGTTCCCAGAGGAGGAGGAAGGCGGCTGCGAACTG

		CGCGTGAAATTCAGCCGCAGCGCAGATGCTCCAGCCTACAAGCAGGGGCAGAA CCAGCTCTACAACGAACTCAATCTTGGTCGGAGAGAGGAGTACGACGTGCTGG ACAAGCGGAGAGGACGGGACCCAGAAATGGGCGGGAAGCCGCGCAGAAAGAAT CCCCAAGAGGGCCTGTACAACGAGCTCCAAAAGGATAAGATGGCAGAAGCCTA TAGCGAGATTGGTATGAAAGGGGAACGCAGAAGAGGCAAAGGCCACGACGGAC TGTACCAGGGACTCAGCACCGCCACCAAGGACACCTATGACGCTCTTCACATG CAGGCCCTGCCGCCTCGG
139103		
139103- aa ScFv domain	39	QVQLVESGGGLVQPGRSLRLSCAASGFTFSNYAMSWVRQAPGKGLGWVSGISR SGENTYYADSVKGRFTISRDNSKNTLYLQMNSLRDEDTAVYYCARSPAHYYGG MDVWGQGTTVTVSSASGGGGSGGRASGGGGSDIVLTQSPGTLSLSPGERATLS CRASQSISSSFLAWYQQKPGQAPRLLIYGASRRATGIPDRFSGSGSGTDFTLT ISRLEPEDSAVYYCQQYHSSPSWTFGQGTKLEIK
139103- nt ScFv domain	54	CAAGTGCAACTCGTGGAATCTGGTGGAGGACTCGTGCAACCCGGAAGATCGCT TAGACTGTCGTGTGCCGCCAGCGGGTTCACTTTCTCGAACTACGCGATGTCCT GGGTCCGCCAGGCACCCGGAAAGGGACTCGGTTGGGTGTCCGGCATTTCCCGG TCCGGCGAAAATACCTACTACGCCGACTCCGTGAAGGGCCGCTTCACCATCTC AAGGGACAACAGCAAAAACACCCTGTACTTGCAAATGAACTCCCTGCGGGATG AAGATACAGCCGTGTACTATTGCGCCCGGTCGCCTGCCCATTACTACGGCGGA ATGGACGTCTGGGGACAGGGAACCACTGTGACTGTCAGCAGCGCGTCGGGTGG CGGCGGCTCAGGGGGTCGGGCCTCCGGGGGGGGAGGGTCCGACATCGTGCTGA CCCAGTCCCCGGGAACCCTGAGCCTGAGCCCGGGAGAGCGCGCGACCCTGTCA TGCCGGGCATCCCAGAGCATTAGCTCCTCCTTTCTCGCCTGGTATCAGCAGAA GCCCGGACAGGCCCCGAGGCTGCTGATCTACGGCGCTAGCAGAAGGGCTACCG GAATCCCAGACCGGTTCTCCGGCTCCGGTTCCGGGACCGATTTCACCCTTACT ATCTCGCGCCTGGAACCTGAGGACTCCGCCGTCTACTACTGCCAGCAGTACCA CTCATCCCCGTCGTGGACGTTCGGACAGGGCACCAAGCTGGAGATTAAG
139103- aa VH	69	QVQLVESGGGLVQPGRSLRLSCAASGFTFSNYAMSWVRQAPGKGLGWVSGISR SGENTYYADSVKGRFTISRDNSKNTLYLQMNSLRDEDTAVYYCARSPAHYYGG MDVWGQGTTVTVSS
139103- aa VL	84	DIVLTQSPGTLSLSPGERATLSCRASQSISSSFLAWYQQKPGQAPRLLIYGAS RRATGIPDRFSGSGSGTDFTLTISRLEPEDSAVYYCQQYHSSPSWTFGQGTKL EIK
139103- aa Full CAR	99	MALPVTALLLPLALLLHAARPQVQLVESGGGLVQPGRSLRLSCAASGFTFSNY AMSWVRQAPGKGLGWVSGISRSGENTYYADSVKGRFTISRDNSKNTLYLQMNS LRDEDTAVYYCARSPAHYYGGMDVWGQGTTVTVSSASGGGGSGGRASGGGGSD IVLTQSPGTLSLSPGERATLSCRASQSISSSFLAWYQQKPGQAPRLLIYGASR RATGIPDRFSGSGSGTDFTLTISRLEPEDSAVYYCQQYHSSPSWTFGQGTKLE IKTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYIWAP LAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEE EEGGCELRVKFSRSADAPAYKQGQNQLYNELNLGRREEYDVLDKRRGRDPEMG GKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKD TYDALHMQALPPR
139103- nt Full CAR	114	ATGGCCCTCCCTGTCACCGCCCTGCTGCTTCCGCTGGCTCTTCTGCTCCACGC CGCTCGGCCCCAAGTGCAACTCGTGGAATCTGGTGGAGGACTCGTGCAACCCG GAAGATCGCTTAGACTGTCGTGTGCCGCCAGCGGGTTCACTTTCTCGAACTAC GCGATGTCCTGGGTCCGCCAGGCACCCGGAAAGGGACTCGGTTGGGTGTCCGG CATTTCCCGGTCCGGCGAAAATACCTACTACGCCGACTCCGTGAAGGGCCGCT TCACCATCTCAAGGGACAACAGCAAAAACACCCTGTACTTGCAAATGAACTCC CTGCGGGATGAAGATACAGCCGTGTACTATTGCGCCCGGTCGCCTGCCCATTA CTACGGCGGAATGGACGTCTGGGGACAGGGAACCACTGTGACTGTCAGCAGCG CGTCGGGTGGCGGCGGCTCAGGGGGTCGGGCCTCCGGGGGGGGAGGGTCCGAC

		ATCGTGCTGACCCAGTCCCCGGGAACCCTGAGCCTGAGCCCGGGAGAGCGCGC GACCCTGTCATGCCGGGCATCCCAGAGCATTAGCTCCTCCTTTCTCGCCTGGT ATCAGCAGAAGCCCGGACAGGCCCCGAGGCTGCTGATCTACGGCGCTAGCAGA AGGGCTACCGGAATCCCAGACCGGTTCTCCGGCTCCGGTTCCGGGACCGATTT CACCCTTACTATCTCGCGCCTGGAACCTGAGGACTCCGCCGTCTACTACTGCC AGCAGTACCACTCATCCCCGTCGTGGACGTTCGGACAGGGCACCAAGCTGGAG ATTAAGACCACTACCCCAGCACCGAGGCCACCCACCCCGGCTCCTACCATCGC CTCCCAGCCTCTGTCCCTGCGTCCGGAGGCATGTAGACCCGCAGCTGGTGGGG CCGTGCATACCCGGGGTCTTGACTTCGCCTGCGATATCTACATTTGGGCCCCT CTGGCTGGTACTTGCGGGGTCCTGCTGCTTTCACTCGTGATCACTCTTTACTG TAAGCGCGGTCGGAAGAAGCTGCTGTACATCTTTAAGCAACCCTTCATGAGGC CTGTGCAGACTACTCAAGAGGAGGACGGCTGTTCATGCCGGTTCCCAGAGGAG GAGGAAGGCGGCTGCGAACTGCGCGTGAAATTCAGCCGCAGCGCAGATGCTCC AGCCTACAAGCAGGGGCAGAACCAGCTCTACAACGAACTCAATCTTGGTCGGA GAGAGGAGTACGACGTGCTGGACAAGCGGAGAGGACGGGACCCAGAAATGGGC GGGAAGCCGCGCAGAAAGAATCCCCAAGAGGGCCTGTACAACGAGCTCCAAAA GGATAAGATGGCAGAAGCCTATAGCGAGATTGGTATGAAAGGGGAACGCAGAA GAGGCAAAGGCCACGACGGACTGTACCAGGGACTCAGCACCGCCACCAAGGAC ACCTATGACGCTCTTCACATGCAGGCCCTGCCGCCTCGG
139105		
139105- aa ScFv domain	40	QVQLVESGGGLVQPGRSLRLSCAASGFTFDDYAMHWVRQAPGKGLEWVSGISW NSGSIGYADSVKGRFTISRDNAKNSLYLQMNSLRAEDTALYYCSVHSFLAYWG QGTLVTVSSASGGGGSGGRASGGGGSDIVMTQTPLSLPVTPGEPASISCRSSQ SLLHSNGYNYLDWYLQKPGQSPQLLIYLGSNRASGVPDRFSGSGSGTDFTLKI SRVEAEDVGVYYCMQALQTPYTFGQGTKVEIK
139105- nt ScFv domain	55	CAAGTGCAACTCGTCGAATCCGGTGGAGGTCTGGTCCAACCTGGTAGAAGCCT GAGACTGTCGTGTGCGGCCAGCGGATTCACCTTTGATGACTATGCTATGCACT GGGTGCGGCAGGCCCCAGGAAAGGGCCTGGAATGGGTGTCGGGAATTAGCTGG AACTCCGGGTCCATTGGCTACGCCGACTCCGTGAAGGGCCGCTTCACCATCTC CCGCGACAACGCAAAGAACTCCCTGTACTTGCAAATGAACTCGCTCAGGGCTG AGGATACCGCGCTGTACTACTGCTCCGTGCATTCCTTCCTGGCCTACTGGGGA CAGGGAACTCTGGTCACCGTGTCGAGCGCCTCCGGCGGCGGGGGCTCGGGTGG ACGGGCCTCGGGCGGAGGGGGGTCCGACATCGTGATGACCCAGACCCCGCTGA GCTTGCCCGTGACTCCCGGAGAGCCTGCATCCATCTCCTGCCGGTCATCCCAG TCCCTTCTCCACTCCAACGGATACAACTACCTCGACTGGTACCTCCAGAAGCC GGGACAGAGCCCTCAGCTTCTGATCTACCTGGGGTCAAATAGAGCCTCAGGAG TGCCGGATCGGTTCAGCGGATCTGGTTCGGGAACTGATTTCACTCTGAAGATT TCCCGCGTGGAAGCCGAGGACGTGGGCGTCTACTACTGTATGCAGGCGCTGCA GACCCCCTATACCTTCGGCCAAGGGACGAAAGTGGAGATCAAG
139105- aa VH	70	QVQLVESGGGLVQPGRSLRLSCAASGFTFDDYAMHWVRQAPGKGLEWVSGISW NSGSIGYADSVKGRFTISRDNAKNSLYLQMNSLRAEDTALYYCSVHSFLAYWG QGTLVTVSS
139105- aa VL	85	DIVMTQTPLSLPVTPGEPASISCRSSQSLLHSNGYNYLDWYLQKPGQSPQLLI YLGSNRASGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCMQALQTPYTFGQG TKVEIK
139105- aa Full CAR	100	MALPVTALLLPLALLLHAARPQVQLVESGGGLVQPGRSLRLSCAASGFTFDDY AMHWVRQAPGKGLEWVSGISWNSGSIGYADSVKGRFTISRDNAKNSLYLQMNS LRAEDTALYYCSVHSFLAYWGQGTLVTVSSASGGGGSGGRASGGGGSDIVMTQ TPLSLPVTPGEPASISCRSSQSLLHSNGYNYLDWYLQKPGQSPQLLIYLGSNR ASGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCMQALQTPYTFGQGTKVEIK TTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYIWAPLA GTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEE

		GGCELRVKFSRSADAPAYKQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGK PRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTY DALHMQALPPR
139105- nt Full CAR	115	ATGGCCCTCCCTGTCACCGCCCTGCTGCTTCCGCTGGCTCTTCTGCTCCACGC CGCTCGGCCCCAAGTGCAACTCGTCGAATCCGGTGGAGGTCTGGTCCAACCTG GTAGAAGCCTGAGACTGTCGTGTGCGGCCAGCGGATTCACCTTTGATGACTAT GCTATGCACTGGGTGCGGCAGGCCCCAGGAAAGGGCCTGGAATGGGTGTCGGG AATTAGCTGGAACTCCGGGTCCATTGGCTACGCCGACTCCGTGAAGGGCCGCT TCACCATCTCCCGCGACAACGCAAAGAACTCCCTGTACTTGCAAATGAACTCG CTCAGGGCTGAGGATACCGCGCTGTACTACTGCTCCGTGCATTCCTTCCTGGC CTACTGGGGACAGGGAACTCTGGTCACCGTGTCGAGCGCCTCCGGCGGCGGGG GCTCGGGTGGACGGGCCTCGGGCGGAGGGGGGTCCGACATCGTGATGACCCAG ACCCCGCTGAGCTTGCCCGTGACTCCCGGAGAGCCTGCATCCATCTCCTGCCG GTCATCCCAGTCCCTTCTCCACTCCAACGGATACAACTACCTCGACTGGTACC TCCAGAAGCCGGGACAGAGCCCTCAGCTTCTGATCTACCTGGGGTCAAATAGA GCCTCAGGAGTGCCGGATCGGTTCAGCGGATCTGGTTCGGGAACTGATTTCAC TCTGAAGATTTCCCGCGTGGAAGCCGAGGACGTGGGCGTCTACTACTGTATGC AGGCGCTGCAGACCCCCTATACCTTCGGCCAAGGGACGAAAGTGGAGATCAAG ACCACTACCCCAGCACCGAGGCCACCCACCCCGGCTCCTACCATCGCCTCCCA GCCTCTGTCCCTGCGTCCGGAGGCATGTAGACCCGCAGCTGGTGGGGCCGTGC ATACCCGGGGTCTTGACTTCGCCTGCGATATCTACATTTGGGCCCCTCTGGCT GGTACTTGCGGGGTCCTGCTGCTTTCACTCGTGATCACTCTTTACTGTAAGCG CGGTCGGAAGAAGCTGCTGTACATCTTTAAGCAACCCTTCATGAGGCCTGTGC AGACTACTCAAGAGGAGGACGGCTGTTCATGCCGGTTCCCAGAGGAGGAGGAA GGCGGCTGCGAACTGCGCGTGAAATTCAGCCGCAGCGCAGATGCTCCAGCCTA CAAGCAGGGGCAGAACCAGCTCTACAACGAACTCAATCTTGGTCGGAGAGAGG AGTACGACGTGCTGGACAAGCGGAGAGGACGGGACCCAGAAATGGGCGGGAAG CCGCGCAGAAAGAATCCCCAAGAGGGCCTGTACAACGAGCTCCAAAAGGATAA GATGGCAGAAGCCTATAGCGAGATTGGTATGAAAGGGGAACGCAGAAGAGGCA AAGGCCACGACGGACTGTACCAGGGACTCAGCACCGCCACCAAGGACACCTAT GACGCTCTTCACATGCAGGCCCTGCCGCCTCGG
139111		
139111- aa ScFv domain	41	EVQLLESGGGLVQPGGSLRLSCAVSGFALSNHGMSWVRRAPGKGLEWVSGIVY SGSTYYAASVKGRFTISRDNSRNTLYLQMNSLRPEDTAIYYCSAHGGESDVWG QGTTVTVSSASGGGGSGGRASGGGGSDIVMTQTPLSLSVTPGQPASISCKSSQ SLLRNDGKTPLYWYLQKAGQPPQLLIYEVSNRFSGVPDRFSGSGSGTDFTLKI SRVEAEDVGAYYCMQNIQFPSFGGGTKLEIK
139111- nt ScFv domain	56	GAAGTGCAATTGTTGGAATCTGGAGGAGGACTTGTGCAGCCTGGAGGATCACT GAGACTTTCGTGTGCGGTGTCAGGCTTCGCCCTGAGCAACCACGGCATGAGCT GGGTGCGGAGAGCCCCGGGGAAGGGTCTGGAATGGGTGTCCGGGATCGTCTAC TCCGGTTCAACTTACTACGCCGCAAGCGTGAAGGGTCGCTTCACCATTTCCCG CGATAACTCCCGGAACACCCTGTACCTCCAAATGAACTCCCTGCGGCCCGAGG ACACCGCCATCTACTACTGTTCCGCGCATGGAGGAGAGTCCGATGTCTGGGGA CAGGGCACTACCGTGACCGTGTCGAGCGCCTCGGGGGGAGGAGGCTCCGGCGG TCGCGCCTCCGGGGGGGGTGGCAGCGACATTGTGATGACGCAGACTCCACTCT CGCTGTCCGTGACCCCGGGACAGCCCGCGTCCATCTCGTGCAAGAGCTCCCAG AGCCTGCTGAGGAACGACGGAAAGACTCCTCTGTATTGGTACCTCCAGAAGGC TGGACAGCCCCCGCAACTGCTCATCTACGAAGTGTCAAATCGCTTCTCCGGGG TGCCGGATCGGTTTTCCGGCTCGGGATCGGGCACCGACTTCACCCTGAAAATC TCCAGGGTCGAGGCCGAGGACGTGGGAGCCTACTACTGCATGCAAAACATCCA GTTCCCTTCCTTCGGCGGCGGCACAAAGCTGGAGATTAAG
139111- aa	71	EVQLLESGGGLVQPGGSLRLSCAVSGFALSNHGMSWVRRAPGKGLEWVSGIVY

VH		SGSTYYAASVKGRFTISRDNSRNTLYLQMNSLRPEDTAIYYCSAHGGESDVWG QGTTVTVSS
139111- aa VL	86	DIVMTQTPLSLSVTPGQPASISCKSSQSLLRNDGKTPLYWYLQKAGQPPQLLI YEVSNRFSGVPDRFSGSGSGTDFTLKISRVEAEDVGAYYCMQNIQFPSFGGGT KLEIK
139111- aa Full CAR	101	MALPVTALLLPLALLLHAARPEVQLLESGGGLVQPGGSLRLSCAVSGFALSNH GMSWVRRAPGKGLEWVSGIVYSGSTYYAASVKGRFTISRDNSRNTLYLQMNSL RPEDTAIYYCSAHGGESDVWGQGTTVTVSSASGGGGSGGRASGGGGSDIVMTQ TPLSLSVTPGQPASISCKSSQSLLRNDGKTPLYWYLQKAGQPPQLLIYEVSNR FSGVPDRFSGSGSGTDFTLKISRVEAEDVGAYYCMQNIQFPSFGGGTKLEIKT TTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYIWAPLAG TCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEG GCELRVKFSRSADAPAYKQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKP RRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYD ALHMQALPPR
139111- nt Full CAR	116	ATGGCCCTCCCTGTCACCGCCCTGCTGCTTCCGCTGGCTCTTCTGCTCCACGC CGCTCGGCCCGAAGTGCAATTGTTGGAATCTGGAGGAGGACTTGTGCAGCCTG GAGGATCACTGAGACTTTCGTGTGCGGTGTCAGGCTTCGCCCTGAGCAACCAC GGCATGAGCTGGGTGCGGAGAGCCCCGGGGAAGGGTCTGGAATGGGTGTCCGG GATCGTCTACTCCGGTTCAACTTACTACGCCGCAAGCGTGAAGGGTCGCTTCA CCATTTCCCGCGATAACTCCCGGAACACCCTGTACCTCCAAATGAACTCCCTG CGGCCCGAGGACACCGCCATCTACTACTGTTCCGCGCATGGAGGAGAGTCCGA TGTCTGGGGACAGGGCACTACCGTGACCGTGTCGAGCGCCTCGGGGGGAGGAG GCTCCGGCGGTCGCGCCTCCGGGGGGGGTGGCAGCGACATTGTGATGACGCAG ACTCCACTCTCGCTGTCCGTGACCCCGGGACAGCCCGCGTCCATCTCGTGCAA GAGCTCCCAGAGCCTGCTGAGGAACGACGGAAAGACTCCTCTGTATTGGTACC TCCAGAAGGCTGGACAGCCCCCGCAACTGCTCATCTACGAAGTGTCAAATCGC TTCTCCGGGGTGCCGGATCGGTTTTCCGGCTCGGGATCGGGCACCGACTTCAC CCTGAAAATCTCCAGGGTCGAGGCCGAGGACGTGGGAGCCTACTACTGCATGC AAAACATCCAGTTCCCTTCCTTCGGCGGCGGCACAAAGCTGGAGATTAAGACC ACTACCCCAGCACCGAGGCCACCCACCCCGGCTCCTACCATCGCCTCCCAGCC TCTGTCCCTGCGTCCGGAGGCATGTAGACCCGCAGCTGGTGGGGCCGTGCATA CCCGGGGTCTTGACTTCGCCTGCGATATCTACATTTGGGCCCCTCTGGCTGGT ACTTGCGGGGTCCTGCTGCTTTCACTCGTGATCACTCTTTACTGTAAGCGCGG TCGGAAGAAGCTGCTGTACATCTTTAAGCAACCCTTCATGAGGCCTGTGCAGA CTACTCAAGAGGAGGACGGCTGTTCATGCCGGTTCCCAGAGGAGGAGGAAGGC GGCTGCGAACTGCGCGTGAAATTCAGCCGCAGCGCAGATGCTCCAGCCTACAA GCAGGGGCAGAACCAGCTCTACAACGAACTCAATCTTGGTCGGAGAGAGGAGT ACGACGTGCTGGACAAGCGGAGAGGACGGGACCCAGAAATGGGCGGGAAGCCG CGCAGAAAGAATCCCCAAGAGGGCCTGTACAACGAGCTCCAAAAGGATAAGAT GGCAGAAGCCTATAGCGAGATTGGTATGAAAGGGGAACGCAGAAGAGGCAAAG GCCACGACGGACTGTACCAGGGACTCAGCACCGCCACCAAGGACACCTATGAC GCTCTTCACATGCAGGCCCTGCCGCCTCGG
139100		
139100- aa ScFv domain	42	QVQLVQSGAEVRKTGASVKVSCKASGYIFDNFGINWVRQAPGQGLEWMGWINP KNNNTNYAQKFQGRVTITADESTNTAYMEVSSLRSEDTAVYYCARGPYYYQSY MDVWGQGTMVTVSSASGGGGSGGRASGGGGSDIVMTQTPLSLPVTPGEPASIS CRSSQSLLHSNGYNYLNWYLQKPGQSPQLLIYLGSKRASGVPDRFSGSGSGTD FTLHITRVGAEDVGVYYCMQALQTPYTFGQGTKLEIK
139100- nt ScFv domain	57	CAAGTCCAACTCGTCCAGTCCGGCGCAGAAGTCAGAAAAACCGGTGCTAGCGT GAAAGTGTCCTGCAAGGCCTCCGGCTACATTTTCGATAACTTCGGAATCAACT GGGTCAGACAGGCCCCGGGCCAGGGGCTGGAATGGATGGGATGGATCAACCCC

		AAGAACAACAACACCAACTACGCACAGAAGTTCCAGGGCCGCGTGACTATCAC CGCCGATGAATCGACCAATACCGCCTACATGGAGGTGTCCTCCCTGCGGTCGG AGGACACTGCCGTGTATTACTGCGCGAGGGGCCCATACTACTACCAAAGCTAC ATGGACGTCTGGGGACAGGGAACCATGGTGACCGTGTCATCCGCCTCCGGTGG TGGAGGCTCCGGGGGGCGGGCTTCAGGAGGCGGAGGAAGCGATATTGTGATGA CCCAGACTCCGCTTAGCCTGCCCGTGACTCCTGGAGAACCGGCCTCCATTTCC TGCCGGTCCTCGCAATCACTCCTGCATTCCAACGGTTACAACTACCTGAATTG GTACCTCCAGAAGCCTGGCCAGTCGCCCCAGTTGCTGATCTATCTGGGCTCGA AGCGCGCCTCCGGGGTGCCTGACCGGTTTAGCGGATCTGGGAGCGGCACGGAC TTCACTCTCCACATCACCCGCGTGGGAGCGGAGGACGTGGGAGTGTACTACTG TATGCAGGCGCTGCAGACTCCGTACACATTCGGACAGGGCACCAAGCTGGAGA TCAAG
139100- aa VH	72	QVQLVQSGAEVRKTGASVKVSCKASGYIFDNFGINWVRQAPGQGLEWMGWINP KNNNTNYAQKFQGRVTITADESTNTAYMEVSSLRSEDTAVYYCARGPYYYQSY MDVWGQGTMVTVSS
139100- aa VL	87	DIVMTQTPLSLPVTPGEPASISCRSSQSLLHSNGYNYLNWYLQKPGQSPQLLI YLGSKRASGVPDRFSGSGSGTDFTLHITRVGAEDVGVYYCMQALQTPYTFGQG TKLEIK
139100- aa Full CAR	102	MALPVTALLLPLALLLHAARPQVQLVQSGAEVRKTGASVKVSCKASGYIFDNF GINWVRQAPGQGLEWMGWINPKNNNTNYAQKFQGRVTITADESTNTAYMEVSS LRSEDTAVYYCARGPYYYQSYMDVWGQGTMVTVSSASGGGGSGGRASGGGGSD IVMTQTPLSLPVTPGEPASISCRSSQSLLHSNGYNYLNWYLQKPGQSPQLLIY LGSKRASGVPDRFSGSGSGTDFTLHITRVGAEDVGVYYCMQALQTPYTFGQGT KLEIKTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYI WAPLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRF PEEEEGGCELRVKFSRSADAPAYKQGQNQLYNELNLGRREEYDVLDKRRGRDP EMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTA TKDTYDALHMQALPPR
139100- nt Full CAR	117	ATGGCCCTCCCTGTCACCGCCCTGCTGCTTCCGCTGGCTCTTCTGCTCCACGC CGCTCGGCCCCAAGTCCAACTCGTCCAGTCCGGCGCAGAAGTCAGAAAAACCG GTGCTAGCGTGAAAGTGTCCTGCAAGGCCTCCGGCTACATTTTCGATAACTTC GGAATCAACTGGGTCAGACAGGCCCCGGGCCAGGGGCTGGAATGGATGGGATG GATCAACCCCAAGAACAACAACACCAACTACGCACAGAAGTTCCAGGGCCGCG TGACTATCACCGCCGATGAATCGACCAATACCGCCTACATGGAGGTGTCCTCC CTGCGGTCGGAGGACACTGCCGTGTATTACTGCGCGAGGGGCCCATACTACTA CCAAAGCTACATGGACGTCTGGGGACAGGGAACCATGGTGACCGTGTCATCCG CCTCCGGTGGTGGAGGCTCCGGGGGGCGGGCTTCAGGAGGCGGAGGAAGCGAT ATTGTGATGACCCAGACTCCGCTTAGCCTGCCCGTGACTCCTGGAGAACCGGC CTCCATTTCCTGCCGGTCCTCGCAATCACTCCTGCATTCCAACGGTTACAACT ACCTGAATTGGTACCTCCAGAAGCCTGGCCAGTCGCCCCAGTTGCTGATCTAT CTGGGCTCGAAGCGCGCCTCCGGGGTGCCTGACCGGTTTAGCGGATCTGGGAG CGGCACGGACTTCACTCTCCACATCACCCGCGTGGGAGCGGAGGACGTGGGAG TGTACTACTGTATGCAGGCGCTGCAGACTCCGTACACATTCGGACAGGGCACC AAGCTGGAGATCAAGACCACTACCCCAGCACCGAGGCCACCCACCCCGGCTCC TACCATCGCCTCCCAGCCTCTGTCCCTGCGTCCGGAGGCATGTAGACCCGCAG CTGGTGGGGCCGTGCATACCCGGGGTCTTGACTTCGCCTGCGATATCTACATT TGGGCCCCTCTGGCTGGTACTTGCGGGGTCCTGCTGCTTTCACTCGTGATCAC TCTTTACTGTAAGCGCGGTCGGAAGAAGCTGCTGTACATCTTTAAGCAACCCT TCATGAGGCCTGTGCAGACTACTCAAGAGGAGGACGGCTGTTCATGCCGGTTC CCAGAGGAGGAGGAAGGCGGCTGCGAACTGCGCGTGAAATTCAGCCGCAGCGC AGATGCTCCAGCCTACAAGCAGGGGCAGAACCAGCTCTACAACGAACTCAATC TTGGTCGGAGAGAGGAGTACGACGTGCTGGACAAGCGGAGAGGACGGGACCCA

		GAAATGGGCGGGAAGCCGCGCAGAAAGAATCCCCAAGAGGGCCTGTACAACGA GCTCCAAAAGGATAAGATGGCAGAAGCCTATAGCGAGATTGGTATGAAAGGGG AACGCAGAAGAGGCAAAGGCCACGACGGACTGTACCAGGGACTCAGCACCGCC ACCAAGGACACCTATGACGCTCTTCACATGCAGGCCCTGCCGCCTCGG
139101		
139101- aa ScFv domain	43	QVQLQESGGGLVQPGGSLRLSCAASGFTFSSDAMTWVRQAPGKGLEWVSVISG SGGTTYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAKLDSSGYYY ARGPRYWGQGTLVTVSSASGGGGSGGRASGGGGSDIQLTQSPSSLSASVGDRV TITCRASQSISSYLNWYQQKPGKAPKLLIYGASTLASGVPARFSGSGSGTHFT LTINSLQSEDSATYYCQQSYKRASFGQGTKVEIK
139101- nt ScFv domain	58	CAAGTGCAACTTCAAGAATCAGGCGGAGGACTCGTGCAGCCCGGAGGATCATT GCGGCTCTCGTGCGCCGCCTCGGGCTTCACCTTCTCGAGCGACGCCATGACCT GGGTCCGCCAGGCCCCGGGGAAGGGGCTGGAATGGGTGTCTGTGATTTCCGGC TCCGGGGGAACTACGTACTACGCCGATTCCGTGAAAGGTCGCTTCACTATCTC CCGGGACAACAGCAAGAACACCCTTTATCTGCAAATGAATTCCCTCCGCGCCG AGGACACCGCCGTGTACTACTGCGCCAAGCTGGACTCCTCGGGCTACTACTAT GCCCGGGGTCCGAGATACTGGGGACAGGGAACCCTCGTGACCGTGTCCTCCGC GTCCGGCGGAGGAGGGTCGGGAGGGCGGGCCTCCGGCGGCGGCGGTTCGGACA TCCAGCTGACCCAGTCCCCATCCTCACTGAGCGCAAGCGTGGGCGACAGAGTC ACCATTACATGCAGGGCGTCCCAGAGCATCAGCTCCTACCTGAACTGGTACCA ACAGAAGCCTGGAAAGGCTCCTAAGCTGTTGATCTACGGGGCTTCGACCCTGG CATCCGGGGTGCCCGCGAGGTTTAGCGGAAGCGGTAGCGGCACTCACTTCACT CTGACCATTAACAGCCTCCAGTCCGAGGATTCAGCCACTTACTACTGTCAGCA GTCCTACAAGCGGGCCAGCTTCGGACAGGGCACTAAGGTCGAGATCAAG
139101- aa VH	73	QVQLQESGGGLVQPGGSLRLSCAASGFTFSSDAMTWVRQAPGKGLEWVSVISG SGGTTYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAKLDSSGYYY ARGPRYWGQGTLVTVSS
139101- aa VL	88	DIQLTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKLLIYGAST LASGVPARFSGSGSGTHFTLTINSLQSEDSATYYCQQSYKRASFGQGTKVEIK
139101- aa Full CAR	103	MALPVTALLLPLALLLHAARPQVQLQESGGGLVQPGGSLRLSCAASGFTFSSD AMTWVRQAPGKGLEWVSVISGSGGTTYYADSVKGRFTISRDNSKNTLYLQMNS LRAEDTAVYYCAKLDSSGYYYARGPRYWGQGTLVTVSSASGGGGSGGRASGGG GSDIQLTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKLLIYGA STLASGVPARFSGSGSGTHFTLTINSLQSEDSATYYCQQSYKRASFGQGTKVE IKTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYIWAP LAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEE EEGGCELRVKFSRSADAPAYKQGQNQLYNELNLGRREEYDVLDKRRGRDPEMG GKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKD TYDALHMQALPPR
139101- nt Full CAR	118	ATGGCCCTCCCTGTCACCGCCCTGCTGCTTCCGCTGGCTCTTCTGCTCCACGC CGCTCGGCCCCAAGTGCAACTTCAAGAATCAGGCGGAGGACTCGTGCAGCCCG GAGGATCATTGCGGCTCTCGTGCGCCGCCTCGGGCTTCACCTTCTCGAGCGAC GCCATGACCTGGGTCCGCCAGGCCCCGGGGAAGGGGCTGGAATGGGTGTCTGT GATTTCCGGCTCCGGGGGAACTACGTACTACGCCGATTCCGTGAAAGGTCGCT TCACTATCTCCCGGGACAACAGCAAGAACACCCTTTATCTGCAAATGAATTCC CTCCGCGCCGAGGACACCGCCGTGTACTACTGCGCCAAGCTGGACTCCTCGGG CTACTACTATGCCCGGGGTCCGAGATACTGGGGACAGGGAACCCTCGTGACCG TGTCCTCCGCGTCCGGCGGAGGAGGGTCGGGAGGGCGGGCCTCCGGCGGCGGC GGTTCGGACATCCAGCTGACCCAGTCCCCATCCTCACTGAGCGCAAGCGTGGG CGACAGAGTCACCATTACATGCAGGGCGTCCCAGAGCATCAGCTCCTACCTGA ACTGGTACCAACAGAAGCCTGGAAAGGCTCCTAAGCTGTTGATCTACGGGGCT TCGACCCTGGCATCCGGGGTGCCCGCGAGGTTTAGCGGAAGCGGTAGCGGCAC

		TCACTTCACTCTGACCATTAACAGCCTCCAGTCCGAGGATTCAGCCACTTACT ACTGTCAGCAGTCCTACAAGCGGGCCAGCTTCGGACAGGGCACTAAGGTCGAG ATCAAGACCACTACCCCAGCACCGAGGCCACCCACCCCGGCTCCTACCATCGC CTCCCAGCCTCTGTCCCTGCGTCCGGAGGCATGTAGACCCGCAGCTGGTGGGG CCGTGCATACCCGGGGTCTTGACTTCGCCTGCGATATCTACATTTGGGCCCCT CTGGCTGGTACTTGCGGGGTCCTGCTGCTTTCACTCGTGATCACTCTTTACTG TAAGCGCGGTCGGAAGAAGCTGCTGTACATCTTTAAGCAACCCTTCATGAGGC CTGTGCAGACTACTCAAGAGGAGGACGGCTGTTCATGCCGGTTCCCAGAGGAG GAGGAAGGCGGCTGCGAACTGCGCGTGAAATTCAGCCGCAGCGCAGATGCTCC AGCCTACAAGCAGGGGCAGAACCAGCTCTACAACGAACTCAATCTTGGTCGGA GAGAGGAGTACGACGTGCTGGACAAGCGGAGAGGACGGGACCCAGAAATGGGC GGGAAGCCGCGCAGAAAGAATCCCCAAGAGGGCCTGTACAACGAGCTCCAAAA GGATAAGATGGCAGAAGCCTATAGCGAGATTGGTATGAAAGGGGAACGCAGAA GAGGCAAAGGCCACGACGGACTGTACCAGGGACTCAGCACCGCCACCAAGGAC ACCTATGACGCTCTTCACATGCAGGCCCTGCCGCCTCGG
139102		
139102- aa ScFv domain	44	QVQLVQSGAEVKKPGASVKVSCKASGYTFSNYGITWVRQAPGQGLEWMGWISA YNGNTNYAQKFQGRVTMTRNTSISTAYMELSSLRSEDTAVYYCARGPYYYYMD VWGKGTMVTVSSASGGGGSGGRASGGGGSEIVMTQSPLSLPVTPGEPASISCR SSQSLLYSNGYNYVDWYLQKPGQSPQLLIYLGSNRASGVPDRFSGSGSGTDFK LQISRVEAEDVGIYYCMQGRQFPYSFGQGTKVEIK
139102- nt ScFv domain	59	CAAGTCCAACTGGTCCAGAGCGGTGCAGAAGTGAAGAAGCCCGGAGCGAGCGT GAAAGTGTCCTGCAAGGCTTCCGGGTACACCTTCTCCAACTACGGCATCACTT GGGTGCGCCAGGCCCCGGGACAGGGCCTGGAATGGATGGGGTGGATTTCCGCG TACAACGGCAATACGAACTACGCTCAGAAGTTCCAGGGTAGAGTGACCATGAC TAGGAACACCTCCATTTCCACCGCCTACATGGAACTGTCCTCCCTGCGGAGCG AGGACACCGCCGTGTACTATTGCGCCCGGGGACCATACTACTACTACATGGAT GTCTGGGGGAAGGGGACTATGGTCACCGTGTCATCCGCCTCGGGAGGCGGCGG ATCAGGAGGACGCGCCTCTGGTGGTGGAGGATCGGAGATCGTGATGACCCAGA GCCCTCTCTCCTTGCCCGTGACTCCTGGGGAGCCCGCATCCATTTCATGCCGG AGCTCCCAGTCACTTCTCTACTCCAACGGCTATAACTACGTGGATTGGTACCT CCAAAAGCCGGGCCAGAGCCCGCAGCTGCTGATCTACCTGGGCTCGAACAGGG CCAGCGGAGTGCCTGACCGGTTCTCCGGGTCGGGAAGCGGGACCGACTTCAAG CTGCAAATCTCGAGAGTGGAGGCCGAGGACGTGGGAATCTACTACTGTATGCA GGGCCGCCAGTTTCCGTACTCGTTCGGACAGGGCACCAAAGTGGAAATCAAG
139102- aa VH	74	QVQLVQSGAEVKKPGASVKVSCKASGYTFSNYGITWVRQAPGQGLEWMGWISA YNGNTNYAQKFQGRVTMTRNTSISTAYMELSSLRSEDTAVYYCARGPYYYYMD VWGKGTMVTVSS
139102- aa VL	89	EIVMTQSPLSLPVTPGEPASISCRSSQSLLYSNGYNYVDWYLQKPGQSPQLLI YLGSNRASGVPDRFSGSGSGTDFKLQISRVEAEDVGIYYCMQGRQFPYSFGQG TKVEIK
139102- aa Full CAR	104	MALPVTALLLPLALLLHAARPQVQLVQSGAEVKKPGASVKVSCKASGYTFSNY GITWVRQAPGQGLEWMGWISAYNGNTNYAQKFQGRVTMTRNTSISTAYMELSS LRSEDTAVYYCARGPYYYYMDVWGKGTMVTVSSASGGGGSGGRASGGGGSEIV MTQSPLSLPVTPGEPASISCRSSQSLLYSNGYNYVDWYLQKPGQSPQLLIYLG SNRASGVPDRFSGSGSGTDFKLQISRVEAEDVGIYYCMQGRQFPYSFGQGTKV EIKTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYIWA PLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPE EEEGGCELRVKFSRSADAPAYKQGQNQLYNELNLGRREEYDVLDKRRGRDPEM GGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATK DTYDALHMQALPPR
139102- nt	119	ATGGCCCTCCCTGTCACCGCCCTGCTGCTTCCGCTGGCTCTTCTGCTCCACGC

Full CAR		CGCTCGGCCCCAAGTCCAACTGGTCCAGAGCGGTGCAGAAGTGAAGAAGCCCG GAGCGAGCGTGAAAGTGTCCTGCAAGGCTTCCGGGTACACCTTCTCCAACTAC GGCATCACTTGGGTGCGCCAGGCCCCGGGACAGGGCCTGGAATGGATGGGGTG GATTTCCGCGTACAACGGCAATACGAACTACGCTCAGAAGTTCCAGGGTAGAG TGACCATGACTAGGAACACCTCCATTTCCACCGCCTACATGGAACTGTCCTCC CTGCGGAGCGAGGACACCGCCGTGTACTATTGCGCCCGGGGACCATACTACTA CTACATGGATGTCTGGGGGAAGGGGACTATGGTCACCGTGTCATCCGCCTCGG GAGGCGGCGGATCAGGAGGACGCGCCTCTGGTGGTGGAGGATCGGAGATCGTG ATGACCCAGAGCCCTCTCTCCTTGCCCGTGACTCCTGGGGAGCCCGCATCCAT TTCATGCCGGAGCTCCCAGTCACTTCTCTACTCCAACGGCTATAACTACGTGG ATTGGTACCTCCAAAAGCCGGGCCAGAGCCCGCAGCTGCTGATCTACCTGGGC TCGAACAGGGCCAGCGGAGTGCCTGACCGGTTCTCCGGGTCGGGAAGCGGGAC CGACTTCAAGCTGCAAATCTCGAGAGTGGAGGCCGAGGACGTGGGAATCTACT ACTGTATGCAGGGCCGCCAGTTTCCGTACTCGTTCGGACAGGGCACCAAAGTG GAAATCAAGACCACTACCCCAGCACCGAGGCCACCCACCCCGGCTCCTACCAT CGCCTCCCAGCCTCTGTCCCTGCGTCCGGAGGCATGTAGACCCGCAGCTGGTG GGGCCGTGCATACCCGGGGTCTTGACTTCGCCTGCGATATCTACATTTGGGCC CCTCTGGCTGGTACTTGCGGGGTCCTGCTGCTTTCACTCGTGATCACTCTTTA CTGTAAGCGCGGTCGGAAGAAGCTGCTGTACATCTTTAAGCAACCCTTCATGA GGCCTGTGCAGACTACTCAAGAGGAGGACGGCTGTTCATGCCGGTTCCCAGAG GAGGAGGAAGGCGGCTGCGAACTGCGCGTGAAATTCAGCCGCAGCGCAGATGC TCCAGCCTACAAGCAGGGGCAGAACCAGCTCTACAACGAACTCAATCTTGGTC GGAGAGAGGAGTACGACGTGCTGGACAAGCGGAGAGGACGGGACCCAGAAATG GGCGGGAAGCCGCGCAGAAAGAATCCCCAAGAGGGCCTGTACAACGAGCTCCA AAAGGATAAGATGGCAGAAGCCTATAGCGAGATTGGTATGAAAGGGGAACGCA GAAGAGGCAAAGGCCACGACGGACTGTACCAGGGACTCAGCACCGCCACCAAG GACACCTATGACGCTCTTCACATGCAGGCCCTGCCGCCTCGG
139104		
139104- aa ScFv domain	45	EVQLLETGGGLVQPGGSLRLSCAVSGFALSNHGMSWVRRAPGKGLEWVSGIVY SGSTYYAASVKGRFTISRDNSRNTLYLQMNSLRPEDTAIYYCSAHGGESDVWG QGTTVTVSSASGGGGSGGRASGGGGSEIVLTQSPATLSVSPGESATLSCRASQ SVSSNLAWYQQKPGQAPRLLIYGASTRASGIPDRFSGSGSGTDFTLTISSLQA EDVAVYYCQQYGSSLTFGGGTKVEIK
139104- nt ScFv domain	60	GAAGTGCAATTGCTCGAAACTGGAGGAGGTCTGGTGCAACCTGGAGGATCACT TCGCCTGTCCTGCGCCGTGTCGGGCTTTGCCCTGTCCAACCATGGAATGAGCT GGGTCCGCCGCGCGCCGGGGAAGGGCCTCGAATGGGTGTCCGGCATCGTCTAC TCCGGCTCCACCTACTACGCCGCGTCCGTGAAGGGCCGGTTCACGATTTCACG GGACAACTCGCGGAACACCCTGTACCTCCAAATGAATTCCCTTCGGCCGGAGG ATACTGCCATCTACTACTGCTCCGCCCACGGTGGCGAATCCGACGTCTGGGGC CAGGGAACCACCGTGACCGTGTCCAGCGCGTCCGGGGGAGGAGGAAGCGGGGG TAGAGCATCGGGTGGAGGCGGATCAGAGATCGTGCTGACCCAGTCCCCCGCCA CCTTGAGCGTGTCACCAGGAGAGTCCGCCACCCTGTCATGCCGCGCCAGCCAG TCCGTGTCCTCCAACCTGGCTTGGTACCAGCAGAAGCCGGGGCAGGCCCCTAG ACTCCTGATCTATGGGGCGTCGACCCGGGCATCTGGAATTCCCGATAGGTTCA GCGGATCGGGCTCGGGCACTGACTTCACTCTGACCATCTCCTCGCTGCAAGCC GAGGACGTGGCTGTGTACTACTGTCAGCAGTACGGAAGCTCCCTGACTTTCGG TGGCGGGACCAAAGTCGAGATTAAG
139104- aa VH	75	EVQLLETGGGLVQPGGSLRLSCAVSGFALSNHGMSWVRRAPGKGLEWVSGIVY SGSTYYAASVKGRFTISRDNSRNTLYLQMNSLRPEDTAIYYCSAHGGESDVWG QGTTVTVSS
139104- aa VL	90	EIVLTQSPATLSVSPGESATLSCRASQSVSSNLAWYQQKPGQAPRLLIYGAST RASGIPDRFSGSGSGTDFTLTISSLQAEDVAVYYCQQYGSSLTFGGGTKVEIK

139104- aa Full CAR	105	MALPVTALLLPLALLLHAARPEVQLLETGGGLVQPGGSLRLSCAVSGFALSNH GMSWVRRAPGKGLEWVSGIVYSGSTYYAASVKGRFTISRDNSRNTLYLQMNSL RPEDTAIYYCSAHGGESDVWGQGTTVTVSSASGGGGSGGRASGGGGSEIVLTQ SPATLSVSPGESATLSCRASQSVSSNLAWYQQKPGQAPRLLIYGASTRASGIP DRFSGSGSGTDFTLTISSLQAEDVAVYYCQQYGSSLTFGGGTKVEIKTTTPAP RPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYIWAPLAGTCGVL LLSLVITLYCKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCELR VKFSRSADAPAYKQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNP QEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQ ALPPR
139104- nt Full CAR	120	ATGGCCCTCCCTGTCACCGCCCTGCTGCTTCCGCTGGCTCTTCTGCTCCACGC CGCTCGGCCCGAAGTGCAATTGCTCGAAACTGGAGGAGGTCTGGTGCAACCTG GAGGATCACTTCGCCTGTCCTGCGCCGTGTCGGGCTTTGCCCTGTCCAACCAT GGAATGAGCTGGGTCCGCCGCGCGCCGGGGAAGGGCCTCGAATGGGTGTCCGG CATCGTCTACTCCGGCTCCACCTACTACGCCGCGTCCGTGAAGGGCCGGTTCA CGATTTCACGGGACAACTCGCGGAACACCCTGTACCTCCAAATGAATTCCCTT CGGCCGGAGGATACTGCCATCTACTACTGCTCCGCCCACGGTGGCGAATCCGA CGTCTGGGGCCAGGGAACCACCGTGACCGTGTCCAGCGCGTCCGGGGGAGGAG GAAGCGGGGGTAGAGCATCGGGTGGAGGCGGATCAGAGATCGTGCTGACCCAG TCCCCCGCCACCTTGAGCGTGTCACCAGGAGAGTCCGCCACCCTGTCATGCCG CGCCAGCCAGTCCGTGTCCTCCAACCTGGCTTGGTACCAGCAGAAGCCGGGGC AGGCCCCTAGACTCCTGATCTATGGGGCGTCGACCCGGGCATCTGGAATTCCC GATAGGTTCAGCGGATCGGGCTCGGGCACTGACTTCACTCTGACCATCTCCTC GCTGCAAGCCGAGGACGTGGCTGTGTACTACTGTCAGCAGTACGGAAGCTCCC TGACTTTCGGTGGCGGGACCAAAGTCGAGATTAAGACCACTACCCCAGCACCG AGGCCACCCACCCCGGCTCCTACCATCGCCTCCCAGCCTCTGTCCCTGCGTCC GGAGGCATGTAGACCCGCAGCTGGTGGGGCCGTGCATACCCGGGGTCTTGACT TCGCCTGCGATATCTACATTTGGGCCCCTCTGGCTGGTACTTGCGGGGTCCTG CTGCTTTCACTCGTGATCACTCTTTACTGTAAGCGCGGTCGGAAGAAGCTGCT GTACATCTTTAAGCAACCCTTCATGAGGCCTGTGCAGACTACTCAAGAGGAGG ACGGCTGTTCATGCCGGTTCCCAGAGGAGGAGGAAGGCGGCTGCGAACTGCGC GTGAAATTCAGCCGCAGCGCAGATGCTCCAGCCTACAAGCAGGGGCAGAACCA GCTCTACAACGAACTCAATCTTGGTCGGAGAGAGGAGTACGACGTGCTGGACA AGCGGAGAGGACGGGACCCAGAAATGGGCGGGAAGCCGCGCAGAAAGAATCCC CAAGAGGGCCTGTACAACGAGCTCCAAAAGGATAAGATGGCAGAAGCCTATAG CGAGATTGGTATGAAAGGGGAACGCAGAAGAGGCAAAGGCCACGACGGACTGT ACCAGGGACTCAGCACCGCCACCAAGGACACCTATGACGCTCTTCACATGCAG GCCCTGCCGCCTCGG
139106		
139106- aa ScFv domain	46	EVQLVETGGGLVQPGGSLRLSCAVSGFALSNHGMSWVRRAPGKGLEWVSGIVY SGSTYYAASVKGRFTISRDNSRNTLYLQMNSLRPEDTAIYYCSAHGGESDVWG QGTTVTVSSASGGGGSGGRASGGGGSEIVMTQSPATLSVSPGERATLSCRASQ SVSSKLAWYQQKPGQAPRLLMYGASIRATGIPDRFSGSGSGTEFTLTISSLEP EDFAVYYCQQYGSSSWTFGQGTKVEIK
139106- nt ScFv domain	61	GAAGTGCAATTGGTGGAAACTGGAGGAGGACTTGTGCAACCTGGAGGATCATT GAGACTGAGCTGCGCAGTGTCGGGATTCGCCCTGAGCAACCATGGAATGTCCT GGGTCAGAAGGGCCCCTGGAAAAGGCCTCGAATGGGTGTCAGGGATCGTGTAC TCCGGTTCCACTTACTACGCCGCCTCCGTGAAGGGGCGCTTCACTATCTCACG GGATAACTCCCGCAATACCCTGTACCTCCAAATGAACAGCCTGCGGCCGGAGG ATACCGCCATCTACTACTGTTCCGCCCACGGTGGAGAGTCTGACGTCTGGGGC CAGGGAACTACCGTGACCGTGTCCTCCGCGTCCGGCGGTGGAGGGAGCGGCGG CCGCGCCAGCGGCGGCGGAGGCTCCGAGATCGTGATGACCCAGAGCCCCGCTA

		CTCTGTCGGTGTCGCCCGGAGAAAGGGCGACCCTGTCCTGCCGGGCGTCGCAG TCCGTGAGCAGCAAGCTGGCTTGGTACCAGCAGAAGCCGGGCCAGGCACCACG CCTGCTTATGTACGGTGCCTCCATTCGGGCCACCGGAATCCCGGACCGGTTCT CGGGGTCGGGGTCCGGTACCGAGTTCACACTGACCATTTCCTCGCTCGAGCCC GAGGACTTTGCCGTCTATTACTGCCAGCAGTACGGCTCCTCCTCATGGACGTT CGGCCAGGGGACCAAGGTCGAAATCAAG
139106- aa VH	76	EVQLVETGGGLVQPGGSLRLSCAVSGFALSNHGMSWVRRAPGKGLEWVSGIVY SGSTYYAASVKGRFTISRDNSRNTLYLQMNSLRPEDTAIYYCSAHGGESDVWG QGTTVTVSS
139106- aa VL	91	EIVMTQSPATLSVSPGERATLSCRASQSVSSKLAWYQQKPGQAPRLLMYGASI RATGIPDRFSGSGSGTEFTLTISSLEPEDFAVYYCQQYGSSSWTFGQGTKVEI K
139106- aa Full CAR	106	MALPVTALLLPLALLLHAARPEVQLVETGGGLVQPGGSLRLSCAVSGFALSNH GMSWVRRAPGKGLEWVSGIVYSGSTYYAASVKGRFTISRDNSRNTLYLQMNSL RPEDTAIYYCSAHGGESDVWGQGTTVTVSSASGGGGSGGRASGGGGSEIVMTQ SPATLSVSPGERATLSCRASQSVSSKLAWYQQKPGQAPRLLMYGASIRATGIP DRFSGSGSGTEFTLTISSLEPEDFAVYYCQQYGSSSWTFGQGTKVEIKTTTPA PRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYIWAPLAGTCGV LLLSLVITLYCKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCEL RVKFSRSADAPAYKQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKN PQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHM QALPPR
139106- nt Full CAR	121	ATGGCCCTCCCTGTCACCGCCCTGCTGCTTCCGCTGGCTCTTCTGCTCCACGC CGCTCGGCCCGAAGTGCAATTGGTGGAAACTGGAGGAGGACTTGTGCAACCTG GAGGATCATTGAGACTGAGCTGCGCAGTGTCGGGATTCGCCCTGAGCAACCAT GGAATGTCCTGGGTCAGAAGGGCCCCTGGAAAAGGCCTCGAATGGGTGTCAGG GATCGTGTACTCCGGTTCCACTTACTACGCCGCCTCCGTGAAGGGGCGCTTCA CTATCTCACGGGATAACTCCCGCAATACCCTGTACCTCCAAATGAACAGCCTG CGGCCGGAGGATACCGCCATCTACTACTGTTCCGCCCACGGTGGAGAGTCTGA CGTCTGGGGCCAGGGAACTACCGTGACCGTGTCCTCCGCGTCCGGCGGTGGAG GGAGCGGCGGCCGCGCCAGCGGCGGCGGAGGCTCCGAGATCGTGATGACCCAG AGCCCCGCTACTCTGTCGGTGTCGCCCGGAGAAAGGGCGACCCTGTCCTGCCG GGCGTCGCAGTCCGTGAGCAGCAAGCTGGCTTGGTACCAGCAGAAGCCGGGCC AGGCACCACGCCTGCTTATGTACGGTGCCTCCATTCGGGCCACCGGAATCCCG GACCGGTTCTCGGGGTCGGGGTCCGGTACCGAGTTCACACTGACCATTTCCTC GCTCGAGCCCGAGGACTTTGCCGTCTATTACTGCCAGCAGTACGGCTCCTCCT CATGGACGTTCGGCCAGGGGACCAAGGTCGAAATCAAGACCACTACCCCAGCA CCGAGGCCACCCACCCCGGCTCCTACCATCGCCTCCCAGCCTCTGTCCCTGCG TCCGGAGGCATGTAGACCCGCAGCTGGTGGGGCCGTGCATACCCGGGGTCTTG ACTTCGCCTGCGATATCTACATTTGGGCCCCTCTGGCTGGTACTTGCGGGGTC CTGCTGCTTTCACTCGTGATCACTCTTTACTGTAAGCGCGGTCGGAAGAAGCT GCTGTACATCTTTAAGCAACCCTTCATGAGGCCTGTGCAGACTACTCAAGAGG AGGACGGCTGTTCATGCCGGTTCCCAGAGGAGGAGGAAGGCGGCTGCGAACTG CGCGTGAAATTCAGCCGCAGCGCAGATGCTCCAGCCTACAAGCAGGGGCAGAA CCAGCTCTACAACGAACTCAATCTTGGTCGGAGAGAGGAGTACGACGTGCTGG ACAAGCGGAGAGGACGGGACCCAGAAATGGGCGGGAAGCCGCGCAGAAAGAAT CCCCAAGAGGGCCTGTACAACGAGCTCCAAAAGGATAAGATGGCAGAAGCCTA TAGCGAGATTGGTATGAAAGGGGAACGCAGAAGAGGCAAAGGCCACGACGGAC TGTACCAGGGACTCAGCACCGCCACCAAGGACACCTATGACGCTCTTCACATG CAGGCCCTGCCGCCTCGG
139107		
139107- aa	47	EVQLVETGGGVVQPGGSLRLSCAVSGFALSNHGMSWVRRAPGKGLEWVSGIVY

ScFv domain		SGSTYYAASVKGRFTISRDNSRNTLYLQMNSLRPEDTAIYYCSAHGGESDVWG QGTTVTVSSASGGGGSGGRASGGGGSEIVLTQSPGTLSLSPGERATLSCRASQ SVGSTNLAWYQQKPGQAPRLLIYDASNRATGIPDRFSGGGSGTDFTLTISRLE PEDFAVYYCQQYGSSPPWTFGQGTKVEIK
139107- nt ScFv domain	62	GAAGTGCAATTGGTGGAGACTGGAGGAGGAGTGGTGCAACCTGGAGGAAGCCT GAGACTGTCATGCGCGGTGTCGGGCTTCGCCCTCTCCAACCACGGAATGTCCT GGGTCCGCCGGGCCCCTGGGAAAGGACTTGAATGGGTGTCCGGCATCGTGTAC TCGGGTTCCACCTACTACGCGGCCTCAGTGAAGGGCCGGTTTACTATTAGCCG CGACAACTCCAGAAACACACTGTACCTCCAAATGAACTCGCTGCGGCCGGAAG ATACCGCTATCTACTACTGCTCCGCCCATGGGGGAGAGTCGGACGTCTGGGGA CAGGGCACCACTGTCACTGTGTCCAGCGCTTCCGGCGGTGGTGGAAGCGGGGG ACGGGCCTCAGGAGGCGGTGGCAGCGAGATTGTGCTGACCCAGTCCCCCGGGA CCCTGAGCCTGTCCCCGGGAGAAAGGGCCACCCTCTCCTGTCGGGCATCCCAG TCCGTGGGGTCTACTAACCTTGCATGGTACCAGCAGAAGCCCGGCCAGGCCCC TCGCCTGCTGATCTACGACGCGTCCAATAGAGCCACCGGCATCCCGGATCGCT TCAGCGGAGGCGGATCGGGCACCGACTTCACCCTCACCATTTCAAGGCTGGAA CCGGAGGACTTCGCCGTGTACTACTGCCAGCAGTATGGTTCGTCCCCACCCTG GACGTTCGGCCAGGGGACTAAGGTCGAGATCAAG
139107- aa VH	77	EVQLVETGGGVVQPGGSLRLSCAVSGFALSNHGMSWVRRAPGKGLEWVSGIVY SGSTYYAASVKGRFTISRDNSRNTLYLQMNSLRPEDTAIYYCSAHGGESDVWG QGTTVTVSS
139107- aa VL	92	EIVLTQSPGTLSLSPGERATLSCRASQSVGSTNLAWYQQKPGQAPRLLIYDAS NRATGIPDRFSGGGSGTDFTLTISRLEPEDFAVYYCQQYGSSPPWTFGQGTKV EIK
139107- aa Full CAR	107	MALPVTALLLPLALLLHAARPEVQLVETGGGVVQPGGSLRLSCAVSGFALSNH GMSWVRRAPGKGLEWVSGIVYSGSTYYAASVKGRFTISRDNSRNTLYLQMNSL RPEDTAIYYCSAHGGESDVWGQGTTVTVSSASGGGGSGGRASGGGGSEIVLTQ SPGTLSLSPGERATLSCRASQSVGSTNLAWYQQKPGQAPRLLIYDASNRATGI PDRFSGGGSGTDFTLTISRLEPEDFAVYYCQQYGSSPPWTFGQGTKVEIKTTT PAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYIWAPLAGTC GVLLLSLVITLYCKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGC ELRVKFSRSADAPAYKQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRR KNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDAL HMQALPPR
139107- nt Full CAR	122	ATGGCCCTCCCTGTCACCGCCCTGCTGCTTCCGCTGGCTCTTCTGCTCCACGC CGCTCGGCCCGAAGTGCAATTGGTGGAGACTGGAGGAGGAGTGGTGCAACCTG GAGGAAGCCTGAGACTGTCATGCGCGGTGTCGGGCTTCGCCCTCTCCAACCAC GGAATGTCCTGGGTCCGCCGGGCCCCTGGGAAAGGACTTGAATGGGTGTCCGG CATCGTGTACTCGGGTTCCACCTACTACGCGGCCTCAGTGAAGGGCCGGTTTA CTATTAGCCGCGACAACTCCAGAAACACACTGTACCTCCAAATGAACTCGCTG CGGCCGGAAGATACCGCTATCTACTACTGCTCCGCCCATGGGGGAGAGTCGGA CGTCTGGGGACAGGGCACCACTGTCACTGTGTCCAGCGCTTCCGGCGGTGGTG GAAGCGGGGGACGGGCCTCAGGAGGCGGTGGCAGCGAGATTGTGCTGACCCAG TCCCCCGGGACCCTGAGCCTGTCCCCGGGAGAAAGGGCCACCCTCTCCTGTCG GGCATCCCAGTCCGTGGGGTCTACTAACCTTGCATGGTACCAGCAGAAGCCCG GCCAGGCCCCTCGCCTGCTGATCTACGACGCGTCCAATAGAGCCACCGGCATC CCGGATCGCTTCAGCGGAGGCGGATCGGGCACCGACTTCACCCTCACCATTTC AAGGCTGGAACCGGAGGACTTCGCCGTGTACTACTGCCAGCAGTATGGTTCGT CCCCACCCTGGACGTTCGGCCAGGGGACTAAGGTCGAGATCAAGACCACTACC CCAGCACCGAGGCCACCCACCCCGGCTCCTACCATCGCCTCCCAGCCTCTGTC CCTGCGTCCGGAGGCATGTAGACCCGCAGCTGGTGGGGCCGTGCATACCCGGG GTCTTGACTTCGCCTGCGATATCTACATTTGGGCCCCTCTGGCTGGTACTTGC

		GGGGTCCTGCTGCTTTCACTCGTGATCACTCTTTACTGTAAGCGCGGTCGGAA GAAGCTGCTGTACATCTTTAAGCAACCCTTCATGAGGCCTGTGCAGACTACTC AAGAGGAGGACGGCTGTTCATGCCGGTTCCCAGAGGAGGAGGAAGGCGGCTGC GAACTGCGCGTGAAATTCAGCCGCAGCGCAGATGCTCCAGCCTACAAGCAGGG GCAGAACCAGCTCTACAACGAACTCAATCTTGGTCGGAGAGAGGAGTACGACG TGCTGGACAAGCGGAGAGGACGGGACCCAGAAATGGGCGGGAAGCCGCGCAGA AAGAATCCCCAAGAGGGCCTGTACAACGAGCTCCAAAAGGATAAGATGGCAGA AGCCTATAGCGAGATTGGTATGAAAGGGGAACGCAGAAGAGGCAAAGGCCACG ACGGACTGTACCAGGGACTCAGCACCGCCACCAAGGACACCTATGACGCTCTT CACATGCAGGCCCTGCCGCCTCGG
139108		
139108- aa ScFv domain	48	QVQLVESGGGLVKPGGSLRLSCAASGFTFSDYYMSWIRQAPGKGLEWVSYISS SGSTIYYADSVKGRFTISRDNAKNSLYLQMNSLRAEDTAVYYCARESGDGMDV WGQGTTVTVSSASGGGGSGGRASGGGGSDIQMTQSPSSLSASVGDRVTITCRA SQSISSYLNWYQQKPGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLTISSL QPEDFATYYCQQSYTLAFGQGTKVDIK
139108- nt ScFv domain	63	CAAGTGCAACTCGTGGAATCTGGTGGAGGACTCGTGAAACCTGGAGGATCATT GAGACTGTCATGCGCGGCCTCGGGATTCACGTTCTCCGATTACTACATGAGCT GGATTCGCCAGGCTCCGGGGAAGGGACTGGAATGGGTGTCCTACATTTCCTCA TCCGGCTCCACCATCTACTACGCGGACTCCGTGAAGGGGAGATTCACCATTAG CCGCGATAACGCCAAGAACAGCCTGTACCTTCAGATGAACTCCCTGCGGGCTG AAGATACTGCCGTCTACTACTGCGCAAGGGAGAGCGGAGATGGGATGGACGTC TGGGGACAGGGTACCACTGTGACCGTGTCGTCGGCCTCCGGCGGAGGGGGTTC GGGTGGAAGGGCCAGCGGCGGCGGAGGCAGCGACATCCAGATGACCCAGTCCC CCTCATCGCTGTCCGCCTCCGTGGGCGACCGCGTCACCATCACATGCCGGGCC TCACAGTCGATCTCCTCCTACCTCAATTGGTATCAGCAGAAGCCCGGAAAGGC CCCTAAGCTTCTGATCTACGCAGCGTCCTCCCTGCAATCCGGGGTCCCATCTC GGTTCTCCGGCTCGGGCAGCGGTACCGACTTCACTCTGACCATCTCGAGCCTG CAGCCGGAGGACTTCGCCACTTACTACTGTCAGCAAAGCTACACCCTCGCGTT TGGCCAGGGCACCAAAGTGGACATCAAG
139108- aa VH	78	QVQLVESGGGLVKPGGSLRLSCAASGFTFSDYYMSWIRQAPGKGLEWVSYISS SGSTIYYADSVKGRFTISRDNAKNSLYLQMNSLRAEDTAVYYCARESGDGMDV WGQGTTVTVSS
139108- aa VL	93	DIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKLLIYAASS LQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQSYTLAFGQGTKVDIK
139108- aa Full CAR	108	MALPVTALLLPLALLLHAARPQVQLVESGGGLVKPGGSLRLSCAASGFTFSDY YMSWIRQAPGKGLEWVSYISSSGSTIYYADSVKGRFTISRDNAKNSLYLQMNS LRAEDTAVYYCARESGDGMDVWGQGTTVTVSSASGGGGSGGRASGGGGSDIQM TQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKLLIYAASSLQSG VPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQSYTLAFGQGTKVDIKTTTPA PRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYIWAPLAGTCGV LLLSLVITLYCKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCEL RVKFSRSADAPAYKQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKN PQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHM QALPPR
139108- nt Full CAR	123	ATGGCCCTCCCTGTCACCGCCCTGCTGCTTCCGCTGGCTCTTCTGCTCCACGC CGCTCGGCCCCAAGTGCAACTCGTGGAATCTGGTGGAGGACTCGTGAAACCTG GAGGATCATTGAGACTGTCATGCGCGGCCTCGGGATTCACGTTCTCCGATTAC TACATGAGCTGGATTCGCCAGGCTCCGGGGAAGGGACTGGAATGGGTGTCCTA CATTTCCTCATCCGGCTCCACCATCTACTACGCGGACTCCGTGAAGGGGAGAT TCACCATTAGCCGCGATAACGCCAAGAACAGCCTGTACCTTCAGATGAACTCC CTGCGGGCTGAAGATACTGCCGTCTACTACTGCGCAAGGGAGAGCGGAGATGG

		GATGGACGTCTGGGGACAGGGTACCACTGTGACCGTGTCGTCGGCCTCCGGCG GAGGGGGTTCGGGTGGAAGGGCCAGCGGCGGCGGAGGCAGCGACATCCAGATG ACCCAGTCCCCCTCATCGCTGTCCGCCTCCGTGGGCGACCGCGTCACCATCAC ATGCCGGGCCTCACAGTCGATCTCCTCCTACCTCAATTGGTATCAGCAGAAGC CCGGAAAGGCCCCTAAGCTTCTGATCTACGCAGCGTCCTCCCTGCAATCCGGG GTCCCATCTCGGTTCTCCGGCTCGGGCAGCGGTACCGACTTCACTCTGACCAT CTCGAGCCTGCAGCCGGAGGACTTCGCCACTTACTACTGTCAGCAAAGCTACA CCCTCGCGTTTGGCCAGGGCACCAAAGTGGACATCAAGACCACTACCCCAGCA CCGAGGCCACCCACCCCGGCTCCTACCATCGCCTCCCAGCCTCTGTCCCTGCG TCCGGAGGCATGTAGACCCGCAGCTGGTGGGGCCGTGCATACCCGGGGTCTTG ACTTCGCCTGCGATATCTACATTTGGGCCCCTCTGGCTGGTACTTGCGGGGTC CTGCTGCTTTCACTCGTGATCACTCTTTACTGTAAGCGCGGTCGGAAGAAGCT GCTGTACATCTTTAAGCAACCCTTCATGAGGCCTGTGCAGACTACTCAAGAGG AGGACGGCTGTTCATGCCGGTTCCCAGAGGAGGAGGAAGGCGGCTGCGAACTG CGCGTGAAATTCAGCCGCAGCGCAGATGCTCCAGCCTACAAGCAGGGGCAGAA CCAGCTCTACAACGAACTCAATCTTGGTCGGAGAGAGGAGTACGACGTGCTGG ACAAGCGGAGAGGACGGGACCCAGAAATGGGCGGGAAGCCGCGCAGAAAGAAT CCCCAAGAGGGCCTGTACAACGAGCTCCAAAAGGATAAGATGGCAGAAGCCTA TAGCGAGATTGGTATGAAAGGGGAACGCAGAAGAGGCAAAGGCCACGACGGAC TGTACCAGGGACTCAGCACCGCCACCAAGGACACCTATGACGCTCTTCACATG CAGGCCCTGCCGCCTCGG
139110		
139110- aa ScFv domain	50	QVQLVQSGGGLVKPGGSLRLSCAASGFTFSDYYMSWIRQAPGKGLEWVSYISS SGNTIYYADSVKGRFTISRDNAKNSLYLQMNSLRAEDTAVYYCARSTMVREDY WGQGTLVTVSSASGGGGSGGRASGGGGSDIVLTQSPLSLPVTLGQPASISCKS SESLVHNSGKTYLNWFHQRPGQSPRRLIYEVSNRDSGVPDRFTGSGSGTDFTL KISRVEAEDVGVYYCMQGTHWPGTFGQGTKLEIK
139110- nt ScFv domain	65	CAAGTGCAACTGGTGCAAAGCGGAGGAGGATTGGTCAAACCCGGAGGAAGCCT GAGACTGTCATGCGCGGCCTCTGGATTCACCTTCTCCGATTACTACATGTCAT GGATCAGACAGGCCCCGGGGAAGGGCCTCGAATGGGTGTCCTACATCTCGTCC TCCGGGAACACCATCTACTACGCCGACAGCGTGAAGGGCCGCTTTACCATTTC CCGCGACAACGCAAAGAACTCGCTGTACCTTCAGATGAATTCCCTGCGGGCTG AAGATACCGCGGTGTACTATTGCGCCCGGTCCACTATGGTCCGGGAGGACTAC TGGGGACAGGGCACACTCGTGACCGTGTCCAGCGCGAGCGGGGGTGGAGGCAG CGGTGGACGCGCCTCCGGCGGCGGCGGTTCAGACATCGTGCTGACTCAGTCGC CCCTGTCGCTGCCGGTCACCCTGGGCCAACCGGCCTCAATTAGCTGCAAGTCC TCGGAGAGCCTGGTGCACAACTCAGGAAAGACTTACCTGAACTGGTTCCATCA GCGGCCTGGACAGTCCCCACGGAGGCTCATCTATGAAGTGTCCAACAGGGATT CGGGGGTGCCCGACCGCTTCACTGGCTCCGGGTCCGGCACCGACTTCACCTTG AAAATCTCCAGAGTGGAAGCCGAGGACGTGGGCGTGTACTACTGTATGCAGGG TACCCACTGGCCTGGAACCTTTGGACAAGGAACTAAGCTCGAGATTAAG
139110- aa VH	80	QVQLVQSGGGLVKPGGSLRLSCAASGFTFSDYYMSWIRQAPGKGLEWVSYISS SGNTIYYADSVKGRFTISRDNAKNSLYLQMNSLRAEDTAVYYCARSTMVREDY WGQGTLVTVSS
139110- aa VL	95	DIVLTQSPLSLPVTLGQPASISCKSSESLVHNSGKTYLNWFHQRPGQSPRRLI YEVSNRDSGVPDRFTGSGSGTDFTLKISRVEAEDVGVYYCMQGTHWPGTFGQG TKLEIK
139110- aa Full CAR	110	MALPVTALLLPLALLLHAARPQVQLVQSGGGLVKPGGSLRLSCAASGFTFSDY YMSWIRQAPGKGLEWVSYISSSGNTIYYADSVKGRFTISRDNAKNSLYLQMNS LRAEDTAVYYCARSTMVREDYWGQGTLVTVSSASGGGGSGGRASGGGGSDIVL TQSPLSLPVTLGQPASISCKSSESLVHNSGKTYLNWFHQRPGQSPRRLIYEVS NRDSGVPDRFTGSGSGTDFTLKISRVEAEDVGVYYCMQGTHWPGTFGQGTKLE

		IKTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYIWAP LAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEE EEGGCELRVKFSRSADAPAYKQGQNQLYNELNLGRREEYDVLDKRRGRDPEMG GKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKD TYDALHMQALPPR
139110- nt Full CAR	125	ATGGCCCTCCCTGTCACCGCCCTGCTGCTTCCGCTGGCTCTTCTGCTCCACGC CGCTCGGCCCCAAGTGCAACTGGTGCAAAGCGGAGGAGGATTGGTCAAACCCG GAGGAAGCCTGAGACTGTCATGCGCGGCCTCTGGATTCACCTTCTCCGATTAC TACATGTCATGGATCAGACAGGCCCCGGGGAAGGGCCTCGAATGGGTGTCCTA CATCTCGTCCTCCGGGAACACCATCTACTACGCCGACAGCGTGAAGGGCCGCT TTACCATTTCCCGCGACAACGCAAAGAACTCGCTGTACCTTCAGATGAATTCC CTGCGGGCTGAAGATACCGCGGTGTACTATTGCGCCCGGTCCACTATGGTCCG GGAGGACTACTGGGGACAGGGCACACTCGTGACCGTGTCCAGCGCGAGCGGGG GTGGAGGCAGCGGTGGACGCGCCTCCGGCGGCGGCGGTTCAGACATCGTGCTG ACTCAGTCGCCCCTGTCGCTGCCGGTCACCCTGGGCCAACCGGCCTCAATTAG CTGCAAGTCCTCGGAGAGCCTGGTGCACAACTCAGGAAAGACTTACCTGAACT GGTTCCATCAGCGGCCTGGACAGTCCCCACGGAGGCTCATCTATGAAGTGTCC AACAGGGATTCGGGGGTGCCCGACCGCTTCACTGGCTCCGGGTCCGGCACCGA CTTCACCTTGAAAATCTCCAGAGTGGAAGCCGAGGACGTGGGCGTGTACTACT GTATGCAGGGTACCCACTGGCCTGGAACCTTTGGACAAGGAACTAAGCTCGAG ATTAAGACCACTACCCCAGCACCGAGGCCACCCACCCCGGCTCCTACCATCGC CTCCCAGCCTCTGTCCCTGCGTCCGGAGGCATGTAGACCCGCAGCTGGTGGGG CCGTGCATACCCGGGGTCTTGACTTCGCCTGCGATATCTACATTTGGGCCCCT CTGGCTGGTACTTGCGGGGTCCTGCTGCTTTCACTCGTGATCACTCTTTACTG TAAGCGCGGTCGGAAGAAGCTGCTGTACATCTTTAAGCAACCCTTCATGAGGC CTGTGCAGACTACTCAAGAGGAGGACGGCTGTTCATGCCGGTTCCCAGAGGAG GAGGAAGGCGGCTGCGAACTGCGCGTGAAATTCAGCCGCAGCGCAGATGCTCC AGCCTACAAGCAGGGGCAGAACCAGCTCTACAACGAACTCAATCTTGGTCGGA GAGAGGAGTACGACGTGCTGGACAAGCGGAGAGGACGGGACCCAGAAATGGGC GGGAAGCCGCGCAGAAAGAATCCCCAAGAGGGCCTGTACAACGAGCTCCAAAA GGATAAGATGGCAGAAGCCTATAGCGAGATTGGTATGAAAGGGGAACGCAGAA GAGGCAAAGGCCACGACGGACTGTACCAGGGACTCAGCACCGCCACCAAGGAC ACCTATGACGCTCTTCACATGCAGGCCCTGCCGCCTCGG
139112		
139112- aa ScFv domain	51	QVQLVESGGGLVQPGGSLRLSCAVSGFALSNHGMSWVRRAPGKGLEWVSGIVY SGSTYYAASVKGRFTISRDNSRNTLYLQMNSLRPEDTAIYYCSAHGGESDVWG QGTTVTVSSASGGGGSGGRASGGGGSDIRLTQSPSPLSASVGDRVTITCQASE DINKFLNWYHQTPGKAPKLLIYDASTLQTGVPSRFSGSGSGTDFTLTINSLQP EDIGTYYCQQYESLPLTFGGGTKVEIK
139112- nt ScFv domain	66	CAAGTGCAACTCGTGGAATCTGGTGGAGGACTCGTGCAACCCGGTGGAAGCCT TAGGCTGTCGTGCGCCGTCAGCGGGTTTGCTCTGAGCAACCATGGAATGTCCT GGGTCCGCCGGGCACCGGGAAAAGGGCTGGAATGGGTGTCCGGCATCGTGTAC AGCGGGTCAACCTATTACGCCGCGTCCGTGAAGGGCAGATTCACTATCTCAAG AGACAACAGCCGGAACACCCTGTACTTGCAAATGAATTCCCTGCGCCCCGAGG ACACCGCCATCTACTACTGCTCCGCCCACGGAGGAGAGTCGGACGTGTGGGGC CAGGGAACGACTGTGACTGTGTCCAGCGCATCAGGAGGGGGTGGTTCGGGCGG CCGGGCCTCGGGGGGAGGAGGTTCCGACATTCGGCTGACCCAGTCCCCGTCCC CACTGTCGGCCTCCGTCGGCGACCGCGTGACCATCACTTGTCAGGCGTCCGAG GACATTAACAAGTTCCTGAACTGGTACCACCAGACCCCTGGAAAGGCCCCCAA GCTGCTGATCTACGATGCCTCGACCCTTCAAACTGGAGTGCCTAGCCGGTTCT CCGGGTCCGGCTCCGGCACTGATTTCACTCTGACCATCAACTCATTGCAGCCG GAAGATATCGGGACCTACTATTGCCAGCAGTACGAATCCCTCCCGCTCACATT

		CGGCGGGGGAACCAAGGTCGAGATTAAG
139112- aa VH	81	QVQLVESGGGLVQPGGSLRLSCAVSGFALSNHGMSWVRRAPGKGLEWVSGIVY SGSTYYAASVKGRFTISRDNSRNTLYLQMNSLRPEDTAIYYCSAHGGESDVWG QGTTVTVSS
139112- aa VL	96	DIRLTQSPSPLSASVGDRVTITCQASEDINKFLNWYHQTPGKAPKLLIYDAST LQTGVPSRFSGSGSGTDFTLTINSLQPEDIGTYYCQQYESLPLTFGGGTKVEI K
139112- aa Full CAR	111	MALPVTALLLPLALLLHAARPQVQLVESGGGLVQPGGSLRLSCAVSGFALSNH GMSWVRRAPGKGLEWVSGIVYSGSTYYAASVKGRFTISRDNSRNTLYLQMNSL RPEDTAIYYCSAHGGESDVWGQGTTVTVSSASGGGGSGGRASGGGGSDIRLTQ SPSPLSASVGDRVTITCQASEDINKFLNWYHQTPGKAPKLLIYDASTLQTGVP SRFSGSGSGTDFTLTINSLQPEDIGTYYCQQYESLPLTFGGGTKVEIKTTTPA PRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYIWAPLAGTCGV LLLSLVITLYCKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCEL RVKFSRSADAPAYKQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKN PQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHM QALPPR
139112- nt Full CAR	126	ATGGCCCTCCCTGTCACCGCCCTGCTGCTTCCGCTGGCTCTTCTGCTCCACGC CGCTCGGCCCCAAGTGCAACTCGTGGAATCTGGTGGAGGACTCGTGCAACCCG GTGGAAGCCTTAGGCTGTCGTGCGCCGTCAGCGGGTTTGCTCTGAGCAACCAT GGAATGTCCTGGGTCCGCCGGGCACCGGGAAAAGGGCTGGAATGGGTGTCCGG CATCGTGTACAGCGGGTCAACCTATTACGCCGCGTCCGTGAAGGGCAGATTCA CTATCTCAAGAGACAACAGCCGGAACACCCTGTACTTGCAAATGAATTCCCTG CGCCCCGAGGACACCGCCATCTACTACTGCTCCGCCCACGGAGGAGAGTCGGA CGTGTGGGGCCAGGGAACGACTGTGACTGTGTCCAGCGCATCAGGAGGGGGTG GTTCGGGCGGCCGGGCCTCGGGGGGAGGAGGTTCCGACATTCGGCTGACCCAG TCCCCGTCCCCACTGTCGGCCTCCGTCGGCGACCGCGTGACCATCACTTGTCA GGCGTCCGAGGACATTAACAAGTTCCTGAACTGGTACCACCAGACCCCTGGAA AGGCCCCCAAGCTGCTGATCTACGATGCCTCGACCCTTCAAACTGGAGTGCCT AGCCGGTTCTCCGGGTCCGGCTCCGGCACTGATTTCACTCTGACCATCAACTC ATTGCAGCCGGAAGATATCGGGACCTACTATTGCCAGCAGTACGAATCCCTCC CGCTCACATTCGGCGGGGGAACCAAGGTCGAGATTAAGACCACTACCCCAGCA CCGAGGCCACCCACCCCGGCTCCTACCATCGCCTCCCAGCCTCTGTCCCTGCG TCCGGAGGCATGTAGACCCGCAGCTGGTGGGGCCGTGCATACCCGGGGTCTTG ACTTCGCCTGCGATATCTACATTTGGGCCCCTCTGGCTGGTACTTGCGGGGTC CTGCTGCTTTCACTCGTGATCACTCTTTACTGTAAGCGCGGTCGGAAGAAGCT GCTGTACATCTTTAAGCAACCCTTCATGAGGCCTGTGCAGACTACTCAAGAGG AGGACGGCTGTTCATGCCGGTTCCCAGAGGAGGAGGAAGGCGGCTGCGAACTG CGCGTGAAATTCAGCCGCAGCGCAGATGCTCCAGCCTACAAGCAGGGGCAGAA CCAGCTCTACAACGAACTCAATCTTGGTCGGAGAGAGGAGTACGACGTGCTGG ACAAGCGGAGAGGACGGGACCCAGAAATGGGCGGGAAGCCGCGCAGAAAGAAT CCCCAAGAGGGCCTGTACAACGAGCTCCAAAAGGATAAGATGGCAGAAGCCTA TAGCGAGATTGGTATGAAAGGGGAACGCAGAAGAGGCAAAGGCCACGACGGAC TGTACCAGGGACTCAGCACCGCCACCAAGGACACCTATGACGCTCTTCACATG CAGGCCCTGCCGCCTCGG
139113		
139113- aa ScFv domain	52	EVQLVETGGGLVQPGGSLRLSCAVSGFALSNHGMSWVRRAPGKGLEWVSGIVY SGSTYYAASVKGRFTISRDNSRNTLYLQMNSLRPEDTAIYYCSAHGGESDVWG QGTTVTVSSASGGGGSGGRASGGGGSETTLTQSPATLSVSPGERATLSCRASQ SVGSNLAWYQQKPGQGPRLLIYGASTRATGIPARFSGSGSGTEFTLTISSLQP EDFAVYYCQQYNDWLPVTFGQGTKVEIK
139113- nt	67	GAAGTGCAATTGGTGGAAACTGGAGGAGGACTTGTGCAACCTGGAGGATCATT

ScFv domain		GCGGCTCTCATGCGCTGTCTCCGGCTTCGCCCTGTCAAATCACGGGATGTCGT GGGTCAGACGGGCCCCGGGAAAGGGTCTGGAATGGGTGTCGGGGATTGTGTAC AGCGGCTCCACCTACTACGCCGCTTCGGTCAAGGGCCGCTTCACTATTTCACG GGACAACAGCCGCAACACCCTCTATCTGCAAATGAACTCTCTCCGCCCGGAGG ATACCGCCATCTACTACTGCTCCGCACACGGCGGCGAATCCGACGTGTGGGGA CAGGGAACCACTGTCACCGTGTCGTCCGCATCCGGTGGCGGAGGATCGGGTGG CCGGGCCTCCGGGGGCGGCGGCAGCGAGACTACCCTGACCCAGTCCCCTGCCA CTCTGTCCGTGAGCCCGGGAGAGAGAGCCACCCTTAGCTGCCGGGCCAGCCAG AGCGTGGGCTCCAACCTGGCCTGGTACCAGCAGAAGCCAGGACAGGGTCCCAG GCTGCTGATCTACGGAGCCTCCACTCGCGCGACCGGCATCCCCGCGAGGTTCT CCGGGTCGGGTTCCGGGACCGAGTTCACCCTGACCATCTCCTCCCTCCAACCG GAGGACTTCGCGGTGTACTACTGTCAGCAGTACAACGATTGGCTGCCCGTGAC ATTTGGACAGGGGACGAAGGTGGAAATCAAA
139113- aa VH	82	EVQLVETGGGLVQPGGSLRLSCAVSGFALSNHGMSWVRRAPGKGLEWVSGIVY SGSTYYAASVKGRFTISRDNSRNTLYLQMNSLRPEDTAIYYCSAHGGESDVWG QGTTVTVSS
139113- aa VL	97	ETTLTQSPATLSVSPGERATLSCRASQSVGSNLAWYQQKPGQGPRLLIYGAST RATGIPARFSGSGSGTEFTLTISSLQPEDFAVYYCQQYNDWLPVTFGQGTKVE IK
139113- aa Full CAR	112	MALPVTALLLPLALLLHAARPEVQLVETGGGLVQPGGSLRLSCAVSGFALSNH GMSWVRRAPGKGLEWVSGIVYSGSTYYAASVKGRFTISRDNSRNTLYLQMNSL RPEDTAIYYCSAHGGESDVWGQGTTVTVSSASGGGGSGGRASGGGGSETTLTQ SPATLSVSPGERATLSCRASQSVGSNLAWYQQKPGQGPRLLIYGASTRATGIP ARFSGSGSGTEFTLTISSLQPEDFAVYYCQQYNDWLPVTFGQGTKVEIKTTTP APRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYIWAPLAGTCG VLLLSLVITLYCKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCE LRVKFSRSADAPAYKQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRK NPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALH MQALPPR
139113- nt Full CAR	127	ATGGCCCTCCCTGTCACCGCCCTGCTGCTTCCGCTGGCTCTTCTGCTCCACGC CGCTCGGCCCGAAGTGCAATTGGTGGAAACTGGAGGAGGACTTGTGCAACCTG GAGGATCATTGCGGCTCTCATGCGCTGTCTCCGGCTTCGCCCTGTCAAATCAC GGGATGTCGTGGGTCAGACGGGCCCCGGGAAAGGGTCTGGAATGGGTGTCGGG GATTGTGTACAGCGGCTCCACCTACTACGCCGCTTCGGTCAAGGGCCGCTTCA CTATTTCACGGGACAACAGCCGCAACACCCTCTATCTGCAAATGAACTCTCTC CGCCCGGAGGATACCGCCATCTACTACTGCTCCGCACACGGCGGCGAATCCGA CGTGTGGGGACAGGGAACCACTGTCACCGTGTCGTCCGCATCCGGTGGCGGAG GATCGGGTGGCCGGGCCTCCGGGGGCGGCGGCAGCGAGACTACCCTGACCCAG TCCCCTGCCACTCTGTCCGTGAGCCCGGGAGAGAGAGCCACCCTTAGCTGCCG GGCCAGCCAGAGCGTGGGCTCCAACCTGGCCTGGTACCAGCAGAAGCCAGGAC AGGGTCCCAGGCTGCTGATCTACGGAGCCTCCACTCGCGCGACCGGCATCCCC GCGAGGTTCTCCGGGTCGGGTTCCGGGACCGAGTTCACCCTGACCATCTCCTC CCTCCAACCGGAGGACTTCGCGGTGTACTACTGTCAGCAGTACAACGATTGGC TGCCCGTGACATTTGGACAGGGGACGAAGGTGGAAATCAAAACCACTACCCCA GCACCGAGGCCACCCACCCCGGCTCCTACCATCGCCTCCCAGCCTCTGTCCCT GCGTCCGGAGGCATGTAGACCCGCAGCTGGTGGGGCCGTGCATACCCGGGGTC TTGACTTCGCCTGCGATATCTACATTTGGGCCCCTCTGGCTGGTACTTGCGGG GTCCTGCTGCTTTCACTCGTGATCACTCTTTACTGTAAGCGCGGTCGGAAGAA GCTGCTGTACATCTTTAAGCAACCCTTCATGAGGCCTGTGCAGACTACTCAAG AGGAGGACGGCTGTTCATGCCGGTTCCCAGAGGAGGAGGAAGGCGGCTGCGAA CTGCGCGTGAAATTCAGCCGCAGCGCAGATGCTCCAGCCTACAAGCAGGGGCA GAACCAGCTCTACAACGAACTCAATCTTGGTCGGAGAGAGGAGTACGACGTGC

		TGGACAAGCGGAGAGGACGGGACCCAGAAATGGGCGGGAAGCCGCGCAGAAAG AATCCCCAAGAGGGCCTGTACAACGAGCTCCAAAAGGATAAGATGGCAGAAGC CTATAGCGAGATTGGTATGAAAGGGGAACGCAGAAGAGGCAAAGGCCACGACG GACTGTACCAGGGACTCAGCACCGCCACCAAGGACACCTATGACGCTCTTCAC ATGCAGGCCCTGCCGCCTCGG
139114		
139114- aa ScFv domain	53	EVQLVESGGGLVQPGGSLRLSCAVSGFALSNHGMSWVRRAPGKGLEWVSGIVY SGSTYYAASVKGRFTISRDNSRNTLYLQMNSLRPEDTAIYYCSAHGGESDVWG QGTTVTVSSASGGGGSGGRASGGGGSEIVLTQSPGTLSLSPGERATLSCRASQ SIGSSSLAWYQQKPGQAPRLLMYGASSRASGIPDRFSGSGSGTDFTLTISRLE PEDFAVYYCQQYAGSPPFTFGQGTKVEIK
139114- nt ScFv domain	68	GAAGTGCAATTGGTGGAATCTGGTGGAGGACTTGTGCAACCTGGAGGATCACT GAGACTGTCATGCGCGGTGTCCGGTTTTGCCCTGAGCAATCATGGGATGTCGT GGGTCCGGCGCGCCCCCGGAAAGGGTCTGGAATGGGTGTCGGGTATCGTCTAC TCCGGGAGCACTTACTACGCCGCGAGCGTGAAGGGCCGCTTCACCATTTCCCG CGATAACTCCCGCAACACCCTGTACTTGCAAATGAACTCGCTCCGGCCTGAGG ACACTGCCATCTACTACTGCTCCGCACACGGAGGAGAATCCGACGTGTGGGGC CAGGGAACTACCGTGACCGTCAGCAGCGCCTCCGGCGGCGGGGGCTCAGGCGG ACGGGCTAGCGGCGGCGGTGGCTCCGAGATCGTGCTGACCCAGTCGCCTGGCA CTCTCTCGCTGAGCCCCGGGGAAAGGGCAACCCTGTCCTGTCGGGCCAGCCAG TCCATTGGATCATCCTCCCTCGCCTGGTATCAGCAGAAACCGGGACAGGCTCC GCGGCTGCTTATGTATGGGGCCAGCTCAAGAGCCTCCGGCATTCCCGACCGGT TCTCCGGGTCCGGTTCCGGCACCGATTTCACCCTGACTATCTCGAGGCTGGAG CCAGAGGACTTCGCCGTGTACTACTGCCAGCAGTACGCGGGGTCCCCGCCGTT CACGTTCGGACAGGGAACCAAGGTCGAGATCAAG
139114- aa VH	83	EVQLVESGGGLVQPGGSLRLSCAVSGFALSNHGMSWVRRAPGKGLEWVSGIVY SGSTYYAASVKGRFTISRDNSRNTLYLQMNSLRPEDTAIYYCSAHGGESDVWG QGTTVTVSS
139114- aa VL	98	EIVLTQSPGTLSLSPGERATLSCRASQSIGSSSLAWYQQKPGQAPRLLMYGAS SRASGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQYAGSPPFTFGQGTKV EIK
139114- aa Full CAR	113	MALPVTALLLPLALLLHAARPEVQLVESGGGLVQPGGSLRLSCAVSGFALSNH GMSWVRRAPGKGLEWVSGIVYSGSTYYAASVKGRFTISRDNSRNTLYLQMNSL RPEDTAIYYCSAHGGESDVWGQGTTVTVSSASGGGGSGGRASGGGGSEIVLTQ SPGTLSLSPGERATLSCRASQSIGSSSLAWYQQKPGQAPRLLMYGASSRASGI PDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQYAGSPPFTFGQGTKVEIKTTT PAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYIWAPLAGTC GVLLLSLVITLYCKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGC ELRVKFSRSADAPAYKQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRR KNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDAL HMQALPPR
139114- nt Full CAR	128	ATGGCCCTCCCTGTCACCGCCCTGCTGCTTCCGCTGGCTCTTCTGCTCCACGC CGCTCGGCCCGAAGTGCAATTGGTGGAATCTGGTGGAGGACTTGTGCAACCTG GAGGATCACTGAGACTGTCATGCGCGGTGTCCGGTTTTGCCCTGAGCAATCAT GGGATGTCGTGGGTCCGGCGCGCCCCCGGAAAGGGTCTGGAATGGGTGTCGGG TATCGTCTACTCCGGGAGCACTTACTACGCCGCGAGCGTGAAGGGCCGCTTCA CCATTTCCCGCGATAACTCCCGCAACACCCTGTACTTGCAAATGAACTCGCTC CGGCCTGAGGACACTGCCATCTACTACTGCTCCGCACACGGAGGAGAATCCGA CGTGTGGGGCCAGGGAACTACCGTGACCGTCAGCAGCGCCTCCGGCGGCGGGG GCTCAGGCGGACGGGCTAGCGGCGGCGGTGGCTCCGAGATCGTGCTGACCCAG TCGCCTGGCACTCTCTCGCTGAGCCCCGGGGAAAGGGCAACCCTGTCCTGTCG GGCCAGCCAGTCCATTGGATCATCCTCCCTCGCCTGGTATCAGCAGAAACCGG

		GACAGGCTCCGCGGCTGCTTATGTATGGGGCCAGCTCAAGAGCCTCCGGCATT CCCGACCGGTTCTCCGGGTCCGGTTCCGGCACCGATTTCACCCTGACTATCTC GAGGCTGGAGCCAGAGGACTTCGCCGTGTACTACTGCCAGCAGTACGCGGGGT CCCCGCCGTTCACGTTCGGACAGGGAACCAAGGTCGAGATCAAGACCACTACC CCAGCACCGAGGCCACCCACCCCGGCTCCTACCATCGCCTCCCAGCCTCTGTC CCTGCGTCCGGAGGCATGTAGACCCGCAGCTGGTGGGGCCGTGCATACCCGGG GTCTTGACTTCGCCTGCGATATCTACATTTGGGCCCCTCTGGCTGGTACTTGC GGGGTCCTGCTGCTTTCACTCGTGATCACTCTTTACTGTAAGCGCGGTCGGAA GAAGCTGCTGTACATCTTTAAGCAACCCTTCATGAGGCCTGTGCAGACTACTC AAGAGGAGGACGGCTGTTCATGCCGGTTCCCAGAGGAGGAGGAAGGCGGCTGC GAACTGCGCGTGAAATTCAGCCGCAGCGCAGATGCTCCAGCCTACAAGCAGGG GCAGAACCAGCTCTACAACGAACTCAATCTTGGTCGGAGAGAGGAGTACGACG TGCTGGACAAGCGGAGAGGACGGGACCCAGAAATGGGCGGGAAGCCGCGCAGA AAGAATCCCCAAGAGGGCCTGTACAACGAGCTCCAAAAGGATAAGATGGCAGA AGCCTATAGCGAGATTGGTATGAAAGGGGAACGCAGAAGAGGCAAAGGCCACG ACGGACTGTACCAGGGACTCAGCACCGCCACCAAGGACACCTATGACGCTCTT CACATGCAGGCCCTGCCGCCTCGG
149362		
149362-aa ScFv domain	129	QVQLQESGPGLVKPSETLSLTCTVSGGSISSSYYYWGWIRQPPGKGLEWIGSI YYSGSAYYNPSLKSRVTISVDTSKNQFSLRLSSVTAADTAVYYCARHWQEWPD AFDIWGQGTMVTVSSGGGGSGGGGSGGGGSETTLTQSPAFMSATPGDKVIISC KASQDIDDAMNWYQQKPGEAPLFIIQSATSPVPGIPPRFSGSGFGTDFSLTIN NIESEDAAYYFCLQHDNFPLTFGQGTKLEIK
149362-nt ScFv domain	150	CAAGTGCAGCTTCAGGAAAGCGGACCGGGCCTGGTCAAGCCATCCGAAACTCT CTCCCTGACTTGCACTGTGTCTGGCGGTTCCATCTCATCGTCGTACTACTACT GGGGCTGGATTAGGCAGCCGCCCGGAAAGGGACTGGAGTGGATCGGAAGCATC TACTATTCCGGCTCGGCGTACTACAACCCTAGCCTCAAGTCGAGAGTGACCAT CTCCGTGGATACCTCCAAGAACCAGTTTTCCCTGCGCCTGAGCTCCGTGACCG CCGCTGACACCGCCGTGTACTACTGTGCTCGGCATTGGCAGGAATGGCCCGAT GCCTTCGACATTTGGGGCCAGGGCACTATGGTCACTGTGTCATCCGGGGGTGG AGGCAGCGGGGGAGGAGGGTCCGGGGGGGGAGGTTCAGAGACAACCTTGACCC AGTCACCCGCATTCATGTCCGCCACTCCGGGAGACAAGGTCATCATCTCGTGC AAAGCGTCCCAGGATATCGACGATGCCATGAATTGGTACCAGCAGAAGCCTGG CGAAGCGCCGCTGTTCATTATCCAATCCGCAACCTCGCCCGTGCCTGGAATCC CACCGCGGTTCAGCGGCAGCGGTTTCGGAACCGACTTTTCCCTGACCATTAAC AACATTGAGTCCGAGGACGCCGCCTACTACTTCTGCCTGCAACACGACAACTT CCCTCTCACGTTCGGCCAGGGAACCAAGCTGGAAATCAAG
149362-aa VH	171	QVQLQESGPGLVKPSETLSLTCTVSGGSISSSYYYWGWIRQPPGKGLEWIGSI YYSGSAYYNPSLKSRVTISVDTSKNQFSLRLSSVTAADTAVYYCARHWQEWPD AFDIWGQGTMVTVSS
149362-aa VL	192	ETTLTQSPAFMSATPGDKVIISCKASQDIDDAMNWYQQKPGEAPLFIIQSATS PVPGIPPRFSGSGFGTDFSLTINNIESEDAAYYFCLQHDNFPLTFGQGTKLEI K
149362-aa Full CAR	213	MALPVTALLLPLALLLHAARPQVQLQESGPGLVKPSETLSLTCTVSGGSISSS YYYWGWIRQPPGKGLEWIGSIYYSGSAYYNPSLKSRVTISVDTSKNQFSLRLS SVTAADTAVYYCARHWQEWPDAFDIWGQGTMVTVSSGGGGSGGGGSGGGGSET TLTQSPAFMSATPGDKVIISCKASQDIDDAMNWYQQKPGEAPLFIIQSATSPV PGIPPRFSGSGFGTDFSLTINNIESEDAAYYFCLQHDNFPLTFGQGTKLEIKT TTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYIWAPLAG TCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEG GCELRVKFSRSADAPAYKQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKP RRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYD

		ALHMQALPPR
149362-nt Full CAR	234	ATGGCCCTCCCTGTCACCGCCCTGCTGCTTCCGCTGGCTCTTCTGCTCCACGC CGCTCGGCCCCAAGTGCAGCTTCAGGAAAGCGGACCGGGCCTGGTCAAGCCAT CCGAAACTCTCTCCCTGACTTGCACTGTGTCTGGCGGTTCCATCTCATCGTCG TACTACTACTGGGGCTGGATTAGGCAGCCGCCCGGAAAGGGACTGGAGTGGAT CGGAAGCATCTACTATTCCGGCTCGGCGTACTACAACCCTAGCCTCAAGTCGA GAGTGACCATCTCCGTGGATACCTCCAAGAACCAGTTTTCCCTGCGCCTGAGC TCCGTGACCGCCGCTGACACCGCCGTGTACTACTGTGCTCGGCATTGGCAGGA ATGGCCCGATGCCTTCGACATTTGGGGCCAGGGCACTATGGTCACTGTGTCAT CCGGGGGTGGAGGCAGCGGGGGAGGAGGGTCCGGGGGGGGAGGTTCAGAGACA ACCTTGACCCAGTCACCCGCATTCATGTCCGCCACTCCGGGAGACAAGGTCAT CATCTCGTGCAAAGCGTCCCAGGATATCGACGATGCCATGAATTGGTACCAGC AGAAGCCTGGCGAAGCGCCGCTGTTCATTATCCAATCCGCAACCTCGCCCGTG CCTGGAATCCCACCGCGGTTCAGCGGCAGCGGTTTCGGAACCGACTTTTCCCT GACCATTAACAACATTGAGTCCGAGGACGCCGCCTACTACTTCTGCCTGCAAC ACGACAACTTCCCTCTCACGTTCGGCCAGGGAACCAAGCTGGAAATCAAGACC ACTACCCCAGCACCGAGGCCACCCACCCCGGCTCCTACCATCGCCTCCCAGCC TCTGTCCCTGCGTCCGGAGGCATGTAGACCCGCAGCTGGTGGGGCCGTGCATA CCCGGGGTCTTGACTTCGCCTGCGATATCTACATTTGGGCCCCTCTGGCTGGT ACTTGCGGGGTCCTGCTGCTTTCACTCGTGATCACTCTTTACTGTAAGCGCGG TCGGAAGAAGCTGCTGTACATCTTTAAGCAACCCTTCATGAGGCCTGTGCAGA CTACTCAAGAGGAGGACGGCTGTTCATGCCGGTTCCCAGAGGAGGAGGAAGGC GGCTGCGAACTGCGCGTGAAATTCAGCCGCAGCGCAGATGCTCCAGCCTACAA GCAGGGGCAGAACCAGCTCTACAACGAACTCAATCTTGGTCGGAGAGAGGAGT ACGACGTGCTGGACAAGCGGAGAGGACGGGACCCAGAAATGGGCGGGAAGCCG CGCAGAAAGAATCCCCAAGAGGGCCTGTACAACGAGCTCCAAAAGGATAAGAT GGCAGAAGCCTATAGCGAGATTGGTATGAAAGGGGAACGCAGAAGAGGCAAAG GCCACGACGGACTGTACCAGGGACTCAGCACCGCCACCAAGGACACCTATGAC GCTCTTCACATGCAGGCCCTGCCGCCTCGG
149363		
149363-aa ScFv domain	130	VNLRESGPALVKPTQTLTLTCTFSGFSLRTSGMCVSWIRQPPGKALEWLARID WDEDKFYSTSLKTRLTISKDTSDNQVVLRMTNMDPADTATYYCARSGAGGTSA TAFDIWGPGTMVTVSSGGGGSGGGGSGGGGSDIQMTQSPSSLSASVGDRVTIT CRASQDIYNNLAWFQLKPGSAPRSLMYAANKSQSGVPSRFSGSASGTDFTLTI SSLQPEDFATYYCQHYYRFPYSFGQGTKLEIK
149363-nt ScFv domain	151	CAAGTCAATCTGCGCGAATCCGGCCCCGCCTTGGTCAAGCCTACCCAGACCCT CACTCTGACCTGTACTTTCTCCGGCTTCTCCCTGCGGACTTCCGGGATGTGCG TGTCCTGGATCAGACAGCCTCCGGGAAAGGCCCTGGAGTGGCTCGCTCGCATT GACTGGGATGAGGACAAGTTCTACTCCACCTCACTCAAGACCAGGCTGACCAT CAGCAAAGATACCTCTGACAACCAAGTGGTGCTCCGCATGACCAACATGGACC CAGCCGACACTGCCACTTACTACTGCGCGAGGAGCGGAGCGGGCGGAACCTCC GCCACCGCCTTCGATATTTGGGGCCCGGGTACCATGGTCACCGTGTCAAGCGG AGGAGGGGGGTCCGGGGGCGGCGGTTCCGGGGGAGGCGGATCGGACATTCAGA TGACTCAGTCACCATCGTCCCTGAGCGCTAGCGTGGGCGACAGAGTGACAATC ACTTGCCGGGCATCCCAGGACATCTATAACAACCTTGCGTGGTTCCAGCTGAA GCCTGGTTCCGCACCGCGGTCACTTATGTACGCCGCCAACAAGAGCCAGTCGG GAGTGCCGTCCCGGTTTTCCGGTTCGGCCTCGGGAACTGACTTCACCCTGACG ATCTCCAGCCTGCAACCCGAGGATTTCGCCACCTACTACTGCCAGCACTACTA CCGCTTTCCCTACTCGTTCGGACAGGGAACCAAGCTGGAAATCAAG
149363-aa VH	172	QVNLRESGPALVKPTQTLTLTCTFSGFSLRTSGMCVSWIRQPPGKALEWLARI DWDEDKFYSTSLKTRLTISKDTSDNQVVLRMTNMDPADTATYYCARSGAGGTS ATAFDIWGPGTMVTVSS

149363-aa VL	193	DIQMTQSPSSLSASVGDRVTITCRASQDIYNNLAWFQLKPGSAPRSLMYAANK SQSGVPSRFSGSASGTDFTLTISSLQPEDFATYYCQHYYRFPYSFGQGTKLEI K
149363-aa Full CAR	214	MALPVTALLLPLALLLHAARPQVNLRESGPALVKPTQTLTLTCTFSGFSLRTS GMCVSWIRQPPGKALEWLARIDWDEDKFYSTSLKTRLTISKDTSDNQVVLRMT NMDPADTATYYCARSGAGGTSATAFDIWGPGTMVTVSSGGGGSGGGGSGGGGS DIQMTQSPSSLSASVGDRVTITCRASQDIYNNLAWFQLKPGSAPRSLMYAANK SQSGVPSRFSGSASGTDFTLTISSLQPEDFATYYCQHYYRFPYSFGQGTKLEI KTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYIWAPL AGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEE EGGCELRVKFSRSADAPAYKQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGG KPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDT YDALHMQALPPR
149363-nt Full CAR	235	ATGGCCCTCCCTGTCACCGCCCTGCTGCTTCCGCTGGCTCTTCTGCTCCACGC CGCTCGGCCCCAAGTCAATCTGCGCGAATCCGGCCCCGCCTTGGTCAAGCCTA CCCAGACCCTCACTCTGACCTGTACTTTCTCCGGCTTCTCCCTGCGGACTTCC GGGATGTGCGTGTCCTGGATCAGACAGCCTCCGGGAAAGGCCCTGGAGTGGCT CGCTCGCATTGACTGGGATGAGGACAAGTTCTACTCCACCTCACTCAAGACCA GGCTGACCATCAGCAAAGATACCTCTGACAACCAAGTGGTGCTCCGCATGACC AACATGGACCCAGCCGACACTGCCACTTACTACTGCGCGAGGAGCGGAGCGGG CGGAACCTCCGCCACCGCCTTCGATATTTGGGGCCCGGGTACCATGGTCACCG TGTCAAGCGGAGGAGGGGGGTCCGGGGGCGGCGGTTCCGGGGGAGGCGGATCG GACATTCAGATGACTCAGTCACCATCGTCCCTGAGCGCTAGCGTGGGCGACAG AGTGACAATCACTTGCCGGGCATCCCAGGACATCTATAACAACCTTGCGTGGT TCCAGCTGAAGCCTGGTTCCGCACCGCGGTCACTTATGTACGCCGCCAACAAG AGCCAGTCGGGAGTGCCGTCCCGGTTTTCCGGTTCGGCCTCGGGAACTGACTT CACCCTGACGATCTCCAGCCTGCAACCCGAGGATTTCGCCACCTACTACTGCC AGCACTACTACCGCTTTCCCTACTCGTTCGGACAGGGAACCAAGCTGGAAATC AAGACCACTACCCCAGCACCGAGGCCACCCACCCCGGCTCCTACCATCGCCTC CCAGCCTCTGTCCCTGCGTCCGGAGGCATGTAGACCCGCAGCTGGTGGGGCCG TGCATACCCGGGGTCTTGACTTCGCCTGCGATATCTACATTTGGGCCCCTCTG GCTGGTACTTGCGGGGTCCTGCTGCTTTCACTCGTGATCACTCTTTACTGTAA GCGCGGTCGGAAGAAGCTGCTGTACATCTTTAAGCAACCCTTCATGAGGCCTG TGCAGACTACTCAAGAGGAGGACGGCTGTTCATGCCGGTTCCCAGAGGAGGAG GAAGGCGGCTGCGAACTGCGCGTGAAATTCAGCCGCAGCGCAGATGCTCCAGC CTACAAGCAGGGGCAGAACCAGCTCTACAACGAACTCAATCTTGGTCGGAGAG AGGAGTACGACGTGCTGGACAAGCGGAGAGGACGGGACCCAGAAATGGGCGGG AAGCCGCGCAGAAAGAATCCCCAAGAGGGCCTGTACAACGAGCTCCAAAAGGA TAAGATGGCAGAAGCCTATAGCGAGATTGGTATGAAAGGGGAACGCAGAAGAG GCAAAGGCCACGACGGACTGTACCAGGGACTCAGCACCGCCACCAAGGACACC TATGACGCTCTTCACATGCAGGCCCTGCCGCCTCGG
149364		
149364-aa ScFv domain	131	EVQLVESGGGLVKPGGSLRLSCAASGFTFSSYSMNWVRQAPGKGLEWVSSISS SSSYIYYADSVKGRFTISRDNAKNSLYLQMNSLRAEDTAVYYCAKTIAAVYAF DIWGQGTTVTVSSGGGGSGGGGSGGGGSEIVLTQSPLSLPVTPEEPASISCRS SQSLLHSNGYNYLDWYLQKPGQSPQLLIYLGSNRASGVPDRFSGSGSGTDFTL KISRVEAEDVGVYYCMQALQTPYTFGQGTKLEIK
149364-nt ScFv domain	152	GAAGTGCAGCTTGTCGAATCCGGGGGGGGACTGGTCAAGCCGGGCGGATCACT GAGACTGTCCTGCGCCGCGAGCGGCTTCACGTTCTCCTCCTACTCCATGAACT GGGTCCGCCAAGCCCCCGGGAAGGGACTGGAATGGGTGTCCTCTATCTCCTCG TCGTCGTCCTACATCTACTACGCCGACTCCGTGAAGGGAAGATTCACCATTTC CCGCGACAACGCAAAGAACTCACTGTACTTGCAAATGAACTCACTCCGGGCCG

		AAGATACTGCTGTGTACTATTGCGCCAAGACTATTGCCGCCGTCTACGCTTTC GACATCTGGGGCCAGGGAACCACCGTGACTGTGTCGTCCGGTGGTGGTGGCTC GGGCGGAGGAGGAAGCGGCGGCGGGGGGTCCGAGATTGTGCTGACCCAGTCGC CACTGAGCCTCCCTGTGACCCCCGAGGAACCCGCCAGCATCAGCTGCCGGTCC AGCCAGTCCCTGCTCCACTCCAACGGATACAATTACCTCGATTGGTACCTTCA GAAGCCTGGACAAAGCCCGCAGCTGCTCATCTACTTGGGATCAAACCGCGCGT CAGGAGTGCCTGACCGGTTCTCCGGCTCGGGCAGCGGTACCGATTTCACCCTG AAAATCTCCAGGGTGGAGGCAGAGGACGTGGGAGTGTATTACTGTATGCAGGC GCTGCAGACTCCGTACACATTTGGGCAGGGCACCAAGCTGGAGATCAAG
149364-aa VH	173	EVQLVESGGGLVKPGGSLRLSCAASGFTFSSYSMNWVRQAPGKGLEWVSSISS SSSYIYYADSVKGRFTISRDNAKNSLYLQMNSLRAEDTAVYYCAKTIAAVYAF DIWGQGTTVTVSS
149364-aa VL	194	EIVLTQSPLSLPVTPEEPASISCRSSQSLLHSNGYNYLDWYLQKPGQSPQLLI YLGSNRASGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCMQALQTPYTFGQG TKLEIK
149364-aa Full CAR	215	MALPVTALLLPLALLLHAARPEVQLVESGGGLVKPGGSLRLSCAASGFTFSSY SMNWVRQAPGKGLEWVSSISSSSSYIYYADSVKGRFTISRDNAKNSLYLQMNS LRAEDTAVYYCAKTIAAVYAFDIWGQGTTVTVSSGGGGSGGGGSGGGGSEIVL TQSPLSLPVTPEEPASISCRSSQSLLHSNGYNYLDWYLQKPGQSPQLLIYLGS NRASGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCMQALQTPYTFGQGTKLE IKTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYIWAP LAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEE EEGGCELRVKFSRSADAPAYKQGQNQLYNELNLGRREEYDVLDKRRGRDPEMG GKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKD TYDALHMQALPPR
149364-nt Full CAR	236	ATGGCCCTCCCTGTCACCGCCCTGCTGCTTCCGCTGGCTCTTCTGCTCCACGC CGCTCGGCCCGAAGTGCAGCTTGTCGAATCCGGGGGGGGACTGGTCAAGCCGG GCGGATCACTGAGACTGTCCTGCGCCGCGAGCGGCTTCACGTTCTCCTCCTAC TCCATGAACTGGGTCCGCCAAGCCCCCGGGAAGGGACTGGAATGGGTGTCCTC TATCTCCTCGTCGTCGTCCTACATCTACTACGCCGACTCCGTGAAGGGAAGAT TCACCATTTCCCGCGACAACGCAAAGAACTCACTGTACTTGCAAATGAACTCA CTCCGGGCCGAAGATACTGCTGTGTACTATTGCGCCAAGACTATTGCCGCCGT CTACGCTTTCGACATCTGGGGCCAGGGAACCACCGTGACTGTGTCGTCCGGTG GTGGTGGCTCGGGCGGAGGAGGAAGCGGCGGCGGGGGGTCCGAGATTGTGCTG ACCCAGTCGCCACTGAGCCTCCCTGTGACCCCCGAGGAACCCGCCAGCATCAG CTGCCGGTCCAGCCAGTCCCTGCTCCACTCCAACGGATACAATTACCTCGATT GGTACCTTCAGAAGCCTGGACAAAGCCCGCAGCTGCTCATCTACTTGGGATCA AACCGCGCGTCAGGAGTGCCTGACCGGTTCTCCGGCTCGGGCAGCGGTACCGA TTTCACCCTGAAAATCTCCAGGGTGGAGGCAGAGGACGTGGGAGTGTATTACT GTATGCAGGCGCTGCAGACTCCGTACACATTTGGGCAGGGCACCAAGCTGGAG ATCAAGACCACTACCCCAGCACCGAGGCCACCCACCCCGGCTCCTACCATCGC CTCCCAGCCTCTGTCCCTGCGTCCGGAGGCATGTAGACCCGCAGCTGGTGGGG CCGTGCATACCCGGGGTCTTGACTTCGCCTGCGATATCTACATTTGGGCCCCT CTGGCTGGTACTTGCGGGGTCCTGCTGCTTTCACTCGTGATCACTCTTTACTG TAAGCGCGGTCGGAAGAAGCTGCTGTACATCTTTAAGCAACCCTTCATGAGGC CTGTGCAGACTACTCAAGAGGAGGACGGCTGTTCATGCCGGTTCCCAGAGGAG GAGGAAGGCGGCTGCGAACTGCGCGTGAAATTCAGCCGCAGCGCAGATGCTCC AGCCTACAAGCAGGGGCAGAACCAGCTCTACAACGAACTCAATCTTGGTCGGA GAGAGGAGTACGACGTGCTGGACAAGCGGAGAGGACGGGACCCAGAAATGGGC GGGAAGCCGCGCAGAAAGAATCCCCAAGAGGGCCTGTACAACGAGCTCCAAAA GGATAAGATGGCAGAAGCCTATAGCGAGATTGGTATGAAAGGGGAACGCAGAA GAGGCAAAGGCCACGACGGACTGTACCAGGGACTCAGCACCGCCACCAAGGAC

		ACCTATGACGCTCTTCACATGCAGGCCCTGCCGCCTCGG
149365		
149365-aa ScFv domain	132	EVQLVESGGGLVKPGGSLRLSCAASGFTFSDYYMSWIRQAPGKGLEWVSYISS SGSTIYYADSVKGRFTISRDNAKNSLYLQMNSLRAEDTAVYYCARDLRGAFDI WGQGTMVTVSSGGGGSGGGGSGGGGSSYVLTQSPSVSAAPGYTATISCGGNNI GTKSVHWYQQKPGQAPLLVIRDDSVRPSKIPGRFSGSNSGNMATLTISGVQAG DEADFYCQVWDSDSEHVVFGGGTKLTVL
149365-nt ScFv domain	153	GAAGTCCAGCTCGTGGAGTCCGGCGGAGGCCTTGTGAAGCCTGGAGGTTCGCT GAGACTGTCCTGCGCCGCCTCCGGCTTCACCTTCTCCGACTACTACATGTCCT GGATCAGACAGGCCCCGGGAAAGGGCCTGGAATGGGTGTCCTACATCTCGTCA TCGGGCAGCACTATCTACTACGCGGACTCAGTGAAGGGGCGGTTCACCATTTC CCGGGATAACGCGAAGAACTCGCTGTATCTGCAAATGAACTCACTGAGGGCCG AGGACACCGCCGTGTACTACTGCGCCCGCGATCTCCGCGGGGCATTTGACATC TGGGGACAGGGAACCATGGTCACAGTGTCCAGCGGAGGGGGAGGATCGGGTGG CGGAGGTTCCGGGGGTGGAGGCTCCTCCTACGTGCTGACTCAGAGCCCAAGCG TCAGCGCTGCGCCCGGTTACACGGCAACCATCTCCTGTGGCGGAAACAACATT GGGACCAAGTCTGTGCACTGGTATCAGCAGAAGCCGGGCCAAGCTCCCCTGTT GGTGATCCGCGATGACTCCGTGCGGCCTAGCAAAATTCCGGGACGGTTCTCCG GCTCCAACAGCGGCAATATGGCCACTCTCACCATCTCGGGAGTGCAGGCCGGA GATGAAGCCGACTTCTACTGCCAAGTCTGGGACTCAGACTCCGAGCATGTGGT GTTCGGGGGCGGAACCAAGCTGACTGTGCTC
149365-aa VH	174	EVQLVESGGGLVKPGGSLRLSCAASGFTFSDYYMSWIRQAPGKGLEWVSYISS SGSTIYYADSVKGRFTISRDNAKNSLYLQMNSLRAEDTAVYYCARDLRGAFDI WGQGTMVTVSS
149365-aa VL	195	SYVLTQSPSVSAAPGYTATISCGGNNIGTKSVHWYQQKPGQAPLLVIRDDSVR PSKIPGRFSGSNSGNMATLTISGVQAGDEADFYCQVWDSDSEHVVFGGGTKLT VL
149365-aa Full CAR	216	MALPVTALLLPLALLLHAARPEVQLVESGGGLVKPGGSLRLSCAASGFTFSDY YMSWIRQAPGKGLEWVSYISSSGSTIYYADSVKGRFTISRDNAKNSLYLQMNS LRAEDTAVYYCARDLRGAFDIWGQGTMVTVSSGGGGSGGGGSGGGGSSYVLTQ SPSVSAAPGYTATISCGGNNIGTKSVHWYQQKPGQAPLLVIRDDSVRPSKIPG RFSGSNSGNMATLTISGVQAGDEADFYCQVWDSDSEHVVFGGGTKLTVLTTTP APRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYIWAPLAGTCG VLLLSLVITLYCKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCE LRVKFSRSADAPAYKQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRK NPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALH MQALPPR
149365-nt Full CAR	237	ATGGCCCTCCCTGTCACCGCCCTGCTGCTTCCGCTGGCTCTTCTGCTCCACGC CGCTCGGCCCGAAGTCCAGCTCGTGGAGTCCGGCGGAGGCCTTGTGAAGCCTG GAGGTTCGCTGAGACTGTCCTGCGCCGCCTCCGGCTTCACCTTCTCCGACTAC TACATGTCCTGGATCAGACAGGCCCCGGGAAAGGGCCTGGAATGGGTGTCCTA CATCTCGTCATCGGGCAGCACTATCTACTACGCGGACTCAGTGAAGGGGCGGT TCACCATTTCCCGGGATAACGCGAAGAACTCGCTGTATCTGCAAATGAACTCA CTGAGGGCCGAGGACACCGCCGTGTACTACTGCGCCCGCGATCTCCGCGGGGC ATTTGACATCTGGGGACAGGGAACCATGGTCACAGTGTCCAGCGGAGGGGGAG GATCGGGTGGCGGAGGTTCCGGGGGTGGAGGCTCCTCCTACGTGCTGACTCAG AGCCCAAGCGTCAGCGCTGCGCCCGGTTACACGGCAACCATCTCCTGTGGCGG AAACAACATTGGGACCAAGTCTGTGCACTGGTATCAGCAGAAGCCGGGCCAAG CTCCCCTGTTGGTGATCCGCGATGACTCCGTGCGGCCTAGCAAAATTCCGGGA CGGTTCTCCGGCTCCAACAGCGGCAATATGGCCACTCTCACCATCTCGGGAGT GCAGGCCGGAGATGAAGCCGACTTCTACTGCCAAGTCTGGGACTCAGACTCCG AGCATGTGGTGTTCGGGGGCGGAACCAAGCTGACTGTGCTCACCACTACCCCA

		GCACCGAGGCCACCCACCCCGGCTCCTACCATCGCCTCCCAGCCTCTGTCCCT GCGTCCGGAGGCATGTAGACCCGCAGCTGGTGGGGCCGTGCATACCCGGGGTC TTGACTTCGCCTGCGATATCTACATTTGGGCCCCTCTGGCTGGTACTTGCGGG GTCCTGCTGCTTTCACTCGTGATCACTCTTTACTGTAAGCGCGGTCGGAAGAA GCTGCTGTACATCTTTAAGCAACCCTTCATGAGGCCTGTGCAGACTACTCAAG AGGAGGACGGCTGTTCATGCCGGTTCCCAGAGGAGGAGGAAGGCGGCTGCGAA CTGCGCGTGAAATTCAGCCGCAGCGCAGATGCTCCAGCCTACAAGCAGGGGCA GAACCAGCTCTACAACGAACTCAATCTTGGTCGGAGAGAGGAGTACGACGTGC TGGACAAGCGGAGAGGACGGGACCCAGAAATGGGCGGGAAGCCGCGCAGAAAG AATCCCCAAGAGGGCCTGTACAACGAGCTCCAAAAGGATAAGATGGCAGAAGC CTATAGCGAGATTGGTATGAAAGGGGAACGCAGAAGAGGCAAAGGCCACGACG GACTGTACCAGGGACTCAGCACCGCCACCAAGGACACCTATGACGCTCTTCAC ATGCAGGCCCTGCCGCCTCGG
149366		
149366-aa ScFv domain	133	QVQLVQSGAEVKKPGASVKVSCKPSGYTVTSHYIHWVRRAPGQGLEWMGMINP SGGVTAYSQTLQGRVTMTSDTSSSTVYMELSSLRSEDTAMYYCAREGSGSGWY FDFWGRGTLVTVSSGGGGSGGGGSGGGGSSYVLTQPPSVSVSPGQTASITCSG DGLSKKYVSWYQQKAGQSPVVLISRDKERPSGIPDRFSGSNSADTATLTISGT QAMDEADYYCQAWDDTTVVFGGGTKLTVL
149366-nt ScFv domain	154	CAAGTGCAGCTGGTGCAGAGCGGGGCCGAAGTCAAGAAGCCGGGAGCCTCCGT GAAAGTGTCCTGCAAGCCTTCGGGATACACCGTGACCTCCCACTACATTCATT GGGTCCGCCGCGCCCCCGGCCAAGGACTCGAGTGGATGGGCATGATCAACCCT AGCGGCGGAGTGACCGCGTACAGCCAGACGCTGCAGGGACGCGTGACTATGAC CTCGGATACCTCCTCCTCCACCGTCTATATGGAACTGTCCAGCCTGCGGTCCG AGGATACCGCCATGTACTACTGCGCCCGGGAAGGATCAGGCTCCGGGTGGTAT TTCGACTTCTGGGGAAGAGGCACCCTCGTGACTGTGTCATCTGGGGGAGGGGG TTCCGGTGGTGGCGGATCGGGAGGAGGCGGTTCATCCTACGTGCTGACCCAGC CACCCTCCGTGTCCGTGAGCCCCGGCCAGACTGCATCGATTACATGTAGCGGC GACGGCCTCTCCAAGAAATACGTGTCGTGGTACCAGCAGAAGGCCGGACAGAG CCCGGTGGTGCTGATCTCAAGAGATAAGGAGCGGCCTAGCGGAATCCCGGACA GGTTCTCGGGTTCCAACTCCGCGGACACTGCTACTCTGACCATCTCGGGGACC CAGGCTATGGACGAAGCCGATTACTACTGCCAAGCCTGGGACGACACTACTGT CGTGTTTGGAGGGGGCACCAAGTTGACCGTCCTT
149366-aa VH	175	QVQLVQSGAEVKKPGASVKVSCKPSGYTVTSHYIHWVRRAPGQGLEWMGMINP SGGVTAYSQTLQGRVTMTSDTSSSTVYMELSSLRSEDTAMYYCAREGSGSGWY FDFWGRGTLVTVSS
149366-aa VL	196	SYVLTQPPSVSVSPGQTASITCSGDGLSKKYVSWYQQKAGQSPVVLISRDKER PSGIPDRFSGSNSADTATLTISGTQAMDEADYYCQAWDDTTVVFGGGTKLTVL
149366-aa Full CAR	217	MALPVTALLLPLALLLHAARPQVQLVQSGAEVKKPGASVKVSCKPSGYTVTSH YIHWVRRAPGQGLEWMGMINPSGGVTAYSQTLQGRVTMTSDTSSSTVYMELSS LRSEDTAMYYCAREGSGSGWYFDFWGRGTLVTVSSGGGGSGGGGSGGGGSSYV LTQPPSVSVSPGQTASITCSGDGLSKKYVSWYQQKAGQSPVVLISRDKERPSG IPDRFSGSNSADTATLTISGTQAMDEADYYCQAWDDTTVVFGGGTKLTVLTTT PAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYIWAPLAGTC GVLLLSLVITLYCKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGC ELRVKFSRSADAPAYKQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRR KNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDAL HMQALPPR
149366-nt Full CAR	238	ATGGCCCTCCCTGTCACCGCCCTGCTGCTTCCGCTGGCTCTTCTGCTCCACGC CGCTCGGCCCCAAGTGCAGCTGGTGCAGAGCGGGGCCGAAGTCAAGAAGCCGG GAGCCTCCGTGAAAGTGTCCTGCAAGCCTTCGGGATACACCGTGACCTCCCAC TACATTCATTGGGTCCGCCGCGCCCCCGGCCAAGGACTCGAGTGGATGGGCAT

		GATCAACCCTAGCGGCGGAGTGACCGCGTACAGCCAGACGCTGCAGGGACGCG TGACTATGACCTCGGATACCTCCTCCTCCACCGTCTATATGGAACTGTCCAGC CTGCGGTCCGAGGATACCGCCATGTACTACTGCGCCCGGGAAGGATCAGGCTC CGGGTGGTATTTCGACTTCTGGGGAAGAGGCACCCTCGTGACTGTGTCATCTG GGGGAGGGGGTTCCGGTGGTGGCGGATCGGGAGGAGGCGGTTCATCCTACGTG CTGACCCAGCCACCCTCCGTGTCCGTGAGCCCCGGCCAGACTGCATCGATTAC ATGTAGCGGCGACGGCCTCTCCAAGAAATACGTGTCGTGGTACCAGCAGAAGG CCGGACAGAGCCCGGTGGTGCTGATCTCAAGAGATAAGGAGCGGCCTAGCGGA ATCCCGGACAGGTTCTCGGGTTCCAACTCCGCGGACACTGCTACTCTGACCAT CTCGGGGACCCAGGCTATGGACGAAGCCGATTACTACTGCCAAGCCTGGGACG ACACTACTGTCGTGTTTGGAGGGGGCACCAAGTTGACCGTCCTTACCACTACC CCAGCACCGAGGCCACCCACCCCGGCTCCTACCATCGCCTCCCAGCCTCTGTC CCTGCGTCCGGAGGCATGTAGACCCGCAGCTGGTGGGGCCGTGCATACCCGGG GTCTTGACTTCGCCTGCGATATCTACATTTGGGCCCCTCTGGCTGGTACTTGC GGGGTCCTGCTGCTTTCACTCGTGATCACTCTTTACTGTAAGCGCGGTCGGAA GAAGCTGCTGTACATCTTTAAGCAACCCTTCATGAGGCCTGTGCAGACTACTC AAGAGGAGGACGGCTGTTCATGCCGGTTCCCAGAGGAGGAGGAAGGCGGCTGC GAACTGCGCGTGAAATTCAGCCGCAGCGCAGATGCTCCAGCCTACAAGCAGGG GCAGAACCAGCTCTACAACGAACTCAATCTTGGTCGGAGAGAGGAGTACGACG TGCTGGACAAGCGGAGAGGACGGGACCCAGAAATGGGCGGGAAGCCGCGCAGA AAGAATCCCCAAGAGGGCCTGTACAACGAGCTCCAAAAGGATAAGATGGCAGA AGCCTATAGCGAGATTGGTATGAAAGGGGAACGCAGAAGAGGCAAAGGCCACG ACGGACTGTACCAGGGACTCAGCACCGCCACCAAGGACACCTATGACGCTCTT CACATGCAGGCCCTGCCGCCTCGG
149367		
149367-aa ScFv domain	134	QVQLQESGPGLVKPSQTLSLTCTVSGGSISSGGYYWSWIRQHPGKGLEWIGYI YYSGSTYYNPSLKSRVTISVDTSKNQFSLKLSSVTAADTAVYYCARAGIAARL RGAFDIWGQGTMVTVSSGGGGSGGGGSGGGGSDIVMTQSPSSVSASVGDRVII TCRASQGIRNWLAWYQQKPGKAPNLLIYAASNLQSGVPSRFSGSGSGADFTLT ISSLQPEDVATYYCQKYNSAPFTFGPGTKVDIK
149367-nt ScFv domain	155	CAAGTGCAGCTTCAGGAGAGCGGCCCGGGACTCGTGAAGCCGTCCCAGACCCT GTCCCTGACTTGCACCGTGTCGGGAGGAAGCATCTCGAGCGGAGGCTACTATT GGTCGTGGATTCGGCAGCACCCTGGAAAGGGCCTGGAATGGATCGGCTACATC TACTACTCCGGCTCGACCTACTACAACCCATCGCTGAAGTCCAGAGTGACAAT CTCAGTGGACACGTCCAAGAATCAGTTCAGCCTGAAGCTCTCTTCCGTGACTG CGGCCGACACCGCCGTGTACTACTGCGCACGCGCTGGAATTGCCGCCCGGCTG AGGGGTGCCTTCGACATTTGGGGACAGGGCACCATGGTCACCGTGTCCTCCGG CGGCGGAGGTTCCGGGGGTGGAGGCTCAGGAGGAGGGGGGTCCGACATCGTCA TGACTCAGTCGCCCTCAAGCGTCAGCGCGTCCGTCGGGGACAGAGTGATCATC ACCTGTCGGGCGTCCCAGGGAATTCGCAACTGGCTGGCCTGGTATCAGCAGAA GCCCGGAAAGGCCCCCAACCTGTTGATCTACGCCGCCTCAAACCTCCAATCCG GGGTGCCGAGCCGCTTCAGCGGCTCCGGTTCGGGTGCCGATTTCACTCTGACC ATCTCCTCCCTGCAACCTGAAGATGTGGCTACCTACTACTGCCAAAAGTACAA CTCCGCACCTTTTACTTTCGGACCGGGGACCAAAGTGGACATTAAG
149367-aa VH	176	QVQLQESGPGLVKPSQTLSLTCTVSGGSISSGGYYWSWIRQHPGKGLEWIGYI YYSGSTYYNPSLKSRVTISVDTSKNQFSLKLSSVTAADTAVYYCARAGIAARL RGAFDIWGQGTMVTVSS
149367-aa VL	197	DIVMTQSPSSVSASVGDRVIITCRASQGIRNWLAWYQQKPGKAPNLLIYAASN LQSGVPSRFSGSGSGADFTLTISSLQPEDVATYYCQKYNSAPFTFGPGTKVDI K
149367-aa Full CAR	218	MALPVTALLLPLALLLHAARPQVQLQESGPGLVKPSQTLSLTCTVSGGSISSG GYYWSWIRQHPGKGLEWIGYIYYSGSTYYNPSLKSRVTISVDTSKNQFSLKLS

		SVTAADTAVYYCARAGIAARLRGAFDIWGQGTMVTVSSGGGGSGGGGSGGGGS DIVMTQSPSSVSASVGDRVIITCRASQGIRNWLAWYQQKPGKAPNLLIYAASN LQSGVPSRFSGSGSGADFTLTISSLQPEDVATYYCQKYNSAPFTFGPGTKVDI KTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYIWAPL AGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEE EGGCELRVKFSRSADAPAYKQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGG KPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDT YDALHMQALPPR
149367-nt Full CAR	239	ATGGCCCTCCCTGTCACCGCCCTGCTGCTTCCGCTGGCTCTTCTGCTCCACGC CGCTCGGCCCCAAGTGCAGCTTCAGGAGAGCGGCCCGGGACTCGTGAAGCCGT CCCAGACCCTGTCCCTGACTTGCACCGTGTCGGGAGGAAGCATCTCGAGCGGA GGCTACTATTGGTCGTGGATTCGGCAGCACCCTGGAAAGGGCCTGGAATGGAT CGGCTACATCTACTACTCCGGCTCGACCTACTACAACCCATCGCTGAAGTCCA GAGTGACAATCTCAGTGGACACGTCCAAGAATCAGTTCAGCCTGAAGCTCTCT TCCGTGACTGCGGCCGACACCGCCGTGTACTACTGCGCACGCGCTGGAATTGC CGCCCGGCTGAGGGGTGCCTTCGACATTTGGGGACAGGGCACCATGGTCACCG TGTCCTCCGGCGGCGGAGGTTCCGGGGGTGGAGGCTCAGGAGGAGGGGGGTCC GACATCGTCATGACTCAGTCGCCCTCAAGCGTCAGCGCGTCCGTCGGGGACAG AGTGATCATCACCTGTCGGGCGTCCCAGGGAATTCGCAACTGGCTGGCCTGGT ATCAGCAGAAGCCCGGAAAGGCCCCCAACCTGTTGATCTACGCCGCCTCAAAC CTCCAATCCGGGGTGCCGAGCCGCTTCAGCGGCTCCGGTTCGGGTGCCGATTT CACTCTGACCATCTCCTCCCTGCAACCTGAAGATGTGGCTACCTACTACTGCC AAAAGTACAACTCCGCACCTTTTACTTTCGGACCGGGGACCAAAGTGGACATT AAGACCACTACCCCAGCACCGAGGCCACCCACCCCGGCTCCTACCATCGCCTC CCAGCCTCTGTCCCTGCGTCCGGAGGCATGTAGACCCGCAGCTGGTGGGGCCG TGCATACCCGGGGTCTTGACTTCGCCTGCGATATCTACATTTGGGCCCCTCTG GCTGGTACTTGCGGGGTCCTGCTGCTTTCACTCGTGATCACTCTTTACTGTAA GCGCGGTCGGAAGAAGCTGCTGTACATCTTTAAGCAACCCTTCATGAGGCCTG TGCAGACTACTCAAGAGGAGGACGGCTGTTCATGCCGGTTCCCAGAGGAGGAG GAAGGCGGCTGCGAACTGCGCGTGAAATTCAGCCGCAGCGCAGATGCTCCAGC CTACAAGCAGGGGCAGAACCAGCTCTACAACGAACTCAATCTTGGTCGGAGAG AGGAGTACGACGTGCTGGACAAGCGGAGAGGACGGGACCCAGAAATGGGCGGG AAGCCGCGCAGAAAGAATCCCCAAGAGGGCCTGTACAACGAGCTCCAAAAGGA TAAGATGGCAGAAGCCTATAGCGAGATTGGTATGAAAGGGGAACGCAGAAGAG GCAAAGGCCACGACGGACTGTACCAGGGACTCAGCACCGCCACCAAGGACACC TATGACGCTCTTCACATGCAGGCCCTGCCGCCTCGG
149368		
149368-aa ScFv domain	135	QVQLVQSGAEVKKPGSSVKVSCKASGGTFSSYAISWVRQAPGQGLEWMGGIIP IFGTANYAQKFQGRVTITADESTSTAYMELSSLRSEDTAVYYCARRGGYQLLR WDVGLLRSAFDIWGQGTMVTVSSGGGGSGGGGSGGGGSSYVLTQPPSVSVAPG QTARITCGGNNIGSKSVHWYQQKPGQAPVLVLYGKNNRPSGVPDRFSGSRSGT TASLTITGAQAEDEADYYCSSRDSSGDHLRVFGTGTKVTVL
149368-nt ScFv domain	156	CAAGTGCAGCTGGTCCAGTCGGGCGCCGAGGTCAAGAAGCCCGGGAGCTCTGT GAAAGTGTCCTGCAAGGCCTCCGGGGGCACCTTTAGCTCCTACGCCATCTCCT GGGTCCGCCAAGCACCGGGTCAAGGCCTGGAGTGGATGGGGGGAATTATCCCT ATCTTCGGCACTGCCAACTACGCCCAGAAGTTCCAGGGACGCGTGACCATTAC CGCGGACGAATCCACCTCCACCGCTTATATGGAGCTGTCCAGCTTGCGCTCGG AAGATACCGCCGTGTACTACTGCGCCCGGAGGGGTGGATACCAGCTGCTGAGA TGGGACGTGGGCCTCCTGCGGTCGGCGTTCGACATCTGGGGCCAGGGCACTAT GGTCACTGTGTCCAGCGGAGGAGGCGGATCGGGAGGCGGCGGATCAGGGGGAG GCGGTTCCAGCTACGTGCTTACTCAACCCCCTTCGGTGTCCGTGGCCCCGGGA CAGACCGCCAGAATCACTTGCGGAGGAAACAACATTGGGTCCAAGAGCGTGCA

		TTGGTACCAGCAGAAGCCAGGACAGGCCCCTGTGCTGGTGCTCTACGGGAAGA ACAATCGGCCCAGCGGAGTGCCGGACAGGTTCTCGGGTTCACGCTCCGGTACA ACCGCTTCACTGACTATCACCGGGGCCCAGGCAGAGGATGAAGCGGACTACTA CTGTTCCTCCCGGGATTCATCCGGCGACCACCTCCGGGTGTTCGGAACCGGAA CGAAGGTCACCGTGCTG
149368-aa VH	177	QVQLVQSGAEVKKPGSSVKVSCKASGGTFSSYAISWVRQAPGQGLEWMGGIIP IFGTANYAQKFQGRVTITADESTSTAYMELSSLRSEDTAVYYCARRGGYQLLR WDVGLLRSAFDIWGQGTMVTVSS
149368-aa VL	198	SYVLTQPPSVSVAPGQTARITCGGNNIGSKSVHWYQQKPGQAPVLVLYGKNNR PSGVPDRFSGSRSGTTASLTITGAQAEDEADYYCSSRDSSGDHLRVFGTGTKV TVL
149368-aa Full CAR	219	MALPVTALLLPLALLLHAARPQVQLVQSGAEVKKPGSSVKVSCKASGGTFSSY AISWVRQAPGQGLEWMGGIIPIFGTANYAQKFQGRVTITADESTSTAYMELSS LRSEDTAVYYCARRGGYQLLRWDVGLLRSAFDIWGQGTMVTVSSGGGGSGGGG SGGGGSSYVLTQPPSVSVAPGQTARITCGGNNIGSKSVHWYQQKPGQAPVLVL YGKNNRPSGVPDRFSGSRSGTTASLTITGAQAEDEADYYCSSRDSSGDHLRVF GTGTKVTVLTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFAC DIYIWAPLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPVQTTQEEDGC SCRFPEEEEGGCELRVKFSRSADAPAYKQGQNQLYNELNLGRREEYDVLDKRR GRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQG LSTATKDTYDALHMQALPPR
149368-nt Full CAR	240	ATGGCCCTCCCTGTCACCGCCCTGCTGCTTCCGCTGGCTCTTCTGCTCCACGC CGCTCGGCCCCAAGTGCAGCTGGTCCAGTCGGGCGCCGAGGTCAAGAAGCCCG GGAGCTCTGTGAAAGTGTCCTGCAAGGCCTCCGGGGGCACCTTTAGCTCCTAC GCCATCTCCTGGGTCCGCCAAGCACCGGGTCAAGGCCTGGAGTGGATGGGGGG AATTATCCCTATCTTCGGCACTGCCAACTACGCCCAGAAGTTCCAGGGACGCG TGACCATTACCGCGGACGAATCCACCTCCACCGCTTATATGGAGCTGTCCAGC TTGCGCTCGGAAGATACCGCCGTGTACTACTGCGCCCGGAGGGGTGGATACCA GCTGCTGAGATGGGACGTGGGCCTCCTGCGGTCGGCGTTCGACATCTGGGGCC AGGGCACTATGGTCACTGTGTCCAGCGGAGGAGGCGGATCGGGAGGCGGCGGA TCAGGGGGAGGCGGTTCCAGCTACGTGCTTACTCAACCCCCTTCGGTGTCCGT GGCCCCGGGACAGACCGCCAGAATCACTTGCGGAGGAAACAACATTGGGTCCA AGAGCGTGCATTGGTACCAGCAGAAGCCAGGACAGGCCCCTGTGCTGGTGCTC TACGGGAAGAACAATCGGCCCAGCGGAGTGCCGGACAGGTTCTCGGGTTCACG CTCCGGTACAACCGCTTCACTGACTATCACCGGGGCCCAGGCAGAGGATGAAG CGGACTACTACTGTTCCTCCCGGGATTCATCCGGCGACCACCTCCGGGTGTTC GGAACCGGAACGAAGGTCACCGTGCTGACCACTACCCCAGCACCGAGGCCACC CACCCCGGCTCCTACCATCGCCTCCCAGCCTCTGTCCCTGCGTCCGGAGGCAT GTAGACCCGCAGCTGGTGGGGCCGTGCATACCCGGGGTCTTGACTTCGCCTGC GATATCTACATTTGGGCCCCTCTGGCTGGTACTTGCGGGGTCCTGCTGCTTTC ACTCGTGATCACTCTTTACTGTAAGCGCGGTCGGAAGAAGCTGCTGTACATCT TTAAGCAACCCTTCATGAGGCCTGTGCAGACTACTCAAGAGGAGGACGGCTGT TCATGCCGGTTCCCAGAGGAGGAGGAAGGCGGCTGCGAACTGCGCGTGAAATT CAGCCGCAGCGCAGATGCTCCAGCCTACAAGCAGGGGCAGAACCAGCTCTACA ACGAACTCAATCTTGGTCGGAGAGAGGAGTACGACGTGCTGGACAAGCGGAGA GGACGGGACCCAGAAATGGGCGGGAAGCCGCGCAGAAAGAATCCCCAAGAGGG CCTGTACAACGAGCTCCAAAAGGATAAGATGGCAGAAGCCTATAGCGAGATTG GTATGAAAGGGGAACGCAGAAGAGGCAAAGGCCACGACGGACTGTACCAGGGA CTCAGCACCGCCACCAAGGACACCTATGACGCTCTTCACATGCAGGCCCTGCC GCCTCGG
149369		
149369-aa	136	EVQLQQSGPGLVKPSQTLSLTCAISGDSVSSNSAAWNWIRQSPSRGLEWLGRT

ScFv domain		YYRSKWYSFYAISLKSRIIINPDTSKNQFSLQLKSVTPEDTAVYYCARSSPEG LFLYWFDPWGQGTLVTVSSGGDGSGGGGSGGGGSSSELTQDPAVSVALGQTIR ITCQGDSLGNYYATWYQQKPGQAPVLVIYGTNNRPSGIPDRFSASSSGNTASL TITGAQAEDEADYYCNSRDSSGHHLLFGTGTKVTVL
149369-nt ScFv domain	157	GAAGTGCAGCTCCAACAGTCAGGACCGGGGCTCGTGAAGCCATCCCAGACCCT GTCCCTGACTTGTGCCATCTCGGGAGATAGCGTGTCATCGAACTCCGCCGCCT GGAACTGGATTCGGCAGAGCCCGTCCCGCGGACTGGAGTGGCTTGGAAGGACC TACTACCGGTCCAAGTGGTACTCTTTCTACGCGATCTCGCTGAAGTCCCGCAT TATCATTAACCCTGATACCTCCAAGAATCAGTTCTCCCTCCAACTGAAATCCG TCACCCCCGAGGACACAGCAGTGTATTACTGCGCACGGAGCAGCCCCGAAGGA CTGTTCCTGTATTGGTTTGACCCCTGGGGCCAGGGGACTCTTGTGACCGTGTC GAGCGGCGGAGATGGGTCCGGTGGCGGTGGTTCGGGGGGCGGCGGATCATCAT CCGAACTGACCCAGGACCCGGCTGTGTCCGTGGCGCTGGGACAAACCATCCGC ATTACGTGCCAGGGAGACTCCCTGGGCAACTACTACGCCACTTGGTACCAGCA GAAGCCGGGCCAAGCCCCTGTGTTGGTCATCTACGGGACCAACAACAGACCTT CCGGCATCCCCGACCGGTTCAGCGCTTCGTCCTCCGGCAACACTGCCAGCCTG ACCATCACTGGAGCGCAGGCCGAAGATGAGGCCGACTACTACTGCAACAGCAG AGACTCCTCGGGTCATCACCTCTTGTTCGGAACTGGAACCAAGGTCACCGTGC TG
149369-aa VH	178	EVQLQQSGPGLVKPSQTLSLTCAISGDSVSSNSAAWNWIRQSPSRGLEWLGRT YYRSKWYSFYAISLKSRIIINPDTSKNQFSLQLKSVTPEDTAVYYCARSSPEG LFLYWFDPWGQGTLVTVSS
149369-aa VL	199	SSELTQDPAVSVALGQTIRITCQGDSLGNYYATWYQQKPGQAPVLVIYGTNNR PSGIPDRFSASSSGNTASLTITGAQAEDEADYYCNSRDSSGHHLLFGTGTKVT VL
149369-aa Full CAR	220	MALPVTALLLPLALLLHAARPEVQLQQSGPGLVKPSQTLSLTCAISGDSVSSN SAAWNWIRQSPSRGLEWLGRTYYRSKWYSFYAISLKSRIIINPDTSKNQFSLQ LKSVTPEDTAVYYCARSSPEGLFLYWFDPWGQGTLVTVSSGGDGSGGGGSGGG GSSSELTQDPAVSVALGQTIRITCQGDSLGNYYATWYQQKPGQAPVLVIYGTN NRPSGIPDRFSASSSGNTASLTITGAQAEDEADYYCNSRDSSGHHLLFGTGTK VTVLTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYIW APLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFP EEEEGGCELRVKFSRSADAPAYKQGQNQLYNELNLGRREEYDVLDKRRGRDPE MGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTAT KDTYDALHMQALPPR
149369-nt Full CAR	241	ATGGCCCTCCCTGTCACCGCCCTGCTGCTTCCGCTGGCTCTTCTGCTCCACGC CGCTCGGCCCGAAGTGCAGCTCCAACAGTCAGGACCGGGGCTCGTGAAGCCAT CCCAGACCCTGTCCCTGACTTGTGCCATCTCGGGAGATAGCGTGTCATCGAAC TCCGCCGCCTGGAACTGGATTCGGCAGAGCCCGTCCCGCGGACTGGAGTGGCT TGGAAGGACCTACTACCGGTCCAAGTGGTACTCTTTCTACGCGATCTCGCTGA AGTCCCGCATTATCATTAACCCTGATACCTCCAAGAATCAGTTCTCCCTCCAA CTGAAATCCGTCACCCCCGAGGACACAGCAGTGTATTACTGCGCACGGAGCAG CCCCGAAGGACTGTTCCTGTATTGGTTTGACCCCTGGGGCCAGGGGACTCTTG TGACCGTGTCGAGCGGCGGAGATGGGTCCGGTGGCGGTGGTTCGGGGGGCGGC GGATCATCATCCGAACTGACCCAGGACCCGGCTGTGTCCGTGGCGCTGGGACA AACCATCCGCATTACGTGCCAGGGAGACTCCCTGGGCAACTACTACGCCACTT GGTACCAGCAGAAGCCGGGCCAAGCCCCTGTGTTGGTCATCTACGGGACCAAC AACAGACCTTCCGGCATCCCCGACCGGTTCAGCGCTTCGTCCTCCGGCAACAC TGCCAGCCTGACCATCACTGGAGCGCAGGCCGAAGATGAGGCCGACTACTACT GCAACAGCAGAGACTCCTCGGGTCATCACCTCTTGTTCGGAACTGGAACCAAG GTCACCGTGCTGACCACTACCCCAGCACCGAGGCCACCCACCCCGGCTCCTAC CATCGCCTCCCAGCCTCTGTCCCTGCGTCCGGAGGCATGTAGACCCGCAGCTG

		GTGGGGCCGTGCATACCCGGGGTCTTGACTTCGCCTGCGATATCTACATTTGG GCCCCTCTGGCTGGTACTTGCGGGGTCCTGCTGCTTTCACTCGTGATCACTCT TTACTGTAAGCGCGGTCGGAAGAAGCTGCTGTACATCTTTAAGCAACCCTTCA TGAGGCCTGTGCAGACTACTCAAGAGGAGGACGGCTGTTCATGCCGGTTCCCA GAGGAGGAGGAAGGCGGCTGCGAACTGCGCGTGAAATTCAGCCGCAGCGCAGA TGCTCCAGCCTACAAGCAGGGGCAGAACCAGCTCTACAACGAACTCAATCTTG GTCGGAGAGAGGAGTACGACGTGCTGGACAAGCGGAGAGGACGGGACCCAGAA ATGGGCGGGAAGCCGCGCAGAAAGAATCCCCAAGAGGGCCTGTACAACGAGCT CCAAAAGGATAAGATGGCAGAAGCCTATAGCGAGATTGGTATGAAAGGGGAAC GCAGAAGAGGCAAAGGCCACGACGGACTGTACCAGGGACTCAGCACCGCCACC AAGGACACCTATGACGCTCTTCACATGCAGGCCCTGCCGCCTCGG
BCMA_EBB-C1978-A4		
BCMA_EBB-C1978-A4 - aa ScFv domain	137	EVQLVESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPGKGLEWVSAISG SGGSTYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAKVEGSGSLD YWGQGTLVTVSSGGGGSGGGGSGGGGSEIVMTQSPGTLSLSPGERATLSCRAS QSVSSAYLAWYQQKPGQPPRLLISGASTRATGIPDRFGGSGSGTDFTLTISRL EPEDFAVYYCQHYGSSFNGSSLFTFGQGTRLEIK
BCMA_EBB-C1978-A4 - nt ScFv domain	158	GAAGTGCAGCTCGTGGAGTCAGGAGGCGGCCTGGTCCAGCCGGGAGGGTCCCT TAGACTGTCATGCGCCGCAAGCGGATTCACTTTCTCCTCCTATGCCATGAGCT GGGTCCGCCAAGCCCCCGGAAAGGGACTGGAATGGGTGTCCGCCATCTCGGGG TCTGGAGGCTCAACTTACTACGCTGACTCCGTGAAGGGACGGTTCACCATTAG CCGCGACAACTCCAAGAACACCCTCTACCTCCAAATGAACTCCCTGCGGGCCG AGGATACCGCCGTCTACTACTGCGCCAAAGTGGAAGGTTCAGGATCGCTGGAC TACTGGGGACAGGGTACTCTCGTGACCGTGTCATCGGGCGGAGGAGGTTCCGG CGGTGGCGGCTCCGGCGGCGGAGGGTCGGAGATCGTGATGACCCAGAGCCCTG GTACTCTGAGCCTTTCGCCGGGAGAAAGGGCCACCCTGTCCTGCCGCGCTTCC CAATCCGTGTCCTCCGCGTACTTGGCGTGGTACCAGCAGAAGCCGGGACAGCC CCCTCGGCTGCTGATCAGCGGGGCCAGCACCCGGGCAACCGGAATCCCAGACA GATTCGGGGGTTCCGGCAGCGGCACAGATTTCACCCTGACTATTTCGAGGTTG GAGCCCGAGGACTTTGCGGTGTATTACTGTCAGCACTACGGGTCGTCCTTTAA TGGCTCCAGCCTGTTCACGTTCGGACAGGGGACCCGCCTGGAAATCAAG
BCMA_EBB-C1978-A4 - aa VH	179	EVQLVESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPGKGLEWVSAISG SGGSTYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAKVEGSGSLD YWGQGTLVTVSS
BCMA_EBB-C1978-A4 - aa VL	200	EIVMTQSPGTLSLSPGERATLSCRASQSVSSAYLAWYQQKPGQPPRLLISGAS TRATGIPDRFGGSGSGTDFTLTISRLEPEDFAVYYCQHYGSSFNGSSLFTFGQ GTRLEIK
BCMA_EBB-C1978-A4 - aa Full CART	221	MALPVTALLLPLALLLHAARPEVQLVESGGGLVQPGGSLRLSCAASGFTFSSY AMSWVRQAPGKGLEWVSAISGSGGSTYYADSVKGRFTISRDNSKNTLYLQMNS LRAEDTAVYYCAKVEGSGSLDYWGQGTLVTVSSGGGGSGGGGSGGGGSEIVMT QSPGTLSLSPGERATLSCRASQSVSSAYLAWYQQKPGQPPRLLISGASTRATG IPDRFGGSGSGTDFTLTISRLEPEDFAVYYCQHYGSSFNGSSLFTFGQGTRLE IKTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYIWAP LAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEE EEGGCELRVKFSRSADAPAYKQGQNQLYNELNLGRREEYDVLDKRRGRDPEMG GKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKD TYDALHMQALPPR
BCMA_EBB-C1978-A4 - nt Full CART	242	ATGGCCCTCCCTGTCACCGCCCTGCTGCTTCCGCTGGCTCTTCTGCTCCACGC CGCTCGGCCCGAAGTGCAGCTCGTGGAGTCAGGAGGCGGCCTGGTCCAGCCGG GAGGGTCCCTTAGACTGTCATGCGCCGCAAGCGGATTCACTTTCTCCTCCTAT

		GCCATGAGCTGGGTCCGCCAAGCCCCCGGAAAGGGACTGGAATGGGTGTCCGC CATCTCGGGGTCTGGAGGCTCAACTTACTACGCTGACTCCGTGAAGGGACGGT TCACCATTAGCCGCGACAACTCCAAGAACACCCTCTACCTCCAAATGAACTCC CTGCGGGCCGAGGATACCGCCGTCTACTACTGCGCCAAAGTGGAAGGTTCAGG ATCGCTGGACTACTGGGGACAGGGTACTCTCGTGACCGTGTCATCGGGCGGAG GAGGTTCCGGCGGTGGCGGCTCCGGCGGCGGAGGGTCGGAGATCGTGATGACC CAGAGCCCTGGTACTCTGAGCCTTTCGCCGGGAGAAAGGGCCACCCTGTCCTG CCGCGCTTCCCAATCCGTGTCCTCCGCGTACTTGGCGTGGTACCAGCAGAAGC CGGGACAGCCCCCTCGGCTGCTGATCAGCGGGGCCAGCACCCGGGCAACCGGA ATCCCAGACAGATTCGGGGGTTCCGGCAGCGGCACAGATTTCACCCTGACTAT TTCGAGGTTGGAGCCCGAGGACTTTGCGGTGTATTACTGTCAGCACTACGGGT CGTCCTTTAATGGCTCCAGCCTGTTCACGTTCGGACAGGGGACCCGCCTGGAA ATCAAGACCACTACCCCAGCACCGAGGCCACCCACCCCGGCTCCTACCATCGC CTCCCAGCCTCTGTCCCTGCGTCCGGAGGCATGTAGACCCGCAGCTGGTGGGG CCGTGCATACCCGGGGTCTTGACTTCGCCTGCGATATCTACATTTGGGCCCCT CTGGCTGGTACTTGCGGGGTCCTGCTGCTTTCACTCGTGATCACTCTTTACTG TAAGCGCGGTCGGAAGAAGCTGCTGTACATCTTTAAGCAACCCTTCATGAGGC CTGTGCAGACTACTCAAGAGGAGGACGGCTGTTCATGCCGGTTCCCAGAGGAG GAGGAAGGCGGCTGCGAACTGCGCGTGAAATTCAGCCGCAGCGCAGATGCTCC AGCCTACAAGCAGGGGCAGAACCAGCTCTACAACGAACTCAATCTTGGTCGGA GAGAGGAGTACGACGTGCTGGACAAGCGGAGAGGACGGGACCCAGAAATGGGC GGGAAGCCGCGCAGAAAGAATCCCCAAGAGGGCCTGTACAACGAGCTCCAAAA GGATAAGATGGCAGAAGCCTATAGCGAGATTGGTATGAAAGGGGAACGCAGAA GAGGCAAAGGCCACGACGGACTGTACCAGGGACTCAGCACCGCCACCAAGGAC ACCTATGACGCTCTTCACATGCAGGCCCTGCCGCCTCGG
BCMA_EBB-C1978-G1		
BCMA_EBB-C1978-G1 - aa ScFv domain	138	EVQLVETGGGLVQPGGSLRLSCAASGITFSRYPMSWVRQAPGKGLEWVSGISD SGVSTYYADSAKGRFTISRDNSKNTLFLQMSSLRDEDTAVYYCVTRAGSEASD IWGQGTMVTVSSGGGGSGGGGSGGGGSEIVLTQSPATLSLSPGERATLSCRAS QSVSNSLAWYQQKPGQAPRLLIYDASSRATGIPDRFSGSGSGTDFTLTISRLE PEDFAIYYCQQFGTSSGLTFGGGTKLEIK
BCMA_EBB-C1978-G1 - nt ScFv domain	159	GAAGTGCAACTGGTGGAAACCGGTGGCGGCCTGGTGCAGCCTGGAGGATCATT GAGGCTGTCATGCGCGGCCAGCGGTATTACCTTCTCCCGGTACCCCATGTCCT GGGTCAGACAGGCCCCGGGGAAAGGGCTTGAATGGGTGTCCGGGATCTCGGAC TCCGGTGTCAGCACTTACTACGCCGACTCCGCCAAGGGACGCTTCACCATTTC CCGGGACAACTCGAAGAACACCCTGTTCCTCCAAATGAGCTCCCTCCGGGACG AGGATACTGCAGTGTACTACTGCGTGACCCGCGCCGGGTCCGAGGCGTCTGAC ATTTGGGGACAGGGCACTATGGTCACCGTGTCGTCCGGCGGAGGGGGCTCGGG AGGCGGTGGCAGCGGAGGAGGAGGGTCCGAGATCGTGCTGACCCAATCCCCGG CCACCCTCTCGCTGAGCCCTGGAGAAAGGGCAACCTTGTCCTGTCGCGCGAGC CAGTCCGTGAGCAACTCCCTGGCCTGGTACCAGCAGAAGCCCGGACAGGCTCC GAGACTTCTGATCTACGACGCTTCGAGCCGGGCCACTGGAATCCCCGACCGCT TTTCGGGGTCCGGCTCAGGAACCGATTTCACCCTGACAATCTCACGGCTGGAG CCAGAGGATTTCGCCATCTATTACTGCCAGCAGTTCGGTACTTCCTCCGGCCT GACTTTCGGAGGCGGCACGAAGCTCGAAATCAAG
BCMA_EBB-C1978-G1 - aa VH	180	EVQLVETGGGLVQPGGSLRLSCAASGITFSRYPMSWVRQAPGKGLEWVSGISD SGVSTYYADSAKGRFTISRDNSKNTLFLQMSSLRDEDTAVYYCVTRAGSEASD IWGQGTMVTVSS
BCMA_EBB-C1978-G1 - aa	201	EIVLTQSPATLSLSPGERATLSCRASQSVSNSLAWYQQKPGQAPRLLIYDASS RATGIPDRFSGSGSGTDFTLTISRLEPEDFAIYYCQQFGTSSGLTFGGGTKLE IK

VL		
BCMA_EBB-C1978-G1 - aa Full CART	222	MALPVTALLLPLALLLHAARPEVQLVETGGGLVQPGGSLRLSCAASGITFSRY PMSWVRQAPGKGLEWVSGISDSGVSTYYADSAKGRFTISRDNSKNTLFLQMSS LRDEDTAVYYCVTRAGSEASDIWGQGTMVTVSSGGGGSGGGGSGGGGSEIVLT QSPATLSLSPGERATLSCRASQSVSNSLAWYQQKPGQAPRLLIYDASSRATGI PDRFSGSGSGTDFTLTISRLEPEDFAIYYCQQFGTSSGLTFGGGTKLEIKTTT PAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYIWAPLAGTC GVLLLSLVITLYCKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGC ELRVKFSRSADAPAYKQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRR KNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDAL HMQALPPR
BCMA_EBB-C1978-G1 - nt Full CART	243	ATGGCCCTCCCTGTCACCGCCCTGCTGCTTCCGCTGGCTCTTCTGCTCCACGC CGCTCGGCCCGAAGTGCAACTGGTGGAAACCGGTGGCGGCCTGGTGCAGCCTG GAGGATCATTGAGGCTGTCATGCGCGGCCAGCGGTATTACCTTCTCCCGGTAC CCCATGTCCTGGGTCAGACAGGCCCCGGGGAAAGGGCTTGAATGGGTGTCCGG GATCTCGGACTCCGGTGTCAGCACTTACTACGCCGACTCCGCCAAGGGACGCT TCACCATTTCCCGGGACAACTCGAAGAACACCCTGTTCCTCCAAATGAGCTCC CTCCGGGACGAGGATACTGCAGTGTACTACTGCGTGACCCGCGCCGGGTCCGA GGCGTCTGACATTTGGGGACAGGGCACTATGGTCACCGTGTCGTCCGGCGGAG GGGGCTCGGGAGGCGGTGGCAGCGGAGGAGGAGGGTCCGAGATCGTGCTGACC CAATCCCCGGCCACCCTCTCGCTGAGCCCTGGAGAAAGGGCAACCTTGTCCTG TCGCGCGAGCCAGTCCGTGAGCAACTCCCTGGCCTGGTACCAGCAGAAGCCCG GACAGGCTCCGAGACTTCTGATCTACGACGCTTCGAGCCGGGCCACTGGAATC CCCGACCGCTTTTCGGGGTCCGGCTCAGGAACCGATTTCACCCTGACAATCTC ACGGCTGGAGCCAGAGGATTTCGCCATCTATTACTGCCAGCAGTTCGGTACTT CCTCCGGCCTGACTTTCGGAGGCGGCACGAAGCTCGAAATCAAGACCACTACC CCAGCACCGAGGCCACCCACCCCGGCTCCTACCATCGCCTCCCAGCCTCTGTC CCTGCGTCCGGAGGCATGTAGACCCGCAGCTGGTGGGGCCGTGCATACCCGGG GTCTTGACTTCGCCTGCGATATCTACATTTGGGCCCCTCTGGCTGGTACTTGC GGGGTCCTGCTGCTTTCACTCGTGATCACTCTTTACTGTAAGCGCGGTCGGAA GAAGCTGCTGTACATCTTTAAGCAACCCTTCATGAGGCCTGTGCAGACTACTC AAGAGGAGGACGGCTGTTCATGCCGGTTCCCAGAGGAGGAGGAAGGCGGCTGC GAACTGCGCGTGAAATTCAGCCGCAGCGCAGATGCTCCAGCCTACAAGCAGGG GCAGAACCAGCTCTACAACGAACTCAATCTTGGTCGGAGAGAGGAGTACGACG TGCTGGACAAGCGGAGAGGACGGGACCCAGAAATGGGCGGGAAGCCGCGCAGA AAGAATCCCCAAGAGGGCCTGTACAACGAGCTCCAAAAGGATAAGATGGCAGA AGCCTATAGCGAGATTGGTATGAAAGGGGAACGCAGAAGAGGCAAAGGCCACG ACGGACTGTACCAGGGACTCAGCACCGCCACCAAGGACACCTATGACGCTCTT CACATGCAGGCCCTGCCGCCTCGG
BCMA_EBB-C1979-C1		
BCMA_EBB-C1979-C1 - aa ScFv domain	139	QVQLVESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPGKGLEWVSAISG SGGSTYYADSVKGRFTISRDNAKNSLYLQMNSLRAEDTAIYYCARATYKRELR YYYGMDVWGQGTMVTVSSGGGGSGGGGSGGGGSEIVMTQSPGTVSLSPGERAT LSCRASQSVSSSFLAWYQQKPGQAPRLLIYGASSRATGIPDRFSGSGSGTDFT LTISRLEPEDSAVYYCQQYHSSPSWTFGQGTRLEIK
BCMA_EBB-C1979-C1 - nt ScFv domain	160	CAAGTGCAGCTCGTGGAATCGGGTGGCGGACTGGTGCAGCCGGGGGGCTCACT TAGACTGTCCTGCGCGGCCAGCGGATTCACTTTCTCCTCCTACGCCATGTCCT GGGTCAGACAGGCCCCTGGAAAGGGCCTGGAATGGGTGTCCGCAATCAGCGGC AGCGGCGGCTCGACCTATTACGCGGATTCAGTGAAGGGCAGATTCACCATTTC CCGGGACAACGCCAAGAACTCCTTGTACCTTCAAATGAACTCCCTCCGCGCGG AAGATACCGCAATCTACTACTGCGCTCGGGCCACTTACAAGAGGGAACTGCGC TACTACTACGGGATGGACGTCTGGGGCCAGGGAACCATGGTCACCGTGTCCAG

		CGGAGGAGGAGGATCGGGAGGAGGCGGTAGCGGGGGTGGAGGGTCGGAGATCG TGATGACCCAGTCCCCCGGCACTGTGTCGCTGTCCCCCGGCGAACGGGCCACC CTGTCATGTCGGGCCAGCCAGTCAGTGTCGTCAAGCTTCCTCGCCTGGTACCA GCAGAAACCGGGACAAGCTCCCCGCCTGCTGATCTACGGAGCCAGCAGCCGGG CCACCGGTATTCCTGACCGGTTCTCCGGTTCGGGGTCCGGGACCGACTTTACT CTGACTATCTCTCGCCTCGAGCCAGAGGACTCCGCCGTGTATTACTGCCAGCA GTACCACTCCTCCCCGTCCTGGACGTTCGGACAGGGCACAAGGCTGGAGATTA AG
BCMA_EBB-C1979-C1 - aa VH	181	QVQLVESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPGKGLEWVSAISG SGGSTYYADSVKGRFTISRDNAKNSLYLQMNSLRAEDTAIYYCARATYKRELR YYYGMDVWGQGTMVTVSS
BCMA_EBB-C1979-C1 - aa VL	202	EIVMTQSPGTVSLSPGERATLSCRASQSVSSSFLAWYQQKPGQAPRLLIYGAS SRATGIPDRFSGSGSGTDFTLTISRLEPEDSAVYYCQQYHSSPSWTFGQGTRL EIK
BCMA_EBB-C1979-C1 - aa Full CART	223	MALPVTALLLPLALLLHAARPQVQLVESGGGLVQPGGSLRLSCAASGFTFSSY AMSWVRQAPGKGLEWVSAISGSGGSTYYADSVKGRFTISRDNAKNSLYLQMNS LRAEDTAIYYCARATYKRELRYYYGMDVWGQGTMVTVSSGGGGSGGGGSGGGG SEIVMTQSPGTVSLSPGERATLSCRASQSVSSSFLAWYQQKPGQAPRLLIYGA SSRATGIPDRFSGSGSGTDFTLTISRLEPEDSAVYYCQQYHSSPSWTFGQGTR LEIKTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYIW APLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFP EEEEGGCELRVKFSRSADAPAYKQGQNQLYNELNLGRREEYDVLDKRRGRDPE MGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTAT KDTYDALHMQALPPR
BCMA_EBB-C1979-C1 - nt Full CART	244	ATGGCCCTCCCTGTCACCGCCCTGCTGCTTCCGCTGGCTCTTCTGCTCCACGC CGCTCGGCCCCAAGTGCAGCTCGTGGAATCGGGTGGCGGACTGGTGCAGCCGG GGGGCTCACTTAGACTGTCCTGCGCGGCCAGCGGATTCACTTTCTCCTCCTAC GCCATGTCCTGGGTCAGACAGGCCCCTGGAAAGGGCCTGGAATGGGTGTCCGC AATCAGCGGCAGCGGCGGCTCGACCTATTACGCGGATTCAGTGAAGGGCAGAT TCACCATTTCCCGGGACAACGCCAAGAACTCCTTGTACCTTCAAATGAACTCC CTCCGCGCGGAAGATACCGCAATCTACTACTGCGCTCGGGCCACTTACAAGAG GGAACTGCGCTACTACTACGGGATGGACGTCTGGGGCCAGGGAACCATGGTCA CCGTGTCCAGCGGAGGAGGAGGATCGGGAGGAGGCGGTAGCGGGGGTGGAGGG TCGGAGATCGTGATGACCCAGTCCCCCGGCACTGTGTCGCTGTCCCCCGGCGA ACGGGCCACCCTGTCATGTCGGGCCAGCCAGTCAGTGTCGTCAAGCTTCCTCG CCTGGTACCAGCAGAAACCGGGACAAGCTCCCCGCCTGCTGATCTACGGAGCC AGCAGCCGGGCCACCGGTATTCCTGACCGGTTCTCCGGTTCGGGGTCCGGGAC CGACTTTACTCTGACTATCTCTCGCCTCGAGCCAGAGGACTCCGCCGTGTATT ACTGCCAGCAGTACCACTCCTCCCCGTCCTGGACGTTCGGACAGGGCACAAGG CTGGAGATTAAGACCACTACCCCAGCACCGAGGCCACCCACCCCGGCTCCTAC CATCGCCTCCCAGCCTCTGTCCCTGCGTCCGGAGGCATGTAGACCCGCAGCTG GTGGGGCCGTGCATACCCGGGGTCTTGACTTCGCCTGCGATATCTACATTTGG GCCCCTCTGGCTGGTACTTGCGGGGTCCTGCTGCTTTCACTCGTGATCACTCT TTACTGTAAGCGCGGTCGGAAGAAGCTGCTGTACATCTTTAAGCAACCCTTCA TGAGGCCTGTGCAGACTACTCAAGAGGAGGACGGCTGTTCATGCCGGTTCCCA GAGGAGGAGGAAGGCGGCTGCGAACTGCGCGTGAAATTCAGCCGCAGCGCAGA TGCTCCAGCCTACAAGCAGGGGCAGAACCAGCTCTACAACGAACTCAATCTTG GTCGGAGAGAGGAGTACGACGTGCTGGACAAGCGGAGAGGACGGGACCCAGAA ATGGGCGGGAAGCCGCGCAGAAAGAATCCCCAAGAGGGCCTGTACAACGAGCT CCAAAAGGATAAGATGGCAGAAGCCTATAGCGAGATTGGTATGAAAGGGGAAC

		GCAGAAGAGGCAAAGGCCACGACGGACTGTACCAGGGACTCAGCACCGCCACC AAGGACACCTATGACGCTCTTCACATGCAGGCCCTGCCGCCTCGG
BCMA_EBB-C1978-C7		
BCMA_EBB-C1978-C7 - aa ScFv domain	140	EVQLVETGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPGKGLEWVSAISG SGGSTYYADSVKGRFTISRDNSKNTLYLQMNTLKAEDTAVYYCARATYKRELR YYYGMDVWGQGTTVTVSSGGGGSGGGGSGGGGSEIVLTQSPSTLSLSPGESAT LSCRASQSVSTTFLAWYQQKPGQAPRLLIYGSSNRATGIPDRFSGSGSGTDFT LTIRRLEPEDFAVYYCQQYHSSPSWTFGQGTKVEIK
BCMA_EBB-C1978-C7 - nt ScFv domain	161	GAGGTGCAGCTTGTGGAAACCGGTGGCGGACTGGTGCAGCCCGGAGGAAGCCT CAGGCTGTCCTGCGCCGCGTCCGGCTTCACCTTCTCCTCGTACGCCATGTCCT GGGTCCGCCAGGCCCCCGGAAAGGGCCTGGAATGGGTGTCCGCCATCTCTGGA AGCGGAGGTTCCACGTACTACGCGGACAGCGTCAAGGGAAGGTTCACAATCTC CCGCGATAATTCGAAGAACACTCTGTACCTTCAAATGAACACCCTGAAGGCCG AGGACACTGCTGTGTACTACTGCGCACGGGCCACCTACAAGAGAGAGCTCCGG TACTACTACGGAATGGACGTCTGGGGCCAGGGAACTACTGTGACCGTGTCCTC GGGAGGGGGTGGCTCCGGGGGGGGCGGCTCCGGCGGAGGCGGTTCCGAGATTG TGCTGACCCAGTCACCTTCAACTCTGTCGCTGTCCCCGGGAGAGAGCGCTACT CTGAGCTGCCGGGCCAGCCAGTCCGTGTCCACCACCTTCCTCGCCTGGTATCA GCAGAAGCCGGGGCAGGCACCACGGCTCTTGATCTACGGGTCAAGCAACAGAG CGACCGGAATTCCTGACCGCTTCTCGGGGAGCGGTTCAGGCACCGACTTCACC CTGACTATCCGGCGCCTGGAACCCGAAGATTTCGCCGTGTATTACTGTCAACA GTACCACTCCTCGCCGTCCTGGACCTTTGGCCAAGGAACCAAAGTGGAAATCA AG
BCMA_EBB-C1978-C7 - aa VH	182	EVQLVETGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPGKGLEWVSAISG SGGSTYYADSVKGRFTISRDNSKNTLYLQMNTLKAEDTAVYYCARATYKRELR YYYGMDVWGQGTTVTVSS
BCMA_EBB-C1978-C7 - aa VL	203	EIVLTQSPSTLSLSPGESATLSCRASQSVSTTFLAWYQQKPGQAPRLLIYGSS NRATGIPDRFSGSGSGTDFTLTIRRLEPEDFAVYYCQQYHSSPSWTFGQGTKV EIK
BCMA_EBB-C1978-C7 - aa Full CART	224	MALPVTALLLPLALLLHAARPEVQLVETGGGLVQPGGSLRLSCAASGFTFSSY AMSWVRQAPGKGLEWVSAISGSGGSTYYADSVKGRFTISRDNSKNTLYLQMNT LKAEDTAVYYCARATYKRELRYYYGMDVWGQGTTVTVSSGGGGSGGGGSGGGG SEIVLTQSPSTLSLSPGESATLSCRASQSVSTTFLAWYQQKPGQAPRLLIYGS SNRATGIPDRFSGSGSGTDFTLTIRRLEPEDFAVYYCQQYHSSPSWTFGQGTK VEIKTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYIW APLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFP EEEEGGCELRVKFSRSADAPAYKQGQNQLYNELNLGRREEYDVLDKRRGRDPE MGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTAT KDTYDALHMQALPPR
BCMA_EBB-C1978-C7 - nt Full CART	245	ATGGCCCTCCCTGTCACCGCCCTGCTGCTTCCGCTGGCTCTTCTGCTCCACGC CGCTCGGCCCGAGGTGCAGCTTGTGGAAACCGGTGGCGGACTGGTGCAGCCCG GAGGAAGCCTCAGGCTGTCCTGCGCCGCGTCCGGCTTCACCTTCTCCTCGTAC GCCATGTCCTGGGTCCGCCAGGCCCCCGGAAAGGGCCTGGAATGGGTGTCCGC CATCTCTGGAAGCGGAGGTTCCACGTACTACGCGGACAGCGTCAAGGGAAGGT TCACAATCTCCCGCGATAATTCGAAGAACACTCTGTACCTTCAAATGAACACC CTGAAGGCCGAGGACACTGCTGTGTACTACTGCGCACGGGCCACCTACAAGAG AGAGCTCCGGTACTACTACGGAATGGACGTCTGGGGCCAGGGAACTACTGTGA CCGTGTCCTCGGGAGGGGGTGGCTCCGGGGGGGGCGGCTCCGGCGGAGGCGGT TCCGAGATTGTGCTGACCCAGTCACCTTCAACTCTGTCGCTGTCCCCGGGAGA

		GAGCGCTACTCTGAGCTGCCGGGCCAGCCAGTCCGTGTCCACCACCTTCCTCG CCTGGTATCAGCAGAAGCCGGGGCAGGCACCACGGCTCTTGATCTACGGGTCA AGCAACAGAGCGACCGGAATTCCTGACCGCTTCTCGGGGAGCGGTTCAGGCAC CGACTTCACCCTGACTATCCGGCGCCTGGAACCCGAAGATTTCGCCGTGTATT ACTGTCAACAGTACCACTCCTCGCCGTCCTGGACCTTTGGCCAAGGAACCAAA GTGGAAATCAAGACCACTACCCCAGCACCGAGGCCACCCACCCCGGCTCCTAC CATCGCCTCCCAGCCTCTGTCCCTGCGTCCGGAGGCATGTAGACCCGCAGCTG GTGGGGCCGTGCATACCCGGGGTCTTGACTTCGCCTGCGATATCTACATTTGG GCCCCTCTGGCTGGTACTTGCGGGGTCCTGCTGCTTTCACTCGTGATCACTCT TTACTGTAAGCGCGGTCGGAAGAAGCTGCTGTACATCTTTAAGCAACCCTTCA TGAGGCCTGTGCAGACTACTCAAGAGGAGGACGGCTGTTCATGCCGGTTCCCA GAGGAGGAGGAAGGCGGCTGCGAACTGCGCGTGAAATTCAGCCGCAGCGCAGA TGCTCCAGCCTACAAGCAGGGGCAGAACCAGCTCTACAACGAACTCAATCTTG GTCGGAGAGAGGAGTACGACGTGCTGGACAAGCGGAGAGGACGGGACCCAGAA ATGGGCGGGAAGCCGCGCAGAAAGAATCCCCAAGAGGGCCTGTACAACGAGCT CCAAAAGGATAAGATGGCAGAAGCCTATAGCGAGATTGGTATGAAAGGGGAAC GCAGAAGAGGCAAAGGCCACGACGGACTGTACCAGGGACTCAGCACCGCCACC AAGGACACCTATGACGCTCTTCACATGCAGGCCCTGCCGCCTCGG
BCMA_EBB-C1978-D10		
BCMA_EBB-C1978-D10 - aa ScFv domain	141	EVQLVETGGGLVQPGRSLRLSCAASGFTFDDYAMHWVRQAPGKGLEWVSGISW NSGSIGYADSVKGRFTISRDNAKNSLYLQMNSLRDEDTAVYYCARVGKAVPDV WGQGTTVTVSSGGGGSGGGGSGGGGSDIVMTQTPSSLSASVGDRVTITCRASQ SISSYLNWYQQKPGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLTISSLQP EDFATYYCQQSYSTPYSFGQGTRLEIK
BCMA_EBB-C1978-D10- nt ScFv domain	162	GAAGTGCAGCTCGTGGAAACTGGAGGTGGACTCGTGCAGCCTGGACGGTCGCT GCGGCTGAGCTGCGCTGCATCCGGCTTCACCTTCGACGATTATGCCATGCACT GGGTCAGACAGGCGCCAGGGAAGGGACTTGAGTGGGTGTCCGGTATCAGCTGG AATAGCGGCTCAATCGGATACGCGGACTCCGTGAAGGGAAGGTTCACCATTTC CCGCGACAACGCCAAGAACTCCCTGTACTTGCAAATGAACAGCCTCCGGGATG AGGACACTGCCGTGTACTACTGCGCCCGCGTCGGAAAAGCTGTGCCCGACGTC TGGGGCCAGGGAACCACTGTGACCGTGTCCAGCGGCGGGGGTGGATCGGGCGG TGGAGGGTCCGGTGGAGGGGGCTCAGATATTGTGATGACCCAGACCCCCTCGT CCCTGTCCGCCTCGGTCGGCGACCGCGTGACTATCACATGTAGAGCCTCGCAG AGCATCTCCAGCTACCTGAACTGGTATCAGCAGAAGCCGGGGAAGGCCCCGAA GCTCCTGATCTACGCGGCATCATCACTGCAATCGGGAGTGCCGAGCCGGTTTT CCGGGTCCGGCTCCGGCACCGACTTCACGCTGACCATTTCTTCCCTGCAACCC GAGGACTTCGCCACTTACTACTGCCAGCAGTCCTACTCCACCCCTTACTCCTT CGGCCAAGGAACCAGGCTGGAAATCAAG
BCMA_EBB-C1978-D10 - aa VH	183	EVQLVETGGGLVQPGRSLRLSCAASGFTFDDYAMHWVRQAPGKGLEWVSGISW NSGSIGYADSVKGRFTISRDNAKNSLYLQMNSLRDEDTAVYYCARVGKAVPDV WGQGTTVTVSS
BCMA_EBB-C1978-D10- aa VL	204	DIVMTQTPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKLLIYAASS LQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQSYSTPYSFGQGTRLEI K
BCMA_EBB-C1978-D10 - aa Full CART	225	MALPVTALLLPLALLLHAARPEVQLVETGGGLVQPGRSLRLSCAASGFTFDDY AMHWVRQAPGKGLEWVSGISWNSGSIGYADSVKGRFTISRDNAKNSLYLQMNS LRDEDTAVYYCARVGKAVPDVWGQGTTVTVSSGGGGSGGGGSGGGGSDIVMTQ TPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKLLIYAASSLQSGVP SRFSGSGSGTDFTLTISSLQPEDFATYYCQQSYSTPYSFGQGTRLEIKTTTPA

		PRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYIWAPLAGTCGV LLLSLVITLYCKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCEL RVKFSRSADAPAYKQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKN PQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHM QALPPR
BCMA_EBB-C1978-D10 - nt Full CART	246	ATGGCCCTCCCTGTCACCGCCCTGCTGCTTCCGCTGGCTCTTCTGCTCCACGC CGCTCGGCCCGAAGTGCAGCTCGTGGAAACTGGAGGTGGACTCGTGCAGCCTG GACGGTCGCTGCGGCTGAGCTGCGCTGCATCCGGCTTCACCTTCGACGATTAT GCCATGCACTGGGTCAGACAGGCGCCAGGGAAGGGACTTGAGTGGGTGTCCGG TATCAGCTGGAATAGCGGCTCAATCGGATACGCGGACTCCGTGAAGGGAAGGT TCACCATTTCCCGCGACAACGCCAAGAACTCCCTGTACTTGCAAATGAACAGC CTCCGGGATGAGGACACTGCCGTGTACTACTGCGCCCGCGTCGGAAAAGCTGT GCCCGACGTCTGGGGCCAGGGAACCACTGTGACCGTGTCCAGCGGCGGGGGTG GATCGGGCGGTGGAGGGTCCGGTGGAGGGGGCTCAGATATTGTGATGACCCAG ACCCCCTCGTCCCTGTCCGCCTCGGTCGGCGACCGCGTGACTATCACATGTAG AGCCTCGCAGAGCATCTCCAGCTACCTGAACTGGTATCAGCAGAAGCCGGGGA AGGCCCCGAAGCTCCTGATCTACGCGGCATCATCACTGCAATCGGGAGTGCCG AGCCGGTTTTCCGGGTCCGGCTCCGGCACCGACTTCACGCTGACCATTTCTTC CCTGCAACCCGAGGACTTCGCCACTTACTACTGCCAGCAGTCCTACTCCACCC CTTACTCCTTCGGCCAAGGAACCAGGCTGGAAATCAAGACCACTACCCCAGCA CCGAGGCCACCCACCCCGGCTCCTACCATCGCCTCCCAGCCTCTGTCCCTGCG TCCGGAGGCATGTAGACCCGCAGCTGGTGGGGCCGTGCATACCCGGGGTCTTG ACTTCGCCTGCGATATCTACATTTGGGCCCCTCTGGCTGGTACTTGCGGGGTC CTGCTGCTTTCACTCGTGATCACTCTTTACTGTAAGCGCGGTCGGAAGAAGCT GCTGTACATCTTTAAGCAACCCTTCATGAGGCCTGTGCAGACTACTCAAGAGG AGGACGGCTGTTCATGCCGGTTCCCAGAGGAGGAGGAAGGCGGCTGCGAACTG CGCGTGAAATTCAGCCGCAGCGCAGATGCTCCAGCCTACAAGCAGGGGCAGAA CCAGCTCTACAACGAACTCAATCTTGGTCGGAGAGAGGAGTACGACGTGCTGG ACAAGCGGAGAGGACGGGACCCAGAAATGGGCGGGAAGCCGCGCAGAAAGAAT CCCCAAGAGGGCCTGTACAACGAGCTCCAAAAGGATAAGATGGCAGAAGCCTA TAGCGAGATTGGTATGAAAGGGGAACGCAGAAGAGGCAAAGGCCACGACGGAC TGTACCAGGGACTCAGCACCGCCACCAAGGACACCTATGACGCTCTTCACATG CAGGCCCTGCCGCCTCGG
BCMA_EBB-C1979-C12		
BCMA_EBB-C1979-C12-aa ScFv domain	142	EVQLVESGGGLVQPGRSLRLSCTASGFTFDDYAMHWVRQRPGKGLEWVASINW KGNSLAYGDSVKGRFAISRDNAKNTVFLQMNSLRTEDTAVYYCASHQGVAYYN YAMDVWGRGTLVTVSSGGGGSGGGGSGGGGSEIVLTQSPGTLSLSPGERATLS CRATQSIGSSFLAWYQQRPGQAPRLLIYGASQRATGIPDRFSGRGSGTDFTLT ISRVEPEDSAVYYCQHYESSPSWTFGQGTKVEIK
BCMA_EBB-C1979-C12 - nt ScFv domain	163	GAAGTGCAGCTCGTGGAGAGCGGGGGAGGATTGGTGCAGCCCGGAAGGTCCCT GCGGCTCTCCTGCACTGCGTCTGGCTTCACCTTCGACGACTACGCGATGCACT GGGTCAGACAGCGCCCGGGAAAGGGCCTGGAATGGGTCGCCTCAATCAACTGG AAGGGAAACTCCCTGGCCTATGGCGACAGCGTGAAGGGCCGCTTCGCCATTTC GCGCGACAACGCCAAGAACACCGTGTTTCTGCAAATGAATTCCCTGCGGACCG AGGATACCGCTGTGTACTACTGCGCCAGCCACCAGGGCGTGGCATACTATAAC TACGCCATGGACGTGTGGGGAAGAGGGACGCTCGTCACCGTGTCCTCCGGGGG CGGTGGATCGGGTGGAGGAGGAAGCGGTGGCGGGGGCAGCGAAATCGTGCTGA CTCAGAGCCCGGGAACTCTTTCACTGTCCCCGGGAGAACGGGCCACTCTCTCG TGCCGGGCCACCCAGTCCATCGGCTCCTCCTTCCTTGCCTGGTACCAGCAGAG GCCAGGACAGGCGCCCCGCCTGCTGATCTACGGTGCTTCCCAACGCGCCACTG GCATTCCTGACCGGTTCAGCGGCAGAGGGTCGGGAACCGATTTCACACTGACC ATTTCCCGGGTGGAGCCCGAAGATTCGGCAGTCTACTACTGTCAGCATTACGA

		GTCCTCCCCTTCATGGACCTTCGGTCAAGGGACCAAAGTGGAGATCAAG
BCMA_EBB-C1979-C12 - aa VH	184	EVQLVESGGGLVQPGRSLRLSCTASGFTFDDYAMHWVRQRPGKGLEWVASINW KGNSLAYGDSVKGRFAISRDNAKNTVFLQMNSLRTEDTAVYYCASHQGVAYYN YAMDVWGRGTLVTVSS
BCMA_EBB-C1979-C12 - aa VL	205	EIVLTQSPGTLSLSPGERATLSCRATQSIGSSFLAWYQQRPGQAPRLLIYGAS QRATGIPDRFSGRGSGTDFTLTISRVEPEDSAVYYCQHYESSPSWTFGQGTKV EIK
BCMA_EBB-C1979-C12 - aa Full CART	226	MALPVTALLLPLALLLHAARPEVQLVESGGGLVQPGRSLRLSCTASGFTFDDY AMHWVRQRPGKGLEWVASINWKGNSLAYGDSVKGRFAISRDNAKNTVFLQMNS LRTEDTAVYYCASHQGVAYYNYAMDVWGRGTLVTVSSGGGGSGGGGSGGGGSE IVLTQSPGTLSLSPGERATLSCRATQSIGSSFLAWYQQRPGQAPRLLIYGASQ RATGIPDRFSGRGSGTDFTLTISRVEPEDSAVYYCQHYESSPSWTFGQGTKVE IKTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYIWAP LAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEE EEGGCELRVKFSRSADAPAYKQGQNQLYNELNLGRREEYDVLDKRRGRDPEMG GKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKD TYDALHMQALPPR
BCMA_EBB-C1979-C12 - nt Full CART	247	ATGGCCCTCCCTGTCACCGCCCTGCTGCTTCCGCTGGCTCTTCTGCTCCACGC CGCTCGGCCCGAAGTGCAGCTCGTGGAGAGCGGGGGAGGATTGGTGCAGCCCG GAAGGTCCCTGCGGCTCTCCTGCACTGCGTCTGGCTTCACCTTCGACGACTAC GCGATGCACTGGGTCAGACAGCGCCCGGGAAAGGGCCTGGAATGGGTCGCCTC AATCAACTGGAAGGGAAACTCCCTGGCCTATGGCGACAGCGTGAAGGGCCGCT TCGCCATTTCGCGCGACAACGCCAAGAACACCGTGTTTCTGCAAATGAATTCC CTGCGGACCGAGGATACCGCTGTGTACTACTGCGCCAGCCACCAGGGCGTGGC ATACTATAACTACGCCATGGACGTGTGGGGAAGAGGGACGCTCGTCACCGTGT CCTCCGGGGGCGGTGGATCGGGTGGAGGAGGAAGCGGTGGCGGGGGCAGCGAA ATCGTGCTGACTCAGAGCCCGGGAACTCTTTCACTGTCCCCGGGAGAACGGGC CACTCTCTCGTGCCGGGCCACCCAGTCCATCGGCTCCTCCTTCCTTGCCTGGT ACCAGCAGAGGCCAGGACAGGCGCCCCGCCTGCTGATCTACGGTGCTTCCCAA CGCGCCACTGGCATTCCTGACCGGTTCAGCGGCAGAGGGTCGGGAACCGATTT CACACTGACCATTTCCCGGGTGGAGCCCGAAGATTCGGCAGTCTACTACTGTC AGCATTACGAGTCCTCCCCTTCATGGACCTTCGGTCAAGGGACCAAAGTGGAG ATCAAGACCACTACCCCAGCACCGAGGCCACCCACCCCGGCTCCTACCATCGC CTCCCAGCCTCTGTCCCTGCGTCCGGAGGCATGTAGACCCGCAGCTGGTGGGG CCGTGCATACCCGGGGTCTTGACTTCGCCTGCGATATCTACATTTGGGCCCCT CTGGCTGGTACTTGCGGGGTCCTGCTGCTTTCACTCGTGATCACTCTTTACTG TAAGCGCGGTCGGAAGAAGCTGCTGTACATCTTTAAGCAACCCTTCATGAGGC CTGTGCAGACTACTCAAGAGGAGGACGGCTGTTCATGCCGGTTCCCAGAGGAG GAGGAAGGCGGCTGCGAACTGCGCGTGAAATTCAGCCGCAGCGCAGATGCTCC AGCCTACAAGCAGGGGCAGAACCAGCTCTACAACGAACTCAATCTTGGTCGGA GAGAGGAGTACGACGTGCTGGACAAGCGGAGAGGACGGGACCCAGAAATGGGC GGGAAGCCGCGCAGAAAGAATCCCCAAGAGGGCCTGTACAACGAGCTCCAAAA GGATAAGATGGCAGAAGCCTATAGCGAGATTGGTATGAAAGGGGAACGCAGAA GAGGCAAAGGCCACGACGGACTGTACCAGGGACTCAGCACCGCCACCAAGGAC ACCTATGACGCTCTTCACATGCAGGCCCTGCCGCCTCGG
BCMA_EBB-C1980-G4		
BCMA_EBB-C1980-G4- aa ScFv domain	143	EVQLVESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPGKGLEWVSAISG SGGSTYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAKVVRDGMDV WGQGTTVTVSSGGGGSGGGGSGGGGSEIVLTQSPATLSLSPGERATLSCRASQ SVSSSYLAWYQQKPGQAPRLLIYGASSRATGIPDRFSGNGSGTDFTLTISRLE

		PEDFAVYYCQQYGSPPRFTFGPGTKVDIK
BCMA_EBB-C1980-G4- nt ScFv domain	164	GAGGTGCAGTTGGTCGAAAGCGGGGGCGGGCTTGTGCAGCCTGGCGGATCACT GCGGCTGTCCTGCGCGGCATCAGGCTTCACGTTTTCTTCCTACGCCATGTCCT GGGTGCGCCAGGCCCCTGGAAAGGGACTGGAATGGGTGTCCGCGATTTCGGGG TCCGGCGGGAGCACCTACTACGCCGATTCCGTGAAGGGCCGCTTCACTATCTC GCGGGACAACTCCAAGAACACCCTCTACCTCCAAATGAATAGCCTGCGGGCCG AGGATACCGCCGTCTACTATTGCGCTAAGGTCGTGCGCGACGGAATGGACGTG TGGGGACAGGGTACCACCGTGACAGTGTCCTCGGGGGGAGGCGGTAGCGGCGG AGGAGGAAGCGGTGGTGGAGGTTCCGAGATTGTGCTGACTCAATCACCCGCGA CCCTGAGCCTGTCCCCCGGCGAAAGGGCCACTCTGTCCTGTCGGGCCAGCCAA TCAGTCTCCTCCTCGTACCTGGCCTGGTACCAGCAGAAGCCAGGACAGGCTCC GAGACTCCTTATCTATGGCGCATCCTCCCGCGCCACCGGAATCCCGGATAGGT TCTCGGGAAACGGATCGGGGACCGACTTCACTCTCACCATCTCCCGGCTGGAA CCGGAGGACTTCGCCGTGTACTACTGCCAGCAGTACGGCAGCCCGCCTAGATT CACTTTCGGCCCCGGCACCAAAGTGGACATCAAG
BCMA_EBB-C1980-G4- aa VH	185	EVQLVESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPGKGLEWVSAISG SGGSTYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAKVVRDGMDV WGQGTTVTVSS
BCMA_EBB-C1980-G4- aa VL	206	EIVLTQSPATLSLSPGERATLSCRASQSVSSSYLAWYQQKPGQAPRLLIYGAS SRATGIPDRFSGNGSGTDFTLTISRLEPEDFAVYYCQQYGSPPRFTFGPGTKV DIK
BCMA_EBB-C1980-G4- aa Full CART	227	MALPVTALLLPLALLLHAARPEVQLVESGGGLVQPGGSLRLSCAASGFTFSSY AMSWVRQAPGKGLEWVSAISGSGGSTYYADSVKGRFTISRDNSKNTLYLQMNS LRAEDTAVYYCAKVVRDGMDVWGQGTTVTVSSGGGGSGGGGSGGGGSEIVLTQ SPATLSLSPGERATLSCRASQSVSSSYLAWYQQKPGQAPRLLIYGASSRATGI PDRFSGNGSGTDFTLTISRLEPEDFAVYYCQQYGSPPRFTFGPGTKVDIKTTT PAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYIWAPLAGTC GVLLLSLVITLYCKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGC ELRVKFSRSADAPAYKQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRR KNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDAL HMQALPPR
BCMA_EBB-C1980-G4- nt Full CART	248	ATGGCCCTCCCTGTCACCGCCCTGCTGCTTCCGCTGGCTCTTCTGCTCCACGC CGCTCGGCCCGAGGTGCAGTTGGTCGAAAGCGGGGGCGGGCTTGTGCAGCCTG GCGGATCACTGCGGCTGTCCTGCGCGGCATCAGGCTTCACGTTTTCTTCCTAC GCCATGTCCTGGGTGCGCCAGGCCCCTGGAAAGGGACTGGAATGGGTGTCCGC GATTTCGGGGTCCGGCGGGAGCACCTACTACGCCGATTCCGTGAAGGGCCGCT TCACTATCTCGCGGGACAACTCCAAGAACACCCTCTACCTCCAAATGAATAGC CTGCGGGCCGAGGATACCGCCGTCTACTATTGCGCTAAGGTCGTGCGCGACGG AATGGACGTGTGGGGACAGGGTACCACCGTGACAGTGTCCTCGGGGGGAGGCG GTAGCGGCGGAGGAGGAAGCGGTGGTGGAGGTTCCGAGATTGTGCTGACTCAA TCACCCGCGACCCTGAGCCTGTCCCCCGGCGAAAGGGCCACTCTGTCCTGTCG GGCCAGCCAATCAGTCTCCTCCTCGTACCTGGCCTGGTACCAGCAGAAGCCAG GACAGGCTCCGAGACTCCTTATCTATGGCGCATCCTCCCGCGCCACCGGAATC CCGGATAGGTTCTCGGGAAACGGATCGGGGACCGACTTCACTCTCACCATCTC CCGGCTGGAACCGGAGGACTTCGCCGTGTACTACTGCCAGCAGTACGGCAGCC CGCCTAGATTCACTTTCGGCCCCGGCACCAAAGTGGACATCAAGACCACTACC CCAGCACCGAGGCCACCCACCCCGGCTCCTACCATCGCCTCCCAGCCTCTGTC CCTGCGTCCGGAGGCATGTAGACCCGCAGCTGGTGGGGCCGTGCATACCCGGG GTCTTGACTTCGCCTGCGATATCTACATTTGGGCCCCTCTGGCTGGTACTTGC GGGGTCCTGCTGCTTTCACTCGTGATCACTCTTTACTGTAAGCGCGGTCGGAA GAAGCTGCTGTACATCTTTAAGCAACCCTTCATGAGGCCTGTGCAGACTACTC AAGAGGAGGACGGCTGTTCATGCCGGTTCCCAGAGGAGGAGGAAGGCGGCTGC

		GAACTGCGCGTGAAATTCAGCCGCAGCGCAGATGCTCCAGCCTACAAGCAGGG GCAGAACCAGCTCTACAACGAACTCAATCTTGGTCGGAGAGAGGAGTACGACG TGCTGGACAAGCGGAGAGGACGGGACCCAGAAATGGGCGGGAAGCCGCGCAGA AAGAATCCCCAAGAGGGCCTGTACAACGAGCTCCAAAAGGATAAGATGGCAGA AGCCTATAGCGAGATTGGTATGAAAGGGGAACGCAGAAGAGGCAAAGGCCACG ACGGACTGTACCAGGGACTCAGCACCGCCACCAAGGACACCTATGACGCTCTT CACATGCAGGCCCTGCCGCCTCGG
BCMA_EBB-C1980-D2		
BCMA_EBB-C1980-D2- aa ScFv domain	144	EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPGKGLEWVSAISG SGGSTYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAKIPQTGTFD YWGQGTLVTVSSGGGGSGGGGSGGGGSEIVLTQSPGTLSLSPGERATLSCRAS QSVSSSYLAWYQQRPGQAPRLLIYGASSRATGIPDRFSGSGSGTDFTLTISRL EPEDFAVYYCQHYGSSPSWTFGQGTRLEIK
BCMA_EBB-C1980-D2- nt ScFv domain	165	GAAGTGCAGCTGCTGGAGTCCGGCGGTGGATTGGTGCAACCGGGGGGATCGCT CAGACTGTCCTGTGCGGCGTCAGGCTTCACCTTCTCGAGCTACGCCATGTCAT GGGTCAGACAGGCCCCTGGAAAGGGTCTGGAATGGGTGTCCGCCATTTCCGGG AGCGGGGGATCTACATACTACGCCGATAGCGTGAAGGGCCGCTTCACCATTTC CCGGGACAACTCCAAGAACACTCTCTATCTGCAAATGAACTCCCTCCGCGCTG AGGACACTGCCGTGTACTACTGCGCCAAAATCCCTCAGACCGGCACCTTCGAC TACTGGGGACAGGGGACTCTGGTCACCGTCAGCAGCGGTGGCGGAGGTTCGGG GGGAGGAGGAAGCGGCGGCGGAGGGTCCGAGATTGTGCTGACCCAGTCACCCG GCACTTTGTCCCTGTCGCCTGGAGAAAGGGCCACCCTTTCCTGCCGGGCATCC CAATCCGTGTCCTCCTCGTACCTGGCCTGGTACCAGCAGAGGCCCGGACAGGC CCCACGGCTTCTGATCTACGGAGCAAGCAGCCGCGCGACCGGTATCCCGGACC GGTTTTCGGGCTCGGGCTCAGGAACTGACTTCACCCTCACCATCTCCCGCCTG GAACCCGAAGATTTCGCTGTGTATTACTGCCAGCACTACGGCAGCTCCCCGTC CTGGACGTTCGGCCAGGGAACTCGGCTGGAGATCAAG
BCMA_EBB-C1980-D2- aa VH	186	EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPGKGLEWVSAISG SGGSTYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAKIPQTGTFD YWGQGTLVTVSS
BCMA_EBB-C1980-D2- aa VL	207	EIVLTQSPGTLSLSPGERATLSCRASQSVSSSYLAWYQQRPGQAPRLLIYGAS SRATGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQHYGSSPSWTFGQGTRL EIK
BCMA_EBB-C1980-D2- aa Full CART	228	MALPVTALLLPLALLLHAARPEVQLLESGGGLVQPGGSLRLSCAASGFTFSSY AMSWVRQAPGKGLEWVSAISGSGGSTYYADSVKGRFTISRDNSKNTLYLQMNS LRAEDTAVYYCAKIPQTGTFDYWGQGTLVTVSSGGGGSGGGGSGGGGSEIVLT QSPGTLSLSPGERATLSCRASQSVSSSYLAWYQQRPGQAPRLLIYGASSRATG IPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQHYGSSPSWTFGQGTRLEIKTT TPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYIWAPLAGT CGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGG CELRVKFSRSADAPAYKQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPR RKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDA LHMQALPPR
BCMA_EBB-C1980-D2- nt Full CART	249	ATGGCCCTCCCTGTCACCGCCCTGCTGCTTCCGCTGGCTCTTCTGCTCCACGC CGCTCGGCCCGAAGTGCAGCTGCTGGAGTCCGGCGGTGGATTGGTGCAACCGG GGGGATCGCTCAGACTGTCCTGTGCGGCGTCAGGCTTCACCTTCTCGAGCTAC GCCATGTCATGGGTCAGACAGGCCCCTGGAAAGGGTCTGGAATGGGTGTCCGC CATTTCCGGGAGCGGGGGATCTACATACTACGCCGATAGCGTGAAGGGCCGCT TCACCATTTCCCGGGACAACTCCAAGAACACTCTCTATCTGCAAATGAACTCC CTCCGCGCTGAGGACACTGCCGTGTACTACTGCGCCAAAATCCCTCAGACCGG CACCTTCGACTACTGGGGACAGGGGACTCTGGTCACCGTCAGCAGCGGTGGCG GAGGTTCGGGGGGAGGAGGAAGCGGCGGCGGAGGGTCCGAGATTGTGCTGACC

		CAGTCACCCGGCACTTTGTCCCTGTCGCCTGGAGAAAGGGCCACCCTTTCCTG CCGGGCATCCCAATCCGTGTCCTCCTCGTACCTGGCCTGGTACCAGCAGAGGC CCGGACAGGCCCCACGGCTTCTGATCTACGGAGCAAGCAGCCGCGCGACCGGT ATCCCGGACCGGTTTTCGGGCTCGGGCTCAGGAACTGACTTCACCCTCACCAT CTCCCGCCTGGAACCCGAAGATTTCGCTGTGTATTACTGCCAGCACTACGGCA GCTCCCCGTCCTGGACGTTCGGCCAGGGAACTCGGCTGGAGATCAAGACCACT ACCCCAGCACCGAGGCCACCCACCCCGGCTCCTACCATCGCCTCCCAGCCTCT GTCCCTGCGTCCGGAGGCATGTAGACCCGCAGCTGGTGGGGCCGTGCATACCC GGGGTCTTGACTTCGCCTGCGATATCTACATTTGGGCCCCTCTGGCTGGTACT TGCGGGGTCCTGCTGCTTTCACTCGTGATCACTCTTTACTGTAAGCGCGGTCG GAAGAAGCTGCTGTACATCTTTAAGCAACCCTTCATGAGGCCTGTGCAGACTA CTCAAGAGGAGGACGGCTGTTCATGCCGGTTCCCAGAGGAGGAGGAAGGCGGC TGCGAACTGCGCGTGAAATTCAGCCGCAGCGCAGATGCTCCAGCCTACAAGCA GGGGCAGAACCAGCTCTACAACGAACTCAATCTTGGTCGGAGAGAGGAGTACG ACGTGCTGGACAAGCGGAGAGGACGGGACCCAGAAATGGGCGGGAAGCCGCGC AGAAAGAATCCCCAAGAGGGCCTGTACAACGAGCTCCAAAAGGATAAGATGGC AGAAGCCTATAGCGAGATTGGTATGAAAGGGGAACGCAGAAGAGGCAAAGGCC ACGACGGACTGTACCAGGGACTCAGCACCGCCACCAAGGACACCTATGACGCT CTTCACATGCAGGCCCTGCCGCCTCGG
BCMA_EBB-C1978-A10		
BCMA_EBB-C1978-A10-aa ScFv domain	145	EVQLVETGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPGKGLEWVSAISG SGGSTYYADSVKGRFTMSRENDKNSVFLQMNSLRVEDTGVYYCARANYKRELR YYYGMDVWGQGTMVTVSSGGGGSGGGGSGGGGSEIVMTQSPGTLSLSPGESAT LSCRASQRVASNYLAWYQHKPGQAPSLLISGASSRATGVPDRFSGSGSGTDFT LAISRLEPEDSAVYYCQHYDSSPSWTFGQGTKVEIK
BCMA_EBB-C1978-A10-nt ScFv domain	166	GAAGTGCAACTGGTGGAAACCGGTGGAGGACTCGTGCAGCCTGGCGGCAGCCT CCGGCTGAGCTGCGCCGCTTCGGGATTCACCTTTTCCTCCTACGCGATGTCTT GGGTCAGACAGGCCCCCGGAAAGGGGCTGGAATGGGTGTCAGCCATCTCCGGC TCCGGCGGATCAACGTACTACGCCGACTCCGTGAAAGGCCGGTTCACCATGTC GCGCGAGAATGACAAGAACTCCGTGTTCCTGCAAATGAACTCCCTGAGGGTGG AGGACACCGGAGTGTACTATTGTGCGCGCGCCAACTACAAGAGAGAGCTGCGG TACTACTACGGAATGGACGTCTGGGGACAGGGAACTATGGTGACCGTGTCATC CGGTGGAGGGGGAAGCGGCGGTGGAGGCAGCGGGGGCGGGGGTTCAGAAATTG TCATGACCCAGTCCCCGGGAACTCTTTCCCTCTCCCCCGGGGAATCCGCGACT TTGTCCTGCCGGGCCAGCCAGCGCGTGGCCTCGAACTACCTCGCATGGTACCA GCATAAGCCAGGCCAAGCCCCTTCCCTGCTGATTTCCGGGGCTAGCAGCCGCG CCACTGGCGTGCCGGATAGGTTCTCGGGAAGCGGCTCGGGTACCGATTTCACC CTGGCAATCTCGCGGCTGGAACCGGAGGATTCGGCCGTGTACTACTGCCAGCA CTATGACTCATCCCCCTCCTGGACATTCGGACAGGGCACCAAGGTCGAGATCA AG
BCMA_EBB-C1978-A10-aa VH	187	EVQLVETGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPGKGLEWVSAISG SGGSTYYADSVKGRFTMSRENDKNSVFLQMNSLRVEDTGVYYCARANYKRELR YYYGMDVWGQGTMVTVSS
BCMA_EBB-C1978-A10-aa VL	208	EIVMTQSPGTLSLSPGESATLSCRASQRVASNYLAWYQHKPGQAPSLLISGAS SRATGVPDRFSGSGSGTDFTLAISRLEPEDSAVYYCQHYDSSPSWTFGQGTKV EIK
BCMA_EBB-C1978-A10-aa Full CART	229	MALPVTALLLPLALLLHAARPEVQLVETGGGLVQPGGSLRLSCAASGFTFSSY AMSWVRQAPGKGLEWVSAISGSGGSTYYADSVKGRFTMSRENDKNSVFLQMNS LRVEDTGVYYCARANYKRELRYYYGMDVWGQGTMVTVSSGGGGSGGGGSGGGG SEIVMTQSPGTLSLSPGESATLSCRASQRVASNYLAWYQHKPGQAPSLLISGA

		SSRATGVPDRFSGSGSGTDFTLAISRLEPEDSAVYYCQHYDSSPSWTFGQGTK VEIKTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYIW APLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFP EEEEGGCELRVKFSRSADAPAYKQGQNQLYNELNLGRREEYDVLDKRRGRDPE MGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTAT KDTYDALHMQALPPR
BCMA_EBB-C1978-A10-nt Full CART	250	ATGGCCCTCCCTGTCACCGCCCTGCTGCTTCCGCTGGCTCTTCTGCTCCACGC CGCTCGGCCCGAAGTGCAACTGGTGGAAACCGGTGGAGGACTCGTGCAGCCTG GCGGCAGCCTCCGGCTGAGCTGCGCCGCTTCGGGATTCACCTTTTCCTCCTAC GCGATGTCTTGGGTCAGACAGGCCCCCGGAAAGGGGCTGGAATGGGTGTCAGC CATCTCCGGCTCCGGCGGATCAACGTACTACGCCGACTCCGTGAAAGGCCGGT TCACCATGTCGCGCGAGAATGACAAGAACTCCGTGTTCCTGCAAATGAACTCC CTGAGGGTGGAGGACACCGGAGTGTACTATTGTGCGCGCGCCAACTACAAGAG AGAGCTGCGGTACTACTACGGAATGGACGTCTGGGGACAGGGAACTATGGTGA CCGTGTCATCCGGTGGAGGGGGAAGCGGCGGTGGAGGCAGCGGGGGCGGGGGT TCAGAAATTGTCATGACCCAGTCCCCGGGAACTCTTTCCCTCTCCCCCGGGGA ATCCGCGACTTTGTCCTGCCGGGCCAGCCAGCGCGTGGCCTCGAACTACCTCG CATGGTACCAGCATAAGCCAGGCCAAGCCCCTTCCCTGCTGATTTCCGGGGCT AGCAGCCGCGCCACTGGCGTGCCGGATAGGTTCTCGGGAAGCGGCTCGGGTAC CGATTTCACCCTGGCAATCTCGCGGCTGGAACCGGAGGATTCGGCCGTGTACT ACTGCCAGCACTATGACTCATCCCCCTCCTGGACATTCGGACAGGGCACCAAG GTCGAGATCAAGACCACTACCCCAGCACCGAGGCCACCCACCCCGGCTCCTAC CATCGCCTCCCAGCCTCTGTCCCTGCGTCCGGAGGCATGTAGACCCGCAGCTG GTGGGGCCGTGCATACCCGGGGTCTTGACTTCGCCTGCGATATCTACATTTGG GCCCCTCTGGCTGGTACTTGCGGGGTCCTGCTGCTTTCACTCGTGATCACTCT TTACTGTAAGCGCGGTCGGAAGAAGCTGCTGTACATCTTTAAGCAACCCTTCA TGAGGCCTGTGCAGACTACTCAAGAGGAGGACGGCTGTTCATGCCGGTTCCCA GAGGAGGAGGAAGGCGGCTGCGAACTGCGCGTGAAATTCAGCCGCAGCGCAGA TGCTCCAGCCTACAAGCAGGGGCAGAACCAGCTCTACAACGAACTCAATCTTG GTCGGAGAGAGGAGTACGACGTGCTGGACAAGCGGAGAGGACGGGACCCAGAA ATGGGCGGGAAGCCGCGCAGAAAGAATCCCCAAGAGGGCCTGTACAACGAGCT CCAAAAGGATAAGATGGCAGAAGCCTATAGCGAGATTGGTATGAAAGGGGAAC GCAGAAGAGGCAAAGGCCACGACGGACTGTACCAGGGACTCAGCACCGCCACC AAGGACACCTATGACGCTCTTCACATGCAGGCCCTGCCGCCTCGG
BCMA_EBB-C1978-D4		
BCMA_EBB-C1978-D4- aa ScFv domain	146	EVQLLETGGGLVQPGGSLRLSCAASGFSFSSYAMSWVRQAPGKGLEWVSAISG SGGSTYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAKALVGATGA FDIWGQGTLVTVSSGGGGSGGGGSGGGGSEIVLTQSPGTLSLSPGERATLSCR ASQSLSSNFLAWYQQKPGQAPGLLIYGASNWATGTPDRFSGSGSGTDFTLTIT RLEPEDFAVYYCQYYGTSPMYTFGQGTKVEIK
BCMA_EBB-C1978-D4- nt ScFv domain	167	GAAGTGCAGCTGCTCGAAACCGGTGGAGGGCTGGTGCAGCCAGGGGGCTCCCT GAGGCTTTCATGCGCCGCTAGCGGATTCTCCTTCTCCTCTTACGCCATGTCGT GGGTCCGCCAAGCCCCTGGAAAAGGCCTGGAATGGGTGTCCGCGATTTCCGGG AGCGGAGGTTCGACCTATTACGCCGACTCCGTGAAGGGCCGCTTTACCATCTC CCGGGATAACTCCAAGAACACTCTGTACCTCCAAATGAACTCGCTGAGAGCCG AGGACACCGCCGTGTATTACTGCGCGAAGGCGCTGGTCGGCGCGACTGGGGCA TTCGACATCTGGGGACAGGGAACTCTTGTGACCGTGTCGAGCGGAGGCGGCGG CTCCGGCGGAGGAGGGAGCGGGGGCGGTGGTTCCGAAATCGTGTTGACTCAGT CCCCGGGAACCCTGAGCTTGTCACCCGGGGAGCGGGCCACTCTCTCCTGTCGC GCCTCCCAATCGCTCTCATCCAATTTCCTGGCCTGGTACCAGCAGAAGCCCGG ACAGGCCCCGGGCCTGCTCATCTACGGCGCTTCAAACTGGGCAACGGGAACCC CTGATCGGTTCAGCGGAAGCGGATCGGGTACTGACTTTACCCTGACCATCACC

		AGACTGGAACCGGAGGACTTCGCCGTGTACTACTGCCAGTACTACGGCACCTC CCCCATGTACACATTCGGACAGGGTACCAAGGTCGAGATTAAG
BCMA_EBB-C1978-D4- aa VH	188	EVQLLETGGGLVQPGGSLRLSCAASGFSFSSYAMSWVRQAPGKGLEWVSAISG SGGSTYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAKALVGATGA FDIWGQGTLVTVSS
BCMA_EBB-C1978-D4- aa VL	209	EIVLTQSPGTLSLSPGERATLSCRASQSLSSNFLAWYQQKPGQAPGLLIYGAS NWATGTPDRFSGSGSGTDFTLTITRLEPEDFAVYYCQYYGTSPMYTFGQGTKV EIK
BCMA_EBB-C1978-D4- aa Full CART	230	MALPVTALLLPLALLLHAARPEVQLLETGGGLVQPGGSLRLSCAASGFSFSSY AMSWVRQAPGKGLEWVSAISGSGGSTYYADSVKGRFTISRDNSKNTLYLQMNS LRAEDTAVYYCAKALVGATGAFDIWGQGTLVTVSSGGGGSGGGGSGGGGSEIV LTQSPGTLSLSPGERATLSCRASQSLSSNFLAWYQQKPGQAPGLLIYGASNWA TGTPDRFSGSGSGTDFTLTITRLEPEDFAVYYCQYYGTSPMYTFGQGTKVEIK TTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYIWAPLA GTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEE GGCELRVKFSRSADAPAYKQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGK PRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTY DALHMQALPPR
BCMA_EBB-C1978-D4- nt Full CART	251	ATGGCCCTCCCTGTCACCGCCCTGCTGCTTCCGCTGGCTCTTCTGCTCCACGC CGCTCGGCCCGAAGTGCAGCTGCTCGAAACCGGTGGAGGGCTGGTGCAGCCAG GGGGCTCCCTGAGGCTTTCATGCGCCGCTAGCGGATTCTCCTTCTCCTCTTAC GCCATGTCGTGGGTCCGCCAAGCCCCTGGAAAAGGCCTGGAATGGGTGTCCGC GATTTCCGGGAGCGGAGGTTCGACCTATTACGCCGACTCCGTGAAGGGCCGCT TTACCATCTCCCGGGATAACTCCAAGAACACTCTGTACCTCCAAATGAACTCG CTGAGAGCCGAGGACACCGCCGTGTATTACTGCGCGAAGGCGCTGGTCGGCGC GACTGGGGCATTCGACATCTGGGGACAGGGAACTCTTGTGACCGTGTCGAGCG GAGGCGGCGGCTCCGGCGGAGGAGGGAGCGGGGGCGGTGGTTCCGAAATCGTG TTGACTCAGTCCCCGGGAACCCTGAGCTTGTCACCCGGGGAGCGGGCCACTCT CTCCTGTCGCGCCTCCCAATCGCTCTCATCCAATTTCCTGGCCTGGTACCAGC AGAAGCCCGGACAGGCCCCGGGCCTGCTCATCTACGGCGCTTCAAACTGGGCA ACGGGAACCCCTGATCGGTTCAGCGGAAGCGGATCGGGTACTGACTTTACCCT GACCATCACCAGACTGGAACCGGAGGACTTCGCCGTGTACTACTGCCAGTACT ACGGCACCTCCCCCATGTACACATTCGGACAGGGTACCAAGGTCGAGATTAAG ACCACTACCCCAGCACCGAGGCCACCCACCCCGGCTCCTACCATCGCCTCCCA GCCTCTGTCCCTGCGTCCGGAGGCATGTAGACCCGCAGCTGGTGGGGCCGTGC ATACCCGGGGTCTTGACTTCGCCTGCGATATCTACATTTGGGCCCCTCTGGCT GGTACTTGCGGGGTCCTGCTGCTTTCACTCGTGATCACTCTTTACTGTAAGCG CGGTCGGAAGAAGCTGCTGTACATCTTTAAGCAACCCTTCATGAGGCCTGTGC AGACTACTCAAGAGGAGGACGGCTGTTCATGCCGGTTCCCAGAGGAGGAGGAA GGCGGCTGCGAACTGCGCGTGAAATTCAGCCGCAGCGCAGATGCTCCAGCCTA CAAGCAGGGGCAGAACCAGCTCTACAACGAACTCAATCTTGGTCGGAGAGAGG AGTACGACGTGCTGGACAAGCGGAGAGGACGGGACCCAGAAATGGGCGGGAAG CCGCGCAGAAAGAATCCCCAAGAGGGCCTGTACAACGAGCTCCAAAAGGATAA GATGGCAGAAGCCTATAGCGAGATTGGTATGAAAGGGGAACGCAGAAGAGGCA AAGGCCACGACGGACTGTACCAGGGACTCAGCACCGCCACCAAGGACACCTAT GACGCTCTTCACATGCAGGCCCTGCCGCCTCGG
BCMA_EBB-C1980-A2		
BCMA_EBB-C1980-A2- aa ScFv domain	147	EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPGKGLEWVSAISG SGGSTYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCVLWFGEGFDP WGQGTLVTVSSGGGGSGGGGSGGGGSDIVLTQSPLSLPVTPGEPASISCRSSQ

		SLLHSNGYNYLDWYLQKPGQSPQLLIYLGSNRASGVPDRFSGSGSGTDFTLKI SRVEAEDVGVYYCMQALQTPLTFGGGTKVDIK
BCMA_EBB-C1980-A2- nt ScFv domain	168	GAAGTGCAGCTGCTTGAGAGCGGTGGAGGTCTGGTGCAGCCCGGGGGATCACT GCGCCTGTCCTGTGCCGCGTCCGGTTTCACTTTCTCCTCGTACGCCATGTCGT GGGTCAGACAGGCACCGGGAAAGGGACTGGAATGGGTGTCAGCCATTTCGGGT TCGGGGGGCAGCACCTACTACGCTGACTCCGTGAAGGGCCGGTTCACCATTTC CCGCGACAACTCCAAGAACACCTTGTACCTCCAAATGAACTCCCTGCGGGCCG AAGATACCGCCGTGTATTACTGCGTGCTGTGGTTCGGAGAGGGATTCGACCCG TGGGGACAAGGAACACTCGTGACTGTGTCATCCGGCGGAGGCGGCAGCGGTGG CGGCGGTTCCGGCGGCGGCGGATCTGACATCGTGTTGACCCAGTCCCCTCTGA GCCTGCCGGTCACTCCTGGCGAACCAGCCAGCATCTCCTGCCGGTCGAGCCAG TCCCTCCTGCACTCCAATGGGTACAACTACCTCGATTGGTATCTGCAAAAGCC GGGCCAGAGCCCCCAGCTGCTGATCTACCTTGGGTCAAACCGCGCTTCCGGGG TGCCTGATAGATTCTCCGGGTCCGGGAGCGGAACCGACTTTACCCTGAAAATC TCGAGGGTGGAGGCCGAGGACGTCGGAGTGTACTACTGCATGCAGGCGCTCCA GACTCCCCTGACCTTCGGAGGAGGAACGAAGGTCGACATCAAGA
BCMA_EBB-C1980-A2- aa VH	189	EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPGKGLEWVSAISG SGGSTYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCVLWFGEGFDP WGQGTLVTVSS
BCMA_EBB-C1980-A2- aa VL	210	DIVLTQSPLSLPVTPGEPASISCRSSQSLLHSNGYNYLDWYLQKPGQSPQLLI YLGSNRASGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCMQALQTPLTFGGG TKVDIK
BCMA_EBB-C1980-A2- aa Full CART	231	MALPVTALLLPLALLLHAARPEVQLLESGGGLVQPGGSLRLSCAASGFTFSSY AMSWVRQAPGKGLEWVSAISGSGGGSTYYADSVKGRFTISRDNSKNTLYLQMNS LRAEDTAVYYCVLWFGEGFDPWGQGTLVTVSSGGGGSGGGGSGGGGSDIVLTQ SPLSLPVTPGEPASISCRSSQSLLHSNGYNYLDWYLQKPGQSPQLLIYLGSNR ASGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCMQALQTPLTFGGGTKVDIK TTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYIWAPLA GTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEE GGCELRVKFSRSADAPAYKQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGK PRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTY DALHMQALPPR
BCMA_EBB-C1980-A2- nt Full CART	252	ATGGCCCTCCCTGTCACCGCCCTGCTGCTTCCGCTGGCTCTTCTGCTCCACGC CGCTCGGCCCGAAGTGCAGCTGCTTGAGAGCGGTGGAGGTCTGGTGCAGCCCG GGGGATCACTGCGCCTGTCCTGTGCCGCGTCCGGTTTCACTTTCTCCTCGTAC GCCATGTCGTGGGTCAGACAGGCACCGGGAAAGGGACTGGAATGGGTGTCAGC CATTTCGGGTTCGGGGGGCAGCACCTACTACGCTGACTCCGTGAAGGGCCGGT TCACCATTTCCCGCGACAACTCCAAGAACACCTTGTACCTCCAAATGAACTCC CTGCGGGCCGAAGATACCGCCGTGTATTACTGCGTGCTGTGGTTCGGAGAGGG ATTCGACCCGTGGGGACAAGGAACACTCGTGACTGTGTCATCCGGCGGAGGCG GCAGCGGTGGCGGCGGTTCCGGCGGCGGCGGATCTGACATCGTGTTGACCCAG TCCCCTCTGAGCCTGCCGGTCACTCCTGGCGAACCAGCCAGCATCTCCTGCCG GTCGAGCCAGTCCCTCCTGCACTCCAATGGGTACAACTACCTCGATTGGTATC TGCAAAAGCCGGGCCAGAGCCCCCAGCTGCTGATCTACCTTGGGTCAAACCGC GCTTCCGGGGTGCCTGATAGATTCTCCGGGTCCGGGAGCGGAACCGACTTTAC CCTGAAAATCTCGAGGGTGGAGGCCGAGGACGTCGGAGTGTACTACTGCATGC AGGCGCTCCAGACTCCCCTGACCTTCGGAGGAGGAACGAAGGTCGACATCAAG ACCACTACCCCAGCACCGAGGCCACCCACCCCGGCTCCTACCATCGCCTCCCA GCCTCTGTCCCTGCGTCCGGAGGCATGTAGACCCGCAGCTGGTGGGGCCGTGC ATACCCGGGGTCTTGACTTCGCCTGCGATATCTACATTTGGGCCCCTCTGGCT GGTACTTGCGGGGTCCTGCTGCTTTCACTCGTGATCACTCTTTACTGTAAGCG CGGTCGGAAGAAGCTGCTGTACATCTTTAAGCAACCCTTCATGAGGCCTGTGC

		AGACTACTCAAGAGGAGGACGGCTGTTCATGCCGGTTCCCAGAGGAGGAGGAA GGCGGCTGCGAACTGCGCGTGAAATTCAGCCGCAGCGCAGATGCTCCAGCCTA CAAGCAGGGGCAGAACCAGCTCTACAACGAACTCAATCTTGGTCGGAGAGAGG AGTACGACGTGCTGGACAAGCGGAGAGGACGGGACCCAGAAATGGGCGGGAAG CCGCGCAGAAAGAATCCCCAAGAGGGCCTGTACAACGAGCTCCAAAAGGATAA GATGGCAGAAGCCTATAGCGAGATTGGTATGAAAGGGGAACGCAGAAGAGGCA AAGGCCACGACGGACTGTACCAGGGACTCAGCACCGCCACCAAGGACACCTAT GACGCTCTTCACATGCAGGCCCTGCCGCCTCGG
BCMA_EBB-C1981-C3		
BCMA_EBB-C1981-C3- aa ScFv domain	148	QVQLVESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPGKGLEWVSAISG SGGSTYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAKVGYDSSGY YRDYYGMDVWGQGTTVTVSSGGGGSGGGGSGGGGSEIVLTQSPGTLSLSPGER ATLSCRASQSVSSSYLAWYQQKPGQAPRLLIYGTSSRATGISDRFSGSGSGTD FTLTISRLEPEDFAVYYCQHYGNSPPKFTFGPGTKLEIK
BCMA_EBB-C1981-C3- nt ScFv domain	169	CAAGTGCAGCTCGTGGAGTCAGGCGGAGGACTGGTGCAGCCCGGGGGCTCCCT GAGACTTTCCTGCGCGGCATCGGGTTTTACCTTCTCCTCCTATGCTATGTCCT GGGTGCGCCAGGCCCCGGGAAAGGGACTGGAATGGGTGTCCGCAATCAGCGGT AGCGGGGGCTCAACATACTACGCCGACTCCGTCAAGGGTCGCTTCACTATTTC CCGGGACAACTCCAAGAATACCCTGTACCTCCAAATGAACAGCCTCAGGGCCG AGGATACTGCCGTGTACTACTGCGCCAAAGTCGGATACGATAGCTCCGGTTAC TACCGGGACTACTACGGAATGGACGTGTGGGGACAGGGCACCACCGTGACCGT GTCAAGCGGCGGAGGCGGTTCAGGAGGGGGAGGCTCCGGCGGTGGAGGGTCCG AAATCGTCCTGACTCAGTCGCCTGGCACTCTGTCGTTGTCCCCGGGGGAGCGC GCTACCCTGTCGTGTCGGGCGTCGCAGTCCGTGTCGAGCTCCTACCTCGCGTG GTACCAGCAGAAGCCCGGACAGGCCCCTAGACTTCTGATCTACGGCACTTCTT CACGCGCCACCGGGATCAGCGACAGGTTCAGCGGCTCCGGCTCCGGGACCGAC TTCACCCTGACCATTAGCCGGCTGGAGCCTGAAGATTTCGCCGTGTATTACTG CCAACACTACGGAAACTCGCCGCCAAAGTTCACGTTCGGACCCGGAACCAAGC TGGAAATCAAG
BCMA_EBB-C1981-C3- aa VH	190	QVQLVESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPGKGLEWVSAISG SGGSTYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAKVGYDSSGY YRDYYGMDVWGQGTTVTVSS
BCMA_EBB-C1981-C3- aa VL	211	EIVLTQSPGTLSLSPGERATLSCRASQSVSSSYLAWYQQKPGQAPRLLIYGTS SRATGISDRFSGSGSGTDFTLTISRLEPEDFAVYYCQHYGNSPPKFTFGPGTK LEIK
BCMA_EBB-C1981-C3- aa Full CART	232	MALPVTALLLPLALLLHAARPQVQLVESGGGLVQPGGSLRLSCAASGFTFSSY AMSWVRQAPGKGLEWVSAISGSGGSTYYADSVKGRFTISRDNSKNTLYLQMNS LRAEDTAVYYCAKVGYDSSGYYRDYYGMDVWGQGTTVTVSSGGGGSGGGGSGG GGSEIVLTQSPGTLSLSPGERATLSCRASQSVSSSYLAWYQQKPGQAPRLLIY GTSSRATGISDRFSGSGSGTDFTLTISRLEPEDFAVYYCQHYGNSPPKFTFGP GTKLEIKTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDI YIWAPLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPVQTTQEEDGCSC RFPEEEEGGCELRVKFSRSADAPAYKQGQNQLYNELNLGRREEYDVLDKRRGR DPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLS

		TATKDTYDALHMQALPPR
BCMA_EBB-C1981-C3- nt Full CART	253	ATGGCCCTCCCTGTCACCGCCCTGCTGCTTCCGCTGGCTCTTCTGCTCCACGC CGCTCGGCCCCAAGTGCAGCTCGTGGAGTCAGGCGGAGGACTGGTGCAGCCCG GGGGCTCCCTGAGACTTTCCTGCGCGGCATCGGGTTTTACCTTCTCCTCCTAT GCTATGTCCTGGGTGCGCCAGGCCCCGGGAAAGGGACTGGAATGGGTGTCCGC AATCAGCGGTAGCGGGGGCTCAACATACTACGCCGACTCCGTCAAGGGTCGCT TCACTATTTCCCGGGACAACTCCAAGAATACCCTGTACCTCCAAATGAACAGC CTCAGGGCCGAGGATACTGCCGTGTACTACTGCGCCAAAGTCGGATACGATAG CTCCGGTTACTACCGGGACTACTACGGAATGGACGTGTGGGGACAGGGCACCA CCGTGACCGTGTCAAGCGGCGGAGGCGGTTCAGGAGGGGGAGGCTCCGGCGGT GGAGGGTCCGAAATCGTCCTGACTCAGTCGCCTGGCACTCTGTCGTTGTCCCC GGGGGAGCGCGCTACCCTGTCGTGTCGGGCGTCGCAGTCCGTGTCGAGCTCCT ACCTCGCGTGGTACCAGCAGAAGCCCGGACAGGCCCCTAGACTTCTGATCTAC GGCACTTCTTCACGCGCCACCGGGATCAGCGACAGGTTCAGCGGCTCCGGCTC CGGGACCGACTTCACCCTGACCATTAGCCGGCTGGAGCCTGAAGATTTCGCCG TGTATTACTGCCAACACTACGGAAACTCGCCGCCAAAGTTCACGTTCGGACCC GGAACCAAGCTGGAAATCAAGACCACTACCCCAGCACCGAGGCCACCCACCCC GGCTCCTACCATCGCCTCCCAGCCTCTGTCCCTGCGTCCGGAGGCATGTAGAC CCGCAGCTGGTGGGGCCGTGCATACCCGGGGTCTTGACTTCGCCTGCGATATC TACATTTGGGCCCCTCTGGCTGGTACTTGCGGGGTCCTGCTGCTTTCACTCGT GATCACTCTTTACTGTAAGCGCGGTCGGAAGAAGCTGCTGTACATCTTTAAGC AACCCTTCATGAGGCCTGTGCAGACTACTCAAGAGGAGGACGGCTGTTCATGC CGGTTCCCAGAGGAGGAGGAAGGCGGCTGCGAACTGCGCGTGAAATTCAGCCG CAGCGCAGATGCTCCAGCCTACAAGCAGGGGCAGAACCAGCTCTACAACGAAC TCAATCTTGGTCGGAGAGAGGAGTACGACGTGCTGGACAAGCGGAGAGGACGG GACCCAGAAATGGGCGGGAAGCCGCGCAGAAAGAATCCCCAAGAGGGCCTGTA CAACGAGCTCCAAAAGGATAAGATGGCAGAAGCCTATAGCGAGATTGGTATGA AAGGGGAACGCAGAAGAGGCAAAGGCCACGACGGACTGTACCAGGGACTCAGC ACCGCCACCAAGGACACCTATGACGCTCTTCACATGCAGGCCCTGCCGCCTCG G
BCMA_EBB-C1978-G4		
BCMA_EBB-C1978-G4- aa ScFv domain	149	EVQLVESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPGKGLEWVSAISG SGGSTYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAKMGWSSGYL GAFDIWGQGTTVTVSSGGGGSGGGGSGGGGSEIVLTQSPGTLSLSPGERATLS CRASQSVASSFLAWYQQKPGQAPRLLIYGASGRATGIPDRFSGSGSGTDFTLT ISRLEPEDFAVYYCQHYGGSPRLTFGGGTKVDIK
BCMA_EBB-C1978-G4- nt ScFv domain	170	GAAGTCCAACTGGTGGAGTCCGGGGGAGGGCTCGTGCAGCCCGGAGGCAGCCT TCGGCTGTCGTGCGCCGCCTCCGGGTTCACGTTCTCATCCTACGCGATGTCGT GGGTCAGACAGGCACCAGGAAAGGGACTGGAATGGGTGTCCGCCATTAGCGGC TCCGGCGGTAGCACCTACTATGCCGACTCAGTGAAGGGAAGGTTCACTATCTC CCGCGACAACAGCAAGAACACCCTGTACCTCCAAATGAACTCTCTGCGGGCCG AGGATACCGCGGTGTACTATTGCGCCAAGATGGGTTGGTCCAGCGGATACTTG GGAGCCTTCGACATTTGGGGACAGGGCACTACTGTGACCGTGTCCTCCGGGGG TGGCGGATCGGGAGGCGGCGGCTCGGGTGGAGGGGGTTCCGAAATCGTGTTGA CCCAGTCACCGGGAACCCTCTCGCTGTCCCCGGGAGAACGGGCTACACTGTCA TGTAGAGCGTCCCAGTCCGTGGCTTCCTCGTTCCTGGCCTGGTACCAGCAGAA GCCGGGACAGGCACCCCGCCTGCTCATCTACGGAGCCAGCGGCCGGGCGACCG GCATCCCTGACCGCTTCTCCGGTTCCGGCTCGGGCACCGACTTTACTCTGACC ATTAGCAGGCTTGAGCCCGAGGATTTTGCCGTGTACTACTGCCAACACTACGG GGGGAGCCCTCGCCTGACCTTCGGAGGCGGAACTAAGGTCGATATCAAAA
BCMA_EBB-C1978-G4- aa	191	EVQLVESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPGKGLEWVSAISG SGGSTYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAKMGWSSGYL

VH		GAFDIWGQGTTVTVSS
BCMA_EBB-C1978-G4- aa VL	212	EIVLTQSPGTLSLSPGERATLSCRASQSVASSFLAWYQQKPGQAPRLLIYGAS GRATGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQHYGGSPRLTFGGGTKV DIK
BCMA_EBB-C1978-G4- aa Full CART	233	MALPVTALLLPLALLLHAARPEVQLVESGGGLVQPGGSLRLSCAASGFTFSSY AMSWVRQAPGKGLEWVSAISGSGGSTYYADSVKGRFTISRDNSKNTLYLQMNS LRAEDTAVYYCAKMGWSSGYLGAFDIWGQGTTVTVSSGGGGSGGGGSGGGGSE IVLTQSPGTLSLSPGERATLSCRASQSVASSFLAWYQQKPGQAPRLLIYGASG RATGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQHYGGSPRLTFGGGTKVD IKTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYIWAP LAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEE EEGGCELRVKFSRSADAPAYKQGQNQLYNELNLGRREEYDVLDKRRGRDPEMG GKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKD TYDALHMQALPPR
BCMA_EBB-C1978-G4- nt Full CART	254	ATGGCCCTCCCTGTCACCGCCCTGCTGCTTCCGCTGGCTCTTCTGCTCCACGC CGCTCGGCCCGAAGTCCAACTGGTGGAGTCCGGGGGAGGGCTCGTGCAGCCCG GAGGCAGCCTTCGGCTGTCGTGCGCCGCCTCCGGGTTCACGTTCTCATCCTAC GCGATGTCGTGGGTCAGACAGGCACCAGGAAAGGGACTGGAATGGGTGTCCGC CATTAGCGGCTCCGGCGGTAGCACCTACTATGCCGACTCAGTGAAGGGAAGGT TCACTATCTCCCGCGACAACAGCAAGAACACCCTGTACCTCCAAATGAACTCT CTGCGGGCCGAGGATACCGCGGTGTACTATTGCGCCAAGATGGGTTGGTCCAG CGGATACTTGGGAGCCTTCGACATTTGGGGACAGGGCACTACTGTGACCGTGT CCTCCGGGGGTGGCGGATCGGGAGGCGGCGGCTCGGGTGGAGGGGGTTCCGAA ATCGTGTTGACCCAGTCACCGGGAACCCTCTCGCTGTCCCCGGGAGAACGGGC TACACTGTCATGTAGAGCGTCCCAGTCCGTGGCTTCCTCGTTCCTGGCCTGGT ACCAGCAGAAGCCGGGACAGGCACCCCGCCTGCTCATCTACGGAGCCAGCGGC CGGGCGACCGGCATCCCTGACCGCTTCTCCGGTTCCGGCTCGGGCACCGACTT TACTCTGACCATTAGCAGGCTTGAGCCCGAGGATTTTGCCGTGTACTACTGCC AACACTACGGGGGGAGCCCTCGCCTGACCTTCGGAGGCGGAACTAAGGTCGAT ATCAAAACCACTACCCCAGCACCGAGGCCACCCACCCCGGCTCCTACCATCGC CTCCCAGCCTCTGTCCCTGCGTCCGGAGGCATGTAGACCCGCAGCTGGTGGGG CCGTGCATACCCGGGGTCTTGACTTCGCCTGCGATATCTACATTTGGGCCCCT CTGGCTGGTACTTGCGGGGTCCTGCTGCTTTCACTCGTGATCACTCTTTACTG TAAGCGCGGTCGGAAGAAGCTGCTGTACATCTTTAAGCAACCCTTCATGAGGC CTGTGCAGACTACTCAAGAGGAGGACGGCTGTTCATGCCGGTTCCCAGAGGAG GAGGAAGGCGGCTGCGAACTGCGCGTGAAATTCAGCCGCAGCGCAGATGCTCC AGCCTACAAGCAGGGGCAGAACCAGCTCTACAACGAACTCAATCTTGGTCGGA GAGAGGAGTACGACGTGCTGGACAAGCGGAGAGGACGGGACCCAGAAATGGGC GGGAAGCCGCGCAGAAAGAATCCCCAAGAGGGCCTGTACAACGAGCTCCAAAA GGATAAGATGGCAGAAGCCTATAGCGAGATTGGTATGAAAGGGGAACGCAGAA GAGGCAAAGGCCACGACGGACTGTACCAGGGACTCAGCACCGCCACCAAGGAC ACCTATGACGCTCTTCACATGCAGGCCCTGCCGCCTCGG

Table 26. Amino acid sequences of exemplary BCMA binding domains

ER26		
SEQ ID NO: 1118	J6M0 VH	QVQLVQSGAEVKKPGSSVKVSCKASGGTFSNYWMHWVRQ APGQGLEWMGATYRGHSDTYYNQKFKGRVTITADKSTSTA YMELSSLRSEDTAVYYCARGAIYDGYDVLDNWGQGTLVTV SS
SEQ ID NO: 1119	J6M0 VL	DIQMTQSPSSLSASVGDRVTITCSASQDISNYLNWYQQKPGK APKLLIYYTSNLHSGVPSRFSGSGSGTDFTLTISSLQPEDFATY

		YCQQYRKLPWTFGQGTKLEIKR
SEQ ID NO: 1120	Anti-BCMA heavy chain of ER26	QVQLVQSGAEVKKPGSSVKVSCKASGGTFSNYWMHWVRQ APGQGLEWMGATYRGHSDTYYNQKFKGRVTITADKSTSTA YMELSSLRSEDTAVYYCARGAIYNGYDVLDNWGQGTLVTV SSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVS WNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYI CNVNHKPSNTKVDKRVEPKSCDKTHTCPPCPAPELLGGPSVF LFPPKPKDTLMISRTPEVTCVVVAVSHEDPEVKFNWYVDGV EVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCK VSNKALAAPIEKTISKAKGQPREPQVCTLPPSREEMTKNQVS LSCAVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFL VSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG X, wherein X is K or absent
SEQ ID NO: 1121	Anti-BCMA light chain of ER26	DIQMTQSPSSLSASVGDRVTITCSASQDISNYLNWYQQKPGK APKLLIYYTSNLHSGVPSRFSGSGSGTDFTLTISSLQPEDFATY YCQQYRKLPWTFGQGTKLEIKRTVAAPSVFIFPPSDEQLKSG TASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDS KDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFN RGEC
BQ76		
SEQ ID NO: 1123	17A5 VH	EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAP GKGLEWVSAISGSGGSTYYADSVKGRFTISRDNSKNTLYLQ MNSLRAEDTAVYYCAKVAPYFAPFDYWGQGTLVTVSS
SEQ ID NO: 1124	17A5 VL	EIVLTQSPGTLSLSPGERATLSCRASQSVSSSYLAWYQQKPG QAPRLLIYGASSRATGIPDRFSGSGSGTDFTLTISRLEPEDFAV YYCQQYGNPPLYTFGQGTKVEIK
SEQ ID NO: 1125	Anti-BCMA heavy chain of BQ76	EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAP GKGLEWVSAISGSGGSTYYADSVKGRFTISRDNSKNTLYLQ MNSLRAEDTAVYYCAKVAPYFAPFDYWGQGTLVTVSSAST KGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGA LTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNH KPSNTKVDKRVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKP KDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNA KTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKA LPAPIEKTISKAKGQPREPQVCTLPPSREEMTKNQVSLSCAVK GFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLVSKLTV DKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGX, wherein X is K or absent
SEQ ID NO: 1126	Anti-BCMA light chain of BQ76	EIVLTQSPGTLSLSPGERATLSCRASQSVSSSYLAWYQQKPG QAPRLLIYGASSRATGIPDRFSGSGSGTDFTLTISRLEPEDFAV YYCQQYGNPPLYTFGQGTKVEIKRTVAAPSVFIFPPSDEQLK SGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQ DSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKS FNRGEC
BU76		
SEQ ID NO: 1127	C11D5 VH	QIQLVQSGPELKKPGETVKISCKASGYTFTDYSINWVKRAPG KGLKWMGWINTETREPAYAYDFRGRFAFSLETSASTAYLQI NNLKYEDTATYFCALDYSYAMDYWGQGTSVTVSS
SEQ ID NO: 1128	C11D5 VL	DIVLTQSPPSLAMSLGKRATISCRASESVTILGSHLIHWYQQK PGQPPTLLIQLASNVQTGVPARFSGSGSRTDFTLTIDPVEEDD VAVYYCLQSRTIPRTFGGGTKLEIK

SEQ ID NO: 1129	Anti-BCMA heavy chain of BU76	QIQLVQSGPELKKPGETVKISCKASGYTFTDYSINWVKRAPG KGLKWMGWINTETREPAYAYDFRGRFAFSLETSASTAYLQI NNLKYEDTATYFCALDYSYAMDYWGQGTSVTVSSASTKGP SVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTS GVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPS NTKVDKRVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKD TLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKT KPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALP APIEKTISKAKGQPREPQVCTLPPSREEMTKNQVSLSCAVKG FYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLVSKLTVD KSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGX, wherein X is K or absent
SEQ ID NO: 1130	Anti-BCMA light chain of BU76	DIVLTQSPASLAMSLGKRATISCRASESVSVIGAHLIHWYQQ KPGQPPKLLIYLASNLETGVPARFSGSGSGTDFTLTIDPVEED DVAIYSCLQSRIFPRTFGGGTKLEIKRTVAAPSVFIFPPSDEQL KSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTE QDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVT KSFNRGEC
EE11		
SEQ ID NO: 1131	83A10 VH	EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAP GKGLEWVSAISGSGGSTYYADSVKGRFTISRDNSKNTLYLQ MNSLRAEDTAVYYCAKVLGWFDYWGQGTLVTVSS
SEQ ID NO: 1132	83A10 VL	EIVLTQSPGTLSLSPGERATLSCRASQSVSSSYLAWYQQKPG QAPRLLIYGASSRATGIPDRFSGSGSGTDFTLTISRLEPEDFAV YYCQQYGYPPDFTFGQGTKVEIK
SEQ ID NO: 1133	Anti-BCMA scFv-Fc of EE11	EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAP GKGLEWVSAISGSGGSTYYADSVKGRFTISRDNSKNTLYLQ MNSLRAEDTAVYYCAKVLGWFDYWGQGTLVTVSSGGGGS GGGGSGGGGSGGGGSEIVLTQSPGTLSLSPGERATLSCRASQ SVSSSYLAWYQQKPGQAPRLLIYGASSRATGIPDRFSGSGSG TDFTLTISRLEPEDFAVYYCQQYGYPPDFTFGQGTKVEIKGG GGSDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVT CVVVAVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTY RVVSVLTVLHQDWLNGKEYKCKVSNKALAAPIEKTISKAKG QPREPQVYTLPPCRDELTKNQVSLWCLVKGFYPSDIAVEWE SNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVF SCSVMHEALHNHYTQKSLSLSPGX, wherein X is K or absent
EM90		
SEQ ID NO: 1153	Comment light chain of EM90	QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQEK PGQAFRGLIGGTNKRAPGTPARFSGSLLGGKAALTLSGAQPE DEAEYYCALWYSNLWVFGGGTKLTVLGQPKAAPSVTLFPPS SEELQANKATLVCLISDFYPGAVTVAWKADSSPVKAGVETT TPSKQSNNKYAASSYLSLTPEQWKSHRSYSCQVTHEGSTVE KTVAPTECS
SEQ ID NO: 1154	Anti-BCMA heavy chain of EM90	EVQLVESGGGLVKPGGSLRLSCAASGFTFSNSGMIWVRQAP GKGLEWVGHIRSKTDGGTTDYAAPVKGRFTISRDDSKNTLY LQMNSLKTEDTAVYYCTTGGSGSFDYWGQGTLVTVSSAST KGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGA LTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNH KPSNTKVDKRVEPKSCDKTHTCPPCPAPEAAGGPSVFLFPPK PKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHN

		AKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNK ALPAPIEKTISKAKGQPREPQVCTLPPSREEMTKNQVSLSCAV KGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLVSKLT VDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGX, wherein X is K or absent

Additional exemplary BCMA-targeting sequences that can be used in the anti-BCMA CAR constructs are disclosed in WO 2017/021450, WO 2017/011804, WO 2017/025038, WO 2016/090327, WO 2016/130598, WO 2016/210293, WO 2016/090320, WO 2016/014789, WO 2016/094304, WO 2016/154055, WO 2015/166073, WO 2015/188119, WO 2015/158671, US 9,243,058, US 8,920,776, US 9,273,141, US 7,083,785, US 9,034,324, US 2007/0049735, US 2015/0284467, US 2015/0051266, US 2015/0344844, US 2016/0131655, US 2016/0297884, US 2016/0297885, US 2017/0051308, US 2017/0051252, US 2017/0051252, WO 2016/020332, WO 2016/087531, WO 2016/079177, WO 2015/172800, WO 2017/008169, US 9,340,621, US 2013/0273055, US 2016/0176973, US 2015/0368351, US 2017/0051068, US 2016/0368988, US 2015/0232557, herein incorporated by reference in their entirety.

In embodiments, additional exemplary BCMA CAR constructs are generated using the VH and VL sequences from PCT Publication WO2012/0163805 (the contents of which are hereby incorporated by reference in its entirety). Exemplary BCMA CAR constructs and their corresponding DNA ID are shown below in Table 3.

Table 3. Tool CAR construct IDs

Nickname	Novartis ID	DNA2.0 ID
BCMA-3NP		126022
BCMA-4NP		126021

In embodiments, additional exemplary BCMA CAR constructs can also be generated using the VH and VL sequences found in Table 16. The amino acid sequences of exemplary scFv domains comprising the VH and VL domains and a linker sequence, and full-length CARs are also found in Table 16.

Table 16. Additional exemplary BCMA CAR sequences

Name	**Sequence**	**SEQ ID NO:**
A7D12.2 VH	QIQLVQSGPDLKKPGETVKLSCKASGYTFTNFGMNWVKQAPGKGFKWMAWINTYT GESYFADDFKGRFAFSVETSATTAYLQINNLKTEDTATYFCARGEIYYGYDGGFA YWGQGTLVTVSA	255

A7D12.2 VL	DVVMTQSHRFMSTSVGDRVSITCRASQDVNTAVSWYQQKPGQSPKLLIFSASYRY TGVPDRFTGSGSGADFTLTISSVQAEDLAVYYCQQHYSTPWTFGGGTKLDIK	259
A7D12.2 scFv domain	QIQLVQSGPDLKKPGETVKLSCKASGYTFTNFGMNWVKQAPGKGFKWMAWINTYT GESYFADDFKGRFAFSVETSATTAYLQINNLKTEDTATYFCARGEIYYGYDGGFA YWGQGTLVTVSAGGGGSGGGGSGGGGSDVVMTQSHRFMSTSVGDRVSITCRASQD VNTAVSWYQQKPGQSPKLLIFSASYRYTGVPDRFTGSGSGADFTLTISSVQAEDL AVYYCQQHYSTPWTFGGGTKLDIK	263
A7D12.2 Full CART	QIQLVQSGPDLKKPGETVKLSCKASGYTFTNFGMNWVKQAPGKGFKWMAWINTYT GESYFADDFKGRFAFSVETSATTAYLQINNLKTEDTATYFCARGEIYYGYDGGFA YWGQGTLVTVSAGGGGSGGGGSGGGGSDVVMTQSHRFMSTSVGDRVSITCRASQD VNTAVSWYQQKPGQSPKLLIFSASYRYTGVPDRFTGSGSGADFTLTISSVQAEDL AVYYCQQHYSTPWTFGGGTKLDIKTTTPAPRPPTPAPTIASQPLSLRPEACRPAA GGAVHTRGLDFACDIYIWAPLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMR PVQTTQEEDGCSCRFPEEEEGGCELRVKFSRSADAPAYKQGQNQLYNELNLGRRE EYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKG HDGLYQGLSTATKDTYDALHMQALPPR	267
C11D5.3 VH	QIQLVQSGPELKKPGETVKISCKASGYTFTDYSINWVKRAPGKGLKWMGWINTET REPAYAYDFRGRFAFSLETSASTAYLQINNLKYEDTATYFCALDYSYAMDYWGQG TSVTVSS	256
C11D5.3 VL	DIVLTQSPASLAMSLGKRATISCRASESVSVIGAHLIHWYQQKPGQPPKLLIYLA SNLETGVPARFSGSGSGTDFTLTIDPVEEDDVAIYSCLQSRIFPRTFGGGTKLEI K	260
C11D5.3 scFv domain	QIQLVQSGPELKKPGETVKISCKASGYTFTDYSINWVKRAPGKGLKWMGWINTET REPAYAYDFRGRFAFSLETSASTAYLQINNLKYEDTATYFCALDYSYAMDYWGQG TSVTVSSGGGGSGGGGSGGGGSQIQLVQSGPELKKPGETVKISCKASGYTFTDYS INWVKRAPGKGLKWMGWINTETREPAYAYDFRGRFAFSLETSASTAYLQINNLKY EDTATYFCALDYSYAMDYWGQGTSVTVSS	264
C11D5.3 Full CART	QIQLVQSGPELKKPGETVKISCKASGYTFTDYSINWVKRAPGKGLKWMGWINTET REPAYAYDFRGRFAFSLETSASTAYLQINNLKYEDTATYFCALDYSYAMDYWGQG TSVTVSSGGGGSGGGGSGGGGSQIQLVQSGPELKKPGETVKISCKASGYTFTDYS INWVKRAPGKGLKWMGWINTETREPAYAYDFRGRFAFSLETSASTAYLQINNLKY EDTATYFCALDYSYAMDYWGQGTSVTVSSTTTPAPRPPTPAPTIASQPLSLRPEA CRPAAGGAVHTRGLDFACDIYIWAPLAGTCGVLLLSLVITLYCKRGRKKLLYIFK QPFMRPVQTTQEEDGCSCRFPEEEEGGCELRVKFSRSADAPAYKQGQNQLYNELN LGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGER RRGKGHDGLYQGLSTATKDTYDALHMQALPPR	268
C12A3.2 VH	QIQLVQSGPELKKPGETVKISCKASGYTFRHYSMNWVKQAPGKGLKWMGRINTES GVPIYADDFKGRFAFSVETSASTAYLVINNLKDEDTASYFCSNDYLYSLDFWGQG TALTVSS	257
C12A3.2 VL	DIVLTQSPPSLAMSLGKRATISCRASESVTILGSHLIYWYQQKPGQPPTLLIQLA SNVQTGVPARFSGSGSRTDFTLTIDPVEEDDVAVYYCLQSRTIPRTFGGGTKLEI K	261
C12A3.2 scFv domain	QIQLVQSGPELKKPGETVKISCKASGYTFRHYSMNWVKQAPGKGLKWMGRINTES GVPIYADDFKGRFAFSVETSASTAYLVINNLKDEDTASYFCSNDYLYSLDFWGQG TALTVSSGGGGSGGGGSGGGGSDIVLTQSPPSLAMSLGKRATISCRASESVTILG SHLIYWYQQKPGQPPTLLIQLASNVQTGVPARFSGSGSRTDFTLTIDPVEEDDVA VYYCLQSRTIPRTFGGGTKLEIK	265
C12A3.2 Full CART	QIQLVQSGPELKKPGETVKISCKASGYTFRHYSMNWVKQAPGKGLKWMGRINTES GVPIYADDFKGRFAFSVETSASTAYLVINNLKDEDTASYFCSNDYLYSLDFWGQG TALTVSSGGGGSGGGGSGGGGSDIVLTQSPPSLAMSLGKRATISCRASESVTILG SHLIYWYQQKPGQPPTLLIQLASNVQTGVPARFSGSGSRTDFTLTIDPVEEDDVA	269

	VYYCLQSRTIPRTFGGGTKLEIKTTTPAPRPPTPAPTIASQPLSLRPEACRPAAG GAVHTRGLDFACDIYIWAPLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRP VQTTQEEDGCSCRFPEEEEGGCELRVKFSRSADAPAYKQGQNQLYNELNLGRREE YDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGH DGLYQGLSTATKDTYDALHMQALPPR	
C13F12.1 VH	QIQLVQSGPELKKPGETVKISCKASGYTFTHYSMNWVKQAPGKGLKWMGRINTET GEPLYADDFKGRFAFSLETSASTAYLVINNLKNEDTATFFCSNDYLYSCDYWGQG TTLTVSS	258
C13F12.1 VL	DIVLTQSPPSLAMSLGKRATISCRASESVTILGSHLIYWYQQKPGQPPTLLIQLA SNVQTGVPARFSGSGSRTDFTLTIDPVEEDDVAVYYCLQSRTIPRTFGGGTKLEI K	262
C13F12.1 scFv domain	QIQLVQSGPELKKPGETVKISCKASGYTFTHYSMNWVKQAPGKGLKWMGRINTET GEPLYADDFKGRFAFSLETSASTAYLVINNLKNEDTATFFCSNDYLYSCDYWGQG TTLTVSSGGGGSGGGGSGGGGSDIVLTQSPPSLAMSLGKRATISCRASESVTILG SHLIYWYQQKPGQPPTLLIQLASNVQTGVPARFSGSGSRTDFTLTIDPVEEDDVA VYYCLQSRTIPRTFGGGTKLEIK	266
C13F12.1 Full CART	QIQLVQSGPELKKPGETVKISCKASGYTFTHYSMNWVKQAPGKGLKWMGRINTET GEPLYADDFKGRFAFSLETSASTAYLVINNLKNEDTATFFCSNDYLYSCDYWGQG TTLTVSSGGGGSGGGGSGGGGSDIVLTQSPPSLAMSLGKRATISCRASESVTILG SHLIYWYQQKPGQPPTLLIQLASNVQTGVPARFSGSGSRTDFTLTIDPVEEDDVA VYYCLQSRTIPRTFGGGTKLEIKTTTPAPRPPTPAPTIASQPLSLRPEACRPAAG GAVHTRGLDFACDIYIWAPLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRP VQTTQEEDGCSCRFPEEEEGGCELRVKFSRSADAPAYKQGQNQLYNELNLGRREE YDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGH DGLYQGLSTATKDTYDALHMQALPPR	270

In embodiments, the nucleic acid sequence of an exemplary humanized anti-BCMA scFv in which VH precedes the VL (H2L, e.g., pBCMA 2 and pBCMA 4) is as follows:

CAGGTGCAGCTGGTCCAGAGCGGCGCCGAAGTGAAGAAGCCCGGCAGCTCCGTGAAAGTGAGCTGCAAG
GCCAGCGGCGGCACCTTCAGCAACTACTGGATGCACTGGGTGAGGCAGGCCCCCGGACAGGGCCTGGAG
TGGATGGGCGCCACCTACAGGGGCCACAGCGACACCTACTACAACCAGAAGTTCAAGGGCCGGGTGACC
ATCACCGCCGACAAGAGCACCAGCACCGCCTACATGGAACTGAGCAGCCTCAGGAGCGAGGACACCGCT
GTGTATTACTGCGCCAGGGGCGCCATCTACAACGGCTACGACGTGCTGGACAACTGGGGCCAGGGCACA
CTAGTGACCGTGTCCAGCGGTGGAGGAGGTAGCGGAGGAGGCGGGAGCGGTGGAGGTGGCTCTGGAGGT
GGCGGAAGCGACATCCAGATGACCCAGAGCCCTAGCTCACTGAGCGCCAGCGTGGGCGACAGGGTGACC
ATTACCTGCTCCGCCAGCCAGGACATCAGCAACTACCTGAACTGGTACCAGCAGAAGCCCGGCAAGGCC
CCCAAGCTGCTGATCTACTACACCTCCAACCTGCACTCCGGCGTGCCCAGCAGGTTCAGCGGAAGCGGC
AGCGGCACCGATTTCACCCTGACCATCTCCAGCCTGCAGCCCGAGGACTTCGCCACCTACTACTGCCAG
CAGTACAGGAAGCTCCCCTGGACTTTCGGCCAGGGCACCAAACTGGAGATCAAGCGT
(SEQ ID NO: 272)

The corresponding amino acid sequence for the exemplary humanized anti-BCMA scFv in which Vh precedes the VL (H2L, e.g., pBCMA 2 and pBCMA 4) is as follows:

QVQLVQSGAEVKKPGSSVKVSCKASGGTFSNYWMHWVRQAPGQGLEWMGATYRGHSDTYYNQKF
KGRVTITADKSTSTAYMELSSLRSEDTAVYYCARGAIYNGYDVLDNWGQGTLVTVSSGGGGSGGGGSGG
GGSGGGGSDIQMTQSPSSLSASVGDRVTITCSASQDISNYLNWYQQKPGKAPKLLIYYTSNLHSGVPSR
FSGSGSGTDFTLTISSLQPEDFATYYCQQYRKLPWTFGQGTKLEIKR (SEQ ID NO: 271)

In embodiments, the nucleic acid sequence of an exemplary humanized anti-BCMA scFv in which VL precedes the VH (L2H, e.g., pBCMA1 and pBCMA3) is as follows:

GACATCCAGATGACCCAGAGCCCTAGCTCACTGAGCGCCAGCGTGGGCGACAGGGTGACCATTACCTGC
TCCGCCAGCCAGGACATCAGCAACTACCTGAACTGGTACCAGCAGAAGCCCGGCAAGGCCCCCAAGCTG
CTGATCTACTACACCTCCAACCTGCACTCCGGCGTGCCCAGCAGGTTCAGCGGAAGCGGCAGCGGCACC
GATTTCACCCTGACCATCTCCAGCCTGCAGCCCGAGGACTTCGCCACCTACTACTGCCAGCAGTACAGG
AAGCTCCCCTGGACTTTCGGCCAGGGCACCAAACTGGAGATCAAGCGTGGTGGAGGAGGTAGCGGAGGA
GGCGGGAGCGGTGGAGGTGGCTCTGGAGGTGGCGGAAGCCAGGTGCAGCTGGTCCAGAGCGGCGCCGAA
GTGAAGAAGCCCGGCAGCTCCGTGAAAGTGAGCTGCAAGGCCAGCGGCGGCACCTTCAGCAACTACTGG
ATGCACTGGGTGAGGCAGGCCCCCGGACAGGGCCTGGAGTGGATGGGCGCCACCTACAGGGGCCACAGC
GACACCTACTACAACCAGAAGTTCAAGGGCCGGGTGACCATCACCGCCGACAAGAGCACCAGCACCGCC
TACATGGAACTGAGCAGCCTCAGGAGCGAGGACACCGCTGTGTATTACTGCGCCAGGGGCGCCATCTAC
AACGGCTACGACGTGCTGGACAACTGGGGCCAGGGCACACTAGTGACCGTGTCCAGC
(SEQ ID NO: 274)

The corresponding amino acid sequence of the exemplary humanized anti-BCMA scFv in which VL precedes the VH (L2H, e.g., pBCMA1 and pBCMA3) is as follows:

DIQMTQSPSS LSASVGDRVT ITCSASQDIS NYLNWYQQKP GKAPKLLIYY
TSNLHSGVPSRFSGSGSGTD FTLTISSLQP EDFATYYCQQ YRKLPWTFGQ GTKLEIKRGG
GGSGGGGSGGGGSGGGGSQV QLVQSGAEVK KPGSSVKVSC KASGGTFSNY
WMHWVRQAPG QGLEWMGATYRGHSDTYYNQ KFKGRVTITA DKSTSTAYME
LSSLRSEDTA VYYCARGAIYNGYDVLDNWGQGTLVTVSS (SEQ ID NO: 273)

The CAR scFv fragments can be cloned into lentiviral vectors to create a full length CAR construct in a single coding frame, and using the EF1 alpha promoter for expression (SEQ ID NO: 11).

The CAR construct can include a Gly/Ser linker having one or more of the following sequences: GGGGS (SEQ ID NO:25); encompassing 1-6 "Gly Gly Gly Gly Ser" repeating units, e.g., GGGGSGGGGS GGGGSGGGGS GGGGSGGGGS (SEQ ID NO:26); GGGGSGGGGS GGGGSGGGGS (SEQ ID NO:27); GGGGSGGGGS GGGGS (SEQ ID NO:28); GGGS (SEQ ID NO:29); or encompassing 1-10 "Gly Gly Gly Ser" repeating units, e.g., GGGSGGGSGG GSGGGSGGGS GGGSGGGSGG GSGGGSGGGS (SEQ ID NO:38).

In embodiments, the CAR construct include a poly A sequence, e.g., a sequence encompassing 50-5000 or 100-5000 adenines (e.g., SEQ ID NO:30, SEQ ID NO:33, SEQ ID NO:34 or SEQ ID NO:35), or a sequence encompassing 50-5000 thymines (e.g., SEQ ID NO:31, SEQ ID NO:32). Alternatively, the CAR construct can include, for example, a linker including the sequence GSTSGSGKPGSGEGSTKG (SEQ ID NO: 1108)

Multispecific CARs

In some embodiments, the CAR construct is multispecific (e.g., bispecific). In some embodiment, the CAR construct comprises a multispecific antibody molecule (e.g., a bispecific antibody molecule).

In an embodiment a multispecific antibody molecule is a bispecific antibody molecule. A bispecific antibody has specificity for no more than two antigens. A bispecific antibody molecule is characterized by a first immunoglobulin variable domain sequence which has binding specificity for a first epitope and a second immunoglobulin variable domain sequence that has binding specificity for a second epitope. In an embodiment the first and second epitopes are on the same antigen, e.g., the same protein (or subunit of a multimeric protein). In an embodiment the first and second epitopes overlap. In an embodiment the first and second epitopes do not overlap. In an embodiment the first and second epitopes are on different antigens, e.g., different proteins (or different subunits of a multimeric protein). In an embodiment a bispecific antibody molecule comprises a heavy chain variable domain sequence and a light chain variable domain sequence which have binding specificity for a first epitope and a heavy chain variable domain sequence and a light chain variable domain sequence which have binding specificity for a second epitope. In an embodiment a bispecific antibody molecule comprises a half antibody having binding specificity for a first epitope and a half antibody having binding specificity for a second epitope. In an embodiment a bispecific antibody molecule comprises a half antibody, or fragment thereof, having binding specificity for a first epitope and a half antibody, or fragment thereof, having binding specificity for a second epitope. In an embodiment a bispecific antibody molecule comprises a scFv, or fragment thereof, have binding specificity for a first epitope and a scFv, or fragment thereof, have binding specificity for a second epitope.

In certain embodiments, the antibody molecule is a multi-specific (*e.g.*, a bispecific or a trispecific) antibody molecule. Protocols for generating bispecific or heterodimeric antibody molecules are known in the art; including but not limited to, for example, the "knob in a hole" approach described in, *e.g.*, US 5731168; the electrostatic steering Fc pairing as described in, *e.g.*, WO 09/089004, WO 06/106905 and WO 2010/129304; Strand Exchange Engineered Domains (SEED) heterodimer formation as described in, *e.g.*, WO 07/110205; Fab arm exchange as described in, *e.g.*, WO 08/119353, WO 2011/131746, and WO 2013/060867; double antibody conjugate, *e.g.*, by antibody cross-linking to generate a bi-specific structure using a heterobifunctional reagent having an amine-reactive group and a sulfhydryl reactive group as described in, *e.g.*, US 4433059; bispecific antibody determinants generated by recombining half antibodies (heavy-light chain pairs or Fabs) from different antibodies through cycle of reduction and oxidation of disulfide bonds between the two heavy chains, as described in, *e.g.*, US 4444878; trifunctional antibodies, *e.g.*, three Fab' fragments cross-linked through sulfhdryl reactive groups, as described in, *e.g.*, US5273743; biosynthetic binding proteins, *e.g.*, pair of scFvs cross-linked through C-terminal tails preferably through disulfide or amine-reactive chemical cross-linking, as

described in, *e.g.*, US5534254; bifunctional antibodies, *e.g.*, Fab fragments with different binding specificities dimerized through leucine zippers (*e.g.*, c-fos and c-jun) that have replaced the constant domain, as described in, *e.g.*, US5582996; bispecific and oligospecific mono-and oligovalent receptors, *e.g.*, VH-CH1 regions of two antibodies (two Fab fragments) linked through a polypeptide spacer between the CH1 region of one antibody and the VH region of the other antibody typically with associated light chains, as described in, *e.g.*, US5591828; bispecific DNA-antibody conjugates, *e.g.*, crosslinking of antibodies or Fab fragments through a double stranded piece of DNA, as described in, *e.g.*, US5635602; bispecific fusion proteins, *e.g.*, an expression construct containing two scFvs with a hydrophilic helical peptide linker between them and a full constant region, as described in, *e.g.*, US5637481; multivalent and multispecific binding proteins, *e.g.*, dimer of polypeptides having first domain with binding region of Ig heavy chain variable region, and second domain with binding region of Ig light chain variable region, generally termed diabodies (higher order structures are also encompassed creating for bispecifc, trispecific, or tetraspecific molecules, as described in, *e.g.*, US5837242; minibody constructs with linked VL and VH chains further connected with peptide spacers to an antibody hinge region and CH3 region, which can be dimerized to form bispecific/multivalent molecules, as described in, *e.g.*, US5837821; VH and VL domains linked with a short peptide linker (*e.g.*, 5 or 10 amino acids) or no linker at all in either orientation, which can form dimers to form bispecific diabodies; trimers and tetramers, as described in, *e.g.*, US5844094; String of VH domains (or VL domains in family members) connected by peptide linkages with crosslinkable groups at the C-terminus futher associated with VL domains to form a series of FVs (or scFvs), as described in, *e.g.*, US5864019; and single chain binding polypeptides with both a VH and a VL domain linked through a peptide linker are combined into multivalent structures through non-covalent or chemical crosslinking to form, *e.g.*, homobivalent, heterobivalent, trivalent, and tetravalent structures using both scFV or diabody type format, as described in, *e.g.*, US5869620. Additional exemplary multispecific and bispecific molecules and methods of making the same are found, for example, in US5910573, US5932448, US5959083, US5989830, US6005079, US6239259, US6294353, US6333396, US6476198, US6511663, US6670453, US6743896, US6809185, US6833441, US7129330, US7183076, US7521056, US7527787, US7534866, US7612181, US2002004587A1, US2002076406A1, US2002103345A1, US2003207346A1, US2003211078A1, US2004219643A1, US2004220388A1, US2004242847A1, US2005003403A1, US2005004352A1, US2005069552A1, US2005079170A1, US2005100543A1, US2005136049A1, US2005136051A1, US2005163782A1, US2005266425A1, US2006083747A1, US2006120960A1, US2006204493A1, US2006263367A1, US2007004909A1, US2007087381A1, US2007128150A1, US2007141049A1, US2007154901A1, US2007274985A1, US2008050370A1, US2008069820A1, US2008152645A1, US2008171855A1, US2008241884A1, US2008254512A1, US2008260738A1, US2009130106A1, US2009148905A1, US2009155275A1,

US2009162359A1, US2009162360A1, US2009175851A1, US2009175867A1, US2009232811A1, US2009234105A1, US2009263392A1, US2009274649A1, EP346087A2, WO0006605A2, WO02072635A2, WO04081051A1, WO06020258A2, WO2007044887A2, WO2007095338A2, WO2007137760A2, WO2008119353A1, WO2009021754A2, WO2009068630A1, WO9103493A1, WO9323537A1, WO9409131A1, WO9412625A2, WO9509917A1, WO9637621A2, WO9964460A1. The contents of the above-referenced applications are incorporated herein by reference in their entireties.

Within each antibody or antibody fragment (e.g., scFv) of a bispecific antibody molecule, the VH can be upstream or downstream of the VL. In some embodiments, the upstream antibody or antibody fragment (e.g., scFv) is arranged with its VH (VH_1) upstream of its VL (VL_1) and the downstream antibody or antibody fragment (e.g., scFv) is arranged with its VL (VL_2) upstream of its VH (VH_2), such that the overall bispecific antibody molecule has the arrangement VH_1-VL_1-VL_2-VH_2. In other embodiments, the upstream antibody or antibody fragment (e.g., scFv) is arranged with its VL (VL_1) upstream of its VH (VH_1) and the downstream antibody or antibody fragment (e.g., scFv) is arranged with its VH (VH_2) upstream of its VL (VL_2), such that the overall bispecific antibody molecule has the arrangement VL_1-VH_1-VH_2-VL_2. Optionally, a linker is disposed between the two antibodies or antibody fragments (e.g., scFvs), e.g., between VL_1 and VL_2 if the construct is arranged as VH_1-VL_1-VL_2-VH_2, or between VH_1 and VH_2 if the construct is arranged as VL_1-VH_1-VH_2-VL_2. The linker may be a linker as described herein, e.g., a (Gly_4-Ser)n linker, wherein n is 1, 2, 3, 4, 5, or 6, preferably 4 (SEQ ID NO: 26). In general, the linker between the two scFvs should be long enough to avoid mispairing between the domains of the two scFvs. Optionally, a linker is disposed between the VL and VH of the first scFv. Optionally, a linker is disposed between the VL and VH of the second scFv. In constructs that have multiple linkers, any two or more of the linkers can be the same or different. Accordingly, in some embodiments, a bispecific CAR comprises VLs, VHs, and optionally one or more linkers in an arrangement as described herein.

In one aspect, the bispecific antibody molecule is characterized by a first immunoglobulin variable domain sequence, e.g., a scFv, which has binding specificity for BCMA, e.g., comprises a scFv as described herein, e.g., as described in Table 1 or Table 16, or comprises the light chain CDRs and/or heavy chain CDRs from a BCMA scFv described herein, and a second immunoglobulin variable domain sequence that has binding specificity for a second epitope on a different antigen. In some aspects the second immunoglobulin variable domain sequence has binding specificity for an antigen expressed on AML cells, e.g., an antigen other than BCMA. For example, the second immunoglobulin variable domain sequence has binding specificity for CD123. As another example, the second immunoglobulin variable domain sequence has binding specificity for CLL-1. As another example, the second immunoglobulin variable domain sequence has binding specificity for CD34. As another example, the

second immunoglobulin variable domain sequence has binding specificity for FLT3. For example, the second immunoglobulin variable domain sequence has binding specificity for folate receptor beta. In some aspects, the second immunoglobulin variable domain sequence has binding specificity for an antigen expressed on B-cells, for example, CD10, CD19, CD20, CD22, CD34, CD123, FLT-3, ROR1, CD79b, CD179b, or CD79a.

Chimeric TCR

In one aspect, the anti-BCMA antibodies and antibody fragments of the present invention (for example, those disclosed in Tables 1 and 16) can be grafted to one or more constant domain of a T cell receptor ("TCR") chain, for example, a TCR alpha or TCR beta chain, to create an chimeric TCR that binds specificity to BCMA. Without being bound by theory, it is believed that chimeric TCRs will signal through the TCR complex upon antigen binding. For example, a BCMA scFv as disclosed herein, can be grafted to the constant domain, e.g., at least a portion of the extracellular constant domain, the transmembrane domain and the cytoplasmic domain, of a TCR chain, for example, the TCR alpha chain and/or the TCR beta chain. As another example, a BCMA antibody fragment, for example a VL domain as described herein, can be grafted to the constant domain of a TCR alpha chain, and a BCMA antibody fragment, for example a VH domain as described herein, can be grafted to the constant domain of a TCR beta chain (or alternatively, a VL domain may be grafted to the constant domain of the TCR beta chain and a VH domain may be grafted to a TCR alpha chain). As another example, the CDRs of an anti-BCMA antibody or antibody fragment, e.g., the CDRs of an anti-BCMA antibody or antibody fragment as described in Tables 20, 21, 22, 23, 24, or 25 may be grafted into a TCR alpha and/or beta chain to create a chimeric TCR that binds specifically to BCMA. For example, the LCDRs disclosed herein may be grafted into the variable domain of a TCR alpha chain and the HCDRs disclosed herein may be grafted to the variable domain of a TCR beta chain, or vice versa. Such chimeric TCRs may be produced by methods known in the art (For example, Willemsen RA et al, Gene Therapy 2000; 7: 1369–1377; Zhang T et al, Cancer Gene Ther 2004; 11: 487–496; Aggen et al, Gene Ther. 2012 Apr;19(4):365-74).

Transmembrane domain

With respect to the transmembrane domain, in various embodiments, a CAR can be designed to comprise a transmembrane domain that is attached to the extracellular domain of the CAR. A transmembrane domain can include one or more additional amino acids adjacent to the transmembrane region, e.g., one or more amino acid associated with the extracellular region of the protein from which

the transmembrane was derived (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 up to 15 amino acids of the extracellular region) and/or one or more additional amino acids associated with the intracellular region of the protein from which the transmembrane protein is derived (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 up to 15 amino acids of the intracellular region). In one aspect, the transmembrane domain is one that is associated with one of the other domains of the CAR is used. In some instances, the transmembrane domain can be selected or modified by amino acid substitution to avoid binding of such domains to the transmembrane domains of the same or different surface membrane proteins, e.g., to minimize interactions with other members of the receptor complex. In one aspect, the transmembrane domain is capable of homodimerization with another CAR on the CAR-expressing cell, e.g., CART cell, surface. In a different aspect the amino acid sequence of the transmembrane domain may be modified or substituted so as to minimize interactions with the binding domains of the native binding partner present in the same CAR-expressing cell, e.g., CART.

The transmembrane domain may be derived either from a natural or from a recombinant source. Where the source is natural, the domain may be derived from any membrane-bound or transmembrane protein. In one aspect the transmembrane domain is capable of signaling to the intracellular domain(s) whenever the CAR has bound to a target. A transmembrane domain of particular use in this invention may include at least the transmembrane region(s) of e.g., the alpha, beta or zeta chain of the T-cell receptor, CD28, CD3 epsilon, CD45, CD4, CD5, CD8 (e.g., CD8 alpha, CD8 beta), CD9, CD16, CD22, CD33, CD37, CD64, CD80, CD86, CD134, CD137, CD154. In some embodiments, a transmembrane domain may include at least the transmembrane region(s) of a costimulatory molecule, e.g., MHC class I molecule, TNF receptor proteins, Immunoglobulin-like proteins, cytokine receptors, integrins, signaling lymphocytic activation molecules (SLAM proteins), activating NK cell receptors, BTLA, a Toll ligand receptor, OX40, CD2, CD7, CD27, CD28, CD30, CD40, CDS, ICAM-1, LFA-1 (CD11a/CD18), 4-1BB (CD137), B7-H3, CDS, ICAM-1, ICOS (CD278), GITR, BAFFR, LIGHT, HVEM (LIGHTR), KIRDS2, SLAMF7, NKp80 (KLRF1), NKp44, NKp30, NKp46, CD19, CD4, CD8alpha, CD8beta, IL2R beta, IL2R gamma, IL7R alpha, ITGA4, VLA1, CD49a, ITGA4, IA4, CD49D, ITGA6, VLA-6, CD49f, ITGAD, CD11d, ITGAE, CD103, ITGAL, CD11a, LFA-1, ITGAM, CD11b, ITGAX, CD11c, ITGB1, CD29, ITGB2, CD18, LFA-1, ITGB7, NKG2D, NKG2C, TNFR2, TRANCE/RANKL, DNAM1 (CD226), SLAMF4 (CD244, 2B4), CD84, CD96 (Tactile), CEACAM1, CRTAM, Ly9 (CD229), CD160 (BY55), PSGL1, CD100 (SEMA4D), CD69, SLAMF6 (NTB-A, Ly108), SLAM (SLAMF1, CD150, IPO-3), BLAME (SLAMF8), SELPLG (CD162), LTBR, LAT, GADS, SLP-76, PAG/Cbp, CD19a, and a ligand that specifically binds with CD83.

In some instances, the transmembrane domain can be attached to the extracellular region of the CAR, e.g., the antigen binding domain of the CAR, via a hinge, e.g., a hinge from a human protein. For example, in one embodiment, the hinge can be a human Ig (immunoglobulin) hinge, e.g., an IgG4 hinge, or a CD8a hinge. In one embodiment, the hinge or spacer comprises (e.g., consists of) the amino acid sequence of SEQ ID NO:2. In one aspect, the transmembrane domain comprises (e.g., consists of) a transmembrane domain of SEQ ID NO: 6.

In one aspect, the hinge or spacer comprises an IgG4 hinge. For example, in one embodiment, the hinge or spacer comprises a hinge of the amino acid sequence ESKYGPPCPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGV EVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPRE PQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSR LTVDKSRWQEGNVFSCSVMHEALHNHYTQKSLSLSLGKM (SEQ ID NO:3). In some embodiments, the hinge or spacer comprises a hinge encoded by a nucleotide sequence of GAGAGCAAGTACGGCCCTCCCTGCCCCCCTTGCCCTGCCCCCGAGTTCCTGGGCGGACCCA GCGTGTTCCTGTTCCCCCCCAAGCCCAAGGACACCCTGATGATCAGCCGGACCCCCGAGGT GACCTGTGTGGTGGTGGACGTGTCCCAGGAGGACCCCGAGGTCCAGTTCAACTGGTACGTG GACGGCGTGGAGGTGCACAACGCCAAGACCAAGCCCCGGGAGGAGCAGTTCAATAGCACC TACCGGGTGGTGTCCGTGCTGACCGTGCTGCACCAGGACTGGCTGAACGGCAAGGAATAC AAGTGTAAGGTGTCCAACAAGGGCCTGCCCAGCAGCATCGAGAAAACCATCAGCAAGGCC AAGGGCCAGCCTCGGGAGCCCCAGGTGTACACCCTGCCCCCTAGCCAAGAGGAGATGACC AAGAACCAGGTGTCCCTGACCTGCCTGGTGAAGGGCTTCTACCCCAGCGACATCGCCGTGG AGTGGGAGAGCAACGGCCAGCCCGAGAACAACTACAAGACCACCCCCCCTGTGCTGGACA GCGACGGCAGCTTCTTCCTGTACAGCCGGCTGACCGTGGACAAGAGCCGGTGGCAGGAGG GCAACGTCTTTAGCTGCTCCGTGATGCACGAGGCCCTGCACAACCACTACACCCAGAAGAG CCTGAGCCTGTCCCTGGGCAAGATG (SEQ ID NO:14).

In one aspect, the hinge or spacer comprises an IgD hinge. For example, in one embodiment, the hinge or spacer comprises a hinge of the amino acid sequence RWPESPKAQASSVPTAQPQAEGSLAKATTAPATTRNTGRGGEEKKKEKEKEEQEERETKTPEC PSHTQPLGVYLLTPAVQDLWLRDKATFTCFVVGSDLKDAHLTWEVAGKVPTGGVEEGLLERH SNGSQSQHSRLTLPRSLWNAGTSVTCTLNHPSLPPQRLMALREPAAQAPVKLSLNLLASSDPPE AASWLLCEVSGFSPPNILLMWLEDQREVNTSGFAPARPPPQPGSTTFWAWSVLRVPAPPSPQPA TYTCVVSHEDSRTLLNASRSLEVSYVTDH (SEQ ID NO:4). In some embodiments, the hinge or spacer comprises a hinge encoded by a nucleotide sequence of AGGTGGCCCGAAAGTCCCAAGGCCCAGGCATCTAGTGTTCCTACTGCACAGCCCCAGGCA

GAAGGCAGCCTAGCCAAAGCTACTACTGCACCTGCCACTACGCGCAATACTGGCCGTGGC
GGGGAGGAGAAGAAAAAGGAGAAAGAGAAAGAAGAACAGGAAGAGAGGGAGACCAAGA
CCCCTGAATGTCCATCCCATACCCAGCCGCTGGGCGTCTATCTCTTGACTCCCGCAGTACAG
GACTTGTGGCTTAGAGATAAGGCCACCTTTACATGTTTCGTCGTGGGCTCTGACCTGAAGG
ATGCCCATTTGACTTGGGAGGTTGCCGGAAAGGTACCCACAGGGGGGGTTGAGGAAGGGT
TGCTGGAGCGCCATTCCAATGGCTCTCAGAGCCAGCACTCAAGACTCACCCTTCCGAGATC
CCTGTGGAACGCCGGGACCTCTGTCACATGTACTCTAAATCATCCTAGCCTGCCCCCACAG
CGTCTGATGGCCCTTAGAGAGCCAGCCGCCCAGGCACCAGTTAAGCTTAGCCTGAATCTGC
TCGCCAGTAGTGATCCCCCAGAGGCCGCCAGCTGGCTCTTATGCGAAGTGTCCGGCTTTAG
CCCGCCCAACATCTTGCTCATGTGGCTGGAGGACCAGCGAGAAGTGAACACCAGCGGCTTC
GCTCCAGCCCGGCCCCCACCCCAGCCGGGTTCTACCACATTCTGGGCCTGGAGTGTCTTAA
GGGTCCCAGCACCACCTAGCCCCCAGCCAGCCACATACACCTGTGTTGTGTCCCATGAAGA
TAGCAGGACCCTGCTAAATGCTTCTAGGAGTCTGGAGGTTTCCTACGTGACTGACCATT
(SEQ ID NO:15).

In one aspect, the transmembrane domain may be recombinant, in which case it will comprise predominantly hydrophobic residues such as leucine and valine. In one aspect a triplet of phenylalanine, tryptophan and valine can be found at each end of a recombinant transmembrane domain.

Optionally, a short oligo- or polypeptide linker, between 2 and 10 amino acids in length may form the linkage between the transmembrane domain and the cytoplasmic region of the CAR. A glycine-serine doublet provides a particularly suitable linker. For example, in one aspect, the linker comprises the amino acid sequence of GGGGSGGGGS (SEQ ID NO:5). In some embodiments, the linker is encoded by a nucleotide sequence of GGTGGCGGAGGTTCTGGAGGTGGAGGTTCC (SEQ ID NO:16).

In one aspect, the hinge or spacer comprises a KIR2DS2 hinge.

Cytoplasmic domain

The cytoplasmic domain or region of a CAR of the present invention includes an intracellular signaling domain. An intracellular signaling domain is generally responsible for activation of at least one of the normal effector functions of the immune cell in which the CAR has been introduced.

Examples of intracellular signaling domains for use in the CAR of the invention include the cytoplasmic sequences of the T cell receptor (TCR) and co-receptors that act in concert to initiate signal

transduction following antigen receptor engagement, as well as any derivative or variant of these sequences and any recombinant sequence that has the same functional capability.

It is known that signals generated through the TCR alone are insufficient for full activation of the T cell and that a secondary and/or costimulatory signal is also required. Thus, T cell activation can be said to be mediated by two distinct classes of cytoplasmic signaling sequences: those that initiate antigen-dependent primary activation through the TCR (primary intracellular signaling domains) and those that act in an antigen-independent manner to provide a secondary or costimulatory signal (secondary cytoplasmic domain, e.g., a costimulatory domain).

A primary signaling domain regulates primary activation of the TCR complex either in a stimulatory way, or in an inhibitory way. Primary intracellular signaling domains that act in a stimulatory manner may contain signaling motifs which are known as immunoreceptor tyrosine-based activation motifs or ITAMs.

Examples of ITAM containing primary intracellular signaling domains that are of particular use in the invention include those of TCR zeta, FcR gamma, FcR beta, CD3 gamma, CD3 delta , CD3 epsilon, CD5, CD22, CD79a, CD79b, CD278 (also known as "ICOS"), FcεRI, DAP10, DAP12, and CD66d. In one embodiment, a CAR of the invention comprises an intracellular signaling domain, e.g., a primary signaling domain of CD3-zeta.

In one embodiment, a primary signaling domain comprises a modified ITAM domain, e.g., a mutated ITAM domain which has altered (e.g., increased or decreased) activity as compared to the native ITAM domain. In one embodiment, a primary signaling domain comprises a modified ITAM-containing primary intracellular signaling domain, e.g., an optimized and/or truncated ITAM-containing primary intracellular signaling domain. In an embodiment, a primary signaling domain comprises one, two, three, four or more ITAM motifs.

Further examples of molecules containing a primary intracellular signaling domain that are of particular use in the invention include those of DAP10, DAP12, and CD32.

The intracellular signalling domain of the CAR can comprise the primary signalling domain, e.g., CD3-zeta signaling domain, by itself or it can be combined with any other desired intracellular signaling domain(s) useful in the context of a CAR of the invention. For example, the intracellular signaling domain of the CAR can comprise a primary signalling domain, e.g., CD3 zeta chain portion, and a costimulatory signaling domain. The costimulatory signaling domain refers to a portion of the CAR comprising the intracellular domain of a costimulatory molecule. A costimulatory molecule is a cell surface molecule other than an antigen receptor or its ligands that is required for an efficient response of lymphocytes to an antigen. Examples of such molecules include MHC class I molecule,

TNF receptor proteins, Immunoglobulin-like proteins, cytokine receptors, integrins, signaling lymphocytic activation molecules (SLAM proteins), activating NK cell receptors, BTLA, a Toll ligand receptor, OX40, CD2, CD7, CD27, CD28, CD30, CD40, CDS, ICAM-1, LFA-1 (CD11a/CD18), 4-1BB (CD137), B7-H3, CDS, ICAM-1, ICOS (CD278), GITR, BAFFR, LIGHT, HVEM (LIGHTR), KIRDS2, SLAMF7, NKp80 (KLRF1), NKp44, NKp30, NKp46, CD19, CD4, CD8alpha, CD8beta, IL2R beta, IL2R gamma, IL7R alpha, ITGA4, VLA1, CD49a, ITGA4, IA4, CD49D, ITGA6, VLA-6, CD49f, ITGAD, CD11d, ITGAE, CD103, ITGAL, CD11a, LFA-1, ITGAM, CD11b, ITGAX, CD11c, ITGB1, CD29, ITGB2, CD18, LFA-1, ITGB7, NKG2D, NKG2C, TNFR2, TRANCE/RANKL, DNAM1 (CD226), SLAMF4 (CD244, 2B4), CD84, CD96 (Tactile), CEACAM1, CRTAM, Ly9 (CD229), CD160 (BY55), PSGL1, CD100 (SEMA4D), CD69, SLAMF6 (NTB-A, Ly108), SLAM (SLAMF1, CD150, IPO-3), BLAME (SLAMF8), SELPLG (CD162), LTBR, LAT, GADS, SLP-76, PAG/Cbp, CD19a, and a ligand that specifically binds with CD83, and the like. For example, CD27 costimulation has been demonstrated to enhance expansion, effector function, and survival of human CART cells in vitro and augments human T cell persistence and antitumor activity in vivo (Song et al. Blood. 2012; 119(3):696-706). The intracellular signaling sequences within the cytoplasmic portion of the CAR of the invention may be linked to each other in a random or specified order. Optionally, a short oligo- or polypeptide linker, for example, between 2 and 10 amino acids (e.g., 2, 3, 4, 5, 6, 7, 8, 9, or 10 amino acids) in length may form the linkage between intracellular signaling sequence. In one embodiment, a glycine-serine doublet can be used as a suitable linker. In one embodiment, a single amino acid, e.g., an alanine, a glycine, can be used as a suitable linker.

In one aspect, the intracellular signaling domain is designed to comprise two or more, e.g., 2, 3, 4, 5, or more, costimulatory signaling domains. In an embodiment, the two or more, e.g., 2, 3, 4, 5, or more, costimulatory signaling domains, are separated by a linker molecule, e.g., a linker molecule described herein. In one embodiment, the intracellular signaling domain comprises two costimulatory signaling domains. In some embodiments, the linker molecule is a glycine residue. In some embodiments, the linker is an alanine residue.

In one aspect, the intracellular signaling domain is designed to comprise the signaling domain of CD3-zeta and the signaling domain of CD28. In one aspect, the intracellular signaling domain is designed to comprise the signaling domain of CD3-zeta and the signaling domain of 4-1BB. In one aspect, the signaling domain of 4-1BB is a signaling domain of SEQ ID NO: 7. In one aspect, the signaling domain of CD3-zeta is a signaling domain of SEQ ID NO: 9 (mutant CD3zeta) or SEQ ID NO: 10 (wild type human CD3zeta).

In one aspect, the intracellular signaling domain is designed to comprise the signaling domain of CD3-zeta and the signaling domain of CD27. In one aspect, the signaling domain of CD27

comprises an amino acid sequence of QRRKYRSNKGESPVEPAEPCRYSCPREEEGSTIPIQEDYRKPEPACSP (SEQ ID NO:8). In one aspect, the signalling domain of CD27 is encoded by a nucleic acid sequence of AGGAGTAAGAGGAGCAGGCTCCTGCACAGTGACTACATGAACATGACTCCCCGCCGCCCC GGGCCCACCCGCAAGCATTACCAGCCCTATGCCCCACCACGCGACTTCGCAGCCTATCGCT CC (SEQ ID NO:19).

In one aspect, the intracellular is designed to comprise the signaling domain of CD3-zeta and the signaling domain of CD28. In one aspect, the signaling domain of CD28 comprises an amino acid sequence of SEQ ID NO: 1104. In one aspect, the signaling domain of CD28 is encoded by a nucleic acid sequence of SEQ ID NO: 1105.

In one aspect, the intracellular is designed to comprise the signaling domain of CD3-zeta and the signaling domain of ICOS. In one aspect, the signaling domain of ICOS comprises an amino acid sequence of SEQ ID NO: 1106. In one aspect, the signaling domain of ICOS is encoded by a nucleic acid sequence of SEQ ID NO: 1107.

In one aspect, the CAR-expressing cell described herein can further comprise a second CAR, e.g., a second CAR that includes a different antigen binding domain, e.g., to the same target (BCMA) or a different target (e.g., CD19, CD20, or CS-1, or other multiple myeloma targets, e.g., kappa light chain, CD138, Lewis Y antigen, or CD38 (Garfall et al., Discovery Medicine, 2014, 17(91):37-46)). In one embodiment, the CAR-expressing cell comprises a first CAR that targets a first antigen and includes an intracellular signaling domain having a costimulatory signaling domain but not a primary signaling domain, and a second CAR that targets a second, different, antigen and includes an intracellular signaling domain having a primary signaling domain but not a costimulatory signaling domain. While not wishing to be bound by theory, placement of a costimulatory signaling domain, e.g., 4-1BB, CD28, CD27 ICOS, or OX-40, onto the first CAR, and the primary signaling domain, e.g., CD3 zeta, on the second CAR can limit the CAR activity to cells where both targets are expressed. In one embodiment, the CAR expressing cell comprises a first BCMA CAR that includes a BCMA binding domain, a transmembrane domain and a costimulatory domain and a second CAR that targets an antigen other than BCMA (e.g., an antigen expressed on leukemia or lymphoma cells, e.g., CD19, CD20, CS-1, kappa light chain, CD139, Lewis Y antigen, or CD38) and includes an antigen binding domain, a transmembrane domain and a primary signaling domain. In another embodiment, the CAR expressing cell comprises a first BCMA CAR that includes a BCMA binding domain, a transmembrane domain and a primary signaling domain and a second CAR that targets an antigen other than BCMA (e.g., an

antigen expressed on leukemia or lymphoma cells, e.g., CD19, CD20, CS-1, kappa light chain, CD139, Lewis Y antigen, or CD38) and includes an antigen binding domain to the antigen, a transmembrane domain and a costimulatory signaling domain. In one embodiment, the CAR-expressing cell comprises a BCMA CAR described herein and a CAR that targets CD19 (CD19 CAR).

In one embodiment, the CAR-expressing cell comprises a BCMA CAR described herein and an inhibitory CAR. In one embodiment, the inhibitory CAR comprises an antigen binding domain that binds an antigen found on normal cells but not cancer cells, e.g., normal cells that also express mesothelin. In one embodiment, the inhibitory CAR comprises the antigen binding domain, a transmembrane domain and an intracellular domain of an inhibitory molecule. For example, the intracellular domain of the inhibitory CAR can be an intracellular domain of PD1, PD-L1, PD-L2, CTLA4, TIM3, CEACAM (e.g., CEACAM-1, CEACAM-3 and/or CEACAM-5), LAG3, VISTA, BTLA, TIGIT, LAIR1, CD160, 2B4, CD80, CD86, B7-H3 (CD276), B7-H4 (VTCN1), HVEM (TNFRSF14 or CD270), KIR, A2aR, MHC class I, MHC class II, GAL9, adenosine, and TGFR beta.

In one embodiment, when the CAR-expressing cell comprises two or more different CARs, the antigen binding domains of the different CARs can be such that the antigen binding domains do not interact with one another. For example, a cell expressing a first and second CAR can have an antigen binding domain of the first CAR, e.g., as a fragment, e.g., an scFv, that does not form an association with the antigen binding domain of the second CAR, e.g., the antigen binding domain of the second CAR is a VHH.

In some embodiments, the antigen binding domain comprises a single domain antigen binding (SDAB) molecules include molecules whose complementary determining regions are part of a single domain polypeptide. Examples include, but are not limited to, heavy chain variable domains, binding molecules naturally devoid of light chains, single domains derived from conventional 4-chain antibodies, engineered domains and single domain scaffolds other than those derived from antibodies. SDAB molecules may be any of the art, or any future single domain molecules. SDAB molecules may be derived from any species including, but not limited to mouse, human, camel, llama, lamprey, fish, shark, goat, rabbit, and bovine. This term also includes naturally occurring single domain antibody molecules from species other than Camelidae and sharks.

In one aspect, an SDAB molecule can be derived from a variable region of the immunoglobulin found in fish, such as, for example, that which is derived from the immunoglobulin isotype known as Novel Antigen Receptor (NAR) found in the serum of shark. Methods of producing single domain molecules derived from a variable region of NAR ("IgNARs") are described in WO 03/014161 and Streltsov (2005) Protein Sci. 14:2901-2909.

According to another aspect, an SDAB molecule is a naturally occurring single domain antigen binding molecule known as heavy chain devoid of light chains. Such single domain molecules are disclosed in WO 9404678 and Hamers-Casterman, C. et al. (1993) Nature 363:446-448, for example. For clarity reasons, this variable domain derived from a heavy chain molecule naturally devoid of light chain is known herein as a VHH or nanobody to distinguish it from the conventional VH of four chain immunoglobulins. Such a VHH molecule can be derived from Camelidae species, for example in camel, llama, dromedary, alpaca and guanaco. Other species besides Camelidae may produce heavy chain molecules naturally devoid of light chain; such VHHs are within the scope of the invention.

The SDAB molecules can be recombinant, CDR-grafted, humanized, camelized, de-immunized and/or in vitro generated (e.g., selected by phage display).

It has also been discovered, that cells having a plurality of chimeric membrane embedded receptors comprising an antigen binding domain that interactions between the antigen binding domain of the receptors can be undesirable, e.g., because it inhibits the ability of one or more of the antigen binding domains to bind its cognate antigen. Accordingly, disclosed herein are cells having a first and a second non-naturally occurring chimeric membrane embedded receptor comprising antigen binding domains that minimize such interactions. Also disclosed herein are nucleic acids encoding a first and a second non-naturally occurring chimeric membrane embedded receptor comprising a antigen binding domains that minimize such interactions, as well as methods of making and using such cells and nucleic acids. In an embodiment the antigen binding domain of one of said first said second non-naturally occurring chimeric membrane embedded receptor, comprises an scFv, and the other comprises a single VH domain, e.g., a camelid, shark, or lamprey single VH domain, or a single VH domain derived from a human or mouse sequence.

In some embodiments, the claimed invention comprises a first and second CAR, wherein the antigen binding domain of one of said first CAR said second CAR does not comprise a variable light domain and a variable heavy domain. In some embodiments, the antigen binding domain of one of said first CAR said second CAR is an scFv, and the other is not an scFv. In some embodiments, the antigen binding domain of one of said first CAR said second CAR comprises a single VH domain, e.g., a camelid, shark, or lamprey single VH domain, or a single VH domain derived from a human or mouse sequence. In some embodiments, the antigen binding domain of one of said first CAR said second CAR comprises a nanobody. In some embodiments, the antigen binding domain of one of said first CAR said second CAR comprises a camelid VHH domain.

In some embodiments, the antigen binding domain of one of said first CAR said second CAR comprises an scFv, and the other comprises a single VH domain, e.g., a camelid, shark, or lamprey

single VH domain, or a single VH domain derived from a human or mouse sequence. In some embodiments, the antigen binding domain of one of said first CAR said second CAR comprises an scFv, and the other comprises a nanobody. In some embodiments, the antigen binding domain of one of said first CAR said second CAR comprises comprises an scFv, and the other comprises a camelid VHH domain.

In some embodiments, when present on the surface of a cell, binding of the antigen binding domain of said first CAR to its cognate antigen is not substantially reduced by the presence of said second CAR. In some embodiments, binding of the antigen binding domain of said first CAR to its cognate antigen in the presence of said second CAR is 85%, 90%, 95%, 96%, 97%, 98% or 99% of binding of the antigen binding domain of said first CAR to its cognate antigen in the absence of said second CAR.

In some embodiments, when present on the surface of a cell, the antigen binding domains of said first CAR said second CAR, associate with one another less than if both were scFv antigen binding domains. In some embodiments, the antigen binding domains of said first CAR said second CAR, associate with one another 85%, 90%, 95%, 96%, 97%, 98% or 99% less than if both were scFv antigen binding domains.

In another aspect, the CAR-expressing cell described herein can further express another agent, e.g., an agent which enhances the activity of a CAR-expressing cell. For example, in one embodiment, the agent can be an agent which inhibits an inhibitory molecule, e.g., an agent described herein. Inhibitory molecules, e.g., PD1, can, in some embodiments, decrease the ability of a CAR-expressing cell to mount an immune effector response. Examples of inhibitory molecules include PD1, PD-L1, PD-L2, CTLA4, TIM3, CEACAM (e.g., CEACAM-1, CEACAM-3 and/or CEACAM-5), LAG3, VISTA, BTLA, TIGIT, LAIR1, CD160, 2B4, CD80, CD86, B7-H3 (CD276), B7-H4 (VTCN1), HVEM (TNFRSF14 or CD270), KIR, A2aR, MHC class I, MHC class II, GAL9, adenosine, and TGFR beta.In one embodiment, the agent which inhibits an inhibitory molecule comprises a first polypeptide, e.g., an inhibitory molecule, associated with a second polypeptide that provides a positive signal to the cell, e.g., an intracellular signaling domain described herein. In one embodiment, the agent comprises a first polypeptide, e.g., of an inhibitory molecule such as PD1, PD-L1, PD-L2, CTLA4, TIM3, CEACAM (e.g., CEACAM-1, CEACAM-3 and/or CEACAM-5), LAG3, VISTA, BTLA, TIGIT, LAIR1, CD160, 2B4, CD80, CD86, B7-H3 (CD276), B7-H4 (VTCN1), HVEM (TNFRSF14 or CD270), KIR, A2aR, MHC class I, MHC class II, GAL9, adenosine, and TGFR beta, or a fragment of any of these (e.g., at least a portion of an extracellular domain of any of these), and a second polypeptide which is an intracellular signaling domain described herein (e.g., comprising a costimulatory domain (e.g., 41BB, CD27 ICOS, or CD28, e.g., as described herein) and/or a primary signaling domain (e.g., a CD3 zeta

signaling domain described herein). In one embodiment, the agent comprises a first polypeptide of PD1 or a fragment thereof (e.g., at least a portion of an extracellular domain of PD1), and a second polypeptide of an intracellular signaling domain described herein (e.g., a CD28 signaling domain described herein and/or a CD3 zeta signaling domain described herein). In embodiments, the CAR-expressing cell described herein comprises a switch costimulatory receptor, e.g., as described in WO 2013/019615, which is incorporated herein by reference in its entirety. PD1 is an inhibitory member of the CD28 family of receptors that also includes CD28, CTLA-4, ICOS, and BTLA. PD-1 is expressed on activated B cells, T cells and myeloid cells (Agata et al. 1996 Int. Immunol 8:765-75). Two ligands for PD1, PD-L1 and PD-L2 have been shown to downregulate T cell activation upon binding to PD1 (Freeman et a. 2000 J Exp Med 192:1027-34; Latchman et al. 2001 Nat Immunol 2:261-8; Carter et al. 2002 Eur J Immunol 32:634-43). PD-L1 is abundant in human cancers (Dong et al. 2003 J Mol Med 81:281-7; Blank et al. 2005 Cancer Immunol. Immunother 54:307-314; Konishi et al. 2004 Clin Cancer Res 10:5094). Immune suppression can be reversed by inhibiting the local interaction of PD1 with PD-L1.

In one embodiment, the agent comprises the extracellular domain (ECD) of an inhibitory molecule, e.g., Programmed Death 1 (PD1), can be fused to a transmembrane domain and intracellular signaling domains such as 41BB and CD3 zeta (also referred to herein as a PD1 CAR). In one embodiment, the PD1 CAR, when used incombinations with a BCMA CAR described herein, improves the persistence of the CAR-expressing cell, e.g., T cell or NK cell. In one embodiment, the CAR is a PD1 CAR comprising the extracellular domain of PD1 indicated as underlined in SEQ ID NO: 24. In one embodiment, the PD1 CAR comprises the amino acid sequence of SEQ ID NO:24.

Malpvtalllplalllhaarppgwfldspdrpwnpptfspallvvtegdnatftcsfsntsesfvlnwyrmspsnqtdklaafpedrsqpgqdcrfrvtqlpngrdfhmsvvrarrndsgtylcgaislapkaqikeslraelrvterraevptahpspsprpagqfqtlvtttpaprpptpaptiasqplslrpeacrpaaggavhtrgldfacdiyiwaplagtcgvlllslvitlyckrgrkkllyifkqpfmrpvqttqeedgcscrfpeeeeggcelrvkfsrsadapaykqgqnqlynelnlgrreeydvldkrrgrdpemggkprrknpqeglynelqkdkmaeayseigmkgerrrgkghdglyqglstatkdtydalhmqalppr (SEQ ID NO:24).

In one embodiment, the PD1 CAR comprises the amino acid sequence provided below (SEQ ID NO:22).

pgwfldspdrpwnpptfspallvvtegdnatftcsfsntsesfvlnwyrmspsnqtdklaafpedrsqpgqdcrfrvtqlpngrdfhmsvvrarrndsgtylcgaislapkaqikeslraelrvterraevptahpspsprpagqfqtlvtttpaprpptpaptiasqplslrpeacrpaaggavhtrgldfacdiyiwaplagtcgvlllslvitlyckrgrkkllyifkqpfmrpvqttqeedgcscrfpeeeeggcelrvkfsrsadapaykqgqnqlynelnlgrreeydvldkrrgrdpemggkprrknpqeglynelqkdkmaeayseigmkgerrrgkghdglyqglstatkdtydalhmqalppr (SEQ ID NO:22).

In one embodiment, the agent comprises a nucleic acid sequence encoding the PD1 CAR, e.g., the PD1 CAR described herein. In one embodiment, the nucleic acid sequence for the PD1 CAR is shown below, with the PD1 ECD underlined below in SEQ ID NO: 23

atggccctccctgtcactgccctgcttctccccctcgcactcctgctccacgccgctagaccacccggatggtttctggactctccggatcg
cccgtggaatcccccaaccttctcaccggcactcttggttgtgactgagggcgataatgcgaccttcacgtgctcgttctccaacacctccgaatcattcg
tgctgaactggtaccgcatgagcccgtcaaaccagaccgacaagctcgccgcgtttccggaagatcggtcgcaaccgggacaggattgtcggttccg
cgtgactcaactgccgaatggcagagacttccacatgagcgtggtccgcgctaggcgaaacgactccgggacctacctgtgcggagccatctcgctg
gcgcctaaggcccaaatcaaagagagcttgagggccgaactgagagtgaccgagcgcagagctgaggtgccaactgcacatccatccccatcgcc
tcggcctgcggggcagtttcagaccctggtcacgaccactccggcgccgcgcccaccgactccggccccaactatcgcgagccagcccctgtcgct
gaggccggaagcatgccgccctgccgccggaggtgctgtgcatacccggggattggacttcgcatgcgacatctacatttgggctcctctcgccgga
acttgtggcgtgctccttctgtccctggtcatcaccctgtactgcaagcggggtcggaaaaagcttctgtacattttcaagcagcccttcatgaggcccgt
gcaaaccacccaggaggaggacggttgctcctgccggttccccgaagaggaagaaggaggttgcgagctgcgcgtgaagttctcccggagcgcc
gacgcccccgcctataagcagggccagaaccagctgtacaacgaactgaacctgggacggcgggaagagtacgatgtgctggacaagcggcgcg
gccgggaccccgaaatgggcgggaagcctagaagaaagaaccctcaggaaggcctgtataacgagctgcagaaggacaagatggccgaggcct
actccgaaattgggatgaagggagagcggcggaggggaaaggggcacgacggcctgtaccaaggactgtccaccgccaccaaggacacatacg
atgccctgcacatgcaggcccttcccccctcgc (SEQ ID NO: 23).

In another aspect, the present invention provides a population of CAR-expressing cells, e.g., CART cells or CAR-expressing NK cells. In some embodiments, the population of CAR-expressing cells comprises a mixture of cells expressing different CARs. For example, in one embodiment, the population of CAR-expressing cells (e.g., CART cells or CAR-expressing NK cells) can include a first cell expressing a CAR having an anti-BCMA binding domain described herein, and a second cell expressing a CAR having a different anti- BCMA binding domain, e.g., an anti-BCMA binding domain described herein that differs from the anti-BCMA binding domain in the CAR expressed by the first cell. As another example, the population of CAR-expressing cells can include a first cell expressing a CAR that includes an anti-BCMA binding domain, e.g., as described herein, and a second cell expressing a CAR that includes an antigen binding domain to a target other than BCMA (e.g., CD19, CD20, CS-1, kappa light chain, CD139, Lewis Y antigen, or CD38). In one embodiment, the population of CAR-expressing cells includes a first cell expressing a CAR comprising an anti-BCMA binding domain, e.g., as described herein, and a second cell expressing a CAR comprising an antigen binding domain that targets CD19 (CD19 CAR). In one embodiment, the population of CAR-expressing cells includes, e.g., a first cell expressing a CAR that includes a primary intracellular signaling domain, and a second cell expressing a CAR that includes a secondary signaling domain.

In another aspect, the present invention provides a population of cells wherein at least one cell in the population expresses a CAR having an anti-BCMA domain described herein, and a second cell

expressing another agent, e.g., an agent which enhances the activity of a CAR-expressing cell. For example, in one embodiment, the agent can be an agent which inhibits an inhibitory molecule. Inhibitory molecules, e.g., can, in some embodiments, decrease the ability of a CAR-expressing cell to mount an immune effector response. Examples of inhibitory molecules include PD1, PD-L1, PD-L2, CTLA4, TIM3, CEACAM (e.g., CEACAM-1, CEACAM-3 and/or CEACAM-5), LAG3, VISTA, BTLA, TIGIT, LAIR1, CD160, 2B4, CD80, CD86, B7-H3 (CD276), B7-H4 (VTCN1), HVEM (TNFRSF14 or CD270), KIR, A2aR, MHC class I, MHC class II, GAL9, adenosine, and TGFR beta. In one embodiment, the agent which inhibits an inhibitory molecule comprises a first polypeptide, e.g., an inhibitory molecule, associated with a second polypeptide that provides a positive signal to the cell, e.g., an intracellular signaling domain described herein. In one embodiment, the agent comprises a first polypeptide, e.g., of an inhibitory molecule such as PD1, PD-L1, PD-L2, CTLA4, TIM3, CEACAM (e.g., CEACAM-1, CEACAM-3 and/or CEACAM-5), LAG3, VISTA, BTLA, TIGIT, LAIR1, CD160, 2B4, CD80, CD86, B7-H3 (CD276), B7-H4 (VTCN1), HVEM (TNFRSF14 or CD270), KIR, A2aR, MHC class I, MHC class II, GAL9, adenosine, and TGFR beta, or a fragment of any of these (e.g., at least a portion of an extracellular domain of any of these), and a second polypeptide which is an intracellular signaling domain described herein (e.g., comprising a costimulatory domain (e.g., 41BB, CD27, ICOS, or CD28, e.g., as described herein) and/or a primary signaling domain (e.g., a CD3 zeta signaling domain described herein). In one embodiment, the agent comprises a first polypeptide of PD1 or a fragment thereof (e.g., at least a portion of the extracellular domain of PD1), and a second polypeptide of an intracellular signaling domain described herein (e.g., a CD28 signaling domain described herein and/or a CD3 zeta signaling domain described herein).

In one aspect, the present invention provides methods comprising administering a population of CAR-expressing cells (e.g., CART cells or CAR-expressing NK cells), e.g., a mixture of cells expressing different CARs, in combination with another agent, e.g., a kinase inhibitor, such as a kinase inhibitor described herein. In another aspect, the present invention provides methods comprising administering a population of cells wherein at least one cell in the population expresses a CAR having an anti- cancer associated antigen binding domain as described herein, and a second cell expressing another agent, e.g., an agent which enhances the activity of a CAR-expressing cell, in combination with another agent, e.g., a kinase inhibitor, such as a kinase inhibitor described herein.

Natural Killer Cell Receptor (NKR) CARs

In an embodiment, the CAR molecule described herein comprises one or more components of a natural killer cell receptor (NKR), thereby forming an NKR-CAR. The NKR component can be a transmembrane domain, a hinge domain, or a cytoplasmic domain from any of the following natural

killer cell receptors: killer cell immunoglobulin-like receptor (KIR), e.g., KIR2DL1, KIR2DL2/L3, KIR2DL4, KIR2DL5A, KIR2DL5B, KIR2DS1, KIR2DS2, KIR2DS3, KIR2DS4, DIR2DS5, KIR3DL1/S1, KIR3DL2, KIR3DL3, KIR2DP1, and KIR3DP1; natural cytoxicity receptor (NCR), e.g., NKp30, NKp44, NKp46; signaling lymphocyte activation molecule (SLAM) family of immune cell receptors, e.g., CD48, CD229, 2B4, CD84, NTB-A, CRACC, BLAME, and CD2F-10; Fc receptor (FcR), e.g., CD16, and CD64; and Ly49 receptors, e.g., LY49A, LY49C. The NKR-CAR molecules described herein may interact with an adaptor molecule or intracellular signaling domain, e.g., DAP12. Exemplary configurations and sequences of CAR molecules comprising NKR components are described in International Publication No. WO2014/145252, the contents of which are hereby incorporated by reference.

Strategies for Regulating Chimeric Antigen Receptors

There are many ways CAR activities can be regulated. In some embodiments, a regulatable CAR (RCAR) where the CAR activity can be controlled is desirable to optimize the safety and efficacy of a CAR therapy. For example, inducing apoptosis using, e.g., a caspase fused to a dimerization domain (see, e.g., Di et al., N Engl. J. Med. 2011 Nov. 3; 365(18):1673-1683), can be used as a safety switch in the CAR therapy of the instant invention. In another example, CAR-expressing cells can also express an inducible Caspase-9 (iCaspase-9) molecule that, upon administration of a dimerizer drug (e.g., rimiducid (also called AP1903 (Bellicum Pharmaceuticals) or AP20187 (Ariad)) leads to activation of the Caspase-9 and apoptosis of the cells. The iCaspase-9 molecule contains a chemical inducer of dimerization (CID) binding domain that mediates dimerization in the presence of a CID. This results in inducible and selective depletion of CAR-expressing cells. In some cases, the iCaspase-9 molecule is encoded by a nucleic acid molecule separate from the CAR-encoding vector(s). In some cases, the iCaspase-9 molecule is encoded by the same nucleic acid molecule as the CAR-encoding vector. The iCaspase-9 can provide a safety switch to avoid any toxicity of CAR-expressing cells. See, e.g., Song et al. Cancer Gene Ther. 2008; 15(10):667-75; Clinical Trial Id. No. NCT02107963; and Di Stasi et al. N. Engl. J. Med. 2011; 365:1673-83.

Alternative strategies for regulating the CAR therapy of the instant invention include utilizing small molecules or antibodies that deactivate or turn off CAR activity, e.g., by deleting CAR-expressing cells, e.g., by inducing antibody dependent cell-mediated cytotoxicity (ADCC). For example, CAR-expressing cells described herein may also express an antigen that is recognized by molecules capable of inducing cell death, e.g., ADCC or compliment-induced cell death. For example, CAR expressing cells described herein may also express a receptor capable of being targeted by an antibody or antibody fragment. Examples of such receptors include EpCAM, VEGFR, integrins (e.g., integrins

αvβ3, α4, αI¾β3, α4β7, α5β1, αvβ3, αv), members of the TNF receptor superfamily (e.g., TRAIL-R1 , TRAIL-R2), PDGF Receptor, interferon receptor, folate receptor, GPNMB, ICAM-1 , HLA-DR, CEA, CA-125, MUC1 , TAG-72, IL-6 receptor, 5T4, GD2, GD3, CD2, CD3, CD4, CD5, CD1 1 , CD1 1 a/LFA-1 , CD15, CD18/ITGB2, CD19, CD20, CD22, CD23/lgE Receptor, CD25, CD28, CD30, CD33, CD38, CD40, CD41 , CD44, CD51 , CD52, CD62L, CD74, CD80, CD125, CD147/basigin, CD152/CTLA-4, CD154/CD40L, CD195/CCR5, CD319/SLAMF7, and EGFR, and truncated versions thereof (e.g., versions preserving one or more extracellular epitopes but lacking one or more regions within the cytoplasmic domain). For example, CAR-expressing cells described herein may also express a truncated epidermal growth factor receptor (EGFR) which lacks signaling capacity but retains the epitope that is recognized by molecules capable of inducing ADCC, e.g., cetuximab (ERBITUX®), such that administration of cetuximab induces ADCC and subsequent depletion of the CAR-expressing cells (see, e.g., WO2011/056894, and Jonnalagadda et al., Gene Ther. 2013; 20(8)853-860). Another strategy includes expressing a highly compact marker/suicide gene that combines target epitopes from both CD32 and CD20 antigens in the CAR-expressing cells described herein, which binds rituximab, resulting in selective depletion of the CAR-expressing cells, e.g., by ADCC (see, e.g., Philip et al., Blood. 2014; 124(8)1277-1287). Other methods for depleting CAR-expressing cells described herein include administration of CAMPATH®, a monoclonal anti-CD52 antibody that selectively binds and targets mature lymphocytes, e.g., CAR-expressing cells, for destruction, e.g., by inducing ADCC. In other embodiments, CAR-expressing cells can be selectively targeted using a CAR ligand, e.g., an anti-idiotypic antibody. In some embodiments, the anti-idiotypic antibody can cause effector cell activity, e.g, ADCC or ADC activities, thereby reducing the number of CAR-expressing cells. In other embodiments, the CAR ligand, e.g., the anti-idiotypic antibody, can be coupled to an agent that induces cell killing, e.g., a toxin, thereby reducing the number of CAR-expressing cells. Alternatively, the CAR molecules themselves can be configured such that the activity can be regulated, e.g., turned on and off, as described below.

In some embodiments, a RCAR comprises a set of polypeptides, typically two in the simplest embodiments, in which the components of a standard CAR described herein, e.g., an antigen binding domain and an intracellular signaling domain, are partitioned on separate polypeptides or members. In some embodiments, the set of polypeptides include a dimerization switch that, upon the presence of a dimerization molecule, can couple the polypeptides to one another, e.g., can couple an antigen binding domain to an intracellular signaling domain. Additional description and exemplary configurations of such regulatable CARs are provided herein and in International Publiciation No. WO 2015/090229, hereby incorporated by reference in its entirety.

In an embodiment, an RCAR comprises two polypeptides or members: 1) an intracellular signaling member comprising an intracellular signaling domain, e.g., a primary intracellular signaling domain described herein, and a first switch domain; 2) an antigen binding member comprising an antigen binding domain, e.g., that targets a tumor antigen described herein, as described herein and a second switch domain. Optionally, the RCAR comprises a transmembrane domain described herein. In an embodiment, a transmembrane domain can be disposed on the intracellular signaling member, on the antigen binding member, or on both. (Unless otherwise indicated, when members or elements of an RCAR are described herein, the order can be as provided, but other orders are included as well. In other words, in an embodiment, the order is as set out in the text, but in other embodiments, the order can be different. E.g., the order of elements on one side of a transmembrane region can be different from the example, e.g., the placement of a switch domain relative to a intracellular signaling domain can be different, e.g., reversed).

In an embodiment, the first and second switch domains can form an intracellular or an extracellular dimerization switch. In an embodiment, the dimerization switch can be a homodimerization switch, e.g., where the first and second switch domain are the same, or a heterodimerization switch, e.g., where the first and second switch domain are different from one another.

In embodiments, an RCAR can comprise a "multi switch." A multi switch can comprise heterodimerization switch domains or homodimerization switch domains. A multi switch comprises a plurality of, e.g., 2, 3, 4, 5, 6, 7, 8, 9, or 10, switch domains, independently, on a first member, e.g., an antigen binding member, and a second member, e.g., an intracellular signaling member. In an embodiment, the first member can comprise a plurality of first switch domains, e.g., FKBP-based switch domains, and the second member can comprise a plurality of second switch domains, e.g., FRB-based switch domains. In an embodiment, the first member can comprise a first and a second switch domain, e.g., a FKBP-based switch domain and a FRB-based switch domain, and the second member can comprise a first and a second switch domain, e.g., a FKBP-based switch domain and a FRB-based switch domain.

In an embodiment, the intracellular signaling member comprises one or more intracellular signaling domains, e.g., a primary intracellular signaling domain and one or more costimulatory signaling domains.

In an embodiment, the antigen binding member may comprise one or more intracellular signaling domains, e.g., one or more costimulatory signaling domains. In an embodiment, the antigen binding member comprises a plurality, e.g., 2 or 3 costimulatory signaling domains described herein,

e.g., selected from 4-1BB, CD28, CD27, ICOS, and OX40, and in embodiments, no primary intracellular signaling domain. In an embodiment, the antigen binding member comprises the following costimulatory signaling domains, from the extracellular to intracellular direction: 4-1BB-CD27; 4-1BB-CD27; CD27-4-1BB; 4-1BB-CD28; CD28-4-1BB; OX40-CD28; CD28-OX40; CD28-4-1BB; or 4-1BB-CD28. In such embodiments, the intracellular binding member comprises a CD3zeta domain. In one such embodiment the RCAR comprises (1) an antigen binding member comprising, an antigen binding domain, a transmembrane domain, and two costimulatory domains and a first switch domain; and (2) an intracellular signaling domain comprising a transmembrane domain or membrane tethering domain and at least one primary intracellular signaling domain, and a second switch domain.

An embodiment provides RCARs wherein the antigen binding member is not tethered to the surface of the CAR cell. This allows a cell having an intracellular signaling member to be conveniently paired with one or more antigen binding domains, without transforming the cell with a sequence that encodes the antigen binding member. In such embodiments, the RCAR comprises: 1) an intracellular signaling member comprising: a first switch domain, a transmembrane domain, an intracellular signaling domain, e.g., a primary intracellular signaling domain, and a first switch domain; and 2) an antigen binding member comprising: an antigen binding domain, and a second switch domain, wherein the antigen binding member does not comprise a transmembrane domain or membrane tethering domain, and, optionally, does not comprise an intracellular signaling domain. In some embodiments, the RCAR may further comprise 3) a second antigen binding member comprising: a second antigen binding domain, e.g., a second antigen binding domain that binds a different antigen than is bound by the antigen binding domain; and a second switch domain.

Also provided herein are RCARs wherein the antigen binding member comprises bispecific activation and targeting capacity. In this embodiment, the antigen binding member can comprise a plurality, e.g., 2, 3, 4, or 5 antigen binding domains, e.g., scFvs, wherein each antigen binding domain binds to a target antigen, e.g. different antigens or the same antigen, e.g., the same or different epitopes on the same antigen. In an embodiment, the plurality of antigen binding domains are in tandem, and optionally, a linker or hinge region is disposed between each of the antigen binding domains. Suitable linkers and hinge regions are described herein.

An embodiment provides RCARs having a configuration that allows switching of proliferation. In this embodiment, the RCAR comprises: 1) an intracellular signaling member comprising: optionally, a transmembrane domain or membrane tethering domain; one or more co-stimulatory signaling domain, e.g., selected from 4-1BB, CD28, CD27, ICOS, and OX40, and a switch domain; and 2) an antigen binding member comprising: an antigen binding domain, a transmembrane domain, and a primary intracellular signaling domain, e.g., a CD3zeta domain, wherein the antigen binding member does not

comprise a switch domain, or does not comprise a switch domain that dimerizes with a switch domain on the intracellular signaling member. In an embodiment, the antigen binding member does not comprise a co-stimulatory signaling domain. In an embodiment, the intracellular signaling member comprises a switch domain from a homodimerization switch. In an embodiment, the intracellular signaling member comprises a first switch domain of a heterodimerization switch and the RCAR comprises a second intracellular signaling member which comprises a second switch domain of the heterodimerization switch. In such embodiments, the second intracellular signaling member comprises the same intracellular signaling domains as the intracellular signaling member. In an embodiment, the dimerization switch is intracellular. In an embodiment, the dimerization switch is extracellular.

In any of the RCAR configurations described here, the first and second switch domains comprise a FKBP-FRB based switch as described herein.

Also provided herein are cells comprising an RCAR described herein. Any cell that is engineered to express a RCAR can be used as a RCARX cell. In an embodiment the RCARX cell is a T cell, and is referred to as a RCART cell. In an embodiment the RCARX cell is an NK cell, and is referred to as a RCARN cell.

Also provided herein are nucleic acids and vectors comprising RCAR encoding sequences. Sequence encoding various elements of an RCAR can be disposed on the same nucleic acid molecule, e.g., the same plasmid or vector, e.g., viral vector, e.g., lentiviral vector. In an embodiment, (i) sequence encoding an antigen binding member and (ii) sequence encoding an intracellular signaling member, can be present on the same nucleic acid, e.g., vector. Production of the corresponding proteins can be achieved, e.g., by the use of separate promoters, or by the use of a bicistronic transcription product (which can result in the production of two proteins by cleavage of a single translation product or by the translation of two separate protein products). In an embodiment, a sequence encoding a cleavable peptide, e.g., a P2A or F2A sequence, is disposed between (i) and (ii). In an embodiment, a sequence encoding an IRES, e.g., an EMCV or EV71 IRES, is disposed between (i) and (ii). In these embodiments, (i) and (ii) are transcribed as a single RNA. In an embodiment, a first promoter is operably linked to (i) and a second promoter is operably linked to (ii), such that (i) and (ii) are transcribed as separate mRNAs.

Alternatively, the sequence encoding various elements of an RCAR can be disposed on the different nucleic acid molecules, e.g., different plasmids or vectors, e.g., viral vector, e.g., lentiviral vector. E.g., the (i) sequence encoding an antigen binding member can be present on a first nucleic acid, e.g., a first vector, and the (ii) sequence encoding an intracellular signaling member can be present on the second nucleic acid, e.g., the second vector.

Dimerization switches

Dimerization switches can be non-covalent or covalent. In a non-covalent dimerization switch, the dimerization molecule promotes a non-covalent interaction between the switch domains. In a covalent dimerization switch, the dimerization molecule promotes a covalent interaction between the switch domains.

In an embodiment, the RCAR comprises a FKBP/FRAP, or FKBP/FRB,-based dimerization switch. FKBP12 (FKBP, or FK506 binding protein) is an abundant cytoplasmic protein that serves as the initial intracellular target for the natural product immunosuppressive drug, rapamycin. Rapamycin binds to FKBP and to the large PI3K homolog FRAP (RAFT, mTOR). FRB is a 93 amino acid portion of FRAP, that is sufficient for binding the FKBP-rapamycin complex (Chen, J., Zheng, X. F., Brown, E. J. & Schreiber, S. L. (1995) *Identification of an 11-kDa FKBP12-rapamycin-binding domain within the 289-kDa FKBP12-rapamycin-associated protein and characterization of a critical serine residue*. Proc Natl Acad Sci U S A 92: 4947-51.)

In embodiments, an FKBP/FRAP, e.g., an FKBP/FRB, based switch can use a dimerization molecule, e.g., rapamycin or a rapamycin analog.

The amino acid sequence of FKBP is as follows:

DVPDYASLGGPSSPKKKRKVSRGVQVETISPGDGRTFPKRGQTCVVHYTGMLEDGKKF DSSRDRNKPFKFMLGKQEVIRGWEEGVAQMSVGQRAKLTISPDYAYGATGHPGIIPPHATLVF DVELLKLETSY (SEQ ID NO: 275)

In embodiments, an FKBP switch domain can comprise a fragment of FKBP having the ability to bind with FRB, or a fragment or analog thereof, in the presence of rapamycin or a rapalog, e.g., the underlined portion of SEQ ID NO: 275, which is:

VQVETISPGDGRTFPKRGQTCVVHYTGMLEDGKKFDSSRDRNKPFKFMLGKQEVIRG WEEGVAQMSVGQRAKLTISPDYAYGATGHPGIIPPHATLVFDVELLKLETS (SEQ ID NO:276)

The amino acid sequence of FRB is as follows:

ILWHEMWHEGLEEASRLYFGERNVKGMFEVLEPLHAMMERGPQTLKETSFNQAYGRDLMEA QEWCRKYMKSGNVKDLTQAWDLYYHVFRRISK (SEQ ID NO: 277)

"FKBP/FRAP, e.g., an FKBP/FRB, based switch" as that term is used herein, refers to a dimerization switch comprising: a first switch domain, which comprises an FKBP fragment or analog thereof having the ability to bind with FRB, or a fragment or analog thereof, in the presence of rapamycin or a rapalog, e.g., RAD001, and has at least 70, 75, 80, 85, 90, 95, 96, 97, 98, or 99% identity

with, or differs by no more than 30, 25, 20, 15, 10, 5, 4, 3, 2, or 1 amino acid residues from, the FKBP sequence of SEQ ID NO: 275 or 276; and a second switch domain, which comprises an FRB fragment or analog thereof having the ability to bind with FRB, or a fragment or analog thereof, in the presence of rapamycin or a rapalog, and has at least 70, 75, 80, 85, 90, 95, 96, 97, 98, or 99% identity with, or differs by no more than 30, 25, 20, 15, 10, 5, 4, 3, 2, or 1 amino acid residues from, the FRB sequence of SEQ ID NO: 277. In an embodiment, a RCAR described herein comprises one switch domain comprises amino acid residues disclosed in SEQ ID NO: 275 (or SEQ ID NO: 276), and one switch domain comprises amino acid residues disclosed in SEQ ID NO: 277.

In embodiments, the FKBP/FRB dimerization switch comprises a modified FRB switch domain that exhibits altered, e.g., enhanced, complex formation between an FRB-based switch domain, e.g., the modified FRB switch domain, a FKBP-based switch domain, and the dimerization molecule, e.g., rapamycin or a rapalogue, e.g., RAD001. In an embodiment, the modified FRB switch domain comprises one or more mutations, e.g., 2, 3, 4, 5, 6, 7, 8, 9, 10 or more, selected from mutations at amino acid position(s) L2031, E2032, S2035, R2036, F2039, G2040, T2098, W2101, D2102, Y2105, and F2108, where the wild-type amino acid is mutated to any other naturally-occurring amino acid. In an embodiment, a mutant FRB comprises a mutation at E2032, where E2032 is mutated to phenylalanine (E2032F), methionine (E2032M), arginine (E2032R), valine (E2032V), tyrosine (E2032Y), isoleucine (E2032I), e.g., SEQ ID NO: 278, or leucine (E2032L), e.g., SEQ ID NO: 279. In an embodiment, a mutant FRB comprises a mutation at T2098, where T2098 is mutated to phenylalanine (T2098F) or leucine (T2098L), e.g., SEQ ID NO: 280. In an embodiment, a mutant FRB comprises a mutation at E2032 and at T2098, where E2032 is mutated to any amino acid, and where T2098 is mutated to any amino acid, e.g., SEQ ID NO: 281. In an embodiment, a mutant FRB comprises an E2032I and a T2098L mutation, e.g., SEQ ID NO: 282. In an embodiment, a mutant FRB comprises an E2032L and a T2098L mutation, e.g., SEQ ID NO: 283.

Table 17. Exemplary mutant FRB having increased affinity for a dimerization molecule.

FRB mutant	Amino Acid Sequence	EQ ID NO:
E2032I mutant	ILWHEMWHEGLIEASRLYFGERNVKGMFEVLEPLHAMMERGPQTLKETSF NQAYGRDLMEAQEWCRKYMKSGNVKDLTQAWDLYYHVFRRISKTS	78
E2032L mutant	ILWHEMWHEGLLEASRLYFGERNVKGMFEVLEPLHAMMERGPQTLKETSF NQAYGRDLMEAQEWCRKYMKSGNVKDLTQAWDLYYHVFRRISKTS	79
T2098L mutant	ILWHEMWHEGLEEASRLYFGERNVKGMFEVLEPLHAMMERGPQTLKETSF NQAYGRDLMEAQEWCRKYMKSGNVKDLLQAWDLYYHVFRRISKTS	80
E2032, T2098	ILWHEMWHEGL**X**EASRLYFGERNVKGMFEVLEPLHAMMERGPQTLKETSF	

mutant	NQAYGRDLMEAQEWCRKYMKSGNVKDL**X**QAWDLYYHVFRRISKTS	81
E2032I, T2098L mutant	ILWHEMWHEGLIEASRLYFGERNVKGMFEVLEPLHAMMERGPQTLKETSF NQAYGRDLMEAQEWCRKYMKSGNVKDLLQAWDLYYHVFRRISKTS	82
E2032L, T2098L mutant	ILWHEMWHEGLLEASRLYFGERNVKGMFEVLEPLHAMMERGPQTLKETSF NQAYGRDLMEAQEWCRKYMKSGNVKDLLQAWDLYYHVFRRISKTS	83

Other suitable dimerization switches include a GyrB-GyrB based dimerization switch, a Gibberellin-based dimerization switch, a tag/binder dimerization switch, and a halo-tag/snap-tag dimerization switch. Following the guidance provided herein, such switches and relevant dimerization molecules will be apparent to one of ordinary skill.

Dimerization molecule

Association between the switch domains is promoted by the dimerization molecule. In the presence of dimerization molecule interaction or association between switch domains allows for signal transduction between a polypeptide associated with, e.g., fused to, a first switch domain, and a polypeptide associated with, e.g., fused to, a second switch domain. In the presence of non-limiting levels of dimerization molecule signal transduction is increased by 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9, 2, 5, 10, 50, 100 fold, e.g., as measured in a system described herein.

Rapamycin and rapamycin analogs (sometimes referred to as rapalogues), e.g., RAD001, can be used as dimerization molecules in a FKBP/FRB-based dimerization switch described herein. In an embodiment the dimerization molecule can be selected from rapamycin (sirolimus), RAD001 (everolimus), zotarolimus, temsirolimus, AP-23573 (ridaforolimus), biolimus and AP21967. Additional rapamycin analogs suitable for use with FKBP/FRB-based dimerization switches are further described in the section entitled “Combination Therapies”, or in the subsection entitled “Combination with a Low, Immune Enhancing, Dose of an mTOR inhibitor”.

Split CAR

In some embodiments, the CAR-expressing cell uses a split CAR. The split CAR approach is described in more detail in publications WO2014/055442 and WO2014/055657, incorporated herein by reference. Briefly, a split CAR system comprises a cell expressing a first CAR having a first antigen binding domain and a costimulatory domain (e.g., 41BB), and the cell also expresses a second CAR having a second antigen binding domain and an intracellular signaling domain (e.g., CD3 zeta). When the cell encounters the first antigen, the costimulatory domain is activated, and the cell proliferates. When the cell encounters the second antigen, the intracellular signaling domain is activated and cell-killing activity begins. Thus, the CAR-expressing cell is only fully activated in the presence of both antigens. In embodiments the first antigen binding domain recognizes BCMA, e.g., comprises an antigen binding domain described herein, and the second antigen binding domain recognizes an antigen

expressed on acute myeloid leukemia cells, e.g., CD123, CLL-1, CD34, FLT3, or folate receptor beta. In embodiments the first antigen binding domain recognizes BCMA, e.g., comprises an antigen binding domain described herein, and the second antigen binding domain recognizes an antigen expressed on B-cells, e.g., CD10, CD19, CD20, CD22, CD34, CD123, FLT-3, ROR1, CD79b, CD179b, or CD79a.

Stability and Mutations

The stability of an anti-BCMA binding domain, e.g., scFv molecules (e.g., soluble scFv) can be evaluated in reference to the biophysical properties (e.g., thermal stability) of a conventional control scFv molecule or a full length antibody. In one embodiment, the humanized scFv has a thermal stability that is greater than about 0.1, about 0.25, about 0.5, about 0.75, about 1, about 1.25, about 1.5, about 1.75, about 2, about 2.5, about 3, about 3.5, about 4, about 4.5, about 5, about 5.5, about 6, about 6.5, about 7, about 7.5, about 8, about 8.5, about 9, about 9.5, about 10 degrees, about 11 degrees, about 12 degrees, about 13 degrees, about 14 degrees, or about 15 degrees Celsius than a control binding molecule (e.g. a conventional scFv molecule) in the described assays.

The improved thermal stability of the anti-BCMA binding domain, e.g., scFv is subsequently conferred to the entire CART-BCMA construct, leading to improved therapeutic properties of the CART-BCMA construct. The thermal stability of the anti-BCMA binding domain, e.g., scFv can be improved by at least about 2°C or 3°C as compared to a conventional antibody. In one embodiment, the anti-BCMA binding domain, e.g., scFv has a 1°C improved thermal stability as compared to a conventional antibody. In another embodiment, the anti-BCMA binding domain, e.g., scFv has a 2°C improved thermal stability as compared to a conventional antibody. In another embodiment, the scFv has a 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15°C improved thermal stability as compared to a conventional antibody. Comparisons can be made, for example, between the scFv molecules disclosed herein and scFv molecules or Fab fragments of an antibody from which the scFv VH and VL were derived. Thermal stability can be measured using methods known in the art. For example, in one embodiment, Tm can be measured. Methods for measuring Tm and other methods of determining protein stability are described in more detail below.

Mutations in scFv (arising through humanization or direct mutagenesis of the soluble scFv) alter the stability of the scFv and improve the overall stability of the scFv and the CART33 construct. Stability of the human scFv can be compared against the murine scFv using measurements such as Tm, temperature denaturation and temperature aggregation.

The binding capacity of the mutant scFvs can be determined using assays described in the Examples.

In one embodiment, the anti-BCMA binding domain, e.g., scFv comprises at least one mutation arising from the humanization process such that the mutated scFv confers improved stability to the CART-BCMA construct. In another embodiment, the anti-BCMA binding domain, e.g., scFv comprises at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 mutations arising from the humanization process such that the mutated scFv confers improved stability to the CART-BCMA construct.

Methods of Evaluating Protein Stability

The stability of an antigen binding domain may be assessed using, e.g., the methods described below. Such methods allow for the determination of multiple thermal unfolding transitions where the least stable domain either unfolds first or limits the overall stability threshold of a multidomain unit that unfolds cooperatively (e.g., a multidomain protein which exhibits a single unfolding transition). The least stable domain can be identified in a number of additional ways. Mutagenesis can be performed to probe which domain limits the overall stability. Additionally, protease resistance of a multidomain protein can be performed under conditions where the least stable domain is known to be intrinsically unfolded via DSC or other spectroscopic methods (Fontana, *et al.*, (1997) Fold. Des., 2: R17-26; Dimasi *et al.* (2009) J. Mol. Biol. 393: 672-692). Once the least stable domain is identified, the sequence encoding this domain (or a portion thereof) may be employed as a test sequence in the methods. Methods of evaluating protein stability have been described in, e.g., pages 191-198 of WO 2016/014565, herein incorporated by reference in its entirety.

RNA Transfection

Disclosed herein are methods for producing an in vitro transcribed RNA CAR. The present invention also includes a CAR encoding RNA construct that can be directly transfected into a cell. A method for generating mRNA for use in transfection can involve in vitro transcription (IVT) of a template with specially designed primers, followed by polyA addition, to produce a construct containing 3' and 5' untranslated sequence ("UTR"), a 5' cap and/or Internal Ribosome Entry Site (IRES), the nucleic acid to be expressed, and a polyA tail, typically 50-2000 bases in length (SEQ ID NO:35). RNA so produced can efficiently transfect different kinds of cells. In one aspect, the template includes sequences for the CAR.

In one aspect the anti-BCMA CAR is encoded by a messenger RNA (mRNA). In one aspect the mRNA encoding the anti-BCMA CAR is introduced into an immune effector cell, e.g., a T cell or a NK cell, for production of a CAR-expressing cell (e.g., CART cell or CAR-expressing NK cell).

In one embodiment, the in vitro transcribed RNA CAR can be introduced to a cell as a form of transient transfection. The RNA is produced by in vitro transcription using a polymerase chain reaction

(PCR)-generated template. DNA of interest from any source can be directly converted by PCR into a template for in vitro mRNA synthesis using appropriate primers and RNA polymerase. The source of the DNA can be, for example, genomic DNA, plasmid DNA, phage DNA, cDNA, synthetic DNA sequence or any other appropriate source of DNA. The desired temple for in vitro transcription is a CAR of the present invention. For example, the template for the RNA CAR comprises an extracellular region comprising a single chain variable domain of an anti-tumor antibody; a hinge region, a transmembrane domain (e.g., a transmembrane domain of CD8a); and a cytoplasmic region that includes an intracellular signaling domain, e.g., comprising the signaling domain of CD3-zeta and the signaling domain of 4-1BB.

In one embodiment, the DNA to be used for PCR contains an open reading frame. The DNA can be from a naturally occurring DNA sequence from the genome of an organism. In one embodiment, the nucleic acid can include some or all of the 5' and/or 3' untranslated regions (UTRs). The nucleic acid can include exons and introns. In one embodiment, the DNA to be used for PCR is a human nucleic acid sequence. In another embodiment, the DNA to be used for PCR is a human nucleic acid sequence including the 5' and 3' UTRs. The DNA can alternatively be an artificial DNA sequence that is not normally expressed in a naturally occurring organism. An exemplary artificial DNA sequence is one that contains portions of genes that are ligated together to form an open reading frame that encodes a fusion protein. The portions of DNA that are ligated together can be from a single organism or from more than one organism.

PCR is used to generate a template for in vitro transcription of mRNA which is used for transfection. Methods for performing PCR are well known in the art. Primers for use in PCR are designed to have regions that are substantially complementary to regions of the DNA to be used as a template for the PCR. "Substantially complementary," as used herein, refers to sequences of nucleotides where a majority or all of the bases in the primer sequence are complementary, or one or more bases are non-complementary, or mismatched. Substantially complementary sequences are able to anneal or hybridize with the intended DNA target under annealing conditions used for PCR. The primers can be designed to be substantially complementary to any portion of the DNA template. For example, the primers can be designed to amplify the portion of a nucleic acid that is normally transcribed in cells (the open reading frame), including 5' and 3' UTRs. The primers can also be designed to amplify a portion of a nucleic acid that encodes a particular domain of interest. In one embodiment, the primers are designed to amplify the coding region of a human cDNA, including all or portions of the 5' and 3' UTRs. Primers useful for PCR can be generated by synthetic methods that are well known in the art. "Forward primers" are primers that contain a region of nucleotides that are substantially complementary to nucleotides on the DNA template that are upstream of the DNA sequence that is to be amplified. "Upstream" is used

herein to refer to a location 5, to the DNA sequence to be amplified relative to the coding strand. "Reverse primers" are primers that contain a region of nucleotides that are substantially complementary to a double-stranded DNA template that are downstream of the DNA sequence that is to be amplified. "Downstream" is used herein to refer to a location 3' to the DNA sequence to be amplified relative to the coding strand.

Any DNA polymerase useful for PCR can be used in the methods disclosed herein. The reagents and polymerase are commercially available from a number of sources.

Chemical structures with the ability to promote stability and/or translation efficiency may also be used. The RNA preferably has 5' and 3' UTRs. In one embodiment, the 5' UTR is between one and 3000 nucleotides in length. The length of 5' and 3' UTR sequences to be added to the coding region can be altered by different methods, including, but not limited to, designing primers for PCR that anneal to different regions of the UTRs. Using this approach, one of ordinary skill in the art can modify the 5' and 3' UTR lengths required to achieve optimal translation efficiency following transfection of the transcribed RNA.

The 5' and 3' UTRs can be the naturally occurring, endogenous 5' and 3' UTRs for the nucleic acid of interest. Alternatively, UTR sequences that are not endogenous to the nucleic acid of interest can be added by incorporating the UTR sequences into the forward and reverse primers or by any other modifications of the template. The use of UTR sequences that are not endogenous to the nucleic acid of interest can be useful for modifying the stability and/or translation efficiency of the RNA. For example, it is known that AU-rich elements in 3' UTR sequences can decrease the stability of mRNA. Therefore, 3' UTRs can be selected or designed to increase the stability of the transcribed RNA based on properties of UTRs that are well known in the art.

In one embodiment, the 5' UTR can contain the Kozak sequence of the endogenous nucleic acid. Alternatively, when a 5' UTR that is not endogenous to the nucleic acid of interest is being added by PCR as described above, a consensus Kozak sequence can be redesigned by adding the 5' UTR sequence. Kozak sequences can increase the efficiency of translation of some RNA transcripts, but does not appear to be required for all RNAs to enable efficient translation. The requirement for Kozak sequences for many mRNAs is known in the art. In other embodiments the 5' UTR can be 5'UTR of an RNA virus whose RNA genome is stable in cells. In other embodiments various nucleotide analogues can be used in the 3' or 5' UTR to impede exonuclease degradation of the mRNA.

To enable synthesis of RNA from a DNA template without the need for gene cloning, a promoter of transcription should be attached to the DNA template upstream of the sequence to be transcribed. When a sequence that functions as a promoter for an RNA polymerase is added to the 5' end

of the forward primer, the RNA polymerase promoter becomes incorporated into the PCR product upstream of the open reading frame that is to be transcribed. In one preferred embodiment, the promoter is a T7 polymerase promoter, as described elsewhere herein. Other useful promoters include, but are not limited to, T3 and SP6 RNA polymerase promoters. Consensus nucleotide sequences for T7, T3 and SP6 promoters are known in the art.

In a preferred embodiment, the mRNA has both a cap on the 5' end and a 3' poly(A) tail which determine ribosome binding, initiation of translation and stability mRNA in the cell. On a circular DNA template, for instance, plasmid DNA, RNA polymerase produces a long concatameric product which is not suitable for expression in eukaryotic cells. The transcription of plasmid DNA linearized at the end of the 3' UTR results in normal sized mRNA which is not effective in eukaryotic transfection even if it is polyadenylated after transcription.

On a linear DNA template, phage T7 RNA polymerase can extend the 3' end of the transcript beyond the last base of the template (Schenborn and Mierendorf, Nuc Acids Res., 13:6223-36 (1985); Nacheva and Berzal-Herranz, Eur. J. Biochem., 270:1485-65 (2003).

The conventional method of integration of polyA/T stretches into a DNA template is molecular cloning. However polyA/T sequence integrated into plasmid DNA can cause plasmid instability, which is why plasmid DNA templates obtained from bacterial cells are often highly contaminated with deletions and other aberrations. This makes cloning procedures not only laborious and time consuming but often not reliable. That is why a method which allows construction of DNA templates with polyA/T 3' stretch without cloning highly desirable.

The polyA/T segment of the transcriptional DNA template can be produced during PCR by using a reverse primer containing a polyT tail, such as 100T tail (SEQ ID NO: 31) (size can be 50-5000 T (SEQ ID NO: 32)), or after PCR by any other method, including, but not limited to, DNA ligation or in vitro recombination. Poly(A) tails also provide stability to RNAs and reduce their degradation. Generally, the length of a poly(A) tail positively correlates with the stability of the transcribed RNA. In one embodiment, the poly(A) tail is between 100 and 5000 adenosines (SEQ ID NO: 33).

Poly(A) tails of RNAs can be further extended following in vitro transcription with the use of a poly(A) polymerase, such as E. coli polyA polymerase (E-PAP). In one embodiment, increasing the length of a poly(A) tail from 100 nucleotides to between 300 and 400 nucleotides (SEQ ID NO: 34) results in about a two-fold increase in the translation efficiency of the RNA. Additionally, the attachment of different chemical groups to the 3' end can increase mRNA stability. Such attachment can contain modified/artificial nucleotides, aptamers and other compounds. For example, ATP analogs can

be incorporated into the poly(A) tail using poly(A) polymerase. ATP analogs can further increase the stability of the RNA.

5' caps on also provide stability to RNA molecules. In a preferred embodiment, RNAs produced by the methods disclosed herein include a 5' cap. The 5' cap is provided using techniques known in the art and described herein (Cougot, et al., Trends in Biochem. Sci., 29:436-444 (2001); Stepinski, et al., RNA, 7:1468-95 (2001); Elango, et al., Biochim. Biophys. Res. Commun., 330:958-966 (2005)).

The RNAs produced by the methods disclosed herein can also contain an internal ribosome entry site (IRES) sequence. The IRES sequence may be any viral, chromosomal or artificially designed sequence which initiates cap-independent ribosome binding to mRNA and facilitates the initiation of translation. Any solutes suitable for cell electroporation, which can contain factors facilitating cellular permeability and viability such as sugars, peptides, lipids, proteins, antioxidants, and surfactants can be included.

RNA can be introduced into target cells using any of a number of different methods, for instance, commercially available methods which include, but are not limited to, electroporation (Amaxa Nucleofector-II (Amaxa Biosystems, Cologne, Germany)), (ECM 830 (BTX) (Harvard Instruments, Boston, Mass.) or the Gene Pulser II (BioRad, Denver, Colo.), Multiporator (Eppendort, Hamburg Germany), cationic liposome mediated transfection using lipofection, polymer encapsulation, peptide mediated transfection, or biolistic particle delivery systems such as “gene guns” (see, for example, Nishikawa, et al. Hum Gene Ther., 12(8):861-70 (2001).

Non-viral delivery methods

In some aspects, non-viral methods can be used to deliver a nucleic acid encoding a CAR described herein into a cell or tissue or a subject.

In some embodiments, the non-viral method includes the use of a transposon (also called a transposable element). In some embodiments, a transposon is a piece of DNA that can insert itself at a location in a genome, for example, a piece of DNA that is capable of self-replicating and inserting its copy into a genome, or a piece of DNA that can be spliced out of a longer nucleic acid and inserted into another place in a genome. For example, a transposon comprises a DNA sequence made up of inverted repeats flanking genes for transposition.

Exemplary methods of nucleic acid delivery using a transposon include a Sleeping Beauty transposon system (SBTS) and a piggyBac (PB) transposon system. See, e.g., Aronovich et al. Hum. Mol. Genet. 20.R1(2011):R14-20; Singh et al. Cancer Res. 15(2008):2961–2971; Huang et al. Mol. Ther. 16(2008):580–589; Grabundzija et al. Mol. Ther. 18(2010):1200–1209; Kebriaei et al. Blood.

122.21(2013):166; Williams. Molecular Therapy 16.9(2008):1515–16; Bell et al. Nat. Protoc. 2.12(2007):3153-65; and Ding et al. Cell. 122.3(2005):473-83, all of which are incorporated herein by reference.

The SBTS includes two components: 1) a transposon containing a transgene and 2) a source of transposase enzyme. The transposase can transpose the transposon from a carrier plasmid (or other donor DNA) to a target DNA, such as a host cell chromosome/genome. For example, the transposase binds to the carrier plasmid/donor DNA, cuts the transposon (including transgene(s)) out of the plasmid, and inserts it into the genome of the host cell. See, e.g., Aronovich et al. *supra.*

Exemplary transposons include a pT2-based transposon. See, e.g., Grabundzija et al. Nucleic Acids Res. 41.3(2013):1829-47; and Singh et al. Cancer Res. 68.8(2008): 2961–2971, all of which are incorporated herein by reference. Exemplary transposases include a Tc1/mariner-type transposase, e.g., the SB10 transposase or the SB11 transposase (a hyperactive transposase which can be expressed, e.g., from a cytomegalovirus promoter). See, e.g., Aronovich et al.; Kebriaei et al.; and Grabundzija et al., all of which are incorporated herein by reference.

Use of the SBTS permits efficient integration and expression of a transgene, e.g., a nucleic acid encoding a CAR described herein. Provided herein are methods of generating a cell, e.g., T cell or NK cell, that stably expresses a CAR described herein, e.g., using a transposon system such as SBTS.

In accordance with methods described herein, in some embodiments, one or more nucleic acids, e.g., plasmids, containing the SBTS components are delivered to a cell (e.g., T or NK cell). For example, the nucleic acid(s) are delivered by standard methods of nucleic acid (e.g., plasmid DNA) delivery, e.g., methods described herein, e.g., electroporation, transfection, or lipofection. In some embodiments, the nucleic acid contains a transposon comprising a transgene, e.g., a nucleic acid encoding a CAR described herein. In some embodiments, the nucleic acid contains a transposon comprising a transgene (e.g., a nucleic acid encoding a CAR described herein) as well as a nucleic acid sequence encoding a transposase enzyme. In other embodiments, a system with two nucleic acids is provided, e.g., a dual-plasmid system, e.g., where a first plasmid contains a transposon comprising a transgene, and a second plasmid contains a nucleic acid sequence encoding a transposase enzyme. For example, the first and the second nucleic acids are co-delivered into a host cell.

In some embodiments, cells, e.g., T or NK cells, are generated that express a CAR described herein by using a combination of gene insertion using the SBTS and genetic editing using a nuclease (e.g., Zinc finger nucleases (ZFNs), Transcription Activator-Like Effector Nucleases (TALENs), the CRISPR/Cas system, or engineered meganuclease re-engineered homing endonucleases).

In some embodiments, use of a non-viral method of delivery permits reprogramming of cells,

e.g., T or NK cells, and direct infusion of the cells into a subject. Advantages of non-viral vectors include but are not limited to the ease and relatively low cost of producing sufficient amounts required to meet a patient population, stability during storage, and lack of immunogenicity.

Nucleic Acid Constructs Encoding a CAR

The present invention also provides nucleic acid molecules encoding one or more CAR constructs described herein. In one aspect, the nucleic acid molecule is provided as a messenger RNA transcript. In one aspect, the nucleic acid molecule is provided as a DNA construct.

Accordingly, in one aspect, the invention pertains to an isolated nucleic acid molecule encoding a chimeric antigen receptor (CAR), wherein the CAR comprises a anti-BCMA binding domain (e.g., a human anti-BCMA binding domain), a transmembrane domain, and an intracellular signaling domain comprising a stimulatory domain, e.g., a costimulatory signaling domain and/or a primary signaling domain, e.g., zeta chain. In one embodiment, the anti-BCMA binding domain is an anti-BCMA binding domain described herein, e.g., an anti-BCMA binding domain which comprises a sequence selected from a group consisting of SEQ ID NO: 39, SEQ ID NO: 40, SEQ ID NO: 41, SEQ ID NO: 42, SEQ ID NO: 43, SEQ ID NO: 44, SEQ ID NO: 45, SEQ ID NO: 46, SEQ ID NO: 47, SEQ ID NO: 48, SEQ ID NO: 49, SEQ ID NO: 50, SEQ ID NO: 51, SEQ ID NO: 52, SEQ ID NO: 53, SEQ ID NO: 129, SEQ ID NO: 130, SEQ ID NO: 131, SEQ ID NO: 132, SEQ ID NO: 133, SEQ ID NO: 134, SEQ ID NO: 135, SEQ ID NO: 136, SEQ ID NO: 137, SEQ ID NO: 138, SEQ ID NO: 139, SEQ ID NO: 140, SEQ ID NO: 141, SEQ ID NO: 142, SEQ ID NO: 143, SEQ ID NO:144, SEQ ID NO:145, SEQ ID NO:146, SEQ ID NO:147, SEQ ID NO:148 or SEQ ID NO:149, or a sequence with 95-99% identify thereof. In one embodiment, the transmembrane domain is transmembrane domain of a protein selected from the group consisting of the alpha, beta or zeta chain of the T-cell receptor, CD28, CD3 epsilon, CD45, CD4, CD5, CD8, CD9, CD16, CD22, CD33, CD37, CD64, CD80, CD86, CD134, CD137 and CD154. In one embodiment, the transmembrane domain comprises a sequence of SEQ ID NO: 6, or a sequence with 95-99% identity thereof. In one embodiment, the anti-BCMA binding domain is connected to the transmembrane domain by a hinge region, e.g., a hinge described herein. In one embodiment, the hinge region comprises SEQ ID NO:2 or SEQ ID NO:3 or SEQ ID NO:4 or SEQ ID NO:5, or a sequence with 95-99% identity thereof. In one embodiment, the isolated nucleic acid molecule further comprises a sequence encoding a costimulatory domain. In one embodiment, the costimulatory domain is a functional signaling domain of a protein selected from the group consisting of MHC class I molecule, TNF receptor proteins, Immunoglobulin-like proteins, cytokine receptors, integrins, signaling lymphocytic activation molecules (SLAM proteins), activating NK cell receptors, BTLA, a Toll ligand receptor, OX40, CD2, CD7, CD27, CD28, CD30, CD40, CDS, ICAM-1, LFA-1 (CD11a/CD18), 4-1BB (CD137), B7-H3, CDS, ICAM-1, ICOS (CD278), GITR, BAFFR, LIGHT,

HVEM (LIGHTR), KIRDS2, SLAMF7, NKp80 (KLRF1), NKp44, NKp30, NKp46, CD19, CD4, CD8alpha, CD8beta, IL2R beta, IL2R gamma, IL7R alpha, ITGA4, VLA1, CD49a, ITGA4, IA4, CD49D, ITGA6, VLA-6, CD49f, ITGAD, CD11d, ITGAE, CD103, ITGAL, CD11a, LFA-1, ITGAM, CD11b, ITGAX, CD11c, ITGB1, CD29, ITGB2, CD18, LFA-1, ITGB7, NKG2D, NKG2C, TNFR2, TRANCE/RANKL, DNAM1 (CD226), SLAMF4 (CD244, 2B4), CD84, CD96 (Tactile), CEACAM1, CRTAM, Ly9 (CD229), CD160 (BY55), PSGL1, CD100 (SEMA4D), CD69, SLAMF6 (NTB-A, Ly108), SLAM (SLAMF1, CD150, IPO-3), BLAME (SLAMF8), SELPLG (CD162), LTBR, LAT, GADS, SLP-76, PAG/Cbp, CD19a, and a ligand that specifically binds with CD83. In one embodiment, the costimulatory domain comprises a sequence of SEQ ID NO:7, or a sequence with 95-99% identity thereof or a CD27 costimulatory domain having a sequence of SEQ ID NO:8 (or a sequence with 95-99% identity thereof) or a CD28 costimulatory domain having a sequence of SEQ ID NO:379 (or a sequence with 95-99% identity thereof) or a ICOS costimulatory domain having a sequence of SEQ ID NO: 381 (or a sequence with 95-99% identity thereof). In one embodiment, the intracellular signaling domain comprises a functional signaling domain of 4-1BB and a functional signaling domain of CD3 zeta. In one embodiment, the intracellular signaling domain comprises the sequence of SEQ ID NO: 7 or SEQ ID NO: 8, or a sequence with 95-99% identity thereof, and the sequence of SEQ ID NO: 9 or SEQ ID NO:10, or a sequence with 95-99% identity thereof, wherein the sequences comprising the intracellular signaling domain are expressed in the same frame and as a single polypeptide chain.

In another aspect, the invention pertains to an isolated nucleic acid molecule encoding a CAR construct comprising a leader sequence of SEQ ID NO: 1, a scFv domain having a sequence selected from the group consisting of SEQ ID NO: 39, SEQ ID NO: 40, SEQ ID NO: 41, SEQ ID NO: 42, SEQ ID NO: 43, SEQ ID NO: 44, SEQ ID NO: 45, SEQ ID NO: 46, SEQ ID NO: 47, SEQ ID NO: 48, SEQ ID NO: 49, SEQ ID NO: 50, SEQ ID NO: 51, SEQ ID NO: 52, SEQ ID NO: 53, SEQ ID NO: 129, SEQ ID NO: 130, SEQ ID NO: 131, SEQ ID NO: 132, SEQ ID NO: 133, SEQ ID NO: 134, SEQ ID NO: 135, SEQ ID NO: 136, SEQ ID NO: 137, SEQ ID NO: 138, SEQ ID NO: 139, SEQ ID NO: 140, SEQ ID NO: 141, SEQ ID NO: 142, SEQ ID NO: 143, SEQ ID NO:144, SEQ ID NO:145, SEQ ID NO:146, SEQ ID NO:147, SEQ ID NO:148 or SEQ ID NO:149 (or a sequence with 95-99% identify thereof), a hinge region of SEQ ID NO:2 or SEQ ID NO:3 or SEQ ID NO:4 or SEQ ID NO:5 (or a sequence with 95-99% identity thereof), a transmembrane domain having a sequence of SEQ ID NO: 6 (or a sequence with 95-99% identity thereof), a 4-1BB costimulatory domain having a sequence of SEQ ID NO:7 or a CD27 costimulatory domain having a sequence of SEQ ID NO:8 (or a sequence with 95-99% identity thereof) or a CD28 costimulatory domain having a sequence of SEQ ID NO:1104 (or a sequence with 95-99% identity thereof) or a ICOS costimulatory domain having a sequence of SEQ ID NO: 1106 (or a

sequence with 95-99% identity thereof), and a CD3 zeta stimulatory domain having a sequence of SEQ ID NO:9 or SEQ ID NO:10 (or a sequence with 95-99% identity thereof).

In another aspect, the invention pertains to an isolated polypeptide molecule encoded by the nucleic acid molecule. In one embodiment, the isolated polypeptide molecule comprises a sequence selected from the group consisting of SEQ ID NO: 39, SEQ ID NO: 40, SEQ ID NO: 41, SEQ ID NO: 42, SEQ ID NO: 43, SEQ ID NO: 44, SEQ ID NO: 45, SEQ ID NO: 46, SEQ ID NO: 47, SEQ ID NO: 48, SEQ ID NO: 49, SEQ ID NO: 50, SEQ ID NO: 51, SEQ ID NO: 52, SEQ ID NO: 53, SEQ ID NO: 129, SEQ ID NO: 130, SEQ ID NO: 131, SEQ ID NO: 132, SEQ ID NO: 133, SEQ ID NO: 134, SEQ ID NO: 135, SEQ ID NO: 136, SEQ ID NO: 137, SEQ ID NO: 138, SEQ ID NO: 139, SEQ ID NO: 140, SEQ ID NO: 141, SEQ ID NO: 142, SEQ ID NO: 143, SEQ ID NO:144, SEQ ID NO:145, SEQ ID NO:146, SEQ ID NO:147, SEQ ID NO:148 and SEQ ID NO:149, or a sequence with 95-99% identify thereof.

In another aspect, the invention pertains to a nucleic acid molecule encoding a chimeric antigen receptor (CAR) molecule that comprises an anti-BCMA binding domain, a transmembrane domain, and an intracellular signaling domain comprising a stimulatory domain, and wherein said anti-BCMA binding domain comprises a sequence selected from the group consisting of SEQ ID NO: 39, SEQ ID NO: 40, SEQ ID NO: 41, SEQ ID NO: 42, SEQ ID NO: 43, SEQ ID NO: 44, SEQ ID NO: 45, SEQ ID NO: 46, SEQ ID NO: 47, SEQ ID NO: 48, SEQ ID NO: 49, SEQ ID NO: 50, SEQ ID NO: 51, SEQ ID NO: 52, SEQ ID NO: 53, SEQ ID NO: 129, SEQ ID NO: 130, SEQ ID NO: 131, SEQ ID NO: 132, SEQ ID NO: 133, SEQ ID NO: 134, SEQ ID NO: 135, SEQ ID NO: 136, SEQ ID NO: 137, SEQ ID NO: 138, SEQ ID NO: 139, SEQ ID NO: 140, SEQ ID NO: 141, SEQ ID NO: 142, SEQ ID NO: 143, SEQ ID NO:144, SEQ ID NO:145, SEQ ID NO:146, SEQ ID NO:147, SEQ ID NO:148 and SEQ ID NO:149, or a sequence with 95-99% identify thereof.

In one embodiment, the encoded CAR molecule further comprises a sequence encoding a costimulatory domain. In one embodiment, the costimulatory domain is a functional signaling domain of a protein, e.g., described herein, e.g., selected from the group consisting of MHC class I molecule, TNF receptor proteins, Immunoglobulin-like proteins, cytokine receptors, integrins, signaling lymphocytic activation molecules (SLAM proteins), activating NK cell receptors, BTLA, a Toll ligand receptor, OX40, CD2, CD7, CD27, CD28, CD30, CD40, CDS, ICAM-1, LFA-1 (CD11a/CD18), 4-1BB (CD137), B7-H3, CDS, ICAM-1, ICOS (CD278), GITR, BAFFR, LIGHT, HVEM (LIGHTR), KIRDS2, SLAMF7, NKp80 (KLRF1), NKp44, NKp30, NKp46, CD19, CD4, CD8alpha, CD8beta, IL2R beta, IL2R gamma, IL7R alpha, ITGA4, VLA1, CD49a, ITGA4, IA4, CD49D, ITGA6, VLA-6, CD49f, ITGAD, CD11d, ITGAE, CD103, ITGAL, CD11a, LFA-1, ITGAM, CD11b, ITGAX, CD11c, ITGB1, CD29, ITGB2, CD18, LFA-1, ITGB7, NKG2D, NKG2C, TNFR2, TRANCE/RANKL,

DNAM1 (CD226), SLAMF4 (CD244, 2B4), CD84, CD96 (Tactile), CEACAM1, CRTAM, Ly9 (CD229), CD160 (BY55), PSGL1, CD100 (SEMA4D), CD69, SLAMF6 (NTB-A, Ly108), SLAM (SLAMF1, CD150, IPO-3), BLAME (SLAMF8), SELPLG (CD162), LTBR, LAT, GADS, SLP-76, PAG/Cbp, CD19a, and a ligand that specifically binds with CD83. In one embodiment, the costimulatory domain comprises a sequence of SEQ ID NO:7. In one embodiment, the transmembrane domain is a transmembrane domain of a protein, e.g., described herein, e.g., selected from the group consisting of the alpha, beta or zeta chain of the T-cell receptor, CD28, CD3 epsilon, CD45, CD4, CD5, CD8, CD9, CD16, CD22, CD33, CD37, CD64, CD80, CD86, CD134, CD137 and CD154. In one embodiment, the transmembrane domain comprises a sequence of SEQ ID NO:6. In one embodiment, the intracellular signaling domain comprises a functional signaling domain of 4-1BB and a functional signaling domain of zeta. In one embodiment, the intracellular signaling domain comprises the sequence of SEQ ID NO: 7 and the sequence of SEQ ID NO: 9, wherein the sequences comprising the intracellular signaling domain are expressed in the same frame and as a single polypeptide chain. In one embodiment, the anti-BCMAbinding domain is connected to the transmembrane domain by a hinge region. In one embodiment, the hinge region comprises SEQ ID NO:2. In one embodiment, the hinge region comprises SEQ ID NO:3 or SEQ ID NO:4 or SEQ ID NO:5.

In another aspect, the invention pertains to an encoded CAR molecule comprising a leader sequence of SEQ ID NO: 1, a scFv domain having a sequence selected from the group consisting of SEQ ID NO: 39, SEQ ID NO: 40, SEQ ID NO: 41, SEQ ID NO: 42, SEQ ID NO: 43, SEQ ID NO: 44, SEQ ID NO: 45, SEQ ID NO: 46, SEQ ID NO: 47, SEQ ID NO: 48, SEQ ID NO: 49, SEQ ID NO: 50, SEQ ID NO: 51, SEQ ID NO: 52, SEQ ID NO: 53, SEQ ID NO: 129, SEQ ID NO: 130, SEQ ID NO: 131, SEQ ID NO: 132, SEQ ID NO: 133, SEQ ID NO: 134, SEQ ID NO: 135, SEQ ID NO: 136, SEQ ID NO: 137, SEQ ID NO: 138, SEQ ID NO: 139, SEQ ID NO: 140, SEQ ID NO: 141, SEQ ID NO: 142, SEQ ID NO: 143, SEQ ID NO:144, SEQ ID NO:145, SEQ ID NO:146, SEQ ID NO:147, SEQ ID NO:148 and SEQ ID NO:149, or a sequence with 95-99% identify thereof, a hinge region of SEQ ID NO:2 or SEQ ID NO:3 or SEQ ID NO:4 or SEQ ID NO:5, a transmembrane domain having a sequence of SEQ ID NO: 6, a 4-1BB costimulatory domain having a sequence of SEQ ID NO:7, or a CD27 costimulatory domain having a sequence of SEQ ID NO:8, or a CD28 costimulatory domain having a sequence of SEQ ID NO:1104 (or a sequence with 95-99% identity thereof) or a ICOS costimulatory domain having a sequence of SEQ ID NO: 1106 (or a sequence with 95-99% identity thereof), and a CD3 zeta stimulatory domain having a sequence of SEQ ID NO:9 or SEQ ID NO:10. In one embodiment, the encoded CAR molecule comprises a sequence selected from a group consisting of residues 22-483 of SEQ ID NO: 109, residues 22-490 of SEQ ID NO: 99, residues 22-488 of SEQ ID NO: 100, residues 22-487 of SEQ ID NO: 101, residues 22-493 of SEQ ID NO: 102, residues 22-490 of

SEQ ID NO: 103, residues 22-491 of SEQ ID NO: 104, residues 22-482 of SEQ ID NO: 105, residues 22-483 of SEQ ID NO: 106, residues 22-485 of SEQ ID NO: 107, residues 22-483 of SEQ ID NO: 108, residues 22-490 of SEQ ID NO: 110, residues 22-483 of SEQ ID NO: 111, residues 22-484 of SEQ ID NO: 112, residues 22-485 of SEQ ID NO: 113, residues 22-487 of SEQ ID NO: 213, residues 23-489 of SEQ ID NO: 214, residues 22-490 of SEQ ID NO: 215, residues 22-484 of SEQ ID NO: 216, residues 22-485 of SEQ ID NO: 217, residues 22-489 of SEQ ID NO: 218, residues 22-497 of SEQ ID NO: 219, residues 22-492 of SEQ ID NO: 220, residues 22-490 of SEQ ID NO: 221, residues 22-485 of SEQ ID NO: 222, residues 22-492 of SEQ ID NO: 223, residues 22-492 of SEQ ID NO: 224, residues 22-483 of SEQ ID NO: 225, residues 22-490 of SEQ ID NO: 226, residues 22-485 of SEQ ID NO: 227, residues 22-486 of SEQ ID NO: 228, residues 22-492 of SEQ ID NO: 229, residues 22-488 of SEQ ID NO: 230, residues 22-488 of SEQ ID NO: 231, residues 22-495 of SEQ ID NO: 232, residues 22-490 of SEQ ID NO: 233, or a sequence with 95-99% identify thereof.

The nucleic acid sequences coding for the desired molecules can be obtained using recombinant methods known in the art, such as, for example by screening libraries from cells expressing the gene, by deriving the gene from a vector known to include the same, or by isolating directly from cells and tissues containing the same, using standard techniques. Alternatively, the gene of interest can be produced synthetically, rather than cloned.

The present invention also provides vectors in which a DNA of the present invention is inserted. Vectors derived from retroviruses such as the lentivirus are suitable tools to achieve long-term gene transfer since they allow long-term, stable integration of a transgene and its propagation in daughter cells. Lentiviral vectors have the added advantage over vectors derived from onco-retroviruses such as murine leukemia viruses in that they can transduce non-proliferating cells, such as hepatocytes. They also have the added advantage of low immunogenicity. A retroviral vector may also be, e.g., a gammaretroviral vector. A gammaretroviral vector may include, e.g., a promoter, a packaging signal (ψ), a primer binding site (PBS), one or more (e.g., two) long terminal repeats (LTR), and a transgene of interest, e.g., a gene encoding a CAR. A gammaretroviral vector may lack viral structural gens such as gag, pol, and env. Exemplary gammaretroviral vectors include Murine Leukemia Virus (MLV), Spleen-Focus Forming Virus (SFFV), and Myeloproliferative Sarcoma Virus (MPSV), and vectors derived therefrom. Other gammaretroviral vectors are described, e.g., in Tobias Maetzig et al., "Gammaretroviral Vectors: Biology, Technology and Application" Viruses. 2011 Jun; 3(6): 677–713.

In another embodiment, the vector comprising the nucleic acid encoding the desired CAR of the invention is an adenoviral vector (A5/35). In another embodiment, the expression of nucleic acids encoding CARs can be accomplished using of transposons such as sleeping beauty, CRISPR, CAS9,

and zinc finger nucleases. See below June et al. 2009*Nature Reviews Immunology* 9.10: 704-716, is incorporated herein by reference.

In brief summary, the expression of natural or synthetic nucleic acids encoding CARs is typically achieved by operably linking a nucleic acid encoding the CAR polypeptide or portions thereof to a promoter, and incorporating the construct into an expression vector. The vectors can be suitable for replication and integration eukaryotes. Typical cloning vectors contain transcription and translation terminators, initiation sequences, and promoters useful for regulation of the expression of the desired nucleic acid sequence.

The expression constructs of the present invention may also be used for nucleic acid immunization and gene therapy, using standard gene delivery protocols. Methods for gene delivery are known in the art. See, e.g., U.S. Pat. Nos. 5,399,346, 5,580,859, 5,589,466, incorporated by reference herein in their entireties. In another embodiment, the invention provides a gene therapy vector.

The nucleic acid can be cloned into a number of types of vectors. For example, the nucleic acid can be cloned into a vector including, but not limited to a plasmid, a phagemid, a phage derivative, an animal virus, and a cosmid. Vectors of particular interest include expression vectors, replication vectors, probe generation vectors, and sequencing vectors.

Further, the expression vector may be provided to a cell in the form of a viral vector. Viral vector technology is well known in the art and is described, for example, in Sambrook et al., 2012, MOLECULAR CLONING: A LABORATORY MANUAL, volumes 1 -4, Cold Spring Harbor Press, NY), and in other virology and molecular biology manuals. Viruses, which are useful as vectors include, but are not limited to, retroviruses, adenoviruses, adeno- associated viruses, herpes viruses, and lentiviruses. In general, a suitable vector contains an origin of replication functional in at least one organism, a promoter sequence, convenient restriction endonuclease sites, and one or more selectable markers, (e.g., WO 01/96584; WO 01/29058; and U.S. Pat. No. 6,326,193).

A number of viral based systems have been developed for gene transfer into mammalian cells. For example, retroviruses provide a convenient platform for gene delivery systems. A selected gene can be inserted into a vector and packaged in retroviral particles using techniques known in the art. The recombinant virus can then be isolated and delivered to cells of the subject either in vivo or ex vivo. A number of retroviral systems are known in the art. In some embodiments, adenovirus vectors are used. A number of adenovirus vectors are known in the art. In one embodiment, lentivirus vectors are used.

Additional promoter elements, e.g., enhancers, regulate the frequency of transcriptional initiation. Typically, these are located in the region 30-110 bp upstream of the start site, although a number of promoters have been shown to contain functional elements downstream of the start site as

well. The spacing between promoter elements frequently is flexible, so that promoter function is preserved when elements are inverted or moved relative to one another. In the thymidine kinase (tk) promoter, the spacing between promoter elements can be increased to 50 bp apart before activity begins to decline. Depending on the promoter, it appears that individual elements can function either cooperatively or independently to activate transcription.

An example of a promoter that is capable of expressing a CAR transgene in a mammalian T cell is the EF1a promoter. The native EF1a promoter drives expression of the alpha subunit of the elongation factor-1 complex, which is responsible for the enzymatic delivery of aminoacyl tRNAs to the ribosome. The EF1a promoter has been extensively used in mammalian expression plasmids and has been shown to be effective in driving CAR expression from transgenes cloned into a lentiviral vector. See, e.g., Milone et al., Mol. Ther. 17(8): 1453–1464 (2009). In one aspect, the EF1a promoter comprises the sequence provided as SEQ ID NO:11.

Another example of a promoter is the immediate early cytomegalovirus (CMV) promoter sequence. This promoter sequence is a strong constitutive promoter sequence capable of driving high levels of expression of any polynucleotide sequence operatively linked thereto. However, other constitutive promoter sequences may also be used, including, but not limited to the simian virus 40 (SV40) early promoter, mouse mammary tumor virus (MMTV), human immunodeficiency virus (HIV) long terminal repeat (LTR) promoter, MoMuLV promoter, an avian leukemia virus promoter, an Epstein-Barr virus immediate early promoter, a Rous sarcoma virus promoter, as well as human gene promoters such as, but not limited to, the actin promoter, the myosin promoter, the elongation factor-1α promoter, the hemoglobin promoter, and the creatine kinase promoter. Further, the invention should not be limited to the use of constitutive promoters. Inducible promoters are also contemplated as part of the invention. The use of an inducible promoter provides a molecular switch capable of turning on expression of the polynucleotide sequence which it is operatively linked when such expression is desired, or turning off the expression when expression is not desired. Examples of inducible promoters include, but are not limited to a metallothionine promoter, a glucocorticoid promoter, a progesterone promoter, and a tetracycline promoter.

Another example of a promoter is the phosphoglycerate kinase (PGK) promoter. In embodiments, a truncated PGK promoter (e.g., a PGK promoter with one or more, e.g., 1, 2, 5, 10, 100, 200, 300, or 400, nucleotide deletions when compared to the wild-type PGK promoter sequence) may be desired. The nucleotide sequences of exemplary PGK promoters are provided below.

WT PGK Promoter

ACCCCTCTCTCCAGCCACTAAGCCAGTTGCTCCCTCGGCTGACGGCTGCACGCGAGGCCTCCGAACG
TCTTACGCCTTGTGGCGCGCCCGTCCTTGTCCCGGGTGTGATGGCGGGGTGTGGGGCGGAGGGCGTG
GCGGGGAAGGGCCGGCGACGAGAGCCGCGCGGGACGACTCGTCGGCGATAACCGGTGTCGGGTAGCG
CCAGCCGCGCGACGGTAACGAGGGACCGCGACAGGCAGACGCTCCCATGATCACTCTGCACGCCGAA
GGCAAATAGTGCAGGCCGTGCGGCGCTTGGCGTTCCTTGGAAGGGCTGAATCCCCGCCTCGTCCTTC
GCAGCGGCCCCCCGGGTGTTCCCATCGCCGCTTCTAGGCCCACTGCGACGCTTGCCTGCACTTCTTA
CACGCTCTGGGTCCCAGCCGCGGCGACGCAAAGGGCCTTGGTGCGGGTCTCGTCGGCGCAGGGACGC
GTTTGGGTCCCGACGGAACCTTTTCCGCGTTGGGGTTGGGGCACCATAAGCT
(SEQ ID NO: 1109)

Exemplary truncated PGK Promoters:

PGK100:
ACCCCTCTCTCCAGCCACTAAGCCAGTTGCTCCCTCGGCTGACGGCTGCACGCGAGGCCTCCGAACG
TCTTACGCCTTGTGGCGCGCCCGTCCTTGTCCCGGGTGTGATGGCGGGGTG
(SEQ ID NO: 1110)

PGK200:
ACCCCTCTCTCCAGCCACTAAGCCAGTTGCTCCCTCGGCTGACGGCTGCACGCGAGGCCTCCGAACG
TCTTACGCCTTGTGGCGCGCCCGTCCTTGTCCCGGGTGTGATGGCGGGGTGTGGGGCGGAGGGCGTG
GCGGGGAAGGGCCGGCGACGAGAGCCGCGCGGGACGACTCGTCGGCGATAACCGGTGTCGGGTAGCG
CCAGCCGCGCGACGGTAACG
(SEQ ID NO: 1111)

PGK300:
ACCCCTCTCTCCAGCCACTAAGCCAGTTGCTCCCTCGGCTGACGGCTGCACGCGAGGCCTCCGAACG
TCTTACGCCTTGTGGCGCGCCCGTCCTTGTCCCGGGTGTGATGGCGGGGTGTGGGGCGGAGGGCGTG
GCGGGGAAGGGCCGGCGACGAGAGCCGCGCGGGACGACTCGTCGGCGATAACCGGTGTCGGGTAGCG
CCAGCCGCGCGACGGTAACGAGGGACCGCGACAGGCAGACGCTCCCATGATCACTCTGCACGCCGAA
GGCAAATAGTGCAGGCCGTGCGGCGCTTGGCGTTCCTTGGAAGGGCTGAATCCCCG
(SEQ ID NO: 1112)

PGK400:
ACCCCTCTCTCCAGCCACTAAGCCAGTTGCTCCCTCGGCTGACGGCTGCACGCGAGGCCTCCGAACG
TCTTACGCCTTGTGGCGCGCCCGTCCTTGTCCCGGGTGTGATGGCGGGGTGTGGGGCGGAGGGCGTG
GCGGGGAAGGGCCGGCGACGAGAGCCGCGCGGGACGACTCGTCGGCGATAACCGGTGTCGGGTAGCG
CCAGCCGCGCGACGGTAACGAGGGACCGCGACAGGCAGACGCTCCCATGATCACTCTGCACGCCGAA
GGCAAATAGTGCAGGCCGTGCGGCGCTTGGCGTTCCTTGGAAGGGCTGAATCCCCGCCTCGTCCTTC
GCAGCGGCCCCCCGGGTGTTCCCATCGCCGCTTCTAGGCCCACTGCGACGCTTGCCTGCACTTCTTA
CACGCTCTGGGTCCCAGCCG
(SEQ ID NO: 1113)

A vector may also include, e.g., a signal sequence to facilitate secretion, a polyadenylation signal and transcription terminator (e.g., from Bovine Growth Hormone (BGH) gene), an element allowing episomal replication and replication in prokaryotes (e.g. SV40 origin and ColE1 or others known in the art) and/or elements to allow selection (e.g., ampicillin resistance gene and/or zeocin marker).

In order to assess the expression of a CAR polypeptide or portions thereof, the expression vector to be introduced into a cell can also contain either a selectable marker gene or a reporter gene or both to facilitate identification and selection of expressing cells from the population of cells sought to be transfected or infected through viral vectors. In other aspects, the selectable marker may be carried on a separate piece of DNA and used in a co- transfection procedure. Both selectable markers and reporter genes may be flanked with appropriate regulatory sequences to enable expression in the host cells. Useful selectable markers include, for example, antibiotic-resistance genes, such as neo and the like.

Reporter genes are used for identifying potentially transfected cells and for evaluating the functionality of regulatory sequences. In general, a reporter gene is a gene that is not present in or expressed by the recipient organism or tissue and that encodes a polypeptide whose expression is manifested by some easily detectable property, e.g., enzymatic activity. Expression of the reporter gene is assayed at a suitable time after the DNA has been introduced into the recipient cells. Suitable reporter genes may include genes encoding luciferase, beta-galactosidase, chloramphenicol acetyl transferase, secreted alkaline phosphatase, or the green fluorescent protein gene (e.g., Ui-Tei et al., 2000 FEBS Letters 479: 79-82). Suitable expression systems are well known and may be prepared using known techniques or obtained commercially. In general, the construct with the minimal 5' flanking region showing the highest level of expression of reporter gene is identified as the promoter. Such promoter regions may be linked to a reporter gene and used to evaluate agents for the ability to modulate promoter- driven transcription.

In one embodiment, the vector can further comprise a nucleic acid encoding a second CAR. In one embodiment, the second CAR includes an antigen binding domain to a target expressed on acute myeloid leukemia cells, such as, e.g., CD123, CD34, CLL-1, folate receptor beta, or FLT3; or a target expressed on a B cell, e.g., CD10, CD19, CD20, CD22, CD34, CD123, FLT-3, ROR1, CD79b, CD179b, or CD79a. In one embodiment, the vector comprises a nucleic acid sequence encoding a first CAR that specifically binds a first antigen and includes an intracellular signaling domain having a costimulatory signaling domain but not a primary signaling domain, and a nucleic acid encoding a second CAR that specifically binds a second, different, antigen and includes an intracellular signaling domain having a primary signaling domain but not a costimulatory signaling domain. In one embodiment, the vector comprises a nucleic acid encoding a first BCMA CAR that includes a BCMA binding domain, a transmembrane domain and a costimulatory domain and a nucleic acid encoding a second CAR that targets an antigen other than BCMA (e.g., an antigen expressed on AML cells, e.g., CD123, CD34, CLL-1, folate receptor beta, or FLT3; or an antigen expressed on a B cell, e.g., CD10, CD19, CD20, CD22, CD34, CD123, FLT-3, ROR1, CD79b, CD179b, or CD79a) and includes an antigen binding domain, a transmembrane domain and a primary signaling domain. In another

embodiment, the vector comprises a nucleic acid encoding a first BCMA CAR that includes a BCMA binding domain, a transmembrane domain and a primary signaling domain and a nucleic acid encoding a second CAR that specifically binds an antigen other than BCMA (e.g., an antigen expressed on AML cells, e.g., CD123, CD34, CLL-1, folate receptor beta, or FLT3; or an antigen expressed on a B cell, e.g., CD10, CD19, CD20, CD22, CD34, CD123, FLT-3, ROR1, CD79b, CD179b, or CD79a) and includes an antigen binding domain to the antigen, a transmembrane domain and a costimulatory signaling domain.

In one embodiment, the vector comprises a nucleic acid encoding a BCMA CAR described herein and a nucleic acid encoding an inhibitory CAR. In one embodiment, the inhibitory CAR comprises an antigen binding domain that binds an antigen found on normal cells but not cancer cells, e.g., normal cells that also express BCMA. In one embodiment, the inhibitory CAR comprises the antigen binding domain, a transmembrane domain and an intracellular domain of an inhibitory molecule. For example, the intracellular domain of the inhibitory CAR can be an intracellular domain of PD1, PD-L1, PD-L2, CTLA4, TIM3, CEACAM (e.g., CEACAM-1, CEACAM-3 and/or CEACAM-5), LAG3, VISTA, BTLA, TIGIT, LAIR1, CD160, 2B4, CD80, CD86, B7-H3 (CD276), B7-H4 (VTCN1), HVEM (TNFRSF14 or CD270), KIR, A2aR, MHC class I, MHC class II, GAL9, adenosine, and TGFR beta.

In embodiments, the vector may comprise two or more nucleic acid sequences encoding a CAR, e.g., a BCMA CAR described herein and a second CAR, e.g., an inhibitory CAR or a CAR that specifically binds to an antigen other than BCMA (e.g., an antigen expressed on AML cells, e.g., CD123, CLL-1, CD34, FLT3, or folate receptor beta; or antigen expresson B cells, e.g., CD10, CD19, CD20, CD22, CD34, CD123, FLT-3, ROR1, CD79b, CD179b, or CD79a). In such embodiments, the two or more nucleic acid sequences encoding the CAR are encoded by a single nucleic molecule in the same frame and as a single polypeptide chain. In this aspect, the two or more CARs, can, e.g., be separated by one or more peptide cleavage sites. (e.g., an auto-cleavage site or a substrate for an intracellular protease). Examples of peptide cleavage sites include the following, wherein the GSG residues are optional:

T2A: (GSG) E G R G S L L T C G D V E E N P G P (SEQ ID NO: 1114)

P2A: (GSG) A T N F S L L K Q A G D V E E N P G P (SEQ ID NO: 1115)

E2A: (GSG) Q C T N Y A L L K L A G D V E S N P G P (SEQ ID NO: 1116)

F2A: (GSG) V K Q T L N F D L L K L A G D V E S N P G P (SEQ ID NO: 1117)

Methods of introducing and expressing genes into a cell are known in the art. In the context of an expression vector, the vector can be readily introduced into a host cell, e.g., mammalian, bacterial,

yeast, or insect cell by any method in the art. For example, the expression vector can be transferred into a host cell by physical, chemical, or biological means.

Physical methods for introducing a polynucleotide into a host cell include calcium phosphate precipitation, lipofection, particle bombardment, microinjection, electroporation, and the like. Methods for producing cells comprising vectors and/or exogenous nucleic acids are well-known in the art. See, for example, Sambrook et al., 2012, MOLECULAR CLONING: A LABORATORY MANUAL, volumes 1 -4, Cold Spring Harbor Press, NY). A preferred method for the introduction of a polynucleotide into a host cell is calcium phosphate transfection

Biological methods for introducing a polynucleotide of interest into a host cell include the use of DNA and RNA vectors. Viral vectors, and especially retroviral vectors, have become the most widely used method for inserting genes into mammalian, e.g., human cells. Other viral vectors can be derived from lentivirus, poxviruses, herpes simplex virus I, adenoviruses and adeno-associated viruses, and the like. See, for example, U.S. Pat. Nos. 5,350,674 and 5,585,362.

Chemical means for introducing a polynucleotide into a host cell include colloidal dispersion systems, such as macromolecule complexes, nanocapsules, microspheres, beads, and lipid-based systems including oil-in-water emulsions, micelles, mixed micelles, and liposomes. An exemplary colloidal system for use as a delivery vehicle in vitro and in vivo is a liposome (e.g. , an artificial membrane vesicle). Other methods of state-of-the-art targeted delivery of nucleic acids are available, such as delivery of polynucleotides with targeted nanoparticles or other suitable sub-micron sized delivery system.

In the case where a non-viral delivery system is utilized, an exemplary delivery vehicle is a liposome. The use of lipid formulations is contemplated for the introduction of the nucleic acids into a host cell (in vitro, ex vivo or in vivo). In another aspect, the nucleic acid may be associated with a lipid. The nucleic acid associated with a lipid may be encapsulated in the aqueous interior of a liposome, interspersed within the lipid bilayer of a liposome, attached to a liposome via a linking molecule that is associated with both the liposome and the oligonucleotide, entrapped in a liposome, complexed with a liposome, dispersed in a solution containing a lipid, mixed with a lipid, combined with a lipid, contained as a suspension in a lipid, contained or complexed with a micelle, or otherwise associated with a lipid. Lipid, lipid/DNA or lipid/expression vector associated compositions are not limited to any particular structure in solution. For example, they may be present in a bilayer structure, as micelles, or with a "collapsed" structure. They may also simply be interspersed in a solution, possibly forming aggregates that are not uniform in size or shape. Lipids are fatty substances which may be naturally occurring or synthetic lipids. For example, lipids include the fatty droplets that naturally occur in the cytoplasm as

well as the class of compounds which contain long-chain aliphatic hydrocarbons and their derivatives, such as fatty acids, alcohols, amines, amino alcohols, and aldehydes.

Lipids suitable for use can be obtained from commercial sources. For example, dimyristyl phosphatidylcholine ("DMPC") can be obtained from Sigma, St. Louis, MO; dicetyl phosphate ("DCP") can be obtained from K & K Laboratories (Plainview, NY); cholesterol ("Choi") can be obtained from Calbiochem-Behring; dimyristyl phosphatidylglycerol ("DMPG") and other lipids may be obtained from Avanti Polar Lipids, Inc. (Birmingham, AL.). Stock solutions of lipids in chloroform or chloroform/methanol can be stored at about -20°C. Chloroform is used as the only solvent since it is more readily evaporated than methanol. "Liposome" is a generic term encompassing a variety of single and multilamellar lipid vehicles formed by the generation of enclosed lipid bilayers or aggregates. Liposomes can be characterized as having vesicular structures with a phospholipid bilayer membrane and an inner aqueous medium. Multilamellar liposomes have multiple lipid layers separated by aqueous medium. They form spontaneously when phospholipids are suspended in an excess of aqueous solution. The lipid components undergo self-rearrangement before the formation of closed structures and entrap water and dissolved solutes between the lipid bilayers (Ghosh et al., 1991 Glycobiology 5: 505-10). However, compositions that have different structures in solution than the normal vesicular structure are also encompassed. For example, the lipids may assume a micellar structure or merely exist as nonuniform aggregates of lipid molecules. Also contemplated are lipofectamine-nucleic acid complexes.

Regardless of the method used to introduce exogenous nucleic acids into a host cell or otherwise expose a cell to the inhibitor of the present invention, in order to confirm the presence of the recombinant DNA sequence in the host cell, a variety of assays may be performed. Such assays include, for example, "molecular biological" assays well known to those of skill in the art, such as Southern and Northern blotting, RT-PCR and PCR; "biochemical" assays, such as detecting the presence or absence of a particular peptide, e.g., by immunological means (ELISAs and Western blots) or by assays described herein to identify agents falling within the scope of the invention.

The present invention further provides a vector comprising a CAR encoding nucleic acid molecule. In one aspect, a CAR vector can be directly transduced into a cell, *e.g.,* a T cell or NK cell. In one aspect, the vector is a cloning or expression vector, *e.g.,* a vector including, but not limited to, one or more plasmids (*e.g.*, expression plasmids, cloning vectors, minicircles, minivectors, double minute chromosomes), retroviral and lentiviral vector constructs. In one aspect, the vector is capable of expressing the CAR construct in mammalian T cells or NK cells. In one aspect, the mammalian T cell is a human T cell. In one aspect, the mammalian NK cell is a human NK cell.

Sources of cells

Prior to expansion and genetic modification, a source of cells, e.g., immune effector cells (e.g., T cells or NK cells), is obtained from a subject. The term "subject" is intended to include living organisms in which an immune response can be elicited (e.g., mammals). Examples of subjects include humans, dogs, cats, mice, rats, and transgenic species thereof. T cells can be obtained from a number of sources, including peripheral blood mononuclear cells, bone marrow, lymph node tissue, cord blood, thymus tissue, tissue from a site of infection, ascites, pleural effusion, spleen tissue, and tumors.

In certain aspects of the present invention, any number of immune effector cell (e.g., T cell or NK cell) lines available in the art, may be used. In certain aspects of the present invention, T cells can be obtained from a unit of blood collected from a subject using any number of techniques known to the skilled artisan, such as Ficoll™ separation. In one preferred aspect, cells from the circulating blood of an individual are obtained by apheresis. The apheresis product typically contains lymphocytes, including T cells, monocytes, granulocytes, B cells, other nucleated white blood cells, red blood cells, and platelets. In one aspect, the cells collected by apheresis may be washed to remove the plasma fraction and to place the cells in an appropriate buffer or media for subsequent processing steps. In one aspect of the invention, the cells are washed with phosphate buffered saline (PBS). In an alternative aspect, the wash solution lacks calcium and may lack magnesium or may lack many if not all divalent cations.

Initial activation steps in the absence of calcium can lead to magnified activation. As those of ordinary skill in the art would readily appreciate a washing step may be accomplished by methods known to those in the art, such as by using a semi-automated "flow-through" centrifuge (for example, the Cobe 2991 cell processor, the Baxter CytoMate, or the Haemonetics Cell Saver 5) according to the manufacturer's instructions. After washing, the cells may be resuspended in a variety of biocompatible buffers, such as, for example, Ca-free, Mg-free PBS, PlasmaLyte A, or other saline solution with or without buffer. Alternatively, the undesirable components of the apheresis sample may be removed and the cells directly resuspended in culture media.

It is recognized that the methods of the application can utilize culture media conditions comprising 5% or less, for example 2%, human AB serum, and employ known culture media conditions and compositions, for example those described in Smith *et al.*, "Ex vivo expansion of human T cells for adoptive immunotherapy using the novel Xeno-free CTS Immune Cell Serum Replacement" *Clinical & Translational Immunology* (2015) 4, e31; doi:10.1038/cti.2014.31.

In one aspect, T cells are isolated from peripheral blood lymphocytes by lysing the red blood cells and depleting the monocytes, for example, by centrifugation through a PERCOLLTM gradient or

by counterflow centrifugal elutriation. A specific subpopulation of T cells, such as CD3+, CD4+, CD8+, CD45RA+, and/or CD45RO+T cells, can be further isolated by positive or negative selection techniques. For example, in one aspect, T cells are isolated by incubation with anti-CD3/anti-CD28 (e.g., 3x28)-conjugated beads, such as DYNABEADS® M-450 CD3/CD28 T, for a time period sufficient for positive selection of the desired T cells. In one aspect, the time period is about 30 minutes. In a further aspect, the time period ranges from 30 minutes to 36 hours or longer and all integer values there between. In a further aspect, the time period is at least 1, 2, 3, 4, 5, or 6 hours. In yet another preferred aspect, the time period is 10 to 24 hours. In one aspect, the incubation time period is 24 hours. Longer incubation times may be used to isolate T cells in any situation where there are few T cells as compared to other cell types, such in isolating tumor infiltrating lymphocytes (TIL) from tumor tissue or from immunocompromised individuals. Further, use of longer incubation times can increase the efficiency of capture of CD8+ T cells. Thus, by simply shortening or lengthening the time T cells are allowed to bind to the CD3/CD28 beads and/or by increasing or decreasing the ratio of beads to T cells (as described further herein), subpopulations of T cells can be preferentially selected for or against at culture initiation or at other time points during the process. Additionally, by increasing or decreasing the ratio of anti-CD3 and/or anti-CD28 antibodies on the beads or other surface, subpopulations of T cells can be preferentially selected for or against at culture initiation or at other desired time points. The skilled artisan would recognize that multiple rounds of selection can also be used in the context of this invention. In certain aspects, it may be desirable to perform the selection procedure and use the "unselected" cells in the activation and expansion process. "Unselected" cells can also be subjected to further rounds of selection.

Enrichment of a T cell population by negative selection can be accomplished with a combination of antibodies directed to surface markers unique to the negatively selected cells. One method is cell sorting and/or selection via negative magnetic immunoadherence or flow cytometry that uses a cocktail of monoclonal antibodies directed to cell surface markers present on the cells negatively selected. For example, to enrich for CD4+ cells by negative selection, a monoclonal antibody cocktail typically includes antibodies to CD14, CD20, CD11b, CD16, HLA-DR, and CD8. In certain aspects, it may be desirable to enrich for or positively select for regulatory T cells which typically express CD4+, CD25+, CD62Lhi, GITR+, and FoxP3+. In certain aspects, it may be desirable to enrich for cells that are CD127low. Alternatively, in certain aspects, T regulatory cells are depleted by anti-C25 conjugated beads or other similar method of selection.

The methods described herein can include, e.g., selection of a specific subpopulation of immune effector cells, e.g., T cells, that are a T regulatory cell-depleted population, CD25+ depleted cells, using,

e.g., a negative selection technique, e.g., described herein. Preferably, the population of T regulatory depleted cells contains less than 30%, 25%, 20%, 15%, 10%, 5%, 4%, 3%, 2%, 1% of CD25+ cells.

In one embodiment, T regulatory cells, e.g., CD25+ T cells, are removed from the population using an anti-CD25 antibody, or fragment thereof, or a CD25-binding ligand, IL-2. In one embodiment, the anti-CD25 antibody, or fragment thereof, or CD25-binding ligand is conjugated to a substrate, e.g., a bead, or is otherwise coated on a substrate, e.g., a bead. In one embodiment, the anti-CD25 antibody, or fragment thereof, is conjugated to a substrate as described herein.

In one embodiment, the T regulatory cells, e.g., CD25+ T cells, are removed from the population using CD25 depletion reagent from Miltenyi™. In one embodiment, the ratio of cells to CD25 depletion reagent is 1e7 cells to 20 uL, or 1e7 cells to15 uL, or 1e7 cells to 10 uL, or 1e7 cells to 5 uL, or 1e7 cells to 2.5 uL, or 1e7 cells to 1.25 uL. In one embodiment, e.g., for T regulatory cells, e.g., CD25+ depletion, greater than 500 million cells/ml is used. In a further aspect, a concentration of cells of 600, 700, 800, or 900 million cells/ml is used.

In one embodiment, the population of immune effector cells to be depleted includes about 6 x 10^9 CD25+ T cells. In other aspects, the population of immune effector cells to be depleted include about 1 x 10^9 to 1x 10^{10} CD25+ T cell, and any integer value in between. In one embodiment, the resulting population T regulatory depleted cells has 2 x 10^9 T regulatory cells, e.g., CD25+ cells, or less (e.g., 1 x 10^9, 5 x 10^8, 1 x 10^8, 5 x 10^7, 1 x 10^7, or less CD25+ cells).

In one embodiment, the T regulatory cells, e.g., CD25+ cells, are removed from the population using the CliniMAC system with a depletion tubing set, such as, e.g., tubing 162-01. In one embodiment, the CliniMAC system is run on a depletion setting such as, e.g., DEPLETION2.1.

Without wishing to be bound by a particular theory, decreasing the level of negative regulators of immune cells (e.g., decreasing the number of unwanted immune cells, e.g., T_{REG} cells), in a subject prior to apheresis or during manufacturing of a CAR-expressing cell product can reduce the risk of subject relapse. For example, methods of depleting T_{REG} cells are known in the art. Methods of decreasing T_{REG} cells include, but are not limited to, cyclophosphamide, anti-GITR antibody (an anti-GITR antibody described herein), CD25-depletion, and combinations thereof.

In some embodiments, the manufacturing methods comprise reducing the number of (e.g., depleting) T_{REG} cells prior to manufacturing of the CAR-expressing cell. For example, manufacturing methods comprise contacting the sample, e.g., the apheresis sample, with an anti-GITR antibody and/or an anti-CD25 antibody (or fragment thereof, or a CD25-binding ligand), e.g., to deplete T_{REG} cells prior to manufacturing of the CAR-expressing cell (e.g., T cell, NK cell) product.

In an embodiment, a subject is pre-treated with one or more therapies that reduce T_{REG} cells prior to collection of cells for CAR-expressing cell product manufacturing, thereby reducing the risk of subject relapse to CAR-expressing cell treatment. In an embodiment, methods of decreasing T_{REG} cells include, but are not limited to, administration to the subject of one or more of cyclophosphamide, anti-GITR antibody, CD25-depletion, or a combination thereof. Administration of one or more of cyclophosphamide, anti-GITR antibody, CD25-depletion, or a combination thereof, can occur before, during or after an infusion of the CAR-expressing cell product.

In an embodiment, a subject is pre-treated with cyclophosphamide prior to collection of cells for CAR-expressing cell product manufacturing, thereby reducing the risk of subject relapse to CAR-expressing cell treatment. In an embodiment, a subject is pre-treated with an anti-GITR antibody prior to collection of cells for CAR-expressing cell product manufacturing, thereby reducing the risk of subject relapse to CAR-expressing cell treatment.

In one embodiment, the population of cells to be removed are neither the regulatory T cells or tumor cells, but cells that otherwise negatively affect the expansion and/or function of CART cells, e.g. cells expressing CD14, CD11b, CD33, CD15, or other markers expressed by potentially immune suppressive cells. In one embodiment, such cells are envisioned to be removed concurrently with regulatory T cells and/or tumor cells, or following said depletion, or in another order.

The methods described herein can include more than one selection step, e.g., more than one depletion step. Enrichment of a T cell population by negative selection can be accomplished, e.g., with a combination of antibodies directed to surface markers unique to the negatively selected cells. One method is cell sorting and/or selection via negative magnetic immunoadherence or flow cytometry that uses a cocktail of monoclonal antibodies directed to cell surface markers present on the cells negatively selected. For example, to enrich for CD4+ cells by negative selection, a monoclonal antibody cocktail can include antibodies to CD14, CD20, CD11b, CD16, HLA-DR, and CD8.

The methods described herein can further include removing cells from the population which express a tumor antigen, e.g., a tumor antigen that does not comprise CD25, e.g., CD19, CD30, CD38, CD123, CD20, CD14 or CD11b, to thereby provide a population of T regulatory depleted, e.g., CD25+ depleted, and tumor antigen depleted cells that are suitable for expression of a CAR, e.g., a CAR described herein. In one embodiment, tumor antigen expressing cells are removed simultaneously with the T regulatory, e.g., CD25+ cells. For example, an anti-CD25 antibody, or fragment thereof, and an anti-tumor antigen antibody, or fragment thereof, can be attached to the same substrate, e.g., bead, which can be used to remove the cells or an anti-CD25 antibody, or fragment thereof, or the anti-tumor antigen antibody, or fragment thereof, can be attached to separate beads, a mixture of which can be used

to remove the cells. In other embodiments, the removal of T regulatory cells, e.g., CD25+ cells, and the removal of the tumor antigen expressing cells is sequential, and can occur, e.g., in either order.

Also provided are methods that include removing cells from the population which express a check point inhibitor, e.g., a check point inhibitor described herein, e.g., one or more of PD1+ cells, LAG3+ cells, and TIM3+ cells, to thereby provide a population of T regulatory depleted, e.g., CD25+ depleted cells, and check point inhibitor depleted cells, e.g., PD1+, LAG3+ and/or TIM3+ depleted cells. Exemplary check point inhibitors include PD1, PD-L1, PD-L2, CTLA4, TIM3, CEACAM (e.g., CEACAM-1, CEACAM-3 and/or CEACAM-5), LAG3, VISTA, BTLA, TIGIT, LAIR1, CD160, 2B4, CD80, CD86, B7-H3 (CD276), B7-H4 (VTCN1), HVEM (TNFRSF14 or CD270), KIR, A2aR, MHC class I, MHC class II, GAL9, adenosine, and TGFR beta. In embodiments, the checkpoint inhibitor is PD1 or PD-L1. In one embodiment, check point inhibitor expressing cells are removed simultaneously with the T regulatory, e.g., CD25+ cells. For example, an anti-CD25 antibody, or fragment thereof, and an anti-check point inhibitor antibody, or fragment thereof, can be attached to the same bead which can be used to remove the cells, or an anti-CD25 antibody, or fragment thereof, and the anti-check point inhibitor antibody, or fragment there, can be attached to separate beads, a mixture of which can be used to remove the cells. In other embodiments, the removal of T regulatory cells, e.g., CD25+ cells, and the removal of the check point inhibitor expressing cells is sequential, and can occur, e.g., in either order.

In one embodiment, a T cell population can be selected that expresses one or more of IFN-γ, TNFα, IL-17A, IL-2, IL-3, IL-4, GM-CSF, IL-10, IL-13, granzyme B, and perforin, or other appropriate molecules, e.g., other cytokines. Methods for screening for cell expression can be determined, e.g., by the methods described in PCT Publication No.: WO 2013/126712.

For isolation of a desired population of cells by positive or negative selection, the concentration of cells and surface (e.g., particles such as beads) can be varied. In certain aspects, it may be desirable to significantly decrease the volume in which beads and cells are mixed together (e.g., increase the concentration of cells), to ensure maximum contact of cells and beads. For example, in one aspect, a concentration of 2 billion cells/ml is used. In one aspect, a concentration of 1 billion cells/ml is used. In a further aspect, greater than 100 million cells/ml is used. In a further aspect, a concentration of cells of 10, 15, 20, 25, 30, 35, 40, 45, or 50 million cells/ml is used. In yet one aspect, a concentration of cells from 75, 80, 85, 90, 95, or 100 million cells/ml is used. In further aspects, concentrations of 125 or 150 million cells/ml can be used. Using high concentrations can result in increased cell yield, cell activation, and cell expansion. Further, use of high cell concentrations allows more efficient capture of cells that may weakly express target antigens of interest, such as CD28-negative T cells, or from samples where there are many tumor cells present (e.g., leukemic blood, tumor tissue, etc.). Such populations of cells

may have therapeutic value and would be desirable to obtain. For example, using high concentration of cells allows more efficient selection of CD8+ T cells that normally have weaker CD28 expression.

In a related aspect, it may be desirable to use lower concentrations of cells. By significantly diluting the mixture of T cells and surface (e.g., particles such as beads), interactions between the particles and cells is minimized. This selects for cells that express high amounts of desired antigens to be bound to the particles. For example, CD4+ T cells express higher levels of CD28 and are more efficiently captured than CD8+ T cells in dilute concentrations. In one aspect, the concentration of cells used is 5 X 10e6/ml. In other aspects, the concentration used can be from about 1 X 10^5/ml to 1 X 10^6/ml, and any integer value in between.

In other aspects, the cells may be incubated on a rotator for varying lengths of time at varying speeds at either 2-10°C or at room temperature.

T cells for stimulation can also be frozen after a washing step. Wishing not to be bound by theory, the freeze and subsequent thaw step provides a more uniform product by removing granulocytes and to some extent monocytes in the cell population. After the washing step that removes plasma and platelets, the cells may be suspended in a freezing solution. While many freezing solutions and parameters are known in the art and will be useful in this context, one method involves using PBS containing 20% DMSO and 8% human serum albumin, or culture media containing 10% Dextran 40 and 5% Dextrose, 20% Human Serum Albumin and 7.5% DMSO, or 31.25% Plasmalyte-A, 31.25% Dextrose 5%, 0.45% NaCl, 10% Dextran 40 and 5% Dextrose, 20% Human Serum Albumin, and 7.5% DMSO or other suitable cell freezing media containing for example, Hespan and PlasmaLyte A, the cells then are frozen to -80°C at a rate of 1° per minute and stored in the vapor phase of a liquid nitrogen storage tank. Other methods of controlled freezing may be used as well as uncontrolled freezing immediately at -20° C or in liquid nitrogen.

In certain aspects, cryopreserved cells are thawed and washed as described herein and allowed to rest for one hour at room temperature prior to activation using the methods of the present invention.

Also contemplated in the context of the invention is the collection of blood samples or apheresis product from a subject at a time period prior to when the expanded cells as described herein might be needed. As such, the source of the cells to be expanded can be collected at any time point necessary, and desired cells, such as immune effector cells, e.g., T cells or NK cells, isolated and frozen for later use in cell therapy, e.g., T cell therapy, for any number of diseases or conditions that would benefit from cell therapy, e.g., T cell therapy, such as those described herein. In one aspect a blood sample or an apheresis is taken from a generally healthy subject. In certain aspects, a blood sample or an apheresis is taken from a generally healthy subject who is at risk of developing a disease, but who has not yet

developed a disease, and the cells of interest are isolated and frozen for later use. In certain aspects, the immune effector cells (e.g., T cells or NK cells) may be expanded, frozen, and used at a later time. In certain aspects, samples are collected from a patient shortly after diagnosis of a particular disease as described herein but prior to any treatments. In a further aspect, the cells are isolated from a blood sample or an apheresis from a subject prior to any number of relevant treatment modalities, including but not limited to treatment with agents such as natalizumab, efalizumab, antiviral agents, chemotherapy, radiation, immunosuppressive agents, such as cyclosporin, azathioprine, methotrexate, mycophenolate, and FK506, antibodies, or other immunoablative agents such as CAMPATH, anti-CD3 antibodies, cytoxan, fludarabine, cyclosporin, FK506, rapamycin, mycophenolic acid, steroids, FR901228, and irradiation.

In a further aspect of the present invention, T cells are obtained from a patient directly following treatment that leaves the subject with functional T cells. In this regard, it has been observed that following certain cancer treatments, in particular treatments with drugs that damage the immune system, shortly after treatment during the period when patients would normally be recovering from the treatment, the quality of T cells obtained may be optimal or improved for their ability to expand ex vivo. Likewise, following ex vivo manipulation using the methods described herein, these cells may be in a preferred state for enhanced engraftment and in vivo expansion. Thus, it is contemplated within the context of the present invention to collect blood cells, including T cells, dendritic cells, or other cells of the hematopoietic lineage, during this recovery phase. Further, in certain aspects, mobilization (for example, mobilization with GM-CSF) and conditioning regimens can be used to create a condition in a subject wherein repopulation, recirculation, regeneration, and/or expansion of particular cell types is favored, especially during a defined window of time following therapy. Illustrative cell types include T cells, B cells, dendritic cells, and other cells of the immune system.

In one embodiment, the immune effector cells expressing a CAR molecule, e.g., a CAR molecule described herein, are obtained from a subject that has received a low, immune enhancing dose of an mTOR inhibitor. In an embodiment, the population of immune effector cells, e.g., T cells, to be engineered to express a CAR, are harvested after a sufficient time, or after sufficient dosing of the low, immune enhancing, dose of an mTOR inhibitor, such that the level of PD1 negative immune effector cells, e.g., T cells, or the ratio of PD1 negative immune effector cells, e.g., T cells/ PD1 positive immune effector cells, e.g., T cells, in the subject or harvested from the subject has been, at least transiently, increased.

In other embodiments, population of immune effector cells, e.g., T cells, which have, or will be engineered to express a CAR, can be treated ex vivo by contact with an amount of an mTOR inhibitor

that increases the number of PD1 negative immune effector cells, e.g., T cells or increases the ratio of PD1 negative immune effector cells, e.g., T cells/ PD1 positive immune effector cells, e.g., T cells.

In one embodiment, a T cell population is diaglycerol kinase (DGK)-deficient. DGK-deficient cells include cells that do not express DGK RNA or protein, or have reduced or inhibited DGK activity. DGK-deficient cells can be generated by genetic approaches, e.g., administering RNA-interfering agents, e.g., siRNA, shRNA, miRNA, to reduce or prevent DGK expression. Alternatively, DGK-deficient cells can be generated by treatment with DGK inhibitors described herein.

In one embodiment, a T cell population is Ikaros-deficient. Ikaros-deficient cells include cells that do not express Ikaros RNA or protein, or have reduced or inhibited Ikaros activity, Ikaros-deficient cells can be generated by genetic approaches, e.g., administering RNA-interfering agents, e.g., siRNA, shRNA, miRNA, to reduce or prevent Ikaros expression. Alternatively, Ikaros-deficient cells can be generated by treatment with Ikaros inhibitors, e.g., lenalidomide.

In embodiments, a T cell population is DGK-deficient and Ikaros-deficient, e.g., does not express DGK and Ikaros, or has reduced or inhibited DGK and Ikaros activity. Such DGK and Ikaros-deficient cells can be generated by any of the methods described herein.

In an embodiment, the NK cells are obtained from the subject. In another embodiment, the NK cells are an NK cell line, e.g., NK-92 cell line (Conkwest).

Modifications of CAR cells, including allogeneic CAR cells

In embodiments described herein, the immune effector cell can be an allogeneic immune effector cell, e.g., T cell or NK cell. For example, the cell can be an allogeneic T cell, e.g., an allogeneic T cell lacking expression of a functional T cell receptor (TCR) and/or human leukocyte antigen (HLA), e.g., HLA class I and/or HLA class II, and/or beta-2 microglobulin (β_2m). Compositions of allogeneic CAR and methods thereof have been described in, e.g., pages 227-237 of WO 2016/014565, incorporated herein by reference in its entirety.

In some embodiments, a cell, e.g., a T cell or a NK cell, is modified to reduce the expression of a TCR, and/or HLA, and/or β_2m, and/or an inhibitory molecule described herein (e.g., PD1, PD-L1, PD-L2, CTLA4, TIM3, CEACAM (e.g., CEACAM-1, CEACAM-3 and/or CEACAM-5), LAG3, VISTA, BTLA, TIGIT, LAIR1, CD160, 2B4, CD80, CD86, B7-H3 (CD276), B7-H4 (VTCN1), HVEM (TNFRSF14 or CD270), KIR, A2aR, MHC class I, MHC class II, GAL9, adenosine, and TGFR beta), using, e.g., a method described herein, e.g., siRNA, shRNA, clustered regularly interspaced short palindromic repeats (CRISPR) transcription-activator like effector nuclease (TALEN), or zinc finger endonuclease (ZFN).

In some embodiments, a cell, e.g., a T cell or a NK cell is engineered to express a telomerase subunit, e.g., the catalytic subunit of telomerase, e.g., TERT, e.g., hTERT. In one embodiment, such modification improves persistence of the cell in a patient.

Activation and Expansion of T Cells

T cells may be activated and expanded generally using methods as described, for example, in U.S. Patents 6,352,694; 6,534,055; 6,905,680; 6,692,964; 5,858,358; 6,887,466; 6,905,681; 7,144,575; 7,067,318; 7,172,869; 7,232,566; 7,175,843; 5,883,223; 6,905,874; 6,797,514; 6,867,041; and U.S. Patent Application Publication No. 20060121005.

Generally, the T cells of the invention may be expanded by contact with a surface having attached thereto an agent that stimulates a CD3/TCR complex associated signal and a ligand that stimulates a costimulatory molecule on the surface of the T cells. In particular, T cell populations may be stimulated as described herein, such as by contact with an anti-CD3 antibody, or antigen-binding fragment thereof, or an anti-CD2 antibody immobilized on a surface, or by contact with a protein kinase C activator (e.g., bryostatin) in conjunction with a calcium ionophore. For co-stimulation of an accessory molecule on the surface of the T cells, a ligand that binds the accessory molecule is used. For example, a population of T cells can be contacted with an anti-CD3 antibody and an anti-CD28 antibody, under conditions appropriate for stimulating proliferation of the T cells. To stimulate proliferation of either CD4+ T cells or CD8+ T cells, an anti-CD3 antibody and an anti-CD28 antibody can be used. Examples of an anti-CD28 antibody include 9.3, B-T3, XR-CD28 (Diaclone, Besançon, France) can be used as can other methods commonly known in the art (Berg et al., Transplant Proc. 30(8):3975-3977, 1998; Haanen et al., J. Exp. Med. 190(9):13191328, 1999; Garland et al., J. Immunol Meth. 227(1-2):53-63, 1999).

In certain aspects, the primary stimulatory signal and the costimulatory signal for the T cell may be provided by different protocols. For example, the agents providing each signal may be in solution or coupled to a surface. When coupled to a surface, the agents may be coupled to the same surface (i.e., in "cis" formation) or to separate surfaces (i.e., in "trans" formation). Alternatively, one agent may be coupled to a surface and the other agent in solution. In one aspect, the agent providing the costimulatory signal is bound to a cell surface and the agent providing the primary activation signal is in solution or coupled to a surface. In certain aspects, both agents can be in solution. In one aspect, the agents may be in soluble form, and then cross-linked to a surface, such as a cell expressing Fc receptors or an antibody or other binding agent which will bind to the agents. In this regard, see for example, U.S. Patent

Application Publication Nos. 20040101519 and 20060034810 for artificial antigen presenting cells (aAPCs) that are contemplated for use in activating and expanding T cells in the present invention.

In one aspect, the two agents are immobilized on beads, either on the same bead, i.e., "cis," or to separate beads, i.e., "trans." By way of example, the agent providing the primary activation signal is an anti-CD3 antibody or an antigen-binding fragment thereof and the agent providing the costimulatory signal is an anti-CD28 antibody or antigen-binding fragment thereof; and both agents are co-immobilized to the same bead in equivalent molecular amounts. In one aspect, a 1:1 ratio of each antibody bound to the beads for CD4+ T cell expansion and T cell growth is used. In certain aspects of the present invention, a ratio of anti CD3:CD28 antibodies bound to the beads is used such that an increase in T cell expansion is observed as compared to the expansion observed using a ratio of 1:1. In one particular aspect an increase of from about 1 to about 3 fold is observed as compared to the expansion observed using a ratio of 1:1. In one aspect, the ratio of CD3:CD28 antibody bound to the beads ranges from 100:1 to 1:100 and all integer values there between. In one aspect of the present invention, more anti-CD28 antibody is bound to the particles than anti-CD3 antibody, i.e., the ratio of CD3:CD28 is less than one. In certain aspects of the invention, the ratio of anti CD28 antibody to anti CD3 antibody bound to the beads is greater than 2:1. In one particular aspect, a 1:100 CD3:CD28 ratio of antibody bound to beads is used. In one aspect, a 1:75 CD3:CD28 ratio of antibody bound to beads is used. In a further aspect, a 1:50 CD3:CD28 ratio of antibody bound to beads is used. In one aspect, a 1:30 CD3:CD28 ratio of antibody bound to beads is used. In one preferred aspect, a 1:10 CD3:CD28 ratio of antibody bound to beads is used. In one aspect, a 1:3 CD3:CD28 ratio of antibody bound to the beads is used. In yet one aspect, a 3:1 CD3:CD28 ratio of antibody bound to the beads is used.

Ratios of particles to cells from 1:500 to 500:1 and any integer values in between may be used to stimulate T cells or other target cells. As those of ordinary skill in the art can readily appreciate, the ratio of particles to cells may depend on particle size relative to the target cell. For example, small sized beads could only bind a few cells, while larger beads could bind many. In certain aspects the ratio of cells to particles ranges from 1:100 to 100:1 and any integer values in-between and in further aspects the ratio comprises 1:9 to 9:1 and any integer values in between, can also be used to stimulate T cells. The ratio of anti-CD3- and anti-CD28-coupled particles to T cells that result in T cell stimulation can vary as noted above, however certain preferred values include 1:100, 1:50, 1:40, 1:30, 1:20, 1:10, 1:9, 1:8, 1:7, 1:6, 1:5, 1:4, 1:3, 1:2, 1:1, 2:1, 3:1, 4:1, 5:1, 6:1, 7:1, 8:1, 9:1, 10:1, and 15:1 with one preferred ratio being at least 1:1 particles per T cell. In one aspect, a ratio of particles to cells of 1:1 or less is used. In one particular aspect, a preferred particle: cell ratio is 1:5. In further aspects, the ratio of particles to cells can be varied depending on the day of stimulation. For example, in one aspect, the ratio of particles to cells is from 1:1 to 10:1 on the first day and additional particles are added to the cells every day or

every other day thereafter for up to 10 days, at final ratios of from 1:1 to 1:10 (based on cell counts on the day of addition). In one particular aspect, the ratio of particles to cells is 1:1 on the first day of stimulation and adjusted to 1:5 on the third and fifth days of stimulation. In one aspect, particles are added on a daily or every other day basis to a final ratio of 1:1 on the first day, and 1:5 on the third and fifth days of stimulation. In one aspect, the ratio of particles to cells is 2:1 on the first day of stimulation and adjusted to 1:10 on the third and fifth days of stimulation. In one aspect, particles are added on a daily or every other day basis to a final ratio of 1:1 on the first day, and 1:10 on the third and fifth days of stimulation. One of skill in the art will appreciate that a variety of other ratios may be suitable for use in the present invention. In particular, ratios will vary depending on particle size and on cell size and type. In one aspect, the most typical ratios for use are in the neighborhood of 1:1, 2:1 and 3:1 on the first day.

In further aspects of the present invention, the cells, such as T cells, are combined with agent-coated beads, the beads and the cells are subsequently separated, and then the cells are cultured. In an alternative aspect, prior to culture, the agent-coated beads and cells are not separated but are cultured together. In a further aspect, the beads and cells are first concentrated by application of a force, such as a magnetic force, resulting in increased ligation of cell surface markers, thereby inducing cell stimulation.

By way of example, cell surface proteins may be ligated by allowing paramagnetic beads to which anti-CD3 and anti-CD28 are attached (3x28 beads) to contact the T cells. In one aspect the cells (for example, 10^4 to 10^9 T cells) and beads (for example, DYNABEADS® M-450 CD3/CD28 T paramagnetic beads at a ratio of 1:1) are combined in a buffer, for example PBS (without divalent cations such as, calcium and magnesium). Again, those of ordinary skill in the art can readily appreciate any cell concentration may be used. For example, the target cell may be very rare in the sample and comprise only 0.01% of the sample or the entire sample (i.e., 100%) may comprise the target cell of interest. Accordingly, any cell number is within the context of the present invention. In certain aspects, it may be desirable to significantly decrease the volume in which particles and cells are mixed together (i.e., increase the concentration of cells), to ensure maximum contact of cells and particles. For example, in one aspect, a concentration of about 10 billion cells/ml, 9 billion/ml, 8 billion/ml, 7 billion/ml, 6 billion/ml, 5 billion/ml, or 2 billion cells/ml is used. In one aspect, greater than 100 million cells/ml is used. In a further aspect, a concentration of cells of 10, 15, 20, 25, 30, 35, 40, 45, or 50 million cells/ml is used. In yet one aspect, a concentration of cells from 75, 80, 85, 90, 95, or 100 million cells/ml is used. In further aspects, concentrations of 125 or 150 million cells/ml can be used. Using high concentrations can result in increased cell yield, cell activation, and cell expansion. Further, use of high cell concentrations allows more efficient capture of cells that may weakly express target antigens of interest, such as CD28-negative T cells. Such populations of cells may have therapeutic value and

would be desirable to obtain in certain aspects. For example, using high concentration of cells allows more efficient selection of CD8+ T cells that normally have weaker CD28 expression.

In one embodiment, cells transduced with a nucleic acid encoding a CAR, e.g., a CAR described herein, are expanded, e.g., by a method described herein. In one embodiment, the cells are expanded in culture for a period of several hours (e.g., about 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 18, 21 hours) to about 14 days (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13 or 14 days). In one embodiment, the cells are expanded for a period of 4 to 9 days. In one embodiment, the cells are expanded for a period of 8 days or less, e.g., 7, 6 or 5 days. In one embodiment, the cells, e.g., a BCMA CAR cell described herein, are expanded in culture for 5 days, and the resulting cells are more potent than the same cells expanded in culture for 9 days under the same culture conditions. Potency can be defined, e.g., by various T cell functions, e.g. proliferation, target cell killing, cytokine production, activation, migration, or combinations thereof. In one embodiment, the cells, e.g., a BCMA CAR cell described herein, expanded for 5 days show at least a one, two, three or four fold increase in cells doublings upon antigen stimulation as compared to the same cells expanded in culture for 9 days under the same culture conditions. In one embodiment, the cells, e.g., the cells expressing a BCMA CAR described herein, are expanded in culture for 5 days, and the resulting cells exhibit higher proinflammatory cytokine production, e.g., IFN-γ and/or GM-CSF levels, as compared to the same cells expanded in culture for 9 days under the same culture conditions. In one embodiment, the cells, e.g., a BCMA CAR cell described herein, expanded for 5 days show at least a one, two, three, four, five, ten fold or more increase in pg/ml of proinflammatory cytokine production, e.g., IFN-γ and/or GM-CSF levels, as compared to the same cells expanded in culture for 9 days under the same culture conditions.

In one aspect of the present invention, the mixture may be cultured for several hours (about 3 hours) to about 14 days or any hourly integer value in between. In one aspect, the mixture may be cultured for 21 days. In one aspect of the invention the beads and the T cells are cultured together for about eight days. In one aspect, the beads and T cells are cultured together for 2-3 days. Several cycles of stimulation may also be desired such that culture time of T cells can be 60 days or more. Conditions appropriate for T cell culture include an appropriate media (e.g., Minimal Essential Media or RPMI Media 1640 or, X-vivo 15, (Lonza)) that may contain factors necessary for proliferation and viability, including serum (e.g., fetal bovine or human serum), interleukin-2 (IL-2), insulin, IFN-γ, IL-4, IL-7, GM-CSF, IL-10, IL-12, IL-15, TGFβ, and TNF-α or any other additives for the growth of cells known to the skilled artisan. Other additives for the growth of cells include, but are not limited to, surfactant, plasmanate, and reducing agents such as N-acetyl-cysteine and 2-mercaptoethanol. Media can include RPMI 1640, AIM-V, DMEM, MEM, α-MEM, F-12, X-Vivo 15, and X-Vivo 20, Optimizer, with added amino acids, sodium pyruvate, and vitamins, either serum-free or supplemented with an appropriate

amount of serum (or plasma) or a defined set of hormones, and/or an amount of cytokine(s) sufficient for the growth and expansion of T cells. Antibiotics, e.g., penicillin and streptomycin, are included only in experimental cultures, not in cultures of cells that are to be infused into a subject. The target cells are maintained under conditions necessary to support growth, for example, an appropriate temperature (e.g., 37° C) and atmosphere (e.g., air plus 5% CO_2).

In one embodiment, the cells are expanded in an appropriate media (e.g., media described herein) that includes one or more interleukin that result in at least a 200-fold (e.g., 200-fold, 250-fold, 300-fold, 350-fold) increase in cells over a 14 day expansion period, e.g., as measured by a method described herein such as flow cytometry. In one embodiment, the cells are expanded in the presence of IL-15 and/or IL-7 (e.g., IL-15 and IL-7).

In embodiments, methods described herein, e.g., CAR-expressing cell manufacturing methods, comprise removing T regulatory cells, e.g., CD25+ T cells, from a cell population, e.g., using an anti-CD25 antibody, or fragment thereof, or a CD25-binding ligand, IL-2. Methods of removing T regulatory cells, e.g., CD25+ T cells, from a cell population are described herein. In embodiments, the methods, e.g., manufacturing methods, further comprise contacting a cell population (e.g., a cell population in which T regulatory cells, such as CD25+ T cells, have been depleted; or a cell population that has previously contacted an anti-CD25 antibody, fragment thereof, or CD25-binding ligand) with IL-15 and/or IL-7. For example, the cell population (e.g., that has previously contacted an anti-CD25 antibody, fragment thereof, or CD25-binding ligand) is expanded in the presence of IL-15 and/or IL-7.

In some embodiments a CAR-expressing cell described herein is contacted with a composition comprising a interleukin-15 (IL-15) polypeptide, a interleukin-15 receptor alpha (IL-15Ra) polypeptide, or a combination of both a IL-15 polypeptide and a IL-15Ra polypeptide e.g., hetIL-15, during the manufacturing of the CAR-expressing cell, e.g., ex vivo. In embodiments, a CAR-expressing cell described herein is contacted with a composition comprising a IL-15 polypeptide during the manufacturing of the CAR-expressing cell, e.g., ex vivo. In embodiments, a CAR-expressing cell described herein is contacted with a composition comprising a combination of both a IL-15 polypeptide and a IL-15 Ra polypeptide during the manufacturing of the CAR-expressing cell, e.g., ex vivo. In embodiments, a CAR-expressing cell described herein is contacted with a composition comprising hetIL-15 during the manufacturing of the CAR-expressing cell, e.g., ex vivo.

In one embodiment the CAR-expressing cell described herein is contacted with a composition comprising hetIL-15 during ex vivo expansion. In an embodiment, the CAR-expressing cell described herein is contacted with a composition comprising an IL-15 polypeptide during ex vivo expansion. In an embodiment, the CAR-expressing cell described herein is contacted with a composition comprising

both an IL-15 polypeptide and an IL-15Ra polypeptide during ex vivo expansion. In one embodiment the contacting results in the survival and proliferation of a lymphocyte subpopulation, e.g., CD8+ T cells.

T cells that have been exposed to varied stimulation times may exhibit different characteristics. For example, typical blood or apheresed peripheral blood mononuclear cell products have a helper T cell population (TH, CD4+) that is greater than the cytotoxic or suppressor T cell population. Ex vivo expansion of T cells by stimulating CD3 and CD28 receptors produces a population of T cells that prior to about days 8-9 consists predominately of TH cells, while after about days 8-9, the population of T cells comprises an increasingly greater population of TC cells. Accordingly, depending on the purpose of treatment, infusing a subject with a T cell population comprising predominately of TH cells may be advantageous. Similarly, if an antigen-specific subset of TC cells has been isolated it may be beneficial to expand this subset to a greater degree.

Further, in addition to CD4 and CD8 markers, other phenotypic markers vary significantly, but in large part, reproducibly during the course of the cell expansion process. Thus, such reproducibility enables the ability to tailor an activated T cell product for specific purposes.

Once a BCMA CAR is constructed, various assays can be used to evaluate the activity of the molecule, such as but not limited to, the ability to expand T cells following antigen stimulation, sustain T cell expansion in the absence of re-stimulation, and anti-cancer activities in appropriate in vitro and animal models. Assays to evaluate the effects of a BCMA CAR are described in further detail below

Western blot analysis of CAR expression in primary T cells can be used to detect the presence of monomers and dimers. See, *e.g.*, Milone *et al.*, Molecular Therapy 17(8): 1453-1464 (2009). Very briefly, T cells (1:1 mixture of $CD4^+$ and $CD8^+$ T cells) expressing the CARs are expanded *in vitro* for more than 10 days followed by lysis and SDS-PAGE under reducing conditions. CARs containing the full length TCR-ζ cytoplasmic domain and the endogenous TCR-ζ chain are detected by western blotting using an antibody to the TCR-ζ chain. The same T cell subsets are used for SDS-PAGE analysis under non-reducing conditions to permit evaluation of covalent dimer formation.

In vitro expansion of CAR^+ T cells following antigen stimulation can be measured by flow cytometry. For example, a mixture of $CD4^+$ and $CD8^+$ T cells are stimulated with αCD3/αCD28 aAPCs followed by transduction with lentiviral vectors expressing GFP under the control of the promoters to be analyzed. Exemplary promoters include the CMV IE gene, EF-1α, ubiquitin C, or phosphoglycerokinase (PGK) promoters. GFP fluorescence is evaluated on day 6 of culture in the $CD4^+$ and/or $CD8^+$ T cell subsets by flow cytometry. See, *e.g.*, Milone *et al.*, Molecular Therapy 17(8): 1453-1464 (2009). Alternatively, a mixture of $CD4^+$ and $CD8^+$ T cells are stimulated with αCD3/αCD28

coated magnetic beads on day 0, and transduced with CAR on day 1 using a bicistronic lentiviral vector expressing CAR along with eGFP using a 2A ribosomal skipping sequence. Cultures are re-stimulated with BCMA-expressing cells, such as multiple myeloma cell lines or K562-BCMA, following washing. Exogenous IL-2 is added to the cultures every other day at 100 IU/ml. GFP^+ T cells are enumerated by flow cytometry using bead-based counting. See, *e.g.*, Milone *et al.*, Molecular Therapy 17(8): 1453-1464 (2009).

Sustained CAR^+ T cell expansion in the absence of re-stimulation can also be measured. See, *e.g.*, Milone *et al.*, Molecular Therapy 17(8): 1453-1464 (2009). Briefly, mean T cell volume (fl) is measured on day 8 of culture using a Coulter Multisizer III particle counter, a Nexcelom Cellometer Vision or Millipore Scepter, following stimulation with αCD3/αCD28 coated magnetic beads on day 0, and transduction with the indicated CAR on day 1.

Animal models can also be used to measure a CART activity. For example, xenograft model using human BCMA-specific CAR^+ T cells to treat a primary human multiple myeloma in immunodeficient mice can be used. See, *e.g.*, Milone *et al.*, Molecular Therapy 17(8): 1453-1464 (2009). Very briefly, after establishment of MM, mice are randomized as to treatment groups. Different numbers of BCMA CART cells can be injected into immunodeficient mice bearing MM. Animals are assessed for disease progression and tumor burden at weekly intervals. Survival curves for the groups are compared using the log-rank test. In addition, absolute peripheral blood $CD4^+$ and $CD8^+$ T cell counts 4 weeks following T cell injection in the immunodeficient mice can also be analyzed. Mice are injected with multiple myeloma cells and 3 weeks later are injected with T cells engineered to express BCMA CAR, e.g., by a bicistronic lentiviral vector that encodes the CAR linked to eGFP. T cells are normalized to 45–50% input GFP^+ T cells by mixing with mock-transduced cells prior to injection, and confirmed by flow cytometry. Animals are assessed for leukemia at 1-week intervals. Survival curves for the CAR^+ T cell groups are compared using the log-rank test.

Assessment of cell proliferation and cytokine production has been previously described, *e.g.*, at Milone *et al.*, Molecular Therapy 17(8): 1453-1464 (2009). Briefly, assessment of CAR-mediated proliferation is performed in microtiter plates by mixing washed T cells with K562 cells expressing BCMA or other BCMA-expressing myeloma cells are irradiated with gamma-radiation prior to use. Anti-CD3 (clone OKT3) and anti- CD28 (clone 9.3) monoclonal antibodies are added to cultures with KT32-BBL cells to serve as a positive control for stimulating T-cell proliferation since these signals support long-term $CD8^+$ T cell expansion *ex vivo*. T cells are enumerated in cultures using CountBright™ fluorescent beads (Invitrogen, Carlsbad, CA) and flow cytometry as described by the manufacturer. CAR^+ T cells are identified by GFP expression using T cells that are engineered with eGFP-2A linked CAR-expressing lentiviral vectors. For CAR+ T cells not expressing GFP, the CAR+ T

cells are detected with biotinylated recombinant BCMA protein and a secondary avidin-PE conjugate. CD4+ and $CD8^+$ expression on T cells are also simultaneously detected with specific monoclonal antibodies (BD Biosciences). Cytokine measurements are performed on supernatants collected 24 hours following re-stimulation using the human TH1/TH2 cytokine cytometric bead array kit (BD Biosciences, San Diego, CA) according the manufacturer's instructions. Fluorescence is assessed using a FACScalibur flow cytometer, and data is analyzed according to the manufacturer's instructions.

Cytotoxicity can be assessed by a standard 51Cr-release assay. See, *e.g.*, Milone *et al.*, Molecular Therapy 17(8): 1453-1464 (2009). Briefly, target cells (e.g., K562 lines expressing BCMA and primary multiple myeloma cells) are loaded with 51Cr (as NaCrO4, New England Nuclear, Boston, MA) at 37°C for 2 hours with frequent agitation, washed twice in complete RPMI and plated into microtiter plates. Effector T cells are mixed with target cells in the wells in complete RPMI at varying ratios of effector cell:target cell (E:T). Additional wells containing media only (spontaneous release, SR) or a 1% solution of triton-X 100 detergent (total release, TR) are also prepared. After 4 hours of incubation at 37°C, supernatant from each well is harvested. Released 51Cr is then measured using a gamma particle counter (Packard Instrument Co., Waltham, MA). Each condition is performed in at least triplicate, and the percentage of lysis is calculated using the formula: % Lysis = (ER– SR) / (TR – SR), where ER represents the average 51Cr released for each experimental condition. Alternatively, cytotoxicity can also be assessed using a Bright-Glo™ Luciferase Assay.

Imaging technologies can be used to evaluate specific trafficking and proliferation of CARs in tumor-bearing animal models. Such assays have been described, for example, in Barrett *et al.*, Human Gene Therapy 22:1575-1586 (2011). Briefly, NOD/SCID/$\gamma c^{-/-}$ (NSG) mice or other immunodeficient are injected IV with multiple myeloma cells followed 7 days later with BCMA CART cells 4 hour after electroporation with the CAR constructs. The T cells are stably transfected with a lentiviral construct to express firefly luciferase, and mice are imaged for bioluminescence. Alternatively, therapeutic efficacy and specificity of a single injection of CAR^+ T cells in a multiple myeloma xenograft model can be measured as the following: NSG mice are injected with multiple myeloma cells transduced to stably express firefly luciferase, followed by a single tail-vein injection of T cells electroporated with BCMA CAR construct days later. Animals are imaged at various time points post injection. For example, photon-density heat maps of firefly luciferasepositive tumors in representative mice at day 5 (2 days before treatment) and day 8 (24 hr post CAR^+ PBLs) can be generated.

Alternatively, or in combination to the methods disclosed herein, methods and compositions for one or more of: detection and/or quantification of CAR-expressing cells (*e.g.*, *in vitro* or *in vivo* (e.g., clinical monitoring)); immune cell expansion and/or activation; and/or CAR-specific selection, that involve the use of a CAR ligand, are disclosed. In one exemplary embodiment, the CAR ligand is an

antibody that binds to the CAR molecule, e.g., binds to the extracellular antigen binding domain of CAR (e.g., an antibody that binds to the antigen binding domain, e.g., an anti-idiotypic antibody; or an antibody that binds to a constant region of the extracellular binding domain). In other embodiments, the CAR ligand is a CAR antigen molecule (e.g., a CAR antigen molecule as described herein).

In one aspect, a method for detecting and/or quantifying CAR-expressing cells is disclosed. For example, the CAR ligand can be used to detect and/or quantify CAR-expressing cells *in vitro* or *in vivo* (e.g., clinical monitoring of CAR-expressing cells in a patient, or dosing a patient). The method includes:

providing the CAR ligand (optionally, a labelled CAR ligand, e.g., a CAR ligand that includes a tag, a bead, a radioactive or fluorescent label);

acquiring the CAR-expressing cell (e.g., acquiring a sample containing CAR-expressing cells, such as a manufacturing sample or a clinical sample);

contacting the CAR-expressing cell with the CAR ligand under conditions where binding occurs, thereby detecting the level (e.g., amount) of the CAR-expressing cells present. Binding of the CAR-expressing cell with the CAR ligand can be detected using standard techniques such as FACS, ELISA and the like.

In another aspect, a method of expanding and/or activating cells (e.g., immune effector cells) is disclosed. The method includes:

providing a CAR-expressing cell (e.g., a first CAR-expressing cell or a transiently expressing CAR cell);

contacting said CAR-expressing cell with a CAR ligand, e.g., a CAR ligand as described herein), under conditions where immune cell expansion and/or proliferation occurs, thereby producing the activated and/or expanded cell population.

In certain embodiments, the CAR ligand is present on (e.g., is immobilized or attached to a substrate, e.g., a non-naturally occurring substrate). In some embodiments, the substrate is a non-cellular substrate. The non-cellular substrate can be a solid support chosen from, e.g., a plate (e.g., a microtiter plate), a membrane (e.g., a nitrocellulose membrane), a matrix, a chip or a bead. In embodiments, the CAR ligand is present in the substrate (e.g., on the substrate surface). The CAR ligand can be immobilized, attached, or associated covalently or non-covalently (e.g., cross-linked) to the substrate. In one embodiment, the CAR ligand is attached (e.g., covalently attached) to a bead. In the aforesaid embodiments, the immune cell population can be expanded *in vitro* or *ex vivo*. The

method can further include culturing the population of immune cells in the presence of the ligand of the CAR molecule, e.g., using any of the methods described herein.

In other embodiments, the method of expanding and/or activating the cells further comprises addition of a second stimulatory molecule, e.g., CD28. For example, the CAR ligand and the second stimulatory molecule can be immobilized to a substrate, e.g., one or more beads, thereby providing increased cell expansion and/or activation.

In yet another aspect, a method for selecting or enriching for a CAR expressing cell is provided. The method includes contacting the CAR expressing cell with a CAR ligand as described herein; and selecting the cell on the basis of binding of the CAR ligand.

In yet other embodiments, a method for depleting, reducing and/or killing a CAR expressing cell is provided. The method includes contacting the CAR expressing cell with a CAR ligand as described herein; and targeting the cell on the basis of binding of the CAR ligand, thereby reducing the number, and/or killing, the CAR-expressing cell. In one embodiment, the CAR ligand is coupled to a toxic agent (e.g., a toxin or a cell ablative drug). In another embodiment, the anti-idiotypic antibody can cause effector cell activity, e.g., ADCC or ADC activities.

Exemplary anti-CAR antibodies that can be used in the methods disclosed herein are described, e.g., in WO 2014/190273 and by Jena et al., "Chimeric Antigen Receptor (CAR)-Specific Monoclonal Antibody to Detect CD19-Specific T cells in Clinical Trials", PLOS March 2013 8:3 e57838, the contents of which are incorporated by reference. In one embodiment, the anti-idiotypic antibody molecule recognizes an anti-CD19 antibody molecule, e.g., an anti-CD19 scFv. For instance, the anti-idiotypic antibody molecule can compete for binding with the CD19-specific CAR mAb clone no. 136.20.1 described in Jena et al., PLOS March 2013 8:3 e57838; may have the same CDRs (e.g., one or more of, e.g., all of, VH CDR1, VH CDR2, CH CDR3, VL CDR1, VL CDR2, and VL CDR3, using the Kabat definition, the Chothia definition, or a combination of tthe Kabat and Chothia definitions) as the CD19-specific CAR mAb clone no. 136.20.1; may have one or more (e.g., 2) variable regions as the CD19-specific CAR mAb clone no. 136.20.1, or may comprise the CD19-specific CAR mAb clone no. 136.20.1. In some embodiments, the anti-idiotypic antibody was made according to a method described in Jena et al. In another embodiment, the anti-idiotypic antibody molecule is an anti-idiotypic antibody molecule described in WO 2014/190273. In some embodiments, the anti-idiotypic antibody molecule has the same CDRs (e.g., one or more of, e.g., all of, VH CDR1, VH CDR2, CH CDR3, VL CDR1, VL CDR2, and VL CDR3) as an antibody molecule of WO 2014/190273 such as 136.20.1; may have one or more (e.g., 2) variable regions of an antibody molecule of WO 2014/190273, or may comprise an antibody molecule of WO 2014/190273 such as

136.20.1. In other embodiments, the anti-CAR antibody binds to a constant region of the extracellular binding domain of the CAR molecule, e.g., as described in WO 2014/190273. In some embodiments, the anti-CAR antibody binds to a constant region of the extracellular binding domain of the CAR molecule, e.g., a heavy chain constant region (e.g., a CH2-CH3 hinge region) or light chain constant region. For instance, in some embodiments the anti-CAR antibody competes for binding with the 2D3 monoclonal antibody described in WO 2014/190273, has the same CDRs (e.g., one or more of, e.g., all of, VH CDR1, VH CDR2, CH CDR3, VL CDR1, VL CDR2, and VL CDR3) as 2D3, or has one or more (e.g., 2) variable regions of 2D3, or comprises 2D3 as described in WO 2014/190273.

In some aspects and embodiments, the compositions and methods herein are optimized for a specific subset of T cells, e.g., as described in US Serial No. 62/031,699 filed July 31, 2014, the contents of which are incorporated herein by reference in their entirety. In some embodiments, the optimized subsets of T cells display an enhanced persistence compared to a control T cell, e.g., a T cell of a different type (e.g., $CD8^+$ or $CD4^+$) expressing the same construct.

In some embodiments, a $CD4^+$ T cell comprises a CAR described herein, which CAR comprises an intracellular signaling domain suitable for (e.g., optimized for, e.g., leading to enhanced persistence in) a $CD4^+$ T cell, e.g., an ICOS domain. In some embodiments, a $CD8^+$ T cell comprises a CAR described herein, which CAR comprises an intracellular signaling domain suitable for (e.g., optimized for, e.g., leading to enhanced persistence of) a $CD8^+$ T cell, e.g., a 4-1BB domain, a CD28 domain, or another costimulatory domain other than an ICOS domain. In some embodiments, the CAR described herein comprises an antigen binding domain described herein, e.g., a CAR comprising an antigen binding domain that targets BCMA, e.g., a CAR of Table 1 or 16).

In an aspect, described herein is a method of treating a subject, e.g., a subject having cancer. The method includes administering to said subject, an effective amount of:

1) a $CD4^+$ T cell comprising a CAR (the CAR^{CD4+})

comprising:

an antigen binding domain, e.g., an antigen binding domain described herein, e.g., an antigen binding domain that targets BCMA, e.g., an antigen-binding domain of Table 1 or 16;

a transmembrane domain; and

an intracellular signaling domain, e.g., a first costimulatory domain, e.g., an ICOS domain; and

2) a $CD8^+$ T cell comprising a CAR (the CAR^{CD8+}) comprising:

an antigen binding domain, e.g., an antigen binding domain described herein, e.g., an antigen binding domain that targets BCMA, e.g., an antigen-binding domain of Table 1 or 16;

a transmembrane domain; and

an intracellular signaling domain, e.g., a second costimulatory domain, e.g., a 4-1BB domain, a CD28 domain, or another costimulatory domain other than an ICOS domain;

wherein the CAR^{CD4+} and the CAR^{CD8+} differ from one another.

Optionally, the method further includes administering:

3) a second CD8+ T cell comprising a CAR (the second CAR^{CD8+}) comprising:

an antigen binding domain, e.g., an antigen binding domain described herein, e.g., an antigen binding domain that specifically binds BCMA, e.g., an antigen-binding domain of Table 1 or 16;

a transmembrane domain; and

an intracellular signaling domain, wherein the second CAR^{CD8+} comprises an intracellular signaling domain, e.g., a costimulatory signaling domain, not present on the CAR^{CD8+}, and, optionally, does not comprise an ICOS signaling domain.

Other assays, including those that are known in the art can also be used to evaluate the BCMA CAR constructs of the invention.

Therapeutic Application

BCMA Associated Diseases and/or Disorders

In one aspect, the invention provides methods for treating a disease associated with BCMA expression. In one aspect, the invention provides methods for treating a disease wherein part of the tumor is negative for BCMA and part of the tumor is positive for BCMA For example, the CAR of the invention is useful for treating subjects that have undergone treatment for a disease associated with elevated expression of BCMA, wherein the subject that has undergone treatment for elevated levels of BCMA exhibits a disease associated with elevated levels of BCMA. In embodiments, the CAR of the invention is useful for treating subjects that have undergone treatment for a disease associated with expression of BCMA, wherein the subject that has undergone treatment related to expression of BCMA exhibits a disease associated with expression of BCMA.

In one embodiment, the invention provides methods for treating a disease wherein BCMA is expressed on both normal cells and cancers cells, but is expressed at lower levels on normal cells. In one embodiment, the method further comprises selecting a CAR that binds of the invention with an affinity that allows the BCMA CAR to bind and kill the cancer cells expressing BCMA but less than 30%, 25%, 20%, 15%, 10%, 5% or less of the normal cells expressing BCMA are killed, e.g., as determined by an assay described herein. For example, a killing assay such as flow cytometry based on

Cr51 CTL can be used. In one embodiment, the BCMA CAR has an antigen binding domain that has a binding affinity KD of 10^{-4} M to 10^{-8} M, e.g., 10^{-5} M to 10^{-7} M, e.g., 10^{-6} M or 10^{-7} M, for the target antigen. In one embodiment, the BCMA antigen binding domain has a binding affinity that is at least five-fold, 10-fold, 20-fold, 30-fold, 50-fold, 100-fold or 1,000-fold less than a reference antibody, e.g., an antibody described herein.

In one aspect, the invention pertains to a vector comprising BCMA CAR operably linked to promoter for expression in mammalian immune effector cells, e.g., T cells or NK cells. In one aspect, the invention provides a recombinant immune effector cell, e.g., T cell or NK cell, expressing the BCMA CAR for use in treating BCMA-expressing tumors, wherein the recombinant immune effector cell (e.g., T cell or NK cell) expressing the BCMA CAR is termed a BCMA CAR-expressing cell (e.g., BCMA CART or BCMA CAR-expressing NK cell). In one aspect, the BCMA CAR-expressing cell (e.g., BCMA CART or BCMA CAR-expressing NK cell)of the invention is capable of contacting a tumor cell with at least one BCMA CAR of the invention expressed on its surface such that the BCMA CAR-expressing cell (e.g., BCMA CART or BCMA CAR-expressing NK cell)targets the tumor cell and growth of the tumor is inhibited.

In one aspect, the invention pertains to a method of inhibiting growth of a BCMA-expressing tumor cell, comprising contacting the tumor cell with a BCMA CAR-expressing cell (e.g., BCMA CART or BCMA CAR-expressing NK cell)of the present invention such that the BCMA CAR-expressing cell (e.g., BCMA CART or BCMA CAR-expressing NK cell) is activated in response to the antigen and targets the cancer cell, wherein the growth of the tumor is inhibited.

In one aspect, the invention pertains to a method of treating cancer in a subject. The method comprises administering to the subject a BCMA CAR-expressing cell (e.g., BCMA CART or BCMA CAR-expressing NK cell)of the present invention such that the cancer is treated in the subject. An example of a cancer that is treatable by the BCMA CAR-expressing cell (e.g., BCMA CART or BCMA CAR-expressing NK cell)of the invention is a cancer associated with expression of BCMA.

The invention includes a type of cellular therapy where immune effector cells (e.g., T cells or NK cells) are genetically modified to express a chimeric antigen receptor (CAR) and the BCMA CAR-expressing cell (e.g., BCMA CART or BCMA CAR-expressing NK cell)is infused to a recipient in need thereof. The infused cell is able to kill tumor cells in the recipient. Unlike antibody therapies, CAR-modified cells, e.g., T cells or NK cells, are able to replicate in vivo resulting in long-term persistence that can lead to sustained tumor control. In various aspects, the cells (e.g., T cells or NK cells) administered to the patient, or their progeny, persist in the patient for at least four months, five months, six months, seven months, eight months, nine months, ten months, eleven months, twelve months,

thirteen months, fourteen month, fifteen months, sixteen months, seventeen months, eighteen months, nineteen months, twenty months, twenty-one months, twenty-two months, twenty-three months, two years, three years, four years, or five years after administration of the cell (e.g., T cell or NK cell) to the patient.

The invention also includes a type of cellular therapy where immune effector cells (e.g., T cells or NK cells) are modified, e.g., by in vitro transcribed RNA, to transiently express a chimeric antigen receptor (CAR) and the immune effector cell (e.g., T cell or NK cell) is infused to a recipient in need thereof. The infused cell is able to kill tumor cells in the recipient. Thus, in various aspects, the immune effector cells (e.g., T cells or NK cells) administered to the patient, is present for less than one month, e.g., three weeks, two weeks, one week, after administration of the immune effector cell (e.g., T cell or NK cell) to the patient.

Without wishing to be bound by any particular theory, the anti-tumor immunity response elicited by the CAR-modified immune effector cells (e.g., T cells or NK cells) may be an active or a passive immune response, or alternatively may be due to a direct vs indirect immune response. In one aspect, the CAR transduced immune effector cells (e.g., T cells or NK cells) exhibit specific proinflammatory cytokine secretion and potent cytolytic activity in response to human cancer cells expressing the BCMA, resist soluble BCMA inhibition, mediate bystander killing and mediate regression of an established human tumor. For example, antigen-less tumor cells within a heterogeneous field of BCMA-expressing tumor may be susceptible to indirect destruction by BCMA-redirected immune effector cells (e.g., T cells or NK cells) that has previously reacted against adjacent antigen-positive cancer cells.

In one aspect, the fully-human CAR-modified immune effector cells (e.g., T cells or NK cells) of the invention may be a type of vaccine for ex vivo immunization and/or in vivo therapy in a mammal. In one aspect, the mammal is a human.

With respect to ex vivo immunization, at least one of the following occurs in vitro prior to administering the cell into a mammal: i) expansion of the cells, ii) introducing a nucleic acid encoding a CAR to the cells or iii) cryopreservation of the cells.

Ex vivo procedures are well known in the art and are discussed more fully below. Briefly, cells are isolated from a mammal (e.g., a human) and genetically modified (i.e., transduced or transfected in vitro) with a vector expressing a CAR disclosed herein. The CAR-modified cell can be administered to a mammalian recipient to provide a therapeutic benefit. The mammalian recipient may be a human and the CAR-modified cell can be autologous with respect to the recipient. Alternatively, the cells can be allogeneic, syngeneic or xenogeneic with respect to the recipient.

The procedure for ex vivo expansion of hematopoietic stem and progenitor cells is described in U.S. Pat. No. 5,199,942, incorporated herein by reference, can be applied to the cells of the present invention. Other suitable methods are known in the art, therefore the present invention is not limited to any particular method of ex vivo expansion of the cells. Briefly, ex vivo culture and expansion of T cells comprises: (1) collecting CD34+ hematopoietic stem and progenitor cells from a mammal from peripheral blood harvest or bone marrow explants; and (2) expanding such cells ex vivo. In addition to the cellular growth factors described in U.S. Pat. No. 5,199,942, other factors such as flt3-L, IL-1, IL-3 and c-kit ligand, can be used for culturing and expansion of the cells.

In addition to using a cell-based vaccine in terms of ex vivo immunization, the present invention also provides compositions and methods for in vivo immunization to elicit an immune response directed against an antigen in a patient.

Generally, the cells activated and expanded as described herein may be utilized in the treatment and prevention of diseases that arise in individuals who are immunocompromised. In particular, the CAR-modified immune effector cells (e.g., T cells or NK cells) of the invention are used in the treatment of diseases, disorders and conditions associated with expression of BCMA. In certain aspects, the cells of the invention are used in the treatment of patients at risk for developing diseases, disorders and conditions associated with expression of BCMA. Thus, the present invention provides methods for the treatment or prevention of diseases, disorders and conditions associated with expression of BCMA comprising administering to a subject in need thereof, a therapeutically effective amount of the CAR-modified immune effector cells (e.g., T cells or NK cells) of the invention.

In one aspect the CAR-expressing cells (e.g., CART cells or CAR-expressing NK cells) of the inventions may be used to treat a proliferative disease such as a cancer or malignancy or is a precancerous condition such as a myelodysplasia, a myelodysplastic syndrome or a preleukemia. In one aspect, the cancer is a hematolical cancer. Hematological cancer conditions are the types of cancer such as leukemia and malignant lymphoproliferative conditions that affect blood, bone marrow and the lymphatic system.In one aspect, the hematological cancer is a leukemia or a hematological. An example of a disease or disorder associated with BCMA is multiple myeloma (also known as MM) (See Claudio et al., *Blood*. 2002, 100(6):2175-86; and Novak et al., *Blood*. 2004, 103(2):689-94). Multiple myeloma, also known as plasma cell myeloma or Kahler's disease, is a cancer characterized by an accumulation of abnormal or malignant plasma B-cells in the bone marrow. Frequently, the cancer cells invade adjacent bone, destroying skeletal structures and resulting in bone pain and fractures. Most cases of myeloma also features the production of a paraprotein (also known as M proteins or myeloma proteins), which is an abnormal immunoglobulin produced in excess by the clonal proliferation of the malignant plasma cells. Blood serum paraprotein levels of more than 30g/L is diagnostic of multiple myeloma, according

to the diagnostic criteria of the International Myeloma Working Group (IMWG) (*See* Kyle et al. (2009), Leukemia. 23:3-9). Other symptoms or signs of multiple myeloma include reduced kidney function or renal failure, bone lesions, anemia, hypercalcemia, and neurological symptoms.

Criteria for distinguishing multiple myeloma from other plasma cell proliferative disorders have been established by the International Myeloma Working Group (*See* Kyle et al. (2009), Leukemia. 23:3-9). All three of the following criteria must be met:

- Clonal bone marrow plasma cells ≥10%
- Present of serum and/or urinary monoclonal protein (except in patients with true non-secretory multiple myeloma)
- Evidence of end-organ damage attributable to the underlying plasma cell proliferative disorder, specifically:
    - Hypercalcemia: serum calcium ≥11.5 mg/100 ml
    - Renal insufficienty: serum creatinine > 1.73 mmol/l
    - Anemia: normochromic, normocytic with a hemoglobin value of >2g/100 ml below the lower limit of normal, or a hemoglobin value <10g/100ml
    - Bone lesions: lytic lesions, severe osteopenia, or pathologic fractures.

Other plasma cell proliferative disorders that can be treated by the compositions and methods described herein include, but are not limited to, asymptomatic myeloma (smoldering multiple myeloma or indolent myeloma), monoclonal gammapathy of undetermined significance (MGUS), Waldenstrom's macroglobulinemia, plasmacytomas (e.g., plasma cell dyscrasia, solitary myeloma, solitary plasmacytoma, extramedullary plasmacytoma, and multiple plasmacytoma), systemic amyloid light chain amyloidosis, and POEMS syndrome (also known as Crow-Fukase syndrome, Takatsuki disease, and PEP syndrome).

Two staging systems are used in the staging of multiple myeloma: the International Staging System (ISS) (*See* Greipp et al. (2005), J. Clin. Oncol. 23 (15):3412-3420) and the Durie-Salmon Staging system (DSS) (*See* Durie et al. (1975), Cancer 36 (3): 842-854). The two staging systems are summarized in the table below:

Table 18

Stage	International Staging System		Durie-Salmon Staging System	
	Criteria	Median survival	Criteria	Median survival*
I	β_2M <3.5 mg/l and serum albumin ≥3.5 g/dL	62 months	All of the following: Hemoglobin level >10g/dL Serum calcium, normal or <12 mg/dL Bone x-ray, normal or 1 plasmacytoma	IA: 62 months IB: 22 months

			only Low monoclonal protein production (IgG <5g/dL, IgA<3g/dL, Bence Jones protein <4g/dL per 24 hours	
II	Neither stage I or stage III	44 months	Neither stage I or stage III	IIA: 58 months IIB: 354 months
III	β_2M ≥5.5 mg/l	29 months	One or more of the following: Hemoglobin level <8.5g/dL Serum calcium, normal or >12 mg/dL Advanced osteolytic lesions High monoclonal protein production (IgG >7g/dL, IgA>5g/dL, Bence Jones protein >12g/dL per 24 hours	IIIA: 45 months IIIB: 24 months

*The Durie-Salmon Staging system also includes a subclassification that designates the status of renal function. The designation of "A" or "B" is added after the stage number, wherein "A" indicates relatively normal renal function (serum creatinine value <2.0 mg/dL), and B indicates abnormal renal function (serum creatinine value >2.0 mg/dL).

Standard treatment for multiple myeloma and associated diseases includes chemotherapy, stem cell transplant (autologous or allogeneic), radiation therapy, and other drug therapies. Frequently used anti-myeloma drugs include alkylating agents (e.g., bendamustine, cyclophosphamide and melphalan), proteasome inhibitors (e.g., bortezomib), corticosteroids (e.g., dexamethasone and prednisone), and immunomodulators (e.g., thalidomide and lenalidomide or Revlimid®), or any combination thereof. Biphosphonate drugs are also frequently administered in combination with the standard anti-MM treamtents to prevent bone loss. Patients older than 65-70 years of age are unlikely candidates for stem cell transplant. In some cases, double-autologous stem cell transplants are options for patients less than 60 years of age with suboptimal response to the first transplant. The compositions and methods of the present invention may be administered in combination with any of the currently prescribed treatments for multiple myeloma.

Another example of a disease or disorder associated with BCMA is Hodgkin's lymphoma and non-Hodgkin's lymphoma (*See* Chiu et al., *Blood*. 2007, 109(2):729-39; He et al., *J Immunol.* 2004, 172(5):3268-79).

Hodgkin's lymphoma (HL), also known as Hodgkin's disease, is a cancer of the lymphatic system that originates from white blood cells, or lymphocytes. The abnormal cells that comprise the lymphoma are called Reed-Sternberg cells. In Hodgkin's lymphoma, the cancer spreads from one lymph node group to another. Hodgkin's lymphoma can be subclassified into four pathologic subtypes based upon Reed-Sternberg cell morphology and the cell composition around the Reed-Sternberg cells (as

determined through lymph node biopsy): nodular sclerosing HL, mixed-cellularity subtype, lymphocyte-rich or lymphocytic predominance, lymphocyte depleted. Some Hodgkin's lymphoma can also be nodular lymphocyte predominant Hodgkin's lymphoma, or can be unspecified. Symptoms and signs of Hodgkin's lymphoma include painless swelling in the lymph nodes in the neck, armpits, or groin, fever, night sweats, weight loss, fatigue, itching, or abdominal pain.

Non-Hodgkin's lymphoma (NHL) comprises a diverse group of blood cancers that include any kind of lymphoma other than Hodgkin's lymphoma. Subtypes of non-Hodgkin's lymphoma are classified primarily by cell morphology, chromosomal aberrations, and surface markers. NHL subtypes (or NHL-associated cancers) include B cell lymphomas such as, but not limited to, Burkitt's lymphoma, B-cell chronic lymphocytic leukemia (B-CLL), B-cell prolymphocytic leukemia (B-PLL), chronic lymphocytic leukemia (CLL), diffuse large B-cell lymphoma (DLBCL) (e.g., intravascular large B-cell lymphoma and primary mediastinal B-cell lymphoma), follicular lymphoma (e.g., follicle center lymphoma, follicular small cleaved cell), hair cell leukemia, high grade B-cell lymphoma (Burkitt's like), lymphoplasmacytic lymphoma (Waldenstrom's macroglublinemia), mantle cell lymphoma, marginal zone B-cell lymphomas (e.g., extranodal marginal zone B-cell lymphoma or mucosa-associated lymphoid tissue (MALT) lymphoma, nodal marginal zone B-cell lymphoma, and splenic marginal zone B-cell lymphoma), plasmacytoma/myeloma, precursor B-lymphoblastic leukemia/lymphoma (PB-LBL/L), primary central nervous system (CNS) lymphoma, primary intraocular lymphoma, small lymphocytic lymphoma (SLL); and T cell lymphomas, such as, but not limited to, anaplastic large cell lymphoma (ALCL), adult T-cell lymphoma/leukemia (e.g., smoldering, chronic, acute and lymphomatous), angiocentric lymphoma, angioimmunoblastic T-cell lymphoma, cutaneous T-cell lymphomas (e.g., mycosis fungoides, Sezary syndrome, etc.), extranodal natural killer/T-cell lymphoma (nasal-type), enteropathy type intestinal T-cell lymphoma, large granular lymphocyte leukemia, precursor T-lymphoblastic lymphoma/leukemia (T-LBL/L), T-cell chronic lymphocytic leukemia/prolymphocytic leukemia (T-CLL/PLL), and unspecified peripheral T-cell lymphoma. Symptoms and signs of Hodgkin's lymphoma include painless swelling in the lymph nodes in the neck, armpits, or groin, fever, night sweats, weight loss, fatigue, itching, abdominal pain, coughing, or chest pain.

The staging is the same for both Hodgkin's and non-Hodgkin's lymphoma, and refers to the extent of spread of the cancer cells within the body. In stage I, the lymphoma cells are in one lymph node group. In stage II, lymphoma cells are present in at least two lymph node groups, but both groups are on the same side of the diaphragm, or in one part of a tissue or organ and the lymph nodes near that organ on the same side of the diaphragm. In stage III, lymphoma cells are in lymph nodes on both sides of the diaphragm, or in one part of a tissue or organ near these lymph node groups or in the spleen. In

stage IV, lymphoma cells are found in several parts of at least one organ or tissue, or lymphoma cells are in an organ and in lymph nodes on the other side of the diaphragm. In addition to the Roman numeral staging designation, the stages of can also be described by letters A, B, E, and S, wherein A refers to patients without symptoms, B refers to patients with symptoms, E refers to patients in which lymphoma is found in tissues outside the lymph system, and S refers to patients in which lymphoma is found in the spleen.

Hodgkin's lymphoma is commonly treated with radiation therapy, chemotherapy, or hematopoietic stem cell transplantation. The most common therapy for non-Hodgkin's lymphoma is R-CHOP, which consists of four different chemotherapies (cyclophosphamide, doxorubicin, vincristine, and prenisolone) and rituximab (Rituxan®). Other therapies commonly used to treat NHL include other chemotherapeutic agents, radiation therapy, stem cell transplantation (autologous or allogeneic bone marrow transplantation), or biological therapy, such as immunotherapy. Other examples of biological therapeutic agents include, but are not limited to, rituximab (Rituxan®), tositumomab (Bexxar®), epratuzumab (LymphoCide®), and alemtuzumab (MabCampath®). The compositions and methods of the present invention may be administered in combination with any of the currently prescribed treatments for Hodgkin's lymphoma or non-Hodgkin's lymphoma.

BCMA expression has also been associated Waldenstrom's macroglobulinemia (WM), also known as lymphoplasmacytic lymphoma (LPL). (See Elsawa et al., *Blood.* 2006, 107(7):2882-8). Waldenstrom's macroglobulinemia was previously considered to be related to multiple myeloma, but has more recently been classified as a subtype of non-Hodgkin's lymphoma. WM is characterized by uncontrolled B-cell lymphocyte proliferation, resulting in anemia and production of excess amounts of paraprotein, or immunoglobulin M (IgM), which thickens the blood and results in hyperviscosity syndrome. Other symptoms or signs of WM include fever, night sweats, fatigue, anemia, weight loss, lymphadenopathy or splenomegaly, blurred vision, dizziness, nose bleeds, bleeding gums, unusual bruises, renal impairment or failure, amyloidosis, or peripheral neuropathy.

Standard treatment for WM consists of chemotherapy, specifically with rituximab (Rituxan®). Other chemotherapeutic drugs can be used in combination, such as chlorambucil (Leukeran®), cyclophosphamide (Neosar®), fludarabine (Fludara®), cladribine (Leustatin®), vincristine, and/or thalidomide. Corticosteriods, such as prednisone, can also be administered in combination with the chemotherapy. Plasmapheresis, or plasma exchange, is commonly used throughout treatment of the patient to alleviate some symptoms by removing the paraprotein from the blood. In some cases, stem cell transplantation is an option for some patients.

Another example of a disease or disorder associated with BCMA is brain cancer. Specifically, expression of BCMA has been associated with astrocytoma or glioblastoma (*See* Deshayes et al, *Oncogene*. 2004, 23(17):3005-12, Pelekanou et al., *PLoS One*. 2013, 8(12):e83250). Astrocytomas are tumors that arise from astrocytes, which are a type of glial cell in the brain. Glioblastoma (also known as glioblastoma multiforme or GBM) is the most malignant form of astrocytoma, and is considered the most advanced stage of brain cancer (stage IV). There are two variants of glioblastoma: giant cell glioblastoma and gliosarcoma. Other astrocytomas include juvenile pilocytic astrocytoma (JPA), fibrillary astrocytoma, pleomorphic xantroastrocytoma (PXA), desembryoplastic neuroepithelial tumor (DNET), and anaplastic astrocytoma (AA).

Symptoms or signs associated with glioblastoma or astrocytoma include increased pressure in the brain, headaches, seizures, memory loss, changes in behavior, loss in movement or sensation on one side of the body, language dysfunction, cognitive impairments, visual impairment, nausea, vomiting, and weakness in the arms or legs.

Surgical removal of the tumor (or resection) is the standard treatment for removal of as much of the glioma as possible without damaging or with minimal damage to the normal, surrounding brain. Radiation therapy and/or chemotherapy are often used after surgery to suppress and slow recurrent disease from any remaining cancer cells or satellite lesions. Radiation therapy includes whole brain radiotherapy (conventional external beam radiation), targeted three-dimensional conformal radiotherapy, and targeted radionuclides. Chemotherapeutic agents commonly used to treat glioblastoma include temozolomide, gefitinib or erlotinib, and cisplatin. Angiogenesis inhibitors, such as Bevacizumab (Avastin®), are also commonly used in combination with chemotherapy and/or radiotherapy.

Supportive treatment is also frequently used to relieve neurological symptoms and improve neurologic function, and is administered in combination any of the cancer therapies described herein. The primary supportive agents include anticonvulsants and corticosteroids. Thus, the compositions and methods of the present invention may be used in combination with any of the standard or supportive treatments to treat a glioblastoma or astrocytoma.

Non-cancer related diseases and disorders associated with BCMA expression can also be treated by the compositions and methods disclosed herein. Examples of non-cancer related diseases and disorders associated with BCMA expression include, but are not limited to: viral infections; e.g., HIV, fungal invections, e.g.,*C. neoformans*; irritable bowel disease; ulcerative colitis, and disorders related to mucosal immunity.

The CAR-modified immune effector cells (e.g., T cells or NK cells) of the present invention may be administered either alone, or as a pharmaceutical composition in combination with diluents and/or with other components such as IL-2 or other cytokines or cell populations.

The present invention provides for compositions and methods for treating cancer. In one aspect, the cancer is a hematologic cancer including but is not limited to hematolical cancer is a leukemia or a lymphoma. In one aspect, the CAR-expressing cells (e.g., CART cells or CAR-expressing NK cells)of the invention may be used to treat cancers and malignancies such as, but not limited to, e.g., acute leukemias including but not limited to, e.g., B-cell acute lymphoid leukemia ("BALL"), T-cell acute lymphoid leukemia ("TALL"), acute lymphoid leukemia (ALL); one or more chronic leukemias including but not limited to, e.g., chronic myelogenous leukemia (CML), chronic lymphocytic leukemia (CLL); additional hematologic cancers or hematologic conditions including, but not limited to, e.g., B cell prolymphocytic leukemia, blastic plasmacytoid dendritic cell neoplasm, Burkitt's lymphoma, diffuse large B cell lymphoma, Follicular lymphoma, Hairy cell leukemia, small cell- or a large cell-follicular lymphoma, malignant lymphoproliferative conditions, MALT lymphoma, mantle cell lymphoma, Marginal zone lymphoma, multiple myeloma, myelodysplasia and myelodysplastic syndrome, non-Hodgkin's lymphoma, plasmablastic lymphoma, plasmacytoid dendritic cell neoplasm, Waldenstrom macroglobulinemia, and "preleukemia" which are a diverse collection of hematological conditions united by ineffective production (or dysplasia) of myeloid blood cells, and the like. Further a disease associated with BCMA expression includes, but not limited to, e.g., atypical and/or non-classical cancers, malignancies, precancerous conditions or proliferative diseases expressing BCMA.

In embodiments, a composition described herein can be used to treat a disease including but not limited to a plasma cell proliferative disorder, e.g., asymptomatic myeloma (smoldering multiple myeloma or indolent myeloma), monoclonal gammapathy of undetermined significance (MGUS), Waldenstrom's macroglobulinemia, plasmacytomas (e.g., plasma cell dyscrasia, solitary myeloma, solitary plasmacytoma, extramedullary plasmacytoma, and multiple plasmacytoma), systemic amyloid light chain amyloidosis, and POEMS syndrome (also known as Crow-Fukase syndrome, Takatsuki disease, and PEP syndrome).

In embodiments, a composition described herein can be used to treat a disease including but not limited to a cancer, e.g., a cancer described herein, e.g., a prostate cancer (e.g., castrate-resistant or therapy-resistant prostate cancer, or metastatic prostate cancer), pancreatic cancer, or lung cancer.

The present invention also provides methods for inhibiting the proliferation or reducing a BCMA-expressing cell population, the methods comprising contacting a population of cells comprising a BMCA-expressing cell with an anti-BCMA CAR-expressing cell (e.g., BCMA CART cell or BCMA CAR-expressing NK cell)of the invention that binds to the BCMA-expressing cell. In a specific aspect,

the present invention provides methods for inhibiting the proliferation or reducing the population of cancer cells expressing BCMA, the methods comprising contacting the BCMA-expressing cancer cell population with an anti-BCMA CAR-expressing cell (e.g., BCMA CART cell or BCMA CAR-expressing NK cell)of the invention that binds to the BCMA-expressing cell. In one aspect, the present invention provides methods for inhibiting the proliferation or reducing the population of cancer cells expressing BCMA, the methods comprising contacting the BMCA-expressing cancer cell population with an anti-BCMA CAR-expressing cell (e.g., BCMA CART cell or BCMA CAR-expressing NK cell)of the invention that binds to the BCMA-expressing cell. In certain aspects, the anti-BCMA CAR-expressing cell (e.g., BCMA CART cell or BCMA CAR-expressing NK cell)of the invention reduces the quantity, number, amount or percentage of cells and/or cancer cells by at least 25%, at least 30%, at least 40%, at least 50%, at least 65%, at least 75%, at least 85%, at least 95%, or at least 99% in a subject with or animal model for myeloid leukemia or another cancer associated with BCMA-expressing cells relative to a negative control. In one aspect, the subject is a human.

The present invention also provides methods for preventing, treating and/or managing a disease associated with BCMA-expressing cells (e.g., a hematologic cancer or atypical cancer expessing BCMA), the methods comprising administering to a subject in need an anti-BCMA CAR-expressing cell (e.g., BCMA CART cell or BCMA CAR-expressing NK cell)of the invention that binds to the BCMA-expressing cell. In one aspect, the subject is a human. Non-limiting examples of disorders associated with BCMA-expressing cells include viral or fungal infections, and disorders related to mucosal immunity.

The present invention also provides methods for preventing, treating and/or managing a disease associated with BCMA-expressing cells, the methods comprising administering to a subject in need an anti-BCMA CAR-expressing cell (e.g., BCMA CART cell or BCMA CAR-expressing NK cell)of the invention that binds to the BCMA-expressing cell. In one aspect, the subject is a human.

The present invention provides methods for preventing relapse of cancer associated with BCMA-expressing cells, the methods comprising administering to a subject in need thereof an anti-BCMA CAR-expressing cell (e.g., BCMA CART cell or BCMA CAR-expressing NK cell)of the invention that binds to the BCMA-expressing cell. In one aspect, the methods comprise administering to the subject in need thereof an effective amount of an anti-BCMA CAR-expressing cell (e.g., BCMA CART cell or BCMA CAR-expressing NK cell)described herein that binds to the BCMA-expressing cell in combination with an effective amount of another therapy.

Combination Therapies

A CAR-expressing cell described herein may be used in combination with other known agents and therapies. Administered "in combination", as used herein, means that two (or more) different treatments are delivered to the subject during the course of the subject's affliction with the disorder, e.g., the two or more treatments are delivered after the subject has been diagnosed with the disorder and before the disorder has been cured or eliminated or treatment has ceased for other reasons. In some embodiments, the delivery of one treatment is still occurring when the delivery of the second begins, so that there is overlap in terms of administration. This is sometimes referred to herein as "simultaneous" or "concurrent delivery". In other embodiments, the delivery of one treatment ends before the delivery of the other treatment begins. In some embodiments of either case, the treatment is more effective because of combined administration. For example, the second treatment is more effective, e.g., an equivalent effect is seen with less of the second treatment, or the second treatment reduces symptoms to a greater extent, than would be seen if the second treatment were administered in the absence of the first treatment, or the analogous situation is seen with the first treatment. In some embodiments, delivery is such that the reduction in a symptom, or other parameter related to the disorder is greater than what would be observed with one treatment delivered in the absence of the other. The effect of the two treatments can be partially additive, wholly additive, or greater than additive. The delivery can be such that an effect of the first treatment delivered is still detectable when the second is delivered.

A CAR-expressing cell described herein and the at least one additional therapeutic agent can be administered simultaneously, in the same or in separate compositions, or sequentially. For sequential administration, the CAR-expressing cell described herein can be administered first, and the additional agent can be administered second, or the order of administration can be reversed.

The CAR therapy and/or other therapeutic agents, procedures or modalities can be administered during periods of active disorder, or during a period of remission or less active disease. The CAR therapy can be administered before the other treatment, concurrently with the treatment, post-treatment, or during remission of the disorder.

When administered in combination, the CAR therapy and the additional agent (e.g., second or third agent), or all, can be administered in an amount or dose that is higher, lower or the same than the amount or dosage of each agent used individually, e.g., as a monotherapy. In certain embodiments, the administered amount or dosage of the CAR therapy, the additional agent (e.g., second or third agent), or all, is lower (e.g., at least 20%, at least 30%, at least 40%, or at least 50%) than the amount or dosage of each agent used individually, e.g., as a monotherapy. In other embodiments, the amount or dosage of the CAR therapy, the additional agent (e.g., second or third agent), or all, that results in a desired effect (e.g., treatment of cancer) is lower (e.g., at least 20%, at least 30%, at least 40%, or at least 50% lower) than the amount or dosage of each agent used individually, e.g., as a monotherapy, required to achieve

the same therapeutic effect.

In further aspects, a CAR-expressing cell described herein may be used in a treatment regimen in combination with surgery, chemotherapy, radiation, immunosuppressive agents, such as cyclosporin, azathioprine, methotrexate, mycophenolate, and FK506, antibodies, or other immunoablative agents such as CAMPATH, anti-CD3 antibodies or other antibody therapies, cytoxin, fludarabine, cyclosporin, FK506, rapamycin, mycophenolic acid, steroids, FR901228, cytokines, and irradiation. peptide vaccine, such as that described in Izumoto et al. 2008 J Neurosurg 108:963-971.

In certain instances, compounds of the present invention are combined with other therapeutic agents, such as other anti-cancer agents, anti-allergic agents, anti-nausea agents (or anti-emetics), pain relievers, cytoprotective agents, and combinations thereof.

In one embodiment, a first CAR-expressing cell described herein, e.g., a BCMA CAR-expressing cell described herein, may be used in combination with a second CAR-expressing cell. In one embodiment, the second CAR-expressing cell expresses a CAR comprising a different anti-BMCA binding domain, e.g., an anti-BCMA binding domain described herein that differs from the anti-BCMA binding domain in the CAR expressed by the first CAR-expressing cell. In one embodiment, the second CAR-expressing cell expresses a CAR comprising an antigen-binding domain that targets an antigen other than BCMA (e.g., CD19, CD20, CS-1, kappa light chain, CD139, Lewis Y antigen, or CD38). In one embodiment, a first CAR-expressing cell described herein, e.g., a BCMA CAR-expressing cell described herein, is used in combination with a second CAR-expressing cell comprising a CD19 CAR. In one embodiment, a BCMA CAR-expressing cell described herein is used in combination with a CD19 CAR-expressing cell to treat a BCMA-associated cancer described herein, e.g., multiple myeloma. In some embodiments, the multiple myeloma is CD19-negative, e.g., having a vast majority (e.g., at least 98%, 99%, 99.5%, 99.9%, or 99.95%) of the neoplastic plasma cells with a CD19-negative phenotype, e.g., as detected flow cytometry, RT-PCR, or both flow cytometry and RT-PCR. A CD19 CAR can be effective even against a CD19-negative multiple myeloma. While not wishing to be bound by theory, the CD19 CAR may act on a small but important CD19-positive population of neoplastic cells, by targeting a cell that expresses levels of CD19 that fall below the detection threshold of the assays described herein, or by targeting a non-neoplastic cell that supports the neoplastic cells. In embodiments, a CD19 CAR can remove B cells, e.g., B regulatory B cells.

For example, in one embodiment, the first CAR-expressing cell described herein, e.g., a BCMA CAR-expressing cell, and the second CAR-expressing cell described herein, e.g., a CD19 CAR-expressing cell, are prepared in the same composition and are administered simultaneously. In another embodiment, the first CAR-expressing cell described herein, e.g., a BCMA CAR-expressing cell, and the second CAR-expressing cell described herein, e.g., a CD19 CAR-expressing cell, are prepared in

separate compositions, and the separate compositions are administered simultaneously or sequentially. When the BCMA CAR-expressing cell and the second CAR-expressing cell are prepared in separate compositions, the BCMA CAR-expressing cell can be administered first, and the second CAR-expressing cell can be administered second, or the order of administration can be reversed.

In one embodiment, a CD19 CAR is a CD19 CAR, e.g., a humanized CD19 CAR, described in WO2014/153270, filed March 15, 2014 (which is incorporated by reference herein in its entirety) or a sequence at least 95%, e.g., 95-99%, identical thereto. In some embodiments, the CD19 CAR construct is a CAR19 construct provided in PCT publication WO2012/079000 (which is incorporated by reference herein in its entirety) or a sequence at least 95%, e.g., 95-99%, identical thereto. In one embodiment, the anti-CD19 binding domain is a scFv described in WO2012/079000, or a sequence at least 95%, e.g., 95-99%, identical thereto.

In embodiments, a first CAR-expressing cell is administered to a subject, and a second CAR-expressing cell is administered to the subject. In embodiments, the first CAR-expressing cell comprises a CAR (e.g., BCMA or CD19 CAR) comprising a CD27 costimulatory domain and a CD3zeta (mutant or wild type) primary signaling domain. In embodiments, the second CAR-expressing cell comprises a CAR (e.g., BCMA CAR) comprising a 4-1BB costimulatory domain and a CD3zeta (mutant or wild type) primary signaling domain. Without wishing to be bound by theory, in embodiments, the first CAR-expressing cell can be less toxic than the second CAR-expressing cell and be used to debulk a tumor.

In one embodiment, a CAR-expressing cell described herein can be used in combination with a chemotherapeutic agent. Exemplary chemotherapeutic agents include an anthracycline (e.g., doxorubicin (e.g., liposomal doxorubicin)), a vinca alkaloid (e.g., vinblastine, vincristine, vindesine, vinorelbine), an alkylating agent (e.g., cyclophosphamide, decarbazine, melphalan, ifosfamide, temozolomide), an immune cell antibody (e.g., alemtuzamab, gemtuzumab, rituximab, tositumomab), an antimetabolite (including, e.g., folic acid antagonists, pyrimidine analogs, purine analogs and adenosine deaminase inhibitors (e.g., fludarabine)), an mTOR inhibitor, a TNFR glucocorticoid induced TNFR related protein (GITR) agonist, a proteasome inhibitor (e.g., aclacinomycin A, gliotoxin or bortezomib), an immunomodulator such as thalidomide or a thalidomide derivative (e.g., lenalidomide).

General Chemotherapeutic agents considered for use in combination therapies include anastrozole (Arimidex®), bicalutamide (Casodex®), bleomycin sulfate (Blenoxane®), busulfan (Myleran®), busulfan injection (Busulfex®), capecitabine (Xeloda®), N4-pentoxycarbonyl-5-deoxy-5-fluorocytidine, carboplatin (Paraplatin®), carmustine (BiCNU®), chlorambucil (Leukeran®), cisplatin (Platinol®), cladribine (Leustatin®), cyclophosphamide (Cytoxan® or Neosar®), cytarabine, cytosine

arabinoside (Cytosar-U®), cytarabine liposome injection (DepoCyt®), dacarbazine (DTIC-Dome®), dactinomycin (Actinomycin D, Cosmegan), daunorubicin hydrochloride (Cerubidine®), daunorubicin citrate liposome injection (DaunoXome®), dexamethasone, docetaxel (Taxotere®), doxorubicin hydrochloride (Adriamycin®, Rubex®), etoposide (Vepesid®), fludarabine phosphate (Fludara®), 5-fluorouracil (Adrucil®, Efudex®), flutamide (Eulexin®), tezacitibine, Gemcitabine (difluorodeoxycitidine), hydroxyurea (Hydrea®), Idarubicin (Idamycin®), ifosfamide (IFEX®), irinotecan (Camptosar®), L-asparaginase (ELSPAR®), leucovorin calcium, melphalan (Alkeran®), 6-mercaptopurine (Purinethol®), methotrexate (Folex®), mitoxantrone (Novantrone®), mylotarg, paclitaxel (Taxol®), phoenix (Yttrium90/MX-DTPA), pentostatin, polifeprosan 20 with carmustine implant (Gliadel®), tamoxifen citrate (Nolvadex®), teniposide (Vumon®), 6-thioguanine, thiotepa, tirapazamine (Tirazone®), topotecan hydrochloride for injection (Hycamptin®), vinblastine (Velban®), vincristine (Oncovin®), and vinorelbine (Navelbine®).

Anti-cancer agents of particular interest for combinations with the compounds of the present invention include: anthracyclines; alkylating agents; antimetabolites; drugs that inhibit either the calcium dependent phosphatase calcineurin or the p70S6 kinase FK506) or inhibit the p70S6 kinase; mTOR inhibitors; immunomodulators; anthracyclines; vinca alkaloids; proteosome inhibitors; GITR agonists; protein tyrosine phosphatase inhibitors; a CDK4 kinase inhibitor; a BTK inhibitor; a MKN kinase inhibitor; a DGK kinase inhibitor; or an oncolytic virus.

Exemplary alkylating agents include, without limitation, nitrogen mustards, ethylenimine derivatives, alkyl sulfonates, nitrosoureas and triazenes): uracil mustard (Aminouracil Mustard®, Chlorethaminacil®, Demethyldopan®, Desmethyldopan®, Haemanthamine®, Nordopan®, Uracil nitrogen mustard®, Uracillost®, Uracilmostaza®, Uramustin®, Uramustine®), chlormethine (Mustargen®), cyclophosphamide (Cytoxan®, Neosar®, Clafen®, Endoxan®, Procytox®, Revimmune™), ifosfamide (Mitoxana®), melphalan (Alkeran®), Chlorambucil (Leukeran®), pipobroman (Amedel®, Vercyte®), triethylenemelamine (Hemel®, Hexalen®, Hexastat®), triethylenethiophosphoramine, Temozolomide (Temodar®), thiotepa (Thioplex®), busulfan (Busilvex®, Myleran®), carmustine (BiCNU®), lomustine (CeeNU®), streptozocin (Zanosar®), and Dacarbazine (DTIC-Dome®). Additional exemplary alkylating agents include, without limitation, Oxaliplatin (Eloxatin®); Temozolomide (Temodar® and Temodal®); Dactinomycin (also known as actinomycin-D, Cosmegen®); Melphalan (also known as L-PAM, L-sarcolysin, and phenylalanine mustard, Alkeran®); Altretamine (also known as hexamethylmelamine (HMM), Hexalen®); Carmustine (BiCNU®); Bendamustine (Treanda®); Busulfan (Busulfex® and Myleran®); Carboplatin (Paraplatin®); Lomustine (also known as CCNU, CeeNU®); Cisplatin (also known as CDDP, Platinol® and Platinol®-AQ); Chlorambucil (Leukeran®); Cyclophosphamide (Cytoxan® and

Neosar®); Dacarbazine (also known as DTIC, DIC and imidazole carboxamide, DTIC-Dome®); Altretamine (also known as hexamethylmelamine (HMM), Hexalen®); Ifosfamide (Ifex®); Prednumustine; Procarbazine (Matulane®); Mechlorethamine (also known as nitrogen mustard, mustine and mechloroethamine hydrochloride, Mustargen®); Streptozocin (Zanosar®); Thiotepa (also known as thiophosphoamide, TESPA and TSPA, Thioplex®); Cyclophosphamide (Endoxan®, Cytoxan®, Neosar®, Procytox®, Revimmune®); and Bendamustine HCl (Treanda®).

Exemplary mTOR inhibitors include, e.g., temsirolimus; ridaforolimus (formally known as deferolimus, (1*R*,2*R*,4*S*)-4-[(2*R*)-2 [(1*R*,9*S*,12*S*,15*R*,16*E*,18*R*,19*R*,21*R*, 23*S*,24*E*,26*E*,28*Z*,30*S*,32*S*,35*R*)-1,18-dihydroxy-19,30-dimethoxy-15,17,21,23, 29,35-hexamethyl-2,3,10,14,20-pentaoxo-11,36-dioxa-4-azatricyclo[30.3.1.$0^{4,9}$] hexatriaconta-16,24,26,28-tetraen-12-yl]propyl]-2-methoxycyclohexyl dimethylphosphinate, also known as AP23573 and MK8669, and described in PCT Publication No. WO 03/064383); everolimus (Afinitor® or RAD001); rapamycin (AY22989, Sirolimus®); simapimod (CAS 164301-51-3); emsirolimus, (5-{2,4-Bis[(3*S*)-3-methylmorpholin-4-yl]pyrido[2,3-*d*]pyrimidin-7-yl}-2-methoxyphenyl)methanol (AZD8055); 2-Amino-8-[*trans*-4-(2-hydroxyethoxy)cyclohexyl]-6-(6-methoxy-3-pyridinyl)-4-methyl-pyrido[2,3-*d*]pyrimidin-7(8*H*)-one (PF04691502, CAS 1013101-36-4); and N^2-[1,4-dioxo-4-[[4-(4-oxo-8-phenyl-4*H*-1-benzopyran-2-yl)morpholinium-4-yl]methoxy]butyl]-L-arginylglycyl-L-α-aspartylL-serine- (SEQ ID NO: 383), inner salt (SF1126, CAS 936487-67-1), and XL765.

Exemplary immunomodulators include, e.g., afutuzumab (available from Roche®); pegfilgrastim (Neulasta®); lenalidomide (CC-5013, Revlimid®); thalidomide (Thalomid®), actimid (CC4047); and IRX-2 (mixture of human cytokines including interleukin 1, interleukin 2, and interferon γ, CAS 951209-71-5, available from IRX Therapeutics).

Exemplary anthracyclines include, e.g., doxorubicin (Adriamycin® and Rubex®); bleomycin (lenoxane®); daunorubicin (dauorubicin hydrochloride, daunomycin, and rubidomycin hydrochloride, Cerubidine®); daunorubicin liposomal (daunorubicin citrate liposome, DaunoXome®); mitoxantrone (DHAD, Novantrone®); epirubicin (Ellence™); idarubicin (Idamycin®, Idamycin PFS®); mitomycin C (Mutamycin®); geldanamycin; herbimycin; ravidomycin; and desacetylravidomycin.

Exemplary vinca alkaloids include, e.g., vinorelbine tartrate (Navelbine®), Vincristine (Oncovin®), and Vindesine (Eldisine®)); vinblastine (also known as vinblastine sulfate, vincaleukoblastine and VLB, Alkaban-AQ® and Velban®); and vinorelbine (Navelbine®).

Exemplary proteosome inhibitors include bortezomib (Velcade®); carfilzomib (PX-171-007, (*S*)-4-Methyl-*N*-((*S*)-1-(((*S*)-4-methyl-1-((*R*)-2-methyloxiran-2-yl)-1-oxopentan-2-yl)amino)-1-oxo-3-phenylpropan-2-yl)-2-((*S*)-2-(2-morpholinoacetamido)-4-phenylbutanamido)-pentanamide); marizomib

(NPI-0052); ixazomib citrate (MLN-9708); delanzomib (CEP-18770); and *O*-Methyl-*N*-[(2-methyl-5-thiazolyl)carbonyl]-L-seryl-*O*-methyl-*N*-[(1*S*)-2-[(2*R*)-2-methyl-2-oxiranyl]-2-oxo-1-(phenylmethyl)ethyl]- L-serinamide (ONX-0912).

In embodiments, a CAR-expressing cell described herein is administered to a subject in combination with fludarabine, cyclophosphamide, and/or rituximab. In embodiments, a CAR-expressing cell described herein is administered to a subject in combination with fludarabine, cyclophosphamide, and rituximab (FCR). In embodiments, the subject has CLL. For example, the subject has a deletion in the short arm of chromosome 17 (del(17p), e.g., in a leukemic cell). In other examples, the subject does not have a del(17p). In embodiments, the subject comprises a leukemic cell comprising a mutation in the immunoglobulin heavy-chain variable-region (IgV_H) gene. In other embodiments, the subject does not comprise a leukemic cell comprising a mutation in the immunoglobulin heavy-chain variable-region (IgV_H) gene. In embodiments, the fludarabine is administered at a dosage of about 10-50 mg/m^2 (e.g., about 10-15, 15-20, 20-25, 25-30, 30-35, 35-40, 40-45, or 45-50 mg/m^2), e.g., intravenously. In embodiments, the cyclophosphamide is administered at a dosage of about 200-300 mg/m^2 (e.g., about 200-225, 225-250, 250-275, or 275-300 mg/m^2), e.g., intravenously. In embodiments, the rituximab is administered at a dosage of about 400-600 mg/m2 (e.g., 400-450, 450-500, 500-550, or 550-600 mg/m^2), e.g., intravenously.

In embodiments, a CAR-expressing cell described herein is administered to a subject in combination with bendamustine and rituximab. In embodiments, the subject has CLL. For example, the subject has a deletion in the short arm of chromosome 17 (del(17p), e.g., in a leukemic cell). In other examples, the subject does not have a del(17p). In embodiments, the subject comprises a leukemic cell comprising a mutation in the immunoglobulin heavy-chain variable-region (IgV_H) gene. In other embodiments, the subject does not comprise a leukemic cell comprising a mutation in the immunoglobulin heavy-chain variable-region (IgV_H) gene. In embodiments, the bendamustine is administered at a dosage of about 70-110 mg/m2 (e.g., 70-80, 80-90, 90-100, or 100-110 mg/m2), e.g., intravenously. In embodiments, the rituximab is administered at a dosage of about 400-600 mg/m2 (e.g., 400-450, 450-500, 500-550, or 550-600 mg/m^2), e.g., intravenously.

In embodiments, a CAR-expressing cell described herein is administered to a subject in combination with rituximab, cyclophosphamide, doxorubicine, vincristine, and/or a corticosteroid (e.g., prednisone). In embodiments, a CAR-expressing cell described herein is administered to a subject in combination with rituximab, cyclophosphamide, doxorubicine, vincristine, and prednisone (R-CHOP). In embodiments, the subject has diffuse large B-cell lymphoma (DLBCL). In embodiments, the subject has nonbulky limited-stage DLBCL (e.g., comprises a tumor having a size/diameter of less than 7 cm). In embodiments, the subject is treated with radiation in combination with the R-CHOP. For example,

the subject is administered R-CHOP (e.g., 1-6 cycles, e.g., 1, 2, 3, 4, 5, or 6 cycles of R-CHOP), followed by radiation. In some cases, the subject is administered R-CHOP (e.g., 1-6 cycles, e.g., 1, 2, 3, 4, 5, or 6 cycles of R-CHOP) following radiation.

In embodiments, a CAR-expressing cell described herein is administered to a subject in combination with etoposide, prednisone, vincristine, cyclophosphamide, doxorubicin, and/or rituximab. In embodiments, a CAR-expressing cell described herein is administered to a subject in combination with etoposide, prednisone, vincristine, cyclophosphamide, doxorubicin, and rituximab (EPOCH-R). In embodiments, a CAR-expressing cell described herein is administered to a subject in combination with dose-adjusted EPOCH-R (DA-EPOCH-R). In embodiments, the subject has a B cell lymphoma, e.g., a Myc-rearranged aggressive B cell lymphoma.

In embodiments, a CAR-expressing cell described herein is administered to a subject in combination with rituximab and/or lenalidomide. Lenalidomide ((*RS*)-3-(4-Amino-1-oxo 1,3-dihydro-2*H*-isoindol- 2-yl)piperidine-2,6-dione) is an immunomodulator. In embodiments, a CAR-expressing cell described herein is administered to a subject in combination with rituximab and lenalidomide. In embodiments, the subject has follicular lymphoma (FL) or mantle cell lymphoma (MCL). In embodiments, the subject has FL and has not previously been treated with a cancer therapy. In embodiments, lenalidomide is administered at a dosage of about 10-20 mg (e.g., 10-15 or 15-20 mg), e.g., daily. In embodiments, rituximab is administered at a dosage of about 350-550 mg/m^2 (e.g., 350-375, 375-400, 400-425, 425-450, 450-475, or 475-500 mg/m^2), e.g., intravenously.

In embodiments, a CAR-expressing cell described herein is administered to a subject in combination with brentuximab. Brentuximab is an antibody-drug conjugate of anti-CD30 antibody and monomethyl auristatin E. In embodiments, the subject has Hodgkin's lymphoma (HL), e.g., relapsed or refractory HL. In embodiments, the subject comprises CD30+ HL. In embodiments, the subject has undergone an autologous stem cell transplant (ASCT). In embodiments, the subject has not undergone an ASCT. In embodiments, brentuximab is administered at a dosage of about 1-3 mg/kg (e.g., about 1-1.5, 1.5-2, 2-2.5, or 2.5-3 mg/kg), e.g., intravenously, e.g., every 3 weeks.

In embodiments, a CAR-expressing cell described herein is administered to a subject in combination with brentuximab and dacarbazine or in combination with brentuximab and bendamustine. Dacarbazine is an alkylating agent with a chemical name of 5-(3,3-Dimethyl-1-triazenyl)imidazole-4-carboxamide. Bendamustine is an alkylating agent with a chemical name of 4-[5-[Bis(2-chloroethyl)amino]-1-methylbenzimidazol-2-yl]butanoic acid. In embodiments, the subject has Hodgkin's lymphoma (HL). In embodiments, the subject has not previously been treated with a cancer therapy. In embodiments, the subject is at least 60 years of age, e.g., 60, 65, 70, 75, 80, 85, or older. In

embodiments, dacarbazine is administered at a dosage of about 300-450 mg/m^2 (e.g., about 300-325, 325-350, 350-375, 375-400, 400-425, or 425-450 mg/m^2), e.g., intravenously. In embodiments, bendamustine is administered at a dosage of about 75-125 mg/m2 (e.g., 75-100 or 100-125 mg/m^2, e.g., about 90 mg/m^2), e.g., intravenously. In embodiments, brentuximab is administered at a dosage of about 1-3 mg/kg (e.g., about 1-1.5, 1.5-2, 2-2.5, or 2.5-3 mg/kg), e.g., intravenously, e.g., every 3 weeks.

In some embodiments, a CAR-expressing cell described herein is administered to a subject in combination with a CD20 inhibitor, e.g., an anti-CD20 antibody (e.g., an anti-CD20 mono- or bispecific antibody) or a fragment thereof. Exemplary anti-CD20 antibodies include but are not limited to rituximab, ofatumumab, ocrelizumab, veltuzumab, obinutuzumab, TRU-015 (Trubion Pharmaceuticals), ocaratuzumab, and Pro131921 (Genentech). See, e.g., Lim et al. Haematologica. 95.1(2010):135-43.

In some embodiments, the anti-CD20 antibody comprises rituximab. Rituximab is a chimeric mouse/human monoclonal antibody IgG1 kappa that binds to CD20 and causes cytolysis of a CD20 expressing cell, e.g., as described in www.accessdata.fda.gov/drugsatfda_docs/label/2010/103705s5311lbl.pdf. In embodiments, a CAR-expressing cell described herein is administered to a subject in combination with rituximab. In embodiments, the subject has CLL or SLL.

In some embodiments, rituximab is administered intravenously, e.g., as an intravenous infusion. For example, each infusion provides about 500-2000 mg (e.g., about 500-550, 550-600, 600-650, 650-700, 700-750, 750-800, 800-850, 850-900, 900-950, 950-1000, 1000-1100, 1100-1200, 1200-1300, 1300-1400, 1400-1500, 1500-1600, 1600-1700, 1700-1800, 1800-1900, or 1900-2000 mg) of rituximab. In some embodiments, rituximab is administered at a dose of 150 mg/m^2 to 750 mg/m^2, e.g., about 150-175 mg/m^2, 175-200 mg/m^2, 200-225 mg/m^2, 225-250 mg/m^2, 250-300 mg/m^2, 300-325 mg/m^2, 325-350 mg/m^2, 350-375 mg/m^2, 375-400 mg/m^2, 400-425 mg/m^2, 425-450 mg/m^2, 450-475 mg/m^2, 475-500 mg/m^2, 500-525 mg/m^2, 525-550 mg/m^2, 550-575 mg/m^2, 575-600 mg/m^2, 600-625 mg/m^2, 625-650 mg/m^2, 650-675 mg/m^2, or 675-700 mg/m^2, where m^2 indicates the body surface area of the subject. In some embodiments, rituximab is administered at a dosing interval of at least 4 days, e.g., 4, 7, 14, 21, 28, 35 days, or more. For example, rituximab is administered at a dosing interval of at least 0.5 weeks, e.g., 0.5, 1, 2, 3, 4, 5, 6, 7, 8 weeks, or more. In some embodiments, rituximab is administered at a dose and dosing interval described herein for a period of time, e.g., at least 2 weeks, e.g., at least 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20 weeks, or greater. For example, rituximab is administered at a dose and dosing interval described herein for a total of at least 4 doses per treatment cycle (e.g., at least 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, or more doses per treatment cycle).

In some embodiments, the anti-CD20 antibody comprises ofatumumab. Ofatumumab is an anti-CD20 IgG1κ human monoclonal antibody with a molecular weight of approximately 149 kDa. For example, ofatumumab is generated using transgenic mouse and hybridoma technology and is expressed and purified from a recombinant murine cell line (NS0). See, e.g., www.accessdata.fda.gov/drugsatfda_docs/label/2009/125326lbl.pdf; and Clinical Trial Identifier number NCT01363128, NCT01515176, NCT01626352, and NCT01397591. In embodiments, a CAR-expressing cell described herein is administered to a subject in combination with ofatumumab. In embodiments, the subject has CLL or SLL.

In some embodiments, ofatumumab is administered as an intravenous infusion. For example, each infusion provides about 150-3000 mg (e.g., about 150-200, 200-250, 250-300, 300-350, 350-400, 400-450, 450-500, 500-550, 550-600, 600-650, 650-700, 700-750, 750-800, 800-850, 850-900, 900-950, 950-1000, 1000-1200, 1200-1400, 1400-1600, 1600-1800, 1800-2000, 2000-2200, 2200-2400, 2400-2600, 2600-2800, or 2800-3000 mg) of ofatumumab. In embodiments, ofatumumab is administered at a starting dosage of about 300 mg, followed by 2000 mg, e.g., for about 11 doses, e.g., for 24 weeks. In some embodiments, ofatumumab is administered at a dosing interval of at least 4 days, e.g., 4, 7, 14, 21, 28, 35 days, or more. For example, ofatumumab is administered at a dosing interval of at least 1 week, e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 24, 26, 28, 20, 22, 24, 26, 28, 30 weeks, or more. In some embodiments, ofatumumab is administered at a dose and dosing interval described herein for a period of time, e.g., at least 1 week, e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 22, 24, 26, 28, 30, 40, 50, 60 weeks or greater, or 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12 months or greater, or 1, 2, 3, 4, 5 years or greater. For example, ofatumumab is administered at a dose and dosing interval described herein for a total of at least 2 doses per treatment cycle (e.g., at least 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 18, 20, or more doses per treatment cycle).

In some cases, the anti-CD20 antibody comprises ocrelizumab. Ocrelizumab is a humanized anti-CD20 monoclonal antibody, e.g., as described in Clinical Trials Identifier Nos. NCT00077870, NCT01412333, NCT00779220, NCT00673920, NCT01194570, and Kappos et al. Lancet. 19.378(2011):1779-87.

In some cases, the anti-CD20 antibody comprises veltuzumab. Veltuzumab is a humanized monoclonal antibody against CD20. See, e.g., Clinical Trial Identifier No. NCT00547066, NCT00546793, NCT01101581, and Goldenberg et al. Leuk Lymphoma. 51(5)(2010):747-55.

In some cases, the anti-CD20 antibody comprises GA101. GA101 (also called obinutuzumab or RO5072759) is a humanized and glyco-engineered anti-CD20 monoclonal antibody. See, e.g., Robak. Curr. Opin. Investig. Drugs. 10.6(2009):588-96; Clinical Trial Identifier Numbers: NCT01995669,

NCT01889797, NCT02229422, and NCT01414205; and www.accessdata.fda.gov/drugsatfda_docs/label/2013/125486s000lbl.pdf.

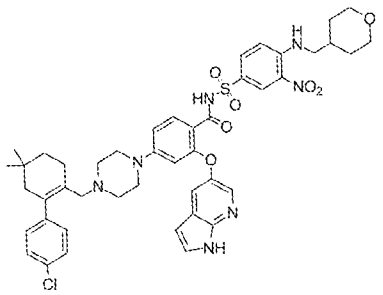
In some cases, the anti-CD20 antibody comprises AME-133v. AME-133v (also called LY2469298 or ocaratuzumab) is a humanized IgG1 monoclonal antibody against CD20 with increased affinity for the FcγRIIIa receptor and an enhanced antibody dependent cellular cytotoxicity (ADCC) activity compared with rituximab. See, e.g., Robak et al. BioDrugs 25.1(2011):13-25; and Forero-Torres et al. Clin Cancer Res. 18.5(2012):1395-403.

In some cases, the anti-CD20 antibody comprises PRO131921. PRO131921 is a humanized anti-CD20 monoclonal antibody engineered to have better binding to FcγRIIIa and enhanced ADCC compared with rituximab. See, e.g., Robak et al. BioDrugs 25.1(2011):13-25; and Casulo et al. Clin Immunol. 154.1(2014):37-46; and Clinical Trial Identifier No. NCT00452127.

In some cases, the anti-CD20 antibody comprises TRU-015. TRU-015 is an anti-CD20 fusion protein derived from domains of an antibody against CD20. TRU-015 is smaller than monoclonal antibodies, but retains Fc-mediated effector functions. See, e.g., Robak et al. BioDrugs 25.1(2011):13-25. TRU-015 contains an anti-CD20 single-chain variable fragment (scFv) linked to human IgG1 hinge, CH2, and CH3 domains but lacks CH1 and CL domains.

In some embodiments, an anti-CD20 antibody described herein is conjugated or otherwise bound to a therapeutic agent, e.g., a chemotherapeutic agent (e.g., cytoxan, fludarabine, histone deacetylase inhibitor, demethylating agent, peptide vaccine, anti-tumor antibiotic, tyrosine kinase inhibitor, alkylating agent, anti-microtubule or anti-mitotic agent), anti-allergic agent, anti-nausea agent (or anti-emetic), pain reliever, or cytoprotective agent described herein.

In embodiments, a CAR-expressing cell described herein is administered to a subject in combination with a B-cell lymphoma 2 (BCL-2) inhibitor (e.g., venetoclax, also called ABT-199 or GDC-0199;) and/or rituximab. In embodiments, a CAR-expressing cell described herein is administered to a subject in combination with venetoclax and rituximab. Venetoclax is a small molecule that inhibits the anti-apoptotic protein, BCL-2. The structure of venetoclax (4-(4-{[2-(4-chlorophenyl)-4,4-dimethylcyclohex-1-en-1-yl]methyl}piperazin-1-yl)-*N*-({3-nitro-4-[(tetrahydro-2*H*-pyran-4-ylmethyl)amino]phenyl}sulfonyl)-2-(1*H*-pyrrolo[2,3-*b*]pyridin-5-yloxy)benzamide) is shown below.



In embodiments, the subject has CLL. In embodiments, the subject has relapsed CLL, e.g., the subject has previously been administered a cancer therapy. In embodiments, venetoclax is administered at a dosage of about 15-600 mg (e.g., 15-20, 20-50, 50-75, 75-100, 100-200, 200-300, 300-400, 400-500, or 500-600 mg), e.g., daily. In embodiments, rituximab is administered at a dosage of about 350-550 mg/m2 (e.g., 350-375, 375-400, 400-425, 425-450, 450-475, or 475-500 mg/m2), e.g., intravenously, e.g., monthly.

Without being bound by theory, it is believed that in some cancers, B cells (e.g., B regulatory cells) can suppress T cells. Further, it is believed that a combination of oxiplatin and the B cell depleting agent may reduce tumor size and/or eliminate tumors in a subject. In some embodiments, a CAR-expressing cell described herein (e.g., BCMA CAR) is administered in combination with a B cell depleting agent (e.g., a CD19 CAR-expressing cell, a CD20 CAR-expressing cell, rituximab, ocrelizumab, epratuzumab, or belimumab) and oxiplatin. In embodiments, the cancer cell can be CD19 negative or CD19 positive; or BCMA negative or BMCA positive. In embodiments, a CAR-expressing cell described herein (e.g., BCMA CAR) is administered in combination with a B cell depleting agent and oxiplatin to treat a cancer, e.g., a cancer described herein, e.g., solid cancer, e.g., prostate cancer, pancreatic cancer, or lung cancer.

In embodiments, a CAR-expressing cell described herein (e.g., BCMA CAR) may deplete B cells (e.g., B cells having a plasma cell-like phenotype, e.g., that express BCMA, CD19, and/or CD20) in a subject. In embodiments, the B cell can be CD19 negative or CD19 positive; or BCMA negative or BMCA positive. In some embodiments, a CAR-expressing cell described herein (e.g., BCMA CAR) is administered in combination with oxiplatin. In embodiments, a CAR-expressing cell described herein is administered in combination with oxiplatin is used to treat a cancer, e.g., solid cancer, e.g., prostate cancer, pancreatic cancer, or lung cancer.In some embodiments, a CAR-expressing cell described herein is administered in combination with an oncolytic virus. In embodiments, oncolytic viruses are capable of selectively replicating in and triggering the death of or slowing the growth of a cancer cell. In some cases, oncolytic viruses have no effect or a minimal effect on non-cancer cells. An oncolytic virus includes but is not limited to an oncolytic adenovirus, oncolytic Herpes Simplex Viruses, oncolytic retrovirus, oncolytic parvovirus, oncolytic vaccinia virus, oncolytic Sinbis virus, oncolytic influenza

virus, or oncolytic RNA virus (e.g., oncolytic reovirus, oncolytic Newcastle Disease Virus (NDV), oncolytic measles virus, or oncolytic vesicular stomatitis virus (VSV)).

In some embodiments, the oncolytic virus is a virus, e.g., recombinant oncolytic virus, described in US2010/0178684 A1, which is incorporated herein by reference in its entirety. In some embodiments, a recombinant oncolytic virus comprises a nucleic acid sequence (e.g., heterologous nucleic acid sequence) encoding an inhibitor of an immune or inflammatory response, e.g., as described in US2010/0178684 A1, incorporated herein by reference in its entirety. In embodiments, the recombinant oncolytic virus, e.g., oncolytic NDV, comprises a pro-apoptotic protein (e.g., apoptin), a cytokine (e.g., GM-CSF, interferon-gamma, interleukin-2 (IL-2), tumor necrosis factor-alpha), an immunoglobulin (e.g., an antibody against ED-B firbonectin), tumor associated antigen, a bispecific adapter protein (e.g., bispecific antibody or antibody fragment directed against NDV HN protein and a T cell co-stimulatory receptor, such as CD3 or CD28; or fusion protein between human IL-2 and single chain antibody directed against NDV HN protein). See, e.g., Zamarin et al. Future Microbiol. 7.3(2012):347-67, incorporated herein by reference in its entirety. In some embodiments, the oncolytic virus is a chimeric oncolytic NDV described in US 8591881 B2, US 2012/0122185 A1, or US 2014/0271677 A1, each of which is incorporated herein by reference in their entireties.

In some embodiments, the oncolytic virus comprises a conditionally replicative adenovirus (CRAd), which is designed to replicate exclusively in cancer cells. See, e.g., Alemany et al. Nature Biotechnol. 18(2000):723-27. In some embodiments, an oncolytic adenovirus comprises one described in Table 1 on page 725 of Alemany et al., incorporated herein by reference in its entirety.

Exemplary oncolytic viruses include but are not limited to the following:

Group B Oncolytic Adenovirus (ColoAd1) (PsiOxus Therapeutics Ltd.) (see, e.g., Clinical Trial Identifier: NCT02053220);

ONCOS-102 (previously called CGTG-102), which is an adenovirus comprising granulocyte-macrophage colony stimulating factor (GM-CSF) (Oncos Therapeutics) (see, e.g., Clinical Trial Identifier: NCT01598129);

VCN-01, which is a genetically modified oncolytic human adenovirus encoding human PH20 hyaluronidase (VCN Biosciences, S.L.) (see, e.g., Clinical Trial Identifiers: NCT02045602 and NCT02045589);

Conditionally Replicative Adenovirus ICOVIR-5, which is a virus derived from wild-type human adenovirus serotype 5 (Had5) that has been modified to selectively replicate in cancer cells with a deregulated retinoblastoma/E2F pathway (Institut Català d'Oncologia) (see, e.g., Clinical Trial Identifier: NCT01864759);

Celyvir, which comprises bone marrow-derived autologous mesenchymal stem cells (MSCs) infected with ICOVIR5, an oncolytic adenovirus (Hospital Infantil Universitario Niño Jesús, Madrid, Spain/ Ramon Alemany) (see, e.g., Clinical Trial Identifier: NCT01844661);

CG0070, which is a conditionally replicating oncolytic serotype 5 adenovirus (Ad5) in which human E2F-1 promoter drives expression of the essential E1a viral genes, thereby restricting viral replication and cytotoxicity to Rb pathway-defective tumor cells (Cold Genesys, Inc.) (see, e.g., Clinical Trial Identifier: NCT02143804); or

DNX-2401 (formerly named Delta-24-RGD), which is an adenovirus that has been engineered to replicate selectively in retinoblastoma (Rb)-pathway deficient cells and to infect cells that express certain RGD-binding integrins more efficiently (Clinica Universidad de Navarra, Universidad de Navarra/ DNAtrix, Inc.) (see, e.g., Clinical Trial Identifier: NCT01956734).

In some embodiments, an oncolytic virus described herein is administering by injection, e.g., subcutaneous, intra-arterial, intravenous, intramuscular, intrathecal, or intraperitoneal injection. In embodiments, an oncolytic virus described herein is administered intratumorally, transdermally, transmucosally, orally, intranasally, or via pulmonary administration.

In an embodiment, cells expressing a CAR described herein are administered to a subject in combination with a molecule that decreases the Treg cell population. Methods that decrease the number of (e.g., deplete) Treg cells are known in the art and include, e.g., CD25 depletion, cyclophosphamide administration, modulating GITR function. Without wishing to be bound by theory, it is believed that reducing the number of Treg cells in a subject prior to apheresis or prior to administration of a CAR-expressing cell described herein reduces the number of unwanted immune cells (e.g., Tregs) in the tumor microenvironment and reduces the subject's risk of relapse. In one embodiment, a CAR expressing cell described herein is administered to a subject in combination with a a molecule targeting GITR and/or modulating GITR functions, such as a GITR agonist and/or a GITR antibody that depletes regulatory T cells (Tregs). In embodiments, cells expressing a CAR described herein are administered to a subject in combination with cyclophosphamide. In one embodiment, the GITR binding molecules and/or molecules modulating GITR functions (e.g., GITR agonist and/or Treg depleting GITR antibodies) are administered prior to administration of the CAR-expressing cell. For example, in one embodiment, the GITR agonist can be administered prior to apheresis of the cells. In embodiments, cyclophosphamide is administered to the subject prior to administration (e.g., infusion or re-infusion) of the CAR-expressing cell or prior to aphersis of the cells. In embodiments, cyclophosphamide and an anti-GITR antibody are administered to the subject prior to administration (e.g., infusion or re-infusion) of the CAR-expressing cell or prior to apheresis of the cells. In one embodiment, the subject has cancer (e.g., a solid cancer or a hematological cancer such as multiple myeloma, ALL or CLL). In an

embodiment, the subject has CLL. In embodiments, the subject has multiple myeloma. In embodiments, the subject has a solid cancer, e.g., a solid cancer described herein. Exemplary GITR agonists include, e.g., GITR fusion proteins and anti-GITR antibodies (e.g., bivalent anti-GITR antibodies) such as, e.g., a GITR fusion protein described in U.S. Patent No.: 6,111,090, European Patent No.: 090505B1, U.S Patent No.: 8,586,023, PCT Publication Nos.: WO 2010/003118 and 2011/090754, or an anti-GITR antibody described, e.g., in U.S. Patent No.: 7,025,962, European Patent No.: 1947183B1, U.S. Patent No.: 7,812,135, U.S. Patent No.: 8,388,967, U.S. Patent No.: 8,591,886, European Patent No.: EP 1866339, PCT Publication No.: WO 2011/028683, PCT Publication No.:WO 2013/039954, PCT Publication No.: WO2005/007190, PCT Publication No.: WO 2007/133822, PCT Publication No.: WO2005/055808, PCT Publication No.: WO 99/40196, PCT Publication No.: WO 2001/03720, PCT Publication No.: WO99/20758, PCT Publication No.: WO2006/083289, PCT Publication No.: WO 2005/115451, U.S. Patent No.: 7,618,632, and PCT Publication No.: WO 2011/051726.

In one embodiment, a CAR expressing cell described herein is administered to a subject in combination with an mTOR inhibitor, e.g., an mTOR inhibitor described herein, e.g., a rapalog such as everolimus. In one embodiment, the mTOR inhibitor is administered prior to the CAR-expressing cell. For example, in one embodiment, the mTOR inhibitor can be administered prior to apheresis of the cells.

In one embodiment, a CAR expressing cell described herein is administered to a subject in combination with a GITR agonist, e.g., a GITR agonist described herein. In one embodiment, the GITR agonist is administered prior to the CAR-expressing cell. For example, in one embodiment, the GITR agonist can be administered prior to apheresis of the cells.

In one embodiment, a CAR expressing cell described herein is administered to a subject in combination with a protein tyrosine phosphatase inhibitor, e.g., a protein tyrosine phosphatase inhibitor described herein. In one embodiment, the protein tyrosine phosphatase inhibitor is an SHP-1 inhibitor, e.g., an SHP-1 inhibitor described herein, such as, e.g., sodium stibogluconate. In one embodiment, the protein tyrosine phosphatase inhibitor is an SHP-2 inhibitor.

In one embodiment, a CAR-expressing cell described herein can be used in combination with a kinase inhibitor. In one embodiment, the kinase inhibitor is a CDK4 inhibitor, e.g., a CDK4 inhibitor described herein, e.g., a CDK4/6 inhibitor, such as, e.g., 6-Acetyl-8-cyclopentyl-5-methyl-2-(5-piperazin-1-yl-pyridin-2-ylamino)-8*H*-pyrido[2,3-*d*]pyrimidin-7-one, hydrochloride (also referred to as palbociclib or PD0332991). In one embodiment, the kinase inhibitor is a BTK inhibitor, e.g., a BTK inhibitor described herein, such as, e.g., ibrutinib. In one embodiment, the kinase inhibitor is an mTOR

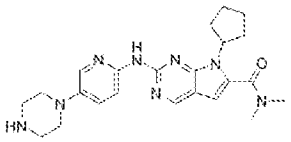
inhibitor, e.g., an mTOR inhibitor described herein, such as, e.g., rapamycin, a rapamycin analog, OSI-027. The mTOR inhibitor can be, e.g., an mTORC1 inhibitor and/or an mTORC2 inhibitor, e.g., an mTORC1 inhibitor and/or mTORC2 inhibitor described herein. In one embodiment, the kinase inhibitor is a MNK inhibitor, e.g., a MNK inhibitor described herein, such as, e.g., 4-amino-5-(4-fluoroanilino)-pyrazolo [3,4-*d*] pyrimidine. The MNK inhibitor can be, e.g., a MNK1a, MNK1b, MNK2a and/or MNK2b inhibitor. In one embodiment, the kinase inhibitor is a dual PI3K/mTOR inhibitor described herein, such as, e.g., PF-04695102. In one embodiment, the kinase inhibitor is a DGK inhibitor, e.g., a DGK inhibitor described herein, such as, e.g., DGKinh1 (D5919) or DGKinh2 (D5794).

In one embodiment, the kinase inhibitor is a CDK4 inhibitor selected from aloisine A; flavopiridol or HMR-1275, 2-(2-chlorophenyl)-5,7-dihydroxy-8-[(3S,4R)-3-hydroxy-1-methyl-4-piperidinyl]-4-chromenone; crizotinib (PF-02341066; 2-(2-Chlorophenyl)-5,7-dihydroxy-8-[(2*R*,3*S*)-2-(hydroxymethyl)-1-methyl-3-pyrrolidinyl]- 4*H*-1-benzopyran-4-one, hydrochloride (P276-00); 1-methyl-5-[[2-[5-(trifluoromethyl)-1*H*-imidazol-2-yl]-4-pyridinyl]oxy]-*N*-[4-(trifluoromethyl)phenyl]-1*H*-benzimidazol-2-amine (RAF265); indisulam (E7070); roscovitine (CYC202); palbociclib (PD0332991); dinaciclib (SCH727965); N-[5-[[(5-*tert*-butyloxazol-2-yl)methyl]thio]thiazol-2-yl]piperidine-4-carboxamide (BMS 387032); 4-[[9-chloro-7-(2,6-difluorophenyl)-5*H*-pyrimido[5,4-*d*][2]benzazepin-2-yl]amino]-benzoic acid (MLN8054); 5-[3-(4,6-difluoro-1H-benzimidazol-2-yl)-1H-indazol-5-yl]-N-ethyl-4-methyl-3-pyridinemethanamine (AG-024322); 4-(2,6-dichlorobenzoylamino)-1H-pyrazole-3-carboxylic acid N-(piperidin-4-yl)amide (AT7519); 4-[2-methyl-1-(1-methylethyl)-1*H*-imidazol-5-yl]-*N*-[4-(methylsulfonyl)phenyl]- 2-pyrimidinamine (AZD5438); and XL281 (BMS908662).

In one embodiment, the kinase inhibitor is a CDK4 inhibitor, e.g., palbociclib (PD0332991), and the palbociclib is administered at a dose of about 50 mg, 60 mg, 70 mg, 75 mg, 80 mg, 90 mg, 100 mg, 105 mg, 110 mg, 115 mg, 120 mg, 125 mg, 130 mg, 135 mg (e.g., 75 mg, 100 mg or 125 mg) daily for a period of time, e.g., daily for 14-21 days of a 28 day cycle, or daily for 7-12 days of a 21 day cycle. In one embodiment, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12 or more cycles of palbociclib are administered.

In embodiments, a CAR-expressing cell described herein is administered to a subject in combination with a cyclin-dependent kinase (CDK) 4 or 6 inhibitor, e.g., a CDK4 inhibitor or a CDK6 inhibitor described herein. In embodiments, a CAR-expressing cell described herein is administered to a subject in combination with a CDK4/6 inhibitor (e.g., an inhibitor that targets both CDK4 and CDK6), e.g., a CDK4/6 inhibitor described herein. In an embodiment, the subject has MCL. MCL is an aggressive cancer that is poorly responsive to currently available therapies, i.e., essentially incurable. In many cases of MCL, cyclin D1 (a regulator of CDK4/6) is expressed (e.g., due to chromosomal

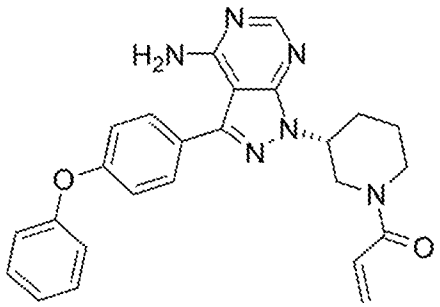
translocation involving immunoglobulin and Cyclin D1 genes) in MCL cells. Thus, without being bound by theory, it is thought that MCL cells are highly sensitive to CDK4/6 inhibition with high specificity (i.e., minimal effect on normal immune cells). CDK4/6 inhibitors alone have had some efficacy in treating MCL, but have only achieved partial remission with a high relapse rate. An exemplary CDK4/6 inhibitor is LEE011 (also called ribociclib), the structure of which is shown below.



Without being bound by theory, it is believed that administration of a CAR-expressing cell described herein with a CDK4/6 inhibitor (e.g., LEE011 or other CDK4/6 inhibitor described herein) can achieve higher responsiveness, e.g., with higher remission rates and/or lower relapse rates, e.g., compared to a CDK4/6 inhibitor alone.

In one embodiment, the kinase inhibitor is a BTK inhibitor selected from ibrutinib (PCI-32765); GDC-0834; RN-486; CGI-560; CGI-1764; HM-71224; CC-292; ONO-4059; CNX-774; and LFM-A13. In a preferred embodiment, the BTK inhibitor does not reduce or inhibit the kinase activity of interleukin-2-inducible kinase (ITK), and is selected from GDC-0834; RN-486; CGI-560; CGI-1764; HM-71224; CC-292; ONO-4059; CNX-774; and LFM-A13.

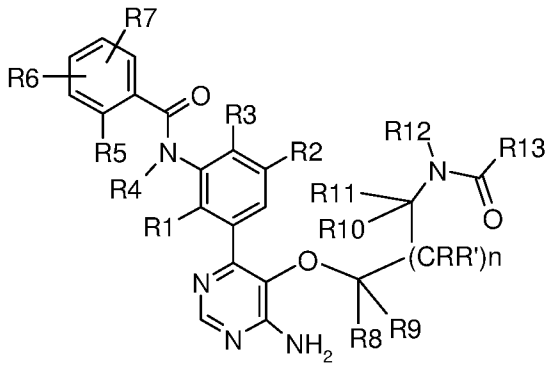
In one embodiment, the kinase inhibitor is a BTK inhibitor, e.g., ibrutinib (PCI-32765). In embodiments, a CAR-expressing cell described herein is administered to a subject in combination with a BTK inhibitor (e.g., ibrutinib). In embodiments, a CAR-expressing cell described herein is administered to a subject in combination with ibrutinib (also called PCI-32765). The structure of ibrutinib (1-[(3*R*)-3-[4-Amino-3-(4-phenoxyphenyl)-1*H*-pyrazolo[3,4-d]pyrimidin-1-yl]piperidin-1-yl]prop-2-en-1-one) is shown below.



In embodiments, the subject has CLL, mantle cell lymphoma (MCL), or small lymphocytic lymphoma (SLL). For example, the subject has a deletion in the short arm of chromosome 17 (del(17p),

e.g., in a leukemic cell). In other examples, the subject does not have a del(17p). In embodiments, the subject has relapsed CLL or SLL, e.g., the subject has previously been administered a cancer therapy (e.g., previously been administered one, two, three, or four prior cancer therapies). In embodiments, the subject has refractory CLL or SLL. In other embodiments, the subject has follicular lymphoma, e.g., relapse or refractory follicular lymphoma. In some embodiments, ibrutinib is administered at a dosage of about 300-600 mg/day (e.g., about 300-350, 350-400, 400-450, 450-500, 500-550, or 550-600 mg/day, e.g., about 420 mg/day or about 560 mg/day), e.g., orally. In embodiments, the ibrutinib is administered at a dose of about 250 mg, 300 mg, 350 mg, 400 mg, 420 mg, 440 mg, 460 mg, 480 mg, 500 mg, 520 mg, 540 mg, 560 mg, 580 mg, 600 mg (e.g., 250 mg, 420 mg or 560 mg) daily for a period of time, e.g., daily for 21 day cycle cycle, or daily for 28 day cycle. In one embodiment, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12 or more cycles of ibrutinib are administered. In some embodiments, ibrutinib is administered in combination with rituximab. See, e.g., Burger et al. (2013) Ibrutinib In Combination With Rituximab (iR) Is Well Tolerated and Induces a High Rate Of Durable Remissions In Patients With High-Risk Chronic Lymphocytic Leukemia (CLL): New, Updated Results Of a Phase II Trial In 40 Patients, Abstract 675 presented at 55th ASH Annual Meeting and Exposition, New Orleans, LA 7-10 Dec. Without being bound by theory, it is thought that the addition of ibrutinib enhances the T cell proliferative response and may shift T cells from a T-helper-2 (Th2) to T-helper-1 (Th1) phenotype. Th1 and Th2 are phenotypes of helper T cells, with Th1 versus Th2 directing different immune response pathways. A Th1 phenotype is associated with proinflammatory responses, e.g., for killing cells, such as intracellular pathogens/viruses or cancerous cells, or perpetuating autoimmune responses. A Th2 phenotype is associated with eosinophil accumulation and anti-inflammatory responses.

In some embodiments of the methods, uses, and compositions herein, the BTK inhibitor is a BTK inhibitor described in International Application WO/2015/079417, which is herein incorporated by reference in its entirety. For instance, in some embodiments, the BTK inhibitor is a compound of formula (I) or a pharmaceutically acceptable salt thereof;



(I)

wherein,

R1 is hydrogen, C1-C6 alkyl optionally substituted by hydroxy;

R2 is hydrogen or halogen;

R3 is hydrogen or halogen;

R4 is hydrogen;

R5 is hydrogen or halogen;

or R4 and R5 are attached to each other and stand for a bond, -CH2-, -CH2-CH2- , -CH=CH-, -CH=CH-CH2-; -CH2-CH=CH-; or -CH2-CH2-CH2-;

R6 and R7 stand independently from each other for H, C1-C6 alkyl optionally substituted by hydroxyl, C3-C6 cycloalkyl optionally substituted by halogen or hydroxy, or halogen;

R8, R9, R, R', R10 and R11 independently from each other stand for H, or C1-C6 alkyl optionally substituted by C1-C6 alkoxy; or any two of R8, R9, R, R', R10 and R11 together with the carbon atom to which they are bound may form a 3 – 6 membered saturated carbocyclic ring;

R12 is hydrogen or C1-C6 alkyl optionally substituted by halogen or C1-C6 alkoxy;

or R12 and any one of R8, R9, R, R', R10 or R11 together with the atoms to which they are bound may form a 4, 5, 6 or 7 membered azacyclic ring, which ring may optionally be substituted by halogen, cyano, hydroxyl, C1-C6 alkyl or C1-C6 alkoxy;

n is 0 or 1; and

R13 is C2-C6 alkenyl optionally substituted by C1-C6 alkyl, C1-C6 alkoxy or N,N-di-C1-C6 alkyl amino; C2-C6 alkynyl optionally substituted by C1-C6 alkyl or C1-C6 alkoxy; or C2-C6 alkylenyl oxide optionally substituted by C1-C6 alkyl.

In some embodiments, the BTK inhibitor of Formula I is chosen from: N-(3-(5-((1-Acryloylazetidin-3-yl)oxy)-6-aminopyrimidin-4-yl)-5-fluoro-2-methylphenyl)-4-cyclopropyl-2-fluorobenzamide; (E)-N-(3-(6-Amino-5-((1-(but-2-enoyl)azetidin-3-yl)oxy)pyrimidin-4-yl)-5-fluoro-2-methylphenyl)-4-cyclopropyl-2-fluorobenzamide; N-(3-(6-Amino-5-((1-propioloylazetidin-3-yl)oxy)pyrimidin-4-yl)-5-fluoro-2-methylphenyl)-4-cyclopropyl-2-fluorobenzamide; N-(3-(6-Amino-5-((1-(but-2-ynoyl)azetidin-3-yl)oxy)pyrimidin-4-yl)-5-fluoro-2-methylphenyl)-4-cyclopropyl-2-fluorobenzamide; N-(3-(5-((1-Acryloylpiperidin-4-yl)oxy)-6-aminopyrimidin-4-yl)-5-fluoro-2-methylphenyl)-4-cyclopropyl-2-fluorobenzamide; N-(3-(6-Amino-5-(2-(N-methylacrylamido)ethoxy)pyrimidin-4-yl)-5-fluoro-2-methylphenyl)-4-cyclopropyl-2-fluorobenzamide; (E)-N-(3-(6-Amino-5-(2-(N-methylbut-2-enamido)ethoxy)pyrimidin-4-yl)-5-fluoro-2-methylphenyl)-4-cyclopropyl-2-fluorobenzamide; N-(3-(6-Amino-5-(2-(N-methylpropiolamido)ethoxy)pyrimidin-4-yl)-5-fluoro-2-methylphenyl)-4-cyclopropyl-2-fluorobenzamide; (E)-N-(3-(6-Amino-5-(2-(4-methoxy-N-methylbut-2-enamido)ethoxy)pyrimidin-4-yl)-5-fluoro-2-methylphenyl)-4-cyclopropyl-2-fluorobenzamide; N-(3-(6-Amino-5-(2-(N-methylbut-2-ynamido)ethoxy)pyrimidin-4-yl)-5-fluoro-2-methylphenyl)-4-cyclopropyl-2-fluorobenzamide; N-(2-((4-Amino-6-(3-(4-cyclopropyl-2-

fluorobenzamido)-5-fluoro-2-methylphenyl)pyrimidin-5-yl)oxy)ethyl)-N-methyloxirane-2-carboxamide; N-(2-((4-Amino-6-(3-(6-cyclopropyl-8-fluoro-1-oxoisoquinolin-2(1H)-yl)phenyl)pyrimidin-5-yl)oxy)ethyl)-N-methylacrylamide; N-(3-(5-(2-Acrylamidoethoxy)-6-aminopyrimidin-4-yl)-5-fluoro-2-methylphenyl)-4-cyclopropyl-2-fluorobenzamide; N-(3-(6-Amino-5-(2-(N-ethylacrylamido)ethoxy)pyrimidin-4-yl)-5-fluoro-2-methylphenyl)-4-cyclopropyl-2-fluorobenzamide; N-(3-(6-Amino-5-(2-(N-(2-fluoroethyl)acrylamido)ethoxy)pyrimidin-4-yl)-5-fluoro-2-methylphenyl)-4-cyclopropyl-2-fluorobenzamide; N-(3-(5-((1-Acrylamidocyclopropyl)methoxy)-6-aminopyrimidin-4-yl)-5-fluoro-2-methylphenyl)-4-cyclopropyl-2-fluorobenzamide; (S)-N-(3-(5-(2-Acrylamidopropoxy)-6-aminopyrimidin-4-yl)-5-fluoro-2-methylphenyl)-4-cyclopropyl-2-fluorobenzamide; (S)-N-(3-(6-Amino-5-(2-(but-2-ynamido)propoxy)pyrimidin-4-yl)-5-fluoro-2-methylphenyl)-4-cyclopropyl-2-fluorobenzamide; (S)-N-(3-(6-Amino-5-(2-(N-methylacrylamido)propoxy)pyrimidin-4-yl)-5-fluoro-2-methylphenyl)-4-cyclopropyl-2-fluorobenzamide; (S)-N-(3-(6-Amino-5-(2-(N-methylbut-2-ynamido)propoxy)pyrimidin-4-yl)-5-fluoro-2-methylphenyl)-4-cyclopropyl-2-fluorobenzamide; N-(3-(6-Amino-5-(3-(N-methylacrylamido)propoxy)pyrimidin-4-yl)-5-fluoro-2-methylphenyl)-4-cyclopropyl-2-fluorobenzamide; (S)-N-(3-(5-((1-Acryloylpyrrolidin-2-yl)methoxy)-6-aminopyrimidin-4-yl)-5-fluoro-2-methylphenyl)-4-cyclopropyl-2-fluorobenzamide; (S)-N-(3-(6-Amino-5-((1-(but-2-ynoyl)pyrrolidin-2-yl)methoxy)pyrimidin-4-yl)-5-fluoro-2-methylphenyl)-4-cyclopropyl-2-fluorobenzamide; (S)-2-(3-(5-((1-Acryloylpyrrolidin-2-yl)methoxy)-6-aminopyrimidin-4-yl)-5-fluoro-2-(hydroxymethyl)phenyl)-6-cyclopropyl-3,4-dihydroisoquinolin-1(2H)-one; N-(2-((4-Amino-6-(3-(6-cyclopropyl-1-oxo-3,4-dihydroisoquinolin-2(1H)-yl)-5-fluoro-2-(hydroxymethyl)phenyl)pyrimidin-5-yl)oxy)ethyl)-N-methylacrylamide; N-(3-(5-(((2S,4R)-1-Acryloyl-4-methoxypyrrolidin-2-yl)methoxy)-6-aminopyrimidin-4-yl)-5-fluoro-2-methylphenyl)-4-cyclopropyl-2-fluorobenzamide; N-(3-(6-Amino-5-(((2S,4R)-1-(but-2-ynoyl)-4-methoxypyrrolidin-2-yl)methoxy)pyrimidin-4-yl)-5-fluoro-2-methylphenyl)-4-cyclopropyl-2-fluorobenzamide; 2-(3-(5-(((2S,4R)-1-Acryloyl-4-methoxypyrrolidin-2-yl)methoxy)-6-aminopyrimidin-4-yl)-5-fluoro-2-(hydroxymethyl)phenyl)-6-cyclopropyl-3,4-dihydroisoquinolin-1(2H)-one; N-(3-(5-(((2S,4S)-1-Acryloyl-4-methoxypyrrolidin-2-yl)methoxy)-6-aminopyrimidin-4-yl)-5-fluoro-2-methylphenyl)-4-cyclopropyl-2-fluorobenzamide; N-(3-(6-Amino-5-(((2S,4S)-1-(but-2-ynoyl)-4-methoxypyrrolidin-2-yl)methoxy)pyrimidin-4-yl)-5-fluoro-2-methylphenyl)-4-cyclopropyl-2-fluorobenzamide; N-(3-(5-(((2S,4R)-1-Acryloyl-4-fluoropyrrolidin-2-yl)methoxy)-6-aminopyrimidin-4-yl)-5-fluoro-2-methylphenyl)-4-cyclopropyl-2-fluorobenzamide; N-(3-(6-Amino-5-(((2S,4R)-1-(but-2-ynoyl)-4-fluoropyrrolidin-2-yl)methoxy)pyrimidin-4-yl)-5-fluoro-2-methylphenyl)-4-cyclopropyl-2-fluorobenzamide; (S)-N-(3-(5-((1-Acryloylazetidin-2-yl)methoxy)-6-aminopyrimidin-4-yl)-5-fluoro-2-methylphenyl)-4-cyclopropyl-2-fluorobenzamide; (S)-N-(3-(6-Amino-5-((1-propioloylazetidin-2-yl)methoxy)pyrimidin-4-yl)-5-fluoro-2-methylphenyl)-4-cyclopropyl-2-

fluorobenzamide; (S)-2-(3-(5-((1-Acryloylazetidin-2-yl)methoxy)-6-aminopyrimidin-4-yl)-5-fluoro-2-(hydroxymethyl)phenyl)-6-cyclopropyl-3,4-dihydroisoquinolin-1(2H)-one; (R)-N-(3-(5-((1-Acryloylazetidin-2-yl)methoxy)-6-aminopyrimidin-4-yl)-5-fluoro-2-methylphenyl)-4-cyclopropyl-2-fluorobenzamide; (R)-N-(3-(5-((1-Acryloylpiperidin-3-yl)methoxy)-6-aminopyrimidin-4-yl)-5-fluoro-2-methylphenyl)-4-cyclopropyl-2-fluorobenzamide; N-(3-(5-(((2R,3S)-1-Acryloyl-3-methoxypyrrolidin-2-yl)methoxy)-6-aminopyrimidin-4-yl)-5-fluoro-2-methylphenyl)-4-cyclopropyl-2-fluorobenzamide; N-(3-(5-(((2S,4R)-1-Acryloyl-4-cyanopyrrolidin-2-yl)methoxy)-6-aminopyrimidin-4-yl)-5-fluoro-2-methylphenyl)-4-cyclopropyl-2-fluorobenzamide; or N-(3-(5-(((2S,4S)-1-Acryloyl-4-cyanopyrrolidin-2-yl)methoxy)-6-aminopyrimidin-4-yl)-5-fluoro-2-methylphenyl)-4-cyclopropyl-2-fluorobenzamide.

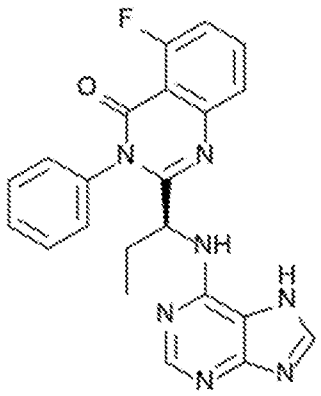
Unless otherwise provided, the chemical terms used above in describing the BTK inhibitor of Formula I are used according to their meanings as set out in International Application WO/2015/079417, which is herein incorporated by reference in its entirety.

In one embodiment, the kinase inhibitor is an mTOR inhibitor selected from temsirolimus; ridaforolimus (1*R*,2*R*,4*S*)-4-[(2*R*)-2 [(1*R*,9*S*,12*S*,15*R*,16*E*,18*R*,19*R*,21*R*, 23*S*,24*E*,26*E*,28*Z*,30*S*,32*S*,35*R*)-1,18-dihydroxy-19,30-dimethoxy-15,17,21,23, 29,35-hexamethyl-2,3,10,14,20-pentaoxo-11,36-dioxa-4-azatricyclo[30.3.1.$0^{4,9}$] hexatriaconta-16,24,26,28-tetraen-12-yl]propyl]-2-methoxycyclohexyl dimethylphosphinate, also known as AP23573 and MK8669; everolimus (RAD001); rapamycin (AY22989); simapimod; (5-{2,4-bis[(3*S*)-3-methylmorpholin-4-yl]pyrido[2,3-*d*]pyrimidin-7-yl}-2-methoxyphenyl)methanol (AZD8055); 2-amino-8-[*trans*-4-(2-hydroxyethoxy)cyclohexyl]-6-(6-methoxy-3-pyridinyl)-4-methyl-pyrido[2,3-*d*]pyrimidin-7(8*H*)-one (PF04691502); and N^2-[1,4-dioxo-4-[[4-(4-oxo-8-phenyl-4*H*-1-benzopyran-2-yl)morpholinium-4-yl]methoxy]butyl]-L-arginylglycyl-L-α-aspartylL-serine- (SEQ ID NO: 383), inner salt (SF1126); and XL765.

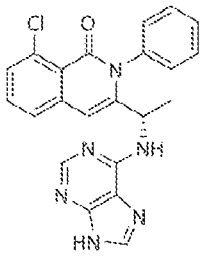
In one embodiment, the kinase inhibitor is an mTOR inhibitor, e.g., rapamycin, and the rapamycin is administered at a dose of about 3 mg, 4 mg, 5 mg, 6 mg, 7 mg, 8 mg, 9 mg, 10 mg (e.g., 6 mg) daily for a period of time, e.g., daily for 21 day cycle cycle, or daily for 28 day cycle. In one embodiment, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12 or more cycles of rapamycin are administered. In one embodiment, the kinase inhibitor is an mTOR inhibitor, e.g., everolimus and the everolimus is administered at a dose of about 2 mg, 2.5 mg, 3 mg, 4 mg, 5 mg, 6 mg, 7 mg, 8 mg, 9 mg, 10 mg, 11 mg, 12 mg, 13 mg, 14 mg, 15 mg (e.g., 10 mg) daily for a period of time, e.g., daily for 28 day cycle. In one embodiment, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12 or more cycles of everolimus are administered.

In one embodiment, the kinase inhibitor is an MNK inhibitor selected from CGP052088; 4-amino-3-(p-fluorophenylamino)-pyrazolo [3,4-*d*] pyrimidine (CGP57380); cercosporamide; ETC-1780445-2; and 4-amino-5-(4-fluoroanilino)-pyrazolo [3,4-*d*] pyrimidine.

In embodiments, a CAR-expressing cell described herein is administered to a subject in combination with a phosphoinositide 3-kinase (PI3K) inhibitor (e.g., a PI3K inhibitor described herein, e.g., idelalisib or duvelisib) and/or rituximab. In embodiments, a CAR-expressing cell described herein is administered to a subject in combination with idelalisib and rituximab. In embodiments, a CAR-expressing cell described herein is administered to a subject in combination with duvelisib and rituximab. Idelalisib (also called GS-1101 or CAL-101; Gilead) is a small molecule that blocks the delta isoform of PI3K. The structure of idelalisib (5-Fluoro-3-phenyl-2-[(1*S*)-1-(7*H*-purin-6-ylamino)propyl]-4(3*H*)-quinazolinone) is shown below.



Duvelisib (also called IPI-145; Infinity Pharmaceuticals and Abbvie) is a small molecule that blocks PI3K-δ,γ. The structure of duvelisib (8-Chloro-2-phenyl-3-[(1S)-1-(9H-purin-6-ylamino)ethyl]-1(2H)-isoquinolinone) is shown below.



In embodiments, the subject has CLL. In embodiments, the subject has relapsed CLL, e.g., the subject has previously been administered a cancer therapy (e.g., previously been administered an anti-CD20 antibody or previously been administered ibrutinib). For example, the subject has a deletion in

the short arm of chromosome 17 (del(17p), e.g., in a leukemic cell). In other examples, the subject does not have a del(17p). In embodiments, the subject comprises a leukemic cell comprising a mutation in the immunoglobulin heavy-chain variable-region (IgV_H) gene. In other embodiments, the subject does not comprise a leukemic cell comprising a mutation in the immunoglobulin heavy-chain variable-region (IgV_H) gene. In embodiments, the subject has a deletion in the long arm of chromosome 11 (del(11q)). In other embodiments, the subject does not have a del(11q). In embodiments, idelalisib is administered at a dosage of about 100-400 mg (e.g., 100-125, 125-150, 150-175, 175-200, 200-225, 225-250, 250-275, 275-300, 325-350, 350-375, or 375-400 mg), e.g., BID. In embodiments, duvelisib is administered at a dosage of about 15-100 mg (e.g., about 15-25, 25-50, 50-75, or 75-100 mg), e.g., twice a day. In embodiments, rituximab is administered at a dosage of about 350-550 mg/m^2 (e.g., 350-375, 375-400, 400-425, 425-450, 450-475, or 475-500 mg/m^2), e.g., intravenously.

In embodiments, a CAR-expressing cell described herein is administered to a subject in combination with an anaplastic lymphoma kinase (ALK) inhibitor. Exemplary ALK kinases include but are not limited to crizotinib (Pfizer), ceritinib (Novartis), alectinib (Chugai), brigatinib (also called AP26113; Ariad), entrectinib (Ignyta), PF-06463922 (Pfizer), TSR-011 (Tesaro) (see, e.g., Clinical Trial Identifier No. NCT02048488), CEP-37440 (Teva), and X-396 (Xcovery). In some embodiments, the subject has a solid cancer, e.g., a solid cancer described herein, e.g., lung cancer.

The chemical name of crizotinib is 3-[(1*R*)-1-(2,6-dichloro-3-fluorophenyl)ethoxy]-5-(1-piperidin-4-ylpyrazol-4-yl)pyridin-2-amine. The chemical name of ceritinib is 5-Chloro-*N*^{2}-[2-isopropoxy-5-methyl-4-(4-piperidinyl)phenyl]-*N*^{4}-[2-(isopropylsulfonyl)phenyl]-2,4-pyrimidinediamine. The chemical name of alectinib is 9-ethyl-6,6-dimethyl-8-(4-morpholinopiperidin-1-yl)-11-oxo-6,11-dihydro-5H-benzo[b]carbazole-3-carbonitrile. The chemical name of brigatinib is 5-Chloro-N^2-{4-[4-(dimethylamino)-1-piperidinyl]-2-methoxyphenyl}-N^4-[2-(dimethylphosphoryl)phenyl]-2,4-pyrimidinediamine. The chemical name of entrectinib is N-(5-(3,5-difluorobenzyl)-1H-indazol-3-yl)-4-(4-methylpiperazin-1-yl)-2-((tetrahydro-2H-pyran-4-yl)amino)benzamide. The chemical name of PF-06463922 is (10R)-7-Amino-12-fluoro-2,10,16-trimethyl-15-oxo-10,15,16,17-tetrahydro-2H-8,4-(metheno)pyrazolo[4,3-h][2,5,11]-benzoxadiazacyclotetradecine-3-carbonitrile. The chemical structure of CEP-37440 is (S)-2-((5-chloro-2-((6-(4-(2-hydroxyethyl)piperazin-1-yl)-1-methoxy-6,7,8,9-tetrahydro-5H-benzo[7]annulen-2-yl)amino)pyrimidin-4-yl)amino)-N-methylbenzamide. The chemical name of X-396 is (R)-6-amino-5-(1-(2,6-dichloro-3-fluorophenyl)ethoxy)-N-(4-(4-methylpiperazine-1-carbonyl)phenyl)pyridazine-3-carboxamide.

In one embodiment, the kinase inhibitor is a dual phosphatidylinositol 3-kinase (PI3K) and mTOR inhibitor selected from 2-Amino-8-[*trans*-4-(2-hydroxyethoxy)cyclohexyl]-6-(6-methoxy-3-

pyridinyl)-4-methyl-pyrido[2,3-*d*]pyrimidin-7(8*H*)-one (PF-04691502); *N*-[4-[[4-(Dimethylamino)-1-piperidinyl]carbonyl]phenyl]-*N*'-[4-(4,6-di-4-morpholinyl-1,3,5-triazin-2-yl)phenyl]urea (PF-05212384, PKI-587); 2-Methyl-2-{4-[3-methyl-2-oxo-8-(quinolin-3-yl)-2,3-dihydro-1*H*-imidazo[4,5-c]quinolin-1-yl]phenyl}propanenitrile (BEZ-235); apitolisib (GDC-0980, RG7422); 2,4-Difluoro-N-{2-(methyloxy)-5-[4-(4-pyridazinyl)-6-quinolinyl]-3-pyridinyl}benzenesulfonamide (GSK2126458); 8-(6-methoxypyridin-3-yl)-3-methyl-1-(4-(piperazin-1-yl)-3-(trifluoromethyl)phenyl)-1H-imidazo[4,5-c]quinolin-2(3H)-one Maleic acid (NVP-BGT226); 3-[4-(4-Morpholinylpyrido[3',2':4,5]furo[3,2-d]pyrimidin-2-yl]phenol (PI-103); 5-(9-isopropyl-8-methyl-2-morpholino-9H-purin-6-yl)pyrimidin-2-amine (VS-5584, SB2343); and N-[2-[(3,5-Dimethoxyphenyl)amino]quinoxalin-3-yl]-4-[(4-methyl-3-methoxyphenyl)carbonyl]aminophenylsulfonamide (XL765).

Drugs that inhibit either the calcium dependent phosphatase calcineurin (cyclosporine and FK506) or inhibit the p70S6 kinase that is important for growth factor induced signaling (rapamycin). (Liu *et al.*, Cell 66:807-815, 1991; Henderson *et al.*, Immun. 73:316-321, 1991; Bierer *et al.*, Curr. Opin. Immun. 5:763-773, 1993) can also be used. In a further aspect, the cell compositions of the present invention may be administered to a patient in conjunction with (e.g., before, simultaneously or following) bone marrow transplantation, T cell ablative therapy using chemotherapy agents such as, fludarabine, external-beam radiation therapy (XRT), cyclophosphamide, and/or antibodies such as OKT3 or CAMPATH. In one aspect, the cell compositions of the present invention are administered following B-cell ablative therapy such as agents that react with CD20, e.g., Rituxan. For example, in one embodiment, subjects may undergo standard treatment with high dose chemotherapy followed by peripheral blood stem cell transplantation. In certain embodiments, following the transplant, subjects receive an infusion of the expanded immune cells of the present invention. In an additional embodiment, expanded cells are administered before or following surgery.

In one embodiment, a CAR expressing cell described herein is administered to a subject in combination with a biphosphonate, e.g., Pamidronate (Aredia®); Zoledronic acid or Zoledronate (Zometa®, Zomera®, Aclasta®, or Reclast®); Alendronate (Fosamax®); Risedronate (Actonel®); Ibandronate (Boniva®); Clondronate (Bonefos®); Etidronate (Didronel®); Tiludronate (Skelid®); Pamidronate (Aredia®); Neridronate (Nerixia®); Strontiun ranelate (Protelos®, or Protos®); and Teriparatide (Forteo®).

In one embodiment, a CAR expressing cell described herein is administered to a subject in combination with a corticosteroid, e.g., dexamethasone (e.g., Decadron®), beclomethasone (e.g., Beclovent®), hydrocortisone (also known as cortisone, hydrocortisone sodium succinate, hydrocortisone sodium phosphate, and sold under the tradenames Ala-Cort®, hydrocortisone phosphate, Solu-Cortef®, Hydrocort Acetate® and Lanacort®), prednisolone (sold under the tradenames Delta-

Cortel®, Orapred®, Pediapred® and Prelone®), prednisone (sold under the tradenames Deltasone®, Liquid Red®, Meticorten® and Orasone®), methylprednisolone (also known as 6-methylprednisolone, methylprednisolone acetate, methylprednisolone sodium succinate, sold under the tradenames Duralone®, Medralone®, Medrol®, M-Prednisol® and Solu-Medrol®); antihistamines, such as diphenhydramine (e.g., Benadryl®), hydroxyzine, and cyproheptadine; and bronchodilators, such as the beta-adrenergic receptor agonists, albuterol (e.g., Proventil®), and terbutaline (Brethine®).

In one embodiment, a CAR expressing cell described herein is administered to a subject in combination with an immunomodulator, e.g., Afutuzumab (available from Roche®); Pegfilgrastim (Neulasta®); Lenalidomide (CC-5013, Revlimid®); Thalidomide (Thalomid®), Actimid (CC4047); and IRX-2 (mixture of human cytokines including interleukin 1, interleukin 2, and interferon γ, CAS 951209-71-5, available from IRX Therapeutics.

In one embodiment, a CAR expressing cell described herein is administered to a subject in combination with a proteasome inhibitor, e.g., Bortezomib (Velcade®); Ixazomib citrate (MLN9708, CAS 1201902-80-8); Danoprevir (RG7227, CAS 850876-88-9); Ixazomib (MLN2238, CAS 1072833-77-2); and (S)-N-[(phenylmethoxy)carbonyl]-L-leucyl-N-(1-formyl-3-methylbutyl)- L-Leucinamide (MG-132, CAS 133407-82-6).

In one embodiment, a CAR expressing cell described herein is administered to a subject in combination with a vascular endothelial growth factor (VEGF) receptor, e.g., Bevacizumab (Avastin®), axitinib (Inlyta®); Brivanib alaninate (BMS-582664, (*S*)-((*R*)-1-(4-(4-Fluoro-2-methyl-1*H*-indol-5-yloxy)-5-methylpyrrolo[2,1-*f*][1,2,4]triazin-6-yloxy)propan-2-yl)2-aminopropanoate); Sorafenib (Nexavar®); Pazopanib (Votrient®); Sunitinib malate (Sutent®); Cediranib (AZD2171, CAS 288383-20-1); Vargatef (BIBF1120, CAS 928326-83-4); Foretinib (GSK1363089); Telatinib (BAY57-9352, CAS 332012-40-5); Apatinib (YN968D1, CAS 811803-05-1); Imatinib (Gleevec®); Ponatinib (AP24534, CAS 943319-70-8); Tivozanib (AV951, CAS 475108-18-0); Regorafenib (BAY73-4506, CAS 755037-03-7); Vatalanib dihydrochloride (PTK787, CAS 212141-51-0); Brivanib (BMS-540215, CAS 649735-46-6); Vandetanib (Caprelsa® or AZD6474); Motesanib diphosphate (AMG706, CAS 857876-30-3, N-(2,3-dihydro-3,3-dimethyl-1H-indol-6-yl)-2-[(4-pyridinylmethyl)amino]-3-pyridinecarboxamide, described in PCT Publication No. WO 02/066470); Dovitinib dilactic acid (TKI258, CAS 852433-84-2); Linfanib (ABT869, CAS 796967-16-3); Cabozantinib (XL184, CAS 849217-68-1); Lestaurtinib (CAS 111358-88-4); N-[5-[[[5-(1,1-Dimethylethyl)-2-oxazolyl]methyl]thio]-2-thiazolyl]-4-piperidinecarboxamide (BMS38703, CAS 345627-80-7); (3R,4R)-4-Amino-1-((4-((3-methoxyphenyl)amino)pyrrolo[2,1-f][1,2,4]triazin-5-yl)methyl)piperidin-3-ol (BMS690514); *N*-(3,4-Dichloro-2-fluorophenyl)-6-methoxy-7-[[(3aα,5β,6aα)-octahydro-2-methylcyclopenta[*c*]pyrrol-5-yl]methoxy]- 4-quinazolinamine (XL647, CAS 781613-23-8); 4-Methyl-

3-[[1-methyl-6-(3-pyridinyl)-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-yl]amino]-*N*-[3-(trifluoromethyl)phenyl]-benzamide (BHG712, CAS 940310-85-0); . and Aflibercept (Eylea®).

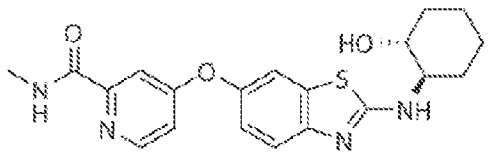
In one embodiment, a CAR expressing cell described herein is administered to a subject in combination with a CD20 antibody or a conjugate thereof, e.g.,: Rituximab (Riuxan® and MabThera®); and Tositumomab (Bexxar®); and Ofatumumab (Arzerra®), Ibritumomab tiuxetan (Zevalin®); and Tositumomab,

In one embodiment, a CAR expressing cell described herein is administered to a subject in combination with an anticonvulsant, e.g.,Anticonvulsants (antiepileptic or antiseizure drugs): aldehydes, e.g., paraldehyde; aromatic allylic alcohols, e.g., stiripentol (Diacomit®); barbiturates, e.g., phenobarbital (Luminal®), methylphenobarbital (Mebaral®), barbexaclone (Maliasin®), benzodiazepines, e.g., clobazam (Onfi®), clonazepam (Klonopin®), clorazepate (Tranxene® and Novo-Clopate®), diazepam (Valium®, Lembrol®, Diastat®), midazolam (Versed®), lorazepam (Ativan® and Orfidal®), nitrazepam (Alodorm®, Arem®, Insoma®), temazepam (restoril®, Normison®), nimetzepam (Erimin®), bromides, e.g., potassium bromide; carbamates, e.g., felbamate (Felbatol®); carboxamides, e.g., carbamazepine (Tegretol®, Equetro®), oxcarbazepine (Trileptal®, Oxcarb®), eslicarbazepine acetate (Aptiom®); fatty acids, e.g., valproates (valproic acid, sodium valproate, divalproex sodium), vigabatrin (Sabril®), progabide (Gabren®), tiagabine (Gabitril®); fructose derivatives, e.g., topiramate (Topamax®); GABA analogs, e.g., gabapentin (Neurontin®), pregabalin (Lyrica®); hydantoins, e.g., ethotoin (Peganone®), phenytoin (Dilantin®), mephenytoin (Mesantoin®), fosphenytoin (Cerebyx®, Prodilantin®); oxazolidinediones, e.g., paramethadione (Paradione®), trimethadione (Tridione®); propionates, e.g., beclamide (Choracon®, Hibicon®, Posedrine®); pyrimidinediones, e.g., primidone (Mysoline®); pyrrolidines, e.g., brivaracetam, levetiracetam, seletracetam (Keppra®); succinimides, e.g., ethosuximide (Zarontin®), phensuximide (Milontin®), mesuximide (Celontin®, Petinutin®); sulfonamides, e.g., acetazolamide (Diamox®), sultiame (Ospolot®), methazolamide (Neptazane®), zonisamide (Zonegran®); triazines, e.g., lamotrigine (Lamictal®); ureas, e.g., pheneturide, phenacemide (Phenurone®); valproylamides (amide derivaties of valproate), e.g., valpromide (Depamide®), valnoctamide; AMPA receptor antagonist, e.g., perampanel (Fycompa®).

In embodiments, a CAR-expressing cell described herein is administered to a subject in combination with an indoleamine 2,3-dioxygenase (IDO) inhibitor. IDO is an enzyme that catalyzes the degradation of the amino acid, L-tryptophan, to kynurenine. Many cancers overexpress IDO, e.g., prostatic, colorectal, pancreatic, cervical, gastric, ovarian, head, and lung cancer. pDCs, macrophages, and dendritic cells (DCs) can express IDO. Without being bound by theory, it is thought that a decrease in L-tryptophan (e.g., catalyzed by IDO) results in an immunosuppressive milieu by inducing T-cell

anergy and apoptosis. Thus, without being bound by theory, it is thought that an IDO inhibitor can enhance the efficacy of a CAR-expressing cell described herein, e.g., by decreasing the suppression or death of a CAR-expressing immune cell. In embodiments, the subject has a solid tumor, e.g., a solid tumor described herein, e.g., prostatic, colorectal, pancreatic, cervical, gastric, ovarian, head, or lung cancer. Exemplary inhibitors of IDO include but are not limited to 1-methyl-tryptophan, indoximod (NewLink Genetics) (see, e.g., Clinical Trial Identifier Nos. NCT01191216; NCT01792050), and INCB024360 (Incyte Corp.) (see, e.g., Clinical Trial Identifier Nos. NCT01604889; NCT01685255)

In embodiments, a CAR-expressing cell described herein is administered to a subject in combination with a modulator of myeloid-derived suppressor cells (MDSCs). MDSCs accumulate in the periphery and at the tumor site of many solid tumors. These cells suppress T cell responses, thereby hindering the efficacy of CAR-expressing cell therapy. Without being bound by theory, it is thought that administration of a MDSC modulator enhances the efficacy of a CAR-expressing cell described herein. In an embodiment, the subject has a solid tumor, e.g., a solid tumor described herein, e.g., glioblastoma. Exemplary modulators of MDSCs include but are not limited to MCS110 and BLZ945. MCS110 is a monoclonal antibody (mAb) against macrophage colony-stimulating factor (M-CSF). See, e.g., Clinical Trial Identifier No. NCT00757757. BLZ945 is a small molecule inhibitor of colony stimulating factor 1 receptor (CSF1R). See, e.g., Pyonteck et al. Nat. Med. 19(2013):1264-72. The structure of BLZ945 is shown below.



In embodiments, a CAR-expressing cell described herein is administered to a subject in combination with a CD19 CART cell (e.g., CTL019, e.g., as described in WO2012/079000, incorporated herein by reference). In embodiments, the subject has acute myeloid leukemia (AML), e.g., a CD19 positive AML or a CD19 negative AML. In embodiments, the subject has a CD19+ lymphoma, e.g., a CD19+ Non-Hodgkin's Lymphoma (NHL), a CD19+ FL, or a CD19+ DLBCL. In embodiments, the subject has a relapsed or refractory CD19+ lymphoma. In embodiments, a lymphodepleting chemotherapy is administered to the subject prior to, concurrently with, or after administration (e.g., infusion) of CD19 CART cells. In an example, the lymphodepleting chemotherapy is administered to the subject prior to administration of CD19 CART cells. For example, the

lymphodepleting chemotherapy ends 1-4 days (e.g., 1, 2, 3, or 4 days) prior to CD19 CART cell infusion. In embodiments, multiple doses of CD19 CART cells are administered, e.g., as described herein. For example, a single dose comprises about 5 x 10^8 CD19 CART cells. In embodiments, a lymphodepleting chemotherapy is administered to the subject prior to, concurrently with, or after administration (e.g., infusion) of a CAR-expressing cell described herein, e.g., a non-CD19 CAR-expresing cell. In embodiments, a CD19 CART is administered to the subject prior to, concurrently with, or after administration (e.g., infusion) of a non-CD19 CAR-expressing cell, e.g., a non-CD19 CAR-expressing cell described herein.

In some embodiments, a CAR-expressing cell described herein is administered to a subject in combination with a CD19 CAR-expressing cell, e.g., CTL019, e.g., as described in WO2012/079000, incorporated herein by reference, for treatment of a disease associated with the expression of BCMA, e.g., a cancer described herein. Without being bound by theory, it is believed that administering a CD19 CAR-expressing cell in combination with a CAR-expressing cell improves the efficacy of a CAR-expressing cell described herein by targeting early lineage cancer cells, e.g., cancer stem cells, modulating the immune response, depleting regulatory B cells, and/or improving the tumor microenvironment. For example, a CD19 CAR-expressing cell targets cancer cells that express early lineage markers, e.g., cancer stem cells and CD19-expressing cells, while the CAR-expressing cell described herein targets cancer cells that express later lineage markers, e.g., BCMA. This preconditioning approach can improve the efficacy of the CAR-expressing cell described herein. In such embodiments, the CD19 CAR-expressing cell is administered prior to, concurrently with, or after administration (e.g., infusion) of a CAR-expressing cell described herein.

In embodiments, a CAR-expressing cell described herein also expresses a CAR targeting CD19, e.g., a CD19 CAR. In an embodiment, the cell expressing a CAR described herein and a CD19 CAR is administered to a subject for treatment of a cancer described herein, e.g., AML. In an embodiment, the configurations of one or both of the CAR molecules comprise a primary intracellular signaling domain and a costimulatory signaling domain. In another embodiment, the configurations of one or both of the CAR molecules comprise a primary intracellular signaling domain and two or more, e.g., 2, 3, 4, or 5 or more, costimulatory signaling domains. In such embodiments, the CAR molecule described herein and the CD19 CAR may have the same or a different primary intracellular signaling domain, the same or different costimulatory signaling domains, or the same number or a different number of costimulatory signaling domains. Alternatively, the CAR described herein and the CD19 CAR are configured as a split CAR, in which one of the CAR molecules comprises an antigen binding domain and a costimulatory domain (e.g., 4-1BB), while the other CAR molecule comprises an antigen binding domain and a primary intracellular signaling domain (e.g., CD3 zeta).

In some embodiments , a CAR-expressing cell described herein is administered to a subject in combination with a interleukin-15 (IL-15) polypeptide, a interleukin-15 receptor alpha (IL-15Ra) polypeptide, or a combination of both a IL-15 polypeptide and a IL-15Ra polypeptide e.g., hetIL-15 (Admune Therapeutics, LLC). hetIL-15 is a heterodimeric non-covalent complex of IL-15 and IL-15Ra. hetIL-15 is described in, e.g., U.S. 8,124,084, U.S. 2012/0177598, U.S. 2009/0082299, U.S. 2012/0141413, and U.S. 2011/0081311, incorporated herein by reference. In embodiments, het-IL-15 is administered subcutaneously. In embodiments, the subject has a cancer, e.g., solid cancer, e.g., melanoma or colon cancer. In embodiments, the subject has a metastatic cancer.

In one embodiment, the subject can be administered an agent which reduces or ameliorates a side effect associated with the administration of a CAR-expressing cell. Side effects associated with the administration of a CAR-expressing cell include, but are not limited to CRS, and hemophagocytic lymphohistiocytosis (HLH), also termed Macrophage Activation Syndrome (MAS). Symptoms of CRS include high fevers, nausea, transient hypotension, hypoxia, and the like. CRS may include clinical constitutional signs and symptoms such as fever, fatigue, anorexia, myalgias, arthalgias, nausea, vomiting, and headache. CRS may include clinical skin signs and symptoms such as rash. CRS may include clinical gastrointestinal signs and symsptoms such as nausea, vomiting and diarrhea. CRS may include clinical respiratory signs and symptoms such as tachypnea and hypoxemia. CRS may include clinical cardiovascular signs and symptoms such as tachycardia, widened pulse pressure, hypotension, increased cardac output (early) and potentially diminished cardiac output (late). CRS may include clinical coagulation signs and symptoms such as elevated d-dimer, hypofibrinogenemia with or without bleeding. CRS may include clinical renal signs and symptoms such as azotemia. CRS may include clinical hepatic signs and symptoms such as transaminitis and hyperbilirubinemia. CRS may include clinical neurologic signs and symptoms such as headache, mental status changes, confusion, delirium, word finding difficulty or frank aphasia, hallucinations, tremor, dymetria, altered gait, and seizures.

Accordingly, the methods described herein can comprise administering a CAR-expressing cell described herein to a subject and further administering one or more agents to manage elevated levels of a soluble factor resulting from treatment with a CAR-expressing cell. In one embodiment, the soluble factor elevated in the subject is one or more of IFN-γ, TNFα, IL-2 and IL-6. In an embodiment, the factor elevated in the subject is one or more of IL-1, GM-CSF, IL-10, IL-8, IL-5 and fraktalkine. Therefore, an agent administered to treat this side effect can be an agent that neutralizes one or more of these soluble factors. In one embodiment, the agent that neutralizes one or more of these soluble forms is an antibody or antibody fragment. Examples of such agents include, but are not limited to a steroid (e.g., corticosteroid), an inhibitor of TNFα, and an inhibitor of IL-6. An example of a TNFα inhibitor is an anti-TNFα antibody molecule such as, infliximab, adalimumab, certolizumab pegol, and golimumab.

Another example of a TNFα inhibitor is a fusion protein such as entanercept. Small molecule inhibitors of TNFα include, but are not limited to, xanthine derivatives (e.g. pentoxifylline) and bupropion. An example of an IL-6 inhibitor is an anti-IL-6 antibody molecule such as tocilizumab (toc), sarilumab, elsilimomab, CNTO 328, ALD518/BMS-945429, CNTO 136, CPSI-2364, CDP6038, VX30, ARGX-109, FE301, and FM101. In one embodiment, the anti-IL-6 antibody molecule is tocilizumab. An example of an IL-1R based inhibitor is anakinra.

In some embodiment, the subject is administered a corticosteroid, such as, e.g., methylprednisolone, hydrocortisone, among others.

In some embodiments, the subject is administered a vasopressor, such as, e.g., norepinephrine, dopamine, phenylephrine, epinephrine, vasopressin, or a combination thereof.

In an embodiment, the subject can be administered an antipyretic agent. In an embodiment, the subject can be administered an analgesic agent.

In one embodiment, the subject can be administered an agent that prevents trafficking of the BCMA CAR-expressing cell to the brain, e.g., natalizumab (TYSABRI®). BCMA expression, e.g., a splice variant thereof, has been detected in some parts of the brain, e.g., the cerebellum or medulla oblongata. Without being bound by any particular theory, prevention of trafficking of the BCMA CAR-expressing cells to the brain is preferred to prevent any BCMA CAR-expressing cells from interacting with or acting on BCMA-expressing brain tissue.

In one embodiment, the subject can be administered an agent which enhances the activity of a CAR-expressing cell. For example, in one embodiment, the agent can be an agent which inhibits an inhibitory molecule, e.g., the agent is a checkpoint inhibitor. Inhibitory molecules, e.g., Programmed Death 1 (PD1), can, in some embodiments, decrease the ability of a CAR-expressing cell to mount an immune effector response. Examples of inhibitory molecules include PD1, PD-L1, PD-L2, CTLA4, TIM3, CEACAM (e.g., CEACAM-1, CEACAM-3 and/or CEACAM-5), LAG3, VISTA, BTLA, TIGIT, LAIR1, CD160, 2B4, CD80, CD86, B7-H3 (CD276), B7-H4 (VTCN1), HVEM (TNFRSF14 or CD270), KIR, A2aR, MHC class I, MHC class II, GAL9, adenosine, and TGFR beta. Inhibition of an inhibitory molecule, e.g., by inhibition at the DNA, RNA or protein level, can optimize a CAR-expressing cell performance. In embodiments, an inhibitory nucleic acid, e.g., an inhibitory nucleic acid, e.g., a dsRNA, e.g., an siRNA or shRNA, a clustered regularly interspaced short palindromic repeats (CRISPR), a transcription-activator like effector nuclease (TALEN), or a zinc finger endonuclease (ZFN), e.g., as described herein, can be used to inhibit expression of an inhibitory molecule in the CAR-expressing cell. In an embodiment the inhibitor is an shRNA. In an embodiment, the inhibitory molecule is inhibited within a CAR-expressing cell. In these embodiments, a dsRNA

molecule that inhibits expression of the inhibitory molecule is linked to the nucleic acid that encodes a component, e.g., all of the components, of the CAR. In embodiments, a CAR-expressing cell described herein is administered in combination with an inhibitor of an inhibitory molecule, e.g., in combination with a checkpoint inhibitor, e.g., in combination with an inhibitor of PD1 and/or PD-L1. In embodiments, a CAR- expressing cell described herein is administered in combination with an inhibitor of PD1. In embodiments, a CAR- expressing cell described herein is administered in combination with an inhibitor of PD-L1.

In an embodiment, a nucleic acid molecule that encodes a dsRNA molecule that inhibits expression of the molecule that modulates or regulates, e.g., inhibits, T-cell function is operably linked to a promoter, e.g., a H1- or a U6-derived promoter such that the dsRNA molecule that inhibits expression of the molecule that modulates or regulates, e.g., inhibits, T-cell function is expressed, e.g., is expressed within a CAR-expressing cell. See e.g., Tiscornia *G.,* "Development of Lentiviral Vectors Expressing siRNA," Chapter 3, in *Gene Transfer: Delivery and Expression of DNA and RNA* (eds. Friedmann and Rossi). Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, USA, 2007; Brummelkamp T*R, et al.* (2002) *Science* 296: 550–553; Miyagishi *M, et al.* (2002) *Nat. Biotechnol.* 19: 497–500. In an embodiment the nucleic acid molecule that encodes a dsRNA molecule that inhibits expression of the molecule that modulates or regulates, e.g., inhibits, T-cell function is present on the same vector, e.g., a lentiviral vector, that comprises a nucleic acid molecule that encodes a component, e.g., all of the components, of the CAR. In such an embodiment, the nucleic acid molecule that encodes a dsRNA molecule that inhibits expression of the molecule that modulates or regulates, e.g., inhibits, T-cell function is located on the vector, e.g., the lentiviral vector, 5'- or 3'- to the nucleic acid that encodes a component, e.g., all of the components, of the CAR. The nucleic acid molecule that encodes a dsRNA molecule that inhibits expression of the molecule that modulates or regulates, e.g., inhibits, T-cell function can be transcribed in the same or different direction as the nucleic acid that encodes a component, e.g., all of the components, of the CAR. In an embodiment the nucleic acid molecule that encodes a dsRNA molecule that inhibits expression of the molecule that modulates or regulates, e.g., inhibits, T-cell function is present on a vector other than the vector that comprises a nucleic acid molecule that encodes a component, e.g., all of the components, of the CAR. In an embodiment, the nucleic acid molecule that encodes a dsRNA molecule that inhibits expression of the molecule that modulates or regulates, e.g., inhibits, T-cell function it transiently expressed within a CAR-expressing cell. In an embodiment, the nucleic acid molecule that encodes a dsRNA molecule that inhibits expression of the molecule that modulates or regulates, e.g., inhibits, T-cell function is stably integrated into the genome of a CAR-expressing cell.

Examples of dsRNA molecules useful for inhibiting expression of a molecule that modulates or regulates, e.g., inhibits, T-cell function, wherein the molecule that modulates or regulates, e.g., inhibits, T-cell function is PD-1 are provided below.

Provided in Table 18 below are the names of PDCD1 (PD1) RNAi agents (derived from their position in the mouse PDCD1 gene sequence NM_008798.2), along with the SEQ ID NOs: 286-333 representing the DNA sequence. Both sense (S) and antisense (AS) sequences are presented as 19mer and 21mer sequences are in this table. Also note that the position (PoS, e.g., 176) is derived from the position number in the mouse PDCD1 gene sequence NM_008798.2. SEQ ID NOs are indicated in groups of 12 that correspond with "sense 19" SEQ ID NOs: 286-297; "sense 21" SEQ ID NOs: 298-309; "asense 21" SEQ ID NOs: 310-321; "asense 19" SEQ ID NOs: 322-333.

Table 18. Mouse PDCD1 (PD1) shRNA sequences

Position on NM_008798.2	Target region	Sense19	Sense21	Asense21	Asense19
176	CDS	GGAGGTCCCT CACCTTCTA (SEQ ID NO: 286)	CTGGAGGTCC CTCACCTTCT A (SEQ ID NO: 298)	TAGAAGGTGA GGGACCTCCA G (SEQ ID NO: 310)	TAGAAGGTGA GGGACCTCC (SEQ ID NO: 322)
260	CDS	CGGAGGATCT TATGCTGAA (SEQ ID NO: 287)	GTCGGAGGAT CTTATGCTGA A (SEQ ID NO: 299)	TTCAGCATAA GATCCTCCGA C (SEQ ID NO: 311)	TTCAGCATAA GATCCTCCG (SEQ ID NO: 323)
359	CDS	CCCGCTTCCA GATCATACA (SEQ ID NO: 288)	TGCCCGCTTC CAGATCATAC A (SEQ ID NO: 300)	TGTATGATCT GGAAGCGGGC A (SEQ ID NO: 312)	TGTATGATCT GGAAGCGGG (SEQ ID NO: 324)
528	CDS	GGAGACCTCA ACAAGATAT (SEQ ID NO: 289)	CTGGAGACCT CAACAAGATA T (SEQ ID NO: 301)	ATATCTTGTT GAGGTCTCCA G (SEQ ID NO: 313)	ATATCTTGTT GAGGTCTCC (SEQ ID NO: 325)
581	CDS	AAGGCATGGT CATTGGTAT (SEQ ID NO: 290)	TCAAGGCATG GTCATTGGTA T (SEQ ID NO: 302)	ATACCAATGA CCATGCCTTG A (SEQ ID NO: 314)	ATACCAATGA CCATGCCTT (SEQ ID NO: 326)
584	CDS	GCATGGTCAT TGGTATCAT (SEQ ID NO: 291)	AGGCATGGTC ATTGGTATCA T (SEQ ID NO:	ATGATACCAA TGACCATGCC T (SEQ ID NO:	ATGATACCAA TGACCATGC (SEQ ID NO: 327)

			303)	315)	
588	CDS	GGTCATTGGT ATCATGAGT (SEQ ID NO: 292)	ATGGTCATTG GTATCATGAG T (SEQ ID NO: 304)	ATGGTCATTG GTATCATGAG T (SEQ ID NO: 316)	ATGGTCATTG GTATCATGA (SEQ ID NO: 328)
609	CDS	CCTAGTGGGT ATCCCTGTA (SEQ ID NO: 293)	GCCCTAGTGG GTATCCCTGT A (SEQ ID NO: 305)	GCCCTAGTGG GTATCCCTGT A (SEQ ID NO: 317)	GCCCTAGTGG GTATCCCTG (SEQ ID NO: 329)
919	CDS	GAGGATGGAC ATTGTTCTT (SEQ ID NO: 294)	ATGAGGATGG ACATTGTTCTT (SEQ ID NO: 306)	ATGAGGATGG ACATTGTTCTT (SEQ ID NO: 318)	ATGAGGATGG ACATTGTTC (SEQ ID NO: 330)
1021	3'UTR	GCATGCAGGC TACAGTTCA (SEQ ID NO: 295)	GAGCATGCAG GCTACAGTTC A (SEQ ID NO: 307)	GAGCATGCAG GCTACAGTTC A (SEQ ID NO: 319)	GAGCATGCAG GCTACAGTT (SEQ ID NO: 331)
1097	3'UTR	CCAGCACATG CACTGTTGA (SEQ ID NO: 296)	TTCCAGCACA TGCACTGTTG A (SEQ ID NO: 308)	TTCCAGCACA TGCACTGTTG A (SEQ ID NO: 320)	TTCCAGCACA TGCACTGTT (SEQ ID NO: 332)
1101	3'UTR	CACATGCACT GTTGAGTGA (SEQ ID NO: 297)	AGCACATGCA CTGTTGAGTG A (SEQ ID NO: 309)	AGCACATGCA CTGTTGAGTG A (SEQ ID NO: 321)	AGCACATGCA CTGTTGAGT (SEQ ID NO: 333)

Provided in Table 19 below are the names of PDCD1 (PD1) RNAi agents (derived from their position in the human PDCD1 gene sequence, along with the SEQ ID NOs. 334-381 representing the DNA sequence. Both sense (S) and antisense (AS) sequences are presented as 19mer and 21mer sequences. SEQ ID NOs are indicated in groups of 12 that correspond with "sense 19" SEQ ID NOs: 334-345; "sense 21" SEQ ID NOs: 346-357; "asense 21" SEQ ID NOs: 358-369; "asense 19" SEQ ID NOs: 370-381.

Table 19. Human PDCD1 (PD1) shRNA sequences

Position on NM_005 018.2	Target region	Sense19	Asense19	Sense21	Asense21
145	CDS	GGCCAGGATG GTTCTTAGA (SEQ ID NO: 334)	TCTAAGAACC ATCCTGGCC (SEQ ID NO: 346)	GCGGCCAGGA TGGTTCTTAG A (SEQ ID NO:	TCTAAGAACC ATCCTGGCCG C (SEQ ID NO:

				358)	370)
271	CDS	GCTTCGTGCT AAACTGGTA (SEQ ID NO: 335)	TACCAGTTTA GCACGAAGC (SEQ ID NO: 347)	GAGCTTCGTG CTAAACTGGT A (SEQ ID NO: 359)	TACCAGTTTA GCACGAAGCT C (SEQ ID NO: 371)
393	CDS	GGGCGTGACT TCCACATGA (SEQ ID NO: 336)	TCATGTGGAA GTCACGCCC (SEQ ID NO: 348)	ACGGGCGTGA CTTCCACATG A (SEQ ID NO: 360)	TCATGTGGAA GTCACGCCCG T (SEQ ID NO: 372)
1497	3'UTR	CAGGCCTAGA GAAGTTTCA (SEQ ID NO: 337)	TGAAACTTCT CTAGGCCTG (SEQ ID NO: 349)	TGCAGGCCTA GAGAAGTTTC A (SEQ ID NO: 361)	TGAAACTTCT CTAGGCCTGC A (SEQ ID NO: 373)
1863	3'UTR	CTTGGAACCC ATTCCTGAA (SEQ ID NO: 338)	TTCAGGAATG GGTTCCAAG (SEQ ID NO: 350)	TCCTTGGAAC CCATTCCTGA A (SEQ ID NO: 362)	TTCAGGAATG GGTTCCAAGG A (SEQ ID NO: 374)
1866	3'UTR	GGAACCCATT CCTGAAATT (SEQ ID NO: 339)	AATTTCAGGA ATGGGTTCC (SEQ ID NO: 351)	TTGGAACCCA TTCCTGAAAT T (SEQ ID NO: 363)	AATTTCAGGA ATGGGTTCCA A (SEQ ID NO: 375)
1867	3'UTR	GAACCCATTC CTGAAATTA (SEQ ID NO: 340)	TAATTTCAGG AATGGGTTC (SEQ ID NO: 352)	TGGAACCCAT TCCTGAAATT A (SEQ ID NO: 364)	TAATTTCAGG AATGGGTTCC A (SEQ ID NO: 376)
1868	3'UTR	AACCCATTCC TGAAATTAT (SEQ ID NO: 341)	ATAATTTCAG GAATGGGTT (SEQ ID NO: 353)	GGAACCCATT CCTGAAATTA T (SEQ ID NO: 365)	ATAATTTCAG GAATGGGTTC C (SEQ ID NO:377)
1869	3'UTR	ACCCATTCCT GAAATTATT (SEQ ID NO: 342)	AATAATTTCA GGAATGGGT (SEQ ID NO: 354)	GAACCCATTC CTGAAATTAT T (SEQ ID NO: 366)	AATAATTTCA GGAATGGGTT C (SEQ ID NO: 378)
1870	3'UTR	CCCATTCCTG AAATTATTT (SEQ ID NO: 343)	AAATAATTTC AGGAATGGG (SEQ ID NO: 355)	AACCCATTCC TGAAATTATT T (SEQ ID NO: 367)	AAATAATTTC AGGAATGGGT T (SEQ ID NO: 379)
2079	3'UTR	CTGTGGTTCT ATTATATTA (SEQ ID NO: 344)	TAATATAATA GAACCACAG (SEQ ID NO: 356)	CCCTGTGGTT CTATTATATT A (SEQ ID NO: 368)	TAATATAATA GAACCACAGG G (SEQ ID NO: 380)

2109	3'UTR	AAATATGAGAGCATGCTAA (SEQ ID NO: 345)	TTAGCATGCTCTCATATTT (SEQ ID NO: 357)	TTAAATATGAGAGCATGCTAA (SEQ ID NO: 369)	TTAGCATGCTCTCATATTTAA (SEQ ID NO: 381)

In one embodiment, the inhibitor of an inhibitory signal can be, e.g., an antibody or antibody fragment that binds to an inhibitory molecule. For example, the agent can be an antibody or antibody fragment that binds to PD1, PD-L1, PD-L2 or CTLA4 (e.g., ipilimumab (also referred to as MDX-010 and MDX-101, and marketed as Yervoy®; Bristol-Myers Squibb; Tremelimumab (IgG2 monoclonal antibody available from Pfizer, formerly known as ticilimumab, CP-675,206).). In an embodiment, the agent is an antibody or antibody fragment that binds to TIM3. In an embodiment, the agent is an antibody or antibody fragment that binds to LAG3. In embodiments, the agent that enhances the activity of a CAR-expressing cell, e.g., inhibitor of an inhibitory molecule, is administered in combination with an allogeneic CAR, e.g., an allogeneic CAR described herein (e.g., described in the Allogeneic CAR section herein).

PD-1 is an inhibitory member of the CD28 family of receptors that also includes CD28, CTLA-4, ICOS, and BTLA. PD-1 is expressed on activated B cells, T cells and myeloid cells (Agata et al. 1996 Int. Immunol 8:765-75). Two ligands for PD-1, PD-L1 and PD-L2 have been shown to downregulate T cell activation upon binding to PD-1 (Freeman et a. 2000 J Exp Med 192:1027-34; Latchman et al. 2001 Nat Immunol 2:261-8; Carter et al. 2002 Eur J Immunol 32:634-43). PD-L1 is abundant in human cancers (Dong et al. 2003 J Mol Med 81:281-7; Blank et al. 2005 Cancer Immunol. Immunother 54:307-314; Konishi et al. 2004 Clin Cancer Res 10:5094). Immune suppression can be reversed by inhibiting the local interaction of PD-1 with PD-L1. Antibodies, antibody fragments, and other inhibitors of PD-1, PD-L1 and PD-L2 are available in the art and may be used combination with a cars of the present invention described herein. For example, nivolumab (also referred to as BMS-936558 or MDX1106; Bristol-Myers Squibb) is a fully human IgG4 monoclonal antibody which specifically blocks PD-1. Nivolumab (clone 5C4) and other human monoclonal antibodies that specifically bind to PD-1 are disclosed in US 8,008,449 and WO2006/121168. Pidilizumab (CT-011; Cure Tech) is a humanized IgG1k monoclonal antibody that binds to PD-1. Pidilizumab and other humanized anti-PD-1 monoclonal antibodies are disclosed in WO2009/101611. Pembrolizumab (formerly known as lambrolizumab, and also referred to as MK03475; Merck) is a humanized IgG4 monoclonal antibody that binds to PD-1. Pembrolizumab and other humanized anti-PD-1 antibodies are disclosed in US 8,354,509 and WO2009/114335. MEDI4736 (Medimmune) is a human monoclonal antibody that binds to PDL1, and inhibits interaction of the ligand with PD1. MDPL3280A (Genentech / Roche) is a human Fc optimized IgG1 monoclonal antibody that binds to PD-L1. MDPL3280A and

other human monoclonal antibodies to PD-L1 are disclosed in U.S. Patent No.: 7,943,743 and U.S Publication No.: 20120039906. Other anti-PD-L1 binding agents include YW243.55.S70 (heavy and light chain variable regions are shown in SEQ ID NOs 20 and 21 in WO2010/077634) and MDX-1 105 (also referred to as BMS-936559, and, e.g., anti-PD-L1 binding agents disclosed in WO2007/005874). AMP-224 (B7-DCIg; Amplimmune; e.g., disclosed in WO2010/027827 and WO2011/066342), is a PD-L2 Fc fusion soluble receptor that blocks the interaction between PD-1 and B7-H1. Other anti-PD-1 antibodies include AMP 514 (Amplimmune), among others, e.g., anti-PD-1 antibodies disclosed in US 8,609,089, US 2010028330, and/or US 20120114649.

TIM3 (T cell immunoglobulin-3) also negatively regulates T cell function, particularly in IFN-g-secreting CD4+ T helper 1 and CD8+ T cytotoxic 1 cells, and plays a critical role in T cell exhaustion. Inhibition of the interaction between TIM3 and its ligands, e.g., galectin-9 (Gal9), phosphotidylserine (PS), and HMGB1, can increase immune response. Antibodies, antibody fragments, and other inhibitors of TIM3 and its ligands are available in the art and may be used combination with a CD19 or BCMA CAR described herein. For example, antibodies, antibody fragments, small molecules, or peptide inhibitors that target TIM3 binds to the IgV domain of TIM3 to inhibit interaction with its ligands. Antibodies and peptides that inhibit TIM3 are disclosed in WO2013/006490 and US20100247521. Other anti-TIM3 antibodies include humanized versions of RMT3-23 (disclosed in Ngiow et al., 2011, Cancer Res, 71:3540-3551), and clone 8B.2C12 (disclosed in Monney et al., 2002, Nature, 415:536-541). Bi-specific antibodies that inhibit TIM3 and PD-1 are disclosed in US20130156774.

In other embodiments, the agent which enhances the activity of a CAR-expressing cell is a CEACAM inhibitor (*e.g.*, CEACAM-1, CEACAM-3, and/or CEACAM-5 inhibitor). In one embodiment, the inhibitor of CEACAM is an anti-CEACAM antibody molecule. Exemplary anti-CEACAM-1 antibodies are described in WO 2010/125571, WO 2013/082366 WO 2014/059251 and WO 2014/022332, *e.g.,* a monoclonal antibody 34B1, 26H7, and 5F4; or a recombinant form thereof, as described in, *e.g.*, US 2004/0047858, US 7,132,255 and WO 99/052552. In other embodiments, the anti-CEACAM antibody binds to CEACAM-5 as described in, *e.g.*, Zheng et al. *PLoS One*. 2010 Sep 2;5(9). pii: e12529 (DOI:10:1371/journal.pone.0021146), or crossreacts with CEACAM-1 and CEACAM-5 as described in, *e.g.*, WO 2013/054331 and US 2014/0271618.

Without wishing to be bound by theory, carcinoembryonic antigen cell adhesion molecules (CEACAM), such as CEACAM-1 and CEACAM-5, are believed to mediate, at least in part, inhibition of an anti-tumor immune response (*see e.g.*, Markel *et al. J Immunol*. 2002 Mar 15;168(6):2803-10; Markel et al. *J Immunol*. 2006 Nov 1;177(9):6062-71; Markel et al. *Immunology*. 2009 Feb;126(2):186-200; Markel et al. *Cancer Immunol Immunother*. 2010 Feb;59(2):215-30; Ortenberg et al. *Mol Cancer Ther*. 2012 Jun;11(6):1300-10; Stern et al. *J Immunol*. 2005 Jun 1;174(11):6692-701; Zheng et al. *PLoS*

One. 2010 Sep 2;5(9). pii: e12529). For example, CEACAM-1 has been described as a heterophilic ligand for TIM-3 and as playing a role in TIM-3-mediated T cell tolerance and exhaustion (*see e.g.*, WO 2014/022332; Huang, *et al.* (2014) *Nature* doi:10.1038/nature13848). In embodiments, co-blockade of CEACAM-1 and TIM-3 has been shown to enhance an anti-tumor immune response in xenograft colorectal cancer models (*see e.g.*, WO 2014/022332; Huang, *et al.* (2014), *supra*). In other embodiments, co-blockade of CEACAM-1 and PD-1 reduce T cell tolerance as described, *e.g.*, in WO 2014/059251. Thus, CEACAM inhibitors can be used with the other immunomodulators described herein (*e.g.*, anti-PD-1 and/or anti-TIM-3 inhibitors) to enhance an immune response against a cancer, *e.g.*, a melanoma, a lung cancer (e.g., NSCLC), a bladder cancer, a colon cancer an ovarian cancer, and other cancers as described herein.

LAG3 (lymphocyte activation gene-3 or CD223) is a cell surface molecule expressed on activated T cells and B cells that has been shown to play a role in CD8+ T cell exhaustion. Antibodies, antibody fragments, and other inhibitors of LAG3 and its ligands are available in the art and may be used combination with a CD19 or BCMA CAR described herein. For example, BMS-986016 (Bristol-Myers Squib) is a monoclonal antibody that targets LAG3. IMP701 (Immutep) is an antagonist LAG3 antibody and IMP731 (Immutep and GlaxoSmithKline) is a depleting LAG3 antibody. Other LAG3 inhibitors include IMP321 (Immutep), which is a recombinant fusion protein of a soluble portion of LAG3 and Ig that binds to MHC class II molecules and activates antigen presenting cells (APC). Other antibodies are disclosed, e.g., in WO2010/019570.

In some embodiments, the agent which enhances the activity of a CAR-expressing cell can be, e.g., a fusion protein comprising a first domain and a second domain, wherein the first domain is an inhibitory molecule, or fragment thereof, and the second domain is a polypeptide that is associated with a positive signal, e.g., a polypeptide comrpsing an antracellular signaling domain as described herein. In some embodiments, the polypeptide that is associated with a positive signal can include a costimulatory domain of CD28, CD27, ICOS, e.g., an intracellular signaling domain of CD28, CD27 and/or ICOS, and/or a primary signaling domain, e.g., of CD3 zeta, e.g., described herein. In one embodiment, the fusion protein is expressed by the same cell that expressed the CAR. In another embodiment, the fusion protein is expressed by a cell, e.g., a T cell or NK cell that does not express an anti-BCMA CAR.

In one embodiment, the agent which enhances activity of a CAR-expressing cell described herein is miR-17-92.

In one embodiment, the agent which enhances activity of a CAR-described herein is a cytokine. Cytokines have important functions related to T cell expansion, differentiation, survival, and

homeostatis. Cytokines that can be administered to the subject receiving a CAR-expressing cell described herein include: IL-2, IL-4, IL-7, IL-9, IL-15, IL-18, and IL-21, or a combination thereof. In preferred embodiments, the cytokine administered is IL-7, IL-15, or IL-21, or a combination thereof. The cytokine can be administered once a day or more than once a day, e.g., twice a day, three times a day, or four times a day. The cytokine can be administered for more than one day, e.g. the cytokine is administered for 2 days, 3 days, 4 days, 5 days, 6 days, 1 week, 2 weeks, 3 weeks, or 4 weeks. For example, the cytokine is administered once a day for 7 days.

In embodiments, the cytokine is administered in combination with CAR-expressing T cells. The cytokine can be administered simultaneously or concurrently with the CAR-expressing T cells, e.g., administered on the same day. The cytokine may be prepared in the same pharmaceutical composition as the CAR-expressing T cells, or may be prepared in a separate pharmaceutical composition. Alternatively, the cytokine can be administered shortly after administration of the CAR-expressing T cells, e.g., 1 day, 2 days, 3 days, 4 days, 5 days, 6 days, or 7 days after administration of the CAR-expressing T cells. In embodiments where the cytokine is administered in a dosing regimen that occurs over more than one day, the first day of the cytokine dosing regimen can be on the same day as administration with the CAR-expressing T cells, or the first day of the cytokine dosing regimen can be 1 day, 2 days, 3 days, 4 days, 5 days, 6 days, or 7 days after administration of the CAR-expressing T cells. In one embodiment, on the first day, the CAR-expressing T cells are administered to the subject, and on the second day, a cytokine is administered once a day for the next 7 days. In a preferred embodiment, the cytokine to be administered in combination with CAR-expressing T cells is IL-7, IL-15, or IL-21.

In other embodiments, the cytokine is administered a period of time after administration of CAR-expressing cells, e.g., at least 2 weeks, 3 weeks, 4 weeks, 6 weeks, 8 weeks, 10 weeks, 12 weeks, 4 months, 5 months, 6 months, 7 months, 8 months, 9 months, 10 months, 11 months, or 1 year or more after administration of CAR-expressing cells. In one embodiment, the cytokine is administered after assessment of the subject's response to the CAR-expressing cells. For example, the subject is administered CAR-expressing cells according to the dosage and regimens described herein. The response of the subject to CAR-expressing cell therapy is assessed at 2 weeks, 3 weeks, 4 weeks, 6 weeks, 8 weeks, 10 weeks, 12 weeks, 4 months, 5 months, 6 months, 7 months, 8 months, 9 months, 10 months, 11 months, or 1 year or more after administration of CAR-expressing cells, using any of the methods described herein, including inhibition of tumor growth, reduction of circulating tumor cells, or tumor regression. Subjects that do not exhibit a sufficient response to CAR-expressing cell therapy can be administered a cytokine. Administration of the cytokine to the subject that has sub-optimal response to the CAR-expressing cell therapy improves CAR-expressing cell efficacy or anti-cancer activity. In a preferred embodiment, the cytokine administered after administration of CAR-expressing cells is IL-7.

5

COMBINATION WITH A LOW, IMMUNE ENHANCING, DOSE OF AN MTOR INHIBITOR

Methods described herein use low, immune enhancing, doses of mTOR inhibitors, e.g., allosteric mTOR inhibitors, including rapalogs such as RAD001. Administration of a low, immune enhancing, dose of an mTOR inhibitor (e.g., a dose that is insufficient to completely suppress the 10 immune system, but sufficient to improve immune function) can optimize the performance of immune effector cells, e.g., T cells or CAR-expressing cells, in the subject. Methods for measuring mTOR inhibition, dosages, treatment regimens, and suitable pharmaceutical compositions are described in U.S. Patent Application No. 2015/01240036, hereby incorporated by reference.

In an embodiment, administration of a low, immune enhancing, dose of an mTOR inhibitor 15 results in one or more of the following:

i) a decrease in the number of PD-1 positive immune effector cells;

ii) an increase in the number of PD-1 negative immune effector cells;

iii) an increase in the ratio of PD-1 negative immune effector cells / PD-1 positive immune effector cells;

20 iv) an increase in the number of naive T cells;

v) an increase in the expression of one or more of the following markers: $CD62L^{high}$, $CD127^{high}$, $CD27^{+}$, and BCL2, e.g., on memory T cells, e.g., memory T cell precursors;

vi) a decrease in the expression of KLRG1, e.g., on memory T cells, e.g., memory T cell precursors; or

25 vii) an increase in the number of memory T cell precursors, e.g., cells with any one or combination of the following characteristics: increased $CD62L^{high}$, increased $CD127^{high}$, increased $CD27^{+}$, decreased KLRG1, and increased BCL2;

and wherein any of the foregoing, e.g., i), ii), iii), iv), v), vi), or vii), occurs e.g., at least transiently, e.g., as compared to a non-treated subject.

30 In another embodiment, administration of a low, immune enhancing, dose of an mTOR inhibitor results in increased or prolonged proliferation or persistence of CAR-expressing cells, e.g., in culture or in a subject, e.g., as compared to non-treated CAR-expressing cells or a non-treated subject. In embodiments, increased proliferation or persistence is associated with in an increase in the number of CAR-expressing cells. In another embodiment, administration of a low, immune enhancing, dose of an 35 mTOR inhibitor results in increased killing of cancer cells by CAR-expressing cells, e.g., in culture or in a subject, e.g., as compared to non-treated CAR-expressing cells or a non-treated subject. In embodiments, increased killing of cancer cells is associated with in a decrease in tumor volume. In one embodiment, the cells expressing a CAR molecule, e.g., a CAR molecule described herein, are

administered in combination with a low, immune enhancing dose of an mTOR inhibitor, e.g., an allosteric mTOR inhibitor, e.g., RAD001, or a catalytic mTOR inhibitor. For example, administration of the low, immune enhancing, dose of the mTOR inhibitor can be initiated prior to administration of a CAR-expressing cell described herein; completed prior to administration of a CAR-expressing cell described herein; initiated at the same time as administration of a CAR-expressing cell described herein; overlapping with administration of a CAR-expressing cell described herein; or continuing after administration of a CAR-expressing cell described herein.

Alternatively or in addition, administration of a low, immune enhancing, dose of an mTOR inhibitor can optimize immune effector cells to be engineered to express a CAR molecule described herein. In such embodiments, administration of a low, immune enhancing, dose of an mTOR inhibitor, e.g., an allosteric inhibitor, e.g., RAD001, or a catalytic inhibitor, is initiated or completed prior to harvest of immune effector cells, e.g., T cells or NK cells, to be engineered to express a CAR molecule described herein, from a subject.

In another embodiment, immune effector cells, e.g., T cells or NK cells, to be engineered to express a CAR molecule described herein, e.g., after harvest from a subject, or CAR-expressing immune effector cells, e.g., T cells or NK cells, e.g., prior to administration to a subject, can be cultured in the presence of a low, immune enhancing, dose of an mTOR inhibitor.

In an embodiment, administering to the subject a low, immune enhancing, dose of an mTOR inhibitor comprises administering, e.g., once per week, e.g., in an immediate release dosage form, 0.1 to 20, 0.5 to 10, 2.5 to 7.5, 3 to 6, or about 5, mgs of RAD001, or a bioequivalent dose thereof. In an embodiment, administering to the subject a low, immune enhancing, dose of an mTOR inhibitor comprises administering, e.g., once per week, e.g., in a sustained release dosage form, 0.3 to 60, 1.5 to 30, 7.5 to 22.5, 9 to 18, or about 15 mgs of RAD001, or a bioequivalent dose thereof.

In an embodiment, a dose of an mTOR inhibitor is associated with, or provides, mTOR inhibition of at least 5 but no more than 90%, at least 10 but no more than 90%, at least 15, but no more than 90%, at least 20 but no more than 90%, at least 30 but no more than 90%, at least 40 but no more than 90%, at least 50 but no more than 90%, at least 60 but no more than 90%, at least 70 but no more than 90%, at least 5 but no more than 80%, at least 10 but no more than 80%, at least 15, but no more than 80%, at least 20 but no more than 80%, at least 30 but no more than 80%, at least 40 but no more than 80%, at least 50 but no more than 80%, at least 60 but no more than 80%, at least 5 but no more than 70%, at least 10 but no more than 70%, at least 15, but no more than 70%, at least 20 but no more than 70%, at least 30 but no more than 70%, at least 40 but no more than 70%, at least 50 but no more than 70%, at least 5 but no more than 60%, at least 10 but no more than 60%, at least 15, but no more

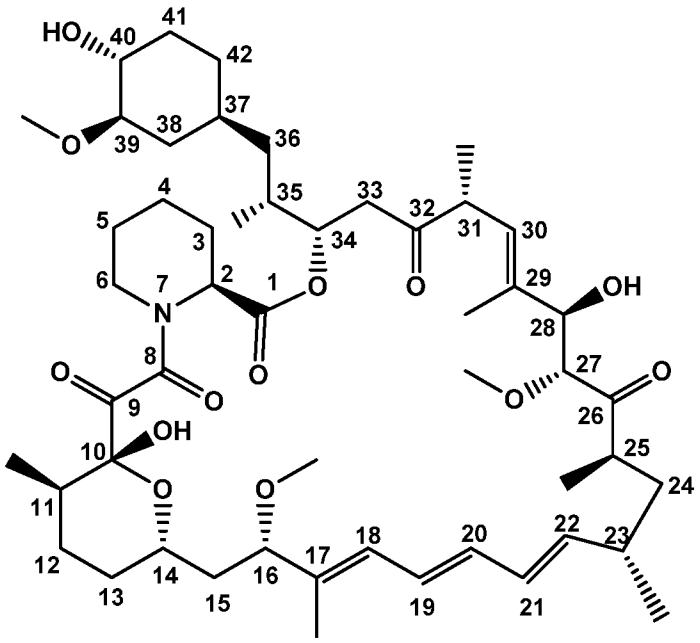
than 60%, at least 20 but no more than 60%, at least 30 but no more than 60%, at least 40 but no more than 60%, at least 5 but no more than 50%, at least 10 but no more than 50%, at least 15, but no more than 50%, at least 20 but no more than 50%, at least 30 but no more than 50%, at least 40 but no more than 50%, at least 5 but no more than 40%, at least 10 but no more than 40%, at least 15, but no more than 40%, at least 20 but no more than 40%, at least 30 but no more than 40%, at least 35 but no more than 40%, at least 5 but no more than 30%, at least 10 but no more than 30%, at least 15, but no more than 30%, at least 20 but no more than 30%, or at least 25 but no more than 30%.

In an embodiment, administering to the subject a low, immune enhancing, dose of an mTOR inhibitor comprises administering, e.g., once per week, e.g., in an immediate release dosage form, 0.1 to 20, 0.5 to 10, 2.5 to 7.5, 3 to 6, or about 5, mgs of RAD001, or a bioequivalent dose thereof. In an embodiment, administering to the subject a low, immune enhancing, dose of an mTOR inhibitor comprises administering, e.g., once per week, e.g., in a sustained release dosage form, 0.3 to 60, 1.5 to 30, 7.5 to 22.5, 9 to 18, or about 15 mgs of RAD001, or a bioequivalent dose thereof.

The extent of mTOR inhibition can be conveyed as, or corresponds to, the extent of P70 S6 kinase inhibition, e.g., the extent of mTOR inhibition can be determined by the level of decrease in P70 S6 kinase activity, e.g., by the decrease in phosphorylation of a P70 S6 kinase substrate. The level of mTOR inhibition can be evaluated by various methods, such as measuring P70 S6 kinase activity by the Boulay assay, as described in U.S. Patent Application No. 2015/01240036, hereby incorporated by reference, or as described in U.S. Patent No. 7,727,950, hereby incorporated by reference; measuring the level of phosphorylated S6 by western blot; or evaluating a change in the ratio of PD1 negative immune effector cells to PD1 positive immune effector cells.

As used herein, the term "mTOR inhibitor" refers to a compound or ligand, or a pharmaceutically acceptable salt thereof, which inhibits the mTOR kinase in a cell. In an embodiment, an mTOR inhibitor is an allosteric inhibitor. Allosteric mTOR inhibitors include the neutral tricyclic compound rapamycin (sirolimus), rapamycin-related compounds, that is compounds having structural and functional similarity to rapamycin including, e.g., rapamycin derivatives, rapamycin analogs (also referred to as rapalogs) and other macrolide compounds that inhibit mTOR activity. In an embodiment, an mTOR inhibitor is a catalytic inhibitor.

Rapamycin is a known macrolide antibiotic produced by Streptomyces hygroscopicus having the structure shown in Formula A.



(A)

See, e.g., McAlpine, J.B., et al., J. Antibiotics (1991) 44: 688; Schreiber, S.L., et al., J. Am. Chem. Soc. (1991) 113: 7433; U.S. Patent No. 3,929,992. There are various numbering schemes proposed for rapamycin. To avoid confusion, when specific rapamycin analogs are named herein, the names are given with reference to rapamycin using the numbering scheme of formula A.

Rapamycin analogs useful in the invention are, for example, O-substituted analogs in which the hydroxyl group on the cyclohexyl ring of rapamycin is replaced by OR_1 in which R_1 is hydroxyalkyl, hydroxyalkoxyalkyl, acylaminoalkyl, or aminoalkyl; e.g. RAD001, also known as everolimus, as described in US 5,665,772 and WO94/09010, the contents of each are incorporated by reference.

Other suitable rapamycin analogs include those substituted at the 26- or 28-position. The rapamycin analog may be an epimer of an analog mentioned above, particularly an epimer of an analog substituted in position 40, 28 or 26, and may optionally be further hydrogenated, e.g. as described in US 6,015,815, WO95/14023 and WO99/15530 the contents of which are incorporated by reference, e.g. ABT578 also known as zotarolimus or a rapamycin analog described in US 7,091,213, WO98/02441 and WO01/14387 the contents of which are incorporated by reference, e.g. AP23573 also known as ridaforolimus.

Examples of rapamycin analogs suitable for use in the present invention from US 5,665,772 include, but are not limited to, 40-O-benzyl-rapamycin, 40-O-(4'-hydroxymethyl)benzyl-rapamycin, 40-O-[4'-(1,2-dihydroxyethyl)]benzyl-rapamycin, 40-O-allyl-rapamycin, 40-O-[3'-(2,2-dimethyl-1,3-dioxolan-4(S)-yl)-prop-2'-en-1'-yl]-rapamycin, (2'E,4'S)-40-O-(4',5'-dihydroxypent-2'-en-1'-yl)-rapamycin, 40-O-(2-hydroxy)ethoxycarbonylmethyl-rapamycin, 40-O-(2-hydroxy)ethyl-rapamycin , 40-

O-(3-hydroxy)propyl-rapamycin, 40-O-(6-hydroxy)hexyl-rapamycin, 40-O-[2-(2-hydroxy)ethoxy]ethyl-rapamycin, 40-O-[(3S)-2,2-dimethyldioxolan-3-yl]methyl-rapamycin, 40-O-[(2S)-2,3-dihydroxyprop-1-yl]-rapamycin, 40-O-(2-acetoxy)ethyl-rapamycin, 40-O-(2-nicotinoyloxy)ethyl-rapamycin, 40-O-[2-(N-morpholino)acetoxy]ethyl-rapamycin, 40-O-(2-N-imidazolylacetoxy)ethyl-rapamycin, 40-O-[2-(N-methyl-N'-piperazinyl)acetoxy]ethyl-rapamycin, 39-O-desmethyl-39,40-O,O-ethylene-rapamycin, (26R)-26-dihydro-40-O-(2-hydroxy)ethyl-rapamycin, 40-O-(2-aminoethyl)-rapamycin, 40-O-(2-acetaminoethyl)-rapamycin, 40-O-(2-nicotinamidoethyl)-rapamycin, 40-O-(2-(N-methyl-imidazo-2'-ylcarbethoxamido)ethyl)-rapamycin, 40-O-(2-ethoxycarbonylaminoethyl)-rapamycin, 40-O-(2-tolylsulfonamidoethyl)-rapamycin and 40-O-[2-(4',5'-dicarboethoxy-1',2',3'-triazol-1'-yl)-ethyl]-rapamycin.

Other rapamycin analogs useful in the present invention are analogs where the hydroxyl group on the cyclohexyl ring of rapamycin and/or the hydroxy group at the 28 position is replaced with an hydroxyester group are known, for example, rapamycin analogs found in US RE44,768, e.g. temsirolimus.

Other rapamycin analogs useful in the preset invention include those wherein the methoxy group at the 16 position is replaced with another substituent, preferably (optionally hydroxy-substituted) alkynyloxy, benzyl, orthomethoxybenzyl or chlorobenzyl and/or wherein the mexthoxy group at the 39 position is deleted together with the 39 carbon so that the cyclohexyl ring of rapamycin becomes a cyclopentyl ring lacking the 39 position methyoxy group; e.g. as described in WO95/16691 and WO96/41807, the contents of which are incorporated by reference. The analogs can be further modified such that the hydroxy at the 40-position of rapamycin is alkylated and/or the 32-carbonyl is reduced.

Rapamycin analogs from WO95/16691 include, but are not limited to, 16-demthoxy-16-(pent-2-ynyl)oxy-rapamycin, 16-demthoxy-16-(but-2-ynyl)oxy-rapamycin, 16-demthoxy-16-(propargyl)oxy-rapamycin, 16-demethoxy-16-(4-hydroxy-but-2-ynyl)oxy-rapamycin, 16-demthoxy-16-benzyloxy-40-O-(2-hydroxyethyl)-rapamycin, 16-demthoxy-16-benzyloxy-rapamycin, 16-demethoxy-16-ortho-methoxybenzyl-rapamycin, 16-demethoxy-40-O-(2-methoxyethyl)-16-pent-2-ynyl)oxy-rapamycin, 39-demethoxy-40-desoxy-39-formyl-42-nor-rapamycin, 39-demethoxy-40-desoxy-39-hydroxymethyl-42-nor-rapamycin, 39-demethoxy-40-desoxy-39-carboxy-42-nor-rapamycin, 39-demethoxy-40-desoxy-39-(4-methyl-piperazin-1-yl)carbonyl-42-nor-rapamycin, 39-demethoxy-40-desoxy-39-(morpholin-4-yl)carbonyl-42-nor-rapamycin, 39-demethoxy-40-desoxy-39-[N-methyl, N-(2-pyridin-2-yl-ethyl)]carbamoyl-42-nor-rapamycin and 39-demethoxy-40-desoxy-39-(p-toluenesulfonylhydrazonomethyl)-42-nor-rapamycin.

Rapamycin analogs from WO96/41807 include, but are not limited to, 32-deoxo-rapamycin, 16-O-pent-2-ynyl-32-deoxo-rapamycin, 16-O-pent-2-ynyl-32-deoxo-40-O-(2-hydroxy-ethyl)-rapamycin,

16-O-pent-2-ynyl-32-(S)-dihydro-40-O-(2-hydroxyethyl)-rapamycin, 32(S)-dihydro-40-O-(2-methoxy)ethyl-rapamycin and 32(S)-dihydro-40-O-(2-hydroxyethyl)-rapamycin.

Another suitable rapamycin analog is umirolimus as described in US2005/0101624 the contents of which are incorporated by reference.

RAD001, otherwise known as everolimus (Afinitor®), has the chemical name (1R,9S,12S,15R,16E,18R,19R,21R,23S,24E,26E,28E,30S,32S,35R)-1,18-dihydroxy-12-{(1R)-2-[(1S,3R,4R)-4-(2-hydroxyethoxy)-3-methoxycyclohexyl]-1-methylethyl}-19,30-dimethoxy-15,17,21,23,29,35-hexamethyl-11,36-dioxa-4-aza-tricyclo[30.3.1.04,9]hexatriaconta-16,24,26,28-tetraene-2,3,10,14,20-pentaone, as described in US 5,665,772 and WO94/09010, the contents of each are incorporated by reference.

Further examples of allosteric mTOR inhibitors include sirolimus (rapamycin, AY-22989), 40-[3-hydroxy-2-(hydroxymethyl)-2-methylpropanoate]-rapamycin (also called temsirolimus or CCI-779) and ridaforolimus (AP-23573/MK-8669). Other examples of allosteric mTor inhibtors include zotarolimus (ABT578) and umirolimus.

Alternatively or additionally, catalytic, ATP-competitive mTOR inhibitors have been found to target the mTOR kinase domain directly and target both mTORC1 and mTORC2. These are also more effective inhibitors of mTORC1 than such allosteric mTOR inhibitors as rapamycin, because they modulate rapamycin-resistant mTORC1 outputs such as 4EBP1-T37/46 phosphorylation and cap-dependent translation.

Catalytic inhibitors include: BEZ235 or 2-methyl-2-[4-(3-methyl-2-oxo-8-quinolin-3-yl-2,3-dihydro-imidazo[4,5-c]quinolin-1-yl)-phenyl]-propionitrile, or the monotosylate salt form (the synthesis of BEZ235 is described in WO2006/122806); CCG168 (otherwise known as AZD-8055, Chresta, C.M., et al., Cancer Res, 2010, 70(1), 288-298) which has the chemical name {5-[2,4-bis-((S)-3-methyl-morpholin-4-yl)-pyrido[2,3d]pyrimidin-7-yl]-2-methoxy-phenyl}-methanol; 3-[2,4-bis[(3S)-3-methylmorpholin-4-yl]pyrido[2,3-d]pyrimidin-7-yl]-N-methylbenzamide (WO09104019); 3-(2-aminobenzo[d]oxazol-5-yl)-1-isopropyl-1H-pyrazolo[3,4-d]pyrimidin-4-amine (WO10051043 and WO2013023184); A N-(3-(N-(3-((3,5-dimethoxyphenyl)amino)quinoxaline-2-yl)sulfamoyl)phenyl)-3-methoxy-4-methylbenzamide (WO07044729 and WO12006552); PKI-587 (Venkatesan, A.M., J. Med.Chem., 2010, 53, 2636-2645) which has the chemical name 1-[4-[4-(dimethylamino)piperidine-1-carbonyl]phenyl]-3-[4-(4,6-dimorpholino-1,3,5-triazin-2-yl)phenyl]urea; GSK-2126458 (ACS Med. Chem. Lett., 2010, 1, 39-43) which has the chemical name 2,4-difluoro-N-{2-methoxy-5-[4-(4-pyridazinyl)-6-quinolinyl]-3-pyridinyl}benzenesulfonamide; 5-(9-isopropyl-8-methyl-2-morpholino-9H-purin-6-yl)pyrimidin-2-amine (WO10114484); and (E)-N-(8-(6-amino-5-(trifluoromethyl)pyridin-3-yl)-1-(6-(2-cyanopropan-2-yl)pyridin-3-yl)-3-methyl-1H-imidazo[4,5-c]quinolin-2(3H)-ylidene)cyanamide (WO12007926).

Further examples of catalytic mTOR inhibitors include 8-(6-methoxy-pyridin-3-yl)-3-methyl-1-(4-piperazin-1-yl-3-trifluoromethyl-phenyl)-1,3-dihydro-imidazo[4,5-c]quinolin-2-one (WO2006/122806) and Ku-0063794 (Garcia-Martinez JM, et al.,Biochem J., 2009, 421(1), 29-42. Ku-0063794 is a specific inhibitor of the mammalian target of rapamycin (mTOR).) WYE-354 is another example of a catalytic mTOR inhibitor (Yu K, et al. (2009). Biochemical, Cellular, and In vivo Activity of Novel ATP-Competitive and Selective Inhibitors of the Mammalian Target of Rapamycin. Cancer Res. 69(15): 6232-6240).

mTOR inhibitors useful according to the present invention also include prodrugs, derivatives, pharmaceutically acceptable salts, or analogs thereof of any of the foregoing.

mTOR inhibitors, such as RAD001, may be formulated for delivery based on well-established methods in the art based on the particular dosages described herein. In particular, US Patent No. 6,004,973 (incorporated herein by reference) provides examples of formulations useable with the mTOR inhibitors described herein.

Methods and Biomarkers for Evaluating CAR-Effectiveness or Sample Suitability

In another aspect, the invention features a method of evaluating or monitoring the effectiveness of a CAR-expressing cell therapy (e.g., a BCMA CAR therapy), in a subject (e.g., a subject having a cancer, e.g., a hematological cancer), or the suitability of a sample (e.g., an apheresis sample) for a CAR therapy (e.g., a BCMA CAR therapy). The method includes acquiring a value of effectiveness to the CAR therapy, or sample suitability, wherein said value is indicative of the effectiveness or suitability of the CAR-expressing cell therapy.

In embodiments, the value of effectiveness to the CAR therapy, or sample suitability, comprises a measure of one, two, three, four, five, six or more (all) of the following:

(i) the level or activity of one, two, three, or more (*e.g.*, all) of resting T_{EFF} cells, resting T_{REG} cells, younger T cells (*e.g.*, younger CD4 or CD8 cells, or gamma/delta T cells), or early memory T cells, or a combination thereof, in a sample (e.g., an apheresis sample or a manufactured CAR-expressing cell product sample);

(ii) the level or activity of one, two, three, or more (*e.g.*, all) of activated T_{EFF} cells, activated T_{REG} cells, older T cells (*e.g.*, older CD4 or CD8 cells), or late memory T cells, or a combination thereof, in a sample (e.g., an apheresis sample or a manufactured CAR-expressing cell product sample);

(iii) the level or activity of an immune cell exhaustion marker, *e.g.*, one, two or more immune checkpoint inhibitors (e.g., PD-1, PD-L1, TIM-3 and/or LAG-3) in a sample (e.g., an apheresis sample or a manufactured CAR-expressing cell product sample). In one embodiment, an immune cell has an exhausted phenotype, *e.g.*, co-expresses at least two exhaustion markers, *e.g.*, co-expresses PD-1 and

TIM-3. In other embodiments, an immune cell has an exhausted phenotype, *e.g.*, co-expresses at least two exhaustion markers, *e.g.*, co-expresses PD-1 and LAG-3;

(iv) the level or activity of CD27 and/or CD45RO- (e.g., CD27+ CD45RO-) immune effector cells, e.g., in a CD4+ or a CD8+ T cell population, in a sample (e.g., an apheresis sample or a manufactured CAR-expressing cell product sample);

(v) the level or activity of one, two, three, four, five, ten, twenty or more of the biomarkers chosen from CCL20, IL-17a and/or IL-6, PD-1, PD-L1, LAG-3, TIM-3, CD57, CD27, CD122, CD62L, KLRG1;

(vi) a cytokine level or activity (*e.g.*, quality of cytokine reportoire) in a CAR-expressing cell product sample, e.g., BCMA- expressing cell product sample; or

(vii) a transduction efficiency of a CAR-expressing cell in a manufactured CAR-expressing cell product sample.

In some embodiments of any of the methods disclosed herein, the CAR-expressing cell therapy comprises a plurality (e.g., a population) of CAR-expressing immune effector cells, e.g., a plurality (e.g., a population) of T cells or NK cells, or a combination thereof. In one embodiment, the CAR-expressing cell therapy is a BCMACAR therapy.

In some embodiments of any of the methods disclosed herein, the measure of one or more of (i)-(vii) is obtained from an apheresis sample acquired from the subject. The apheresis sample can be evaluated prior to infusion or re-infusion.

In some embodiments of any of the methods disclosed herein, the measure of one or more of (i)-(vii) is obtained from a manufactured CAR-expressing cell product sample, e.g., BCMACAR-expressing cell product sample. The manufactured CAR-expressing cell product can be evaluated prior to infusion or re-infusion.

In some embodiments of any of the methods disclosed herein, the subject is evaluated prior to receiving, during, or after receiving, the CAR-expressing cell therapy.

In some embodiments of any of the methods disclosed herein, the measure of one or more of (i)-(vii) evaluates a profile for one or more of gene expression, flow cytometry or protein expression.

In some embodiments of any of the methods disclosed herein, the method further comprises identifying the subject as a responder, a non-responder, a relapser or a non-relapser, based on a measure of one or more of (i)-(vii).

In some embodiments of any of the methods disclosed herein, a responder (e.g., a complete responder) has, or is identified as having, a greater level or activity of one, two, or more (all) of GZMK, PPF1BP2, or naïve T cells as compared to a non-responder.

In some embodiments of any of the methods disclosed herein, a non-responder has, or is identified as having, a greater level or activity of one, two, three, four, five, six, seven, or more (e.g., all) of IL22, IL-2RA, IL-21, IRF8, IL8, CCL17, CCL22, effector T cells, or regulatory T cells, as compared to a responder.

In an embodiment, a relapser is a patient having, or who is identified as having, an increased level of expression of one or more of (e.g., 2, 3, 4, or all of) the following genes, compared to non relapsers: MIR199A1, MIR1203, uc021ovp, ITM2C, and HLA-DQB1 and/or a decreased levels of expression of one or more of (e.g., 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, or all of) the following genes, compared to non relapsers: PPIAL4D, TTTY10, TXLNG2P, MIR4650-1, KDM5D, USP9Y, PRKY, RPS4Y2, RPS4Y1, NCRNA00185, SULT1E1, and EIF1AY.

In some embodiments of any of the methods disclosed herein, a complete responder has, or is identified as having, a greater, e.g., a statistically significant greater, percentage of CD8+ T cells compared to a reference value, e.g., a non-responder percentage of CD8+ T cells.

In some embodiments of any of the methods disclosed herein, a complete responder has, or is identified as having, a greater percentage of CD27+ CD45RO- immune effector cells, e.g., in the CD8+ population, compared to a reference value, e.g., a non-responder number of CD27+ CD45RO- immune effector cells.

In some embodiments of any of the methods disclosed herein, a complete responder or a partial responder has, or is identified as having, a greater, e.g., a statistically significant greater, percentage of CD4+ T cells compared to a reference value, e.g., a non-responder percentage of CD4+ T cells.

In some embodiments of any of the methods disclosed herein, a complete responder has, or is identified as having, a greater percentage of one, two, three, or more (*e.g.*, all) of resting T_{EFF} cells, resting T_{REG} cells, younger T cells (*e.g.*, younger CD4 or CD8 cells, or gamma/delta T cells), or early memory T cells, or a combination thereof, compared to a reference value, e.g., a non-responder number of resting T_{EFF} cells, resting T_{REG} cells, younger T cells (*e.g.*, younger CD4 or CD8 cells), or early memory T cells.

In some embodiments of any of the methods disclosed herein, a non-responder has, or is identified as having, a greater percentage of one, two, three, or more (*e.g.*, all) of activated T_{EFF} cells, activated T_{REG} cells, older T cells (*e.g.*, older CD4 or CD8 cells), or late memory T cells, or a combination thereof, compared to a reference value, e.g., a responder number of activated T_{EFF} cells, activated T_{REG} cells, older T cells (*e.g.*, older CD4 or CD8 cells), or late memory T cells.

In some embodiments of any of the methods disclosed herein, a non-responder has, or is identified as having, a greater percentage of an immune cell exhaustion marker, *e.g.*, one, two or more immune checkpoint inhibitors (e.g., PD-1, PD-L1, TIM-3 and/or LAG-3). In one embodiment, a non-responder has, or is identified as having, a greater percentage of PD-1, PD-L1, or LAG-3 expressing immune effector cells (e.g., CD4+ T cells and/or CD8+ T cells) (e.g., CAR-expressing CD4+ cells and/or CD8+ T cells) compared to the percentage of PD-1 or LAG-3 expressing immune effector cells from a responder.

In one embodiment, a non-responder has, or is identified as having, a greater percentage of immune cells having an exhausted phenotype, *e.g.*, immune cells that co-express at least two exhaustion markers, *e.g.*, co-expresses PD-1, PD-L1 and/or TIM-3. In other embodiments, a non-responder has, or is identified as having, a greater percentage of immune cells having an exhausted phenotype, *e.g.*, immune cells that co-express at least two exhaustion markers, *e.g.*, co-expresses PD-1 and LAG-3.

In some embodiments of any of the methods disclosed herein, a non-responder has, or is identified as having, a greater percentage of PD-1/ PD-L1+/LAG-3+ cells in the CAR-expressing cell population (e.g., a BCMACAR+ cell population) compared to a responder (e.g., a complete responder) to the CAR-expressing cell therapy.

In some embodiments of any of the methods disclosed herein, a partial responder has, or is identified as having, a higher percentages of PD-1/ PD-L1+/LAG-3+ cells, than a responder, in the CAR-expressing cell population (e.g., a BCMACAR+ cell population).

In some embodiments of any of the methods disclosed herein, a non-responder has, or is identified as having, an exhausted phenotype of PD1/ PD-L1+ CAR+ and co-expression of LAG3 in the CAR-expressing cell population (e.g., a BCMACAR + cell population).

In some embodiments of any of the methods disclosed herein, a non-responder has, or is identified as having, a greater percentage of PD-1/ PD-L1+/TIM-3+ cells in the CAR-expressing cell population (e.g., a BCMACAR + cell population) compared to the responder (e.g., a complete responder).

In some embodiments of any of the methods disclosed herein, a partial responders has, or is identified as having, a higher percentage of PD-1/ PD-L1+/TIM-3+ cells, than responders, in the CAR-expressing cell population (e.g., a BCMACAR + cell population).

In some embodiments of any of the methods disclosed herein, the presence of CD8+ CD27+ CD45RO- T cells in an apheresis sample is a positive predictor of the subject response to a CAR-expressing cell therapy (e.g., a BCMACAR therapy).

In some embodiments of any of the methods disclosed herein, a high percentage of PD1+ CAR+ and LAG3+ or TIM3+ T cells in an apheresis sample is a poor prognostic predictor of the subject response to a CAR-expressing cell therapy (e.g., a BCMACAR therapy).

In some embodiments of any of the methods disclosed herein, the responder (e.g., the complete or partial responder) has one, two, three or more (or all) of the following profile:

(i) has a greater number of CD27+ immune effector cells compared to a reference value, e.g., a non-responder number of CD27+ immune effector cells;

(ii) (i) has a greater number of CD8+ T cells compared to a reference value, e.g., a non-responder number of CD8+ T cells;

(iii) has a lower number of immune cells expressing one or more checkpoint inhibitors, e.g., a checkpoint inhibitor chosen from PD-1, PD-L1, LAG-3, TIM-3, or KLRG-1, or a combination, compared to a reference value, e.g., a non-responder number of cells expressing one or more checkpoint inhibitors; or

(iv) has a greater number of one, two, three, four or more (all) of resting T_{EFF} cells, resting T_{REG} cells, naïve CD4 cells, unstimulated memory cells or early memory T cells, or a combination thereof, compared to a reference value, e.g., a non-responder number of resting T_{EFF} cells, resting T_{REG} cells, naïve CD4 cells, unstimulated memory cells or early memory T cells.

In some embodiments of any of the methods disclosed herein, the cytokine level or activity of (vi) is chosen from one, two, three, four, five, six, seven, eight, or more (or all) of cytokine CCL20/MIP3a, IL17A, IL6, GM-CSF, IFNγ, IL10, IL13, IL2, IL21, IL4, IL5, IL9 or TNFα, or a combination thereof. The cytokine can be chosen from one, two, three, four or more (all) of IL-17a, CCL20, IL2, IL6, or TNFa. In one embodiment, an increased level or activity of a cytokine is chosen from one or both of IL-17a and CCL20, is indicative of increased responsiveness or decreased relapse.

In some embodiments of any of the methods disclosed herein, a transduction efficiency of 15% or higher in (vii) is indicative of increased responsiveness or decreased relapse.

In some embodiments of any of the methods disclosed herein, a transduction efficiency of less than 15% in (vii) is indicative of decreased responsiveness or increased relapse.

In embodiments, the responder, a non-responder, a relapser or a non-relapser identified by the methods herein can be further evaluated according to clinical criteria. For example, a complete responder has, or is identified as, a subject having a disease, e.g., a cancer, who exhibits a complete response, e.g., a complete remission, to a treatment. A complete response may be identified, e.g., using the NCCN Guidelines®, or Cheson et al, J Clin Oncol 17:1244 (1999) and Cheson et al., "Revised Response Criteria for Malignant Lymphoma", J Clin Oncol 25:579-586 (2007) (both of which are incorporated by reference herein in their entireties), as described herein. A partial responder has, or is identified as, a subject having a disease, e.g., a cancer, who exhibits a partial response, e.g., a partial remission, to a treatment. A partial response may be identified, e.g., using the NCCN Guidelines®, or Cheson criteria as described herein. A non-responder has, or is identified as, a subject having a disease, e.g., a cancer, who does not exhibit a response to a treatment, e.g., the patient has stable disease or

progressive disease. A non-responder may be identified, e.g., using the NCCN Guidelines®, or Cheson criteria as described herein.

Alternatively, or in combination with the methods disclosed herein, responsive to said value, performing one, two, three four or more of:

administering e.g., to a responder or a non-relapser, a CAR-expressing cell therapy;

administered an altered dosing of a CAR-expressing cell therapy;

altering the schedule or time course of a CAR-expressing cell therapy;

administering, e.g., to a non-responder or a partial responder, an additional agent in combination with a CAR-expressing cell therapy, e.g., a checkpoint inhibitor, e.g., a checkpoint inhibitor described herein;

administering to a non-responder or partial responder a therapy that increases the number of younger T cells in the subject prior to treatment with a CAR-expressing cell therapy;

modifying a manufacturing process of a CAR-expressing cell therapy, e.g., enriching for younger T cells prior to introducing a nucleic acid encoding a CAR, or increasing the transduction efficiency, e.g., for a subject identified as a non-responder or a partial responder;

administering an alternative therapy, e.g., for a non-responder or partial responder or relapser; or

if the subject is, or is identified as, a non-responder or a relapser, decreasing the T_{REG} cell population and/or T_{REG} gene signature, *e.g.*, by one or more of CD25 depletion, administration of cyclophosphamide, anti-GITR antibody, or a combination thereof.

In certain embodiments, the subject is pre-treated with an anti-GITR antibody. In certain embodiment, the subject is treated with an anti-GITR antibody prior to infusion or re-infusion.

Biopolymer delivery methods

In some embodiments, one or more CAR-expressing cells as disclosed herein can be administered or delivered to the subject via a biopolymer scaffold, e.g., a biopolymer implant. Biopolymer scaffolds can support or enhance the delivery, expansion, and/or dispersion of the CAR-expressing cells described herein. A biopolymer scaffold comprises a biocompatible (e.g., does not substantially induce an inflammatory or immune response) and/or a biodegradable polymer that can be naturally occurring or synthetic.

Examples of suitable biopolymers include, but are not limited to, agar, agarose, alginate, alginate/calcium phosphate cement (CPC), beta-galactosidase (β-GAL), (1 ,2,3,4,6-pentaacetyl a-D-galactose), cellulose, chitin, chitosan, collagen, elastin, gelatin, hyaluronic acid collagen, hydroxyapatite, poly(3-hydroxybutyrate-co-3-hydroxy-hexanoate) (PHBHHx), poly(lactide), poly(caprolactone) (PCL), poly(lactide-co-glycolide) (PLG), polyethylene oxide (PEO), poly(lactic-co-glycolic acid) (PLGA), polypropylene oxide (PPO), polyvinyl alcohol) (PVA), silk, soy protein, and soy protein isolate, alone or in combination with any other polymer composition, in any concentration and in any ratio. The biopolymer can be augmented or modified with adhesion- or migration-promoting molecules, e.g., collagen-mimetic peptides that bind to the collagen receptor of lymphocytes, and/or stimulatory molecules to enhance the delivery, expansion, or function, e.g., anti-cancer activity, of the cells to be delivered. The biopolymer scaffold can be an injectable, e.g., a gel or a semi-solid, or a solid composition.

In some embodiments, CAR-expressing cells described herein are seeded onto the biopolymer scaffold prior to delivery to the subject. In embodiments, the biopolymer scaffold further comprises one or more additional therapeutic agents described herein (e.g., another CAR-expressing cell, an antibody, or a small molecule) or agents that enhance the activity of a CAR-expressing cell, e.g., incorporated or conjugated to the biopolymers of the scaffold. In embodiments, the biopolymer scaffold is injected, e.g., intratumorally, or surgically implanted at the tumor or within a proximity of the tumor sufficient to mediate an anti-tumor effect. Additional examples of biopolymer compositions and methods for their delivery are described in Stephan et al., *Nature Biotechnology*, 2015, 33:97-101; and WO2014/110591.

Pharmaceutical compositions and treatments

Pharmaceutical compositions of the present invention may comprise a CAR-expressing cell, e.g., a plurality of CAR-expressing cells, as described herein, in combination with one or more pharmaceutically or physiologically acceptable carriers, diluents or excipients. Such compositions may comprise buffers such as neutral buffered saline, phosphate buffered saline and the like; carbohydrates such as glucose, mannose, sucrose or dextrans, mannitol; proteins; polypeptides or amino acids such as glycine; antioxidants; chelating agents such as EDTA or glutathione; adjuvants (e.g., aluminum hydroxide); and preservatives. Compositions of the present invention are in one aspect formulated for intravenous administration.

Pharmaceutical compositions of the present invention may be administered in a manner appropriate to the disease to be treated (or prevented). The quantity and frequency of administration will

be determined by such factors as the condition of the patient, and the type and severity of the patient's disease, although appropriate dosages may be determined by clinical trials.

In one embodiment, the pharmaceutical composition is substantially free of, e.g., there are no detectable levels of a contaminant, e.g., selected from the group consisting of endotoxin, mycoplasma, replication competent lentivirus (RCL), p24, VSV-G nucleic acid, HIV gag, residual anti-CD3/anti-CD28 coated beads, mouse antibodies, pooled human serum, bovine serum albumin, bovine serum, culture media components, vector packaging cell or plasmid components, a bacterium and a fungus. In one embodiment, the bacterium is at least one selected from the group consisting of Alcaligenes faecalis, Candida albicans, Escherichia coli, Haemophilus influenza, Neisseria meningitides, Pseudomonas aeruginosa, Staphylococcus aureus, Streptococcus pneumonia, and Streptococcus pyogenes group A.

When "an immunologically effective amount," "an anti-tumor effective amount," "a tumor-inhibiting effective amount," or "therapeutic amount" is indicated, the precise amount of the compositions of the present invention to be administered can be determined by a physician with consideration of individual differences in age, weight, tumor size, extent of infection or metastasis, and condition of the patient (subject). It can generally be stated that a pharmaceutical composition comprising the T cells described herein may be administered at a dosage of 10^4 to 10^9 cells/kg body weight, in some instances 10^5 to 10^6 cells/kg body weight, including all integer values within those ranges. T cell compositions may also be administered multiple times at these dosages. The cells can be administered by using infusion techniques that are commonly known in immunotherapy (see, e.g., Rosenberg et al., New Eng. J. of Med. 319:1676, 1988).

In certain aspects, it may be desired to administer activated T cells to a subject and then subsequently redraw blood (or have an apheresis performed), activate T cells therefrom according to the present invention, and reinfuse the patient with these activated and expanded T cells. This process can be carried out multiple times every few weeks. In certain aspects, T cells can be activated from blood draws of from 10cc to 400cc. In certain aspects, T cells are activated from blood draws of 20cc, 30cc, 40cc, 50cc, 60cc, 70cc, 80cc, 90cc, or 100cc.

The administration of the subject compositions may be carried out in any convenient manner, including by aerosol inhalation, injection, ingestion, transfusion, implantation or transplantation. The compositions described herein may be administered to a patient trans arterially, subcutaneously, intradermally, intratumorally, intranodally, intramedullary, intramuscularly, by intravenous (i.v.) injection, or intraperitoneally. In one aspect, the T cell compositions of the present invention are administered to a patient by intradermal or subcutaneous injection. In one aspect, the CAR-expressing

cell (e.g., T cell or NK cell) compositions of the present invention are administered by i.v. injection. The compositions of CAR-expressing cells (e.g., T cells or NK cells) may be injected directly into a tumor, lymph node, or site of infection.

In a particular exemplary aspect, subjects may undergo leukapheresis, wherein leukocytes are collected, enriched, or depleted ex vivo to select and/or isolate the cells of interest, e.g., immune effector cells (e.g., T cells or NK cells). These immune effector cell (e.g., T cell or NK cell) isolates may be expanded by methods known in the art and treated such that one or more CAR constructs of the invention may be introduced, thereby creating a CAR-expressing cell (e.g., CAR T cell or CAR-expressing NK cell)of the invention. Subjects in need thereof may subsequently undergo standard treatment with high dose chemotherapy followed by peripheral blood stem cell transplantation. In certain aspects, following or concurrent with the transplant, subjects receive an infusion of the expanded CAR-expressing cells (e.g., CAR T cells or NK cells) of the present invention. In an additional aspect, expanded cells are administered before or following surgery.

In embodiments, lymphodepletion is performed on a subject, e.g., prior to administering one or more cells that express a CAR described herein, e.g., a BCMA-binding CAR described herein. In embodiments, the lymphodepletion comprises administering one or more of melphalan, cytoxan, cyclophosphamide, and fludarabine.

The dosage of the above treatments to be administered to a patient will vary with the precise nature of the condition being treated and the recipient of the treatment. The scaling of dosages for human administration can be performed according to art-accepted practices. The dose for CAMPATH, for example, will generally be in the range 1 to about 100 mg for an adult patient, usually administered daily for a period between 1 and 30 days. The preferred daily dose is 1 to 10 mg per day although in some instances larger doses of up to 40 mg per day may be used (described in U.S. Patent No. 6,120,766).

In one embodiment, the CAR is introduced into immune effector cells (e.g., T cells or NK cells), e.g., using in vitro transcription, and the subject (e.g., human) receives an initial administration of CAR immune effector cells (e.g., T cells or NK cells)of the invention, and one or more subsequent administrations of the CAR immune effector cells (e.g., T cells or NK cells) of the invention, wherein the one or more subsequent administrations are administered less than 15 days, e.g., 14, 13, 12, 11, 10, 9, 8, 7, 6, 5, 4, 3, or 2 days after the previous administration. In one embodiment, more than one administration of the CAR immune effector cells (e.g., T cells or NK cells) of the invention are administered to the subject (e.g., human) per week, e.g., 2, 3, or 4 administrations of the CAR immune effector cells (e.g., T cells or NK cells) of the invention are administered per week. In one embodiment,

the subject (e.g., human subject) receives more than one administration of the CAR immune effector cells (e.g., T cells or NK cells) per week (e.g., 2, 3 or 4 administrations per week) (also referred to herein as a cycle), followed by a week of no CAR immune effector cells (e.g., T cells or NK cells) administrations, and then one or more additional administration of the CAR immune effector cells (e.g., T cells or NK cells) (e.g., more than one administration of the CAR immune effector cells (e.g., T cells or NK cells) per week) is administered to the subject. In another embodiment, the subject (e.g., human subject) receives more than one cycle of CAR immune effector cells (e.g., T cells or NK cells), and the time between each cycle is less than 10, 9, 8, 7, 6, 5, 4, or 3 days. In one embodiment, the CAR immune effector cells (e.g., T cells or NK cells) are administered every other day for 3 administrations per week. In one embodiment, the CAR immune effector cells (e.g., T cells or NK cells) of the invention are administered for at least two, three, four, five, six, seven, eight or more weeks.

In one aspect, BCMA CAR-expressing cells (e.g., BCMA CARTs or BCMA CAR-expressing NK cells) are generated using lentiviral viral vectors, such as lentivirus. CAR-expressing cells (e.g., CARTs or CAR-expressing NK cells) generated that way will have stable CAR expression.

In one aspect, CAR-expressing cells, e.g., CARTs, are generated using a viral vector such as a gammaretroviral vector, e.g., a gammaretroviral vector described herein. CARTs generated using these vectors can have stable CAR expression.

In one aspect, CAR-expressing cells (e.g., CARTs or CAR-expressing NK cells) transiently express CAR vectors for 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15 days after transduction. Transient expression of CARs can be effected by RNA CAR vector delivery. In one aspect, the CAR RNA is transduced into the cell, e.g., T cell or NK cell, by electroporation.

A potential issue that can arise in patients being treated using transiently expressing CAR-expressing cells (e.g., CARTs or CAR-expressing NK cells) (particularly with murine scFv bearing CAR-expressing cells (e.g., CARTs or CAR-expressing NK cells)) is anaphylaxis after multiple treatments.

Without being bound by this theory, it is believed that such an anaphylactic response might be caused by a patient developing humoral anti-CAR response, i.e., anti-CAR antibodies having an anti-IgE isotype. It is thought that a patient's antibody producing cells undergo a class switch from IgG isotype (that does not cause anaphylaxis) to IgE isotype when there is a ten to fourteen day break in exposure to antigen.

If a patient is at high risk of generating an anti-CAR antibody response during the course of transient CAR therapy (such as those generated by RNA transductions), CAR-expressing cell (e.g., CART or CAR-expressing NK cell) infusion breaks should not last more than ten to fourteen days.

EXAMPLES

The invention is further described in detail by reference to the following experimental examples. These examples are provided for purposes of illustration only, and are not intended to be limiting unless otherwise specified. Thus, the invention should in no way be construed as being limited to the following examples, but rather, should be construed to encompass any and all variations which become evident as a result of the teaching provided herein.

Without further description, it is believed that one of ordinary skill in the art can, using the preceding description and the following illustrative examples, make and utilize the molecules of the present invention and practice the claimed methods. The following working examples specifically point out various aspects of the present invention, and are not to be construed as limiting in any way the remainder of the disclosure.

Example 1

Summary

In this example, the effect of gamma secretase inhibitors (GSIs) on BCMA cell surface expression and BCMA shedding in vitro was examined. Treatment of BCMA-expressing cells with a GSI prevented shedding of BCMA into cell culture supernatants, which in turn increased cell surface expression of BCMA in vitro.

Materials and Methods

Human cancer cell lines expressing various levels of human BCMA (NCI-H929 $^{[High]}$, MM1S $^{[Med]}$, and U266B1 $^{[low]}$) were all transduced to constitutively express luciferase, which is used to measure cell viability/survival with the BrightGlo reagent (Promega # E2650).

BCMA expressing cell lines were treated overnight with and without GSIs. Cell culture supernatants were collected and quantified for levels of soluble BCMA by ELISA (R&D Systems, # DY193). Additionally, remaining cells were pelleted, stained with a PE conjugated BCMA antibody (clone 19F2, Biolegend # 357504) and analyzed by flow cytometry for BCMA cell surface expression levels.

Results

The level of soluble (shed) BCMA in cell culture supernatants was measured by ELISA following 24-hour treatment with or without 0.5μM DAPT. GSI treatment decreased levels of soluble BCMA in cultures to levels below the assay detection limit (Table 28).

Table 28. Gamma secretase inhibitor DAPT inhibits shedding of BCMA in vitro

ng/mL BMCA	NCI-H929	MM1S	RPM-8226
Untreated	137.5	40.8	49.8
0.5μM DAPT	<0.03	<0.03	<0.03

BCMA cell surface expression level was determined by flow cytometry on cell lines with or without GSI treatment. As shown in FIG. 1, GSI treatment resulted in a 3-15 fold increase in cell surface expression of BCMA compared to non-treated cells (3 fold in NCI-H929, 10 fold in MM1S, and 15 fold in U266B1).

Discussion

Table 28 shows the efficiency of DAPT at preventing the shedding of BCMA from the cell surface into the culture supernatant. This would be expected to enhance BCMA-directed therapies by removing a potential drug sink: binding of the therapies to soluble BCMA may neutralize their ability to bind and target cancer cells. FIG. 1 demonstrates that treatment of BCMA-expressing multiple myeloma cell lines with two different GSIs, DAPT and LY-411,575, strongly enhances cell surface expression of BCMA. Increased cell surface expression of BCMA provides more potential binding events of BCMA-directed therapies, thereby enhancing target cell killing.

The data strongly support the use of GSIs in combination with BCMA-targeted therapies, including but not limited to BCMA-targeting chimeric antigen receptor T cell therapy.

Example 2

In this example, the effect of gamma secretase inhibitors on BCMA cell surface expression and BCMA shedding was investigated in vitro. Treatment of BCMA expressing cells with a gamma secretase inhibitor (GSI) prevented shedding of the receptor into cell culture supernatants, which in turn increased cell surface expression of the receptor in vitro.

Materials and Methods

Target human cancer cell lines expressing various levels of human BCMA (NCI-H929$^{[High]}$, MM1S $^{[Med]}$, U266B1 $^{[low]}$) were all transduced to constitutively express luciferase, which is used to measure cell viability/survival with the BrightGlo reagent (Promega E2650). Target cells were plated

30,000 cells per well in a 96 well plate (Costar 3904) together with serial dilutions of a panel of gamma secretase inhibitors (Table 29). The plate was incubated at 37°C/5% CO_2 for 72 hours, followed by measurements of cell viability (BrightGlo, Promega # E2650) according to vendor protocol. In a replicate set of assay plates, cell culture supernatants were collected by centrifugation and quantified for levels of soluble BCMA by ELISA (R&D Systems, DY193), and the remaining cells were stained with a PE conjugated BCMA antibody (clone 19F2, Biolegend 357504) and analyzed by flow cytometry for BCMA cell surface expression levels.

Table 29. Gamma secretase inhibitors

Gamma Secretase Inhibitor (GSI)	**Developer**	**Supplier**	**item #**
LY-450139 (Semangacestat)	Lilly/Elan	Novartis	NVP-ANJ-889
PF-5212362 (Begacestat, GSI-953)	Pfizer (Wyeth)	Novartis	NVP-CGX-400
BMS-708163 (Avagacestat)	BMS	Novartis	NVP-CHG-662
LY-411575	Lilly	Sigma	SML0506-5mg
PF-3084014	Pfizer	Novartis	NVP-CHE-743
BMS-299897	BMS	R&D Systems	2870/10
DAPT	Lilly	Enzo	ALX-270-416-M005
RO4929097	Roche	Selleck	S1575-5MG
MK-0752	Merck	Novartis	NVP-LJR-137

Results

Luminescence values obtained from the BrightGlo assay were normalized to percent viability by taking the ratio of values from treated over untreated and plotted in Spotfire. All the GSI compounds were well tolerated at all the concentrations used (FIG. 2).

Raw OD450 values from the BCMA ELISA were plotted together with the mean fluorescent intensity values from flow cytometry analysis of cells stained with anti-BCMA antibody, and are shown for MM1S cells treated with the various GSIs in FIG. 3. Table 30 summarizes the EC50 results of all the GSIs on the three BCMA-expressing cell lines.

Table 30. EC50 [nM] summary of GSI inhibition of shed/soluble BCMA, and enhancement of membrane BCMA expression.

EC50, nM	H929		MM1S		U266B1	
	sBCMA	mBCMA	sBCMA	mBCMA	sBCMA	mBCMA
LY-450139	99.9	54.8	65.0	40.7	236.0	190.4
PF-5212362	136.6	13.8	120.9	52.2	583.0	560.0
BMS-708163	2.5	3.6	13.3	7.4	29.8	32.4
LY-411575	0.2	0.1	0.1	0.0	0.1	0.1
PF-3084014	34.8	31.6	32.2	36.6	67.2	70.4
BMS-299897	528.1	151.6	799.8	484.9		1687.7
DAPT	220.4	144.7	144.2	97.4	458.9	401.3

RO4929097	3.0	4.4	4.6	3.2	6.2	10.1
MK-0752	269.1	69.3	565.7	331.6	1056.8	1208.3

Discussion

It was demonstrated here that a broad panel of gamma secretase inhibitors, some reaching Phase III clinical trials, are all effective at inhibiting cleavage, and thereby shedding, of BCMA in vitro. This inhibition has been shown to result in increased membrane expression of BCMA in three different multiple myeloma cell lines, ideally maximizing the clinical efficacy of BCMA targeted therapies when used in combination. FIG. 2 suggests all of these inhibitors are well tolerated in vitro at effective concentrations.

Interestingly, all of the inhibitors show a correlation between effective concentrations that inhibit shedding of BCMA and that enhance membrane expression of BCMA. This correlation is exhibited by similar EC50 values in Table 30, as well as the overlapping infection points in FIG. 3. Among the panel of GSIs, efficacious concentrations vary considerably, with LY-411575 being the most potent, and BMS-299897 being the least. Effective concentrations also vary among the different cell lines tested, and may not necessarily be based on level of BCMA expression.

Taken together, these data strongly support the further development and use of gamma secretase inhibitors in combination with BCMA targeted therapies, including but not limited to BCMA-targeting chimeric antigen receptor T cell therapy.

Example 3

Part I- BCMA surface expression is enhanced whereas soluble BCMA is reduced after GSI treatment in Myeloma cell lines and primary samples.

A- Analysis of BCMA expression in MM cell lines and primary samples by flow cytometry

Materials and Methods:

Multiple myeloma (MM) cell lines U266 or RPMI 8226 were incubated with several concentrations (3μM-25μM) of Ly 411575 gamma secretase inhibitor (Miltenyi) for 48 hrs. Primary samples from MM patients (bone marrow) were incubated with 50μM of Ly 411575 gamma secretase inhibitor for 4 hrs (Miltenyi). Cells were stained with Human BCMA/ CD269-PE (clone 19F2, Biolegend 357503) or mouse IgG2a, κ PE isotype control. Primary samples from multiple myeloma patients were also stained with Live/dead dye (LifeTechnologies, L34960), and CD138-APC (eBioscience 17-1389-42). Data from stained samples were collected using a BD Fortessa cytometer. Flow cytometric analysis was performed using Flowjo v10 (Tree Star Inc).

Results:

BCMA was detected on the surface of the MM cell lines and primary samples as shown in FIGs. 4A and 4B. Moreover, BCMA expression was enhanced after 48hr treatment on MM cell lines or 4 hrs in primary samples, suggesting that utilizing the inhibitor one can regulate the amount of BCMA on the surface of MM cell lines (FIGs. 4A and 4B). These results provide strong support for the relevance of BCMA as a target in MM.

B- Analysis of soluble BCMA in MM cell lines by ELISA

Materials and Methods:

MM cell lines U266 or RPMI 8226 were incubated with several concentrations (6μM-100μM) of Ly 411575 gamma secretase inhibitor (Miltenyi) for 48 hrs. Supernatants were collected and secreted BCMA was measured using a sandwich ELISA (BCMA/TNFRSF17 ELISA Duo set, R&D Systems).

Results:

The ELISA showed that the gamma secretase inhibitor LY-411575 blocked the production of soluble BCMA, although the magnitude of its effect depended on the cell line that was tested. On the U266 cell line, LY-411575 slightly reduced the amount of soluble BCMA in the supernatant (FIG. 5A). Cells without treatment secreted 1250 pg/mL of BCMA, whereas cells treated secreted between 800 and 1100 pg/ml (FIG. 5A).On the RPMI 8226 cell line, LY-411575 drastically reduced the amount of soluble BCMA in the supernatant (FIG. 5B). Cells without treatment secreted 1350 pg/mL of BCMA, whereas cells treated secreted 200 pg/ml (FIG. 5B).These results showed that soluble BCMA would vary depending on the type of cell line and can be regulated by GSI treatment.

Part II- *in vitro* evaluation of GSI treatment on T cell proliferation.

Material and Method:

Fresh isolated human T cells from healthy donors were cultured in RPMI 1640 medium with 10% FBS and stimulated with anti-CD3/anti-CD28 Dynabeads (invitrogen) in the presence or absence of several concentrations of GSI (12μM-100μM) during the culture expansion. Stimulated T cells were fed and split every 2-3 days to $0.8x10^6$ cells/ml and GSI was added to the cultures during the expansion (7-9 days) or until cells were rested as determined by decreased rate of cell division and a decrease in mean cellular volume to < ~300fl. Cellular volume (FL) and number of cells were measured every 3 days using a Coulter Multisizer 3 (Beckman Coulter).

Result:

Proliferation and number of T cells were not affected when incubated with GSI at doses lower than 100μM (FIGs. 6A and 6B).

Part III- in vitro evaluation of GSI treatment on T cell function.

A- BCMA-CARs are efficiently expressed on T cells

Material and Method:

Fresh isolated human T cells from healthy donors were transduced with lentiviral vector supernatants encoding the BCMA3NP CAR and anti-BCMA CAR expression was evaluated by flow cytometry. Briefly, T cells were cultured in RPMI 1640 medium with 10% FBS and stimulated with anti-CD3/anti-CD28 Dynabeads (invitrogen). 24 hrs after stimulation, T cells were transduced with the BCMA CAR lentiviral vector supernatants. Mock-transduced T cells (NTD) were used as a negative control. 4-6 days after lentiviral transduction, T cells were stained with Biotin Goat-anti mouse followed by SAV-PE and CAR expression was evaluated by flow cytometry (FortessaC, BD). Flow cytometric analysis was carried out by using Flowjo v10 (Tree Star Inc).

Result:

After transduction, BCMA CARs were efficiently expressed on the cell surface of the transduced T cells (data not shown).

B- In the presence of cognate target antigen, anti-BCMA CAR-transduced T cells (BCMA CARTs) show enhanced cytotoxic activity on Myeloma cell lines.

Material and Method:

The ability of BCMA CAR T cells to kill BCMA-expressing target cells was evaluated using a 51Cr release-assay. Briefly, target MM cell line U266, a positive control cell line K562-BCMA (ectopically expressing human BCMA), or a negative control cell line K562 were cultured for 48hr in presence or absence of 10μM of GSI, then washed and labeled with 51Cr (Sodium Dichromate salt), washed and co-cultured with effector BCMA CAR T cells or NTD T cells at different effector/target ratios. Supernatants were collected at 4-hrs, and placed into 96 well Lumaplates (Perkin Elmer). The amount of 51Cr released from the labeled target cells was measured on a liquid scintillation counter (MicroBeta trilux, Perkin Elmer). Target cells incubated in medium alone or with 1% SDS were used to determine spontaneous (S) or maximum (M) 51Cr release. Percentage of specific lysis was calculated as follow: 100x (cpm experimental release- cpm S release)/ (cpm M release- cpm S release).

Result:

BCMA-CAR-transduced T cells were able to induce lysis of K562-BCMA cells and BCMA-expressing multiple myeloma cells lines with little activity towards the BCMA negative cell lineK562. γ-secretase inhibitor treatment enhanced in vitro cytotoxicity of CAR T cells (FIGs. 7A-7C).

Part IV- in vivo evaluation of BCMA-CAR T cells for MM

Material and Method:

Based upon in vitro data supporting enhanced expression of BCMA on cell surface after GSI treatment, the anti-tumor activity before and after GSI treatment was evaluated in a preclinical animal model of multiple myeloma using the U266 cell line. U266 cells were engineered to express Click-beetle Green luciferase (CB-G Luc+) to track tumor progression by bioluminescent in vivo imaging (IVIS) and Living Image software (Perkin Elmer) (FIG. 8). 4 weeks after injection of CB-G Luc+ U266 NSG recipients were treated with GSI (10mg/kg) by IP injection every day for 4 days. On day 5, mice were IV injected with BCMA or NTD T and the tumor burden was evaluated by optical imaging (FIGs. 9A-9D).

Result:

T cells expressing BCMA3NP induced reduction in the tumor burden of mice bearing U266 (FIG. 9C). After GSI treatment, tumor burden was further reduced and improved clinical disease activity was observed (FIG. 9D).

EQUIVALENTS

The disclosures of each and every patent, patent application, and publication cited herein are hereby incorporated herein by reference in their entirety. While this invention has been disclosed with reference to specific aspects, it is apparent that other aspects and variations of this invention may be devised by others skilled in the art without departing from the true spirit and scope of the invention. The appended claims are intended to be construed to include all such aspects and equivalent variations.

What is claimed is:

1. A composition comprising a cell (e.g., a population of cells) that expresses a CAR molecule that binds BCMA (a "BCMA CAR-expressing cell") for use, in combination with a gamma secretase inhibitor (GSI), in the treatment of a subject having a disease associated with expression of BCMA, wherein:

the CAR molecule comprises an anti-BCMA binding domain, a transmembrane domain, and an intracellular signaling domain, and wherein:

the GSI has one or more (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, or all) of the following properties:

(i) the GSI reduces gamma secretase-mediated cleavage of BCMA;

(ii) the GSI, when incubated with BCMA-expressing cells, increases cell surface expression of BCMA, e.g., by at least 2, 4, 6, 8, 10, 15, or 20-fold, e.g., as measured by a method described herein, e.g., a flow cytometry assay, e.g., as measured using methods described in Example 1 with respect to FIG. 1;

(iii) the GSI, when incubated with BCMA-expressing cells, changes conformation and/or posttranslational modification of the extracellular domain of cell surface-expressed BCMA;

(iv) the GSI, when incubated with BCMA-expressing cells, decreases the level of soluble BCMA in the cell supernatant, e.g., by at least 80, 85, 90, 95, 99, or 99.5%, e.g., as measured by a method described herein, e.g., an ELISA assay, e.g., as measured using methods described in Example 1 with respect to Table 28;

(v) the GSI, when administered in vivo, increases cell surface expression of BCMA, e.g., as measured by a method described herein, e.g., a flow cytometry assay;

(vi) the GSI, when administered in vivo, changes conformation and/or posttranslational modification of the extracellular domain of cell surface-expressed BCMA;

(vii) the GSI, when administered in vivo, decreases the level of soluble BCMA in the serum and/or bone marrow, e.g., as measured by a method described herein, e.g., an ELISA assay;

(viii) the GSI is capable of increasing the activity of the BCMA CAR-expressing cell, e.g., increasing the cytotoxicity of the BCMA CAR-expressing cell, e.g., as measured by a method described herein, e.g., as measured using methods described in Example 3 with res pect to FIGs. 7B and 7C;

(ix) the GSI is capable of increasing the activity of the BCMA CAR-expressing cell, e.g., increasing the anti-tumor activity of the BCMA CAR-expressing cell, e.g., as measured by a method described herein, e.g., as measured using methods described in Example 3 with respect to FIG. 9D;

(x) the GSI does not reduce gamma secretase-mediated cleavage of Notch, or reduces gamma secretase-mediated cleavage of Notch less efficiently, e.g., at least 2-fold, 5-fold, 10-fold, 50-fold, or 100-fold less efficiently, than the GSI reduces gamma secretase-mediated cleavage of BCMA;

(xi) the GSI reduces gamma secretase-mediated cleavage of BCMA more efficiently, e.g., at least 2-fold, 5-fold, 10-fold, 50-fold, or 100-fold more efficiently, than the GSI reduces gamma secretase-mediated cleavage of another substrate of gamma secretase, e.g., Cadherins, ErbB, or CD44;

(xii) the GSI specifically binds to Presenilin-1, e.g., the GSI binds to Presenilin-1 with higher affinity, e.g., at least 2-fold, 5-fold, 10-fold, 50-fold, or 100-fold higher affinity, than the GSI binds to another subunit of gamma secretase, e.g., nicastrin, anterior pharynx-defective 1, or presenilin enhancer 2; or

(xiii) the GSI exhibits low gastrointestinal toxicity.

2. A method of treating a subject having a disease associated with expression of B-cell maturation antigen (BCMA) comprising administering to the subject an effective amount of a cell (e.g., a population of cells) that expresses a chimeric antigen receptor (CAR) molecule that binds BCMA (a "BCMA CAR-expressing cell"), in combination with a gamma secretase inhibitor (GSI), wherein:

the CAR molecule comprises an anti-BCMA binding domain, a transmembrane domain, and an intracellular signaling domain, and wherein:

the GSI has one or more (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, or all) of the following properties:

(i) the GSI reduces gamma secretase-mediated cleavage of BCMA;

(ii) the GSI, when incubated with BCMA-expressing cells, increases cell surface expression of BCMA, e.g., by at least 2, 4, 6, 8, 10, 15, or 20-fold, e.g., as measured by a method described herein, e.g., a flow cytometry assay, e.g., as measured using methods described in Example 1 with respect to FIG. 1;

(iii) the GSI, when incubated with BCMA-expressing cells, changes conformation and/or posttranslational modification of the extracellular domain of cell surface-expressed BCMA;

(iv) the GSI, when incubated with BCMA-expressing cells, decreases the level of soluble BCMA in the cell supernatant, e.g., by at least 80, 85, 90, 95, 99, or 99.5%, e.g., as measured by a method described herein, e.g., an ELISA assay, e.g., as measured using methods described in Example 1 with respect to Table 28;

(v) the GSI, when administered in vivo, increases cell surface expression of BCMA, e.g., as measured by a method described herein, e.g., a flow cytometry assay;

(vi) the GSI, when administered in vivo, changes conformation and/or posttranslational modification of the extracellular domain of cell surface-expressed BCMA;

(vii) the GSI, when administered in vivo, decreases the level of soluble BCMA in the serum and/or bone marrow, e.g., as measured by a method described herein, e.g., an ELISA assay;

(viii) the GSI is capable of increasing the activity of the BCMA CAR-expressing cell, e.g., increasing the cytotoxicity of the BCMA CAR-expressing cell, e.g., as measured by a method described herein, e.g., as measured using methods described in Example 3 with respect to FIGs. 7B and 7C;

(ix) the GSI is capable of increasing the activity of the BCMA CAR-expressing cell, e.g., increasing the anti-tumor activity of the BCMA CAR-expressing cell, e.g., as measured by a method described herein, e.g., as measured using methods described in Example 3 with respect to FIG. 9D;

(x) the GSI does not reduce gamma secretase-mediated cleavage of Notch, or reduces gamma secretase-mediated cleavage of Notch less efficiently, e.g., at least 2-fold, 5-fold, 10-fold, 50-fold, or 100-fold less efficiently, than the GSI reduces gamma secretase-mediated cleavage of BCMA;

(xi) the GSI reduces gamma secretase-mediated cleavage of BCMA more efficiently, e.g., at least 2-fold, 5-fold, 10-fold, 50-fold, or 100-fold more efficiently, than the GSI reduces gamma secretase-mediated cleavage of another substrate of gamma secretase, e.g., Cadherins, ErbB, or CD44;

(xii) the GSI specifically binds to Presenilin-1, e.g., the GSI binds to Presenilin-1 with higher affinity, e.g., at least 2-fold, 5-fold, 10-fold, 50-fold, or 100-fold higher affinity, than the GSI binds to another subunit of gamma secretase, e.g., nicastrin, anterior pharynx-defective 1, or presenilin enhancer 2; or

(xiii) the GSI exhibits low gastrointestinal toxicity.

3. A composition comprising a cell (e.g., a population of cells) that expresses a CAR molecule that binds BCMA (a "BCMA CAR-expressing cell") for use, in combination with a gamma secretase

inhibitor (GSI), in the treatment of a subject having a disease associated with expression of BCMA, wherein:

the CAR molecule comprises an anti-BCMA binding domain comprising:

a heavy chain variable region (VH) comprising a heavy chain complementarity determining region 1 (VHCDR1), a VHCDR2, and a VHCDR3 of any anti-BCMA heavy chain binding domain amino acid sequence listed in Tables 1, 20, 22, 24, and 26 (or a sequence at least about 85%, 90%, 95%, 99% or more identical thereto, and/or having one, two, three or more substitutions, insertions or deletions, *e.g.*, conserved substitutions), and/or

a light chain variable region (VL) comprising a light chain complementarity determining region 1 (VLCDR1), a VLCDR2, and a VLCDR3 of any anti-BCMA light chain binding domain amino acid sequence listed in Tables 1, 21, 23, 25, and 26 (or a sequence at least about 85%, 90%, 95%, 99% or more identical thereto, and/or having one, two, three or more substitutions, insertions or deletions, *e.g.*, conserved substitutions).

4. A method of treating a subject having a disease associated with expression of B-cell maturation antigen (BCMA) comprising administering to the subject an effective amount of a cell (e.g., a population of cells) that expresses a chimeric antigen receptor (CAR) molecule that binds BCMA (a "BCMA CAR-expressing cell"), in combination with a gamma secretase inhibitor (GSI), wherein:

the CAR molecule comprises an anti-BCMA binding domain comprising:

a heavy chain variable region (VH) comprising a heavy chain complementarity determining region 1 (VHCDR1), a VHCDR2, and a VHCDR3 of any anti-BCMA heavy chain binding domain amino acid sequence listed in Tables 1, 20, 22, 24, and 26 (or a sequence at least about 85%, 90%, 95%, 99% or more identical thereto, and/or having one, two, three or more substitutions, insertions or deletions, *e.g.*, conserved substitutions), and/or

a light chain variable region (VL) comprising a light chain complementarity determining region 1 (VLCDR1), a VLCDR2, and a VLCDR3 of any anti-BCMA light chain binding domain amino acid sequence listed in Tables 1, 21, 23, 25, and 26 (or a sequence at least about 85%, 90%, 95%, 99% or more identical thereto, and/or having one, two, three or more substitutions, insertions or deletions, *e.g.*, conserved substitutions).

5. A method of treating a subject having a disease associated with expression of B-cell maturation antigen (BCMA) comprising administering to the subject an effective amount of a cell (e.g., a population of cells) that expresses a chimeric antigen receptor (CAR) molecule that binds BCMA (a "BCMA CAR-expressing cell"), in combination with a gamma secretase inhibitor (GSI), wherein:

(i) the GSI is an antibody molecule that reduces the expression and/or function of gamma secretase, optionally wherein the GSI is an antibody molecule that specifically binds to a subunit of gamma secretase (e.g., presenilin, nicastrin, APH-1, or PEN-2);

(ii) the GSI is (1) a gene editing system targeted to one or more sites within a gene encoding a subunit of gamma secretase (e.g., presenilin, nicastrin, APH-1, or PEN-2) or a regulatory element thereof; (2) a nucleic acid encoding one or more components of the gene editing system; or (3) a combination thereof; or

(iii) the GSI is an agent that mediates RNA interference, e.g., an siRNA or shRNA specific for a gene encoding a subunit of gamma secretase (e.g., presenilin, nicastrin, APH-1, or PEN-2), or a nucleic acid encoding the siRNA or shRNA.

6. The method or use of any of claims 3-5, wherein the GSI has one or more (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, or all) of the following properties:

(i) the GSI reduces gamma secretase-mediated cleavage of BCMA;

(ii) the GSI, when incubated with BCMA-expressing cells, increases cell surface expression of BCMA, e.g., by at least 2, 4, 6, 8, 10, 15, or 20-fold, e.g., as measured by a method described herein, e.g., a flow cytometry assay, e.g., as measured using methods described in Example 1 with respect to FIG. 1;

(iii) the GSI, when incubated with BCMA-expressing cells, changes conformation and/or posttranslational modification of the extracellular domain of cell surface-expressed BCMA;

(iv) the GSI, when incubated with BCMA-expressing cells, decreases the level of soluble BCMA in the cell supernatant, e.g., by at least 80, 85, 90, 95, 99, or 99.5%, e.g., as measured by a method described herein, e.g., an ELISA assay, e.g., as measured using methods described in Example 1 with respect to Table 28;

(v) the GSI, when administered in vivo, increases cell surface expression of BCMA, e.g., as measured by a method described herein, e.g., a flow cytometry assay;

(vi) the GSI, when administered in vivo, changes conformation and/or posttranslational modification of the extracellular domain of cell surface-expressed BCMA;

(vii) the GSI, when administered in vivo, decreases the level of soluble BCMA in the serum and/or bone marrow, e.g., as measured by a method described herein, e.g., an ELISA assay;

(viii) the GSI is capable of increasing the activity of the BCMA CAR-expressing cell, e.g., increasing the cytotoxicity of the BCMA CAR-expressing cell, e.g., as measured by a method described herein, e.g., as measured using methods described in Example 3 with respect to FIGs. 7B and 7C;

(ix) the GSI is capable of increasing the activity of the BCMA CAR-expressing cell, e.g., increasing the anti-tumor activity of the BCMA CAR-expressing cell, e.g., as measured by a method described herein, e.g., as measured using methods described in Example 3 with respect to FIG. 9D;

(x) the GSI does not reduce gamma secretase-mediated cleavage of Notch, or reduces gamma secretase-mediated cleavage of Notch less efficiently, e.g., at least 2-fold, 5-fold, 10-fold, 50-fold, or 100-fold less efficiently, than the GSI reduces gamma secretase-mediated cleavage of BCMA;

(xi) the GSI reduces gamma secretase-mediated cleavage of BCMA more efficiently, e.g., at least 2-fold, 5-fold, 10-fold, 50-fold, or 100-fold more efficiently, than the GSI reduces gamma secretase-mediated cleavage of another substrate of gamma secretase, e.g., Cadherins, ErbB, or CD44;

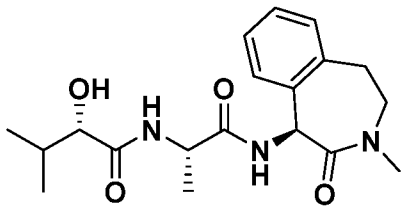
(xii) the GSI specifically binds to Presenilin-1, e.g., the GSI binds to Presenilin-1 with higher affinity, e.g., at least 2-fold, 5-fold, 10-fold, 50-fold, or 100-fold higher affinity, than the GSI binds to another subunit of gamma secretase, e.g., nicastrin, anterior pharynx-defective 1, or presenilin enhancer 2; or

(xiii) the GSI exhibits low gastrointestinal toxicity.

7. The method or use of any of claims 1-4 or 6, wherein the GSI is a small molecule that reduces the expression and/or function of gamma secretase.

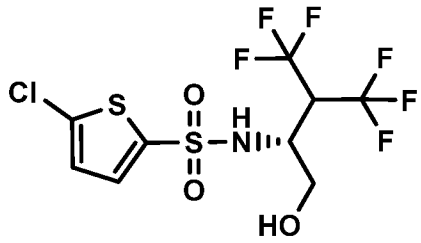
8. The method or use of claim 7, wherein the GSI is chosen from LY-450139, PF-5212362, BMS-708163, MK-0752, ELN-318463, BMS-299897, LY-411575, DAPT, BMS-906024, PF-3084014, RO4929097, or LY3039478, optionally wherein the GSI is chosen from PF-5212362, ELN-318463, BMS-906024, or LY3039478.

9. The method or use of claim 7, wherein the GSI is



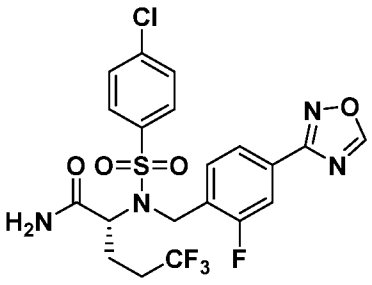
, or a pharmaceutically acceptable salt thereof.

10. The method or use of claim 7, wherein the GSI is



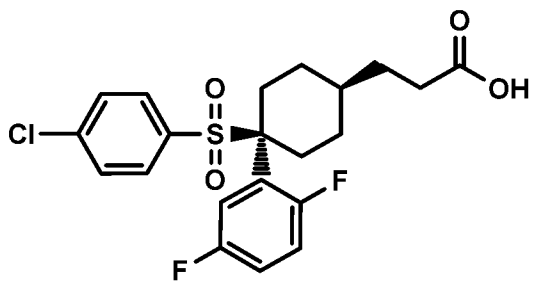
, or a pharmaceutically acceptable salt thereof.

11. The method or use of claim 7, wherein the GSI is



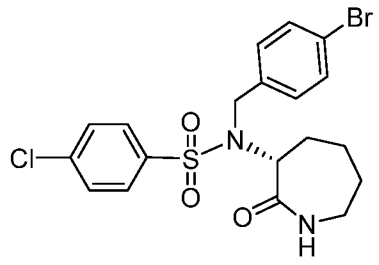
, or a pharmaceutically acceptable salt thereof.

12. The method or use of claim 7, wherein the GSI is



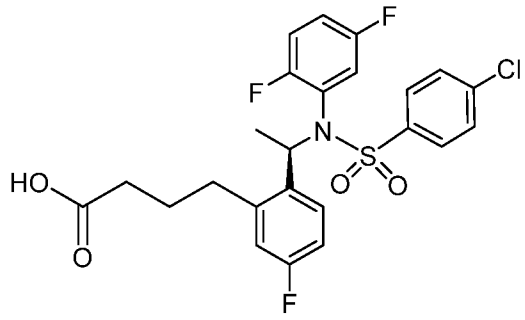
, or a pharmaceutically acceptable salt thereof.

13. The method or use of claim 7, wherein the GSI is



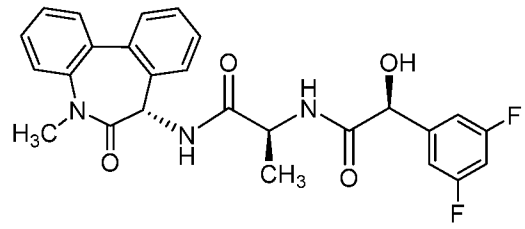
, or a pharmaceutically acceptable salt thereof.

14. The method or use of claim 7, wherein the GSI is



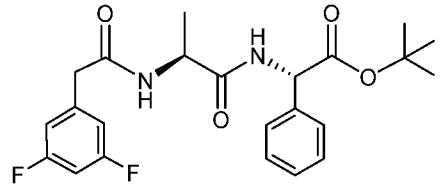
, or a pharmaceutically acceptable salt thereof.

15. The method or use of claim 7, wherein the GSI is



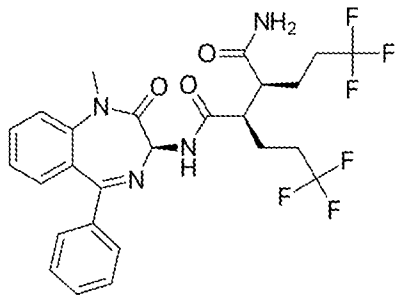
, or a pharmaceutically acceptable salt thereof.

16. The method or use of claim 7, wherein the GSI is



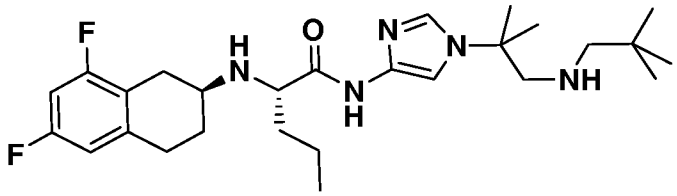
, or a pharmaceutically acceptable salt thereof.

17. The method or use of claim 7, wherein the GSI is



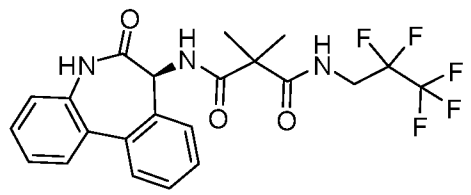
, or a pharmaceutically acceptable salt thereof.

18. The method or use of claim 7, wherein the GSI is



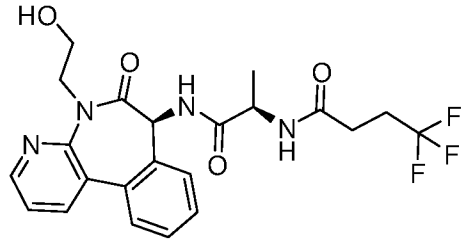
, or a pharmaceutically acceptable salt thereof.

19. The method or use of claim 7, wherein the GSI is



, or a pharmaceutically acceptable salt thereof.

20. The method or use of claim 7, wherein the GSI is



, or a pharmaceutically acceptable salt thereof.

21. The method or use of any of claims 1-6, wherein the GSI is an antibody molecule that reduces the expression and/or function of gamma secretase, optionally wherein the GSI is an antibody molecule that specifically binds to a subunit of gamma secretase (e.g., presenilin, nicastrin, APH-1, or PEN-2).

22. The method or use of any of claims 1-6, wherein the GSI is (1) a gene editing system targeted to one or more sites within a gene encoding a subunit of gamma secretase (e.g., presenilin, nicastrin, APH-1, or PEN-2) or a regulatory element thereof; (2) a nucleic acid encoding one or more components of the gene editing system; or (3) a combination thereof.

23. The method or use of claim 22, wherein the gene editing system is chosen from a CRISPR/Cas9 system, a zinc finger nuclease system, a TALEN system, or a meganuclease system.

24. The method or use of any of claims 1-6, wherein the GSI is an agent that mediates RNA interference, e.g., an siRNA or shRNA specific for a gene encoding a subunit of gamma secretase (e.g., presenilin, nicastrin, APH-1, or PEN-2), or a nucleic acid encoding the siRNA or shRNA.

25. The method or use of claim 24, wherein the siRNA or shRNA comprises a sequence complementary to a sequence of an mRNA of the gene encoding a subunit of gamma secretase (e.g., presenilin, nicastrin, APH-1, or PEN-2).

26. The method or use of any of claims 1-25, wherein the BCMA CAR-expressing cell comprises a nucleic acid encoding a CAR molecule, wherein the CAR molecule comprises an anti-BCMA binding domain, a transmembrane domain, and an intracellular signaling domain.

27. The method or use of claim 26, wherein the encoded anti-BCMA binding domain comprises a heavy chain variable region (VH) comprising a heavy chain complementarity determining region 1 (VHCDR1), a VHCDR2, and a VHCDR3 of any anti-BCMA heavy chain binding domain amino acid sequence listed in Tables 1, 20, 22, 24, and 26 (or a sequence at least about 85%, 90%, 95%, 99% or

more identical thereto, and/or having one, two, three or more substitutions, insertions or deletions, *e.g.*, conserved substitutions).

28. The method or use of claim 26 or 27, wherein the encoded anti-BCMA binding domain comprises a light chain variable region (VL) comprising a light chain complementarity determining region 1 (VLCDR1), a VLCDR2, and a VLCDR3 of any anti-BCMA light chain binding domain amino acid sequence listed in Tables 1, 21, 23, 25, and 26 (or a sequence at least about 85%, 90%, 95%, 99% or more identical thereto, and/or having one, two, three or more substitutions, insertions or deletions, e.g., conserved substitutions).

29. The method or use of any of claims 26-28, wherein the encoded anti-BCMA binding domain comprises:

a heavy chain variable region (VH) comprising a heavy chain complementarity determining region 1 (VHCDR1), a VHCDR2, and a VHCDR3 of any anti-BCMA heavy chain binding domain amino acid sequence listed in Tables 1, 20, 22, 24, and 26 (or a sequence at least about 85%, 90%, 95%, 99% or more identical thereto, and/or having one, two, three or more substitutions, insertions or deletions, *e.g.*, conserved substitutions), and

a light chain variable region (VL) comprising a light chain complementarity determining region 1 (VLCDR1), a VLCDR2, and a VLCDR3 of any anti-BCMA light chain binding domain amino acid sequence listed in Tables 1, 21, 23, 25, and 26 (or a sequence at least about 85%, 90%, 95%, 99% or more identical thereto, and/or having one, two, three or more substitutions, insertions or deletions, e.g., conserved substitutions).

30. The method or use of any of claims 26-29, wherein the encoded anti-BCMA binding domain comprises a VH comprising a VH of any anti-BCMA heavy chain binding domain amino acid sequence listed in Tables 1 and 26 (or a sequence at least about 85%, 90%, 95%, 99% or more identical thereto, and/or having one, two, three or more substitutions, insertions or deletions, *e.g.*, conserved substitutions).

31. The method or use of any of claims 26-30, wherein the encoded anti-BCMA binding domain comprises a VL comprising a VL of any anti-BCMA light chain binding domain amino acid

sequence listed in Tables 1 and 26 (or a sequence at least about 85%, 90%, 95%, 99% or more identical thereto, and/or having one, two, three or more substitutions, insertions or deletions, *e.g.*, conserved substitutions).

32. The method or use of any of claims 26-31, wherein the encoded anti-BCMA binding domain comprises:

a VH comprising a VH of any anti-BCMA heavy chain binding domain amino acid sequence listed in Tables 1 and 26 (or a sequence at least about 85%, 90%, 95%, 99% or more identical thereto, and/or having one, two, three or more substitutions, insertions or deletions, *e.g.*, conserved substitutions), and

a VL comprising a VL of any anti-BCMA light chain binding domain amino acid sequence listed in Tables 1 and 26 (or a sequence at least about 85%, 90%, 95%, 99% or more identical thereto, and/or having one, two, three or more substitutions, insertions or deletions, *e.g.*, conserved substitutions).

33. The method or use of any of claims 26-32, wherein:

(i) the encoded anti-BCMA binding domain comprises an scFv comprising an scFv amino acid sequence listed in Table 1 (or a sequence at least about 85%, 90%, 95%, 99% or more identical thereto, and/or having one, two, three or more substitutions, insertions or deletions, *e.g.*, conserved substitutions);

(ii) the encoded anti-BCMA binding domain comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 39, SEQ ID NO: 40, SEQ ID NO: 41, SEQ ID NO: 42, SEQ ID NO: 43, SEQ ID NO: 44, SEQ ID NO: 45, SEQ ID NO: 46, SEQ ID NO: 47, SEQ ID NO: 48, SEQ ID NO: 49, SEQ ID NO: 50, SEQ ID NO: 51, SEQ ID NO: 52, SEQ ID NO: 53, SEQ ID NO: 129, SEQ ID NO: 130, SEQ ID NO: 131, SEQ ID NO: 132, SEQ ID NO: 133, SEQ ID NO: 134, SEQ ID NO: 135, SEQ ID NO: 136, SEQ ID NO: 137, SEQ ID NO: 138, SEQ ID NO: 139, SEQ ID NO: 140, SEQ ID NO: 141, SEQ ID NO: 142, SEQ ID NO: 143, SEQ ID NO: 144, SEQ ID NO: 145, SEQ ID NO: 146, SEQ ID NO: 147, SEQ ID NO: 148, and SEQ ID NO: 149, or a sequence with 95-99% identify thereof; or

(iii) the nucleic acid encoding the CAR molecule comprises a nucleotide sequence selected from the group consisting of SEQ ID NO: 54, SEQ ID NO: 55, SEQ ID NO: 56, SEQ ID NO: 57, SEQ

ID NO: 58, SEQ ID NO: 59, SEQ ID NO: 60, SEQ ID NO: 61, SEQ ID NO: 62, SEQ ID NO: 63, SEQ ID NO: 64, SEQ ID NO: 65, SEQ ID NO: 66, SEQ ID NO: 67, SEQ ID NO: 68, SEQ ID NO: 150, SEQ ID NO: 151, SEQ ID NO: 152, SEQ ID NO: 153, SEQ ID NO: 154, SEQ ID NO: 155, SEQ ID NO: 156, SEQ ID NO: 157, SEQ ID NO: 158, SEQ ID NO: 159, SEQ ID NO: 160, SEQ ID NO: 161, SEQ ID NO: 162, SEQ ID NO: 163, SEQ ID NO: 164, SEQ ID NO: 165, SEQ ID NO: 166, SEQ ID NO: 167, SEQ ID NO: 168, SEQ ID NO: 169, SEQ ID NO: 170, or a sequence with 95-99% identify thereof.

34. The method or use of any of claims 26-33, wherein:

(i) the encoded CAR molecule comprises a full CAR amino acid sequence listed in Table 1 (or a sequence at least about 85%, 90%, 95%, 99% or more identical thereto, and/or having one, two, three or more substitutions, insertions or deletions, *e.g.*, conserved substitutions);

(ii) the encoded CAR molecule comprises an amino acid sequence selected from the group consisting of residues 22-483 of SEQ ID NO: 109, residues 22-490 of SEQ ID NO: 99, residues 22-488 of SEQ ID NO: 100, residues 22-487 of SEQ ID NO: 101, residues 22-493 of SEQ ID NO: 102, residues 22-490 of SEQ ID NO: 103, residues 22-491 of SEQ ID NO: 104, residues 22-482 of SEQ ID NO: 105, residues 22-483 of SEQ ID NO: 106, residues 22-485 of SEQ ID NO: 107, residues 22-483 of SEQ ID NO: 108, residues 22-490 of SEQ ID NO: 110, residues 22-483 of SEQ ID NO: 111, residues 22-484 of SEQ ID NO: 112, residues 22-485 of SEQ ID NO: 113, residues 22-487 of SEQ ID NO: 213, residues 23-489 of SEQ ID NO: 214, residues 22-490 of SEQ ID NO: 215, residues 22-484 of SEQ ID NO: 216, residues 22-485 of SEQ ID NO: 217, residues 22-489 of SEQ ID NO: 218, residues 22-497 of SEQ ID NO: 219, residues 22-492 of SEQ ID NO: 220, residues 22-490 of SEQ ID NO: 221, residues 22-485 of SEQ ID NO: 222, residues 22-492 of SEQ ID NO: 223, residues 22-492 of SEQ ID NO: 224, residues 22-483 of SEQ ID NO: 225, residues 22-490 of SEQ ID NO: 226, residues 22-485 of SEQ ID NO: 227, residues 22-486 of SEQ ID NO: 228, residues 22-492 of SEQ ID NO: 229, residues 22-488 of SEQ ID NO: 230, residues 22-488 of SEQ ID NO: 231, residues 22-495 of SEQ ID NO: 232, residues 22-490 of SEQ ID NO: 233, or a sequence with 95-99% identify thereof; or

(iii) the nucleic acid encoding the CAR molecule comprises a nucleotide sequence selected from the group consisting of SEQ ID NO: 114, SEQ ID NO: 115, SEQ ID NO: 116, SEQ ID NO: 117, SEQ ID NO: 118, SEQ ID NO: 119, SEQ ID NO: 120, SEQ ID NO: 121, SEQ ID NO: 122, SEQ ID NO: 123, SEQ ID NO: 124, SEQ ID NO: 125, SEQ ID NO: 126, SEQ ID NO: 127, SEQ ID NO: 128, SEQ ID NO: 234, SEQ ID NO: 235, SEQ ID NO: 236, SEQ ID NO: 237, SEQ ID NO: 238, SEQ ID

NO: 239, SEQ ID NO: 240, SEQ ID NO: 241, SEQ ID NO: 242, SEQ ID NO: 243, SEQ ID NO: 244, SEQ ID NO: 245, SEQ ID NO: 246, SEQ ID NO: 247, SEQ ID NO: 248, SEQ ID NO: 249, SEQ ID NO: 250, SEQ ID NO: 251, SEQ ID NO: 252, SEQ ID NO: 253, and SEQ ID NO: 254, or a sequence with 95-99% identify thereof.

35. The method or use of any of claims 26-34, wherein:

(i) the encoded transmembrane domain comprises a transmembrane domain of a protein selected from the group consisting of the alpha, beta or zeta chain of a T-cell receptor, CD28, CD3 epsilon, CD45, CD4, CD5, CD8, CD9, CD16, CD22, CD33, CD37, CD64, CD80, CD86, CD134, CD137 and CD154;

(ii) the encoded transmembrane domain comprises the amino acid sequence of SEQ ID NO: 6 (or a sequence at least about 85%, 90%, 95%, 99% or more identical thereto, and/or having one, two, three or more substitutions, insertions or deletions, *e.g.*, conserved substitutions); or

(iii) the nucleic acid encoding the CAR molecule comprises the nucleotide sequence of SEQ ID NO: 17, or a sequence with 95-99% identify thereof.

36. The method or use of any of claims 26-35, wherein the encoded anti-BCMA binding domain is connected to the transmembrane domain by a hinge region.

37. The method or use of claim 36, wherein:

(i) the encoded hinge region comprises the amino acid sequence of SEQ ID NO: 2 or 36 (or a sequence at least about 85%, 90%, 95%, 99% or more identical thereto, and/or having one, two, three or more substitutions, insertions or deletions, *e.g.*, conserved substitutions); or

(ii) the nucleic acid encoding the CAR molecule comprises the nucleotide sequence of SEQ ID NO: 13 or 37, or a sequence with 95-99% identify thereof.

38. The method or use of any of claims 26-37, wherein the encoded intracellular signaling domain is a functional signaling domain obtained from a protein chosen from an MHC class I molecule, a TNF receptor, an immunoglobulin-like protein, a cytokine receptor, integrin, signaling lymphocytic activation molecule (SLAM), an activating NK cell receptor, BTLA, a Toll ligand receptor, CD3,

OX40, CD2, CD7, CD27, CD28, CD30, CD40, CDS, ICAM-1, 4-1BB (CD137), B7-H3, ICOS (CD278), GITR, BAFFR, LIGHT, HVEM (LIGHTR), KIRDS2, SLAMF7, NKp80 (KLRF1), NKp44, NKp30, NKp46, CD19, CD4, CD8alpha, CD8beta, IL2R beta, IL2R gamma, IL7R alpha, ITGA4, VLA1, CD49a, ITGA4, IA4, CD49D, ITGA6, VLA-6, CD49f, ITGAD, CD11d, ITGAE, CD103, ITGAL, CD11a, LFA-1, ITGAM, CD11b, ITGAX, CD11c, ITGB1, CD29, ITGB2, CD18, ITGB7, NKG2D, NKG2C, TNFR2, TRANCE/RANKL, DNAM1 (CD226), SLAMF4 (CD244, 2B4), CD84, CD96 (Tactile), CEACAM1, CRTAM, Ly9 (CD229), CD160 (BY55), PSGL1, CD100 (SEMA4D), CD69, SLAMF6 (NTB-A, Ly108), SLAM (SLAMF1, CD150, IPO-3), BLAME (SLAMF8), SELPLG (CD162), LTBR, LAT, GADS, SLP-76, PAG/Cbp, CD19a, or a ligand that specifically binds with CD83.

39. The method or use of claim 38, wherein the encoded intracellular signaling domain is a functional signaling domain of a protein chosen from 4-1BB, CD3 zeta, CD28, or ICOS.

40. The method or use of any of claims 26-39, wherein:

(i) the encoded intracellular signaling domain comprises the amino acid sequence of SEQ ID NO: 7, 9, 10, 1104, or 1106 (or a sequence at least about 85%, 90%, 95%, 99% or more identical thereto, and/or having one, two, three or more substitutions, insertions or deletions, *e.g.*, conserved substitutions); or

(ii) the nucleic acid molecule encoding the CAR molecule comprises the nucleotide sequence of SEQ ID NO: 18, 20, 21, 1105, or 1107, or a sequence with 95-99% identify thereof.

41. The method or use of any of claims 26-40, wherein the encoded intracellular signaling domain comprises the amino acid sequence of SEQ ID NO: 7 and the amino acid sequence of SEQ ID NO: 9 or 10, or an amino acid sequence at least about 85%, 90%, 95%, 99% or more identical to SEQ ID NO: 7 and an amino acid sequence at least about 85%, 90%, 95%, 99% or more identical to SEQ ID NO: 9 or 10.

42. The method or use of any of claims 26-41, wherein the nucleic acid encoding the CAR molecule comprises:

(i) a leader sequence encoding the amino acid sequence of SEQ ID NO: 1 (or a sequence at least about 85%, 90%, 95%, 99% or more identical thereto, and/or having one, two, three or more substitutions, insertions or deletions, *e.g.*, conserved substitutions); or

(ii) the nucleotide sequence of SEQ ID NO: 12, or a sequence with 95-99% identify thereof.

43. The method or use of any of claims 26-42, wherein the nucleic acid encoding the CAR molecule is a DNA molecule, optionally wherein the DNA molecule is transcribed under an EF-1 promoter comprising the sequence of SEQ ID NO: 11.

44. The method or use of any of claims 26-42, wherein the nucleic acid encoding the CAR molecule is an RNA molecule.

45. The method or use of any of claims 1-44, wherein the cell is an autologous cell or an allogeneic cell.

46. The method or use of any of claims 1-45, wherein the cell is a T cell or a natural killer (NK) cell.

47. The method or use of any of claims 1-46, wherein the disease associated with expression of BCMA is:

(i) a cancer or malignancy, or a precancerous condition chosen from one or more of a myelodysplasia, a myelodysplastic syndrome or a preleukemia, or

(ii) a non-cancer related indication associated with expression of BCMA.

48. The method or use of any of claims 1-47, wherein the disease is chosen from acute leukemia, B-cell acute lymphoid leukemia (BALL), T-cell acute lymphoid leukemia (TALL), acute lymphoid leukemia (ALL), chronic myelogenous leukemia (CML), chronic lymphocytic leukemia (CLL), B cell prolymphocytic leukemia, blastic plasmacytoid dendritic cell neoplasm, Burkitt's lymphoma, diffuse large B cell lymphoma, follicular lymphoma, hairy cell leukemia, small cell- or large

cell-follicular lymphoma, a malignant lymphoproliferative condition, mucosa associated lymphoid tissue (MALT) lymphoma, mantle cell lymphoma, marginal zone lymphoma, multiple myeloma, myelodysplasia and myelodysplastic syndrome, non-Hodgkin's lymphoma, plasmablastic lymphoma, plasmacytoid dendritic cell neoplasm, Waldenstrom macroglobulinemia, a plasma cell proliferative disorder (e.g., asymptomatic myeloma (smoldering multiple myeloma or indolent myeloma), monoclonal gammapathy of undetermined significance (MGUS), Waldenstrom's macroglobulinemia, plasmacytomas (e.g., plasma cell dyscrasia, solitary myeloma, solitary plasmacytoma, extramedullary plasmacytoma, and multiple plasmacytoma), systemic amyloid light chain amyloidosis, and POEMS syndrome (also known as Crow-Fukase syndrome, Takatsuki disease, and PEP syndrome)), prostate cancer (e.g., castrate-resistant or therapy-resistant prostate cancer, or metastatic prostate cancer), pancreatic cancer, or lung cancer.

49. The method or use of any of claims 1-48, wherein the disease is a hematologic cancer.

50. The method or use of any of claims 1-49, wherein the disease is multiple myeloma.

51. The method or use of any of claims 1-50, wherein the BCMA CAR-expressing cell and the GSI are administered simultaneously or sequentially.

52. The method or use of claim 51, wherein the GSI is administered prior to the administration of the BCMA CAR-expressing cell (e.g., GSI is administered 1, 2, 3, 4, or 5 days prior to the administration of the BCMA CAR-expressing cell), optionally wherein after the administration of the GSI and prior to the administration of the BCMA CAR-expressing cell, the subject shows an increase in cell surface BCMA expression levels and/or a decrease in soluble BCMA levels.

53. The method or use of any of claims 1-50, comprising a first treatment regimen and a second treatment regimen, wherein the first treatment regimen is performed prior to the second treatment regimen, wherein:

(i) the first treatment regimen comprises administering a first dose of the BCMA CAR-expressing cell, and

(ii) the second treatment regimen comprises administering a dose of GSI followed by a second dose of the BCMA CAR-expressing cell,

optionally wherein after the administration of the dose of GSI and prior to the administration of the second dose of the BCMA CAR-expressing cell, the subject shows an increase in cell surface BCMA expression levels and/or a decrease in soluble BCMA levels.

54. The method or use of any of claims 1-53, wherein the BCMA CAR-expressing cell and the GSI are administered in combination with a third therapeutic agent or procedure, wherein the third therapeutic agent or procedure is chosen from one or more of chemotherapy, a targeted anti-cancer therapy, an oncolytic drug, a cytotoxic agent, an immune-based therapy, a cytokine, surgical procedure, a radiation procedure, an activator of a costimulatory molecule, an inhibitor of an inhibitory molecule, a vaccine, or a cellular immunotherapy.

55. The method or use of claim 54, wherein the third therapeutic agent or procedure is chosen from:

(i) Dexamethasone;

(ii) a PD-1 inhibitor, optionally wherein the PD-1 inhibitor is selected from the group consisting of PDR001, Nivolumab, Pembrolizumab, Pidilizumab, MEDI0680, REGN2810, TSR-042, PF-06801591, and AMP-224;

(iii) a PD-L1 inhibitor, optionally wherein the PD-L1 inhibitor is selected from the group consisting of FAZ053, Atezolizumab, Avelumab, Durvalumab, and BMS-936559;

(iv) a CTLA-4 inhibitor, optionally wherein the CTLA-4 inhibitor is Ipilimumab or Tremelimumab;

(v) a TIM-3 inhibitor, optionally wherein the TIM-3 inhibitor is MGB453 or TSR-022;

(vi) a LAG-3 inhibitor, optionally wherein the LAG-3 inhibitor is selected from the group consisting of LAG525, BMS-986016, and TSR-033;

(vii) an mTOR inhibitor, optionally wherein the mTOR inhibitor is RAD001 or rapamycin; or

(viii) an agent chosen from HetIL-15, an anti-TGFβ antibody, an anti-CD47 antibody, an IDO inhibitor, a STING agonist, a TLR agonist, an immunomodulatory drug (IMiD) (e.g., Thalidomide, Lenalidomide, or Pomalidomide), a proteasome inhibitor (e.g., Bortezomib), or an ADCC-competent antibody (e.g., Daratumumab or Elotuzumab).

56. A composition comprising a cell (e.g., a population of cells) that expresses a CAR molecule that binds BCMA (a "BCMA CAR-expressing cell") and a GSI, optionally wherein:

the CAR molecule comprises an anti-BCMA binding domain, a transmembrane domain, and an intracellular signaling domain, and optionally wherein:

the GSI has one or more (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, or all) of the following properties:

(i) the GSI reduces gamma secretase-mediated cleavage of BCMA;

(ii) the GSI, when incubated with BCMA-expressing cells, increases cell surface expression of BCMA, e.g., by at least 2, 4, 6, 8, 10, 15, or 20-fold, e.g., as measured by a method described herein, e.g., a flow cytometry assay, e.g., as measured using methods described in Example 1 with respect to FIG. 1;

(iii) the GSI, when incubated with BCMA-expressing cells, changes conformation and/or posttranslational modification of the extracellular domain of cell surface-expressed BCMA;

(iv) the GSI, when incubated with BCMA-expressing cells, decreases the level of soluble BCMA in the cell supernatant, e.g., by at least 80, 85, 90, 95, 99, or 99.5%, e.g., as measured by a method described herein, e.g., an ELISA assay, e.g., as measured using methods described in Example 1 with respect to Table 28;

(v) the GSI, when administered in vivo, increases cell surface expression of BCMA, e.g., as measured by a method described herein, e.g., a flow cytometry assay;

(vi) the GSI, when administered in vivo, changes conformation and/or posttranslational modification of the extracellular domain of cell surface-expressed BCMA;

(vii) the GSI, when administered in vivo, decreases the level of soluble BCMA in the serum and/or bone marrow, e.g., as measured by a method described herein, e.g., an ELISA assay;

(viii) the GSI is capable of increasing the activity of the BCMA CAR-expressing cell, e.g., increasing the cytotoxicity of the BCMA CAR-expressing cell, e.g., as measured by a method described herein, e.g., as measured using methods described in Example 3 with respect to FIGs. 7B and 7C;

(ix) the GSI is capable of increasing the activity of the BCMA CAR-expressing cell, e.g., increasing the anti-tumor activity of the BCMA CAR-expressing cell, e.g., as measured by a method described herein, e.g., as measured using methods described in Example 3 with respect to FIG. 9D;

(x) the GSI does not reduce gamma secretase-mediated cleavage of Notch, or reduces gamma secretase-mediated cleavage of Notch less efficiently, e.g., at least 2-fold, 5-fold, 10-fold, 50-fold, or 100-fold less efficiently, than the GSI reduces gamma secretase-mediated cleavage of BCMA;

(xi) the GSI reduces gamma secretase-mediated cleavage of BCMA more efficiently, e.g., at least 2-fold, 5-fold, 10-fold, 50-fold, or 100-fold more efficiently, than the GSI reduces gamma secretase-mediated cleavage of another substrate of gamma secretase, e.g., Cadherins, ErbB, or CD44;

(xii) the GSI specifically binds to Presenilin-1, e.g., the GSI binds to Presenilin-1 with higher affinity, e.g., at least 2-fold, 5-fold, 10-fold, 50-fold, or 100-fold higher affinity, than the GSI binds to another subunit of gamma secretase, e.g., nicastrin, anterior pharynx-defective 1, or presenilin enhancer 2; or

(xiii) the GSI exhibits low gastrointestinal toxicity.

57. The composition of claim 56, wherein the BCMA CAR-expressing cell and the GSI are present in a single dose form, or as two or more dose forms.

58. The composition of claim 56 or 57 for use as a medicament.

59. The composition of claim 56 or 57 for use in the treatment of a disease associated with expression of BCMA.

60. A kit comprising a cell (e.g., a population of cells) that expresses a CAR molecule that binds BCMA (a "BCMA CAR-expressing cell") and a GSI, optionally wherein:

the CAR molecule comprises an anti-BCMA binding domain, a transmembrane domain, and an intracellular signaling domain, and optionally wherein:

the GSI has one or more (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, or all) of the following properties:

(i) the GSI reduces gamma secretase-mediated cleavage of BCMA;

(ii) the GSI, when incubated with BCMA-expressing cells, increases cell surface expression of BCMA, e.g., by at least 2, 4, 6, 8, 10, 15, or 20-fold, e.g., as measured by a method described herein, e.g., a flow cytometry assay, e.g., as measured using methods described in Example 1 with respect to FIG. 1;

(iii) the GSI, when incubated with BCMA-expressing cells, changes conformation and/or posttranslational modification of the extracellular domain of cell surface-expressed BCMA;

(iv) the GSI, when incubated with BCMA-expressing cells, decreases the level of soluble BCMA in the cell supernatant, e.g., by at least 80, 85, 90, 95, 99, or 99.5%, e.g., as measured by a method described herein, e.g., an ELISA assay, e.g., as measured using methods described in Example 1 with respect to Table 28;

(v) the GSI, when administered in vivo, increases cell surface expression of BCMA, e.g., as measured by a method described herein, e.g., a flow cytometry assay;

(vi) the GSI, when administered in vivo, changes conformation and/or posttranslational modification of the extracellular domain of cell surface-expressed BCMA;

(vii) the GSI, when administered in vivo, decreases the level of soluble BCMA in the serum and/or bone marrow, e.g., as measured by a method described herein, e.g., an ELISA assay;

(viii) the GSI is capable of increasing the activity of the BCMA CAR-expressing cell, e.g., increasing the cytotoxicity of the BCMA CAR-expressing cell, e.g., as measured by a method described herein, e.g., as measured using methods described in Example 3 with respect to FIGs. 7B and 7C;

(ix) the GSI is capable of increasing the activity of the BCMA CAR-expressing cell, e.g., increasing the anti-tumor activity of the BCMA CAR-expressing cell, e.g., as measured by a method described herein, e.g., as measured using methods described in Example 3 with respect to FIG. 9D;

(x) the GSI does not reduce gamma secretase-mediated cleavage of Notch, or reduces gamma secretase-mediated cleavage of Notch less efficiently, e.g., at least 2-fold, 5-fold, 10-fold, 50-fold, or 100-fold less efficiently, than the GSI reduces gamma secretase-mediated cleavage of BCMA;

(xi) the GSI reduces gamma secretase-mediated cleavage of BCMA more efficiently, e.g., at least 2-fold, 5-fold, 10-fold, 50-fold, or 100-fold more efficiently, than the GSI reduces gamma secretase-mediated cleavage of another substrate of gamma secretase, e.g., Cadherins, ErbB, or CD44;

(xii) the GSI specifically binds to Presenilin-1, e.g., the GSI binds to Presenilin-1 with higher affinity, e.g., at least 2-fold, 5-fold, 10-fold, 50-fold, or 100-fold higher affinity, than the GSI binds to another subunit of gamma secretase, e.g., nicastrin, anterior pharynx-defective 1, or presenilin enhancer 2; or

(xiii) the GSI exhibits low gastrointestinal toxicity.

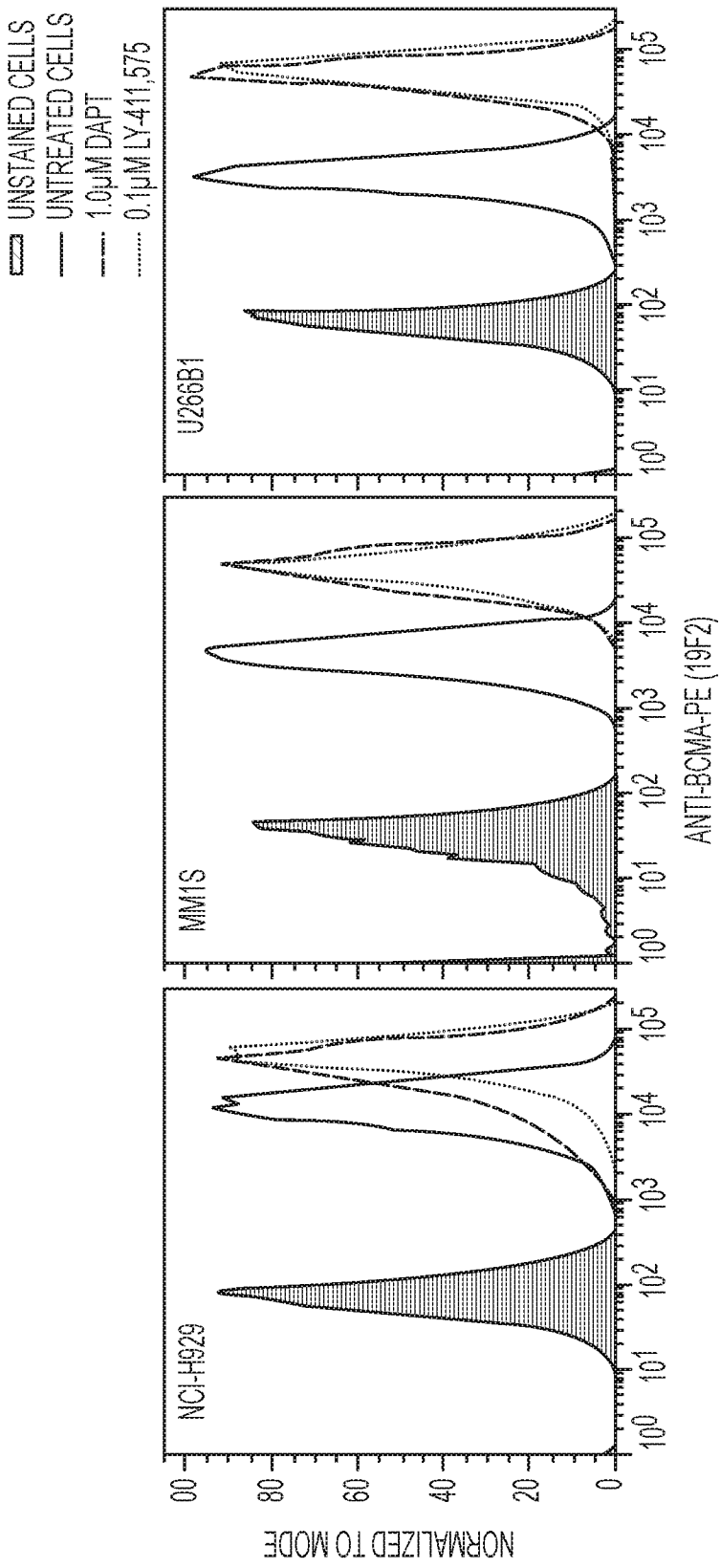

UNSTAINED CELLS
UNTREATED CELLS
1.0μM DAPT
0.1μM LY-411,575
NCI-H929
MM1S
U266B1
NORMALIZED TO MODE
ANTI-BCMA-PE (19F2)

FIG. 1

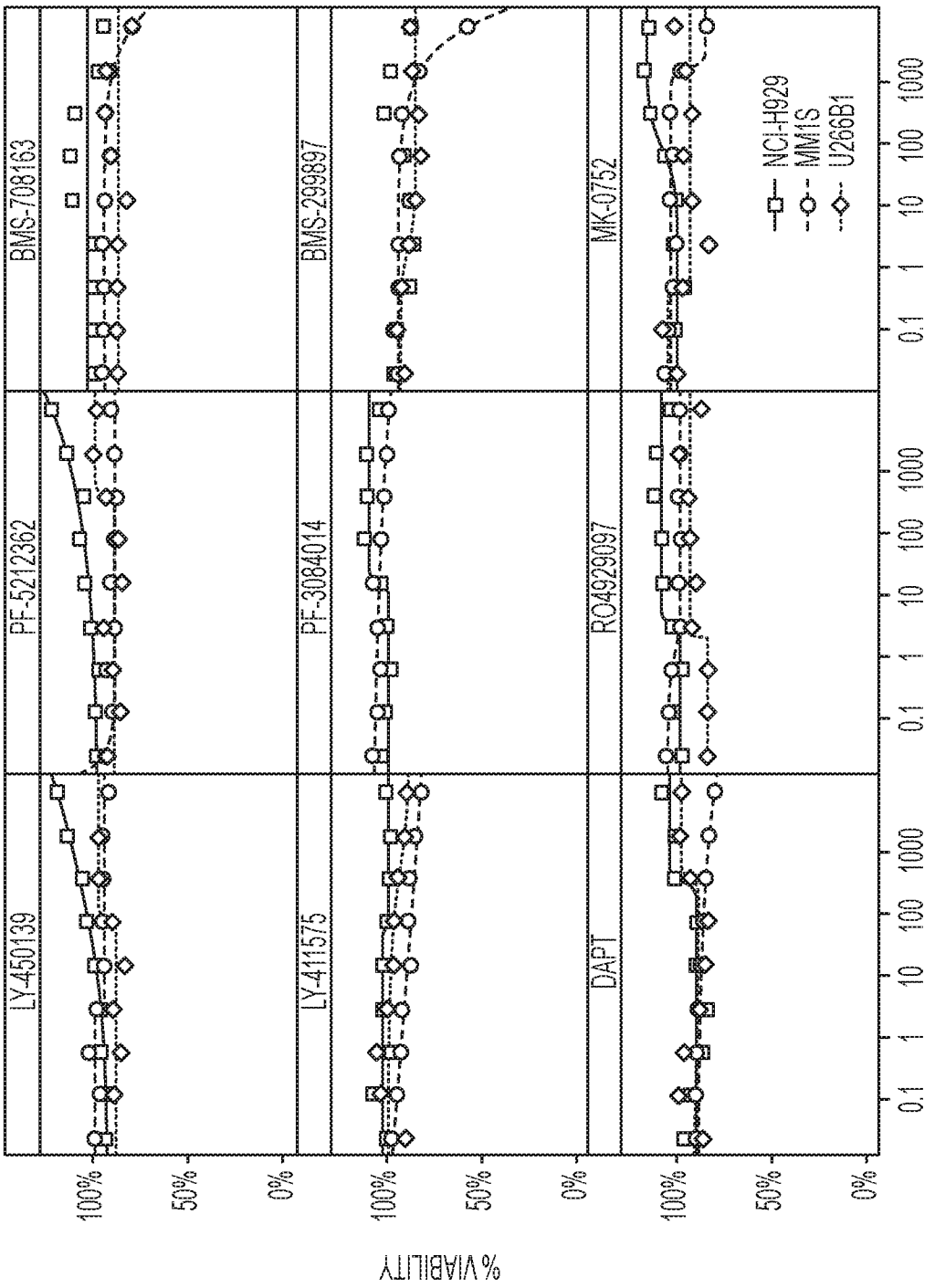
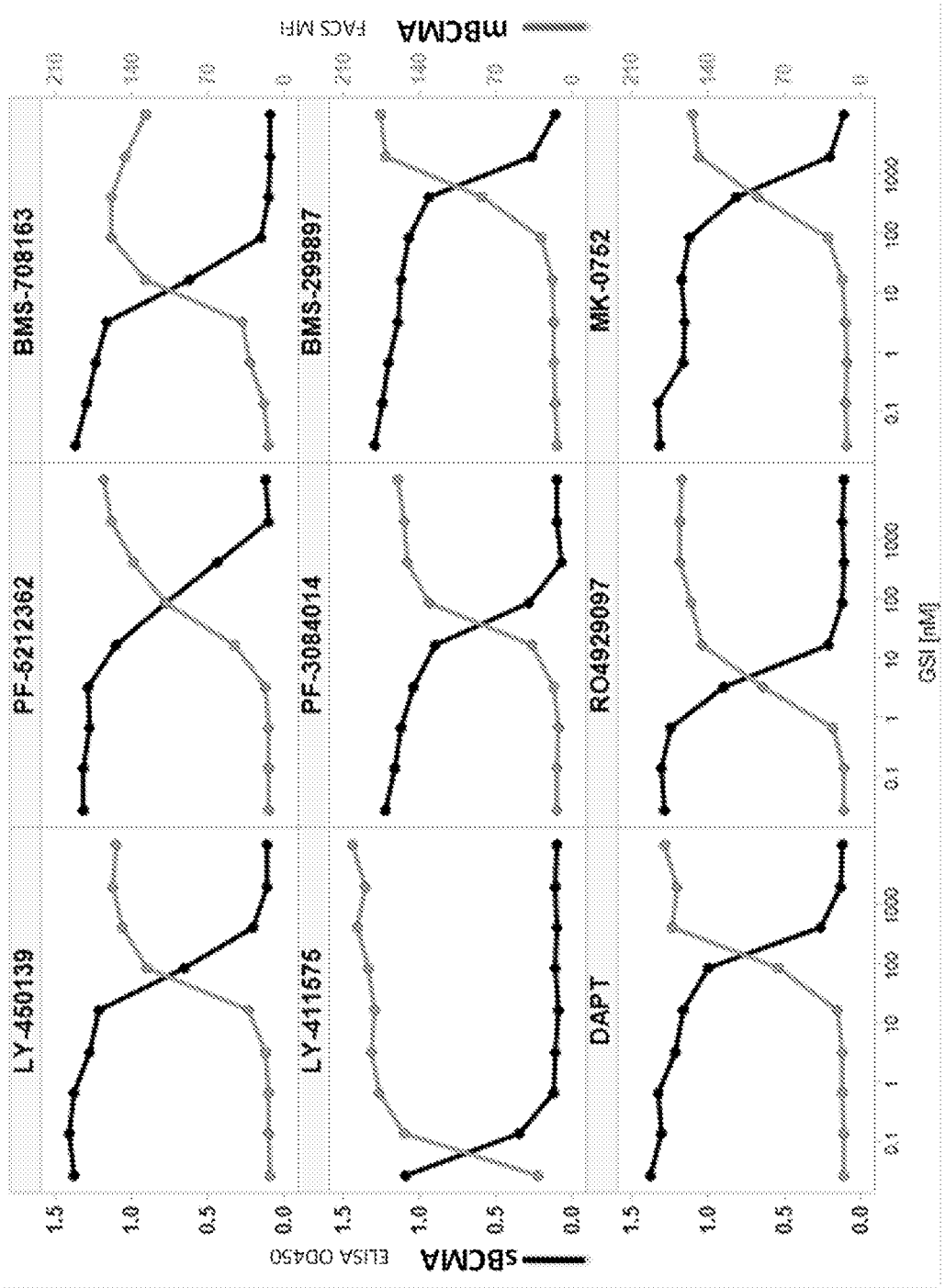

LY-450139
PF-5212362
BMS-708163
LY-411575
PF-3084014
BMS-299897
DAPT
RO4929097
MK-0752
NCI-H929
MM1S
U266B1
% VIABILITY
100%
50%
0%
0.1
1
10
100
1000
GSI [nM]

FIG. 2

FIG. 3


LY-450139
PF-5212362
BMS-708163
LY-411575
PF-3084014
BMS-299897
DAPT
RO4929097
MK-0752
sBCMA
ELISA OD450
mBCMA
FACS MFI
GSI [nM]

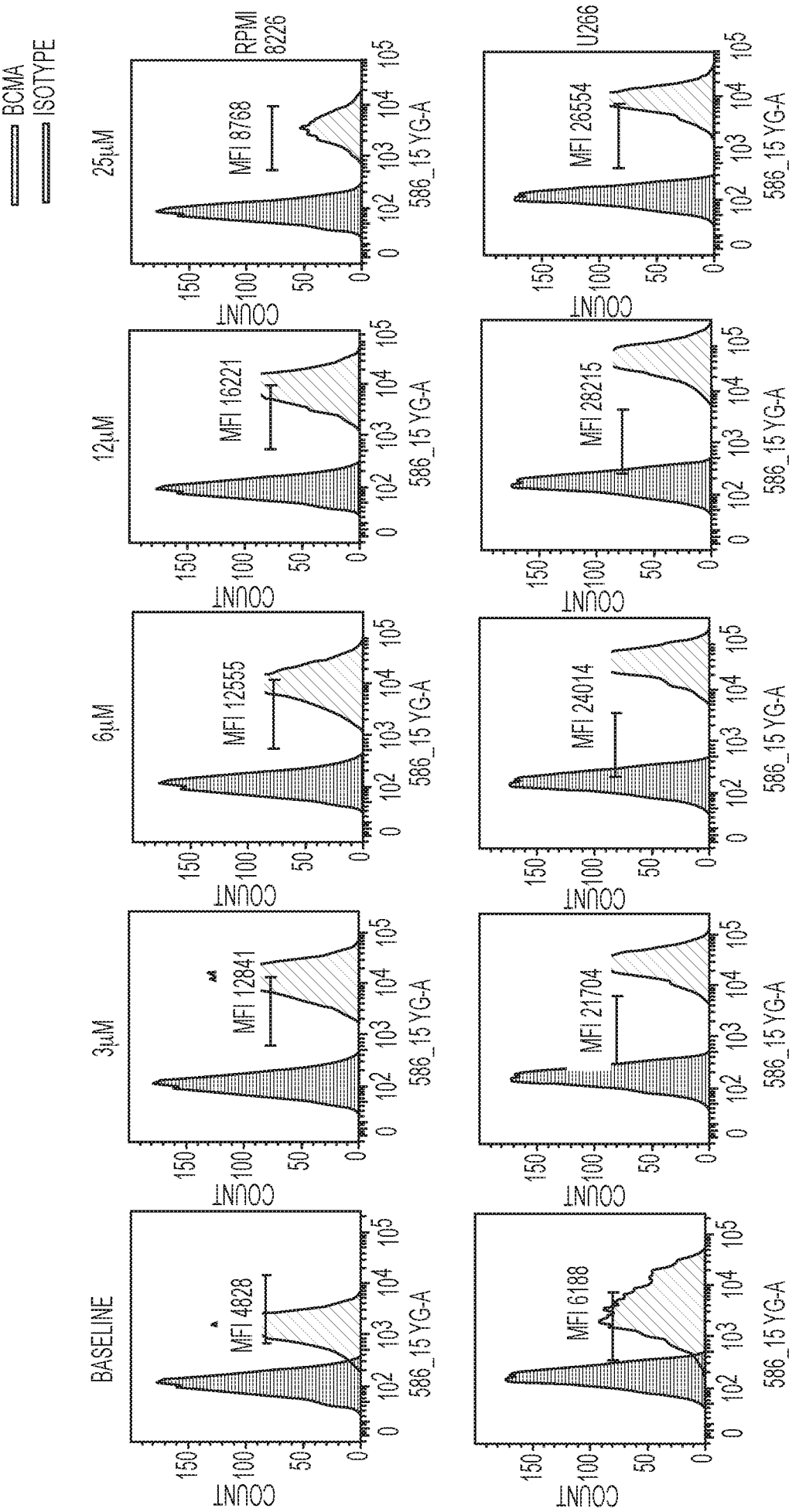

BCMA
ISOTYPE
BASELINE
3μM
6μM
12μM
25μM
RPMI 8226
U266
MFI 4828
MFI 12841
MFI 12555
MFI 16221
MFI 8768
MFI 6188
MFI 21704
MFI 24014
MFI 28215
MFI 26554
COUNT
586_15 YG-A

FIG. 4A

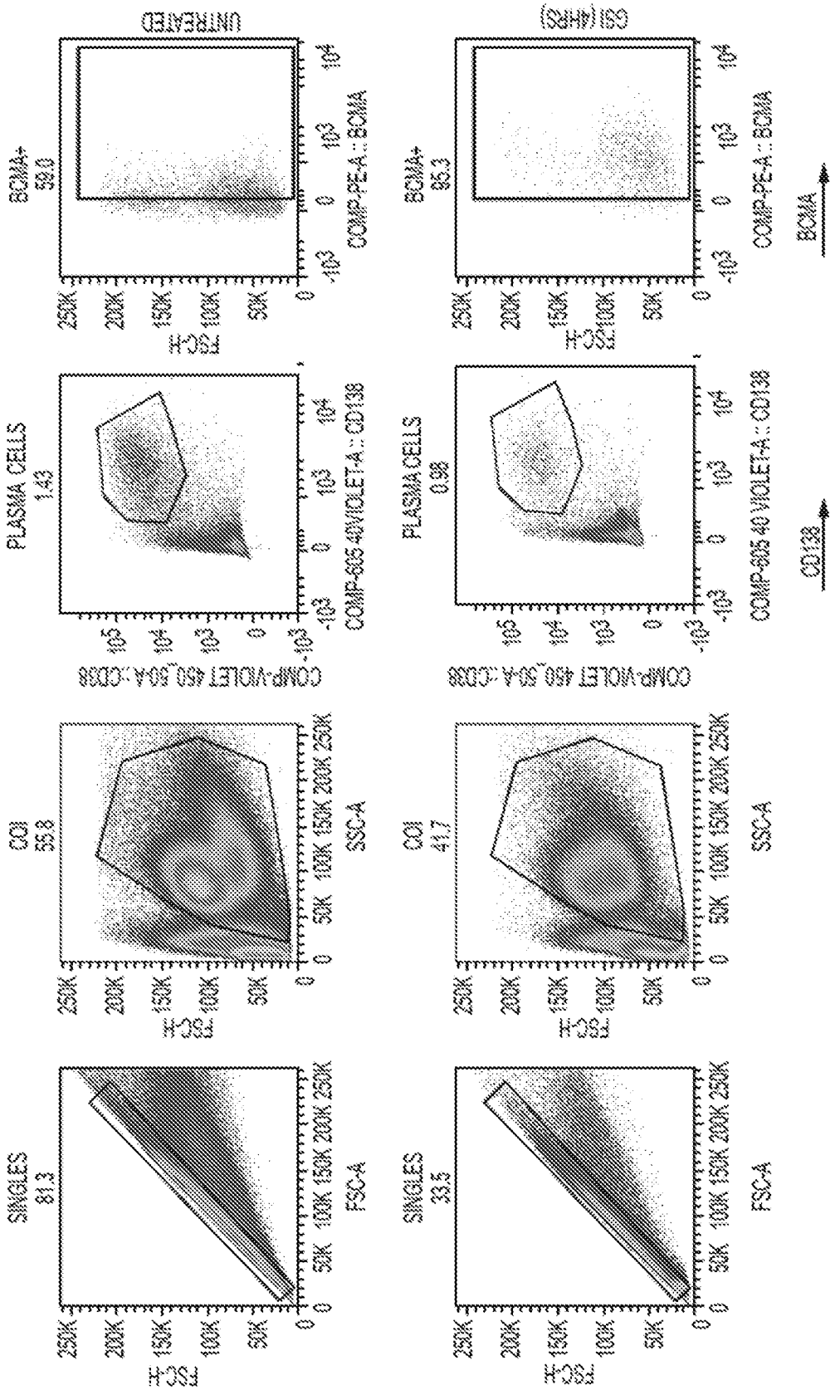

SINGLES
81.3
FSC-H
FSC-A
COI
55.8
SSC-H
SSC-A
PLASMA CELLS
1.43
COMP-VIOLET 450_50-A :: CD38
COMP-605 40-VIOLET-A :: CD138
BCMA+
59.0
COMP-PE-A :: BCMA
UNTREATED
SINGLES
33.5
COI
41.7
PLASMA CELLS
0.98
BCMA+
85.3
GSI (4HRS)
CD138
BCMA

FIG. 4B

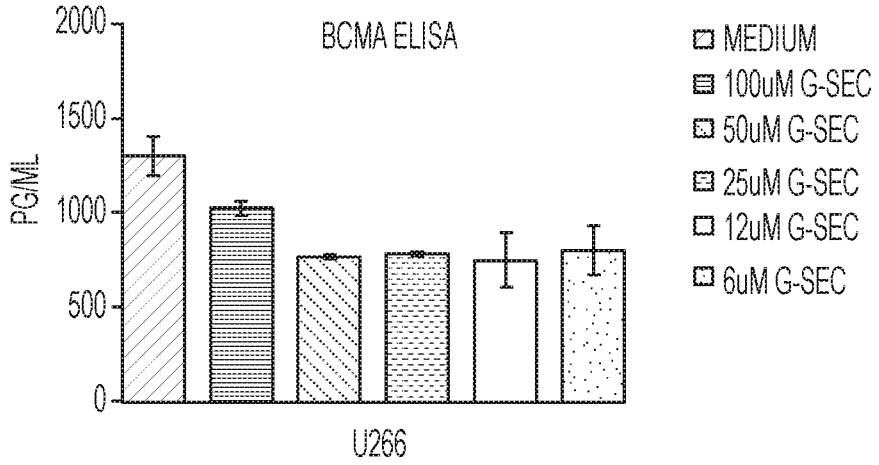

BCMA ELISA
PG/ML
2000
1500
1000
500
0
U266
MEDIUM
100uM G-SEC
50uM G-SEC
25uM G-SEC
12uM G-SEC
6uM G-SEC

FIG. 5A

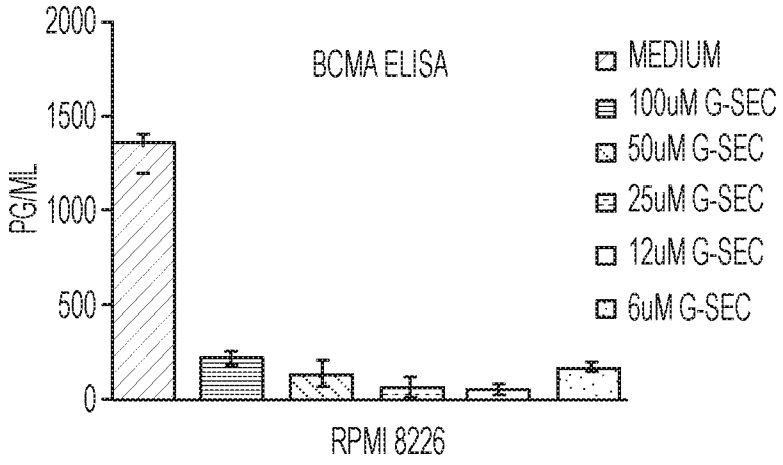

BCMA ELISA
PG/ML
2000
1500
1000
500
0
RPMI 8226
MEDIUM
100uM G-SEC
50uM G-SEC
25uM G-SEC
12uM G-SEC
6uM G-SEC

FIG. 5B

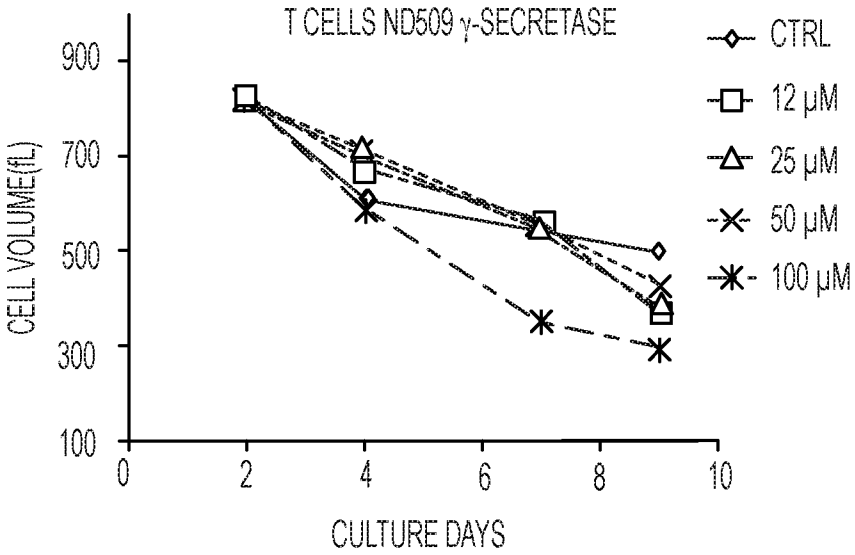

T CELLS ND509 γ-SECRETASE
CELL VOLUME(fL)
900
700
500
300
100
0
2
4
6
8
10
CULTURE DAYS
CTRL
12 μM
25 μM
50 μM
100 μM

FIG. 6A

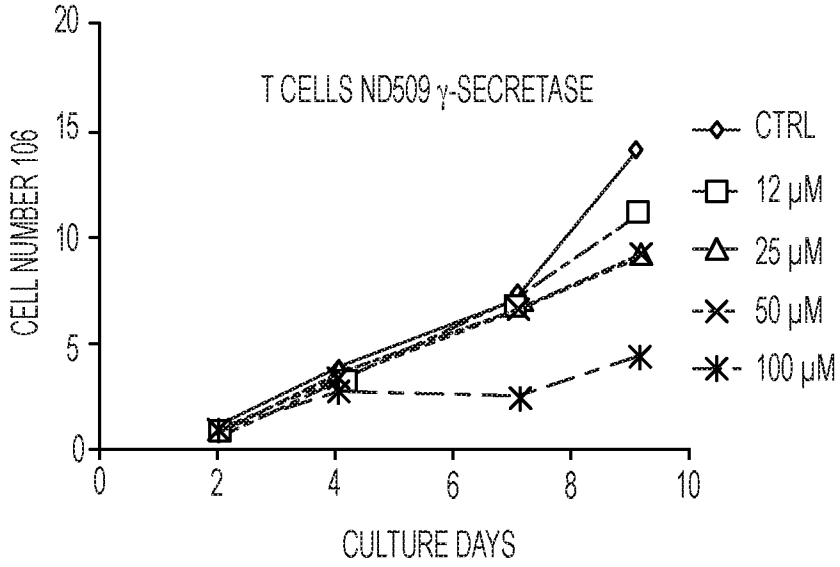

T CELLS ND509 γ-SECRETASE
CELL NUMBER 10^6
20
15
10
5
0
0
2
4
6
8
10
CULTURE DAYS
CTRL
12 μM
25 μM
50 μM
100 μM

FIG. 6B

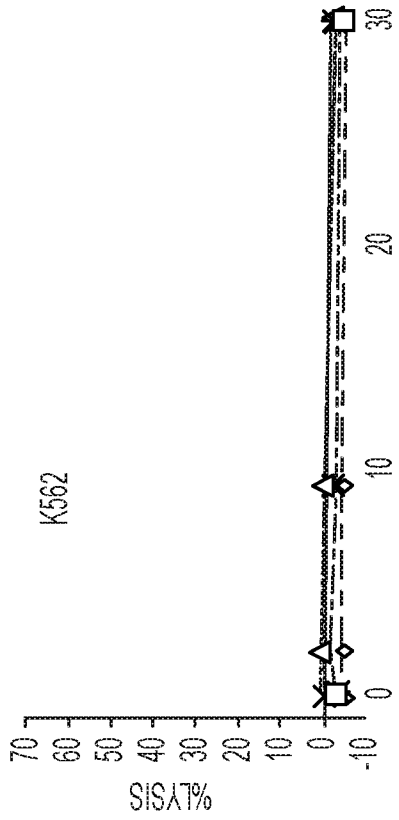


FIG. 7A

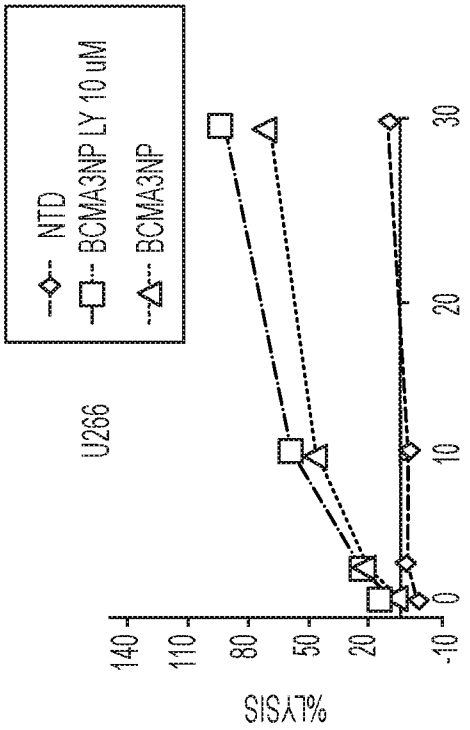


FIG. 7C

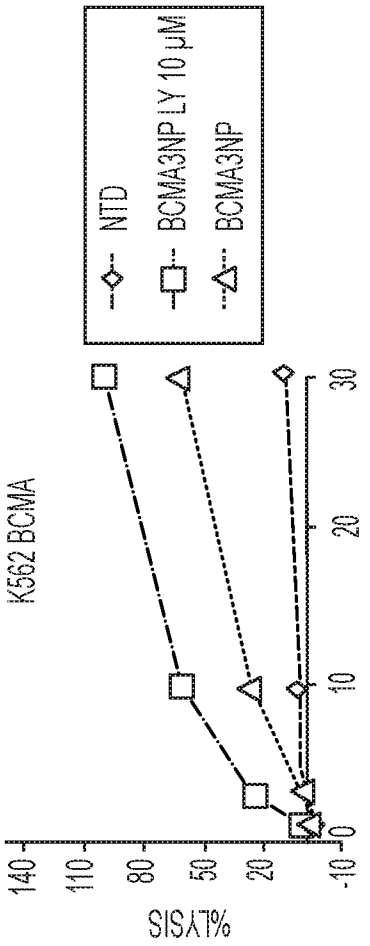


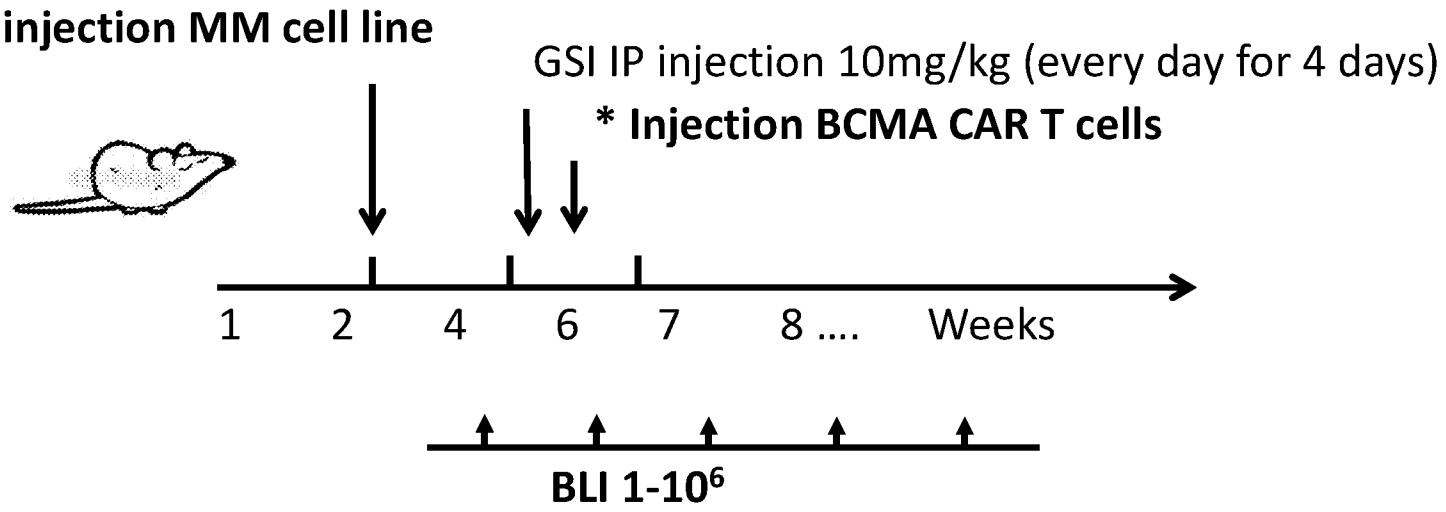
FIG. 7B

FIG. 8

Study Design: BCMA3NP CAR T cells + γ-secretase inhibitor

Goal: Combinatorial low CAR T cells doses / GSI treatment increases antitumor response in an aggressive MM mouse model.

GROUP	U266/route	CART cells/route	MICE
1.BCMA 3NP + GSI	5.10^6/ IV	$2x10^6$ / IV	N=10
2.BCMA 3NP	5.10^6/ IV	$2x10^6$ / IV	N=8
3.NTD + GSI	5.10^6/ IV	$2x10^6$ / IV	N=9
4.NTD	5.10^6/ IV	$2x10^6$ / IV	N=8



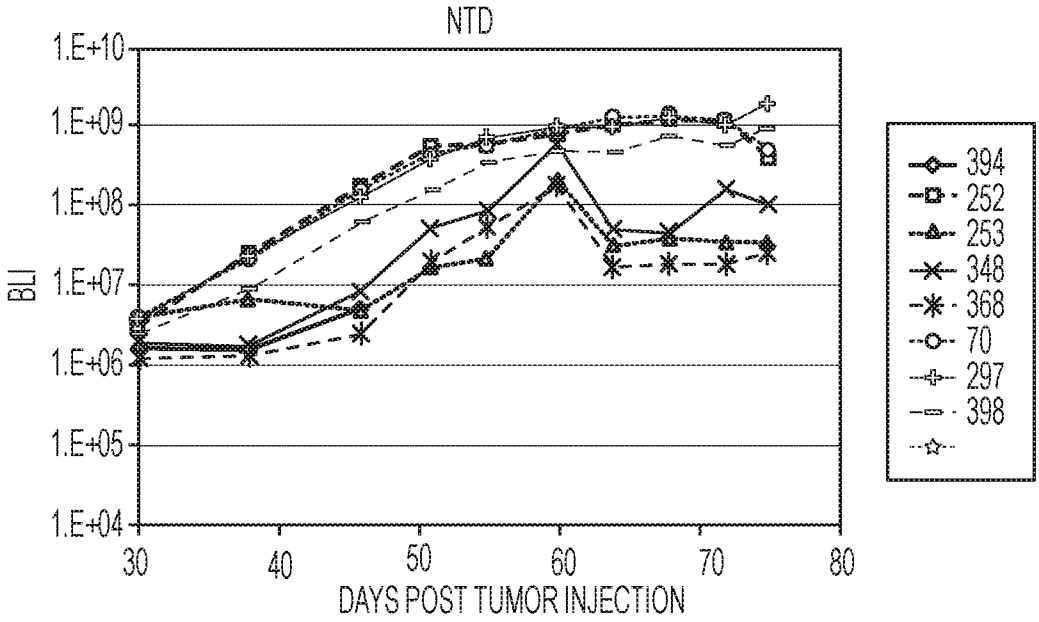

NTD
1.E+10
1.E+09
1.E+08
1.E+07
1.E+06
1.E+05
1.E+04
BLI
30
40
50
60
70
80
DAYS POST TUMOR INJECTION
394
252
253
348
368
70
297
398

FIG. 9A

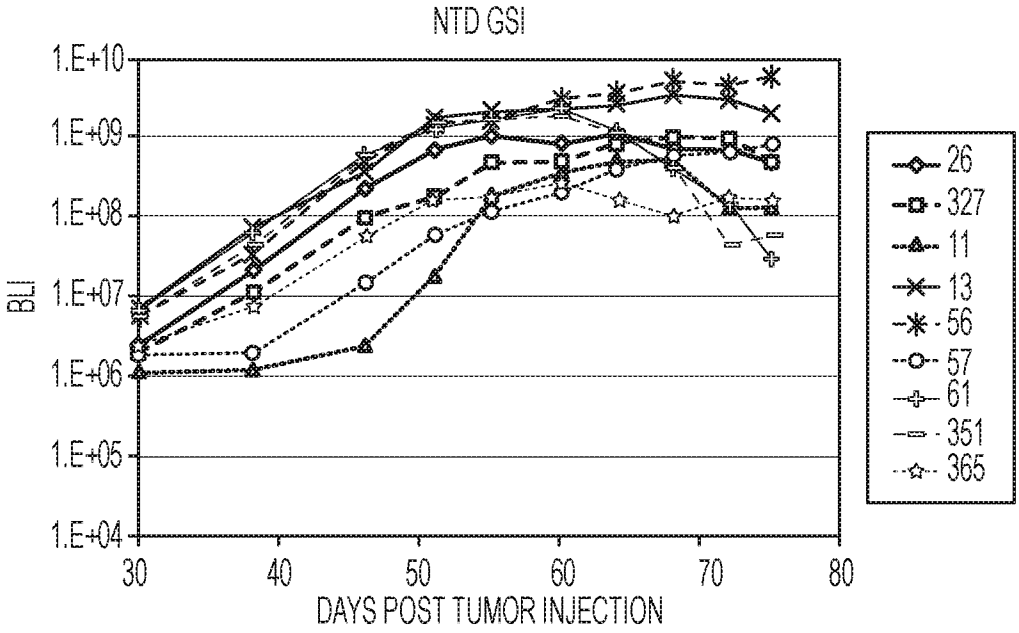

NTD GSI
1.E+10
1.E+09
1.E+08
1.E+07
1.E+06
1.E+05
1.E+04
BLI
30
40
50
60
70
80
DAYS POST TUMOR INJECTION
26
327
11
13
56
57
61
351
365

FIG. 9B

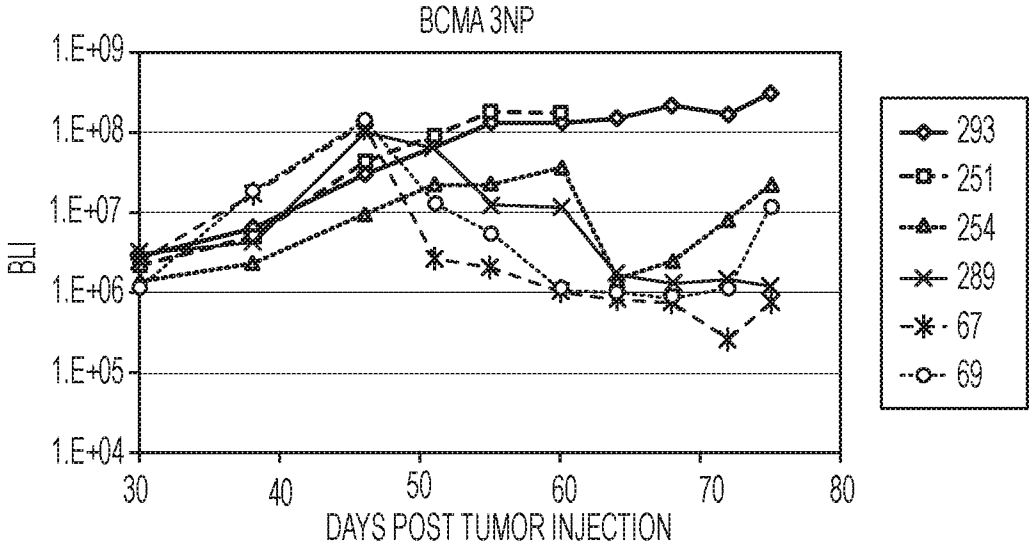

BCMA 3NP
1.E+09
1.E+08
1.E+07
1.E+06
1.E+05
1.E+04
BLI
30
40
50
60
70
80
DAYS POST TUMOR INJECTION
293
251
254
289
67
69

FIG. 9C

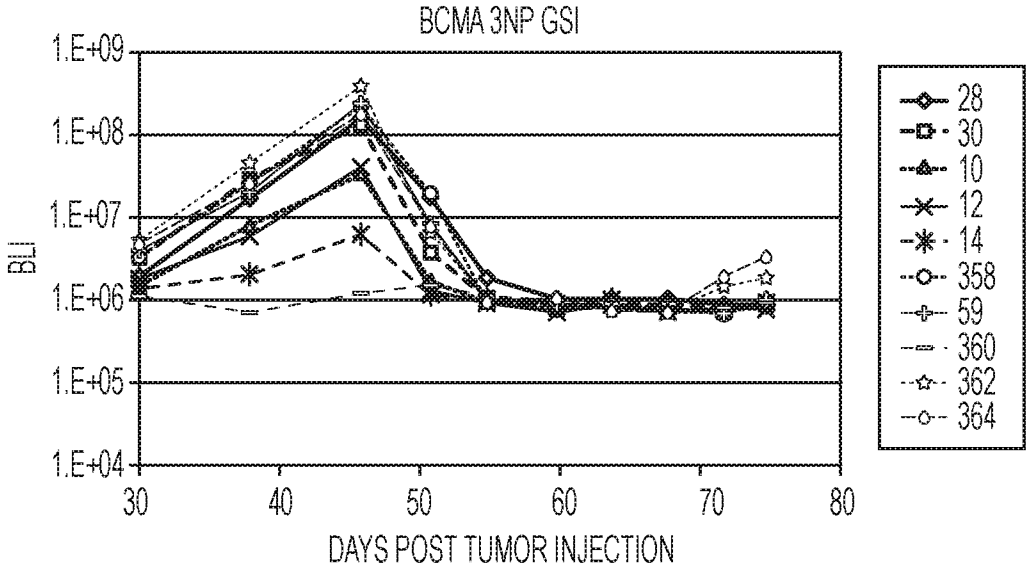

BCMA 3NP GSI
1.E+09
1.E+08
1.E+07
1.E+06
1.E+05
1.E+04
BLI
30
40
50
60
70
80
DAYS POST TUMOR INJECTION
28
30
10
12
14
358
59
360
362
364

FIG. 9D

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2018/029963

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61K38/17 A61K35/17 C07K14/725 C07K16/28 C12N5/0783
ADD.

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)
A61K C07K C12N

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

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2017/019496 A1 (BERENSON JAMES RICHARD [US]) 2 February 2017 (2017-02-02) page 10, line 5 - line 15 page 16, line 13 - page 19, line 24 -----	1-4, 6-20, 26-60
A	WO 2016/014565 A2 (NOVARTIS AG [CH]; UNIV PENNSYLVANIA [US]; BROGDON JENNIFER [US]; CHOI) 28 January 2016 (2016-01-28) the whole document -----	3,4, 26-55
	-/--	

[X] Further documents are listed in the continuation of Box C.

[X] 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
19 June 2018	29/06/2018

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Galli, Ivo

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Form PCT/ISA/210 (second sheet) (April 2005)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2018/029963

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.: 5, 21-25(completely); 1-4, 6-20, 26-60(partially)
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:
see FURTHER INFORMATION sheet PCT/ISA/210

3. ☐ Claims Nos.:
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:

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 an 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.:

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.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box II.2

Claims Nos.: 5, 21-25(completely); 1-4, 6-20, 26-60(partially)

Claims 1-4, 6-7, 56, 60 define the GSI in terms of results to be achieved, covering GSIs that have not even been developed so far. Such desiderata cannot be searched. The claims have been interpreted as to refer to any known GSI.

Claims 5, 21-25 refer to specific GSIs that have neither been disclosed in the application or developed in prior art. These claims so lack disclosure that they cannot be searched.

The term *BCMA CAR expressing cell" is vague. It covers any cell that expresses a BCMA-binding moiety fused to anything. The application makes it plausible that BCMA-CAR-T can be used. For the rest, the application so lacks clarity, support and disclosure under Art. 5-6 PCT that a complete search is impossible. The search of the claims has been restricted to BCMA-CAR-T.

Claims 26-55 cover a myriad of variations in terms of CAR constructs (BCMA binding part, TM, intracellular part, hinges,...) The disclosure, however, is remarkably scant as to which constructs are useful and which are not. The number, breadth and complexity of the claims is unreasonable in consideration of the nature of the invention disclosed and claimed (Rule 6.1(a) PCT). The search has been limited to the constructs already developed by the Applicants in WO2016014565.

The applicant's attention is drawn to the fact that claims relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure. If the application proceeds into the regional phase before the EPO, the applicant is reminded that a search may be carried out during examination before the EPO (see EPO Guidelines C-IV, 7.2), should the problems which led to the Article 17(2) declaration be overcome.

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X,P	Worcester S.: "GSI inhibition may boost BCMA CAR T-cell therapy efficacy in myeloma", Hematology News , 27 November 2017 (2017-11-27), XP002782175, Retrieved from the Internet: URL:https://www.mdedge.com/hematologynews/article/152733/multiple-myeloma/gsi-inhibition-may-boost-bcma-car-t-cell-therapy [retrieved on 2018-06-19] the whole document -----	1-4,8, 19,26, 47-52, 58-60

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INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/US2018/029963

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2017019496 A1	02-02-2017	AU 2016297793 A1	08-02-2018
		CA 2992557 A1	02-02-2017
		CN 108025001 A	11-05-2018
		EP 3324965 A1	30-05-2018
		KR 20180033232 A	02-04-2018
		WO 2017019496 A1	02-02-2017
WO 2016014565 A2	28-01-2016	AU 2015292744 A1	02-02-2017
		CA 2955386 A1	28-01-2016
		CN 106687483 A	17-05-2017
		EP 3172237 A2	31-05-2017
		JP 2017527271 A	21-09-2017
		KR 20170037626 A	04-04-2017
		SG 11201700476V A	27-02-2017
		TW 201615832 A	01-05-2016
		US 2016046724 A1	18-02-2016
		WO 2016014565 A2	28-01-2016