METHODS FOR TREATMENT USING CHIMERIC ANTIGEN RECEPTORS SPECIFIC FOR B-CELL MATURATION ANTIGEN

Cross-Reference to Related Applications

[0001] This application claims priority from U.S. provisional application No. 62/754,577, filed November 1, 2018, entitled "METHODS FOR TREATMENT USING CHIMERIC ANTIGEN RECEPTORS SPECFIFIC FOR B-CELL MATURATION ANTIGEN," U.S. provisional application No. 62/774,167, filed November 30, 2018, entitled "METHODS FOR TREATMENT USING CHIMERIC ANTIGEN RECEPTORS SPECFIFIC FOR B-CELL MATURATION ANTIGEN," U.S. provisional application No. 62/774,856, filed December 3, 2018, entitled "METHODS FOR TREATMENT USING CHIMERIC ANTIGEN RECEPTORS SPECFIFIC FOR B-CELL MATURATION ANTIGEN," U.S. provisional application No. 67/777,066 filed December 7, 2018, entitled "METHODS FOR TREATMENT USING CHIMERIC ANTIGEN RECEPTORS SPECFIFIC FOR B-CELL MATURATION ANTIGEN," U.S. provisional application No. 62/845,817 filed May 9, 2019, entitled "METHODS FOR TREATMENT USING CHIMERIC ANTIGEN RECEPTORS SPECFIFIC FOR B-CELL MATURATION ANTIGEN," the contents of which are incorporated by reference in their entirety.

Incorporation by Reference of Sequence Listing

[0002] The present application is being filed along with a Sequence Listing in electronic format. The Sequence Listing is provided as a file entitled 735042019140SEQLIST.txt, created October 31, 2019, which is 166 kilobytes in size. The information in the electronic format of the Sequence Listing is incorporated by reference in its entirety.

Field

[0003] The present disclosure relates in some aspects to adoptive cell therapy involving the administration of doses of cells for treating disease and conditions, including certain plasma cell malignancy. The cells generally express recombinant receptors such as chimeric antigen receptors (CARs) specific to B-cell maturation antigen (BCMA). In some embodiments, the methods are for treating subjects with multiple myeloma (MM). The disclosure further relates to genetically engineered cells containing such BCMA-binding receptors for uses in adoptive cell therapy.

Background

[0004] B-cell maturation antigen (BCMA) is a transmembrane type III protein expressed on mature B lymphocytes. Following binding of BCMA to its ligands, B cell activator of the TNF family (BAFF) or a proliferation inducing ligand (APRIL), a pro-survival cell signal is delivered to the B cell which has been found to be required for plasma cell survival. The expression of BCMA has been linked to several

diseases including cancer, autoimmune disorders and infectious diseases Due to the role of BCMA in various diseases and conditions, including cancer, BCMA is a therapeutic target. Various BCMA-binding chimeric antigen receptors (CARs), and cells expressing such CARs, are available. However, there remains a need for improved BCMA-binding CARs and engineered BCMA-CAR expressing targeting cells, such as for use in adoptive cell therapy. Provided herein are embodiments that meet such needs.

Summary

[0005] Provided herein are methods of treating a subject having or suspected of having multiple myeloma (MM), the method comprising administering to the subject a dose of engineered T cells comprising a chimeric antigen receptor (CAR), the CAR including: (a) a variable heavy chain (V_H) comprising a heavy chain complementarity determining region 1 (CDR-H1), a heavy chain complementarity determining region 2 (CDR-H2) and a heavy chain complementarity determining region 3 (CDR-H3) contained within the sequence set forth in SEQ ID NO: 116 and a variable light chain (V_L) comprising a light chain complementarity determining region 1 (CDR-L1), a light chain complementarity determining region 2 (CDR-L2) and a light chain complementarity determining region 3 (CDR-L3) contained within the sequence set forth in SEQ ID NO: 119; a V_H comprising a CDR-H1, a CDR-H2 and a CDR-H3 sequences set forth in SEQ ID NOS:97, 101 and 103, respectively, and a V_L comprising a CDR-L1, a CDR-L2 and a CDR-L3 sequences set forth in SEQ ID NOS:105, 107 and 108, respectively; a V_H comprising a CDR-H1, a CDR-H2 and a CDR-H3 sequences set forth in SEQ ID NOS:96, 100 and 103, respectively, and a V_L comprising a CDR-L1, a CDR-L2 and a CDR-L3 sequences set forth in SEQ ID NOS:105, 107 and 108, respectively; a V_H comprising a CDR-H1, a CDR-H2 and a CDR-H3 sequences set forth in SEQ ID NOS:95, 99 and 103, respectively, and a V_L comprising a CDR-L1, a CDR-L2 and a CDR-L3 sequences set forth in SEQ ID NOS: 105, 107 and 108, respectively; a V_H comprising a CDR-H1, a CDR-H2 and a CDR-H3 sequences set forth in SEQ ID NOS:94, 98 and 102, respectively, and a V_L comprising a CDR-L1, a CDR-L2 and a CDR-L3 sequences set forth in SEQ ID NOS: 104, 106 and 108, respectively; or a V_H comprising the amino acid sequence of SEQ ID NO: 116 and a V_L comprising the amino acid sequence of SEQ ID NO: 119; (b) a spacer comprising an IgG4/2 chimeric hinge or a modified IgG4 hinge; an IgG2/4 chimeric C_H2 region; and an IgG4 C_H3 region, which optionally is about 228 amino acids in length; or a spacer set forth in SEQ ID NO: 174; (c) a transmembrane domain, optionally a transmembrane domain from a human CD28; and (d) an intracellular signaling region that includes a cytoplasmic signaling domain of a CD3-zeta (CD3ζ) chain and a costimulatory signaling region that includes an intracellular signaling domain of a T cell costimulatory molecule or a signaling portion thereof; wherein, prior to the administration, the subject has received a lymphodepleting therapy comprising the administration of fludarabine at or about 20-40 mg/m^2 body surface area of the subject, optionally at or about 30 mg/m^2, daily, for 2-4 days, and/or cyclophosphamide at or about 200-400 mg/m^2 body surface area of the subject, optionally at or about 300

mg/m^2, daily, for 2-4 days.

[0006] Provided herein are methods of treating a subject having or suspected of having multiple myeloma (MM), the method comprising administering to the subject a dose of engineered T cells comprising a chimeric antigen receptor (CAR), the CAR including: (a) a variable heavy chain (V_H) comprising a heavy chain complementarity determining region 1 (CDR-H1), a heavy chain complementarity determining region 2 (CDR-H2) and a heavy chain complementarity determining region 3 (CDR-H3) contained within the sequence set forth in SEQ ID NO: 116 and a variable light chain (V_L) comprising a light chain complementarity determining region 1 (CDR-L1), a light chain complementarity determining region 2 (CDR-L2) and a light chain complementarity determining region 3 (CDR-L3) contained within the sequence set forth in SEQ ID NO: 119; a V_H comprising a CDR-H1, a CDR-H2 and a CDR-H3 sequences set forth in SEQ ID NOS:97, 101 and 103, respectively, and a V_L comprising a CDR-L1, a CDR-L2 and a CDR-L3 sequences set forth in SEQ ID NOS:105, 107 and 108, respectively; a V_H comprising a CDR-H1, a CDR-H2 and a CDR-H3 sequences set forth in SEQ ID NOS:96, 100 and 103, respectively, and a V_L comprising a CDR-L1, a CDR-L2 and a CDR-L3 sequences set forth in SEQ ID NOS:105, 107 and 108, respectively; a V_H comprising a CDR-H1, a CDR-H2 and a CDR-H3 sequences set forth in SEQ ID NOS:95, 99 and 103, respectively, and a V_L comprising a CDR-L1, a CDR-L2 and a CDR-L3 sequences set forth in SEQ ID NOS: 105, 107 and 108, respectively; a V_H comprising a CDR-H1, a CDR-H2 and a CDR-H3 sequences set forth in SEQ ID NOS:94, 98 and 102, respectively, and a V_L comprising a CDR-L1, a CDR-L2 and a CDR-L3 sequences set forth in SEQ ID NOS: 104, 106 and 108, respectively; or a V_H comprising the amino acid sequence of SEQ ID NO: 116 and a V_L comprising the amino acid sequence of SEQ ID NO: 119; (b) a spacer comprising an IgG4/2 chimeric hinge or a modified IgG4 hinge; an IgG2/4 chimeric C_H2 region; and an IgG4 C_H3 region, which optionally is about 228 amino acids in length; or a spacer set forth in SEQ ID NO: 174; (c) a transmembrane domain, optionally a transmembrane domain from a human CD28; and (d) an intracellular signaling region that includes a cytoplasmic signaling domain of a CD3-zeta (CD3ζ) chain and a costimulatory signaling region that includes an intracellular signaling domain of a T cell costimulatory molecule or a signaling portion thereof; wherein at or prior to the administration of the dose of engineered T cells, the subject has received three or more therapies selected from among: autologous stem cell transplant (ASCT); an immunomodulatory agent; a proteasome inhibitor; and an anti-CD38 antibody; unless the subject was not a candidate for or was contraindicated for one or more of the therapies.

[0007] Provided herein are methods of treating a subject having or suspected of having multiple myeloma (MM), the method comprising administering to the subject a dose of engineered T cells comprising a chimeric antigen receptor (CAR), the CAR including: (a) a variable heavy chain (V_H) comprising a heavy chain complementarity determining region 1 (CDR-H1), a heavy chain complementarity determining region 2 (CDR-H2) and a heavy chain complementarity determining region

3 (CDR-H3) contained within the sequence set forth in SEQ ID NO: 116 and a variable light chain (V_L) comprising a light chain complementarity determining region 1 (CDR-L1), a light chain complementarity determining region 2 (CDR-L2) and a light chain complementarity determining region 3 (CDR-L3) contained within the sequence set forth in SEQ ID NO: 119; a V_H comprising a CDR-H1, a CDR-H2 and a CDR-H3 sequences set forth in SEQ ID NOS:97, 101 and 103, respectively, and a V_L comprising a CDR-L1, a CDR-L2 and a CDR-L3 sequences set forth in SEQ ID NOS:105, 107 and 108, respectively; a V_H comprising a CDR-H1, a CDR-H2 and a CDR-H3 sequences set forth in SEQ ID NOS:96, 100 and 103, respectively, and a V_L comprising a CDR-L1, a CDR-L2 and a CDR-L3 sequences set forth in SEQ ID NOS:105, 107 and 108, respectively; a V_H comprising a CDR-H1, a CDR-H2 and a CDR-H3 sequences set forth in SEQ ID NOS:95, 99 and 103, respectively, and a V_L comprising a CDR-L1, a CDR-L2 and a CDR-L3 sequences set forth in SEQ ID NOS: 105, 107 and 108, respectively; a V_H comprising a CDR-H1, a CDR-H2 and a CDR-H3 sequences set forth in SEQ ID NOS:94, 98 and 102, respectively, and a V_L comprising a CDR-L1, a CDR-L2 and a CDR-L3 sequences set forth in SEQ ID NOS: 104, 106 and 108, respectively; or a V_H comprising the amino acid sequence of SEQ ID NO: 116 and a V_L comprising the amino acid sequence of SEQ ID NO: 119; (b) a spacer comprising an IgG4/2 chimeric hinge or a modified IgG4 hinge; an IgG2/4 chimeric C_H2 region; and an IgG4 C_H3 region, which optionally is about 228 amino acids in length; or a spacer set forth in SEQ ID NO: 174; (c) a transmembrane domain, optionally a transmembrane domain from a human CD28; and (d) an intracellular signaling region that includes a cytoplasmic signaling domain of a CD3-zeta (CD3ζ) chain and a costimulatory signaling region that includes an intracellular signaling domain of a T cell costimulatory molecule or a signaling portion thereof; wherein at the administration of the dose of engineered T cells, the subject has not had active or history of plasma cell leukemia (PCL).

[0008] Provided herein are methods of treating a subject having or suspected of having multiple myeloma (MM), the method comprising administering to the subject a dose of engineered T cells comprising a chimeric antigen receptor (CAR), the CAR including: (a) a variable heavy chain (V_H) comprising a heavy chain complementarity determining region 1 (CDR-H1), a heavy chain complementarity determining region 2 (CDR-H2) and a heavy chain complementarity determining region 3 (CDR-H3) contained within the sequence set forth in SEQ ID NO: 116 and a variable light chain (V_L) comprising a light chain complementarity determining region 1 (CDR-L1), a light chain complementarity determining region 2 (CDR-L2) and a light chain complementarity determining region 3 (CDR-L3) contained within the sequence set forth in SEQ ID NO: 119; a V_H comprising a CDR-H1, a CDR-H2 and a CDR-H3 sequences set forth in SEQ ID NOS:97, 101 and 103, respectively, and a V_L comprising a CDR-L1, a CDR-L2 and a CDR-L3 sequences set forth in SEQ ID NOS:105, 107 and 108, respectively; a V_H comprising a CDR-H1, a CDR-H2 and a CDR-H3 sequences set forth in SEQ ID NOS:96, 100 and 103, respectively, and a V_L comprising a CDR-L1, a CDR-L2 and a CDR-L3 sequences set forth in SEQ ID NOS:105, 107 and 108, respectively; a V_H comprising a CDR-H1, a CDR-H2 and a CDR-H3

sequences set forth in SEQ ID NOS:95, 99 and 103, respectively, and a V_L comprising a CDR-L1, a CDR-L2 and a CDR-L3 sequences set forth in SEQ ID NOS: 105, 107 and 108, respectively; a V_H comprising a CDR-H1, a CDR-H2 and a CDR-H3 sequences set forth in SEQ ID NOS:94, 98 and 102, respectively, and a V_L comprising a CDR-L1, a CDR-L2 and a CDR-L3 sequences set forth in SEQ ID NOS: 104, 106 and 108, respectively; or a V_H comprising the amino acid sequence of SEQ ID NO: 116 and a V_L comprising the amino acid sequence of SEQ ID NO: 119; (b) a spacer comprising an IgG4/2 chimeric hinge or a modified IgG4 hinge; an IgG2/4 chimeric C_H2 region; and an IgG4 C_H3 region, which optionally is about 228 amino acids in length; or a spacer set forth in SEQ ID NO: 174; (c) a transmembrane domain, optionally a transmembrane domain from a human CD28; and (d) an intracellular signaling region that includes a cytoplasmic signaling domain of a CD3-zeta (CD3ζ) chain and a costimulatory signaling region that includes an intracellular signaling domain of a T cell costimulatory molecule or a signaling portion thereof; wherein the dose of engineered T cells comprises: between at or about 1 x 10^7 CAR-expressing (CAR+) T cells and 2 x 10^9 CAR-expressing T cells; a combination of $CD4^+$ T cells and $CD8^+$ T cells, at a defined ratio of $CD4^+$ CAR-expressing T cells to $CD8^+$ CAR-expressing T cells and/or of $CD4^+$ T cells to $CD8^+$ T cells, that is or is approximately 1:1 or is between approximately 1:3 and approximately 3:1; and less than 25%, 20%, 15%, 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2% or 1% of the CAR-expressing T cells in the dose express a marker of apoptosis, optionally Annexin V or active Caspase 3.

[0009] In some of any embodiments, the extracellular antigen-binding domain of the CAR specifically binds to a B cell maturation antigen (BCMA).

[0010] In some of any embodiments, the V_H is or comprises the amino acid sequence of SEQ ID NO: 116; and the V_L is or comprises the amino acid sequence of SEQ ID NO: 119. In some of any embodiments, the extracellular antigen-binding domain comprises an scFv. In some of any embodiments, the V_H and the V_L are joined by a flexible linker. In some of any embodiments, the scFv comprises a linker comprising the amino acid sequence GGGGSGGGGSGGGGS (SEQ ID NO:1). In some of any embodiments, the V_H is amino-terminal to the V_L.

[0011] In some of any embodiments, the antigen-binding domain comprises the amino acid sequence of SEQ ID NO: 114 or an amino acid sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to the amino acid sequence of SEQ ID NO: 114. In some of any embodiments, the antigen-binding domain comprises the amino acid sequence of SEQ ID NO: 114. In some of any embodiments, a nucleic acid encoding the antigen-binding domain comprises (a) the sequence of nucleotides of SEQ ID NO:113; (b) a sequence of nucleotides that has at least 90% sequence identity thereto; or (c) a degenerate sequence of (a) or (b). In some of any embodiments, the nucleic acid encoding the antigen-binding domain comprises the sequence of nucleotides of SEQ ID NO:115.

[0012] In some of any embodiments, the V_H is carboxy-terminal to the V_L.

[0013] In some of any embodiments, the cytoplasmic signaling domain is or comprises the sequence set forth in SEQ ID NO:143 or a sequence of amino acids that has at least 90% sequence identity to SEQ ID NO:143.

[0014] In some of any embodiments, the costimulatory signaling region comprises an intracellular signaling domain of CD28, 4-1BB, or ICOS, or a signaling portion thereof. In some of any embodiments, the costimulatory signaling region comprises an intracellular signaling domain of 4-1BB, optionally human 4-1BB. In some of any embodiments, the costimulatory signaling region is or comprises the sequence set forth in SEQ ID NO:4 or a sequence of amino acids that exhibits at least 90% sequence identity to the sequence set forth in SEQ ID NO: 4.

[0015] In some of any embodiments, the costimulatory signaling region is between the transmembrane domain and the cytoplasmic signaling domain of a CD3-zeta (CD3ζ) chain.

[0016] In some of any embodiments, the transmembrane domain is or comprises a transmembrane domain from human CD28. In some of any embodiments, the transmembrane domain is or comprises the sequence set forth in SEQ ID NO:138 or a sequence of amino acids that exhibits at least 90% sequence identity to SEQ ID NO:138.

[0017] In some of any embodiments, the CAR includes from its N to C terminus in order: the antigen-binding domain, the spacer, the transmembrane domain and the intracellular signaling region.

[0018] In some of any embodiments, the CAR comprises (a) an extracellular antigen-binding domain, comprising: a variable heavy chain (V_H) comprising a heavy chain complementarity determining region 1 (CDR-H1), a heavy chain complementarity determining region 2 (CDR-H2) and a heavy chain complementarity determining region 3 (CDR-H3) contained within the sequence set forth in SEQ ID NO: 116 and a variable light chain (V_L) comprising a light chain complementarity determining region 1 (CDR-L1), a light chain complementarity determining region 2 (CDR-L2) and a light chain complementarity determining region 3 (CDR-L3) contained within the sequence set forth in SEQ ID NO: 119; (b) a spacer comprising a modified IgG4 hinge; an IgG2/4 chimeric C_H2 region; and an IgG4 C_H3 region, that is about 228 amino acids in length; (c) a transmembrane domain from a human CD28; and (d) an intracellular signaling region comprising a cytoplasmic signaling domain of a CD3-zeta (CD3ζ) chain and a costimulatory signaling region comprising an intracellular signaling domain of a 4-1BB.

[0019] In some of any embodiments, the CAR comprises (a) an extracellular antigen-binding domain, comprising the sequence set forth in SEQ ID NO: 114 or a sequence of amino acids having at least 90% sequence identity to the amino acid sequence of SEQ ID NO: 114; (b) a spacer comprising the sequence set forth in SEQ ID NO: 174 or a sequence of amino acids that has at least 90% sequence identity to SEQ ID NO:174; (c) a transmembrane domain comprising the sequence set forth in SEQ ID NO:138 or a sequence of amino acids that has at least 90% sequence identity to SEQ ID NO:138; and (d) an intracellular signaling region comprising a cytoplasmic signaling comprising the sequence set forth in SEQ ID NO:143 or a sequence of amino acids that has at least 90% sequence identity to SEQ ID NO:143

and a costimulatory signaling region comprising the sequence set forth in SEQ ID NO:4 or a sequence of amino acids that has at least 90% sequence identity to the sequence set forth in SEQ ID NO: 4.

[0020] In some of any embodiments, the CAR comprises (a) an extracellular antigen-binding domain, comprising the sequence set forth in SEQ ID NO: 114; (b) a spacer comprising the sequence set forth in SEQ ID NO: 174; (c) a transmembrane domain comprising the sequence set forth in SEQ ID NO:138; and (d) an intracellular signaling region comprising a cytoplasmic signaling comprising the sequence set forth in SEQ ID NO:143 and a costimulatory signaling region comprising the sequence set forth in SEQ ID NO:4.

[0021] In some of any embodiments, the CAR comprises the sequence set forth in SEQ ID NO:19.

[0022] In some of any embodiments, the binding of the antigen-binding domain and/or the CAR or a measure indicative of function or activity of the CAR following exposure to cells expressing surface BCMA, is not reduced or blocked or is not substantially reduced or blocked in the presence of a soluble or shed form of BCMA. In some of any embodiments, the concentration or amount of the soluble or shed form of the BCMA corresponds to a concentration or amount present in serum or blood or plasma of the subject or of a multiple myeloma patient, or on average in a multiple myeloma patient population, or at a concentration or amount of the soluble or shed BCMA at which the binding or measure is reduced or blocked, or is substantially reduced or blocked, for cells expressing a reference anti-BCMA recombinant receptor, optionally a reference anti-BCMA CAR, in the same assay.

[0023] In some of any embodiments, the CAR is encoded by a polynucleotide sequence comprising the sequence set forth in SEQ ID NO: 13 or a sequence that exhibits at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity thereto. In some of any embodiments, the CAR expressed by T cells in the provided method is encoded by a polynucleotide sequence comprising the sequence set forth in SEQ ID NO: 13.

[0024] In some of any embodiments, following expression of a polynucleotide encoding the CAR in a human cell, optionally a human T cell, the transcribed RNA, optionally messenger RNA (mRNA), from the polynucleotide, exhibits at least 70%, 75%, 80%, 85%, 90%, or 95% RNA homogeneity.

[0025] In some of any embodiments, the dose of engineered T cells comprises between at or about 1 x 10^7 CAR-expressing (CAR+) T cells and at or about 2 x 10^9 CAR-expressing T cells. In some of any embodiments, the dose of engineered T cells comprise between at or about 2.5 x 10^7 CAR-expressing T cells and at or about 1.2 x 10^9 CAR-expressing T cells, between at or about 5.0 x 10^7 CAR-expressing T cells and at or about 4.5 x 10^8 CAR-expressing T cells, or between at or about 1.5 x 10^8 CAR-expressing T cells and at or about 3.0 x 10^8 CAR-expressing T cells. In some of any embodiments, the dose of engineered T cells comprise at or about 2.5 x 10^7, at or about 5.0 x 10^7, at or about 1.5 x 10^8, at or about 3.0 x 10^8, at or about 4.5 x 10^8, at or about 6.0 x 10^8, at or about 8.0 x 10^8 or at or about 1.2 x 10^9 CAR-expressing T cells. In some of any embodiments, the dose of engineered T cells comprise at or about 5.0 x 10^7, at or about 1.5 x 10^8, at or about 3.0 x 10^8 or at or about 4.5 x 10^8 CAR-expressing T cells.

[0026] In some of any embodiments, the dose of engineered T cells is less than 1.5 x 10^8 cells or less than 1.5 x 10^8 CAR+ T cells or less than 3 x 10^8 CAR+ T cells or less than 4.5 x 10^8 CAR+ T cells. In some of any embodiments, the dose of engineered T cells is at or less than 1.5 x 10^8 cells or less than 1.5 x 10^8 CAR+ T cells. In some of any embodiments, the dose of engineered T cells is at or about 5 x 10^7 cells or CAR+ T cells. In some of any embodiments, the dose of engineered T cells is at or about 1.5 x 10^8 cells or CAR+ T cells. In some of any embodiments, the dose of engineered T cells is at or about 3 x 10^8 cells or CAR+ T cells. In some of any embodiments, the dose of engineered T cells is at or about 4.5 x 10^8 cells or CAR+ T cells. In some of any embodiments, the dose of engineered T cells is at or about 6 x 10^8 cells or CAR+ T cells.

[0027] In some of any embodiments, the dose of engineered T cells is less than 1.5 x 10^8 CAR+ T cells or less than 3 x 10^8 CAR+ T cells or less than 4.5 x 10^8 CAR+ T cells. In some of any embodiments, the dose of engineered T cells is at or less than 1.5 x 10^8 CAR+ T cells. In some of any embodiments, the dose of engineered T cells is at or about 5 x 10^7 CAR+ T cells. In some of any embodiments, the dose of engineered T cells is at or about 1.5 x 10^8 CAR+ T cells. In some of any embodiments, the dose of engineered T cells is at or about 3 x 10^8 CAR+ T cells. In some of any embodiments, the dose of engineered T cells is at or about 4.5 x 10^8 CAR+ T cells. In some of any embodiments, the dose of engineered T cells is at or about 6 x 10^8 CAR+ T cells.

[0028] In some of any embodiments, the dose of engineered T cells comprises a combination of $CD4^+$ T cells and $CD8^+$ T cells and/or a combination of $CD4^+$ CAR-expressing T cells and $CD8^+$ CAR-expressing T cells. In some embodiments, the ratio of $CD4^+$ CAR-expressing T cells to $CD8^+$ CAR-expressing T cells and/or of $CD4^+$ T cells to $CD8^+$ T cells, is or is approximately 1:1 or is between at or approximately 1:3 and at or approximately 3:1. In some embodiments, the ratio of $CD4^+$ T cells to $CD8^+$ T cells is or is approximately 1:1 or is between at or approximately 1:3 and at or approximately 3:1. In some embodiments, the dose of engineered T cells comprises $CD3^+$ CAR-expressing T cells.

[0029] In some of any embodiments, less than at or about 25%, 20%, 15%, 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2% or 1% of the CAR-expressing T cells in the dose of engineered T cells express a marker of apoptosis, optionally Annexin V or active Caspase 3. In some of any embodiments, less than at or about 5%, 4%, 3%, 2% or 1% of the CAR-expressing T cells in the dose of engineered T cells express Annexin V or active Caspase 3.

[0030] In some of any embodiments, prior to the administration, the subject has received a lymphodepleting therapy comprising the administration of fludarabine at or about 20-40 mg/m^2 body surface area of the subject, optionally at or about 30 mg/m^2, daily, for 2-4 days, and/or cyclophosphamide at or about 200-400 mg/m^2 body surface area of the subject, optionally at or about 300 mg/m^2, daily, for 2-4 days. In some of any embodiments, the subject has received a lymphodepleting therapy comprising the administration of fludarabine at or about 30 mg/m^2 body surface area of the subject, daily, and cyclophosphamide at or about 300 mg/m^2 body surface area of the subject, daily, for 3 days.

[0031] In some of any embodiments, prior to the administration, the subject has received a lymphodepleting therapy comprising the administration of fludarabine at or about 20-40 mg/m^2 body surface area of the subject, optionally at or about 30 mg/m^2, daily, for 2-4 days.

[0032] In some of any embodiments, prior to the administration, the subject has received a lymphodepleting therapy comprising the administration of cyclophosphamide at or about 200-400 mg/m^2 body surface area of the subject, optionally at or about 300 mg/m^2, daily, for 2-4 days.

[0033] In some of any embodiments, the subject has or is suspected of having a relapsed or refractory multiple myeloma (R/R MM).

[0034] In some of any embodiments, at or prior to the administration of the dose of cells, the subject has received three or more prior therapies for the disease or disorder, optionally four or more prior therapies, optionally selected from among: autologous stem cell transplant (ASCT); an immunomodulatory agent; a proteasome inhibitor; and an anti-CD38 antibody. In some of any embodiments, the subject has relapsed or been refractory following the three or more prior therapies.

[0035] In some of any embodiments, at or prior to the administration of the dose of cells, the subject has received three or more prior therapies for the disease or disorder selected from among: autologous stem cell transplant (ASCT); an immunomodulatory agent or a proteasome inhibitor, or a combination thereof; and an anti-CD38 antibody. In some of any embodiments, the immunomodulatory agent is selected from among thalidomide, lenalidomide and pomalidomide. In some of any embodiments, the proteasome inhibitor is selected from among bortezomib, carfilzomib and ixazomib. In some of any embodiments, the anti-CD38 antibody is or comprises daratumumab.

[0036] In some of any embodiments, at the time of the administration of the dose of cells, and/or at the time of lymphodepleting chemotherapy or leukapheresis, the subject has not had active or history of plasma cell leukemia (PCL). In some of any embodiments, at the time of the administration of the dose of cells the subject has developed secondary plasma cell leukemia (PCL).

[0037] In some of any embodiments, at the time of administration, the subject: has relapsed or has been refractory following at least 3 or at least 4 prior therapies for multiple myeloma. In some of any embodiments, at the time of administration, the subject is an adult subject or is 25 or 35 years of age or older. In some of any embodiments, at the time of administration, the subject has a time from diagnosis of multiple myeloma of approximately 4 years or between 2 and 15 or 2 and 12 years. In some of any embodiments, at the time of administration, the subject has received about 10 or between 3 and 15 or between 4 and 15 prior regimens for multiple myeloma. In some of any embodiments, at the time of administration, the subject has been refractory to or not responded to bortezomib, carfilzomib, lenalidomide, pomalidomide and/or an anti-CD38 monoclonal antibody. In some of any embodiments, at the time of administration, the subject has had prior autologous stem cell transplant or has not had prior autologous stem cell transplant. In some of any embodiments, at the time of administration, the subject has IMWG high risk cytogenetics. In some of any embodiments, at the time of administration of the dose

of engineered T cells comprising a chimeric antigen receptor (CAR) the subject has relapsed or been refractory to at least 3 or at least 4 prior therapies that include bortezomib, carfilzomib, lenalidomide, pomalidomide and/or an anti-CD38 monoclonal antibody. In some of any embodiments, at the time of administration, the subject has had a prior autologous stem cell transplant.

[0038] In some of any embodiments, at the time of administration, the subject: has relapsed or been refractory following at least 3 or at least 4 prior therapies for multiple myeloma; is an adult subject or is 25 or 35 years of age or older; has a time from diagnosis of multiple myeloma of approximately 4 years or between 2 and 15 or 2 and 12 years; has received about 10 or between 3 and 15 or between 4 and 15 prior regimens for multiple myeloma; has been refractory to or not responded to bortezomib, carfilzomib, lenalidomide, pomalidomide and/or an anti-CD38 monoclonal antibody; has had prior autologous stem cell transplant or has not had prior autologous stem cell transplant; and/or has IMWG high risk cytogenetics.

[0039] In some of any embodiments, the method is capable of achieving a specified response or outcome, optionally at a designated timepoint following initiation of the administration, in at least one or in at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, or at least 95% of subjects in a cohort of subjects having the disease or disorder of the subject, wherein: the response is selected from the group consisting of objective response (OR), complete response (CR), stringent complete response (sCR), very good partial response (VGPR), partial response (PR) and minimal response (MR); the response or outcome is or comprises an OR; and/or the response or outcome is or comprises a CR. In some of any embodiments, the cohort of subjects has at least the same number of prior therapies, prognosis or prognostic factor, sub-type, secondary involvement or other specified patient characteristic or characteristics, as the subject treated by the method.

[0040] In some of any embodiments, the method is capable of achieving a specified response or outcome, optionally at a designated timepoint following initiation of the administration, in at least one of or in at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, or at least 95% of subjects in a cohort of subjects having the disease or disorder of the subject, optionally wherein the cohort of subjects has at least the same number of prior therapies, prognosis or prognostic factor, sub-type, secondary involvement or other specified patient characteristic or characteristics, as the subject treated by the method, wherein: the response is selected from the group consisting of objective response (OR), complete response (CR), stringent complete response (sCR), very good partial response (VGPR), partial response (PR) and minimal response (MR); the response or outcome is or comprises an OR; and/or the response or outcome is or comprises a CR.

[0041] In some embodiments, the designated timepoint is at or about 1, 2, 3, 6, 9, 12, 18, 24, 30 or 36 months following initiation of the administration, or within a range defined by any of the foregoing. In some embodiments, the designated timepoint is 4, 8, 12, 16, 20, 24, 28, 32, 36, 48 or 52 weeks months following initiation of the administration, or within a range defined by any of the foregoing. In some of

any embodiments, the designated timepoint is at or about 1 month following initiation of the administration. In some of any embodiments, the designated timepoint is at or about 3 months following initiation of the administration. In some of any embodiments, the designated timepoint is at or about 6 months following initiation of the administration. In some of any embodiments, the designated timepoint is at or about 9 months following initiation of the administration. In some of any embodiments, the designated timepoint is at or about 12 months following initiation of the administration.

[0042] In some of any embodiments, the response or outcome is an OR and is achieved in at least 40%, at least 50%, at least 60%, at least 70%, or at least 80% of subjects of the cohort. In some of any embodiments, the response or outcome is a VGPR, a CR or an sCR and is achieved in at least 30%, 35%, 40%, 45% or 50% of subjects of the cohort. In some of any embodiments, the response or outcome is or comprises a CR or an sCR and is achieved in at least 20%, 30%, or 40% of subjects of the cohort. In some of any embodiments, the response or outcome is or comprises an OR and is achieved in at least 50%, 60%, 70%, or 80% of subjects of the cohort. In some of any embodiments, the response or outcome is or comprises a VGPR, a CR or an sCR and is achieved in at least 40%, 45% or 50% of subjects of the cohort.

[0043] In some of any of the provided embodiments, the response or outcome is durable for greater than at or about 3, 6, 9 or 12 months. In some of any of the provided embodiments, the response or outcome determined at or about 3, 6, 9 or 12 months after the designated timepoint is equal to or improved compared to the response or outcome determined at the designated timepoint.

[0044] In some embodiments, subjects treated according to the provided methods, such as at a designated timepoint following the initiation of the administration, do not exhibit a response or outcome with any sign or symptom of neurotoxicity or CRS (absence of neurotoxicity or CRS). In some of any embodiments, the response or outcome comprises or further comprises the absence of neurotoxicity or the absence of cytokine release syndrome (CRS). In some of any embodiments, the response or outcome comprises or further comprises the absence of neurotoxicity, and is achieved in at least 40%, 50%, 60%, 70% or 80% of the subject in the cohort. In some of any embodiments, the response or outcome comprises or further comprises the absence of CRS, and is achieved in at least 10%, 15%, 20%, 25% or 30% of the subject in the cohort.

[0045] In some embodiments, subjects treated according to the provided methods, such as at a designated timepoint following the initiation of the administration, do not exhibit a response or outcome with grade 3 or higher or grade 4 or higher neurotoxicity (absence of grade 3 or higher or grade 4 or higher neurotoxicity). In some embodiments, subjects treated according to the provided methods, such as at a designated timepoint following the initiation of the administration, do not exhibit a response or outcome with grade 3 or higher or grade 4 or higher CRS (absence of grade 3 or higher or grade 4 or higher CRS. In some of any embodiments, the response or outcome comprises or further comprises the absence of grade 3 or higher, or grade 4 or higher, neurotoxicity, the absence of grade 3 or higher, or

grade 4 or higher, cytokine release syndrome (CRS). In some of any embodiments, the response or outcome comprises or further comprises the absence of grade 3 or higher neurotoxicity, and is achieved in at least 80%, 85%, 90% or 95% of the subjects in the cohort. In some of any embodiments, the response or outcome comprises or further comprises the absence of grade 3 or higher CRS, and is achieved in at least 80%, 85%, 90% or 95% of the subjects in the cohort.

[0046] In some of any embodiments, the method does not result in a specified toxicity outcome, optionally at a designated timepoint following initiation of the administration, in at least one of or in at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, or at least 95% of subjects in the cohort of subjects having the disease or disorder.

[0047] In some of any embodiments, the specified toxicity outcome is neurotoxicity. In some of any embodiments, the specified toxicity outcome is neurotoxicity, and neurotoxicity does not result in at least 60%, 70% or 80% of the subject in the cohort. In some of any embodiments, the specified toxicity outcome is grade 3 or higher, or grade 4 or higher, neurotoxicity. In some of any embodiments, the specified toxicity outcome is grade 3 or higher neurotoxicity, and grade 3 or higher neurotoxicity does not result in in at least 80%, 85%, 90% or 95% of the subjects in the cohort.

[0048] In some of any embodiments, the specified toxicity outcome is cytokine release syndrome (CRS). In some of any embodiments, the specified toxicity outcome is CRS, and CRS does not result in at least 15%, 20%, 25% or 30% of the subject in the cohort. In some of any embodiments, the specified toxicity outcome is grade 3 or higher, or grade 4 or higher, cytokine release syndrome (CRS). In some of any embodiments, the specified toxicity outcome is grade 3 or higher CRS, and grade 3 or higher CRS does not result in achieved in at least 80%, 85%, 90% or 95% of the subjects in the cohort.

[0049] In some of any embodiments, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or greater than 95% of the cells in the dose are of a memory phenotype. In some of any embodiments, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or greater than 95% of the cells in the dose are of a central memory phenotype. In some of any embodiments, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or greater than 95% of the cells in the dose are CD27+, CD28+, CCR7+, CD45RA-, CD45RO+, CD62L+, CD3+, granzyme B-, and/or CD127+. In some of any embodiments, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or greater than 95% of the cells in the dose are CCR7+/CD45RA- or are CCR7+/CD45RO+.

[0050] In some of any embodiments, the cells in the administered dose are produced by a method to produce an output composition exhibiting a predetermined feature, wherein iterations of the method produce a plurality of the output compositions, optionally from human biological samples, when carried out among a plurality of different individual subjects. In some of any embodiments, the predetermined

feature of the output composition among the plurality of output compositions is the mean percentage of cells of a memory phenotype in the plurality of the output compositions is between about 40% and about 65%, between about 40% and about 45%, between about 45% and about 50%, between about 50% and about 55%, between about 55% and about 60%, or between about 60% and about 65%. In some of any embodiments, the predetermined feature of the output composition among the plurality of output compositions is the mean percentage of cells of a central memory phenotype in the plurality of the output compositions is between about 40% and about 65%, between about 40% and about 45%, between about 45% and about 50%, between about 50% and about 55%, between about 55% and about 60%, or between about 60% and about 65%. In some of any embodiments, the predetermined feature of the output composition among the plurality of output compositions is the mean percentage of cells that are CD27+, CD28+, CCR7+, CD45RA-, CD45RO+, CD62L+, CD3+, CD95+, granzyme B-, and/or CD127+ in the plurality of the output compositions is between about 40% and about 65%, between about 40% and about 45%, between about 45% and about 50%, between about 50% and about 55%, between about 55% and about 60%, or between about 60% and about 65%. In some of any embodiments, the predetermined feature of the output composition among the plurality of output compositions is the mean percentage of cells that are CCR7+/CD45RA- or CCR7+/CD45RO+ in the plurality of the output compositions is between about 40% and about 65%, between about 40% and about 45%, between about 45% and about 50%, between about 50% and about 55%, between about 55% and about 60%, or between about 60% and about 65%. In some of any embodiments, the predetermined feature of the output composition among the plurality of output compositions is the mean percentage of central memory CD4+ T cells in the engineered CD4+ T cells, optionally CAR+CD4+ T cells, of the plurality of the output compositions is between about 40% and about 65%, between about 40% and about 45%, between about 45% and about 50%, between about 50% and about 55%, between about 55% and about 60%, or between about 60% and about 65%. In some of any embodiments, the predetermined feature of the output composition among the plurality of output compositions is the mean percentage of central memory CD8+ T cells in the engineered CD8+ T cells, optionally CAR+CD8+ T cells, of the plurality of the output compositions is between about 40% and about 65%, between about 40% and about 45%, between about 45% and about 50%, between about 50% and about 55%, between about 55% and about 60%, or between about 60% and about 65%. In some of any embodiments, the predetermined feature of the output composition among the plurality of output compositions is the mean percentage of central memory T cells, optionally CD4+ central memory T cells and CD8+ central memory T cells, in the engineered T cells, optionally CAR+ T cells, of the plurality of the output compositions is between about 40% and about 65%, between about 40% and about 45%, between about 45% and about 50%, between about 50% and about 55%, between about 55% and about 60%, or between about 60% and about 65%.

[0051] In some of any embodiments, the administered dose is produced by a method to produce an output composition exhibiting a predetermined feature, optionally a threshold number of cells expressing

the CAR in the output composition, in at least about 80%, about 90%, about 95%, about 97%, about 99%, about 100%, or is 100% of the human biological samples in which it is carried out among a plurality of different individual subjects. In some of any embodiments, the plurality of different individual subject comprise subjects having a disease or condition. In some of any embodiments, the disease or condition is a cancer. In some of any embodiments, the cancer is a hematological cancer, optionally multiple myeloma. In some embodiments, the cancer is relapsed or refractory multiple myeloma (R/R MM).

[0052] In some of any embodiments, the dose of engineered T cells comprise at or about 5.0×10^7, at or about 1.5×10^8, at or about 3.0×10^8 or at or about 4.5×10^8 CAR-expressing T cells. In some of any embodiments, the dose of the engineered T cells comprise at or about 5.0×10^7 CAR-expressing T cells. In some of any embodiments, the dose of the engineered T cells comprise at or about 1.5×10^8 CAR-expressing T cells. In some of any embodiments, the dose of the engineered T cells comprise at or about 3×10^8 CAR-expressing T cells. In some of any embodiments, the dose of the engineered T cells comprise at or about 4.5×10^8 CAR-expressing T cells.

[0053] Also provided are the engineered T cell or a dose of engineered T cells administered in any of the provided methods or uses, or engineered T cells or a dose of engineered T cells for use in accordance with any of the methods provided herein. In some of any embodiments, wherein the engineered T cell or the dose of engineered T cells, following administration at a dose of engineered T cells is capable of achieving, optionally at a designated time following initiation of the administration, a specified response or outcome in at least one of, or in at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, or at least 95% of subjects within a cohort of subjects or evaluable subjects thereof, wherein the cohort of subjects is a cohort having multiple myeloma. In any of the engineered T cells or dose of engineered T cells for use, the engineered T cells or dose of engineered T cells are administered in accordance with any of the methods provided herein.

[0054] Also provided are a dose of engineered T cells for use in or for use in accordance with any of the embodiments of the methods provided herein. In some of any embodiments, the dose of engineered T cells, following administration, is capable of achieving, optionally at a designated time following initiation of the administration, a specified response or outcome in at least one of, or in at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, or at least 95% of subjects within a cohort of subjects or evaluable subjects thereof, wherein the cohort of subjects is a cohort having multiple myeloma. In any of the provided dose of engineered T cells for use, the dose of engineered T cells are administered in accordance with any of the methods provided herein.

[0055] Also provided are A dose of engineered T cells for use in or for use in accordance with any of the embodiments of the methods provided herein that comprises one or more engineered T cells comprising a chimeric antigen receptor (CAR) in a treatment regimen for a subject having or suspected

of having multiple myeloma (MM) comprising administering to the subject the dose of engineered T cells, wherein the CAR comprises: (a) an extracellular antigen-binding domain, comprising: a variable heavy chain (V_H) comprising a heavy chain complementarity determining region 1 (CDR-H1), a heavy chain complementarity determining region 2 (CDR-H2) and a heavy chain complementarity determining region 3 (CDR-H3) contained within the sequence set forth in SEQ ID NO: 116 and a variable light chain (V_L) comprising a light chain complementarity determining region 1 (CDR-L1), a light chain complementarity determining region 2 (CDR-L2) and a light chain complementarity determining region 3 (CDR-L3) contained within the sequence set forth in SEQ ID NO: 119; a V_H comprising a CDR-H1, a CDR-H2 and a CDR-H3 sequences set forth in SEQ ID NOS:97, 101 and 103, respectively, and a V_L comprising a CDR-L1, a CDR-L2 and a CDR-L3 sequences set forth in SEQ ID NOS:105, 107 and 108, respectively; a V_H comprising a CDR-H1, a CDR-H2 and a CDR-H3 sequences set forth in SEQ ID NOS:96, 100 and 103, respectively, and a V_L comprising a CDR-L1, a CDR-L2 and a CDR-L3 sequences set forth in SEQ ID NOS:105, 107 and 108, respectively; a V_H comprising a CDR-H1, a CDR-H2 and a CDR-H3 sequences set forth in SEQ ID NOS:95, 99 and 103, respectively, and a V_L comprising a CDR-L1, a CDR-L2 and a CDR-L3 sequences set forth in SEQ ID NOS: 105, 107 and 108, respectively; a V_H comprising a CDR-H1, a CDR-H2 and a CDR-H3 sequences set forth in SEQ ID NOS:94, 98 and 102, respectively, and a V_L comprising a CDR-L1, a CDR-L2 and a CDR-L3 sequences set forth in SEQ ID NOS: 104, 106 and 108, respectively; or a V_H comprising the amino acid sequence of SEQ ID NO: 116 and a V_L comprising the amino acid sequence of SEQ ID NO: 119; (b) a spacer comprising an IgG4/2 chimeric hinge or a modified IgG4 hinge; an IgG2/4 chimeric C_H2 region; and an IgG4 C_H3 region, which optionally is about 228 amino acids in length; or a spacer set forth in SEQ ID NO: 174; (c) a transmembrane domain, optionally a transmembrane domain from a human CD28; and (d) an intracellular signaling region comprising a cytoplasmic signaling domain of a CD3-zeta (CD3ζ) chain and a costimulatory signaling region comprising an intracellular signaling domain of a T cell costimulatory molecule or a signaling portion thereof; and the dose of engineered T cells, following administration, is capable of achieving, optionally at a designated time following initiation of the administration, a specified response or outcome in at least one of, or in at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, or at least 95% of subjects within a cohort of subjects or evaluable subjects thereof, wherein the cohort of subjects is a cohort having multiple myeloma.

[0056] In some of any embodiments, the CAR comprises (a) an extracellular antigen-binding domain, comprising: a variable heavy chain (V_H) comprising a heavy chain complementarity determining region 1 (CDR-H1), a heavy chain complementarity determining region 2 (CDR-H2) and a heavy chain complementarity determining region 3 (CDR-H3) contained within the sequence set forth in SEQ ID NO: 116 and a variable light chain (V_L) comprising a light chain complementarity determining region 1 (CDR-L1), a light chain complementarity determining region 2 (CDR-L2) and a light chain

complementarity determining region 3 (CDR-L3) contained within the sequence set forth in SEQ ID NO: 119; (b) a spacer comprising a modified IgG4 hinge; an IgG2/4 chimeric C_H2 region; and an IgG4 C_H3 region, that is about 228 amino acids in length; (c) a transmembrane domain from a human CD28; and (d) an intracellular signaling region comprising a cytoplasmic signaling domain of a CD3-zeta (CD3ζ) chain and a costimulatory signaling region comprising an intracellular signaling domain of a 4-1BB.

[0057] In some of any embodiments, the CAR comprises (a) an extracellular antigen-binding domain, comprising the sequence set forth in SEQ ID NO: 114 or a sequence of amino acids having at least 90% sequence identity to the amino acid sequence of SEQ ID NO: 114; (b) a spacer comprising the sequence set forth in SEQ ID NO: 174 or a sequence of amino acids that has at least 90% sequence identity to SEQ ID NO:174; (c) a transmembrane domain comprising the sequence set forth in SEQ ID NO:138 or a sequence of amino acids that has at least 90% sequence identity to SEQ ID NO:138; and (d) an intracellular signaling region comprising a cytoplasmic signaling comprising the sequence set forth in SEQ ID NO:143 or a sequence of amino acids that has at least 90% sequence identity to SEQ ID NO:143 and a costimulatory signaling region comprising the sequence set forth in SEQ ID NO:4 or a sequence of amino acids that has at least 90% sequence identity to the sequence set forth in SEQ ID NO: 4.

[0058] In some of any embodiments, the CAR comprises (a) an extracellular antigen-binding domain, comprising the sequence set forth in SEQ ID NO: 114; (b) a spacer comprising the sequence set forth in SEQ ID NO: 174; (c) a transmembrane domain comprising the sequence set forth in SEQ ID NO:138; and (d) an intracellular signaling region comprising a cytoplasmic signaling comprising the sequence set forth in SEQ ID NO:143 and a costimulatory signaling region comprising the sequence set forth in SEQ ID NO:4.

[0059] In some of any embodiments, the CAR comprises the sequence set forth in SEQ ID NO:19.

[0060] In some of any embodiments, the achievement of the response or outcome is at the designated time following initiation of administration, which is at or about 1, 2, 3, 6, 9, 12, 18, 24, 30 or 36 months following said initiation. In some of any embodiments, the achievement of the response or outcome is at the designated time following initiation of administration, which is at 1, 2, 3, 6, 9 or 12 months following said initiation. In some of any embodiments, the achievement of the response or outcome is at the designated time following initiation of administration, which is at 1 or 2 or 3 months following said initiation. In some of any embodiments, the achievement of the response or outcome is at the designated timepoint following initiation of administration, which is at or about 1 month following said initiation. In some of any embodiments, the achievement of the response or outcome is at the designated timepoint following initiation of administration, which is at or about 3 months following said initiation. In some of any embodiments, the achievement of the response or outcome is at the designated timepoint following initiation of administration, which is at or about 6 months following said initiation. In some of any embodiments, the achievement of the response or outcome is at the designated timepoint following initiation of administration, which is at or about 9 months following said initiation. In some of

any embodiments, the achievement of the response or outcome is at the designated timepoint following initiation of administration, which is at or about 12 months following said initiation.

[0061] In some of any embodiments, the cohort of subjects is subjects having relapsed or refractory multiple myeloma. In some of any embodiments, the cohort of subjects is subjects having relapsed or refractory multiple myeloma having been administered, and relapsed or been refractory following, at least 3 prior therapies for multiple myeloma, said prior therapies optionally including an autologous stem cell transplant (ASCT); an immunomodulatory agent; a proteasome inhibitor; and/or an anti-CD38 antibody. In some of any embodiments, the cohort of subjects is subjects having relapsed or refractory multiple myeloma having been administered, and relapsed or been refractory following, at least 3 prior therapies for multiple myeloma, said prior therapies optionally including an immunomodulatory agent; a proteasome inhibitor; and/or an anti-CD38 antibody and/or an autologous stem cell transplant. In some of any embodiments, the cohort of subjects is subjects has no active plasma cell leukemia (PCL) or no history of PCL at the time of said administration. In some of any embodiments, the cohort of subjects is subjects has developed secondary plasma cell leukemia (PCL) prior to administration of the cells. In some of any embodiments, the cohort of subjects is or includes subjects having relapsed or refractory multiple myeloma having been administered, and relapsed or been refractory following, at least 4 or an average of at least 10 prior therapies for multiple myeloma. In some of any embodiments, the cohort of subjects consists of or includes adult subjects. In some of any embodiments, the cohort of subjects has a median time from diagnosis of 4 years and/or a range of time from diagnosis from 2 to 12 years. In some of any embodiments, the cohort of subjects has received a median of 10 prior regimens or between 3 and 15 or 4 and 15 prior therapies for multiple myeloma. In some of any embodiments, the cohort of subjects includes subjects refractory to bortezomib, carfilzomib, lenalidomide, pomalidomide and an anti-CD38 monoclonal antibody. In some of any embodiments, the cohort of subjects includes subjects having had prior autologous stem cell transplant. In some of any embodiments, the cohort of subjects includes subjects having IMWG high risk cytogenetics. In some of any embodiments, the at least 3 prior therapies comprise autologous stem cell transplant (ASCT); an immunomodulatory agent or a proteasome inhibitor, or a combination thereof; and an anti-CD38 antibody.

[0062] In some of any embodiments, the cohort of subjects is subjects having relapsed or refractory multiple myeloma; the cohort of subjects is subjects having relapsed or refractory multiple myeloma having been administered, and relapsed or been refractory following, at least 3 prior therapies for multiple myeloma, said prior therapies optionally including an autologous stem cell transplant (ASCT); an immunomodulatory agent; a proteasome inhibitor; and/or an anti-CD38 antibody; the cohort of subjects is subjects having relapsed or refractory multiple myeloma having been administered, and relapsed or been refractory following, at least 3 prior therapies for multiple myeloma, said prior therapies optionally including an immunomodulatory agent; a proteasome inhibitor; and/or an anti-CD38 antibody and/or an autologous stem cell transplant; and/or the cohort of subjects is subjects has no active plasma

cell leukemia (PCL) or no history of PCL at the time of said administration; the cohort of subjects is subjects has developed secondary plasma cell leukemia (PCL) prior to administration of the cells the cohort of subjects is or includes subjects having relapsed or refractory multiple myeloma having been administered, and relapsed or been refractory following, at least 4 or an average of at least 10 prior therapies for multiple myeloma; the cohort of subjects consists of or includes adult subjects; the cohort of subjects has a median time from diagnosis of 4 years and/or a range of time from diagnosis from 2 to 12 years; the cohort of subjects has received a median of 10 prior regimens or between 3 and 15 or 4 and 15 prior therapies for multiple myeloma; the cohort of subjects includes subjects refractory to bortezomib, carfilzomib, lenalidomide, pomalidomide and an anti-CD38 monoclonal antibody; the cohort of subjects includes subjects having had prior autologous stem cell transplant; and/or the cohort of subjects includes subjects having IMWG high risk cytogenetics. In some of any embodiments, thee at least 3 prior therapies comprise autologous stem cell transplant (ASCT); an immunomodulatory agent or a proteasome inhibitor, or a combination thereof; and an anti-CD38 antibody.

[0063] In some of any embodiments, the immunomodulatory agent is selected from among thalidomide, lenalidomide and pomalidomide, the proteasome inhibitor is selected from among bortezomib, carfilzomib and ixazomib, and/or the anti-CD38 antibody is or comprises daratumumab.

[0064] In some of any embodiments, the response or outcome is selected from the group consisting of objective response (OR), complete response (CR), stringent complete response (sCR), very good partial response (VGPR), partial response (PR) and minimal response (MR), optionally based on the International Myeloma Working Group (IMWG) uniform response criteria; the response or outcome is or comprises an OR, optionally based on the International Myeloma Working Group (IMWG) uniform response criteria; or the response or outcome is or comprises a CR, optionally based on the International Myeloma Working Group (IMWG) uniform response criteria.

[0065] In some of any embodiments, the response or outcome is or comprises an OR. In some of any embodiments, the dose is capable of achieving the response or outcome in at least 40%, at least 50%, at least 60%, at least 70%, or at least 80% of subjects of the cohort.

[0066] In some of any embodiments, the response or outcome is or comprises a VGPR, a CR or an sCR. In some of any embodiments, the dose is capable of achieving the response or outcome in at least 30%, 35%, 40%, 45% or 50% of subjects of the cohort.

[0067] In some of any embodiments, the response or outcome is or comprises a CR or an sCR. In some of any embodiments, the dose is capable of achieving the response or outcome in at least 20%, 30%, or 40% of subjects of the cohort.

[0068] In some of any embodiments, the response or outcome is or comprises an OR and the dose is capable of achieving the response or outcome in at least 50%, 60%, 70%, or 80% of subjects of the cohort. In some of any embodiments, the response or outcome is or comprises a VGPR, a CR or an sCR, and the dose is capable of achieving the response or outcome in at least 40%, 45% or 50% of subjects of

the cohort. In some of any embodiments, the response or outcome is or comprises a CR or an sCR, and the dose is capable of achieving the response or outcome in at least 20%, 30%, or 40% of subjects of the cohort.

[0069] In some of any embodiments, the response or outcome is durable for greater than at or about 3, 6, 9 or 12 months. In some of any embodiments, the response or outcome determined at or about 3, 6, 9 or 12 months after the designated time is equal to or improved compared to the response or outcome determined at the designated time.

[0070] In some of any embodiments, the dose capable of achieving said response or outcome is less than 1.5 x 10^8 cells. In some of any embodiments, the dose capable of achieving said response or outcome is less than 1.5 x 10^8 CAR+ T cells. In some of any embodiments, the dose capable of achieving said response or outcome is less than 3 x 10^8 CAR+ T cells. In some of any embodiments, the dose capable of achieving said response or outcome is less than or less than 4.5 x 10^8 CAR+ T cells. In some of any embodiments, the dose capable of achieving said response or outcome is less than 1.5 x 10^8 cells; or the dose capable of achieving said response or outcome is less than 1.5 x 10^8 CAR+ T cells. In some of any embodiments, the dose capable of achieving said response or outcome is less than 1 x 10^8 cells. In some of any embodiments, the dose capable of achieving said response or outcome is less than 1 x 10^8 CAR+ T cells. In some of any embodiments, the dose capable of achieving said response or outcome is at or about 5 x 10^7 cells. In some of any embodiments, at or about 5 x 10^7 CAR+ T cells. In some of any embodiments, the dose capable of achieving said response or outcome is at or about 1.5 x 10^8 cells or CAR+ T cells. In some of any embodiments, the dose capable of achieving said response or outcome is at or about 3 x 10^8 cells or CAR+ T cells. In some of any embodiments, the dose capable of achieving said response or outcome is at or about 4.5 x 10^8 cells or CAR+ T cells. In some of any embodiments, the dose capable of achieving said response or outcome is at or about 6.0 x 10^8 cells or CAR+ T cells.

[0071] In some of any embodiments, the dose capable of achieving said response or outcome comprises a combination of $CD4^+$ T cells and $CD8^+$ T cells. In some of any embodiments, the dose capable of achieving said response or outcome comprises a combination of a combination of $CD4^+$ CAR-expressing T cells and $CD8^+$ CAR-expressing T cells. In some of any embodiments, the ratio of $CD4^+$ CAR-expressing T cells to $CD8^+$ CAR-expressing T cells and/or of $CD4^+$ T cells to $CD8^+$ T cells, is or is approximately 1:1 or is between at or approximately 1:3 and at or approximately 3:1. In some of any embodiments, the dose capable of achieving said response or outcome comprises $CD3^+$ CAR-expressing T cells.

[0072] In some of any embodiments, the response or outcome comprises or further comprises the absence of neurotoxicity or the absence of cytokine release syndrome (CRS). In some of any embodiments, the response or outcome comprises or further comprises the absence of neurotoxicity, and is achieved in at least 40%, 50%, 60%, 70% or 80% of the subject in the cohort. In some of any

embodiments, the response or outcome comprises or further comprises the absence of CRS, and is achieved in at least 10%, 15%, 20%, 25% or 30% of the subject in the cohort. In some of any embodiments, the response or outcome comprises or further comprises the absence of grade 3 or higher, or grade 4 or higher, neurotoxicity, the absence of grade 3 or higher, or grade 4 or higher, cytokine release syndrome. In some of any embodiments, the response or outcome comprises or further comprises the absence of grade 3 or higher neurotoxicity, and is achieved in at least 80%, 85%, 90% or 95% of the subjects in the cohort. In some of any embodiments, the response or outcome comprises or further comprises the absence of grade 3 or higher CRS, and is achieved in at least 80%, 85%, 90% or 95% of the subjects in the cohort.

[0073] In some of any embodiments, administration of the dose of engineered T cell does not result in a specified toxicity outcome, optionally at a designated timepoint following initiation of the administration, in at least one of or in at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 90%, at least 70%, at least 80%, at least 90%, or at least 95% of subjects in the cohort of subjects having the disease or disorder.

[0074] In some of any embodiments, n the specified toxicity outcome is neurotoxicity. In some of any embodiments, the specified toxicity outcome is neurotoxicity, and neurotoxicity does not result in at least 90%, 70% or 80% of the subject in the cohort. In some of any embodiments, the specified toxicity outcome is grade 3 or higher, or grade 4 or higher, neurotoxicity. In some of any embodiments, the specified toxicity outcome is grade 3 or higher neurotoxicity, and grade 3 or higher neurotoxicity does not result in in at least 80%, 85%, 90% or 95% of the subjects in the cohort.

[0075] In some of any embodiments, the specified toxicity outcome is cytokine release syndrome (CRS). In some of any embodiments, the specified toxicity outcome is CRS, and CRS does not result in at least 15%, 20%, 25% or 30% of the subject in the cohort. In some of any embodiments, the specified toxicity outcome is grade 3 or higher, or grade 4 or higher, cytokine release syndrome (CRS). In some of any embodiments, the specified toxicity outcome is grade 3 or higher CRS, and grade 3 or higher CRS does not result in achieved in at least 80%, 85%, 90% or 95% of the subjects in the cohort.

[0076] In some of any embodiments, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or greater than 95% of the cells in the dose are of a memory phenotype. In some of any embodiments, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or greater than 95% of the cells in the dose are of a central memory phenotype. In some of any embodiments, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or greater than 95% of the cells in the dose are CD27+, CD28+, CCR7+, CD45RA-, CD45RO+, CD62L+, CD3+, granzyme B-, and/or CD127+. In some of any embodiments, wherein at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or greater than 95% of the cells in the dose are

CCR7+/CD45RA- or are CCR7+/CD45RO+.

[0077] In some of any embodiments, the dose of engineered T cells is produced by a method exhibiting a predetermined feature, wherein iterations of the method produce a plurality of output compositions, optionally from human biological samples in which the method is carried out among a plurality of different individual subjects. In some of any embodiments, the cells in the administered dose are produced by a method to produce an output composition exhibiting a predetermined feature, wherein iterations of the method produce a plurality of the output compositions, optionally from human biological samples, when carried out among a plurality of different individual subjects.

[0078] In some of any embodiments, the predetermined feature of the output composition among the plurality of output compositions includes the mean percentage of cells of a memory phenotype in the plurality of the output compositions is between about 40% and about 65%, between about 40% and about 45%, between about 45% and about 50%, between about 50% and about 55%, between about 55% and about 60%, or between about 60% and about 65%. In some of any embodiments, the predetermined feature of the output composition among the plurality of output compositions includes the mean percentage of cells of a central memory phenotype in the plurality of the output compositions is between about 40% and about 65%, between about 40% and about 45%, between about 45% and about 50%, between about 50% and about 55%, between about 55% and about 60%, or between about 60% and about 65%. In some of any embodiments, the predetermined feature of the output composition among the plurality of output compositions includes the mean percentage of cells that are CD27+, CD28+, CCR7+, CD45RA-, CD45RO+, CD62L+, CD3+, CD95+, granzyme B-, and/or CD127+ in the plurality of the output compositions is between about 40% and about 65%, between about 40% and about 45%, between about 45% and about 50%, between about 50% and about 55%, between about 55% and about 60%, or between about 60% and about 65%. In some of any embodiments, the predetermined feature of the output composition among the plurality of output compositions includes the mean percentage of cells that are CCR7+/CD45RA- or CCR7+/CD45RO+ in the plurality of the output compositions is between about 40% and about 65%, between about 40% and about 45%, between about 45% and about 50%, between about 50% and about 55%, between about 55% and about 60%, or between about 60% and about 65%; the mean percentage of central memory CD4+ T cells in the engineered CD4+ T cells, optionally CAR+CD4+ T cells, of the plurality of the output compositions is between about 40% and about 65%, between about 40% and about 45%, between about 45% and about 50%, between about 50% and about 55%, between about 55% and about 60%, or between about 60% and about 65%. In some of any embodiments, the predetermined feature of the output composition among the plurality of output compositions includes the mean percentage of central memory CD8+ T cells in the engineered CD8+ T cells, optionally CAR+CD8+ T cells, of the plurality of the output compositions is between about 40% and about 65%, between about 40% and about 45%, between about 45% and about 50%, between about 50% and about 55%, between about 55% and about 60%, or between about 60% and about 65%; and/or

the mean percentage of central memory T cells, optionally CD4+ central memory T cells and CD8+ central memory T cells, in the engineered T cells, optionally CAR+ T cells, of the plurality of the output compositions is between about 40% and about 65%, between about 40% and about 45%, between about 45% and about 50%, between about 50% and about 55%, between about 55% and about 60%, or between about 60% and about 65%.

[0079] In some of any embodiments, the dose is produced by a method to produce an output composition exhibiting a predetermined feature, optionally a threshold number of cells expressing the CAR in the output composition, in at least about 80%, about 90%, about 95%, about 97%, about 99%, about 100%, or is 100% of the human biological samples in which it is carried out among a plurality of different individual subjects.

[0080] In some of any embodiments, the plurality of different individual subject comprise subjects having a disease or condition. In some of any embodiments, the disease or condition is a cancer. In some of any embodiments, the cancer is a hematological cancer, optionally multiple myeloma. In particular embodiments, the disease or condition is a cancer that is multiple myeloma. In some of any embodiments, the disease or condition is a relapsed or refractory multiple myeloma (R/R MM).

[0081] Provided are uses of a dose of engineered T cells comprising a chimeric antigen receptor (CAR) in a treatment regimen for a subject having or suspected of having multiple myeloma (MM) comprising administering to the subject the dose of engineered T cells, wherein the CAR includes: (a) a variable heavy chain (V_H) comprising a heavy chain complementarity determining region 1 (CDR-H1), a heavy chain complementarity determining region 2 (CDR-H2) and a heavy chain complementarity determining region 3 (CDR-H3) contained within the sequence set forth in SEQ ID NO: 116 and a variable light chain (V_L) comprising a light chain complementarity determining region 1 (CDR-L1), a light chain complementarity determining region 2 (CDR-L2) and a light chain complementarity determining region 3 (CDR-L3) contained within the sequence set forth in SEQ ID NO: 119; a V_H comprising a CDR-H1, a CDR-H2 and a CDR-H3 sequences set forth in SEQ ID NOS:97, 101 and 103, respectively, and a V_L comprising a CDR-L1, a CDR-L2 and a CDR-L3 sequences set forth in SEQ ID NOS:105, 107 and 108, respectively; a V_H comprising a CDR-H1, a CDR-H2 and a CDR-H3 sequences set forth in SEQ ID NOS:96, 100 and 103, respectively, and a V_L comprising a CDR-L1, a CDR-L2 and a CDR-L3 sequences set forth in SEQ ID NOS:105, 107 and 108, respectively; a V_H comprising a CDR-H1, a CDR-H2 and a CDR-H3 sequences set forth in SEQ ID NOS:95, 99 and 103, respectively, and a V_L comprising a CDR-L1, a CDR-L2 and a CDR-L3 sequences set forth in SEQ ID NOS: 105, 107 and 108, respectively; a V_H comprising a CDR-H1, a CDR-H2 and a CDR-H3 sequences set forth in SEQ ID NOS:94, 98 and 102, respectively, and a V_L comprising a CDR-L1, a CDR-L2 and a CDR-L3 sequences set forth in SEQ ID NOS: 104, 106 and 108, respectively; or a V_H comprising the amino acid sequence of SEQ ID NO: 116 and a V_L comprising the amino acid sequence of SEQ ID NO: 119; (b) a spacer comprising an IgG4/2 chimeric hinge or a modified IgG4 hinge; an IgG2/4 chimeric C_H2 region; and an

IgG4 C_H3 region, which optionally is about 228 amino acids in length; or a spacer set forth in SEQ ID NO: 174; (c) a transmembrane domain, optionally a transmembrane domain from a human CD28; and (d) an intracellular signaling region that includes a cytoplasmic signaling domain of a CD3-zeta (CD3ζ) chain and a costimulatory signaling region that includes an intracellular signaling domain of a T cell costimulatory molecule or a signaling portion thereof; wherein, prior to the administration, the subject has received a lymphodepleting therapy comprising the administration of fludarabine at or about 20-40 mg/m^2 body surface area of the subject, optionally at or about 30 mg/m^2, daily, for 2-4 days, and/or cyclophosphamide at or about 200-400 mg/m^2 body surface area of the subject, optionally at or about 300 mg/m^2, daily, for 2-4 days.

[0082] Provided are uses of a dose of engineered T cells comprising a chimeric antigen receptor (CAR) in a treatment regimen for a subject having or suspected of having multiple myeloma (MM) comprising administering to the subject the dose of engineered T cells, wherein the CAR includes: (a) a variable heavy chain (V_H) comprising a heavy chain complementarity determining region 1 (CDR-H1), a heavy chain complementarity determining region 2 (CDR-H2) and a heavy chain complementarity determining region 3 (CDR-H3) contained within the sequence set forth in SEQ ID NO: 116 and a variable light chain (V_L) comprising a light chain complementarity determining region 1 (CDR-L1), a light chain complementarity determining region 2 (CDR-L2) and a light chain complementarity determining region 3 (CDR-L3) contained within the sequence set forth in SEQ ID NO: 119; a V_H comprising a CDR-H1, a CDR-H2 and a CDR-H3 sequences set forth in SEQ ID NOS:97, 101 and 103, respectively, and a V_L comprising a CDR-L1, a CDR-L2 and a CDR-L3 sequences set forth in SEQ ID NOS:105, 107 and 108, respectively; a V_H comprising a CDR-H1, a CDR-H2 and a CDR-H3 sequences set forth in SEQ ID NOS:96, 100 and 103, respectively, and a V_L comprising a CDR-L1, a CDR-L2 and a CDR-L3 sequences set forth in SEQ ID NOS:105, 107 and 108, respectively; a V_H comprising a CDR-H1, a CDR-H2 and a CDR-H3 sequences set forth in SEQ ID NOS:95, 99 and 103, respectively, and a V_L comprising a CDR-L1, a CDR-L2 and a CDR-L3 sequences set forth in SEQ ID NOS: 105, 107 and 108, respectively; a V_H comprising a CDR-H1, a CDR-H2 and a CDR-H3 sequences set forth in SEQ ID NOS:94, 98 and 102, respectively, and a V_L comprising a CDR-L1, a CDR-L2 and a CDR-L3 sequences set forth in SEQ ID NOS: 104, 106 and 108, respectively; or a V_H comprising the amino acid sequence of SEQ ID NO: 116 and a V_L comprising the amino acid sequence of SEQ ID NO: 119; (b) a spacer comprising an IgG4/2 chimeric hinge or a modified IgG4 hinge; an IgG2/4 chimeric C_H2 region; and an IgG4 C_H3 region, which optionally is about 228 amino acids in length; or a spacer set forth in SEQ ID NO: 174; (c) a transmembrane domain, optionally a transmembrane domain from a human CD28; and (d) an intracellular signaling region that includes a cytoplasmic signaling domain of a CD3-zeta (CD3ζ) chain and a costimulatory signaling region that includes an intracellular signaling domain of a T cell costimulatory molecule or a signaling portion thereof; wherein at or prior to the administration of the dose of engineered T cells, the subject has received three or more therapies selected from among:

autologous stem cell transplant (ASCT); an immunomodulatory agent; a proteasome inhibitor; and an anti-CD38 antibody; unless the subject was not a candidate for or was contraindicated for one or more of the therapies.

[0083] Provided are uses of a dose of engineered T cells comprising a chimeric antigen receptor (CAR) in a treatment regimen for a subject having or suspected of having multiple myeloma (MM) comprising administering to the subject the dose of engineered T cells, wherein the CAR includes: (a) a variable heavy chain (V_H) comprising a heavy chain complementarity determining region 1 (CDR-H1), a heavy chain complementarity determining region 2 (CDR-H2) and a heavy chain complementarity determining region 3 (CDR-H3) contained within the sequence set forth in SEQ ID NO: 116 and a variable light chain (V_L) comprising a light chain complementarity determining region 1 (CDR-L1), a light chain complementarity determining region 2 (CDR-L2) and a light chain complementarity determining region 3 (CDR-L3) contained within the sequence set forth in SEQ ID NO: 119; a V_H comprising a CDR-H1, a CDR-H2 and a CDR-H3 sequences set forth in SEQ ID NOS:97, 101 and 103, respectively, and a V_L comprising a CDR-L1, a CDR-L2 and a CDR-L3 sequences set forth in SEQ ID NOS:105, 107 and 108, respectively; a V_H comprising a CDR-H1, a CDR-H2 and a CDR-H3 sequences set forth in SEQ ID NOS:96, 100 and 103, respectively, and a V_L comprising a CDR-L1, a CDR-L2 and a CDR-L3 sequences set forth in SEQ ID NOS:105, 107 and 108, respectively; a V_H comprising a CDR-H1, a CDR-H2 and a CDR-H3 sequences set forth in SEQ ID NOS:95, 99 and 103, respectively, and a V_L comprising a CDR-L1, a CDR-L2 and a CDR-L3 sequences set forth in SEQ ID NOS: 105, 107 and 108, respectively; a V_H comprising a CDR-H1, a CDR-H2 and a CDR-H3 sequences set forth in SEQ ID NOS:94, 98 and 102, respectively, and a V_L comprising a CDR-L1, a CDR-L2 and a CDR-L3 sequences set forth in SEQ ID NOS: 104, 106 and 108, respectively; or a V_H comprising the amino acid sequence of SEQ ID NO: 116 and a V_L comprising the amino acid sequence of SEQ ID NO: 119; (b) a spacer comprising an IgG4/2 chimeric hinge or a modified IgG4 hinge; an IgG2/4 chimeric C_H2 region; and an IgG4 C_H3 region, which optionally is about 228 amino acids in length; or a spacer set forth in SEQ ID NO: 174; (c) a transmembrane domain, optionally a transmembrane domain from a human CD28; and (d) an intracellular signaling region that includes a cytoplasmic signaling domain of a CD3-zeta (CD3ζ) chain and a costimulatory signaling region that includes an intracellular signaling domain of a T cell costimulatory molecule or a signaling portion thereof; wherein at the administration of the dose of engineered T cells, the subject has not had active or history of plasma cell leukemia (PCL).

[0084] Provided are uses of a dose of engineered T cells comprising a chimeric antigen receptor (CAR) in a treatment regimen for a subject having or suspected of having multiple myeloma (MM) comprising administering to the subject the dose of engineered T cells, wherein the CAR includes: (a) a variable heavy chain (V_H) comprising a heavy chain complementarity determining region 1 (CDR-H1), a heavy chain complementarity determining region 2 (CDR-H2) and a heavy chain complementarity determining region 3 (CDR-H3) contained within the sequence set forth in SEQ ID NO: 116 and a

variable light chain (V_L) comprising a light chain complementarity determining region 1 (CDR-L1), a light chain complementarity determining region 2 (CDR-L2) and a light chain complementarity determining region 3 (CDR-L3) contained within the sequence set forth in SEQ ID NO: 119; a V_H comprising a CDR-H1, a CDR-H2 and a CDR-H3 sequences set forth in SEQ ID NOS:97, 101 and 103, respectively, and a V_L comprising a CDR-L1, a CDR-L2 and a CDR-L3 sequences set forth in SEQ ID NOS:105, 107 and 108, respectively; a V_H comprising a CDR-H1, a CDR-H2 and a CDR-H3 sequences set forth in SEQ ID NOS:96, 100 and 103, respectively, and a V_L comprising a CDR-L1, a CDR-L2 and a CDR-L3 sequences set forth in SEQ ID NOS:105, 107 and 108, respectively; a V_H comprising a CDR-H1, a CDR-H2 and a CDR-H3 sequences set forth in SEQ ID NOS:95, 99 and 103, respectively, and a V_L comprising a CDR-L1, a CDR-L2 and a CDR-L3 sequences set forth in SEQ ID NOS: 105, 107 and 108, respectively; a V_H comprising a CDR-H1, a CDR-H2 and a CDR-H3 sequences set forth in SEQ ID NOS:94, 98 and 102, respectively, and a V_L comprising a CDR-L1, a CDR-L2 and a CDR-L3 sequences set forth in SEQ ID NOS: 104, 106 and 108, respectively; or a V_H comprising the amino acid sequence of SEQ ID NO: 116 and a V_L comprising the amino acid sequence of SEQ ID NO: 119; (b) a spacer comprising an IgG4/2 chimeric hinge or a modified IgG4 hinge; an IgG2/4 chimeric C_H2 region; and an IgG4 C_H3 region, which optionally is about 228 amino acids in length; or a spacer set forth in SEQ ID NO: 174; (c) a transmembrane domain, optionally a transmembrane domain from a human CD28; and (d) an intracellular signaling region that includes a cytoplasmic signaling domain of a CD3-zeta (CD3ζ) chain and a costimulatory signaling region that includes an intracellular signaling domain of a T cell costimulatory molecule or a signaling portion thereof; wherein the dose of engineered T cells comprises: between at or about 1 x 10^7 CAR-expressing T cells and 2 x 10^9 CAR-expressing T cells; a combination of $CD4^+$ T cells and $CD8^+$ T cells, at a defined ratio of $CD4^+$ CAR-expressing T cells to $CD8^+$ CAR-expressing T cells and/or of $CD4^+$ T cells to $CD8^+$ T cells, that is or is approximately 1:1 or is between approximately 1:3 and approximately 3:1; and less than 25%, 20%, 15%, 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2% or 1% of the CAR-expressing T cells in the dose express a marker of apoptosis, optionally Annexin V or active Caspase 3.

[0085] Provided are uses of a dose of engineered T cells comprising a chimeric antigen receptor (CAR) for the manufacture of a medicament for the treatment for a subject having or suspected of having multiple myeloma (MM), wherein the CAR includes: (a) a variable heavy chain (V_H) comprising a heavy chain complementarity determining region 1 (CDR-H1), a heavy chain complementarity determining region 2 (CDR-H2) and a heavy chain complementarity determining region 3 (CDR-H3) contained within the sequence set forth in SEQ ID NO: 116 and a variable light chain (V_L) comprising a light chain complementarity determining region 1 (CDR-L1), a light chain complementarity determining region 2 (CDR-L2) and a light chain complementarity determining region 3 (CDR-L3) contained within the sequence set forth in SEQ ID NO: 119; a V_H comprising a CDR-H1, a CDR-H2 and a CDR-H3 sequences set forth in SEQ ID NOS:97, 101 and 103, respectively, and a V_L comprising a CDR-L1, a

CDR-L2 and a CDR-L3 sequences set forth in SEQ ID NOS:105, 107 and 108, respectively; a V_H comprising a CDR-H1, a CDR-H2 and a CDR-H3 sequences set forth in SEQ ID NOS:96, 100 and 103, respectively, and a V_L comprising a CDR-L1, a CDR-L2 and a CDR-L3 sequences set forth in SEQ ID NOS:105, 107 and 108, respectively; a V_H comprising a CDR-H1, a CDR-H2 and a CDR-H3 sequences set forth in SEQ ID NOS:95, 99 and 103, respectively, and a V_L comprising a CDR-L1, a CDR-L2 and a CDR-L3 sequences set forth in SEQ ID NOS: 105, 107 and 108, respectively; a V_H comprising a CDR-H1, a CDR-H2 and a CDR-H3 sequences set forth in SEQ ID NOS:94, 98 and 102, respectively, and a V_L comprising a CDR-L1, a CDR-L2 and a CDR-L3 sequences set forth in SEQ ID NOS: 104, 106 and 108, respectively; or a V_H comprising the amino acid sequence of SEQ ID NO: 116 and a V_L comprising the amino acid sequence of SEQ ID NO: 119; (b) a spacer comprising an IgG4/2 chimeric hinge or a modified IgG4 hinge; an IgG2/4 chimeric C_H2 region; and an IgG4 C_H3 region, which optionally is about 228 amino acids in length; or a spacer set forth in SEQ ID NO: 174; (c) a transmembrane domain, optionally a transmembrane domain from a human CD28; and (d) an intracellular signaling region that includes a cytoplasmic signaling domain of a CD3-zeta (CD3ζ) chain and a costimulatory signaling region that includes an intracellular signaling domain of a T cell costimulatory molecule or a signaling portion thereof; wherein, prior to the administration of the dose of engineered T cells, the subject has received a lymphodepleting therapy comprising the administration of fludarabine at or about 20-40 mg/m^2 body surface area of the subject, optionally at or about 30 mg/m^2, daily, for 2-4 days, and/or cyclophosphamide at or about 200-400 mg/m^2 body surface area of the subject, optionally at or about 300 mg/m^2, daily, for 2-4 days.

[0086] Provided are uses of a dose of engineered T cells comprising a chimeric antigen receptor (CAR) for the manufacture of a medicament for the treatment for a subject having or suspected of having multiple myeloma (MM), wherein the CAR includes: (a) a variable heavy chain (V_H) comprising a heavy chain complementarity determining region 1 (CDR-H1), a heavy chain complementarity determining region 2 (CDR-H2) and a heavy chain complementarity determining region 3 (CDR-H3) contained within the sequence set forth in SEQ ID NO: 116 and a variable light chain (V_L) comprising a light chain complementarity determining region 1 (CDR-L1), a light chain complementarity determining region 2 (CDR-L2) and a light chain complementarity determining region 3 (CDR-L3) contained within the sequence set forth in SEQ ID NO: 119; a V_H comprising a CDR-H1, a CDR-H2 and a CDR-H3 sequences set forth in SEQ ID NOS:97, 101 and 103, respectively, and a V_L comprising a CDR-L1, a CDR-L2 and a CDR-L3 sequences set forth in SEQ ID NOS:105, 107 and 108, respectively; a V_H comprising a CDR-H1, a CDR-H2 and a CDR-H3 sequences set forth in SEQ ID NOS:96, 100 and 103, respectively, and a V_L comprising a CDR-L1, a CDR-L2 and a CDR-L3 sequences set forth in SEQ ID NOS:105, 107 and 108, respectively; a V_H comprising a CDR-H1, a CDR-H2 and a CDR-H3 sequences set forth in SEQ ID NOS:95, 99 and 103, respectively, and a V_L comprising a CDR-L1, a CDR-L2 and a CDR-L3 sequences set forth in SEQ ID NOS: 105, 107 and 108, respectively; a V_H comprising a CDR-

H1, a CDR-H2 and a CDR-H3 sequences set forth in SEQ ID NOS:94, 98 and 102, respectively, and a V_L comprising a CDR-L1, a CDR-L2 and a CDR-L3 sequences set forth in SEQ ID NOS: 104, 106 and 108, respectively; or a V_H comprising the amino acid sequence of SEQ ID NO: 116 and a V_L comprising the amino acid sequence of SEQ ID NO: 119; (b) a spacer comprising an IgG4/2 chimeric hinge or a modified IgG4 hinge; an IgG2/4 chimeric C_H2 region; and an IgG4 C_H3 region, which optionally is about 228 amino acids in length; or a spacer set forth in SEQ ID NO: 174; (c) a transmembrane domain, optionally a transmembrane domain from a human CD28; and (d) an intracellular signaling region that includes a cytoplasmic signaling domain of a CD3-zeta (CD3ζ) chain and a costimulatory signaling region that includes an intracellular signaling domain of a T cell costimulatory molecule or a signaling portion thereof; wherein at or prior to the administration of the dose of engineered T cells, the subject has received three or more therapies selected from among: autologous stem cell transplant (ASCT); an immunomodulatory agent; a proteasome inhibitor; and an anti-CD38 antibody; unless the subject was not a candidate for or was contraindicated for one or more of the therapies.

[0087] Provided are uses of a dose of engineered T cells comprising a chimeric antigen receptor (CAR) for the manufacture of a medicament for the treatment for a subject having or suspected of having multiple myeloma (MM), wherein the CAR includes: (a) a variable heavy chain (V_H) comprising a heavy chain complementarity determining region 1 (CDR-H1), a heavy chain complementarity determining region 2 (CDR-H2) and a heavy chain complementarity determining region 3 (CDR-H3) contained within the sequence set forth in SEQ ID NO: 116 and a variable light chain (V_L) comprising a light chain complementarity determining region 1 (CDR-L1), a light chain complementarity determining region 2 (CDR-L2) and a light chain complementarity determining region 3 (CDR-L3) contained within the sequence set forth in SEQ ID NO: 119; a V_H comprising a CDR-H1, a CDR-H2 and a CDR-H3 sequences set forth in SEQ ID NOS:97, 101 and 103, respectively, and a V_L comprising a CDR-L1, a CDR-L2 and a CDR-L3 sequences set forth in SEQ ID NOS:105, 107 and 108, respectively; a V_H comprising a CDR-H1, a CDR-H2 and a CDR-H3 sequences set forth in SEQ ID NOS:96, 100 and 103, respectively, and a V_L comprising a CDR-L1, a CDR-L2 and a CDR-L3 sequences set forth in SEQ ID NOS:105, 107 and 108, respectively; a V_H comprising a CDR-H1, a CDR-H2 and a CDR-H3 sequences set forth in SEQ ID NOS:95, 99 and 103, respectively, and a V_L comprising a CDR-L1, a CDR-L2 and a CDR-L3 sequences set forth in SEQ ID NOS: 105, 107 and 108, respectively; a V_H comprising a CDR-H1, a CDR-H2 and a CDR-H3 sequences set forth in SEQ ID NOS:94, 98 and 102, respectively, and a V_L comprising a CDR-L1, a CDR-L2 and a CDR-L3 sequences set forth in SEQ ID NOS: 104, 106 and 108, respectively; or a V_H comprising the amino acid sequence of SEQ ID NO: 116 and a V_L comprising the amino acid sequence of SEQ ID NO: 119; (b) a spacer comprising an IgG4/2 chimeric hinge or a modified IgG4 hinge; an IgG2/4 chimeric C_H2 region; and an IgG4 C_H3 region, which optionally is about 228 amino acids in length; or a spacer set forth in SEQ ID NO: 174; (c) a transmembrane domain, optionally a transmembrane domain from a human CD28; and (d) an intracellular signaling region that

includes a cytoplasmic signaling domain of a CD3-zeta (CD3ζ) chain and a costimulatory signaling region that includes an intracellular signaling domain of a T cell costimulatory molecule or a signaling portion thereof; wherein at the administration of the dose of engineered T cells, the subject has not had active or history of plasma cell leukemia (PCL).

[0088] Provided are uses of a dose of engineered T cells comprising a chimeric antigen receptor (CAR) for the manufacture of a medicament for the treatment for a subject having or suspected of having multiple myeloma (MM), wherein the CAR includes: (a) a variable heavy chain (V_H) comprising a heavy chain complementarity determining region 1 (CDR-H1), a heavy chain complementarity determining region 2 (CDR-H2) and a heavy chain complementarity determining region 3 (CDR-H3) contained within the sequence set forth in SEQ ID NO: 116 and a variable light chain (V_L) comprising a light chain complementarity determining region 1 (CDR-L1), a light chain complementarity determining region 2 (CDR-L2) and a light chain complementarity determining region 3 (CDR-L3) contained within the sequence set forth in SEQ ID NO: 119; a V_H comprising a CDR-H1, a CDR-H2 and a CDR-H3 sequences set forth in SEQ ID NOS:97, 101 and 103, respectively, and a V_L comprising a CDR-L1, a CDR-L2 and a CDR-L3 sequences set forth in SEQ ID NOS:105, 107 and 108, respectively; a V_H comprising a CDR-H1, a CDR-H2 and a CDR-H3 sequences set forth in SEQ ID NOS:96, 100 and 103, respectively, and a V_L comprising a CDR-L1, a CDR-L2 and a CDR-L3 sequences set forth in SEQ ID NOS:105, 107 and 108, respectively; a V_H comprising a CDR-H1, a CDR-H2 and a CDR-H3 sequences set forth in SEQ ID NOS:95, 99 and 103, respectively, and a V_L comprising a CDR-L1, a CDR-L2 and a CDR-L3 sequences set forth in SEQ ID NOS: 105, 107 and 108, respectively; a V_H comprising a CDR-H1, a CDR-H2 and a CDR-H3 sequences set forth in SEQ ID NOS:94, 98 and 102, respectively, and a V_L comprising a CDR-L1, a CDR-L2 and a CDR-L3 sequences set forth in SEQ ID NOS: 104, 106 and 108, respectively; or a V_H comprising the amino acid sequence of SEQ ID NO: 116 and a V_L comprising the amino acid sequence of SEQ ID NO: 119; (b) a spacer comprising an IgG4/2 chimeric hinge or a modified IgG4 hinge; an IgG2/4 chimeric C_H2 region; and an IgG4 C_H3 region, which optionally is about 228 amino acids in length; or a spacer set forth in SEQ ID NO: 174; (c) a transmembrane domain, optionally a transmembrane domain from a human CD28; and (d) an intracellular signaling region that includes a cytoplasmic signaling domain of a CD3-zeta (CD3ζ) chain and a costimulatory signaling region that includes an intracellular signaling domain of a T cell costimulatory molecule or a signaling portion thereof; wherein the dose of engineered T cells comprises: between at or about 1 x 10^7 CAR-expressing T cells and 2 x 10^9 CAR-expressing T cells; a combination of $CD4^+$ T cells and $CD8^+$ T cells, at a defined ratio of $CD4^+$ CAR-expressing T cells to $CD8^+$ CAR-expressing T cells and/or of $CD4^+$ T cells to $CD8^+$ T cells, that is or is approximately 1:1 or is between approximately 1:3 and approximately 3:1; and less than 25%, 20%, 15%, 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2% or 1% of the CAR-expressing T cells in the dose express a marker of apoptosis, optionally Annexin V or active Caspase 3.

[0089] In some of any embodiments, the extracellular antigen-binding domain specifically binds to a

B cell maturation antigen (BCMA). In some of any embodiments, the V_H is or comprises the amino acid sequence of SEQ ID NO: 116; and the V_L is or comprises the amino acid sequence of SEQ ID NO: 119.

[0090] In some of any embodiments, the CAR comprises (a) an extracellular antigen-binding domain, comprising: a variable heavy chain (V_H) comprising a heavy chain complementarity determining region 1 (CDR-H1), a heavy chain complementarity determining region 2 (CDR-H2) and a heavy chain complementarity determining region 3 (CDR-H3) contained within the sequence set forth in SEQ ID NO: 116 and a variable light chain (V_L) comprising a light chain complementarity determining region 1 (CDR-L1), a light chain complementarity determining region 2 (CDR-L2) and a light chain complementarity determining region 3 (CDR-L3) contained within the sequence set forth in SEQ ID NO: 119; (b) a spacer comprising a modified IgG4 hinge; an IgG2/4 chimeric C_H2 region; and an IgG4 C_H3 region, that is about 228 amino acids in length; (c) a transmembrane domain from a human CD28; and (d) an intracellular signaling region comprising a cytoplasmic signaling domain of a CD3-zeta (CD3ζ) chain and a costimulatory signaling region comprising an intracellular signaling domain of a 4-1BB.

[0091] In some of any embodiments, the CAR comprises (a) an extracellular antigen-binding domain, comprising the sequence set forth in SEQ ID NO: 114 or a sequence of amino acids having at least 90% sequence identity to the amino acid sequence of SEQ ID NO: 114; (b) a spacer comprising the sequence set forth in SEQ ID NO: 174 or a sequence of amino acids that has at least 90% sequence identity to SEQ ID NO:174; (c) a transmembrane domain comprising the sequence set forth in SEQ ID NO:138 or a sequence of amino acids that has at least 90% sequence identity to SEQ ID NO:138; and (d) an intracellular signaling region comprising a cytoplasmic signaling comprising the sequence set forth in SEQ ID NO:143 or a sequence of amino acids that has at least 90% sequence identity to SEQ ID NO:143 and a costimulatory signaling region comprising the sequence set forth in SEQ ID NO:4 or a sequence of amino acids that has at least 90% sequence identity to the sequence set forth in SEQ ID NO: 4.

[0092] In some of any embodiments, the CAR comprises (a) an extracellular antigen-binding domain, comprising the sequence set forth in SEQ ID NO: 114; (b) a spacer comprising the sequence set forth in SEQ ID NO: 174; (c) a transmembrane domain comprising the sequence set forth in SEQ ID NO:138; and (d) an intracellular signaling region comprising a cytoplasmic signaling comprising the sequence set forth in SEQ ID NO:143 and a costimulatory signaling region comprising the sequence set forth in SEQ ID NO:4.

[0093] In some of any embodiments, the CAR comprises the sequence set forth in SEQ ID NO:19.

[0094] In some of any embodiments, the dose of engineered T cells comprises between at or about 1 x 10^7 CAR-expressing T cells and at or about 2 x 10^9 CAR-expressing T cells. In some of any embodiments, the dose of engineered T cells comprise between at or about 2.5 x 10^7 CAR-expressing T cells and at or about 1.2 x 10^9 CAR-expressing T cells. In some of any embodiments, the dose of engineered T cells comprises between at or about 5.0 x 10^7 CAR-expressing T cells and at or about 4.5 x 10^8 CAR-expressing T cells. In some of any embodiments, the dose of engineered T cells comprises

between at or about 1.5 x 10^8 CAR-expressing (CAR+) T cells and at or about 3.0 x 10^8 CAR-expressing T cells. In some of any embodiments, the dose of engineered T cells comprise at or about 2.5 x 10^7 CAR-expressing (CAR+) T cells. In some of any embodiments, the dose of engineered T cells comprises at or about 5.0 x 10^7 CAR+ T cells. In some of any embodiments, the dose of engineered T cells comprises at or about 1.5 x 10^8 CAR+ T cells. In some of any embodiments, the dose of engineered T cells comprises at or about 3.0 x 10^8 CAR+ T cells. In some of any embodiments, the dose of engineered T cells comprises at or about 4.5 x 10^8 CAR+ T cells. In some of any embodiments, the dose of engineered T cells comprises at or about 6.0 x 10^8 CAR+ T cells. In some of any embodiments, the dose of engineered T cells comprises at or about 8.0 x 10^8 or at or about 1.2 x 10^9 CAR-expressing T (CAR+) cells. In some of any embodiments, the dose of engineered T cells comprise at or about 5.0 x 10^7, at or about 1.5 x 10^8, at or about 3.0 x 10^8 or at or about 4.5 x 10^8 CAR-expressing (CAR+) T cells.

[0095] In some of any embodiments, the dose of engineered T cells is less than 1.5 x 10^8 cells or less than 1.5 x 10^8 CAR+ T cells or less than 3 x 10^8 CAR+ T cells or less than 4.5 x 10^8 CAR+ T cells. In some of any embodiments, the dose of engineered T cells is at or less than 1.5 x 10^8 cells or less than 1.5 x 10^8 CAR+ T cells.

[0096] In some of any embodiments, the dose of engineered T cells is at or about 5 x 10^7 cells or CAR+ T cells. In some of any embodiments, the dose of engineered T cells is at or about 1.5 x 10^8 cells or CAR+ T cells. In some of any embodiments, the dose of engineered T cells is at or about 3 x 10^8 cells or CAR+ T cells. In some of any embodiments, the dose of engineered T cells is at or about 4.5 x 10^8 cells or CAR+ T cells. In some of any embodiments, the dose of engineered T cells is at or about 6 x 10^8 cells or CAR+ T cells.

[0097] In some of any embodiments, the dose of engineered T cells comprises a combination of $CD4^+$ T cells and $CD8^+$ T cells. In some of any embodiments, the dose of engineered T cells comprises a combination of $CD4^+$ CAR-expressing T cells and $CD8^+$ CAR-expressing T cells, In some of any embodiments, the ratio of $CD4^+$ CAR-expressing T cells to $CD8^+$ CAR-expressing T cells and/or of $CD4^+$ T cells to $CD8^+$ T cells is or is approximately 1:1 or is between at or approximately 1:3 and at or approximately 3:1. In some of any embodiments, the dose of engineered T cells comprises $CD3^+$ CAR-expressing T cells.

[0098] In some of any embodiments, less than at or about 25%, 20%, 15%, 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2% or 1% of the CAR-expressing T cells in the dose of engineered T cells express a marker of apoptosis. In some of any embodiments, the marker is Annexin V or active Caspase 3. In some of any embodiments, less than at or about 5%, 4%, 3%, 2% or 1% of the CAR-expressing T cells in the dose of engineered T cells express Annexin V or active Caspase 3.

[0099] In some of any of the methods or uses provided herein, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or greater than 95% of the cells in the administered dose are of a memory phenotype. In some of any of the

methods or uses provided herein, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or greater than 95% of the cells in the administered dose are of a central memory phenotype. In some of any of the methods or uses provided herein, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or greater than 95% of the cells in the administered dose are CD27+, CD28+, CCR7+, CD45RA-, CD45RO+, CD62L+, CD3+, granzyme B-, and/or CD127+. In some of any of the methods or uses provided herein, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or greater than 95% of the cells in the administered dose are CCR7+/CD45RA- or are CCR7+/CD45RO+.

[0100] In some of any of the methods or uses provided herein, the cells in the administered dose are produced by a method that produces a plurality of output compositions, optionally from human biological samples in which the method is carried out among a plurality of different individual subjects. In some of any of the methods or uses provided herein, cells in the administered dose are produced by a method to produce an output composition exhibiting a predetermined feature, wherein iterations of the method produce a plurality of the output compositions, optionally from human biological samples, when carried out among a plurality of different individual subjects.

[0101] In some of any embodiments, the predetermined feature of the output composition among the plurality of output compositions includes the mean percentage of cells of a memory phenotype in the plurality of the output compositions includes the mean percentage of cells of a memory phenotype in the plurality of the output compositions is between about 40% and about 65%, between about 40% and about 45%, between about 45% and about 50%, between about 50% and about 55%, between about 55% and about 60%, or between about 60% and about 65%. In some of any embodiments, the predetermined feature of the output composition among the plurality of output compositions includes the mean percentage of cells of a central memory phenotype in the plurality of the output compositions is between about 40% and about 65%, between about 40% and about 45%, between about 45% and about 50%, between about 50% and about 55%, between about 55% and about 60%, or between about 60% and about 65%. In some of any embodiments, the predetermined feature of the output composition among the plurality of output compositions includes the mean percentage of cells that are CD27+, CD28+, CCR7+, CD45RA-, CD45RO+, CD62L+, CD3+, CD95+, granzyme B-, and/or CD127+ in the plurality of the output compositions is between about 40% and about 65%, between about 40% and about 45%, between about 45% and about 50%, between about 50% and about 55%, between about 55% and about 60%, or between about 60% and about 65%. In some of any embodiments, the predetermined feature of the output composition among the plurality of output compositions includes the mean percentage of cells that are CCR7+/CD45RA- or CCR7+/CD45RO+ in the plurality of the output compositions is between about 40% and about 65%, between about 40% and about 45%, between about 45% and about 50%, between about 50% and about 55%, between about 55% and about 60%, or between about 60% and about 65%. In

some of any embodiments, the predetermined feature of the output composition among the plurality of output compositions includes the mean percentage of central memory CD4+ T cells in the engineered CD4+ T cells, optionally CAR+CD4+ T cells, of the plurality of the output compositions is between about 40% and about 65%, between about 40% and about 45%, between about 45% and about 50%, between about 50% and about 55%, between about 55% and about 60%, or between about 60% and about 65%. In some of any embodiments, the predetermined feature of the output composition among the plurality of output compositions includes the mean percentage of central memory CD8+ T cells in the engineered CD8+ T cells, optionally CAR+CD8+ T cells, of the plurality of the output compositions is between about 40% and about 65%, between about 40% and about 45%, between about 45% and about 50%, between about 50% and about 55%, between about 55% and about 60%, or between about 60% and about 65%. In some of any embodiments, the predetermined feature of the output composition among the plurality of output compositions includes the mean percentage of central memory T cells, optionally CD4+ central memory T cells and CD8+ central memory T cells, in the engineered T cells, optionally CAR+ T cells, of the plurality of the output compositions is between about 40% and about 65%, between about 40% and about 45%, between about 45% and about 50%, between about 50% and about 55%, between about 55% and about 60%, or between about 60% and about 65%.

[0102] In some of any of the methods or uses provided herein, the administered dose is produced by a method to produce an output composition exhibiting a predetermined feature, optionally a threshold number of cells expressing the CAR in the output composition, in at least about 80%, about 90%, about 95%, about 97%, about 99%, about 100%, or is 100% of the human biological samples in which it is carried out among a plurality of different individual subjects. In some of any embodiments, the plurality of different individual subject comprise subjects having a disease or condition. In some of any embodiments, the disease or condition is a cancer. In some of any embodiments, the cancer is a hematological cancer, optionally multiple myeloma. In some embodiments, the cancer is relapsed or refractory multiple myeloma (R/R MM).

Brief Description of the Drawings

[0103] **FIGS. 1A-1B** depict results of an assay assessing RNA heterogeneity as assessed by agarose gel electrophoresis. **FIG. 1A** depicts the RNA heterogeneity of several anti-BCMA-CARs, containing a long spacer (LS) region, or a shorter CD28 spacer region. **FIG. 1B** depicts RNA heterogeneity of three different anti-BCMA CAR encoding sequences, containing the long spacer (LS) region, before and after coding sequence optimization and splice site elimination (O/SSE).

[0104] **FIG. 2** depicts results of an assay assessing levels of BCMA-LS CAR expression on the surface of transduced T cells before (Non-SSE) and after (O/SSE) optimization and splice site elimination of the coding sequence.

[0105] **FIG. 3** depicts the comparison of transduction efficiency of lentiviral vectors encoding

BCMA-LS CAR constructs and lentiviral vectors encoding BCMA-LS CAR constructs that have been codon optimized and modified to eliminate predicted splice sites (O/SSE).

[0106] FIG. 4A depicts results of an assay assessing the cytolytic activity of BCMA-LS CAR-expressing T cells against cell lines that express high (K562/BCMA) or low (RPMI 8226) levels of BCMA at several effector:target cell (E:T) ratios. **FIG. 4B** depicts the cytolytic activity of several BCMA-LS CAR-expressing T cells against RPMI-8226 cells at an E:T ratio of 3:1. **FIGS. 4C-4D** depict the cytolytic activity of non-optimized BCMA-LS CAR-expressing T cells and optimized (O/SSE) BCMA-LS CAR-expressing T cells on various BCMA-expressing cell lines.

[0107] FIG. 5A depicts results of an assay assessing IFNγ, IL-2, and TNFα cytokine release of BCMA-LS CAR-expressing T cells in response to incubation with cell lines that express high (K562/BCMA) or low (RPMI 8226) levels of BCMA at several effector:target cell (E:T) ratios (5:1, 2.5:1, 1.25:1 and 0.6:1 indicated as a, b, c and d, respectively, in the figure). **FIG. 5B** depicts the IFNγ, and IL-2 cytokine release of non-optimized BCMA-LS CAR-expressing T cells and optimized (O/SSE) BCMA-LS CAR-expressing T cells in response to incubation with BCMA-expressing K562/BCMA and RPMI 8226 cells at different E:T ratios (3:1, 1.5:1, 0.75:1 and 0.375:1 indicated as a, b, c and d, respectively, in the figure).

[0108] FIG. 6 depicts results of an assay assessing cytolytic activity following incubation of BCMA-55-LS-O/SSE CAR-expressing T cells, from two donors, with BCMA-expressing cells that express varying levels of BCMA.

[0109] FIG. 7 depicts results of an assay assessing IFNγ release following incubation of BCMA-55-LS CAR O/SSE-expressing T cells, from two donors, with BCMA-expressing cells that express varying levels of BCMA.

[0110] FIG. 8 depicts results of an assay assessing cytolytic activity of anti-BCMA-expressing CAR T cells that express CARs containing different spacer regions, on OPM2 target cells.

[0111] FIGS. 9A-9B depict results of an assay assessing cytolytic activity of anti-BCMA CAR-expressing T cells following incubation of anti-BCMA CAR-expressing T cells with OPM2 target cells in the presence of soluble BCMA-Fc.

[0112] FIG. 10A depicts results of an assay assessing cytolytic activity of optimized (O/SSE) anti-BCMA CAR-expressing T cells in the presence of supernatant from the H929 multiple myeloma cell line. **FIG. 10B** depicts results of an assay assessing cytolytic activity of optimize (O/SSE) anti-BCMA CAR-expressing T cells in the presence of recombinant B-cell activating factor (BAFF).

[0113] FIGS. 11A-11B depict results of an assay assessing IFNγ, IL-2, and TNFα cytokine release following incubation of anti-BCMA CAR-expressing T cells with OPM2 target cells in the presence of soluble BCMA-Fc (**FIG. 11A**) or supernatant from a multiple myeloma cell line H929 (**FIG. 11B**) at different concentrations (0 ng/mL, 111 ng/mL, 333 ng/mL and 1000 ng/mL indicated as a, b, c and d, respectively, in the figures).

[0114] FIG. 12A depicts results of an assay assessing tumor growth in an OPM2 human multiple myeloma xenograft mouse model, following a single intravenous injection of CAR T cells expressing optimized (O/SSE) anti-BCMA CARs. **FIG. 12B** depicts results of an assay assessing survival in an OPM2 human multiple myeloma xenograft mouse model, following a single intravenous injection of CAR T cells expressing optimized (O/SSE) anti-BCMA CARs.

[0115] FIG. 13A depicts results of an assay assessing tumor growth in an RPMI-8226 (subcutaneous) xenograft mouse model, following a single intravenous injection of CAR T cells expressing optimized (O/SSE) anti-BCMA CARs. **FIG. 13B** depicts survival in an RPMI-8226 (subcutaneous) xenograft mouse model, following a single intravenous injection of CAR T cells expressing optimized (O/SSE) anti-BCMA CARs.

[0116] FIGS. 14A-14B depict results of an assay assessing the number of CD4+ (**FIG. 14A**) and CD8+ (**FIG. 14B**) CAR-positive T cells in the blood from RPMI-8226 (subcutaneous) xenograft mice treated with optimized (O/SSE) anti-BCMA CAR T cells derived from a single donor (Donor 2).

[0117] FIGS. 15A-15B depict results of an assay assessing the number of CD4+ (**FIG. 15A**) and CD8+ (**FIG. 15B**) CAR-positive T cells in the blood from RPMI-8226 (subcutaneous) xenograft mice treated with optimized (O/SSE) anti-BCMA CAR T cells derived from a single donor (Donor 1).

[0118] FIG. 16A depicts results of an assay assessing expression level of tdTomato and a truncated receptor (surrogate marker for CAR expression), as detected by flow cytometry, in BCMA-55-LS-O/SSE CAR-expressing cells, incubated for 6 hours in 96-well cell culture plates coated overnight with (0.008 µg/mL, 0.04 µg/mL, 0.2 µg/mL, 1 µg/mL and 5 µg/mL) of BCMA-Fc (soluble human BCMA fused at its C-terminus to an Fc region of IgG) fusion polypeptide. A recombinant Fc polypeptide was used as a control (Fc Control). **FIG. 16B** depicts results of an assay assessing percentage of tdTomato+ cells among cells expressing the truncated receptor, in reporter cells expressing BCMA-55-LS-O/SSE CAR, BCMA-26-LS-O/SSE CAR, BCMA-23-LS-O/SSE CAR, and BCMA-25-LS-O/SSE CAR, incubated with ten (10) 2-fold serial dilution of BCMA-Fc. Cells expressing a CAR specific for a different antigen (anti-CD19 CAR) was used as control.

[0119] FIG. 17 depicts the percentage of tdTomato+ cells among reporter cells expressing BCMA-55-LS-O/SSE CAR or BCMA-55-SS CAR, following co-cultured with human BCMA-expressing K562 target cells (BCMA.K562) target cells at various E:T ratios.

[0120] FIG. 18 depicts the expression level of tdTomato and GFP (surrogate marker for CAR expression), as detected by flow cytometry, in reporter cells expressing an anti-CD19 CAR, BCMA-55-LS-O/SSE CAR, BCMA-26-LS-O/SSE CAR, BCMA-23-LS-O/SSE CAR, or BCMA-52-LS-O/SSE CAR, incubated without antigen stimulation to assess the degree of antigen-independent (tonic) signaling for 3 days.

[0121] FIGS. 19A-19B depict the expression level of tdTomato and truncated receptor (surrogate marker for CAR expression), as detected by flow cytometry, in reporter cells expressing an anti-CD19

CAR, BCMA-55-LS-O/SSE CAR, BCMA-26-LS-O/SSE CAR, BCMA-23-LS-O/SSE CAR, or BCMA-52-LS-O/SSE CAR that contain intracellular domains derived from 4-1BB or CD28 incubated without antigen stimulation to assess the degree of antigen-independent (tonic) signaling.

[0122] FIG. 20A depicts the percentage of tdTomato+ cells, as assessed by flow cytometry, among the Nur77-tdTomato reporter cells engineered to express BCMA-55-LS-O/SSE CAR, specific for human BCMA, co-cultured with K562 human myelogenous leukemia cells expressing human BCMA (huBCMA), murine BCMA (muBCMA) or cynomolgus monkey BCMA (cynoBCMA), at an E:T ratio of 2:1 or 5:1. **FIGS. 20B-20C** depict the percentage (**FIG. 20B**) and mean fluorescence intensity (MFI; **FIG. 20C**) of tdTomato+ cells, as assessed by flow cytometry, among reporter cells expressing BCMA-55-LS-O/SSE CAR, incubated with increasing concentrations (0, 0.1, 0.25, 1, 2.5, 10, 25 and 100 μg/mL) of huBCMA and cynoBCMA coated on 96-well flat-bottom plates.

[0123] FIG. 21A depicts an exemplary amplification strategy for a transcript and predicted amplified product. **FIG. 21B** depicts exemplary amplified products resulting from amplification of a transcript known and unknown (cryptic) splice sites. **FIG. 21C** depicts exemplary sliding window amplification of a transcript using nested primer pairs.

[0124] FIGS. 22A-22D depict exemplary phenotypical profiles of 40 engineered CAR+ T cell compositions, each from a multiple myeloma patient. CD45RA×CCR7 expression profiles among the CAR+ T cell compositions are shown for the CD4+ populations (**FIG. 22A**) and the CD8+ populations (**FIG. 22B**). CD27×CD28 expression profiles among the CAR+ T cell compositions are shown for the CD4+ populations (**FIG. 22C**) and the CD8+ populations (**FIG. 22D**). Each CAR+ T cell composition is shown by a dot (●), a cross (×), a diamond (◊), or a triangle (Δ).

[0125] FIG. 23 shows the objective response rates (ORR) and complete response (CR) and stringent complete response (sCR), very good partial response (VGPR) and partial response (PR) in human subjects with relapsed and/or refractory multiple myeloma (MM) that have been administered compositions containing autologous T cells expressing a CAR specific for B-cell maturation antigen (BCMA), at a single dose of dose level 1 (DL1) containing 5 x 10^7 total CAR+ T cells, a single dose of dose level 2 (DL2) containing 1.5 x 10^8 total CAR+ T cells, or a single dose of dose level 3 (DL3) containing 4.5 x 10^8 total CAR+ T cells. [b]:One subject in the DL3 cohort was not evaluable for efficacy due to the lack of post-baseline response evaluation at Day 29.

[0126] FIG. 24 shows the assessment of response over time, in subjects in the DL1 cohorts at the longest follow-up, after administration of the CAR-expressing T cells (n = 14)

[0127] FIG. 25 shows the expansion and long-term persistence of CAR^+ T cells in the peripheral blood of subjects in the DL1, DL2, and DL3 cohorts, as measured by quantitative polymerase chain reaction (qPCR) of genomic DNA preparations from whole blood samples to detect vector sequences encoding the CAR (vector copies/μg genomic DNA). LLOQ, lower limit of quantification; LLOD, lower limit of detection.

[0128] **FIG. 26A** shows the level of soluble BCMA (sBCMA) (ng/mL) in the serum of the subjects prior to CAR+ T cell administration and at various timepoints after administration (day 29, month 2 and month 3) in various subjects with an overall response of PR or better (PR, VGPR, CR or sCR; responders) as compared to subjects with an overall response that is worse than PR (MR or SD; non-responders). **FIG. 26B** shows the level of sBCMA prior to CAR+ T cell administration (pre-treatment) in subjects who exhibited an overall response of PR or better (responders) and in subjects who exhibited a response worse than PR (MR or SD; non-responders).

Detailed Description

[0129] Among the provided embodiments are compositions, articles of manufacture, compounds, methods and uses including those targeting or directed to BCMA and BCMA-expressing cells and diseases. It is observed that BCMA is expressed, e.g., heterogeneously expressed, on certain diseases and conditions such as malignancies or tissues or cells thereof, e.g., on malignant plasma cells such as from all relapsed or newly diagnosed myeloma patients, for example, with little expression on normal tissues. Among the provided embodiments are approaches useful in the treatment of such diseases and conditions and/or for targeting such cell types, including nucleic acid molecules that encode BCMA-binding receptors, including chimeric antigen receptors (CARs), and the encoded receptors such as the encoded CARs, and compositions and articles of manufacture comprising the same. The receptors generally can contain antigen-binding domains that include antibodies (including antigen-binding antibody fragments, such as heavy chain variable (V_H) regions, single domain antibody fragments and single chain fragments, including scFvs) specific for BCMA. Also provided are cells, such as engineered or recombinant cells expressing such BCMA-binding receptors, *e.g.*, anti-BCMA CARs and/or containing nucleic acids encoding such receptors, and compositions and articles of manufacture and therapeutic doses containing such cells. Also provided are methods of evaluating, optimizing, making and using nucleic acid sequence(s), for example, nucleic acid sequences encoding recombinant BCMA-binding receptors. Also provided are methods of making and using (such as in the treatment or amelioration of BCMA-expressing diseases and conditions) cells (*e.g.*, engineered cells) expressing or containing the recombinant BCMA-binding receptors and recombinant BCMA-binding receptor-encoding polynucleotides or compositions containing such cells.

[0130] Adoptive cell therapies (including those involving the administration of cells expressing chimeric receptors specific for a disease or disorder of interest, such as chimeric antigen receptors (CARs) and/or other recombinant antigen receptors, as well as other adoptive immune cell and adoptive T cell therapies) can be effective in the treatment of cancer and other diseases and disorders. In certain contexts, available approaches to adoptive cell therapy may not always be entirely satisfactory. In some aspects, the ability of the administered cells to recognize and bind to a target, *e.g.*, target antigen such as BCMA, to traffic, localize to and successfully enter appropriate sites within the subject, tumors, and

environments thereof, to become activated, expand, to exert various effector functions, including cytotoxic killing and secretion of various factors such as cytokines, to persist, including long-term, to differentiate, transition or engage in reprogramming into certain phenotypic states to provide effective and robust recall responses following clearance and re-exposure to target ligand or antigen, and avoid or reduce exhaustion, anergy, terminal differentiation, and/or differentiation into a suppressive state.

[0131] In some aspects, available approaches for treatment of diseases or disorders such as multiple myeloma is complex and may not always be entirely satisfactory. In some aspects, choosing a treatment regimen can depend on numerous factors including drug availability, response to prior therapy, aggressiveness of the relapse, eligibility for autologous stem cell transplantation (ASCT), and whether the relapse occurred on or off therapy. In some aspects, MM results in relapses and remissions, and existing regimen in some cases can result in relapse and/or toxicity from the treatment. In some cases, subjects with particularly aggressive disease, such as subjects that have persistent or relapsed disease after various therapies, subjects with a high disease burden, such as a high tumor burden, and/or subjects with particularly aggressive types of disease, such as plasmacytoma, can be particularly difficult to treat, and responses to certain therapies in these subjects can be poor or have a short duration. In some cases, subjects who have been heavily pre-treated, e.g., subjects who have relapsed after several different prior therapies, can exhibit a low response rate and/or high incidence of adverse events. In some aspects, the provided embodiments are based on an observation that treatment according to the provided embodiments results in a high response rate, low incidences of adverse events (e.g., toxicity), prolonged response, and in some cases, improvement in the response over time.

[0132] The provided embodiments, in some contexts, are based on an observation from a clinical study, that administration of engineered cells expressing a particular recombinant receptor, such as those described herein, results in a high response rate and a low rate of adverse events such as cytokine release syndrome (CRS) or neurological events (NE; or neurotoxicity; NT). In some aspects, the provided cells, methods and uses result in a cell therapy that exhibits prolonged persistence of the cells after administration of the cells, along with a high response rate and a low rate of toxicity (e.g., CRS or NE, such as grade 3 or higher CRS or grade 3 or higher neurotoxicity). In some aspects, such high response and low rate of toxicity (e.g., grade 3 or higher CRS or grade 3 or higher neurotoxicity), is achieved from employing various different doses of cells. For example, even at a relatively low dose of cells, a high rate of objective response and high level of response (e.g., very good partial response, VGPR, or better) is achieved. In some cases, a relatively high dose of cells can be administered, and such doses are observed to result in a high rate of objective response with low rate of toxicity (e.g., grade 3 or higher CRS or grade 3 or higher neurotoxicity). In some cases, the provided embodiments also permit improved expansion and/or persistence of the administered engineered cells, and in some cases result in prolonged response and/or response that is improved over time. In some aspects, treatment of subjects with aggressive or refractory disease (e.g., heavily pre-treated subjects, subjects with a high tumor burden

and/or subjects with aggressive disease types) according to the provided embodiments, was observed to provide a safe, effective and durable treatment.

[0133] In some contexts, optimal response to therapy can depend on the ability of the engineered recombinant receptors such as CARs, to be consistently and reliably expressed on the surface of the cells and/or bind the target antigen. For example, in some cases, heterogeneity of the transcribed RNA from an introduced transgene (e.g., encoding the recombinant receptor) can affect the expression and/or activity of the recombinant receptor, in some cases when expressed in a cell, such as a human T cell, used in cell therapy. In some contexts, the length and type of spacer in the recombinant receptor, such as a CAR, can affect the expression, activity and/or function of the receptor.

[0134] Also, in some contexts, certain recombinant receptors can exhibit antigen-independent activity or signaling (also known as "tonic signaling"), which could lead to undesirable effects, such as due to increased differentiation and/or exhaustion of T cells that express the recombinant receptor. In some aspects, such activities may limit the T cell's activity, effect or potency. In some cases, during engineering and ex vivo expansion of the cells for recombinant receptor expression, the cells may exhibit phenotypes indicative of exhaustion, due to tonic signaling through the recombinant receptor.

[0135] In some contexts, properties of particular target antigens that the recombinant receptors specifically bind, recognize or target, can that affect the activity of the receptor. In some contexts, B-cell maturation antigen (BCMA), is typically expressed on malignant plasma cells and is an attractive therapeutic target for cell therapy. In some cases, BCMA is can be cleaved by gamma secretase, generating a soluble BCMA (sBCMA), or "shed" form of BCMA, reducing the BCMA expressed on the surface of target cells. In some cases, the activity of the BCMA-binding molecules, such as anti-BCMA chimeric antigen receptors, can be blocked or inhibited by the presence of soluble BCMA. Improved strategies are needed for optimal responses to cell therapies, in particular, for recombinant receptors that specifically bind, recognize or target BCMA, such as BCMA expressed on the surface of the target cells.

[0136] The provided embodiments, in some contexts, are based on the observation that particular spacers and optimization of the nucleic acid sequences can lead to consistent and robust expression of the recombinant receptor. The provided BCMA-binding recombinant receptors offer advantages over available approaches for cell therapies, in particular, BCMA-targeting cell therapy. In some embodiments, provided BCMA-binding recombinant receptors contain fully human antigen-binding domains, with low affinity for binding soluble BCMA. In some embodiments, provided BCMA-binding recombinant receptors contain a modified spacer that result in enhanced binding to BCMA expressed on the surface of target cells. In some embodiments, provided BCMA-binding recombinant receptors are observed to exhibit reduced antigen-independent, tonic signaling, which in some cases can result in reduced exhaustion of the cells from antigen-independent signaling, and lack of inhibition by soluble BCMA. In some embodiments, provided BCMA-binding recombinant receptors exhibit activity or potency against target cells that express a low density or low level of BCMA.

[0137] In various aspects, the provided BCMA-binding recombinant receptors, polynucleotides encoding such receptors, engineered cells and cell compositions, exhibit certain desired properties that can overcome or counteract certain limitations that can reduce optimal responses to cell therapy, for example, cell therapy with engineered cells expressing a BCMA-binding recombinant receptor. In some aspects, compositions containing engineered cells expressing an exemplary BCMA-binding recombinant receptor provided herein was observed to exhibit consistency of cell health of the engineered cells, and was associated with improved clinical response. In some aspects, compositions containing the engineered cells expressing an exemplary BCMA-binding recombinant receptor provided herein was observed to be enriched for immune cell subtypes, e.g., CD4+ or CD8+ T cell subtypes, that were associated with central memory T cell (T_{CM}) phenotype, which, in some aspects is associated with increased persistence and durability of the engineered cells. In some contexts, the provided embodiments, including the recombinant receptors, polynucleotides encoding such receptors, engineered cells and cell compositions, can provide various advantages over available therapies targeting BCMA, to improve the activity of the recombinant receptors and response to BCMA-targeting cell therapies. In addition, the provided methods and uses of the engineered cells or compositions comprising the engineered cells, has been observed to provide an advantage in treating subjects, that results in a high response rate, a durable response, and low rate of adverse events, at various different dose levels tested. Further, the provided methods and uses of the engineered cells or compositions comprising the engineered cells, has been observed to provide an advantage in treating subjects with particularly aggressive and/or refractory disease, or subjects who have relapsed and/or are refractory to numerous different prior treatments for the disease.

[0138] All publications, including patent documents, scientific articles and databases, referred to in this application are incorporated by reference in their entirety for all purposes to the same extent as if each individual publication were individually incorporated by reference. If a definition set forth herein is contrary to or otherwise inconsistent with a definition set forth in the patents, applications, published applications and other publications that are herein incorporated by reference, the definition set forth herein prevails over the definition that is incorporated herein by reference.

[0139] The section headings used herein are for organizational purposes only and are not to be construed as limiting the subject matter described.

I. BCMA-BINDING RECEPTORS AND ENCODING POLYNUCLEOTIDES

[0140] Provided in some aspects are BCMA-binding agents, such as cell surface proteins, such as recombinant receptors or chimeric antigen receptors that bind or recognize BCMA molecules and polynucleotides encoding BCMA-binding cell surface proteins, such as recombinant receptors (e.g., chimeric antigen receptors; CARs), and cells expressing such receptors. The BCMA-binding cell surface proteins generally contain antibodies (*e.g.*, antigen-binding antibody fragments), and/or other binding peptides that specifically recognize, such as specifically bind to BCMA, such as to BCMA proteins, such

as human BCMA protein. In some aspects, the agents bind to an extracellular portion of BCMA. Also provided are cells, e.g., engineered cells, comprising such polynucleotides or expressing such receptors, and compositions comprising such engineered cells. In some aspects, also provided are methods employing such cells and compositions, and uses thereof, such as in therapeutic methods.

[0141] In some embodiments, the polynucleotides are optimized, or contain certain features designed for optimization, such as for codon usage, to reduce RNA heterogeneity and/or to modify, e.g., increase or render more consistent among cell product lots, expression, such as surface expression, of the encoded receptor. In some embodiments, polynucleotides, encoding BCMA-binding cell surface proteins, are modified as compared to a reference polynucleotide, such as to remove cryptic or hidden splice sites, to reduce RNA heterogeneity. In some embodiments, polynucleotides, encoding BCMA-binding cell surface proteins, are codon optimized, such as for expression in a mammalian, e.g., human, cell such as in a human T cell. In some aspects, the modified polynucleotides result in in improved, e.g., increased or more uniform or more consistent level of, expression, e.g., surface expression, when expressed in a cell. Such polynucleotides can be utilized in constructs for generation of engineered cells that express the encoded BCMA-binding cell surface protein. Thus, also provided are cells expressing the recombinant receptors encoded by the polynucleotides provided herein and uses thereof in adoptive cell therapy, such as treatment of diseases and disorders associated with BCMA expression, such as multiple myeloma.

[0142] Among the provided polynucleotides are those that encode recombinant receptors, such as antigen receptors, that specifically recognize, such as specifically bind, BCMA, such as a human BCMA. In some aspects, the encoded receptors, such as those containing BCMA-binding polypeptides, and compositions and articles of manufacture and uses of the same, also are provided.

[0143] Among the BCMA-binding polypeptides are antibodies, such as single-chain antibodies (e.g., antigen binding antibody fragments), or portions thereof. In some examples, the recombinant receptors are chimeric antigen receptors, such as those containing anti-BCMA antibodies or antigen-binding fragments thereof. In any of the embodiments, an antibody or antigen binding fragment, in the provided CARs, that specifically recognizes an antigen, e.g. BCMA, specifically binds to the antigen. The provided polynucleotides can be incorporated into constructs, such as deoxyribonucleic acid (DNA) or RNA constructs, such as those that can be introduced into cells for expression of the encoded recombinant BCMA-binding receptors.

[0144] In some cases, the polynucleotide encoding the BCMA-binding receptor contains a signal sequence that encodes a signal peptide, in some cases encoded upstream of the nucleic acid sequences encoding the BCMA-binding receptor, or joined at the 5' terminus of the nucleic acid sequences encoding the antigen-binding domain. In some cases, the polynucleotide containing nucleic acid sequences encoding the BCMA-binding receptor, e.g., chimeric antigen receptor (CAR), contains a signal sequence that encodes a signal peptide. In some aspects, the signal sequence may encode a signal peptide derived from a native polypeptide. In other aspects, the signal sequence may encode a

heterologous or non-native signal peptide. In some aspects, non-limiting exemplary signal peptide include a signal peptide of the IgG kappa chain set forth in SEQ ID NO: 166, or encoded by the nucleotide sequence set forth in SEQ ID NO: 167 or 168-171; a GMCSFR alpha chain set forth in SEQ ID NO:154 and encoded by the nucleotide sequence set forth in SEQ ID NO:155; a CD8 alpha signal peptide set forth in SEQ ID NO:146; or a CD33 signal peptide set forth in SEQ ID NO:142. In some cases, the polynucleotide encoding the BCMA-binding receptor can contain nucleic acid sequence encoding additional molecules, such as a surrogate marker or other markers, or can contain additional components, such as promoters, regulatory elements and/or multicistronic elements. In some embodiments, the nucleic acid sequence encoding the BCMA-binding receptor can be operably linked to any of the additional components.

A. Components of Encoded Recombinant BCMA-Binding Receptors

[0145] The provided BCMA-binding receptors, e.g., expressed in the cells employed in the methods and uses provided herein, generally contain an extracellular binding molecule and an intracellular signaling domain. Among the provided binding molecules are polypeptides containing antibodies, including single chain cell surface proteins, *e.g.*, recombinant receptors such as chimeric antigen receptors, containing such antibodies.

[0146] Among the provided binding molecules (*e.g.*, BCMA-binding molecules) are single chain cell surface proteins, such as recombinant receptors (*e.g.*, antigen receptors), that include one of the provided antibodies or fragment thereof (*e.g.*, BCMA-binding fragment). The recombinant receptors include antigen receptors that specifically bind to or specifically recognize BCMA, such as antigen receptors containing the provided anti-BCMA antibodies, *e.g.*, antigen-binding fragments. Among the antigen receptors are functional non-TCR antigen receptors, such as chimeric antigen receptors (CARs). Also provided are cells expressing the recombinant receptors and uses thereof in adoptive cell therapy, such as treatment of diseases and disorders associated with BCMA expression.

[0147] Exemplary antigen receptors, including CARs, and methods for engineering and introducing such antigen receptors into cells, include those described, for example, in international patent application publication Nos. WO200014257, WO2013126726, WO2012/129514, WO2014031687, WO2013166321, WO2013071154, WO2013123061 U.S. patent application publication Nos. US2002131960, US2013287748, US20130149337, U.S. Patent Nos. 6,451,995, 7,446,190, 8,252,592, 8,339,645, 8,398,282, 7,446,179, 6,410,319, 7,070,995, 7,265,209, 7,354,762, 7,446,191, 8,324,353, and 8,479,118, and European patent application No. EP2537416, and/or those described by Sadelain *et al.*, Cancer Discov. 2013 April; 3(4): 388–398; Davila *et al.* (2013) PLoS ONE 8(4): e61338; Turtle *et al.*, Curr. Opin. Immunol., 2012 October; 24(5): 633-39; Wu *et al.*, Cancer, 2012 March 18(2): 160-75. In some aspects, the antigen receptors include a CAR as described in U.S. Patent No. 7,446,190, and those described in International Patent Application Publication No. WO2014055668. Exemplary CARs include CARs as disclosed in any of the aforementioned publications, such as WO2014031687, US

8,339,645, US 7,446,179, US 2013/0149337, US 7,446,190, and US 8,389,282, and in which the antigen-binding portion, *e.g.*, scFv, is replaced by an antibody or an antigen-binding fragment thereof, as provided herein.

[0148] In some embodiments, the provided CAR has an amino acid sequence selected from among SEQ ID NOs: 15-20, or an amino acid sequence that exhibits at least or about at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% sequence identity to the amino acid sequence set forth in any of SEQ ID NOs 15-20. In some embodiments, the provided CAR has an amino acid sequence set forth in SEQ ID NO: 19, or an amino acid sequence that exhibits at least or about at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% sequence identity to the amino acid sequence set forth in SEQ ID NO:19.

[0149] In some embodiments, the provided CAR is encoded by a polynucleotide, such as an polynucleotide with the nucleic acid sequence set forth in any of SEQ ID NOs 9-14, or a sequences that exhibits at least or at least about 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% sequence identity to the nucleic acid sequence set forth in any of SEQ ID NOs: 9-14. In some embodiments, the provided CAR is encoded by a polynucleotide, such as an polynucleotide with the nucleic acid sequence set forth in any of SEQ ID NOs:13 and 14, or a sequences that exhibits at least or at least about 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% sequence identity to the nucleic acid sequence set forth in any of SEQ ID NOs: 13 and 14. In some embodiments, the provided CAR is encoded by a polynucleotide, such as an polynucleotide with the nucleic acid sequence set forth in SEQ ID NO:13 or a sequences that exhibits at least or at least about 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% sequence identity thereto. In some embodiments, the provided CAR is encoded by a polynucleotide, such as an polynucleotide with the nucleic acid sequence set forth in SEQ ID NO:13.

[0150] In some embodiments, the nucleic acid encoding the antigen-binding domain comprises (a) the sequence of nucleotides set forth in any of SEQ ID NOS: 30, 31, 50, 51, 59, 60, 82, 84, 113, 115; (b) a sequence of nucleotides that has at least 90% sequence identity to any of SEQ ID NOS: 30, 31, 50, 51, 59, 60, 82, 84, 113, 115; or (c) a degenerate sequence of (a) or (b). In some embodiments, the nucleic acid encoding the antigen-binding domain comprises (a) a sequence of nucleotides encoding the amino acid sequence set forth in any of SEQ ID NOS: 29, 49, 58, 83, 114, 127, 128, 129, 130; (b) a sequence of nucleotides that has at least 90% sequence identity to a sequence of nucleotides encoding the amino acid sequence set forth in any of SEQ ID NOS: 29, 49, 58, 83, 114, 126, 127, 129, 130; or (c) a degenerate sequence of (a) or (b).

1. Antigen-binding domain

[0151] Among the chimeric receptors are chimeric antigen receptors (CARs). The chimeric receptors, such as CARs, generally include an extracellular antigen binding domain that includes, is, or is comprised within or comprises, one of the provided anti-BCMA antibodies. Thus, the chimeric receptors, *e.g.*, CARs, typically include in their extracellular portions one or more BCMA-binding

molecules, such as one or more antigen-binding fragment, domain, or portion, or one or more antibody variable regions, and/or antibody molecules, such as those described herein.

[0152] The term "antibody" herein is used in the broadest sense and includes polyclonal and monoclonal antibodies, including intact antibodies and functional (antigen-binding) antibody fragments, including fragment antigen binding (Fab) fragments, F(ab')$_2$ fragments, Fab' fragments, Fv fragments, recombinant IgG (rIgG) fragments, heavy chain variable (V_H) regions capable of specifically binding the antigen, single chain antibody fragments, including single chain variable fragments (scFv), and single domain antibodies (*e.g.*, sdAb, sdFv, nanobody) fragments. The term encompasses genetically engineered and/or otherwise modified forms of immunoglobulins, such as intrabodies, peptibodies, chimeric antibodies, fully human antibodies, humanized antibodies, and heteroconjugate antibodies, multispecific, *e.g.*, bispecific or trispecific, antibodies, diabodies, triabodies, and tetrabodies, tandem di-scFv, tandem tri-scFv. Unless otherwise stated, the term "antibody" should be understood to encompass functional antibody fragments thereof also referred to herein as "antigen-binding fragments." The term also encompasses intact or full-length antibodies, including antibodies of any class or sub-class, including IgG and sub-classes thereof, IgM, IgE, IgA, and IgD.

[0153] The terms "complementarity determining region," and "CDR," synonymous with "hypervariable region" or "HVR," are known in the art to refer to non-contiguous sequences of amino acids within antibody variable regions, which confer antigen specificity and/or binding affinity. In general, there are three CDRs in each heavy chain variable region (CDR-H1, CDR-H2, CDR-H3) and three CDRs in each light chain variable region (CDR-L1, CDR-L2, CDR-L3). "Framework regions" and "FR" are known in the art to refer to the non-CDR portions of the variable regions of the heavy and light chains. In general, there are four FRs in each full-length heavy chain variable region (FR-H1, FR-H2, FR-H3, and FR-H4), and four FRs in each full-length light chain variable region (FR-L1, FR-L2, FR-L3, and FR-L4).

[0154] The precise amino acid sequence boundaries of a given CDR or FR can be readily 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); MacCallum *et al.*, J. Mol. Biol. 262:732-745 (1996), "Antibody-antigen interactions: Contact analysis and binding site topography," J. Mol. Biol. 262, 732-745." ("Contact" numbering scheme); Lefranc MP *et al.*, "IMGT unique numbering for immunoglobulin and T cell receptor variable domains and Ig superfamily V-like domains," Dev Comp Immunol, 2003 Jan;27(1):55-77 ("IMGT" numbering scheme); Honegger A and Plückthun A, "Yet another numbering scheme for immunoglobulin variable domains: an automatic modeling and analysis tool," J Mol Biol, 2001 Jun 8;309(3):657-70, ("Aho" numbering scheme); and Martin *et al.*, "Modeling antibody hypervariable loops: a combined algorithm," PNAS, 1989, 86(23):9268-9272, ("AbM" numbering

scheme).

[0155] The boundaries of a given CDR or FR may vary depending on the scheme used for identification. For example, the Kabat scheme is based on structural alignments, while the Chothia scheme is based on structural information. Numbering for both the Kabat and Chothia schemes is based upon the most common antibody region sequence lengths, with insertions accommodated by insertion letters, for example, "30a," and deletions appearing in some antibodies. The two schemes place certain insertions and deletions ("indels") at different positions, resulting in differential numbering. The Contact scheme is based on analysis of complex crystal structures and is similar in many respects to the Chothia numbering scheme. The AbM scheme is a compromise between Kabat and Chothia definitions based on that used by Oxford Molecular's AbM antibody modeling software.

[0156] **Table 1**, below, lists exemplary position boundaries of CDR-L1, CDR-L2, CDR-L3 and CDR-H1, CDR-H2, CDR-H3 as identified by Kabat, Chothia, AbM, and Contact schemes, respectively. For CDR-H1, residue numbering is listed using both the Kabat and Chothia numbering schemes. FRs are located between CDRs, for example, with FR-L1 located before CDR-L1, FR-L2 located between CDR-L1 and CDR-L2, FR-L3 located between CDR-L2 and CDR-L3 and so forth. It is noted that because the shown Kabat numbering scheme places insertions at H35A and H35B, the end of the Chothia CDR-H1 loop when numbered using the shown Kabat numbering convention varies between H32 and H34, depending on the length of the loop.

Table 1. Boundaries of CDRs according to various numbering schemes.

CDR	**Kabat**	**Chothia**	**AbM**	**Contact**
CDR-L1	L24--L34	L24--L34	L24--L34	L30--L36
CDR-L2	L50--L56	L50--L56	L50--L56	L46--L55
CDR-L3	L89--L97	L89--L97	L89--L97	L89--L96
CDR-H1 (Kabat Numbering[1])	H31--H35B	H26--H32.34	H26--H35B	H30--H35B
CDR-H1 (Chothia Numbering[2])	H31--H35	H26--H32	H26--H35	H30--H35
CDR-H2	H50--H65	H52--H56	H50--H58	H47--H58
CDR-H3	H95--H102	H95--H102	H95--H102	H93--H101

1 - Kabat *et al.* (1991), "Sequences of Proteins of Immunological Interest," 5th Ed. Public Health Service, National Institutes of Health, Bethesda, MD
2 - Al-Lazikani *et al.*, (1997) JMB 273,927-948

[0157] Thus, unless otherwise specified, a "CDR" or "complementary determining region," or individual specified CDRs (*e.g.*, CDR-H1, CDR-H2, CDR-H3), of a given antibody or region thereof, such as a variable region thereof, should be understood to encompass a (or the specific) complementary determining region as defined by any of the aforementioned schemes, or other known schemes. For example, where it is stated that a particular CDR (*e.g.*, a CDR-H3) contains the amino acid sequence of a corresponding CDR in a given V_H or V_L region amino acid sequence, it is understood that such a CDR has a sequence of the corresponding CDR (*e.g.*, CDR-H3) within the variable region, as defined by any

of the aforementioned schemes, or other known schemes. In some embodiments, specific CDR sequences are specified. Exemplary CDR sequences of provided antibodies are described using various numbering schemes, although it is understood that a provided antibody can include CDRs as described according to any of the other aforementioned numbering schemes or other numbering schemes known to a skilled artisan.

[0158] Likewise, unless otherwise specified, a FR or individual specified FR(s) (*e.g.*, FR-H1, FR-H2, FR-H3, FR-H4), of a given antibody or region thereof, such as a variable region thereof, should be understood to encompass a (or the specific) framework region as defined by any of the known schemes. In some instances, the scheme for identification of a particular CDR, FR, or FRs or CDRs is specified, such as the CDR as defined by the Kabat, Chothia, AbM, IMGT or Contact method, or other known schemes. In other cases, the particular amino acid sequence of a CDR or FR is given.

[0159] The term "variable region" or "variable domain" refers to the domain of an antibody heavy or light chain that is involved in binding the antibody to antigen. The variable regions of the heavy chain and light chain (V_H and V_L, respectively) of a native antibody generally have similar structures, with each domain comprising four conserved framework regions (FRs) and three CDRs. (See, *e.g.*, Kindt *et al.* Kuby Immunology, 6th ed., W.H. Freeman and Co., page 91 (2007). A single V_H or V_L domain may be sufficient to confer antigen-binding specificity. Furthermore, antibodies that bind a particular antigen may be isolated using a V_H or V_L domain from an antibody that binds the antigen to screen a library of complementary V_L or V_H domains, respectively. See, *e.g.*, Portolano *et al.*, J. Immunol. 150:880-887 (1993); Clarkson *et al.*, Nature 352:624-628 (1991).

[0160] Among the antibodies included in the provided CARs are antibody fragments. An "antibody fragment" or "antigen-binding fragment" refers to a molecule other than an intact antibody that comprises a portion of an intact antibody that binds the antigen to which the intact antibody binds. Examples of antibody fragments include but are not limited to Fv, Fab, Fab', Fab'-SH, $F(ab')_2$; diabodies; linear antibodies; heavy chain variable (V_H) regions, single-chain antibody molecules such as scFvs and single-domain antibodies comprising only the V_H region; and multispecific antibodies formed from antibody fragments. In some embodiments, the antigen-binding domain in the provided CARs is or comprises an antibody fragment comprising a variable heavy chain (V_H) and a variable light chain (V_L) region. In particular embodiments, the antibodies are single-chain antibody fragments comprising a heavy chain variable (V_H) region and/or a light chain variable (V_L) region, such as scFvs.

[0161] Single-domain antibodies (sdAbs) are antibody fragments comprising all or a portion of the heavy chain variable region or all or a portion of the light chain variable region of an antibody. In certain embodiments, a single-domain antibody is a human single-domain antibody.

[0162] Antibody fragments can be made by various techniques, including but not limited to proteolytic digestion of an intact antibody as well as production by recombinant host cells. In some embodiments, the antibodies are recombinantly-produced fragments, such as fragments comprising

arrangements that do not occur naturally, such as those with two or more antibody regions or chains joined by synthetic linkers, *e.g.*, peptide linkers, and/or that are may not be produced by enzyme digestion of a naturally-occurring intact antibody. In some aspects, the antibody fragments are scFvs.

[0163] A "humanized" antibody is an antibody in which all or substantially all CDR amino acid residues are derived from non-human CDRs and all or substantially all FR amino acid residues are derived from human FRs. A humanized antibody optionally may include at least a portion of an antibody constant region derived from a human antibody. A "humanized form" of a non-human antibody, refers to a variant of the non-human antibody that has undergone humanization, typically to reduce immunogenicity to humans, while retaining the specificity and affinity of the parental non-human antibody. In some embodiments, some FR residues in a humanized antibody are substituted with corresponding residues from a non-human antibody (*e.g.*, the antibody from which the CDR residues are derived), *e.g.*, to restore or improve antibody specificity or affinity.

[0164] Among the anti-BCMA antibodies included in the provided CARs are human antibodies. A "human antibody" is an antibody with an amino acid sequence corresponding to that of an antibody produced by a human or a human cell, or non-human source that utilizes human antibody repertoires or other human antibody-encoding sequences, including human antibody libraries. The term excludes humanized forms of non-human antibodies comprising non-human antigen-binding regions, such as those in which all or substantially all CDRs are non-human. The term includes antigen-binding fragments of human antibodies.

[0165] Human antibodies may be prepared by administering an immunogen to a transgenic animal that has been modified to produce intact human antibodies or intact antibodies with human variable regions in response to antigenic challenge. Such animals typically contain all or a portion of the human immunoglobulin loci, which replace the endogenous immunoglobulin loci, or which are present extrachromosomally or integrated randomly into the animal's chromosomes. In such transgenic animals, the endogenous immunoglobulin loci have generally been inactivated. Human antibodies also may be derived from human antibody libraries, including phage display and cell-free libraries, containing antibody-encoding sequences derived from a human repertoire.

[0166] Among the antibodies included in the provided CARs are those that are monoclonal antibodies, including monoclonal antibody fragments. The term "monoclonal antibody" as used herein refers to an antibody obtained from or within a population of substantially homogeneous antibodies, *i.e.*, the individual antibodies comprising the population are identical, except for possible variants containing naturally occurring mutations or arising during production of a monoclonal antibody preparation, such variants generally being present in minor amounts. In contrast to polyclonal antibody preparations, which typically include different antibodies directed against different epitopes, each monoclonal antibody of a monoclonal antibody preparation is directed against a single epitope on an antigen. The term is not to be construed as requiring production of the antibody by any particular method. A monoclonal

antibody may be made by a variety of techniques, including but not limited to generation from a hybridoma, recombinant DNA methods, phage-display and other antibody display methods.

[0167] In some embodiments, the CAR includes a BCMA-binding portion or portions of the antibody molecule, such as a heavy chain variable (V_H) region and/or light chain variable (V_L) region of the antibody, *e.g.*, an scFv antibody fragment. In some embodiments, the provided BCMA-binding CARs contain an antibody, such as an anti-BCMA antibody, or an antigen-binding fragment thereof that confers the BCMA-binding properties of the provided CAR. In some embodiments, the antibody or antigen-binding domain can be any anti-BCMA antibody described or derived from any anti-BCMA antibody described. See, *e.g.,* Carpenter *et al.*, *Clin Cancer Res.*, 2013, 19(8):2048-2060, WO 2016090320, WO2016090327, WO2010104949 and WO2017173256. Any of such anti-BCMA antibodies or antigen-binding fragments can be used in the provided CARs. In some embodiments, the anti-BCMA CAR contains an antigen-binding domain that is an scFv containing a variable heavy (V_H) and/or a variable light (V_L) region derived from an antibody described in WO 2016090320 or WO2016090327.

[0168] In some embodiments, the antibody, *e.g.*, the anti-BCMA antibody or antigen-binding fragment, contains a heavy and/or light chain variable (V_H or V_L) region sequence as described, or a sufficient antigen-binding portion thereof. In some embodiments, the anti-BCMA antibody, *e.g.*, antigen-binding fragment, contains a V_H region sequence or sufficient antigen-binding portion thereof that contains a CDR-H1, CDR-H2 and/or CDR-H3 as described. In some embodiments, the anti-BCMA antibody, *e.g.*, antigen-binding fragment, contains a V_L region sequence or sufficient antigen-binding portion that contains a CDR-L1, CDR-L2 and/or CDR-L3 as described. In some embodiments, the anti-BCMA antibody, *e.g.*, antigen-binding fragment, contains a V_H region sequence that contains a CDR-H1, CDR-H2 and/or CDR-H3 as described and contains a V_L region sequence that contains a CDR-L1, CDR-L2 and/or CDR-L3 as described. Also among the antibodies are those having sequences at least at or about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, or about 99% identical to such a sequence.

[0169] In some embodiments, the antibody is a single domain antibody (sdAb) comprising only a V_H region sequence or a sufficient antigen-binding portion thereof, such as any of the above described V_H sequences (*e.g.*, a CDR-H1, a CDR-H2, a CDR-H3 and/or a CDR-H4).

[0170] In some embodiments, an antibody provided herein (*e.g.*, an anti-BCMA antibody) or antigen-binding fragment thereof comprising a V_H region further comprises a light chain or a sufficient antigen binding portion thereof. For example, in some embodiments, the antibody or antigen-binding fragment thereof contains a V_H region and a V_L region, or a sufficient antigen-binding portion of a V_H and V_L region. In such embodiments, a V_H region sequence can be any of the above described V_H sequence. In some such embodiments, the antibody is an antigen-binding fragment, such as a Fab or an scFv. In some such embodiments, the antibody is a full-length antibody that also contains a constant region.

[0171] In some embodiments, the antibody, *e.g.*, antigen-binding fragment thereof, in the provided CAR, has a heavy chain variable (V_H) region having the amino acid sequence selected from any one of SEQ ID NOs: 32, 52, 61, 85, 116, 125, 131, or an amino acid sequence that has at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to the V_H region amino acid selected from any one of SEQ ID NOs: 32, 52, 61, 85, 116, 125, 131, or contains a CDR-H1, CDR-H2, and/or CDR-H3 present in such a V_H sequence. In some embodiments, the antibody or antibody fragment, in the provided CAR, has a V_H region of any of the antibodies or antibody binding fragments described in WO 2016/090327, WO 2016/090320, or WO 2017/173256.

[0172] In some embodiments, the antibody, *e.g.*, antigen-binding fragment thereof, in the provided CAR, has a light chain variable (V_L) region having the amino acid sequence selected from any one of SEQ ID NOs: 33, 53, 62, 88, 119, 127, 132, or an amino acid sequence that has at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to the V_L region amino acid selected from any one of SEQ ID NOs: 33, 53, 62, 88, 119, 127, 132, or contains a CDR-L1, CDR-L2, and/or CDR-L3 present in such a V_L sequence. In some embodiments, the antibody or antibody fragment, in the provided CAR, has a V_L region of any of the antibodies or antibody binding fragments described in WO 2016/090327, WO 2016/090320, or WO 2017/173256.

[0173] In some embodiments, the V_H and V_L regions of the antibody, *e.g.*, antigen-binding fragment thereof, in the provided CAR, comprises: the amino acid sequence of SEQ ID NOS:32 and 33, respectively, or a sequence of amino acids having at least 90% identity to SEQ ID NOS:32 and 33, respectively; the amino acid sequence of SEQ ID NOS:52 and 53, respectively, or a sequence of amino acids having at least 90% identity to SEQ ID NOS:52 and 53, respectively; the amino acid sequence of SEQ ID NOS:61 and 62, respectively, or a sequence of amino acids having at least 90% identity to SEQ ID NOS:61 and 62, respectively; the amino acid sequence of SEQ ID NOS:85 and 88, respectively, or a sequence of amino acids having at least 90% identity to SEQ ID NOS:85 and 88, respectively; the amino acid sequence of SEQ ID NOS:116 and 119, respectively, or a sequence of amino acids having at least 90% identity to SEQ ID NOS:116 and 119, respectively; the amino acid sequence of SEQ ID NOS:125 and 127, respectively, or a sequence of amino acids having at least 90% identity to SEQ ID NOS:125 and 127, respectively; the amino acid sequence of SEQ ID NOS:131 and 132, respectively, or a sequence of amino acids having at least 90% identity to SEQ ID NOS:131 and 132, respectively.

[0174] In some embodiments, the V_H and V_L regions of the antibody or antigen-binding fragment thereof, in the provided CAR, comprises: the amino acid sequence of SEQ ID NOS:32 and 33, respectively, or a sequence of amino acids having at least 90% identity to SEQ ID NOS:32 and 33, respectively. In some embodiments, the V_H and V_L regions of the antibody or antigen-binding fragment thereof comprises the amino acid sequence of SEQ ID NOS:52 and 53, respectively, or a sequence of amino acids having at least 90% identity to SEQ ID NOS:52 and 53, respectively. In some embodiments, the V_H and V_L regions of the antibody or antigen-binding fragment thereof comprises the amino acid

sequence of SEQ ID NOS:61 and 62, respectively, or a sequence of amino acids having at least 90% identity to SEQ ID NOS:61 and 62, respectively. In some embodiments, the V_H and V_L regions of the antibody or antigen-binding fragment thereof comprises the amino acid sequence of SEQ ID NOS:85 and 88, respectively, or a sequence of amino acids having at least 90% identity to SEQ ID NOS:85 and 88, respectively. In some embodiments, the V_H and V_L regions of the antibody or antigen-binding fragment thereof comprises the amino acid sequence of SEQ ID NOS:116 and 119, respectively, or a sequence of amino acids having at least 90% identity to SEQ ID NOS:116 and 119, respectively. In some embodiments, the V_H and V_L regions of the antibody or antigen-binding fragment thereof comprises the amino acid sequence of SEQ ID NOS:125 and 127, respectively, or a sequence of amino acids having at least 90% identity to SEQ ID NOS:125 and 127, respectively. In some embodiments, the V_H and V_L regions of the antibody or antigen-binding fragment thereof comprises the amino acid sequence of SEQ ID NOS:131 and 132, respectively, or a sequence of amino acids having at least 90% identity to SEQ ID NOS:131 and 132, respectively.

[0175] In some embodiments, in the provided CAR, the antibody or antigen-binding fragment thereof comprises a V_H and a V_L region, and the V_H region comprises a heavy chain complementarity determining region 1 (CDR-H1), a heavy chain complementarity determining region 2 (CDR-H2) and a heavy chain complementarity determining region 3 (CDR-H3) contained within the V_H region amino acid sequence selected from any one of SEQ ID NOs: 32, 52, 61, 85, 116, 125, 131; and the V_L region comprises a light chain complementarity determining region 1 (CDR-L1), a light chain complementarity determining region 2 (CDR-L2) and a light chain complementarity determining region 3 (CDR-L3) contained within the V_L region amino acid sequence selected from any one of SEQ ID NOs: 33, 53, 62, 88, 119, 127, 132.

[0176] In some embodiments, in the provided CAR, the antibody or antigen-binding fragment thereof comprises a V_H and a V_L region, and the V_H region comprises a CDR-H1, a CDR-H2 and a CDR-H3 contained within the amino acid sequence of SEQ ID NO:32, and the V_L region comprises a CDR-L1, a CDR-L2 and a CDR-L3 contained within the amino acid sequence of SEQ ID NO:33; the V_H region comprises a CDR-H1, a CDR-H2 and a CDR-H3 contained within the amino acid sequence of SEQ ID NO:52, and the V_L region comprises a CDR-L1, a CDR-L2 and a CDR-L3 contained within the amino acid sequence of SEQ ID NO:53; the V_H region comprises a CDR-H1, a CDR-H2 and a CDR-H3 contained within the amino acid sequence of SEQ ID NO:61, and the V_L region comprises a CDR-L1, a CDR-L2 and a CDR-L3 contained within the amino acid sequence of SEQ ID NO:62; the V_H region comprises a CDR-H1, a CDR-H2 and a CDR-H3 contained within the amino acid sequence of SEQ ID NO:85, and the V_L region comprises a CDR-L1, a CDR-L2 and a CDR-L3 contained within the amino acid sequence of SEQ ID NO:88; the V_H region comprises a CDR-H1, a CDR-H2 and a CDR-H3 contained within the amino acid sequence of SEQ ID NO:116, and the V_L region comprises a CDR-L1, a CDR-L2 and a CDR-L3 contained within the amino acid sequence of SEQ ID NO:119; the V_H region

comprises a CDR-H1, a CDR-H2 and a CDR-H3 contained within the amino acid sequence of SEQ ID NO:125, and the V_L region comprises a CDR-L1, a CDR-L2 and a CDR-L3 contained within the amino acid sequence of SEQ ID NO:127; the V_H region comprises a CDR-H1, a CDR-H2 and a CDR-H3 contained within the amino acid sequence of SEQ ID NO:131, and the V_L region comprises a CDR-L1, a CDR-L2 and a CDR-L3 contained within the amino acid sequence of SEQ ID NO:132;

[0177] In some embodiments, the V_H and V_L regions of the antibody or antigen-binding fragment thereof, in the provided CAR, comprises: the amino acid sequence of SEQ ID NOS:32 and 33, respectively. In some embodiments, the V_H and V_L regions of the antibody or antigen-binding fragment thereof comprises the amino acid sequence of SEQ ID NOS:52 and 53, respectively. In some embodiments, the V_H and V_L regions of the antibody or antigen-binding fragment thereof comprises the amino acid sequence of SEQ ID NOS:61 and 62, respectively. In some embodiments, the V_H and V_L regions of the antibody or antigen-binding fragment thereof comprises the amino acid sequence of SEQ ID NOS:85 and 88, respectively. In some embodiments, the V_H and V_L regions of the antibody or antigen-binding fragment thereof comprises the amino acid sequence of SEQ ID NOS:116 and 119, respectively. In some embodiments, the V_H and V_L regions of the antibody or antigen-binding fragment thereof comprises the amino acid sequence of SEQ ID NOS:125 and 127, respectively. In some embodiments, the V_H and V_L regions of the antibody or antigen-binding fragment thereof comprises the amino acid sequence of SEQ ID NOS:131 and 132, respectively.

[0178] In some embodiments, the V_H and V_L regions of the antibody or antigen-binding fragment thereof provided therein comprise the amino acid sequences selected from: SEQ ID NOS:116 and 119, or any antibody or antigen-binding fragment thereof that has at least 90% sequence identity to any of the above V_H and V_L, such as at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity thereto, or any antibody or antigen-binding fragment thereof that comprises a CDR-H1, CDR-H2 and CDR-H3 contained within the V_H region and a CDR-L1, CDR-L2 and CDR-L3 contained within the V_L region of any of the above V_H and V_L.

[0179] In some embodiments, the antibody or antigen-binding fragment thereof is a single-chain antibody fragment, such as a single chain variable fragment (scFv) or a diabody or a single domain antibody (sdAb). In some embodiments, the antibody or antigen-binding fragment is a single domain antibody comprising only the V_H region. In some embodiments, the antibody or antigen binding fragment is an scFv comprising a heavy chain variable (V_H) region and a light chain variable (V_L) region. In some embodiments, the single-chain antibody fragment (*e.g.* scFv) includes one or more linkers joining two antibody domains or regions, such as a heavy chain variable (V_H) region and a light chain variable (V_L) region. The linker typically is a peptide linker, *e.g.*, a flexible and/or soluble peptide linker. Among the linkers are those rich in glycine and serine and/or in some cases threonine. In some embodiments, the linkers further include charged residues such as lysine and/or glutamate, which can improve solubility. In some embodiments, the linkers further include one or more proline.

[0180] Accordingly, the provided anti-BCMA antibodies include single-chain antibody fragments, such as scFvs and diabodies, particularly human single-chain antibody fragments, typically comprising linker(s) joining two antibody domains or regions, such V_H and V_L regions. The linker typically is a peptide linker, *e.g.*, a flexible and/or soluble peptide linker, such as one rich in glycine and serine.

[0181] In some aspects, the linkers rich in glycine and serine (and/or threonine) include at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% such amino acid(s). In some embodiments, they include at least at or about 50%, 55%, 60%, 70%, or 75%, glycine, serine, and/or threonine. In some embodiments, the linker is comprised substantially entirely of glycine, serine, and/or threonine. The linkers generally are between about 5 and about 50 amino acids in length, typically between at or about 10 and at or about 30, *e.g.*, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30, and in some examples between 10 and 25 amino acids in length. Exemplary linkers include linkers having various numbers of repeats of the sequence GGGGS (4GS; SEQ ID NO:7) or GGGS (3GS; SEQ ID NO:2), such as between 2, 3, 4, and 5 repeats of such a sequence. Exemplary linkers include those having or consisting of an sequence set forth in SEQ ID NO:1 (GGGGSGGGGSGGGGS). Exemplary linkers further include those having or consisting of the sequence set forth in SEQ ID NO:176 (GSTSGSGKPGSGEGSTKG). Exemplary linkers further include those having or consisting of the sequence set forth in SEQ ID NO:255 (SRGGGGSGGGGSGGGGSLEMA).

[0182] Accordingly, in some embodiments, the provided embodiments include single-chain antibody fragments, *e.g.*, scFvs, comprising one or more of the aforementioned linkers, such as glycine/serine rich linkers, including linkers having repeats of GGGS (SEQ ID NO: 2) or GGGGS (SEQ ID NO: 7), such as the linker set forth in SEQ ID NO:1.

[0183] In some embodiments, the linker has an amino acid sequence containing the sequence set forth in SEQ ID NO:1. The fragment, *e.g.*, scFv, may include a V_H region or portion thereof, followed by the linker, followed by a V_L region or portion thereof. The fragment, *e.g.*, the scFv, may include the V_L region or portion thereof, followed by the linker, followed by the V_H region or portion thereof.

[0184] **Table 2** provides the SEQ ID NOS: of exemplary antigen-binding domains, such as antibodies or antigen-binding fragments, that can be comprised in the provided BCMA-binding receptors, such as anti-BCMA chimeric antigen receptors (CARs). In some embodiments, the BCMA-binding receptor contains a BCMA-binding antibody or fragment thereof, comprising a V_H region that comprises the CDR-H1, CDR-H2, and CDR-H3 sequence and a V_L region that comprises the CDR-L1, CDR-L2 and CDR-L3 sequence set forth in the SEQ ID NOS: listed in each row of **Table 2** below (by Kabat numbering). In some embodiments, the BCMA-binding receptor contains a BCMA-binding antibody or fragment thereof, comprising a V_H region sequence and a V_L region sequence set forth in the SEQ ID NOS: listed in each row of **Table 2** below, or an antibody comprising a V_H and V_L region amino acid sequence that has at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity

to the V_H region sequence and the V_L region sequence set forth in the SEQ ID NOS: listed in each row of **Table 2** below. In some embodiments, the BCMA-binding receptor contains a BCMA-binding antibody or fragment thereof, comprising a V_H region sequence and a V_L region sequence set forth in the SEQ ID NOS: listed in each row of **Table 2** below. In some embodiments, the BCMA-binding receptor contains a BCMA-binding antibody or fragment thereof, comprising an scFv sequence set forth in the SEQ ID NOS: listed in each row of **Table 2** below, or an antibody comprising an scFv amino acid sequence that has at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to the scFv sequence set forth in the SEQ ID NOS: listed in each row of **Table 2** below. In some embodiments, the BCMA-binding receptor contains a BCMA-binding antibody or fragment thereof, comprising an scFv sequence set forth in SEQ ID NO:114 or an antibody comprising an scFv amino acid sequence that has at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity thereto. In some embodiments, the BCMA-binding receptor contains a BCMA-binding antibody or fragment thereof, comprising an scFv sequence set forth in the SEQ ID NOS: listed in each row of **Table 2** below. In some embodiments, the BCMA-binding receptor contains a BCMA-binding antibody or fragment thereof, comprising an scFv sequence set forth in SEQ ID NO: 114.

Table 2. Sequence identifier (SEQ ID NO) for Exemplary Antigen-binding Domains

Antigen-binding domain	CDR-H1	CDR-H2	CDR-H3	CDR-L1	CDR-L2	CDR-L3	V_H	V_L	scFv
BCMA-23	34	35	36	22	23	24	32	33	29
BCMA-25	37	38	39	40	41	42	52	53	49
BCMA-26	34	35	54	55	56	57	61	62	58
BCMA-52	66	70	72	74	76	77	85	88	83
BCMA-55	97	101	103	105	107	108	116	119	114
BCMA-C1, V_H-V_L							125	127	126
BCMA-C1, V_L-V_H							125	127	128
BCMA-C2, V_H-V_L							131	132	129
BCMA-C2, V_L-V_H							131	132	130

[0185] Among the antibodies, *e.g.* antigen-binding fragments, in the provided CARs, are human antibodies. In some embodiments of a provided human anti-BCMA antibody, *e.g.*, antigen-binding fragments, the human antibody contains a V_H region that comprises a portion having at least 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to an amino acid sequence encoded by a germline nucleotide human heavy chain V segment, a portion having at least 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to an amino acid sequence encoded by a germline nucleotide human heavy chain D segment, and/or a portion having at least 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to an amino acid sequence encoded by a germline nucleotide human heavy chain J segment; and/or contains a V_L region that comprises a portion having at least 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to an amino acid sequence encoded by a germline nucleotide human kappa or lambda chain V segment, and/or a portion having at least 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to an amino acid sequence encoded by a germline nucleotide human kappa or lambda chain J segment. In some

embodiments, the portion of the V_H region corresponds to the CDR-H1, CDR-H2 and/or CDR-H3. In some embodiments, the portion of the V_H region corresponds to the framework region 1 (FR1), FR2, FR2 and/or FR4. In some embodiments, the portion of the V_L region corresponds to the CDR-L1, CDR-L2 and/or CDR-L3. In some embodiments, the portion of the V_L region corresponds to the FR1, FR2, FR2 and/or FR4.

[0186] In some embodiments, the human antibody, *e.g.*, antigen-binding fragment, contains a CDR-H1 having at least 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to an amino acid sequence of the corresponding CDR-H1 region within a sequence encoded by a germline nucleotide human heavy chain V segment. For example, the human antibody in some embodiments contains a CDR-H1 having a sequence that is 100% identical or with no more than one, two or three amino acid differences as compared to the corresponding CDR-H1 region within a sequence encoded by a germline nucleotide human heavy chain V segment.

[0187] In some embodiments, the human antibody, *e.g.*, antigen-binding fragment, contains a CDR-H2 having at least 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to an amino acid sequence of the corresponding CDR-H2 region within a sequence encoded by a germline nucleotide human heavy chain V segment. For example, the human antibody in some embodiments contains a CDR-H2 having a sequence that is 100% identical or with no more than one, two or three amino acid difference as compared to the corresponding CDR-H2 region within a sequence encoded by a germline nucleotide human heavy chain V segment.

[0188] In some embodiments, the human antibody, *e.g.*, antigen-binding fragment, contains a CDR-H3 having at least 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to an amino acid sequence of the corresponding CDR-H3 region within a sequence encoded by a germline nucleotide human heavy chain V segment, D segment and J segment. For example, the human antibody in some embodiments contains a CDR-H3 having a sequence that is 100% identical or with no more than one, two or three amino acid differences as compared to the corresponding CDR-H3 region within a sequence encoded by a germline nucleotide human heavy chain V segment, D segment and J segment.

[0189] In some embodiments, the human antibody, *e.g.*, antigen-binding fragment, contains a CDR-L1 having at least 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to an amino acid sequence of the corresponding CDR-L1 region within a sequence encoded by a germline nucleotide human light chain V segment. For example, the human antibody in some embodiments contains a CDR-L1 having a sequence that is 100% identical or with no more than one, two or three amino acid differences as compared to the corresponding CDR-L1 region within a sequence encoded by a germline nucleotide human light chain V segment.

[0190] In some embodiments, the human antibody, *e.g.*, antigen-binding fragment, contains a CDR-L2 having at least 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to an amino acid sequence of the corresponding CDR-L2 region within a sequence encoded by a germline nucleotide human light

chain V segment. For example, the human antibody in some embodiments contains a CDR-L2 having a sequence that is 100% identical or with no more than one, two or three amino acid difference as compared to the corresponding CDR-L2 region within a sequence encoded by a germline nucleotide human light chain V segment.

[0191] In some embodiments, the human antibody, *e.g.*, antigen-binding fragment, contains a CDR-L3 having at least 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to an amino acid sequence of the corresponding CDR-L3 region within a sequence encoded by a germline nucleotide human light chain V segment and J segment. For example, the human antibody in some embodiments contains a CDR-L3 having a sequence that is 100% identical or with no more than one, two or three amino acid differences as compared to the corresponding CDR-L3 region within a sequence encoded by a germline nucleotide human light chain V segment and J segment.

[0192] In some embodiments, the human antibody, *e.g.*, antigen-binding fragment, contains a framework region that contains human germline gene segment sequences. For example, in some embodiments, the human antibody contains a V_H region in which the framework region, *e.g.* FR1, FR2, FR3 and FR4, has at least 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to a framework region encoded by a human germline antibody segment, such as a V segment and/or J segment. In some embodiments, the human antibody contains a V_L region in which the framework region *e.g.* FR1, FR2, FR3 and FR4, has at least 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to a framework region encoded by a human germline antibody segment, such as a V segment and/or J segment. For example, in some such embodiments, the framework region sequence contained within the V_H region and/or V_L region differs by no more than 10 amino acids, such as no more than 9, 8, 7, 6, 5, 4, 3, 2 or 1 amino acid, compared to the framework region sequence encoded by a human germline antibody segment.

[0193] In some embodiments, the reference antibody can be a mouse anti-BCMA scFv described in International Patent App. Pub. No. WO 2010/104949.

[0194] The antibody, *e.g.*, antigen-binding fragment, may contain at least a portion of an immunoglobulin constant region, such as one or more constant region domain. In some embodiments, the constant regions include a light chain constant region and/or a heavy chain constant region 1 (C_H1). In some embodiments, the antibody includes a C_H2 and/or C_H3 domain, such as an Fc region. In some embodiments, the Fc region is an Fc region of a human IgG, such as an IgG1 or IgG4.

2. Spacer

[0195] In some embodiments, the recombinant receptor such as a CAR comprising an antibody (*e.g.*, antigen-binding fragment) provided herein, such as those expressed by engineered cells employed in the methods and uses provided herein, further includes a spacer or spacer region. The spacer typically is a polypeptide spacer and in general is located within the CAR between the antigen binding domain and the transmembrane domain of the CAR. In some aspects, the spacer may be or include at least a portion

of an immunoglobulin constant region or variant or modified version thereof, such as a hinge region of an immunoglobulin, such as an IgG hinge region, *e.g.*, an IgG4 or IgG4-derived hinge region, and/or a C_H1/C_L and/or Fc region. In some embodiments, the constant region or one or more of the portion(s) thereof is of a human IgG, such as of a human IgG4 or IgG1 or IgG2. In general, the spacer, such as the portion of the constant region, serves as a spacer region between the antigen-recognition component (*e.g.*, scFv) and transmembrane domain. In some embodiments, the length and/or composition of the spacer is designed to optimize or promote certain features of the interaction between the CAR and its target; in some aspects, it is designed to optimize the biophysical synapse distance between the CAR-expressing cell and the cell expressing the target of the CAR during or upon or following binding of the CAR to its target on the target-expressing cell; in some aspects, the target expressing cell is a BCMA-expressing tumor cell. In some embodiments, The CAR is expressed by a T-cell, and the length of the spacer is of a length that is compatible for T-cell activation or to optimize CAR T-cell performance. In some embodiments, the spacer is a spacer region, located between the ligand-binding domain and the transmembrane domain, of the recombinant receptor, e.g., CAR. In some embodiments, the spacer region is a region located between the ligand-binding domain and the transmembrane domain, of the recombinant receptor, e.g., CAR.

[0196] In some embodiments, the spacer can be of a length that provides for increased responsiveness of the cell following antigen binding, as compared to in the absence of the spacer and/or in the presence of a different spacer, such as one different only in length. In some embodiments, the spacer is at least 100 amino acids in length, such as at least 110, 125, 130, 135, 140, 145, 150, 160, 170, 180, 190, 200, 210, 220, 230, 240, or 250 amino acids in length. In some examples, the spacer is at or about 12 amino acids in length or is no more than 12 amino acids in length. Exemplary spacers include those having at least about 10 to 300 amino acids, about 10 to 200 amino acids, about 50 to 175 amino acids, about 50 to 150 amino acids, about 10 to 125 amino acids, about 50 to 100 amino acids, about 100 to 300 amino acids, about 100 to 250 amino acids, about 125 to 250 amino acids, or about 200 to 250 amino acids, and including any integer between the endpoints of any of the listed ranges. In some embodiments, a spacer or a spacer region is at least about 12 amino acids, at least about 119 amino acids or less, at least about 125 amino acids, at least about 200 amino acids, or at least about 220 amino acids, or at least about 225 amino acids in length.

[0197] In some embodiments, the spacer has a length of 125 to 300 amino acids in length, 125 to 250 amino acids in length, 125 to 230 amino acids in length, 125 to 200 amino acids in length, 125 to 180 amino acids in length, 125 to 150 amino acids in length, 150 to 300 amino acids in length, 150 to 250 amino acids in length, 150 to 230 amino acids in length, 150 to 200 amino acids in length, 150 to 180 amino acids in length, 180 to 300 amino acids in length, 180 to 250 amino acids in length, 180 to 230 amino acids in length, 180 to 200 amino acids in length, 200 to 300 amino acids in length, 200 to 250 amino acids in length, 200 to 230 amino acids in length, 230 to 300 amino acids in length, 230 to 250

amino acids in length or 250 to 300 amino acids in length. In some embodiments, the spacer is at least or at least about or is or is about 130, 140, 150, 160, 170, 180, 190, 200, 210, 220, 221, 222, 223, 224, 225, 226, 227, 228 or 229 amino acids in length, or a length between any of the foregoing.

[0198] Exemplary spacers include those containing portion(s) of an immunoglobulin constant region such as those containing an Ig hinge, such as an IgG hinge domain. In some aspects, the spacer includes an IgG hinge alone, an IgG hinge linked to one or more of a C_H2 and C_H3 domain, or IgG hinge linked to the C_H3 domain. In some embodiments, the IgG hinge, C_H2 and/or C_H3 can be derived all or in part from IgG4 or IgG2. In some embodiments, the spacer can be a chimeric polypeptide containing one or more of a hinge, C_H2 and/or C_H3 sequence(s) derived from IgG4, IgG2, and/or IgG2 and IgG4. In some embodiments, the hinge region comprises all or a portion of an IgG4 hinge region and/or of an IgG2 hinge region, wherein the IgG4 hinge region is optionally a human IgG4 hinge region and the IgG2 hinge region is optionally a human IgG2 hinge region; the C_H2 region comprises all or a portion of an IgG4 C_H2 region and/or of an IgG2 C_H2 region, wherein the IgG4 C_H2 region is optionally a human IgG4 C_H2 region and the IgG2 C_H2 region is optionally a human IgG2 C_H2 region; and/or the C_H3 region comprises all or a portion of an IgG4 C_H3 region and/or of an IgG2 C_H3 region, wherein the IgG4 C_H3 region is optionally a human IgG4 C_H3 region and the IgG2 C_H3 region is optionally a human IgG2 C_H3 region. In some embodiments, the hinge, C_H2 and C_H3 comprises all or a portion of each of a hinge region, C_H2 and C_H3 from IgG4. In some embodiments, the hinge region is chimeric and comprises a hinge region from human IgG4 and human IgG2; the C_H2 region is chimeric and comprises a C_H2 region from human IgG4 and human IgG2; and/or the C_H3 region is chimeric and comprises a C_H3 region from human IgG4 and human IgG2. In some embodiments, the spacer comprises an IgG4/2 chimeric hinge or a modified IgG4 hinge comprising at least one amino acid replacement compared to human IgG4 hinge region; an human IgG2/4 chimeric C_H2 region; and a human IgG4 C_H3 region.

[0199] In some embodiments, the spacer can be derived all or in part from IgG4 and/or IgG2 and can contain mutations, such as one or more single amino acid mutations in one or more domains. In some examples, the amino acid modification is a substitution of a proline (P) for a serine (S) in the hinge region of an IgG4. In some embodiments, the amino acid modification is a substitution of a glutamine (Q) for an asparagine (N) to reduce glycosylation heterogeneity, such as an N177Q mutation at position 177, in the C_H2 region, of the full-length IgG4 Fc sequence set forth in SEQ ID NO: 173 or an N176Q. at position 176, in the C_H2 region, of the full-length IgG2 Fc sequence set forth in SEQ ID NO: 172. In some embodiments, the spacer is or comprises an IgG4/2 chimeric hinge or a modified IgG4 hinge; an IgG2/4 chimeric C_H2 region; and an IgG4 C_H3 region and optionally is about 228 amino acids in length; or a spacer set forth in SEQ ID NO: 174. In some embodiments, the spacer comprises the amino acid sequence

ESKYGPPCPPCPAPPVAGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDG
VEVHNAKTKPREEQFQSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQ

PREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSF FLYSRLTVDKSRWQEGNVFSCSVMHEALHNHYTQKSLSLSLGK (SEQ ID NO: 174)

encoded by a polynucleotide that has been optimized for codon expression and/or to eliminate splice sites such as cryptic splice sites. In some embodiments, the coding sequence for the spacer comprises the nucleic acid sequence set forth in SEQ ID NO: 200. In some embodiments, the coding sequence for the spacer comprises the nucleic acid sequence set forth in SEQ ID NO: 236 or 8.

[0200] Additional exemplary spacers include, but are not limited to, those described in Hudecek *et al.* (2013) *Clin. Cancer Res.*, 19:3153, Hudecek *et al.* (2015) *Cancer Immunol. Res.*, 3(2):125-135, or international patent application publication number WO2014031687. In some embodiments, the nucleotide sequence of the spacer is optimized to reduce RNA heterogeneity following expression. In some embodiments, the nucleotide sequence of the spacer is optimized to reduce cryptic splice sites or reduce the likelihood of a splice event at a splice site.

[0201] In some embodiments, the spacer has the amino acid sequence set forth in SEQ ID NO:237, and is encoded by the polynucleotide sequence set forth in SEQ ID NO:238. In some embodiments, the spacer has the amino acid sequence set forth in SEQ ID NO:157. In some embodiments, the spacer has the amino acid sequence set forth in SEQ ID NO:156. In some embodiments, the spacer has the amino acid sequence set forth in SEQ ID NO: 134, and is encoded by the polynucleotide sequence set forth in SEQ ID NO: 135. In some embodiments, the spacer has an amino acid sequence set forth in SEQ ID NO: 174, encoded by the polynucleotide sequence set forth in SEQ ID NO: 175, 200, 236 or 8 or a polynucleotide that exhibits at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to SEQ ID NO: 175, 200, 236 or 8. In some embodiments, the spacer has an amino acid sequence that exhibits at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to SEQ ID NO: 174, encoded by a polynucleotide that has been optionally optimized for codon usage and/or to reduce RNA heterogeneity.

[0202] In some embodiments, the spacer is or comprises an amino acid sequence encoded by the nucleotide sequence set forth in SEQ ID NO:200.

3. Transmembrane domain and intracellular signaling components

[0203] The antigen-recognition component (e.g., antigen-binding domain) generally is linked to one or more intracellular signaling regions containing signaling components, such as signaling components that mimic stimulation and/or activation through an antigen receptor complex, such as a TCR complex, in the case of a CAR, and/or signal via another cell surface receptor. Thus, in some embodiments, the BCMA-binding molecule (*e.g.*, antibody or antigen binding fragment thereof) is linked to one or more transmembrane domains such as those described herein and intracellular signaling regions or domains comprising one or more intracellular components such as those described herein. In some embodiments, the transmembrane domain is fused to the extracellular domain. In one embodiment, a transmembrane domain that naturally is associated with one of the domains in the receptor, *e.g.*, CAR, is used. In some

instances, the transmembrane domain is 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 to minimize interactions with other members of the receptor complex.

[0204] The transmembrane domain in some embodiments is derived either from a natural or from a synthetic source. Where the source is natural, the domain in some aspects is derived from any membrane-bound or transmembrane protein. Transmembrane domains include those derived from (*i.e.* comprise at least the transmembrane domain(s) of) the alpha, beta or zeta chain of the T-cell receptor, CD3 epsilon, CD4, CD5, CD8, CD9, CD16, CD22, CD28, CD33, CD37, CD45, CD64, CD80, CD86, CD134, CD137, and/or CD154. For example, the transmembrane domain can be a CD28 transmembrane domain that comprises the sequence of amino acids set forth in SEQ ID NO: 138, encoded by the nucleic acid sequence set forth in SEQ ID NO: 139 or SEQ ID NO:140. Alternatively the transmembrane domain in some embodiments is synthetic. In some aspects, the synthetic transmembrane domain comprises predominantly hydrophobic residues such as leucine and valine. In some aspects, a triplet of phenylalanine, tryptophan and valine will be found at each end of a synthetic transmembrane domain. In some embodiments, the linkage is by linkers, spacers, and/or transmembrane domain(s).

[0205] Among the intracellular signaling regions or domains are those that mimic or approximate a signal through a natural antigen receptor, a signal through such a receptor in combination with a costimulatory receptor, and/or a signal through a costimulatory receptor alone. In some embodiments, a short oligo- or polypeptide linker, for example, a linker of between 2 and 10 amino acids in length, such as one containing glycines and serines, *e.g.*, glycine-serine doublet, is present and forms a linkage between the transmembrane domain and the intracellular signaling domain of the CAR.

[0206] The receptor, *e.g.*, the CAR, generally includes an intracellular signaling region comprising at least one intracellular signaling component or components. In some embodiments, the receptor includes an intracellular component or signaling domain of a TCR complex, such as a TCR CD3 chain that mediates T-cell activation and cytotoxicity, *e.g.*, CD3 zeta chain. Thus, in some aspects, the BCMA-binding antibody is linked to one or more cell signaling modules. In some embodiments, cell signaling modules include CD3 transmembrane domain, CD3 intracellular signaling domains, and/or other CD transmembrane domains. In some embodiments, the receptor, *e.g.*, CAR, further includes a portion of one or more additional molecules such as Fc receptor γ, CD8, CD4, CD25, or CD16. For example, in some aspects, the CAR includes a chimeric molecule between CD3-zeta (CD3-ζ) or Fc receptor γ and CD8, CD4, CD25 or CD16.

[0207] In some embodiments, upon or following ligation of the CAR, the cytoplasmic domain or intracellular signaling domain of the CAR stimulates and/or activates at least one of the normal effector functions or responses of the immune cell, *e.g.*, T cell engineered to express the CAR. For example, in some contexts, the CAR induces a function of a T cell such as cytolytic activity or T-helper activity, such as secretion of cytokines or other factors. In some embodiments, a truncated portion of an intracellular

signaling domain of an antigen receptor component or costimulatory molecule is used in place of an intact immunostimulatory chain, for example, if it transduces the effector function signal. In some embodiments, the intracellular signaling domain or domains include the cytoplasmic sequences of the T cell receptor (TCR), and in some aspects also those of co-receptors that in the natural context act in concert with such receptor to initiate signal transduction following antigen receptor engagement, and/or any derivative or variant of such molecules, and/or any synthetic sequence that has the same functional capability.

[0208] In the context of a natural TCR, full activation generally requires not only signaling through the TCR, but also a costimulatory signal. Thus, in some embodiments, to promote full activation, a component for generating secondary or co-stimulatory signal is also included in the CAR. In other embodiments, the CAR does not include a component for generating a costimulatory signal. In some aspects, an additional CAR is expressed in the same cell and provides the component for generating the secondary or costimulatory signal.

[0209] T cell activation is in some aspects described as being mediated by two classes of cytoplasmic signaling sequences: those that initiate antigen-dependent primary activation through the TCR (primary cytoplasmic signaling sequences), and those that act in an antigen-independent manner to provide a secondary or co-stimulatory signal (secondary cytoplasmic signaling sequences). In some aspects, the CAR includes one or both of such classes of cytoplasmic signaling sequences.

[0210] In some aspects, the CAR includes a primary cytoplasmic signaling sequence that regulates primary stimulation and/or activation of the TCR complex. Primary cytoplasmic signaling sequences 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 cytoplasmic signaling sequences include those derived from TCR or CD3 zeta, FcR gamma, CD3 gamma, CD3 delta and CD3 epsilon. In some embodiments, the intracellular signaling region or domain in the CAR contain(s) a cytoplasmic signaling domain, portion thereof, or sequence derived from CD3 zeta. In some embodiments the CD3 zeta comprises the sequence of amino acids set forth in SEQ ID NO: 143, encoded by the nucleic acid sequence set forth in SEQ ID NO: 144 or SEQ ID NO: 145.

[0211] In some embodiments, the CAR includes a signaling domain (*e.g.*, an intracellular or cytoplasmic signaling domain) and/or transmembrane portion of a costimulatory molecule, such as a T cell costimulatory molecule. Exemplary costimulatory molecules include CD28, 4-1BB, OX40, DAP10, and ICOS. For example, a costimulatory molecule can be derived from 4-1BB and can comprise the amino acid sequence set forth in SEQ ID NO: 4, encoded by the nucleotide sequence set forth in SEQ ID NO: 5 or SEQ ID NO: 6. In some aspects, the same CAR includes both the stimulatory or activating components (*e.g.*, cytoplasmic signaling sequence) and costimulatory components.

[0212] In some embodiments, the stimulatory or activating components are included within one CAR, whereas the costimulatory component is provided by another CAR recognizing another antigen. In

some embodiments, the CARs include activating or stimulatory CARs, and costimulatory CARs, both expressed on the same cell (see WO2014/055668). In some aspects, the BCMA-targeting CAR is the stimulatory or activating CAR; in other aspects, it is the costimulatory CAR. In some embodiments, the cells further include inhibitory CARs (iCARs, see Fedorov *et al.*, Sci. Transl. Medicine, 5(215) (December, 2013), such as a CAR recognizing an antigen other than BCMA, whereby a stimulatory or an activating signal delivered through the BCMA-targeting CAR is diminished or inhibited by binding of the inhibitory CAR to its ligand, *e.g.*, to reduce off-target effects.

[0213] In certain embodiments, the intracellular signaling region comprises a CD28 transmembrane and signaling domain linked to a CD3 (*e.g.*, CD3-zeta) intracellular domain. In some embodiments, the intracellular signaling domain comprises a chimeric CD28 and CD137 (4-1BB, TNFRSF9) co-stimulatory domains, linked to a CD3 zeta intracellular domain.

[0214] In some embodiments, the CAR encompasses one or more, *e.g.*, two or more, costimulatory domains and a stimulatory or activation domain, *e.g.*, primary activation domain, in the cytoplasmic portion. Exemplary CARs include intracellular components of CD3-zeta, CD28, and 4-1BB.

[0215] In some embodiments, the provided chimeric antigen receptor comprises: (a) an extracellular antigen-binding domain that specifically recognizes B cell maturation antigen (BCMA), such as any antigen-binding domain described herein; (b) a spacer of at least 125 amino acids in length; (c) a transmembrane domain; and (d) an intracellular signaling region. In some embodiments, the antigen-binding domain of such receptor, comprising a V_H region and a V_L region comprising the amino acid sequence of SEQ ID NOs:116 and 119, respectively, or a sequence of amino acids having at least 90% identity to SEQ ID NOS:116 and 119, respectively. In some embodiments, the antigen-binding domain of such receptor, comprising a V_H region that is or comprises a CDR-H1, CDR-H2 and CDR-H3 contained within the V_H region amino acid sequence of SEQ ID NO: 116 and a V_L region that is or comprises a CDR-L1, CDR-L2 and CDR-L3 contained within the V_L region amino acid sequence of SEQ ID NO: 119. In some embodiments, the antigen-binding domain of such receptor, comprising a V_H region comprising a CDR-H1, CDR-H2, and CDR-H3 comprising SEQ ID NOS:97, 101 and 103, respectively, and a V_L region comprising a CDR-L1, CDR-L2, and CDR-L3 comprising SEQ ID NOS:105, 107 and 108, respectively. In some embodiments, the antigen-binding domain of such receptor, comprising a V_H region comprising a CDR-H1, CDR-H2, and CDR-H3 comprising SEQ ID NOS:96, 100 and 103, respectively, and a V_L region comprising a CDR-L1, CDR-L2, and CDR-L3 comprising SEQ ID NOS:105, 107 and 108, respectively. In some embodiments, the antigen-binding domain of such receptor, comprising a V_H region comprising a CDR-H1, CDR-H2, and CDR-H3 comprising SEQ ID NOS: 95, 99 and 103, respectively, and a V_L region comprising a CDR-L1, CDR-L2, and CDR-L3 comprising SEQ ID NOS:105, 107 and 108, respectively. In some embodiments, the antigen-binding domain of such receptor, comprising a V_H region comprising a CDR-H1, CDR-H2, and CDR-H3 comprising SEQ ID NOS: 94, 98 and 102, respectively, and a V_L region comprising a CDR-L1,

CDR-L2, and CDR-L3 comprising SEQ ID NOS: 104, 106 and 108, respectively. In some embodiments, the antigen-binding domain of such receptor, comprises a V_H region that is or comprises the amino acid sequence of SEQ ID NO: 116 and a V_L region that is or comprises the amino acid sequence of SEQ ID NO: 119. In some embodiments, the antigen-binding domain of such receptor, comprises the amino acid sequence of SEQ ID NO: 114.

[0216] In some embodiments, the intracellular signaling region includes an stimulating cytoplasmic signaling domain. In some embodiments, the stimulating cytoplasmic signaling domain is capable of inducing a primary activation signal in a T cell, is a T cell receptor (TCR) component and/or includes an immunoreceptor tyrosine-based activation motif (ITAM). In some embodiments, the stimulating cytoplasmic signaling domain is or includes a cytoplasmic signaling domain of a CD3-zeta (CD3ζ) chain or a functional variant or signaling portion thereof. In some embodiments, the stimulating cytoplasmic domain is human or is derived from a human protein. In some embodiments, the stimulating cytoplasmic domain is or includes the sequence set forth in SEQ ID NO:143 or a sequence of amino acids that has at least 90% sequence identity to SEQ ID NO:143. In some embodiments, the nucleic acid encoding the stimulating cytoplasmic domain is or includes the sequence set forth in SEQ ID NO:144 or is a codon-optimized sequence and/or degenerate sequence thereof. In other embodiments, the nucleic acid encoding the stimulating cytoplasmic signaling domain is or includes the sequence set forth in SEQ ID NO:145. In some embodiments, the intracellular signaling region further includes a costimulatory signaling region. In some embodiments, the costimulatory signaling region includes an intracellular signaling domain of a T cell costimulatory molecule or a signaling portion thereof. In some embodiments, the costimulatory signaling region includes an intracellular signaling domain of a CD28, a 4-1BB or an ICOS or a signaling portion thereof. In some embodiments, the costimulatory signaling region includes an intracellular signaling domain of 4-1BB. In some embodiments, the costimulatory signaling region is human or is derived from a human protein. In other embodiments, the costimulatory signaling region is or includes the sequence set forth in SEQ ID NO:4 or a sequence of amino acids that exhibits at least 90% sequence identity to the sequence set forth in SEQ ID NO: 4. In some embodiments, the nucleic acid encoding the costimulatory region is or includes the sequence set forth in SEQ ID NO:5 or is a codon-optimized sequence and/or degenerate sequence thereof. In some embodiments, the nucleic acid encoding the costimulatory signaling region includes the sequence set forth in SEQ ID NO:6. In some embodiments, the costimulatory signaling region is between the transmembrane domain and the intracellular signaling region. In some embodiments, the transmembrane domain is or includes a transmembrane domain derived from CD4, CD28, or CD8. In some embodiments, the transmembrane domain is or includes a transmembrane domain derived from a CD28. In some embodiments, the transmembrane domain is human or is derived from a human protein. In other embodiments, the transmembrane domain is or includes the sequence set forth in SEQ ID NO:138 or a sequence of amino acids that exhibits at least 90% sequence identity to SEQ ID NO:138.

[0217] Provided are chimeric antigen receptors, comprising: (1) an extracellular antigen-binding domain that specifically binds human B cell maturation antigen (BCMA), wherein the extracellular antigen-binding domain comprises: (i) a variable heavy chain (V_H) comprising an amino acid sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to the V_H region sequence of SEQ ID NO: 116; and (ii) a variable light chain (V_L) region comprising an amino acid sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to the V_L region sequence of any of SEQ ID NO: 119; (2) a spacer set forth in SEQ ID NO: 174 or wherein the nucleic acid encoding the spacer is or comprises the sequence set forth in SEQ ID NO:200; (3) a transmembrane domain, optionally a transmembrane domain from a human CD28; and (4) an intracellular signaling region comprising a cytoplasmic signaling domain of a CD3-zeta (CD3ζ) chain and an intracellular signaling domain of a T cell costimulatory molecule. Also provided are polynucleotides encoding such a chimeric antigen receptor.

[0218] In some embodiments, the V_H region comprises a CDR-H1, CDR-H2 and CDR-H3 contained within the V_H region sequence of SEQ ID NO: 116; and the V_L region comprises a CDR-L1, CDR-L2 and CDR-L3 contained within the V_L region sequence of SEQ ID NO: 119; or the V_H region comprises a CDR-H1, CDR-H2, and CDR-H3 comprising the sequence of SEQ ID NOS:97, 101 and 103, respectively, and the V_L region comprises a CDR-L1, CDR-L2, and CDR-L3 comprising the sequence of SEQ ID NOS:105, 107 and 108, respectively; the V_H region comprises a CDR-H1, CDR-H2, and CDR-H3 comprising the sequence of SEQ ID NOS:96, 100 and 103, respectively, and the V_L region comprises a CDR-L1, CDR-L2, and CDR-L3 comprising the sequence of SEQ ID NOS:105, 107 and 108, respectively; the V_H region comprises a CDR-H1, CDR-H2, and CDR-H3 comprising the sequence of SEQ ID NOS:95, 99 and 103, respectively, and the V_L region comprises a CDR-L1, CDR-L2, and CDR-L3 comprising the sequence of SEQ ID NOS:105, 107 and 108, respectively; or the V_H region comprises a CDR-H1, CDR-H2, and CDR-H3 comprising the sequence of SEQ ID NOS:94, 98 and 102, respectively, and the V_L region comprises a CDR-L1, CDR-L2, and CDR-L3 comprising the sequence of SEQ ID NOS:104, 106 and 108, respectively.

[0219] Provided are chimeric antigen receptors, comprising: (1) an extracellular antigen-binding domain that specifically binds human B cell maturation antigen (BCMA), wherein the extracellular antigen-binding domain comprises: a variable heavy (V_H) region comprising a CDR-H1, CDR-H2 and CDR-H3 contained within the V_H region sequence of SEQ ID NO: 116 and a variable light (V_L) region comprising a CDR-L1, CDR-L2 and CDR-L3 contained within the V_L region sequence of SEQ ID NO: 119; or the V_H region comprises a CDR-H1, CDR-H2 and CDR-H3 contained within the V_H region sequence of SEQ ID NO: 116; and the V_L region comprises a CDR-L1, CDR-L2 and CDR-L3 contained within the V_L region sequence of SEQ ID NO: 119; or the V_H region comprises a CDR-H1, CDR-H2, and CDR-H3 comprising the sequence of SEQ ID NOS:97, 101 and 103, respectively, and the V_L region comprises a CDR-L1, CDR-L2, and CDR-L3 comprising the sequence of SEQ ID NOS:105, 107 and

108, respectively; the V_H region comprises a CDR-H1, CDR-H2, and CDR-H3 comprising the sequence of SEQ ID NOS:96, 100 and 103, respectively, and the V_L region comprises a CDR-L1, CDR-L2, and CDR-L3 comprising the sequence of SEQ ID NOS:105, 107 and 108, respectively; the V_H region comprises a CDR-H1, CDR-H2, and CDR-H3 comprising the sequence of SEQ ID NOS:95, 99 and 103, respectively, and the V_L region comprises a CDR-L1, CDR-L2, and CDR-L3 comprising the sequence of SEQ ID NOS:105, 107 and 108, respectively; or the V_H region comprises a CDR-H1, CDR-H2, and CDR-H3 comprising the sequence of SEQ ID NOS:94, 98 and 102, respectively, and the V_L region comprises a CDR-L1, CDR-L2, and CDR-L3 comprising the sequence of SEQ ID NOS:104, 106 and 108, respectively; (2) a spacer set forth in SEQ ID NO: 174 or wherein the nucleic acid encoding the spacer is or comprises the sequence set forth in SEQ ID NO:200; (3) a transmembrane domain, optionally a transmembrane domain from a human CD28; and (4) an intracellular signaling region comprising a cytoplasmic signaling domain of a human CD3-zeta (CD3ζ) chain and an intracellular signaling domain of a T cell costimulatory molecule, optionally from a human 4-1BB or a human CD28. Also provided are polynucleotides encoding such a chimeric antigen receptor. In some embodiments, the extracellular antigen-binding domain comprises the V_H region sequence of SEQ ID NO:116 and the V_L region sequence of SEQ ID NO:119. In some embodiments, the antigen-binding domain of such receptor, comprises the amino acid sequence of SEQ ID NO: 114. In some embodiments, other domains, regions, or components of the chimeric antigen receptor includes any domains, regions, or components described herein.

4. Surrogate marker

[0220] In some embodiments, the CAR, or the polynucleotide that encodes the CAR, further includes a surrogate marker, such as a cell surface marker (e.g., a truncated cell surface marker), which may be used to confirm transduction or engineering of the cell to express the receptor. For example, in some aspects, extrinsic marker genes are utilized in connection with engineered cell therapies to permit detection or selection of cells and, in some cases, also to promote cell suicide by ADCC. Exemplary marker genes include truncated epidermal growth factor receptor (EGFRt), which can be co-expressed with a transgene of interest (e.g., a CAR or TCR) in transduced cells (see, e.g., U.S. Patent No. 8,802,374). EGFRt contains an epitope recognized by the antibody cetuximab (Erbitux®). For this reason, Erbitux® can be used to identify or select cells that have been engineered with the EGFRt construct, including in cells also co-engineered with another recombinant receptor, such as a chimeric antigen receptor (CAR). Additionally, EGFRt is commonly used as a suicide mechanism in connection with cell therapies. In some aspects, when EGFRt is co-expressed in cells with a transgene of interest (e.g. CAR or TCR), it can be targeted by the cetuximab monoclonal antibody to reduce or deplete the transferred gene-modified cells via ADCC (see U.S. Patent No. 8,802,374 and Liu et al., Nature Biotech. 2016 April; 34(4): 430–434). Importantly, the suicide killing approach using tEGFR requires availability of the antibody epitope. Another example of such a marker gene is prostate-specific membrane antigen

(PSMA) or a modified form thereof. PSMA or modified forms thereof may comprise a sequence of amino acids bound by or recognized by a PSMA-targeting molecule, such as an antibody or an antigen-binding fragment thereof. PSMA-targeting molecules can be used to identify or select cells that have been engineered with a PSMA or modified construct, including in cells also co-engineered with another recombinant receptor, such as a chimeric antigen receptor (CAR) provided herein. In some aspects, the marker includes all or part (*e.g.*, truncated form) of CD34, a nerve growth factor receptor (NGFR), epidermal growth factor receptor (*e.g.*, EGFR), or PSMA.

[0221] Exemplary surrogate markers can include truncated forms of cell surface polypeptides, such as truncated forms that are non-functional and to not transduce or are not capable of transducing a signal or a signal ordinarily transduced by the full-length form of the cell surface polypeptide, and/or do not or are not capable of internalizing. Exemplary truncated cell surface polypeptides including truncated forms of growth factors or other receptors such as a truncated human epidermal growth factor receptor 2 (tHER2), a truncated epidermal growth factor receptor (tEGFR, exemplary tEGFR sequence set forth in SEQ ID NO:246) or a prostate-specific membrane antigen (PSMA) or modified form thereof. tEGFR may contain an epitope recognized by the antibody cetuximab (Erbitux®) or other therapeutic anti-EGFR antibody or binding molecule, which can be used to identify or select cells that have been engineered with the tEGFR construct and an encoded exogenous protein, and/or to eliminate or separate cells expressing the encoded exogenous protein. See U.S. Patent No. 8,802,374 and Liu et al., Nature Biotech. 2016 April; 34(4): 430–434). In some aspects, the marker, *e.g.* surrogate marker, includes all or part (*e.g.*, truncated form) of CD34, a NGFR, a CD19 or a truncated CD19, e.g., a truncated non-human CD19, or epidermal growth factor receptor (*e.g.*, tEGFR). In some embodiments, the marker is or comprises a fluorescent protein, such as green fluorescent protein (GFP), enhanced green fluorescent protein (EGFP), such as super-fold GFP (sfGFP), red fluorescent protein (RFP), such as tdTomato, mCherry, mStrawberry, AsRed2, DsRed or DsRed2, cyan fluorescent protein (CFP), blue green fluorescent protein (BFP), enhanced blue fluorescent protein (EBFP), and yellow fluorescent protein (YFP), and variants thereof, including species variants, monomeric variants, and codon-optimized and/or enhanced variants of the fluorescent proteins. In some embodiments, the marker is or comprises an enzyme, such as a luciferase, the lacZ gene from *E. coli*, alkaline phosphatase, secreted embryonic alkaline phosphatase (SEAP), chloramphenicol acetyl transferase (CAT). Exemplary light-emitting reporter genes include luciferase (luc), β-galactosidase, chloramphenicol acetyltransferase (CAT), β-glucuronidase (GUS) or variants thereof.

[0222] In some embodiments, the marker is a selection marker. In some embodiments, the selection marker is or comprises a polypeptide that confers resistance to exogenous agents or drugs. In some embodiments, the selection marker is an antibiotic resistance gene. In some embodiments, the selection marker is an antibiotic resistance gene confers antibiotic resistance to a mammalian cell. In some embodiments, the selection marker is or comprises a Puromycin resistance gene, a Hygromycin

resistance gene, a Blasticidin resistance gene, a Neomycin resistance gene, a Geneticin resistance gene or a Zeocin resistance gene or a modified form thereof.

[0223] In some embodiments, the nucleic acid encoding the marker is operably linked to a polynucleotide encoding for a linker sequence, such as a cleavable linker sequence, *e.g.*, T2A. See WO2014031687. In some embodiments, introduction of a construct encoding the CAR and surrogate marker, separated by a T2A ribosome switch, can express two proteins from the same construct, such that the surrogate marker can be used as a marker to detect cells expressing such construct. In some embodiments, the surrogate marker, and optionally a linker sequence, can be any as disclosed in international publication no. WO2014031687. For example, the marker can be a truncated EGFR (tEGFR) or PSMA that is, optionally, linked to a linker sequence, such as a 2A cleavable linker sequence (e.g., a T2A, P2A, E2A or F2A cleavable linker, described elsewhere herein). An exemplary polypeptide for a truncated EGFR surrogate marker comprises the sequence of amino acids set forth in SEQ ID NO:246 or a sequence of amino acids that exhibits at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to SEQ ID NO:246. In some embodiments, the spacer is or comprises a glycine-serine rich sequence or other flexible linker such as known flexible linkers.

[0224] In some embodiments, the marker is a molecule, *e.g.*, cell surface protein, not naturally found on T cells or not naturally found on the surface of T cells, or a portion thereof.

[0225] In some embodiments, the molecule is a non-self molecule, *e.g.*, non-self protein, *i.e.*, one that is not recognized as "self" by the immune system of the host into which the cells will be adoptively transferred.

[0226] In some embodiments, the marker serves no therapeutic function and/or produces no effect other than to be used as a marker for genetic engineering, *e.g.*, for selecting cells successfully engineered. In other embodiments, the marker may be a therapeutic molecule or molecule otherwise exerting some desired effect, such as a ligand for a cell to be encountered *in vivo*, such as a costimulatory or immune checkpoint molecule to enhance and/or dampen responses of the cells following adoptive transfer and encounter with ligand.

[0227] In some cases, CARs are referred to as first, second, and/or third generation CARs. In some aspects, a first generation CAR is one that solely provides a CD3-chain induced signal upon or in response to antigen binding; in some aspects, a second-generation CARs is one that provides such a signal and costimulatory signal, such as one including an intracellular signaling domain from a costimulatory receptor such as CD28 or CD137; in some aspects, a third generation CAR in some aspects is one that includes multiple costimulatory domains of different costimulatory receptors.

[0228] In some embodiments, the chimeric antigen receptor includes an extracellular portion containing the antibody or fragment described herein. In some aspects, the chimeric antigen receptor includes an extracellular portion containing the antibody or fragment described herein and an

intracellular signaling domain. In some embodiments, the antibody or fragment includes an scFv or a single-domain antibody comprising only the V_H region and the intracellular signaling domain contains an ITAM. In some aspects, the intracellular signaling domain includes a signaling domain of a zeta chain of a CD3-zeta (CD3ζ) chain. In some embodiments, the chimeric antigen receptor includes a transmembrane domain linking the extracellular domain and the intracellular signaling domain. In some aspects, the transmembrane domain contains a transmembrane portion of CD28. The extracellular domain and transmembrane can be linked directly or indirectly. In some embodiments, the extracellular domain and transmembrane are linked by a spacer, such as any described herein. In some embodiments, the chimeric antigen receptor contains an intracellular domain of a co-stimulatory molecule (*e.g.*, T cell costimulatory molecule), such as between the transmembrane domain and intracellular signaling domain. In some aspects, the T cell costimulatory molecule is CD28 or 4-1BB.

[0229] In some embodiments, the transmembrane domain of the receptor (*e.g.*, CAR) is a transmembrane domain of human CD28 or variant thereof, *e.g.*, a 27-amino acid transmembrane domain of a human CD28 (Accession No.: P10747.1). In some embodiments, the intracellular signaling domain comprises an intracellular costimulatory signaling domain of human CD28 or functional variant thereof, such as a 41 amino acid domain thereof and/or such a domain with an LL to GG substitution at positions 186-187 of a native CD28 protein. In some embodiments, the intracellular domain comprises an intracellular costimulatory signaling domain of 4-1BB or functional variant thereof, such as a 42-amino acid cytoplasmic domain of a human 4-1BB (Accession No. Q07011.1). In some embodiments, the intracellular signaling domain comprises a human CD3 zeta stimulatory signaling domain or functional variant thereof, such as an 112 AA cytoplasmic domain of isoform 3 of human CD3ζ (Accession No.: P20963.2) or a CD3 zeta signaling domain as described in U.S. Patent No.: 7,446,190.

[0230] For example, in some embodiments, the CAR includes a BCMA antibody or fragment, such as any of the human BCMA antibodies, including sdAbs and scFvs, described herein, a spacer such as any of the Ig-hinge containing spacers, a CD28 transmembrane domain, a CD28 intracellular signaling domain, and a CD3 zeta signaling domain. In some embodiments, the CAR includes the BCMA antibody or fragment, such as any of the human BCMA antibodies, including sdAbs and scFvs described herein, a spacer such as any of the Ig-hinge containing spacers, a CD28 transmembrane domain, a 4-1BB intracellular signaling domain, and a CD3 zeta signaling domain. In some embodiments, such CAR constructs further includes a T2A ribosomal skip element and/or a tEGFR sequence, *e.g.*, downstream of the CAR.

[0231] In certain embodiments, multispecific binding molecules, e.g., multispecific chimeric receptors, such as multispecific CARs, can contain any of the multispecific antibodies, including, *e.g.* bispecific antibodies, multispecific single-chain antibodies, *e.g.*, diabodies, triabodies, and tetrabodies, tandem di-scFvs, and tandem tri-scFvs, such as any described above in Section I.A.

B. Exemplary features

[0232] In some aspects, the antibodies or antigen-binding fragments thereof, in the provided CARs, have one or more specified functional features, such as binding properties, including recognizing or binding to particular epitopes, such as to epitopes that are similar to or overlap with those specifically bound by other antibodies such as reference antibodies, or epitopes that are different from those specifically bound by other antibodies such as reference antibodies, the ability to compete for binding with other antibodies such as reference antibodies, and/or particular binding affinities. In other embodiments, the antibodies or antigen-binding fragments thereof, in the provided CARs, recognize, such as specifically recognize, or bind, *e.g.*, specifically bind, to epitopes that are different from, or do not overlap with those specifically bound by other antibodies such as reference antibodies. For example, the epitopes specifically bound by the antibodies, in the provided CARs, are different from those specifically bound by other antibodies such as reference antibodies. In some embodiments, the antibodies and antigen binding fragments thereof do not directly compete for, or compete to a lower degree, with binding with other antibodies such as reference antibodies.

[0233] In some embodiments, the antibodies or antigen-binding fragments thereof specifically recognize or specifically bind to BCMA protein. In any of the embodiments, an antibody or antigen binding fragment, in the provided CARs, that specifically recognize BCMA, specifically binds BCMA. In some embodiments provided herein, BCMA protein refers to human BCMA, a mouse BCMA protein, or a non-human primate (*e.g.*, cynomolgus monkey) BCMA protein. In some embodiments of any of the embodiments herein, BCMA protein refers to human BCMA protein. The observation that an antibody or other binding molecule binds to BCMA protein or specifically binds to BCMA protein does not necessarily mean that it binds to a BCMA protein of every species. For example, in some embodiments, features of binding to BCMA protein, such as the ability to specifically bind thereto and/or to compete for binding thereto with a reference antibody, and/or to bind with a particular affinity or compete to a particular degree, in some embodiments, refers to the ability with respect to a human BCMA protein and the antibody may not have this feature with respect to a BCMA protein of another species, such as mouse.

[0234] In some embodiments, the antibody or antigen-binding fragment binds to a mammalian BCMA protein, including to naturally occurring variants of BCMA, such as certain splice variants or allelic variants.

[0235] In some embodiments, the antibodies specifically bind to human BCMA protein, such as to an epitope or region of human BCMA protein, such as the human BCMA protein comprising the amino acid sequence of SEQ ID NO:164 (GenBank No. BAB60895.1), or SEQ ID NO:165 (NCBI No. NP_001183.2) or an allelic variant or splice variant thereof. In one embodiment, the human BCMA protein is encoded by a transcript variant or is an isoform that has the sequence of amino acids forth in SEQ ID NO:163. In some embodiments, the antibodies bind to cynomolgus monkey BCMA protein, such as the cynomolgus monkey BCMA protein set forth in SEQ ID NO:147 (GenBank No.

EHH60172.1). In some embodiments, the antibodies bind to human BCMA but do not bind to or bind in a lower level or degree or affinity to cynomolgus monkey BCMA protein, such as the cynomolgus monkey BCMA protein set forth in SEQ ID NO:147 (GenBank No. EHH60172.1). In some embodiments, the antibodies do not bind to or bind in a lower level or degree or affinity to mouse BCMA protein, such as the mouse BCMA protein set forth in SEQ ID NO:179 (NCBI No. NP_035738.1). In some embodiments, the antibodies bind to mouse BCMA protein, such as the mouse BCMA protein set forth in SEQ ID NO:179 (NCBI No. NP_035738.1). In some embodiments, the antibodies bind to mouse BCMA protein, with lower affinity than its binding to a human BCMA protein and/or a cynomolgus monkey BCMA protein. In some embodiments, the antibodies bind to mouse BCMA protein and/or a cynomolgus monkey BCMA protein with lower affinity than its binding to a human BCMA protein. In some embodiments, the antibodies bind to mouse BCMA protein and/or a cynomolgus monkey BCMA protein with similar binding affinity compared to its binding to a human BCMA protein.

[0236] In some embodiments, the provided antigen-binding domain or CAR exhibits preferential binding to membrane-bound BCMA as compared to soluble BCMA. In some embodiments, the provided antigen-binding domain or CAR exhibits greater binding affinity for, membrane-bound BCMA compared to soluble BCMA.

[0237] In one embodiment, the extent of binding of an anti-BCMA antibody or antigen-binding domain or CAR to an unrelated, non-BCMA protein, such as a non-human BCMA protein or other non-BCMA protein, is less than at or about 10% of the binding of the antibody or antigen-binding domain or CAR to human BCMA protein or human membrane-bound BCMA as measured, *e.g.*, by a radioimmunoassay (RIA). In some embodiments, among the antibodies or antigen-binding domains in the provided CARs, are antibodies or antigen-binding domains or CARs in which binding to mouse BCMA protein is less than or at or about 10% of the binding of the antibody to human BCMA protein. In some embodiments, among the antibodies or antigen-binding domains in the provided CARs, are antibodies in which binding to cynomolgus monkey BCMA protein is less than or at or about 10% of the binding of the antibody to human BCMA protein. In some embodiments, among the antibodies or antigen-binding domains in the provided CARs, are antibodies in which binding to cynomolgus monkey BCMA protein and/or a mouse BCMA protein is similar to or about the same as the binding of the antibody to human BCMA protein. In some embodiments, among the antibodies or antigen-binding domains in the provided CARs, are antibodies or antigen-binding domains or CARs in which binding to soluble BCMA protein is less than or at or about 10% of the binding of the antibody to membrane-bound BCMA protein.

[0238] In some embodiments, the antibody specifically binds to, and/or competes for binding thereto with a reference antibody, and/or binds with a particular affinity or competes to a particular degree, to a BCMA protein, *e.g.*, human BCMA, a mouse BCMA protein, or a non-human primate (*e.g.*, cynomolgus monkey) BCMA protein.

[0239] In some embodiments, the antibodies, in the provided CARs, are capable of binding BCMA protein, such as human BCMA protein, with at least a certain affinity, as measured by any of a number of known methods. In some embodiments, the affinity is represented by an equilibrium dissociation constant (K_D); in some embodiments, the affinity is represented by EC_{50}.

[0240] A variety of assays are known for assessing binding affinity and/or determining whether a binding molecule (*e.g.*, an antibody or fragment thereof) specifically binds to a particular ligand (*e.g.*, an antigen, such as a BCMA protein). It is within the level of a skilled artisan to determine the binding affinity of a binding molecule, *e.g.*, an antibody, for an antigen, *e.g.*, BCMA, such as human BCMA or cynomolgus BCMA or mouse BCMA, such as by using any of a number of binding assays that are well known in the art. For example, in some embodiments, a BIAcore® instrument can be used to determine the binding kinetics and constants of a complex between two proteins (*e.g.*, an antibody or fragment thereof, and an antigen, such as a BCMA protein), using surface plasmon resonance (SPR) analysis (*see, e.g.*, Scatchard *et al.*, *Ann. N.Y. Acad. Sci. 51*:660, 1949; Wilson, *Science 295*:2103, 2002; Wolff *et al.*, *Cancer Res. 53*:2560, 1993; and U.S. Patent Nos. 5,283,173, 5,468,614, or the equivalent).

[0241] SPR measures changes in the concentration of molecules at a sensor surface as molecules bind to or dissociate from the surface. The change in the SPR signal is directly proportional to the change in mass concentration close to the surface, thereby allowing measurement of binding kinetics between two molecules. The dissociation constant for the complex can be determined by monitoring changes in the refractive index with respect to time as buffer is passed over the chip. Other suitable assays for measuring the binding of one protein to another include, for example, immunoassays such as enzyme linked immunosorbent assays (ELISA) and radioimmunoassays (RIA), or determination of binding by monitoring the change in the spectroscopic or optical properties of the proteins through fluorescence, UV absorption, circular dichroism, or nuclear magnetic resonance (NMR). Other exemplary assays include, but are not limited to, Western blot, ELISA, analytical ultracentrifugation, spectroscopy, flow cytometry, sequencing and other methods for detection of expressed polynucleotides or binding of proteins.

[0242] In some embodiments, the binding molecule, *e.g.*, antibody or fragment thereof or antigen-binding domain of a CAR, binds, such as specifically binds, to an antigen, *e.g.*, a BCMA protein or an epitope therein, with an affinity or K_A (*i.e.*, an equilibrium association constant of a particular binding interaction with units of 1/M; equal to the ratio of the on-rate [k_{on} or k_a] to the off-rate [k_{off} or k_d] for this association reaction, assuming bimolecular interaction) equal to or greater than 10^5 M^{-1}. In some embodiments, the antibody or fragment thereof or antigen-binding domain of a CAR exhibits a binding affinity for the peptide epitope with a K_D (*i.e.*, an equilibrium dissociation constant of a particular binding interaction with units of M; equal to the ratio of the off-rate [k_{off} or k_d] to the on-rate [k_{on} or k_a] for this association reaction, assuming bimolecular interaction) of equal to or less than 10^{-5} M. For example, the equilibrium dissociation constant K_D ranges from 10^{-5} M to 10^{-13} M, such as 10^{-7} M to 10^{-11} M, 10^{-8} M to

10^{-10} M, or 10^{-9} M to 10^{-10} M. The on-rate (association rate constant; k_{on} or k_a; units of 1/Ms) and the off-rate (dissociation rate constant; k_{off} or k_d; units of 1/s) can be determined using any of the assay methods known in the art, for example, surface plasmon resonance (SPR).

[0243] In some embodiments, the binding affinity (EC_{50}) and/or the dissociation constant of the antibody (*e.g.* antigen-binding fragment) or antigen-binding domain of a CAR to about BCMA protein, such as human BCMA protein, is from or from about 0.01 nM to about 500 nM, from or from about 0.01 nM to about 400 nM, from or from about 0.01 nM to about 100 nM, from or from about 0.01 nM to about 50 nM, from or from about 0.01 nM to about 10 nM, from or from about 0.01 nM to about 1 nM, from or from about 0.01 nM to about 0.1 nM, is from or from about 0.1 nM to about 500 nM, from or from about 0.1 nM to about 400 nM, from or from about 0.1 nM to about 100 nM, from or from about 0.1 nM to about 50 nM, from or from about 0.1 nM to about 10 nM, from or from about 0.1 nM to about 1 nM, from or from about 0.5 nM to about 200 nM, from or from about 1 nM to about 500 nM, from or from about 1 nM to about 100 nM, from or from about 1 nM to about 50 nM, from or from about 1 nM to about 10 nM, from or from about 2 nM to about 50 nM, from or from about 10 nM to about 500 nM, from or from about 10 nM to about 100 nM, from or from about 10 nM to about 50 nM, from or from about 50 nM to about 500 nM, from or from about 50 nM to about 100 nM or from or from about 100 nM to about 500 nM. In certain embodiments, the binding affinity (EC_{50}) and/or the equilibrium dissociation constant, K_D, of the antibody to a BCMA protein, such as human BCMA protein, is at or less than or about 400 nM, 300 nM, 200 nM, 100 nM, 50 nM, 40 nM, 30 nM, 25 nM, 20 nM, 19 nM, 18 nM, 17 nM, 16 nM, 15 nM, 14 nM, 13 nM, 12 nM, 11 nM, 10 nM, 9 nM, 8 nM, 7 nM, 6 nM, 5 nM, 4 nM, 3 nM, 2 nM, or 1 nM or less. In some embodiments, the antibodies bind to a BCMA protein, such as human BCMA protein, with a sub-nanomolar binding affinity, for example, with a binding affinity less than about 1 nM, such as less than about 0.9 nM, about 0.8 nM, about 0.7 nM, about 0.6 nM, about 0.5 nM, about 0.4 nM, about 0.3 nM, about 0.2 nM or about 0.1 nM or less.

[0244] In some embodiments, the binding affinity may be classified as high affinity or as low affinity. In some cases, the binding molecule (*e.g.* antibody or fragment thereof) or antigen-binding domain of a CAR that exhibits low to moderate affinity binding exhibits a K_A of up to 10^7 M^{-1}, up to 10^6 M^{-1}, up to 10^5 M^{-1}. In some cases, a binding molecule (*e.g.* antibody or fragment thereof) that exhibits high affinity binding to a particular epitope interacts with such epitope with a K_A of at least 10^7 M^{-1}, at least 10^8 M^{-1}, at least 10^9 M^{-1}, at least 10^{10} M^{-1}, at least 10^{11} M^{-1}, at least 10^{12} M^{-1}, or at least 10^{13} M^{-1}. In some embodiments, the binding affinity (EC_{50}) and/or the equilibrium dissociation constant, K_D, of the binding molecule, *e.g.*, anti-BCMA antibody or fragment thereof or antigen-binding domain of a CAR, to a BCMA protein, is from or from about 0.01 nM to about 1 μM, 0.1 nM to 1 μM, 1 nM to 1 μM, 1 nM to 500 nM, 1 nM to 100 nM, 1 nM to 50 nM, 1 nM to 10 nM, 10 nM to 500 nM, 10 nM to 100 nM, 10 nM to 50 nM, 50 nM to 500 nM, 50 nM to 100 nM or 100 nM to 500 nM. In certain embodiments, the binding affinity (EC_{50}) and/or the dissociation constant of the equilibrium dissociation constant, K_D,

of the binding molecule, *e.g.*, anti-BCMA antibody or fragment thereof or antigen-binding domain of a CAR, to a BCMA protein, is at or about or less than at or about 1 μM, 500 nM, 100 nM, 50 nM, 40 nM, 30 nM, 25 nM, 20 nM, 19 nM, 18 nM, 17 nM, 16 nM, 15 nM, 14 nM, 13 nM, 12 nM, 11 nM, 10 nM, 9 nM, 8 nM, 7 nM, 6 nM, 5 nM, 4 nM, 3 nM, 2 nM, or 1 nM or less. The degree of affinity of a particular antibody can be compared with the affinity of a known antibody, such as a reference antibody.

[0245] In some embodiments, the binding affinity of a binding molecule, such as an anti-BCMA antibody or antigen-binding domain of a CAR, for different antigens, *e.g.*, BCMA proteins from different species can be compared to determine the species cross-reactivity. For example, species cross-reactivity can be classified as high cross reactivity or low cross reactivity. In some embodiments, the equilibrium dissociation constant, K_D, for different antigens, *e.g.*, BCMA proteins from different species such as human, cynomolgus monkey or mouse, can be compared to determine species cross-reactivity. In some embodiments, the species cross-reactivity of an anti-BCMA antibody or antigen-binding domain of a CAR can be high, *e.g.*, the anti-BCMA antibody binds to human BCMA and a species variant BCMA to a similar degree, *e.g.*, the ratio of K_D for human BCMA and K_D for the species variant BCMA is or is about 1. In some embodiments, the species cross-reactivity of an anti-BCMA antibody or antigen-binding domain of a CAR can be low, *e.g.*, the anti-BCMA antibody has a high affinity for human BCMA but a low affinity for a species variant BCMA, or vice versa. For example, the ratio of K_D for the species variant BCMA and K_D for the human BCMA is more than 10, 15, 20, 25, 30, 40, 50, 60, 70, 80, 90, 100, 200, 500, 1000, 2000 or more, and the anti-BCMA antibody has low species cross-reactivity. The degree of species cross-reactivity can be compared with the species cross-reactivity of a known antibody, such as a reference antibody.

[0246] In some embodiments, the binding affinity of the anti-BCMA antibody or antigen-binding domain of a CAR, for different form or topological type of antigens, *e.g.*, soluble BCMA protein compared to the binding affinity to a membrane-bound BCMA, to determine the preferential binding or relative affinity for a particular form or topological type. For example, in some aspects, the provided anti-BCMA antibodies or antigen-binding domains can exhibit preferential binding to membrane-bound BCMA as compared to soluble BCMA and/or exhibit greater binding affinity for, membrane-bound BCMA compared to soluble BCMA. In some embodiments, the equilibrium dissociation constant, K_D, for different form or topological type of BCMA proteins, can be compared to determine preferential binding or relative binding affinity. In some embodiments, the preferential binding or relative affinity to a membrane-bound BCMA compared to soluble BCMA can be high. For example, in some cases, the ratio of K_D for soluble BCMA and the K_D for membrane-bound BCMA is more than 10, 15, 20, 25, 30, 40, 50, 60, 70, 80, 90, 100, 200, 500, 1000, 2000 or more and the antibody or antigen-binding domain preferentially binds or has higher binding affinity for membrane-bound BCMA. In some cases, the ratio of K_A for membrane-bound BCMA and the K_A for soluble BCMA is more than 10, 15, 20, 25, 30, 40, 50, 60, 70, 80, 90, 100, 200, 500, 1000, 2000 or more and the antibody or antigen-binding domain

preferentially binds or has higher binding affinity for membrane-bound BCMA. In some cases, the antibody or antigen-binding domain of CAR binds soluble BCMA and membrane-bound BCMA to a similar degree, *e.g.*, the ratio of K_D for soluble BCMA and K_D for membrane-bound BCMA is or is about 1. In some cases, the antibody or antigen-binding domain of CAR binds soluble BCMA and membrane-bound BCMA to a similar degree, *e.g.*, the ratio of K_A for soluble BCMA and K_A for membrane-bound BCMA is or is about 1. The degree of preferential binding or relative affinity for membrane-bound BCMA or soluble BCMA can be compared with that of a known antibody, such as a reference antibody.

[0247] In some embodiments, the antibodies or antigen binding fragments thereof, in the provided CARs, bind to a similar degree to a human BCMA protein and a non-human BCMA protein or other non-BCMA proteins. For example, in some embodiments, the antibodies or antigen binding fragments thereof or antigen-binding domain of a CAR bind to a human BCMA protein, such as the human BCMA protein comprising the amino acid sequence of SEQ ID NO:164 (GenBank No. BAB60895.1), or SEQ ID NO:165 (NCBI No. NP_001183.2) or an allelic variant or splice variant thereof, with an equilibrium dissociation constant (K_D), and to a non-human BCMA, such as a cynomolgus monkey BCMA, such as the cynomolgus monkey BCMA protein set forth in SEQ ID NO:147 (GenBank No. EHH60172.1), with a K_D that is similar, or about the same, or less than 2-fold different, or less than 5-fold different.

[0248] In some embodiments, the antibodies or antigen binding fragments thereof, in the provided CARs, bind to a similar degree to a soluble BCMA protein and a membrane-bound BCMA protein, with an equilibrium dissociation constant (K_D) that is similar, or about the same, or less than 2-fold different, or less than 5-fold different.

[0249] For example, in some embodiments, the antibodies, in the provided CARs, or antigen binding fragments thereof bind to a human BCMA with a K_D of about or less than at or about 1 μM, 500 nM, 100 nM, 50 nM, 40 nM, 30 nM, 25 nM, 20 nM, 19 nM, 18 nM, 17 nM, 16 nM, 15 nM, 14 nM, 13 nM, 12 nM, 11 nM, 10 nM, 9 nM, 8 nM, 7 nM, 6 nM, 5 nM, 4 nM, 3 nM, 2 nM, or 1 nM or less, and binds to a cynomolgus monkey BCMA with a K_D of about or less than at or about 1 μM, 500 nM, 100 nM, 50 nM, 40 nM, 30 nM, 25 nM, 20 nM, 19 nM, 18 nM, 17 nM, 16 nM, 15 nM, 14 nM, 13 nM, 12 nM, 11 nM, 10 nM, 9 nM, 8 nM, 7 nM, 6 nM, 5 nM, 4 nM, 3 nM, 2 nM, or 1 nM or less. In some embodiments, the antibodies or antigen binding fragments thereof bind to a mouse BCMA protein with a K_D of about or less than at or about 1 μM, 500 nM, 100 nM, 50 nM, 40 nM, 30 nM, 25 nM, 20 nM, 19 nM, 18 nM, 17 nM, 16 nM, 15 nM, 14 nM, 13 nM, 12 nM, 11 nM, 10 nM, 9 nM, 8 nM, 7 nM, 6 nM, 5 nM, 4 nM, 3 nM, 2 nM, or 1 nM or less. In some embodiments, the antibodies or antigen binding fragments thereof, in the provided CARs, bind to a human BCMA, a cynomolgus monkey BCMA and a mouse BCMA with high affinity. In some embodiments, the antibodies or antigen binding fragments thereof bind to a human BCMA and cynomolgus monkey BCMA with a high affinity, and to a mouse BCMA with low affinity. In some embodiments, the antibodies or antigen binding fragments thereof bind to a human BCMA and BCMA from other species, or other variants of the BCMA protein, with high

affinity.

[0250] In some embodiments, the total binding capacity (R_{max}), as measured using particular surface plasmon resonance (SPR) conditions, is used to determine the ability or capacity of binding of the antibody or antigen binding fragment thereof, to the antigen, *e.g.*, a BCMA protein, such as a human BCMA protein. For SPR analysis, the "ligand" is the immobilized target molecule on the surface of the sensor, for example, a BCMA protein, and the "analyte" is the tested molecule, *e.g.*, antibody, for binding to the "ligand". For example, the "analyte" can be any of the antibodies, or antigen binding fragments thereof, that binds to a BCMA protein. For a particular ligand and analyte pair in SPR, the R_{max} can be determined assuming a 1:1 binding stoichiometry model, for a particular condition. Binding capacity (R_{max}) was determined using the following formula: R_{max} (RU) = (analyte molecular weight)/(ligand molecular weight) × immobilized ligand level (RU). For example, in a particular SPR conditions, the R_{max} of binding between any of the antibody or antigen binding fragment thereof and a BCMA protein, such as a human BCMA or a cynomolgus BCMA, is at least or at least about 50 resonance units (RU), such as about 25 RU, 20 RU, 15 RU, 10 RU, 5 RU or 1 RU.

[0251] In some embodiments, the antibodies, such as the human antibodies, in the provided CAR, specifically bind to a particular epitope or region of BCMA protein, such as generally an extracellular epitope or region. BCMA protein is a type III membrane 184 amino acid protein that contains an extracellular domain, a transmembrane domain, and a cytoplasmic domain. With reference to a human BCMA amino acid sequence set forth in SEQ ID NO:164, the extracellular domain corresponds to amino acids 1-54, amino acids 55-77 correspond to the transmembrane domain, and amino acids 78-184 correspond to the cytoplasmic domain.

[0252] Among the provided CARs are CARs that exhibit antigen-dependent activity or signaling, i.e. signaling activity that is measurably absent or at background levels in the absence of the antigen, e.g. BCMA. Thus, in some aspects, provided CARs do not exhibit, or exhibit no more than background or a tolerable or low level of, tonic signaling or antigen-independent activity or signaling in the absence of antigen, e.g. BCMA, being present. In some embodiments, the provided anti-BCMA CAR-expressing cells exhibit biological activity or function, including cytotoxic activity, cytokine production, and ability to proliferate.

[0253] In some embodiments, biological activity or functional activity of a chimeric receptor, such as cytotoxic activity, can be measured using any of a number of known methods. The activity can be assessed or determined either in vitro or in vivo. In some embodiments, activity can be assessed once the cells are administered to the subject (e.g., human). Parameters to assess include specific binding of an engineered or natural T cell or other immune cell to antigen, e.g., in vivo, e.g., by imaging, or ex vivo, e.g., by ELISA or flow cytometry. In certain embodiments, the ability of the engineered cells to destroy target cells can be measured using any suitable method known in the art, such as cytotoxicity assays described in, for example, Kochenderfer et al., J. Immunotherapy, 32(7): 689-702 (2009), and Herman et

al. J. Immunological Methods, 285(1): 25-40 (2004). In certain embodiments, the biological activity of the cells also can be measured by assaying expression and/or secretion of certain cytokines, such as interlekukin-2 (IL-2), interferon-gamma (IFN□), interleukin-4 (IL-4), TNF-alpha (TNFα), interleukin-6 (IL-6), interleukin-10 (IL-10), interleukin-12 (IL-12), granulocyte-macrophage colony-stimulating factor (GM-CSF), CD107a, and/or TGF-beta (TGFβ). Assays to measure cytokines are well known in the art, and include but are not limited to, ELISA, intracellular cytokine staining, cytometric bead array, RT-PCR, ELISPOT, flow cytometry and bio-assays in which cells responsive to the relevant cytokine are tested for responsiveness (*e.g.* proliferation) in the presence of a test sample. In some aspects the biological activity is measured by assessing clinical outcome, such as reduction in tumor burden or load.

[0254] In some aspects, a reporter cell line can be employed to monitor antigen-independent activity and/or tonic signaling through anti-BCMA CAR-expressing cells. In some embodiments, a T cell line, such as a Jurkat cell line, contains a reporter molecule, such as a fluorescent protein or other detectable molecule, such as a red fluorescent protein, expressed under the control of the endogenous Nur77 transcriptional regulatory elements. In some embodiments, the Nur77 reporter expression is cell intrinsic and dependent upon signaling through a recombinant reporter containing a primary activation signal in a T cell, a signaling domain of a T cell receptor (TCR) component, and/or a signaling domain comprising an immunoreceptor tyrosine-based activation motif (ITAM), such as a CD3ζ chain. Nur77 expression is generally not affected by other signaling pathways such as cytokine signaling or toll-like receptor (TLR) signaling, which may act in a cell extrinsic manner and may not depend on signaling through the recombinant receptor. Thus, only cells that express the exogenous recombinant receptor, e.g. anti-BCMA CAR, containing the appropriate signaling regions is capable of expressing Nur77 upon stimulation (e.g., binding of the specific antigen). In some cases, Nur77 expression also can show a dose-dependent response to the amount of stimulation (e.g., antigen).

[0255] In some embodiments, the provided anti-BCMA CARs exhibit improved expression on the surface of cells, such as compared to an alternative CAR that has an identical amino acid sequence but that is encoded by non-splice site eliminated and/or a codon-optimized nucleotide sequence. In some embodiments, the expression of the recombinant receptor on the surface of the cell can be assessed. Approaches for determining expression of the recombinant receptor on the surface of the cell may include use of chimeric antigen receptor (CAR)-specific antibodies (e.g., Brentjens et al., Sci. Transl. Med. 2013 Mar; 5(177): 177ra38), Protein L (Zheng et al., J. Transl. Med. 2012 Feb; 10:29), epitope tags, and monoclonal antibodies that specifically bind to a CAR polypeptide (see international patent application Pub. No. WO2014190273). In some embodiments, the expression of the recombinant receptor on the surface of the cell, e.g., primary T cell, can be assessed, for example, by flow cytometry, using binding molecules that can bind to the recombinant receptor or a portion thereof that can be detected. In some embodiments, the binding molecules used for detecting expression of the recombinant receptor an anti-idiotypic antibody, e.g., an anti-idiotypic agonist antibody specific for a binding domain,

e.g., scFv, or a portion thereof. In some embodiments, the binding molecule is or comprises an isolated or purified antigen, e.g., recombinantly expressed antigen.

C. Multispecific antibodies

[0256] In certain embodiments, the BCMA-binding molecules, *e.g.*, antibodies or polypeptides, such as chimeric receptors containing the same, are multispecific. Among the multispecific binding molecules are multispecific antibodies, including, *e.g.* bispecific antibodies. Multispecific binding partners, *e.g.*, antibodies, have binding specificities for at least two different sites, which may be in the same or different antigens. In certain embodiments, one of the binding specificities is for BCMA and the other is for another antigen. In some embodiments, additional binding molecules bind to and/or recognize a third, or more antigens. In certain embodiments, bispecific antibodies may bind to two different epitopes of BCMA. Bispecific antibodies may also be used to localize cytotoxic agents to cells which express BCMA. Bispecific antibodies can be prepared as full length antibodies or antibody fragments. Among the multispecific antibodies are multispecific single-chain antibodies, *e.g.*, diabodies, triabodies, and tetrabodies, tandem di-scFvs, and tandem tri-scFvs. Also provided are multispecific chimeric receptors, such as multispecific CARs, containing the antibodies (*e.g.*, antigen-binding fragments). Also provided are multispecific cells containing the antibodies or polypeptides including the same, such as cells containing a cell surface protein including the anti-BCMA antibody and an additional cell surface protein, such as an additional chimeric receptor, which binds to a different antigen or a different epitope on BCMA.

[0257] Exemplary antigens include B cell specific antigens, other tumor-specific antigens, such as antigens expressed specifically on or associated with a leukemia (*e.g.*, B cell leukemia), lymphoma (*e.g.*, Hodgkin's lymphoma, non-Hodgkin's lymphoma, etc.), or a myeloma, *e.g.*, a multiple myeloma (MM), a plasma cell malignancy (*e.g.*, plasmacytoma). For example, antigens include those expressed specifically on or associated with B cell chronic lymphocytic leukemia (CLL), a diffuse large B-cell lymphoma (DLBCL), acute myeloid leukemia (AML), acute lymphocytic leukemia (ALL), Burkitt's lymphoma (*e.g.*, endemic Burkitt's lymphoma or sporadic Burkitt's lymphoma), mantle cell lymphoma (MCL), non-small cell lung cancer (NSCLC), chronic myeloid (or myelogenous) leukemia (CML), hairy cell leukemia (HCL), small lymphocytic lymphoma (SLL), Marginal zone lymphoma, Hodgkin lymphoma (HL), non-Hodgkin lymphoma (NHL), Anaplastic large cell lymphoma (ALCL), refractory follicular lymphoma, Waldenstrom macroglobulinemia, follicular lymphoma, small non-cleaved cell lymphoma, mucosa-associated lymphatic tissue lymphoma (MALT), marginal zone lymphoma, nodal monocytoid B cell lymphoma, immunoblastic lymphoma, large cell lymphoma, diffuse mixed cell lymphoma, pulmonary B cell angiocentric lymphoma, small lymphocytic lymphoma, primary mediastinal B cell lymphoma, lymphoplasmacytic lymphoma (LPL), neuroblastoma, renal cell carcinoma, colon cancer, colorectal cancer, breast cancer, epithelial squamous cell cancer, melanoma, myeloma such as multiple myeloma (*e.g.*, non-secretory multiple myeloma, smoldering multiple myeloma), stomach cancer,

esophageal cancer, brain cancer, lung cancer (*e.g.*, small-cell lung cancer), pancreatic cancer, cervical cancer, ovarian cancer, liver cancer (*e.g.*, hepatic carcinoma, hepatoma, etc.), bladder cancer, prostate cancer, testicular cancer, thyroid cancer, uterine cancer, spleen cancer (*e.g.*, splenic lymphoma), adrenal cancer and/or head and neck cancer, and antigens expressed on T cells.

[0258] In some embodiments, among the second or additional antigens for multi-targeting strategies includes those in which at least one of the antigens is a universal tumor antigen, or a family member thereof. In some embodiments, the second or additional antigen is an antigen expressed on a tumor. In some embodiments, the BCMA-binding molecules provided herein target an antigen on the same tumor type as the second or additional antigen. In some embodiments, the second or additional antigen may also be a universal tumor antigen or may be a tumor antigen specific to a tumor type.

[0259] Exemplary second or additional antigens include CD4, CD5, CD8, CD14, CD15, CD19, CD20, CD21, CD22, CD23, CD25, CD33, CD37, CD38, CD40, CD40L, CD46, CD52, CD54, CD74, CD80, CD126, CD138, B7, MUC-1, Ia, HM1.24, HLA-DR, tenascin, an angiogenesis factor, VEGF, PIGF, ED-B fibronectin, an oncogene, an oncogene product, CD66a-d, necrosis antigens, Ii, IL-2, T101, TAC, IL-6, ROR1, TRAIL-R1 (DR4), TRAIL-R2 (DR5), Her2, L1-CAM, mesothelin, CEA, hepatitis B surface antigen, anti-folate receptor, CD24, CD30, CD44, EGFR, EGP-2, EGP-4, EPHa2, ErbB2, ErbB3, ErbB4, erbB dimers, EGFR vIII, FBP, FCRL5, FCRH5, fetal acetylcholine receptor, GD2, GD3, G protein-coupled receptor class C group 5 member D (GPRC5D), HMW-MAA, IL-22R-alpha, IL-13R-alpha2, kdr, kappa light chain, Lewis Y, L1-cell adhesion molecule (L1-CAM), Melanoma-associated antigen (MAGE)-A1, MAGE-A3, MAGE-A6, Preferentially expressed antigen of melanoma (PRAME), survivin, EGP2, EGP40, TAG72, B7-H6, IL-13 receptor a2 (IL-13Ra2), CA9, CD171, G250/CAIX, HLA-AI MAGE Al, HLA-A2 NY-ESO-1, PSCA, folate receptor-a, CD44v6, CD44v7/8, avb6 integrin, 8H9, NCAM, VEGF receptors, 5T4, Foetal AchR, NKG2D ligands, dual antigen, an antigen associated with a universal tag, a cancer-testes antigen, MUC1, MUC16, NY-ESO-1, MART-1, gp100, oncofetal antigen, VEGF-R2, carcinoembryonic antigen (CEA), prostate specific antigen, PSMA, Her2/neu, estrogen receptor, progesterone receptor, ephrinB2, CD123, c-Met, GD-2, O-acetylated GD2 (OGD2), CE7, Wilms Tumor 1 (WT-1), a cyclin, cyclin A2, CCL-1, hTERT, MDM2, CYP1B, WT1, livin, AFP, p53, cyclin (D1), CS-1, BAFF-R, TACI, CD56, TIM-3, CD123, L1-cell adhesion molecule, MAGE-A1, MAGE A3, a cyclin, such as cyclin A1 (CCNA1) and/or a pathogen-specific antigen, biotinylated molecules, molecules expressed by HIV, HCV, HBV and/or other pathogens, and/or in some aspects, neoepitopes or neoantigens thereof. In some embodiments, the antigen is associated with or is a universal tag.

[0260] In some aspects, the antigen, e.g., the second or additional antigen, such as the disease-specific antigen and/or related antigen, is expressed on multiple myeloma, such as G protein-coupled receptor class C group 5 member D (GPRC5D), CD38 (cyclic ADP ribose hydrolase), CD138 (syndecan-1, syndecan, SYN-1), CS-1 (CS1, CD2 subset 1, CRACC, SLAMF7, CD319, and 19A24), BAFF-R,

TACI and/or FcRH5. Other exemplary multiple myeloma antigens include CD56, TIM-3, CD33, CD123, CD44, CD20, CD40, CD74, CD200, EGFR, β2-Microglobulin, HM1.24, IGF-1R, IL-6R, TRAIL-R1, and the activin receptor type IIA (ActRIIA). See Benson and Byrd, J. Clin. Oncol. (2012) 30(16): 2013-15; Tao and Anderson, Bone Marrow Research (2011):924058; Chu et al., Leukemia (2013) 28(4):917-27; Garfall et al., Discov Med. (2014) 17(91):37-46. In some embodiments, the antigens include those present on lymphoid tumors, myeloma, AIDS-associated lymphoma, and/or post-transplant lymphoproliferations, such as CD38. Antibodies or antigen-binding fragments directed against such antigens are known and include, for example, those described in U.S. Patent No. 8,153,765; 8,603477, 8,008,450; U.S. Pub. No. US20120189622 or US20100260748; and/or International PCT Publication Nos. WO2006099875, WO2009080829 or WO2012092612 or WO2014210064. In some embodiments, such antibodies or antigen-binding fragments thereof (e.g. scFv) are contained in multispecific antibodies, multispecific chimeric receptors, such as multispecific CARs, and/or multispecific cells.

II. METHODS OF OPTIMIZING AND PRODUCING POLYNUCLEOTIDES, E.G., POLYNUCLEOTIDES ENCODING BCMA CARS, AND OPTIMIZED POLYNUCLEOTIDES

[0261] Provided herein are methods for optimizing polynucleotides for expression and/or therapeutic use, and polynucleotides optimized, e.g., according to the methods. In some aspects, in the provided methods and uses, such as methods and uses for cell therapy, employs cells, such as immune cells, that are engineered by introducing optimized polynucleotides. In some embodiments, the provided methods or optimizations reduce heterogeneity and/or increase homogeneity of transcribed RNA, such as messenger RNA (mRNA), for example, when the polynucleotide is expressed in a cell, such as in a particular cell type, such as in a mammalian, e.g., human cell type such as a human T cell such as a primary human T cell or T cell line. In some embodiments, the methods for optimizing polynucleotides include methods to identify and remove or alter the sequence of one or more cryptic splice site, such as one or both of a donor splice site or an acceptor splice site. In some embodiments, the methods can additionally or further include codon optimization. In some embodiments, codon optimization can be performed prior to and/or after methods of reducing heterogeneity of transcribed RNA (e.g., mRNA), such as by removal or elimination of predicted splice sites. In some embodiments, codon optimization is integrated in any one or more steps of the method of reducing heterogeneity of transcribed RNAs. In some embodiments, methods of reducing heterogeneity, such as by removal or elimination of predicted splice sites, can be performed after codon optimization. In some embodiments, provided are methods in which a polynucleotide encoding a transgene, including a polynucleotide encoding any of the provided anti-BCMA CAR polypeptides, can be optimized for expression and/or for therapeutic use. In some embodiments, the polynucleotides are modified to optimize codon usage. In some embodiments, the polynucleotides are codon optimized for expression in a human cell such as a human T cell such as a primary human T cell. In some embodiments, the polynucleotides, such as those encoding any of the antibodies, receptors (such as antigen receptors such as chimeric antigen receptors) and/or BCMA-

specific binding proteins provided herein, are or have been modified to reduce heterogeneity or contain one or more nucleic acid sequences observed herein (such as by the optimization methods) to result in improved features of the polypeptides, such as the CARs, as compared to those containing distinct, reference, sequences or that have not been optimized. Among such features include improvements in RNA heterogeneity, such as that resulting from the presence of one or more splice sites, such as one or more cryptic splice sites, and/or improved expression and/or surface expression of the encoded protein, such as increased levels, uniformity, or consistency of expression among cells or different therapeutic cell compositions engineered to express the polypeptides. In some embodiments, the polynucleotides can be codon optimized for expression in human cells.

[0262] Genomic nucleic acid sequences generally, in nature, in a mammalian cell, undergo processing co-transcriptionally or immediately following transcription, wherein a nascent precursor messenger ribonucleic acid (pre-mRNA), transcribed from a genomic deoxyribonucleic acid (DNA) sequence, is in some cases edited by way of splicing, to remove introns, followed by ligation of the exons in eukaryotic cells. Consensus sequences for splice sites are known, but in some aspects, specific nucleotide information defining a splice site may be complex and may not be readily apparent based on available methods. Cryptic splice sites are splice sites that are not predicted based on the standard consensus sequences and are variably activated. Hence, variable splicing of pre-mRNA at cryptic splice sites leads to heterogeneity in the transcribed mRNA products following expression in eukaryotic cells.

[0263] Polynucleotides generated for the expression of transgenes are typically constructed from nucleic acid sequences, such as complementary DNA (cDNA), or portions thereof, that do not contain introns. Thus, splicing of such sequences is not expected to occur. However, the presence of cryptic splice sites within the cDNA sequence can lead to unintended or undesired splicing reactions and heterogeneity in the transcribed mRNA. Such heterogeneity results in translation of unintended protein products, such as truncated protein products with variable amino acid sequences that exhibit modified expression and/or activity.

[0264] Also provided are methods and approaches for determining the heterogeneity of a transcribed nucleic acid such as one encoding or containing a transgene or encoding a recombinant protein. In some embodiments, the methods include determining the heterogeneity of a transcribed nucleic acid sequence that includes all or a portion of the 5′ untranslated region (5′ UTR), and/or all or a portion of the 3′ untranslated region (3′ UTR), of the transcribed nucleic acid. Also provided herein are methods of identifying the presence of splice sites, such as cryptic splice sites, based on the heterogeneity of the transcribed nucleic acid. Also provided are methods of identifying a transgene candidate for the removal of splice sites, such as cryptic splice sites, using the provided methods of determining the heterogeneity of the transcribed nucleic acid of the transgene. Also provided are methods of reducing the heterogeneity of an expressed transgene transcript.

[0265] Also provided herein are methods of identifying a transgene or recombinant protein or

nucleic acid candidate for the removal or modification of one or more splice sites, such as cryptic splice sites, such as based on the determined heterogeneity of the transcribed nucleic acid, e.g., of the transgene.

[0266] Also provided are methods and approaches for reducing the heterogeneity of a transcribed nucleic acid (e.g., transcript) of a transgene (e.g., an expressed transgene transcript) or other nucleic acid. Such methods and approaches can include identifying a transgene candidate for the removal of splice sites (such as cryptic splice sites) according to the provided methods and identifying one or more potential splice donor and/or splice acceptor sites within the transgene. In embodiments of the provided methods the splice donor and/or splice acceptor sites can be in the translated and/or untranslated regions of the transcribed nucleic acid (e.g., transcript).

[0267] In some embodiments, eliminating splice sites, such as cryptic splice sites, can improve or optimize expression of a transgene product, such as a polypeptide translated from the transgene, such as an anti-BCMA CAR polypeptide. Splicing at cryptic splice sites of an encoded transgene, such as an encoded BCMA CAR molecule, can lead to reduced protein expression, e.g., expression on cell surfaces, and/or reduced function, e.g., reduced intracellular signaling. Provided herein are polynucleotides, encoding anti-BCMA CAR proteins that have been optimized to reduce or eliminate cryptic splice sites. Also provided herein are polynucleotides encoding anti-BCMA CAR proteins that have been optimized for codon expression and/or in which one or more sequence, such as one identified by the methods or observations herein regarding splice sites, is present, and/or in which an identified splice site, such as any of the identified splice sites herein, is not present. Among the provided polynucleotides are those exhibiting below a certain degree of RNA heterogeneity or splice forms when expressed under certain conditions and/or introduced into a specified cell type, such as a human T cell, such as a primary human T cell, and cells and compositions and articles of manufacture containing such polypeptides and/or exhibiting such properties.

[0268] In some embodiments, reducing RNA heterogeneity or removing potential splice site comprises modifying a polynucleotide. In some embodiments, the modification includes one or more nucleotide modifications, such as a replacement or substitution, compared to a reference polynucleotide such as an unmodified polynucleotide that encodes the same polypeptide. In some embodiments, the reference polynucleotide is one in which the transcribed RNA (e.g. mRNA), when expressed in a cell, exhibits greater than or greater than about 10%, 15%, 20%, 25%, 30%, 40%, 50% or more RNA heterogeneity. In some embodiments, the provided methods can result in polynucleotides in which RNA heterogeneity of transcribed RNA is reduced by greater than or greater than about 10%, 15%, 20%, 25%, 30%, 40%, 50% or more. In some embodiments, the provided methods produce polynucleotides in which RNA homogeneity of transcribed RNA is at least 70%, 75%, 80%, 85%, 90%, or 95% or greater.

A. Methods of Measuring and Reducing RNA Heterogeneity

[0269] Provided herein are methods, approaches, and strategies for measuring, evaluating and/or reducing RNA heterogeneity of a nucleic acid, such as of a transcribed RNA, e.g., when expressed in a

particular cell type or context, as well as polynucleotides exhibiting reduction in such heterogeneity and/or risk thereof, as compared to a reference polynucleotide. In some embodiments, a reference polynucleotide can be assessed for RNA heterogeneity, such as by methods as described in this Section. In some embodiments, the provided approaches involve identifying RNA (e.g., mRNA) heterogeneity or likelihood thereof, such as in a particular cell or context, such as due to cryptic splice sites. In some aspects, such heterogeneity is identified by amplifying RNA transcripts using a first primer specific to the 5′ untranslated region (5′ UTR), corresponding to a portion of an element located upstream of the transgene in the transcribed RNA, such as a promoter, and a second primer specific to a 3′ untranslated region (3′ UTR), located downstream of the expressed transgene in the transcribed RNA sequence or specific to a sequence within the transgene. In some embodiments, the methods involve amplifying a transcribed nucleic acid using at least one 5′ and 3′ primer pair, wherein at least one pair comprises a 5′ primer that is complementary to a nucleic acid sequence within the 5′ untranslated region (5′ UTR) of the transcribed nucleic acid and a 3′ primer that is complementary to a nucleic acid sequence within the 3′ untranslated region (3′ UTR) of the transcribed nucleic acid to generate one or more amplified products. In some embodiments, the methods involve detecting the amplified products, wherein the presence of two or more amplified products from at least one 5′ and 3′ primer pair indicates heterogeneity in the amplified products. In some embodiments, the detected difference in transcripts are different lengths of the amplified transcript. In some embodiments, the detected difference in transcripts are differences in chromatographic profiles. Exemplary methods for identifying a polynucleotide with RNA heterogeneity are described below. In some embodiments, the methods comprise evaluating RNA heterogeneity for the need of being modified to reduce heterogeneity. In some embodiments, polynucleotides that exhibit RNA heterogeneity greater than or greater than about 10%, 15%, 20%, 25%, 30%, 40%, 50% or more are selected for nucleotide modification to remove one or more splice sites, such as one or more cryptic splice sites.

1. Measuring RNA Heterogeneity

[0270] RNA heterogeneity can be determined by any of a number of methods provided herein or described or known. In some embodiments, RNA heterogeneity of a transcribed nucleic acid is determined by amplifying the transcribed nucleic acid, such as by reverse transcriptase polymerase chain reaction (RT-PCR) followed by detecting one or more differences, such as differences in size, in the one or more amplified products. In some embodiments, the RNA heterogeneity is determined based on the number of differently sized amplified products, or the proportion of various differently sized amplified products. For example, in some embodiments, RNA heterogeneity is quantified by determining the number, amount or proportion of differently sized amplified product compared to the number or amount of total amplified products. In some cases, all or substantially all of a particular transcript is determined to be equal in size, and in this case, the RNA heterogeneity is low. In some cases, a variety of differently sized transcripts are present, or a large proportion of a particular transcript is of a different size compared

to the predicted size of the amplified product without cryptic or undesired splicing events. In some embodiments, RNA heterogeneity can be calculated by dividing the total number or amount of all of amplified products that are of a different size compared to the predicted size of the amplified product by the total number or amount of all amplified products. In some embodiments, the predicted size of the transcript or amplified product is from an RNA that does not contain or is not predicted to contain a cryptic splice site. In some embodiments, the predicted size of the transcript or amplified product takes into account one or more splice sites that are desired or intentionally placed.

[0271] In some embodiments, RNA, such as total RNA or cytoplasmic polyadenylated RNA, is harvested from cells, expressing the transgene to be optimized, and amplified by reverse transcriptase polymerase chain reaction (RT-PCR) using a primer specific to the 5′ untranslated region (5′ UTR), in some cases corresponding to a portion of the promoter sequence in the expression vector, located upstream of the transgene in the transcribed RNA, and a primer specific to the 3′ untranslated region (3′ UTR), located downstream of the expressed transgene in the transcribed RNA sequence or a primer specific to a sequence within the transgene. In particular embodiments, at least one primer complementary to a sequence in the 5′ untranslated region (UTR) and at least one primer complementary to a sequence in the 3′ untranslated region (UTR) are employed to amplify the transgene. An exemplary depiction of the amplification of a transcript and resulting product using a forward primer specific to the 5′ UTR and a primer specific to a nucleotide sequence in the 3′ UTR and a predicted amplified product, where no splice events have occurred, is provided in **FIG. 21A**. An exemplary depiction of exemplary multiple amplified products (i.e., heterogeneity) resulting from amplification of a transcript that has a 5′ UTR, with a transcribed promoter sequence that contains a known splice donor site (P-SD) and a known splice acceptor site (P-SD), a transcribed transgene containing an unknown (cryptic) splice donor site (T-SD) and two unknown (cryptic) splice acceptor sites (T-SA) and a 3′ UTR, using primers specific to regions of the 5′ UTR and 3′ UTR, is shown in **FIG. 21B**.

[0272] Exemplary primers specific for the 5′ untranslated region (UTR) include primers directed to sequences within the promoter of the transgene. In some examples, a primer specific to an EF1a/HTLV promoter. An exemplary forward primer, specific to an EF1a-HTLV promoter is set forth in SEQ ID NO: 150.

[0273] Exemplary primers specific for the 3′ untranslated region (UTR) include primers directed to 3′ posttranscriptional regulatory elements located downstream of the transgene. Exemplary 3′ posttranscriptional regulatory elements include the woodchuck hepatitis virus (WHP) posttranscriptional regulatory element (WPRE), set forth in SEQ ID NO:253. An exemplary forward primer, specific to a WPRE is set forth in SEQ ID NO: 235.

[0274] In some embodiments, multiple primer pairs can be used to amplify the transgene, such as for long transgenes. In some embodiments, sequential or nested pairs of forward and reverse primers, to crease a sliding window of amplified products, can be used to gain full and overlapping coverage of the

sequence. Typically, the primers are designed to amplify a length of transgene that is approximately 1.5-6 kb, 2-6 kb, or 3-6 kb. An exemplary depiction of the amplification of a transcript using nested primer pairs is provided in **FIG. 21C**.

[0275] The amplified nucleic acid sequence is then analyzed for heterogeneity in terms of amplified transcript lengths. In some examples, heterogeneity is determined by the number and intensity of the bands for the expressed sequence. In some embodiments, RNA sequences having splice events upon expression generate multiple bands with different mobilities. In some embodiments, a major band is detected at the predicted mobility for a sequence not having any unpredicted splice events, and 1 or more additional bands of varying intensities and mobilities indicate the occurrence of one or more cryptic splice events within the transgene sequence.

[0276] The skilled artisan can resolve RNA, such as messenger RNA, and analyze the heterogeneity thereof by several methods. Non-limiting, exemplary methods include agarose gel electrophoresis, chip-based capillary electrophoresis, analytical centrifugation, field flow fractionation, and chromatography, such as size exclusion chromatography or liquid chromatography.

[0277] One or more steps of the above techniques can be performed under denaturing conditions, partially denaturing conditions, or non-denaturing conditions. The denaturing conditions can include conditions that cause denaturing of the nucleic acid transcript (e.g., mRNA) due to temperature, chaotropic agents (including salts), organic agents, among other mechanisms for denaturing. With thermal denaturing conditions, an elevated temperature can be applied. The elevated temperature can be one that is sufficient to denature intramolecular hydrogen bonds, to cause a change in or loss of secondary or tertiary structure, and so forth. For example, the temperature or thermal denaturing conditions can include a temperature of 25 degrees Celsius to 95 degrees Celsius, 35 to 85 degrees Celsius, 55 to 75 degrees Celsius, or of another range within those ranges. Similarly, higher or lower temperatures can be used as appropriate to cause the desired level of denaturing. The temperature or thermal denaturing conditions can also be dependent on the identity of the nucleic acid transcript, such that different temperatures are used for different nucleic acid transcripts or types of nucleic acid transcripts. The denaturing conditions can also include using chaotropic agents, such as lithium perchlorate and other perchlorate salts, guanidinium chloride and other guanidinium salts, urea, butanol, ethanol, lithium acetate, magnesium chloride, phenol, propanol, sodium dodecyl sulfate, thiourea, or others. The denaturing conditions can further include organic denaturing agents, such as dimethyl sulfoxide (DMSO), acetonitrile, and glyoxal. In addition, the denaturing conditions can include a combination of two or more of these types of denaturing conditions. Any one or more of the steps of the RNA heterogeneity determining techniques can be performed at an elevated temperature or at ambient temperature, with or without chaotropic or organic agents.

a) Gel Electrophoresis

[0278] In some embodiments, RNA transcript topology and apparent (hydrodynamic) size can be

analyzed by gel electrophoresis, such as agarose gel electrophoresis. In some examples, RNA transcript can be resolved on a 0.05% to 2% agarose gel, such as a 1.2% agarose gel, and visualized by staining or using probes that are specific to a particular sequence. In some embodiments, RNA transcripts can be directly assessed by gel electrophoresis, or can be assessed after amplification, such as quantitative amplification methods. Nucleic acid stains for visualizing nucleic acid on agarose gel are well known. Exemplary stains include BlueView™ Nucleic Acid Stain (Millipore Sigma), SYBR® Gold Nucleic Acid Stain (ThermoFisher), SYBR® Green Nucleic Acid Stain (Millipore Sigma), SYBR® Green II (ThermoFisher), PicoGreen® nucleic acid stain (Invitrogen), and ethidium bromide: 0.5 µg/mL prepared in distilled water, or incorporated into the gel. In some examples, the nucleic acid is stained using Quant-iT™ PicoGreen ® binding followed by fluorescence detection and quantitation of the amplified products. The agarose gel method gives a more quantitative, but less resolving, measure of size distribution. In some embodiments, the nucleic acid fragments, resolved by agarose gel electrophoresis can be visualized by Northern blot for RNA or Southern blot for amplified reverse transcriptase-polymerase chain reaction (RT-PCR) products.

b) Chip-based Capillary Electrophoresis

[0279] Chip-based capillary electrophoresis (e.g., with the AGILENT 2100 BIOANALYZER™) can be used a rapid and routine method for monitoring RNA transcript integrity and its size distribution. The separation is based on hydrodynamic size and charge, and is affected by the nucleotide length and folded structure of the RNA transcript. In one embodiment, the method includes delivering the sample into a channel of a chip with an electrolyte medium and applying an electric field to the chip that causes the RNA transcript and the impurities migrate through the channel. The RNA transcript has a different electrophoretic mobility than the impurities such that the RNA transcript migrates through the channel at rate that is different from a rate at which the impurities migrate through the channel. The electrophoretic mobility of the RNA transcript is proportional to an ionic charge the RNA transcript and inversely proportional to frictional forces in the electrolyte medium. The method also includes collecting from the chip the sample comprising the RNA transcript and one or more separate portions of the sample comprising the impurities. In addition, the method includes characterizing an aspect of at least one of the portion of the sample comprising the RNA transcript and the one or more separate portions of the sample comprising the impurities. The characterizing can include, for example, quantifying charge variants.

c) Analytical Ultracentrifugation (AUC)

[0280] Analytical ultracentrifugation (AUC) is a solution phase method for measuring molecular weight distribution, without the potential artifacts that could be introduced by matrix (resin or gel) interaction in the SEC, agarose, or other methods. Both equilibrium AUC and sedimentation ultracentrifugation are used, and the latter provides sedimentation coefficients that are related to both size and shape of the RNA transcript. A BECKMAN™ analytical ultracentrifuge equipped with a scanning

UV/visible optics is used for analysis of the RNA transcript.

d) Field Flow Fractionation (FFF)

[0281] Another solution phase method for assessing hydrodynamic size distribution is field flow fractionation (FFF). FFF is a separation technique where a field is applied to a fluid suspension or solution pumped through a long and narrow channel, perpendicular to the direction of flow, to cause separation of the polynucleotides (RNA transcripts) present in the fluid, under the force exerted by the field. The field can be asymmetrical flow through a semi-permeable membrane, gravitational, centrifugal, thermal-gradient, electrical, magnetic etc.

e) Chromatography

[0282] Chromatography also can be used to detect heterogeneity of RNA transcript lengths. Methods of size exclusion chromatography and liquid chromatography for determining mRNA heterogeneity are described in WO2014144711 which is incorporated herein by reference.

B. Methods of Optimizing Polynucleotides, e.g., Polynucleotides Encoding BCMA CARs

[0283] In some embodiments, the provided methods include optimizing and/or modifying the polynucleotide, for example, to reduce RNA heterogeneity and/or removing or eliminating cryptic or undesired splice sites. In some aspects, provided are methods of reducing the heterogeneity of an expressed transgene transcript that involves identifying a transgene candidate for the removal of splice sites, such as by the methods described above in Section I.A; identifying one or more potential splice donor and/or splice acceptor sites; and modifying the nucleic acid sequence at or near the one or more identified splice donor sites that were identified, thereby generating a modified polynucleotide. In some aspects, the methods also involve assessing the transgene candidacy for the removal of splice sites. In some embodiments, the methods also include repeating one or more steps above until the heterogeneity of the transcript is reduced compared to the initial heterogeneity of the transcript as determined (such as before modification).

[0284] In some embodiments, methods of reducing heterogeneity, such as by removal or elimination of predicted splice sites, can be performed after codon optimization, or on non codon-optimized RNA. In some aspects, the methods involve identifying splice sites, such as one or more potential splice donor and/or acceptor sites, and modifying or change the RNA sequence (e.g., by replacing or substituting one or more nucleotides at or near the splice site. In some embodiments, codon optimization can be performed prior to and/or after methods of reducing heterogeneity of transcribed RNA (e.g., mRNA), such as by removal or elimination of predicted splice sites. In some embodiments, whether a transcript is a candidate for reducing RNA heterogeneity is determined based on the method of measuring RNA heterogeneity, e.g., as described in Section II.A herein. In some aspects, a transcribed nucleic acid that is detected as having heterogeneity is identified as a transgene candidate for removal of one or more splice

site. In some embodiments, a transgene sequence can be a candidate for reducing heterogeneity when the transcribed nucleic acid of the transgene candidate exhibits at least or at least about 5%, 10%, 15%, 20%, 25%, 30%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75% or more heterogeneity following expression in a cell. In some embodiments, following transcription and processing of the polynucleotide in a human cell, optionally a human T cell, the messenger RNA (mRNA) from the polynucleotide, exhibits at least 70%, 75%, 80%, 85%, 90%, or 95% RNA homogeneity.

1. Methods of Reducing RNA Heterogeneity

[0285] Provided are methods of reducing heterogeneity of an expressed transgene transcript. In some embodiments, the methods involve identifying one or more potential splice donor and/or splice acceptor sites and modifying the nucleic acid sequence at or near the one or more of the identified splice donor sites. In some embodiments, the methods also involve assessing the transgene candidacy for removal of splice sites. In some aspects, one or more steps described herein can be repeated, for example, until the potential RNA heterogeneity is reduced compared to the starting or unmodified transcript.

a) Splice Site Identification

[0286] In some aspects, the presence of potential cryptic splice sites (splice donor and/or acceptor sites that are present in a transcript, such as a transgene transcript, can result in RNA heterogeneity of the transcript following expression in a cell. In some embodiments, the methods involve identifying one or more potential splice sites that can be present in the transgene transcript, that are not desired and/or that may be created in a transgene transcript from various underlying sequences, following codon optimization of a transcript and/or by mutation or mistake or error in transcription. In some aspects of the provided embodiments, the splice donor sites and splice acceptor sites are identified independently. In some embodiments, the splice acceptor and/or donor site(s) is/are canonical, non-canonical, and/or cryptic splice acceptor and/or donor site(s).

[0287] In some embodiments, the provided methods include identifying one or more potential splice site (e.g., canonical, non-canonical, and/or cryptic splice acceptor and/or donor site(s) or branch sites) in a polynucleotide, such as a polynucleotide encoding a transgene, such as a recombinant receptor, that may exhibit RNA heterogeneity or contain undesired. Also provided are polypeptides having reduced numbers of such splice sites as compared to such reference polynucleotides.

[0288] In some aspects, identification of the one or more splice sites in a nucleic acid sequence is an iterative process. In some embodiments, splice sites can be identified using a splice site and/or codon optimization prediction tool, such as by submitting the starting or reference sequence encoding the transgene, such as a BCMA-binding receptor, e.g., anti-BCMA CAR, to a database, a gene synthesis vendor or other source able to computationally or algorithmically compare the starting or reference sequence to identify or predict splice sites and/or for codon optimization and/or splice site removal. In

some embodiments, after modifying the sequence for codon optimization and/or splice site removal, one or more further assessment of a sequence, such as a revised or modified nucleic acid sequence, is carried out to further evaluate for splice site removal, such as cryptic splice sites, using one or more other or additional splice site prediction tool(s).

[0289] In some aspects, RNA heterogeneity can be a result of the activity of the spliceosome present in a eukaryotic cell. In some aspects, splicing is typically carried out in a series of reactions catalyzed by the spliceosome. Consensus sequences for splice sites are known, but in some aspects, specific nucleotide information defining a splice site may be complex and may not be readily apparent based on available methods. Cryptic splice sites are splice sites that are not predicted based on the standard consensus sequences and are variably activated. Hence, variable splicing of pre-mRNA at cryptic splice sites leads to heterogeneity in the transcribed mRNA products following expression in eukaryotic cells. In some cases, within spliceosomal introns, a donor site (usually at the 5' end of the intron), a branch site (near the 3' end of the intron) and an acceptor site (3' end of the intron) are required for a splicing event. The splice donor site can include a GU sequence at the 5' end of the intron, with a large less highly conserved region. The splice acceptor site at the 3' end of the intron can terminate with an AG sequence.

[0290] In some embodiments, splice sites, including potential cryptic splice sites can be identified by comparing sequences to known splice site sequences, such as those in a sequence database. In some embodiments, splice sites can be identified by computationally by submitting nucleotide sequences for analysis by splice site prediction tools, such as Human Splice Finder (Desmet et al., Nucl. Acids Res. 37(9):e67 (2009)), a neural network splice site prediction tool, NNSplice (Reese et al., *J. Comput. Biol.*, 4(4):311 (1997)), GeneSplicer (Pertea et al., Nucleic Acids Res. 2001 29(5): 1185–1190) or NetUTR (Eden and Brunak, Nucleic Acids Res. 32(3):1131 (2004)), which identify potential splice sites and the probability of a splicing event at such sites. Additional splice prediction tools include RegRNA, ESEfinder, and MIT splice predictor. Splice site prediction tools such as GeneSplicer has been trained and/or tested successfully on databases for different species, such as human, Drosophila melanogaster, Plasmodium falciparum, Arabidopsis thaliana, and rice. In some embodiments, different prediction tools may be adapted for different extents on different database and/or for different species. In some embodiments, the one or more prediction tools are selected based upon their utility in certain database and/or for certain species. *See*, e.g., Saxonov et al., (2000) Nucleic Acids Res., 28, 185-190.

[0291] In some embodiments, one or more splice site prediction tools are selected for use in the determination of potential splice donor and/or acceptor sites. In some embodiments, splice site prediction tools that can be run locally; that can be retrained with a set of data at the user site; that can use databases for particular species (such as human), that can be compiled for multiple platforms, that allow real-time predictions for sequence selections, and/or that is an OSI certified open source software such that particular tools or plugins can be modified, can be employed. Exemplary tools that can be employed include NNSplice, GeneSplicer or both..

[0292] In some aspects, the splice site prediction tools be used to identify a list of potential splice donor and/or splice acceptor sites in a sequence such as a polynucleotide sequence containing transgene sequences. In some aspects, the prediction tools also can generate one or more prediction scores for one or more sequences in the polynucleotide, that can indicate the likelihoods of the one or more sequences being a splice donor or acceptor site sequence.

[0293] In some embodiments, the method involves comparing the prediction score for a particular splice site with a threshold score or reference score to determine or identify a particular splice sites that are candidate for elimination or removal. For example, in some embodiments, the predicted splice site is identified as a potential splice site when the prediction score is greater or no less than the threshold score or reference score. In some aspects, considerations for eliminating or removing a particular splice site include the prediction score as compared to a reference score or a threshold score; and whether a particular splice site is desired or intentional (for example, when the splicing event is more advantageous or is required for regulation of transcription and/or translation). In some aspects, the likelihood that the resulting splice variant loses the desired function or has compromised function can also be considered when determining particular donor and/or acceptor sites for elimination or removal. In some aspects, the one or more potential splice donor and/or splice acceptor sites exhibit a score about or at least about 0.7, 0.75, 0.8, 0.85, 0.9, 0.95, or 1.0 (e.g., on a scale with a maximum of 1.0) of a splice event or probability of a splice event, and the site can be a candidate for splice site elimination or removal. In some aspects, the score, e.g., used by GeneSplicer, at the one or more potential splice donor and/or splice site is based on the difference between the log-odds score returned for that sequence by the true Markov model and the score is computed by the false Markov model. In particular embodiments, the splice donor sites and splice acceptor sites are evaluated independently, or individually. In some embodiments, splice donor sites and splice acceptor sites are evaluated as a splice donor/acceptor pair.

b) Splice Site Elimination

[0294] In some embodiments, the provided methods involve eliminating or eliminating one or more splice donor and/or splice acceptor site(s), such as the potential splice donor and/or acceptor sites that may be involved in a cryptic splicing event that is not desired or that results in undesired RNA heterogeneity. In some embodiments, eliminating one or more splice sites comprises modifying one or more nucleotides (e.g., by substitution or replacement) in at, containing or near the splice donor and/or acceptor sites that are candidates for removal. In some aspects, a particular nucleotide within a codon that is at, contains or is near the splice site is modified (e.g., substituted or replaced). In some aspects, the modification (such as substitution or replacement) retains or preserves the amino acid encoded by the particular codon at the site, at the same time removing the potential splice donor and/or acceptor sites.

[0295] In some embodiments, the codon at or near the splice site for modification comprises one or more codons that involve one or both of the two nucleotides at the potential splice site (in some cases referred to as "splice site codon"). When the potential splicing is predicted to occur between two

nucleotides in a codon, the codon is the only splice site codon for this splice site. If the potential splicing is predicted to occur between two adjacent codons, for example, between the last nucleotide of the first codon and the first nucleotide of the next codon, the two codons are splice site codons. For example, for splice sites that are predicted to be at boundaries of two codons, the two adjacent codons can be candidates for nucleotide modification. In some embodiments, the one or more codons comprise one splice site codon. In some embodiments, the one or more codons comprise both splice site codons. In some embodiments, the method involves eliminating potential splice donor site by modifying one or both splice site codons. In some embodiments, the method involves eliminating a potential splice acceptor donor site by modifying one or both splice site codons. In some embodiments, the one or both codons at the splice site is not modified, for example, when there are no synonymous codon for the splice site codon. In some embodiments, if there are no synonymous codons available for the particular splice site codon, one or more nucleotides in a nearby codon can be modified. In some embodiments, one or more codons that are modified include a splice site codon, wherein the modification comprises changing one or both nucleotides at the splice site to a different nucleotide or different nucleotides. In some embodiments, In some embodiments, the method involves eliminating the splice donor site by modifying one or both splice site codons, wherein the modification does not change one or two of the nucleotides of the at the splice site to a different nucleotide, but a nearby nucleotide, e.g., a part of a codon adjacent to the splice site, is modified. In some embodiments, the nearby or adjacent nucleotides that can be modified include modification of a nucleotide that is a part of a nearby or adjacent codon, such as a codon that is within one, two, three, four, five, six, seven, eight, nine, or ten codons upstream or downstream of the splice site codon.

[0296] In some cases, manual modification of the polynucleotides can be employed, while preserving the encoded amino acid sequence, to reduce the probability of a predicted splice site. In some embodiments, one or more of the predicted splice sites having at least 80%, 85%, 90%, or 95% probability of a splice site are manually modified to reduce the probability of the splicing event. In some embodiments, the one or more modification(s) is/are by nucleotide replacement or substitution of 1, 2, 3, 4, 5, 6 or 7 nucleotides. In some embodiments, the modification(s) is/are at the junction of the splice donor site or are at the junction of the splice acceptor site. In some embodiments, at least one of the one or more nucleotide modifications is within 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 residues of the splice site junction of the splice acceptor and/or splice donor site. In some embodiments, libraries of modified nucleic acid sequences can be generated with reduced probability of cryptic splice sites. In some embodiments, splice donor sites and splice acceptor sites are evaluated as a splice donor/acceptor pair. In particular embodiments, the splice donor sites and splice acceptor sites are evaluated independently, or individually, and not part as a splice donor/acceptor pair. In some embodiments, one or more predicted splice sites are not eliminated. In some embodiments, splice sites, such as known or predicted splice sites, within the promoter region of the transcript are not eliminated.

[0297] In some embodiments, the method involves eliminating one or more potential donor splice site by modifying one or two splice site codons or one or more nearby or adjacent codons (for example, if a synonymous codon is not available for the splice site codon). In some embodiments, the method involves eliminating one or more potential acceptor splice site by modifying one or two splice site codons or one or more nearby or adjacent codons (for example, if a synonymous codon is not available for the splice site codon). In some embodiments, the nearby or adjacent codon that is subject to modification include a codon that is within one, two, three, four, five, six, seven, eight, nine or ten codons upstream or downstream of the splice site codon, such as a codon that is within one, two or three codons from the splice site. In some embodiments, the methods can include removal or elimination of a potential branch site for splicing. In some aspects, a nucleotide within the codon at or near the branch site can be modified, e.g., substituted or replaced, to eliminate cryptic splicing and/or reduce RNA heterogeneity. In some embodiments, the modification of the one or more nucleotides can involve a substitution or replacement of one of the nucleotides that may be involved in splicing (such as at the splice donor site, splice acceptor site or splice branch site), such that the amino acid encoded by the codon is preserved, and the nucleotide substitution or replacement does not change the polypeptide sequence that is encoded by the polynucleotide. In some cases, the third position in the codon is more degenerate than the other two positions. Thus, various synonymous codons can encode a particular amino acid (see, e.g., Section II.B.2 below). In some embodiments, the modification includes replacing the codon with a synonymous codon used in the species of the cell into which the polynucleotide is introduced (e.g., human). In some embodiments, the species is human. In some embodiments, the one or more codon is replaced with a corresponding synonymous codons that the most frequently used in the species or synonymous codons that have a similar frequency of usage (e.g., most closest frequency of usage) as the corresponding codon (see, e.g., Section II.B.2 below).

[0298] In some embodiments, the methods also involve assessing the transgene candidacy for the removal of splice sites, after initial proposed modification. In some aspects, the proposed modification can be evaluated again, to assess the proposed modification and identify any further potential splice sites after modification and/or codon optimization. In some aspects, after modifying the sequence for codon optimization and/or splice site removal, one or more further assessment of a sequence, such as a revised or modified nucleic acid sequence, is carried out to further evaluate for splice site removal, such as cryptic splice sites, using the same or one or more other or additional splice site prediction tool(s). In some aspects, proposed modifications are considered for subsequent steps, and iterative optimization can be used. In some aspects, the methods also include repeating any of the identification and/or modification step, for example, until heterogeneity of the transcript is reduced compared to the heterogeneity of the transcript as initially determined. In some embodiments, a further or a different modification, such as with a different nucleotide replacement at the same codon or a modification at a different position or codon, can be done after an iterative evaluation and assessment. In some embodiments, corresponding

different synonymous codon can be used, such as the second most frequently used in the particular species or a codon that has a similar frequency of usage (e.g., the next closest frequency of usage) as the corresponding codon (see, e.g., Section II.B.2 below).

[0299] In some aspects, a proposed modification can be further evaluated, for example, to assess whether the modification generates an undesired or additional restriction site in the polynucleotide. In some aspects, an additional restriction site may not be desired, and a further or a different modification (e.g., with a different nucleotide replacement at the same codon or a modification at a different position or codon) can be considered. In some aspects, particular restriction site, such as a designated restriction site, is avoided. In some aspects, if the modification does not substantially reduce or, the splice site prediction score, an additional or alternative modification can be proposed. In some embodiments, the splice site prediction score can be is reduced or lowered by at least about 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70% or 75%, after one or more iteration of the methods.

[0300] In some embodiments of any of the methods provided herein, a computer system can be used to execute one or more steps, tools, functions, processes or scripts. In certain embodiments, methods provided herein are computer implemented methods and/or are performed with the aid of a computer. In some embodiments, the splice site prediction, evaluation and modification for elimination or removal of a splice site can be performed by computer implemented methods and/or by methods which include steps that are computer implemented steps. In some embodiments, comparison of the sequences to a known database, calculating a splice site prediction score, determining potential nucleotide modifications, codon optimization and/or any one of the iterative steps can be implemented by a computer or using a computer-implemented steps, tools, functions, processes or scripts. In particular embodiments, a computer system comprising a processor and memory is provided, wherein the memory contains instructions operable to cause the processor to carry out any one or more of steps of the methods provided herein. In some embodiments, the methods include steps, functions, processes or scripts that are performed computationally, e.g., performed using one or more computer programs and/or via the use of computational algorithms.

[0301] Exemplary steps, functions, processes or scripts of the provided methods for identifying and/or removing possible splice sites include one or more steps of: selecting sequence, writing FASTA format sequences, loading codon table (e.g., from www.kazusa.or.jp/codon), running GeneSplicer, loading predictions, parsing codons, determining overlaps in prediction, identifying next highest usage synonymous codon, reviewing for restriction site, creating annotations or assessing other codons. Particular steps can assess both forward and reverse strands. In some aspects, previously annotated splice site modifications can also be considered, to allow for iterative optimization. In some embodiments, any one or more of the steps, functions, processes or scripts can be repeated.

[0302] In certain embodiments, methods provided herein may be practiced, at least in part, with computer system configurations, including single-processor or multi-processor computer systems,

minicomputers, mainframe computers, as well as personal computers, hand-held computing devices, microprocessor-based and/or programmable consumer electronics and the like, each of which may operatively communicate with one or more associated devices. In particular embodiments, the methods provided herein may be practiced, at least in part, in distributed computing environments such that certain tasks are performed by remote processing devices that are linked through a communications network. In a distributed computing environment, program modules may be located in local and/or remote memory storage devices. In particular embodiments, some or all steps of the methods provided herein may be practiced on stand-alone computers.

[0303] In particular embodiments, some or all of the steps of the methods provided herein can operate in the general context of computer-executable instructions, such as program modules, plugins and/or scripts executed by one or more components. Generally, program modules include routines, programs, objects, data structures and/or scripts, that perform particular tasks or implement particular abstract data types. Typically, the functionality of the program modules may be combined or distributed as desired. In certain embodiments, instructions operable to cause the processor to carry out any one or more steps of the methods provided herein can be embodied on a computer-readable medium having computer-executable instructions and transmitted as signals manufactured to transmit such instructions as well as the results of performing the instructions, for instance, on a network. In some embodiments, also provided are computer systems, computer readable instructions, software, systems, networks and/or devices for carrying out or performing one or more steps of the methods provided herein.

2. Codon optimization

[0304] In some embodiments the polynucleotides are modified by optimization of the codons for expression in humans. In some aspects, codon optimization can be considered before and/or after the steps for splice site identification and/or splice site elimination, and/or at each of the iterative steps for reducing RNA heterogeneity. Codon optimization generally involves balancing the percentages of codons selected with the abundance, e.g., published abundance, of human transfer RNAs, for example, so that none is overloaded or limiting. In some cases, such balancing is necessary or useful because most amino acids are encoded by more than one codon, and codon usage generally varies from organism to organism. Differences in codon usage between transfected or transduced genes or nucleic acids and host cells can have effects on protein expression from the nucleic acid molecule. **Table 3** below sets forth an exemplary human codon usage frequency table. In some embodiments, to generate codon-optimized nucleic acid sequences, codons are chosen to select for those codons that are in balance with human usage frequency. The redundancy of the codons for amino acids is such that different codons code for one amino acid, such as depicted in **Table 3**. In selecting a codon for replacement, it is desired that the resulting mutation is a silent mutation such that the codon change does not affect the amino acid sequence. Generally, the last nucleotide of the codon (e.g., at the third position) can remain unchanged without affecting the amino acid sequence.

Table 3. Human Codon Usage Frequency

Human codon	amino acid	freq./ 1000	number		Human codon	amino acid	freq./ 1000	number
TTT	F	17.6	714298		TCT	S	15.2	618711
TTC	F	20.3	824692		TCC	S	17.7	718892
TTA	L	7.7	311881		TCA	S	12.2	496448
TTG	L	12.9	525688		TCG	S	4.4	179419
CTT	L	13.2	536515		CCT	P	17.5	713233
CTC	L	19.6	796638		CCC	P	19.8	804620
CTA	L	7.2	290751		CCA	P	16.9	688038
CTG	L	39.6	1611801		CCG	P	6.9	281570
ATT	I	16	650473		ACT	T	13.1	533609
ATC	I	20.8	846466		ACC	T	18.9	768147
ATA	I	7.5	304565		ACA	T	15.1	614523
ATG	M	22	896005		ACG	T	6.1	246105
GTT	V	11	448607		GCT	A	18.4	750096
GTC	V	14.5	588138		GCC	A	27.7	1127679
GTA	V	7.1	287712		GCA	A	15.8	643471
GTG	V	28.1	1143534		GCG	A	7.4	299495
TAT	Y	12.2	495699		TGT	C	10.6	430311
TAC	Y	15.3	622407		TGC	C	12.6	513028
TAA	*	1	40285		TGA	*	1.6	63237
TAG	*	0.8	32109		TGG	W	13.2	535595
CAT	H	10.9	441711		CGT	R	4.5	184609
CAC	H	15.1	613713		CGC	R	10.4	423516
CAA	Q	12.3	501911		CGA	R	6.2	250760
CAG	Q	34.2	1391973		CGG	R	11.4	464485
AAT	N	17	689701		AGT	S	12.1	493429
AAC	N	19.1	776603		AGC	S	19.5	791383
AAA	K	24.4	993621		AGA	R	12.2	494682
AAG	K	31.9	1295568		AGG	R	12	486463
GAT	D	21.8	885429		GGT	G	10.8	437126
GAC	D	25.1	1020595		GGC	G	22.2	903565
GAA	E	29	1177632		GGA	G	16.5	669873
GAG	E	39.6	1609975		GGG	G	16.5	669768

[0305] For example, the codons TCT, TCC, TCA, TCG, AGT and AGC all code for Serine (note that T in the DNA equivalent to the U in RNA). From a human codon usage frequency, such as set forth in **Table 3** above, the corresponding usage frequencies for these codons are 15.2, 17.7, 12.2, 4.4, 12.1, and 19.5, respectively. Since TCG corresponds to 4.4%, if this codon were commonly used in a gene synthesis, the tRNA for this codon would be limiting. In codon optimization, the goal is to balance the usage of each codon with the normal frequency of usage in the species of animal in which the transgene

is intended to be expressed.

C. Optimized Anti-BCMA CAR

[0306] In some embodiments, a starting or reference sequence encoding a transgene, such as a BCMA-binding receptor, e.g., anti-BCMA CAR, is assessed for codon optimization and/or splice site removal.

[0307] In some embodiments, the methods are carried out on an anti-BCMA CAR, such as a CAR containing an scFv antigen-binding domain specific to BCMA, a spacer, such as a spacer set forth in SEQ ID NO:174, a costimulatory signaling region, such as a costimulatory signaling domain from 4-1BB and a CD3 zeta signaling region. Exemplary identified splice donor sites and splice acceptor sites, and their corresponding scores, are listed in Tables 3 and 4 below for exemplary anti-BCMA CARs.

Table 4. Predicted Splice Donor Sites

	STARTING SEQUENCE			O/SSE SEQUENCE		
Region of Construct	**splice donor site**	**SEQ ID NO**	**Splice score**	**optimized splice donor site**	**SEQ ID NO**	**Splice score**
promoter	cgtctag**gt**aagttt	206	1	no change		<0.7
scFv-encoding						
BCMA-23	gaccaag**gt**gaccgt	207	N/A	caccaag**gt**gaccgt	215	0.54
BCMA-26	tgcactg**gt**accagc	208	0.55	no change		
BCMA-52	taaactg**gt**accagc	209	0.76	tgaactg**gt**atcagc	216	<0.7
BCMA-52	atctcct**gt**aaggt	210	0.79	atctct**ga**aatggt	217	<0.7
BCMA-52	ggtcaag**gt**actctg	211	0.85	ggccagg**gc**cacactg	218	<0.7
BCMA-55	gaggacag**gt**aagcgg	212	0.66	gaggacag**gc**aagagg	219	<0.5
BCMA-55	ggtcaag**gt**actctg	213	0.85	ggccagg**ga**accctg	220	<0.5
BCMA-55	tgcctcc**gt**gtctgc	214	<0.50	tgccagc**gt**tagtgc	221	0.60
Spacer-encoding						
	aatctaa**gt**acggac	222	0.65	agtctaa**at**acggac	189	<0.7
	tcaactg**gt**acgtgg	223	0.96	tcaactg**gt**atgtgg	190	<0.7
	tcaattg**gt**acgtgg	254	0.97	tcaactg**gt**atgtgg	190	<0.7
	acaatta**gt**aaggca	224	0.43	accatct**cc**aaggcc	191	<0.7
	accacag**gt**gtatac	225	0.42	gccccag**gt**ttacac	192	<0.7
CD3zeta signaling region-encoding	ttccagg**gt**ccgccg	226	0.74	tcagcag**at**ccgccg	193	<0.7
Truncated receptor surrogate marker - encoding						
	ctgctct**gt**gagtta	227	0.56	ctcctgt**gt**gaactc	194	<0.7
	acgcaaa**gt**gtgtaa	228	0.5	tcggaaa**gt**gtgcaa	195	<0.7
	caacatg**gt**cagttt	229	0.71	cagcacg**gc**cagttt	196	<0.7
	aacagag**gt**gaaaac	230	0.42	aaccggg**gc**gagaac	197	<0.7

Table 4. Predicted Splice Donor Sites

STARTING SEQUENCE			O/SSE SEQUENCE		
Region of Construct	**splice donor site**	**SEQ ID NO**	**Splice score**	**optimized splice donor site**	**SEQ ID NO**	**Splice score**
	ctggaggtgagcca	231	0.82	ctggaaggcgagcccc	198	<0.7
	tcttcatgtgagcgg	252	0.84	tgttcatgtgagcgg	199	<0.7

Table 5. Predicted Splice Acceptor Sites

STARTING SEQUENCE				O/SSE SEQUENCE		
Region of Construct	**splice acceptor site**	**SEQ ID NO**	**splice score**	**optimized splice acceptor site**	**SEQ ID NO**	**Splice score**
Promoter						
	tggctccgccttttcccgaggtggg ggagaaccgtatat	232	0.50	no change		
	tgaactgcgtccgccgtctaggtaagtt taaagctcaggtc	233	0.71	no change		
	ttctgttctgcgccgttacaggatccaag ctgtgaccggcgc	234	0.89	no change		
scFv-encoding						
BCMA-23	ctactacatgagctggatccgccaggc tccagggaaggggc	26	N/A	ctactatatgtcctggatcaggacaggca cctggcaagggcc	27	0.46
BCMA-23	ggctgattattattgtagctccatatggag gtagtaggtctt	25	N/A	ggcagattactattgttctaggctacggc ggcagcagatcct	28	0.55
BCMA-25	ctatgccatgtcctggttcaggcaggc accaggcaagggcc	43	0.95	ctatgccatgtcctggttcaagcaggc accaggcaagggcc	48	<0.7
BCMA-25	gtccgcctctgtgggcgataggggtgac cgtgacatgtcgcg	44	0.50	no change		
BCMA-25	gtgggctttatcgctctaaggcctacg gcggcaccacaga	45	0.55	no change		
BCMA-25	gtgacatgtcgcgcctcccaggcatc tctaactacctggc	46	0.67	no change		

Table 5. Predicted Splice Acceptor Sites

	STARTING SEQUENCE			O/SSE SEQUENCE		
Region of Construct	**splice acceptor site**	**SEQ ID NO**	**splice score**	**optimized splice acceptor site**	**SEQ ID NO**	**Splice score**
BCMA-25	tacagcgcctccaccctgc**ag**agcgg agtgccctcccggtt	47	0.66	no change		
BCMA-52	ctggccatcagtggcctcc**ag**tctgag gatgaggctgatta	78	<0.50	ctggctatttctggactgc**ag**agcgag gacgaggccgacta	80	0.62
BCMA-52	agatacagcccgtcctccaaggcca cgtcaccatctcagc	79	<0.50	agatacagccctagctttc**ag**ggccac gtgaccatcagcgc	81	0.67
BCMA-55	cgaggctgattattactgc**ag**ctcaaat acaagaagcagca	110	0.79	cgaggccgattactactgc**ag**cagca acacccggtccagca	111	<0.40
BCMA-55	gccctcaggggtttctaatc**g**cttctctg gctccaagtctg	109	<0.50	gcccagcggcgtgtccaat**ag**attcag cggcagcaagagcg	112	0.40
Spacer-encoding						
	cgccttgtcctcctgtcc**ag**ctcctcct gttgccggacct	203	0.84	cgccttgtcctcctgtccg**c**gctcctcct gttgccggacct	188	<0.7
	aagtttcttctgtattcc**ag**gctgaccg tggataaatctc	239	0.97	cagtttcttctgtatagt**ag**actcaccg tggataaatcaa	180	<0.7
	aagtttcttctgtattcc**ag**gctgaccg tggataaatctc	239	0.97	aagtttcttctgtattcc**ag**actgaccgt ggataaatctc	187	
	gggcaacgtgttctcttgc**ag**tgtcatg cacgaagccctgc	240	0.55	gggcaacgtgttcagctgc**ag**cgtgat gcacgaggccctgc	181	<0.7
	cagtttctcctgtatagt**ag**actcaccg tggataaatcaa	204	0.74	No change		
CD28 TM - encoding	aggggtgctggcctgttac**ag**cctgct ggtgacagtcgctt	141	0.4	cggagtgctggcctgttac**ag**cctgct ggttaccgtggcct	182	0.75

Table 5. Predicted Splice Acceptor Sites

	STARTING SEQUENCE			O/SSE SEQUENCE		
Region of Construct	**splice acceptor site**	**SEQ ID NO**	**splice score**	**optimized splice acceptor site**	**SEQ ID NO**	**Splice score**
4-1BB/ CD3zeta signaling region-encoding	gctgagagtcaagtttccag**g**tccgc cgacgctccagcct	3	0.55	gctgagagtgaagttcagc**ag**atccg ccgacgctccagcct	183	<0.7
Truncated Receptor Surrogate Marker-encoding						
	actcctcctctggatccacag**g**aactg gatattctgaaaac	249	0.74	acacctccactggatcccc**aa**gagct ggatatcctgaaaac	184	<0.7
	acagggttttgctgattc**ag**gcttggc ctgaaaacaggac	250	0.73	accggattcctcctgatcc**aa**gcctgg ccagagaacagaac	185	<0.7
	accggattcctcctgattc**ag**gcctgg ccagagaacagaac	205	0.82	accggattcctcctgatcc**aa**gcctgg ccagagaacagaac	185	<0.7
	atggtcagttttctcttgc**ag**tcgtcagc ctgaacataaca	251	0.89	acggccagtttagcctggc**tg**tggtgt ctctgaacatcacc	186	<0.7

[0308] In some embodiments, the resulting modified nucleic acid sequence(s) is/are then synthesized and used to transduce cells to test for splicing as indicated by RNA heterogeneity. Exemplary methods are as follows and described in the Examples. Briefly, RNA is harvested from the expressing cells, amplified by reverse transcriptase polymerase chain reaction (RT-PCR) and resolved by agarose gel electrophoresis to determine the heterogeneity of the RNA, compared to the starting sequence. In some cases, improved sequences can be resubmitted to the gene synthesis vendor for further codon optimization and splice site removal, followed by further cryptic splice site evaluation, modification, synthesis and testing, until the RNA on the agarose gel exhibits minimal RNA heterogeneity.

[0309] In some embodiments, the provided methods for optimizing a coding nucleic acid sequence encoding a transgene, such as an anti-BCMA CAR provided herein, or a construct provided herein, is to both reduce or eliminate cryptic splice sites (see, e.g., SEQ ID NO: 200 for an exemplary codon optimized and splice site eliminated spacer sequence) and optimize human codon usage (see, e.g., SEQ ID NO: 236 for an exemplary codon optimized and spacer sequence). An exemplary optimization strategy is described in the Examples.

[0310] In some embodiments, provided are polynucleotides encoding a chimeric antigen receptor, comprising nucleic acid encoding: (a) an extracellular antigen-binding domain that specifically recognizes BCMA, including any of the antigen-binding domains described below; (b) a spacer of at least 125 amino acids in length; (c) a transmembrane domain; and (d) an intracellular signaling region, wherein following expression of the polynucleotide in a cell, the transcribed RNA, optionally messenger RNA (mRNA), from the polynucleotide, exhibits at least 70%, 75%, 80%, 85%, 90%, or 95% RNA homogeneity. In some embodiments the antigen-binding domain comprises a V_H region and a V_L region comprising the amino acid sequence set forth in SEQ ID NOs:116 and 119, respectively, or a sequence of amino acids having at least 90% identity to SEQ ID NOS:116 and 119, respectively. In some embodiments, the antigen-binding domain comprises a V_H region that is or comprises a CDR-H1, CDR-H2 and CDR-H3 contained within the V_H region amino acid sequence selected from SEQ ID NO: 116 and a V_L region that is or comprises a CDR-L1, CDR-L2 and CDR-L3 contained within the V_L region amino acid sequence selected from SEQ ID NO: 119. In some embodiments, In some embodiments, the antigen-binding domain comprises a V_H region comprising a CDR-H1, CDR-H2, and CDR-H3 comprising the amino acid sequence of SEQ ID NOS:97, 101 and 103, respectively, and a V_L region comprising a CDR-L1, CDR-L2, and CDR-L3 comprising the amino acid sequence of SEQ ID NOS:105, 107 and 108, respectively; or a V_H region comprising a CDR-H1, CDR-H2, and CDR-H3 comprising the amino acid sequence of SEQ ID NOS:96, 100 and 103, respectively, and a V_L region comprising a CDR-L1, CDR-L2, and CDR-L3 comprising the amino acid sequence of SEQ ID NOS:105, 107 and 108, respectively; or a V_H region comprising a CDR-H1, CDR-H2, and CDR-H3

comprising the amino acid sequence of SEQ ID NOS: 95, 99 and 103, respectively, and a V_L region comprising a CDR-L1, CDR-L2, and CDR-L3 comprising the amino acid sequence of SEQ ID NOS:105, 107 and 108, respectively; or a V_H region comprising a CDR-H1, CDR-H2, and CDR-H3 comprising the amino acid sequence of SEQ ID NOS: 94, 98 and 102, respectively, and a V_L region comprising a CDR-L1, CDR-L2, and CDR-L3 comprising the amino acid sequence of SEQ ID NOS: 104, 106 and 108, respectively; or a V_H region that is or comprises the amino acid sequence set forth in SEQ ID NO: 116 and a V_L region that is or comprises the amino acid sequence set forth in SEQ ID NO: 119. In some embodiments, exemplary antigen-binding domain in the chimeric antigen receptor encoded by the polynucleotide include those described in each row of **Table 2** herein. In any of such embodiments, the transmembrane domain of the CAR is or comprises a transmembrane domain derived from a CD28; the intracellular signaling region comprises a cytoplasmic signaling domain of a CD3-zeta (CD3ζ) chain or a functional variant or signaling portion thereof and a costimulatory signaling region comprises an intracellular signaling domain of 4-1BB.

[0311] In some embodiments, provided are polynucleotides encoding a chimeric antigen receptor, comprising nucleic acid encoding: (a) an extracellular antigen-binding domain that specifically recognizes BCMA, including any of the antigen-binding domains described below; (b) (b) a spacer, wherein the encoding nucleic acid is or comprises, or consists or consists essentially of, the sequence set forth in SEQ ID NO:200 or encodes a sequence of amino acids set forth in SEQ ID NO:174; (c) a transmembrane domain; and (d) an intracellular signaling region. In some embodiments the antigen-binding domain comprises a V_H region and a V_L region comprising the amino acid sequence set forth in SEQ ID NOs:116 and 119, respectively, or a sequence of amino acids having at least 90% identity to SEQ ID NOS:116 and 119, respectively. In some embodiments, the antigen-binding domain comprises a V_H region that is or comprises a CDR-H1, CDR-H2 and CDR-H3 contained within the V_H region amino acid sequence selected from SEQ ID NO: 116 and a V_L region that is or comprises a CDR-L1, CDR-L2 and CDR-L3 contained within the V_L region amino acid sequence selected from SEQ ID NO: 119. In some embodiments, In some embodiments, the antigen-binding domain comprises a V_H region comprising a CDR-H1, CDR-H2, and CDR-H3 comprising the amino acid sequence of SEQ ID NOS:97, 101 and 103, respectively, and a V_L region comprising a CDR-L1, CDR-L2, and CDR-L3 comprising the amino acid sequence of SEQ ID NOS:105, 107 and 108, respectively; or a V_H region comprising a CDR-H1, CDR-H2, and CDR-H3 comprising the amino acid sequence of SEQ ID NOS:96, 100 and 103, respectively, and a V_L region comprising a CDR-L1, CDR-L2, and CDR-L3 comprising the amino acid sequence of SEQ ID NOS:105, 107 and 108, respectively; or a V_H region comprising a CDR-H1, CDR-H2, and CDR-H3 comprising the amino acid sequence of SEQ ID NOS: 95, 99 and 103, respectively, and a V_L region comprising a CDR-L1, CDR-L2, and CDR-L3 comprising the amino acid sequence of SEQ ID NOS:105, 107 and 108, respectively; or a V_H region

comprising a CDR-H1, CDR-H2, and CDR-H3 comprising the amino acid sequence of SEQ ID NOS: 94, 98 and 102, respectively, and a V_L region comprising a CDR-L1, CDR-L2, and CDR-L3 comprising the amino acid sequence of SEQ ID NOS: 104, 106 and 108, respectively; or a V_H region that is or comprises the amino acid sequence set forth in SEQ ID NO: 116 and a V_L region that is or comprises the amino acid sequence set forth in SEQ ID NO: 119. In some embodiments, exemplary antigen-binding domain in the chimeric antigen receptor encoded by the polynucleotide include those described in each row of **Table 2** herein. In any of such embodiments, the transmembrane domain of the CAR is or comprises a transmembrane domain derived from a CD28; the intracellular signaling region comprises a cytoplasmic signaling domain of a CD3-zeta (CD3ζ) chain or a functional variant or signaling portion thereof and a costimulatory signaling region comprises an intracellular signaling domain of 4-1BB.

[0312] Also provided herein are exemplary modified polynucleotides, including polynucleotides that were modified for codon optimization (O) and/or splice site elimination (SSE). Examples of such polynucleotides are set forth in **Table 6**, wherein exemplary nucleotide (nt) sequences for the components of the exemplary CAR constructs prior to splice site elimination and codon optimization (non-opt), nucleic acid (nt) sequences for the components of the CAR constructs following splice site elimination and optimization (O/SSE), and the corresponding amino acid (aa) sequences encoded by the nucleic acid sequences are provided. The components include the IgG-kappa signaling sequence (ss), the anti-BCMA scFv, spacer region, transmembrane (tm) domain, co-signaling sequence (4-1BB co-sig or CD28 co-sig), CD3-ζ signaling domain (CD3-ζ), T2A ribosomal skip element (T2A) and truncated EGF receptor (EGFRt) sequence. Polynucleotide sequences of exemplary CAR constructs are set forth in SEQ ID NOs: 9-14, encoding the amino acid sequences set forth in SEQ ID NOs: 15-20.

Table 6. Exemplary BCMA CAR components (SEQ ID NOs)

Construct	Sequence	ss	scFv	spacer	TM	4-1BB co-stim	CD3-ζ
BCMA-23-L CAR	non-opt (nt)	167	30	175	139	5	144
BCMA-23-L CAR CO/SSE	O/SSE (nt)	171	31	200 or 8	140	6	145
both	aa	166	29	174	138	4	143
BCMA-25-L CAR	non-opt (nt)	167	50	175	139	5	144
BCMA-25-L CAR CO/SSE	O/SSE (nt)	169	51	200 or 8	140	6	145
both	Aa	166	49	174	138	4	143
BCMA-26-L CAR	non-opt (nt)	167	59	175	139	5	144
BCMA-26-L CAR CO/SSE	O/SSE (nt)	168	60	200 or 8	140	6	145
both	aa	166	58	174	138	4	143
BCMA-52-L CAR	non-opt (nt)	167	82	175	139	5	144
BCMA-52-L CAR CO/SSE	O/SSE (nt)	169	84	200 or 8	140	6	145

Table 6. Exemplary BCMA CAR components (SEQ ID NOs)							
Construct	**Sequence**	**ss**	**scFv**	**spacer**	**TM**	**4-1BB co-stim**	**CD3-ζ**
both	Aa	166	83	174	138	4	143
BCMA-55-L CAR	non-opt (nt)	167	113	175	139	5	144
BCMA-55-L CAR CO/SSE	O/SSE (nt)	170	115	200 or 8	140	6	145
both	aa	166	114	174	138	4	143
Construct	**Sequence**	**ss**	**scFv**	**spacer**	**TM**	**CD28 co-stim**	**CD3-ζ**
BCMA-55-L-CD28 CAR	non-opt (nt)	167	113	175	139	137	144
BCMA-55-L-CD28 CAR CO/SSE	O/SSE (nt)	170	115	200 or 8	140	137	145
both	aa	166	114	174	138	136	143

III. ENGINEERED CELLS AND PROCESSES FOR PRODUCING ENGINEERED CELLS

[0313] Also provided are cells such as engineered cells that contain a recombinant receptor (*e.g.*, a chimeric antigen receptor) such as one that contains an extracellular domain including an anti-BCMA antibody or fragment as described herein. Also provided are populations of such cells, compositions containing such cells and/or enriched for such cells, such as in which cells expressing the BCMA-binding molecule make up at least 50, 60, 70, 80, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99 % or more of the total cells in the composition or cells of a certain type such as T cells or CD8+ or CD4+ cells. Among the compositions are pharmaceutical compositions and formulations for administration, such as for adoptive cell therapy. Also provided are therapeutic methods for administering the cells and compositions to subjects, *e.g.*, patients, and cells and pharmaceutical compositions for use in such methods.

[0314] Thus also provided are genetically engineered cells expressing the recombinant receptors containing the antibodies, *e.g.*, cells containing the CARs. The cells generally are eukaryotic cells, such as mammalian cells, and typically are human cells. In some embodiments, the cells are derived from the blood, bone marrow, lymph, or lymphoid organs, are cells of the immune system, such as cells of the innate or adaptive immunity, *e.g.*, myeloid or lymphoid cells, including lymphocytes, typically T cells and/or NK cells. Other exemplary cells include stem cells, such as multipotent and pluripotent stem cells, including induced pluripotent stem cells (iPSCs). The cells typically are primary cells, such as those isolated directly from a subject and/or isolated from a subject and frozen. In some embodiments, the cells include one or more subsets of T cells or other cell types, such as whole T cell populations, CD4+ cells, CD8+ cells, and subpopulations thereof, such as those defined by function, activation state, maturity, potential for

differentiation, expansion, recirculation, localization, and/or persistence capacities, antigen-specificity, type of antigen receptor, presence in a particular organ or compartment, marker or cytokine secretion profile, and/or degree of differentiation. With reference to the subject to be treated, the cells may be allogeneic and/or autologous. Among the methods include off-the-shelf methods. In some aspects, such as for off-the-shelf technologies, the cells are pluripotent and/or multipotent, such as stem cells, such as induced pluripotent stem cells (iPSCs). In some embodiments, the methods include isolating cells from the subject, preparing, processing, culturing, and/or engineering them, as described herein, and re-introducing them into the same patient, before or after cryopreservation.

[0315] Among the sub-types and subpopulations of T cells and/or of CD4+ and/or of CD8+ T cells are naïve T (T_N) cells, effector T cells (T_{EFF}), memory T cells and sub-types thereof, such as stem cell memory T (T_{SCM}), central memory T (T_{CM}), effector memory T (T_{EM}), or terminally differentiated effector memory T cells, tumor-infiltrating lymphocytes (TIL), immature T cells, mature T cells, helper T cells, cytotoxic T cells, mucosa-associated invariant T (MAIT) cells, naturally occurring and adaptive regulatory T (Treg) cells, helper T cells, such as TH1 cells, TH2 cells, TH3 cells, TH17 cells, TH9 cells, TH22 cells, follicular helper T cells, alpha/beta T cells, and delta/gamma T cells.

[0316] In some embodiments, the cells are natural killer (NK) cells. In some embodiments, the cells are monocytes or granulocytes, *e.g.*, myeloid cells, macrophages, neutrophils, dendritic cells, mast cells, eosinophils, and/or basophils.

[0317] In some embodiments, the cells include one or more polynucleotides introduced via genetic engineering, and thereby express recombinant or genetically engineered products of such polynucleotides. In some embodiments, the polynucleotides are heterologous, *i.e.*, normally not present in a cell or sample obtained from the cell, such as one obtained from another organism or cell, which for example, is not ordinarily found in the cell being engineered and/or an organism from which such cell is derived. In some embodiments, the polynucleotides are not naturally occurring, such as a polynucleotide not found in nature, including one comprising chimeric combinations of polynucleotides encoding various domains from multiple different cell types. In some embodiments, the cells (*e.g.*, engineered cells) comprise a vector (*e.g.*, a viral vector, expression vector, etc.) as described herein such as a vector comprising a nucleic acid encoding a recombinant receptor described herein.

[0318] In particular examples immune cells, such as human immune cells are used to express the provided polypeptides encoding chimeric antigen receptors. In some examples, the immune cells are T cells, such as CD4+ and/or CD8+ immune cells, including primary cells, such as primary CD4+ and CD8+ cells.

[0319] In particular embodiments, the engineered cells are produced by a process that generates an output composition of enriched T cells from one or more input compositions and/or from a single biological

sample. In certain embodiments, the output composition contains cells that express a recombinant receptor, e.g., a CAR, such as an anti-BCMA CAR. In particular embodiments, the cells of the output compositions are suitable for administration to a subject as a therapy, e.g., an autologous cell therapy. In some embodiments, the output composition is a composition of enriched CD4+ and CD8+ T cells.

[0320] In some embodiments, the process for generating or producing engineered cells is by a process that includes some or all of the steps of: collecting or obtaining a biological sample; isolating, selecting, or enriching input cells from the biological sample; cryopreserving and storing the input cells; thawing and/or incubating the input cells under stimulating conditions; engineering the stimulated cells to express or contain a recombinant polynucleotide, e.g., a polynucleotide encoding a recombinant receptor such as a CAR; cultivating the engineered cells to a threshold amount, density, or expansion; formulating the cultivated cells in an output composition; and/or cryopreserving and storing the formulated output cells until the cells are released for infusion and/or are suitable to be administered to a subject. In some embodiments, the entire process is performed with a single composition of enriched T cells, e.g., CD4+ and CD8+ T cells. In certain embodiments, the process is performed with two or more input compositions of enriched T cells that are combined prior to and/or during the process to generate or produce a single output composition of enriched T cells. In some embodiments, the enriched T cells are or include engineered T cells, e.g., T cells transduced to express a recombinant receptor.

[0321] In particular embodiments, an output composition of engineered cells expressing a recombinant receptor (e.g. anti-BCMA CAR) is produced from an initial and/or input composition of cells. In some embodiments, the input composition is a composition of enriched T cells, enriched CD4+ T cells, and/or enriched CD8+ T cells (herein after also referred to as compositions of enriched T cells, compositions of enriched CD4+ T cells, and compositions of enriched CD8+ T cells, respectively). In some embodiments, a composition enriched in CD4+ T cells contains at least 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 98%, 99%, or 99.9% CD4+ T cells. In particular embodiments, the composition of enriched CD4+ T cells contains 100% CD4+ T cells contains about 100% CD4+ T cells. In certain embodiments, the composition of enriched T cells includes or contains less than 20%, less than 10%, less than 5%, less than 1%, less than 0.1%, or less than 0.01% CD8+ T cells, and/or contains no CD8+ T cells, and/or is free or substantially free of CD8+ T cells. In some embodiments, the populations of cells consist essentially of CD4+ T cells. In some embodiments, a composition enriched in CD8+ T cells contains at least 75%, 80%, 85%, 90%, 95%, 98%, 99%, or 99.9% CD8+ T cells, or contains or contains about 100% CD8+ T cells. In certain embodiments, the composition of enriched CD8+ T cells includes or contains less than 20%, less than 10%, less than 5%, less than 1%, less than 0.1%, or less than 0.01% CD4+ T cells, and/or contains no CD4+ T cells, and/or is free or substantially free of CD4+ T cells. In some embodiments, the populations of cells consist essentially of

CD8+ T cells.

[0322] In particular embodiments, an output composition of engineered cells is produced from an initial or input composition of cell that is generated and/or made by combining, mixing, and/or pooling cells including from composition of cells containing enriched T cells, enriched CD4+ T cells, and/or enriched CD8+ T cells. In some embodiments, the input composition of cells is a composition of combined, mixed, and/or pooled CD4+ and CD8+ T cells. In particular embodiments, the input composition contains between 30% and 70%, between 35% and 65%, between 40% and 60%, between 45% and 55%, or about 50% or 50% CD4+ T cells and between 30% and 70%, between 35% and 65%, between 40% and 60%, between 45% and 55%, or about 50% or 50% CD8+ T cells. In certain embodiments, the input composition contains between 45% and 55%, about 50%, or 50% CD4+ T cells and between 45% and 55%, about 50%, or 50% CD8+ T cells.

[0323] In certain embodiments, the process for producing engineered cells further can include one or more of: activating and/or stimulating a cells, e.g., cells of an input composition; genetically engineering the activated and/or stimulated cells, e.g., to introduce a polynucleotide encoding a recombinant protein by transduction or transfection; and/or cultivating the engineered cells, e.g., under conditions that promote proliferation and/or expansion. In particular embodiments, the provided methods may be used in connection with harvesting, collecting, and/or formulating output compositions produced after the cells have been incubated, activated, stimulated, engineered, transduced, transfected, and/or cultivated.

[0324] In some embodiments, the one or more process steps are carried out, at least in part, in serum free media. In some embodiments, the serum free media is a defined or well-defined cell culture media. In certain embodiments, the serum free media is a controlled culture media that has been processed, e.g., filtered to remove inhibitors and/or growth factors. In some embodiments, the serum free media contains proteins. In certain embodiments, the serum-free media may contain serum albumin, hydrolysates, growth factors, hormones, carrier proteins, and/or attachment factors. In some embodiments, the serum free media includes cytokines. In some embodiments, the serum free media includes cytokines or recombinant cytokines. In some embodiments, the serum free media includes recombinant IL-2, IL-15, and/or IL-7. In some embodiments, the serum free media includes glutamine. In some embodiments, the serum free media includes glutamine and recombinant IL-2, IL-15, and IL-7.

[0325] In some embodiments, the serum-free media includes a basal media that contains one or more proteins or other additives. In some embodiments, all or a portion of the incubation is performed in basal media. In some embodiments, the basal medium contains a mixture of inorganic salts, sugars, amino acids, and, optionally, vitamins, organic acids and/or buffers or other well-known cell culture nutrients. In addition to nutrients, the medium also helps maintain pH and osmolality. In some aspects, the components of the

serum-free media support cell growth, proliferation and/or expansion.

[0326] A wide variety of commercially available basal media are well known to those skilled in the art, and include Dulbecco's Modified Eagles Medium (DMEM), Roswell Park Memorial Institute Medium (RPMI), Iscove modified Dulbecco's medium and Hams medium. In some embodiments, the basal medium is Iscove's Modified Dulbecco's Medium, RPMI- 1640, or α-MEM. In some embodiments, the basal media is a balanced salt solution (e.g., PBS, DPBS, HBSS, EBSS). In some embodiments, the basal media is selected from Dulbecco's Modified Eagle's Medium (DMEM), Minimal Essential Medium (MEM), Basal Medium Eagle (BME), F-10, F-12, RPMI 1640, Glasgow's Minimal Essential Medium (GMEM), alpha Minimal Essential Medium (alpha MEM), Iscove's Modified Dulbecco's Medium, and M199. In some embodiments, the base media is a complex medium (e.g., RPMI-1640, IMDM). In some embodiments, the base medium is OpTmizer™ CTS™ T-Cell Expansion Basal Medium (ThermoFisher).

[0327] In some embodiments, the basal medium further may comprises a protein or a peptide. In some embodiments, the at least one protein is not of non-mammalian origin. In some embodiments, the at least one protein is human or derived from human. In some embodiments, the at least one protein is recombinant. In some embodiments, the at least one protein includes albumin, transferrin, insulin, fibronectin, aprotinin or fetuin. In some embodiments, the protein comprises one or more of albumin, insulin or transferrin, optionally one or more of a human or recombinant albumin, insulin or transferrin.

[0328] In some embodiments, the protein is an albumin or albumin substitute. In some embodiments, the albumin is a human derived albumin. In some embodiments, the albumin is a recombinant albumin. In some embodiments, the albumin is a natural human serum albumin. In some embodiments, the albumin is a recombinant human serum albumin. In some embodiments, the albumin is a recombinant albumin from a non-human source. Albumin substitutes may be any protein or polypeptide source. Examples of such protein or polypeptide samples include but are not limited to bovine pituitary extract, plant hydrolysate (e.g., rice hydrolysate), fetal calf albumin (fetuin), egg albumin, human serum albumin (HSA), or another animal-derived albumins, chick extract, bovine embryo extract, AlbuMAX® I, and AlbuMAX® II. In some embodiments, the protein or peptide comprises a transferrin. In some embodiments, the protein or peptide comprises a fibronectin. In some embodiments, the protein or peptide comprises aprotinin. In some embodiments, the protein comprises fetuin.

[0329] In some embodiments, the one or more additional protein is part of a serum replacement supplement that is added to the basal medium. Examples of serum replacement supplements include, for example, Immune Cell Serum Replacement (ThermoFisher, #A2598101) or those described in Smith *et al. Clin Transl Immunology*. 2015 Jan; 4(1): e31.

[0330] In certain embodiments, the basal media is supplemented with additional additives. Additives to

cell culture media may include, but is not limited to nutrients, sugars, e.g., glucose, amino acids, vitamins, or additives such as ATP and NADH.

[0331] In some embodiments, the basal medium further comprises glutamine, such as L-glutamine. In some aspects, the glutamine is a free form of glutamine, such as L-glutamine. In some embodiments, the concentration of the glutamine, such as L-glutamine, in the basal medium is less than 200 mM, such as less than 150 mM, 100 mM or less, such as 20 mM to 120 mM, or 40 mM to 100 mM, such as or about 80 mM. In some embodiments, the concentration of L-glutamine is about 0.5 mM to about 5 mM (such as 2mM)..

[0332] In some embodiments, the basal medium further contains a synthetic amino acid, such as a dipeptide form of L-glutamine, e.g. L-alanyl-L-glutamine. In some embodiments, the concentration of the dipeptide form of L-glutamine (e.g., L-alanyl-L-glutamine) in the basal medium is about 0.5 mM-5mM. In some embodiments, the concentration of the dipeptide form of L-glutamine (e.g., L-alanyl-L-glutamine) in the basal medium is about 2 mM.

[0333] In some embodiments, the provided methods are carried out such that one, more, or all steps in the preparation of cells for clinical use, e.g., in adoptive cell therapy, are carried out without exposing the cells to non-sterile conditions. In some embodiments, the cells are selected, stimulated, transduced, washed, and formulated, all within a closed, sterile system or device. In some embodiments, the one or more of the steps are carried out apart from the closed system or device. In some such embodiments, the cells are transferred apart from the closed system or device under sterile conditions, such as by sterile transfer to a separate closed system.

A. Preparation of Cells for Engineering

[0334] In some embodiments, preparation of the engineered cells includes one or more culture and/or preparation steps. The cells for introduction of the recombinant receptor (*e.g.*, CAR) may be isolated from a sample, such as a biological sample, *e.g.*, one obtained from or derived from a subject. In some embodiments, the subject from which the cell is isolated is one having the disease or condition or in need of a cell therapy or to which cell therapy will be administered. The subject in some embodiments is a human in need of a particular therapeutic intervention, such as the adoptive cell therapy for which cells are being isolated, processed, and/or engineered.

[0335] Accordingly, the cells in some embodiments are primary cells, *e.g.*, primary human cells. The samples include tissue, fluid, and other samples taken directly from the subject, as well as samples resulting from one or more processing steps, such as separation, centrifugation, genetic engineering (*e.g.* transduction with viral vector), washing, and/or incubation. The biological sample can be a sample obtained directly from a biological source or a sample that is processed. Biological samples include, but are not limited to, body

fluids, such as blood, plasma, serum, cerebrospinal fluid, synovial fluid, urine and sweat, tissue and organ samples, including processed samples derived therefrom.

[0336] In some aspects, the sample from which the cells are derived or isolated is blood or a blood-derived sample, or is or is derived from an apheresis or leukapheresis product. Exemplary samples include whole blood, peripheral blood mononuclear cells (PBMCs), leukocytes, bone marrow, thymus, tissue biopsy, tumor, leukemia, lymphoma, lymph node, gut associated lymphoid tissue, mucosa associated lymphoid tissue, spleen, other lymphoid tissues, liver, lung, stomach, intestine, colon, kidney, pancreas, breast, bone, prostate, cervix, testes, ovaries, tonsil, or other organ, and/or cells derived therefrom. Samples include, in the context of cell therapy, *e.g.*, adoptive cell therapy, samples from autologous and allogeneic sources.

[0337] In some embodiments, the cells are derived from cell lines, *e.g.*, T cell lines. The cells in some embodiments are obtained from a xenogeneic source, for example, from mouse, rat, non-human primate, or pig.

[0338] In some embodiments, isolation of the cells includes one or more preparation and/or non-affinity based cell separation steps. In some examples, cells are washed, centrifuged, and/or incubated in the presence of one or more reagents, for example, to remove unwanted components, enrich for desired components, lyse or remove cells sensitive to particular reagents. In some examples, cells are separated based on one or more property, such as density, adherent properties, size, sensitivity and/or resistance to particular components.

[0339] In some examples, cells from the circulating blood of a subject are obtained, *e.g.*, by apheresis or leukapheresis. The samples, in some aspects, contain lymphocytes, including T cells, monocytes, granulocytes, B cells, other nucleated white blood cells, red blood cells, and/or platelets, and in some aspects contain cells other than red blood cells and platelets.

[0340] In some embodiments, the blood cells collected from the subject are washed, *e.g.*, to remove the plasma fraction and to place the cells in an appropriate buffer or media for subsequent processing steps. In some embodiments, the cells are washed with phosphate buffered saline (PBS). In some embodiments, the wash solution lacks calcium and/or magnesium and/or many or all divalent cations. In some aspects, a washing step is accomplished a semi-automated "flow-through" centrifuge (for example, the Cobe 2991 cell processor, Baxter) according to the manufacturer's instructions. In some aspects, a washing step is accomplished by tangential flow filtration (TFF) according to the manufacturer's instructions. In some embodiments, the cells are resuspended in a variety of biocompatible buffers after washing, such as, for example, Ca^{++}/Mg^{++} free PBS. In certain embodiments, components of a blood cell sample are removed and the cells directly resuspended in culture media.

[0341] In some aspects, for production of isolated or secreted polypeptides, in addition to prokaryotes, eukaryotic microbes such as filamentous fungi or yeast are suitable cloning or expression hosts for antibody-

encoding vectors, including fungi and yeast strains whose glycosylation pathways have been modified to mimic or approximate those in human cells, resulting in the production of an antibody with a partially or fully human glycosylation pattern. See Gerngross, Nat. Biotech. 22:1409-1414 (2004), and Li *et al.*, Nat. Biotech. 24:210-215 (2006).

[0342] Exemplary eukaryotic cells that may be used to express polypeptides, including isolated or secreted polypeptides, include, but are not limited to, COS cells, including COS 7 cells; 293 cells, including 293-6E cells; CHO cells, including CHO-S, DG44. Lec13 CHO cells, and FUT8 CHO cells; PER.C6® cells; and NSO cells. In some embodiments, the antibody heavy chains and/or light chains (*e.g.*, V_H region and/or V_L region) may be expressed in yeast. See, *e.g.*, U.S. Publication No. US 2006/0270045 A1. In some embodiments, a particular eukaryotic host cell is selected based on its ability to make desired post-translational modifications to the heavy chains and/or light chains (*e.g.*, V_H region and/or V_L region). For example, in some embodiments, CHO cells produce polypeptides that have a higher level of sialylation than the same polypeptide produced in 293 cells.

[0343] In some embodiments, the preparation methods include steps for freezing, e.g., cryopreserving, the cells, either before or after isolation, selection and/or enrichment and/or incubation for transduction and engineering, and/or after cultivation and/or harvesting of the engineered cells. In some embodiments, the freeze and subsequent thaw step removes granulocytes and, to some extent, monocytes in the cell population. In some embodiments, the cells are suspended in a freezing solution, e.g., following a washing step to remove plasma and platelets. Any of a variety of known freezing solutions and parameters in some aspects may be used. In some embodiments, the cells are frozen, e.g., cryoprotected or cryopreserved, in media and/or solution with a final concentration of or of about 12.5%, 12.0%, 11.5%, 11.0%, 10.5%, 10.0%, 9.5%, 9.0%, 8.5%, 8.0%, 7.5%, 7.0%, 6.5%, 6.0%, 5.5%, or 5.0% DMSO, or between 1% and 15%, between 6% and 12%, between 5% and 10%, or between 6% and 8% DMSO. In particular embodiments, the cells are frozen, e.g., cryoprotected or cryopreserved, in media and/or solution with a final concentration of or of about 5.0%, 4.5%, 4.0%, 3.5%, 3.0%, 2.5%, 2.0%, 1.5%, 1.25%, 1.0%, 0.75%, 0.5%, or 0.25% HSA, or between 0.1% and -5%, between 0.25% and 4%, between 0.5% and 2%, or between 1% and 2% HSA. One example involves using PBS containing 20% DMSO and 8% human serum albumin (HSA), or other suitable cell freezing media. This is then diluted 1:1 with media so that the final concentration of DMSO and HSA are 10% and 4%, respectively. The cells are generally then frozen to or to about −80 degrees Celsius at a rate of or of about 1 degree Celsius per minute and stored in the vapor phase of a liquid nitrogen storage tank.

[0344] In some embodiments, isolation of the cells or populations includes one or more preparation and/or non-affinity based cell separation steps. In some examples, cells are washed, centrifuged, and/or incubated in the presence of one or more reagents, for example, to remove unwanted components, enrich for

desired components, lyse or remove cells sensitive to particular reagents. In some examples, cells are separated based on one or more property, such as density, adherent properties, size, sensitivity and/or resistance to particular components. In some embodiments, the methods include density-based cell separation methods, such as the preparation of white blood cells from peripheral blood by lysing the red blood cells and centrifugation through a Percoll or Ficoll gradient.

[0345] In some embodiments, at least a portion of the selection step includes incubation of cells with a selection reagent. The incubation with a selection reagent or reagents, e.g., as part of selection methods which may be performed using one or more selection reagents for selection of one or more different cell types based on the expression or presence in or on the cell of one or more specific molecules, such as surface markers, e.g., surface proteins, intracellular markers, or nucleic acid. In some embodiments, any known method using a selection reagent or reagents for separation based on such markers may be used. In some embodiments, the selection reagent or reagents result in a separation that is affinity- or immunoaffinity-based separation. For example, the selection in some aspects includes incubation with a reagent or reagents for separation of cells and cell populations based on the cells' expression or expression level of one or more markers, typically cell surface markers, for example, by incubation with an antibody or binding partner that specifically binds to such markers, followed generally by washing steps and separation of cells having bound the antibody or binding partner, from those cells having not bound to the antibody or binding partner.

[0346] In some aspects of such processes, a volume of cells is mixed with an amount of a desired affinity-based selection reagent. The immunoaffinity-based selection can be carried out using any system or method that results in a favorable energetic interaction between the cells being separated and the molecule specifically binding to the marker on the cell, e.g., the antibody or other binding partner on the solid surface, e.g., particle. In some embodiments, methods are carried out using particles such as beads, e.g. magnetic beads, that are coated with a selection agent (e.g. antibody) specific to the marker of the cells. The particles (e.g. beads) can be incubated or mixed with cells in a container, such as a tube or bag, while shaking or mixing, with a constant cell density-to-particle (e.g., bead) ratio to aid in promoting energetically favored interactions. In other cases, the methods include selection of cells in which all or a portion of the selection is carried out in the internal cavity of a centrifugal chamber, for example, under centrifugal rotation. In some embodiments, incubation of cells with selection reagents, such as immunoaffinity-based selection reagents, is performed in a centrifugal chamber. In certain embodiments, the isolation or separation is carried out using a system, device, or apparatus described in International Patent Application, Publication Number WO2009/072003, or US 20110003380 A1. In one example, the system is a system as described in International Publication Number WO2016/073602.

[0347] In some embodiments, by conducting such selection steps or portions thereof (e.g., incubation

with antibody-coated particles, e.g., magnetic beads) in the cavity of a centrifugal chamber, the user is able to control certain parameters, such as volume of various solutions, addition of solution during processing and timing thereof, which can provide advantages compared to other available methods. For example, the ability to decrease the liquid volume in the cavity during the incubation can increase the concentration of the particles (e.g. bead reagent) used in the selection, and thus the chemical potential of the solution, without affecting the total number of cells in the cavity. This in turn can enhance the pairwise interactions between the cells being processed and the particles used for selection. In some embodiments, carrying out the incubation step in the chamber, e.g., when associated with the systems, circuitry, and control as described herein, permits the user to effect agitation of the solution at desired time(s) during the incubation, which also can improve the interaction.

[0348] In some embodiments, at least a portion of the selection step is performed in a centrifugal chamber, which includes incubation of cells with a selection reagent. In some aspects of such processes, a volume of cells is mixed with an amount of a desired affinity-based selection reagent that is far less than is normally employed when performing similar selections in a tube or container for selection of the same number of cells and/or volume of cells according to manufacturer's instructions. In some embodiments, an amount of selection reagent or reagents that is/are no more than 5%, no more than 10%, no more than 15%, no more than 20%, no more than 25%, no more than 50%, no more than 60%, no more than 70% or no more than 80% of the amount of the same selection reagent(s) employed for selection of cells in a tube or container-based incubation for the same number of cells and/or the same volume of cells according to manufacturer's instructions is employed.

[0349] In some embodiments, for selection, e.g., immunoaffinity-based selection of the cells, the cells are incubated in the cavity of the chamber in a composition that also contains the selection buffer with a selection reagent, such as a molecule that specifically binds to a surface marker on a cell that it desired to enrich and/or deplete, but not on other cells in the composition, such as an antibody, which optionally is coupled to a scaffold such as a polymer or surface, e.g., bead, e.g., magnetic bead, such as magnetic beads coupled to monoclonal antibodies specific for CD4 and CD8. In some embodiments, as described, the selection reagent is added to cells in the cavity of the chamber in an amount that is substantially less than (e.g. is no more than 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70% or 80% of the amount) as compared to the amount of the selection reagent that is typically used or would be necessary to achieve about the same or similar efficiency of selection of the same number of cells or the same volume of cells when selection is performed in a tube with shaking or rotation. In some embodiments, the incubation is performed with the addition of a selection buffer to the cells and selection reagent to achieve a target volume with incubation of the reagent of, for example, 10 mL to 200 mL, such as at least or about at least 10 mL, 20 mL, 30 mL, 40

mL, 50 mL, 60 mL, 70 mL, 80 mL, 90 mL, 100 mL, 150 mL or 200 mL. In some embodiments, the selection buffer and selection reagent are pre-mixed before addition to the cells. In some embodiments, the selection buffer and selection reagent are separately added to the cells. In some embodiments, the selection incubation is carried out with periodic gentle mixing condition, which can aid in promoting energetically favored interactions and thereby permit the use of less overall selection reagent while achieving a high selection efficiency.

[0350] In some embodiments, the total duration of the incubation with the selection reagent is from or from about 5 minutes to 6 hours, such as 30 minutes to 3 hours, for example, at least or about at least 30 minutes, 60 minutes, 120 minutes or 180 minutes.

[0351] In some embodiments, the incubation generally is carried out under mixing conditions, such as in the presence of spinning, generally at relatively low force or speed, such as speed lower than that used to pellet the cells, such as from or from about 600 rpm to 1700 rpm (e.g. at or about or at least 600 rpm, 1000 rpm, or 1500 rpm or 1700 rpm), such as at an RCF at the sample or wall of the chamber or other container of from or from about 80g to 100g (e.g. at or about or at least 80 g, 85 g, 90 g, 95 g, or 100 g). In some embodiments, the spin is carried out using repeated intervals of a spin at such low speed followed by a rest period, such as a spin and/or rest for 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 seconds, such as a spin at approximately 1 or 2 seconds followed by a rest for approximately 5, 6, 7, or 8 seconds.

[0352] In some embodiments, such process is carried out within the entirely closed system to which the chamber is integral. In some embodiments, this process (and in some aspects also one or more additional step, such as a previous wash step washing a sample containing the cells, such as an apheresis sample) is carried out in an automated fashion, such that the cells, reagent, and other components are drawn into and pushed out of the chamber at appropriate times and centrifugation effected, so as to complete the wash and binding step in a single closed system using an automated program.

[0353] In some embodiments, after the incubation and/or mixing of the cells and selection reagent and/or reagents, the incubated cells are subjected to a separation to select for cells based on the presence or absence of the particular reagent or reagents. In some embodiments, the separation is performed in the same closed system in which the incubation of cells with the selection reagent was performed. In some embodiments, after incubation with the selection reagents, incubated cells, including cells in which the selection reagent has bound are transferred into a system for immunoaffinity-based separation of the cells. In some embodiments, the system for immunoaffinity-based separation is or contains a magnetic separation column.

[0354] In some embodiments, the isolation methods include the separation of different cell types based on the expression or presence in the cell of one or more specific molecules, such as surface markers, *e.g.*,

surface proteins, intracellular markers, or nucleic acid. In some embodiments, any known method for separation based on such markers may be used. In some embodiments, the separation is affinity- or immunoaffinity-based separation. For example, the isolation in some aspects includes separation of cells and cell populations based on the cells' expression or expression level of one or more markers, typically cell surface markers, for example, by incubation with an antibody or binding partner that specifically binds to such markers, followed generally by washing steps and separation of cells having bound the antibody or binding partner, from those cells having not bound to the antibody or binding partner.

[0355] Such separation steps can be based on positive selection, in which the cells having bound the reagents are retained for further use, and/or negative selection, in which the cells having not bound to the antibody or binding partner are retained. In some examples, both fractions are retained for further use. In some aspects, negative selection can be particularly useful where no antibody is available that specifically identifies a cell type in a heterogeneous population, such that separation is best carried out based on markers expressed by cells other than the desired population.

[0356] In some embodiments, the process steps further include negative and/or positive selection of the incubated cells, such as using a system or apparatus that can perform an affinity-based selection. In some embodiments, isolation is carried out by enrichment for a particular cell population by positive selection, or depletion of a particular cell population, by negative selection. In some embodiments, positive or negative selection is accomplished by incubating cells with one or more antibodies or other binding agent that specifically bind to one or more surface markers expressed or expressed (marker+) at a relatively higher level (marker^{high}) on the positively or negatively selected cells, respectively.

[0357] The separation need not result in 100% enrichment or removal of a particular cell population or cells expressing a particular marker. For example, positive selection of or enrichment for cells of a particular type, such as those expressing a marker, refers to increasing the number or percentage of such cells, but need not result in a complete absence of cells not expressing the marker. Likewise, negative selection, removal, or depletion of cells of a particular type, such as those expressing a marker, refers to decreasing the number or percentage of such cells, but need not result in a complete removal of all such cells.

[0358] In some examples, multiple rounds of separation steps are carried out, where the positively or negatively selected fraction from one step is subjected to another separation step, such as a subsequent positive or negative selection. In some examples, a single separation step can deplete cells expressing multiple markers simultaneously, such as by incubating cells with a plurality of antibodies or binding partners, each specific for a marker targeted for negative selection. Likewise, multiple cell types can simultaneously be positively selected by incubating cells with a plurality of antibodies or binding partners expressed on the various cell types.

[0359] For example, in some aspects, specific subpopulations of T cells, such as cells positive or expressing high levels of one or more surface markers, *e.g.*, CD28+, CD62L+, CCR7+, CD27+, CD127+, CD4+, CD8+, CD45RA+, and/or CD45RO+ T cells, are isolated by positive or negative selection techniques.

[0360] For example, CD3+, CD28+ T cells can be positively selected using anti-CD3/anti-CD28 conjugated magnetic beads (*e.g.*, DYNABEADS® M-450 CD3/CD28 T Cell Expander, MACSiBeads™, etc.).

[0361] In some embodiments, T cells are separated from a PBMC sample by negative selection of markers expressed on non-T cells, such as B cells, monocytes, or other white blood cells, such as CD14. In some aspects, CD4+ and/or CD8+ selection steps are used to separate CD4+ helper and CD8+ cytotoxic T cells from a composition, such as from a PBMC composition such as one obtained via leukapheresis. Such CD4+ and CD8+ populations, in some aspects, can be further sorted into sub-populations by positive or negative selection for markers expressed or expressed to a relatively higher degree on one or more naive, memory, and/or effector T cell subpopulations. In some embodiments, CD4+ and CD8+ cells are mixed at a desired ratio

[0362] In some embodiments, CD8+ cells are further enriched for or depleted of naive, central memory, effector memory, and/or central memory stem cells, such as by positive or negative selection based on surface antigens associated with the respective subpopulation. In some embodiments, enrichment for central memory T (T_{CM}) cells is carried out to increase efficacy, such as to improve long-term survival, expansion, and/or engraftment following administration, which in some aspects is particularly robust in such sub-populations. See Terakura *et al.* (2012) Blood.1:72–82; Wang *et al.* (2012) J Immunother. 35(9):689-701. In some embodiments, combining T_{CM}-enriched CD8+ T cells and CD4+ T cells further enhances efficacy.

[0363] In embodiments, memory T cells are present in both CD62L+ and CD62L- subsets of CD8+ peripheral blood lymphocytes. PBMC can be enriched for or depleted of CD62L-CD8+ and/or CD62L+CD8+ fractions, such as using anti-CD8 and anti-CD62L antibodies.

[0364] In some embodiments, the enrichment for central memory T (T_{CM}) cells is based on positive or high surface expression of CD45RO, CD62L, CCR7, CD27, CD28, CD3, and/or CD127; in some aspects, it is based on negative selection for cells expressing or highly expressing CD45RA and/or granzyme B. In some aspects, isolation of a CD8+ population enriched for T_{CM} cells is carried out by depletion of cells expressing CD4, CD14, CD45RA, and positive selection or enrichment for cells expressing CD62L. In one aspect, enrichment for central memory T (T_{CM}) cells is carried out starting with a negative fraction of cells selected based on CD4 expression, which is subjected to a negative selection based on expression of CD14 and CD45RA, and a positive selection based on CD62L. Such selections in some aspects are carried out

simultaneously and in other aspects are carried out sequentially, in either order. In some aspects, the same CD4 expression-based selection step used in preparing the CD8+ cell population or subpopulation, also is used to generate the CD4+ cell population or sub-population, such that both the positive and negative fractions from the CD4-based separation are retained and used in subsequent steps of the methods, optionally following one or more further positive or negative selection steps.

[0365] In some embodiments, central memory CD8+ cells are CD27+, CD28+, CD62L+, CCR7+, CD45RA-, and/or CD45RO+. In some embodiments, central memory CD8+ cells are CD62L+ and CD45RO+. In some embodiments, central memory CD8+ cells are CCR7+ and CD45RO+. In some embodiments, central memory CD8+ cells are CCR7+ and CD45RA-. In some embodiments, central memory CD8+ cells are CD62L+ and CCR7+. In some embodiments, central memory CD8+ cells are CD62L+/CD45RA-, CCR7+/CD45RA-, CD62L+/CCR7+, or CD62L+/CCR7+/CD45RA-, and have intermediate to high expression of CD44. In some embodiments, central memory CD8+ cells are CD27+/CD28+/CD62L+/CD45RA-, CD27+/CD28+/CCR7+/CD45RA-, CD27+/CD28+/CD62L+/CCR7+, or CD27+/CD28+/CD62L+/CCR7+/CD45RA-.

[0366] In particular embodiments, a biological sample, e.g., a sample of PBMCs or other white blood cells, are subjected to selection of CD4+ T cells, where both the negative and positive fractions are retained. In certain embodiments, CD8+ T cells are selected from the negative fraction. In some embodiments, a biological sample is subjected to selection of CD8+ T cells, where both the negative and positive fractions are retained. In certain embodiments, CD4+ T cells are selected from the negative fraction.

[0367] In a particular example, a sample of PBMCs or other white blood cell sample is subjected to selection of CD4+ cells, where both the negative and positive fractions are retained. The negative fraction then is subjected to negative selection based on expression of CD14 and CD45RA, and positive selection based on a marker characteristic of central memory T cells, such as CD62L or CCR7, where the positive and negative selections are carried out in either order.

[0368] In some embodiments, CD4+ T helper cells are sorted into naïve, central memory, and effector cells by identifying cell populations that have cell surface antigens. CD4+ lymphocytes can be obtained by standard methods. In some embodiments, naive CD4+ T lymphocytes are CD45RO-, CD45RA+, CD62L+, CD4+ T cells. In some embodiments, central memory CD4+ cells are CD62L+ and CD45RO+. In some embodiments, central memory CD4+ cells are CD62L+ and CD45RO+. In some embodiments, central memory CD4+ cells are CD27+, CD28+, CD62L+, CCR7+, CD45RA-, and/or CD45RO+. In some embodiments, central memory CD4+ cells are CD62L+ and CD45RO+. In some embodiments, central memory CD4+ cells are CCR7+ and CD45RO+. In some embodiments, central memory CD4+ cells are CCR7+ and CD45RA-. In some embodiments, central memory CD4+ cells are CD62L+ and CCR7+. In

some embodiments, central memory CD4+ cells are CD62L+/CD45RA-, CCR7+/CD45RA-, CD62L+/CCR7+, or CD62L+/CCR7+/CD45RA-, and have intermediate to high expression of CD44. In some embodiments, central memory CD4+ cells are CD27+/CD28+/CD62L+/CD45RA-, CD27+/CD28+/CCR7+/CD45RA-, CD27+/CD28+/CD62L+/CCR7+, or CD27+/CD28+/CD62L+/CCR7+/CD45RA-. In some embodiments, effector CD4+ cells are CD62L- and CD45RO-.

[0369] In one 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 some embodiments, the antibody or binding partner is bound to a solid support or matrix, such as a magnetic bead or paramagnetic bead, to allow for separation of cells for positive and/or negative selection. For example, in some embodiments, the cells and cell populations are separated or isolated using immunomagnetic (or affinitymagnetic) separation techniques (reviewed in Methods in Molecular Medicine, vol. 58: Metastasis Research Protocols, Vol. 2: Cell Behavior *In vitro* and *In vivo*, p 17-25 Edited by: S. A. Brooks and U. Schumacher © Humana Press Inc., Totowa, NJ).

[0370] In some aspects, the sample or composition of cells to be separated is incubated with small, magnetizable or magnetically responsive material, such as magnetically responsive particles or microparticles, such as paramagnetic beads (*e.g.*, such as Dynabeads® or MACS® beads). The magnetically responsive material, *e.g.*, particle, generally is directly or indirectly attached to a binding partner, *e.g.*, an antibody, that specifically binds to a molecule, *e.g.*, surface marker, present on the cell, cells, or population of cells that it is desired to separate, *e.g.*, that it is desired to negatively or positively select.

[0371] In some embodiments, the magnetic particle or bead comprises a magnetically responsive material bound to a specific binding member, such as an antibody or other binding partner. There are many well-known magnetically responsive materials used in magnetic separation methods. Suitable magnetic particles include those described in Molday, U.S. Pat. No. 4,452,773, and in European Patent Specification EP 452342 B, which are hereby incorporated by reference. Colloidal sized particles, such as those described in Owen U.S. Pat. No. 4,795,698, and Liberti *et al.*, U.S. Pat. No. 5,200,084, are other examples.

[0372] The incubation generally is carried out under conditions whereby the antibodies or binding partners, or molecules, such as secondary antibodies or other reagents, which specifically bind to such antibodies or binding partners, which are attached to the magnetic particle or bead, specifically bind to cell surface molecules if present on cells within the sample.

[0373] In some aspects, the sample is placed in a magnetic field, and those cells having magnetically responsive or magnetizable particles attached thereto will be attracted to the magnet and separated from the unlabeled cells. For positive selection, cells that are attracted to the magnet are retained; for negative

selection, cells that are not attracted (unlabeled cells) are retained. In some aspects, a combination of positive and negative selection is performed during the same selection step, where the positive and negative fractions are retained and further processed or subject to further separation steps.

[0374] In certain embodiments, the magnetically responsive particles are coated in primary antibodies or other binding partners, secondary antibodies, lectins, enzymes, or streptavidin. In certain embodiments, the magnetic particles are attached to cells via a coating of primary antibodies specific for one or more markers. In certain embodiments, the cells, rather than the beads, are labeled with a primary antibody or binding partner, and then cell-type specific secondary antibody- or other binding partner (*e.g.*, streptavidin)-coated magnetic particles, are added. In certain embodiments, streptavidin-coated magnetic particles are used in conjunction with biotinylated primary or secondary antibodies.

[0375] In some embodiments, the magnetically responsive particles are left attached to the cells that are to be subsequently incubated, cultured and/or engineered; in some aspects, the particles are left attached to the cells for administration to a patient. In some embodiments, the magnetizable or magnetically responsive particles are removed from the cells. Methods for removing magnetizable particles from cells are known and include, *e.g.*, the use of competing non-labeled antibodies, magnetizable particles or antibodies conjugated to cleavable linkers, etc. In some embodiments, the magnetizable particles are biodegradable.

[0376] In some embodiments, the affinity-based selection is via magnetic-activated cell sorting (MACS®) (Miltenyi Biotec, Auburn, CA). Magnetic Activated Cell Sorting (MACS®) systems are capable of high-purity selection of cells having magnetized particles attached thereto. In certain embodiments, MACS® operates in a mode wherein the non-target and target species are sequentially eluted after the application of the external magnetic field. That is, the cells attached to magnetized particles are held in place while the unattached species are eluted. Then, after this first elution step is completed, the species that were trapped in the magnetic field and were prevented from being eluted are freed in some manner such that they can be eluted and recovered. In certain embodiments, the non-target cells are labelled and depleted from the heterogeneous population of cells.

[0377] In certain embodiments, the isolation or separation is carried out using a system, device, or apparatus that carries out one or more of the isolation, cell preparation, separation, processing, incubation, culture, and/or formulation steps of the methods. In some aspects, the system is used to carry out each of these steps in a closed or sterile environment, for example, to minimize error, user handling and/or contamination. In one example, the system is a system as described in International Patent Application, Publication Number WO2009/072003, or US 20110003380 A1.

[0378] In some embodiments, the system or apparatus carries out one or more, *e.g.*, all, of the isolation, processing, engineering, and formulation steps in an integrated or self-contained system, and/or in an

automated or programmable fashion. In some aspects, the system or apparatus includes a computer and/or computer program in communication with the system or apparatus, which allows a user to program, control, assess the outcome of, and/or adjust various aspects of the processing, isolation, engineering, and formulation steps.

[0379] In some aspects, the separation and/or other steps is carried out using CliniMACS® system (Miltenyi Biotec), for example, for automated separation of cells on a clinical-scale level in a closed and sterile system. Components can include an integrated microcomputer, magnetic separation unit, peristaltic pump, and various pinch valves. The integrated computer in some aspects controls all components of the instrument and directs the system to perform repeated procedures in a standardized sequence. The magnetic separation unit in some aspects includes a movable permanent magnet and a holder for the selection column. The peristaltic pump controls the flow rate throughout the tubing set and, together with the pinch valves, ensures the controlled flow of buffer through the system and continual suspension of cells.

[0380] The CliniMACS® system in some aspects uses antibody-coupled magnetizable particles that are supplied in a sterile, non-pyrogenic solution. In some embodiments, after labelling of cells with magnetic particles the cells are washed to remove excess particles. A cell preparation bag is then connected to the tubing set, which in turn is connected to a bag containing buffer and a cell collection bag. The tubing set consists of pre-assembled sterile tubing, including a pre-column and a separation column, and are for single use only. After initiation of the separation program, the system automatically applies the cell sample onto the separation column. Labelled cells are retained within the column, while unlabeled cells are removed by a series of washing steps. In some embodiments, the cell populations for use with the methods described herein are unlabeled and are not retained in the column. In some embodiments, the cell populations for use with the methods described herein are labeled and are retained in the column. In some embodiments, the cell populations for use with the methods described herein are eluted from the column after removal of the magnetic field, and are collected within the cell collection bag.

[0381] In certain embodiments, separation and/or other steps are carried out using the CliniMACS Prodigy® system (Miltenyi Biotec). The CliniMACS Prodigy® system in some aspects is equipped with a cell processing unity that permits automated washing and fractionation of cells by centrifugation. The CliniMACS Prodigy® system can also include an onboard camera and image recognition software that determines the optimal cell fractionation endpoint by discerning the macroscopic layers of the source cell product. For example, peripheral blood may be automatically separated into erythrocytes, white blood cells and plasma layers. The CliniMACS Prodigy® system can also include an integrated cell cultivation chamber which accomplishes cell culture protocols such as, *e.g.*, cell differentiation and expansion, antigen loading, and long-term cell culture. Input ports can allow for the sterile removal and replenishment of media and cells

can be monitored using an integrated microscope. See, *e.g.*, Klebanoff *et al.* (2012) J Immunother. 35(9): 651–660, Terakura *et al.* (2012) Blood.1:72–82, and Wang *et al.* (2012) J Immunother. 35(9):689-701.

[0382] In some embodiments, a cell population described herein is collected and enriched (or depleted) via flow cytometry, in which cells stained for multiple cell surface markers are carried in a fluidic stream. In some embodiments, a cell population described herein is collected and enriched (or depleted) via preparative scale (FACS)-sorting. In certain embodiments, a cell population described herein is collected and enriched (or depleted) by use of microelectromechanical systems (MEMS) chips in combination with a FACS-based detection system (see, *e.g.*, WO 2010/033140, Cho *et al.* (2010) Lab Chip 10, 1567-1573; and Godin *et al.* (2008) J Biophoton. 1(5):355–376. In both cases, cells can be labeled with multiple markers, allowing for the isolation of well-defined T cell subsets at high purity.

[0383] In some embodiments, the antibodies or binding partners are labeled with one or more detectable marker, to facilitate separation for positive and/or negative selection. For example, separation may be based on binding to fluorescently labeled antibodies. In some examples, separation of cells based on binding of antibodies or other binding partners specific for one or more cell surface markers are carried in a fluidic stream, such as by fluorescence-activated cell sorting (FACS), including preparative scale (FACS) and/or microelectromechanical systems (MEMS) chips, *e.g.*, in combination with a flow-cytometric detection system. Such methods allow for positive and negative selection based on multiple markers simultaneously.

[0384] In some embodiments, the isolation and/or selection results in one or more input compositions of enriched T cells, e.g., CD3+ T cells, CD4+ T cells, and/or CD8+ T cells. In some embodiments, two or more separate input composition are isolated, selected, enriched, or obtained from a single biological sample. In some embodiments, separate input compositions are isolated, selected, enriched, and/or obtained from separate biological samples collected, taken, and/or obtained from the same subject.

[0385] In certain embodiments, the one or more input compositions is or includes a composition of enriched T cells that includes at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, at least 99.5%, at least 99.9%, or at or at about 100% CD3+ T cells. In particular embodiment, the input composition of enriched T cells consists essentially of CD3+ T cells.

[0386] In certain embodiments, the one or more input compositions is or includes a composition of enriched CD4+ T cells that includes at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, at least 99.5%, at least 99.9%, or at or at about 100% CD4+ T cells. In certain embodiments, the input composition of CD4+ T cells includes less than 40%, less than 35%, less than 30%, less than 25%, less than 20%, less than 15%, less than 10%, less than 5%, less than 1%, less than 0.1%, or less than 0.01% CD8+ T cells, and/or contains no CD8+ T cells,

and/or is free or substantially free of CD8+ T cells. In some embodiments, the composition of enriched T cells consists essentially of CD4+ T cells.

[0387] In certain embodiments, the one or more compositions is or includes a composition of CD8+ T cells that is or includes at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, at least 99.5%, at least 99.9%, or at or at about 100% CD8+ T cells. In certain embodiments, the composition of CD8+ T cells contains less than 40%, less than 35%, less than 30%, less than 25%, less than 20%, less than 15%, less than 10%, less than 5%, less than 1%, less than 0.1%, or less than 0.01% CD4+ T cells, and/or contains no CD4+ T cells, and/or is free of or substantially free of CD4+ T cells. In some embodiments, the composition of enriched T cells consists essentially of CD8+ T cells.

[0388] In some embodiments, the preparation methods include steps for freezing, *e.g.*, cryopreserving, the cells, either before or after isolation, incubation, and/or engineering. In some embodiments, the freeze and subsequent thaw step removes granulocytes and, to some extent, monocytes in the cell population. In some embodiments, the cells are suspended in a freezing solution, *e.g.*, following a washing step to remove plasma and platelets. Any of a variety of known freezing solutions and parameters in some aspects may be used. One example involves using PBS containing 20% DMSO and 8% human serum albumin (HSA), or other suitable cell freezing media. This is then diluted 1:1 with media so that the final concentration of DMSO and HSA are 10% and 4%, respectively. The cells are then frozen to −80 degrees Celsius. at a rate of 1 degrees Celsius per minute and stored in the vapor phase of a liquid nitrogen storage tank.

B. Activation and Stimulation

[0389] In some embodiments, the cells are incubated and/or cultured prior to or in connection with genetic engineering. The incubation steps can include culture, cultivation, stimulation, activation, and/or propagation. In some embodiments, the compositions or cells are incubated in the presence of stimulating conditions or a stimulatory agent. Such conditions include those designed to induce proliferation, expansion, activation, and/or survival of cells in the population, to mimic antigen exposure, and/or to prime the cells for genetic engineering, such as for the introduction of a recombinant antigen receptor.

[0390] In some embodiments, the provided methods include cultivation, incubation, culture, and/or genetic engineering steps. For example, in some embodiments, provided are methods for incubating and/or engineering the depleted cell populations and culture-initiating compositions. Thus, in some embodiments, the cell populations are incubated in a culture-initiating composition.

[0391] The incubation and/or engineering may be carried out in a culture vessel, such as a unit, chamber, well, column, tube, tubing set, valve, vial, culture dish, bag, or other container for culture or

cultivating cells.

[0392] The conditions can include one or more of particular media, temperature, oxygen content, carbon dioxide content, time, agents, *e.g.*, nutrients, amino acids, antibiotics, ions, and/or stimulatory factors, such as cytokines, chemokines, antigens, binding partners, fusion proteins, recombinant soluble receptors, and any other agents designed to activate the cells.

[0393] In some embodiments, the stimulating conditions or agents include one or more agent, e.g., ligand, which is capable of stimulating or activating an intracellular signaling domain of a TCR complex. In some aspects, the agent turns on or initiates TCR/CD3 intracellular signaling cascade in a T cell. Such agents can include antibodies, such as those specific for a TCR, e.g. anti-CD3. In some embodiments, the stimulating conditions include one or more agent, e.g. ligand, which is capable of stimulating a costimulatory receptor, e.g., anti-CD28. In some embodiments, such agents and/or ligands may be, bound to solid support such as a bead, and/or one or more cytokines. Optionally, the expansion method may further comprise the step of adding anti-CD3 and/or anti CD28 antibody to the culture medium (e.g., at a concentration of at least about 0.5 ng/ml). In some embodiments, the stimulating agents include IL-2, IL-15 and/or IL-7. In some aspects, the IL-2 concentration is at least about 10 units/mL.

[0394] In some aspects, incubation is carried out in accordance with techniques such as those described in US Patent No. 6,040,177 to Riddell *et al.*, Klebanoff *et al.* (2012) J Immunother. 35(9): 651–660, Terakura *et al.* (2012) Blood.1:72–82, and/or Wang *et al.* (2012) J Immunother. 35(9):689-701.

[0395] In some embodiments, the T cells are expanded by adding to the culture-initiating composition feeder cells, such as non-dividing peripheral blood mononuclear cells (PBMC), (*e.g.*, such that the resulting population of cells contains at least about 5, 10, 20, or 40 or more PBMC feeder cells for each T lymphocyte in the initial population to be expanded); and incubating the culture (*e.g.* for a time sufficient to expand the numbers of T cells). In some aspects, the non-dividing feeder cells can comprise gamma-irradiated PBMC feeder cells. In some embodiments, the PBMC are irradiated with gamma rays in the range of about 3000 to 3600 rads to prevent cell division. In some aspects, the feeder cells are added to culture medium prior to the addition of the populations of T cells.

[0396] In some embodiments, the stimulating conditions include temperature suitable for the growth of human T lymphocytes, for example, at least about 25 degrees Celsius, generally at least about 30 degrees, and generally at or about 37 degrees Celsius. Optionally, the incubation may further comprise adding non-dividing EBV-transformed lymphoblastoid cells (LCL) as feeder cells. LCL can be irradiated with gamma rays in the range of about 6000 to 10,000 rads. The LCL feeder cells in some aspects is provided in any suitable amount, such as a ratio of LCL feeder cells to initial T lymphocytes of at least about 10:1.

[0397] In embodiments, antigen-specific T cells, such as antigen-specific CD4+ and/or CD8+ T cells,

are obtained by stimulating naive or antigen specific T lymphocytes with antigen. For example, antigen-specific T cell lines or clones can be generated to cytomegalovirus antigens by isolating T cells from infected subjects and stimulating the cells *in vitro* with the same antigen.

[0398] In some embodiments, at least a portion of the incubation in the presence of one or more stimulating conditions or a stimulatory agents is carried out in the internal cavity of a centrifugal chamber, for example, under centrifugal rotation, such as described in International Publication Number WO2016/073602. In some embodiments, at least a portion of the incubation performed in a centrifugal chamber includes mixing with a reagent or reagents to induce stimulation and/or activation. In some embodiments, cells, such as selected cells, are mixed with a stimulating condition or stimulatory agent in the centrifugal chamber. In some aspects of such processes, a volume of cells is mixed with an amount of one or more stimulating conditions or agents that is far less than is normally employed when performing similar stimulations in a cell culture plate or other system.

[0399] In some embodiments, the stimulating agent is added to cells in the cavity of the chamber in an amount that is substantially less than (e.g. is no more than 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70% or 80% of the amount) as compared to the amount of the stimulating agent that is typically used or would be necessary to achieve about the same or similar efficiency of selection of the same number of cells or the same volume of cells when selection is performed without mixing in a centrifugal chamber, e.g. in a tube or bag with periodic shaking or rotation. In some embodiments, the incubation is performed with the addition of an incubation buffer to the cells and stimulating agent to achieve a target volume with incubation of the reagent of, for example, 10 mL to 200 mL, such as at least or about at least or about or 10 mL, 20 mL, 30 mL, 40 mL, 50 mL, 60 mL, 70 mL, 80 mL, 90 mL, 100 mL, 150 mL or 200 mL. In some embodiments, the incubation buffer and stimulating agent are pre-mixed before addition to the cells. In some embodiments, the incubation buffer and stimulating agent are separately added to the cells. In some embodiments, the stimulating incubation is carried out with periodic gentle mixing condition, which can aid in promoting energetically favored interactions and thereby permit the use of less overall stimulating agent while achieving stimulating and activation of cells.

[0400] In some embodiments, the incubation generally is carried out under mixing conditions, such as in the presence of spinning, generally at relatively low force or speed, such as speed lower than that used to pellet the cells, such as from or from about 600 rpm to 1700 rpm (e.g. at or about or at least 600 rpm, 1000 rpm, or 1500 rpm or 1700 rpm), such as at an RCF at the sample or wall of the chamber or other container of from or from about 80g to 100g (e.g. at or about or at least 80 g, 85 g, 90 g, 95 g, or 100 g). In some embodiments, the spin is carried out using repeated intervals of a spin at such low speed followed by a rest period, such as a spin and/or rest for 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 seconds, such as a spin at approximately 1

or 2 seconds followed by a rest for approximately 5, 6, 7, or 8 seconds.

[0401] In some embodiments, the total duration of the incubation, e.g. with the stimulating agent, is between or between about 1 hour and 96 hours, 1 hour and 72 hours, 1 hour and 48 hours, 4 hours and 36 hours, 8 hours and 30 hours or 12 hours and 24 hours, such as at least or about at least 6 hours, 12 hours, 18 hours, 24 hours, 36 hours or 72 hours. In some embodiments, the further incubation is for a time between or about between 1 hour and 48 hours, 4 hours and 36 hours, 8 hours and 30 hours or 12 hours and 24 hours, inclusive.

[0402] In particular embodiments, the stimulating conditions include incubating, culturing, and/or cultivating a composition of enriched T cells with and/or in the presence of one or more cytokines. In particular embodiments, the one or more cytokines are recombinant cytokines. In some embodiments, the one or more cytokines are human recombinant cytokines. In certain embodiments, the one or more cytokines bind to and/or are capable of binding to receptors that are expressed by and/or are endogenous to T cells. In particular embodiments, the one or more cytokines is or includes a member of the 4-alpha-helix bundle family of cytokines. In some embodiments, members of the 4-alpha-helix bundle family of cytokines include, but are not limited to, interleukin-2 (IL-2), interleukin-4 (IL-4), interleukin-7 (IL-7), interleukin-9 (IL-9), interleukin 12 (IL-12), interleukin 15 (IL-15), granulocyte colony-stimulating factor (G-CSF), and granulocyte-macrophage colony-stimulating factor (GM-CSF).

[0403] In some embodiments, the stimulation results in activation and/or proliferation of the cells, for example, prior to transduction.

C. Vectors and Methods for Genetic Engineering

[0404] Also provided are methods, polynucleotides, compositions, and kits, for expressing the binding molecules (*e.g.*, anti-BCMA binding molecules), including recombinant receptors (*e.g.*, CARs) comprising the binding molecules, and for producing the genetically engineered cells expressing such binding molecules. In some embodiments, one or more binding molecules, including recombinant receptors (*e.g.*, CARs) can be genetically engineered into cells or plurality of cells. The genetic engineering generally involves introduction of a nucleic acid encoding the recombinant or engineered component into the cell, such as by retroviral transduction, transfection, or transformation.

[0405] Also provided are polynucleotides encoding the chimeric antigen receptors and/or portions, *e.g.*, chains, thereof. Among the provided polynucleotides are those encoding the anti-BCMA chimeric antigen receptors (*e.g.*, antigen-binding fragment) described herein. Also provided are polynucleotides encoding one or more antibodies and/or portions thereof, e.g., those encoding one or more of the anti-BCMA antibodies (*e.g.*, antigen-binding fragment) described herein and/or other antibodies and/or portions thereof, *e.g.*,

antibodies and/or portions thereof that binds other target antigens. The polynucleotides may include those encompassing natural and/or non-naturally occurring nucleotides and bases, *e.g.*, including those with backbone modifications. The terms "nucleic acid molecule", "nucleic acid" and "polynucleotide" may be used interchangeably, and refer to a polymer of nucleotides. Such polymers of nucleotides may contain natural and/or non-natural nucleotides, and include, but are not limited to, DNA, RNA, and PNA. "Nucleic acid sequence" refers to the linear sequence of nucleotides that comprise the nucleic acid molecule or polynucleotide.

[0406] Also provided are polynucleotides that have been optimized for codon usage and/or to eliminate splice sites, such as cryptic splice sites. Also provided are methods of optimizing and producing the coding sequences of chimeric antigen receptors, such as any of the chimeric antigen receptors described herein. Such methods are described in Section II herein.

[0407] Also provided are vectors containing the polynucleotides, such as any of the polynucleotides described herein, and host cells containing the vectors, *e.g.*, for producing the antibodies or antigen-binding fragments thereof or cells expressing a recombinant receptor (*e.g.* CAR) containing such antibodies or fragments. In some embodiments, the vector is a viral vector. In some embodiments, the vector is a retroviral vector, or a lentiviral vector. Also provided are methods for producing the antibodies or antigen-binding fragments thereof or cells expressing a recombinant receptor (*e.g.* CAR) containing such antibodies or fragments.

[0408] In some embodiments, a nucleic acid may encode an amino acid sequence comprising the V_L region and/or an amino acid sequence comprising the V_H region of the antibody (*e.g.*, the light and/or heavy chains of the antibody). The nucleic acid may encode one or more amino acid sequence comprising the V_L region and/or an amino acid sequence comprising the V_H region of the antibody (*e.g.*, the light and/or heavy chains of the antibody). In a further embodiment, one or more vectors (*e.g.*, expression vectors) comprising such polynucleotides are provided. In a further embodiment, a host cell comprising such polynucleotides is provided. In one such embodiment, a host cell comprises (*e.g.*, has been transformed with) a vector comprising a nucleic acid that encodes an amino acid sequence comprising the V_H region of the antibody. In another such embodiment, a host cell comprises (*e.g.*, has been transformed with) (1) a vector comprising a nucleic acid that encodes an amino acid sequence comprising the V_L region of the antibody and an amino acid sequence comprising the V_H region of the antibody, or (2) a first vector comprising a nucleic acid that encodes an amino acid sequence comprising the V_L region of the antibody and a second vector comprising a nucleic acid that encodes an amino acid sequence comprising the V_H region of the antibody. In some embodiments, a host cell comprises (*e.g.*, has been transformed with) one or more vectors comprising one or more nucleic acid that encodes one or more an amino acid sequence comprising one or more antibodies

and/or portions thereof, *e.g.*, antigen-binding fragments thereof. In some embodiments, one or more such host cells are provided. In some embodiments, a composition containing one or more such host cells are provided. In some embodiments, the one or more host cells can express different antibodies, or the same antibody. In some embodiments, each of the host cells can express more than one antibody.

[0409] Also provided are methods of making the anti-BCMA chimeric antigen receptors. For recombinant production of the chimeric receptors, a nucleic acid sequence encoding a chimeric receptor antibody, *e.g.*, as described herein, may be isolated and inserted into one or more vectors for further cloning and/or expression in a host cell. Such nucleic acid sequences may be readily isolated and sequenced using conventional procedures (*e.g.*, by using oligonucleotide probes that are capable of binding specifically to genes encoding the heavy and light chains of the antibody). In some embodiments, a method of making the anti-BCMA chimeric antigen receptor is provided, wherein the method comprises culturing a host cell comprising a nucleic acid sequence encoding the antibody, as provided above, under conditions suitable for expression of the receptor.

[0410] In some cases, the polynucleotide containing nucleic acid sequences encoding the BCMA-binding receptor, *e.g.*, chimeric antigen receptor (CAR), contains a signal sequence that encodes a signal peptide. In some aspects, the signal sequence may encode a signal peptide derived from a native polypeptide. In other aspects, the signal sequence may encode a heterologous or non-native signal peptide. In some aspects, non-limiting exemplary signal peptide include a signal peptide of the IgG kappa chain set forth in SEQ ID NO: 166, or encoded by the nucleotide sequence set forth in SEQ ID NO: 167 or 168-171; a GMCSFR alpha chain set forth in SEQ ID NO:154 and encoded by the nucleotide sequence set forth in SEQ ID NO:155; a CD8 alpha signal peptide set forth in SEQ ID NO:146; or a CD33 signal peptide set forth in SEQ ID NO:142.

[0411] In some embodiments the vector or construct can contain promoter and/or enhancer or regulatory elements to regulate expression of the encoded recombinant receptor. In some examples the promoter and/or enhancer or regulatory elements can be condition-dependent promoters, enhancers, and/or regulatory elements. In some examples these elements drive expression of the transgene. In some examples, the CAR transgene can be operatively linked to a promoter, such as an EF1alpha promoter with an HTLV1 enhancer (SEQ ID NO: 151). In some examples, the CAR transgene is operatively linked to a Woodchuck Hepatitis Virus (WHP) Posttranscriptional Regulatory Element (WPRE; SEQ ID NO: 253), located downstream of the transgene.

[0412] In some embodiments, the vector or construct can contain a single promoter that drives the expression of one or more nucleic acid molecules. In some embodiments, such nucleic acid molecules, *e.g.*, transcripts, can be multicistronic (bicistronic or tricistronic, see *e.g.*, U.S. Patent No. 6,060,273). For

example, in some embodiments, transcription units can be engineered as a bicistronic unit containing an IRES (internal ribosome entry site), which allows coexpression of gene products (*e.g.* encoding a first and second chimeric receptor) by a message from a single promoter. Alternatively, in some cases, a single promoter may direct expression of an RNA that contains, in a single open reading frame (ORF), two or three genes (*e.g.* encoding a first and second binding molecules, *e.g.*, antibody recombinant receptor) separated from one another by sequences encoding a self-cleavage peptide (*e.g.*, 2A cleavage sequences) or a protease recognition site (*e.g.*, furin). The ORF thus encodes a single polypeptide, which, either during (in the case of T2A) or after translation, is cleaved into the individual proteins. In some cases, the peptide, such as T2A, can cause the ribosome to skip (ribosome skipping) synthesis of a peptide bond at the C-terminus of a 2A element, leading to separation between the end of the 2A sequence and the next peptide downstream (*see*, for example, de Felipe. *Genetic Vaccines and Ther.* 2:13 (2004) and deFelipe *et al. Traffic* 5:616-626 (2004)). Many 2A elements are known. Examples of 2A sequences that can be used in the methods and polynucleotides disclosed herein, without limitation, 2A sequences from the foot-and-mouth disease virus (F2A, *e.g.*, SEQ ID NO: 152 or 153), equine rhinitis A virus (E2A, *e.g.*, SEQ ID NO: 148 or 149), Thosea asigna virus (T2A, *e.g.*, SEQ ID NO: 241, 242 or 243), and porcine teschovirus-1 (P2A, *e.g.*, SEQ ID NO: 201 or 202) as described in U.S. Patent Publication No. 20070116690. In some embodiments, the one or more different or separate promoters drive the expression of one or more nucleic acid molecules encoding the one or more binding molecules, *e.g.*, recombinant receptors.

[0413] Any of the binding molecules, e.g., antibodies and/or recombinant receptors provided herein, *e.g.*, BCMA-binding molecules and/or the additional recombinant receptors, can be encoded by polynucleotides containing one or more nucleic acid molecules encoding the receptors, in any combinations or arrangements. For example, one, two, three or more polynucleotides can encode one, two, three or more different receptors or domains. In some embodiments, one vector or construct contains nucleic acid molecules encoding one or more binding molecules, *e.g.*, antibody and/or recombinant receptor, and a separate vector or construct contains nucleic acid molecules encoding an additional binding molecule, *e.g.*, antibody and/or recombinant receptor. Each of the nucleic acid molecules can also encode one or more marker(s), such as a surface marker, *e.g.*, truncated EGFR (tEGFR).

[0414] Also provided are compositions containing one or more of the nucleic acid molecules, vectors or constructs, such as any described above. In some embodiments, the nucleic acid molecules, vectors, constructs or compositions can be used to engineer cells, such as T cells, to express any of the binding molecules, e.g., antibody or recombinant receptor, and/or the additional binding molecules.

[0415] In some embodiments, one or more binding molecules, including antibodies and/or recombinant receptors (e.g., CARs), can be genetically engineered to be expressed in cells or plurality of cells. In some

embodiments, a first recombinant receptor and a second binding molecule, *e.g.*, recombinant receptor, are encoded by the same or separate nucleic acid molecules. In some embodiments, additional binding molecules are engineered to be expressed in cells or a plurality of cells.

1. Gene Transfer

[0416] In some embodiments, methods for producing engineered cells includes the introduction of a polynucleotide encoding a recombinant receptor (e.g. anti-BCMA CAR) into a cell, *e.g.*, such as a stimulated or activated cell. In particular embodiments, the recombinant proteins are recombinant receptors, such as any described in Section I. Introduction of the nucleic acid molecules encoding the recombinant protein, such as recombinant receptor, in the cell may be carried out using any of a number of known vectors. Such vectors include viral and non-viral systems, including lentiviral and gammaretroviral systems, as well as transposon-based systems such as PiggyBac or Sleeping Beauty-based gene transfer systems. Exemplary methods include those for transfer of nucleic acids encoding the receptors, including via viral, *e.g.*, retroviral or lentiviral, transduction, transposons, and electroporation. In some embodiments, the engineering produces one or more engineered compositions of enriched T cells.

[0417] In certain embodiments, the one or more compositions of stimulated T cells are or include two separate stimulated compositions of enriched T cells. In particular embodiments, two separate compositions of enriched T cells, *e.g.*, two separate compositions of enriched T cells that have been selected, isolated, and/or enriched from the same biological sample, are separately engineered. In certain embodiments, the two separate compositions include a composition of enriched CD4+ T cells. In particular embodiments, the two separate compositions include a composition of enriched CD8+ T cells. In some embodiments, two separate compositions of enriched CD4+ T cells and enriched CD8+ T cells are genetically engineered separately. In some embodiments, a single composition of enriched T cells is genetically engineered. In certain embodiments, the single composition is a composition of enriched CD4+ T cells. In some embodiments, the single composition is a composition of enriched CD4+ and CD8+ T cells that have been combined from separate compositions prior to the engineering.

[0418] In some embodiments, separate compositions of enriched CD4+ and CD8+ T cells are combined into a single composition and are genetically engineered, *e.g.*, transduced or transfected. In certain embodiments, separate engineered compositions of enriched CD4+ and enriched CD8+ T cells are combined into a single composition after the genetic engineering has been performed and/or completed.

[0419] In some embodiments, gene transfer is accomplished by first stimulating the cell, such as by combining it with a stimulus that induces a response such as proliferation, survival, and/or activation, *e.g.*, as measured by expression of a cytokine or activation marker, followed by transduction of the activated cells,

and expansion in culture to numbers sufficient for clinical applications. In certain embodiments, the gene transfer is accomplished by first incubating the cells under stimulating conditions, such as by any of the methods described in Section III-B.

[0420] In some contexts, overexpression of a stimulatory factor (for example, a lymphokine or a cytokine) may be toxic to a subject. Thus, in some contexts, the engineered cells include gene segments that cause the cells to be susceptible to negative selection *in vivo*, such as following administration in adoptive immunotherapy. For example in some aspects, the cells are engineered so that they can be eliminated as a result of a change in the *in vivo* condition of the patient to which they are administered. The negative selectable phenotype may result from the insertion of a gene that confers sensitivity to an administered agent, for example, a compound. Negative selectable genes include the Herpes simplex virus type I thymidine kinase (HSV-I TK) gene (Wigler *et al.*, Cell 2:223, 1977) which confers ganciclovir sensitivity; the cellular hypoxanthine phosphoribosyltransferase (HPRT) gene, the cellular adenine phosphoribosyltransferase (APRT) gene, bacterial cytosine deaminase, (Mullen *et al.*, Proc. Natl. Acad. Sci. USA. 89:33 (1992)).

[0421] In some aspects, the cells further are engineered to promote expression of cytokines or other factors. Various methods for the introduction of genetically engineered components, *e.g.*, antigen receptors, *e.g.*, CARs, are well known and may be used with the provided methods and compositions. Exemplary methods include those for transfer of polynucleotides encoding the receptors, including via viral, *e.g.*, retroviral or lentiviral, transduction, transposons, and electroporation.

[0422] In some embodiments, recombinant polynucleotides are transferred into cells using recombinant infectious virus particles, such as, *e.g.*, vectors derived from simian virus 40 (SV40), adenoviruses, adeno-associated virus (AAV). In some embodiments, recombinant polynucleotides are transferred into T cells using recombinant lentiviral vectors or retroviral vectors, such as gamma-retroviral vectors (see, *e.g.*, Koste *et al.* (2014) Gene Therapy 2014 Apr 3. doi: 10.1038/gt.2014.25; Carlens *et al.* (2000) Exp Hematol 28(10): 1137-46; Alonso-Camino *et al.* (2013) Mol Ther Nucl Acids 2, e93; Park *et al.*, Trends Biotechnol. 2011 November 29(11): 550–557).

[0423] In some embodiments, methods for genetic engineering are carried out by contacting one or more cells of a composition with a nucleic acid molecule encoding the recombinant protein, e.g. recombinant receptor. In some embodiments, the contacting can be effected with centrifugation, such as spinoculation (e.g. centrifugal inoculation). Such methods include any of those as described in International Publication Number WO2016/073602. Exemplary centrifugal chambers include those produced and sold by Biosafe SA, including those for use with the Sepax® and Sepax® 2 system, including an A-200/F and A-200 centrifugal chambers and various kits for use with such systems. Exemplary chambers, systems, and processing instrumentation and cabinets are described, for example, in US Patent No. 6,123,655, US Patent No.

6,733,433 and Published U.S. Patent Application, Publication No.: US 2008/0171951, and published international patent application, publication no. WO 00/38762, the contents of each of which are incorporated herein by reference in their entirety. Exemplary kits for use with such systems include, but are not limited to, single-use kits sold by BioSafe SA under product names CS-430.1, CS-490.1, CS-600.1 or CS-900.2.

[0424] In some embodiments, the contacting can be effected with centrifugation, such as spinoculation (e.g., centrifugal inoculation). In some embodiments, the composition containing cells, viral particles and reagent can be rotated, generally at relatively low force or speed, such as speed lower than that used to pellet the cells, such as from or from about 600 rpm to 1700 rpm (e.g., at or about or at least 600 rpm, 1000 rpm, or 1500 rpm or 1700 rpm). In some embodiments, the rotation is carried at a force, e.g., a relative centrifugal force, of from or from about 100 g to 3200 g (e.g., at or about or at least at or about 100 g, 200 g, 300 g, 400 g, 500 g, 1000 g, 1500 g, 2000 g, 2500 g, 3000 g or 3200 g), as measured for example at an internal or external wall of the chamber or cavity. The term "relative centrifugal force" or RCF is generally understood to be the effective force imparted on an object or substance (such as a cell, sample, or pellet and/or a point in the chamber or other container being rotated), relative to the earth's gravitational force, at a particular point in space as compared to the axis of rotation. The value may be determined using well-known formulas, taking into account the gravitational force, rotation speed and the radius of rotation (distance from the axis of rotation and the object, substance, or particle at which RCF is being measured).

[0425] In some embodiments, the introducing is carried out by contacting one or more cells of a composition with a nucleic acid molecule encoding the recombinant protein, e.g. recombinant receptor. In some embodiments, the contacting can be effected with centrifugation, such as spinoculation (e.g. centrifugal inoculation). Such methods include any of those as described in International Publication Number WO2016/073602. Exemplary centrifugal chambers include those produced and sold by Biosafe SA, including those for use with the Sepax® and Sepax® 2 system, including an A-200/F and A-200 centrifugal chambers and various kits for use with such systems. Exemplary chambers, systems, and processing instrumentation and cabinets are described, for example, in US Patent No. 6,123,655, US Patent No. 6,733,433 and Published U.S. Patent Application, Publication No.: US 2008/0171951, and published international patent application, publication no. WO 00/38762, the contents of each of which are incorporated herein by reference in their entirety. Exemplary kits for use with such systems include, but are not limited to, single-use kits sold by BioSafe SA under product names CS-430.1, CS-490.1, CS-600.1 or CS-900.2.

[0426] In some embodiments, the system is included with and/or placed into association with other instrumentation, including instrumentation to operate, automate, control and/or monitor aspects of the

transduction step and one or more various other processing steps performed in the system, e.g. one or more processing steps that can be carried out with or in connection with the centrifugal chamber system as described herein or in International Publication Number WO2016/073602. This instrumentation in some embodiments is contained within a cabinet. In some embodiments, the instrumentation includes a cabinet, which includes a housing containing control circuitry, a centrifuge, a cover, motors, pumps, sensors, displays, and a user interface. An exemplary device is described in US Patent No. 6,123,655, US Patent No. 6,733,433 and US 2008/0171951.

[0427] In some embodiments, the system comprises a series of containers, e.g., bags, tubing, stopcocks, clamps, connectors, and a centrifuge chamber. In some embodiments, the containers, such as bags, include one or more containers, such as bags, containing the cells to be transduced and the viral vector particles, in the same container or separate containers, such as the same bag or separate bags. In some embodiments, the system further includes one or more containers, such as bags, containing medium, such as diluent and/or wash solution, which is pulled into the chamber and/or other components to dilute, resuspend, and/or wash components and/or compositions during the methods. The containers can be connected at one or more positions in the system, such as at a position corresponding to an input line, diluent line, wash line, waste line and/or output line.

[0428] In some embodiments, the chamber is associated with a centrifuge, which is capable of effecting rotation of the chamber, such as around its axis of rotation. Rotation may occur before, during, and/or after the incubation in connection with transduction of the cells and/or in one or more of the other processing steps. Thus, in some embodiments, one or more of the various processing steps is carried out under rotation, e.g., at a particular force. The chamber is typically capable of vertical or generally vertical rotation, such that the chamber sits vertically during centrifugation and the side wall and axis are vertical or generally vertical, with the end wall(s) horizontal or generally horizontal.

[0429] In some embodiments, the composition containing cells, the vector, e.g., viral particles, and reagent can be rotated, generally at relatively low force or speed, such as speed lower than that used to pellet the cells, such as from or from about 600 rpm to 1700 rpm (e.g. at or about or at least 600 rpm, 1000 rpm, or 1500 rpm or 1700 rpm). In some embodiments, the rotation is carried at a force, e.g., a relative centrifugal force, of from or from about 100 g to 3200 g (e.g. at or about or at least at or about 100 g, 200 g, 300 g, 400 g, 500 g, 1000 g, 1500 g, 2000 g, 2500 g, 3000 g or 3200 g), as measured for example at an internal or external wall of the chamber or cavity. The term "relative centrifugal force" or RCF is generally understood to be the effective force imparted on an object or substance (such as a cell, sample, or pellet and/or a point in the chamber or other container being rotated), relative to the earth's gravitational force, at a particular point in space as compared to the axis of rotation. The value may be determined using well-known formulas,

taking into account the gravitational force, rotation speed and the radius of rotation (distance from the axis of rotation and the object, substance, or particle at which RCF is being measured).

[0430] In some embodiments, during at least a part of the genetic engineering, e.g. transduction, and/or subsequent to the genetic engineering the cells are transferred to a bioreactor bag assembly for culture of the genetically engineered cells, such as for cultivation or expansion of the cells.

2. Viral Vectors

[0431] In some embodiments, recombinant nucleic acids are transferred into cells using recombinant infectious virus particles, such as, e.g., vectors derived from simian virus 40 (SV40), adenoviruses, adeno-associated virus (AAV). In some embodiments, recombinant nucleic acids are transferred into T cells using recombinant lentiviral vectors or retroviral vectors, such as gamma-retroviral vectors (see, e.g., Koste et al. (2014) Gene Therapy 2014 Apr 3. doi: 10.1038/gt.2014.25; Carlens et al. (2000) Exp Hematol 28(10): 1137-46; Alonso-Camino et al. (2013) Mol Ther Nucl Acids 2, e93; Park et al., Trends Biotechnol. 2011 November 29(11): 550–557).

[0432] In some embodiments, the viral vector or the non-viral DNA contains a nucleic acid that encodes a heterologous recombinant protein. In some embodiments, the heterologous recombinant molecule is or includes a recombinant receptor, *e.g.,* an antigen receptor, SB-transposons, *e.g.,* for gene silencing, capsid-enclosed transposons, homologous double stranded nucleic acid, *e.g.,* for genomic recombination or reporter genes (*e.g.,* fluorescent proteins, such as GFP) or luciferase).

[0433] In some embodiments, the retroviral vector has a long terminal repeat sequence (LTR), *e.g.*, a retroviral vector derived from the Moloney murine leukemia virus (MoMLV), myeloproliferative sarcoma virus (MPSV), murine embryonic stem cell virus (MESV), murine stem cell virus (MSCV), spleen focus forming virus (SFFV), or human immunodeficiency virus type 1 (HIV-1). Most retroviral vectors are derived from murine retroviruses. In some embodiments, the retroviruses include those derived from any avian or mammalian cell source. The retroviruses typically are amphotropic, meaning that they are capable of infecting host cells of several species, including humans. In one embodiment, the gene to be expressed replaces the retroviral gag, pol and/or env sequences. A number of illustrative retroviral systems have been described (*e.g.*, U.S. Pat. Nos. 5,219,740; 6,207,453; 5,219,740; Miller and Rosman (1989) BioTechniques 7:980-990; Miller, A. D. (1990) Human Gene Therapy 1:5-14; Scarpa *et al.* (1991) Virology 180:849-852; Burns *et al.* (1993) Proc. Natl. Acad. Sci. USA 90:8033-8037; and Boris-Lawrie and Temin (1993) Cur. Opin. Genet. Develop. 3:102-109.

[0434] Methods of lentiviral transduction are known. Exemplary methods are described in, *e.g.*, Wang *et al.* (2012) J. Immunother. 35(9): 689-701; Cooper *et al.* (2003) Blood. 101:1637–1644; Verhoeyen *et al.*

(2009) Methods Mol Biol. 506: 97-114; and Cavalieri *et al.* (2003) Blood. 102(2): 497-505.

[0435] In some embodiments, the viral vector particles contain a genome derived from a retroviral genome based vector, such as derived from a lentiviral genome based vector. In some aspects of the provided viral vectors, the heterologous nucleic acid encoding a recombinant receptor, such as an antigen receptor, such as a CAR, is contained and/or located between the 5′ LTR and 3′ LTR sequences of the vector genome.

[0436] In some embodiments, the viral vector genome is a lentivirus genome, such as an HIV-1 genome or an SIV genome. For example, lentiviral vectors have been generated by multiply attenuating virulence genes, for example, the genes env, vif, vpu and nef can be deleted, making the vector safer for therapeutic purposes. Lentiviral vectors are known. See Naldini et al., (1996 and 1998); Zufferey et al., (1997); Dull et al., 1998, U.S. Pat. Nos. 6,013,516; and 5,994,136). In some embodiments, these viral vectors are plasmid-based or virus-based, and are configured to carry the essential sequences for incorporating foreign nucleic acid, for selection, and for transfer of the nucleic acid into a host cell. Known lentiviruses can be readily obtained from depositories or collections such as the American Type Culture Collection ("ATCC"; 10801 University Blvd., Manassas, Va. 20110-2209), or isolated from known sources using commonly available techniques.

[0437] Non-limiting examples of lentiviral vectors include those derived from a lentivirus, such as Human Immunodeficiency Virus 1 (HIV-1), HIV-2, an Simian Immunodeficiency Virus (SIV), Human T-lymphotropic virus 1 (HTLV-1), HTLV-2 or equine infection anemia virus (E1AV). For example, lentiviral vectors have been generated by multiply attenuating the HIV virulence genes, for example, the genes env, vif, vpr, vpu and nef are deleted, making the vector safer for therapeutic purposes. Lentiviral vectors are known in the art, see Naldini et al., (1996 and 1998); Zufferey et al., (1997); Dull et al., 1998, U.S. Pat. Nos. 6,013,516; and 5,994,136). In some embodiments, these viral vectors are plasmid-based or virus-based, and are configured to carry the essential sequences for incorporating foreign nucleic acid, for selection, and for transfer of the nucleic acid into a host cell. Known lentiviruses can be readily obtained from depositories or collections such as the American Type Culture Collection ("ATCC"; 10801 University Blvd., Manassas, Va. 20110-2209), or isolated from known sources using commonly available techniques.

[0438] In some embodiments, the viral genome vector can contain sequences of the 5′ and 3′ LTRs of a retrovirus, such as a lentivirus. In some aspects, the viral genome construct may contain sequences from the 5′ and 3′ LTRs of a lentivirus, and in particular can contain the R and U5 sequences from the 5′ LTR of a lentivirus and an inactivated or self-inactivating 3′ LTR from a lentivirus. The LTR sequences can be LTR sequences from any lentivirus from any species. For example, they may be LTR sequences from HIV, SIV, FIV or BIV. Typically, the LTR sequences are HIV LTR sequences.

[0439] In some embodiments, the nucleic acid of a viral vector, such as an HIV viral vector, lacks additional transcriptional units. The vector genome can contain an inactivated or self-inactivating 3′ LTR (Zufferey et al. *J Virol* 72: 9873, 1998; Miyoshi et al., *J Virol* 72:8150, 1998). For example, deletion in the U3 region of the 3′ LTR of the nucleic acid used to produce the viral vector RNA can be used to generate self-inactivating (SIN) vectors. This deletion can then be transferred to the 5′ LTR of the proviral DNA during reverse transcription. A self-inactivating vector generally has a deletion of the enhancer and promoter sequences from the 3′ long terminal repeat (LTR), which is copied over into the 5′ LTR during vector integration. In some embodiments enough sequence can be eliminated, including the removal of a TATA box, to abolish the transcriptional activity of the LTR. This can prevent production of full-length vector RNA in transduced cells. In some aspects, the U3 element of the 3′ LTR contains a deletion of its enhancer sequence, the TATA box, Sp1, and NF-kappa B sites. As a result of the self-inactivating 3′ LTR, the provirus that is generated following entry and reverse transcription contains an inactivated 5′ LTR. This can improve safety by reducing the risk of mobilization of the vector genome and the influence of the LTR on nearby cellular promoters. The self-inactivating 3′ LTR can be constructed by any method known in the art. In some embodiments, this does not affect vector titers or the in vitro or in vivo properties of the vector.

[0440] Optionally, the U3 sequence from the lentiviral 5′ LTR can be replaced with a promoter sequence in the viral construct, such as a heterologous promoter sequence. This can increase the titer of virus recovered from the packaging cell line. An enhancer sequence can also be included. Any enhancer/promoter combination that increases expression of the viral RNA genome in the packaging cell line may be used. In one example, the CMV enhancer/promoter sequence is used (U.S. Pat. No. 5,385,839 and U.S. Pat. No. 5,168,062).

[0441] In certain embodiments, the risk of insertional mutagenesis can be minimized by constructing the retroviral vector genome, such as lentiviral vector genome, to be integration defective. A variety of approaches can be pursued to produce a non-integrating vector genome. In some embodiments, a mutation(s) can be engineered into the integrase enzyme component of the pol gene, such that it encodes a protein with an inactive integrase. In some embodiments, the vector genome itself can be modified to prevent integration by, for example, mutating or deleting one or both attachment sites, or making the 3′ LTR-proximal polypurine tract (PPT) non-functional through deletion or modification. In some embodiments, non-genetic approaches are available; these include pharmacological agents that inhibit one or more functions of integrase. The approaches are not mutually exclusive; that is, more than one of them can be used at a time. For example, both the integrase and attachment sites can be non-functional, or the integrase and PPT site can be non-functional, or the attachment sites and PPT site can be non-functional, or all of them can be non-functional. Such methods and viral vector genomes are known and available (see Philpott and

Thrasher, *Human Gene Therapy* 18:483, 2007; Engelman et al. J Virol 69:2729, 1995; Brown et al *J Virol* 73:9011 (1999); WO 2009/076524; McWilliams et al., *J Virol* 77:11150, 2003; Powell and Levin *J Virol* 70:5288, 1996).

[0442] In some embodiments, the vector contains sequences for propagation in a host cell, such as a prokaryotic host cell. In some embodiments, the nucleic acid of the viral vector contains one or more origins of replication for propagation in a prokaryotic cell, such as a bacterial cell. In some embodiments, vectors that include a prokaryotic origin of replication also may contain a gene whose expression confers a detectable or selectable marker such as drug resistance.

[0443] The viral vector genome is typically constructed in a plasmid form that can be transfected into a packaging or producer cell line. Any of a variety of known methods can be used to produce retroviral particles whose genome contains an RNA copy of the viral vector genome. In some embodiments, at least two components are involved in making a virus-based gene delivery system: first, packaging plasmids, encompassing the structural proteins as well as the enzymes necessary to generate a viral vector particle, and second, the viral vector itself, i.e., the genetic material to be transferred. Biosafety safeguards can be introduced in the design of one or both of these components.

[0444] In some embodiments, the packaging plasmid can contain all retroviral, such as HIV-1, proteins other than envelope proteins (Naldini *et al*., 1998). In other embodiments, viral vectors can lack additional viral genes, such as those that are associated with virulence, *e.g.,* vpr, vif, vpu and nef, and/or Tat, a primary transactivator of HIV. In some embodiments, lentiviral vectors, such as HIV-based lentiviral vectors, comprise only three genes of the parental virus: gag, pol and rev, which reduces or eliminates the possibility of reconstitution of a wild-type virus through recombination.

[0445] In some embodiments, the viral vector genome is introduced into a packaging cell line that contains all the components necessary to package viral genomic RNA, transcribed from the viral vector genome, into viral particles. Alternatively, the viral vector genome may comprise one or more genes encoding viral components in addition to the one or more sequences, e.g., recombinant nucleic acids, of interest. In some aspects, in order to prevent replication of the genome in the target cell, however, endogenous viral genes required for replication are removed and provided separately in the packaging cell line.

[0446] In some embodiments, a packaging cell line is transfected with one or more plasmid vectors containing the components necessary to generate the particles. In some embodiments, a packaging cell line is transfected with a plasmid containing the viral vector genome, including the LTRs, the cis-acting packaging sequence and the sequence of interest, i.e. a nucleic acid encoding an antigen receptor, such as a CAR; and one or more helper plasmids encoding the virus enzymatic and/or structural components, such as

Gag, pol and/or rev. In some embodiments, multiple vectors are utilized to separate the various genetic components that generate the retroviral vector particles. In some such embodiments, providing separate vectors to the packaging cell reduces the chance of recombination events that might otherwise generate replication competent viruses. In some embodiments, a single plasmid vector having all of the retroviral components can be used.

[0447] In some embodiments, the retroviral vector particle, such as lentiviral vector particle, is pseudotyped to increase the transduction efficiency of host cells. For example, a retroviral vector particle, such as a lentiviral vector particle, in some embodiments is pseudotyped with a VSV-G glycoprotein, which provides a broad cell host range extending the cell types that can be transduced. In some embodiments, a packaging cell line is transfected with a plasmid or polynucleotide encoding a non-native envelope glycoprotein, such as to include xenotropic, polytropic or amphotropic envelopes, such as Sindbis virus envelope, GALV or VSV-G.

[0448] In some embodiments, the packaging cell line provides the components, including viral regulatory and structural proteins, that are required in trans for the packaging of the viral genomic RNA into lentiviral vector particles. In some embodiments, the packaging cell line may be any cell line that is capable of expressing lentiviral proteins and producing functional lentiviral vector particles. In some aspects, suitable packaging cell lines include 293 (ATCC CCL X), 293T, HeLA (ATCC CCL 2), D17 (ATCC CCL 183), MDCK (ATCC CCL 34), BHK (ATCC CCL-10) and Cf2Th (ATCC CRL 1430) cells.

[0449] In some embodiments, the packaging cell line stably expresses the viral protein(s). For example, in some aspects, a packaging cell line containing the gag, pol, rev and/or other structural genes but without the LTR and packaging components can be constructed. In some embodiments, a packaging cell line can be transiently transfected with nucleic acid molecules encoding one or more viral proteins along with the viral vector genome containing a nucleic acid molecule encoding a heterologous protein, and/or a nucleic acid encoding an envelope glycoprotein.

[0450] In some embodiments, the viral vectors and the packaging and/or helper plasmids are introduced via transfection or infection into the packaging cell line. The packaging cell line produces viral vector particles that contain the viral vector genome. Methods for transfection or infection are well known. Non-limiting examples include calcium phosphate, DEAE-dextran and lipofection methods, electroporation and microinjection.

[0451] When a recombinant plasmid and the retroviral LTR and packaging sequences are introduced into a special cell line (e.g., by calcium phosphate precipitation for example), the packaging sequences may permit the RNA transcript of the recombinant plasmid to be packaged into viral particles, which then may be secreted into the culture media. The media containing the recombinant retroviruses in some embodiments is

then collected, optionally concentrated, and used for gene transfer. For example, in some aspects, after cotransfection of the packaging plasmids and the transfer vector to the packaging cell line, the viral vector particles are recovered from the culture media and titered by standard methods used by those of skill in the art.

[0452] In some embodiments, a retroviral vector, such as a lentiviral vector, can be produced in a packaging cell line, such as an exemplary HEK 293T cell line, by introduction of plasmids to allow generation of lentiviral particles. In some embodiments, a packaging cell is transfected and/or contains a polynucleotide encoding gag and pol, and a polynucleotide encoding a recombinant receptor, such as an antigen receptor, for example, a CAR. In some embodiments, the packaging cell line is optionally and/or additionally transfected with and/or contains a polynucleotide encoding a rev protein. In some embodiments, the packaging cell line is optionally and/or additionally transfected with and/or contains a polynucleotide encoding a non-native envelope glycoprotein, such as VSV-G. In some such embodiments, approximately two days after transfection of cells, *e.g.*, HEK 293T cells, the cell supernatant contains recombinant lentiviral vectors, which can be recovered and titered.

[0453] Recovered and/or produced retroviral vector particles can be used to transduce target cells using the methods as described. Once in the target cells, the viral RNA is reverse-transcribed, imported into the nucleus and stably integrated into the host genome. One or two days after the integration of the viral RNA, the expression of the recombinant protein, *e.g.*, antigen receptor, such as CAR, can be detected.

[0454] In some embodiments, the provided methods involve methods of transducing cells by contacting, e.g., incubating, a cell composition comprising a plurality of cells with a viral particle. In some embodiments, the cells to be transfected or transduced are or comprise primary cells obtained from a subject, such as cells enriched and/or selected from a subject.

[0455] In some embodiments, the concentration of cells to be transduced of the composition is from or from about 1.0×10^5 cells/mL to 1.0×10^8 cells/mL, such as at least or about at least or about 1.0×10^5 cells/mL, 5×10^5 cells/mL, 1×10^6 cells/mL, 5×10^6 cells/mL, 1×10^7 cells/mL, 5×10^7 cells/mL or 1×10^8 cells/mL.

[0456] In some embodiments, the viral particles are provided at a certain ratio of copies of the viral vector particles or infectious units (IU) thereof, per total number of cells to be transduced (IU/cell). For example, in some embodiments, the viral particles are present during the contacting at or about or at least at or about 0.5, 1, 2, 3, 4, 5, 10, 15, 20, 30, 40, 50, or 60 IU of the viral vector particles per one of the cells.

[0457] In some embodiments, the titer of viral vector particles is between or between about 1×10^6 IU/mL and 1×10^8 IU/mL, such as between or between about 5×10^6 IU/mL and 5×10^7 IU/mL, such as at least 6×10^6 IU/mL, 7×10^6 IU/mL, 8×10^6 IU/mL, 9×10^6 IU/mL, 1×10^7 IU/mL, 2×10^7 IU/mL, 3×10^7

IU/mL, 4 x 10^7 IU/mL, or 5 x10^7 IU/mL.

[0458] In some embodiments, transduction can be achieved at a multiplicity of infection (MOI) of less than 100, such as generally less than 60, 50, 40, 30, 20, 10, 5 or less.

[0459] In some embodiments, the method involves contacting or incubating, the cells with the viral particles. In some embodiments, the contacting is for 30 minutes to 72 hours, such as 30 minute to 48 hours, 30 minutes to 24 hours or 1 hour to 24 hours, such as at least or about at least 30 minutes, 1 hour, 2 hours, 6 hours, 12 hours, 24 hours, 36 hours or more.

[0460] In some embodiments, contacting is performed in solution. In some embodiments, the cells and viral particles are contacted in a volume of from or from about 0.5 mL to 500 mL, such as from or from about 0.5 mL to 200 mL, 0.5 mL to 100 mL, 0.5 mL to 50 mL, 0.5 mL to 10 mL, 0.5 mL to 5 mL, 5 mL to 500 mL, 5 mL to 200 mL, 5 mL to 100 mL, 5 mL to 50 mL, 5 mL to 10 mL, 10 mL to 500 mL, 10 mL to 200 mL, 10 mL to 100 mL, 10 mL to 50 mL, 50 mL to 500 mL, 50 mL to 200 mL, 50 mL to 100 mL, 100 mL to 500 mL, 100 mL to 200 mL or 200 mL to 500 mL.

[0461] In certain embodiments, the input cells are treated, incubated, or contacted with particles that comprise binding molecules that bind to or recognize the recombinant receptor that is encoded by the viral DNA.

[0462] In some embodiments, the incubation of the cells with the viral vector particles results in or produces an output composition comprising cells transduced with the viral vector particles.

[0463] In some embodiments, recombinant polynucleotides are transferred into T cells via electroporation (see, *e.g.*, Chicaybam et al, (2013) PLoS ONE 8(3): e60298 and Van Tedeloo *et al.* (2000) Gene Therapy 7(16): 1431-1437). In some embodiments, recombinant polynucleotides are transferred into T cells via transposition (see, *e.g.*, Manuri *et al.* (2010) Hum Gene Ther 21(4): 427-437; Sharma *et al.* (2013) Molec Ther Nucl Acids 2, e74; and Huang *et al.* (2009) Methods Mol Biol 506: 115-126). Other methods of introducing and expressing genetic material in immune cells include calcium phosphate transfection (*e.g.*, as described in Current Protocols in Molecular Biology, John Wiley & Sons, New York. N.Y.), protoplast fusion, cationic liposome-mediated transfection; tungsten particle-facilitated microparticle bombardment (Johnston, Nature, 346: 776-777 (1990)); and strontium phosphate DNA co-precipitation (Brash *et al.*, Mol. Cell Biol., 7: 2031-2034 (1987)).

[0464] Other approaches and vectors for transfer of the polynucleotides encoding the recombinant products are those described, *e.g.*, in international patent application, Publication No.: WO2014055668, and U.S. Patent No. 7,446,190.

[0465] Among additional polynucleotides, *e.g.*, genes for introduction are those to improve the efficacy

of therapy, such as by promoting viability and/or function of transferred cells; genes to provide a genetic marker for selection and/or evaluation of the cells, such as to assess *in vivo* survival or localization; genes to improve safety, for example, by making the cell susceptible to negative selection *in vivo* as described by Lupton S. D. *et al.*, Mol. and Cell Biol., 11:6 (1991); and Riddell *et al.*, Human Gene Therapy 3:319-338 (1992); see also the publications of PCT/US91/08442 and PCT/US94/05601 by Lupton *et al.* describing the use of bifunctional selectable fusion genes derived from fusing a dominant positive selectable marker with a negative selectable marker. See, *e.g.*, Riddell *et al.*, US Patent No. 6,040,177, at columns 14-17.

3. Engineered Cells, Vectors and Compositions for Multi-Targeting

[0466] Also provided are cells such as engineered cells that can bind to and/or target multiple antigens. In some embodiments, improved selectivity and specificity is achieved through strategies targeting multiple antigens. Such strategies generally involve multiple antigen-binding domains, which typically are present on distinct genetically engineered antigen receptors and specifically bind to distinct antigens. In some embodiments, the cells are engineered with the ability to bind more than one antigen. For example, in some embodiments, the cells are engineered to express multispecific binding molecules. In some embodiments, the cells express multiple binding molecules, e.g., recombinant receptors, each of which can target one antigen or multiple antigens, *e.g.*, one receptor that targets BCMA, such as any described herein, and another receptor that targets another antigen, *e.g.*, tumor antigen. In some aspects, a plurality of genetically engineered antigen receptors are introduced into the cell, which specifically bind to different antigens, each expressed in or on the disease or condition to be targeted with the cells or tissues or cells thereof. Such features can in some aspects address or reduce the likelihood of off-target effects or increase efficacy. For example, where a single antigen expressed in a disease or condition is also expressed on or in non-diseased or normal cells, such multi-targeting approaches can provide selectivity for desired cell types by requiring binding via multiple antigen receptors in order to activate the cell or induce a particular effector function. In some embodiments, a plurality of cells can be engineered to express one or more different binding molecules, e.g., recombinant receptors, each of which can target one antigen or multiple antigens.

[0467] Also provided are multispecific cells containing any of the binding molecules described herein, such as cells containing a cell surface protein including the anti-BCMA antibody and an additional cell surface protein, such as an additional chimeric receptor, which binds to a different antigen or a different epitope on BCMA. In some embodiments, provided are compositions of cells that express recombinant receptors, wherein one or more of the binding molecules, multispecific binding molecules and/or recombinant receptors bind and/or target BCMA. In some embodiments, the multispecific binding molecules and/or recombinant receptors target one or more different epitopes on BCMA.

[0468] In some embodiments, provided are composition of cells, wherein each type of cell expresses one or more binding molecules, *e.g.*, recombinant receptors. In some embodiments, the cell comprises (*e.g.*, has been transformed with) one or more vectors comprising one or more nucleic acid that encodes one or more an amino acid sequence comprising one or more antibodies and/or portions thereof, *e.g.*, antigen-binding fragments thereof. In some embodiments, one or more such cells are provided. In some embodiments, a composition containing one or more such cells is provided. In some embodiments, the one or more cells can express different antibodies, or the same antibody. In some embodiments, each of the cells expresses one or more antibodies, such as more than one antibody. In some embodiments, each of the cells expresses a multispecific binding molecule, *e.g.*, a multispecific receptor, *e.g.*, CAR.

[0469] In some embodiments, the cells include multi-targeting strategies that target BCMA and a second or additional antigen associated with a particular disease or condition. In some embodiments, the second or additional antigen is targeted by a multispecific binding molecule and/or multiple binding molecules and/or a plurality of cells, *e.g.*, one or more cells, each engineered to express one or more recombinant receptors. In some embodiments, a recombinant receptor targeting a second or additional antigen is expressed on the same cell as a BCMA binding molecule, or on a different cell.

[0470] In some embodiments, among the second or additional antigens for multi-targeting strategies includes those in which at least one of the antigens is a universal tumor antigen, or a family member thereof. In some embodiments, the second or additional antigen is an antigen expressed on a tumor. In some embodiments, the BCMA-binding molecules provided herein target an antigen on the same tumor type as the second or additional antigen. In some embodiments, the second or additional antigen may also be a universal tumor antigen or may be a tumor antigen specific to a tumor type. In some embodiments, the cell further comprises an additional genetically engineered antigen receptor that recognizes a second or additional antigen expressed on a disease or condition to be treated and induces a stimulatory or activating signal.

[0471] Exemplary antigens include CD4, CD5, CD8, CD14, CD15, CD19, CD20, CD21, CD22, CD23, CD25, CD33, CD37, CD38, CD40, CD40L, CD46, CD52, CD54, CD74, CD80, CD126, CD138, B7, MUC-1, Ia, HM1.24, HLA-DR, tenascin, an angiogenesis factor, VEGF, PIGF, ED-B fibronectin, an oncogene, an oncogene product, CD66a-d, necrosis antigens, Ii, IL-2, T101, TAC, IL-6, ROR1, TRAIL-R1 (DR4), TRAIL-R2 (DR5), B cell maturation antigen (BCMA), tEGFR, Her2, L1-CAM, mesothelin, CEA, hepatitis B surface antigen, anti-folate receptor, CD24, CD30, CD44, EGFR, EGP-2, EGP-4, EPHa2, ErbB2, ErbB3, ErbB4, erbB dimers, EGFR vIII, FBP, FCRL5, FCRH5, fetal acetylcholine receptor, GD2, GD3, G protein-coupled receptor class C group 5 member D (GPRC5D), HMW-MAA, IL-22R-alpha, IL-13R-alpha2, kdr, kappa light chain, Lewis Y, L1-cell adhesion molecule (L1-CAM), Melanoma-associated antigen (MAGE)-A1, MAGE-A3, MAGE-A6, Preferentially expressed antigen of melanoma (PRAME), survivin, EGP2,

EGP40, TAG72, B7-H6, IL-13 receptor a2 (IL-13Ra2), CA9, CD171, G250/CAIX, HLA-AI MAGE Al, HLA-A2 NY-ESO-1, PSCA, folate receptor-a, CD44v6, CD44v7/8, avb6 integrin, 8H9, NCAM, VEGF receptors, 5T4, Foetal AchR, NKG2D ligands, dual antigen, an antigen associated with a universal tag, a cancer-testes antigen, MUC1, MUC16, NY-ESO-1, MART-1, gp100, oncofetal antigen, VEGF-R2, carcinoembryonic antigen (CEA), prostate specific antigen, PSMA, Her2/neu, estrogen receptor, progesterone receptor, ephrinB2, CD123, c-Met, GD-2, O-acetylated GD2 (OGD2), CE7, Wilms Tumor 1 (WT-1), a cyclin, cyclin A2, CCL-1, hTERT, MDM2, CYP1B, WT1, livin, AFP, p53, cyclin (D1), CS-1, BCMA, BAFF-R, TACI, CD56, TIM-3, CD123, L1-cell adhesion molecule, MAGE-A1, MAGE A3, a cyclin, such as cyclin A1 (CCNA1) and/or a pathogen-specific antigen, biotinylated molecules, molecules expressed by HIV, HCV, HBV and/or other pathogens, and/or in some aspects, neoepitopes or neoantigens thereof. In some embodiments, the antigen is associated with or is a universal tag.

[0472] In some embodiments, the plurality of antigens, *e.g.*, the first antigen, *e.g.*, BCMA, and the second or additional antigens, are expressed on the cell, tissue, or disease or condition being targeted, such as on the cancer cell. In some aspects, the cell, tissue, disease or condition is multiple myeloma or a multiple myeloma cell. One or more of the plurality of antigens generally also is expressed on a cell which it is not desired to target with the cell therapy, such as a normal or non-diseased cell or tissue, and/or the engineered cells themselves. In such embodiments, by requiring ligation of multiple receptors to achieve a response of the cell, specificity and/or efficacy is achieved.

[0473] In some aspects, the antigen, *e.g.*, the second or additional antigen, such as the disease-specific antigen and/or related antigen, is expressed on multiple myeloma, such as G protein-coupled receptor class C group 5 member D (GPRC5D), CD38 (cyclic ADP ribose hydrolase), CD138 (syndecan-1, syndecan, SYN-1), CS-1 (CS1, CD2 subset 1, CRACC, SLAMF7, CD319, and 19A24), BAFF-R, TACI and/or FcRH5. Other exemplary multiple myeloma antigens include CD56, TIM-3, CD33, CD123, CD44, CD20, CD40, CD74, CD200, EGFR, β2-Microglobulin, HM1.24, IGF-1R, IL-6R, TRAIL-R1, and the activin receptor type IIA (ActRIIA). See Benson and Byrd, J. Clin. Oncol. (2012) 30(16): 2013-15; Tao and Anderson, Bone Marrow Research (2011):924058; Chu *et al.*, Leukemia (2013) 28(4):917-27; Garfall *et al.*, Discov Med. (2014) 17(91):37-46. In some embodiments, the antigens include those present on lymphoid tumors, myeloma, AIDS-associated lymphoma, and/or post-transplant lymphoproliferations, such as CD38. Antibodies or antigen-binding fragments directed against such antigens are known and include, for example, those described in U.S. Patent No. 8,153,765; 8,603477, 8,008,450; U.S. Pub. No. US20120189622 or US20100260748; and/or International PCT Publication Nos. WO2006099875, WO2009080829 or WO2012092612 or WO2014210064. In some embodiments, such antibodies or antigen-binding fragments thereof (e.g. scFv) are contained in multispecific antibodies, multispecific chimeric receptors, such as

multispecific CARs, and/or multispecific cells.

[0474] In some embodiments, the cells and methods include multi-targeting strategies, such as expression of two or more genetically engineered receptors on the cell, each recognizing a different antigen and typically each including a different intracellular signaling component. Such multi-targeting strategies are described, for example, in International Patent Application, Publication No.: WO 2014055668 A1 (describing combinations of a stimulatory or activating and costimulatory CARs, e.g., targeting two different antigens present individually on off-target, e.g., normal cells, but present together only on cells of the disease or condition to be treated) and Fedorov et al., *Sci. Transl. Medicine*, 5(215) (December, 2013) (describing cells expressing a stimulatory or an activating and an inhibitory CAR, such as those in which the stimulatory or activating CAR binds to one antigen expressed on both normal or non-diseased cells and cells of the disease or condition to be treated, and the inhibitory CAR binds to another antigen expressed only on the normal cells or cells which it is not desired to treat).

[0475] In some embodiments, a plurality of cells, each engineered to express one or more recombinant receptors, are provided. For example, in some embodiments, one cell is engineered to express a binding molecule that binds and/or targets BCMA, and another cell is engineered to express a binding molecule that binds and/or targets an additional or second antigen. In some embodiments, the cells can each express a multispecific binding molecule, e.g., a multispecific recombinant receptor, where one or more of the target antigen is BCMA. In some of such embodiments, the plurality of cells can be administered together or separately. In some embodiments, the plurality of cells are administered simultaneously or concurrently with the cells, e.g., administered on the same day, and/or sequentially with or intermittently with, in any order, another engineered cell in the plurality. For example, in some embodiments, an engineered cell expressing a BCMA-binding molecule, e.g., CAR, is administered simultaneously with or sequentially with, in any order, another engineered cell expressing a binding molecule that binds a different target antigen or a different epitope on BCMA. In some embodiments, the plurality of cells can be in the same composition. Exemplary compositions of the cells include compositions described in Section II below.

D. Cultivation, Expansion and Formulation of Engineered Cells

[0476] In some embodiments, the provided methods include one or more steps for cultivating cells, e.g., cultivating cells under conditions that promote proliferation and/or expansion. In some embodiments, cells are cultivated under conditions that promote proliferation and/or expansion subsequent to a step of genetically engineering, e.g., introducing a recombinant polypeptide to the cells by transduction or transfection. In particular embodiments, the cells are cultivated after the cells have been incubated under stimulating conditions and transduced or transfected with a recombinant polynucleotide, e.g., a

polynucleotide encoding a recombinant receptor.

[0477] In certain embodiments, the one or more compositions of engineered T cells are or include two separate compositions of enriched T cells. In particular embodiments, two separate compositions of enriched T cells, e.g., two separate compositions of enriched T cells selected, isolated, and/or enriched from the same biological sample, are separately cultivated under stimulating conditions. In certain embodiments, the two separate compositions include a composition of enriched CD4+ T cells. In particular embodiments, the two separate compositions include a composition of enriched CD8+ T cells. In some embodiments, two separate compositions of enriched CD4+ T cells and enriched CD8+ T cells are separately cultivated, e.g., under conditions that promote proliferation and/or expansion.

[0478] In some embodiments, a single composition of enriched T cells is cultivated. In some embodiments, the single composition is a composition of enriched CD4+ and CD8+ T cells that have been combined from separate compositions prior to the cultivation. In some embodiments, separate compositions of enriched CD4+ and CD8+ T cells are combined into a single composition and are cultivated, e.g., under conditions that promote proliferation and/or expansion. In certain embodiments, separate cultivated compositions of enriched CD4+ and enriched CD8+ T cells are combined into a single composition after the cultivation has been performed and/or completed.

[0479] In some embodiments, cultivation is carried out under conditions that promote proliferation and/or expansion. In some embodiments, such conditions may be designed to induce proliferation, expansion, activation, and/or survival of cells in the population. In particular embodiments, the stimulating conditions can include one or more of particular media, temperature, oxygen content, carbon dioxide content, time, agents, e.g., nutrients, amino acids, antibiotics, ions, and/or stimulatory factors, such as cytokines, chemokines, antigens, binding partners, fusion proteins, recombinant soluble receptors, and any other agents designed to promote growth, division, and/or expansion of the cells.

[0480] In particular embodiments, the cells are cultivated in the presence of one or more cytokines. In particular embodiments, the one or more cytokines are recombinant cytokines. In some embodiments, the one or more cytokines are human recombinant cytokines. In certain embodiments, the one or more cytokines bind to and/or are capable of binding to receptors that are expressed by and/or are endogenous to T cells. In particular embodiments, the one or more cytokines, e.g. a recombinant cytokine, is or includes a member of the 4-alpha-helix bundle family of cytokines. In some embodiments, members of the 4-alpha-helix bundle family of cytokines include, but are not limited to, interleukin-2 (IL-2), interleukin-4 (IL-4), interleukin-7 (IL-7), interleukin-9 (IL-9), interleukin 12 (IL-12), interleukin 15 (IL-15), granulocyte colony-stimulating factor (G-CSF), and granulocyte-macrophage colony-stimulating factor (GM-CSF). In some embodiments, the one or more recombinant cytokine includes IL-2, IL-7 and/or IL-15. In some embodiments, the cells, e.g.,

engineered cells, are cultivated in the presence of a cytokine, e.g., a recombinant human cytokine, at a concentration of between 1 IU/mL and 2,000 IU/mL, between 10 IU/mL and 100 IU/mL, between 50 IU/mL and 200 IU/mL, between 100 IU/mL and 500 IU/mL, between 100 IU/mL and 1,000 IU/mL, between 500 IU/mL and 2,000 IU/mL, or between 100 IU/mL and 1,500 IU/mL.

[0481] In some embodiments, the cultivation is performed under conditions that generally include a temperature suitable for the growth of primary immune cells, such as human T lymphocytes, for example, at least about 25 degrees Celsius, generally at least about 30 degrees, and generally at or about 37 degrees Celsius. In some embodiments, the composition of enriched T cells is incubated at a temperature of 25 to 38 degrees Celsius, such as 30 to 37 degrees Celsius, for example at or about 37 degrees Celsius ± 2 degrees Celsius. In some embodiments, the incubation is carried out for a time period until the culture, e.g. cultivation or expansion, results in a desired or threshold density, number or dose of cells. In some embodiments, the incubation is greater than or greater than about or is for about or 24 hours, 48 hours, 72 hours, 96 hours, 5 days, 6 days, 7 days, 8 days, 9 days or more.

[0482] In particular embodiments, the cultivation is performed in a closed system. In certain embodiments, the cultivation is performed in a closed system under sterile conditions. In particular embodiments, the cultivation is performed in the same closed system as one or more steps of the provided systems. In some embodiments the composition of enriched T cells is removed from a closed system and placed in and/or connected to a bioreactor for the cultivation. Examples of suitable bioreactors for the cultivation include, but are not limited to, GE Xuri W25, GE Xuri W5, Sartorius BioSTAT RM 20 | 50, Finesse SmartRocker Bioreactor Systems, and Pall XRS Bioreactor Systems. In some embodiments, the bioreactor is used to perfuse and/or mix the cells during at least a portion of the cultivation step.

[0483] In some embodiments, the mixing is or includes rocking and/or motioning. In some cases, the bioreactor can be subject to motioning or rocking, which, in some aspects, can increase oxygen transfer. Motioning the bioreactor may include, but is not limited to rotating along a horizontal axis, rotating along a vertical axis, a rocking motion along a tilted or inclined horizontal axis of the bioreactor or any combination thereof. In some embodiments, at least a portion of the incubation is carried out with rocking. The rocking speed and rocking angle may be adjusted to achieve a desired agitation. In some embodiments the rock angle is 20°, 19°, 18°, 17°, 16°, 15°, 14°, 13°, 12°, 11°, 10°, 9°, 8°, 7°, 6°, 5°, 4°, 3°, 2° or 1°. In certain embodiments, the rock angle is between 6-16°. In other embodiments, the rock angle is between 7-16°. In other embodiments, the rock angle is between 8-12°. In some embodiments, the rock rate is 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40 rpm. In some embodiments, the rock rate is between 4 and 12 rpm, such as between 4 and 6 rpm, inclusive.

[0484] In some embodiments, the bioreactor maintains the temperature at or near 37°C and CO2 levels at or near 5% with a steady air flow at, at about, or at least 0.01 L/min, 0.05 L/min, 0.1 L/min, 0.2 L/min, 0.3 L/min, 0.4 L/min, 0.5 L/min, 1.0 L/min, 1.5 L/min, or 2.0 L/min or greater than 2.0 L/min. In certain embodiments, at least a portion of the cultivation is performed with perfusion, such as with a rate of 290 ml/day, 580 ml/day, and/or 1160 ml/day, e.g., depending on the timing in relation to the start of the cultivation and/or density of the cultivated cells. In some embodiments, at least a portion of the cell culture expansion is performed with a rocking motion, such as at an angle of between 5° and 10°, such as 6°, at a constant rocking speed, such as a speed of between 5 and 15 RPM, such as 6 RMP or 10 RPM.

[0485] In some embodiments, the provided methods for manufacturing, generating or producing a cell therapy and/or engineered cells may include formulation of cells, such as formulation of genetically engineered cells resulting from the provided processing steps prior to or after the incubating, engineering, and cultivating, and/or one or more other processing steps as described. In some embodiments, one or more of the processing steps, including formulation of cells, can be carried out in a closed system. In some cases, the cells are processed in one or more steps (e.g. carried out in the centrifugal chamber and/or closed system) for manufacturing, generating or producing a cell therapy and/or engineered cells may include formulation of cells, such as formulation of genetically engineered cells resulting from the provided transduction processing steps prior to or after the culturing, e.g. cultivation and expansion, and/or one or more other processing steps as described.

[0486] In some embodiments, the dose of cells comprising cells engineered with a recombinant antigen receptor, e.g. CAR or TCR, is provided as a composition or formulation, such as a pharmaceutical composition or formulation. Such compositions can be used in accord with the provided methods, such as in the prevention or treatment of diseases, conditions, and disorders, or in detection, diagnostic, and prognostic methods. In some cases, the cells can be formulated in an amount for dosage administration, such as for a single unit dosage administration or multiple dosage administration.

[0487] In some embodiments, cells can be formulated into a container, such as a bag or vial.

[0488] In some embodiments, the cells are formulated in a pharmaceutically acceptable buffer, which may, in some aspects, include a pharmaceutically acceptable carrier or excipient. In some embodiments, the processing includes exchange of a medium into a medium or formulation buffer that is pharmaceutically acceptable or desired for administration to a subject. In some embodiments, the processing steps can involve washing the transduced and/or expanded cells to replace the cells in a pharmaceutically acceptable buffer that can include one or more optional pharmaceutically acceptable carriers or excipients. Exemplary of such pharmaceutical forms, including pharmaceutically acceptable carriers or excipients, can be any described below in conjunction with forms acceptable for administering the cells and compositions to a subject. The

pharmaceutical composition in some embodiments contains the cells in amounts effective to treat or prevent the disease or condition, such as a therapeutically effective or prophylactically effective amount.

[0489] In some embodiments, the formulation buffer contains a cryopreservative. In some embodiments, the cell are formulated with a cryopreservative solution that contains 1.0% to 30% DMSO solution, such as a 5% to 20% DMSO solution or a 5% to 10% DMSO solution. In some embodiments, the cryopreservation solution is or contains, for example, PBS containing 20% DMSO and 8% human serum albumin (HSA), or other suitable cell freezing media. In some embodiments, the cryopreservative solution is or contains, for example, at least or about 7.5% DMSO. In some embodiments, the processing steps can involve washing the transduced and/or expanded cells to replace the cells in a cryopreservative solution. In some embodiments, the cells are frozen, e.g., cryoprotected or cryopreserved, in media and/or solution with a final concentration of or of about 12.5%, 12.0%, 11.5%, 11.0%, 10.5%, 10.0%, 9.5%, 9.0%, 8.5%, 8.0%, 7.5%, 7.0%, 6.5%, 6.0%, 5.5%, or 5.0% DMSO, or between 1% and 15%, between 6% and 12%, between 5% and 10%, or between 6% and 8% DMSO. In particular embodiments, the cells are frozen, e.g., cryoprotected or cryopreserved, in media and/or solution with a final concentration of or of about 5.0%, 4.5%, 4.0%, 3.5%, 3.0%, 2.5%, 2.0%, 1.5%, 1.25%, 1.0%, 0.75%, 0.5%, or 0.25% HSA, or between 0.1% and 5%, between 0.25% and 4%, between 0.5% and 2%, or between 1% and 2% HSA.

[0490] In some embodiments, the formulation is carried out using one or more processing step including washing, diluting or concentrating the cells, such as the cultured or expanded cells. In some embodiments, the processing can include dilution or concentration of the cells to a desired concentration or number, such as unit dose form compositions including the number of cells for administration in a given dose or fraction thereof. In some embodiments, the processing steps can include a volume-reduction to thereby increase the concentration of cells as desired. In some embodiments, the processing steps can include a volume-addition to thereby decrease the concentration of cells as desired. In some embodiments, the processing includes adding a volume of a formulation buffer to transduced and/or expanded cells. In some embodiments, the volume of formulation buffer is from or from about 10 mL to 1000 mL, such as at least or about at least or about or 50 mL, 100 mL, 200 mL, 300 mL, 400 mL, 500 mL, 600 mL, 700 mL, 800 mL, 900 mL or 1000 mL.

[0491] In some embodiments, such processing steps for formulating a cell composition is carried out in a closed system. Exemplary of such processing steps can be performed using a centrifugal chamber in conjunction with one or more systems or kits associated with a cell processing system, such as a centrifugal chamber produced and sold by Biosafe SA, including those for use with the Sepax® or Sepax 2® cell processing systems. An exemplary system and process is described in International Publication Number WO2016/073602. In some embodiments, the method includes effecting expression from the internal cavity

of the centrifugal chamber a formulated composition, which is the resulting composition of cells formulated in a formulation buffer, such as pharmaceutically acceptable buffer, in any of the above embodiments as described. In some embodiments, the expression of the formulated composition is to a container, such as the vials of the biomedical material vessels described herein, that is operably linked as part of a closed system with the centrifugal chamber. In some embodiments, the biomedical material vessels are configured for integration and or operable connection and/or is integrated or operably connected, to a closed system or device that carries out one or more processing steps. In some embodiments, the biomedical material vessel is connected to a system at an output line or output position. In some cases, the closed system is connected to the vial of the biomedical material vessel at the inlet tube. Exemplary close systems for use with the biomedical material vessels described herein include the Sepax® and Sepax® 2 system.

[0492] In some embodiments, the closed system, such as associated with a centrifugal chamber or cell processing system, includes a multi-port output kit containing a multi-way tubing manifold associated at each end of a tubing line with a port to which one or a plurality of containers can be connected for expression of the formulated composition. In some aspects, a desired number or plurality of vials, can be sterilely connected to one or more, generally two or more, such as at least 3, 4, 5, 6, 7, 8 or more of the ports of the multi-port output. For example, in some embodiments, one or more containers, e.g., biomedical material vessels, can be attached to the ports, or to fewer than all of the ports. Thus, in some embodiments, the system can effect expression of the output composition into a plurality of vials of the biomedical material vessels.

[0493] In some aspects, cells can be expressed to the one or more of the plurality of output containers, e.g., vials, in an amount for dosage administration, such as for a single unit dosage administration or multiple dosage administration. For example, in some embodiments, the vials, may each contain the number of cells for administration in a given dose or fraction thereof. Thus, each vial, in some aspects, may contain a single unit dose for administration or may contain a fraction of a desired dose such that more than one of the plurality of vials, such as two of the vials, or 3 of the vials, together constitute a dose for administration.

[0494] Thus, the containers, e.g. bags or vials, generally contain the cells to be administered, e.g., one or more unit doses thereof. The unit dose may be an amount or number of the cells to be administered to the subject or twice the number (or more) of the cells to be administered. It may be the lowest dose or lowest possible dose of the cells that would be administered to the subject.

[0495] In some embodiments, each of the containers, e.g. bags or vials, individually comprises a unit dose of the cells. Thus in some embodiments, each of the containers comprises the same or approximately or substantially the same number of cells. In some embodiments, each unit dose contains at least or about at least 1×10^6, 2×10^6, 5×10^6, 1×10^7, 5×10^7, or 1×10^8 engineered cells, total cells, T cells, or PBMCs. In some embodiments, the volume of the formulated cell composition in each container, e.g. bag or vial, is 10

mL to 100 mL, such as at least or about at least 20 mL, 30 mL, 40 mL, 50 mL, 60 mL, 70 mL, 80 mL, 90 mL or 100 mL. In some embodiments, the cells in the container, e.g. bag or vials, can be cryopreserved. In some embodiments, the container, e.g. vials, can be stored in liquid nitrogen until further use.

[0496] In some embodiments, such cells produced by the method, or a composition comprising such cells, are administered to a subject for treating a disease or condition.

E. Exemplary Process and Features

[0497] In some embodiments, engineered cells, such as those that express an anti-BCMA CAR as described, used in accord with the provided methods are produced or generated by a process for selecting, isolating, activating, stimulating, expanding, cultivating, and/or formulating cells. In some embodiments, such methods include any as described.

[0498] In some embodiments, at least one separate composition of enriched CD4+ T cells and at least one separate composition of enriched CD8+ T cells are isolated, selected, enriched, or obtained from a single biological sample, e.g., a sample of PBMCs or other white blood cells from the same donor such as a patient or healthy individual. In some embodiments, a separate composition of enriched CD4+ T cells and a separate composition of enriched CD8+ T cells originated, e.g., were initially isolated, selected, and/or enriched, from the same biological sample, such as a single biological sample obtained, collected, and/or taken from a single subject. In some embodiments, a biological sample is first subjected to selection of CD4+ T cells, where both the negative and positive fractions are retained, and the negative fraction is further subjected to selection of CD8+ T cells. In other embodiments, a biological sample is first subjected to selection of CD8+ T cells, where both the negative and positive fractions are retained, and the negative fraction is further subjected to selection of CD4+ T cells. In some embodiments, methods of selection are carried out as described in International PCT publication No. WO2015/164675. In some aspects, a biological sample is first positively selected for CD8+ T cells to generate at least one composition of enriched CD8+ T cells, and the negative fraction is then positively selected for CD4+ T cells to generate at least one composition of enriched CD4+ T cells, such that the at least one composition of enriched CD8+ T cells and the at least one composition of enriched CD4+ T cells are separate compositions from the same biological sample, e.g., from the same donor patient or healthy individual. In some aspects, two or more separate compositions of enriched T cells, e.g., at least one being a composition of enriched CD4+ T cells and at least one being a separate composition of enriched CD8+ T cells from the same donor, are separately frozen, e.g., cryoprotected or cryopreserved in a cryopreservation media.

[0499] In some embodiments, cells from a composition of enriched CD4+ T cells and cells from a composition of enriched CD8+ T cells are mixed, combined, and/or pooled to generate an input composition

containing CD4+ T cells and CD8+ T cells. In certain embodiments, the compositions of enriched CD4+ T cells and CD8+ T cells are pooled, mixed, and/or combined prior to incubating the cells under stimulating conditions. In certain embodiments, the compositions of enriched CD4+ and CD8+ T cells are pooled, mixed, and/or combined subsequent to isolating, enriching, and/or selecting the CD4+ and CD8+ T cells from a biological sample. In particular embodiments, the compositions of enriched CD4+ and CD8+ T cells are pooled, mixed, and/or combined subsequent to freezing, e.g., cryopreserving, and thawing the compositions of enriched CD4+ and CD8+ T cells.

[0500] In particular embodiments, the input composition contains a ratio of between 3:1 and 1:3, between 2:1 and 1:2, between 1.5 and 0.75, between 1.25 and 0.75, or between 1.2 and 0.8 CD4+ T cells to CD8+ T cells. In certain embodiments, the input composition contains a ratio of or of about 1:1 CD4+ T cells to CD8+ T cells.

[0501] In some aspects, two or more separate compositions of enriched T cells, e.g., at least one being a composition of enriched CD4+ T cells and at least one being a separate composition of enriched CD8+ T cells from the same biological sample, are thawed and mixed, combined, and/or pooled, and the compositions may be optionally washed before or after the mixing, combining, and/or pooling. In some aspects, the mixed, combined, and/or pooled and optionally washed compositions of enriched T cells form an input composition. In some aspects, the input composition (e.g., comprising CD4+ T cells and CD8+ T cells at a ratio of or of about 1:1) is activated and/or stimulated by contacting with a stimulatory reagent (e.g., by incubation with CD3/CD28 conjugated magnetic beads for T cell activation). In some aspects, the activated/stimulated cell composition is engineered, transduced, and/or transfected, e.g., using a viral vector encoding a recombinant protein (e.g. CAR), to express the same recombinant protein in the CD4+ T cells and CD8+ T cells of the cell composition. In some aspects, the method comprises removing the stimulatory reagent, e.g., magnetic beads, from the cell composition. In some aspects, a cell composition containing engineered CD4+ T cells and engineered CD8+ T cells is cultivated, e.g., for expansion of the CD4+ T cell and/or CD8+ T cell populations therein. In certain embodiments, a cell composition from the cultivation is harvested and/or collected and/or formulated, e.g., by washing the cell composition in a formulation buffer. In certain embodiments, a formulated cell composition comprising CD4+ T cells and CD8+ T cells is frozen, e.g., cryoprotected or cryopreserved in a cryopreservation media. In some aspects, engineered CD4+ T cells and CD8+ T cells in the formulation originate from the same donor or biological sample and express the same recombination protein (e.g., CAR), and the formulation is administered to a subject in need thereof such as the same donor.

[0502] In some embodiments, engineered cells, such as those that express an anti-BCMA CAR as described, and compositions containing such cells, such as compositions containing CD4+ and CD8+ T cells

expressing an anti-BCMA chimeric antigen receptor (CAR), used in accord with the provided methods are produced or generated by an exemplary process that includes separately selecting CD4+ and CD8+ T cells from a sample prior to combining the selected cells at a defined ratio for subsequent processing steps.

[0503] In some aspects of an exemplary process, separate compositions of CD4+ and CD8+ cells are selected from isolated PBMCs from a human leukapheresis sample, and the selected cell compositions are cryopreserved. In some embodiments, the human subject is a subject that has multiple myeloma (MM). In some aspects, the selected CD4+ and CD8+ T cell compositions are subsequently thawed and mixed at a ratio of 1:1 of viable CD4+ T cells to viable CD8+ T cells prior to carrying out steps for stimulation, transduction and expansion. In an exemplary embodiment, approximately 300 x 10^6 T cells (150 x 10^6 CD4+ and 150 x 10^6 CD8+ T cells) from the mixed cell composition, at a density of about 3 x 10^6 cells/mL, are stimulated in the presence of paramagnetic polystyrene-coated beads with attached anti-CD3 and anti-CD28 antibodies at a 1:1 bead to cell ratio in serum-free media. In some embodiments, the media also contain recombinant IL-2, IL-7, and IL-15. The stimulation is carried out by incubation for between 18 to 30 hours.

[0504] In some aspects of an exemplary process, following the incubation, approximately 100 x 10^6 viable cells from the stimulated cell composition are washed and resuspended in the exemplary serum free media containing recombinant IL-2, IL-7, and IL-15. In some cases, no transduction adjuvant is added. In some aspects, the cells are transduced with an exemplary lentiviral vector encoding any of the exemplary anti-BCMA CARs described herein (e.g., containing an scFv antigen-binding domain specific for BCMA, a CD28 transmembrane region, a 4-1BB costimulatory signaling region, and a CD3-zeta derived intracellular signaling domain), by spinoculation for 60 minutes followed by incubation for about 18 to 30 hours at about 37 °C. In some aspects, the density of the cells post-spinoculation is about 1 x10^6 cells/mL.

[0505] In some embodiments, the transduced cells are then cultivated for expansion by transfer to a bioreactor (e.g., a rocking motion bioreactor) in about 500 mL of the exemplary serum free media containing twice the concentration of IL-2, IL-7, and IL-15 as used during the incubation and transduction steps. In some exemplary processes, the exemplary media does not contain poloxamer.

[0506] In some aspects, after a threshold cell density of greater than or about 0.6 x 10^6 cells/mL is achieved, media is added step-wise with shots of fresh media being added periodically, such as between about 2 and about 15 minutes to a volume of 1000 mL and the cells are cultivated under steady rocking conditions (non-perfusion) until a threshold viable cell density of greater than or about 0.6 x 10^6 cells/mL is achieved. In some embodiments, if the viable cell density is greater than 0.8 x 10^6cells/mL, a combination fill/perfusion step is initiated wherein first media is added in a step-wise manner, for example, as indicated above, until a target volume of 1000mL, then perfusion is initiated, such as described below. In some aspects, media is then replaced by semi-continuous perfusion with continual mixing. In some embodiments,

the perfusion rate and/or rocking speed are increased at least one time during the expansion phase as cell density increased. In some embodiments, the perfusion rate is increased at least one time during the expansion phase as cell density increased. In some embodiments, media is added to the culture in a step-wise manner with total volume per day determined by viable cell density (such as with higher rates once certain densities are reached), up to a rate, e.g., resulting in approximately 750 mL or 1500 mL of total fresh media added to the culture per day (with higher rates when higher cell concentrations are reached), with shots of fresh media added throughout the day periodically, such as between about every 0.5 and about every 1.5 or 2 hours. In some embodiments, the cells are harvested one day after an exemplary threshold of expansion of about is 3500 x 10^6 or 5500 x 10^6 is achieved. In some embodiments, the cells are harvested at a time one day after the total number of nucleated cells (TNC) had reached at least or at least approximately 3500 x 10^6 and at a point at which the TNC number had reached at least or at least approximately 5500 x 10^6 total nucleated cells. Following harvest, the anti-CD3 and anti-CD28 antibody conjugated beads are removed from the cell composition by exposure to a magnetic field. The cells are then formulated, aliquoted into freezing bags for administration(e.g. CryoStore Freezing Bags) and vials for further analysis, and cryopreserved. In some cases, 30 mL volumes of formulated cell composition is aliquoted per bag. In some instances, cells are cryopreserved at a variable concentration, so long as the target cell number is met for the total output composition.

[0507] In some embodiments, engineered cells, such as those that express an anti-BCMA CAR as described, and compositions containing such cells, such as compositions containing CD4+ and CD8+ T cells expressing an anti-BCMA chimeric antigen receptor (CAR), used in accord with the provided methods are produced or generated by another exemplary process. In the exemplary process, primary CD4+ and CD8+ cells are enriched from biological samples containing PBMCs from a human leukapheresis sample, including from subjects having multiple myeloma (MM). In some aspects, the enriched CD4+ and enriched CD8+ cell compositions are separately cryopreserved and subsequently thawed and mixed at a ratio of 1:1 of viable CD4+ T cells to viable CD8+ T cells, prior to carrying out steps for stimulation, transduction and expansion.

[0508] In some embodiments, approximately 300 x 10^6 T cells (for example, 150 x 10^6 CD4+ and 150 x 10^6 CD8+ T cells) from the mixed cell composition, at a density of about 3 x 10^6 cells/mL, are incubated for between 18 and 30 hours in the presence of paramagnetic polystyrene-coated beads with attached anti-CD3 and anti-CD28 antibodies, at a 1:1 bead to cell ratio in an exemplary serum-free media containing recombinant IL-2, IL-7, and IL-15.

[0509] In some aspects, following the incubation, at least approximately 100 x10^6 and up to approximately 200 x10^6 viable cells from the incubated cell composition are transduced, in the exemplary serum free media with cytokines, with a lentiviral vector encoding any of the exemplary anti-BCMA CARs

described herein (e.g., containing an scFv antigen-binding domain specific for BCMA, a CD28 transmembrane region, a 4-1BB costimulatory signaling region, and a CD3-zeta derived intracellular signaling domain), by spinoculation for 60 minutes followed by incubation for about 18 to 30 hours at about 37 °C.

[0510] In some embodiments, the transduced cells are then expanded by cultivation in a bioreactor (e.g. a rocking motion bioreactor) in about 500 mL of the exemplary serum free media containing twice the concentration of IL-2, IL-7, and IL-15 as used during the incubation and transduction steps. In some aspects, the media does not contain or is free of poloxamer. In some aspects, after a cell density of greater than at or about 0.6 x 10^6 cells/mL is deemed to be achieved, media is added step-wise with shots of fresh media being added periodically, such as between about 2 and about 15 minutes to a volume of 1000 mL and the cells are cultivated under steady rocking conditions (non-perfusion) until a threshold viable cell density of greater than at or about 0.6 x 10^6 cells/mL is achieved. In some aspects, if the viable cell density is greater than 0.8x10^6cells/mL, a combination fill/perfusion step is initiated wherein first media is added in a step-wise manner as indicated above, until a target volume of 1000mL, then perfusion is initiated. In some embodiments, media is replaced by semi-continuous perfusion with continual mixing. In some aspects, the perfusion rate and/or rocking speed are increased at least one time during the expansion phase as cell density increased. In some embodiments, the perfusion rate is increased at least one time during the expansion phase as cell density increased. In some aspects, media is added to the culture in a step-wise manner with total volume per day determined by viable cell density (e.g., with higher rates once certain densities are reached), up to a rate, e.g., resulting in approximately 750 mL or 1500 mL of total fresh media added to the culture per day (e.g., with higher rates when higher cell concentrations are reached), with shots of fresh media added throughout the day periodically, such as between at or about every 0.5 and at or about every 1.5 or 2 hours. In some embodiments, the cells are harvested at a time one day after the total number of nucleated cells (TNC) reaches at least or at least approximately 1000 x 10^6 and at a point at which the TNC number reaches at least or at least approximately 2400 x 10^6 total nucleated cells, with at least 85% viability. In some aspects, following harvest, the anti-CD3 and anti-CD28 antibody conjugated beads are removed from the cell composition.

[0511] In some embodiments, the cells are then formulated and aliquots of the composition transferred into containers, e.g., for downstream storage or use. In some embodiments, formulated compositions or portions thereof are transferred freezing bags appropriate for cryopreservation and storage of cell compositions, e.g., for potential administration to subjects (such as CryoStore Freezing Bags) and/or compositions or portions thereof are transferred to vials or other containers, such as for further analysis of the cells. In some aspects, cells are cryopreserved, such as under conditions appropriate for downstream thawing

and use for administration. In some cases, 30mL volumes of formulated cells are used in individual bags. In some instances, cells are cryopreserved at a variable total cell concentration, for example, to permit a consistent number or concentration of CAR+ T cells in each dose in the context of cells for administration. In some embodiments, the target CAR+CD3+ cell number is at or approximately a desired number (such as at or about 37.5 x 10^6) CAR+CD3+ cells per 30 mL or per bag, which in some embodiments involves varying total cell concentrations among compositions generated from different donors or patients.

[0512] In some aspects, for individual leukapheresis samples obtained from a range of multiple myeloma patients, such an exemplary process to generate engineered cell compositions from such samples, can result in a range of duration of the portion of the process from initiation of activation through harvest of between 5 and 8 days, and an average duration among these samples of 5.5 days. In some aspects, the average number of cumulative population doublings over the process for this group of samples can be approximately 5.

[0513] In some aspects, the exemplary processes described herein can be used to generate engineered T cell compositions from a number of human multiple myeloma leukapheresis samples. In some aspects, various parameters, including those reflective of cell phenotype, function and cell engineering are assessed. In some embodiments, T cell purity, T cell lineage representation, transduction frequency and functionality are observed to be substantially similar as for compositions generated with these leukapheresis products using a different exemplary process (e.g., described above). In some aspects, a reduced number of population doublings and average duration of days between activation initiation and harvest is observed, with production using the exemplary process described above, compared to a different exemplary process (e.g., described above). In some aspects, similar or increased percentages of central memory-phenotype cells (and similar or decreased percentages of effector memory-phenotype cells) are observed in engineered cell compositions produced by the different exemplary processes described herein.

[0514] In some embodiments, the engineered cell compositions are generated using a process that, in some aspects have particular success rates such as high success rates or rates of success greater than a threshold rate, such as those that are able to generate therapeutic cell compositions, such as able to generate such compositions having certain required or desired features, for a large number or percentage of samples, such as for all or a high percentage of samples each derived from a different individual subject or patient, such as a subject or patient to be treated with the therapeutic composition (e.g., in the context of autologous cell therapy). In some aspects, the subjects or patients have a disease or condition such as a cancer such as a blood or hematological cancer such as a multiple myeloma. In some aspects, the samples—from which, for a high percentage thereof, it is possible to generate therapeutic cell compositions—are patient samples including those that are variable for example in terms of cell phenotypes or other parameters of the samples

or cells thereof. In some embodiments, the engineered cell compositions that have improved or high degrees of cell health such as compared to cell compositions generated via other processes. In some embodiments, the compositions include a high percentage of cells that are negative of an apoptotic marker. In some embodiments, the engineered cell compositions are generated by a method which generates a composition comprising polyfunctional cells with robust cytokine production. In some embodiments, the engineered cell compositions are generated by a method which generates T cell compositions that are enriched for a memory phenotype, enriched for a central memory phenotype, and/or enriched for cells that are CD27+, CD28+, CCR7+, CD45RA-, CD45RO+, CD62L+, CD3+, granzyme B-, and/or CD127+. In some embodiments, at least 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, or 80 % or more of the cells in the composition (or at least 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, or 80 % or more of the cells in the composition for at least half or a majority of samples produced using particular methods or, on average, for samples produced using particular methods), of the T cells in the composition, or of the engineered T cells in the composition, are T cells of a central memory phenotype; are CD27+, CD28+; are CCR7+, CD45RA-; and/or are CCR7+, CD45RO+. In some embodiments, at least 50, 55, 60, 65, 70, 75, or 80 or 85 or 90 or 95 % or more of the cells in the composition (or at least 50, 55, 60, 65, 70, 75, or 80 or 85 or 90 or 95 % or more of the cells in the composition for at least half or a majority of samples produced using particular methods or, on average, for samples produced using particular methods), of the T cells in the composition, or of the engineered T cells in the composition, are T cells of a memory phenotype; are CD45RA-; and/or are CD45RO+.

[0515] In certain embodiments, the cells of the composition have a high portion and/or frequency of central memory cells. In some embodiments, at least or at or about 30%, at least or at or about 40%, at least or at or about 50%, at least or at or about 60%, at least or at or about 70%, at least or at or about 75%, at least or at or about 80%, at least or at or about 85%, at least or at or about 90%, at least or at or about 95%, or greater than 95% of the cells of the composition are of a memory phenotype, are of a central memory phenotype, or are central memory T cells. In certain embodiments, at least or at or about 50%, at least or at or about 55%, at least or at or about 60%, or at least or at or about 65% of the cells of the composition are central memory T cells. In certain embodiments, between at or about 40% and at or about 65%, between at or about 40% and at or about 45%, between at or about 45% and at or about 50%, between at or about 50% and at or about 55%, between at or about 55% and at or about 60%, or between at or about 60% and at or about 65% of the cells of the composition are of a memory phenotype, are of a central memory phenotype, or are central memory T cells. In some embodiments, at least or at or about 30%, at least or at or about 40%, at least or at or about 50%, at least or at or about 60%, at least or at or about 70%, at least or at or about 75%, at least or at or about 80%, at least or at or about 85%, at least or at or about 90%, at least or at or about 95%, or greater than 95% of the T cells of the composition are of a memory phenotype, are of a central memory

phenotype, or are central memory T cells. In certain embodiments, at least or at or about 50%, at least or at or about 55%, at least or at or about 60%, or at least or at or about 65% of the T cells of the composition are of a memory phenotype, are of a central memory phenotype, or are central memory T cells. In certain embodiments, between at or about 40% and at or about 65%, between at or about 40% and at or about 45%, between at or about 45% and at or about 50%, between at or about 50% and at or about 55%, between at or about 55% and at or about 60%, or between at or about 60% and at or about 65% of the T cells of the composition are of a memory phenotype, are of a central memory phenotype, or are central memory T cells. In some embodiments, at least or at or about 30%, at least or at or about 40%, at least or at or about 50%, at least or at or about 60%, at least or at or about 70%, at least or at or about 75%, at least or at or about 80%, at least or at or about 85%, at least or at or about 90%, at least or at or about 95%, or greater than 95% of the CD4+ T cells of the composition are central memory CD4+ T cells. In certain embodiments, at least or at or about 50%, at least or at or about 55%, at least or at or about 60%, or at least or at or about 65% of the CD4+ T cells of the composition are central memory CD4+ T cells. In certain embodiments, between at or about 40% and at or about 65%, between at or about 40% and at or about 45%, between at or about 45% and at or about 50%, between at or about 50% and at or about 55%, between at or about 55% and at or about 60%, or between at or about 60% and at or about 65% of the CD4+ T cells of the composition are central memory CD4+ T cells. In some embodiments, at least or at or about 30%, at least or at or about 40%, at least or at or about 50%, at least or at or about 60%, at least or at or about 70%, at least or at or about 75%, at least or at or about 80%, at least or at or about 85%, at least or at or about 90%, at least or at or about 95%, or greater than 95% of the CD4+CAR+ T cells of the composition are central memory CD4+CAR+ T cells. In certain embodiments, at least or at or about 50%, at least or at or about 55%, at least or at or about 60%, or at least or at or about 65% of the CD4+CAR+ T cells of the composition are central memory CD4+CAR+ T cells. In certain embodiments, between at or about 40% and at or about 65%, between at or about 40% and at or about 45%, between at or about 45% and at or about 50%, between at or about 50% and at or about 55%, between at or about 55% and at or about 60%, or between at or about 60% and at or about 65% of the CD4+CAR+ T cells of the composition are central memory CD4+CAR+ T cells. In some embodiments, at least or at or about 30%, at least or at or about 40%, at least or at or about 50%, at least or at or about 60%, at least or at or about 70%, at least or at or about 75%, at least or at or about 80%, at least or at or about 85%, at least or at or about 90%, at least or at or about 95%, or greater than 95% of the CD8+ T cells of the composition are central memory CD8+ T cells. In certain embodiments, at least or at or about 50%, at least or at or about 55%, at least or at or about 60%, or at least or at or about 65% of the CD8+ T cells of the composition are central memory CD8+ T cells. In certain embodiments, between at or about 40% and at or about 65%, between at or about 40% and at or about 45%, between at or about 45% and at or about 50%, between at or

about 50% and at or about 55%, between at or about 55% and at or about 60%, or between at or about 60% and at or about 65% of the CD8+ T cells of the composition are central memory CD8+ T cells. In some embodiments, at least or at or about 30%, at least or at or about 40%, at least or at or about 50%, at least or at or about 60%, at least or at or about 70%, at least or at or about 75%, at least or at or about 80%, at least or at or about 85%, at least or at or about 90%, at least or at or about 95%, or greater than 95% of the CD8+CAR+ T cells of the composition are central memory CD8+CAR+ T cells. In certain embodiments, at least or at or about 50%, at least or at or about 55%, at least or at or about 60%, or at least or at or about 65% of the CD8+CAR+ T cells of the composition are central memory CD8+CAR+ T cells. In certain embodiments, between at or about 40% and at or about 65%, between at or about 40% and at or about 45%, between at or about 45% and at or about 50%, between at or about 50% and at or about 55%, between at or about 55% and at or about 60%, or between at or about 60% and at or about 65% of the CD8+CAR+ T cells of the composition are central memory CD8+CAR+ T cells. In some embodiments, at least or at or about 30%, at least or at or about 40%, at least or at or about 50%, at least or at or about 60%, at least or at or about 70%, at least or at or about 75%, at least or at or about 80%, at least or at or about 85%, at least or at or about 90%, at least or at or about 95%, or greater than 95% of CAR+ T cells (e.g., the CD4+ T cells and CD8+ T cells) of the composition are central memory CD4+ or CD8+ T cells. In certain embodiments, at least or at or about 50%, at least or at or about 55%, at least or at or about 60%, or at least or at or about 65% of the CAR+ T cells (e.g., CD4+ T cells and CD8+ T cells) of the composition are central memory CD4+ or CD8+ T cells. In some embodiments, at least or at or about 30%, at least or at or about 40%, at least or at or about 50%, at least or at or about 60%, at least or at or about 70%, at least or at or about 75%, at least or at or about 80%, at least or at or about 85%, at least or at or about 90%, at least or at or about 95%, or greater than 95% of the cells in the composition are CD27+, CD28+, CCR7+, CD45RA-, CD45RO+, CD62L+, CD3+, CD95+, granzyme B-, and/or CD127+. In some embodiments, at least or at or about 50%, at least or at or about 55%, at least or at or about 60%, or at least or at or about 65% of the CAR+ T cells in the composition are CD27+, CD28+, CCR7+, CD45RA-, CD45RO+, CD62L+, CD3+, CD95+, granzyme B-, and/or CD127+.

[0516] In some embodiments, iterations of the method produce a plurality of the compositions, optionally from human biological samples in which the method is carried out among a plurality of different individual subjects. In some embodiments, the average (i.e., mean) or median percentage of cells of a memory phenotype in the plurality of the compositions is between at or about 40% and at or about 65%, between at or about 40% and at or about 45%, between at or about 45% and at or about 50%, between at or about 50% and at or about 55%, between at or about 55% and at or about 60%, or between at or about 60% and at or about 65%. In some embodiments, the average (i.e., mean) or median percentage of cells of a central memory phenotype in the plurality of the compositions is between at or about 40% and at or about

65%, between at or about 40% and at or about 45%, between at or about 45% and at or about 50%, between at or about 50% and at or about 55%, between at or about 55% and at or about 60%, or between at or about 60% and at or about 65%. In some embodiments, the average (i.e., mean) or median percentage of cells that are CD27+, CD28+, CCR7+, CD45RA-, CD45RO+, CD62L+, CD3+, CD95+, granzyme B-, and/or CD127+ in the plurality of the compositions is between at or about 40% and at or about 65%, between at or about 40% and at or about 45%, between at or about 45% and at or about 50%, between at or about 50% and at or about 55%, between at or about 55% and at or about 60%, or between at or about 60% and at or about 65%. In some embodiments, the average (i.e., mean) or median percentage of cells that are CCR7+/CD45RA- or CCR7+/CD45RO+ in the plurality of the compositions is between at or about 40% and at or about 65%, between at or about 40% and at or about 45%, between at or about 45% and at or about 50%, between at or about 50% and at or about 55%, between at or about 55% and at or about 60%, or between at or about 60% and at or about 65%. In some embodiments, the average (i.e., mean) or median percentage of central memory CD4+ T cells in the engineered CD4+ T cells (e.g., CAR+CD4+ T cells) of the plurality of the compositions is between at or about 40% and at or about 65%, between at or about 40% and at or about 45%, between at or about 45% and at or about 50%, between at or about 50% and at or about 55%, between at or about 55% and at or about 60%, or between at or about 60% and at or about 65%. In some embodiments, the average (i.e., mean) or median percentage of central memory CD8+ T cells in the engineered CD8+ T cells (e.g., CAR+CD8+ T cells) of the plurality of the compositions is between at or about 40% and at or about 65%, between at or about 40% and at or about 45%, between at or about 45% and at or about 50%, between at or about 50% and at or about 55%, between at or about 55% and at or about 60%, or between at or about 60% and at or about 65%. In some embodiments, the average (i.e., mean) or median percentage of central memory T cells (e.g., CD4+ central memory T cells and CD8+ central memory T cells) in the engineered T cells (e.g., CAR+ T cells) of the plurality of the compositions is between at or about 40% and at or about 65%, between at or about 40% and at or about 45%, between at or about 45% and at or about 50%, between at or about 50% and at or about 55%, between at or about 55% and at or about 60%, or between at or about 60% and at or about 65%.

IV. PHARMACEUTICAL COMPOSITIONS

[0517] Also provided are compositions including the BCMA-binding molecules, immunoconjugates, recombinant receptors, and engineered cells, including pharmaceutical compositions and formulations. Among such compositions are those that include engineered cells, such as a plurality of engineered cells, expressing the provided anti-BCMA recombinant receptors (e.g. CARs). In some aspects, also provided are compositions, e.g., cell compositions for use in the provided methods and uses, e.g., therapeutic methods and

uses. In some embodiments, the provided compositions are capable of achieving certain therapeutic outcomes, e.g., response or safety outcomes, when administered to subjects that have a disease or disorder, e.g., multiple myeloma.

[0518] Provided are pharmaceutical formulations comprising a BCMA-binding recombinant chimeric antigen receptors or engineered cells expressing said receptors, a plurality of engineered cells expressing said receptors and/or additional agents for combination treatment or therapy. The pharmaceutical compositions and formulations generally include one or more optional pharmaceutically acceptable carrier(s) or excipient(s). In some embodiments, the composition includes at least one additional therapeutic agent.

[0519] The term "pharmaceutical formulation" refers to a preparation which is in such form as to permit the biological activity of an active ingredient contained therein to be effective, and which contains no additional components which are unacceptably toxic to a subject to which the formulation would be administered.

[0520] A "pharmaceutically acceptable carrier" refers to an ingredient in a pharmaceutical formulation, other than an active ingredient, which is nontoxic to a subject. A pharmaceutically acceptable carrier includes, but is not limited to, a buffer, excipient, stabilizer, or preservative.

[0521] In some aspects, the choice of carrier is determined in part by the particular cell, binding molecule, and/or antibody, and/or by the method of administration. Accordingly, there are a variety of suitable formulations. For example, the pharmaceutical composition can contain preservatives. Suitable preservatives may include, for example, methylparaben, propylparaben, sodium benzoate, and benzalkonium chloride. In some aspects, a mixture of two or more preservatives is used. The preservative or mixtures thereof are typically present in an amount of about 0.0001% to about 2% by weight of the total composition. Carriers are described, *e.g.*, by Remington's Pharmaceutical Sciences 16th edition, Osol, A. Ed. (1980). Pharmaceutically acceptable carriers are generally nontoxic to recipients at the dosages and concentrations employed, and include, but are not limited to: buffers such as phosphate, citrate, and other organic acids; antioxidants including ascorbic acid and methionine; preservatives (such as octadecyldimethylbenzyl ammonium chloride; hexamethonium chloride; benzalkonium chloride; benzethonium chloride; phenol, butyl or benzyl alcohol; alkyl parabens such as methyl or propyl paraben; catechol; resorcinol; cyclohexanol; 3-pentanol; and m-cresol); low molecular weight (less than about 10 residues) polypeptides; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids such as glycine, glutamine, asparagine, histidine, arginine, or lysine; monosaccharides, disaccharides, and other carbohydrates including glucose, mannose, or dextrins; chelating agents such as EDTA; sugars such as sucrose, mannitol, trehalose or sorbitol; salt-forming counter-ions such as sodium; metal complexes (*e.g.* Zn-protein complexes); and/or non-ionic surfactants such as polyethylene glycol (PEG).

[0522] Buffering agents in some aspects are included in the compositions. Suitable buffering agents include, for example, citric acid, sodium citrate, phosphoric acid, potassium phosphate, and various other acids and salts. In some aspects, a mixture of two or more buffering agents is used. The buffering agent or mixtures thereof are typically present in an amount of about 0.001% to about 4% by weight of the total composition. Methods for preparing administrable pharmaceutical compositions are known. Exemplary methods are described in more detail in, for example, Remington: The Science and Practice of Pharmacy, Lippincott Williams & Wilkins; 21st ed. (May 1, 2005).

[0523] Formulations of the antibodies described herein can include lyophilized formulations and aqueous solutions.

[0524] The formulation or composition may also contain more than one active ingredient useful for the particular indication, disease, or condition being treated with the binding molecules or cells, preferably those with activities complementary to the binding molecule or cell, where the respective activities do not adversely affect one another. Such active ingredients are suitably present in combination in amounts that are effective for the purpose intended. Thus, in some embodiments, the pharmaceutical composition further includes other pharmaceutically active agents or drugs, such as chemotherapeutic agents, *e.g.*, asparaginase, busulfan, carboplatin, cisplatin, daunorubicin, doxorubicin, fluorouracil, gemcitabine, hydroxyurea, methotrexate, paclitaxel, rituximab, vinblastine, vincristine, etc. In some embodiments, the cells or antibodies are administered in the form of a salt, *e.g.*, a pharmaceutically acceptable salt. Suitable pharmaceutically acceptable acid addition salts include those derived from mineral acids, such as hydrochloric, hydrobromic, phosphoric, metaphosphoric, nitric, and sulphuric acids, and organic acids, such as tartaric, acetic, citric, malic, lactic, fumaric, benzoic, glycolic, gluconic, succinic, and arylsulphonic acids, for example, p-toluenesulphonic acid.

[0525] Active ingredients may be entrapped in microcapsules, in colloidal drug delivery systems (for example, liposomes, albumin microspheres, microemulsions, nano-particles and nanocapsules) or in macroemulsions. In certain embodiments, the pharmaceutical composition is formulated as an inclusion complex, such as cyclodextrin inclusion complex, or as a liposome. Liposomes can serve to target the host cells (*e.g.*, T-cells or NK cells) to a particular tissue. Many methods are available for preparing liposomes, such as those described in, for example, Szoka *et al.*, Ann. Rev. Biophys. Bioeng., 9: 467 (1980), and U.S. Patents 4,235,871, 4,501,728, 4,837,028, and 5,019,369.

[0526] The pharmaceutical composition in some aspects can employ time-released, delayed release, and sustained release delivery systems such that the delivery of the composition occurs prior to, and with sufficient time to cause, sensitization of the site to be treated. Many types of release delivery systems are available and known. Such systems can avoid repeated administrations of the composition, thereby

increasing convenience to the subject and the physician.

[0527] The pharmaceutical composition in some embodiments contains the binding molecules and/or cells in amounts effective to treat or prevent the disease or condition, such as a therapeutically effective or prophylactically effective amount. Therapeutic or prophylactic efficacy in some embodiments is monitored by periodic assessment of treated subjects. For repeated administrations over several days or longer, depending on the condition, the treatment is repeated until a desired suppression of disease symptoms occurs. However, other dosage regimens may be useful and can be determined. The desired dosage can be delivered by a single bolus administration of the composition, by multiple bolus administrations of the composition, or by continuous infusion administration of the composition.

[0528] In certain embodiments, in the context of genetically engineered cells containing the binding molecules, e.g., CAR, a subject is administered the range of at or about one million to at or about 100 billion cells, such as, *e.g.*, 1 million to at or about 50 billion cells (*e.g.*, at or about 5 million cells, at or about 25 million cells, at or about 50 million cells, at or about 500 million cells, at or about 1 billion cells, at or about 5 billion cells, at or about 20 billion cells, at or about 30 billion cells, at or about 40 billion cells, or a range defined by any two of the foregoing values), such as at or about 10 million to at or about 100 billion cells (*e.g.*, at or about 20 million cells, at or about 30 million cells, at or about 40 million cells, at or about 60 million cells, at or about 70 million cells, at or about 80 million cells, at or about 90 million cells, at or about 10 billion cells, at or about 25 billion cells, at or about 50 billion cells, at or about 75 billion cells, at or about 90 billion cells, or a range defined by any two of the foregoing values), and in some cases at or about 100 million cells to at or about 50 billion cells (*e.g.*, at or about 120 million cells, at or about 150 million cells, at or about 250 million cells, at or about 300 million cells, at or about 350 million cells, at or about 450 million cells, at or about 650 million cells, at or about 800 million cells, at or about 900 million cells, at or about 1.2 billion cells, at or about 3 billion cells, at or about 30 billion cells, at or about 45 billion cells) or any value in between these ranges, and/or such a number of cells per kilogram of body weight of the subject. In some aspects, in the context of genetically engineered cells expressing the binding molecules, e.g., CAR, a composition can contain at least the number of cells for administration for a dose of cell therapy, such as about or at least a number of cells described herein for administration, e.g., in Section V.A.

[0529] The may be administered using standard administration techniques, formulations, and/or devices. Provided are formulations and devices, such as syringes and vials, for storage and administration of the compositions. Administration of the cells can be autologous or heterologous. For example, immunoresponsive cells or progenitors can be obtained from one subject, and administered to the same subject or a different, compatible subject. Peripheral blood derived immunoresponsive cells or their progeny (*e.g.*, *in vivo*, ex vivo or *in vitro* derived) can be administered via localized injection, including catheter

administration, systemic injection, localized injection, intravenous injection, or parenteral administration. When administering a therapeutic composition (*e.g.*, a pharmaceutical composition containing a genetically modified immunoresponsive cell), it will generally be formulated in a unit dosage injectable form (solution, suspension, emulsion).

[0530] Formulations include those for oral, intravenous, intraperitoneal, subcutaneous, pulmonary, transdermal, intramuscular, intranasal, buccal, sublingual, or suppository administration. In some embodiments, the cell populations are administered parenterally. The term "parenteral," as used herein, includes intravenous, intramuscular, subcutaneous, rectal, vaginal, intracranial, intrathoracic, and intraperitoneal administration. In some embodiments, the cell populations are administered to a subject using peripheral systemic delivery by intravenous, intraperitoneal, or subcutaneous injection.

[0531] Compositions in some embodiments are provided as sterile liquid preparations, *e.g.*, isotonic aqueous solutions, suspensions, emulsions, dispersions, or viscous compositions, which may in some aspects be buffered to a selected pH. Liquid preparations are normally easier to prepare than gels, other viscous compositions, and solid compositions. Additionally, liquid compositions are somewhat more convenient to administer, especially by injection. Viscous compositions, on the other hand, can be formulated within the appropriate viscosity range to provide longer contact periods with specific tissues. Liquid or viscous compositions can comprise carriers, which can be a solvent or dispersing medium containing, for example, water, saline, phosphate buffered saline, polyol (for example, glycerol, propylene glycol, liquid polyethylene glycol) and suitable mixtures thereof.

[0532] Sterile injectable solutions can be prepared by incorporating the binding molecule in a solvent, such as in admixture with a suitable carrier, diluent, or excipient such as sterile water, physiological saline, glucose, dextrose, or the like. The compositions can also be lyophilized. The compositions can contain auxiliary substances such as wetting, dispersing, or emulsifying agents (*e.g.*, methylcellulose), pH buffering agents, gelling or viscosity enhancing additives, preservatives, flavoring agents, colors, and the like, depending upon the route of administration and the preparation desired. Standard texts may in some aspects be consulted to prepare suitable preparations.

[0533] Various additives which enhance the stability and sterility of the compositions, including antimicrobial preservatives, antioxidants, chelating agents, and buffers, can be added. Prevention of the action of microorganisms can be ensured by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, sorbic acid, and the like. Prolonged absorption of the injectable pharmaceutical form can be brought about by the use of agents delaying absorption, for example, aluminum monostearate and gelatin.

[0534] Sustained-release preparations may be prepared. Suitable examples of sustained-release

preparations include semipermeable matrices of solid hydrophobic polymers containing the antibody, which matrices are in the form of shaped articles, *e.g.* films, or microcapsules.

[0535] The formulations to be used for *in vivo* administration are generally sterile. Sterility may be readily accomplished, *e.g.*, by filtration through sterile filtration membranes.

[0536] Also provided are pharmaceutical compositions for combination therapy. Any of the additional agents for combination therapy described herein, such as agents described in Section III.B, can be prepared and administered as one or more pharmaceutical compositions, with the BCMA-binding molecule (*e.g.*, antibody), immunoconjugate, recombinant receptor (*e.g.*, chimeric antigen receptor) and/or engineered cells expressing said molecules (*e.g.*, recombinant receptor) described herein. The combination therapy can be administered in one or more pharmaceutical compositions, e.g., where the binding molecules, recombinant receptors and/or cells are in the same pharmaceutical composition as the additional agent, or in separate pharmaceutical compositions. For example, in some embodiments, the additional agent is an additional engineered cell, e.g., cell engineered to express a different recombinant receptor, and is administered in the same composition or in a separate composition. In some embodiments, each of the pharmaceutical composition is formulated in a suitable formulation according to the particular binding molecule, recombinant receptor, cell, e.g., engineered cell, and/or additional agent, and the particular dosage regimen and/or method of delivery.

V. METHODS AND USES

[0537] Also provided methods of using and uses of the BCMA-binding molecules, immunoconjugates, recombinant receptors, engineered cells, and pharmaceutical compositions and formulations thereof, such as in the treatment of diseases, conditions, and disorders in which BCMA is expressed, and/or detection, diagnostic, and prognostic methods. Among such methods, such as methods of treatment, and uses are those that involve administering to a subject engineered cells, such as a plurality of engineered cells, expressing the provided anti-BCMA recombinant receptors (e.g. CARs). Also provided are methods of combination therapy and/or treatment.

A. Therapeutic and prophylactic methods and uses

[0538] Also provided are methods of administering and uses, such as therapeutic and prophylactic uses, of the BCMA-binding molecules, including the anti-BCMA recombinant receptors (*e.g.*, CARs), engineered cells expressing the recombinant receptors (*e.g.*, CARs), plurality of engineered cells expressing the receptors, and/or compositions comprising the same. Such methods and uses include therapeutic methods and uses, for example, involving administration of the molecules (*e.g.*, recombinant receptors), cells (*e.g.*, engineered cells), or compositions containing the same, to a subject having a disease, condition, or disorder

associated with BCMA such as a disease, condition, or disorder associated with BCMA expression, and/or in which cells or tissues express, *e.g.*, specifically express, BCMA. In some embodiments, the molecule, cell, and/or composition is/are administered in an effective amount to effect treatment of the disease or disorder. Provided herein are uses of the recombinant receptors (*e.g.*, CARs), and cells (*e.g.*, engineered cells) in such methods and treatments, and in the manufacture or preparation of a medicament in order to carry out such therapeutic methods. In some embodiments, the methods are carried out by administering the binding molecules or cells, or compositions comprising the same, to the subject having, having had, or suspected of having the disease or condition. In some embodiments, the methods thereby treat the disease or condition or disorder in the subject. Also provided herein are of use of any of the compositions, such as pharmaceutical compositions provided herein, for the treatment of a disease or disorder associated with BCMA, such as use in a treatment regimen.

[0539] As used herein, "treatment" (and grammatical variations thereof such as "treat" or "treating") refers to complete or partial amelioration or reduction of a disease or condition or disorder, or a symptom, adverse effect or outcome, or phenotype associated therewith. Desirable effects of treatment include, but are not limited to, preventing occurrence or recurrence of disease, alleviation of symptoms, diminishment of any direct or indirect pathological consequences of the disease, preventing metastasis, decreasing the rate of disease progression, amelioration or palliation of the disease state, and remission or improved prognosis. The terms do not imply complete curing of a disease or complete elimination of any symptom or effect(s) on all symptoms or outcomes.

[0540] As used herein, "delaying development of a disease" means to defer, hinder, slow, retard, stabilize, suppress and/or postpone development of the disease (such as cancer). This delay can be of varying lengths of time, depending on the history of the disease and/or subject being treated. As sufficient or significant delay can, in effect, encompass prevention, in that the subject does not develop the disease. For example, a late stage cancer, such as development of metastasis, may be delayed.

[0541] "Preventing," as used herein, includes providing prophylaxis with respect to the occurrence or recurrence of a disease in a subject that may be predisposed to the disease but has not yet been diagnosed with the disease. In some embodiments, the provided molecules and compositions are used to delay development of a disease or to slow the progression of a disease.

[0542] As used herein, to "suppress" a function or activity is to reduce the function or activity when compared to otherwise same conditions except for a condition or parameter of interest, or alternatively, as compared to another condition. For example, an antibody or composition or cell which suppresses tumor growth reduces the rate of growth of the tumor compared to the rate of growth of the tumor in the absence of the antibody or composition or cell.

[0543] An "effective amount" of an agent, *e.g.*, a pharmaceutical formulation, binding molecule, antibody, cells, or composition, in the context of administration, refers to an amount effective, at dosages/amounts and for periods of time necessary, to achieve a desired result, such as a therapeutic or prophylactic result.

[0544] A "therapeutically effective amount" of an agent, *e.g.*, a pharmaceutical formulation, binding molecule, antibody, cells, or composition refers to an amount effective, at dosages and for periods of time necessary, to achieve a desired therapeutic result, such as for treatment of a disease, condition, or disorder, and/or pharmacokinetic or pharmacodynamics effect of the treatment. The therapeutically effective amount may vary according to factors such as the disease state, age, sex, and weight of the subject, and the populations of cells administered. In some embodiments, the provided methods involve administering the molecules, antibodies, cells, and/or compositions at effective amounts, *e.g.*, therapeutically effective amounts.

[0545] A "prophylactically effective amount" refers to an amount effective, at dosages and for periods of time necessary, to achieve the desired prophylactic result. Typically but not necessarily, since a prophylactic dose is used in subjects prior to or at an earlier stage of disease, the prophylactically effective amount will be less than the therapeutically effective amount.

[0546] As used herein, a "subject" or an "individual" is a mammal. In some embodiments, a "mammal" includes humans, non-human primates, domestic and farm animals, and zoo, sports, or pet animals, such as dogs, horses, rabbits, cattle, pigs, hamsters, gerbils, mice, ferrets, rats, cats, monkeys, *etc.* In some embodiments, the subject is human.

[0547] Methods for administration of cells for adoptive cell therapy are known and may be used in connection with the provided methods and compositions. For example, adoptive T cell therapy methods are described, e.g., in US Pat. App. Pub. No. 2003/0170238 to Gruenberg et al; US Patent No. 4,690,915 to Rosenberg; Rosenberg (2011) Nat Rev Clin Oncol. 8(10):577-85). See, e.g., Themeli et al. (2013) Nat Biotechnol. 31(10): 928-933; Tsukahara et al. (2013) Biochem Biophys Res Commun 438(1): 84-9; Davila et al. (2013) PLoS ONE 8(4): e61338.

[0548] Among the diseases to be treated is any disease or disorder associated with BCMA or any disease or disorder in which BCMA is specifically expressed and/or in which BCMA has been targeted for treatment (also referred to herein interchangeably as a "BCMA-associated disease or disorder"). Cancers associated with BCMA expression include hematologic malignancies such as multiple myeloma, Waldenstrom macroglobulinemia, as well as both Hodgkin's and non-Hodgkin's lymphomas. *See* Coquery *et al.*, *Crit Rev Immunol.*, 2012, 32(4):287-305 for a review of BCMA. Since BCMA has been implicated in mediating tumor cell survival, it is a potential target for cancer therapy. Chimeric antigen receptors

containing mouse anti-human BCMA antibodies and cells expressing such chimeric receptors have been previously described. *See* Carpenter *et al.*, *Clin Cancer Res.*, 2013, 19(8):2048-2060.

[0549] In some embodiments, the disease or disorder associated with BCMA is a B cell-related disorder. In some embodiments, the disease or disorder associated with BCMA is one or more diseases or conditions from among glioblastoma, lymphomatoid granulomatosis, post-transplant lymphoproliferative disorder, an immunoregulatory disorder, heavy-chain disease, primary or immunocyte-associated amyloidosis, or monoclonal gammopathy of undetermined significance.

[0550] In some embodiments, the disease or disorder associated with BCMA is an autoimmune disease or disorder. Such autoimmune diseases or disorder include, but are not limited to, systemic lupus erythematosus (SLE), lupus nephritis, inflammatory bowel disease, rheumatoid arthritis (*e.g.*, juvenile rheumatoid arthritis), ANCA associated vasculitis, idiopathic thrombocytopenia purpura (ITP), thrombotic thrombocytopenia purpura (TTP), autoimmune thrombocytopenia, Chagas' disease, Grave's disease, Wegener's granulomatosis, polyarteritis nodosa, Sjogren's syndrome, pemphigus vulgaris, scleroderma, multiple sclerosis, psoriasis, IgA nephropathy, IgM polyneuropathies, vasculitis, diabetes mellitus, Reynaud's syndrome, anti-phospholipid syndrome, Goodpasture's disease, Kawasaki disease, autoimmune hemolytic anemia, myasthenia gravis, or progressive glomerulonephritis.

[0551] In certain diseases and conditions, BCMA is expressed on malignant cells and cancers. In some embodiments, the cancer (*e.g.*, a BCMA-expressing cancer) is a B cell malignancy. In some embodiments, the cancer (*e.g.*, a BCMA-expressing cancer) is a lymphoma, a leukemia, or a plasma cell malignancy. Lymphomas contemplated herein include, but are not limited to, Burkitt lymphoma (*e.g.*, endemic Burkitt's lymphoma or sporadic Burkitt's lymphoma), non-Hodgkin's lymphoma (NHL), Hodgkin's lymphoma, Waldenstrom macroglobulinemia, follicular lymphoma, small non-cleaved cell lymphoma, mucosa-associated lymphatic tissue lymphoma (MALT), marginal zone lymphoma, splenic lymphoma, nodal monocytoid B cell lymphoma, immunoblastic lymphoma, large cell lymphoma, diffuse mixed cell lymphoma, pulmonary B cell angiocentric lymphoma, small lymphocytic lymphoma, primary mediastinal B cell lymphoma, lymphoplasmacytic lymphoma (LPL), or mantle cell lymphoma (MCL). Leukemias contemplated here, include, but are not limited to, chronic lymphocytic leukemia (CLL), plasma cell leukemia or acute lymphocytic leukemia (ALL). Also contemplated herein are plasma cell malignancies including, but not limited to, multiple myeloma (*e.g.*, non-secretory multiple myeloma, smoldering multiple myeloma) or plasmacytoma. In some embodiments the disease or condition is multiple myeloma (MM), such as relapsed and/or refractory multiple myeloma (R/R MM). In some embodiments, the disease or condition is a plasmacytoma, such as extramedullary plasmacytoma. In some embodiments, the subject does not have a plasmacytoma, such as extramedullary plasmacytoma. Among the diseases, disorders or

conditions associated with BCMA (*e.g.*, a BCMA-expressing cancer) that can be treated include, but are not limited to, neuroblastoma, renal cell carcinoma, colon cancer, colorectal cancer, breast cancer, epithelial squamous cell cancer, melanoma, myeloma (*e.g.*, multiple myeloma), stomach cancer, brain cancer, lung cancer, pancreatic cancer, cervical cancer, ovarian cancer, liver cancer, bladder cancer, prostate cancer, testicular cancer, thyroid cancer, uterine cancer, adrenal cancer and head and neck cancer.

[0552] In some embodiments, the methods may identify a subject who has, is suspected to have, or is at risk for developing a BCMA-associated disease or disorder. Hence, provided are methods for identifying subjects with diseases or disorders associated with elevated BCMA expression and selecting them for treatment with a provided BCMA-binding recombinant receptors (*e.g.*, CARs), and/or engineered cells expressing the recombinant receptors.

[0553] In some aspects, for example, a subject may be screened for the presence of a disease or disorder associated with elevated BCMA expression, such as a BCMA-expressing cancer. In some embodiments, the methods include screening for or detecting the presence of a BCMA-associated disease, *e.g.* a tumor or a cancer, such as multiple myeloma. Thus, in some aspects, a sample may be obtained from a patient suspected of having a disease or disorder associated with elevated BCMA expression and assayed for the expression level of BCMA. In some aspects, a subject who tests positive for a BCMA-associated disease or disorder may be selected for treatment by the present methods, and may be administered a therapeutically effective amount of a recombinant receptor (*e.g.*, CAR) comprising a BCMA-binding molecule, cells containing a recombinant receptor or a pharmaceutical composition thereof as described herein.

[0554] In some aspects, a subject may be screened for the level of soluble BCMA (sBCMA), e.g., from a biological sample from the subject, such as the blood or serum. In some aspects, a subject may be screened for the level of sBCMA prior to treatment with the cell therapy. In some aspects, the methods include screening for or detecting the level or amount of sBCMA in a subject that has a disease or disorder associated with BCMA expression, e.g., a tumor or a cancer, such as multiple myeloma. In some aspects, a sample may be obtained from a patient suspected of having a disease or disorder associated with BCMA and assayed for the level or amount of sBCMA, for example, using an assay to detect soluble protein levels, such as an enzyme-linked immunosorbent assay (ELISA). In some aspects, in subjects having a multiple myeloma (MM), sBCMA levels can correlate with the proportion of plasma cells in bone marrow biopsies. In some aspects, in subjects having a multiple myeloma (MM), sBCMA levels can correlate with reduced response to treatment or shorter overall survival or progression free survival (see, e.g., Ghermezi et al., Haematologica 2017, 102(4): 785–795). In some aspects, a subject who exhibits low sBCMA levels may be selected for treatment by the present methods, and may be administered a therapeutically effective amount of a recombinant receptor (*e.g.*, CAR) comprising a BCMA-binding molecule, cells containing a recombinant

receptor or a pharmaceutical composition thereof as described herein.

[0555] In some embodiments, the subject has persistent or relapsed disease, *e.g.*, following treatment with another BCMA-specific antibody and/or cells expressing a BCMA-targeting chimeric receptor and/or other therapy, including chemotherapy, radiation, and/or hematopoietic stem cell transplantation (HSCT), *e.g.*, allogeneic HSCT or autologous HSCT. In some embodiments, the administration effectively treats the subject despite the subject having become resistant to another BCMA-targeted therapy. In some embodiments, the subject has not relapsed but is determined to be at risk for relapse, such as at a high risk of relapse, and thus the compound or composition is administered prophylactically, *e.g.*, to reduce the likelihood of or prevent relapse.

[0556] In some embodiments, the subject is one that is eligible for a transplant, such as is eligible for a hematopoietic stem cell transplantation (HSCT), *e.g.*, allogeneic HSCT or autologous HSCT. In some such embodiments, the subject has not previously received a transplant, despite being eligible, prior to administration of the BCMA-binding molecules, including the anti-BCMA recombinant receptors (*e.g.*, CARs), engineered cells expressing the recombinant receptors (*e.g.*, CARs), plurality of engineered cells expressing the receptors, and/or compositions comprising the same, as provided herein.

[0557] In some embodiments, the subject is one that is not eligible for a transplant, such as is not eligible for a hematopoietic stem cell transplantation (HSCT), *e.g.*, allogenic HSCT or autologous HSCT. In some such embodiments, such a subject is administered the BCMA-binding molecules, including the anti-BCMA recombinant receptors (*e.g.*, CARs), engineered cells expressing the recombinant receptors (*e.g.*, CARs), plurality of engineered cells expressing the receptors, and/or compositions comprising the same, according to the provided embodiments herein.

[0558] In some embodiments, prior to the initiation of administration of the engineered cells, the subject has received one or more prior therapies. In some embodiments, the subject has received at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20 or more prior therapies. In some embodiments, the subject has received at least 3, 4, 5, 6, 7, 8, 9, 10 or more prior therapies.

[0559] In some aspects, the subject has relapsed or has been refractory to the one or more prior therapies. In some aspects, the prior therapies include treatment with autologous stem cell transplant (ASCT); an immunomodulatory agent; a proteasome inhibitor; and an anti-CD38 antibody; unless the subject was not a candidate for or was contraindicated for one or more of the therapies. In some aspects, the subject has relapsed or has been refractory to the three or more prior therapies, including treatment with three or more therapies selected from (1) an autologous stem cell transplantation, (2) a proteasome inhibitor and an immunomodulatory agent, either alone or in combination, and (3) an anti-CD38 monoclonal antibody, as a part of a combination therapy or a monotherapy; unless the subject was not a candidate for or was

contraindicated for one or more of the therapies. In some embodiments, the immunomodulatory agent is selected from among thalidomide, lenalidomide or pomalidomide. In some embodiments, the proteasome inhibitor is selected from among bortezomib, carfilzomib or ixazomib. In some embodiments, the anti-CD38 antibody is or comprises daratumumab. In some embodiments, the subject must have undergone at least 2 consecutive cycles of treatment for each regimen unless progressive disease was the best response to the regimen.

[0560] In some embodiments, the method can involve including or excluding particular subjects for therapy with the provided anti-BCMA antibodies, recombinant receptors and/or cells comprising such receptors, based on particular criteria, diagnosis or indication. In some embodiments, at the time of administration of the dose of cells or pre-treatment lymphodepleting chemotherapy, the subject has not had active or history of plasma cell leukemia (PCL). In some embodiments, if the subject had active or a history of PCL at the time of administration, the subject can be excluded from being treated according to the provided methods. In some embodiments, if the subject develops a PCL, such as secondary PCL, at the time of administration, the subject can be excluded from being treated according to the provided methods. In some embodiments, the assessment for the criteria, diagnosis or indication can be performed at the time of screening the subjects for eligibility or suitability of treatment according to the provided methods, at various steps of the treatment regimen, at the time of receiving lymphodepleting therapy, and/or at or immediately prior to the initiation of administration of the engineered cells or composition thereof.

[0561] In some embodiments, the treatment does not induce an immune response by the subject to the therapy, and/or does not induce such a response to a degree that prevents effective treatment of the disease or condition. In some aspects, the degree of immunogenicity and/or graft versus host response is less than that observed with a different but comparable treatment. For example, in the case of adoptive cell therapy using cells expressing CARs including the provided anti-BCMA antibodies, the degree of immunogenicity in some embodiments is reduced compared to CARs including a different antibody that binds to a similar, *e.g.*, overlapping epitope and/or that competes for binding to BCMA with the antibody, such as a mouse or monkey or rabbit or humanized antibody.

[0562] In some embodiments, the methods include adoptive cell therapy, whereby genetically engineered cells expressing the provided recombinant receptors comprising a BCMA-binding molecule (*e.g.*, CARs comprising anti-BCMA antibody or antigen-binding fragment thereof) are administered to subjects. Such administration can promote activation of the cells (*e.g.*, T cell activation) in a BCMA-targeted manner, such that the cells of the disease or disorder are targeted for destruction.

[0563] Thus, the provided methods and uses include methods and uses for adoptive cell therapy. In some embodiments, the methods include administration of the cells or a composition containing the cells to a

subject, tissue, or cell, such as one having, at risk for, or suspected of having the disease, condition or disorder. In some embodiments, the cells, populations, and compositions are administered to a subject having the particular disease or condition to be treated, *e.g.*, via adoptive cell therapy, such as adoptive T cell therapy. In some embodiments, the cells or compositions are administered to the subject, such as a subject having or at risk for the disease or condition. In some aspects, the methods thereby treat, *e.g.*, ameliorate one or more symptom of the disease or condition, such as by lessening tumor burden in a BCMA-expressing cancer.

[0564] Methods for administration of cells for adoptive cell therapy are known and may be used in connection with the provided methods and compositions. For example, adoptive T cell therapy methods are described, *e.g.*, in US Patent Application Publication No. 2003/0170238 to Gruenberg et al; US Patent No. 4,690,915 to Rosenberg; Rosenberg (2011) Nat Rev Clin Oncol. 8(10):577-85). See, *e.g.*, Themeli *et al.* (2013) Nat Biotechnol. 31(10): 928-933; Tsukahara *et al.* (2013) Biochem Biophys Res Commun 438(1): 84-9; Davila *et al.* (2013) PLoS ONE 8(4): e61338.

[0565] In some embodiments, the cell therapy, *e.g.*, adoptive cell therapy, *e.g.*, adoptive T cell therapy, is carried out by autologous transfer, in which the cells are isolated and/or otherwise prepared from the subject who is to receive the cell therapy, or from a sample derived from such a subject. Thus, in some aspects, the cells are derived from a subject, *e.g.*, patient, in need of a treatment and the cells, following isolation and processing are administered to the same subject.

[0566] In some embodiments, the cell therapy, *e.g.*, adoptive cell therapy, *e.g.*, adoptive T cell therapy, is carried out by allogeneic transfer, in which the cells are isolated and/or otherwise prepared from a subject other than a subject who is to receive or who ultimately receives the cell therapy, *e.g.*, a first subject. In such embodiments, the cells then are administered to a different subject, *e.g.*, a second subject, of the same species. In some embodiments, the first and second subjects are genetically identical. In some embodiments, the first and second subjects are genetically similar. In some embodiments, the second subject expresses the same HLA class or supertype as the first subject.

[0567] In some embodiments, the subject, to whom the cells, cell populations, or compositions are administered, is a primate, such as a human. In some embodiments, the subject, to whom the cells, cell populations, or compositions are administered, is a non-human primate. In some embodiments, the non-human primate is a monkey (*e.g.*, cynomolgus monkey) or an ape. The subject can be male or female and can be any suitable age, including infant, juvenile, adolescent, adult, and geriatric subjects. In some embodiments, the subject is a non-primate mammal, such as a rodent (*e.g.*, mouse, rat, etc.). In some examples, the patient or subject is a validated animal model for disease, adoptive cell therapy, and/or for assessing toxic outcomes such as cytokine release syndrome (CRS).

[0568] The BCMA-binding molecules such as recombinant receptors (*e.g.*, CARs) and cells expressing the same, can be administered by any suitable means, for example, by injection, *e.g.*, intravenous or subcutaneous injections, intraocular injection, periocular injection, subretinal injection, intravitreal injection, trans-septal injection, subscleral injection, intrachoroidal injection, intracameral injection, subconjunctival injection, subconjunctival injection, sub-Tenon's injection, retrobulbar injection, peribulbar injection, or posterior juxtascleral delivery. In some embodiments, they are administered by parenteral, intrapulmonary, and intranasal, and, if desired for local treatment, intralesional administration. Parenteral infusions include intramuscular, intravenous, intraarterial, intraperitoneal, intracranial, intrathoracic, or subcutaneous administration. Dosing and administration may depend in part on whether the administration is brief or chronic. Various dosing schedules include but are not limited to single or multiple administrations over various time-points, bolus administration, and pulse infusion.

[0569] For the prevention or treatment of disease, the appropriate dosage of the binding molecule, recombinant receptor or cell may depend on the type of disease to be treated, the type of binding molecule or recombinant receptor, the severity and course of the disease, whether the binding molecule or recombinant receptor is administered for preventive or therapeutic purposes, previous therapy, the patient's clinical history and response to the recombinant receptor or cell, and the discretion of the attending physician. The compositions and molecules and cells are in some embodiments suitably administered to the patient at one time or over a series of treatments.

[0570] In some embodiments, the dose and/or frequency of administration is determined based on efficacy and/or response. In some embodiments, efficacy is determined by evaluating disease status. Exemplary methods for assessing disease status include: measurement of M protein in biological fluids, such as blood and/or urine, by electrophoresis and immunofixation; quantification of sFLC (κ and λ) in blood; skeletal survey; and imaging by positron emission tomography (PET)/computed tomography (CT) in subjects with extramedullary disease. In some embodiments, disease status can be evaluated by bone marrow examination. In some examples, dose and/or frequency of administration is determined by the expansion and persistence of the recombinant receptor or cell in the blood and/or bone marrow. In some embodiments, dose and/or frequency of administration is determined based on the antitumor activity of the recombinant receptor or engineered cell. In some embodiments antitumor activity is determined by the overall response rate (ORR) and/or International Myeloma Working Group (IMWG) Uniform Response Criteria (see Kumar et al. (2016) Lancet Oncol 17(8):e328-346). In some embodiments, response is evaluated using minimal residual disease (MRD) assessment. In some embodiments, MRD can be assessed by methods such as flow cytometry and high-throughput sequencing, e.g., deep sequencing. In some aspects, subjects that have a MRD-negative disease include those exhibiting Absence of aberrant clonal plasma cells on bone marrow aspirate, ruled out

by an assay with a minimum sensitivity of 1 in 10^5 nucleated cells or higher (i.e., 10^{-5} sensitivity), such as flow cytometry (next-generation flow cytometry; NGF) or high-throughput sequencing, e.g., deep sequencing or next-generation sequencing (NGS).

[0571] In some aspects, sustained MRD-negative includes subjects that exhibit MRD negativity in the marrow (NGF or NGS, or both) and by imaging as defined below, confirmed minimum of 1 year apart. Subsequent evaluations can be used to further specify the duration of negativity (e.g., MRD-negative at 5 years). In some aspects, flow MRD-negative includes subjects that exhibit an absence of phenotypically aberrant clonal plasma cells by NGF on bone marrow aspirates using the EuroFlow standard operation procedure for MRD detection in multiple myeloma (or validated equivalent method) with a minimum sensitivity of 1 in 10^5 nucleated cells or higher. In some aspects, sequencing MRD-negative includes subjects that exhibit an absence of clonal plasma cells by NGS on bone marrow aspirate in which presence of a clone is defined as less than two identical sequencing reads obtained after DNA sequencing of bone marrow aspirates using the LymphoSIGHT platform (or validated equivalent method) with a minimum sensitivity of 1 in 10^5 nucleated cells or higher. In some aspects, imaging plus MRD-negative includes subjects that exhibit MRD negativity as assessed by NGF or NGS plus disappearance of every area of increased tracer uptake found at baseline or a preceding PET/CT or decrease to less mediastinal blood pool SUV or decrease to less than that of surrounding normal tissue (see Kumar et al. (2016) Lancet Oncol 17(8):e328-346).

[0572] In some embodiments, response is evaluated based on the duration of response following administration of the recombinant receptor or cells. In some examples, dose and/or frequency of administration can be based on toxicity. In some embodiments, dose and/or frequency can be determined based on health-related quality of life (HRQoL) of the subject to which the recombinant receptor and/or cells is/are administered. In some embodiments, dose and/or frequency of administration can be changed, i.e., increased or decreased, based on any of the above criteria.

[0573] In some aspects, survival of the subject, survival within a certain time period, extent of survival, presence or duration of event-free or symptom-free survival, or relapse-free survival, is assessed. In some embodiments, any symptom of the disease or condition is assessed. In some embodiments, the measure of tumor burden is specified. In some embodiments, exemplary parameters for determination include particular clinical outcomes indicative of amelioration or improvement in the tumor. Such parameters include: duration of disease control, including objective response (OR), complete response (CR), stringent complete response (sCR), very good partial response (VGPR), partial response (PR), minimal response (MR), Stable disease (SD), Progressive disease (PD) or relapse (see, *e.g.*, International Myeloma Working Group (IMWG) Uniform Response Criteria; see Kumar et al. (2016) Lancet Oncol 17(8):e328-346), objective response rate

(ORR), progression-free survival (PFS) and overall survival (OS). In some embodiments, response is evaluated using minimal residual disease (MRD) assessment. Specific thresholds for the parameters can be set to determine the efficacy of the methods provided herein. In some embodiments, the disease or disorder to be treated is multiple myeloma. In some embodiments, measurable disease criteria for multiple myeloma can include (1) serum M-protein 1 g/dL or greater; (2) Urine M-protein 200 mg or greater/24 hour; (3) involved serum free light chain (sFLC) level 10 mg/dL or greater, with abnormal κ to λ ratio. In some cases, light chain disease is acceptable only for subjects without measurable disease in the serum or urine.

[0574] In some aspects, the response to the therapy, e.g., according to the provided embodiments, can be measured at a designated timepoint after the initiation of administration of the cell therapy. In some embodiments, the designated timepoint is at or about 1, 2, 3, 6, 9, 12, 18, 24, 30 or 36 months following initiation of the administration, or within a range defined by any of the foregoing. In some embodiments, the designated time point is 4, 8, 12, 16, 20, 24, 28, 32, 36, 48 or 52 weeks months following initiation of the administration, or within a range defined by any of the foregoing. In some embodiments, the designated timepoint is at or about 1 month following initiation of the administration. In some embodiments, the designated timepoint is at or about 3 months following initiation of the administration. In some embodiments, the designated timepoint is at or about 6 months following initiation of the administration. In some embodiments, the designated timepoint is at or about 9 months following initiation of the administration. In some embodiments, the designated timepoint is at or about 12 months following initiation of the administration.

[0575] In some embodiments, the response or outcome determined at or about 3, 6, 9 or 12 months after the designated timepoint is equal to or improved compared to the response or outcome determined at the initial designated timepoint. For example, in some aspects, if the response or outcome determined at the initial designated timepoint is stable disease (SD), Progressive disease (PD) or relapse, the subject treated according to the provided embodiments can show an equal or improved response or outcome (e.g., exhibiting a better response outcome according to the International Myeloma Working Group (IMWG) Uniform Response Criteria; see Kumar et al. (2016) Lancet Oncol 17(8):e328-346) at a subsequent time point, after at or about 3, 6, 9 or 12 months after the initial designated timepoint, that is equal to the response or outcome at the initial designated timepoint, or a response or outcome that is objective response (OR), complete response (CR), stringent complete response (sCR), very good partial response (VGPR) or partial response (PR). In some aspects, subjects treated according to the provided embodiments can show a response or outcome that is improved between two time point of determination. In some aspects, the subject can exhibit a PR or VGPR in the initial designated timepoint for assessment, e.g., at 4 weeks after the initiation of administration, then exhibit an improved response, such as a CR or an sCR, at a later time point, e.g., at 12 weeks after the

initiation of administration. In some respects, progression-free survival (PFS) is described as the length of time during and after the treatment of a disease, such as cancer, that a subject lives with the disease but it does not get worse. In some aspects, objective response (OR) is described as a measurable response. In some aspects, objective response rate (ORR; also known in some cases as overall response rate) is described as the proportion of patients who achieved CR or PR. In some aspects, overall survival (OS) is described as the length of time from either the date of diagnosis or the start of treatment for a disease, such as cancer, that subjects diagnosed with the disease are still alive. In some aspects, event-free survival (EFS) is described as the length of time after treatment for a cancer ends that the subject remains free of certain complications or events that the treatment was intended to prevent or delay. These events may include the return of the cancer or the onset of certain symptoms, such as bone pain from cancer that has spread to the bone, or death.

[0576] In some embodiments, the measure of duration of response (DOR) includes the time from documentation of tumor response to disease progression. In some embodiments, the parameter for assessing response can include durable response, e.g., response that persists after a period of time from initiation of therapy. In some embodiments, durable response is indicated by the response rate at approximately 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 18 or 24 months after initiation of therapy. In some embodiments, the response or outcome is durable for greater than at or about 3, 6, 9 or 12 months.

[0577] In some embodiments, the Eastern Cooperative Oncology Group (ECOG) performance status indicator can be used to assess or select subjects for treatment, e.g., subjects who have had poor performance from prior therapies (see, e.g., Oken et al. (1982) Am J Clin Oncol. 5:649-655). The ECOG Scale of Performance Status describes a patient's level of functioning in terms of their ability to care for themselves, daily activity, and physical ability (e.g., walking, working, etc.). In some embodiments, an ECOG performance status of 0 indicates that a subject can perform normal activity. In some aspects, subjects with an ECOG performance status of 1 exhibit some restriction in physical activity but the subject is fully ambulatory. In some aspects, patients with an ECOG performance status of 2 is more than 50% ambulatory. In some cases, the subject with an ECOG performance status of 2 may also be capable of self care; see e.g., Sørensen et al., (1993) Br J Cancer 67(4) 773-775. In some embodiments, the subject that are to be administered according to the methods or treatment regimen provided herein include those with an ECOG performance status of 0 or 1.

[0578] In some embodiments, the administration can treat the subject despite the subject having become resistant to another therapy. In some embodiments, when administered to subjects according to the embodiments described herein, the dose or the composition is capable of achieving objective response (OR), in at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, or at least 95% of subjects that were administered. In some embodiments, OR includes subjects who achieve stringent complete response (sCR),

complete response (CR), very good partial response (VGPR), partial response (PR) and minimal response (MR). In some embodiments, when administered to subjects according to the embodiments described herein, the dose or the composition is capable of achieving stringent complete response (sCR), complete response (CR), very good partial response (VGPR) or partial response (PR), in at least 50%, 60%, 70%, 80%, or 85% of subjects that were administered. In some embodiments, when administered to subjects according to the embodiments described herein, the dose or the composition is capable of achieving stringent complete response (sCR) or complete response (CR) at least 20%, 30%, 40% 50%, 60% or 70% of subjects that were administered. In some embodiments, exemplary doses include about 1.0×10^7, 1.5×10^7, 2.0×10^7, 2.5×10^7, 5.0×10^7, 1.5×10^8, 3.0×10^8, 4.5×10^8, 6.0×10^8 or 8.0×10^8 CAR-expressing (CAR+) T cells. In some embodiments, exemplary doses include about 5.0×10^7, 1.5×10^8, 3.0×10^8, 4.5×10^8, 6.0×10^8 or 8.0×10^8 CAR-expressing (CAR+) T cells. In some embodiments, exemplary doses include about 5.0×10^7, 1.5×10^8, 3.0×10^8 or 4.5×10^8 CAR-expressing (CAR+) T cells. In some aspects, particular response to the treatment, e.g., according to the methods provided herein, can be assessed based on the International Myeloma Working Group (IMWG) Uniform Response Criteria (see Kumar et al. (2016) Lancet Oncol 17(8):e328-346).

[0579] In some embodiments, exemplary doses to achieve particular outcomes, such as OR and/or an absence of toxicity or severe toxicity, includes about 5.0×10^7 CAR-expressing (CAR+) T cells. In some embodiments, exemplary doses to achieve particular outcomes, such as OR and/or an absence of toxicity or severe toxicity, includes about 1.5×10^8 CAR+ T cells. In some embodiments, exemplary doses to achieve particular outcomes, such as OR and/or an absence of toxicity or severe toxicity, includes about 3.0×10^8 CAR+ T cells. In some embodiments, exemplary doses to achieve particular outcomes, such as OR and/or an absence of toxicity or severe toxicity, includes about 4.5×10^8 CAR+ T cells. In some embodiments, exemplary doses to achieve particular outcomes, such as OR and/or an absence of toxicity or severe toxicity, includes about 6.0×10^8 CAR+ T cells. In some aspects, the exemplary doses

[0580] In some embodiments, toxicity and/or side-effects of treatment can be monitored and used to adjust dose and/or frequency of administration of the recombinant receptor, e.g., CAR, cells, and or compositions. For example, adverse events and laboratory abnormalities can be monitored and used to adjust dose and/or frequency of administration. Adverse events include infusion reactions, cytokine release syndrome (CRS), neurotoxicity, macrophage activation syndrome, and tumor lysis syndrome (TLS). Any of such events can establish dose-limiting toxicities and warrant decrease in dose and/or a termination of treatment. Other side effects or adverse events which can be used as a guideline for establishing dose and/or frequency of administration include non-hematologic adverse events, which include but are not limited to fatigue, fever or febrile neutropenia, increase in transaminases for a set duration (e.g., less than or equal to 2 weeks or less than or equal to 7 days), headache, bone pain, hypotension, hypoxia, chills, diarrhea,

nausea/vomiting, neurotoxicity (e.g., confusion, aphasia, seizures, convulsions, lethargy, and/or altered mental status), disseminated intravascular coagulation, other asymptomatic non-hematological clinical laboratory abnormalities, such as electrolyte abnormalities. Other side effects or adverse events which can be used as a guideline for establishing dose and/or frequency of administration include hematologic adverse events, which include but are not limited to neutropenia, leukopenia, thrombocytopenia, animal, and/or B-cell aplasia and hypogammaglobinemia.

[0581] In some embodiments, treatment according to the provided methods can result in a lower rate and/or lower degree of toxicity, toxic outcome or symptom, toxicity-promoting profile, factor, or property, such as a symptom or outcome associated with or indicative of cytokine release syndrome (CRS) or neurotoxicity, such as severe CRS or severe neurotoxicity, for example, compared to administration of other therapies. In some embodiments, treatment according to the provided methods can result in both a higher response rate, e.g., higher rate of OR, CR, VGPR or PR, and/or a more durable response, together with a lower rate and/or lower degree of toxicity, toxic outcome or symptom, toxicity-promoting profile, factor, or property, such as a symptom or outcome associated with or indicative of cytokine release syndrome (CRS) or neurotoxicity, such as severe CRS or severe neurotoxicity, for example, compared to administration of other therapies. In some embodiments, treatment according to the provided methods can result in both a higher response rate and a lower rate or degree of toxicity. In some aspects, such results can also be accompanied by higher expansion or prolonged persistence of the administered cells, compared to administration of other therapies.

[0582] In certain embodiments, in the context of genetically engineered cells containing the binding molecules or recombinant receptors, a subject is administered the range of at or about 0.1 million to at or about 100 billion cells and/or that amount of cells per kilogram of body weight of the subject, such as, *e.g.*, 0.1 million to at or about 50 billion cells (*e.g.*, at or about 5 million cells, at or about 25 million cells, at or about 500 million cells, at or about 1 billion cells, at or about 5 billion cells, at or about 20 billion cells, at or about 30 billion cells, at or about 40 billion cells, or a range defined by any two of the foregoing values), 1 million to at or about 50 billion cells (*e.g.*, at or about 5 million cells, at or about 25 million cells, at or about 500 million cells, at or about 1 billion cells, at or about 5 billion cells, at or about 20 billion cells, at or about 30 billion cells, at or about 40 billion cells, or a range defined by any two of the foregoing values), such as at or about 10 million to at or about 100 billion cells (*e.g.*, at or about 20 million cells, at or about 25 million cells, at or about 30 million cells, at or about 40 million cells, at or about 50 million cells, at or about 60 million cells, at or about 70 million cells, at or about 80 million cells, at or about 90 million cells, at or about 10 billion cells, at or about 25 billion cells, at or about 50 billion cells, at or about 75 billion cells, at or about 90 billion cells, or a range defined by any two of the foregoing values), and in some cases at or about 100

million cells to at or about 50 billion cells (*e.g.*, at or about 120 million cells, at or about 150 million cells, at or about 250 million cells, at or about 300 million cells, at or about 350 million cells, at or about 450 million cells, at or about 600 million cells, at or about 650 million cells, at or about 800 million cells, at or about 900 million cells, at or about 1.2 billion cells, at or about 3 billion cells, at or about 30 billion cells, at or about 45 billion cells) or any value in between these ranges and/or per kilogram of body weight of the subject. Again, dosages may vary depending on attributes particular to the disease or disorder and/or patient and/or other treatments.

[0583] In some embodiments, the methods comprises administering a dose of the engineered cells or a composition comprising a dose of the engineered cells. In some embodiments, the engineered cells or compositions containing engineered cells can be used in a treatment regimen, wherein the treatment regimen comprises administering a dose of the engineered cells or a composition comprising a dose of the engineered cells. In some embodiments, the dose can contain, for example, a particular number or range of recombinant receptor-expressing T cells, total T cells, or total peripheral blood mononuclear cells (PBMCs), such as any number of such cells described herein. In some embodiments, a composition containing a dose of the cells can be administered. In some aspects, the number, amount or proportion of CAR-expressing (CAR+) cells in a cell population or a cell composition can be assessed by detection of a surrogate marker, e.g., by flow cytometry or other means, or by detecting binding of a labelled molecule, such as a labelled antigen, that can specifically bind to the binding molecules or receptors provided herein.

[0584] In connection with the provided methods, the cells administered are immune cells engineered to express the BCMA-binding (anti-BCMA) recombinant receptor, e.g., CAR. In some embodiments the immune cells are T cells. In some embodiments, the administered cells are CD4+ T cells. In some embodiments the administered cells are CD8+ T cells. In some embodiments, the administered cells are a combination of CD4+ T cells and CD8+ T cells, such as a combination of CD4+ CAR T cells and CD8+ CAR T cells, which in some aspects are within the same vessel or cell composition or suspension. In some examples the ratio of CD4+ cells to CD8+ cells (CD4:CD8) administered, such as ratio within the suspension or composition or vessel, is 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. In some embodiments, the ratio is between 1:3 and 3:1 or is between at or about 1:4 to at or about 4:1, or between at or about 1:3 to at or about 3:1, or between at or about 1:2 to at or about 2:1, or any of such ratios, within a tolerated error rate. In some aspects, among subjects receiving the therapy and/or among subjects from whom samples are taken and processed to produce the cell compositions, the ratio of CD4+ CAR-T cells to CD8+ CAR-T cells or ratio of CD4+ to CD8+ cells is within a desired range, such as between at or about 1:4 to at or about 4:1, or between at or about 1:3 to at or about 3:1, or between at or about 1:2 to at or about 2:1, or is within such desired ratio for a given percentage of such subjects, such as for

at least 65%, at least 70%, at least 75% or at least 80% or at least 85% or at least 90% or at least 95%, of such subjects.

[0585] In some embodiments, for example, where the subject is a human, the dose includes more than at or about 1 x 10^6 total recombinant receptor (e.g., CAR)-expressing (CAR+) cells, T cells, or peripheral blood mononuclear cells (PBMCs) and fewer than at or about 2 x 10^9 total recombinant receptor (e.g., CAR)-expressing cells, T cells, or peripheral blood mononuclear cells (PBMCs), e.g., in the range of at or about 1.0 x 10^7 to at or about 1.2 x 10^9 such cells, such as at or about 1.0 x 10^7, 1.5 x 10^7, 2.0 x 10^7, 2.5 x 10^7, 5 x 10^7, 1.5 x 10^8, 3 x 10^8, 4.5 x 10^8, 6 x 10^8, 8 x 10^8 or 1.2 x 10^9 total such cells, or the range between any two of the foregoing values. In some embodiments, for example, where the subject is a human, the dose includes more than at or about 1 x 10^6 total recombinant receptor (e.g., CAR)-expressing (CAR+) cells, T cells, or peripheral blood mononuclear cells (PBMCs) and fewer than at or about 2 x 10^9 total recombinant receptor (e.g., CAR)-expressing cells, T cells, or peripheral blood mononuclear cells (PBMCs), e.g., in the range of at or about 2.5 x 10^7 to at or about 1.2 x 10^9 such cells, such as at or about 2.5 x 10^7, 5 x 10^7, 1.5 x 10^8, 3 x 10^8, 4.5 x 10^8, 6 x 10^8, 8 x 10^8 or 1.2 x 10^9 total such cells, or the range between any two of the foregoing values. In some embodiments, for example, where the subject is a human, the dose includes at or about 1.0 x 10^7 total recombinant receptor (e.g., CAR)-expressing cells, T cells, or peripheral blood mononuclear cells (PBMCs). In some embodiments, for example, where the subject is a human, the dose includes at or about 1.5 x 10^7 total recombinant receptor (e.g., CAR)-expressing cells, T cells, or peripheral blood mononuclear cells (PBMCs). In some embodiments, for example, where the subject is a human, the dose includes at or about 2.0 x 10^7 total recombinant receptor (e.g., CAR)-expressing cells, T cells, or peripheral blood mononuclear cells (PBMCs). In some embodiments, for example, where the subject is a human, the dose includes at or about 2.5 x 10^7 total recombinant receptor (e.g., CAR)-expressing cells, T cells, or peripheral blood mononuclear cells (PBMCs). In some embodiments, for example, where the subject is a human, the dose includes at or about 5 x 10^7 total recombinant receptor (e.g., CAR)-expressing cells, T cells, or peripheral blood mononuclear cells (PBMCs). In some embodiments, for example, where the subject is a human, the dose includes at or about 1.5 x 10^8 total recombinant receptor (e.g., CAR)-expressing cells, T cells, or peripheral blood mononuclear cells (PBMCs). In some embodiments, for example, where the subject is a human, the dose includes at or about 3 x 10^8 total recombinant receptor (e.g., CAR)-expressing cells, T cells, or peripheral blood mononuclear cells (PBMCs). In some embodiments, for example, where the subject is a human, the dose includes at or about 4.5 x 10^8 total recombinant receptor (e.g., CAR)-expressing cells, T cells, or peripheral blood mononuclear cells (PBMCs). In some embodiments, for example, where the subject is a human, the dose includes at or about 6 x 10^8 total recombinant receptor (e.g., CAR)-expressing cells, T cells, or peripheral blood mononuclear cells (PBMCs). In some embodiments, for example, where the

subject is a human, the dose includes at or about 8 x 10^8 total recombinant receptor (e.g., CAR)-expressing cells, T cells, or peripheral blood mononuclear cells (PBMCs). In some embodiments, for example, where the subject is a human, the dose includes at or about 1.2 x 10^9 total recombinant receptor (e.g., CAR)-expressing cells, T cells, or peripheral blood mononuclear cells (PBMCs).

[0586] In some embodiments, the dose of genetically engineered cells comprises from at or about 1 x 10^5 to at or about 2 x 10^9 total CAR-expressing (CAR+) T cells, from at or about 1 x 10^5 to at or about 5 x 10^8 total CAR-expressing T cells, from at or about 1 x 10^5 to at or about 2.5 x 10^8 total CAR-expressing T cells, from at or about 1 x 10^5 to at or about 1 x 10^8 total CAR-expressing T cells, from at or about 1 x 10^5 to at or about 5 x 10^7 total CAR-expressing T cells, from at or about 1 x 10^5 to at or about 2.5 x 10^7 total CAR-expressing T cells, from at or about 1 x 10^5 to at or about 1 x 10^7 total CAR-expressing T cells, from at or about 1 x 10^5 to at or about 5 x 10^6 total CAR-expressing T cells, from at or about 1 x 10^5 to at or about 2.5 x 10^6 total CAR-expressing T cells, from at or about 1 x 10^5 to at or about 1 x 10^6 total CAR-expressing T cells, from at or about 1 x 10^6 to at or about 5 x 10^8 total CAR-expressing T cells, from at or about 1 x 10^6 to at or about 2.5 x 10^8 total CAR-expressing T cells, from at or about 1 x 10^6 to at or about 1 x 10^8 total CAR-expressing T cells, from at or about 1 x 10^6 to at or about 5 x 10^7 total CAR-expressing T cells, from at or about 1 x 10^6 to at or about 2.5 x 10^7 total CAR-expressing T cells, from at or about 1 x 10^6 to at or about 1 x 10^7 total CAR-expressing T cells, from at or about 1 x 10^6 to at or about 5 x 10^6 total CAR-expressing T cells, from at or about 1 x 10^6 to at or about 2.5 x 10^6 total CAR-expressing T cells, from at or about 2.5 x 10^6 to at or about 5 x 10^8 total CAR-expressing T cells, from at or about 2.5 x 10^6 to at or about 2.5 x 10^8 total CAR-expressing T cells, from at or about 2.5 x 10^6 to at or about 1 x 10^8 total CAR-expressing T cells, from at or about 2.5 x 10^6 to at or about 5 x 10^7 total CAR-expressing T cells, from at or about 2.5 x 10^6 to at or about 2.5 x 10^7 total CAR-expressing T cells, from at or about 2.5 x 10^6 to at or about 1 x 10^7 total CAR-expressing T cells, from at or about 2.5 x 10^6 to at or about 5 x 10^6 total CAR-expressing T cells, from at or about 5 x 10^6 to at or about 5 x 10^8 total CAR-expressing T cells, from at or about 5 x 10^6 to at or about 2.5 x 10^8 total CAR-expressing T cells, from at or about 5 x 10^6 to at or about 1 x 10^8 total CAR-expressing T cells, from at or about 5 x 10^6 to at or about 5 x 10^7 total CAR-expressing T cells, from at or about 5 x 10^6 to at or about 2.5 x 10^7 total CAR-expressing T cells, from at or about 5 x 10^6 to at or about 1 x 10^7 total CAR-expressing T cells, from at or about 1 x 10^7 to at or about 5 x 10^8 total CAR-expressing T cells, from at or about 1 x 10^7 to at or about 2.5 x 10^8 total CAR-expressing T cells, from at or about 1 x 10^7 to at or about 1 x 10^8 total CAR-expressing T cells, from at or about 1 x 10^7 to at or about 5 x 10^7 total CAR-expressing T cells, from at or about 1 x 10^7 to at or about 2.5 x 10^7 total CAR-expressing T cells, from at or about 2.5 x 10^7 to at or about 5 x 10^8 total CAR-expressing T cells, from at or about 2.5 x 10^7 to at or about 2.5 x 10^8 total CAR-expressing T cells, from at or about 2.5 x 10^7 to at or about 1 x 10^8 total CAR-

expressing T cells, from at or about 2.5 x 10^7 to at or about 5 x 10^7 total CAR-expressing T cells, from at or about 5 x 10^7 to at or about 5 x 10^8 total CAR-expressing T cells, from at or about 5 x 10^7 to at or about 2.5 x 10^8 total CAR-expressing T cells, from at or about 5 x 10^7 to at or about 1 x 10^8 total CAR-expressing T cells, from at or about 1 x 10^8 to at or about 5 x 10^8 total CAR-expressing T cells, from at or about 1 x 10^8 to at or about 2.5 x 10^8 total CAR-expressing T cells, from at or about or 2.5 x 10^8 to at or about 5 x 10^8 total CAR-expressing T cells. In some embodiments, the dose of genetically engineered cells comprises from at or about 1.0 x 10^7 to at or about 8 x 10^8 total CAR-expressing (CAR+) T cells, from at or about 1.0 x 10^7 to at or about 6.5 x 10^8 total CAR+ T cells, from at or about 1.5 x 10^7 to at or about 6.5 x 10^8 total CAR+ T cells, from at or about 1.5 x 10^7 to at or about 6.0 x 10^8 total CAR+ T cells, from at or about 2.5 x 10^7 to at or about 6.0 x 10^8 total CAR+ T cells, or from at or about 5.0 x 10^7 to at or about 6.0 x 10^8 total CAR+ T cells.

[0587] In some embodiments, the dose of genetically engineered cells comprises between at or about 2.5 x 10^7 CAR-expressing (CAR+) T cells, total T cells, or total peripheral blood mononuclear cells (PBMCs) and at or about 1.2 x 10^9 CAR-expressing T cells, total T cells, or total PBMCs, between at or about 5.0 x 10^7 CAR-expressing T cells, total T cells, or total peripheral blood mononuclear cells (PBMCs) and at or about 6.0 x 10^8 CAR-expressing T cells, total T cells, or total PBMCs, between at or about 5.0 x 10^7 CAR-expressing T cells and at or about 4.5 x 10^8 CAR-expressing T cells, total T cells, or total peripheral blood mononuclear cells (PBMCs), between at or about 1.5 x 10^8 CAR-expressing T cells and at or about 3.0 x 10^8 CAR-expressing T cells, total T cells, or total PBMCs, each inclusive. In some embodiments, the number is with reference to the total number of CD3+ or CD8+, in some cases also CAR-expressing (e.g. CAR+) cells. In some embodiments, the dose comprises a number of cell from or from about 2.5 x 10^7 to or to about 1.2 x 10^9 CD3+ or CD8+ total T cells or CD3+ or CD8+ CAR-expressing cells, from or from about 5.0 x 10^7 to or to about 6.0 x 10^8 CD3+ or CD8+ total T cells or CD3+ or CD8+ CAR-expressing cells, from or from about 5.0 x 10^7 to or to about 4.5 x 10^8 CD3+ or CD8+ total T cells or CD3+ or CD8+ CAR-expressing cells, or from or from about 1.5 x 10^8 to or to about 3.0 x 10^8 CD3+ or CD8+ total T cells or CD3+ or CD8+CAR-expressing cells, each inclusive.

[0588] In some embodiments, the dose of genetically engineered cells is with reference to the total number of CD3+ CAR-expressing (CAR+) or CD4+/CD8+ CAR-expressing (CAR+) cells. In some embodiments, the dose comprises a number of genetically engineered cells from or from about 1.0 x 10^7 to or to about 1.2 x 10^9 CD3+ or CD4+/CD8+ total T cells or CD3+ CAR-expressing or CD4+/CD8+ CAR-expressing cells, from or from about 1.5 x 10^7 to or to about 1.2 x 10^9 CD3+ or CD4+/CD8+ total T cells or CD3+ CAR-expressing or CD4+/CD8+ CAR-expressing cells, from or from about 2.0 x 10^7 to or to about 1.2 x 10^9 CD3+ or CD4+/CD8+ total T cells or CD3+ CAR-expressing or CD4+/CD8+ CAR-expressing cells, from or from about 2.5 x 10^7 to or to about 1.2 x 10^9 CD3+ or CD4+/CD8+ total T cells or CD3+

CAR-expressing or CD4+/CD8+ CAR-expressing cells, from or from about 5.0 x 10^7 to or to about 6.0 x 10^8 CD3+ or CD4+/CD8+ total T cells or CD3+ CAR-expressing or CD4+/CD8+ CAR-expressing cells, from or from about 5.0 x 10^7 to or to about 4.5 x 10^8 CD3+ or CD4+/CD8+ total T cells or CD3+ CAR-expressing or CD4+/CD8+ CAR-expressing cells, or from or from about 1.5 x 10^8 to or to about 3.0 x 10^8 CD3+ or CD4+/CD8+ total T cells or CD3+ CAR-expressing or CD4+/CD8+CAR-expressing cells, each inclusive. In some embodiments, the dose comprises at or about 1.0 x 10^7, 1.5 x 10^7, 2.0 x 10^7, 2.5 x 10^7, 5 x 10^7, 1.5 x 10^8, 3 x 10^8, 4.5 x 10^8, 6 x 10^8, 8 x 10^8 or 1.2 x 10^9 CD3+ or CD4+/CD8+ total T cells or CD3+ CAR-expressing or CD4+/CD8+ CAR-expressing cells. In some embodiments, the dose comprises at or about 2.5 x 10^7, 5 x 10^7, 1.5 x 10^8, 3 x 10^8, 4.5 x 10^8, 6 x 10^8, 8 x 10^8 or 1.2 x 10^9 CD3+ CAR-expressing cells. In some embodiments, the dose comprises at or about 1.0 x 10^7, 1.5 x 10^7, 2.0 x 10^7, 2.5 x 10^7, 5 x 10^7, 1.5 x 10^8, 3 x 10^8, 4.5 x 10^8, 6 x 10^8, 8 x 10^8 or 1.2 x 10^9 CD4+/CD8+ CAR-expressing cells.

[0589] In some embodiments, the dose is at or about 1.0 x 10^7 CD4+/CD8+ CAR-expressing cells. In some embodiments, the dose is at or about 1.5 x 10^7 CD4+/CD8+ CAR-expressing cells. In some embodiments, the dose is at or about 2.0 x 10^7 CD4+/CD8+ CAR-expressing cells. In some embodiments, the dose is at or about 2.5 x 10^7 CD4+/CD8+ CAR-expressing cells. In some embodiments, the dose is at or about 5 x 10^7 CD4+/CD8+ CAR-expressing cells. In some embodiments, the dose is at or about 1.5 x 10^8 CD4+/CD8+ CAR-expressing cells. In some embodiments, the dose is at or about 3 x 10^8 CD4+/CD8+ CAR-expressing cells. In some embodiments, the dose is at or about 4.5 x 10^8 CD4+/CD8+ CAR-expressing cells. In some embodiments, the dose is at or about 6 x 10^8 CD4+/CD8+ CAR-expressing cells. In some embodiments, the dose is at or about 8 x 10^8 CD4+/CD8+ CAR-expressing cells. In some embodiments, the dose is at or about 1.2 x 10^9 CD4+/CD8+ CAR-expressing cells. In some embodiments, the dose is at or about 2.5 x 10^7 CD4+ or CD8+ CAR-expressing cells. In some embodiments, the dose is at or about 5 x 10^7 CD4+ or CD8+ CAR-expressing cells. In some embodiments, the dose is at or about 1.5 x 10^8 CD4+ or CD8+ CAR-expressing cells. In some embodiments, the dose is at or about 3 x 10^8 CD4+ or CD8+ CAR-expressing cells. In some embodiments, the dose is at or about 4.5 x 10^8 CD4+ or CD8+ CAR-expressing cells. In some embodiments, the dose is at or about 6 x 10^8 CD4+ or CD8+ CAR-expressing cells. In some embodiments, the dose is at or about 6.5 x 10^8 CD4+ or CD8+ CAR-expressing cells. In some embodiments, the dose is at or about 8 x 10^8 CD4+ or CD8+ CAR-expressing cells. In some embodiments, the dose is at or about 1.2 x 10^9 CD4+ or CD8+ CAR-expressing cells.

[0590] In some embodiments, the T cells of the dose include CD4+ T cells, CD8+ T cells or CD4+ T cells and CD8+ T cells.

[0591] In some embodiments, for example, where the subject is human, the total of CD4+ T cells and CD8+ T cells of the dose includes between at or about 1 x 10^6 and at or about 2 x 10^9 total CAR-

expressing CD4+ cells and CAR-expressing CD8+ cells, e.g., in the range of at or about 2.5 x 10^7 to at or about 1.2 x 10^9 such cells, for example, in the range of at or about 5 x 10^7 to at or about 4.5 x 10^8 such cells; such as at or about 1.0 x 10^7, at or about 2.5 x 10^7, at or about 2.0 x 10^7, at or about 2.5 x 10^7, at or about 5 x 10^7, at or about 1.5 x 10^8, at or about 3 x 10^8, at or about 4.5 x 10^8, at or about 6 x 10^8, at or about 6.5 x 10^8, at or about 8 x 10^8, or at or about 1.2 x 10^9 total such cells, or the range between any two of the foregoing values. In some embodiments, for example, where the subject is human, the CD8+ T cells of the dose, including in a dose including CD4+ T cells and CD8+ T cells, includes between at or about 1 x 10^6 and at or about 2 x 10^9 total recombinant receptor (e.g., CAR)-expressing CD8+cells, e.g., in the range of at or about 2.5 x 10^7 to at or about 1.2 x 10^9 such cells, for example, in the range of at or about 5 x 10^7 to at or about 4.5 x 10^8 such cells; such as at or about 2.5 x 10^7, at or about 5 x 10^7, at or about 1.5 x 10^8, at or about 3 x 10^8, at or about 4.5 x 10^8, at or about 6 x 10^8, at or about 8 x 10^8, or at or about 1.2 x 10^9 total such cells, or the range between any two of the foregoing values.

[0592] In some embodiments, the dose of cells, e.g., recombinant receptor-expressing T cells, is administered to the subject as a single dose or is administered only one time within a period of two weeks, one month, three months, six months, 1 year or more. In some embodiments, the patient is administered multiple doses, and each of the doses or the total dose can be within any of the foregoing values. In some embodiments, the engineered cells for administration or composition of engineered cells for administration, exhibits properties indicative of or consistent with cell health. In some embodiments, at or about or at least at or about 70, 75, 80, 85, or 90% CAR+ cells of such dose exhibit one or more properties or phenotypes indicative of cell health or biologically active CAR cell, such as absence expression of an apoptotic marker.

[0593] In particular embodiments, the phenotype is or includes an absence of apoptosis and/or an indication the cell is undergoing the apoptotic process. Apoptosis is a process of programmed cell death that includes a series of stereotyped morphological and biochemical events that lead to characteristic cell changes and death, including blebbing, cell shrinkage, nuclear fragmentation, chromatin condensation, chromosomal DNA fragmentation, and global mRNA decay. In some aspects, early stages of apoptosis can be indicated by activation of certain caspases, e.g., 2, 8, 9, and 10. In some aspects, middle to late stages of apoptosis are characterized by further loss of membrane integrity, chromatin condensation and DNA fragmentation, include biochemical events such as activation of caspases 3, 6, and 7.

[0594] In particular embodiments, the phenotype is negative expression of one or more factors associated with programmed cell death, for example pro-apoptotic factors known to initiate apoptosis, e.g., members of the death receptor pathway, activated members of the mitochondrial (intrinsic) pathway, such as Bcl-2 family members, e.g., Bax, Bad, and Bid, and caspases. In certain embodiments, the phenotype is the absence of an indicator, e.g., an Annexin V molecule or by TUNEL staining, that will preferentially bind to

cells undergoing apoptosis when incubated with or contacted to a cell composition. In some embodiments, the phenotype is or includes the expression of one or more markers that are indicative of an apoptotic state in the cell. In some embodiments, the phenotype is lack of expression and/or activation of a caspase, such as caspase 3. In some aspects, activation of caspase-3 is indicative of an increase or revival of apoptosis. In certain embodiments, caspase activation can be detected by known methods. In some embodiments, an antibody that binds specifically to an activated caspase (i.e., binds specifically to the cleaved polypeptide) can be used to detect caspase activation. In particular embodiments, the phenotype is or includes active caspase 3-. In some embodiments, the marker of apoptosis is a reagent that detects a feature in a cell that is associated with apoptosis. In certain embodiments, the reagent is an annexin V molecule.

[0595] In some embodiments, the compositions containing the engineered cells for administration contain a certain number or amount of cells that exhibit phenotypes indicative of or consistent with cell health. In some of any embodiments, less than about 25%, 20%, 15%, 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2% or 1% of the CAR-expressing T cells in the dose of engineered T cells express a marker of apoptosis, optionally Annexin V or active Caspase 3. In some of any embodiments, less than 5%, 4%, 3%, 2% or 1% of the CAR-expressing T cells in the dose of engineered T cells express Annexin V or active Caspase 3.

[0596] In some embodiments, the cells, binding molecules, or recombinant receptors are administered as part of a combination treatment, such as simultaneously with or sequentially with, in any order, another therapeutic intervention, such as another antibody or engineered cell or receptor or agent, such as a cytotoxic or therapeutic agent.

[0597] The cells, binding molecules and/or recombinant receptors in some embodiments are co-administered with one or more additional therapeutic agents or in connection with another therapeutic intervention, either simultaneously or sequentially in any order. In some contexts, the cells are co-administered with another therapy sufficiently close in time such that the cell populations enhance the effect of one or more additional therapeutic agents, or vice versa. In some embodiments, the cells, binding molecules and/or recombinant receptors are administered prior to the one or more additional therapeutic agents. In some embodiments, the cells, binding molecules and/or recombinant receptors are administered after to the one or more additional therapeutic agents.

[0598] In some embodiments, the subject may receive a bridging therapy after leukapheresis and before lymphodepleting chemotherapy. A treating physician can determine if bridging therapy is necessary, for example for disease control, during manufacturing of the provided composition or cells. In some embodiments, bridging therapies do not include biological agents, such as antibodies (e.g., Daratumumab). In some embodiments, bridging therapies are discontinued prior to initiation of lymphodepletion. In some embodiments, bridging therapies are discontinued 1 day, 2 days 3 days, 4 days, 5 days, 7 days, 10 days, 14

days, 21 days, 28 days, 45 days, or 60 days before lymphodepletion.

[0599] Once the cells are administered to a mammal (*e.g.*, a human), the biological activity of the engineered cell populations and/or antibodies in some aspects is measured by any of a number of known methods. Parameters to assess include specific binding of an engineered or natural T cell or other immune cell to antigen, *in vivo*, *e.g.*, by imaging, or ex vivo, *e.g.*, by ELISA or flow cytometry. In certain embodiments, the ability of the engineered cells to destroy target cells can be measured using any suitable method known in the art, such as cytotoxicity assays described in, for example, Kochenderfer *et al.*, *J. Immunotherapy*, 32(7): 689-702 (2009), and Herman *et al. J. Immunological Methods*, 285(1): 25-40 (2004). In certain embodiments, the biological activity of the cells also can be measured by assaying expression and/or secretion of certain cytokines, such as CD 107a, IFNγ, IL-2, and TNF. In some aspects the biological activity is measured by assessing clinical outcome, such as reduction in tumor burden or load.

[0600] In certain embodiments, engineered cells are modified in any number of ways, such that their therapeutic or prophylactic efficacy is increased. For example, the engineered CAR or TCR expressed by the population in some embodiments are conjugated either directly or indirectly through a linker to a targeting moiety. The practice of conjugating compounds, *e.g.*, the CAR or TCR, to targeting moieties is known in the art. See, for instance, Wadwa *et al.*, *J. Drug Targeting,* 3(2):111 (1995), and U.S. Patent 5,087,616.

B. Combination Therapy

[0601] Also provided are methods of combination therapy that includes administering and uses, such as therapeutic and prophylactic uses, of the BCMA-binding recombinant receptors (*e.g.*, CARs), engineered cells expressing the recombinant receptors (*e.g.*, CARs), plurality of engineered cells expressing the receptors, and/or compositions comprising the same.

[0602] In some embodiments, the BCMA-binding recombinant receptor (*e.g.*, chimeric antigen receptor) and/or engineered cells expressing said molecules (*e.g.*, recombinant receptor) described herein are administered as part of a combination treatment or combination therapy, such as simultaneously with, sequentially with or intermittently with, in any order, one or more additional therapeutic intervention. In some embodiments, the one or more additional therapeutic intervention includes, for example, an antibody, an engineered cell, a receptor and/or an agent, such as a cell expressing a recombinant receptor, and/or cytotoxic or therapeutic agent, e.g., a chemotherapeutic agent. In some embodiments, the combination therapy includes administration of one or more additional agents, therapies and/or treatments, e.g., any of the additional agents, therapy and/or treatments described herein. In some embodiments, the combination therapy includes administration of one or more additional agents for treatment or therapy, such as an immunomodulatory agent, immune checkpoint inhibitor, adenosine pathway or adenosine receptor antagonist

or agonist and kinase inhibitors. In some embodiments, the combination treatment or combination therapy includes an additional treatment, such as a surgical treatment, transplant, and/or radiation therapy. Also provided are methods of combination treatment or combination therapy that includes BCMA-binding recombinant receptors (e.g., CARs), cells and/or compositions described herein and one or more additional therapeutic interventions.

[0603] In some embodiments, the additional agent for combination treatment or combination therapy enhances, boosts and/or promotes the efficacy and/or safety of the therapeutic effect of binding molecules, recombinant receptors, cells and/or compositions. In some embodiments, the additional agent enhances or improves the efficacy, survival or persistence of the administered cells, e.g., cells expressing the binding molecule or a recombinant receptor. In some embodiments, the additional agent is selected from among a protein phosphatase inhibitor, a kinase inhibitor, a cytokine, an immunomodulator, or an agent that decreases the level or activity of a regulatory T (Treg) cell. In some embodiments, the additional agent enhances safety, by virtue of reducing or ameliorating adverse effects of the administered binding molecules, recombinant receptors, cells and/or compositions. In some embodiments, the additional agent can treat the same disease, condition or a comorbidity. In some embodiments, the additional agent can ameliorate, reduce or eliminate one or more toxicities, adverse effects or side effects that are associated with administration of the recombinant receptors, cells and/or compositions, e.g., CAR-expressing cells.

[0604] In some embodiments, pain management medication such as acetaminophen, or antihistamine, such as diphenhydramine can be administered prior to, during or after administration of the recombinant receptor, engineered T cell or a composition or dose of engineered T cells provided herein, to ameliorate or reduce or eliminate minor side effects associated with treatment. In some examples, red blood cell and platelet transfusions, and/or colony-stimulating factors can be administered reduce or eliminate one or more toxicities, adverse effects or side effects that are associated with administration of the recombinant receptors, cells and/or compositions, e.g., CAR-expressing cells. In some embodiments, prophylactic or empiric anti-infective agents (e.g., trimethoprim/sulfamethoxazole for pneumocystis pneumonia [PCP] prophylaxis, broad spectrum antibiotics, antifungals, or antiviral agents for febrile neutropenia) can be administered to treat side-effects resulting from treatment. In some examples, when necessary, prophylaxis may be provided to treat lymphopenia and/or neutropenia occurring as a result of treatment.

[0605] In some embodiments, the additional therapy, treatment or agent includes chemotherapy, radiation therapy, surgery, transplantation, adoptive cell therapy, antibodies, cytotoxic agents, chemotherapeutic agents, cytokines, growth inhibitory agents, anti-hormonal agents, kinase inhibitors, anti-angiogenic agents, cardioprotectants, immunostimulatory agents, immunosuppressive agents, immune checkpoint inhibitors, antibiotics, angiogenesis inhibitors, metabolic modulators or other therapeutic agents

or any combination thereof. In some embodiments, the additional agent is a protein, a peptide, a nucleic acid, a small molecule agent, a cell, a toxin, a lipid, a carbohydrate or combinations thereof, or any other type of therapeutic agent, e.g. radiation. In some embodiments, the additional therapy, agent or treatment includes surgery, chemotherapy, radiation therapy, transplantation, administration of cells expressing a recombinant receptor, e.g., CAR, kinase inhibitor, immune checkpoint inhibitor, mTOR pathway inhibitor, immunosuppressive agents, immunomodulators, antibodies, immunoablative agents, antibodies and/or antigen binding fragments thereof, antibody conjugates, other antibody therapies, cytotoxins, steroids, cytokines, peptide vaccines, hormone therapy, antimetabolites, metabolic modulators, drugs that inhibit either the calcium dependent phosphatase calcineurin or the p70S6 kinase FK506) or inhibit the p70S6 kinase, alkylating agents, anthracyclines, vinca alkaloids, proteasome inhibitors, GITR agonists, protein tyrosine phosphatase inhibitors, protein kinase inhibitors, an oncolytic virus, and/or other types of immunotherapy. In some embodiments, the additional agent or treatment is bone marrow transplantation, T cell ablative therapy using chemotherapy agents such as, fludarabine, external-beam radiation therapy (XRT), cyclophosphamide, and/or antibody therapy.

[0606] In some embodiments, the cells, BCMA-binding recombinant receptors and/or compositions, e.g., CAR-expressing cells, are administered in combination with other engineered cells, e.g., other CAR-expressing cells. In some embodiments, the additional agent is a kinase inhibitor, e.g., an inhibitor of Bruton's tyrosine kinase (Btk), e.g., ibrutinib. In some embodiments, the additional agent is an adenosine pathway or adenosine receptor antagonist or agonist. In some embodiments, the additional agent is an immunomodulator such as thalidomide or a thalidomide derivative (e.g., lenalidomide). In some embodiments, the additional agent is a gamma secretase inhibitor, such as a gamma secretase inhibitor that inhibits or reduces intramembrane cleavage of a target of a gamma secretase, e.g. BCMA, on a cell (such as a tumor/cancer cell). In some embodiments, the additional therapy, agent or treatment is a cytotoxic or chemotherapy agent, a biologic therapy (e.g., antibody, e.g., monoclonal antibody, or cellular therapy), or an inhibitor (e.g., kinase inhibitor).

[0607] In some embodiments, the additional agent is a chemotherapeutic agent. Exemplary chemotherapeutic agents include an anthracycline (e.g., doxorubicin, such as 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., alemtuzumab, gemtuzumab, rituximab, tositumomab); an antimetabolite (including, e.g., folic acid antagonists, pyrimidine analogs, purine analogs and adenosine deaminase inhibitors such as fludarabine); a TNFR glucocorticoid induced TNFR related protein (GITR) agonist; a proteasome inhibitor (e.g., aclacinomycin A, gliotoxin or bortezomib); an immunomodulatory such as thalidomide or a thalidomide derivative (e.g., lenalidomide).

[0608] In some embodiments, the additional therapy or treatment is cell therapy, e.g., adoptive cell therapy. In some embodiments, the additional therapy includes administration of engineered cells, e.g., additional CAR-expressing cell. In some embodiments, the additional engineered cell is a CAR-expressing cell that expresses the same or different recombinant receptor as the engineered cells provided herein, e.g., anti-BCMA CAR-expressing cells. In some embodiments, the recombinant receptor, e.g., CAR, expressed on the additional engineered cell, recognizes a different antigen and/or epitope. In some embodiments, the recombinant receptor, e.g., CAR, expressed on the additional engineered cell, recognizes a different epitope of the same antigen as the recombinant receptors described herein, e.g., BCMA. In some embodiments, the recombinant receptor, e.g., CAR, expressed on the additional engineered cell, recognizes a different antigen, e.g., a different tumor antigen or combination of antigens. For example, in some embodiments, the recombinant receptor, e.g., CAR, expressed on the additional engineered cell, targets cancer cells that express early lineage markers, e.g., cancer stem cells, while other CAR-expressing cells target cancer cells that express later lineage markers. In such embodiments, the additional engineered cell is administered prior to, concurrently with, or after administration (e.g., infusion) of the CAR-expressing cells described herein. In some embodiments, the additional engineered cell expresses allogeneic CAR.

[0609] In some embodiments, the configurations of one or more 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 some embodiments, the one or more of the CAR molecules 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. In some embodiments, the one or more of the CAR molecules can be 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).

[0610] In some embodiments, the additional agent is any of the cells engineered to express one or more of the anti-BCMA binding molecules and/or cells engineered to express additional binding molecules, e.g., recombinant receptors, e.g., CAR, that target a different antigen. In some embodiments, the additional agent includes any of the cells or plurality of cells described herein, e.g., in Section I.C and III.C. In some embodiments, the additional agent is a cell engineered to express a recombinant receptor, e.g., CAR, targeting a different epitope and/or antigen, e.g., a different antigen associated with a disease or condition. In some embodiments, the additional agent is a cell engineered to express a recombinant receptor, e.g., CAR, targeting a second or additional antigen expressed in multiple myeloma, e.g., GPRC5D, CD38, CD138, CS-1, BAFF-R, TACI and/or FcRH5.

[0611] In some embodiments, the additional agent is an immunomodulatory agent. In some

embodiments, the combination therapy includes an immunomodulatory agent that can stimulate, amplify and/or otherwise enhance an anti-tumor immune response, e.g. anti-tumor immune response from the administered engineered cells, such as by inhibiting immunosuppressive signaling or enhancing immunostimulant signaling. In some embodiments, the immunomodulatory agent is a peptide, protein or is a small molecule. In some embodiments, the protein can be a fusion protein or a recombinant protein. In some embodiments, the immunomodulatory agent binds to an immunologic target, such as a cell surface receptor expressed on immune cells, such a T cells, B cells or antigen-presenting cells. For example, in some embodiments, the immunomodulatory agent is an antibody or antigen-binding antibody fragment, a fusion protein, a small molecule or a polypeptide. In some embodiments, the recombinant receptors, cells and/or compositions are administered in combination with an additional agent that is an antibody or an antigen-binding fragment thereof, such as a monoclonal antibody.

[0612] In some embodiments, the immunomodulatory agent blocks, inhibits or counteracts a component of the immune checkpoint pathway. The immune system has multiple inhibitory pathways that are involved in maintaining self-tolerance and for modulating immune responses. Tumors can use certain immune-checkpoint pathways as a major mechanism of immune resistance, particularly against T cells that are specific for tumor antigens (Pardoll (2012) Nature Reviews Cancer 12:252-264), e.g., engineered cells such as CAR-expressing cells. Because many such immune checkpoints are initiated by ligand-receptor interactions, they can be readily blocked by antibodies against the ligands and/or their receptors.

[0613] Therefore, therapy with antagonistic molecules blocking an immune checkpoint pathway, such as small molecules, nucleic acid inhibitors (*e.g.*, RNAi) or antibody molecules, are becoming promising avenues of immunotherapy for cancer and other diseases. In contrast to the majority of anti-cancer agents, checkpoint inhibitors do not necessarily target tumor cells directly, but rather target lymphocyte receptors or their ligands in order to enhance the endogenous antitumor activity of the immune system.

[0614] As used herein, the term "immune checkpoint inhibitor" refers to molecules that totally or partially reduce, inhibit, interfere with or modulate one or more checkpoint proteins. Checkpoint proteins regulate T-cell activation or function. These proteins are responsible for co-stimulatory or inhibitory interactions of T-cell responses. Immune checkpoint proteins regulate and maintain self-tolerance and the duration and amplitude of physiological immune responses. In some embodiments, the subject can be administered an additional agent that can enhance or boost the immune response, e.g., immune response effected by the BCMA-binding recombinant receptors, cells and/or compositions provided herein, against a disease or condition, e.g., a cancer, such as any described herein.

[0615] Immune checkpoint inhibitors include any agent that blocks or inhibits in a statistically significant manner, the inhibitory pathways of the immune system. Such inhibitors may include small

molecule inhibitors or may include antibodies, or antigen binding fragments thereof, that bind to and block or inhibit immune checkpoint receptors, ligands and/or receptor-ligand interaction. In some embodiments, modulation, enhancement and/or stimulation of particular receptors can overcome immune checkpoint pathway components. Illustrative immune checkpoint molecules that may be targeted for blocking, inhibition, modulation, enhancement and/or stimulation include, but are not limited to, PD-1 (CD279), PD-L1 (CD274, B7-H1), PDL2 (CD273, B7-DC), CTLA-4, LAG-3 (CD223), TIM-3, 4-1BB (CD137), 4-1BBL (CD137L), GITR (TNFRSF18, AITR), CD40, OX40 (CD134, TNFRSF4), CXCR2, tumor associated antigens (TAA), B7-H3, B7-H4, BTLA, HVEM, GAL9, B7H3, B7H4, VISTA, KIR, 2B4 (belongs to the CD2 family of molecules and is expressed on all NK, γδ, and memory CD8+ (αβ) T cells), CD160 (also referred to as BY55), CGEN-15049, CEACAM (e.g., CEACAM-1, CEACAM-3 and/or CEACAM-5), 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 a transforming growth factor receptor (TGFR; e.g., TGFR beta). Immune checkpoint inhibitors include antibodies, or antigen binding fragments thereof, or other binding proteins, that bind to and block or inhibit and/or enhance or stimulate the activity of one or more of any of the said molecules.

[0616] Exemplary immune checkpoint inhibitors include Tremelimumab (CTLA-4 blocking antibody, also known as ticilimumab, CP-675,206), anti-OX40, PD-L1 monoclonal antibody (Anti-B7-H1; MEDI4736), MK-3475 (PD-1 blocker), nivolumab (anti-PD-1 antibody), CT-011 (anti-PD-1 antibody), BY55 monoclonal antibody, AMP224 (anti-PD-L1 antibody), BMS-936559 (anti-PD-L1 antibody), MPLDL3280A (anti-PD-L1 antibody), MSB0010718C (anti-PD-L1 antibody) and ipilimumab (anti-CTLA-4 antibody, also known as Yervoy®, MDX-010 and MDX-101). Exemplary immunomodulatory antibodies include, but are not limited to, Daclizumab (Zenapax), Bevacizumab (Avastin ®), Basiliximab, Ipilimumab, Nivolumab, pembrolizumab, MPDL3280A, Pidilizumab (CT-011), MK-3475, BMS-936559, MPDL3280A (Atezolizumab), tremelimumab, IMP321, BMS-986016, LAG525, urelumab, PF-05082566, TRX518, MK-4166, dacetuzumab (SGN-40), lucatumumab (HCD122), SEA-CD40, CP-870, CP-893, MEDI6469, MEDI6383, MOXR0916, AMP-224, MSB0010718C (Avelumab), MEDI4736, PDR001, rHIgM12B7, Ulocuplumab, BKT140, Varlilumab (CDX-1127), ARGX-110, MGA271, lirilumab (BMS-986015, IPH2101), IPH2201, ARGX-115, Emactuzumab, CC-90002 and MNRP1685A or an antibody-binding fragment thereof. Other 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 gamma, CAS 951209-71-5, available from IRX Therapeutics).

[0617] Programmed cell death 1 (PD-1) is an immune checkpoint protein that is expressed in B cells,

NK cells, and T cells (Shinohara et al., 1995, Genomics 23:704-6; Blank et al., 2007, Cancer Immunol Immunother 56:739-45; Finger et al., 1997, Gene 197:177-87; Pardoll (2012) Nature Reviews Cancer 12:252-264). The major role of PD-1 is to limit the activity of T cells in peripheral tissues during inflammation in response to infection, as well as to limit autoimmunity. PD-1 expression is induced in activated T cells and binding of PD-1 to one of its endogenous ligands acts to inhibit T-cell activation by inhibiting stimulatory kinases. PD-1 also acts to inhibit the TCR "stop signal". PD-1 is highly expressed on Treg cells and may increase their proliferation in the presence of ligand (Pardoll (2012) Nature Reviews Cancer 12:252-264). Anti-PD 1 antibodies have been used for treatment of melanoma, non-small-cell lung cancer, bladder cancer, prostate cancer, colorectal cancer, head and neck cancer, triple-negative breast cancer, leukemia, lymphoma and renal cell cancer (Topalian et al., 2012, N Engl J Med 366:2443-54; Lipson et al., 2013, Clin Cancer Res 19:462-8; Berger et al., 2008, Clin Cancer Res 14:3044-51; Gildener-Leapman et al., 2013, Oral Oncol 49:1089-96; Menzies & Long, 2013, Ther Adv Med Oncol 5:278-85). Exemplary anti-PD-1 antibodies include nivolumab (Opdivo by BMS), pembrolizumab (Keytruda by Merck), pidilizumab (CT-011 by Cure Tech), lambrolizumab (MK-3475 by Merck), and AMP-224 (Merck), nivolumab (also referred to as Opdivo, 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 described 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 described in WO2009/101611. Pembrolizumab (formerly known as lambrolizumab, and also referred to as Keytruda, MK03475; Merck) is a humanized IgG4 monoclonal antibody that binds to PD-1. Pembrolizumab and other humanized anti-PD-1 antibodies are described in US 8,354,509 and WO2009/114335. Other anti-PD-1 antibodies include AMP 514 (Amplimmune), among others, e.g., anti-PD-1 antibodies described in US 8,609,089, US 2010028330, US 20120114649 and/or US 20150210769. AMP-224 (B7-DCIg; Amplimmune; e.g., described in WO2010/027827 and WO2011/066342), is a PD-L2 Fc fusion soluble receptor that blocks the interaction between PD-1 and B7-H1.

[0618] PD-L1 (also known as CD274 and B7-H1) and PD-L2 (also known as CD273 and B7-DC) are ligands for PD-1, found on activated T cells, B cells, myeloid cells, macrophages, and some types of tumor cells. Anti-tumor therapies have focused on anti-PD-L1 antibodies. The complex of PD-1 and PD-L1 inhibits proliferation of CD8+ T cells and reduces the immune response (Topalian et al., 2012, N Engl J Med 366:2443-54; Brahmer et al., 2012, N Eng J Med 366:2455-65). Anti-PD-L1 antibodies have been used for treatment of non-small cell lung cancer, melanoma, colorectal cancer, renal-cell cancer, pancreatic cancer, gastric cancer, ovarian cancer, breast cancer, and hematologic malignancies (Brahmer et al., 2012, N Eng J

Med 366:2455-65; Ott et al., 2013, Clin Cancer Res 19:5300-9; Radvanyi et al., 2013, Clin Cancer Res 19:5541; Menzies & Long, 2013, Ther Adv Med Oncol 5:278-85; Berger et al., 2008, Clin Cancer Res 14:13044-51). Exemplary anti-PD-L1 antibodies include MDX-1105 (Medarex), MEDI4736 (Medimmune) MPDL3280A (Genentech), BMS-935559 (Bristol-Myers Squibb) and MSB0010718C. MEDI4736 (Medimmune) is a human monoclonal antibody that binds to PD-L1, and inhibits interaction of the ligand with PD-1. 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 described in U.S. Patent No. 7,943,743 and U.S Publication No. 20120039906. Other anti-PD-L1 binding agents include YW243.55.S70 (see WO2010/077634) and MDX-1105 (also referred to as BMS-936559, and, e.g., anti-PD-L1 binding agents described in WO2007/005874).

[0619] Cytotoxic T-lymphocyte-associated antigen (CTLA-4), also known as CD152, is a co-inhibitory molecule that functions to regulate T-cell activation. CTLA-4 is a member of the immunoglobulin superfamily that is expressed exclusively on T-cells. CTLA-4 acts to inhibit T-cell activation and is reported to inhibit helper T-cell activity and enhance regulatory T-cell immunosuppressive activity. Although the precise mechanism of action of CTLA-4 remains under investigation, it has been suggested that it inhibits T cell activation by outcompeting CD28 in binding to CD80 and CD86, as well as actively delivering inhibitor signals to the T cell (Pardoll (2012) Nature Reviews Cancer 12:252-264). Anti-CTLA-4 antibodies have been used in clinical trials for the treatment of melanoma, prostate cancer, small cell lung cancer, non-small cell lung cancer (Robert & Ghiringhelli, 2009, Oncologist 14:848-61; Ott et al., 2013, Clin Cancer Res 19:5300; Weber, 2007, Oncologist 12:864-72; Wada et al., 2013, J Transl Med 11:89). A significant feature of anti-CTLA-4 is the kinetics of anti-tumor effect, with a lag period of up to 6 months after initial treatment required for physiologic response. In some cases, tumors may actually increase in size after treatment initiation, before a reduction is seen (Pardoll (2012) Nature Reviews Cancer 12:252-264). Exemplary anti-CTLA-4 antibodies include ipilimumab (Bristol-Myers Squibb) and tremelimumab (Pfizer). Ipilimumab has recently received FDA approval for treatment of metastatic melanoma (Wada et al., 2013, J Transl Med 11:89).

[0620] Lymphocyte activation gene-3 (LAG-3), also known as CD223, is another immune checkpoint protein. LAG-3 has been associated with the inhibition of lymphocyte activity and in some cases the induction of lymphocyte anergy. LAG-3 is expressed on various cells in the immune system including B cells, NK cells, and dendritic cells. LAG-3 is a natural ligand for the MHC class II receptor, which is substantially expressed on melanoma-infiltrating T cells including those endowed with potent immune-suppressive activity. Exemplary anti-LAG-3 antibodies include BMS-986016 (Bristol-Myers Squib), which is a monoclonal antibody that targets LAG-3. IMP701 (Immutep) is an antagonist LAG-3 antibody and

IMP731 (Immutep and GlaxoSmithKline) is a depleting LAG-3 antibody. Other LAG-3 inhibitors include IMP321 (Immutep), which is a recombinant fusion protein of a soluble portion of LAG-3 and Ig that binds to MHC class II molecules and activates antigen presenting cells (APC). Other antibodies are described, e.g., in WO2010/019570 and US 2015/0259420

[0621] T-cell immunoglobulin domain and mucin domain-3 (TIM-3), initially identified on activated Th1 cells, has been shown to be a negative regulator of the immune response. Blockade of TIM-3 promotes T-cell mediated anti-tumor immunity and has anti-tumor activity in a range of mouse tumor models. Combinations of TIM-3 blockade with other immunotherapeutic agents such as TSR-042, anti-CD137 antibodies and others, can be additive or synergistic in increasing anti-tumor effects. TIM-3 expression has been associated with a number of different tumor types including melanoma, NSCLC and renal cancer, and additionally, expression of intratumoral TIM-3 has been shown to correlate with poor prognosis across a range of tumor types including NSCLC, cervical, and gastric cancers. Blockade of TIM-3 is also of interest in promoting increased immunity to a number of chronic viral diseases. TIM-3 has also been shown to interact with a number of ligands including galectin-9, phosphatidylserine and HMGB1, although which of these, if any, are relevant in regulation of anti-tumor responses is not clear at present. In some embodiments, antibodies, antibody fragments, small molecules, or peptide inhibitors that target TIM-3 can bind to the IgV domain of TIM-3 to inhibit interaction with its ligands. Exemplary antibodies and peptides that inhibit TIM-3 are described in US 2015/0218274, WO2013/006490 and US 2010/0247521. Other anti-TIM-3 antibodies include humanized versions of RMT3-23 (Ngiow et al., 2011, Cancer Res, 71:3540-3551), and clone 8B.2C12 (Monney et al., 2002, Nature, 415:536-541). Bi-specific antibodies that inhibit TIM-3 and PD-1 are described in US 2013/0156774.

[0622] In some embodiments, the additional agent is a CEACAM inhibitor (e.g., CEACAM-1, CEACAM-3, and/or CEACAM-5 inhibitor). In some embodiments, 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 some embodiments, the anti-CEACAM antibody binds to CEACAM-5 as described in, e.g., Zheng et al. PLoS One. (2011) 6(6): e21146), or cross reacts with CEACAM-1 and CEACAM-5 as described in, e.g., WO 2013/054331 and US 2014/0271618.

[0623] 4-1BB, also known as CD137, is transmembrane glycoprotein belonging to the TNFR superfamily. 4-1BB receptors are present on activated T cells and B cells and monocytes. An exemplary anti-4-1BB antibody is urelumab (BMS-663513), which has potential immunostimulatory and antineoplastic activities.

[0624] Tumor necrosis factor receptor superfamily, member 4 (TNFRSF4), also known as OX40 and CD134, is another member of the TNFR superfamily. OX40 is not constitutively expressed on resting naïve T cells and acts as a secondary co-stimulatory immune checkpoint molecule. Exemplary anti-OX40 antibodies are MEDI6469 and MOXR0916 (RG7888, Genentech).

[0625] In some embodiments, the additional agent includes a molecule that decreases the regulatory T cell (Treg) 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, and modulating Glucocorticoid-induced TNFR family related gene (GITR) function. GITR is a member of the TNFR superfamily that is upregulated on activated T cells, which enhances the immune system. Reducing the number of Treg cells in a subject prior to apheresis or prior to administration of engineered cells, e.g., CAR-expressing cells, can reduce the number of unwanted immune cells (e.g., Tregs) in the tumor microenvironment and reduces the subject's risk of relapse. In some embodiments, the additional agent includes 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 some embodiments, the additional agent includes cyclophosphamide. In some embodiments, the GITR binding molecule and/or molecule modulating GITR function (e.g., GITR agonist and/or Treg depleting GITR antibodies) is administered prior to the engineered cells, e.g., CAR-expressing cells. For example, in some embodiments, the GITR agonist can be administered prior to apheresis of the cells. In some embodiments, cyclophosphamide is administered to the subject prior to administration (e.g., infusion or re-infusion) of the engineered cells, e.g., CAR-expressing cells or prior to apheresis of the cells. In some embodiments, cyclophosphamide and an anti-GITR antibody are administered to the subject prior to administration (e.g., infusion or re-infusion) of the engineered cells, e.g., CAR-expressing cells or prior to apheresis of the cells.

[0626] In some embodiments, the additional agent is a GITR agonist. 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. 090505B 1, 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. 1947183B 1, 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. An exemplary anti-GITR antibody is TRX518.

[0627] In some embodiments, the additional agent enhances tumor infiltration or transmigration of the administered cells, e.g., CAR-expressing cells. For example, in some embodiments, the additional agent stimulates CD40, such as CD40L, e.g., recombinant human CD40L. Cluster of differentiation 40 (CD40) is also a member of the TNFR superfamily. CD40 is a costimulatory protein found on antigen-presenting cells and mediates a broad variety of immune and inflammatory responses. CD40 is also expressed on some malignancies, where it promotes proliferation. Exemplary anti-CD40 antibodies are dacetuzumab (SGN-40), lucatumumab (Novartis, antagonist), SEA-CD40 (Seattle Genetics), and CP-870,893. In some embodiments, the additional agent that enhances tumor infiltration includes tyrosine kinase inhibitor sunitnib, heparanase, and/or chemokine receptors such as CCR2, CCR4, and CCR7.

[0628] In some embodiments, the additional agent includes thalidomide drugs or analogs thereof and/or derivatives thereof, such as lenalidomide, pomalidomide or apremilast. See, e.g., Bertilaccio et al., Blood (2013) 122:4171, Otahal et al., Oncoimmunology (2016) 5(4):e1115940; Fecteau et al., Blood (2014) 124(10):1637-1644 and Kuramitsu et al., Cancer Gene Therapy (2015) 22:487-495). Lenalidomide ((RS)-3-(4-Amino-1-oxo-1,3-dihydro-2H-isoindol-2-yl)piperidine-2,6-dione; also known as Revlimid) is a synthetic derivative of thalidomide, and has multiple immunomodulatory effects, including enforcement of immune synapse formation between T cell and antigen presenting cells (APCs). For example, in some cases, lenalidomide modulates T cell responses and results in increased interleukin (IL)-2 production in CD4+ and CD8+ T cells, induces the shift of T helper (Th) responses from Th2 to Th1, inhibits expansion of regulatory subset of T cells (Tregs), and improves functioning of immunological synapses in follicular lymphoma and chronic lymphocytic leukemia (CLL) (Otahal et al., Oncoimmunology (2016) 5(4):e1115940). Lenalidomide also has direct tumoricidal activity in patients with multiple myeloma (MM) and directly and indirectly modulates survival of CLL tumor cells by affecting supportive cells, such as nurse-like cells found in the microenvironment of lymphoid tissues. Lenalidomide also can enhance T-cell proliferation and interferon-γ production in response to activation of T cells via CD3 ligation or dendritic cell-mediated activation. Lenalidomide can also induce malignant B cells to express higher levels of immunostimulatory molecules such as CD80, CD86, HLA-DR, CD95, and CD40 (Fecteau et al., Blood (2014) 124(10):1637-1644). In some embodiments, lenalidomide is administered at a dosage of from about 1 mg to about 20 mg daily, e.g., from about 1 mg to about 10 mg, from about 2.5 mg to about 7.5 mg, from about 5 mg to about 15 mg, such as about 5 mg, 10 mg, 15 mg or 20 mg daily. In some embodiments, lenalidomide is administered at a dose of from about 10 μg/kg to 5 mg/kg, e.g., about 100 μg/kg to about 2 mg/kg, about 200 μg/kg to about 1 mg/kg, about 400 μg/kg to about 600 μg/kg, such as about 500 μg/kg. In some 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 some embodiments, lenalidomide is administered at a low dose.

[0629] In some embodiments, the additional agent is a B-cell inhibitor. In some embodiments, the additional agent is one or more B-cell inhibitors selected from among inhibitors of CD10, CD19, CD20, CD22, CD34, CD123, CD79a, CD79b, CD179b, FLT-3, or ROR1, or a combination thereof. In some embodiments, the B-cell inhibitor is an antibody (e.g., a mono- or bispecific antibody) or an antigen binding fragment thereof. In some embodiments, the additional agent is an engineered cell expressing recombinant receptors that target B-cell targets, e.g., CD10, CD19, CD20, CD22, CD34, CD123, CD79a, CD79b, CD179b, FLT-3, or ROR1.

[0630] In some embodiments, the additional agent is a CD20 inhibitor, e.g., an anti-CD20 antibody (e.g., an anti-CD20 mono- or bi-specific antibody) or a fragment thereof. Exemplary anti-CD20 antibodies include but are not limited to rituximab, ofatumumab, ocrelizumab (also known as GA101 or RO5072759), veltuzumab, obinutuzumab, TRU-015 (Trubion Pharmaceuticals), ocaratuzumab (also known as AME-133v or ocaratuzumab), and Pro131921 (Genentech). See, e.g., Lim et al. Haematologica. (2010) 95(1):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. In some embodiments, the additional agent includes rituximab. In some embodiments, the CD20 inhibitor is a small molecule.

[0631] In some embodiments, the additional agent is a CD22 inhibitor, e.g., an anti-CD22 antibody (e.g., an anti-CD22 mono- or bi-specific antibody) or a fragment thereof. Exemplary anti-CD22 antibodies include epratuzumab and RFB4. In some embodiments, the CD22 inhibitor is a small molecule. In some embodiments, the antibody is a monospecific antibody, optionally conjugated to a second agent such as a chemotherapeutic agent. For instance, in some embodiments, the antibody is an anti-CD22 monoclonal antibody-MMAE conjugate (e.g., DCDT2980S). In some embodiments, the antibody is an scFv of an anti-CD22 antibody, e.g., an scFv of antibody RFB4. In some embodiments, the scFv is fused to all of or a fragment of Pseudomonas exotoxin-A (e.g., BL22). In some embodiments, the scFv is fused to all of or a fragment of (e.g., a 38 kDa fragment of) Pseudomonas exotoxin-A (e.g., moxetumomab pasudotox). In some embodiments, the anti-CD22 antibody is an anti-CD19/CD22 bispecific antibody, optionally conjugated to a toxin. For instance, in some embodiments, the anti-CD22 antibody comprises an anti-CD19/CD22 bispecific portion, (e.g., two scFv ligands, recognizing human CD19 and CD22) optionally linked to all of or a portion of diphtheria toxin (DT), e.g., first 389 amino acids of diphtheria toxin (DT), DT 390, e.g., a ligand-directed toxin such as DT2219ARL). In some embodiments, the bispecific portion (e.g., anti-CD 19/anti-CD22) is linked to a toxin such as deglycosylated ricin A chain (e.g., Combotox).

[0632] In some embodiments, the immunomodulatory agent is a cytokine. In some embodiments, the immunomodulatory agent is a cytokine or is an agent that induces increased expression of a cytokine in the

tumor microenvironment. Cytokines have important functions related to T cell expansion, differentiation, survival, and homeostasis. Cytokines that can be administered to the subject receiving the BCMA-binding recombinant receptors, cells and/or compositions provided herein include one or more of IL-2, IL-4, IL-7, IL-9, IL-15, IL-18, and IL-21. In some embodiments, the cytokine administered is IL-7, IL-15, or IL-21, or a combination thereof. In some embodiments, administration of the cytokine to the subject that has sub-optimal response to the administration of the engineered cells, e.g., CAR-expressing cells improves efficacy and/or anti-tumor activity of the administered cells, e.g., CAR-expressing cells.

[0633] By "cytokine" is meant a generic term for proteins released by one cell population that act on another cell as intercellular mediators. Examples of such cytokines are lymphokines, monokines, and traditional polypeptide hormones. Included among the cytokines are growth hormones such as human growth hormone, N-methionyl human growth hormone, and bovine growth hormone; parathyroid hormone; thyroxine; insulin; proinsulin; relaxin; prorelaxin; glycoprotein hormones such as follicle stimulating hormone (FSH), thyroid stimulating hormone (TSH), and luteinizing hormone (LH); hepatic growth factor; fibroblast growth factor; prolactin; placental lactogen; tumor necrosis factor-alpha and -beta; mullerian-inhibiting substance; mouse gonadotropin-associated peptide; inhibin; activin; vascular endothelial growth factor; integrin; thrombopoietin (TPO); nerve growth factors such as NGF-beta; platelet-growth factor; transforming growth factors (TGFs) such as TGF-alpha and TGF-beta; insulin-like growth factor-I and -II; erythropoietin (EPO); osteoinductive factors; interferons such as interferon-alpha, beta, and -gamma; colony stimulating factors (CSFs) such as macrophage-CSF (M-CSF); granulocyte-macrophage-CSF (GM-CSF); and granulocyte-CSF (G-CSF); interleukins (ILs) such as IL-1, IL-1alpha, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-11, IL-12; IL-15, a tumor necrosis factor such as TNF-alpha or TNF-beta; and other polypeptide factors including LIF and kit ligand (KL). As used herein, the term cytokine includes proteins from natural sources or from recombinant cell culture, and biologically active equivalents of the native sequence cytokines. For example, the immunomodulatory agent is a cytokine and the cytokine is IL-4, TNF-α, GM-CSF or IL-2.

[0634] In some embodiments, the additional agent includes an interleukin-15 (IL-15) polypeptide, an interleukin-15 receptor alpha (IL-15Rα) polypeptide, or combination thereof, e.g., hetIL-15 (Admune Therapeutics, LLC). hetIL-15 is a heterodimeric non-covalent complex of IL-15 and IL-15Rα. 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. In some embodiments, the immunomodulatory agent can contain one or more cytokines. For example, the interleukin can include leukocyte interleukin injection (Multikine), which is a combination of natural cytokines. In some embodiments, the immunomodulatory agent is a Toll-like receptor (TLR) agonist, an adjuvant or a cytokine.

[0635] In some embodiments, the additional agent is an agent that ameliorates or neutralizes one or more toxicities or side effects associated with the cell therapy. In some embodiments, the additional agent is selected from among 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, sarilumab, elsilimomab, CNTO 328, ALD518/BMS-945429, CNTO 136, CPSI-2364, CDP6038, VX30, ARGX-109, FE301, and FM101. In some embodiments, the anti-IL-6 antibody molecule is tocilizumab. In some embodiments, the additional agent is an IL-1R inhibitor, such as anakinra.

[0636] In some embodiments, the additional agent is a modulator of adenosine levels and/or an adenosine pathway component. Adenosine can function as an immunomodulatory agent in the body. For example, adenosine and some adenosine analogs that non-selectively activate adenosine receptor subtypes decrease neutrophil production of inflammatory oxidative products (Cronstein et al., Ann. N.Y. Acad. Sci. 451:291, 1985; Roberts et al., Biochem. J., 227:669, 1985; Schrier et al., J. Immunol. 137:3284, 1986; Cronstein et al., Clinical Immunol. Immunopath. 42:76, 1987). In some cases, concentration of extracellular adenosine or adenosine analogs can increase in specific environments, e.g., tumor microenvironment (TME). In some cases, adenosine or adenosine analog signaling depends on hypoxia or factors involved in hypoxia or its regulation, e.g., hypoxia inducible factor (HIF). In some embodiments, increase in adenosine signaling can increase in intracellular cAMP and cAMP-dependent protein kinase that results in inhibition of proinflammatory cytokine production, and can lead to the synthesis of immunosuppressive molecules and development of Tregs (Sitkovsky et al., Cancer Immunol Res (2014) 2(7):598-605). In some embodiments, the additional agent can reduce or reverse immunosuppressive effects of adenosine, adenosine analogs and/or adenosine signaling. In some embodiments, the additional agent can reduce or reverse hypoxia-driven A2-adenosinergic T cell immunosuppression. In some embodiments, the additional agent is selected from among antagonists of adenosine receptors, extracellular adenosine-degrading agents, inhibitors of adenosine generation by CD39/CD73 ectoenzymes, and inhibitors of hypoxia-HIF-1α signaling. In some embodiments, the additional agent is an adenosine receptor antagonist or agonist.

[0637] Inhibition or reduction of extracellular adenosine or the adenosine receptor by virtue of an inhibitor of extracellular adenosine (such as an agent that prevents the formation of, degrades, renders inactive, and/or decreases extracellular adenosine), and/or an adenosine receptor inhibitor (such as an adenosine receptor antagonist) can enhance immune response, such as a macrophage, neutrophil, granulocyte, dendritic cell, T- and/or B cell-mediated response. In addition, inhibitors of the Gs protein

mediated cAMP dependent intracellular pathway and inhibitors of the adenosine receptor-triggered Gi protein mediated intracellular pathways, can also increase acute and chronic inflammation.

[0638] In some embodiments, the additional agent is an adenosine receptor antagonist or agonist, e.g., an antagonist or agonist of one or more of the adenosine receptors A2a, A2b, A1, and A3. A1 and A3 inhibit, and A2a and A2b stimulate, respectively, adenylate cyclase activity. Certain adenosine receptors, such as A2a, A2b, and A3, can suppress or reduce the immune response during inflammation. Thus, antagonizing immunosuppressive adenosine receptors can augment, boost or enhance immune response, e.g., immune response from administered cells, e.g., CAR-expressing T cells. In some embodiments, the additional agent inhibits the production of extracellular adenosine and adenosine-triggered signaling through adenosine receptors. For example, enhancement of an immune response, local tissue inflammation, and targeted tissue destruction can be enhanced by inhibiting or reducing the adenosine-producing local tissue hypoxia; by degrading (or rendering inactive) accumulated extracellular adenosine; by preventing or decreasing expression of adenosine receptors on immune cells; and/or by inhibiting/antagonizing signaling by adenosine ligands through adenosine receptors.

[0639] An antagonist is any substance that tends to nullify the action of another, as an agent that binds to a cell receptor without eliciting a biological response. In some embodiments, the antagonist is a chemical compound that is an antagonist for an adenosine receptor, such as the A2a, A2b, or A3 receptor. In some embodiments, the antagonist is a peptide, or a pepidomimetic, that binds the adenosine receptor but does not trigger a Gi protein dependent intracellular pathway. Exemplary antagonists are described in U.S. Pat. Nos. 5,565,566; 5,545, 627, 5,981,524; 5,861,405; 6,066,642; 6,326,390; 5,670,501; 6,117,998; 6,232,297; 5,786,360; 5,424,297; 6,313,131, 5,504,090; and 6,322,771.

[0640] In some embodiments, the additional agent is an A2 receptor (A2R) antagonist, such as an A2a antagonist. Exemplary A2R antagonists include KW6002 (istradefyline), SCH58261, caffeine, paraxanthine, 3,7-dimethyl-1-propargylxanthine (DMPX), 8-(m-chlorostyryl) caffeine (CSC), MSX-2, MSX-3, MSX-4, CGS-15943, ZM-241385, SCH-442416, preladenant, vipadenant (BII014), V2006, ST-1535, SYN-115, PSB-1115, ZM241365, FSPTP, and an inhibitory nucleic acid targeting A2R expression, e.g., siRNA or shRNA, or any antibodies or antigen-binding fragment thereof that targets an A2R. In some embodiments, the additional agent is an A2R antagonist described in, e.g., Ohta et al., Proc Natl Acad Sci U S A (2006) 103:13132-13137; Jin et al., Cancer Res. (2010) 70(6):2245-2255; Leone et al., Computational and Structural Biotechnology Journal (2015) 13:265-272; Beavis et al., Proc Natl Acad Sci U S A (2013) 110:14711–14716; and Pinna, A., Expert Opin Investig Drugs (2009) 18:1619-1631; Sitkovsky et al., Cancer Immunol Res (2014) 2(7):598-605; US 8,080,554; US 8,716,301; US 20140056922; WO2008/147482; US 8,883,500; US 20140377240; WO02/055083; US 7,141,575; US 7,405,219; US 8,883,500; US 8,450,329 and US

8,987,279).

[0641] In some embodiments, the antagonist is an antisense molecule, inhibitory nucleic acid molecule (e.g., small inhibitory RNA (siRNA)) or catalytic nucleic acid molecule (e.g. a ribozyme) that specifically binds mRNA encoding an adenosine receptor. In some embodiments, the antisense molecule, inhibitory nucleic acid molecule or catalytic nucleic acid molecule binds nucleic acids encoding A2a, A2b, or A3. In some embodiments, an antisense molecule, inhibitory nucleic acid molecule or catalytic nucleic acid targets biochemical pathways downstream of the adenosine receptor. For example, the antisense molecule or catalytic nucleic acid can inhibit an enzyme involved in the Gs protein- or Gi protein-dependent intracellular pathway. In some embodiments, the additional agent includes dominant negative mutant form of an adenosine receptor, such as A2a, A2b, or A3.

[0642] In some embodiments, the additional agent that inhibits extracellular adenosine includes agents that render extracellular adenosine non-functional (or decrease such function), such as a substance that modifies the structure of adenosine to inhibit the ability of adenosine to signal through adenosine receptors. In some embodiments, the additional agent is an extracellular adenosine-generating or adenosine-degrading enzyme, a modified form thereof or a modulator thereof. For example, in some embodiments, the additional agent is an enzyme (e.g. adenosine deaminase) or another catalytic molecule that selectively binds and destroys the adenosine, thereby abolishing or significantly decreasing the ability of endogenously formed adenosine to signal through adenosine receptors and terminate inflammation.

[0643] In some embodiments, the additional agent is an adenosine deaminase (ADA) or a modified form thereof, e.g., recombinant ADA and/or polyethylene glycol-modified ADA (ADA-PEG), which can inhibit local tissue accumulation of extracellular adenosine. ADA-PEG has been used in treatment of patients with ADA SCID (Hershfield (1995) Hum Mutat. 5:107). In some embodiments, an agent that inhibits extracellular adenosine includes agents that prevent or decrease formation of extracellular adenosine, and/or prevent or decrease the accumulation of extracellular adenosine, thereby abolishing, or substantially decreasing, the immunosuppressive effects of adenosine. In some embodiments, the additional agent specifically inhibits enzymes and proteins that are involved in regulation of synthesis and/or secretion of pro-inflammatory molecules, including modulators of nuclear transcription factors. Suppression of adenosine receptor expression or expression of the Gs protein- or Gi protein-dependent intracellular pathway, or the cAMP dependent intracellular pathway, can result in an increase/enhancement of immune response.

[0644] In some embodiments, the additional agent can target ectoenzymes that generate or produce extracellular adenosine. In some embodiments, the additional agent targets CD39 and CD73 ectoenzymes, which function in tandem to generate extracellular adenosine. CD39 (also called ectonucleoside triphosphate diphosphohydrolase) converts extracellular ATP (or ADP) to 5′AMP. Subsequently, CD73 (also called

5′nucleotidase) converts 5′AMP to adenosine. The activity of CD39 is reversible by the actions of NDP kinase and adenylate kinase, whereas the activity of CD73 is irreversible. CD39 and CD73 are expressed on tumor stromal cells, including endothelial cells and Tregs, and also on many cancer cells. For example, the expression of CD39 and CD73 on endothelial cells is increased under the hypoxic conditions of the tumor microenvironment. Tumor hypoxia can result from inadequate blood supply and disorganized tumor vasculature, impairing delivery of oxygen (Carroll and Ashcroft (2005), Expert. Rev. Mol. Med. 7(6):1-16). Hypoxia also inhibits adenylate kinase (AK), which converts adenosine to AMP, leading to very high extracellular adenosine concentration. Thus, adenosine is released at high concentrations in response to hypoxia, which is a condition that frequently occurs the tumor microenvironment (TME), in or around solid tumors. In some embodiments, the additional agent is one or more of anti-CD39 antibody or antigen binding fragment thereof, anti-CD73 antibody or antigen binding fragment thereof, e.g., MEDI9447 or TY/23, α-β-methylene-adenosine diphosphate (ADP), ARL 67156, POM-3, IPH52 (see, e.g., Allard et al. Clin Cancer Res (2013) 19(20):5626-5635; Hausler et al., Am J Transl Res (2014) 6(2):129-139; Zhang, B., Cancer Res. (2010) 70(16):6407-6411).

[0645] In some embodiments, the additional agent is an inhibitor of hypoxia inducible factor 1 alpha (HIF-1α) signaling. Exemplary inhibitors of HIF-1α include digoxin, acriflavine, sirtuin-7 and ganetespib.

[0646] In some embodiments, the additional agent includes a protein tyrosine phosphatase inhibitor, e.g., a protein tyrosine phosphatase inhibitor described herein. In some embodiments, 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 some embodiments, the protein tyrosine phosphatase inhibitor is an SHP-2 inhibitor, e.g., an SHP-2 inhibitor described herein.

[0647] In some embodiments, the additional agent is a kinase inhibitor. Kinase inhibitors, such as a CDK4 kinase inhibitor, a BTK kinase inhibitor, a MNK kinase inhibitor, or a DGK kinase inhibitor, can regulate the constitutively active survival pathways that exist in tumor cells and/or modulate the function of immune cells. In some embodiments, the kinase inhibitor is a Bruton's tyrosine kinase (BTK) inhibitor, e.g., ibrutinib. In some embodiments, the kinase inhibitor is a phosphatidylinositol-4,5-bisphosphate 3-kinase (PI3K) inhibitor. In some embodiments, the kinase inhibitor is a CDK4 inhibitor, e.g., a CDK4/6 inhibitor. In some embodiments, the kinase inhibitor is an mTOR inhibitor, 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. In some embodiments, the kinase inhibitor is an MNK inhibitor, or a dual PI3K/mTOR inhibitor. In some embodiments, other exemplary kinase inhibitors include the AKT inhibitor perifosine, the mTOR inhibitor temsirolimus, the Src kinase inhibitors dasatinib and fostamatinib, the JAK2 inhibitors pacritinib and ruxolitinib, the PKCβ inhibitors enzastaurin and bryostatin,

and the AAK inhibitor alisertib.

[0648] In some embodiments, 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 some embodiments, 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.

[0649] In some embodiments, the kinase inhibitor is a BTK inhibitor, e.g., ibrutinib (1-[(3R)-3-[4-Amino-3-(4-phenoxyphenyl)-1H-pyrazolo[3,4-d]pyrimidin-1-yl]piperidin-1-yl]prop-2-en-1-one; also known as PCI-32765). In some embodiments, the kinase inhibitor is a BTK inhibitor, e.g., ibrutinib (PCI-32765), and 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, or daily for 28 day cycle. In some embodiments, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12 or more cycles of ibrutinib are administered. In some embodiments, the BTK inhibitor is a BTK inhibitor described in International Application WO 2015/079417.

[0650] In some embodiments, the kinase inhibitor is a PI3K inhibitor. PI3K is central to the PI3K/Akt/mTOR pathway involved in cell cycle regulation and lymphoma survival. Exemplary PI3K inhibitor includes idelalisib (PI3Kδ inhibitor). In some embodiments, the additional agent is idelalisib and rituximab.

[0651] In some embodiments, the additional agent is an inhibitor of mammalian target of rapamycin (mTOR). In some embodiments, the kinase inhibitor is an mTOR inhibitor selected from temsirolimus; ridaforolimus (also known as AP23573 and MK8669); everolimus (RAD001); rapamycin (AY22989); simapimod; AZD8055; PF04691502; SF1126; and XL765. In some embodiments, the additional agent is an inhibitor of mitogen-activated protein kinase (MAPK), such as vemurafenib, dabrafenib, and trametinib.

[0652] In some embodiments, the additional agent is an agent that regulates pro- or anti-apoptotic proteins. In some embodiments, the additional agent includes a B-cell lymphoma 2 (BCL-2) inhibitor (e.g., venetoclax, also called ABT-199 or GDC-0199; or ABT-737). Venetoclax is a small molecule (4-(4-{[2-(4-Chlorophenyl)-4,4-dimethyl-1-cyclohexen-1-yl]methyl}-1-piperazinyl)-N-({3-nitro-4-[(tetrahydro-2H-pyran-4-ylmethyl)amino]phenyl}sulfonyl)-2-(1H-pyrrolo[2,3-b]pyridin-5-yloxy)benzamide) that inhibits the anti-apoptotic protein, BCL-2. Other agents that modulate pro- or anti-apoptotic protein include BCL-2 inhibitor ABT-737, navitoclax (ABT-263); Mcl-1 siRNA or Mcl-1 inhibitor retinoid N-(4-hydroxyphenyl) retinamide (4-HPR) for maximal efficacy. In some embodiments, the additional agent provides a pro-apoptotic stimuli, such as recombinant tumor necrosis factor-related apoptosis-inducing ligand (TRAIL), which can activate the apoptosis pathway by binding to TRAIL death receptors DR-4 and DR-5 on tumor

cell surface, or TRAIL-R2 agonistic antibodies.

[0653] In some embodiments, the additional agent includes 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. Plasmacytoid dendritic cells (pDCs), macrophages, and dendritic cells (DCs) can express IDO. In some aspects, a decrease in L-tryptophan (e.g., catalyzed by IDO) results in an immunosuppressive milieu by inducing T-cell anergy and apoptosis. Thus, in some aspects, an IDO inhibitor can enhance the efficacy of the BCMA-binding recombinant receptors, cells and/or compositions described herein, e.g., by decreasing the suppression or death of the administered CAR-expressing cell. Exemplary inhibitors of IDO include but are not limited to 1-methyl-tryptophan, indoximod (New Link Genetics) (see, e.g., Clinical Trial Identifier Nos. NCT01191216; NCT01792050), and INCB024360 (Incyte Corp.) (see, e.g., Clinical Trial Identifier Nos. NCT01604889; NCT01685255).

[0654] In some embodiments, the additional agent includes a cytotoxic agent, e.g., CPX-351 (Celator Pharmaceuticals), cytarabine, daunorubicin, vosaroxin (Sunesis Pharmaceuticals), sapacitabine (Cyclacel Pharmaceuticals), idarubicin, or mitoxantrone. In some embodiments, the additional agent includes a hypomethylating agent, e.g., a DNA methyltransferase inhibitor, e.g., azacitidine or decitabine.

[0655] In another embodiment, the additional therapy is transplantation, e.g., an allogeneic stem cell transplant.

[0656] In some embodiments, the additional therapy is a lymphodepleting therapy. Lymphodepleting chemotherapy is thought to improve engraftment and activity of recombinant receptor-expressing cells, such as CAR T cells. In some embodiments, lymphodepleting chemotherapy may enhance adoptively transferred tumor-specific T cells to proliferate in vivo through homeostatic proliferation (Grossman 2004, Stachel 2004). In some embodiments, chemotherapy may reduce or eliminate CD4+CD25+ regulatory T cells, which can suppress the function of tumor-targeted adoptively transferred T cells (Turk 2004). In some embodiments, lymphodepleting chemotherapy prior to adoptive T-cell therapy may enhance the expression of stromal cell-derived factor 1 (SDF-1) in the bone marrow, enhancing the homing of modified T cells to the primary tumor site through binding of SDF-1 with CXCR-4 expressed on the T-cell surface (Pinthus 2004). In some embodiments, lymphodepleting chemotherapy may further reduce the subject's tumor burden and potentially lower the risk and severity of CRS.

[0657] In some embodiments, lymphodepletion is performed on a subject, e.g., prior to administering engineered cells, e.g., CAR-expressing cells. In some embodiments, the lymphodepletion comprises administering one or more of melphalan, Cytoxan, cyclophosphamide, and/or fludarabine. In some embodiments, a lymphodepleting chemotherapy is administered to the subject prior to, concurrently with, or

after administration (e.g., infusion) of engineered cells, e.g., CAR-expressing cells. In an example, the lymphodepleting chemotherapy is administered to the subject prior to administration of engineered cells, e.g., CAR-expressing cells. In some embodiments the lymphodepleting chemotherapy is administered 1 to 10 days prior to administration of engineered cells, such as 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 days prior to the initiation of administration of engineered cells, or at least 2 days prior, such as at least 3, 4, 5, 6, or 7 days prior, to the initiation of administration of engineered cell. In some embodiments, the subject is administered a preconditioning agent no more than 7 days prior, such as no more than 6, 5, 4, 3, or 2 days prior, to the initiation of administration of engineered cell. The number of days after lymphodepleting chemotherapy that the engineered ells are administered can be determined based on clinical or logistical circumstances. In some examples, dose adjustments or other changes to the lymphodepleting chemotherapy regimen can implemented due to a subject's health, such as the subject's underlying organ function, as determined by the treating physician.

[0658] In some embodiments, lymphodepleting chemotherapy comprises administration of a lymphodepleting agent, such as cyclophosphamide, fludarabine, or combinations thereof, In some embodiments, the subject is administered cyclophosphamide at a dose between or between about 20 mg/kg and 100 mg/kg body weight of the subject, such as between or between about 40 mg/kg and 80 mg/kg. In some aspects, the subject is administered about 60 mg/kg of cyclophosphamide. In some embodiments, the cyclophosphamide is administered once daily for one or two days. In some embodiments, where the lymphodepleting agent comprises cyclophosphamide, the subject is administered cyclophosphamide at a dose between or between about 100 mg/m^2 and 500 mg/m^2 body surface area of the subject, such as between or between about 200 mg/m^2 and 400 mg/m^2, or 250 mg/m^2 and 350 mg/m^2, inclusive. In some instances, the subject is administered about 100 mg/m^2 of cyclophosphamide. In some instances, the subject is administered about 150 mg/m^2 of cyclophosphamide. In some instances, the subject is administered about 200 mg/m^2 of cyclophosphamide. In some instances, the subject is administered about 250 mg/m^2 of cyclophosphamide. In some instances, the subject is administered about 300 mg/m^2 of cyclophosphamide. In some embodiments, the cyclophosphamide can be administered in a single dose or can be administered in a plurality of doses, such as given daily, every other day or every three days. In some embodiments, cyclophosphamide is administered daily, such as for 1-5 days, for example, for 2 to 4 days. In some instances, the subject is administered about 300 mg/m^2 body surface area of the subject, of cyclophosphamide, daily for 3 days, prior to initiation of the cell therapy. In some embodiments, the subject is administered a total of at or about 300 mg/m^2, 400 mg/m^2, 500 mg/m^2, 600 mg/m^2, 700 mg/m^2, 800 mg/m^2, 900 mg/m^2, 1000 mg/m^2, 1200 mg/m^2, 1500 mg/m^2, 1800 mg/m^2, 2000 mg/m^2, 2500 mg/m^2, 2700 mg/m^2, 3000 mg/m^2, 3300 mg/m^2, 3600 mg/m^2, 4000 mg/m^2 or 5000 mg/m^2 cyclophosphamide, or a range defined

by any of the foregoing, prior to initiation of the cell therapy.

[0659] In some embodiments, where the lymphodepleting agent comprises fludarabine, the subject is administered fludarabine at a dose between or between about 1 mg/m^2 and 100 mg/m^2 body surface area of the subject, such as between or between about 10 mg/m^2 and 75 mg/m^2, 15 mg/m^2 and 50 mg/m^2, 20 mg/m^2 and 40 mg/m^2, or 24 mg/m^2 and 35 mg/m^2, inclusive. In some instances, the subject is administered about 10 mg/m^2 of fludarabine. In some instances, the subject is administered about 15 mg/m^2 of fludarabine. In some instances, the subject is administered about 20 mg/m^2 of fludarabine. In some instances, the subject is administered about 25 mg/m^2 of fludarabine. In some instances, the subject is administered about 30 mg/m^2 of fludarabine. In some embodiments, the fludarabine can be administered in a single dose or can be administered in a plurality of doses, such as given daily, every other day or every three days. In some embodiments, fludarabine is administered daily, such as for 1-5 days, for example, for 2 to 4 days. In some instances, the subject is administered about 30 mg/m^2 body surface area of the subject, of fludarabine, daily for 3 days, prior to initiation of the cell therapy. In some embodiments, the subject is administered a total of at or about 10 mg/m^2, 20 mg/m^2, 25 mg/m^2, 30 mg/m^2, 40 mg/m^2, 50 mg/m^2, 60 mg/m^2, 70 mg/m^2, 80 mg/m^2, 90 mg/m^2, 100 mg/m^2, 120 mg/m^2, 150 mg/m^2, 180 mg/m^2, 200 mg/m^2, 250 mg/m^2, 270 mg/m^2, 300 mg/m^2, 330 mg/m^2, 360 mg/m^2, 400 mg/m^2 or 500 mg/m^2 cyclophosphamide, or a range defined by any of the foregoing, prior to initiation of the cell therapy.

[0660] In some embodiments, the lymphodepleting agent comprises a single agent, such as cyclophosphamide or fludarabine. In some embodiments, the subject is administered cyclophosphamide only, without fludarabine or other lymphodepleting agents. In some embodiments, prior to the administration, the subject has received a lymphodepleting therapy comprising the administration of cyclophosphamide at or about 200-400 mg/m^2 body surface area of the subject, optionally at or about 300 mg/m^2, daily, for 2-4 days. In some embodiments, the subject is administered fludarabine only, for example, without cyclophosphamide or other lymphodepleting agents. In some embodiments, prior to the administration, the subject has received a lymphodepleting therapy comprising the administration of fludarabine at or about 20-40 mg/m^2 body surface area of the subject, optionally at or about 30 mg/m^2, daily, for 2-4 days.

[0661] In some embodiments, the lymphodepleting agent comprises a combination of agents, such as a combination of cyclophosphamide and fludarabine. Thus, the combination of agents may include cyclophosphamide at any dose or administration schedule, such as those described above, and fludarabine at any dose or administration schedule, such as those described above. For example, in some aspects, the subject is administered fludarabine at or about 30 mg/m^2 body surface area of the subject, daily, and cyclophosphamide at or about 300 mg/m^2 body surface area of the subject, daily, for 3 days.

[0662] In some embodiments, antiemetic therapy, except dexamethasone or other steroids, may be given prior to lymphodepleting chemotherapy. In some embodiments, Mesna may be used for subjects with a history of hemorrhagic cystitis.

[0663] In some embodiments, the additional agent is an oncolytic virus. In some 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)).

[0664] Other exemplary combination therapy, treatment and/or agents include anti-allergenic agents, anti-emetics, analgesics and adjunct therapies. In some embodiments, the additional agent includes cytoprotective agents, such as neuroprotectants, free-radical scavengers, cardioprotectors, anthracycline extravasation neutralizers and nutrients.

[0665] In some embodiments, an antibody used as an additional agent 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 some embodiments, the additional agent is an antibody-drug conjugate.

[0666] In some embodiments, the additional agent can modulate, inhibit or stimulate particular factors at the DNA, RNA or protein levels, to enhance or boost the efficacy of the BCMA-binding recombinant receptors, cells and/or compositions provided herein. In some embodiments, the additional agent can modulate the factors at the nucleic acid level, e.g., DNA or RNA, within the administered cells, e.g., cells engineered to express recombinant receptors, e.g., CAR. In some embodiments, an inhibitory nucleic acid, e.g., an inhibitory nucleic acid, e.g., a dsRNA, e.g., an siRNA or shRNA, or a clustered regularly interspaced short palindromic repeats (CRISPR), a transcription-activator like effector nuclease (TALEN), or a zinc finger endonuclease (ZFN), can be used to inhibit expression of an inhibitory molecule in the engineered cell, e.g., CAR-expressing cell. In some embodiments the inhibitor is an shRNA. In some embodiments, the inhibitory molecule is inhibited within the engineered cell, e.g., CAR-expressing cell. In some embodiments, 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 HI- or a U6-derived promoter such that the dsRNA molecule that inhibits expression of the inhibitory molecule is

expressed within the engineered cell, e.g., CAR-expressing cell. See, e.g., Brummelkamp TR, et al. (2002) Science 296: 550- 553; Miyagishi M, et al. (2002) Nat. Biotechnol. 19: 497-500.

[0667] In some embodiments, the additional agent is capable of disrupting the gene encoding an inhibitory molecule, such as any immune checkpoint inhibitors described herein. In some embodiments, disruption is by deletion, e.g., deletion of an entire gene, exon, or region, and/or replacement with an exogenous sequence, and/or by mutation, *e.g.*, frameshift or missense mutation, within the gene, typically within an exon of the gene. In some embodiments, the disruption results in a premature stop codon being incorporated into the gene, such that the inhibitory molecule is not expressed or is not expressed in a form that is capable of being expressed on the cells surface and/or capable of mediating cell signaling. The disruption is generally carried out at the DNA level. The disruption generally is permanent, irreversible, or not transient.

[0668] In some aspects, the disruption is carried out by gene editing, such as using a DNA binding protein or DNA-binding nucleic acid, which specifically binds to or hybridizes to the gene at a region targeted for disruption. In some aspects, the protein or nucleic acid is coupled to or complexed with a nuclease, such as in a chimeric or fusion protein. For example, in some embodiments, the disruption is effected using a fusion comprising a DNA-targeting protein and a nuclease, such as a Zinc Finger Nuclease (ZFN) or TAL-effector nuclease (TALEN), or an RNA-guided nuclease such as a clustered regularly interspersed short palindromic nucleic acid (CRISPR)-Cas system, such as CRISPR-Cas9 system, specific for the gene being disrupted. In some embodiments, methods of producing or generating genetically engineered cells, e.g., CAR-expressing cells, include introducing into a population of cells nucleic acid molecules encoding a genetically engineered antigen receptor (e.g. CAR) and nucleic acid molecules encoding an agent targeting an inhibitory molecule that is a gene editing nuclease, such as a fusion of a DNA-targeting protein and a nuclease such as a ZFN or a TALEN, or an RNA-guided nuclease such as of the CRISPR-Cas9 system, specific for an inhibitory molecule.

[0669] Any of the additional agents described herein can be prepared and administered as combination therapy with the BCMA-binding recombinant receptor (*e.g.*, chimeric antigen receptor) and/or engineered cells expressing said molecules (*e.g.*, recombinant receptor) described herein, such as in pharmaceutical compositions comprising one or more agents of the combination therapy and a pharmaceutically acceptable carrier, such as any described herein. In some embodiments, the BCMA-binding recombinant receptor (*e.g.*, chimeric antigen receptor), engineered cells expressing said molecules (*e.g.*, recombinant receptor), plurality of engineered cells expressing said molecules (e.g., recombinant receptor) can be administered simultaneously, concurrently or sequentially, in any order with the additional agents, therapy or treatment, wherein such administration provides therapeutically effective levels each of the agents in the body of the

subject. In some embodiments, the additional agent can be co-administered with the BCMA-binding recombinant receptors, cells and/or compositions described herein, for example, as part of the same pharmaceutical composition or using the same method of delivery. In some embodiments, the additional agent is administered simultaneously with the BCMA-binding recombinant receptors, cells and/or compositions described herein, but in separate compositions. In some embodiments, the additional agent is an additional engineered cell, e.g., cell engineered to express a different recombinant receptor, and is administered in the same composition or in a separate composition. In some embodiments, the additional agent is incubated with the engineered cell, e.g., CAR-expressing cells, prior to administration of the cells.

[0670] In some examples, the one or more additional agents are administered subsequent to or prior to the administration of the BCMA-binding recombinant receptors, cells and/or compositions described herein, separated by a selected time period. In some examples, the time period is 1 day, 2 days, 3 days, 4 days, 5 days, 6 days, 1 week, 2 weeks, 3 weeks, 1 month, 2 months, or 3 months. In some examples, the one or more additional agents are administered multiple times and/or the BCMA-binding recombinant receptors, cells and/or compositions described herein, is administered multiple times. For example, in some embodiments, the additional agent is administered prior to the BCMA-binding recombinant receptors, cells and/or compositions described herein, e.g., two weeks, 12 days, 10 days, 8 days, one week, 6 days, 5 days, 4 days, 3 days, 2 days or 1 day before the administration. For example, in some embodiments, the additional agent is administered after the BCMA-binding recombinant receptors, cells and/or compositions described herein, e.g., two weeks, 12 days, 10 days, 8 days, one week, 6 days, 5 days, 4 days, 3 days, 2 days or 1 day after the administration.

[0671] The dose of the additional agent can be any therapeutically effective amount, e.g., any dose amount described herein, and the appropriate dosage of the additional agent may depend on the type of disease to be treated, the type, dose and/or frequency of the recombinant receptor, cell and/or composition administered, the severity and course of the disease, whether the recombinant receptor, cell and/or composition is administered for preventive or therapeutic purposes, previous therapy, the patient's clinical history and response to the recombinant receptor, cell and/or composition, and the discretion of the attending physician. The recombinant receptor, cell and/or composition and/or the additional agent and/or therapy can be administered to the patient at one time, repeated or administered over a series of treatments.

[0672] In some aspects, administration of a dose of engineered cells and/or a composition containing the engineered cells, is repeated. In some aspects, the subject receives one or more additional doses of the engineered cells and/or a composition containing the engineered cells, that is the same as the initial dose of the engineered cells and/or composition containing the engineered cells. In some aspects, the subject receives one or more additional doses of the engineered cells and/or a composition containing the engineered

cells, that is different from the initial dose of the engineered cells and/or composition containing the engineered cells. In some aspects, the additional dose is higher than the initial dose. In some aspects the additional dose is lower than the initial dose. In some embodiments, the subject is only administered one dose of engineered cells and/or composition containing the engineered cells. In some embodiments, administration of a dose of engineered cells and/or a composition containing the engineered cells, is not repeated.

VI. ARTICLES OF MANUFACTURE OR KITS

[0673] Also provided are articles of manufacture or kit containing the provided recombinant receptors (*e.g.*, CARs), genetically engineered cells, and/or compositions comprising the same. The articles of manufacture may include a container and a label or package insert on or associated with the container. Suitable containers include, for example, bottles, vials, syringes, test tubes, IV solution bags, etc. The containers may be formed from a variety of materials such as glass or plastic. In some embodiments, the container has a sterile access port. Exemplary containers include an intravenous solution bags, vials, including those with stoppers pierceable by a needle for injection. The article of manufacture or kit may further include a package insert indicating that the compositions can be used to treat a particular condition such as a condition described herein (*e.g.*, multiple myeloma). Alternatively, or additionally, the article of manufacture or kit may further include another or the same container comprising a pharmaceutically-acceptable buffer. It may further include other materials such as other buffers, diluents, filters, needles, and/or syringes.

[0674] The label or package insert may indicate that the composition is used for treating the BCMA-expressing or BCMA-associated disease, disorder or condition in an individual. The label or a package insert, which is on or associated with the container, may indicate directions for reconstitution and/or use of the formulation. The label or package insert may further indicate that the formulation is useful or intended for subcutaneous, intravenous, or other modes of administration for treating or preventing a BCMA-expressing or BCMA-associated disease, disorder or condition in an individual.

[0675] The container in some embodiments holds a composition which is by itself or combined with another composition effective for treating, preventing and/or diagnosing the condition. The article of manufacture or kit may include (a) a first container with a composition contained therein (*i.e.*, first medicament), wherein the composition includes the antibody (*e.g.*, anti-BCMA antibody) or antigen-binding fragment thereof or recombinant receptor (*e.g.*, CAR); and (b) a second container with a composition contained therein (*i.e.*, second medicament), wherein the composition includes a further agent, such as a cytotoxic or otherwise therapeutic agent, and which article or kit further comprises instructions on the label

or package insert for treating the subject with the second medicament, in an effective amount.

VII. DEFINITIONS

[0676] As used herein, reference to a "corresponding form" of an antibody means that when comparing a property or activity of two antibodies, the property is compared using the same form of the antibody. For example, if it is stated that an antibody has greater activity compared to the activity of the corresponding form of a first antibody, that means that a particular form, such as an scFv of that antibody, has greater activity compared to the scFv form of the first antibody.

[0677] The term "Fc region" herein is used to define a C-terminal region of an immunoglobulin heavy chain that contains at least a portion of the constant region. The term includes native sequence Fc regions and variant Fc regions. In one embodiment, a human IgG heavy chain Fc region extends from Cys226, or from Pro230, to the carboxyl-terminus of the heavy chain. However, the C-terminal lysine (Lys447) of the Fc region may or may not be present. Unless otherwise specified herein, numbering of amino acid residues in the Fc region or constant region is according to the EU numbering system, also called the EU index, as described in Kabat *et al.*, Sequences of Proteins of Immunological Interest, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, MD, 1991.

[0678] The terms "full length antibody," "intact antibody," and "whole antibody" are used herein interchangeably to refer to an antibody having a structure substantially similar to a native antibody structure or having heavy chains that contain an Fc region as defined herein.

[0679] An "isolated" antibody is one which has been separated from a component of its natural environment. In some embodiments, an antibody is purified to greater than 95% or 99% purity as determined by, for example, electrophoretic (*e.g.*, SDS-PAGE, isoelectric focusing (IEF), capillary electrophoresis) or chromatographic (*e.g.*, ion exchange or reverse phase HPLC). For review of methods for assessment of antibody purity, see, *e.g.*, Flatman *et al.*, J. Chromatogr. B 848:79-87 (2007).

[0680] An "isolated" nucleic acid refers to a nucleic acid molecule that has been separated from a component of its natural environment. An isolated nucleic acid includes a nucleic acid molecule contained in cells that ordinarily contain the nucleic acid molecule, but the nucleic acid molecule is present extrachromosomally or at a chromosomal location that is different from its natural chromosomal location.

[0681] "Isolated nucleic acid encoding an anti-BCMA antibody" refers to one or more nucleic acid molecules encoding antibody heavy and light chains (or fragments thereof), including such nucleic acid molecule(s) in a single vector or separate vectors, and such nucleic acid molecule(s) present at one or more locations in a host cell.

[0682] The terms "host cell," "host cell line," and "host cell culture" are used interchangeably and refer

to cells into which exogenous nucleic acid has been introduced, including the progeny of such cells. Host cells include "transformants" and "transformed cells," which include the primary transformed cell and progeny derived therefrom without regard to the number of passages. Progeny may not be completely identical in nucleic acid content to a parent cell, but may contain mutations. Mutant progeny that have the same function or biological activity as screened or selected for in the originally transformed cell are included herein.

[0683] The terms "polypeptide" and "protein" are used interchangeably to refer to a polymer of amino acid residues, and are not limited to a minimum length. Polypeptides, including the antibodies and antibody chains and other peptides, *e.g.*, linkers and BCMA-binding peptides, may include amino acid residues including natural and/or non-natural amino acid residues. The terms also include post-expression modifications of the polypeptide, for example, glycosylation, sialylation, acetylation, phosphorylation, and the like. In some aspects, the polypeptides may contain modifications with respect to a native or natural sequence, as long as the protein maintains the desired activity. These modifications may be deliberate, as through site-directed mutagenesis, or may be accidental, such as through mutations of hosts which produce the proteins or errors due to PCR amplification.

[0684] As used herein, "percent (%) amino acid sequence identity" and "percent identity" and "sequence identity" when used with respect to an amino acid sequence (reference polypeptide sequence) is defined as the percentage of amino acid residues in a candidate sequence (*e.g.*, the subject antibody or fragment) that are identical with the amino acid residues in the reference polypeptide sequence, after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent sequence identity, and not considering any conservative substitutions as part of the sequence identity. Alignment for purposes of determining percent amino acid sequence identity can be achieved in various ways that are within the skill in the art, for instance, using publicly available computer software such as BLAST, BLAST-2, ALIGN or Megalign (DNASTAR) software. Those skilled in the art can determine appropriate parameters for aligning sequences, including any algorithms needed to achieve maximal alignment over the full length of the sequences being compared.

[0685] An amino acid substitution may include replacement of one amino acid in a polypeptide with another amino acid. Amino acid substitutions may be introduced into a binding molecule, *e.g.*, antibody, of interest and the products screened for a desired activity, *e.g.*, retained/improved antigen binding, or decreased immunogenicity.

[0686] Amino acids generally can be grouped according to the following common side-chain properties:

(1) hydrophobic: Norleucine, Met, Ala, Val, Leu, Ile;

(2) neutral hydrophilic: Cys, Ser, Thr, Asn, Gln;

(3) acidic: Asp, Glu;

(4) basic: His, Lys, Arg;

(5) residues that influence chain orientation: Gly, Pro;

(6) aromatic: Trp, Tyr, Phe.

[0687] Non-conservative amino acid substitutions will involve exchanging a member of one of these classes for another class.

[0688] The term "vector," as used herein, refers to a nucleic acid molecule capable of propagating another nucleic acid to which it is linked. The term includes the vector as a self-replicating nucleic acid structure as well as the vector incorporated into the genome of a host cell into which it has been introduced. Certain vectors are capable of directing the expression of nucleic acids to which they are operatively linked. Such vectors are referred to herein as "expression vectors."

[0689] The term "package insert" is used to refer to instructions customarily included in commercial packages of therapeutic products, that contain information about the indications, usage, dosage, administration, combination therapy, contraindications and/or warnings concerning the use of such therapeutic products.

[0690] As used herein, the singular forms "a," "an," and "the" include plural referents unless the context clearly dictates otherwise. For example, "a" or "an" means "at least one" or "one or more." It is understood that aspects, embodiments, and variations described herein include "comprising," "consisting," and/or "consisting essentially of" aspects, embodiments and variations.

[0691] Throughout this disclosure, various aspects of the claimed subject matter are 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 claimed subject matter. Accordingly, the description of a range should be considered to have specifically disclosed all the possible sub-ranges as well as individual numerical values within that range. For example, where a range of values is provided, it is understood that each intervening value, between the upper and lower limit of that range and any other stated or intervening value in that stated range is encompassed within the claimed subject matter. The upper and lower limits of these smaller ranges may independently be included in the smaller ranges, and are also encompassed within the claimed subject matter, subject to any specifically excluded limit in the stated range. Where the stated range includes one or both of the limits, ranges excluding either or both of those included limits are also included in the claimed subject matter. This applies regardless of the breadth of the range.

[0692] The term "about" as used herein refers to the usual error range for the respective value readily known to the skilled person in this technical field. Reference to "about" a value or parameter herein includes

(and describes) embodiments that are directed to that value or parameter per se. For example, description referring to "about X" includes description of "X".

[0693] As used herein, a "composition" refers to any mixture of two or more products, substances, or compounds, including cells. It may be a solution, a suspension, liquid, powder, a paste, aqueous, non-aqueous or any combination thereof.

[0694] As used herein, a statement that a cell or population of cells is "positive" for a particular marker refers to the detectable presence on or in the cell of a particular marker, typically a surface marker. When referring to a surface marker, the term refers to the presence of surface expression as detected by flow cytometry, for example, by staining with an antibody that specifically binds to the marker and detecting said antibody, wherein the staining is detectable by flow cytometry at a level substantially above the staining detected carrying out the same procedure with an isotype-matched control under otherwise identical conditions and/or at a level substantially similar to that for cell known to be positive for the marker, and/or at a level substantially higher than that for a cell known to be negative for the marker.

[0695] As used herein, a statement that a cell or population of cells is "negative" for a particular marker refers to the absence of substantial detectable presence on or in the cell of a particular marker, typically a surface marker. When referring to a surface marker, the term refers to the absence of surface expression as detected by flow cytometry, for example, by staining with an antibody that specifically binds to the marker and detecting said antibody, wherein the staining is not detected by flow cytometry at a level substantially above the staining detected carrying out the same procedure with an isotype-matched control under otherwise identical conditions, and/or at a level substantially lower than that for cell known to be positive for the marker, and/or at a level substantially similar as compared to that for a cell known to be negative for the marker.

[0696] Unless defined otherwise, all terms of art, notations and other technical and scientific terms or terminology used herein are intended to have the same meaning as is commonly understood by one of ordinary skill in the art to which the claimed subject matter pertains. In some cases, terms with commonly understood meanings are defined herein for clarity and/or for ready reference, and the inclusion of such definitions herein should not necessarily be construed to represent a substantial difference over what is generally understood in the art.

VIII. EXEMPLARY EMBODIMENTS

[0697] Among the embodiments provided herein are:

1. A method of treating a subject having or suspected of having multiple myeloma (MM), the method comprising administering to the subject a dose of engineered T cells comprising a chimeric antigen

receptor (CAR), the CAR comprising:

(a) an extracellular antigen-binding domain, comprising:

a variable heavy chain (V_H) comprising a heavy chain complementarity determining region 1 (CDR-H1), a heavy chain complementarity determining region 2 (CDR-H2) and a heavy chain complementarity determining region 3 (CDR-H3) contained within the amino acid sequence of SEQ ID NO: 116 and a variable light chain (V_L) comprising a light chain complementarity determining region 1 (CDR-L1), a light chain complementarity determining region 2 (CDR-L2) and a light chain complementarity determining region 3 (CDR-L3) contained within the amino acid sequence of SEQ ID NO: 119;

a V_H comprising the amino acid sequences of SEQ ID NOS:97, 101 and 103 and a V_L comprising the amino acid sequences of SEQ ID NOS:105, 107 and 108;

a V_H comprising the amino acid sequences of SEQ ID NOS:96, 100 and 103 and a V_L comprising the amino acid sequences of SEQ ID NOS:105, 107 and 108;

a V_H comprising the amino acid sequences of SEQ ID NOS:95, 99 and 103 and a V_L comprising the amino acid sequences of SEQ ID NOS: 105, 107 and 108;

a V_H comprising the amino acid sequences of SEQ ID NOS:94, 98 and 102 and a V_L comprising the amino acid sequences of SEQ ID NOS: 104, 106 and 108; or

a V_H comprising the amino acid sequence of SEQ ID NO: 116 and a V_L comprising the amino acid sequence of SEQ ID NO: 119;

(b) a spacer comprising an IgG4/2 chimeric hinge or a modified IgG4 hinge; an IgG2/4 chimeric C_H2 region; and an IgG4 C_H3 region, and optionally is about 228 amino acids in length; or a spacer set forth in SEQ ID NO: 174;

(c) a transmembrane domain, optionally a transmembrane domain from a human CD28; and

(d) an intracellular signaling region comprising a cytoplasmic signaling domain of a CD3-zeta (CD3ζ) chain and a costimulatory signaling region comprising an intracellular signaling domain of a T cell costimulatory molecule or a signaling portion thereof;

wherein, prior to the administration, the subject has received a lymphodepleting therapy comprising the administration of fludarabine at or about 20-40 mg/m^2 body surface area of the subject, optionally at or about 30 mg/m^2, daily, for 2-4 days, and/or cyclophosphamide at or about 200-400 mg/m^2 body surface area of the subject, optionally at or about 300 mg/m^2, daily, for 2-4 days.

2. A method of treating a subject having or suspected of having multiple myeloma (MM), the method comprising administering to the subject a dose of engineered T cells comprising a chimeric antigen receptor (CAR), the CAR comprising:

(a) an extracellular antigen-binding domain, comprising:

a variable heavy chain (V_H) comprising a heavy chain complementarity determining region 1 (CDR-H1), a heavy chain complementarity determining region 2 (CDR-H2) and a heavy chain complementarity determining region 3 (CDR-H3) contained within the amino acid sequence of SEQ ID NO: 116 and a variable light chain (V_L) comprising a light chain complementarity determining region 1 (CDR-L1), a light chain complementarity determining region 2 (CDR-L2) and a light chain complementarity determining region 3 (CDR-L3) contained within the amino acid sequence of SEQ ID NO: 119;

a V_H comprising the amino acid sequences of SEQ ID NOS:97, 101 and 103 and a V_L comprising the amino acid sequences of SEQ ID NOS:105, 107 and 108;

a V_H comprising the amino acid sequences of SEQ ID NOS:96, 100 and 103 and a V_L comprising the amino acid sequences of SEQ ID NOS:105, 107 and 108;

a V_H comprising the amino acid sequences of SEQ ID NOS:95, 99 and 103 and a V_L comprising the amino acid sequences of SEQ ID NOS:105, 107 and 108;

a V_H comprising the amino acid sequences of SEQ ID NOS:94, 98 and 102 and a V_L comprising the amino acid sequences of SEQ ID NOS: 104, 106 and 108; or

a V_H comprising the amino acid sequence of SEQ ID NO: 116 and a V_L comprising the amino acid sequence of SEQ ID NO: 119;

(b) a spacer comprising an IgG4/2 chimeric hinge or a modified IgG4 hinge; an IgG2/4 chimeric C_H2 region; and an IgG4 C_H3 region, and optionally is about 228 amino acids in length; or a spacer set forth in SEQ ID NO: 174;

(c) a transmembrane domain, optionally a transmembrane domain from a human CD28; and

(d) an intracellular signaling region comprising a cytoplasmic signaling domain of a CD3-zeta (CD3ζ) chain and a costimulatory signaling region comprising an intracellular signaling domain of a T cell costimulatory molecule or a signaling portion thereof; wherein at or prior to the administration of the dose of engineered T cells, the subject has received three or more therapies selected from among:

autologous stem cell transplant (ASCT);

an immunomodulatory agent;

a proteasome inhibitor; and

an anti-CD38 antibody; unless the subject was not a candidate for or was contraindicated for one or more of the therapies.

3. A method of treating a subject having or suspected of having multiple myeloma (MM), the method comprising administering to the subject a dose of engineered T cells comprising a chimeric antigen receptor (CAR), the CAR comprising:

(a) an extracellular antigen-binding domain, comprising:

a variable heavy chain (V_H) comprising a heavy chain complementarity determining region 1 (CDR-H1), a heavy chain complementarity determining region 2 (CDR-H2) and a heavy chain complementarity determining region 3 (CDR-H3) contained within the amino acid sequence of SEQ ID NO: 116 and a variable light chain (V_L) comprising a light chain complementarity determining region 1 (CDR-L1), a light chain complementarity determining region 2 (CDR-L2) and a light chain complementarity determining region 3 (CDR-L3) contained within the amino acid sequence of SEQ ID NO: 119;

a V_H comprising the amino acid sequences of SEQ ID NOS:97, 101 and 103 and a V_L comprising the amino acid sequences of SEQ ID NOS:105, 107 and 108;

a V_H comprising the amino acid sequences of SEQ ID NOS:96, 100 and 103 and a V_L comprising the amino acid sequences of SEQ ID NOS:105, 107 and 108;

a V_H comprising the amino acid sequences of SEQ ID NOS:95, 99 and 103 and a V_L comprising the amino acid sequences of SEQ ID NOS:105, 107 and 108;

a V_H comprising the amino acid sequences of SEQ ID NOS:94, 98 and 102 and a V_L comprising the amino acid sequences of SEQ ID NOS: 104, 106 and 108; or

a V_H comprising the amino acid sequence of SEQ ID NO: 116 and a V_L comprising the amino acid sequence of SEQ ID NO: 119;

(b) a spacer comprising an IgG4/2 chimeric hinge or a modified IgG4 hinge; an IgG2/4 chimeric C_H2 region; and an IgG4 C_H3 region, and optionally is about 228 amino acids in length; or a spacer set forth in SEQ ID NO: 174;

(c) a transmembrane domain, optionally a transmembrane domain from a human CD28; and

(d) an intracellular signaling region comprising a cytoplasmic signaling domain of a CD3-zeta (CD3ζ) chain and a costimulatory signaling region comprising an intracellular signaling domain of a T cell costimulatory molecule or a signaling portion thereof; wherein at the administration of the dose of engineered T cells, the subject has not had active or history of plasma cell leukemia (PCL).

4. A method of treating a subject having or suspected of having multiple myeloma (MM), the method comprising administering to the subject a dose of engineered T cells comprising a chimeric antigen receptor (CAR), the CAR comprising:

(a) an extracellular antigen-binding domain, comprising:

a variable heavy chain (V_H) comprising a heavy chain complementarity determining region 1 (CDR-H1), a heavy chain complementarity determining region 2 (CDR-H2) and a heavy chain complementarity determining region 3 (CDR-H3) contained within the amino acid sequence of SEQ ID NO: 116 and a variable light chain (V_L) comprising a light chain complementarity determining region 1 (CDR-L1), a light chain complementarity determining region 2 (CDR-L2) and a light chain complementarity determining

region 3 (CDR-L3) contained within the amino acid sequence of SEQ ID NO: 119;

a V_H comprising the amino acid sequences of SEQ ID NOS:97, 101 and 103 and a V_L comprising the amino acid sequences of SEQ ID NOS:105, 107 and 108;

a V_H comprising the amino acid sequences of SEQ ID NOS:96, 100 and 103 and a V_L comprising the amino acid sequences of SEQ ID NOS:105, 107 and 108;

a V_H comprising the amino acid sequences of SEQ ID NOS:95, 99 and 103 and a V_L comprising the amino acid sequences of SEQ ID NOS:105, 107 and 108;

a V_H comprising the amino acid sequences of SEQ ID NOS:94, 98 and 102 and a V_L comprising the amino acid sequences of SEQ ID NOS: 104, 106 and 108; or

a V_H comprising the amino acid sequence of SEQ ID NO: 116 and a V_L comprising the amino acid sequence of SEQ ID NO: 119;

(b) a spacer comprising an IgG4/2 chimeric hinge or a modified IgG4 hinge; an IgG2/4 chimeric C_H2 region; and an IgG4 C_H3 region, and optionally is about 228 amino acids in length; or a spacer set forth in SEQ ID NO: 174;

(c) a transmembrane domain, optionally a transmembrane domain from a human CD28; and

(d) an intracellular signaling region comprising a cytoplasmic signaling domain of a CD3-zeta (CD3ζ) chain and a costimulatory signaling region comprising an intracellular signaling domain of a T cell costimulatory molecule or a signaling portion thereof; wherein the dose of engineered T cells comprises:

between at or about 1 x 10^7 CAR-expressing T cells and 2 x 10^9 CAR-expressing T cells;

a combination of $CD4^+$ T cells and $CD8^+$ T cells, at a defined ratio of $CD4^+$ CAR-expressing T cells to $CD8^+$ CAR-expressing T cells and/or of $CD4^+$ T cells to $CD8^+$ T cells, that is or is approximately 1:1 or is between approximately 1:3 and approximately 3:1; and

less than 25%, 20%, 15%, 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2% or 1% of the CAR-expressing T cells in the dose express a marker of apoptosis, optionally Annexin V or active Caspase 3.

5. The method of any of embodiments 1-4, wherein the extracellular antigen-binding domain specifically binds to a B cell maturation antigen (BCMA).

6. The method of any of embodiments 1-5, wherein the V_H is or comprises the amino acid sequence of SEQ ID NO: 116; and the V_L is or comprises the amino acid sequence of SEQ ID NO: 119.

7. The method of any of embodiments 1-6, wherein the extracellular antigen-binding domain comprises an scFv.

8. The method of any of embodiments 1-7, when the V_H and the V_L are joined by a flexible linker.

9. The method of embodiment 8, wherein the scFv comprises a linker comprising the amino acid sequence GGGGSGGGGSGGGGS (SEQ ID NO:1).

10. The method of any of embodiments 1-9, wherein the V_H is amino-terminal to the V_L.

11. The method of any of embodiments 1-10, wherein the antigen-binding domain comprises the amino acid sequence of SEQ ID NO: 114 or an amino acid sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to the amino acid sequence of SEQ ID NO: 114.

12. The method of any of embodiments 1-11, wherein the antigen-binding domain comprises the amino acid sequence of SEQ ID NO: 114.

13. The method of any of embodiments 1-12, wherein a nucleic acid encoding the antigen-binding domain comprises (a) the sequence of nucleotides of SEQ ID NO:113; (b) a sequence of nucleotides that has at least 90% sequence identity thereto; or (c) a degenerate sequence of (a) or (b).

14. The method of any of embodiments 1-13, wherein the nucleic acid encoding the antigen-binding domain comprises the sequence of nucleotides of SEQ ID NO:115.

15. The method of any of embodiments 1-9, wherein the V_H is carboxy-terminal to the V_L.

16. The method of any of embodiments 1-15, wherein the cytoplasmic signaling domain is or comprises the sequence set forth in SEQ ID NO:143 or a sequence of amino acids that has at least 90% sequence identity to SEQ ID NO:143.

17. The method of any of embodiments 1-16, wherein the costimulatory signaling region comprises an intracellular signaling domain of CD28, 4-1BB, or ICOS, or a signaling portion thereof.

18. The method of any of embodiments 1-17, wherein the costimulatory signaling region comprises an intracellular signaling domain of 4-1BB, optionally human 4-1BB.

19. The method of any of embodiments 1-18, wherein the costimulatory signaling region is or comprises the sequence set forth in SEQ ID NO:4 or a sequence of amino acids that exhibits at least 90% sequence identity to the sequence set forth in SEQ ID NO: 4.

20. The method of any of embodiments 1-19, wherein the costimulatory signaling region is between the transmembrane domain and the cytoplasmic signaling domain of a CD3-zeta (CD3ζ) chain.

21. The method of any of embodiments 1-20, wherein the transmembrane domain is or comprises a transmembrane domain from human CD28.

22. The method of any of embodiments 1-21, wherein the transmembrane domain is or comprises the sequence set forth in SEQ ID NO:138 or a sequence of amino acids that exhibits at least 90% sequence identity to SEQ ID NO:138.

23. The method of any of embodiments 1-22, wherein the CAR comprises from its N to C terminus in order: the antigen-binding domain, the spacer, the transmembrane domain and the intracellular

signaling region.

24. The method of any of embodiments 1-23, wherein the antigen-binding domain and or the CAR, or a measure indicative of function or activity of the CAR following exposure to cells expressing surface BCMA, is not reduced or blocked or is not substantially reduced or blocked in the presence of a soluble or shed form of BCMA.

25. The method of embodiment 24, wherein the concentration or amount of the soluble or shed form of the BCMA corresponds to a concentration or amount present in serum or blood or plasma of the subject or of a multiple myeloma patient, or on average in a multiple myeloma patient population, or at a concentration or amount of the soluble or shed BCMA at which the binding or measure is reduced or blocked, or is substantially reduced or blocked, for cells expressing a reference anti-BCMA recombinant receptor, optionally a reference anti-BCMA CAR, in the same assay.

26. The method of any of embodiments 1-14 and 16-25, wherein the CAR is encoded by a polynucleotide sequence comprising the sequence set forth in SEQ ID NO: 13 or a sequence that exhibits at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity thereto.

27. The method of any of embodiments 1-14 and 16-26, wherein the method is encoded by a polynucleotide sequence comprising the sequence set forth in SEQ ID NO: 13.

28. The method of any of embodiments 1-27, wherein following expression of a polynucleotide encoding the CAR in a human cell, optionally a human T cell, the transcribed RNA, optionally messenger RNA (mRNA), from the polynucleotide, exhibits at least 70%, 75%, 80%, 85%, 90%, or 95% RNA homogeneity.

29 The method of any of embodiments 1-28, wherein the dose of engineered T cells comprises between at or about 1 x 10^7 CAR-expressing T cells and at or about 2 x 10^9 CAR-expressing T cells.

30. The method of any of embodiments 1-29, wherein the dose of engineered T cells comprise between at or about 2.5 x 10^7 CAR-expressing T cells and at or about 1.2 x 10^9 CAR-expressing T cells, between at or about 5.0 x 10^7 CAR-expressing T cells and at or about 4.5 x 10^8 CAR-expressing T cells, or between at or about 1.5 x 10^8 CAR-expressing T cells and at or about 3.0 x 10^8 CAR-expressing T cells.

31. The method of any of embodiments 1-30, wherein the dose of engineered T cells comprise at or about 2.5 x 10^7, at or about 5.0 x 10^7, at or about 1.5 x 10^8, at or about 3.0 x 10^8, at or about 4.5 x 10^8, at or about 8.0 x 10^8 or at or about 1.2 x 10^9 CAR-expressing T cells.

32. The method of any of embodiments 1-31, wherein the dose of engineered T cells comprise at or about 5.0 x 10^7, at or about 1.5 x 10^8, at or about 3.0 x 10^8 or at or about 4.5 x 10^8 CAR-expressing T cells.

33. The method of any of embodiments 1-32, wherein the dose of engineered T cells comprises

a combination of $CD4^+$ T cells and $CD8^+$ T cells, at a ratio of $CD4^+$ CAR-expressing T cells to $CD8^+$ CAR-expressing T cells and/or of $CD4^+$ T cells to $CD8^+$ T cells, that is or is approximately 1:1 or is between at or approximately 1:3 and at or approximately 3:1.

34. The method of any of embodiments 1-33, wherein less than at or about 25%, 20%, 15%, 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2% or 1% of the CAR-expressing T cells in the dose of engineered T cells express a marker of apoptosis, optionally Annexin V or active Caspase 3.

35. The method of any of embodiments 1-34, wherein less than at or about 5%, 4%, 3%, 2% or 1% of the CAR-expressing T cells in the dose of engineered T cells express Annexin V or active Caspase 3.

36. The method of any of embodiments 1-35, wherein prior to the administration, the subject has received a lymphodepleting therapy comprising the administration of fludarabine at or about 20-40 mg/m^2 body surface area of the subject, optionally at or about 30 mg/m^2, daily, for 2-4 days, and/or cyclophosphamide at or about 200-400 mg/m^2 body surface area of the subject, optionally at or about 300 mg/m^2, daily, for 2-4 days.

37. The method of any of embodiments 1-36, wherein the subject has received a lymphodepleting therapy comprising the administration of fludarabine at or about 30 mg/m^2 body surface area of the subject, daily, and cyclophosphamide at or about 300 mg/m^2 body surface area of the subject, daily, for 3 days.

38. The method of any of embodiments 1-37, wherein at or prior to the administration of the dose of cells, the subject has received three or more prior therapies for the disease or disorder, optionally four or more prior therapies, optionally selected from among:

autologous stem cell transplant (ASCT);

an immunomodulatory agent;

a proteasome inhibitor; and

an anti-CD38 antibody.

39. The method of any of embodiments 1-38, wherein at or prior to the administration of the dose of cells, the subject has received three or more prior therapies for the disease or disorder selected from among:

autologous stem cell transplant (ASCT);

an immunomodulatory agent or a proteasome inhibitor, or a combination thereof; and

an anti-CD38 antibody.

40. The method of embodiment 38 or embodiment 39, wherein the immunomodulatory agent is selected from among thalidomide, lenalidomide and pomalidomide.

41. The method of any of embodiments 38-40, wherein the proteasome inhibitor is selected from

among bortezomib, carfilzomib and ixazomib.

42. The method of any of embodiments 38-41, wherein the anti-CD38 antibody is or comprises daratumumab.

43. The method of any of embodiments 1-42, wherein at the time of the administration of the dose of cells, and/or at the time of lymphodepleting chemotherapy or leukapheresis, the subject has not had active or history of plasma cell leukemia (PCL).

44. The method of any of embodiments 1-43, wherein at the time of the administration of the dose of cells the subject has developed secondary plasma cell leukemia (PCL).

45. The method of any of embodiments 1-44, wherein, at the time of administration, the subject:

has relapsed or been refractory following at least 3 or at least 4 prior therapies for multiple myeloma;

is an adult subject or is 25 or 35 years of age or older;

has a time from diagnosis of multiple myeloma of approximately 4 years or between 2 and 15 or 2 and 12 years;

has received about 10 or between 3 and 15 or between 4 and 15 prior regimens for multiple myeloma;

has been refractory to or not responded to bortezomib, carfilzomib, lenalidomide, pomalidomide and/or an anti-CD38 monoclonal antibody;

has had prior autologous stem cell transplant or has not had prior autologous stem cell transplant; and/or

has IMWG high risk cytogenetics.

46. The method of any of embodiments 1-45, wherein the method is capable of achieving a specified response or outcome, optionally at a designated timepoint following initiation of the administration, in at least one or in at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, or at least 95% of subjects in a cohort of subjects having the disease or disorder of the subject, optionally wherein the cohort of subjects has at least the same number of prior therapies, prognosis or prognostic factor, sub-type, secondary involvement or other specified patient characteristic or characteristics, as the subject treated by the method, wherein:

the response is selected from the group consisting of objective response (OR), complete response (CR), stringent complete response (sCR), very good partial response (VGPR), partial response (PR) and minimal response (MR);

the response or outcome is or comprises an OR; and/or

the response or outcome is or comprises a CR.

47. The method of embodiment 46, wherein the response or outcome is an OR and is achieved in

at least 40%, at least 50%, at least 60%, at least 70%, or at least 80% of subjects of the cohort.

48. The method of embodiment 46, wherein the response or outcome is a VGPR, a CR or an sCR and is achieved in at least 30%, 35%, 40%, 45% or 50% of subjects of the cohort.

49. The method of embodiment 46, wherein the response or outcome is a CR or an sCR and is achieved in at least 20%, 30%, or 40% of subjects of the cohort.

50. The method of any of embodiments 1-49, wherein the dose of cells is less than 1.5 x 10^8 cells or less than 1.5 x 10^8 CAR+ T cells or less than 3 x 10^8 CAR+ T cells or less than 4.5 x 10^8 CAR+ T cells.

51. The method of any of embodiments 1-50, wherein the dose of cells is at or less than 1.5 x 10^8 cells or less than 1.5 x 10^8 CAR+ T cells.

52. The method of any of embodiments 1-51, wherein the dose of cells is at or about 5 x 10^7 cells or CAR+ T cells.

53. The method of any of embodiments 1-51, wherein the dose of cells is at or about 1.5 x 10^8 cells or CAR+ T cells.

54. The method of any of embodiments 1-51, wherein the dose of cells is at or about 3 x 10^8 cells or CAR+ T cells.

55. The method of any of embodiments 1-51, wherein the dose of cells is at or about 4.5 x 10^8 cells or CAR+ T cells.

56. The method of any of embodiments 46-55, wherein the response or outcome comprises or further comprises the absence neurotoxicity or the absence of cytokine release syndrome (CRS).

57. The method of any of embodiments 46-55, wherein the response or outcome comprises or further comprises the absence of neurotoxicity, and is achieved in at least 40%, 50%, 60%, 70% or 80% of the subject in the cohort.

58. The method of any of embodiments 46-57, wherein the response or outcome comprises or further comprises the absence of CRS, and is achieved in at least 10%, 15%, 20%, 25% or 30% of the subject in the cohort.

59. The method of any of embodiments 46-58, wherein the response or outcome comprises or further comprises the absence of grade 3 or higher, or grade 4 or higher, neurotoxicity, the absence of grade 3 or higher, or grade 4 or higher, cytokine release syndrome (CRS).

60. The method of any of embodiments 46-59, wherein the response or outcome comprises or further comprises the absence of grade 3 or higher neurotoxicity, and is achieved in at least 80%, 85%, 90% or 95% of the subjects in the cohort.

61. The method of any of embodiments 45-59, wherein the response or outcome comprises or

further comprises the absence of grade 3 or higher CRS, and is achieved in at least 80%, 85%, 90% or 95% of the subjects in the cohort.

62. The method of any of embodiments 1-61, wherein the dose of engineered T cells comprise at or about 5.0 x 10^7, at or about 1.5 x 10^8, at or about 3.0 x 10^8 or at or about 4.5 x 10^8 CAR-expressing T cells.

63. The method of any of embodiments 1-62, wherein the dose of the engineered T cells comprise at or about 5.0 x 10^7 CAR-expressing T cells.

64. The method of any of embodiments 1-62, wherein the dose of the engineered T cells comprise at or about 1.5 x 10^8 CAR-expressing T cells.

65. The method of any of embodiments 1-62, wherein the dose of the engineered T cells comprise at or about 3 x 10^8 CAR-expressing T cells.

66. The method of any of embodiments 1-62, wherein the dose of the engineered T cells comprise at or about 4.5 x 10^8 CAR-expressing T cells.

67. The engineered T cell or a dose of engineered T cells administered in the method of any of embodiments 1-66, wherein the engineered T cell or the dose of engineered T cells, following administration at a dose of engineered T cells is capable of achieving, optionally at a designated time following initiation of the administration, a specified response or outcome in at least one of, or in at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, or at least 95% of subjects within a cohort of subjects or evaluable subjects thereof, wherein the cohort of subjects is a cohort having multiple myeloma.

68. The engineered T cell or the dose of engineered T cells of embodiment 67, wherein the achievement of the response or outcome is at the designated time following initiation of administration, which is at 1, 2, 3, 6, 9 or 12 months following said initiation.

69. The engineered T cell or the dose of engineered T cells of embodiment 68, wherein the achievement of the response or outcome is at the designated time following initiation of administration, which is at 1 or 2 or 3 months following said initiation.

70. The engineered T cell or the dose of engineered T cells of any of embodiments 67-69, wherein:

the cohort of subjects is subjects having relapsed or refractory multiple myeloma;

the cohort of subjects is subjects having relapsed or refractory multiple myeloma having been administered, and relapsed or been refractory following, at least 3 prior therapies for multiple myeloma, said prior therapies optionally including an autologous stem cell transplant (ASCT); an immunomodulatory agent; a proteasome inhibitor; and/or an anti-CD38 antibody;

the cohort of subjects is subjects having relapsed or refractory multiple myeloma having been

administered, and relapsed or been refractory following, at least 3 prior therapies for multiple myeloma, said prior therapies optionally including an immunomodulatory agent; a proteasome inhibitor; and/or an anti-CD38 antibody and/or an autologous stem cell transplant; and/or

the cohort of subjects is subjects has no active plasma cell leukemia (PCL) or no history of PCL at the time of said administration;

the cohort of subjects is subjects has developed secondary plasma cell leukemia (PCL) prior to administration of the cells

the cohort of subjects is or includes subjects having relapsed or refractory multiple myeloma having been administered, and relapsed or been refractory following, at least 4 or an average of at least 10 prior therapies for multiple myeloma;

the cohort of subjects consists of or includes adult subjects;

the cohort of subjects has a median time from diagnosis of 4 years and/or a range of time from diagnosis from 2 to 12 years;

the cohort of subjects has received a median of 10 prior regimens or between 3 and 15 or 4 and 15 prior therapies for multiple myeloma;

the cohort of subjects includes subjects refractory to bortezomib, carfilzomib, lenalidomide, pomalidomide and an anti-CD38 monoclonal antibody;

the cohort of subjects includes subjects having had prior autologous stem cell transplant; and/or

the cohort of subjects includes subjects having IMWG high risk cytogenetics.

71. The engineered T cell or the dose of engineered T cells of embodiment 70, wherein thee at least 3 prior therapies comprise autologous stem cell transplant (ASCT); an immunomodulatory agent or a proteasome inhibitor, or a combination thereof; and an anti-CD38 antibody.

72. The engineered T cell or the dose of engineered T cells of embodiment 70 or embodiment 71, wherein the immunomodulatory agent is selected from among thalidomide, lenalidomide and pomalidomide, the proteasome inhibitor is selected from among bortezomib, carfilzomib and ixazomib, and/or the anti-CD38 antibody is or comprises daratumumab.

73. The engineered T cell or the dose of engineered T cells of any of embodiments 67-72, wherein

the response or outcome is selected from the group consisting of objective response (OR), complete response (CR), stringent complete response (sCR), very good partial response (VGPR), partial response (PR) and minimal response (MR), optionally based on the International Myeloma Working Group (IMWG) uniform response criteria;

the response or outcome is or comprises an OR, optionally based on the International Myeloma

Working Group (IMWG) uniform response criteria; or

the response or outcome is or comprises a CR, optionally based on the International Myeloma Working Group (IMWG) uniform response criteria.

74. The engineered T cell or the dose of engineered T cells of any of embodiments 67-73, wherein the response or outcome is or comprises an OR.

75. The engineered T cell or the dose of engineered T cells of any of embodiments 67-74, wherein the dose is capable of achieving the response or outcome in at least 40%, at least 50%, at least 60%, at least 70%, or at least 80% of subjects of the cohort.

76. The engineered T cell or the dose of engineered T cells of any of embodiments 67-73, wherein the response or outcome is or comprises a VGPR, a CR or an sCR.

77. The engineered T cell or the dose of engineered T cells of any of embodiments 67-73 and 76, wherein the dose is capable of achieving the response or outcome in at least 30%, 35%, 40%, 45% or 50% of subjects of the cohort.

78. The engineered T cell or the dose of engineered T cells of any of embodiments 67-73, wherein the response or outcome is or comprises a CR or an sCR.

79. The engineered T cell or the dose of engineered T cells of any of embodiments 67-78, wherein the dose is capable of achieving the response or outcome in at least 20%, 30%, or 40% of subjects of the cohort.

80. The engineered T cell or the dose of engineered T cells of any of embodiments 67-79, wherein:

the dose capable of achieving said response or outcome is less than 1.5×10^8 cells; or

the dose capable of achieving said response or outcome is less than 1.5×10^8 CAR+ T cells.

81. The engineered T cell or the dose of engineered T cells of any of embodiments 67-80, wherein

the dose capable of achieving said response or outcome is less than 1.5×10^8 cells;

the dose capable of achieving said response or outcome is less than 1.5×10^8 CAR+ T cells;

the dose capable of achieving said response or outcome is less than 3×10^8 CAR+ T cells; or

the dose capable of achieving said response or outcome is less than or less than 4.5×10^8 CAR+ T cells.

82. The engineered T cell or the dose of engineered T cells of any of embodiments 67-81, wherein

the dose capable of achieving said response or outcome is less than 1×10^8 cells

the dose capable of achieving said response or outcome is less than 1×10^8 CAR+ T cells.

83. The engineered T cell or the dose of engineered T cells of any of embodiments 67-82, wherein the dose capable of achieving said response or outcome is at or about 5 x 10^7 cells or at or about 5 x 10^7 CAR+ T cells.

84. The engineered T cell or the dose of engineered T cells of any of embodiments 67-81, wherein the dose capable of achieving said response or outcome is at or about 1.5 x 10^8 cells or CAR+ T cells.

85. The engineered T cell or the dose of engineered T cells of any of embodiments 67-81, wherein the dose capable of achieving said response or outcome is at or about 3 x 10^8 cells or CAR+ T cells.

86. The engineered T cell or the dose of engineered T cells of any of embodiments 67-81, wherein the dose capable of achieving said response or outcome is at or about 4.5 x 10^8 cells or CAR+ T cells.

87. The engineered T cell or the dose of engineered T cells of any of embodiments 67-86, wherein the response or outcome comprises or further comprises the absence neurotoxicity or the absence of cytokine release syndrome (CRS).

88. The engineered T cell or the dose of engineered T cells of any of embodiments 67-87, wherein the response or outcome comprises or further comprises the absence of neurotoxicity, and is achieved in at least 40%, 50%, 60%, 70% or 80% of the subject in the cohort.

89. The engineered T cell or the dose of engineered T cells of any of embodiments 67-87, wherein the response or outcome comprises or further comprises the absence of CRS, and is achieved in at least 10%, 15%, 20%, 25% or 30% of the subject in the cohort.

90. The engineered T cell or the dose of engineered T cells of any of embodiments 67-89, wherein the response or outcome comprises or further comprises the absence of grade 3 or higher, or grade 4 or higher, neurotoxicity, the absence of grade 3 or higher, or grade 4 or higher, cytokine release syndrome.

91. The engineered T cell or the dose of engineered T cells of any of embodiments 67-90, wherein the response or outcome comprises or further comprises the absence of grade 3 or higher neurotoxicity, and is achieved in at least 80%, 85%, 90% or 95% of the subjects in the cohort.

92. The engineered T cell or the dose of engineered T cells of any of embodiments 67-91, wherein the response or outcome comprises or further comprises the absence of grade 3 or higher CRS, and is achieved in at least 80%, 85%, 90% or 95% of the subjects in the cohort.

93. The engineered T cell or a dose of engineered T cells of any of embodiments 67-92, wherein:

at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or greater than 95% of the cells in the dose are of a memory phenotype;

wherein at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or greater than 95% of the cells in the dose are of a central memory phenotype; and/or

wherein at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or greater than 95% of the cells in the dose are CD27+, CD28+, CCR7+, CD45RA-, CD45RO+, CD62L+, CD3+, granzyme B-, and/or CD127+; and/or

wherein at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or greater than 95% of the cells in the dose are CCR7+/CD45RA- or are CCR7+/CD45RO+.

94. The engineered T cell or a dose of engineered T cells of any of embodiments 67-93, wherein: the dose of engineered T cells is produced by a method exhibiting a predetermined feature, wherein iterations of the method produce a plurality of output compositions, optionally from human biological samples in which the method is carried out among a plurality of different individual subjects, wherein:

the mean percentage of cells of a memory phenotype in the plurality of the output compositions is between about 40% and about 65%, between about 40% and about 45%, between about 45% and about 50%, between about 50% and about 55%, between about 55% and about 60%, or between about 60% and about 65%;

the mean percentage of cells of a central memory phenotype in the plurality of the output compositions is between about 40% and about 65%, between about 40% and about 45%, between about 45% and about 50%, between about 50% and about 55%, between about 55% and about 60%, or between about 60% and about 65%;

the mean percentage of cells that are CD27+, CD28+, CCR7+, CD45RA-, CD45RO+, CD62L+, CD3+, CD95+, granzyme B-, and/or CD127+ in the plurality of the output compositions is between about 40% and about 65%, between about 40% and about 45%, between about 45% and about 50%, between about 50% and about 55%, between about 55% and about 60%, or between about 60% and about 65%;

the mean percentage of cells that are CCR7+/CD45RA- or CCR7+/CD45RO+ in the plurality of the output compositions is between about 40% and about 65%, between about 40% and about 45%, between about 45% and about 50%, between about 50% and about 55%, between about 55% and about 60%, or between about 60% and about 65%;

the mean percentage of central memory CD4+ T cells in the engineered CD4+ T cells, optionally CAR+CD4+ T cells, of the plurality of the output compositions is between about 40% and about 65%, between about 40% and about 45%, between about 45% and about 50%, between about 50% and about 55%, between about 55% and about 60%, or between about 60% and about 65%;

the mean percentage of central memory CD8+ T cells in the engineered CD8+ T cells, optionally CAR+CD8+ T cells, of the plurality of the output compositions is between about 40% and about 65%, between about 40% and about 45%, between about 45% and about 50%, between about 50% and about 55%, between about 55% and about 60%, or between about 60% and about 65%; and/or

the mean percentage of central memory T cells, optionally CD4+ central memory T cells and CD8+ central memory T cells, in the engineered T cells, optionally CAR+ T cells, of the plurality of the output compositions is between about 40% and about 65%, between about 40% and about 45%, between about 45% and about 50%, between about 50% and about 55%, between about 55% and about 60%, or between about 60% and about 65%.

95. The engineered T cell or a dose of engineered T cells of any of embodiments 67-94, wherein the dose is produced by a method exhibiting a predetermined feature, optionally a threshold number of cells expressing the CAR in the output composition, in at least about 80%, about 90%, about 95%, about 97%, about 99%, about 100%, or is 100% of the human biological samples in which it is carried out among a plurality of different individual subjects.

96. The engineered T cell or a dose of engineered T cells of embodiment 95, wherein the plurality of different individual subject comprise subjects having a disease or condition.

97. The engineered T cell or a dose of engineered T cells of embodiment 96, wherein the disease or condition is a cancer.

98. The engineered T cell or a dose of engineered T cells of embodiment 97, wherein the cancer is a hematological cancer, optionally multiple myeloma.

99. Use of a dose of engineered T cells comprising a chimeric antigen receptor (CAR) in a treatment regimen for a subject having or suspected of having multiple myeloma (MM) comprising administering to the subject the dose of engineered T cells, wherein the CAR comprises:

(a) an extracellular antigen-binding domain, comprising:

a variable heavy chain (V_H) comprising a heavy chain complementarity determining region 1 (CDR-H1), a heavy chain complementarity determining region 2 (CDR-H2) and a heavy chain complementarity determining region 3 (CDR-H3) contained within the amino acid sequence of SEQ ID NO: 116 and a variable light chain (V_L) comprising a light chain complementarity determining region 1 (CDR-L1), a light chain complementarity determining region 2 (CDR-L2) and a light chain complementarity determining region 3 (CDR-L3) contained within the amino acid sequence of SEQ ID NO: 119;

a V_H comprising the amino acid sequences of SEQ ID NOS:97, 101 and 103 and a V_L comprising the amino acid sequences of SEQ ID NOS:105, 107 and 108;

a V_H comprising the amino acid sequences of SEQ ID NOS:96, 100 and 103 and a V_L comprising

the amino acid sequences of SEQ ID NOS:105, 107 and 108;

a V_H comprising the amino acid sequences of SEQ ID NOS:95, 99 and 103 and a V_L comprising the amino acid sequences of SEQ ID NOS:105, 107 and 108;

a V_H comprising the amino acid sequences of SEQ ID NOS:94, 98 and 102 and a V_L comprising the amino acid sequences of SEQ ID NOS: 104, 106 and 108; or

a V_H comprising the amino acid sequence of SEQ ID NO: 116 and a V_L comprising the amino acid sequence of SEQ ID NO: 119;

(b) a spacer comprising an IgG4/2 chimeric hinge or a modified IgG4 hinge; an IgG2/4 chimeric C_H2 region; and an IgG4 C_H3 region, and optionally is about 228 amino acids in length; or a spacer set forth in SEQ ID NO: 174;

(c) a transmembrane domain, optionally a transmembrane domain from a human CD28; and

(d) an intracellular signaling region comprising a cytoplasmic signaling domain of a CD3-zeta (CD3ζ) chain and a costimulatory signaling region comprising an intracellular signaling domain of a T cell costimulatory molecule or a signaling portion thereof;

wherein, prior to the administration, the subject has received a lymphodepleting therapy comprising the administration of fludarabine at or about 20-40 mg/m^2 body surface area of the subject, optionally at or about 30 mg/m^2, daily, for 2-4 days, and/or cyclophosphamide at or about 200-400 mg/m^2 body surface area of the subject, optionally at or about 300 mg/m^2, daily, for 2-4 days.

100. Use of a dose of engineered T cells comprising a chimeric antigen receptor (CAR) in a treatment regimen for a subject having or suspected of having multiple myeloma (MM) comprising administering to the subject the dose of engineered T cells, wherein the CAR comprises:

(a) an extracellular antigen-binding domain, comprising:

a variable heavy chain (V_H) comprising a heavy chain complementarity determining region 1 (CDR-H1), a heavy chain complementarity determining region 2 (CDR-H2) and a heavy chain complementarity determining region 3 (CDR-H3) contained within the amino acid sequence of SEQ ID NO: 116 and a variable light chain (V_L) comprising a light chain complementarity determining region 1 (CDR-L1), a light chain complementarity determining region 2 (CDR-L2) and a light chain complementarity determining region 3 (CDR-L3) contained within the amino acid sequence of SEQ ID NO: 119;

a V_H comprising the amino acid sequences of SEQ ID NOS:97, 101 and 103 and a V_L comprising the amino acid sequences of SEQ ID NOS:105, 107 and 108;

a V_H comprising the amino acid sequences of SEQ ID NOS:96, 100 and 103 and a V_L comprising the amino acid sequences of SEQ ID NOS:105, 107 and 108;

a V_H comprising the amino acid sequences of SEQ ID NOS:95, 99 and 103 and a V_L comprising the

amino acid sequences of SEQ ID NOS:105, 107 and 108;

a V_H comprising the amino acid sequences of SEQ ID NOS:94, 98 and 102 and a V_L comprising the amino acid sequences of SEQ ID NOS: 104, 106 and 108; or

a V_H comprising the amino acid sequence of SEQ ID NO: 116 and a V_L comprising the amino acid sequence of SEQ ID NO: 119;

(b) a spacer comprising an IgG4/2 chimeric hinge or a modified IgG4 hinge; an IgG2/4 chimeric C_H2 region; and an IgG4 C_H3 region, and optionally is about 228 amino acids in length; or a spacer set forth in SEQ ID NO: 174;

(c) a transmembrane domain, optionally a transmembrane domain from a human CD28; and

(d) an intracellular signaling region comprising a cytoplasmic signaling domain of a CD3-zeta (CD3ζ) chain and a costimulatory signaling region comprising an intracellular signaling domain of a T cell costimulatory molecule or a signaling portion thereof; wherein at or prior to the administration of the dose of engineered T cells, the subject has received three or more therapies selected from among:

autologous stem cell transplant (ASCT);

an immunomodulatory agent;

a proteasome inhibitor; and

an anti-CD38 antibody; unless the subject was not a candidate for or was contraindicated for one or more of the therapies.

101. Use of a dose of engineered T cells comprising a chimeric antigen receptor (CAR) in a treatment regimen for a subject having or suspected of having multiple myeloma (MM) comprising administering to the subject the dose of engineered T cells, wherein the CAR comprises:

(a) an extracellular antigen-binding domain, comprising:

a variable heavy chain (V_H) comprising a heavy chain complementarity determining region 1 (CDR-H1), a heavy chain complementarity determining region 2 (CDR-H2) and a heavy chain complementarity determining region 3 (CDR-H3) contained within the amino acid sequence of SEQ ID NO: 116 and a variable light chain (V_L) comprising a light chain complementarity determining region 1 (CDR-L1), a light chain complementarity determining region 2 (CDR-L2) and a light chain complementarity determining region 3 (CDR-L3) contained within the amino acid sequence of SEQ ID NO: 119;

a V_H comprising the amino acid sequences of SEQ ID NOS:97, 101 and 103 and a V_L comprising the amino acid sequences of SEQ ID NOS:105, 107 and 108;

a V_H comprising the amino acid sequences of SEQ ID NOS:96, 100 and 103 and a V_L comprising the amino acid sequences of SEQ ID NOS:105, 107 and 108;

a V_H comprising the amino acid sequences of SEQ ID NOS:95, 99 and 103 and a V_L comprising the

amino acid sequences of SEQ ID NOS:105, 107 and 108;

a V_H comprising the amino acid sequences of SEQ ID NOS:94, 98 and 102 and a V_L comprising the amino acid sequences of SEQ ID NOS: 104, 106 and 108; or

a V_H comprising the amino acid sequence of SEQ ID NO: 116 and a V_L comprising the amino acid sequence of SEQ ID NO: 119;

(b) a spacer comprising an IgG4/2 chimeric hinge or a modified IgG4 hinge; an IgG2/4 chimeric C_H2 region; and an IgG4 C_H3 region, and optionally is about 228 amino acids in length; or a spacer set forth in SEQ ID NO: 174;

(c) a transmembrane domain, optionally a transmembrane domain from a human CD28; and

(d) an intracellular signaling region comprising a cytoplasmic signaling domain of a CD3-zeta (CD3ζ) chain and a costimulatory signaling region comprising an intracellular signaling domain of a T cell costimulatory molecule or a signaling portion thereof; wherein at the administration of the dose of engineered T cells, the subject has not had active or history of plasma cell leukemia (PCL).

102. Use of a dose of engineered T cells comprising a chimeric antigen receptor (CAR) in a treatment regimen for a subject having or suspected of having multiple myeloma (MM) comprising administering to the subject the dose of engineered T cells, wherein the CAR comprises:

(a) an extracellular antigen-binding domain, comprising:

a variable heavy chain (V_H) comprising a heavy chain complementarity determining region 1 (CDR-H1), a heavy chain complementarity determining region 2 (CDR-H2) and a heavy chain complementarity determining region 3 (CDR-H3) contained within the amino acid sequence of SEQ ID NO: 116 and a variable light chain (V_L) comprising a light chain complementarity determining region 1 (CDR-L1), a light chain complementarity determining region 2 (CDR-L2) and a light chain complementarity determining region 3 (CDR-L3) contained within the amino acid sequence of SEQ ID NO: 119;

a V_H comprising the amino acid sequences of SEQ ID NOS:97, 101 and 103 and a V_L comprising the amino acid sequences of SEQ ID NOS:105, 107 and 108;

a V_H comprising the amino acid sequences of SEQ ID NOS:96, 100 and 103 and a V_L comprising the amino acid sequences of SEQ ID NOS:105, 107 and 108;

a V_H comprising the amino acid sequences of SEQ ID NOS:95, 99 and 103 and a V_L comprising the amino acid sequences of SEQ ID NOS:105, 107 and 108;

a V_H comprising the amino acid sequences of SEQ ID NOS:94, 98 and 102 and a V_L comprising the amino acid sequences of SEQ ID NOS: 104, 106 and 108; or

a V_H comprising the amino acid sequence of SEQ ID NO: 116 and a V_L comprising the amino acid sequence of SEQ ID NO: 119;

(b) a spacer comprising an IgG4/2 chimeric hinge or a modified IgG4 hinge; an IgG2/4 chimeric C_H2 region; and an IgG4 C_H3 region, and optionally is about 228 amino acids in length; or a spacer set forth in SEQ ID NO: 174;

(c) a transmembrane domain, optionally a transmembrane domain from a human CD28; and

(d) an intracellular signaling region comprising a cytoplasmic signaling domain of a CD3-zeta (CD3ζ) chain and a costimulatory signaling region comprising an intracellular signaling domain of a T cell costimulatory molecule or a signaling portion thereof; wherein the dose of engineered T cells comprises:

between at or about 1 x 10^7 CAR-expressing T cells and 2 x 10^9 CAR-expressing T cells;

a combination of $CD4^+$ T cells and $CD8^+$ T cells, at a defined ratio of $CD4^+$ CAR-expressing T cells to $CD8^+$ CAR-expressing T cells and/or of $CD4^+$ T cells to $CD8^+$ T cells, that is or is approximately 1:1 or is between approximately 1:3 and approximately 3:1; and

less than 25%, 20%, 15%, 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2% or 1% of the CAR-expressing T cells in the dose express a marker of apoptosis, optionally Annexin V or active Caspase 3.

103. Use of a dose of engineered T cells comprising a chimeric antigen receptor (CAR) for the manufacture of a medicament for the treatment for a subject having or suspected of having multiple myeloma (MM), wherein the CAR comprises:

(a) an extracellular antigen-binding domain, comprising:

a variable heavy chain (V_H) comprising a heavy chain complementarity determining region 1 (CDR-H1), a heavy chain complementarity determining region 2 (CDR-H2) and a heavy chain complementarity determining region 3 (CDR-H3) contained within the amino acid sequence of SEQ ID NO: 116 and a variable light chain (V_L) comprising a light chain complementarity determining region 1 (CDR-L1), a light chain complementarity determining region 2 (CDR-L2) and a light chain complementarity determining region 3 (CDR-L3) contained within the amino acid sequence of SEQ ID NO: 119;

a V_H comprising the amino acid sequences of SEQ ID NOS:97, 101 and 103 and a V_L comprising the amino acid sequences of SEQ ID NOS:105, 107 and 108;

a V_H comprising the amino acid sequences of SEQ ID NOS:96, 100 and 103 and a V_L comprising the amino acid sequences of SEQ ID NOS:105, 107 and 108;

a V_H comprising the amino acid sequences of SEQ ID NOS:95, 99 and 103 and a V_L comprising the amino acid sequences of SEQ ID NOS:105, 107 and 108;

a V_H comprising the amino acid sequences of SEQ ID NOS:94, 98 and 102 and a V_L comprising the amino acid sequences of SEQ ID NOS: 104, 106 and 108; or

a V_H comprising the amino acid sequence of SEQ ID NO: 116 and a V_L comprising the amino acid

sequence of SEQ ID NO: 119;

(b) a spacer comprising an IgG4/2 chimeric hinge or a modified IgG4 hinge; an IgG2/4 chimeric C_H2 region; and an IgG4 C_H3 region, and optionally is about 228 amino acids in length; or a spacer set forth in SEQ ID NO: 174;

(c) a transmembrane domain, optionally a transmembrane domain from a human CD28; and

(d) an intracellular signaling region comprising a cytoplasmic signaling domain of a CD3-zeta (CD3ζ) chain and a costimulatory signaling region comprising an intracellular signaling domain of a T cell costimulatory molecule or a signaling portion thereof;

wherein, prior to the administration of the dose of engineered T cells, the subject has received a lymphodepleting therapy comprising the administration of fludarabine at or about 20-40 mg/m^2 body surface area of the subject, optionally at or about 30 mg/m^2, daily, for 2-4 days, and/or cyclophosphamide at or about 200-400 mg/m^2 body surface area of the subject, optionally at or about 300 mg/m^2, daily, for 2-4 days.

104. Use of a dose of engineered T cells comprising a chimeric antigen receptor (CAR) for the manufacture of a medicament for the treatment for a subject having or suspected of having multiple myeloma (MM), wherein the CAR comprises:

(a) an extracellular antigen-binding domain, comprising:

a variable heavy chain (V_H) comprising a heavy chain complementarity determining region 1 (CDR-H1), a heavy chain complementarity determining region 2 (CDR-H2) and a heavy chain complementarity determining region 3 (CDR-H3) contained within the amino acid sequence of SEQ ID NO: 116 and a variable light chain (V_L) comprising a light chain complementarity determining region 1 (CDR-L1), a light chain complementarity determining region 2 (CDR-L2) and a light chain complementarity determining region 3 (CDR-L3) contained within the amino acid sequence of SEQ ID NO: 119;

a V_H comprising the amino acid sequences of SEQ ID NOS:97, 101 and 103 and a V_L comprising the amino acid sequences of SEQ ID NOS:105, 107 and 108;

a V_H comprising the amino acid sequences of SEQ ID NOS:96, 100 and 103 and a V_L comprising the amino acid sequences of SEQ ID NOS:105, 107 and 108;

a V_H comprising the amino acid sequences of SEQ ID NOS:95, 99 and 103 and a V_L comprising the amino acid sequences of SEQ ID NOS:105, 107 and 108;

a V_H comprising the amino acid sequences of SEQ ID NOS:94, 98 and 102 and a V_L comprising the amino acid sequences of SEQ ID NOS: 104, 106 and 108; or

a V_H comprising the amino acid sequence of SEQ ID NO: 116 and a V_L comprising the amino acid sequence of SEQ ID NO: 119;

(b) a spacer comprising an IgG4/2 chimeric hinge or a modified IgG4 hinge; an IgG2/4 chimeric C_H2

region; and an IgG4 C_H3 region, and optionally is about 228 amino acids in length; or a spacer set forth in SEQ ID NO: 174;

(c) a transmembrane domain, optionally a transmembrane domain from a human CD28; and

(d) an intracellular signaling region comprising a cytoplasmic signaling domain of a CD3-zeta (CD3ζ) chain and a costimulatory signaling region comprising an intracellular signaling domain of a T cell costimulatory molecule or a signaling portion thereof; wherein at or prior to the administration of the dose of engineered T cells, the subject has received three or more therapies selected from among:

autologous stem cell transplant (ASCT);

an immunomodulatory agent;

a proteasome inhibitor; and

an anti-CD38 antibody; unless the subject was not a candidate for or was contraindicated for one or more of the therapies.

105. Use of a dose of engineered T cells comprising a chimeric antigen receptor (CAR) for the manufacture of a medicament for the treatment for a subject having or suspected of having multiple myeloma (MM), wherein the CAR comprises:

(a) an extracellular antigen-binding domain, comprising:

a variable heavy chain (V_H) comprising a heavy chain complementarity determining region 1 (CDR-H1), a heavy chain complementarity determining region 2 (CDR-H2) and a heavy chain complementarity determining region 3 (CDR-H3) contained within the amino acid sequence of SEQ ID NO: 116 and a variable light chain (V_L) comprising a light chain complementarity determining region 1 (CDR-L1), a light chain complementarity determining region 2 (CDR-L2) and a light chain complementarity determining region 3 (CDR-L3) contained within the amino acid sequence of SEQ ID NO: 119;

a V_H comprising the amino acid sequences of SEQ ID NOS:97, 101 and 103 and a V_L comprising the amino acid sequences of SEQ ID NOS:105, 107 and 108;

a V_H comprising the amino acid sequences of SEQ ID NOS:96, 100 and 103 and a V_L comprising the amino acid sequences of SEQ ID NOS:105, 107 and 108;

a V_H comprising the amino acid sequences of SEQ ID NOS:95, 99 and 103 and a V_L comprising the amino acid sequences of SEQ ID NOS:105, 107 and 108;

a V_H comprising the amino acid sequences of SEQ ID NOS:94, 98 and 102 and a V_L comprising the amino acid sequences of SEQ ID NOS: 104, 106 and 108; or

a V_H comprising the amino acid sequence of SEQ ID NO: 116 and a V_L comprising the amino acid sequence of SEQ ID NO: 119;

(b) a spacer comprising an IgG4/2 chimeric hinge or a modified IgG4 hinge; an IgG2/4 chimeric C_H2

region; and an IgG4 C_H3 region, and optionally is about 228 amino acids in length; or a spacer set forth in SEQ ID NO: 174;

(c) a transmembrane domain, optionally a transmembrane domain from a human CD28; and

(d) an intracellular signaling region comprising a cytoplasmic signaling domain of a CD3-zeta (CD3ζ) chain and a costimulatory signaling region comprising an intracellular signaling domain of a T cell costimulatory molecule or a signaling portion thereof; wherein at the administration of the dose of engineered T cells, the subject has not had active or history of plasma cell leukemia (PCL).

106. Use of a dose of engineered T cells comprising a chimeric antigen receptor (CAR) for the manufacture of a medicament for the treatment for a subject having or suspected of having multiple myeloma (MM), wherein the CAR comprises:

(a) an extracellular antigen-binding domain, comprising:

a variable heavy chain (V_H) comprising a heavy chain complementarity determining region 1 (CDR-H1), a heavy chain complementarity determining region 2 (CDR-H2) and a heavy chain complementarity determining region 3 (CDR-H3) contained within the amino acid sequence of SEQ ID NO: 116 and a variable light chain (V_L) comprising a light chain complementarity determining region 1 (CDR-L1), a light chain complementarity determining region 2 (CDR-L2) and a light chain complementarity determining region 3 (CDR-L3) contained within the amino acid sequence of SEQ ID NO: 119;

a V_H comprising the amino acid sequences of SEQ ID NOS:97, 101 and 103 and a V_L comprising the amino acid sequences of SEQ ID NOS:105, 107 and 108;

a V_H comprising the amino acid sequences of SEQ ID NOS:96, 100 and 103 and a V_L comprising the amino acid sequences of SEQ ID NOS:105, 107 and 108;

a V_H comprising the amino acid sequences of SEQ ID NOS:95, 99 and 103 and a V_L comprising the amino acid sequences of SEQ ID NOS:105, 107 and 108;

a V_H comprising the amino acid sequences of SEQ ID NOS:94, 98 and 102 and a V_L comprising the amino acid sequences of SEQ ID NOS: 104, 106 and 108; or

a V_H comprising the amino acid sequence of SEQ ID NO: 116 and a V_L comprising the amino acid sequence of SEQ ID NO: 119;

(b) a spacer comprising an IgG4/2 chimeric hinge or a modified IgG4 hinge; an IgG2/4 chimeric C_H2 region; and an IgG4 C_H3 region, and optionally is about 228 amino acids in length; or a spacer set forth in SEQ ID NO: 174;

(c) a transmembrane domain, optionally a transmembrane domain from a human CD28; and

(d) an intracellular signaling region comprising a cytoplasmic signaling domain of a CD3-zeta (CD3ζ) chain and a costimulatory signaling region comprising an intracellular signaling domain of a T cell

costimulatory molecule or a signaling portion thereof; wherein the dose of engineered T cells comprises:

between at or about 1 x 10^7 CAR-expressing T cells and 2 x 10^9 CAR-expressing T cells;

a combination of $CD4^+$ T cells and $CD8^+$ T cells, at a defined ratio of $CD4^+$ CAR-expressing T cells to $CD8^+$ CAR-expressing T cells and/or of $CD4^+$ T cells to $CD8^+$ T cells, that is or is approximately 1:1 or is between approximately 1:3 and approximately 3:1; and

less than 25%, 20%, 15%, 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2% or 1% of the CAR-expressing T cells in the dose express a marker of apoptosis, optionally Annexin V or active Caspase 3.

107. The use of any of embodiments 99-106, wherein the extracellular antigen-binding domain specifically binds to a B cell maturation antigen (BCMA).

108. The use of any of embodiments 99-107, wherein the V_H is or comprises the amino acid sequence of SEQ ID NO: 116; and the V_L is or comprises the amino acid sequence of SEQ ID NO: 119.

109. The method or use of any of embodiments 1-66 and 99-108, wherein:

at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or greater than 95% of the cells in the administered dose are of a memory phenotype;

wherein at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or greater than 95% of the cells in the administered dose are of a central memory phenotype; and/or

wherein at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or greater than 95% of the cells in the administered dose are CD27+, CD28+, CCR7+, CD45RA-, CD45RO+, CD62L+, CD3+, granzyme B-, and/or CD127+; and/or

wherein at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or greater than 95% of the cells in the administered dose are CCR7+/CD45RA- or are CCR7+/CD45RO+.

110. The method or use of any of embodiments 1-66 and 99-109, wherein the cells in the administered dose are produced by a method that produces a plurality of output compositions, optionally from human biological samples in which the method is carried out among a plurality of different individual subjects, wherein:

the mean percentage of cells of a memory phenotype in the plurality of the output compositions is between about 40% and about 65%, between about 40% and about 45%, between about 45% and about 50%, between about 50% and about 55%, between about 55% and about 60%, or between about 60% and about 65%;

the mean percentage of cells of a central memory phenotype in the plurality of the output compositions is between about 40% and about 65%, between about 40% and about 45%, between about 45% and about 50%, between about 50% and about 55%, between about 55% and about 60%, or between about 60% and about 65%;

the mean percentage of cells that are CD27+, CD28+, CCR7+, CD45RA-, CD45RO+, CD62L+, CD3+, CD95+, granzyme B-, and/or CD127+ in the plurality of the output compositions is between about 40% and about 65%, between about 40% and about 45%, between about 45% and about 50%, between about 50% and about 55%, between about 55% and about 60%, or between about 60% and about 65%;

the mean percentage of cells that are CCR7+/CD45RA- or CCR7+/CD45RO+ in the plurality of the output compositions is between about 40% and about 65%, between about 40% and about 45%, between about 45% and about 50%, between about 50% and about 55%, between about 55% and about 60%, or between about 60% and about 65%;

the mean percentage of central memory CD4+ T cells in the engineered CD4+ T cells, optionally CAR+CD4+ T cells, of the plurality of the output compositions is between about 40% and about 65%, between about 40% and about 45%, between about 45% and about 50%, between about 50% and about 55%, between about 55% and about 60%, or between about 60% and about 65%;

the mean percentage of central memory CD8+ T cells in the engineered CD8+ T cells, optionally CAR+CD8+ T cells, of the plurality of the output compositions is between about 40% and about 65%, between about 40% and about 45%, between about 45% and about 50%, between about 50% and about 55%, between about 55% and about 60%, or between about 60% and about 65%; and/or

the mean percentage of central memory T cells, optionally CD4+ central memory T cells and CD8+ central memory T cells, in the engineered T cells, optionally CAR+ T cells, of the plurality of the output compositions is between about 40% and about 65%, between about 40% and about 45%, between about 45% and about 50%, between about 50% and about 55%, between about 55% and about 60%, or between about 60% and about 65%.

111. The method or use of any of embodiments 1-66 and 99-110,wherein the administered dose is produced by a method exhibiting a predetermined feature, optionally a threshold number of cells expressing the CAR in the output composition, in at least about 80%, about 90%, about 95%, about 97%, about 99%, about 100%, or is 100% of the human biological samples in which it is carried out among a plurality of different individual subjects.

112. The method or use of embodiment 111, wherein the plurality of different individual subject comprise subjects having a disease or condition.

113. The method or use of embodiment 112, wherein the disease or condition is a cancer.

114. The method or use of embodiment 113, wherein the cancer is a hematological cancer, optionally multiple myeloma.

IX. EXAMPLES

[0698] The following examples are included for illustrative purposes only and are not intended to limit the scope of the invention.

Example 1: Generation of Chimeric Antigen Receptors (CARs) Against BCMA and Cells Expressing Anti-BCMA CARs

[0699] Polynucleotides encoding exemplary chimeric antigen receptors (CARs), each containing a human anti-BCMA scFv antigen-binding domain, were generated. Among the human anti-BCMA scFvs were those described in Example 2. Also among the CARs generated were CARs containing scFvs containing V_H and V_L sequences of antibodies described in WO2016090327. Also generated were anti-BCMA CARs containing scFvs with V_H and V_L sequences of BCMA antibodies described in WO2010104949. In some cases of the scFv, the V_H was amino-terminal to the V_L and in some cases the V_L was amino-terminal to the V_H. Exemplary scFv regions in generated CARs are set forth in **Table E1**.

Table E1. Sequence identifier (SEQ ID NO) for Exemplary scFvs

Antigen-binding domain	CDR-H1	CDR-H2	CDR-H3	CDR-L1	CDR-L2	CDR-L3	V_H	V_L	scFv
BCMA-23	34	35	36	22	23	24	32	33	29
BCMA-25	37	38	39	40	41	42	52	53	49
BCMA-26	34	35	54	55	56	57	61	62	58
BCMA-52	66	70	72	74	76	77	85	88	83
BCMA-55	97	101	103	105	107	108	116	119	114

[0700] Specifically, the exemplary polynucleotide CAR constructs contained nucleic acid encoding a human IgG-kappa signaling sequence (SEQ ID NO: 167, encoding SEQ ID NO: 166), a human anti-BCMA scFv, a spacer (such as a spacer containing a modified IgG4-hinge C_H2-C_H3 (SEQ ID NO:175, encoding SEQ ID NO:174) (which spacer may in some instances be referred to as "LS") or, in some cases, a shorter spacer (which may be referred to as "SS"), such as one derived from an IgG hinge region, such as an IgG4-derived hinge region or modified form thereof, or derived from a CD28 extracellular domain; a human CD28 transmembrane domain; a human 4-1BB-derived intracellular co-signaling sequence; and a human CD3-zeta derived intracellular signaling domain. Exemplary spacers included those derived from an IgG4 hinge region and CD28 ectodomain-derived spacers.

[0701] A polynucleotide encoding another CAR construct also was generated containing nucleic acid encoding a human IgG-kappa signal sequence (SEQ ID NO: 167, encoding SEQ ID NO: 166), a mouse anti-

BCMA scFv, a spacer (SEQ ID NO:175, encoding SEQ ID NO:174), a human CD28 transmembrane domain, a human 4-1BB-derived intracellular co-signaling sequence, and a CD3-zeta derived intracellular signaling domain.

[0702] cDNA clones encoding such CARs, were linked to a downstream ribosomal skip element (such as T2A-encoding sequence SEQ ID NO: 244 or 245, encoding SEQ ID NO: 243) followed by a truncated receptor-encoding sequence, and cloned into a lentiviral expression vector.

[0703] To generate anti-BCMA CAR-expressing T cells, T cells were isolated by immunoaffinity-based enrichment from leukapheresis samples from human donor subjects. Isolated T cells were activated and transduced with lentiviral vectors containing the respective polynucleotides encoding the anti-BCMA CARs. After transduction and expansion, CD4+ and CD8+ T cells were stained with an antibody specific for the truncated receptor and with a fluorescently labeled-recombinant human BCMA and analyzed by flow cytometry, confirming transduction of cells and expression of the anti-BCMA CARs.

Example 2: Assessment of Potential RNA Heterogeneity and Modification

[0704] RNA from cells transduced with exemplary anti-BCMA CARs as described in Example 3 were analyzed for heterogeneity by agarose gel electrophoresis, following reverse transcriptase polymerase chain reaction (RT-PCR) using primers specific to the promoter and the WPRE downstream in the 5′ UTR and 3′ UTR of the exemplary CAR transcripts. Multiple bands were observed for various anti-BCMA CAR constructs containing an exemplary spacer including a modified IgG C_H2-C_H3-hinge region (BCMA-LS CAR) (**FIG. 1A**), indicating RNA heterogeneity. Less RNA heterogeneity was observed for exemplary CARs containing a shorter spacer, such as that including a portion of a human CD28 extracellular region (see, e.g., BCMA-52-SS CAR).

[0705] In the nucleotide sequences encoding various BCMA-LS CARs were assessed for potential splice sites and modified in a conservative manner, including removal of potential predicted splice sites. The sequences prior to modification (starting sequence) and those following modification (optimized sequences) were subjected to analysis to assess the presence of potential cryptic splice sites. Splice donor sites and splice acceptor sites were evaluated independently. Exemplary splice donor and splice acceptor sites of the starting sequences of various regions of the construct were identified (e.g. in promoter region and long spacer region). Exemplary splice donor sites and splice acceptor sites were identified within the long spacer region following initial codon optimization that had a splice site score of > 0.7 (> 70%), e.g. donor sites set forth in SEQ ID NO: 210 (splice site score of 0.96) and 225 (splice site score of 0.97), respectively. Modified constructs were generated containing additional modifications within regions assessed with a splice site score of > 0.7 (> 70%) following initial codon optimization (see, e.g., SEQ ID NO:236 for an exemplary initial

codon-optimized spacer sequence) were made in order to reduce potential for unwanted splice sites. Among such regions further modified after codon optimization/splice site elimination were those within longer spacer region sequences, e.g. final optimized splice site eliminated (O/SSE) sequences of splice donor site and splice acceptor site is set forth in SEQ ID NOS:190 and 180, respectively.

[0706] The modified sequences were constructed and tested for RNA heterogeneity as described above. Electrophoresis confirmed reduction of RNA heterogeneity. Analysis of BCMA-CAR constructs before and after splice site elimination demonstrated reduced RNA heterogeneity (**FIG. 1B**). Exemplary O/SSE CAR constructs were generated containing the modifications of the long spacer region, e.g. BCMA-23-LS-O/SSE CAR, BCMA-25-LS-O/SSE CAR, BCMA-26-LS-O/SSE CAR, BCMA-52-LS-O/SSE CAR, and BCMA-55-LS-O/SSE CAR.

Example 3: Assessment of CAR Expression and Function in Primary T cells

[0707] Lentiviral constructs containing anti-BCMA CAR-encoding polynucleotides with starting and optimized sequences, respectively, as described in Example 3, were transduced into T cells and transduced cells were analyzed for transduction (based on expression of a surrogate marker) and for CAR expression based on binding to recombinant BCMA-Fc fusion protein by flow cytometry. A greater percentage of CD4+ and CD8+ T cells transduced using the optimized sequences, BCMA-52-LS-O/SSE CAR and BCMA-55-LS-O/SSE CAR, expressed the anti-BCMA CAR on the surface, compared to cells transduced to express the same corresponding CAR via the polynucleotide having the starting (non-SSE) sequence. Representative data are set forth in **FIG. 2** and **Table E2** below.

Table E2. Percentage of CD4+ and CD8+ T cells expressing anti-BCMA CAR

	BCMA-25	BCMA-25-O/SSE	BCMA-52	BCMA-52-O/SSE	BCMA-55	BCMA-55-O/SSE
CD4+ T cells	17.9	64.9	36.4	69.6	44.1	50.2
CD8+ T cells	17.7	61.7	31.8	62.4	36.5	43.4

[0708] Various volumes of viral preparations containing lentiviral vectors encoding CAR constructs, BCMA-23-LS CAR, BCMA 26-LS CAR, BCMA 55-LS CAR and BCMA 55-LS-O/SSE CAR, were used to transduce 500,000 donor-derived primary human T cells and transduction efficiency was compared. The percent transduction of T-cells was increased following transduction by optimized sequences (**FIG. 3**, circles) compared to starting sequences (**FIG. 3**, triangles).

Example 4: Characterization of BCMA-52 and BCMA-55 scFvs

A. Immunohistochemistry Staining of Tissues

[0709] Cells and tissues expressing varying levels of BCMA were assessed by immunohistochemistry for binding of exemplary anti-BCMA antibodies. Binding domains (scFvs) of exemplary human-BCMA-targeted CARs, which had been fused to a mouse IgG1 Fc region peptide, were assessed for binding cells and tissues by immunohistochemistry.

B. Assessment of Binding Kinetics

[0710] A CAR with a BCMA-55-derived scFv binding domain, a modified IgG-derived C_H2-C_H3-hinge spacer, a CD28 transmembrane domain, and 41BB and CD3zeta endodomain, was expressed in a Jurkat T cell line. Kinetics of binding by the CAR to recombinant human BCMA-hFc (rhBCMA hFc) was assessed using a kinetics exclusion assay. Affinity of binding of an Fc fusion protein containing the scFv portion of the CAR (scFv-Fc) to recombinant human BCMA fusion protein was also assessed using a Biacore-based assay. In these studies, the K_D for binding by the CAR and scFv-Fc fusion, respectively, were observed to be approximately 1 nM and 10 nM.

[0711] In a further experiment, Jurkat cells were transduced with a polynucleotide encoding a CAR with a BCMA-55-derived scFv binding domain and were cultured to a density of ~2 x 10^6. The cells were harvested and spun at 1500g for 15 minutes at 4 °C. The cell pellet was washed and cells were resuspended and serially diluted in 20 nM or 1 nM biotinylated rhBCMA hFc (also referred to in this assay as the constant binding partner (CBP). After equilibration, cells were spun down and supernatants were harvested for KinExa kinetic exclusion analysis. Briefly, supernatants from equilibrated BCMA-55-LS CAR O/SSE-expressing Jurkat cells containing rhBCMA hFc were flowed over a streptavidin bead flow cell to capture free biotinylated rhBCMA hFc. The rhBCMA was then detected using a secondary anti-hBCMA antibody that was fluorescently labelled. The absorbance of the detected rhBCMA hFc was recorded for each sample, and plotted against the number of cells in each dilution (Darling (2004) Assay Drug. Dev., 2:647-657). In this study, the K_D for the interaction of the BCMA-55-LS-O/SSE CAR-expressing cells binding to rhBCMA hFc in this assay was determined to be approximately 1.46 nM, and the expression level (EL) was determined to be approximately 146,500 CARs per CAR-expressing Jurkat cell.

C. Selectivity of BCMA-55 scFv-Fc

[0712] A membrane proteome array (MPA) assay was used to assess binding specificity of the BCMA-55-derived binding domain, using an scFv-Fc fusion protein. The interactions of BCMA-55-Fc to HEK293 cells expressing over 4400 unique human extracellular proteins, representing over 85% of the human extracellular proteome, and a fluorescent protein were evaluated using the Retrogenix™ platform. Fluorescent protein was detected to verify transfection, and CTLA4-Fc (tested at 0.2 µg/mL), containing a

matched Fc, was also used to screen for CD86 as a positive control. An initial screening involved an scFv binding assay for BCMA-55-scFv against the full protein panel. A follow-up confirmation screen was then performed retesting the interaction of BCMA-55-Fc with a subset of potential hits identified in the initial screen. BCMA was identified as the only strong, specific hit in this assay, consistent with a conclusion that this binding domain is highly selective for BCMA over other extracellular proteins. Some low level signal was observed for Cathepsin G (CTSG), but was observed not to confer functional activity (see Example 16).

Example 5: *In vitro* Functional Assessment of T cells Engineered to Express Various Anti-BCMA Chimeric Antigen Receptor (CARs)

[0713] Genetically engineered human T cells expressing various exemplary anti-BCMA CARs were assessed *in vitro* following co-culture with BCMA-expressing target cells. T cells were transduced with BCMA-52-LS CAR, BCMA-55-LS CAR, BCMA-52-LS-O/SSE CAR, or BCMA-55-LS-O/SSE CAR). Responses were compared to reference anti-BCMA CAR-expressing cells as positive control or mock-processed cells as negative control.

A. Cytolytic Activity Against Target Cells

[0714] BCMA-expressing target cells were incubated with T cells expressing the BCMA-52-LS CAR, BCMA-55-LS CAR, or a reference anti-BCMA CAR at an effector to target (E:T) ratio of 5:1, 2.5:1, 1.25:1 and 0.65:1. As a control, target cells were incubated with T cells not expressing a CAR (mock control). Specifically, BCMA-transduced K562 cells (K562/BCMA, $BCMA^{high}$) or RPMI 8226 cells ($BCMA^{low}$ human multiple myeloma cell line) were used as targets for lysis. Target cells were labeled with NucLight Red (NLR) to permit tracking of target cells by microscopy. Cytolytic activity was assessed by measuring the loss of viable target cells over a period of between 24 and 72 hours, as determined by red fluorescent signal (using the IncuCyte® Live Cell Analysis System, Essen Bioscience). Percent lysis (% Lysis) was normalized to the lysis that occurred in target cells incubated with mock-processed T cells. As shown in **FIG. 4A**, the anti-BCMA CAR-expressing T cells exhibited antigen-specific cytolytic activity against BCMA+ cells. The magnitude of cell lysis differed depending on the particular cell line and CAR.

[0715] In a separate experiment, cytolytic activity was tested with RPMI 8226 target cells at a E:T ratio of 3:1. As shown in **FIG. 4B**, BCMA-52-LS- and BCMA-55-LS-CAR-expressing cells showed approximately 70% lysis, normalized to the lysis by mock-processed cells not expressing a CAR, whereas the cells expressing the CAR containing the reference anti-BCMA antibody binding domain showed approximately 50% lysis. Thus, the results showed that cytolytic activity of cells engineered to express BCMA-52- or BCMA-55-CARs was similar to or higher than that of the reference binding domain-containing CAR.

[0716] To compare cytolytic activity of T cells engineered with the same CAR encoded by an unmodified CAR construct or an optimized CAR construct, T cells were engineered to express an anti-BCMA CAR using a viral vector containing either an unmodified polynucleotide construct (BCMA-52-LS CAR and BCMA-55-LS CAR) or an optimized polynucleotide construct (BCMA-52-LS-O/SSE CAR and BCMA-55-LS-O/SSE CAR). Cytolytic activity of the engineered cells was assayed substantially as described above. The CAR-expressing T cells were incubated with target cells, K562-BCMA, RPMI 8226, MM1.S cells ($BCMA^{med}$ human multiple myeloma cell line) or OPM2 cells ($BCMA^{med}$ human multiple myeloma cell line) target cells, at an E:T ratio of 3:1. As shown in **FIG. 4C** and **FIG. 4D**, CAR-expressing cells transduced with a CO/SSE CAR construct exhibited greater cytolytic activity compared to cells transduced with the corresponding unmodified construct.

B. Cytokine Release

[0717] Cytokine release was assessed following incubation of the various anti-BCMA CAR-expressing cells with antigen-expressing target cells.

[0718] BCMA-expressing target cells, K562/BCMA or RPMI 8226 cells, were incubated with T cells expressing the BCMA-52-LS CAR, BCMA-55-LS CAR, or a reference binding domain-containing anti-BCMA CAR at an E:T ratio of 5:1, 2.5:1, 1.25:1 or 0.6:1. As a control, target cells were incubated with T cells not expressing a CAR (mock control). The co-cultured cells were incubated for about 24 hours, and then supernatants were collected for measurement of IFN-γ, TNF-α and IL-2, using a multiplex cytokine immunoassay. As shown in **FIG. 5A**, the tested anti-BCMA CAR-expressing T cells produced cytokines following antigen stimulation.

[0719] To assess antigen-dependent cytokine production of T cells engineered with the same CAR encoded by an unmodified CAR construct or an optimized CAR construct, T cells were engineered to express an anti-BCMA CAR using a viral vector containing either an unmodified polynucleotide construct (BCMA-52-LS CAR and BCMA-55-LS CAR) or an optimized polynucleotide construct (BCMA-52-LS-O/SSE CAR and BCMA-55-LS-O/SSE CAR). CAR-expressing T cells were incubated with target cells, either K562/BCMA, RPMI 8226 cells, MM1S ($BCMA^{med}$ human multiple myeloma cell line) or OPM2 cells ($BCMA^{med}$ human multiple myeloma cell line) target cells, at an E:T ratio of 3:1, 1.5:1, 0.75:1 and 0.375:1. Production of cytokines IFN-γ, and IL-2 was assessed as described above. As shown in **FIG. 5B**, CAR-expressing cells transduced using O/SSE optimized constructs were observed to exhibit higher cytokine production compared to cells transduced with the corresponding unmodified (starting) construct.

C. Cytolytic Activity, Cytokine Release and Proliferation in Response to Targets Expressing Different Levels of Antigen on their Surfaces

[0720] Cytolytic activity, cytokine release, and proliferation were assessed following incubation of BCMA-55-LS-O/SSE CAR-expressing T cells with BCMA-expressing cells that expressed different levels of BCMA. All activity was evaluated in the presence or absence of soluble BCMA.

[0721] A 1:1 ratio of CD4+ and CD8+ primary T cells, harvested from two human donors (D#1 and D#2), were stimulated with CD3/CD28 beads and transduced with a lentiviral vector to stably express BCMA-55 CAR. Transduced cells were cultured in the presence of BCMA-expressing target cells at an E:T ratio of 1:3, 1:1 or 3:1. Mock-processed T cells from the same donors were also mixed with target cells for use as a control. The BCMA+ target cells, Daudi, RPMI-8226, and K562-BCMA cell, exhibited different levels of BCMA antigen-density of the surface (antigen density: Daudi (<1000 BCMA molecules/cell) < RPMI-8226 < K562-BCMA) and were stained with carboxyfluorescein succinimidyl ester (CFSE) prior to incubation with the T cells. An equal number of target-negative cells, not expressing BCMA and stained with cell trace violet (CTV), were also included in the cultures with the T cells and BCMA+ target cells. After a 24 hour incubation, the remaining BCMA+ vs BCMA- target cells were measured by flow cytometry, and the degree of target cell lysis, indicative of cytotoxicity, was assessed.

[0722] BCMA-55-LS-O/SSE CAR T cells displayed similar cytolytic activity when cultured with target cells, regardless of BCMA expression levels (**FIG. 6**). Additionally, similar results were observed for target cells (NCI-H929) expressing a greater than 100,000 molecules per cell. Mock-processed T cells did not show activity against any of the BCMA+ target cell lines. Target cells negative for BCMA expression were not lysed by the BCMA-55-LS-O/SSE CAR T cells from any of the donors tested (data not shown).

[0723] The supernatants following the incubation were analyzed for accumulated IFN-γ, TNF-α, and IL-2 cytokines. Data were consistent with a conclusion that BCMA-55-LS-O/SSE CAR T cells had released a range of cytokines following engagement with BCMA-expressing target cells; with the level of cytokines released generally corresponding with increasing level of antigen (i.e., Daudi < RPMI 8226 < K562-BCMA). Results for IFN-γ are shown in **FIG. 7**; similar data were observed for TNF-α and IL-2 (data not shown). BCMA-55-LS CAR O/SSE T cells did not release cytokines in response to BCMA-negative targets, nor did they express cytokines without any target cells present, demonstrating specificity for BCMA+ target cells and lack of tonic signaling.

[0724] Activity of BCMA-55-LS-O/SSE CAR-expressing T cells in the presence vs. absence of soluble BCMA was assessed. BCMA-55-LS-O/SSE CAR-expressing T cells were co-cultured with RPMI-8226 tumor cells, with recombinant BCMA-Fc, or with cell culture supernatant derived from NCI-H929 multiple myeloma cells (BCMA-secreting cell line, the supernatant containing soluble BCMA). Neither tumor-cell lysis nor cytokine production was observed to be affected by any of the concentrations of NCI-H929–derived soluble BCMA (up to 1000 ng/mL). Both tumor-cell lysis and cytokine production were only minimally

decreased at similarly high physiological levels of recombinant BCMA.

[0725] Proliferation in response to BCMA was measured in BCMA-55-LS-O/SSE CAR-expressing T cells and mock-processed T cells. Transduced T cells were labeled with cell trace violet (CTV) and cultured in the presence of BCMA-positive target cells, BCMA-negative target cells, or no cells, at an effector to target (E:T) ratio of 1:1, for 72 hours. Proliferation was measured by flow cytometry. Proliferation of T cells (CD4+ and CD8+ T cells) was observed only for BCMA-55-LS-O/SSE CAR-expressing T cells in response to incubation with BCMA-positive target cells.

D. Transduced T cells Harvested from Healthy Donors and a Myeloma Patient

[0726] T cells engineered to express BCMA-55-LS-O/SSE CAR harvested from multiple myeloma patients were compared to those derived from healthy human donors following a 24-hour incubation with BCMA+ and BCMA- K562 target cells. T cells not expressing a CAR were also evaluated as a negative control. CAR T cells derived from multiple myeloma patients demonstrated similar expression, expansion and antigen-specific activities as compared to cells expressing the CAR derived from healthy human donors.

Example 6: Anti-BCMA CARs with Different Spacers

[0727] Polynucleotide constructs encoding anti-BCMA CARs were generated that contained different spacer regions between the scFv and transmembrane segments of the encoded CAR polypeptide. Specifically, CARs were generated containing: (1) a spacer derived from an IgG hinge region (e.g., e.g., BCMA-5-SS, BCMA-9-SS, BCMA-18-SS, BCMA-23-SS, BCMA-25-SS, BCMA-26-SS, BCMA-52-SS, BCMA-55-SS, and Referenc1 (V_H/V_L)-SS); or (2) a short spacer derived from the ectodomain of CD28 (e.g. BCMA-52-SCD28 and BCMA-55-SCD28). T cells expressing such spacer-containing CARs were compared to T cells transduced with polynucleotide constructs encoding exemplary CARs containing spacers as described in Example 3 (e.g. BCMA-1-LS, BCMA-5-LS, BCMA-9-LS, BCMA-18-LS, BCMA-23-LS, BCMA-25-LS, BCMA-26-LS, BCMA-27-LS, BCMA-52-LS, BCMA-55-LS, and Reference1 (V_H/V_L)-LS).

[0728] CAR-expressing cells were assessed for cytolytic activity by monitoring the lysis of OPM2 human multiple myeloma target cells cultured with CAR-expressing T cells at an effector to target (E:T) ratio of 1.25:1 and 0.65:1. Cells that did not express a CAR (mock) were used as a negative control. Cytolytic activity was assessed as described in Example 7. For most assessed CAR-expressing cells, target cell lysis was greater for cells engineered to express a CAR containing a C_H2-C_H3-hinge spacer as compared to cells engineered with a CAR containing a shorter spacer (**FIG. 8**).

Example 7: Assessment of Agents on Blocking Activity of Anti-BCMA CAR Activity

[0729] The function of anti-BCMA CAR-expressing cells was assessed following incubation with

BCMA-expressing target cells and soluble BCMA or other proteins. Cytolytic activity and cytokine production was assessed substantially as described in Example 7.

A. Cytolytic Activity

1. Soluble recombinant BCMA (rBCMA) – OPM2 target cells

[0730] Anti-BCMA CAR-expressing T cells, BCMA-52-LS CAR, BCMA-55-LS CAR or Reference binding domain-containing CAR, were incubated with OPM2 target cells at an E:T ratio of 5:1 in the presence of soluble BCMA-Fc at 0, 0.3, 3, 30 or 300 ng/mL. As shown in **FIG. 9A** cytolytic activity of T cells expressing the Reference binding domain-containing CAR or BCMA-52-LS CAR were substantially reduced in the presence of 3 ng/mL or more BCMA-Fc, however the cytolytic activity of cells expressing BCMA-55-LS CAR was not blocked by the presence of up to 300 ng/mL BCMA-Fc.

[0731] In another experiment, Anti-BCMA CAR-expressing T cells (BCMA-1-LS CAR, BCMA-9-LS CAR, BCMA-23-LS CAR, BCMA-25-LS CAR, BCMA-26-LS CAR, BCMA-55-LS CAR and Reference1 (V_H/V_L)-LS CAR) were incubated with OPM2 target cells at an E:T ratio of 5:1 in the presence of soluble BCMA-Fc at concentrations of 0, 7.8, 15.6, 31.3, 62.5, 125, 250, 500 and 1000 ng/mL. As shown in **FIG. 9B** the cytolytic activity of cells expressing BCMA-55-CAR was not blocked by the presence of BCMA-Fc at any of the concentrations tested; however, the presence of variable concentrations of BCMA-Fc blocked activity of cells expressing other anti-BCMA CARs to different extents.

2. Multiple Myeloma Cell Line (H929) Supernatant – OPM2 target cells

[0732] Optimized, splice site eliminated (O/SSE) anti-BCMA CAR-expressing T cells, BCMA-52-LS-O/SSE CAR, BCMA-55-LS-O/SSE CAR or Reference binding domain-containing CAR, were incubated with OPM2 target cells at an E:T ratio of 5:1 in the presence of 0, 111, 333 and 1000 ng/mL culture supernatant from the H929 multiple myeloma cell line. The concentration of soluble BCMA was quantified from the H929 supernatant by ELISA. As shown in **FIG. 10A** the cytolytic activity of cells expressing BCMA-52-LS-O/SSE CAR, BCMA-55-LS-O/SSE CAR or Reference CAR were not blocked by the presence of H929 supernatant.

3. Soluble recombinant BCMA (rBCMA) and H929 Supernatant – RPMI-8226 target cells

[0733] In a further study, optimized, splice site eliminated (O/SSE) BCMA-55-LS-O/SSE CAR - expressing T cells, were incubated with RPMI-8226 tumor target cells at an E:T ratio of 3:1 in the presence of 0, 111, 333 and 1000 ng/mL soluble BCMA from culture supernatant from the H929 multiple myeloma cell line (soluble BCMA quantitated by ELISA) or BCMA-Fc. The cytolytic activity of cells expressing

BCMA-52-LS-O/SSE CAR, BCMA-55-LS-O/SSE CAR or Reference CAR was not blocked by the presence of H929 supernatant.

4. B-Cell Activating Factor (BAFF)

[0734] Optimized, splice site eliminated (O/SSE) anti-BCMA CAR-expressing T cells, BCMA-52-LS-O/SSE CAR, BCMA-55-LS-O/SSE CAR or Reference CAR, were incubated with OPM2 target cells at an E:T ratio of 5:1 in the presence of 0, 1, 10, 100 and 1000 ng/mL recombinant B-cell activating factor (BAFF), a ligand for BCMA. As shown in **FIG. 10B**, cytolytic activity of T cells expressing BCMA-52-LS-O/SSE CAR, BCMA-55-LS-O/SSE CAR or Reference CAR were not blocked by the presence of BAFF.

B. Cytokine Release

1. BCMA-Fc

[0735] Anti-BCMA CAR-expressing T cells, BCMA-52-LS CAR, BCMA-55-LS CAR or Reference-LS CAR, were incubated with OPM2 target cells at an E:T ratio of 5:1 in the presence of soluble BCMA-Fc at 0, 111, 333 and 1000 ng/mL T cells not expressing a CAR (mock) also were assessed. Cytokine accumulation of IFN-γ, TNF-α and IL-2 in supernatant was assessed. As shown in **FIG. 11A**, cytokine accumulation in cultures containing T cells expressing the Reference CAR or BCMA-52-CAR were substantially reduced in the presence of 111 ng/mL or more BCMA-Fc, however less reduction in cytokine accumulation was observed in cultures containing T cells expressing BCMA-55-CAR in the presence of soluble BCMA-Fc at all concentrations tested.

2. Multiple Myeloma Cell Line (H929) Supernatant

[0736] Anti-BCMA CAR-expressing T cells, BCMA-52-LS CAR, BCMA-55-LS CAR or Reference-LS CAR, were incubated with OPM2 target cells at an E:T ratio of 5:1 in the presence of 0, 111, 333 and 1000 ng/mL culture supernatant from a multiple myeloma cell line H929. Cytokine accumulation in cultures containing T cells expressing BCMA-52-CAR, BCMA-55-CAR or Reference CAR were not blocked by the presence of H929 supernatant (**FIG. 11B**)

Example 8: Anti-Tumor Effect of anti-BCMA CAR-Expressing T cells After Adoptive Transfer *In Vivo* in an Animal Model

[0737] The anti-tumor effects of exemplary engineered anti-BCMA CAR-expressing primary human T cells were assessed by monitoring tumors following adoptive transfer of cells in tumor-bearing animal models, including OPM2 human multiple myeloma xenograft mouse model (orthotopic bone marrow model) and RPMI 8226 human multiple myeloma xenograft mouse model (subcutaneous implant model).

A. OPM2 (Orthotopic/Bone Marrow) Model

[0738] NOD.Cg.Prkdc^{scid}IL2rg^{tm1Wjl}/SzJ (NSG) mice were injected intravenously (i.v.) with 2 x 10^6 OPM2 (multiple myeloma) cells transfected with firefly luciferase (OPM2-ffluc). On day 14, following tumor engraftment, mice received a single intravenous (i.v.) injection of anti-BCMA CAR T cells expressing optimized, splice site eliminated (O/SSE) BCMA-23-LS-O/SSE CAR, BCMA-26-LS-O/SSE CAR or BCMA-55-LS-O/SSE CAR. The anti-BCMA CAR-expressing T cells were administered at a dose of either 1 x 10^6 (low dose, n=8) or 3 x 10^6 (high dose, n=8) CAR-expressing T cells per mouse, and each condition repeated for CAR-expressing T cells derived from two different donors. As a control, mice were administered cells not expressing a CAR (mock, n=8) or were untreated (n=3). Survival and tumor burden were assessed over 90 days.

[0739] Anti-tumor activity of the adoptively transferred CAR-expressing (CAR-T) cells was monitored by bioluminescence imaging every 3 to 6 days post CAR-T cell administration for the length of the study. For bioluminescence imaging, mice received intraperitoneal (i.p.) injections of luciferin substrate (CaliperLife Sciences, Hopkinton, MA) resuspended in PBS (15 µg/g body weight). Mice were anesthetized and imaged essentially as described in WO2015/095895. The total flux (photon/s) was determined at each time point. For the negative control treated mice, animals were sacrificed between 19 and 23 days after CAR-T cell administration, due to high tumor burden. Representative results from one donor-derived CAR-expressing T cells are shown in **FIG. 12A**.

[0740] As shown in **FIG. 12A**, for all treated mice, the tumor in mice receiving mock-processed T cells or no T cells continued to grow over the course of the study. Compared to the control mice, mice that received an adoptive transfer of T cells engineered to express BCMA-23-LS-O/SSE CAR, BCMA-26-LS-O/SSE CAR, or BCMA-55-LS-O/SSE CAR, were observed to generally have a lower degree of bioluminescence signal, indicating a reduction in tumor growth over time and/or a lower degree of tumor growth in the treated animals. The effect on tumor growth was greater with the higher dose of anti-BCMA CAR expressing cells for the exemplary tested anti-BCMA CARs.

[0741] Survival of mice treated as described above were assessed and compared until day 79 post-infusion of CAR-expressing T cells. Representative survival curves, Kaplan-Meier method (GraphPad Prism 7.0, GraphPad Software, La Jolla), from one donor are shown in **FIG. 12B**. As shown, the tested anti-BCMA CAR-T cells at the low and high dose resulted in greater percent survival of mice compared to mice receiving no treatment or mock-processed T cells. Mice also were assessed for presentation of clinical signs associated with tumor burden, including hind limb paralysis (HLP), greater than 20% body weight loss (>20% BWL), and graft-versus-host disease (GVHD). The number of mice with these clinical signs was reduced compared to mice receiving no treatment or mock T cells.

B. RPMI-8226 (Subcutaneous) Model

[0742] NOD.Cg.PrkdcscidIL2rgtm1Wjl/SzJ (NSG) mice were injected subcutaneously with RPMI 8226 (peripheral blood plasmacytoma) cells. On Day 27, the mice were randomized into groups based on a minimum mean tumor volume of approximately 130 mm^3. On Day 29, mice received a single intravenous (i.v.) injection of primary human T cells (CD4+ and CD8+) engineered to express optimized, splice site eliminated (O/SSE) BCMA-23-LS-O/SSE CAR, BCMA-26-LS-O/SSE CAR, or BCMA-55-LS-O/SSE CAR at a dose of 1 x 10^6 (low dose, n=8) or 3 x 10^6 (high dose, n=8) CAR-expressing T cells. Each condition was repeated for CAR-expressing T cells derived from two different donors. Mice that were administered cells that were mock-processed and untreated mice were used as negative controls. Tumor volume was measured by calipers twice weekly up to Day 152 post CAR T-cell transfer and euthanized when moribund, 20% weight loss, or when tumor volume exceeded 1500 mm^3. Survival curves were plotted up to Day 108 post CAR T-cell transfer using the Kaplan-Meier method (GraphPad Prism 7.0, GraphPad)

[0743] Representative results for tumor growth and survival from CAR-expressing T cells derived from one donor are shown in **FIGS. 13A** and **13B**, respectively. As shown in **FIG. 13A**, the tumor continued to grow over the course of the study following adoptive transfer of negative control cells or in mice not receiving treatment. Compared to the control mice, mice that received an adoptive transfer of T cells engineered to express BCMA-23-LS-O/SSE CAR, BCMA-26-LS-O/SSE CAR, or BCMA-55-LS-O/SSE CAR showed substantially reduced tumor volume after receiving the low or high dose of CAR-expressing T cells (**FIG. 13A**). In this model, mice administered both tested doses of anti-BCMA CAR T cells exhibited complete regression of tumor growth by 20 days post CAR T-cell transfer, which continued throughout the duration of the study assessment shown in **FIG. 13A**.

[0744] The percent survival of mice administered anti-BCMA CAR-expressing T cells also was substantially greater than control groups (**FIG. 13B**). At 108 days post-CAR T cell infusion, two animals had been lost post-tumor elimination in the group treated with the high dose of BCMA-26-LS-O/SSE CAR - expressing T cells, although this was likely due to graft versus host disease (GVHD) symptoms in this model. All other CAR-T cell treated mice remained alive up to 108 days post-CAR T cell administration.

[0745] The presence of CAR+ T cells in the blood was monitored to assess pharmacokinetics of CAR-expressing T cells in the mice from treated. The 8 mice of each treatment group were divided into 2 groups of 4 mice. Blood was drawn weekly, by retro-orbital bleeding, alternating between the 2 groups such that each mouse was bled every other week for 4 weeks post CAR-T cell administration (i.e., on days 7, 14, 21 and 28 post CAR-T cell administration). The collected blood was analyzed for the number of CAR-expressing T cells, as determined using an antibody against the surrogate marker or soluble BCMA-Fc, and non-CAR T cells, per μL blood by flow cytometry (FlowJo software, Treestar Inc., Ashland, OR).

[0746] The number of CD4+ and CD8+ T cells per μL of blood at days 7, 14, 21 and 28 or 36 are shown in **FIG. 14A** and **FIG. 14B**, respectively, for one donor and in **FIG. 15A** and **FIG. 15B**, respectively, for the second donor. As shown, CAR-T expansion occurred in high and low dose groups in CD4+ and CD8+ T cells, with maximum or peak expansion observed at day 14 post CAR T-cell transfer for both donors. At all assessed times post CAR-T cell transfer, greater numbers of CD8+ CAR+ T cells were observed compared to CD4+ CAR+ T cells for both donors (compare **FIG. 14A** and **FIG. 14B** or **FIG. 15A** and **FIG. 15B**). T cells engineered to express BCMA-55-LS-O/SSE CAR exhibited greater CAR expression compared to T cells expressing BCMA-23-LS-O/SSE CAR and BCMA-26-LS-O/SSE CAR constructs, which exhibited comparable expression to each other. These results demonstrate BCMA-55-LS CAR expressing T cells can be identified circulating in the blood during tumor clearance.

Example 9: Assessment of Signals through Anti-BCMA Chimeric Antigen Receptor (CAR) in a Nur77-tdTomato Reporter Signal in Reporter Cell Line

[0747] An exemplary stable Jurkat T cell reporter cell line was generated containing a Nur77 knock-in reporter, where the nucleic acid sequences encoding the reporter molecule was knocked-in at the endogenous Nur77 locus via homology dependent repair (HDR). Orphan nuclear hormone receptor Nur77 (also called Nr4a1) is an immediate-early response gene induced by activation of signal from the T cell receptor and/or via molecules containing immunoreceptor tyrosine-based activation motif (ITAM). The Nur77-reporter cell line was used to assess T cell activation in CAR-engineered cells as Nur77 is an immediate early gene product in T lymphocytes; transcription is initiated specifically downstream of CD3 zeta signaling, and is not influenced by cytokine or TLR mediated signals. In a Jurkat T cell clone E6-1 (ATCC® TIB-152™), nucleic acid sequence encoding a red fluorescent protein (RFP; such as the tdTomato fluorescent protein) was targeted for integration in-frame with the endogenous *Nr4a1* (Nur77) gene at the final exon, prior to the stop codon, and after a "self-cleaving" T2A element, to allow for co-expression of RFP as a reporter of Nur77 expression, by introducing a genetic disruption using gene editing and targeting a transgene for integration at a site near the genetic disruption by homology-dependent repair (HDR). The Nur77-tdTomato reporter cell line was engineered to express various anti-BCMA chimeric antigen receptors, and reporter expression was assessed.

[0748] Viral vectors containing polynucleotides encoding the following anti-BCMA chimeric antigen receptors (CARs), described in Example 3, were introduced into the Nur77-tdTomato reporter Jurkat T cell line: BCMA-55-LS-O/SSE CAR, BCMA-26-LS-O/SSE CAR, BCMA-23-LS-O/SSE CAR, and BCMA-25-LS-O/SSE CAR. Anti-BCMA CAR-expressing reporter cells were evaluated for activity of Nur77 signaling in response to increasing amounts of plate-bound recombinant BCMA or in response to exemplary multiple

myeloma cell lines after 20 hours of co-culture.

A. Nur77 Signaling in Response to Plate-Bound Recombinant BCMA

[0749] Reporter cells transduced with a viral vector encoding BCMA-55-LS-O/SSE CAR were incubated for 6 hours in 96-well cell culture plates that had been coated overnight with varying concentrations (0.008 µg/mL, 0.04 µg/mL, 0.2 µg/mL, 1 µg/mL and 5 µg/mL) of BCMA-Fc (soluble human BCMA fused at its C-terminus to an Fc region of IgG) fusion polypeptide. A recombinant Fc polypeptide was used as a control (Fc Control). As shown in **FIG. 16A**, a dose-dependent increase in tdTomato expression was observed following stimulation of anti-BCMA CAR-expressing reporter cells with recombinant antigen.

[0750] In another study, reporter cells engineered to express BCMA-55-LS-O/SSE CAR, BCMA-26-LS-O/SSE CAR, BCMA-23-LS-O/SSE CAR, and BCMA-25-LS-O/SSE CAR were incubated with ten (10) 2-fold serial dilutions of BCMA-Fc. Reporter cells expressing an anti-CD19 CAR was used as a non-target control. The percentage of tdTomato-expressing cells within the population of cells expressing the CAR (as determined based on expression of the surrogate marker) was determined. As shown in **FIG. 16B**, a dose-dependent increase in tdTomato expression was observed following stimulation of with recombinant antigen. No response to stimulation with BCMA-Fc was observed by the control reporter cells expressing a CAR against a non-target antigen.

B. Nur77 Signaling in Response to Multiple Myeloma Cell Lines

[0751] Reporter cells transduced with a viral vector encoding BCMA-55-LS-O/SSE CAR were incubated for 20 hours with NALM6, Daudi, RPMI-8226, MM1S, OPM2, and H929 cells. Different levels of RFP expression were observed depending on the cell line which conferred stimulation of the anti-BCMA CAR-expressing reporter cells.

[0752] To assess the amounts of BCMA expression on the surface of the multiple myeloma cell lines used to stimulate the anti-BCMA CAR-expressing reporter cells, the cells were stained with anti-human BCMA antibody (BioLegend, San Diego, CA), flow cytometry events were collected on an LSRFortessa™ flow cytometer (BD Biosciences, San Jose, CA) and data were analyzed with FlowJo software (Treestar Inc., Ashland, OR). BCMA antigen density (AD) was determined by using Quantum™ Simply Cellular® anti-Mouse IgG microsphere beads coated with the same anti-human BCMA antibody. Microspheres were labeled and BCMA antibody binding capacity was calculated. The results confirmed the detection of a parameter (detectable levels of the reporter) indicative of specific CAR activity in CAR-expressing reporter cells, when incubated with each of the various different BCMA-expressing cells, exhibiting a range of different antigen densities, and not when incubated with target-negative cells. The degree of the RFP

reporter signal generally correlated with levels of surface BCMA expression. When incubat3d with cells in which lower levels of surface BCMA expression were observed, CAR-expressing reporter cells exhibited lower levels of the reporter indicative of activity. Likewise, CAR-expressing reporter cells incubated with cell lines in which higher levels of surface BCMA expression was observed exhibited higher levels of the reporter indicative of activity. Thus, the density of BCMA expression on the surface of the various multiple myeloma cell lines was observed to correlate with the level of a parameter indicative of antigen-specific activity of reporter cells expressing the BCMA-55-LS-O/SSE CAR, indicating that cells expressing the CAR can exhibit activity over a range of antigen densities, and in some aspects can exhibit increased activity with increased antigen levels.

Example 10: Assessment of Nur77-tdTomato Reporter Signal in Reporter Cell Lines Expressing Anti-BCMA Chimeric Antigen Receptors (CARs) Containing Spacers of Different Length

[0753] Expression of the reporter in cells engineered to express anti-BCMA CARs containing the same antigen-binding domain but spacers of different length was determined after co-culture with target cells. The Jurkat Nur77-tdTomato cells, generated as described in Example 11, were engineered to express BCMA-55-LS-O/SSE CAR (containing a longer spacer derived from modified IgG Hinge-C_H2-C_H3, set forth in SEQ ID NO:174) or BCMA-55-SS CAR (containing a shorter spacer derived from IgG4 hinge, set forth in SEQ ID NO:237). The cells were co-cultured with human BCMA-expressing K562 target cells (BCMA-K562) target cells at various E:T ratios. Reporter cells expressing a CAR targeting a different antigen (anti-CD19 CAR), were used as control. As shown in **FIG. 17**, the Nur77-tdTomato expression level was observed to be different in the anti-BCMA CARs containing different spacer lengths, and a dose-dependent response to stimulation with target cells expressing BCMA was observed.

Example 11: Assessment of Antigen-independent (Tonic) Signaling from Different Anti-BCMA Chimeric Antigen Receptors (CARs)

[0754] The Nur77-tdTomato reporter cells were transduced with a viral vector encoding anti-CD19 CAR (control), BCMA-55-LS-O/SSE CAR, BCMA-26-LS-O/SSE CAR, BCMA-23-LS-O/SSE CAR, or BCMA-25-LS-O/SSE CAR as described in Example 11 above, with the exception that the surrogate marker for transduction was super-fold green fluorescent protein, sfGFP. In this model, tonic signaling was indicated by tdTomato expression in the absence of BCMA antigen stimulation.

[0755] A viral vector encoding an anti-BCMA CAR containing a different anti-BCMA scFv, designated as BCMA-52-LS-O/SSE CAR, also was generated and transduced into the reporter cell. The various CAR-expressing cells were incubated without antigen stimulation to assess the degree of antigen-independent

(tonic) signaling for 3 days and evaluated for the expression of tdTomato by flow cytometry.

[0756] As shown in **FIG. 18**, various CAR-expressing cell lines exhibited a varying degree of tdTomato expression in the absence of antigen stimulation. The percentage of tdTomato+ cells (indicative of tonic reporter activation) among CAR-expressing cells (indicated by GFP+ cells) varied from 0.23% to 19.3%, in cells expressing different CARs.

Example 12: Assessment of Antigen-independent (Tonic) Signaling from Anti-BCMA Chimeric Antigen Receptors (CARs) Containing Different Intracellular Domains

[0757] Antigen-independent (tonic) signaling was assessed in reporter cells expressing various CARs containing different intracellular signaling regions. The Nur77-tdTomato reporter cells were transduced with a viral vector encoding anti-CD19 CAR, BCMA-55-LS-O/SSE CAR, BCMA-26-LS-O/SSE CAR, BCMA-23-LS-O/SSE CAR, or BCMA-52-LS-O/SSE CAR, generated generally as described in Example 11 and 13, with the exception that the CARs contained intracellular domains derived from 4-1BB or CD28, and the surrogate marker for transduction was a truncated receptor. The various CAR-expressing cells were incubated without antigen stimulation to assess the degree of antigen-independent (tonic) signaling and evaluated for the expression of tdTomato by flow cytometry.

[0758] As shown in **FIG. 19A** and **FIG. 19B**, the 4-1BB- and CD28-derived intracellular domains in various CARs resulted in different levels of tonic signaling, as indicated by the percentage of tdTomato+ cells among the CAR+ cells (as determined based on expression of the surrogate marker).

Example 13: Assessment of Antigen Cross-Reactivity of Anti-BCMA Chimeric Antigen Receptors (CARs) Using Reporter Cell Line

[0759] The Nur77-tdTomato cell line engineered to express BCMA-55-LS-O/SSE CAR, specific for human BCMA and generated as generally described in Example 11, was employed to assess species cross reactivity of the antigen-binding domains of CARs. The reporter cell line expressing BCMA-55-LS-O/SSE CAR was co-cultured with K562 human myelogenous leukemia cells expressing human BCMA (huBCMA), murine BCMA (muBCMA) or cynomolgus monkey BCMA (cynoBCMA), at an E:T ratio of 2:1 or 5:1. The percentage of tdTomato+ cells were determined by flow cytometry.

[0760] As shown in **FIG. 20A**, more than 90% of the BCMA-55-LS-O/SSE CAR -expressing cells were observed to be tdTomato+ when cultured with target cells expressing huBCMA, at both E:T ratios tested. In comparison, when cultured with target cells expressing muBCMA, very few cells were tdTomato+, indicating very low cross-reactivity. When cultured with target cells expressing cynoBCMA, approximately 10 to 20% of the cells were tdTomato+, indicating some cross-reactivity by cynoBCMA.

[0761] The reporter cell line expressing BCMA-55-LS-O/SSE CAR was incubated with increasing concentrations (0, 0.1, 0.25, 1, 2.5, 10, 25 and 100 μg/mL) of huBCMA and cynoBCMA coated on 96-well flat-bottom plates. The percentage of tdTomato+ cells and the mean fluorescence intensity (MFI) of the tdTomato signal in CAR+ cells were determined.

[0762] As shown in **FIGS. 20B** and **20C,** cynoBCMA did not cross-react with BCMA-55-LS-O/SSE CAR at low concentrations, but did at high concentrations.

Example 14: Assessment of Antigen Specificity of Anti-BCMA Chimeric Antigen Receptors (CARs) Using Reporter Cell Line

[0763] The antigen specificity for activation of BCMA-55-LS-O/SSE CAR-expressing cells was tested by comparing the activation of Jurkat Nur77 reporter cells in response to BCMA-expressing MM1S target cells, with K562 target cells engineered to express a non-BCMA protein shown to be recognized at low levels by BCMA-55-scFv Fc in Example 5C, Cathepsin G (CTSG). As a negative control, parental K542 cells also were assessed. Briefly, Nur77 reporter cells, transduced with a viral vector encoding BCMA-55-LS-O/SSE CAR, were incubated 24 hours with the target cells listed above, at 5:1, 1:1, and 1:5 effector:target cell ratios, and activation was determined by measuring the percentage of cells expressing RFP (RFP+) by flow cytometry. The results demonstrated that BCMA-55-LS-O/SSE CAR -expressing cells were activated by BCMA-expressing MM1S cells, but not BCMA-negative target cells (parental or cells expressing the non-BCMA antigen, CTSG).

Example 15: Determining the Binding Epitope for BCMA-52 and BCMA-55 scFvs

[0764] Epitopes recognized, e.g., specifically bound to, by exemplary anti-BCMA scFv clones (BCMA-1, BCMA-5, BCMA-9, BCMA-23, BCMA-25, BCMA-26, BCMA-52 and BCMA-55 anti-BCMA scFvs), were assessed using full discontinuous epitope mapping by Chemical Linkage of Peptides onto Scaffolds (CLIPS; Pepscan Presto BV, Lelystad, The Netherlands; see, e.g., Timmerman et al., (2007) J. Mol. Recognit. 20: 283-329). Mapping was carried out using anti-BCMA scFv clones, such as those fused with mouse Fc (scFv-mFc).

[0765] Linear and conformational peptide libraries of amino acid residues 1-54 of human BCMA (set forth as amino acid residues 1-54 of SEQ ID NO:164) were generated based on a combinatorial matrix design. Linear peptides and structural mimetics including single loop, α-helix, β-turn, combinatorial and linear disulfide bridge mimics, and discontinuous epitope mimics were used, along with positive and negative control peptides, on an amino-functionalized solid support.

[0766] Affinities for binding to the peptides in the epitope library were determined using ELISA. The

peptide arrays were incubated with a solution containing the scFv overnight at 4 °C. Affinity information was used in iterative screens to define the sequence and conformation of epitopes. Heat maps of affinity information for two or more loops were generated.

[0767] scFvs assessed were observed to recognized conformational epitopes that included several discontinuous peptide stretches of the BCMA peptide sequence. BCMA-1, BCMA-5, BCMA-23 and BCMA-25 scFv were observed to bind to a peptide of $_{30}$SNTPPLTCQR$_{39}$ (set forth in SEQ ID NO:160), which could be recognized in a linear form. In some aspects, such antibodies recognize a non-linear or linear epitope including residues of such peptide of SEQ ID NO: 160, and in some aspects to recognize a non-linear epitope further including residues of $_{21}$CIPCQLR$_{27}$ (set forth in SEQ ID NO:159), $_{30}$SNTPPLTCQR$_{39}$ and/or $_{44}$SVTNSVK$_{50}$ (set forth in SEQ ID NO:161). The BCMA-26 scFv was observed to recognize an epitope comprising residues present in $_{8}$CSQNEYF$_{14}$ (set forth in SEQ ID NO:162) and $_{17}$LLHACIPCQLR$_{27}$ (set forth in SEQ ID NO:158). BCMA-52-scFv-mFc was observed to bind to an epitope containing residues of the following discontinuous peptides: $_{10}$QNEYF$_{14}$ (SEQ ID NO:91), $_{21}$CIPCQL$_{26}$ (SEQ ID NO:92), and $_{7}$CQRYC$_{41}$ (SEQ ID NO:93). BCMA-55-scFv-mFc was observed to specifically bind to an epitope containing residues present in peptides comprising discontinuous portions of the BCMA polypeptide sequence, individually comprising the following sequences: $_{1}$MLMAG$_{6}$ (SEQ ID NO:122), $_{13}$YFDSL$_{17}$ (SEQ ID NO:21), and $_{25}$QLRCSSNTPPL$_{35}$ (SEQ ID NO:124). In some embodiments, the provided antibody or receptor specifically binds to an epitope comprising residues present within one or more of, e.g., each of discontinuous peptides having the sequences of: MLMAG (SEQ ID NO:122), YFDSL (SEQ ID NO:21), and QLRCSSNTPPL (SEQ ID NO:124). In some aspects, the provided antibody or receptor specifically binds to an epitope comprising residues present within one or more of, e.g., each of, the following discontinuous peptides having the sequences of: MLMAG (SEQ ID NO:122), YFDSLL (SEQ ID NO:123), and QLRCSSNTPPL (SEQ ID NO:124); in some aspects, the provided antibody or receptor specifically binds to an epitope comprising residues present within one or more of, e.g., each of, the following discontinuous peptides having the sequences of: MLMAG (SEQ ID NO:233), QNEYFDSLL (SEQ ID NO:133), and QLRCSSNTPPL (SEQ ID NO:124).

Example 16: Administration of Anti-BCMA CAR-Expressing Cells to Subjects with Relapsed or Refractory Multiple Myeloma (MM)

[0768] Chimeric antigen-receptor (CAR)-expressing T cell compositions containing autologous T cells expressing a CAR specific for B-cell maturation antigen (BCMA) were administered to human subjects with relapsed and/or refractory multiple myeloma (MM).

A. Subjects and Treatment

[0769] Compositions containing autologous T cells engineered to express an exemplary CAR specific for BCMA were administered to adult human subjects with relapsed or refractory (R/R) multiple myeloma (MM), who have received 3 or more prior treatments (the 3 or more prior treatments including at least a proteasome inhibitor, an immunomodulatory agent and an anti-CD38 monoclonal antibody, in each case unless the subject was not a candidate to receive such treatment such as by way of it being contraindicated).

[0770] The administered T cell compositions had been generated by a process including immunoaffinity-based enrichment of CD4+ and CD8+ cell populations from leukapheresis samples from individual subjects with MM, combining cells of such populations, such as at or at approximately a 1:1 ratio, and subjecting the cells to processing steps including for stimulation, cell transduction and expansion, in an exemplary serum-free media, and cryopreservation, and generation of cells with a range of CD4+ to CD8+ CAR T cell ratios. The process resulted in a cell composition that was observed to be enriched for a central memory phenotype as compared to the starting samples and to cell compositions generated using a different manufacturing process. The CAR contained a BCMA-55-derived scFv binding domain, a modified IgG-derived C_H2-C_H3-hinge spacer, a CD28 transmembrane domain, and an intracellular signaling region including, in series, a 4-1BB endodomain and a CD3zeta endodomains. The polynucleotide sequence encoding the anti-BCMA CAR did not include identified potential cryptic splice donor and acceptor sites.

[0771] Two to seven days prior to CAR+ T cell infusion (and completed at least 48 hours prior to CAR-T infusion) subjects received a lymphodepleting chemotherapy (LDC) with fludarabine (flu, 30 mg/m^2/day) and cyclophosphamide (Cy, 300mg/m^2/day) for 3 days, the LDC completed at least 48 hours prior to CAR-T infusion. The cryopreserved cell compositions were thawed at bedside prior to intravenous administration, with the day of infusion being designated day 1. On day 1, subjects were administered a dose of CAR-expressing T cells as follows: a single dose of dose level 1 (DL1) containing 5 x 10^7 total CAR-expressing T cells, or a single dose of dose level 2 (DL2) containing 1.5 x 10^8 total CAR-expressing T cells.

[0772] At a particular timepoint of analysis, 19 adult subjects had been enrolled in an ongoing clinical study involving such therapy. Of these 19 subjects at this particular timepoint, 13 subjects had been administered the anti-BCMA CAR+ cells, each either at DL1 or DL2. Of these 13 subjects, at this particular timepoint in the ongoing study, 8 subjects were evaluable for attributes indicative of safety (evaluability based on $\geq$ 1 mo. follow-up) (n = 5 DL1; n = 3 DL2). One subject had been unable to receive CAR+ T cells, due to sepsis after LDC, leading to death before CAR+ T cell administration. Three subjects (all DL1) were evaluable at this timepoint for confirmed response (evaluability based on $\geq$ 2 mo. follow-up) according to International Myeloma Working Group (IMWG) uniform response criteria (Kumar et al. (2016) Lancet Oncol 17(8):e328-346).

[0773] For these 8 subjects assessed at this timepoint, median follow-up was 5 weeks (range 4 – 13

weeks). Median age was 53 years (range 36 – 66) with a median time from diagnosis of 4 years (range 2 – 12). Subjects had received a median of 10 prior regimens (range 4 – 15) for MM. Of the 8 subjects, 4 (50%) had been refractory (no response or progression within 60 days of last therapy) to bortezomib, carfilzomib, lenalidomide, pomalidomide and an anti-CD38 monoclonal antibody. Seven of the 8 subjects (88%) had had prior autologous stem cell transplant and 4 of 8 (50%) had IMWG high risk cytogenetics.

[0774] At the time of the assessment at the timepoint in the ongoing study, no dose-limiting toxicities (DLTs) had been observed in the subjects assessed receiving DL1 or DL2. Cytokine release syndrome (CRS), all grade 1 or 2, had been observed in 6 of the 8 (75%) subjects at the timepoint. Median onset of CRS at the timepoint among the 8 subjects was 9 days (range 4 – 10) with a median duration of 4.5 days (range 2 – 19 days). None of the subjects with grade 2 CRS at the timepoint had required vasopressor support and only 1 subject had received tocilizumab. None of the subjects had exhibited CRS of grade 3 or higher. Three of 8 (38%) subjects had experienced neurologic adverse events (AE). Two of the eight subjects at the timepoint had exhibited grade 1 events, and 1 had exhibited a grade 3 event (lethargy), which had resolved within 24 hours after receiving steroids. Onset of neurologic AEs was 9, 11 and 12 days, with a duration of 2, 3 and 1 days, respectively, for the 3 subjects experiencing neurological AE. The subject who had experienced grade 3 neurotoxicity (NT) as-of the analysis at this timepoint had developed secondary plasma cell leukemia (PCL) just prior to receiving LDC.

[0775] All 8 subjects at the timepoint were observed to have had evidence of objective response, including the subject with secondary PCL. Three subjects, all administered DL1, were observed to have achieved confirmed responses (1 partial response, PR; 2 stringent complete response, sCR), whereas the remaining subjects remained unconfirmed (1 complete response, CR; 2 very good partial response, VGPR; 1 PR, 1 minimal response, MR). As of the timepoint for assessment, no subject had been observed to have progressed.

[0776] The results showed that at the assessed dose levels, administration of the anti-BCMA CAR cell therapy exhibited favorable safety profiles, with no DLTs reported at this timepoint in an ongoing clinical study. The results were consistent with a conclusion that at this timepoint the incidence of grade 3 or higher NT was low, and no grade 3 or higher CRS had been observed with clinical response.

Example 17: Assessment of T Cell Compositions Generated by Exemplary Manufacturing Processes

[0777] In an exemplary process, 50 CAR+ T cell compositions containing autologous T cells expressing an anti-BCMA CAR were generated from apheresis collected from 50 separate human subjects (one apheresis from each subject), including 10 healthy donors and 40 multiple myeloma patients. CD4+ and CD8+ T cells were selected from the apheresis samples and separately cryopreserved. The cells were then

thawed, and the CD4+ T cells and CD8+ T cells were combined at a 1:1 ratio of viable CD4+ to CD8+ cells. The combined CD4+ and CD8+ T cells were stimulated, transduced with a vector encoding a CAR, and expanded, in an exemplary serum-free media, and frozen by cryopreservation, generally as described in Example 16.

[0778] In an exemplary alternative process, therapeutic T cell compositions were generated by a process including immunoaffinity-based selection of T cells from leukapheresis samples from 55 individual human cancer subjects. Bulk T cells were subjected to activation and transduction with a viral vector encoding a CAR, expansion and cryopreservation.

[0779] The cells in the frozen compositions were thawed and assessed by flow cytometry for viability, expression of an apoptotic marker such as active caspase 3 (CAS)), surface expression of CD3, CD4, CD8, CD27, CD28, CCR7, and CD45RA, and CAR. The percentage of CD3+ cells, percentage of CAR+ apoptotic marker negative cells in CD3+CAR+ cells in the compositions, and the percentages of central memory CD4+CAR+ cells and central memory CD8+CAR+ cells in the compositions were determined. Cell phenotypes of the cell compositions generated by the manufacturing process were assessed and in some aspects were compared to those of the cell compositions generated by the alternative process.

[0780] The manufacturing process of this example resulted in engineered cell compositions meeting certain pre-determined features, including threshold numbers of cells expressing the CAR in a cell composition administration to the patients, in 100% of the human biological samples on which it was carried out. **FIGS. 22A** and **22B** show median (horizontal lines), interquartile range (box), and 1.5x interquartile range (whiskers) for percentages of cells of the indicated phenotypes (based on CD45RA and CCR7 surface expression), among CD4+CAR+ cells (**FIG. 22A**) and among CD8+CAR+ cells (**FIG. 22B**) in the compositions, respectively, for compositions individually generated from the group of samples from the 40 multiple myeloma subjects. **FIGS. 22C** and **22D** show median (horizontal lines), interquartile range (box), and 1.5x interquartile range (whiskers) for percentages of cells of the indicated phenotypes (based on CD27 and CD28 surface expression), among CD4+CAR+ cells (**FIG. 22C**) and among CD8+CAR+ cells (**FIG. 22D**) in the compositions, respectively, for compositions individually generated from the group of samples from the 40 multiple myeloma subjects. For individual leukapheresis samples obtained from a range of multiple myeloma patients, using this exemplary process to generate engineered cell compositions from such samples, it was observed that the range of duration of the portion of the process from initiation of activation through harvest was between 7 and 10 days, and an average duration among these samples of approximately 7.5 days. It was further determined that the average number of cumulative population doublings over the course of the process among the different samples was approximately 7.5.

[0781] In this study, engineered T cell populations in the cell compositions produced by the exemplary

process included less than 15 % cells expressing an apoptotic marker, and were enriched for a central memory phenotype as compared to the starting samples and to cell compositions generated using the exemplary alternative process.

Example 18: Further Assessment of Response and Safety Outcomes Following Administration of Anti-BCMA CAR-Expressing Cells to Subjects with Relapsed or Refractory Multiple Myeloma (MM)

[0782] Response and safety outcomes were assessed in patients at subsequent points in time in the clinical study described in Example 16.

A. Subjects and Treatment

[0783] The analysis at the time points presented in this example is based on assessment of a total of 44 subjects that had been administered the anti-BCMA CAR-expressing cells. The 44 subjects were adult human subjects with relapsed or refractory (R/R) multiple myeloma (MM), who have received and failed 3 or more prior treatments (the 3 or more prior treatments including at least (1) an autologous stem cell transplantation, (2) a proteasome inhibitor and an immunomodulatory agent, either alone or in combination, and (3) an anti-CD38 monoclonal antibody, as a part of a combination therapy or a monotherapy, in each case unless the subject was not a candidate to receive such treatment such as by way of it being contraindicated). Among the subjects treated were subjects that have failed the last line of therapy, and having Eastern Cooperative Oncology Group (ECOG) scores of between 0 and 1. The subjects were not selected based on the level of BCMA expression in a sample from the subject.

[0784] On day 1, subjects were administered a dose of CAR+ T cells as follows: a single dose of dose level 1 (DL1) containing 5 x 10^7 total CAR+ T cells, a single dose of dose level 2 (DL2) containing 1.5 x 10^8 total CAR+ T cells, a single dose of dose level 2A (DL2A) containing 3.0 x 10^8 total CAR+ T cells, or a single dose of dose level 3 (DL3) containing 4.5 x 10^8 total CAR+ T cells. On day 15 after administration, a bone marrow examination was performed, and disease was assessed on day 29 after administration.

[0785] Subjects were monitored over time for response, including the objective response rate (ORR), complete response (CR), stringent complete response (sCR), partial response (PR), very good partial response (VGPR), minimal residual disease (MRD), progressive disease (PD), stable disease (SD), and minimal response (MR) (e.g., according to International Myeloma Working Group (IMWG) uniform response criteria; Kumar et al. (2016) Lancet Oncol 17(8):e328-346), and development of any adverse events, such as serious adverse events (SAEs). Minimal residual disease (MRD) was assessed in by next-generation sequencing (NGS), in subjects where the predominant clonotype was identified at screen evaluation.

[0786] Expansion and long-term persistence of the anti-BCMA CAR$^+$T cells in the peripheral blood of

subjects in the DL1, DL2, and DL3 cohorts was assessed at Days 1, 5, 8, 11, 15, 22, 29, 60, and 90 following administration of the CAR-expressing T cells, by quantitative polymerase chain reaction (qPCR) of genomic DNA preparations from whole blood samples from the subjects, using primers specific for the vector encoding the anti-BCMA CAR (vector copies/μg genomic DNA). Levels of soluble BCMA (sBCMA) in serum samples from subjects were also measured prior to administration of CAR+ T cells and at various time points following administration of CAR+ T cells.

[0787] Demographics and baseline characteristics of the total, DL1, DL2, and DL3 cohort subjects at the timepoint are set forth in **Table E3**. The subjects generally had highly refractory myeloma, with 77% of the subjects having high-risk cytogenetics. More than 50% of the subjects who received a bridging therapy exhibited disease progression before receiving administration of the anti-BCMA CAR+ T cells. The subjects were also shown to generally have a high tumor burden prior to administration of the CAR+ T cells, as indicated by serum and urine M-protein levels, serum free light chain (FLC) levels, and the presence of plasma cells in the bone marrow and extramedullary plasmacytomas.

Table E3. Demographics and Baseline Characteristics of Subjects

		CAR+ T cell dose		
Characteristic	**Total (N = 44)**	**DL 1 50×10^6 (N = 14)**	**DL 2 150×10^6 (N = 28)**	**DL 3 450×10^6 (N = 2)**
Median (range) age, y	**62 (36–79)**	56 (36–70)	63 (42–79)	67 (64–69)
Male, n (%)	**25 (57)**	9 (64)	15 (54)	1 (50)
High-risk cytogenetics, n (%)[a]	**34 (77)**	11 (79)	22 (79)	1 (50)
ECOG performance status 0 or 1, n (%)	**43 (98)**	14 (100)	27 (96)	2 (100)
Median (range) time since initial diagnosis, years	**6 (2–17)**	6 (2–15)	5 (2–17)	6 (5–7)
ISS stage III, n (%)	**11 (25)**	1(7)	9 (32)	1 (50)
Measurable serum M-protein spike, n (%)	**24 (55)**	4 (29)	19 (68)	1 (50)
Measurable urine M-protein spike, n (%)	**23 (55)**	8 (62)	14 (52)	1 (50)
Measurable by sFLC only, n (%)	**8 (18)**	4 (29)	3 (11)	1 (50)
Plasma cells in BM before LDC, median (range) %	**40 (0–100)**	50 (3–100)	35 (0–95)	31 (12–50)
EMP, n (%)	**13 (30)**	5 (36)	8 (29)	0
Received bridging chemotherapy, n (%)	**34 (77)**	9 (64)	23 (82)	2 (100)
Progressed on bridging chemotherapy, n (%)	**19 (56)**	5 (56)	12 (52)	2 (100)

BM, bone marrow; ECOG, Eastern Cooperative Oncology Group; EMP extramedullary plasmacytomas; ISS, International Staging System; sFLC, serum free light chain.
[a]High-risk cytogenetics is based on local testing and includes: del(17p), t(4; 14), t(14; 16), 1q21 amp.

[0788] The treatment history of the total, DL1, DL2, and DL3 cohort subjects at the timepoint are set forth in **Table E4**.

Table E4. Treatment History Characteristics of Subjects

		CAR+ T cell dose		
	Total (n = 44)	DL1 50×10^6 (n = 14)	DL2 150×10^6 (n = 28)	DL3 450×10^6 (n = 2)
Median (range) of prior regimens	7 (3-23)	8 (4-23)	7 (3-14)	7 (7-7)
Prior autologous SCT, n (%)				
1	30 (68)	10 (71)	19 (68)	1 (50)
> 1	12 (27)	4 (29)	7 (25)	1 (50)
Cumulative exposure, n (%)				
Prior PI, IMiD, and anti-CD38 agent	44 (100)	14 (100)	28 (100)	2 (100)
Prior 2 PIs, 2 IMiDs, and anti-CD38 agent	38 (86)	12 (86)	24 (86)	2 (100)

SCT, stem cell transplant; IMiD, immunomodulatory drug; PI, proteasome inhibitor.

B. Safety and Response Outcomes after Treatment

[0789] **Table E5** sets forth the serious adverse events (SAEs) that occurred in the total, DL1, DL2, and DL3 cohorts.

Table E5. Safety Outcome After CAR+ Cell Administration

		CAR+ T cell dose		
	Total (n = 44)	DL1 50×10^6 (n = 14)	DL2 150×10^6 (n = 28)	DL3 450×10^6 (n = 2)
Any SAE, n (%)	**12 (27)**	1 (7)	9 (32)	2 (100)
AEs of special interest grade ≥ 3/4, n (%)				
Neutropenia	**38 (86)**	11 (79)	25 (89)	2 (100)
Anemia	**22 (50)**	6 (43)	15 (54)	1 (50)
Thrombocytopenia	**19 (43)**	4 (29)	13 (46)	2 (100)
Febrile neutropenia	**8 (18)**	1 (7)	6 (21)	1 (50)
Infections[a]	**6 (14)**	0	4 (14)	2 (100)
CRS	**4 (9)**	1 (7)	2 (7)	1 (50)
Neurological events[b]	**3 (7)**	0	2 (7)	1 (50)
TLS	**1 (2)**	0	1 (4)	0
DLT, n	**1**	0	0	1

CRS, cytokine release syndrome; DLT, dose limiting toxicity; SAE, serious adverse event; AESI, adverse events of special interest.

[a]Pneumonia, appendicitis, campylobacter infection, cellulitis, sepsis.

[b]Confusional state, agitation, areflexia, lethargy, depressed state of consciousness.

[0790] A DLT of grade 4 CRS occurred in a subject in the DL3 cohort who had a history of chronic kidney disease related to the myeloma, with a neurological event of confusion, as well as a lack of pharyngeal reflex, acute kidney injury, and *Klebsiella pneumonia* sepsis as a nosocomial infection, and deceased on Day 19 following administration of the CAR+ T cells.

[0791] **Table E6** shows the safety outcome of the total, DL1, DL2, and DL3 cohorts with respect to

CRS and neurological events. Neurological events were generally associated with CRS. Grade 1 or 2 CRS occurred in 71% of total subjects, while Grade 3 or higher CRS was observed in only 9% of total subjects. One subject experienced a grade 4 neurological event of areflexia. One subject who had a CRS event required a high-dose vasopressor.

Table E6. Cytokine Release Syndrome and Neurological Events

		CAR+ T cell dose		
	Total (n = 44)	DL1 50×10^6 (n = 14)	DL2 150×10^6 (n = 28)	DL3 450×10^6 (n = 2)
Cytokine release syndrome, n (%)	**35 (80)**	**11 (79)**	**22 (79)**	**2 (100)**
Median time to onset, days (range)	3 (1–10)	7 (3–10)	3 (1–10)	1
Median duration, days (range)	5 (1–19)	3 (2–16)	5 (1–19)	8
Neurological events, n (%)	**11 (25)**	**1 (7)**	**8 (29)**	**2 (100)**
Median time to onset, days (range)	3 (1–12)	11	3 (1–12)	3 (2–3)
Median duration, days (range)	6 (1–58)	3	9 (1–58)	6
Treatment of CRS and neurological events, n (%)				
Tocilizumab	15 (34)	3 (21)	10 (36)	2 (100)
Siltuximab	3 (7)	0	2 (7)	1 (50)
Anakinra	2 (5)	0	1 (4)	1 (50)
Steroids	9 (20)	1 (7)	6 (21)	2 (100)
Tocilizumab and Steroids	8 (18)	1 (7)	5 (18)	2 (100)
Admitted to ICU, n (%)	3 (7)	0	1 (4)	2 (100)

CRS, Cytokine Release Syndrome.

[0792] With respect to prolonged cytopenias, e.g., as determined based on laboratory assessment, Grade 3 or 4 anemia and thrombocytopenia prior to the start of lymphodepleting chemotherapy occurred in 18% of subjects. Grade 3 or 4 cytopenias lasting longer than 29 days occurred in 28/42 subjects (67%). The cytopenias were resolved to grade ≤ 2 by month 3 in 17/24 subjects (71%) with 3 months follow-up. The median time to resolution, in which, in some cases, recovery was defined as grade 2 or lower without transfusion within 1 week of laboratory assessment or without growth factor support within 1 week of lab assessment (2 weeks for pegfilgrastim), was 2.1 months for neutropenia, 2.2 months for anemia, and 3.4 months for thrombocytopenia.

[0793] Objective response rates (ORR) based on the best overall response, among the total, DL1, DL2, and DL3 cohorts are shown in **FIG. 23**. In all subjects, an ORR of 82% was observed, with 48% of the subject exhibiting a response better than VGPR. A complete response (CR) rate of 43% was observed in the lowest dose level of 5×10^7 total CAR-expressing T cells (DL1). One subject in the DL3 cohort was not evaluable for efficacy due to the lack of post-baseline response evaluation at Day 29. **Table E7** sets forth the results of minimal residual disease (MRD) assessment by next-generation sequencing (NGS), in 21 subjects

in which MRD evaluation was possible.

Table E7. MRD Assessment by NGS		
	Day 29 / Month 2 (n = 21)	Month 3 (n = 4)
MRD negative ($\leq 10^{-5}$) subjects, n (%)	9 (43)	3 (75)

[0794] The assessment of response over time, in subjects in the DL1 cohorts at the longest follow-up, after administration of the CAR-expressing T cells (n = 14) is shown in **FIG. 24**. In general, response was observed to continue improve over time, with five (5) out of 14 subjects (36%) showing a deepening of response after day 29. Six (6) out of nine (9) subjects evaluated for MRD, were MRD negative (as assessed by NGS) at day 29, with one subject that had an MRD assessment at month 2.

C. Persistence

[0795] The expansion and long-term persistence of CAR^+ T cells in the peripheral blood of subjects in the DL1, DL2, and DL3 cohorts are shown in **FIG. 25**. The results were consistent with a robust expansion of CAR^+ T cells observed at all dose levels (DL1, DL2, and DL3). In general, an increased persistence past month 2 was observed, in subjects administered a dose of $\geq 150 \times 10^6$ total CAR-expressing T cells (DL2 and DL3).

D. Soluble BCMA

[0796] The level of soluble BCMA (sBCMA) (ng/mL) in the serum of the subjects prior to CAR+ T cell administration and at various timepoints after administration, is shown in **FIG. 26A**. **FIG. 26B** shows the level of sBCMA prior to CAR+ T cell administration (pre-treatment) in subjects who exhibited an overall response of PR or better (responders) and in subjects who exhibited a response worse than PR (MR or SD; non-responders). Responses were observed in subjects across broad range of sBCMA levels, and response or the lack of response did not correlate with sBCMA levels. A decline in sBCMA level was observed after anti-BCMA CAR administration, consistent with tumor killing activity by the anti-BCMA CAR+ cells. The results showed a greater decline of sBCMA observed in subjects with an overall response of PR or better (PR, VGPR, CR or sCR; responders) as compared to subjects with an overall response that is worse than PR (MR or SD; non-responders) at day 29 or later. The results were consistent with the observation that the anti-BCMA CAR+ T cells were not inhibited by high pre-treatment levels of sBCMA.

E. Conclusion

[0797] The results were consistent with an observation of a high overall response rate (ORR) (82%) in response to administration of anti-BCMA CAR+ cells, which express a fully human antigen-binding domain and is generated by a manufacturing process that results in a population enriched for central memory T cell

phenotypes, was high in heavily pre-treated subjects with relapsed/refractory multiple myeloma (R/R MM), 77% of whom had high-risk cytogenetics. A robust expansion of the administered cells were observed at all dose level tested, and approximately 27% of the subject achieved a complete response (CR) or stringent complete response (sCR), with a general observation of deepening response over time. A high rate of CR and sCR of 43% was observed at the lowest dose level administered (50 x 10^6 CAR+ T cells). The results also were consistent with a manageable toxicity profile, including low rates of grade 3 or higher CRS (9%) and grade 3 or higher neurological events (7%). Grade 1 or 2 CRS was observed in approximately 71% of the subjects, and grade 1 or 2 neurological events was observed in 18% of the subjects. The results also showed that the anti-BCMA CAR+ T cells showed activity in subjects with a high pre-treatment level of soluble BCMA.

[0798] At a further time in the clinical study described in Example 16 and in this Example, additional subjects were administered anti-BCMA CAR-expressing cells, including at a single dose of dose level 4 (DL4) containing 6.0 x 10^8 total CAR+ T cells.

[0799] The present invention is not intended to be limited in scope to the particular disclosed embodiments, which are provided, for example, to illustrate various aspects of the invention. Various modifications to the compositions and methods described will become apparent from the description and teachings herein. Such variations may be practiced without departing from the true scope and spirit of the disclosure and are intended to fall within the scope of the present disclosure.

SEQUENCES

SEQ ID NO:	Sequence	description
1	GGGGSGGGGSGGGGS	$(4GS)_3$ linker (aa)
2	GGGS	3GS linker (aa)
3	gctgagagtcaagttttccaggtccgccgacgctccagcct	4-1BB/CD3 zeta predicted splice acceptor site
4	KRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCEL	4-1BB-derived intracellular co-signaling sequence (aa)
5	aagcgggggagaaagaaactgctgtatattttcaaacagccctttatgagacctg tgcagactacccaggaggaagacggatgcagctgtaggtttcccgaggaagagga aggaggctgtgagctg	4-1BB-derived intracellular co-signaling sequence (nt)
6	aagcggggcagaaagaagctgctctacatcttcaagcagcccttcatgcggcccg tgcagaccacacaagaggaagatggctgctcctgcagattccccgaggaagaaga aggcggctgcgagctg	4-1BB-derived intracellular co-signaling sequence (nt)
7	GGGGS	4GS linker (aa)
8	gaatctaagtacggaccgccttgtcctccttgtcccgctcctcctgttgccggac cttccgtgttcctgtttcctccaaagcctaaggacaccctgatgatcagcaggac ccctgaagtgacctgcgtggtggtggatgtgtcccaagaggatcccgaggtgcag ttcaactggtatgtggacggcgtggaagtgcacaacgccaagaccaagcctagag aggaacagttccagagcacctacagagtggtgtccgtgctgacagtgctgcacca ggattggctgaacggcaaagagtacaagtgcaaggtgtccaacaagggcctgcct agcagcatcgagaaaaccatctccaaggccaagggccagccaagagagccccagg tttacacactgcctccaagccaagaggaaatgaccaagaatcaggtgtccctgac atgcctggtcaagggcttctaccctccgatatcgccgtggaatgggagagcaat ggccagcctgagaacaactacaagaccacacctcctgtgctggacagcgacggca gtttcttcctgtatagtagactcaccgtggataaatcaagatggcaagagggcaa cgtgttcagctgcagcgtgatgcacgaggccctgcacaaccactacacccagaaa agcctgagcctgtctctgggcaag	Alternative CO/SSE spacer (nt)
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17	EVQLVESGGGLVKPGGSLRLSCAASGFTFSDYYMSWIRQAPGKGLEWVSYISSSG STIYYADSVKGRFTISRDNAKNSLYLQMNSLRAEDTAVYYCAKVDGPPSFDIWGQ GTMVTVSSGGGGSGGGGSGGGGSSYVLTQPPSVSVAPGQTARITCGANNIGSKSV HWYQQKPGQAPMLVVYDDDDRPSGIPERFSGSNSGNTATLTISGVEAGDEADYFC HLWDRSRDHYVFGTGTKLTVLESKYGPPCPPCPAPPVAGPSVFLFPPKPKDTLMI SRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQFQSTYRVVSVLTV LHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQV SLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQ EGNVFSCSVMHEALHNHYTQKSLSLSLGKMFWVLVVVGGVLACYSLLVTVAFIIF WVKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCELRVKFSRSADAP AYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDK MAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR	anti-BCMA CAR
18	SYELTQPPSASGTPGQRVTMSCSGTSSNIGSHSVNWYQQLPGTAPKLLIYTNNQR PSGVPDRFSGSKSGTSASLAISGLQSEDEADYYCAAWDGSLNGLVFGGGTKLTVL GSRGGGGSGGGGSGGGGSLEMAEVQLVQSGAEVKKPGESLKISCKGSGYSFTSYW IGWVRQMPGKGLEWMGIIYPGDSDTRYSPSFQGHVTISADKSISTAYLQWSSLKA SDTAMYYCARYSGSFDNWGQGTLVTVSSESKYGPPCPPCPAPPVAGPSVFLFPPK PKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQFQSTYR VVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQE EMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSRLT VDKSRWQEGNVFSCSVMHEALHNHYTQKSLSLSLGKMFWVLVVVGGVLACYSLLV TVAFIIFWVKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCELRVKF SRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLY NELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR	anti-BCMA CAR
19	QSALTQPASVSASPGQSIAISCTGTSSDVGWYQQHPGKAPKLMIYEDSKRPSGVS NRFSGSKSGNTASLTISGLQAEDEADYYCSSNTRSSTLVFGGGTKLTVLGSRGGG GSGGGGSGGGGSLEMAEVQLVQSGAEMKKPGASLKLSCKASGYTFIDYYVYWMRQ APGQGLESMGWINPNSGGTNYAQKFQGRVTMTRDTSISTAYMELSRLRSDDTAMY YCARSQRDGYMDYWGQGTLVTVSSESKYGPPCPPCPAPPVAGPSVFLFPPKPKDT LMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQFQSTYRVVSV LTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTK NQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKS RWQEGNVFSCSVMHEALHNHYTQKSLSLSLGKMFWVLVVVGGVLACYSLLVTVAF IIFWVKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCELRVKFSRSA DAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQ KDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR	anti-BCMA CAR
20	QSALTQPASVSASPGQSIAISCTGTSSDVGWYQQHPGKAPKLMIYEDSKRPSGVS NRFSGSKSGNTASLTISGLQAEDEADYYCSSNTRSSTLVFGGGTKLTVLGSRGGG GSGGGGSGGGGSLEMAEVQLVQSGAEMKKPGASLKLSCKASGYTFIDYYVYWMRQ APGQGLESMGWINPNSGGTNYAQKFQGRVTMTRDTSISTAYMELSRLRSDDTAMY YCARSQRDGYMDYWGQGTLVTVSSESKYGPPCPPCPAPPVAGPSVFLFPPKPKDT LMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQFQSTYRVVSV LTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTK NQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKS RWQEGNVFSCSVMHEALHNHYTQKSLSLSLGKMFWVLVVVGGVLACYSLLVTVAF IIFWVRSKRSRLLHSDYMNMTPRRPGPTRKHYQPYAPPRDFAAYRSRVKFSRSAD APAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQK DKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR	anti-BCMA CAR
21	YFDSL	BCMA epitope

22	TGSSSDVGKYNLVS	BCMA-23 CDR-L1 (aa)
23	DVNKRPS	BCMA-23 CDR-L2 (aa)
24	SSYGGSRSYV	BCMA-23 CDR-L3 (aa)
25	ggctgattattattgtagctcatatggaggtagtaggtctt	BCMA-23 predicted splice acceptor site
26	ctactacatgagctggatccgccaggctccagggaaggggc	BCMA-23 predicted splice acceptor site
27	ctactatatgtcctggatcagacaggcacctggcaagggcc	BCMA-23 predicted splice acceptor site (O/SSE)
28	ggcagattactattgttctagctacggcggcagcagatcct	BCMA-23 predicted splice acceptor site (O/SSE)
29	EVQLVESGGGLVKPGGSLRLSCAASGFTFSDYYMSWIRQAPGKGLEWVSYISSSG STIYYADSVKGRFTISRDNAKNSLYLQMNSLRAEDTAVYYCAKVDGDYTEDYWGQ GTLVTVSSGGGGSGGGGSGGGGSQSALTQPASVSGSPGQSITISCTGSSSDVGKY NLVSWYQQPPGKAPKLIIYDVNKRPSGVSNRFSGSKSGNTATLTISGLQGDDEAD YYCSSYGGSRSYVFGTGTKVTVL	BCMA-23 scFv (aa)
30	GAAGTGCAGCTGGTGGAGTCTGGGGGAGGCTTGGTCAAGCCTGGAGGGTCCCTGA GACTCTCCTGTGCAGCCTCTGGATTCACCTTCAGTGACTACTACATGAGCTGGAT CCGCCAGGCTCCAGGGAAGGGGCTGGAGTGGGTTTCATACATTAGTAGTAGTGGT AGTACCATATACTACGCAGACTCTGTGAAGGGCCGATTCACCATCTCCAGGGACA ACGCCAAGAACTCACTGTATCTGCAAATGAACAGCCTGAGAGCCGAGGACACGGC CGTGTATTACTGTGCGAAAGTAGACGGAGACTACACAGAGGACTACTGGGGCCAG GGAACCCTGGTCACCGTCTCCTCAGGTGGAGGCGGTTCAGGCGGAGGTGGCTCTG GCGGTGGCGGATCGCAGTCTGCCCTGACTCAGCCTGCCTCCGTGTCTGGGTCTCC TGGACAGTCGATCACTATCTCCTGCACTGGAAGCAGCAGTGATGTTGGCAAATAT AATCTTGTCTCCTGGTACCAACAGCCCCCAGGCAAAGCCCCCAAGCTCATAATTT ATGACGTCAATAAGCGGCCCTCAGGGGTTTCTAATCGCTTCTCTGGCTCCAAGTC TGGCAACACGGCCACCCTGACAATCTCTGGGCTCCAGGGTGACGACGAGGCTGAT TATTATTGTAGCTCATATGGAGGTAGTAGGTCTTATGTCTTCGGAACTGGGACCA AGGTGACCGTCCTA	BCMA-23 scFv (nt)
31	gaggtgcagctggtggagtccggaggaggcctggtgaagccaggaggctccctga ggctgtcttgcgcagccagcggcttcacctttagcgactactatatgtcctggat cagacaggcacctggcaagggcctggagtgggtgagctacatcagctcctctggc tccacaatctactatgccgactctgtgaagggccggtttaccatcagcagagata acgccaagaattccctgtatctgcagatgaacagcctgagggccgaggacacagc cgtgtactattgcgccaaggtggacggcgattacaccgaggattattggggccag ggcacactggtgaccgtgagctccggcggcggcggctctggaggaggaggcagcg gcggaggaggctcccagtctgccctgacacagccagccagcgtgtccggctctcc cggacagtccatcacaatctcttgtaccggctctagctccgacgtgggcaagtac aacctggtgtcctggtatcagcagcccccctggcaaggcccctaagctgatcatct acgatgtgaacaagaggccatctggcgtgagcaatcgcttcagcggctccaagtc tggcaataccgccacactgaccatcagcggcctgcagggcgacgatgaggcagat tactattgttctagctacggcggcagcagatcctacgtgttcggcacaggcacca aggtgaccgtgctg	BCMA-23 scFv (nt)
32	EVQLVESGGGLVKPGGSLRLSCAASGFTFSDYYMSWIRQAPGKGLEWVSYISSSG STIYYADSVKGRFTISRDNAKNSLYLQMNSLRAEDTAVYYCAKVDGDYTEDYWGQ GTLVTVSS	BCMA-23 V_H Chain (aa)

33	QSALTQPASVSGSPGQSITISCTGSSSDVGKYNLVSWYQQPPGKAPKLIIYDVNK RPSGVSNRFSGSKSGNTATLTISGLQGDDEADYYCSSYGGSRSYVFGTGTKVTVL	BCMA-23 V_L Chain (aa)
34	DYYMS	BCMA-23, -26 CDR-H1 (aa) Kabat numbering
35	YISSSGSTIYYADSVKG	BCMA-23, -26 CDR-H2 (aa) Kabat numbering
36	VDGDYTEDY	BCMA-23 CDR-H3 (aa)
37	DYAMS	BCMA-25 CDR-H1 (aa) Kabat numbering
38	FIRSKAYGGTTEYAASVKG	BCMA-25 CDR-H2 (aa) Kabat numbering
39	WSAPTDY	BCMA-25 CDR-H3 (aa)
40	RASQGISNYLA	BCMA-25 CDR-L1 (aa)
41	SASTLQS	BCMA-25 CDR-L2 (aa)
42	QQSYTSRQT	BCMA-25 CDR-L3 (aa)
43	ctatgccatgtcctggttcaggcaggcaccaggcaagggcc	BCMA-25 predicted splice acceptor site
44	gtccgcctctgtgggcgatagggtgaccgtgacatgtcgcg	BCMA-25 predicted splice acceptor site
45	gtgggctttatccgctctaaggcctacggcggcaccacaga	BCMA-25 predicted splice acceptor site
46	gtgacatgtcgcgcctcccagggcatctctaactacctggc	BCMA-25 predicted splice acceptor site
47	tacagcgcctccaccctgcagagcggagtgccctcccggtt	BCMA-25 predicted splice acceptor site
48	ctatgccatgtcctggttcaagcaggcaccaggcaagggcc	BCMA-25 predicted splice acceptor site (O/SSE)
49	EVQLVQSGGGLVQPGRSLRLSCTASGFTFGDYAMSWFRQAPGKGLEWVGFIRSKA YGGTTEYAASVKGRFTISRDDSKSIAYLQMNSLKTEDTAVYYCAAWSAPTDYWGQ GTLVTVSSGGGGSGGGGSGGGGSDIQMTQSPAFLSASVGDRVTVTCRASQGISNY LAWYQQKPGNAPRLLIYSASTLQSGVPSRFRGTGYGTEFSLTIDSLQPEDFATYY CQQSYTSRQTFGPGTRLDIK	BCMA-25 scFv sequence (aa)
50	gaggtgcagctggtgcagagcggaggaggcctggtgcagcctggcaggtccctgc gcctgtcttgcaccgccagcggcttcacatttggcgactatgccatgtcctggtt caggcaggcaccaggcaagggcctggagtgggtgggctttatccgctctaaggcc tacggcggcaccacagagtatgccgccagcgtgaagggccggttcaccatcagcc gggacgactctaagagcatcgcctacctgcagatgaactctctgaagaccgagga cacagccgtgtactattgcgcagcatggagcgccccaaccgattattggggccag ggcaccctggtgacagtgagctccggcggcggcggctctggaggaggaggaagcg gaggaggaggatccgacatccagatgacacagtcccctgcctttctgtccgcctc	BCMA-25 scFv (nt)

	tgtgggcgatagggtgaccgtgacatgtcgcgcctcccagggcatctctaactac ctggcctggtatcagcagaagcccggcaatgcccctcggctgctgatctacagcg cctccaccctgcagagcggagtgccctcccggttcagaggaaccggctatggcac agagttttctctgaccatcgacagcctgcagccagaggatttcgccacatactat tgtcagcagtcttacaccagccggcagacatttggccccggcacaagactggata tcaag	
51	gaggtgcagctggtgcagagcggaggaggcctggtgcagcctggcaggtccctgc gcctgtcttgcaccgccagcggcttcacatttggcgactatgccatgtcctggtt caagcaggcaccaggcaagggcctggagtgggtgggctttatccgctctaaggcc tacggcggcaccacagagtatgccgccagcgtgaagggccggttcaccatcagcc gggacgactctaagagcatcgcctacctgcagatgaactctctgaagaccgagga cacagccgtgtactattgcgcagcatggagcgccccaaccgattattggggccag ggcaccctggtgacagtgagctccggcggcggcggctctggaggaggaggaagcg gaggaggaggatccgacatccagatgacacagtcccctgcctttctgtccgcctc tgtgggcgatagggtgaccgtgacatgtcgcgcctcccagggcatctctaactac ctggcctggtatcagcagaagcccggcaatgcccctcggctgctgatctacagcg cctccaccctgcagagcggagtgccctcccggttcagaggaaccggctatggcac agagttttctctgaccatcgacagcctgcagccagaggatttcgccacatactat tgtcagcagtcttacaccagccggcagacatttggccccggcacaagactggata tcaag	BCMA-25 scFv (nt) (O/SSE)
52	EVQLVQSGGGLVQPGRSLRLSCTASGFTFGDYAMSWFRQAPGKGLEWVGFIRSKA YGGTTEYAASVKGRFTISRDDSKSIAYLQMNSLKTEDTAVYYCAAWSAPTDYWGQ GTLVTVSS	BCMA-25 VH chain (aa)
53	DIQMTQSPAFLSASVGDRVTVTCRASQGISNYLAWYQQKPGNAPRLLIYSASTLQ SGVPSRFRGTGYGTEFSLTIDSLQPEDFATYYCQQSYTSRQTFGPGTRLDIK	BCMA-25 VL chain (aa)
54	VDGPPSFDI	BCMA-26 CDR-H3 (aa)
55	GANNIGSKSVH	BCMA-26 CDR-L1 (aa)
56	DDDDRPS	BCMA-26 CDR-L2 (aa)
57	HLWDRSRDHYV	BCMA-26 CDR-L3 (aa)
58	EVQLVESGGGLVKPGGSLRLSCAASGFTFSDYYMSWIRQAPGKGLEWVSYISSSG STIYYADSVKGRFTISRDNAKNSLYLQMNSLRAEDTAVYYCAKVDGPPSFDIWGQ GTMVTVSSGGGGSGGGGSGGGGSSYVLTQPPSVSVAPGQTARITCGANNIGSKSV HWYQQKPGQAPMLVVYDDDDRPSGIPERFSGSNSGNTATLTISGVEAGDEADYFC HLWDRSRDHYVFGTGTKLTVL	BCMA-26 scFv sequence (aa)
59	gaggtgcagctggtggagtccggaggaggcctggtgaagccaggaggctctctga ggctgagctgcgcagcctccggcttcacctttctgactactatatgagctggat caggcaggcaccaggcaagggcctggagtgggtgtcttacatcagctcctctggc agcacaatctactatgccgactccgtgaagggcaggttcaccatctctcgcgata acgccaagaatagcctgtatctgcagatgaactccctgcgggccgaggatacagc cgtgtactattgcgccaaggtggacggccccccttcctttgatatctggggccag ggcacaatggtgaccgtgagctccggaggaggaggatccggcggaggaggctctg gcggcggcggctctagctatgtgctgacccagccaccatccgtgtctgtggcacc tggacagacagcaaggatcacctgtggagcaaacaatatcggcagcaagtccgtg cactggtaccagcagaagcctggccaggccccaatgctggtggtgtatgacgatg acgatcggcccagcggcatccctgagagattttctggcagcaactccggcaatac cgccacactgaccatctctggagtggaggcaggcgacgaggcagattacttctgt cacctgtgggaccggagcagagatcactacgtgttcggcacaggcaccaagctga ccgtgctg	BCMA-26 scFv (nt)

60	gaggtgcagctggtggagtccggaggaggcctggtgaagccaggaggctctctga ggctgagctgcgcagcctccggcttcacctttctgactactatatgagctggat caggcaggcaccaggcaagggcctggagtgggtgtcttacatcagctcctctggc agcacaatctactatgccgactccgtgaagggcaggttcaccatctctcgcgata acgccaagaatagcctgtatctgcagatgaactccctgcgggccgaggatacagc cgtgtactattgcgccaaggtggacggcccccttcctttgatatctggggccag ggcacaatggtgaccgtgagctccggaggaggaggatccggcggaggaggctctg gcggcggcggctctagctatgtgctgacccagccaccatccgtgtctgtggcacc tggacagacagcaaggatcacctgtggagcaaacaatatcggcagcaagtccgtg cactggtaccagcagaagcctggccaggccccaatgctggtggtgtatgacgatg acgatcggcccagcggcatccctgagagattttctggcagcaactccggcaatac cgccacactgaccatctctggagtggaggcaggcgacgaggcagattacttctgt cacctgtgggaccggagcagagatcactacgtgttcggcacaggcaccaagctga ccgtgctg	BCMA-26 scFv (nt) (O/SSE)
61	EVQLVESGGGLVKPGGSLRLSCAASGFTFSDYYMSWIRQAPGKGLEWVSYISSSG STIYYADSVKGRFTISRDNAKNSLYLQMNSLRAEDTAVYYCAKVDGPPSFDIWGQ GTMVTVSS	BCMA-26 V_H Chain (aa)
62	SYVLTQPPSVSVAPGQTARITCGANNIGSKSVHWYQQKPGQAPMLVVYDDDDRPS GIPERFSGSNSGNTATLTISGVEAGDEADYFCHLWDRSRDHYVFGTGTKLTVL	BCMA-26 VL chain (aa)
63	GYSFTSYW	BCMA-52 CDR-H1 (aa)
64	GYSFTSYWIG	BCMA-52 CDR-H1 (aa) - AbM numbering
65	GYSFTSY	BCMA-52 CDR-H1 (aa) - Chothia numbering
66	SYWIG	BCMA-52 CDR-H1 (aa) - Kabat numbering
67	IYPGDSDT	BCMA-52 CDR-H2 (aa)
68	IIYPGDSDTR	BCMA-52 CDR-H2 (aa) - AbM numbering
69	YPGDSD	BCMA-52 CDR-H2 (aa) - Chothia numbering
70	IIYPGDSDTRYSPSFQG	BCMA-52 CDR-H2 (aa) - Kabat numbering
71	ARYSGSFDN	BCMA-52 CDR-H3 (aa)
72	YSGSFDN	BCMA-52 CDR-H3 (aa) - Kabat, Chothia, and AbM numbering
73	SSNIGSHS	BCMA-52 CDR-L1 (aa)

74	SGTSSNIGSHSVN	BCMA-52 CDR-L1 (aa) - Kabat, Chothia, and AbM numbering
75	TNN	BCMA-52 CDR-L2 (aa)
76	TNNQRPS	BCMA-52 CDR-L2 (aa) - Kabat, Chothia, and AbM numbering
77	AAWDGSLNGLV	BCMA-52 CDR-L3 (aa) - Kabat, Chothia, and AbM numbering
78	ctggccatcagtggcctccagtctgaggatgaggctgatta	BCMA-52 predicted splice acceptor site
79	agatacagcccgtccttccaaggccacgtcaccatctcagc	BCMA-52 predicted splice acceptor site
80	ctggctatttctggactgcagagcgaggacgaggccgacta	BCMA-52 predicted splice acceptor site (O/SSE)
81	agatacagccctagctttcagggccacgtgaccatcagcgc	BCMA-52 predicted splice acceptor site (O/SSE)
82	tcctatgagctgactcagccaccctcagcgtctgggacccccgggcagagggtca ccatgtcttgttctggaaccagctccaacatcggaagtcactctgtaaactggta ccagcagctcccaggaacggcccccaaactcctcatctatactaataatcagcgg ccctcaggggtccctgaccgattctctggctccaagtctggcacctcagcctccc tggccatcagtggcctccagtctgaggatgaggctgattattactgtgcagcatg ggatggcagcctgaatggtctggtattcggcggagggaccaagctgaccgtccta ggttctagaggtggtggtggtagcggcggcggcggctctggtggtggtggatccc tcgagatggccgaggtgcagctggtgcagtctggagcagaggtgaaaaagcccgg ggagtctctgaagatctcctgtaagggttctggatacagctttaccagctactgg atcggctgggtgcgccagatgcccgggaaaggcctggagtggatggggatcatct atcctggtgactctgataccagatacagcccgtccttccaaggccacgtcaccat ctcagctgacaagtccatcagcactgcctacctgcagtggagcagcctgaaggcc tcggacaccgccatgtattactgtgcgcgctactctggttctttcgataactggg gtcaaggtactctggtgaccgtctcctca	BCMA-52 scFv
83	SYELTQPPSASGTPGQRVTMSCSGTSSNIGSHSVNWYQQLPGTAPKLLIYTNNQR PSGVPDRFSGSKSGTSASLAISGLQSEDEADYYCAAWDGSLNGLVFGGGTKLTVL GSRGGGGSGGGGSGGGGSLEMAEVQLVQSGAEVKKPGESLKISCKGSGYSFTSYW IGWVRQMPGKGLEWMGIIYPGDSDTRYSPSFQGHVTISADKSISTAYLQWSSLKA SDTAMYYCARYSGSFDNWGQGTLVTVSS	BCMA-52 scFv (aa)
84	agctatgagctgacacagcctccaagcgcctctggcacacctggacagcgagtga caatgagctgtagcggcaccagcagcaacatcggcagccacagcgtgaactggta tcagcagctgcctggcacagcccctaaactgctgatctacaccaacaaccagcgg cctagcggcgtgcccgatagattttctggcagcaagagcggcacaagcgccagcc tggctatttctggactgcagagcgaggacgaggccgactattattgtgccgcctg ggacggctctctgaacggccttgtttttggcggaggcaccaagctgacagtgctg ggatctagaggtggcggaggatctggcggcggaggaagcggaggcggcggatctc ttgaaatggctgaagtgcagctggtgcagtctggcgccgaagtgaagaagcctgg	BCMA-52 scFv (nt) (O/SSE)

	cgagagcctgaagatcagctgcaaaggcagcggctacagcttcaccagctactgg atcggctgggtccgacagatgcctggcaaaggccttgagtggatgggcatcatct accccggcgacagcgacaccagatacagccctagctttcagggccacgtgaccat cagcgccgacaagtctatcagcaccgcctacctgcagtggtccagcctgaaggcc tctgacaccgccatgtactactgcgccagatactctggcagcttcgacaattggg gccagggcacactggtcaccgtgtccagc	
85	EVQLVQSGAEVKKPGESLKISCKGSGYSFTSYWIGWVRQMPGKGLEWMGIIYPGD SDTRYSPSFQGHVTISADKSISTAYLQWSSLKASDTAMYYCARYSGSFDNWGQGT LVTVSS	BCMA-52 VH chain (aa)
86	gaggtgcagctggtgcagtctggagcagaggtgaaaaagcccggggagtctctga agatctcctgtaagggttctggatacagctttaccagctactggatcggctgggt gcgccagatgcccgggaaaggcctggagtggatggggatcatctatcctggtgac tctgataccagatacagcccgtccttccaaggccacgtcaccatctcagctgaca agtccatcagcactgcctacctgcagtggagcagcctgaaggcctcggacaccgc catgtattactgtgcgcgctactctggttctttcgataactggggtcaaggtact ctggtgaccgtctcctcagc	BCMA-52 VH chain (nt)
87	gaagtgcagctggtgcagtctggcgccgaagtgaagaagcctggcgagagcctga agatcagctgcaaaggcagcggctacagcttcaccagctactggatcggctgggt ccgacagatgcctggcaaaggccttgagtggatgggcatcatctaccccggcgac agcgacaccagatacagccctagctttcagggccacgtgaccatcagcgccgaca agtctatcagcaccgcctacctgcagtggtccagcctgaaggcctctgacaccgc catgtactactgcgccagatactctggcagcttcgacaattggggccagggcaca ctggtcaccgtgtccagc	BCMA-52 VH chain (nt) (O/SSE)
88	SYELTQPPSASGTPGQRVTMSCSGTSSNIGSHSVNWYQQLPGTAPKLLIYTNNQR PSGVPDRFSGSKSGTSASLAISGLQSEDEADYYCAAWDGSLNGLVFGGGTKLTVL G	BCMA-52 VL chain (aa)
89	tcctatgagctgactcagccaccctcagcgtctgggacccccgggcagagggtca ccatgtcttgttctggaaccagctccaacatcggaagtcactctgtaaactggta ccagcagctcccaggaacggcccccaaactcctcatctatactaataatcagcgg ccctcaggggtccctgaccgattctctggctccaagtctggcacctcagcctccc tggccatcagtggcctccagtctgaggatgaggctgattattactgtgcagcatg gatggcagcctgaatggtctggtattcggcggagggaccaagctgaccgtccta ggt	BCMA-52 VL chain (nt)
90	agctatgagctgacacagcctccaagcgcctctggcacacctggacagcgagtga caatgagctgtagcggcaccagcagcaacatcggcagccacagcgtgaactggta tcagcagctgcctggcacagcccctaaactgctgatctacaccaacaaccagcgg cctagcggcgtgcccgatagattttctggcagcaagagcggcacaagcgccagcc tggctatttctggactgcagagcgaggacgaggccgactattattgtgccgcctg ggacggctctctgaacggccttgtttttggcggaggcaccaagctgacagtgctg gga	BCMA-52 VL chain (nt) (O/SSE)
91	QNEYF	BCMA-52-scFV-mFc BCMA binding epitope 1
92	CIPCQL	BCMA-52-scFV-mFc BCMA binding epitope 2
93	CQRYC	BCMA-52-scFV-mFc BCMA binding epitope 3
94	GYTFIDYY	BCMA-55 CDR-H1 (aa)

95	GYTFIDYYVY	BCMA-55 CDR-H1 (aa) - AbM numbering
96	GYTFIDY	BCMA-55 CDR-H1 (aa) - Chothia numbering
97	DYYVY	BCMA-55 CDR-H1 (aa) - Kabat numbering
98	INPNSGGT	BCMA-55 CDR-H2 (aa)
99	WINPNSGGTN	BCMA-55 CDR-H2 (aa) - AbM numbering
100	NPNSGG	BCMA-55 CDR-H2 (aa) - Chothia numbering
101	WINPNSGGTNYAQKFQG	BCMA-55 CDR-H2 (aa) - Kabat numbering
102	ARSQRDGYMDY	BCMA-55 CDR-H3 (aa)
103	SQRDGYMDY	BCMA-55 CDR-H3 (aa) - Kabat, Chothia, and AbM numbering
104	ISCTGTSSD	BCMA-55 CDR-L1 (aa)
105	TGTSSDVG	BCMA-55 CDR-L1 (aa) - Kabat, Chothia, and AbM numbering
106	EDS	BCMA-55 CDR-L2 (aa)
107	EDSKRPS	BCMA-55 CDR-L2 (aa) - Kabat, Chothia, and AbM numbering
108	SSNTRSSTLV	BCMA-55 CDR-L3 (aa) - Kabat, Chothia, and AbM numbering
109	gccctcaggggtttctaatcgcttctctggctccaagtctg	BCMA-55 predicted splice acceptor site
110	cgaggctgattattactgcagctcaaatacaagaagcagca	BCMA-55 predicted splice acceptor site

111	cgaggccgattactactgcagcagcaacacccggtccagca	BCMA-55 predicted splice acceptor site (O/SSE)
112	gcccagcggcgtgtccaatagattcagcggcagcaagagcg	BCMA-55 predicted splice acceptor site (O/SSE)
113	caatctgccctgactcagcctgcctccgtgtctgcgtctcctggacagtcgatcg ccatctcctgcactggaaccagcagtgacgttggttggtatcaacagcacccagg caaagcccccaaactcatgatttatgaggacagtaagcggccctcaggggtttct aatcgcttctctggctccaagtctggcaacacggcctccctgaccatctctgggc tccaggctgaggacgaggctgattattactgcagctcaaatacaagaagcagcac tttggtgttcggcggagggaccaagctgaccgtcctaggttctagaggtggtggt ggtagcggcggcggcggctctggtggtggtggatccctcgagatggccgaagtgc agctggtgcagtctggggctgagatgaagaagcctggggcctcactgaagctctc ctgcaaggcttctggatacaccttcatcgactactatgtatactggatgcgacag gcccctggacaagggcttgagtccatgggatggatcaaccctaacagtggtggca caaactatgcacagaagtttcagggcagggtcaccatgaccagggacacgtccat cagcacagcctacatggagctgagcaggctgagatctgacgacaccgccatgtat tactgtgcgcgctcccagcgtgacggttacatggattactggggtcaaggtactc tggtgaccgtctcctca	BCMA-55 scFv
114	QSALTQPASVSASPGQSIAISCTGTSSDVGWYQQHPGKAPKLMIYEDSKRPSGVS NRFSGSKSGNTASLTISGLQAEDEADYYCSSNTRSSTLVFGGGTKLTVLGSRGGG GSGGGGSGGGGSLEMAEVQLVQSGAEMKKPGASLKLSCKASGYTFIDYYVYWMRQ APGQGLESMGWINPNSGGTNYAQKFQGRVTMTRDTSISTAYMELSRLRSDDTAMY YCARSQRDGYMDYWGQGTLVTVSS	BCMA-55 scFv (aa)
115	cagtctgccctgacacagcctgccagcgttagtgctagtcccggacagtctatcg ccatcagctgtaccggcaccagctctgacgttggctggtatcagcagcaccctgg caaggcccctaagctgatgatctacgaggacagcaagaggcccagcggcgtgtcc aatagattcagcggcagcaagagcggcaacaccgccagcctgacaattagcggac tgcaggccgaggacgaggccgattactactgcagcagcaacacccggtccagcac actggtttttggcggaggcaccaagctgacagtgctgggatctagaggtggcgga ggatctggcggcggaggaagcggaggcggcggatctcttgaaatggctgaagtgc agctggtgcagtctggcgccgagatgaagaaacctggcgcctctctgaagctgag ctgcaaggccagcggctacaccttcatcgactactacgtgtactggatgcggcag gcccctggacagggactcgaatctatgggctggatcaaccccaatagcggcggca ccaattacgcccagaaattccagggcagagtgaccatgaccagagacaccagcat cagcaccgcctacatggaactgagccggctgagatccgacgacaccgccatgtac tactgcgccagatctcagcgcgacggctacatggattattggggccagggaaccc tggtcaccgtgtccagc	BCMA-55 scFv (nt) (O/SSE)
116	EVQLVQSGAEMKKPGASLKLSCKASGYTFIDYYVYWMRQAPGQGLESMGWINPNS GGTNYAQKFQGRVTMTRDTSISTAYMELSRLRSDDTAMYYCARSQRDGYMDYWGQ GTLVTVSS	BCMA-55 V_H chain (aa)
117	gaagtgcagctggtgcagtctggggctgagatgaagaagcctggggcctcactga agctctcctgcaaggcttctggatacaccttcatcgactactatgtatactggat gcgacaggcccctggacaagggcttgagtccatgggatggatcaaccctaacagt ggtggcacaaactatgcacagaagtttcagggcagggtcaccatgaccagggaca cgtccatcagcacagcctacatggagctgagcaggctgagatctgacgacaccgc catgtattactgtgcgcgctcccagcgtgacggttacatggattactggggtcaa ggtactctggtgaccgtctcctca	BCMA-55 V_H chain (nt)
118	gaagtgcagctggtgcagtctggcgccgagatgaagaaacctggcgcctctctga agctgagctgcaaggccagcggctacaccttcatcgactactacgtgtactggat gcggcaggcccctggacagggactcgaatctatgggctggatcaaccccaatagc ggcggcaccaattacgcccagaaattccagggcagagtgaccatgaccagagaca ccagcatcagcaccgcctacatggaactgagccggctgagatccgacgacaccgc	BCMA-55 V_H chain (nt) (O/SSE)

	catgtactactgcgccagatctcagcgcgacggctacatggattattggggccag ggaaccctggtcaccgtgtccagc	
119	QSALTQPASVSASPGQSIAISCTGTSSDVGWYQQHPGKAPKLMIYEDSKRPSGVS NRFSGSKSGNTASLTISGLQAEDEADYYCSSNTRSSTLVFGGGTKLTVLG	BCMA-55 V_L chain (aa)
120	caatctgccctgactcagcctgcctccgtgtctgcgtctcctggacagtcgatcg ccatctcctgcactggaaccagcagtgacgttggttggtatcaacagcacccagg caaagccccaaactcatgatttatgaggacagtaagcggccctcaggggtttct aatcgcttctctggctccaagtctggcaacacggcctccctgaccatctctgggc tccaggctgaggacgaggctgattattactgcagctcaaatacaagaagcagcac tttggtgttcggcggagggaccaagctgaccgtccta	BCMA-55 V_L chain (nt)
121	cagtctgccctgacacagcctgccagcgttagtgctagtcccggacagtctatcg ccatcagctgtaccggcaccagctctgacgttggctggtatcagcagcaccctgg caaggcccctaagctgatgatctacgaggacagcaagaggcccagcggcgtgtcc aatagattcagcggcagcaagagcggcaacaccgccagcctgacaattagcggac tgcaggccgaggacgaggccgattactactgcagcagcaacacccggtccagcac actggtttttggcggaggcaccaagctgacagtgctg	BCMA-55 V_L chain (nt) (O/SSE)
122	MLMAG	BCMA-55-scFv-mFc BCMA binding epitope 1
123	YFDSLL	BCMA-55-scFv-mFc BCMA binding epitope 2
124	QLRCSSNTPPL	BCMA-55-scFv-mFc BCMA binding epitope 3
125	QIQLVQSGPELKKPGETVKISCKASGYTFTDYSINWVKRAPGKGLKWMGWINTET REPAYAYDFRGRFAFSLETSASTAYLQINNLKYEDTATYFCALDYSYAMDYWGQG TSVTVSS	BCMA-C1 V_H Chain (aa)
126	QIQLVQSGPELKKPGETVKISCKASGYTFTDYSINWVKRAPGKGLKWMGWINTET REPAYAYDFRGRFAFSLETSASTAYLQINNLKYEDTATYFCALDYSYAMDYWGQG TSVTVSSGGGGSGGGGSGGGGSDIVLTQSPPSLAMSLGKRATISCRASESVTILG SHLIHWYQQKPGQPPTLLIQLASNVQTGVPARFSGSGSRTDFTLTIDPVEEDDVA VYYCLQSRTIPRTFGGGTKLEIK	BCMA-C1 V_H-V_L scFv (aa)
127	DIVLTQSPPSLAMSLGKRATISCRASESVTILGSHLIHWYQQKPGQPPTLLIQLA SNVQTGVPARFSGSGSRTDFTLTIDPVEEDDVAVYYCLQSRTIPRTFGGGTKLEI K	BCMA-C1 V_L Chain (aa)
128	DIVLTQSPPSLAMSLGKRATISCRASESVTILGSHLIHWYQQKPGQPPTLLIQLA SNVQTGVPARFSGSGSRTDFTLTIDPVEEDDVAVYYCLQSRTIPRTFGGGTKLEI KGGGGSGGGGSGGGGSQIQLVQSGPELKKPGETVKISCKASGYTFTDYSINWVKR APGKGLKWMGWINTETREPAYAYDFRGRFAFSLETSASTAYLQINNLKYEDTATY FCALDYSYAMDYWGQGTSVTVSS	BCMA-C1 V_L-V_H scFv (aa)
129	QIQLVQSGPDLKKPGETVKLSCKASGYTFTNFGMNWVKQAPGKGFKWMAWINTYT GESYFADDFKGRFAFSVETSATTAYLQINNLKTEDTATYFCARGEIYYGYDGGFA YWGQGTLVTVSAGGGGSGGGGSGGGGSDVVMTQSHRFMSTSVGDRVSITCRASQD VNTAVSWYQQKPGQSPKLLIFSASYRYTGVPDRFTGSGSGADFTLTISSVQAEDL AVYYCQQHYSTPWTFGGGTKLDIK	BCMA-C2 V_H-V_L scFv (aa)
130	DVVMTQSHRFMSTSVGDRVSITCRASQDVNTAVSWYQQKPGQSPKLLIFSASYRY TGVPDRFTGSGSGADFTLTISSVQAEDLAVYYCQQHYSTPWTFGGGTKLDIKGGG GSGGGGSGGGGSQIQLVQSGPDLKKPGETVKLSCKASGYTFTNFGMNWVKQAPGK GFKWMAWINTYTGESYFADDFKGRFAFSVETSATTAYLQINNLKTEDTATYFCAR GEIYYGYDGGFAYWGQGTLVTVSA	BCMA-C2 V_L-V_H scFv (aa)

131	QIQLVQSGPDLKKPGETVKLSCKASGYTFTNFGMNWVKQAPGKGFKWMAWINTYT GESYFADDFKGRFAFSVETSATTAYLQINNLKTEDTATYFCARGEIYYGYDGGFA YWGQGTLVTVSA	BCMA-C2 V_H Chain (aa)
132	DVVMTQSHRFMSTSVGDRVSITCRASQDVNTAVSWYQQKPGQSPKLLIFSASYRY TGVPDRFTGSGSGADFTLTISSVQAEDLAVYYCQQHYSTPWTFGGGTKLDIK	BCMA-C2 V_L Chain (aa)
133	QNEYFDSLL	BCMA epitope
134	IEVMYPPPYLDNEKSNGTIIHVKGKHLCPSPLFPGPSKP	CD28 ectodomain spacer (aa)
135	attgaagttatgtatcctcctccttacctagacaatgagaagagcaatggaacca ttatccatgtgaaagggaaacacctttgtccaagtcccctatttcccggaccttc taagccc	CD28 ectodomain spacer (nt)
136	RSKRSRLLHSDYMNMTPRRPGPTRKHYQPYAPPRDFAAYRS	CD28 endo (aa)
137	aggagtaagaggagcaggctcctgcacagtgactacatgaacatgactccccgcc gccccgggcccacccgcaagcattaccagccctatgccccaccacgcgacttcgc agcctatcgctcc	CD28 endo (nt)
138	MFWVLVVVGGVLACYSLLVTVAFIIFWV	CD28 transmembrane domain (aa)
139	atgttttgggtgctggtcgtggtcggaggggtgctggcctgttacagcctgctgg tgacagtcgctttcatcatcttctgggtg	CD28 transmembrane domain (nt)
140	atgttctgggtgctcgtggtcgttggcggagtgctggcctgttacagcctgctgg ttaccgtggccttcatcatcttttgggtc	CD28 transmembrane domain (nt)
141	aggggtgctggcctgttacagcctgctggtgacagtcgctt	CD28TM predicted splice acceptor site
142	MPLLLLLPLLWAGALA	CD33 signal peptide
143	RVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQ EGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALP PR	CD3-zeta derived intracellular signaling domain (aa)
144	agagtcaagttttccaggtccgccgacgctccagcctaccagcaggggcagaacc agctgtacaacgagctgaacctgggcagaagggaagagtacgacgtcctggataa gcggagaggccgggaccctgagatgggcggcaagcctcggcggaagaacccccag gaaggcctgtataacgaactgcagaaagacaagatggccgaggcctacagcgaga tcggcatgaagggcgagcggaggcggggcaagggccacgacggcctgtatcaggg cctgtccaccgccaccaaggatacctacgacgccctgcacatgcaggccctgccc ccaagg	CD3-zeta derived intracellular signaling domain (nt)
145	agagtgaagttcagcagatccgccgacgctccagcctatcagcagggccaaaacc agctgtacaacgagctgaacctggggagaagagaagagtacgacgtgctggataa gcggagaggcagagatcctgaaatgggcggcaagcccagacggaagaatcctcaa gagggcctgtataatgagctgcagaaagacaagatggccgaggcctacagcgaga tcggaatgaagggcgagcgcagaagaggcaagggacacgatggactgtaccaggg cctgagcaccgccaccaaggatacctatgacgcactgcacatgcaggccctgcca cctaga	CD3-zeta derived intracellular signaling domain (nt)
146	MALPVTALLLPLALLLHA	CD8 alpha signal peptide
147	MLQMARQCSQNEYFDSLLHDCKPCQLRCSSTPPLTCQRYCNASMTNSVKGMNAIL WTCLGLSLIISLAVFVLTFLLRKMSSEPLKDEFKNTGSGLLGMANIDLEKGRTGD EIVLPRGLEYTVEECTCEDCIKNKPKVDSDHCFPLPAMEEGATILVTTKTNDYCN SLSAALSVTEIEKSISAR	Cynomolgus BCMA; GenBank No. EHH60172.1

148	QCTNYALLKLAGDVESNPGP	E2A peptide (aa)
149	GSGQCTNYALLKLAGDVESNPGP	E2A peptide (aa)
150	ctttttcgcaacgggtttgc	EF1a/HTLV promoter forward primer
151	ggatctgcgatcgctccggtgcccgtcagtgggcagagcgcacatcgcccacagt ccccgagaagttgggggggaggggtcggcaattgaaccggtgcctagagaaggtgg cgcggggtaaactgggaaagtgatgtcgtgtactggctccgccttttttcccgagg gtgggggagaaccgtatataagtgcagtagtcgccgtgaacgttctttttcgcaa cgggtttgccgccagaacacagctgaagcttcgaggggctcgcatctctccttca cgcgcccgccgccctacctgaggccgccatccacgccggttgagtcgcgttctgc cgcctcccgcctgtggtgcctcctgaactgcgtccgccgtctaggtaagtttaaa gctcaggtcgagaccgggcctttgtccggcgctcccttggagcctacctagactc agccggctctccacgctttgcctgaccctgcttgctcaactctacgtctttgttt cgttttctgttctgcgccgttacagatccaagctgtgaccggcgcctac	EF1alpha promoter with HTLV1 enhancer
152	VKQTLNFDLLKLAGDVESNPGP	F2A peptide (aa)
153	GSGVKQTLNFDLLKLAGDVESNPGP	F2A peptide (aa)
154	MLLLVTSLLLCELPHPAFLLIP	GMCSFR alpha chain signal peptide
155	atgcttctcctggtgacaagccttctgctctgtgagttaccacacccagcattcc tcctgatccca	GMCSFR alpha chain signal sequence
156	ESKYGPPCPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEV QFNWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGL PSSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWES NGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNHYTQ KSLSLSLGK	Hinge-C_H2-C_H3 spacer (aa)
157	ESKYGPPCPPCPGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWES NGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNHYTQ KSLSLSLGK	Hinge-C_H3 spacer (aa)
158	LLHACIPCQLR	human BCMA epitope (residues 17-27)
159	CIPCQLR	human BCMA epitope (residues 21-27)
160	SNTPPLTCQR	human BCMA epitope (residues 30-39)
161	SVTNSVK	human BCMA epitope (residues 44-50)
162	CSQNEYF	human BCMA epitope (residues 8-15)
163	MLQMAGQCSQNEYFDSLLHACIPCQLRCSSNTPPLTCQRYCNARSGLLGMANIDL EKSRTGDEIILPRGLEYTVEECTCEDCIKSKPKVDSDHCFPLPAMEEGATILVTT KTNDYCKSLPAALSATEIEKSISAR	Human BCMA Variant; GenBank No. ABN42510.1

164	MLQMAGQCSQNEYFDSLLHACIPCQLRCSSNTPPLTCQRYCNASVTNSVKGTNAI LWTCLGLSLIISLAVFVLMFLLRKISSEPLKDEFKNTGSGLLGMANIDLEKSRTG DEIILPRGLEYTVEECTCEDCIKSKPKVDSDHCFPLPAMEEGATILVTTKTNDYC KSLPAALSATEIEKSISAR	Human BCMA; GenBank No. BAB60895.1
165	MLQMAGQCSQNEYFDSLLHACIPCQLRCSSNTPPLTCQRYCNASVTNSVKGTNAI LWTCLGLSLIISLAVFVLMFLLRKINSEPLKDEFKNTGSGLLGMANIDLEKSRTG DEIILPRGLEYTVEECTCEDCIKSKPKVDSDHCFPLPAMEEGATILVTTKTNDYC KSLPAALSATEIEKSISAR	Human BCMA; NCBI No. NP_001183.2
166	MVLQTQVFISLLLWISGAYG	human IgG -kappa signal peptide(aa)
167	atggtgctgcagacccaggtgttcatcagcctgctgctgtggatctccggagcat acgga	human IgG -kappa signal sequence (nt)
168	atggtgctgcagacacaggtgttcatcagcctgctgctgtggatctccggagcat acgga	human IgG -kappa signal sequence (nt)
169	atggtgctgcagacccaggtgttcatcagcctgctgctgtggatctctggcgcct acggc	human IgG -kappa signal sequence (nt)
170	atggtgctgcagacccaggtgttcatcagcctgctgctgtggatctctggcgcct atgga	human IgG -kappa signal sequence (nt)
171	atggtgctgcagacacaggtgttcatctccctgctgctgtggatctctggagcat acgga	human IgG -kappa signal sequence (nt)
172	ASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPA VLQSSGLYSLSSVVTVPSSNFGTQTYTCNVDHKPSNTKVDKTVERKCCVECPPCP APPVAGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVQFNWYVDGVEVHN AKTKPREEQFNSTFRVVSVLTVVHQDWLNGKEYKCKVSNKGLPAPIEKTISKTKG QPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDISVEWESNGQPENNYKTTPP MLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK	Human IgG2 Fc (Uniprot P01859)
173	ASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPA VLQSSGLYSLSSVVTVPSSSLGTKTYTCNVDHKPSNTKVDKRVESKYGPPCPSCP APEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVH NAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAK GQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTP PVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNHYTQKSLSLSLGK	Human IgG4 Fc (Uniprot P01861)
174	ESKYGPPCPPCPAPPVAGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQ FNWYVDGVEVHNAKTKPREEQFQSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLP SSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESN GQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNHYTQK SLSLSLGK	Modified IgG4 hinge- IgG2/IgG4 C_H2- IgG4 C_H3 spacer (aa)
175	gaatctaagtacggaccgccctgccctccctgccctgctcctcctgtggctggac caagcgtgttcctgtttccacctaagcctaaagataccctgatgatttcccgcac acctgaagtgacttgcgtggtcgtggacgtgagccaggaggatccagaagtgcag ttcaactggtacgtggacggcgtggaagtccacaatgctaagactaaaccccgag aggaacagtttcagtcaacttaccgggtcgtgagcgtgctgaccgtcctgcatca ggattggctgaacgggaaggagtataagtgcaaagtgtctaataagggactgcct agctccatcgagaaaacaattagtaaggcaaaagggcagcctcgagaaccacagg tgtataccctgcccctagccaggaggaaatgaccaagaaccaggtgtccctgac atgtctggtcaaaggcttctatccaagtgacatcgccgtggagtgggaatcaaat gggcagcccgagaacaattacaagaccacaccacccgtgctggactctgatggaa gtttctttctgtattccaggctgaccgtggataaatctcgctggcaggagggcaa cgtgttctcttgcagtgtcatgcacgaagccctgcacaatcattatacacagaag tcactgagcctgtccctgggcaaa	Modified IgG4 hinge- IgG2/IgG4 C_H2- IgG4 C_H3 spacer (nt)
176	GSTSGSGKPGSGEGSTKG	Linker (aa)
177	tttatttagtctccagaaaaaggggggaatgaaagaccccacctgtaggtttggc aagctaggatcaaggttaggaacagagagacagcagaatatgggccaaacaggat	MND promoter

	atctgtggtaagcagttcctgccccggctcagggccaagaacagttggaacagca gaatatgggccaaacaggatatctgtggtaagcagttcctgccccggctcagggc caagaacagatggtccccagatgcggtcccgccctcagcagtttctagagaacca tcagatgtttccagggtgccccaaggacctgaaatgaccctgtgccttatttgaa ctaaccaatcagttcgcttctcgcttctgttcgcgcgcttctgctccccgagctc aataaaagagccca	
178	ggatctgcgatcgctccggtgcccgtcagtgggcagagcgcacatcgcccacagt ccccgagaagttgggggggaggggtcggcaattgaaccggtgcctagagaaggtgg cgcggggtaaactgggaaagtgatgtcgtgtactggctccgccttttcccgagg gtgggggagaaccgtatataagtgcagtagtcgccgtgaacgttcttttcgcaa cgggtttgccgccagaacacagctgaagcttcgaggggctcgcatctctccttca cgcgcccgccgccctacctgaggccgccatccacgccggttgagtcgcgttctgc cgcctcccgcctgtggtgcctcctgaactgcgtccgccgtctaggtaagtttaaa gctcaggtcgagaccgggcctttgtccggcgctcccttggagcctacctagactc agccggctctccacgctttgcctgaccctgcttgctcaactctacgtctttgttt cgttttctgttctgcgccgttacagatccaagctgtgaccggcgcctacggctag cgcc	modified EF1 alpha promoter
179	MAQQCFHSEYFDSLLHACKPCHLRCSNPPATCQPYCDPSVTSSVKGTYTVLWIFL GLTLVLSLALFTISFLLRKMNPEALKDEPQSPGQLDGSAQLDKADTELTRIRAGD DRIFPRSLEYTVEECTCEDCVKSKPKGDSDHFFPLPAMEEGATILVTTKTGDYGK SSVPTALQSVMGMEKPTHTR	Mouse BCMA; NCBI No. NP_035738.1
180	cagtttcttcctgtatagtagactcaccgtggataaatcaa	Optimized splice acceptor site
181	gggcaacgtgttcagctgcagcgtgatgcacgaggccctgc	Optimized splice acceptor site
182	cggagtgctggcctgttacagcctgctggttaccgtggcct	Optimized splice acceptor site
183	gctgagagtgaagttcagcagatccgccgacgctccagcct	Optimized splice acceptor site
184	acacctccactggatccccaagagctggatatcctgaaaac	Optimized splice acceptor site
185	accggattcctcctgatccaagcctggccagagaacagaac	Optimized splice acceptor site
186	acggccagtttagcctggctgtggtgtctctgaacatcacc	Optimized splice acceptor site
187	aagtttctttctgtattccagactgaccgtggataaatctc	Optimized splice acceptor site
188	cgccttgtcctccttgtcccgctcctcctgttgccggacct	optimized splice acceptor site
189	agtctaaatacggac	Optimized splice donor site
190	tcaactggtatgtgg	Optimized splice donor site
191	accatctccaaggcc	Optimized splice donor site
192	gccccaggtttacac	Optimized splice donor site
193	tcagcagatccgccg	Optimized splice donor site

194	ctcctgtgtgaactc	Optimized splice donor site
195	tcggaaagtgtgcaa	Optimized splice donor site
196	cagcacggccagttt	Optimized splice donor site
197	aaccggggcgagaac	Optimized splice donor site
198	ctggaaggcgagccc	Optimized splice donor site
199	tgttcatgtgagcgg	Optimized splice donor site (last 4 nt outside of coding region)
200	gagtctaaatacggaccgccttgtcctccttgtcccgctcctcctgttgccggac cttccgtgttcctgtttcctccaaagcctaaggacaccctgatgatcagcaggac ccctgaagtgacctgcgtggtggtggatgtgtcccaagaggatcccgaggtgcag ttcaactggtatgtggacggcgtggaagtgcacaacgccaagaccaagcctagag aggaacagttccagagcacctacagagtggtgtccgtgctgacagtgctgcacca ggattggctgaacggcaaagagtacaagtgcaaggtgtccaacaagggcctgcct agcagcatcgagaaaaccatctccaaggccaagggccagccaagagagccccagg tttacacactgcctccaagccaagaggaaatgaccaagaatcaggtgtccctgac atgcctggtcaagggcttctacccctccgatatcgccgtggaatgggagagcaat ggccagcctgagaacaactacaagaccacacctcctgtgctggacagcgacggca gtttcttcctgtatagtagactcaccgtggataaatcaagatggcaagagggcaa cgtgttcagctgcagcgtgatgcacgaggccctgcacaaccactacacccagaaa agcctgagcctgtctctgggcaag	optimized SSE modified IgG4 hinge- IgG2/IgG4 C_H2- IgG4 C_H3 spacer (nt)
201	ATNFSLLKQAGDVEENPGP	P2A peptide (aa)
202	GSGATNFSLLKQAGDVEENPGP	P2A peptide (aa)
203	cgccttgtcctccttgtccagctcctcctgttgccggacct	predicted splice acceptor site
204	cagtttcttcctgtatagtagactcaccgtggataaatcaa	predicted splice acceptor site
205	accggattcctcctgattcaggcctggccagagaacagaac	predicted splice acceptor site
206	cgtctaggtaagttt	Predicted splice donor site
207	gaccaaggtgaccgt	Predicted splice donor site
208	tgcactggtaccagc	Predicted splice donor site
209	taaactggtaccagc	Predicted splice donor site
210	atctcctgtaagggt	Predicted splice donor site
211	ggtcaaggtactctg	Predicted splice donor site
212	gaggacagtaagcgg	Predicted splice donor site

213	ggtcaaggtactctg	Predicted splice donor site
214	tgcctccgtgtctgc	Predicted splice donor site
215	caccaaggtgaccgt	Predicted splice donor site
216	tgaactggtatcagc	Predicted splice donor site
217	atctcttgaaatggt	Predicted splice donor site
218	ggccagggcacactg	Predicted splice donor site
219	gaggacagcaagagg	Predicted splice donor site
220	ggccagggaaccctg	Predicted splice donor site
221	tgccagcgttagtgc	Predicted splice donor site
222	aatctaagtacggac	Predicted splice donor site
223	tcaactggtacgtgg	Predicted splice donor site
224	acaattagtaaggca	Predicted splice donor site
225	accacaggtgtatac	Predicted splice donor site
226	tttccaggtccgccg	Predicted splice donor site
227	ctgctctgtgagtta	Predicted splice donor site
228	acgcaaagtgtgtaa	Predicted splice donor site
229	caacatggtcagttt	Predicted splice donor site
230	aacagaggtgaaaac	Predicted splice donor site
231	ctggagggtgagcca	Predicted splice donor site
232	tggctccgccttttttcccgagggtgggggagaaccgtatat	promoter predicted splice acceptor site
233	tgaactgcgtccgccgtctaggtaagtttaaagctcaggtc	promoter predicted splice acceptor site
234	ttctgttctgcgccgttacagatccaagctgtgaccggcgc	promoter predicted splice acceptor site
235	gatatcgaattcctgcagcc	Reverse primer just 5′ of WPRE
236	GAGTCTAAATACGGACCGCCTTGTCCTCCTTGTCCAGCTCCTCCTGTTGCCGGAC CTTCCGTGTTCCTGTTTCCTCCAAAGCCTAAGGACACCCTGATGATCAGCAGGAC	Spacer – codon optimized (nt)

	CCCTGAAGTGACCTGCGTGGTGGTGGATGTGTCCCAAGAGGATCCCGAGGTGCAG TTCAATTGGTACGTGGACGGCGTGGAAGTGCACAACGCCAAGACCAAGCCTAGAG AGGAACAGTTCCAGAGCACCTACAGAGTGGTGTCCGTGCTGACAGTGCTGCACCA GGATTGGCTGAACGGCAAAGAGTACAAGTGCAAGGTGTCCAACAAGGGCCTGCCT AGCAGCATCGAGAAAACCATCTCCAAGGCCAAGGGCCAGCCAAGAGAGCCCCAGG TTTACACACTGCCTCCAAGCCAAGAGGAAATGACCAAGAATCAGGTGTCCCTGAC ATGCCTGGTCAAGGGCTTCTACCCCTCCGATATCGCCGTGGAATGGGAGAGCAAT GGCCAGCCTGAGAACAACTACAAGACCACACCTCCTGTGCTGGACAGCGACGGCA GTTTCTTCCTGTATAGTAGACTCACCGTGGATAAATCAAGATGGCAAGAGGGCAA CGTGTTCAGCTGCAGCGTGATGCACGAGGCCCTGCACAACCACTACACCCAGAAA AGCCTGAGCCTGTCTCTGGGCAAA	
237	ESKYGPPCPPCP	Spacer (IgG4hinge) (aa)
238	gaatctaagtacggaccgccctgccccccttgccct	Spacer (IgG4hinge) (nt)
239	aagtttctttctgtattccaggctgaccgtggataaatctc	spacer predicted splice acceptor site
240	gggcaacgtgttctcttgcagtgtcatgcacgaagccctgc	spacer predicted splice acceptor site
241	EGRGSLLTCGDVEENPGP	T2A peptide (aa)
242	GSGEGRGSLLTCGDVEENPGP	T2A peptide (aa)
243	LEGGGEGRGSLLTCGDVEENPGPR	T2A peptide (aa)
244	ctcgagggcggcggagagggcagaggaagtcttctaacatgcggtgacgtggagg agaatcccggccctagg	T2A peptide (nt)
245	cttgaaggtggtggcgaaggcagaggcagcctgcttacatgcggagatgtggaag agaaccccggacctaga	T2A peptide (nt)
246	MLLLVTSLLLCELPHPAFLLIPRKVCNGIGIGEFKDSLSINATNIKHFKNCTSIS GDLHILPVAFRGDSFTHTPPLDPQELDILKTVKEITGFLLIQAWPENRTDLHAFE NLEIIRGRTKQHGQFSLAVVSLNITSLGLRSLKEISDGDVIISGNKNLCYANTIN WKKLFGTSGQKTKIISNRGENSCKATGQVCHALCSPEGCWGPEPRDCVSCRNVSR GRECVDKCNLLEGEPREFVENSECIQCHPECLPQAMNITCTGRGPDNCIQCAHYI DGPHCVKTCPAGVMGENNTLVWKYADAGHVCHLCHPNCTYGCTGPGLEGCPTNGP KIPSIATGMVGALLLLLVVALGIGLFM	truncated EGFR (tEGFR) sequence (aa)
247	atgcttctcctggtgacaagccttctgctctgtgagttaccacacccagcattcc tcctgatcccacgcaaagtgtgtaacggaataggtattggtgaatttaaagactc actctccataaatgctacgaatattaaacacttcaaaaactgcacctccatcagt ggcgatctccacatcctgccggtggcatttaggggtgactccttcacacatactc ctcctctggatccacaggaactggatattctgaaaaccgtaaaggaaatcacagg gtttttgctgattcaggcttggcctgaaaacaggacggacctccatgcctttgag aacctagaaatcatacgcggcaggaccaagcaacatggtcagttttctcttgcag tcgtcagcctgaacataacatccttgggattacgctccctcaaggagataagtga tggagatgtgataatttcaggaaacaaaaatttgtgctatgcaaatacaataaac tggaaaaaactgtttgggacctccggtcagaaaaccaaaattataagcaacagag gtgaaaacagctgcaaggccacaggccaggtctgccatgccttgtgctcccccga gggctgctggggcccggagcccagggactgcgtctcttgccggaatgtcagccga ggcagggaatgcgtggacaagtgcaaccttctggagggtgagccaagggagtttg tggagaactctgagtgcatacagtgccacccagagtgcctgcctcaggccatgaa catcacctgcacaggacggggaccagacaactgtatccagtgtgcccactacatt gacggcccccactgcgtcaagacctgcccggcaggagtcatgggagaaaacaaca ccctggtctggaagtacgcagacgccggccatgtgtgccacctgtgccatccaaa ctgcacctacggatgcactgggccaggtcttgaaggctgtccaacgaatgggcct aagatcccgtccatcgccactgggatggtgggggccctcctcttgctgctggtgg tggccctggggatcggcctcttcatgtga	truncated EGFR (tEGFR) sequence (nt)

248	atgctgctcctcgtgacaagcctgctcctgtgtgaactccctcatccagctttc tgctcattcctcggaaagtgtgcaacggcatcggcatcggagagttcaaggacag cctgagcatcaatgccaccaacatcaagcacttcaagaattgcaccagcatcagc ggcgacctgcacattctgcctgtggcctttagaggcgacagcttcacccacacac ctccactggatccccaagagctggatatcctgaaaaccgtgaaagagattaccgg attcctcctgatccaagcctggccagagaacagaaccgatctgcacgccttcgag aacctcgagatcatcagaggccggaccaaacagcacggccagtttagcctggctg tggtgtctctgaacatcaccagtctgggcctgagaagcctgaaagaaatctccga cggcgacgtgatcatctccggaaacaagaacctgtgctacgccaacaccatcaac tggaagaagctgttcggcacctccggccagaaaacaaagatcatctctaaccggg gcgagaacagctgcaaggccaccggacaagtttgtcacgccctgtgtagccctga aggctgttggggacccgaacctagagactgtgtgtcctgccggaatgtgtcccgg ggcagagaatgtgtggataagtgcaacctgctggaaggcgagccccgcgagtttg tggaaaacagcgagtgcatccagtgtcaccccgagtgtctgccccaggccatgaa cattacatgcaccggcagaggccccgacaactgtattcagtgcgcccactacatc gacggccctcactgcgtgaaaacatgtccagctggcgtgatgggagagaacaaca ccctcgtgtggaagtatgccgacgccggacatgtgtgccacctgtgtcaccctaa ttgcacctacggctgtaccggacctggcctggaaggatgccctacaaacggccct aagatccccagcattgccaccggaatggttggagccctgctgcttctgttggtgg tggccctcggaatcggcctgttcatgtga	truncated EGFR (tEGFR) sequence (nt) (O/SSE)
249	actcctcctctggatccacaggaactggatattctgaaaac	truncated marker predicted splice acceptor site
250	acagggtttttgctgattcaggcttggcctgaaaacaggac	truncated marker predicted splice acceptor site
251	atggtcagttttctcttgcagtcgtcagcctgaacataaca	truncated marker predicted splice acceptor site
252	tcttcatgtgagcgg	truncated marker predicted splice donor site
253	aatcaacctctggattacaaaatttgtgaaagattgactggtattcttaactatg ttgctccttttacgctatgtggatacgctgctttaatgcctttgtatcatgctat tgcttcccgtatggctttcattttctcctccttgtataaatcctggttgctgtct ctttatgaggagttgtggcccgttgtcaggcaacgtggcgtggtgtgcactgtgt ttgctgacgcaacccccactggttggggcattgccaccacctgtcagctcctttc cgggactttcgctttcccctccctattgccacggcggaactcatcgccgcctgc cttgcccgctgctggacaggggctcggctgttgggcactgacaattccgtggtgt tgtcggggaaatcatcgtcctttccttggctgctcgcctgtgttgccacctggat tctgcgcgggacgtccttctgctacgtcccttcggccctcaatccagcggacctt ccttcccgcggcctgctgccggctctgcggcctcttccgcgtcttcgccttcgcc ctcagacgagtcggatctccctttgggccgcctccccgc	Woodchuck Hepatitis Virus (WHP) Posttranscriptional Regulatory Element (WPRE)
254	tcaattggtacgtgg	predicted splice site
255	SRGGGGSGGGGSGGGGSLEMA	Linker (aa)

CLAIMS

WHAT IS CLAIMED:-

1. A method of treating a subject having or suspected of having multiple myeloma (MM), the method comprising administering to the subject a dose of engineered T cells comprising a chimeric antigen receptor (CAR), the CAR comprising:

(a) an extracellular antigen-binding domain, comprising:

a variable heavy chain (V_H) comprising a heavy chain complementarity determining region 1 (CDR-H1), a heavy chain complementarity determining region 2 (CDR-H2) and a heavy chain complementarity determining region 3 (CDR-H3) contained within the sequence set forth in SEQ ID NO: 116 and a variable light chain (V_L) comprising a light chain complementarity determining region 1 (CDR-L1), a light chain complementarity determining region 2 (CDR-L2) and a light chain complementarity determining region 3 (CDR-L3) contained within the sequence set forth in SEQ ID NO: 119;

a V_H comprising a CDR-H1, a CDR-H2 and a CDR-H3 sequences set forth in SEQ ID NOS:97, 101 and 103, respectively, and a V_L comprising a CDR-L1, a CDR-L2 and a CDR-L3 sequences set forth in SEQ ID NOS:105, 107 and 108, respectively;

a V_H comprising a CDR-H1, a CDR-H2 and a CDR-H3 sequences set forth in SEQ ID NOS:96, 100 and 103, respectively, and a V_L comprising a CDR-L1, a CDR-L2 and a CDR-L3 sequences set forth in SEQ ID NOS:105, 107 and 108, respectively;

a V_H comprising a CDR-H1, a CDR-H2 and a CDR-H3 sequences set forth in SEQ ID NOS:95, 99 and 103, respectively, and a V_L comprising a CDR-L1, a CDR-L2 and a CDR-L3 sequences set forth in SEQ ID NOS: 105, 107 and 108, respectively;

a V_H comprising a CDR-H1, a CDR-H2 and a CDR-H3 sequences set forth in SEQ ID NOS:94, 98 and 102, respectively, and a V_L comprising a CDR-L1, a CDR-L2 and a CDR-L3 sequences set forth in SEQ ID NOS: 104, 106 and 108, respectively; or

a V_H comprising the amino acid sequence of SEQ ID NO: 116 and a V_L comprising the amino acid sequence of SEQ ID NO: 119;

(b) a spacer comprising an IgG4/2 chimeric hinge or a modified IgG4 hinge; an IgG2/4 chimeric C_H2 region; and an IgG4 C_H3 region, which optionally is about 228 amino acids in length; or a spacer set forth in SEQ ID NO: 174;

(c) a transmembrane domain, optionally a transmembrane domain from a human CD28; and

(d) an intracellular signaling region comprising a cytoplasmic signaling domain of a CD3-zeta (CD3ζ) chain and a costimulatory signaling region comprising an intracellular signaling domain of a T cell

costimulatory molecule or a signaling portion thereof;

wherein, prior to the administration, the subject has received a lymphodepleting therapy comprising the administration of fludarabine at or about 20-40 mg/m^2 body surface area of the subject, optionally at or about 30 mg/m^2, daily, for 2-4 days, and/or cyclophosphamide at or about 200-400 mg/m^2 body surface area of the subject, optionally at or about 300 mg/m^2, daily, for 2-4 days.

2. A method of treating a subject having or suspected of having multiple myeloma (MM), the method comprising administering to the subject a dose of engineered T cells comprising a chimeric antigen receptor (CAR), the CAR comprising:

(a) an extracellular antigen-binding domain, comprising:

a variable heavy chain (V_H) comprising a heavy chain complementarity determining region 1 (CDR-H1), a heavy chain complementarity determining region 2 (CDR-H2) and a heavy chain complementarity determining region 3 (CDR-H3) contained within the sequence set forth in SEQ ID NO: 116 and a variable light chain (V_L) comprising a light chain complementarity determining region 1 (CDR-L1), a light chain complementarity determining region 2 (CDR-L2) and a light chain complementarity determining region 3 (CDR-L3) contained within the sequence set forth in SEQ ID NO: 119;

a V_H comprising a CDR-H1, a CDR-H2 and a CDR-H3 sequences set forth in SEQ ID NOS:97, 101 and 103, respectively, and a V_L comprising a CDR-L1, a CDR-L2 and a CDR-L3 sequences set forth in SEQ ID NOS:105, 107 and 108, respectively;

a V_H comprising a CDR-H1, a CDR-H2 and a CDR-H3 sequences set forth in SEQ ID NOS:96, 100 and 103, respectively, and a V_L comprising a CDR-L1, a CDR-L2 and a CDR-L3 sequences set forth in SEQ ID NOS:105, 107 and 108, respectively;

a V_H comprising a CDR-H1, a CDR-H2 and a CDR-H3 sequences set forth in SEQ ID NOS:95, 99 and 103, respectively, and a V_L comprising a CDR-L1, a CDR-L2 and a CDR-L3 sequences set forth in SEQ ID NOS: 105, 107 and 108, respectively;

a V_H comprising a CDR-H1, a CDR-H2 and a CDR-H3 sequences set forth in SEQ ID NOS:94, 98 and 102, respectively, and a V_L comprising a CDR-L1, a CDR-L2 and a CDR-L3 sequences set forth in SEQ ID NOS: 104, 106 and 108, respectively; or

a V_H comprising the amino acid sequence of SEQ ID NO: 116 and a V_L comprising the amino acid sequence of SEQ ID NO: 119;

(b) a spacer comprising an IgG4/2 chimeric hinge or a modified IgG4 hinge; an IgG2/4 chimeric C_H2 region; and an IgG4 C_H3 region, which optionally is about 228 amino acids in length; or a spacer set forth in SEQ ID NO: 174;

(c) a transmembrane domain, optionally a transmembrane domain from a human CD28; and

(d) an intracellular signaling region comprising a cytoplasmic signaling domain of a CD3-zeta (CD3ζ) chain and a costimulatory signaling region comprising an intracellular signaling domain of a T cell costimulatory molecule or a signaling portion thereof; wherein at or prior to the administration of the dose of engineered T cells, the subject has received three or more therapies selected from among:

autologous stem cell transplant (ASCT);

an immunomodulatory agent;

a proteasome inhibitor; and

an anti-CD38 antibody; unless the subject was not a candidate for or was contraindicated for one or more of the therapies.

3. A method of treating a subject having or suspected of having multiple myeloma (MM), the method comprising administering to the subject a dose of engineered T cells comprising a chimeric antigen receptor (CAR), the CAR comprising:

(a) an extracellular antigen-binding domain, comprising:

a variable heavy chain (V_H) comprising a heavy chain complementarity determining region 1 (CDR-H1), a heavy chain complementarity determining region 2 (CDR-H2) and a heavy chain complementarity determining region 3 (CDR-H3) contained within the sequence set forth in SEQ ID NO: 116 and a variable light chain (V_L) comprising a light chain complementarity determining region 1 (CDR-L1), a light chain complementarity determining region 2 (CDR-L2) and a light chain complementarity determining region 3 (CDR-L3) contained within the sequence set forth in SEQ ID NO: 119;

a V_H comprising a CDR-H1, a CDR-H2 and a CDR-H3 sequences set forth in SEQ ID NOS:97, 101 and 103, respectively, and a V_L comprising a CDR-L1, a CDR-L2 and a CDR-L3 sequences set forth in SEQ ID NOS:105, 107 and 108, respectively;

a V_H comprising a CDR-H1, a CDR-H2 and a CDR-H3 sequences set forth in SEQ ID NOS:96, 100 and 103, respectively, and a V_L comprising a CDR-L1, a CDR-L2 and a CDR-L3 sequences set forth in SEQ ID NOS:105, 107 and 108, respectively;

a V_H comprising a CDR-H1, a CDR-H2 and a CDR-H3 sequences set forth in SEQ ID NOS:95, 99 and 103, respectively, and a V_L comprising a CDR-L1, a CDR-L2 and a CDR-L3 sequences set forth in SEQ ID NOS: 105, 107 and 108, respectively;

a V_H comprising a CDR-H1, a CDR-H2 and a CDR-H3 sequences set forth in SEQ ID NOS:94, 98 and 102, respectively, and a V_L comprising a CDR-L1, a CDR-L2 and a CDR-L3 sequences set forth in SEQ ID NOS: 104, 106 and 108, respectively; or

a V_H comprising the amino acid sequence of SEQ ID NO: 116 and a V_L comprising the amino acid sequence of SEQ ID NO: 119;

(b) a spacer comprising an IgG4/2 chimeric hinge or a modified IgG4 hinge; an IgG2/4 chimeric C_H2 region; and an IgG4 C_H3 region, which optionally is about 228 amino acids in length; or a spacer set forth in SEQ ID NO: 174;

(c) a transmembrane domain, optionally a transmembrane domain from a human CD28; and

(d) an intracellular signaling region comprising a cytoplasmic signaling domain of a CD3-zeta (CD3ζ) chain and a costimulatory signaling region comprising an intracellular signaling domain of a T cell costimulatory molecule or a signaling portion thereof; wherein at the time of administration of the dose of engineered T cells, the subject has not had an active or a history of plasma cell leukemia (PCL).

4. A method of treating a subject having or suspected of having multiple myeloma (MM), the method comprising administering to the subject a dose of engineered T cells comprising a chimeric antigen receptor (CAR), the CAR comprising:

(a) an extracellular antigen-binding domain, comprising:

a variable heavy chain (V_H) comprising a heavy chain complementarity determining region 1 (CDR-H1), a heavy chain complementarity determining region 2 (CDR-H2) and a heavy chain complementarity determining region 3 (CDR-H3) contained within the sequence set forth in SEQ ID NO: 116 and a variable light chain (V_L) comprising a light chain complementarity determining region 1 (CDR-L1), a light chain complementarity determining region 2 (CDR-L2) and a light chain complementarity determining region 3 (CDR-L3) contained within the sequence set forth in SEQ ID NO: 119;

a V_H comprising a CDR-H1, a CDR-H2 and a CDR-H3 sequences set forth in SEQ ID NOS:97, 101 and 103, respectively, and a V_L comprising a CDR-L1, a CDR-L2 and a CDR-L3 sequences set forth in SEQ ID NOS:105, 107 and 108, respectively;

a V_H comprising a CDR-H1, a CDR-H2 and a CDR-H3 sequences set forth in SEQ ID NOS:96, 100 and 103, respectively, and a V_L comprising a CDR-L1, a CDR-L2 and a CDR-L3 sequences set forth in SEQ ID NOS:105, 107 and 108, respectively;

a V_H comprising a CDR-H1, a CDR-H2 and a CDR-H3 sequences set forth in SEQ ID NOS:95, 99 and 103, respectively, and a V_L comprising a CDR-L1, a CDR-L2 and a CDR-L3 sequences set forth in SEQ ID NOS: 105, 107 and 108, respectively;

a V_H comprising a CDR-H1, a CDR-H2 and a CDR-H3 sequences set forth in SEQ ID NOS:94, 98 and 102, respectively, and a V_L comprising a CDR-L1, a CDR-L2 and a CDR-L3 sequences set forth in SEQ ID NOS: 104, 106 and 108, respectively; or

a V_H comprising the amino acid sequence of SEQ ID NO: 116 and a V_L comprising the amino acid sequence of SEQ ID NO: 119;

(b) a spacer comprising an IgG4/2 chimeric hinge or a modified IgG4 hinge; an IgG2/4 chimeric C_H2 region; and an IgG4 C_H3 region, which optionally is about 228 amino acids in length; or a spacer set forth in SEQ ID NO: 174;

(c) a transmembrane domain, optionally a transmembrane domain from a human CD28; and

(d) an intracellular signaling region comprising a cytoplasmic signaling domain of a CD3-zeta (CD3ζ) chain and a costimulatory signaling region comprising an intracellular signaling domain of a T cell costimulatory molecule or a signaling portion thereof; wherein the dose of engineered T cells comprises:

between at or about 1 x 10^7 CAR-expressing (CAR+) T cells and at or about 2 x 10^9 CAR+ T cells;

a combination of $CD4^+$ T cells and $CD8^+$ T cells, at a defined ratio of $CD4^+$ CAR+ T cells to $CD8^+$ CAR+ T cells and/or of $CD4^+$ T cells to $CD8^+$ T cells, that is or is approximately 1:1 or is between at or approximately 1:3 and at or approximately 3:1; and

less than 25%, 20%, 15%, 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2% or 1% of the CAR+ T cells in the dose express a marker of apoptosis, optionally Annexin V or active Caspase 3.

5. The method of any of claims 1-4, wherein the extracellular antigen-binding domain binds to a B cell maturation antigen (BCMA).

6. The method of any of claims 1-5, wherein the V_H is or comprises the amino acid sequence of SEQ ID NO: 116; and the V_L is or comprises the amino acid sequence of SEQ ID NO: 119.

7. The method of any of claims 1-6, wherein the extracellular antigen-binding domain comprises an scFv.

8. The method of any of claims 1-7, wherein the V_H and the V_L are joined by a flexible linker.

9. The method of claim 8, wherein the scFv comprises a linker comprising the amino acid sequence GGGGSGGGGSGGGGS (SEQ ID NO:1).

10. The method of any of claims 1-9, wherein the V_H is carboxy-terminal to the V_L.

11. The method of any of claims 1-10, wherein the extracellular antigen-binding domain

comprises the amino acid sequence of SEQ ID NO: 114 or an amino acid sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to the amino acid sequence of SEQ ID NO: 114.

12. The method of any of claims 1-11, wherein the extracellular antigen-binding domain comprises the amino acid sequence of SEQ ID NO: 114.

13. The method of any of claims 1-12, wherein a nucleic acid encoding the extracellular antigen-binding domain comprises (a) the sequence of nucleotides of SEQ ID NO:113; (b) a sequence of nucleotides that has at least 90% sequence identity thereto; or (c) a degenerate sequence of (a) or (b).

14. The method of any of claims 1-13, wherein the nucleic acid encoding the extracellular antigen-binding domain comprises the sequence of nucleotides of SEQ ID NO:115.

15. The method of any of claims 1-9, wherein the V_H is amino-terminal to the V_L.

16. The method of any of claims 1-15, wherein the cytoplasmic signaling domain is or comprises the sequence set forth in SEQ ID NO:143 or a sequence of amino acids that has at least 90% sequence identity to SEQ ID NO:143.

17. The method of any of claims 1-16, wherein the costimulatory signaling region comprises an intracellular signaling domain of CD28, 4-1BB, or ICOS, or a signaling portion thereof.

18. The method of any of claims 1-17, wherein the costimulatory signaling region comprises an intracellular signaling domain of 4-1BB, optionally human 4-1BB.

19. The method of any of claims 1-18, wherein the costimulatory signaling region is or comprises the sequence set forth in SEQ ID NO:4 or a sequence of amino acids that has at least 90% sequence identity to the sequence set forth in SEQ ID NO: 4.

20. The method of any of claims 1-19, wherein the costimulatory signaling region is between the transmembrane domain and the cytoplasmic signaling domain of a CD3-zeta (CD3ζ) chain.

21. The method of any of claims 1-20, wherein the transmembrane domain is or comprises a transmembrane domain from human CD28.

22. The method of any of claims 1-21, wherein the transmembrane domain is or comprises the sequence set forth in SEQ ID NO:138 or a sequence of amino acids that has at least 90% sequence identity to SEQ ID NO:138.

23. The method of any of claims 1-22, wherein the CAR comprises from its N to C terminus in order: the extracellular antigen-binding domain, the spacer, the transmembrane domain and the intracellular signaling region.

24. The method of any of claims 1-23, wherein the CAR comprises

(a) an extracellular antigen-binding domain, comprising:

a variable heavy chain (V_H) comprising a heavy chain complementarity determining region 1 (CDR-H1), a heavy chain complementarity determining region 2 (CDR-H2) and a heavy chain complementarity determining region 3 (CDR-H3) contained within the sequence set forth in SEQ ID NO: 116 and a variable light chain (V_L) comprising a light chain complementarity determining region 1 (CDR-L1), a light chain complementarity determining region 2 (CDR-L2) and a light chain complementarity determining region 3 (CDR-L3) contained within the sequence set forth in SEQ ID NO: 119;

(b) a spacer comprising a modified IgG4 hinge; an IgG2/4 chimeric C_H2 region; and an IgG4 C_H3 region, that is about 228 amino acids in length;

(c) a transmembrane domain from a human CD28; and

(d) an intracellular signaling region comprising a cytoplasmic signaling domain of a CD3-zeta (CD3ζ) chain and a costimulatory signaling region comprising an intracellular signaling domain of a 4-1BB.

25. The method of any of claims 1-14 and 16-24, wherein the CAR comprises

(a) an extracellular antigen-binding domain, comprising the sequence set forth in SEQ ID NO: 114 or a sequence of amino acids having at least 90% sequence identity to the amino acid sequence of SEQ ID NO: 114;

(b) a spacer comprising the sequence set forth in SEQ ID NO: 174 or a sequence of amino acids that has at least 90% sequence identity to SEQ ID NO:174;

(c) a transmembrane domain comprising the sequence set forth in SEQ ID NO:138 or a sequence of amino acids that has at least 90% sequence identity to SEQ ID NO:138; and

(d) an intracellular signaling region comprising a cytoplasmic signaling comprising the sequence set forth in SEQ ID NO:143 or a sequence of amino acids that has at least 90% sequence identity to SEQ ID NO:143 and a costimulatory signaling region comprising the sequence set forth in SEQ ID NO:4 or a sequence of amino acids that has at least 90% sequence identity to the sequence set forth in SEQ ID NO: 4.

26. The method of any of claims 1-14 and 16-25, wherein the CAR comprises

(a) an extracellular antigen-binding domain, comprising the sequence set forth in SEQ ID NO: 114;

(b) a spacer comprising the sequence set forth in SEQ ID NO: 174;

(c) a transmembrane domain comprising the sequence set forth in SEQ ID NO:138; and

(d) an intracellular signaling region comprising a cytoplasmic signaling comprising the sequence set forth in SEQ ID NO:143 and a costimulatory signaling region comprising the sequence set forth in SEQ ID NO:4.

27. The method of any of claims 1-14 and 16-26, wherein the CAR comprises the sequence set forth in SEQ ID NO:19.

28. The method of any of claims 1-27, wherein following expression of a polynucleotide encoding the CAR in a human cell, optionally a human T cell, the transcribed RNA, optionally messenger RNA (mRNA), from the polynucleotide, exhibits at least 70%, 75%, 80%, 85%, 90%, or 95% RNA homogeneity.

29. The method of any of claims 1-14 and 16-28, wherein the CAR is encoded by a polynucleotide sequence comprising the sequence set forth in SEQ ID NO: 13 or a sequence that exhibits at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity thereto.

30. The method of any of claims 1-14 and 16-29, wherein the CAR is encoded by a polynucleotide sequence comprising the sequence set forth in SEQ ID NO: 13.

31. The method of any of claims 1-30, wherein the binding of the extracellular antigen-binding domain and/or the CAR, or a measure indicative of function or activity of the CAR following exposure to cells expressing surface BCMA, is not reduced or blocked or is not substantially reduced or blocked in the presence of a soluble or shed form of BCMA.

32. The method of claim 31, wherein the concentration or amount of the soluble or shed form of the BCMA corresponds to a concentration or amount present in serum or blood or plasma of the subject or of a multiple myeloma patient, or on average in a multiple myeloma patient population, or at a concentration or amount of the soluble or shed BCMA at which the binding or measure is reduced or blocked, or is substantially reduced or blocked, for cells expressing a reference anti-BCMA recombinant receptor, optionally a reference anti-BCMA CAR, in the same assay.

33. The method of any of claims 1-3 and 5-32, wherein the dose of engineered T cells comprises between at or about 1 x 10^7 CAR+ T cells and at or about 2 x 10^9 CAR+ T cells.

34. The method of any of claims 1-33, wherein the dose of engineered T cells is at or about 5 x 10^7 cells or CAR+ T cells.

35. The method of any of claims 1-33, wherein the dose of engineered T cells is at or about 1.5 x 10^8 cells or CAR+ T cells.

36. The method of any of claims 1-33, wherein the dose of engineered T cells is at or about 3 x 10^8 cells or CAR+ T cells.

37. The method of any of claims 1-33, wherein the dose of engineered T cells is at or about 4.5 x 10^8 cells or CAR+ T cells.

38. The method of any of claims 1-33, wherein the dose of engineered T cells is at or about 6 x 10^8 cells or CAR+ T cells.

39. The method of any of claims 1-38, wherein the dose of engineered T cells comprises a combination of $CD4^+$ T cells and $CD8^+$ T cells.

40. The method of any of claims 1-39, wherein the dose of engineered T cells comprise a combination of $CD4^+$ CAR+ T cells and $CD8^+$ CAR+ T cells.

41. The method of claim 38 or 39, wherein the ratio of $CD4^+$ CAR+ T cells to $CD8^+$ CAR+ T

cells and/or of $CD4^+$ T cells to $CD8^+$ T cells is or is approximately 1:1 or is between at or approximately 1:3 and at or approximately 3:1.

42. The method of any of claims 1-41, wherein the dose of engineered T cells comprises $CD3^+$ CAR+ T cells.

43. The method of any of claims 1-3 and 5-42, wherein less than at or about 25%, 20%, 15%, 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2% or 1% of the CAR+ T cells in the dose of engineered T cells express a marker of apoptosis, optionally Annexin V or active Caspase 3.

44. The method of any of claims 1-43, wherein less than at or about 5%, 4%, 3%, 2% or 1% of the CAR+ T cells in the dose of engineered T cells express Annexin V or active Caspase 3.

45. The method of any of claims 2-44, wherein prior to the administration, the subject has received a lymphodepleting therapy comprising the administration of fludarabine at or about 20-40 mg/m^2 body surface area of the subject, optionally at or about 30 mg/m^2, daily, for 2-4 days, and/or cyclophosphamide at or about 200-400 mg/m^2 body surface area of the subject, optionally at or about 300 mg/m^2, daily, for 2-4 days.

46. The method of any of claims 1-44, wherein prior to the administration, the subject has received a lymphodepleting therapy comprising the administration of fludarabine at or about 20-40 mg/m^2 body surface area of the subject, optionally at or about 30 mg/m^2, daily, for 2-4 days.

47. The method of any of claims 1-44, wherein prior to the administration, the subject has received a lymphodepleting therapy comprising the administration of cyclophosphamide at or about 200-400 mg/m^2 body surface area of the subject, optionally at or about 300 mg/m^2, daily, for 2-4 days.

48. The method of any of claims 1-47, wherein the subject has received a lymphodepleting therapy comprising the administration of fludarabine at or about 30 mg/m^2 body surface area of the subject, daily, and cyclophosphamide at or about 300 mg/m^2 body surface area of the subject, daily, for 3 days.

49. The method of any of claims 1-48, wherein the subject has or is suspected of having a relapsed or refractory multiple myeloma (R/R MM).

50. The method of any of claims 1 and 3-49, wherein at or prior to the administration of the dose of cells, the subject has received three or more prior therapies for the disease or disorder, optionally four or more prior therapies, optionally selected from among:

autologous stem cell transplant (ASCT);

an immunomodulatory agent;

a proteasome inhibitor; and

an anti-CD38 antibody.

51. The method of any of claims 1-50, wherein at or prior to the administration of the dose of cells, the subject has received three or more prior therapies for the disease or disorder selected from among:

autologous stem cell transplant (ASCT);

an immunomodulatory agent or a proteasome inhibitor, or a combination thereof; and

an anti-CD38 antibody.

52. The method of any of claims 2 and 5-51, wherein the subject has relapsed or been refractory following the three or more prior therapies.

53. The method of any of claims 2 and 5-52, wherein the immunomodulatory agent is selected from among thalidomide, lenalidomide and pomalidomide.

54. The method of any of claims 2 and 5-53, wherein the proteasome inhibitor is selected from among bortezomib, carfilzomib and ixazomib.

55. The method of any of claims 2 and 5-54, wherein the anti-CD38 antibody is or comprises daratumumab.

56. The method of any of claims 1, 2 and 4-55, wherein at the time of the administration of the dose of cells, and/or at the time of lymphodepleting chemotherapy or leukapheresis, the subject has not had an active or a history of plasma cell leukemia (PCL).

57. The method of any of claims 1-56, wherein at the time of the administration of the dose of cells the subject has developed secondary plasma cell leukemia (PCL).

58. The method of any of claims 1-57, wherein, at the time of administration, the subject has relapsed or has been refractory following at least 3 or at least 4 prior therapies for multiple myeloma.

59. The method of any of claims 1-58, wherein, at the time of administration, the subject is an adult subject or is 25 or 35 years of age or older.

60. The method of any of claims 1-59, wherein, at the time of administration, the subject has a time from diagnosis of multiple myeloma of approximately 4 years or between 2 and 15 years or between 2 and 12 years.

61. The method of any of claims 1-60, wherein, at the time of administration, the subject has received about 10 or between 3 and 15 or between 4 and 15 prior regimens for multiple myeloma.

62. The method of any of claims 1-61, wherein, at the time of administration, the subject has been refractory to or not responded to bortezomib, carfilzomib, lenalidomide, pomalidomide and/or an anti-CD38 monoclonal antibody.

63. The method of any of claims 1-62, wherein, at the time of administration, the subject has had prior autologous stem cell transplant

64. The method of any of claims 1-62, wherein, at the time of administration, the subject has not had prior autologous stem cell transplant.

65. The method of any of claims 1-64, wherein, at the time of administration, the subject has IMWG high risk cytogenetics.

66. The method of any of claims 1-65, wherein the method is capable of achieving a specified response or outcome, optionally at a designated timepoint following initiation of the administration, in at least one of or in at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, or at least 95% of subjects in the cohort of subjects having the disease or disorder, wherein:

the response is selected from the group consisting of objective response (OR), complete response (CR), stringent complete response (sCR), very good partial response (VGPR), partial response (PR) and minimal response (MR);

the response or outcome is or comprises an OR; and/or

the response or outcome is or comprises a CR.

67. The method of claim 66, wherein the cohort of subjects has at least the same number of prior therapies, prognosis or prognostic factor, sub-type, secondary involvement or other specified patient characteristic or characteristics, as the subject treated by the method.

68. The method of claim 66 or 67, wherein the response or outcome is or comprises an OR and is achieved in at least 50%, 60%, 70%, or 80% of subjects of the cohort.

69. The method of claim 66 or 67, wherein the response or outcome is or comprises a VGPR, a CR or an sCR and is achieved in at least 40%, 45% or 50% of subjects of the cohort.

70. The method of claim 66 or 67, wherein the response or outcome is or comprises a CR or an sCR and is achieved in at least 20%, 30%, or 40% of subjects of the cohort.

71. The method of any of claims 66-70, wherein the response or outcome is durable for greater than at or about 3, 6, 9 or 12 months.

72. The method of any of claims 66-70, wherein the response or outcome determined at or about 3, 6, 9 or 12 months after the designated timepoint is equal to or improved compared to the response or outcome determined at the designated timepoint.

73. The method of any of claims 66-72, wherein the response or outcome is or comprises or further comprises the absence of neurotoxicity or the absence of cytokine release syndrome (CRS).

74. The method of any of claims 1-73, wherein the method does not result in a specified toxicity outcome, optionally at a designated timepoint following initiation of the administration, in at least one of or in at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, or at least 95% of subjects in the cohort of subjects having the disease or disorder.

75. The method of claim 74, wherein the specified toxicity outcome is neurotoxicity.

76. The method of claim 74 or 75, wherein the specified toxicity outcome is neurotoxicity, and neurotoxicity does not result in at least 60%, 70% or 80% of the subject in the cohort.

77. The method of any of claims 74-76, wherein the specified toxicity outcome is grade 3 or higher, or grade 4 or higher, neurotoxicity.

78. The method of any of claims 74-77, wherein the specified toxicity outcome is grade 3 or higher neurotoxicity, and grade 3 or higher neurotoxicity does not result in in at least 80%, 85%, 90% or 95% of the subjects in the cohort.

79. The method of claim 74, wherein the specified toxicity outcome is cytokine release syndrome (CRS).

80. The method of claim 74 or 79, wherein the specified toxicity outcome is CRS, and CRS does not result in at least 15%, 20%, 25% or 30% of the subject in the cohort.

81. The method of any of claims 74, 79 and 80, wherein the specified toxicity outcome is grade 3 or higher, or grade 4 or higher, cytokine release syndrome (CRS).

82. The method of any of claims 74 and 79-81, wherein the specified toxicity outcome is grade 3 or higher CRS, and grade 3 or higher CRS does not result in achieved in at least 80%, 85%, 90% or 95% of the subjects in the cohort.

83. The method of any of claims 66-82, wherein the designated timepoint is at or about 1 month following initiation of the administration.

84. The method of any of claims 66-82, wherein the designated timepoint is at or about 3 months following initiation of the administration.

85. The method of any of claims 66-82, wherein the designated timepoint is at or about 6 months

following initiation of the administration.

86. The method of any of claims 66-82, wherein the designated timepoint is at or about 9 months following initiation of the administration.

87. The method of any of claims 66-82, wherein the designated timepoint is at or about 12 months following initiation of the administration.

88. The method of any of claims 1-87, wherein at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or greater than 95% of the cells in the dose are of a memory phenotype.

89. The method of any of claims 1-88, wherein at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or greater than 95% of the cells in the dose are of a central memory phenotype.

90. The method of any of claims 1-89, wherein at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or greater than 95% of the cells in the dose are CD27+, CD28+, CCR7+, CD45RA-, CD45RO+, CD62L+, CD3+, granzyme B-, and/or CD127+.

91. The method of any of claims 1-90, wherein at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or greater than 95% of the cells in the dose are CCR7+/CD45RA- or are CCR7+/CD45RO+.

92. The method of any of claims 1-91, wherein the cells in the administered dose are produced by a method to produce an output composition exhibiting a predetermined feature, wherein iterations of the method produce a plurality of the output compositions, optionally from human biological samples, when carried out among a plurality of different individual subjects, in which the predetermined feature of the output composition among the plurality of output compositions is selected from:

the mean percentage of cells of a memory phenotype in the plurality of the output compositions is between about 40% and about 65%, between about 40% and about 45%, between about 45% and about 50%,

between about 50% and about 55%, between about 55% and about 60%, or between about 60% and about 65%;

the mean percentage of cells of a central memory phenotype in the plurality of the output compositions is between about 40% and about 65%, between about 40% and about 45%, between about 45% and about 50%, between about 50% and about 55%, between about 55% and about 60%, or between about 60% and about 65%;

the mean percentage of cells that are CD27+, CD28+, CCR7+, CD45RA-, CD45RO+, CD62L+, CD3+, CD95+, granzyme B-, and/or CD127+ in the plurality of the output compositions is between about 40% and about 65%, between about 40% and about 45%, between about 45% and about 50%, between about 50% and about 55%, between about 55% and about 60%, or between about 60% and about 65%;

the mean percentage of cells that are CCR7+/CD45RA- or CCR7+/CD45RO+ in the plurality of the output compositions is between about 40% and about 65%, between about 40% and about 45%, between about 45% and about 50%, between about 50% and about 55%, between about 55% and about 60%, or between about 60% and about 65%;

the mean percentage of central memory CD4+ T cells in the engineered CD4+ T cells, optionally CAR+CD4+ T cells, of the plurality of the output compositions is between about 40% and about 65%, between about 40% and about 45%, between about 45% and about 50%, between about 50% and about 55%, between about 55% and about 60%, or between about 60% and about 65%;

the mean percentage of central memory CD8+ T cells in the engineered CD8+ T cells, optionally CAR+CD8+ T cells, of the plurality of the output compositions is between about 40% and about 65%, between about 40% and about 45%, between about 45% and about 50%, between about 50% and about 55%, between about 55% and about 60%, or between about 60% and about 65%; and/or

the mean percentage of central memory T cells, optionally CD4+ central memory T cells and CD8+ central memory T cells, in the engineered T cells, optionally CAR+ T cells, of the plurality of the output compositions is between about 40% and about 65%, between about 40% and about 45%, between about 45% and about 50%, between about 50% and about 55%, between about 55% and about 60%, or between about 60% and about 65%.

93. The method of claim 92, wherein the administered dose is produced by a method to produce an output composition exhibiting a predetermined feature, optionally a threshold number of cells expressing the CAR in the output composition, in at least about 80%, about 90%, about 95%, about 97%, about 99%, about 100%, or is 100% of the human biological samples in which it is carried out among a plurality of different individual subjects.

94. The method of claim 93, wherein the plurality of different individual subject comprise subjects having a disease or condition.

95. The method of claim 94, wherein the disease or condition is a cancer.

96. The method of claim 95, wherein the cancer is a hematological cancer, optionally multiple myeloma.

97. A dose of engineered T cells for use in the method of any of claims 1-96, wherein the dose comprises one or more engineered T cells comprising a chimeric antigen receptor (CAR) in a treatment regimen for a subject having or suspected of having multiple myeloma (MM) comprising administering to the subject the dose of engineered T cells, wherein the CAR comprises:

(a) an extracellular antigen-binding domain, comprising:

a variable heavy chain (V_H) comprising a heavy chain complementarity determining region 1 (CDR-H1), a heavy chain complementarity determining region 2 (CDR-H2) and a heavy chain complementarity determining region 3 (CDR-H3) contained within the sequence set forth in SEQ ID NO: 116 and a variable light chain (V_L) comprising a light chain complementarity determining region 1 (CDR-L1), a light chain complementarity determining region 2 (CDR-L2) and a light chain complementarity determining region 3 (CDR-L3) contained within the sequence set forth in SEQ ID NO: 119;

a V_H comprising a CDR-H1, a CDR-H2 and a CDR-H3 sequences set forth in SEQ ID NOS:97, 101 and 103, respectively, and a V_L comprising a CDR-L1, a CDR-L2 and a CDR-L3 sequences set forth in SEQ ID NOS:105, 107 and 108, respectively;

a V_H comprising a CDR-H1, a CDR-H2 and a CDR-H3 sequences set forth in SEQ ID NOS:96, 100 and 103, respectively, and a V_L comprising a CDR-L1, a CDR-L2 and a CDR-L3 sequences set forth in SEQ ID NOS:105, 107 and 108, respectively;

a V_H comprising a CDR-H1, a CDR-H2 and a CDR-H3 sequences set forth in SEQ ID NOS:95, 99 and 103, respectively, and a V_L comprising a CDR-L1, a CDR-L2 and a CDR-L3 sequences set forth in SEQ ID NOS: 105, 107 and 108, respectively;

a V_H comprising a CDR-H1, a CDR-H2 and a CDR-H3 sequences set forth in SEQ ID NOS:94, 98 and 102, respectively, and a V_L comprising a CDR-L1, a CDR-L2 and a CDR-L3 sequences set forth in SEQ ID NOS: 104, 106 and 108, respectively; or

a V_H comprising the amino acid sequence of SEQ ID NO: 116 and a V_L comprising the amino acid

sequence of SEQ ID NO: 119;

(b) a spacer comprising an IgG4/2 chimeric hinge or a modified IgG4 hinge; an IgG2/4 chimeric C_H2 region; and an IgG4 C_H3 region, which optionally is about 228 amino acids in length; or a spacer set forth in SEQ ID NO: 174;

(c) a transmembrane domain, optionally a transmembrane domain from a human CD28; and

(d) an intracellular signaling region comprising a cytoplasmic signaling domain of a CD3-zeta (CD3ζ) chain and a costimulatory signaling region comprising an intracellular signaling domain of a T cell costimulatory molecule or a signaling portion thereof; and

the dose of engineered T cells, following administration, is capable of achieving, optionally at a designated time following initiation of the administration, a specified response or outcome in at least one of, or in at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, or at least 95% of subjects within a cohort of subjects or evaluable subjects thereof, wherein the cohort of subjects is a cohort having multiple myeloma.

98. The dose of engineered T cells for use of claim 97, wherein the CAR comprises

(a) an extracellular antigen-binding domain, comprising:

a variable heavy chain (V_H) comprising a heavy chain complementarity determining region 1 (CDR-H1), a heavy chain complementarity determining region 2 (CDR-H2) and a heavy chain complementarity determining region 3 (CDR-H3) contained within the sequence set forth in SEQ ID NO: 116 and a variable light chain (V_L) comprising a light chain complementarity determining region 1 (CDR-L1), a light chain complementarity determining region 2 (CDR-L2) and a light chain complementarity determining region 3 (CDR-L3) contained within the sequence set forth in SEQ ID NO: 119;

(b) a spacer comprising a modified IgG4 hinge; an IgG2/4 chimeric C_H2 region; and an IgG4 C_H3 region, that is about 228 amino acids in length;

(c) a transmembrane domain from a human CD28; and

(d) an intracellular signaling region comprising a cytoplasmic signaling domain of a CD3-zeta (CD3ζ) chain and a costimulatory signaling region comprising an intracellular signaling domain of a 4-1BB.

99. The dose of engineered T cells for use of claim 97 or 98, wherein the CAR comprises

(a) an extracellular antigen-binding domain, comprising the sequence set forth in SEQ ID NO: 114 or a sequence of amino acids having at least 90% sequence identity to the amino acid sequence of SEQ ID NO: 114;

(b) a spacer comprising the sequence set forth in SEQ ID NO: 174 or a sequence of amino acids that

has at least 90% sequence identity to SEQ ID NO:174;

(c) a transmembrane domain comprising the sequence set forth in SEQ ID NO:138 or a sequence of amino acids that has at least 90% sequence identity to SEQ ID NO:138; and

(d) an intracellular signaling region comprising a cytoplasmic signaling comprising the sequence set forth in SEQ ID NO:143 or a sequence of amino acids that has at least 90% sequence identity to SEQ ID NO:143 and a costimulatory signaling region comprising the sequence set forth in SEQ ID NO:4 or a sequence of amino acids that has at least 90% sequence identity to the sequence set forth in SEQ ID NO: 4.

100. The dose of engineered T cells for use of any of claims 97-99, wherein the CAR comprises

(a) an extracellular antigen-binding domain, comprising the sequence set forth in SEQ ID NO: 114;

(b) a spacer comprising the sequence set forth in SEQ ID NO: 174;

(c) a transmembrane domain comprising the sequence set forth in SEQ ID NO:138; and

(d) an intracellular signaling region comprising a cytoplasmic signaling comprising the sequence set forth in SEQ ID NO:143 and a costimulatory signaling region comprising the sequence set forth in SEQ ID NO:4.

101. The dose of engineered T cells for use of any of claims 97-100, wherein the CAR comprises the sequence set forth in SEQ ID NO:19.

102. The dose of engineered T cells for use of any one of claims 97-101, wherein the achievement of the response or outcome is at the designated timepoint following initiation of administration, which is at or about 1, 2, 3, 6, 9, 12, 18, 24, 30 or 36 months following said initiation.

103. The dose of engineered T cells for use of any one of claims 97-102, wherein the achievement of the response or outcome is at the designated timepoint following initiation of administration, which is at 1, 2, 3, 6, 9 or 12 months following said initiation.

104. The dose of engineered T cells for use of any of claims 97-103, wherein the achievement of the response or outcome is at the designated timepoint following initiation of administration, which is at 1, 2 or 3 months following said initiation.

105. The dose of engineered T cell for use of any of claims 97-103, wherein the achievement of the response or outcome is at the designated timepoint following initiation of administration, which is at or

about 1 month following said initiation.

106. The dose of engineered T cell for use of any of claims 97-103, wherein the achievement of the response or outcome is at the designated timepoint following initiation of administration, which is at or about 3 months following said initiation.

107. The dose of engineered T cell for use of any of claims 97-103, wherein the achievement of the response or outcome is at the designated timepoint following initiation of administration, which is at or about 6 months following said initiation.

108. The dose of engineered T cell for use of any of claims 97-103, wherein the achievement of the response or outcome is at the designated timepoint following initiation of administration, which is at or about 9 months following said initiation.

109. The dose of engineered T cell for use of any of claims 97-103, wherein the achievement of the response or outcome is at the designated timepoint following initiation of administration, which is at or about 12 months following said initiation.

110. The dose of engineered T cells for use of any of claims 97-109, wherein:

the cohort of subjects is subjects having relapsed or refractory multiple myeloma;

the cohort of subjects is subjects having relapsed or refractory multiple myeloma having been administered, and relapsed or has been refractory following, at least 3 prior therapies for multiple myeloma, said prior therapies optionally including an autologous stem cell transplant (ASCT); an immunomodulatory agent; a proteasome inhibitor; and/or an anti-CD38 antibody;

the cohort of subjects is subjects having relapsed or refractory multiple myeloma having been administered, and relapsed or has been refractory following, at least 3 prior therapies for multiple myeloma, said prior therapies optionally including an immunomodulatory agent; a proteasome inhibitor; and/or an anti-CD38 antibody and/or an autologous stem cell transplant;

the cohort of subjects is subjects having no active plasma cell leukemia (PCL) or no history of PCL at the time of said administration;

the cohort of subjects is subjects having developed secondary plasma cell leukemia (PCL) prior to administration of the cells;

the cohort of subjects is or includes subjects having relapsed or refractory multiple myeloma having

been administered, and relapsed or has been refractory following, at least 4 or an average of at least 10 prior therapies for multiple myeloma.

the cohort of subjects has received a median of 10 prior regimens or between 3 and 15 or 4 and 15 prior therapies for multiple myeloma;

the cohort of subjects includes subjects refractory to bortezomib, carfilzomib, lenalidomide, pomalidomide and an anti-CD38 monoclonal antibody; and/or

the cohort of subjects includes subjects having had prior autologous stem cell transplant

111. The dose of engineered T cells for use of any of claims 97-110, wherein:

the cohort of subjects consists of or includes adult subjects;

the cohort of subjects has a median time from diagnosis of 4 years and/or a range of time from diagnosis from 2 to 12 years; and/or

the cohort of subjects includes subjects having IMWG high risk cytogenetics.

112. The dose of engineered T cells for use of claim 110 or 111, wherein the at least 3 prior therapies comprise autologous stem cell transplant (ASCT); an immunomodulatory agent or a proteasome inhibitor, or a combination thereof; and an anti-CD38 antibody.

113. The dose of engineered T cells for use of any one of claims 110-112, wherein the immunomodulatory agent is selected from among thalidomide, lenalidomide and pomalidomide, the proteasome inhibitor is selected from among bortezomib, carfilzomib and ixazomib, and/or the anti-CD38 antibody is or comprises daratumumab.

114. The dose of engineered T cells for use of any of claims 97-113, wherein

the response or outcome is selected from the group consisting of objective response (OR), complete response (CR), stringent complete response (sCR), very good partial response (VGPR), partial response (PR) and minimal response (MR), optionally based on the International Myeloma Working Group (IMWG) uniform response criteria;

the response or outcome is or comprises an OR, optionally based on the International Myeloma Working Group (IMWG) uniform response criteria; or

the response or outcome is or comprises a CR, optionally based on the International Myeloma Working Group (IMWG) uniform response criteria.

115. The dose of engineered T cells for use of any of claims 97-114, wherein the response or outcome is or comprises an OR and the dose is capable of achieving the response or outcome in at least 50%, 60%, 70%, or 80% of subjects of the cohort.

116. The dose of engineered T cells for use of any of claims 97-114, wherein the response or outcome is or comprises a VGPR, a CR or an sCR, and the dose is capable of achieving the response or outcome in at least 40%, 45% or 50% of subjects of the cohort.

117. The dose of engineered T cells for use of any of claims 97-114, wherein the response or outcome is or comprises a CR or an sCR, and the dose is capable of achieving the response or outcome in at least 20%, 30%, or 40% of subjects of the cohort.

118. The dose of engineered T cells for use of any of claims 97-117, wherein the response or outcome is durable for greater than at or about 3, 6, 9 or 12 months.

119. The dose of engineered T cells for use of any of claims 97-117, wherein the response or outcome determined at or about 3, 6, 9 or 12 months after the designated time is equal to or improved compared to the response or outcome determined at the designated time.

120. The dose of engineered T cells for use of any of claims 97-119, wherein the dose capable of achieving said response or outcome comprises a combination of $CD4^+$ T cells and $CD8^+$ T cells and/or a combination of $CD4^+$ CAR+ T cells and $CD8^+$ CAR+ T cells.

121. The dose of engineered T cells for use of claim 120, wherein the ratio of $CD4^+$ CAR+ T cells to $CD8^+$ CAR+ T cells and/or of $CD4^+$ T cells to $CD8^+$ T cells, is or is approximately 1:1 or is between at or approximately 1:3 and at or approximately 3:1.

122. The dose of engineered T cells for use of any of claims 97-121, wherein the dose capable of achieving said response or outcome comprises $CD3^+$ CAR+ T cells.

123. The dose of engineered T cell for use of any of claims 97-122, wherein administration of the dose of engineered T cell does not result in a specified toxicity outcome, optionally at a designated timepoint following initiation of the administration, in at least one of or in at least 10%, at least 20%, at least 30%, at

least 40%, at least 50%, at least 90%, at least 70%, at least 80%, at least 90%, or at least 95% of subjects in the cohort of subjects having the disease or disorder.

124. The dose of engineered T cell for use of claim 123, wherein the specified toxicity outcome is neurotoxicity.

125. The dose of engineered T cell for use of claim 123 or 124, wherein the specified toxicity outcome is neurotoxicity, and neurotoxicity does not result in at least 90%, 70% or 80% of the subject in the cohort.

126. The dose of engineered T cell for use of any of claims 123-125, wherein the specified toxicity outcome is grade 3 or higher, or grade 4 or higher, neurotoxicity.

127. The dose of engineered T cell for use of any of claims 123-126, wherein the specified toxicity outcome is grade 3 or higher neurotoxicity, and grade 3 or higher neurotoxicity does not result in in at least 80%, 85%, 90% or 95% of the subjects in the cohort.

128. The dose of engineered T cell for use of claim 123, wherein the specified toxicity outcome is cytokine release syndrome (CRS).

129. The dose of engineered T cell for use of claim 123 or 128, wherein the specified toxicity outcome is CRS, and CRS does not result in at least 15%, 20%, 25% or 30% of the subject in the cohort.

130. The dose of engineered T cell for use of any of claims 123, 128 and 129, wherein the specified toxicity outcome is grade 3 or higher, or grade 4 or higher, cytokine release syndrome (CRS).

131. The dose of engineered T cell for use of any of claims 123 and 128-130, wherein the specified toxicity outcome is grade 3 or higher CRS, and grade 3 or higher CRS does not result in achieved in at least 80%, 85%, 90% or 95% of the subjects in the cohort.

132. The dose of engineered T cells for use of any of claims 97-131, wherein the dose capable of achieving said response or outcome is at or about 5×10^7 cells or CAR+ T cells.

133. The dose of engineered T cells for use of any of claims 97-131, wherein the dose capable of achieving said response or outcome is at or about 1.5 x 10^8 cells or CAR+ T cells.

134. The dose of engineered T cells for use of any of claims 97-131, wherein the dose capable of achieving said response or outcome is at or about 3 x 10^8 cells or CAR+ T cells.

135. The dose of engineered T cells for use of any of claims 97-131, wherein the dose capable of achieving said response or outcome is at or about 4.5 x 10^8 cells or CAR+ T cells.

136. The dose of engineered T cells for use of any of claims 97-119, wherein the dose capable of achieving said response or outcome is at or about 6.0 x 10^8 cells or CAR+ T cells.

137. The dose of engineered T cells for use of any of claims 97-136, wherein at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or greater than 95% of the cells in the dose are of a memory phenotype.

138. The dose of engineered T cells for use of any of claims 97-137, wherein at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or greater than 95% of the cells in the dose are of a central memory phenotype.

139. The dose of engineered T cells for use of any of claims 97-138, wherein at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or greater than 95% of the cells in the dose are CD27+, CD28+, CCR7+, CD45RA-, CD45RO+, CD62L+, CD3+, granzyme B-, and/or CD127+.

140. The dose of engineered T cells for use of any of claims 97-139, wherein least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or greater than 95% of the cells in the dose are CCR7+/CD45RA- or are CCR7+/CD45RO+.

141. The dose of engineered T cells for use of any of claims 97-140, wherein: the dose of engineered T cells is produced by a method to produce an output composition exhibiting a predetermined feature, wherein iterations of the method produce a plurality of the output compositions, optionally from

human biological samples, when carried out among a plurality of different individual subjects, in which the predetermined feature of the output composition among the plurality of output compositions is selected from:

the mean percentage of cells of a memory phenotype in the plurality of the output compositions is between about 40% and about 65%, between about 40% and about 45%, between about 45% and about 50%, between about 50% and about 55%, between about 55% and about 60%, or between about 60% and about 65%;

the mean percentage of cells of a central memory phenotype in the plurality of the output compositions is between about 40% and about 65%, between about 40% and about 45%, between about 45% and about 50%, between about 50% and about 55%, between about 55% and about 60%, or between about 60% and about 65%;

the mean percentage of cells that are CD27+, CD28+, CCR7+, CD45RA-, CD45RO+, CD62L+, CD3+, CD95+, granzyme B-, and/or CD127+ in the plurality of the output compositions is between about 40% and about 65%, between about 40% and about 45%, between about 45% and about 50%, between about 50% and about 55%, between about 55% and about 60%, or between about 60% and about 65%;

the mean percentage of cells that are CCR7+/CD45RA- or CCR7+/CD45RO+ in the plurality of the output compositions is between about 40% and about 65%, between about 40% and about 45%, between about 45% and about 50%, between about 50% and about 55%, between about 55% and about 60%, or between about 60% and about 65%;

the mean percentage of central memory CD4+ T cells in the engineered CD4+ T cells, optionally CAR+CD4+ T cells, of the plurality of the output compositions is between about 40% and about 65%, between about 40% and about 45%, between about 45% and about 50%, between about 50% and about 55%, between about 55% and about 60%, or between about 60% and about 65%;

the mean percentage of central memory CD8+ T cells in the engineered CD8+ T cells, optionally CAR+CD8+ T cells, of the plurality of the output compositions is between about 40% and about 65%, between about 40% and about 45%, between about 45% and about 50%, between about 50% and about 55%, between about 55% and about 60%, or between about 60% and about 65%; and/or

the mean percentage of central memory T cells, optionally CD4+ central memory T cells and CD8+ central memory T cells, in the engineered T cells, optionally CAR+ T cells, of the plurality of the output compositions is between about 40% and about 65%, between about 40% and about 45%, between about 45% and about 50%, between about 50% and about 55%, between about 55% and about 60%, or between about 60% and about 65%.

142. The dose of engineered T cells for use of claim 141, wherein the dose is produced by a method to produce an output composition exhibiting a predetermined feature, optionally a threshold number of cells expressing the CAR in the output composition, in at least about 80%, about 90%, about 95%, about 97%, about 99%, about 100%, or is 100% of the human biological samples in which it is carried out among a plurality of different individual subjects.

143. The dose of engineered T cells for use of claim 142, wherein the plurality of different individual subject comprise subjects having a disease or condition.

144. The dose of engineered T cells for use of claim 143, wherein the disease or condition is a cancer.

145. The dose of engineered T cells for use of claim 144, wherein the cancer is a hematological cancer, optionally multiple myeloma.

146. Use of a dose of engineered T cells comprising a chimeric antigen receptor (CAR) in a treatment regimen for a subject having or suspected of having multiple myeloma (MM) comprising administering to the subject the dose of engineered T cells, wherein the CAR comprises:

(a) an extracellular antigen-binding domain, comprising:

a variable heavy chain (V_H) comprising a heavy chain complementarity determining region 1 (CDR-H1), a heavy chain complementarity determining region 2 (CDR-H2) and a heavy chain complementarity determining region 3 (CDR-H3) contained within the sequence set forth in SEQ ID NO: 116 and a variable light chain (V_L) comprising a light chain complementarity determining region 1 (CDR-L1), a light chain complementarity determining region 2 (CDR-L2) and a light chain complementarity determining region 3 (CDR-L3) contained within the sequence set forth in SEQ ID NO: 119;

a V_H comprising a CDR-H1, a CDR-H2 and a CDR-H3 sequences set forth in SEQ ID NOS:97, 101 and 103, respectively, and a V_L comprising a CDR-L1, a CDR-L2 and a CDR-L3 sequences set forth in SEQ ID NOS:105, 107 and 108, respectively;

a V_H comprising a CDR-H1, a CDR-H2 and a CDR-H3 sequences set forth in SEQ ID NOS:96, 100 and 103, respectively, and a V_L comprising a CDR-L1, a CDR-L2 and a CDR-L3 sequences set forth in SEQ ID NOS:105, 107 and 108, respectively;

a V_H comprising a CDR-H1, a CDR-H2 and a CDR-H3 sequences set forth in SEQ ID NOS:95, 99 and 103, respectively, and a V_L comprising a CDR-L1, a CDR-L2 and a CDR-L3 sequences set forth in SEQ

ID NOS: 105, 107 and 108, respectively;

a V_H comprising a CDR-H1, a CDR-H2 and a CDR-H3 sequences set forth in SEQ ID NOS:94, 98 and 102, respectively, and a V_L comprising a CDR-L1, a CDR-L2 and a CDR-L3 sequences set forth in SEQ ID NOS: 104, 106 and 108, respectively; or

a V_H comprising the amino acid sequence of SEQ ID NO: 116 and a V_L comprising the amino acid sequence of SEQ ID NO: 119;

(b) a spacer comprising an IgG4/2 chimeric hinge or a modified IgG4 hinge; an IgG2/4 chimeric C_H2 region; and an IgG4 C_H3 region, which optionally is about 228 amino acids in length; or a spacer set forth in SEQ ID NO: 174;

(c) a transmembrane domain, optionally a transmembrane domain from a human CD28; and

(d) an intracellular signaling region comprising a cytoplasmic signaling domain of a CD3-zeta (CD3ζ) chain and a costimulatory signaling region comprising an intracellular signaling domain of a T cell costimulatory molecule or a signaling portion thereof;

wherein, prior to the administration, the subject has received a lymphodepleting therapy comprising the administration of fludarabine at or about 20-40 mg/m^2 body surface area of the subject, optionally at or about 30 mg/m^2, daily, for 2-4 days, and/or cyclophosphamide at or about 200-400 mg/m^2 body surface area of the subject, optionally at or about 300 mg/m^2, daily, for 2-4 days.

147. Use of a dose of engineered T cells comprising a chimeric antigen receptor (CAR) in a treatment regimen for a subject having or suspected of having multiple myeloma (MM) comprising administering to the subject the dose of engineered T cells, wherein the CAR comprises:

(a) an extracellular antigen-binding domain, comprising:

a variable heavy chain (V_H) comprising a heavy chain complementarity determining region 1 (CDR-H1), a heavy chain complementarity determining region 2 (CDR-H2) and a heavy chain complementarity determining region 3 (CDR-H3) contained within the sequence set forth in SEQ ID NO: 116 and a variable light chain (V_L) comprising a light chain complementarity determining region 1 (CDR-L1), a light chain complementarity determining region 2 (CDR-L2) and a light chain complementarity determining region 3 (CDR-L3) contained within the sequence set forth in SEQ ID NO: 119;

a V_H comprising a CDR-H1, a CDR-H2 and a CDR-H3 sequences set forth in SEQ ID NOS:97, 101 and 103, respectively, and a V_L comprising a CDR-L1, a CDR-L2 and a CDR-L3 sequences set forth in SEQ ID NOS:105, 107 and 108, respectively;

a V_H comprising a CDR-H1, a CDR-H2 and a CDR-H3 sequences set forth in SEQ ID NOS:96, 100 and 103, respectively, and a V_L comprising a CDR-L1, a CDR-L2 and a CDR-L3 sequences set forth in SEQ

ID NOS:105, 107 and 108, respectively;

a V_H comprising a CDR-H1, a CDR-H2 and a CDR-H3 sequences set forth in SEQ ID NOS:95, 99 and 103, respectively, and a V_L comprising a CDR-L1, a CDR-L2 and a CDR-L3 sequences set forth in SEQ ID NOS: 105, 107 and 108, respectively;

a V_H comprising a CDR-H1, a CDR-H2 and a CDR-H3 sequences set forth in SEQ ID NOS:94, 98 and 102, respectively, and a V_L comprising a CDR-L1, a CDR-L2 and a CDR-L3 sequences set forth in SEQ ID NOS: 104, 106 and 108, respectively; or

a V_H comprising the amino acid sequence of SEQ ID NO: 116 and a V_L comprising the amino acid sequence of SEQ ID NO: 119;

(b) a spacer comprising an IgG4/2 chimeric hinge or a modified IgG4 hinge; an IgG2/4 chimeric C_H2 region; and an IgG4 C_H3 region, which optionally is about 228 amino acids in length; or a spacer set forth in SEQ ID NO: 174;

(c) a transmembrane domain, optionally a transmembrane domain from a human CD28; and

(d) an intracellular signaling region comprising a cytoplasmic signaling domain of a CD3-zeta (CD3ζ) chain and a costimulatory signaling region comprising an intracellular signaling domain of a T cell costimulatory molecule or a signaling portion thereof; wherein at or prior to the administration of the dose of engineered T cells, the subject has received three or more therapies selected from among:

autologous stem cell transplant (ASCT);

an immunomodulatory agent;

a proteasome inhibitor; and

an anti-CD38 antibody; unless the subject was not a candidate for or was contraindicated for one or more of the therapies.

148. Use of a dose of engineered T cells comprising a chimeric antigen receptor (CAR) in a treatment regimen for a subject having or suspected of having multiple myeloma (MM) comprising administering to the subject the dose of engineered T cells, wherein the CAR comprises:

(a) an extracellular antigen-binding domain, comprising:

a variable heavy chain (V_H) comprising a heavy chain complementarity determining region 1 (CDR-H1), a heavy chain complementarity determining region 2 (CDR-H2) and a heavy chain complementarity determining region 3 (CDR-H3) contained within the sequence set forth in SEQ ID NO: 116 and a variable light chain (V_L) comprising a light chain complementarity determining region 1 (CDR-L1), a light chain complementarity determining region 2 (CDR-L2) and a light chain complementarity determining region 3 (CDR-L3) contained within the sequence set forth in SEQ ID NO: 119;

a V_H comprising a CDR-H1, a CDR-H2 and a CDR-H3 sequences set forth in SEQ ID NOS:97, 101 and 103, respectively, and a V_L comprising a CDR-L1, a CDR-L2 and a CDR-L3 sequences set forth in SEQ ID NOS:105, 107 and 108, respectively;

a V_H comprising a CDR-H1, a CDR-H2 and a CDR-H3 sequences set forth in SEQ ID NOS:96, 100 and 103, respectively, and a V_L comprising a CDR-L1, a CDR-L2 and a CDR-L3 sequences set forth in SEQ ID NOS:105, 107 and 108, respectively;

a V_H comprising a CDR-H1, a CDR-H2 and a CDR-H3 sequences set forth in SEQ ID NOS:95, 99 and 103, respectively, and a V_L comprising a CDR-L1, a CDR-L2 and a CDR-L3 sequences set forth in SEQ ID NOS: 105, 107 and 108, respectively;

a V_H comprising a CDR-H1, a CDR-H2 and a CDR-H3 sequences set forth in SEQ ID NOS:94, 98 and 102, respectively, and a V_L comprising a CDR-L1, a CDR-L2 and a CDR-L3 sequences set forth in SEQ ID NOS: 104, 106 and 108, respectively; or

a V_H comprising the amino acid sequence of SEQ ID NO: 116 and a V_L comprising the amino acid sequence of SEQ ID NO: 119;

(b) a spacer comprising an IgG4/2 chimeric hinge or a modified IgG4 hinge; an IgG2/4 chimeric C_H2 region; and an IgG4 C_H3 region, which optionally is about 228 amino acids in length; or a spacer set forth in SEQ ID NO: 174;

(c) a transmembrane domain, optionally a transmembrane domain from a human CD28; and

(d) an intracellular signaling region comprising a cytoplasmic signaling domain of a CD3-zeta (CD3ζ) chain and a costimulatory signaling region comprising an intracellular signaling domain of a T cell costimulatory molecule or a signaling portion thereof; wherein at the administration of the dose of engineered T cells, the subject has not had active or history of plasma cell leukemia (PCL).

149. Use of a dose of engineered T cells comprising a chimeric antigen receptor (CAR) in a treatment regimen for a subject having or suspected of having multiple myeloma (MM) comprising administering to the subject the dose of engineered T cells, wherein the CAR comprises:

(a) an extracellular antigen-binding domain, comprising:

a variable heavy chain (V_H) comprising a heavy chain complementarity determining region 1 (CDR-H1), a heavy chain complementarity determining region 2 (CDR-H2) and a heavy chain complementarity determining region 3 (CDR-H3) contained within the sequence set forth in SEQ ID NO: 116 and a variable light chain (V_L) comprising a light chain complementarity determining region 1 (CDR-L1), a light chain complementarity determining region 2 (CDR-L2) and a light chain complementarity determining region 3 (CDR-L3) contained within the sequence set forth in SEQ ID NO: 119;

a V_H comprising a CDR-H1, a CDR-H2 and a CDR-H3 sequences set forth in SEQ ID NOS:97, 101 and 103, respectively, and a V_L comprising a CDR-L1, a CDR-L2 and a CDR-L3 sequences set forth in SEQ ID NOS:105, 107 and 108, respectively;

a V_H comprising a CDR-H1, a CDR-H2 and a CDR-H3 sequences set forth in SEQ ID NOS:96, 100 and 103, respectively, and a V_L comprising a CDR-L1, a CDR-L2 and a CDR-L3 sequences set forth in SEQ ID NOS:105, 107 and 108, respectively;

a V_H comprising a CDR-H1, a CDR-H2 and a CDR-H3 sequences set forth in SEQ ID NOS:95, 99 and 103, respectively, and a V_L comprising a CDR-L1, a CDR-L2 and a CDR-L3 sequences set forth in SEQ ID NOS: 105, 107 and 108, respectively;

a V_H comprising a CDR-H1, a CDR-H2 and a CDR-H3 sequences set forth in SEQ ID NOS:94, 98 and 102, respectively, and a V_L comprising a CDR-L1, a CDR-L2 and a CDR-L3 sequences set forth in SEQ ID NOS: 104, 106 and 108, respectively; or

a V_H comprising the amino acid sequence of SEQ ID NO: 116 and a V_L comprising the amino acid sequence of SEQ ID NO: 119;

(b) a spacer comprising an IgG4/2 chimeric hinge or a modified IgG4 hinge; an IgG2/4 chimeric C_H2 region; and an IgG4 C_H3 region, which optionally is about 228 amino acids in length; or a spacer set forth in SEQ ID NO: 174;

(c) a transmembrane domain, optionally a transmembrane domain from a human CD28; and

(d) an intracellular signaling region comprising a cytoplasmic signaling domain of a CD3-zeta (CD3ζ) chain and a costimulatory signaling region comprising an intracellular signaling domain of a T cell costimulatory molecule or a signaling portion thereof; wherein the dose of engineered T cells comprises:

between at or about 1 x 10^7 CAR+ T cells and 2 x 10^9 CAR+ T cells;

a combination of $CD4^+$ T cells and $CD8^+$ T cells, at a defined ratio of $CD4^+$ CAR+ T cells to $CD8^+$ CAR+ T cells and/or of $CD4^+$ T cells to $CD8^+$ T cells, that is or is approximately 1:1 or is between approximately 1:3 and approximately 3:1; and

less than 25%, 20%, 15%, 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2% or 1% of the CAR+ T cells in the dose express a marker of apoptosis, optionally Annexin V or active Caspase 3.

150. Use of a dose of engineered T cells comprising a chimeric antigen receptor (CAR) for the manufacture of a medicament for the treatment for a subject having or suspected of having multiple myeloma (MM), wherein the CAR comprises:

(a) an extracellular antigen-binding domain, comprising:

a variable heavy chain (V_H) comprising a heavy chain complementarity determining region 1 (CDR-

H1), a heavy chain complementarity determining region 2 (CDR-H2) and a heavy chain complementarity determining region 3 (CDR-H3) contained within the sequence set forth in SEQ ID NO: 116 and a variable light chain (V_L) comprising a light chain complementarity determining region 1 (CDR-L1), a light chain complementarity determining region 2 (CDR-L2) and a light chain complementarity determining region 3 (CDR-L3) contained within the sequence set forth in SEQ ID NO: 119;

a V_H comprising a CDR-H1, a CDR-H2 and a CDR-H3 sequences set forth in SEQ ID NOS:97, 101 and 103, respectively, and a V_L comprising a CDR-L1, a CDR-L2 and a CDR-L3 sequences set forth in SEQ ID NOS:105, 107 and 108, respectively;

a V_H comprising a CDR-H1, a CDR-H2 and a CDR-H3 sequences set forth in SEQ ID NOS:96, 100 and 103, respectively, and a V_L comprising a CDR-L1, a CDR-L2 and a CDR-L3 sequences set forth in SEQ ID NOS:105, 107 and 108, respectively;

a V_H comprising a CDR-H1, a CDR-H2 and a CDR-H3 sequences set forth in SEQ ID NOS:95, 99 and 103, respectively, and a V_L comprising a CDR-L1, a CDR-L2 and a CDR-L3 sequences set forth in SEQ ID NOS: 105, 107 and 108, respectively;

a V_H comprising a CDR-H1, a CDR-H2 and a CDR-H3 sequences set forth in SEQ ID NOS:94, 98 and 102, respectively, and a V_L comprising a CDR-L1, a CDR-L2 and a CDR-L3 sequences set forth in SEQ ID NOS: 104, 106 and 108, respectively; or

a V_H comprising the amino acid sequence of SEQ ID NO: 116 and a V_L comprising the amino acid sequence of SEQ ID NO: 119;

(c) a transmembrane domain, optionally a transmembrane domain from a human CD28; and

(d) an intracellular signaling region comprising a cytoplasmic signaling domain of a CD3-zeta (CD3ζ) chain and a costimulatory signaling region comprising an intracellular signaling domain of a T cell costimulatory molecule or a signaling portion thereof;

wherein, prior to the administration of the dose of engineered T cells, the subject has received a lymphodepleting therapy comprising the administration of fludarabine at or about 20-40 mg/m^2 body surface area of the subject, optionally at or about 30 mg/m^2, daily, for 2-4 days, and/or cyclophosphamide at or about 200-400 mg/m^2 body surface area of the subject, optionally at or about 300 mg/m^2, daily, for 2-4 days.

151. Use of a dose of engineered T cells comprising a chimeric antigen receptor (CAR) for the manufacture of a medicament for the treatment for a subject having or suspected of having multiple myeloma (MM), wherein the CAR comprises:

(a) an extracellular antigen-binding domain, comprising:

a variable heavy chain (V_H) comprising a heavy chain complementarity determining region 1 (CDR-

H1), a heavy chain complementarity determining region 2 (CDR-H2) and a heavy chain complementarity determining region 3 (CDR-H3) contained within the sequence set forth in SEQ ID NO: 116 and a variable light chain (V_L) comprising a light chain complementarity determining region 1 (CDR-L1), a light chain complementarity determining region 2 (CDR-L2) and a light chain complementarity determining region 3 (CDR-L3) contained within the sequence set forth in SEQ ID NO: 119;

a V_H comprising a CDR-H1, a CDR-H2 and a CDR-H3 sequences set forth in SEQ ID NOS:97, 101 and 103, respectively, and a V_L comprising a CDR-L1, a CDR-L2 and a CDR-L3 sequences set forth in SEQ ID NOS:105, 107 and 108, respectively;

a V_H comprising a CDR-H1, a CDR-H2 and a CDR-H3 sequences set forth in SEQ ID NOS:96, 100 and 103, respectively, and a V_L comprising a CDR-L1, a CDR-L2 and a CDR-L3 sequences set forth in SEQ ID NOS:105, 107 and 108, respectively;

a V_H comprising a CDR-H1, a CDR-H2 and a CDR-H3 sequences set forth in SEQ ID NOS:95, 99 and 103, respectively, and a V_L comprising a CDR-L1, a CDR-L2 and a CDR-L3 sequences set forth in SEQ ID NOS: 105, 107 and 108, respectively;

a V_H comprising a CDR-H1, a CDR-H2 and a CDR-H3 sequences set forth in SEQ ID NOS:94, 98 and 102, respectively, and a V_L comprising a CDR-L1, a CDR-L2 and a CDR-L3 sequences set forth in SEQ ID NOS: 104, 106 and 108, respectively; or

a V_H comprising the amino acid sequence of SEQ ID NO: 116 and a V_L comprising the amino acid sequence of SEQ ID NO: 119;

(c) a transmembrane domain, optionally a transmembrane domain from a human CD28; and

(d) an intracellular signaling region comprising a cytoplasmic signaling domain of a CD3-zeta (CD3ζ) chain and a costimulatory signaling region comprising an intracellular signaling domain of a T cell costimulatory molecule or a signaling portion thereof; wherein at or prior to the administration of the dose of engineered T cells, the subject has received three or more therapies selected from among:

autologous stem cell transplant (ASCT);

an immunomodulatory agent;

a proteasome inhibitor; and

an anti-CD38 antibody; unless the subject was not a candidate for or was contraindicated for one or more of the therapies.

152. Use of a dose of engineered T cells comprising a chimeric antigen receptor (CAR) for the manufacture of a medicament for the treatment for a subject having or suspected of having multiple myeloma (MM), wherein the CAR comprises:

(a) an extracellular antigen-binding domain, comprising:

a variable heavy chain (V_H) comprising a heavy chain complementarity determining region 1 (CDR-H1), a heavy chain complementarity determining region 2 (CDR-H2) and a heavy chain complementarity determining region 3 (CDR-H3) contained within the sequence set forth in SEQ ID NO: 116 and a variable light chain (V_L) comprising a light chain complementarity determining region 1 (CDR-L1), a light chain complementarity determining region 2 (CDR-L2) and a light chain complementarity determining region 3 (CDR-L3) contained within the sequence set forth in SEQ ID NO: 119;

a V_H comprising a CDR-H1, a CDR-H2 and a CDR-H3 sequences set forth in SEQ ID NOS:97, 101 and 103, respectively, and a V_L comprising a CDR-L1, a CDR-L2 and a CDR-L3 sequences set forth in SEQ ID NOS:105, 107 and 108, respectively;

a V_H comprising a CDR-H1, a CDR-H2 and a CDR-H3 sequences set forth in SEQ ID NOS:96, 100 and 103, respectively, and a V_L comprising a CDR-L1, a CDR-L2 and a CDR-L3 sequences set forth in SEQ ID NOS:105, 107 and 108, respectively;

a V_H comprising a CDR-H1, a CDR-H2 and a CDR-H3 sequences set forth in SEQ ID NOS:95, 99 and 103, respectively, and a V_L comprising a CDR-L1, a CDR-L2 and a CDR-L3 sequences set forth in SEQ ID NOS: 105, 107 and 108, respectively;

a V_H comprising a CDR-H1, a CDR-H2 and a CDR-H3 sequences set forth in SEQ ID NOS:94, 98 and 102, respectively, and a V_L comprising a CDR-L1, a CDR-L2 and a CDR-L3 sequences set forth in SEQ ID NOS: 104, 106 and 108, respectively; or

a V_H comprising the amino acid sequence of SEQ ID NO: 116 and a V_L comprising the amino acid sequence of SEQ ID NO: 119;

(b) a spacer comprising an IgG4/2 chimeric hinge or a modified IgG4 hinge; an IgG2/4 chimeric C_H2 region; and an IgG4 C_H3 region, which optionally is about 228 amino acids in length; or a spacer set forth in SEQ ID NO: 174;

(c) a transmembrane domain, optionally a transmembrane domain from a human CD28; and

(d) an intracellular signaling region comprising a cytoplasmic signaling domain of a CD3-zeta (CD3ζ) chain and a costimulatory signaling region comprising an intracellular signaling domain of a T cell costimulatory molecule or a signaling portion thereof; wherein at the administration of the dose of engineered T cells, the subject has not had active or history of plasma cell leukemia (PCL).

153. Use of a dose of engineered T cells comprising a chimeric antigen receptor (CAR) for the manufacture of a medicament for the treatment for a subject having or suspected of having multiple myeloma (MM), wherein the CAR comprises:

(a) an extracellular antigen-binding domain, comprising:

a variable heavy chain (V_H) comprising a heavy chain complementarity determining region 1 (CDR-H1), a heavy chain complementarity determining region 2 (CDR-H2) and a heavy chain complementarity determining region 3 (CDR-H3) contained within the sequence set forth in SEQ ID NO: 116 and a variable light chain (V_L) comprising a light chain complementarity determining region 1 (CDR-L1), a light chain complementarity determining region 2 (CDR-L2) and a light chain complementarity determining region 3 (CDR-L3) contained within the sequence set forth in SEQ ID NO: 119;

a V_H comprising a CDR-H1, a CDR-H2 and a CDR-H3 sequences set forth in SEQ ID NOS:97, 101 and 103, respectively, and a V_L comprising a CDR-L1, a CDR-L2 and a CDR-L3 sequences set forth in SEQ ID NOS:105, 107 and 108, respectively;

a V_H comprising a CDR-H1, a CDR-H2 and a CDR-H3 sequences set forth in SEQ ID NOS:96, 100 and 103, respectively, and a V_L comprising a CDR-L1, a CDR-L2 and a CDR-L3 sequences set forth in SEQ ID NOS:105, 107 and 108, respectively;

a V_H comprising a CDR-H1, a CDR-H2 and a CDR-H3 sequences set forth in SEQ ID NOS:95, 99 and 103, respectively, and a V_L comprising a CDR-L1, a CDR-L2 and a CDR-L3 sequences set forth in SEQ ID NOS: 105, 107 and 108, respectively;

a V_H comprising a CDR-H1, a CDR-H2 and a CDR-H3 sequences set forth in SEQ ID NOS:94, 98 and 102, respectively, and a V_L comprising a CDR-L1, a CDR-L2 and a CDR-L3 sequences set forth in SEQ ID NOS: 104, 106 and 108, respectively; or

a V_H comprising the amino acid sequence of SEQ ID NO: 116 and a V_L comprising the amino acid sequence of SEQ ID NO: 119;

(b) a spacer comprising an IgG4/2 chimeric hinge or a modified IgG4 hinge; an IgG2/4 chimeric C_H2 region; and an IgG4 C_H3 region, which optionally is about 228 amino acids in length; or a spacer set forth in SEQ ID NO: 174;

(c) a transmembrane domain, optionally a transmembrane domain from a human CD28; and

(d) an intracellular signaling region comprising a cytoplasmic signaling domain of a CD3-zeta (CD3ζ) chain and a costimulatory signaling region comprising an intracellular signaling domain of a T cell costimulatory molecule or a signaling portion thereof; wherein the dose of engineered T cells comprises:

between at or about 1 x 10^7 CAR+ T cells and 2 x 10^9 CAR+ T cells;

a combination of $CD4^+$ T cells and $CD8^+$ T cells, at a defined ratio of $CD4^+$ CAR+ T cells to $CD8^+$ CAR+ T cells and/or of $CD4^+$ T cells to $CD8^+$ T cells, that is or is approximately 1:1 or is between approximately 1:3 and approximately 3:1; and

less than 25%, 20%, 15%, 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2% or 1% of the CAR+ T

cells in the dose express a marker of apoptosis, optionally Annexin V or active Caspase 3.

154. The use of any of claims 146-153, wherein the extracellular antigen-binding domain specifically binds to a B cell maturation antigen (BCMA).

155. The use of any of claims 146-154, wherein the V_H is or comprises the amino acid sequence of SEQ ID NO: 116; and the V_L is or comprises the amino acid sequence of SEQ ID NO: 119.

156. The use of any of claims 146-155, wherein the CAR comprises

(a) an extracellular antigen-binding domain, comprising:

a variable heavy chain (V_H) comprising a heavy chain complementarity determining region 1 (CDR-H1), a heavy chain complementarity determining region 2 (CDR-H2) and a heavy chain complementarity determining region 3 (CDR-H3) contained within the sequence set forth in SEQ ID NO: 116 and a variable light chain (V_L) comprising a light chain complementarity determining region 1 (CDR-L1), a light chain complementarity determining region 2 (CDR-L2) and a light chain complementarity determining region 3 (CDR-L3) contained within the sequence set forth in SEQ ID NO: 119;

(b) a spacer comprising a modified IgG4 hinge; an IgG2/4 chimeric C_H2 region; and an IgG4 C_H3 region, that is about 228 amino acids in length;

(c) a transmembrane domain from a human CD28; and

(d) an intracellular signaling region comprising a cytoplasmic signaling domain of a CD3-zeta (CD3ζ) chain and a costimulatory signaling region comprising an intracellular signaling domain of a 4-1BB.

157. The use of any of claims 146-156, wherein the CAR comprises

(a) an extracellular antigen-binding domain, comprising the sequence set forth in SEQ ID NO: 114 or a sequence of amino acids having at least 90% sequence identity to the amino acid sequence of SEQ ID NO: 114;

(b) a spacer comprising the sequence set forth in SEQ ID NO: 174 or a sequence of amino acids that has at least 90% sequence identity to SEQ ID NO:174;

(c) a transmembrane domain comprising the sequence set forth in SEQ ID NO:138 or a sequence of amino acids that has at least 90% sequence identity to SEQ ID NO:138; and

(d) an intracellular signaling region comprising a cytoplasmic signaling comprising the sequence set forth in SEQ ID NO:143 or a sequence of amino acids that has at least 90% sequence identity to SEQ ID NO:143 and a costimulatory signaling region comprising the sequence set forth in SEQ ID NO:4 or a

sequence of amino acids that has at least 90% sequence identity to the sequence set forth in SEQ ID NO: 4.

158. The use of any of claims 146-157, wherein the CAR comprises

(a) an extracellular antigen-binding domain, comprising the sequence set forth in SEQ ID NO: 114;

(b) a spacer comprising the sequence set forth in SEQ ID NO: 174;

(c) a transmembrane domain comprising the sequence set forth in SEQ ID NO:138; and

(d) an intracellular signaling region comprising a cytoplasmic signaling comprising the sequence set forth in SEQ ID NO:143 and a costimulatory signaling region comprising the sequence set forth in SEQ ID NO:4.

159. The use of any of claims 146-158, wherein the CAR comprises the sequence set forth in SEQ ID NO:19.

160 The use of any of claims 146-148, 150-152 and 154-159, wherein the dose of engineered T cells comprises between at or about 1 x 10^7 CAR+ T cells and at or about 2 x 10^9 CAR+ T cells.

161. The use of any of claims 146-160, wherein the dose of engineered T cells is at or about 5 x 10^7 cells or CAR+ T cells.

162. The use of any of claims 146-160, wherein the dose of engineered T cells is at or about 1.5 x 10^8 cells or CAR+ T cells.

163. The use of any of claims 146-160, wherein the dose of engineered T cells is at or about 3 x 10^8 cells or CAR+ T cells.

164. The use of any of claims 146-160, wherein the dose of engineered T cells is at or about 4.5 x 10^8 cells or CAR+ T cells.

164. The use of any of claims 146-160, wherein the dose of engineered T cells is at or about 6 x 10^8 cells or CAR+ T cells.

165. The use of any of claims 146-164, wherein the dose of engineered T cells comprises a combination of $CD4^+$ T cells and $CD8^+$ T cells and/or a combination of $CD4^+$ CAR+ T cells and $CD8^+$

CAR+ T cells,

166. The use of claim 165, wherein the ratio of $CD4^+$ CAR+ T cells to $CD8^+$ CAR+ T cells and/or of $CD4^+$ T cells to $CD8^+$ T cells is or is approximately 1:1 or is between at or approximately 1:3 and at or approximately 3:1.

167. The use of any of claims 146-166, wherein the dose of engineered T cells comprises $CD3^+$ CAR+ T cells.

168. The use of any of claims 146-148, 150-152 and 154-167, wherein less than at or about 25%, 20%, 15%, 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2% or 1% of the CAR+ T cells in the dose of engineered T cells express a marker of apoptosis, optionally Annexin V or active Caspase 3.

169. The use of any of claims 146-168, wherein less than at or about 5%, 4%, 3%, 2% or 1% of the CAR+ T cells in the dose of engineered T cells express Annexin V or active Caspase 3.

170. The use of any of claims 146-169, wherein at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or greater than 95% of the cells in the dose are of a memory phenotype.

171. The use of any of claims 146-170, wherein at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or greater than 95% of the cells in the dose are of a central memory phenotype.

172. The use of any of claims 146-171, wherein at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or greater than 95% of the cells in the dose are CD27+, CD28+, CCR7+, CD45RA-, CD45RO+, CD62L+, CD3+, granzyme B-, and/or CD127+.

173. The use of any of claims 146-172, wherein at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or greater than 95% of the cells in the dose are CCR7+/CD45RA- or are CCR7+/CD45RO+.

174. The use of any of claims 146-173, wherein the cells in the administered dose are produced by a method to produce an output composition exhibiting a predetermined feature, wherein iterations of the method produce a plurality of the output compositions, optionally from human biological samples, when carried out among a plurality of different individual subjects, in which the predetermined feature of the output composition among the plurality of output compositions is selected from:

the mean percentage of cells of a memory phenotype in the plurality of the output compositions is between about 40% and about 65%, between about 40% and about 45%, between about 45% and about 50%, between about 50% and about 55%, between about 55% and about 60%, or between about 60% and about 65%;

the mean percentage of cells of a central memory phenotype in the plurality of the output compositions is between about 40% and about 65%, between about 40% and about 45%, between about 45% and about 50%, between about 50% and about 55%, between about 55% and about 60%, or between about 60% and about 65%;

the mean percentage of cells that are CD27+, CD28+, CCR7+, CD45RA-, CD45RO+, CD62L+, CD3+, CD95+, granzyme B-, and/or CD127+ in the plurality of the output compositions is between about 40% and about 65%, between about 40% and about 45%, between about 45% and about 50%, between about 50% and about 55%, between about 55% and about 60%, or between about 60% and about 65%;

the mean percentage of cells that are CCR7+/CD45RA- or CCR7+/CD45RO+ in the plurality of the output compositions is between about 40% and about 65%, between about 40% and about 45%, between about 45% and about 50%, between about 50% and about 55%, between about 55% and about 60%, or between about 60% and about 65%;

the mean percentage of central memory CD4+ T cells in the engineered CD4+ T cells, optionally CAR+CD4+ T cells, of the plurality of the output compositions is between about 40% and about 65%, between about 40% and about 45%, between about 45% and about 50%, between about 50% and about 55%, between about 55% and about 60%, or between about 60% and about 65%;

the mean percentage of central memory CD8+ T cells in the engineered CD8+ T cells, optionally CAR+CD8+ T cells, of the plurality of the output compositions is between about 40% and about 65%, between about 40% and about 45%, between about 45% and about 50%, between about 50% and about 55%, between about 55% and about 60%, or between about 60% and about 65%; and/or

the mean percentage of central memory T cells, optionally CD4+ central memory T cells and CD8+ central memory T cells, in the engineered T cells, optionally CAR+ T cells, of the plurality of the output compositions is between about 40% and about 65%, between about 40% and about 45%, between about 45%

and about 50%, between about 50% and about 55%, between about 55% and about 60%, or between about 60% and about 65%.

175. The use of claim 174, wherein the administered dose is produced by a method to produce an output composition exhibiting a predetermined feature, optionally a threshold number of cells expressing the CAR in the output composition, in at least about 80%, about 90%, about 95%, about 97%, about 99%, about 100%, or is 100% of the human biological samples in which it is carried out among a plurality of different individual subjects.

176. The use of claim 175, wherein the plurality of different individual subject comprise subjects having a disease or condition.

177. The use of claim 176, wherein the disease or condition is a cancer.

178. The use of claim 177, wherein the cancer is a hematological cancer, optionally multiple myeloma.