

November 1, 2018, which is 593 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 chimeric antigen receptors (CARs), which contain antibody portions specific to B-cell maturation antigen (BCMA) and polynucleotides that encode CARs specific for BCMA. The disclosure further relates to genetically engineered cells, containing such BCMA-binding receptors, and uses thereof 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 are polynucleotides encoding a chimeric antigen receptor, containing nucleic acid encoding: (a) an extracellular antigen-binding domain that specifically recognizes an antigen; (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 cases, the spacer is derived from an immunoglobulin. In some examples, the spacer includes a sequence of a hinge region, a C_H2 and a C_H3 region. In some examples, one or more of the hinge, C_H2 and C_H3 is derived all or in part from IgG4 or IgG2. In some cases, the hinge, C_H2 and C_H3 is derived from IgG4. In some examples, one or more of the hinge, C_H2 and C_H3 is chimeric and contains sequence derived from IgG4 and IgG2. In some examples, the spacer

contains an IgG4/2 chimeric hinge, an IgG2/4 C_H2, and an IgG4 C_H3 region. In some examples, the encoded spacer is or contains (i) the sequence set forth in SEQ ID NO: 649; (ii) a functional variant of SEQ ID NO:649 that has at least 95%, 96%, 97%, 98% or 99% sequence identity to SEQ ID NO:649; or (iii) a contiguous portion of (i) or (ii) that is at least 125 amino acids in length. In some examples, the encoded spacer is or includes the sequence set forth in SEQ ID NO: 649.

[0006] In some of any examples, 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 examples, 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.

[0007] In some examples of any of the polynucleotides described herein, the nucleic acid encoding the spacer includes at least one modified splice donor and/or splice acceptor site, said modified splice donor and/or acceptor site containing one or more nucleotide modifications corresponding to a reference splice donor site and/or reference splice acceptor site contained in the sequence set forth in SEQ ID NO:621. In some cases, the one or more nucleotide modifications contains an insertion, deletion, substitution or combinations thereof. In some instances, the reference splice acceptor and/or reference splice donor sites are canonical, non-canonical, or cryptic splice sites. In some examples, the reference splice donor and/or reference splice acceptor site(s) has a splice site prediction score of at least or about 0.4, 0.5, 0.6, 0.70, 0.75, 0.80, 0.85, 0.90, 0.95, 0.99, or 1.0; and/or the reference splice donor and/or reference splice acceptor site(s) is/are predicted to be involved in a splice event with a probability of at least 40%, 50%, 60%, 70%, 75%, 80%, 85%, 90%, 95%, 99%, or 100%.

[0008] In some examples of any of the polynucleotides described herein, the reference splice donor site includes the sequence aatctaagtacggac (SEQ ID NO: 705), tcaactggtacgtgg (SEQ ID NO:706), acaattagtaaggca (SEQ ID NO:707) and/or accacaggtgtatac (SEQ ID NO:708); and/or the reference splice acceptor site includes the sequence

aagtttcttctgtattccaggctgaccgtggataaatctc (SEQ ID NO:742) and/or
gggcaacgtgttctcttcagtgatgcacgaagccctgc (SEQ ID NO:743). In some examples, the reference splice donor and/or reference splice acceptor site(s) has a splice site prediction score of at least or about 0.70, 0.75, 0.80, 0.85, 0.90, 0.95, 0.99, or 1.0; and/or the reference splice donor and/or reference splice acceptor site(s) is/are predicted to be involved in a splice event with a probability of at least 70%, 75%, 80%, 85%, 90%, 95%, 99%, or 100%. In some examples, the reference splice donor site contains the sequence tcaactggtatgtgg (SEQ ID NO:706); and/or the reference splice acceptor site contains the sequence aagtttcttctgtattccaggctgaccgtggataaatctc (SEQ ID NO:742).

[0009] In some examples of any of the polynucleotides described herein, at least one of the one or more nucleotide modifications are within 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 residues of the splice site junction of the reference splice acceptor and/or reference splice donor site. In some examples, the one or more nucleotide modifications is silent and/or results in a degenerate codon compared to SEQ ID NO:621 and/or does not change the amino acid sequence of the encoded spacer. In some examples, the modified splice donor site is set forth in agtctaaatacggac (SEQ ID NO:661), tcaactggtatgtgg (SEQ ID NO:662), accatctccaaggcc (SEQ ID NO:663) and/or gccccaggtttacac (SEQ ID NO:664); and/or the modified splice acceptor site is set forth in cagtttcttctgtatagtagactcaccgtggataaatcaa (SEQ ID NO:672)
gggcaacgtgttcagctgcagcgtgatgcacgaggccctgc (SEQ ID NO: 673) and/or
aagtttcttctgtattccagactgaccgtggataaatctc (SEQ ID NO:854). In some cases, the modified splice donor site is set forth in tcaactggtatgtgg (SEQ ID NO:662) and/or the modified acceptor site is set forth in cagtttcttctgtatagtagactcaccgtggataaatcaa (SEQ ID NO:672). In some of any such examples, the spacer is encoded by a sequence of nucleotide set forth in SEQ ID NO:622 or a portion thereof.

[0010] Provided is a polynucleotide encoding a chimeric antigen receptor, wherein the polynucleotide includes nucleic acid encoding: (a) an extracellular antigen-binding domain that specifically recognizes an antigen; (b) a spacer, wherein the encoding nucleic acid is or includes the sequence set forth in SEQ ID NO:622 or encodes a sequence of amino acids set forth in SEQ ID NO:649; (c) a transmembrane domain; and (d) an intracellular signaling region.

[0010a] In one aspect, the present disclosure refers to a polynucleotide encoding the chimeric antigen receptor as disclosed herein.

[0011] Also provided is a polynucleotide encoding a chimeric antigen receptor, wherein the polynucleotide including nucleic acid encoding: (a) an extracellular antigen-binding domain that specifically recognizes an antigen; (b) a spacer, wherein the encoding nucleic acid includes or mostly includes the sequence set forth in SEQ ID NO:622 or encodes a sequence of amino acids

set forth in SEQ ID NO:649; (c) a transmembrane domain; and (d) an intracellular signaling region.

[0012] In some of any of the examples, 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 examples, following expression in a cell, the transcribed RNA, optionally messenger RNA (mRNA), from the polynucleotide exhibits reduced heterogeneity compared to the heterogeneity of the mRNA transcribed from a reference polynucleotide, said reference polynucleotide encoding the same amino acid sequence as the polynucleotide, wherein the reference polynucleotide differs by the presence of one or more splice donor site and/or one or more splice acceptor site in the nucleic acid encoding the spacer and/or includes one or more nucleotide modifications compared to the polynucleotide. In some instances, the RNA heterogeneity is reduced by greater than or greater than about 10%, 15%, 20%, 25%, 30%, 40%, 50% or more. In some cases, the transcribed RNA, optionally messenger RNA (mRNA), from the reference polynucleotide exhibits greater than or greater than about 10%, 15%, 20%, 25%, 30%, 40%, 50% or more RNA heterogeneity. In some of any such examples, the RNA homogeneity and/or heterogeneity is determined by agarose gel electrophoresis, chip-based capillary electrophoresis, analytical ultracentrifugation, field flow fractionation, or liquid chromatography. In some of any such examples, the polynucleotide is codon-optimized.

[0012a] In another aspect, the present disclosure refers to a polynucleotide encoding a chimeric antigen receptor, the polynucleotide comprising a nucleic acid sequence encoding: (a) an extracellular antigen-binding domain that specifically recognizes an antigen; (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 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.

[0012b] In another aspect, the present disclosure refers to a polynucleotide encoding a chimeric antigen receptor, the polynucleotide comprising a nucleic acid sequence encoding: (a) an extracellular antigen-binding domain that specifically recognizes an antigen; (b) a spacer of at least 125 amino acids in length; (c) a transmembrane domain; and (d) an intracellular signaling

region, wherein the nucleic acid sequence encoding the spacer comprises at least one modified splice donor and/or splice acceptor site, the modified splice donor and/or splice acceptor site comprising one or more nucleotide modifications corresponding to a reference splice donor site and/or splice acceptor site contained in the sequence set forth in SEQ ID NO:621.

[0012c] In one aspect, the present disclosure refers to a polynucleotide encoding a chimeric antigen receptor, wherein the polynucleotide comprises a nucleic acid sequence encoding:

(i) (a) an extracellular antigen-binding domain that specifically recognizes an antigen; (b) a spacer, wherein the encoding nucleic acid is or comprises the sequence set forth in SEQ ID NO:622 or encodes a sequence of amino acids set forth in SEQ ID NO:649; (c) a transmembrane domain; and (d) an intracellular signaling region; or

(ii) (a) an extracellular antigen-binding domain that specifically recognizes an antigen; (b) a spacer, wherein the encoding nucleic acid consists or consists essentially of the sequence set forth in SEQ ID NO:622 or encodes a sequence of amino acids set forth in SEQ ID NO:649; (c) a transmembrane domain; and (d) an intracellular signaling region.

[0013] In some examples of any of the polynucleotides described herein, the antigen is associated with the disease or condition or expressed in cells of the environment of a lesion associated with the disease or condition. In some cases, the disease or condition is a cancer. In some examples, the disease or condition is a myeloma, leukemia or lymphoma. In some examples, the antigen is ROR1, B cell maturation antigen (BCMA), carbonic anhydrase 9 (CAIX), tEGFR, Her2/neu (receptor tyrosine kinase erbB2), L1-CAM, CD19, CD20, CD22, mesothelin, CEA, and hepatitis B surface antigen, anti-folate receptor, CD23, CD24, CD30, CD33, CD38, CD44, EGFR, epithelial glycoprotein 2 (EPG-2), epithelial glycoprotein 40 (EPG-40), EPHA2, erb-B2, erb-B3, erb-B4, erbB dimers, EGFR vIII, folate binding protein (FBP), FCRL5, FCRH5, fetal acetylcholine receptor, GD2, GD3, HMW-MAA, IL-22R-alpha, IL-13R-alpha2, kinase insert domain receptor (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, TAG72, B7-H6, IL-13 receptor alpha 2 (IL-13Ra2), CA9, GD3, HMW-MAA, 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, CD44v6, dual antigen, a cancer-testes antigen, mesothelin, murine CMV, mucin 1 (MUC1), MUC16, PSCA, NKG2D, NY-ESO-1, MART-1, gp100, oncofetal antigen, ROR1, TAG72, VEGF-R2, carcinoembryonic antigen (CEA), 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, CD138, a pathogen-specific antigen. In some cases, the antigen is B cell maturation antigen (BCMA).

[0014] In some of any such examples, the antigen-binding domain is an antibody fragment containing a variable heavy chain (V_H) and a variable light chain (V_L) region. In some examples, the V_H region is or includes 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 amino acid sequence set forth in any of SEQ ID NOs:110-115, 247-256, 324, 325, 518-531, 533, 609 617, 772-774, or 814-832; and/or the V_L region is or includes 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 amino acid sequence set forth in any of SEQ ID NOs:116-127, 257-267, 326, 327, 534-550, 552-557, 610, 618, 775-777, or 833-849. In some cases, the V_H region is or includes 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 amino acid sequence set forth in any of SEQ ID NOs: 110, 111, 112, 113, 115, 248, 252, 253, 254, 255, 256, 324, 325, 518, 519, 520, 521, 522, 609, 617, 772-774, or 814-832; and/or the V_L region is or includes 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 amino acid sequence set forth in any of SEQ ID NOs: 116, 117, 118, 120, 121, 124, 125, 258, 262, 263, 264, 265, 266, 267, 326, 327, 534, 535, 536, 537, 538, 610, 618, 775-777, or 833-849.

[0015] In some examples of any of the polynucleotides described herein, the V_H region is or contains a CDR-H1, CDR-H2 and CDR-H3 contained within the V_H region amino acid sequence selected from any one of SEQ ID NOs:110-115, 247-256, 324, 325, 518-531, 533, 609, 617, 772-774, or 814-832; and/or the V_L region is or includes a CDR-L1, CDR-L2 and CDR-L3 contained within the V_L region amino acid sequence selected from any one of SEQ ID NOs:116-127, 257-267, 326, 327, 534-550, 552-557, 610, 618, 775-777, or 833-849. In some examples, the V_H region is or contains a CDR-H1, CDR-H2 and CDR-H3 contained within the V_H region amino acid sequence selected from any one of SEQ ID NOs: 110, 111, 112, 113, 115, 248, 252, 253, 254, 255, 256, 324, 325, 518, 519, 520, 521, 522, 609, 617, 772-774, or 814-832; and/or the V_L region is or includes a CDR-L1, CDR-L2 and CDR-L3 contained within the

V_L region amino acid sequence selected from any one of SEQ ID NOs: 116, 117, 118, 120, 121, 124, 125, 258, 262, 263, 264, 265, 266, 267, 326, 327, 534, 535, 536, 537, 538, 610, 618, 775-777, or 833-849. In some examples, the V_H region is or includes (a) a heavy chain complementarity determining region 1 (CDR-H1) containing the amino acid sequence selected from any one of SEQ ID NOs: 1-3, 140-144, 288, 289, 294, 295, 507, 532, 593, 596, 604, 611; and/or (b) a heavy chain complementarity determining region 2 (CDR-H2) containing the amino acid sequence selected from any one of SEQ ID NOs: 4-6, 145-148, 290, 291, 296, 297, 372-374, 513, 551, 594, 597, 605, or 612; and (c) a heavy chain complementarity determining region 3 (CDR-H3) containing the amino acid sequence selected from any one of SEQ ID NOs: 7-11, 149-157, 279-287, 292, 293, 376-378, 517, 595, 606, 613; and/or the V_L region is or includes (a) a light chain complementarity determining region 1 (CDR-L1) containing the amino acid sequence selected from any one of SEQ ID NOs: 26-36, 174-178, 302, 303, 380-392, 394-398, 589, 601, 607 or 614; (b) a light chain complementarity determining region 2 (CDR-L2) containing the amino acid sequence selected from any one of SEQ ID NOs: 37-46, 179-183, 304, 305, 399-409, 411-414, 590, 602, 608 or 615; and (c) a light chain complementarity determining region 3 (CDR-L3) containing the amino acid sequence selected from any one of SEQ ID NOs: 47-58, 184-194, 306, 307, 415-427, 429-433, 591, or 603.

[0016] In some examples of any of the polynucleotides described herein, the V_H region is or contains (a) a heavy chain complementarity determining region 1 (CDR-H1) containing the amino acid sequence selected from any one of SEQ ID NOs: 1, 2, 3, 141, 143, 144, 288, 289, 507, 593, 604, 611; and/or (b) a heavy chain complementarity determining region 2 (CDR-H2) containing the amino acid sequence selected from any one of SEQ ID NOs: 4, 5, 6, 145, 147, 148, 290, 291, 372, 513, 594, 605 or 612; and (c) a heavy chain complementarity determining region 3 (CDR-H3) containing the amino acid sequence selected from any one of SEQ ID NOs: 7, 8, 9, 10, 149, 153, 154, 155, 156, 157, 292, 293, 376, 517, 595, 606 or 613; and/or the V_L region is or contains (a) a light chain complementarity determining region 1 (CDR-L1) containing the amino acid sequence selected from any one of SEQ ID NOs: 26, 27, 28, 30, 31, 33, 34, 174, 176, 177, 178, 302, 303, 380, 381, 382, 589, 601, 607 or 614; (b) a light chain complementarity determining region 2 (CDR-L2) containing the amino acid sequence selected from any one of SEQ ID NOs: 37, 38, 39, 41, 43, 44, 179, 181, 182, 183, 304, 305, 399, 400, 401, 402, 590, 602, 608 or 615; and (c) a light chain complementarity determining region 3 (CDR-L3) containing the amino acid sequence selected from any one of SEQ ID NOs: 47, 48, 49, 51, 52, 55, 56, 185, 189, 190, 191, 192, 193, 194, 306, 307, 415, 417, 418, 421, 591, or 603.

[0017] In some examples of any of the polynucleotides described herein, the V_H region contains a CDR-H1, CDR-H2, and CDR-H3, selected from: a CDR-H1, CDR-H2, and CDR-H3 containing the amino acid sequence of SEQ ID NOs:1, 4, and 7, respectively; a CDR-H1, CDR-H2, and CDR-H3 containing the amino acid sequence of SEQ ID NOs:2, 5, and 8, respectively; a CDR-H1, CDR-H2, and CDR-H3 containing the amino acid sequence of SEQ ID NOs:2, 5, and 9, respectively; a CDR-H1, CDR-H2, and CDR-H3 containing the amino acid sequence of SEQ ID NOs:2, 5, and 10, respectively; a CDR-H1, CDR-H2, and CDR-H3 containing the amino acid sequence of SEQ ID NOs:3, 6, and 11, respectively; a CDR-H1, CDR-H2, and CDR-H3 containing the amino acid sequence of SEQ ID NOs:140, 145, and 149, respectively; a CDR-H1, CDR-H2, and CDR-H3 containing the amino acid sequence of SEQ ID NOs:141, 145, and 149, respectively; a CDR-H1, CDR-H2, and CDR-H3 containing the amino acid sequence of SEQ ID NOs:141, 145, and 150, respectively; a CDR-H1, CDR-H2, and CDR-H3 containing the amino acid sequence of SEQ ID NOs:142, 146, and 151, respectively; a CDR-H1, CDR-H2, and CDR-H3 containing the amino acid sequence of SEQ ID NOs:2, 5, and 152, respectively; a CDR-H1, CDR-H2, and CDR-H3 containing the amino acid sequence of SEQ ID NOs:143, 147, and 153, respectively; a CDR-H1, CDR-H2, and CDR-H3 containing the amino acid sequence of SEQ ID NOs:144, 148, and 154, respectively; a CDR-H1, CDR-H2, and CDR-H3 containing the amino acid sequence of SEQ ID NOs:3, 6, and 155, respectively; a CDR-H1, CDR-H2, and CDR-H3 containing the amino acid sequence of SEQ ID NOs:2, 5, and 156, respectively; a CDR-H1, CDR-H2, and CDR-H3 containing the amino acid sequence of SEQ ID NOs:2, 5, and 157, respectively; a CDR-H1, CDR-H2, and CDR-H3 containing the amino acid sequence of SEQ ID NOs:2, 6, and 376, respectively; a CDR-H1, CDR-H2, and CDR-H3 containing the amino acid sequence of SEQ ID NOs:3, 6, and 155, respectively; a CDR-H1, CDR-H2, and CDR-H3 containing the amino acid sequence of SEQ ID NOs:3, 372, and 376, respectively; a CDR-H1, CDR-H2, and CDR-H3 containing the amino acid sequence of SEQ ID NOs:3, 6, and 376, respectively; a CDR-H1, CDR-H2, and CDR-H3 containing the amino acid sequence of SEQ ID NOs:3, 6, and 377, respectively; a CDR-H1, CDR-H2, and CDR-H3 containing the amino acid sequence of SEQ ID NOs:2, 373, and 152, respectively; a CDR-H1, CDR-H2, and CDR-H3 containing the amino acid sequence of SEQ ID NOs:2, 5, and 378, respectively; a CDR-H1, CDR-H2, and CDR-H3 containing the amino acid sequence of SEQ ID NOs:2, 374, and 9, respectively; a CDR-H1, CDR-H2, and CDR-H3 containing the amino acid sequence of SEQ ID NOs:593, 594, and 595, respectively; a CDR-H1, CDR-H2, and CDR-H3 containing the amino acid sequence of SEQ ID NOs:611, 612, and 613, respectively; a CDR-H1, CDR-H2, and

CDR-H3 containing the amino acid sequence of SEQ ID NOs:507, 513, and 517, respectively; a CDR-H1, CDR-H2, and CDR-H3 containing the amino acid sequence of SEQ ID NOs:604, 605, and 606, respectively; a CDR-H1, CDR-H2, and CDR-H3 containing the amino acid sequence of SEQ ID NOs:288, 290, and 292, respectively; or a CDR-H1, CDR-H2, and CDR-H3 containing the amino acid sequence of SEQ ID NOs:289, 291, and 293, respectively.

[0018] In some examples of any of the polynucleotides described herein, the V_H region contains a CDR-H1, CDR-H2, and CDR-H3, selected from: a CDR-H1, CDR-H2, and CDR-H3 containing the amino acid sequence of SEQ ID NOs:1, 4, and 7, respectively; a CDR-H1, CDR-H2, and CDR-H3 containing the amino acid sequence of SEQ ID NOs:2, 5, and 8, respectively; a CDR-H1, CDR-H2, and CDR-H3 containing the amino acid sequence of SEQ ID NOs:2, 5, and 9, respectively; a CDR-H1, CDR-H2, and CDR-H3 containing the amino acid sequence of SEQ ID NOs:2, 5, and 10, respectively; a CDR-H1, CDR-H2, and CDR-H3 containing the amino acid sequence of SEQ ID NOs:141, 145, and 149, respectively; a CDR-H1, CDR-H2, and CDR-H3 containing the amino acid sequence of SEQ ID NOs:143, 147, and 153, respectively; a CDR-H1, CDR-H2, and CDR-H3 containing the amino acid sequence of SEQ ID NOs:144, 148, and 154, respectively; a CDR-H1, CDR-H2, and CDR-H3 containing the amino acid sequence of SEQ ID NOs:3, 6, and 155, respectively; a CDR-H1, CDR-H2, and CDR-H3 containing the amino acid sequence of SEQ ID NOs:2, 5, and 156, respectively; a CDR-H1, CDR-H2, and CDR-H3 containing the amino acid sequence of SEQ ID NOs:2, 5, and 157, respectively; a CDR-H1, CDR-H2, and CDR-H3 containing the amino acid sequence of SEQ ID NOs:2, 6, and 376, respectively; a CDR-H1, CDR-H2, and CDR-H3 containing the amino acid sequence of SEQ ID NOs:3, 6, and 155, respectively; a CDR-H1, CDR-H2, and CDR-H3 containing the amino acid sequence of SEQ ID NOs:3, 372, and 376, respectively; a CDR-H1, CDR-H2, and CDR-H3 containing the amino acid sequence of SEQ ID NOs:3, 6, and 376, respectively; a CDR-H1, CDR-H2, and CDR-H3 containing the amino acid sequence of SEQ ID NOs:593, 594, and 595, respectively; a CDR-H1, CDR-H2, and CDR-H3 containing the amino acid sequence of SEQ ID NOs:611, 612, and 613, respectively; a CDR-H1, CDR-H2, and CDR-H3 containing the amino acid sequence of SEQ ID NOs:507, 513, and 517, respectively; a CDR-H1, CDR-H2, and CDR-H3 containing the amino acid sequence of SEQ ID NOs:604, 605, and 606, respectively; a CDR-H1, CDR-H2, and CDR-H3 containing the amino acid sequence of SEQ ID NOs:288, 290, and 292, respectively; or a CDR-H1, CDR-H2, and CDR-H3 containing the amino acid sequence of SEQ ID NOs:289, 291, and 293, respectively;

[0019] In some examples of any of the polynucleotides described herein, the V_H region is or includes the amino acid sequence set forth in any of SEQ ID NOs: 110-115, 247-256, 324, 325, 518-531, 533, 609, 617, 772-774, or 814-832. In some examples, the V_H region is or includes the amino acid sequence set forth in any of SEQ ID NOs: 110, 111, 112, 113, 115, 248, 252, 253, 254, 255, 256, 324, 325, 518, 519, 520, 521, 522, 609 or 617. In some examples, the V_H region contains a CDR-H1, CDR-H2, and CDR-H3 containing the amino acid sequence of SEQ ID NOs:593, 594, and 595, respectively; or the V_H region includes a CDR-H1, CDR-H2, and CDR-H3 containing the amino acid sequence of SEQ ID NOs:611, 612, and 613, respectively. In some examples, the V_H region is or includes the amino acid sequence set forth in SEQ ID NO: 617.

[0020] In some examples of any of the polynucleotides described herein, the V_L region includes a CDR-L1, CDR-L2, and CDR-L3 selected from: a CDR-L1, CDR-L2, and CDR-L3 containing the amino acid sequence of SEQ ID NOs:26, 37, and 47, respectively; a CDR-L1, CDR-L2, and CDR-L3 containing the amino acid sequence of SEQ ID NOs:27, 38, and 48, respectively; a CDR-L1, CDR-L2, and CDR-L3 containing the amino acid sequence of SEQ ID NOs:28, 39, and 49, respectively; a CDR-L1, CDR-L2, and CDR-L3 containing the amino acid sequence of SEQ ID NOs:29, 40, and 50, respectively; a CDR-L1, CDR-L2, and CDR-L3 containing the amino acid sequence of SEQ ID NOs:30, 39, and 51, respectively; a CDR-L1, CDR-L2, and CDR-L3 containing the amino acid sequence of SEQ ID NOs:31, 41, and 52, respectively; a CDR-L1, CDR-L2, and CDR-L3 containing the amino acid sequence of SEQ ID NOs:32, 42, and 53, respectively; a CDR-L1, CDR-L2, and CDR-L3 containing the amino acid sequence of SEQ ID NOs:30, 39, and 54, respectively; a CDR-L1, CDR-L2, and CDR-L3 containing the amino acid sequence of SEQ ID NOs:33, 43, and 55, respectively; a CDR-L1, CDR-L2, and CDR-L3 containing the amino acid sequence of SEQ ID NOs:34, 44, and 56, respectively; a CDR-L1, CDR-L2, and CDR-L3 containing the amino acid sequence of SEQ ID NOs:35, 45, and 57, respectively; a CDR-L1, CDR-L2, and CDR-L3 containing the amino acid sequence of SEQ ID NOs:36, 46, and 58, respectively; a CDR-L1, CDR-L2, and CDR-L3 containing the amino acid sequence of SEQ ID NOs:174, 179, and 184, respectively; a CDR-L1, CDR-L2, and CDR-L3 containing the amino acid sequence of SEQ ID NOs:174, 179, and 185, respectively; a CDR-L1, CDR-L2, and CDR-L3 containing the amino acid sequence of SEQ ID NOs:174, 179, and 186, respectively; a CDR-L1, CDR-L2, and CDR-L3 containing the amino acid sequence of SEQ ID NOs:174, 179, and 187, respectively; a CDR-L1, CDR-L2, and CDR-L3 containing the amino acid sequence of SEQ ID NOs:175, 180, and 188, respectively; a CDR-

amino acid sequence of SEQ ID NOs:396, 412, and 58, respectively; a CDR-L1, CDR-L2, and CDR-L3 containing the amino acid sequence of SEQ ID NOs:397, 413, and 432, respectively; a CDR-L1, CDR-L2, and CDR-L3 containing the amino acid sequence of SEQ ID NOs:398, 414, and 433, respectively; a CDR-L1, CDR-L2, and CDR-L3 containing the amino acid sequence of SEQ ID NOs:601, 602, and 603, respectively; a CDR-L1, CDR-L2, and CDR-L3 containing the amino acid sequence of SEQ ID NOs:614, 615, and 603, respectively; a CDR-L1, CDR-L2, and CDR-L3 containing the amino acid sequence of SEQ ID NOs:589, 590, and 591, respectively; a CDR-L1, CDR-L2, and CDR-L3 containing the amino acid sequence of SEQ ID NOs:607, 608, and 591, respectively; a CDR-L1, CDR-L2, and CDR-L3 containing the amino acid sequence of SEQ ID NOs:302, 304, and 306, respectively; or a CDR-L1, CDR-L2, and CDR-L3 containing the amino acid sequence of SEQ ID NOs:303, 305, and 307, respectively.

[0021] In some examples of any of the polynucleotides described herein, the V_L region includes a CDR-L1, CDR-L2, and CDR-L3 selected from: a CDR-L1, CDR-L2, and CDR-L3 containing the amino acid sequence of SEQ ID NOs:26, 37, and 47, respectively; a CDR-L1, CDR-L2, and CDR-L3 containing the amino acid sequence of SEQ ID NOs:27, 38, and 48, respectively; a CDR-L1, CDR-L2, and CDR-L3 containing the amino acid sequence of SEQ ID NOs:28, 39, and 49, respectively; a CDR-L1, CDR-L2, and CDR-L3 containing the amino acid sequence of SEQ ID NOs:30, 39, and 51, respectively; a CDR-L1, CDR-L2, and CDR-L3 containing the amino acid sequence of SEQ ID NOs:31, 41, and 52, respectively; a CDR-L1, CDR-L2, and CDR-L3 containing the amino acid sequence of SEQ ID NOs:33, 43, and 55, respectively; a CDR-L1, CDR-L2, and CDR-L3 containing the amino acid sequence of SEQ ID NOs:34, 44, and 56, respectively; a CDR-L1, CDR-L2, and CDR-L3 containing the amino acid sequence of SEQ ID NOs:174, 179, and 185, respectively; a CDR-L1, CDR-L2, and CDR-L3 containing the amino acid sequence of SEQ ID NOs:174, 179, and 189, respectively; a CDR-L1, CDR-L2, and CDR-L3 containing the amino acid sequence of SEQ ID NOs:176, 181, and 190, respectively; a CDR-L1, CDR-L2, and CDR-L3 containing the amino acid sequence of SEQ ID NOs:177, 182, and 191, respectively; a CDR-L1, CDR-L2, and CDR-L3 containing the amino acid sequence of SEQ ID NOs:174, 179, and 192, respectively; a CDR-L1, CDR-L2, and CDR-L3 containing the amino acid sequence of SEQ ID NOs:178, 183, and 193, respectively; a CDR-L1, CDR-L2, and CDR-L3 containing the amino acid sequence of SEQ ID NOs:178, 183, and 194, respectively; a CDR-L1, CDR-L2, and CDR-L3 containing the amino acid sequence of SEQ ID NOs:30, 399, and 415, respectively; a CDR-L1, CDR-L2, and CDR-L3 containing the amino acid sequence of SEQ ID NOs:380, 400, and 416, respectively; a CDR-L1, CDR-L2, and

CDR-L3 containing the amino acid sequence of SEQ ID NOs:33, 43, and 421, respectively; a CDR-L1, CDR-L2, and CDR-L3 containing the amino acid sequence of SEQ ID NOs:381, 401, and 417, respectively; a CDR-L1, CDR-L2, and CDR-L3 containing the amino acid sequence of SEQ ID NOs:382, 402, and 418, respectively; a CDR-L1, CDR-L2, and CDR-L3 containing the amino acid sequence of SEQ ID NOs:601, 602, and 603, respectively; a CDR-L1, CDR-L2, and CDR-L3 containing the amino acid sequence of SEQ ID NOs:614, 615, and 603, respectively; a CDR-L1, CDR-L2, and CDR-L3 containing the amino acid sequence of SEQ ID NOs:589, 590, and 591, respectively; a CDR-L1, CDR-L2, and CDR-L3 containing the amino acid sequence of SEQ ID NOs:607, 608, and 591, respectively; a CDR-L1, CDR-L2, and CDR-L3 containing the amino acid sequence of SEQ ID NOs:302, 304, and 306, respectively; or a CDR-L1, CDR-L2, and CDR-L3 containing the amino acid sequence of SEQ ID NOs:303, 305, and 307, respectively.

[0022] In some of any such examples, the V_L region is or includes the amino acid sequence set forth in any of SEQ ID NOs: 116-127, 257-267, 326, 327, 534-550, 552-557, 610, 618, 775-777, or 833-849. In some examples, the V_L region is or contains the amino acid sequence set forth in any of SEQ ID NOs: 116, 117, 118, 120, 121, 124, 125, 258, 262, 263, 264, 265, 266, 267, 326, 327, 534, 535, 536, 537, 538, 610, 618, 775-777, or 833-849.

[0023] In some examples of any of the polynucleotides described herein, the V_L region contains a CDR-L1, CDR-L2, and CDR-L3 including the amino acid sequence of SEQ ID NOs:601, 602, and 603, respectively; or the V_L region contains a CDR-L1, CDR-L2, and CDR-L3 including the amino acid sequence of SEQ ID NOs:614, 615, and 603, respectively. In some cases, the V_L region is or includes the amino acid sequence set forth in SEQ ID NO:618.

[0024] In some of any examples, the V_H region is or comprises 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 any of SEQ ID NOs:617, 110-115, 247-256, 324, 325, 518-531, 533, 609, 772-774, or 814-832; and the V_L region is or comprises 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 NOs: 618, 116-127, 257-267, 326, 327, 534-550, 552-557, 610, 775-777, or 833-849.

[0025] In some of any examples, the V_H region is or comprises a CDR-H1, CDR-H2 and CDR-H3 contained within the V_H region amino acid sequence selected from any one of SEQ ID NOs: 617, 110-115, 247-256, 324, 325, 518-531, 533, 609, 772-774, or 814-832; and the V_L region is or comprises a CDR-L1, CDR-L2 and CDR-L3 contained within the V_L region amino

acid sequence selected from any one of SEQ ID NOs: 618, 116-127, 257-267, 326, 327, 534-550, 552-557, 610, 775-777, or 833-849.

[0026] In some of any examples, the V_H region is or comprises (a) a CDR-H1 comprising the sequence selected from any one of SEQ ID NOs: 593, 611, 1-3, 140-144, 288, 289, 294, 295, 507, 532, 596, or 604; (b) a CDR-H2 comprising the sequence selected from any one of SEQ ID NOs: 594, 612, 4-6, 145-148, 290, 291, 296, 297, 372-374, 513, 551, 597, or 605; and (c) a CDR-H3 comprising the sequence selected from any one of SEQ ID NOs: 595, 613, 7-11, 149-157, 279-287, 292, 293, 376-378, 517, or 606; and the V_L region is or comprises (a) a CDR-L1 comprising the sequence selected from any one of SEQ ID NOs: 601, 614, 26-36, 174-178, 302, 303, 380-392, 394-398, 589, or 607; (b) a CDR-L2 comprising the sequence selected from any one of SEQ ID NOs: 602, 615, 37-46, 179-183, 304, 305, 399-409, 411-414, 590, or 608; and (c) a CDR-L3 comprising the sequence selected from any one of SEQ ID NOs: 603, 47-58, 184-194, 306, 307, 415-427, 429-433, or 591.

[0027] In some of any such examples, the V_H region and the V_L regions includes the amino acid sequence set forth in SEQ ID NOs:110 and 116, respectively, or a sequence of amino acids that has at least 90% identity to SEQ ID NO:110 and 116, respectively; the V_H region and the V_L regions contain the amino acid sequence set forth in SEQ ID NOs:111 and 117, respectively, or a sequence of amino acids that has at least 90% identity to SEQ ID NO:111 and 117, respectively; the V_H region and the V_L regions contain the amino acid sequence set forth in SEQ ID NOs:110 and 118, respectively, or a sequence of amino acids that has at least 90% identity to SEQ ID NO:110 and 118, respectively; the V_H region and the V_L regions contain the amino acid sequence set forth in SEQ ID NOs:110 and 119, respectively, or a sequence of amino acids that has at least 90% identity to SEQ ID NO:110 and 119, respectively; the V_H region and the V_L regions contain the amino acid sequence set forth in SEQ ID NOs:110 and 120, respectively, or a sequence of amino acids that has at least 90% identity to SEQ ID NO:110 and 120, respectively; the V_H region and the V_L regions contain the amino acid sequence set forth in SEQ ID NOs:110 and 121, respectively, or a sequence of amino acids that has at least 90% identity to SEQ ID NO:110 and 121, respectively; the V_H region and the V_L regions contain the amino acid sequence set forth in SEQ ID NOs:110 and 122, respectively, or a sequence of amino acids that has at least 90% identity to SEQ ID NO:110 and 122, respectively; the V_H region and the V_L regions contain the amino acid sequence set forth in SEQ ID NOs:110 and 123, respectively, or a sequence of amino acids that has at least 90% identity to SEQ ID NO:110 and 123, respectively; the V_H region and the V_L regions contain the amino acid sequence set forth in

90% identity to SEQ ID NO:823 and 841, respectively; or the V_H region and the V_L regions contain the amino acid sequence set forth in SEQ ID NOs:824 and 842, respectively, or a sequence of amino acids that has at least 90% identity to SEQ ID NO:824 and 842, respectively; or the V_H region and the V_L regions contain the amino acid sequence set forth in SEQ ID NOs:825 and 843, respectively, or a sequence of amino acids that has at least 90% identity to SEQ ID NO:825 and 843, respectively; or the V_H region and the V_L regions contain the amino acid sequence set forth in SEQ ID NOs:826 and 844, respectively, or a sequence of amino acids that has at least 90% identity to SEQ ID NO:826 and 844, respectively; or the V_H region and the V_L regions contain the amino acid sequence set forth in SEQ ID NOs:827 and 845, respectively, or a sequence of amino acids that has at least 90% identity to SEQ ID NO:827 and 845, respectively; or the V_H region and the V_L regions contain the amino acid sequence set forth in SEQ ID NOs:828 and 846, respectively, or a sequence of amino acids that has at least 90% identity to SEQ ID NO:828 and 846, respectively; or the V_H region and the V_L regions contain the amino acid sequence set forth in SEQ ID NOs:829 and 847, respectively, or a sequence of amino acids that has at least 90% identity to SEQ ID NO:829 and 847, respectively; or the V_H region and the V_L regions contain the amino acid sequence set forth in SEQ ID NOs:830 and 847, respectively, or a sequence of amino acids that has at least 90% identity to SEQ ID NO:830 and 847, respectively; or the V_H region and the V_L regions contain the amino acid sequence set forth in SEQ ID NOs:831 and 848, respectively, or a sequence of amino acids that has at least 90% identity to SEQ ID NO:831 and 848, respectively; or the V_H region and the V_L regions contain the amino acid sequence set forth in SEQ ID NOs:832 and 849, respectively, or a sequence of amino acids that has at least 90% identity to SEQ ID NO:832 and 849, respectively.

[0028] In some examples of any of the polynucleotides described herein, the V_H region and the V_L regions encoded by the polynucleotides include the amino acid sequence set forth in SEQ ID NOs:110 and 116, respectively, or a sequence of amino acids that has at least 90% identity to SEQ ID NO:110 and 116, respectively; the V_H region and the V_L regions contain the amino acid sequence set forth in SEQ ID NOs:111 and 117, respectively, or a sequence of amino acids that has at least 90% identity to SEQ ID NO:111 and 117, respectively; the V_H region and the V_L regions contain the amino acid sequence set forth in SEQ ID NOs:110 and 118, respectively, or a sequence of amino acids that has at least 90% identity to SEQ ID NO:110 and 118, respectively; the V_H region and the V_L regions contain the amino acid sequence set forth in SEQ ID NOs:110 and 120, respectively, or a sequence of amino acids that has at least 90% identity to SEQ ID NO:110 and 120, respectively; the V_H region and the V_L regions contain the

NOs:820 and 838, respectively, or a sequence of amino acids that has at least 90% identity to SEQ ID NO:820 and 838, respectively; or the V_H region and the V_L regions contain the amino acid sequence set forth in SEQ ID NOs:821 and 839, respectively, or a sequence of amino acids that has at least 90% identity to SEQ ID NO:821 and 839, respectively; or the V_H region and the V_L regions contain the amino acid sequence set forth in SEQ ID NOs:822 and 840, respectively, or a sequence of amino acids that has at least 90% identity to SEQ ID NO:822 and 840, respectively; or the V_H region and the V_L regions contain the amino acid sequence set forth in SEQ ID NOs:823 and 841, respectively, or a sequence of amino acids that has at least 90% identity to SEQ ID NO:823 and 841, respectively; or the V_H region and the V_L regions contain the amino acid sequence set forth in SEQ ID NOs:824 and 842, respectively, or a sequence of amino acids that has at least 90% identity to SEQ ID NO:824 and 842, respectively; or the V_H region and the V_L regions contain the amino acid sequence set forth in SEQ ID NOs:825 and 843, respectively, or a sequence of amino acids that has at least 90% identity to SEQ ID NO:825 and 843, respectively; or the V_H region and the V_L regions contain the amino acid sequence set forth in SEQ ID NOs:826 and 844, respectively, or a sequence of amino acids that has at least 90% identity to SEQ ID NO:826 and 844, respectively; or the V_H region and the V_L regions contain the amino acid sequence set forth in SEQ ID NOs:827 and 845, respectively, or a sequence of amino acids that has at least 90% identity to SEQ ID NO:827 and 845, respectively; or the V_H region and the V_L regions contain the amino acid sequence set forth in SEQ ID NOs:828 and 846, respectively, or a sequence of amino acids that has at least 90% identity to SEQ ID NO:828 and 846, respectively; or the V_H region and the V_L regions contain the amino acid sequence set forth in SEQ ID NOs:829 and 847, respectively, or a sequence of amino acids that has at least 90% identity to SEQ ID NO:829 and 847, respectively; or the V_H region and the V_L regions contain the amino acid sequence set forth in SEQ ID NOs:830 and 847, respectively, or a sequence of amino acids that has at least 90% identity to SEQ ID NO:830 and 847, respectively; or the V_H region and the V_L regions contain the amino acid sequence set forth in SEQ ID NOs:831 and 848, respectively, or a sequence of amino acids that has at least 90% identity to SEQ ID NO:831 and 848, respectively; or the V_H region and the V_L regions contain the amino acid sequence set forth in SEQ ID NOs:832 and 849, respectively, or a sequence of amino acids that has at least 90% identity to SEQ ID NO:832 and 849, respectively.

[0029] In some of any examples, the V_H region is or comprises the sequence of any of SEQ ID NOs: 617, 110-115, 247-256, 324, 325, 518-531, 533, 609, 772-774, or 814-832; and

the V_L region is or comprises the sequence of any of SEQ ID NOs: 618, 116-127, 257-267, 326, 327, 534-550, 552-557, 610, 775-777, or 833-849.

[0030] In some examples of any of the polynucleotides described herein, the fragment includes an scFv. In some examples, the V_H region and the V_L region are joined by a flexible linker. In some examples, the scFv includes a linker containing the amino acid sequence GGGGSGGGGSGGGGS (SEQ ID NO:361). In some examples, the V_H region is amino-terminal to the V_L region.

[0031] In some examples of any of the polynucleotides described herein, the antigen-binding domain includes the amino acid sequence selected from any one of SEQ ID NOs:128-139, 268-278, 329, 442, 478, 558-576, 578-583, 585, or 769-771 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 selected from any one of SEQ ID NOs: 128-139, 268-278, 329, 442, 478, 558-576, 578-583, 585, or 769-771. In some examples, the antigen-binding domain includes the amino acid sequence selected from any one of SEQ ID NOs:128-130, 132, 133, 136, 137, 269, 273-278, 329, 442, 478, 558-563 or 585 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 selected from any one of SEQ ID NOs: 128-130, 132, 133, 136, 137, 269, 273-278, 329, 442, 478, 558-563 or 585.

[0032] In some examples of any of the polynucleotides described herein, the nucleic acid encoding the antigen-binding domain includes (a) the sequence of nucleotides set forth in any of SEQ ID NOS: 330-352, 647, 648, 716 or 718; (b) a sequence of nucleotides that has at least 90% sequence identity to any of SEQ ID NOS: 330-352, 647, 648, 716 or 718; or (c) a degenerate sequence of (a) or (b). In some examples, the nucleic acid encoding the antigen-binding domain includes (a) the sequence of nucleotides set forth in any of SEQ ID NOS: 352, 647, 648, 716, or 718; (b) a sequence of nucleotides that has at least 90% sequence identity to any of SEQ ID NOS: 352, 647, 648, 716, or 718; or (c) a degenerate sequence of (a) or (b). In some examples, the nucleic acid encoding the antigen-binding domain is codon-optimized. In some examples, the nucleic acid encoding the antigen-binding domain includes the sequence of nucleotides set forth in any of SEQ ID NO: 440, 460, 715, 717 or 719. In some examples, the nucleic acid encoding the antigen-binding domain includes the sequence of nucleotides set forth in SEQ ID NO:460.

[0033] In some examples of any of the polynucleotides described herein, the V_H region is carboxy-terminal to the V_L region. In some examples, the scFv includes the amino acid

sequence set forth in SEQ ID NOs:328 or 586, 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 set forth in SEQ ID NO:328 or 586.

[0034] 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: 617; 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: 618; (2) a spacer set forth in SEQ ID NO: 649 or wherein the nucleic acid encoding the spacer is or comprises the sequence set forth in SEQ ID NO:622; (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 ($CD3\zeta$) chain and an intracellular signaling domain of a T cell costimulatory molecule. Also provided are polynucleotides encoding such a chimeric antigen receptor. In some of any examples, the V_H region comprises a CDR-H1, CDR-H2 and CDR-H3 contained within the V_H region sequence of SEQ ID NO: 617; 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: 618; or the V_H region comprises a CDR-H1, CDR-H2, and CDR-H3 comprising the sequence of SEQ ID NOS:593, 594, and 595, respectively, and the V_L region comprises a CDR-L1, CDR-L2, and CDR-L3 comprising the sequence of SEQ ID NOS:601, 602, and 603, respectively; the V_H region comprises a CDR-H1, CDR-H2, and CDR-H3 comprising the sequence of SEQ ID NOS:596, 597, and 595, respectively, and the V_L region comprises a CDR-L1, CDR-L2, and CDR-L3 comprising the sequence of SEQ ID NOS:601, 602, and 603, respectively; the V_H region comprises a CDR-H1, CDR-H2, and CDR-H3 comprising the sequence of SEQ ID NOS:598, 599, and 595, respectively, and the V_L region comprises a CDR-L1, CDR-L2, and CDR-L3 comprising the sequence of SEQ ID NOS:601, 602, and 603, respectively; or the V_H region comprises a CDR-H1, CDR-H2, and CDR-H3 comprising the sequence of SEQ ID NOS:611, 612, and 613, respectively, and the V_L region comprises a CDR-L1, CDR-L2, and CDR-L3 comprising the sequence of SEQ ID NOS:614, 615, and 603, respectively.

[0034a] In another aspect, the present disclosure refers to a polynucleotide encoding a chimeric antigen receptor, comprising a nucleic acid encoding:

(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 amino acid sequence set forth in SEQ ID NO: 617; 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 amino acid sequence set forth in any of SEQ ID NO: 618;

(2) 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, optionally that is about 228 amino acids in length; or a spacer set forth in SEQ ID NO: 649;

(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 ($CD3\zeta$) chain and an intracellular signaling domain of a T cell costimulatory molecule.

[0034b] In another aspect, the present disclosure refers to a chimeric antigen receptor, 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 amino acid sequence set forth in SEQ ID NO: 617; 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 amino acid sequence set forth in any of SEQ ID NO: 618;

(2) 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, optionally that is about 228 amino acids in length; or a spacer set forth in SEQ ID NO: 649;

(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 ($CD3\zeta$) chain and an intracellular signaling domain of a T cell costimulatory molecule.

[0035] 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: 617; 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: 618; 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: 617; 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: 618; or the V_H region comprises a CDR-H1, CDR-H2, and CDR-H3 comprising the sequence of SEQ ID NOS:593, 594, and 595, respectively, and the V_L region comprises a CDR-L1, CDR-L2, and CDR-L3 comprising the sequence of SEQ ID NOS:601, 602, and 603, respectively; the V_H region comprises a CDR-H1, CDR-H2, and CDR-H3 comprising the sequence of SEQ ID NOS:596, 597, and 595, respectively, and the V_L region comprises a CDR-L1, CDR-L2, and CDR-L3 comprising the sequence of SEQ ID NOS:601, 602, and 603, respectively; the V_H region comprises a CDR-H1, CDR-H2, and CDR-H3 comprising the sequence of SEQ ID NOS:598, 599, and 595, respectively, and the V_L region comprises a CDR-L1, CDR-L2, and CDR-L3 comprising the sequence of SEQ ID NOS:601, 602, and 603, respectively; or the V_H region comprises a CDR-H1, CDR-H2, and CDR-H3 comprising the sequence of SEQ ID NOS:611, 612, and 613, respectively, and the V_L region comprises a CDR-L1, CDR-L2, and CDR-L3 comprising the sequence of SEQ ID NOS:614, 615, and 603, respectively; (2) a spacer set forth in SEQ ID NO: 649 or wherein the nucleic acid encoding the spacer is or comprises the sequence set forth in SEQ ID NO:622; (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 ($CD3\zeta$) chain and an intracellular signaling domain of a human 4-1BB or a human CD28. Also provided are polynucleotides encoding such a chimeric antigen receptor. In some of any examples, the extracellular antigen-binding domain comprises the V_H region sequence of SEQ ID NO:617 and the V_L region sequence of SEQ ID NO:618.

[0036] In some examples, the receptor includes an antigen-binding domain that binds to the same or substantially the same epitope on BCMA, or competes for binding to BCMA with, any of the antibodies and fragments, or antibodies having the provided combinations of V_H/V_L or CDR sequences, described herein including in any of the foregoing examples. In some examples, the binding domain recognizes an epitope comprising a portion of one or more amino acid sequences within a BCMA polypeptide. In some examples, such one or more amino acid sequences are or comprise: MLMAG (SEQ ID NO:640), YFDSL (SEQ ID NO:779), and

QLRCSSNTPPL (SEQ ID NO:642). In some examples, such one or more amino acid sequences are or comprise: MLMAG (SEQ ID NO:640), YFDSL (SEQ ID NO:641), and QLRCSSNTPPL (SEQ ID NO:642). In some examples, such one or more amino acid sequences are or comprise: MLMAG (SEQ ID NO:640), QNEYFDSL (SEQ ID NO:780), and QLRCSSNTPPL (SEQ ID NO:642). In some examples, such one or more amino acid sequences are or comprise: QNEYF (SEQ ID NO:637), CIPCQL (SEQ ID NO:638), and CQRYC (SEQ ID NO:639). In some examples, such one or more amino acid sequences are or comprise: CSQNEYF (set forth in SEQ ID NO:410) and LLHACIPCQLR (set forth in SEQ ID NO:428).

[0037] In some examples of any of the polynucleotides described herein, the intracellular signaling region includes an activating cytoplasmic signaling domain. In some examples, the activating 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 examples, the activating cytoplasmic signaling domain is or includes a cytoplasmic signaling domain of a zeta chain of a CD3-zeta (CD3 ζ) chain or a functional variant or signaling portion thereof. In some examples, the activating cytoplasmic domain is human or is derived from a human protein. In some examples, the activating cytoplasmic domain is or includes the sequence set forth in SEQ ID NO:628 or a sequence of amino acids that has at least 90% sequence identity to SEQ ID NO:628.

[0038] In some examples of any of the polynucleotides described herein, the nucleic acid encoding the activating cytoplasmic domain is or includes the sequence set forth in SEQ ID NO:627 or is a codon-optimized sequence and/or degenerate sequence thereof. In other examples, the nucleic acid encoding the activating cytoplasmic signaling domain is or includes the sequence set forth in SEQ ID NO:652.

[0039] In some examples of any of the polynucleotides described herein, the intracellular signaling region further includes a costimulatory signaling region. In some examples, the costimulatory signaling region includes an intracellular signaling domain of a T cell costimulatory molecule or a signaling portion thereof. In some examples, 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 examples, the costimulatory signaling region includes an intracellular signaling domain of 4-1BB. In some examples, the costimulatory signaling region is human or is derived from a human protein. In other examples, the costimulatory signaling region is or includes the sequence set forth in SEQ ID

NO:626 or a sequence of amino acids that exhibits at least 90% sequence identity to the sequence set forth in SEQ ID NO: 626.

[0040] In some examples of any of the polynucleotides described herein, the nucleic acid encoding the costimulatory region is or includes the sequence set forth in SEQ ID NO:625 or is a codon-optimized sequence and/or degenerate sequence thereof. In some examples, the nucleic acid encoding the costimulatory signaling region includes the sequence set forth in SEQ ID NO:681. In some examples, the costimulatory signaling region is between the transmembrane domain and the intracellular signaling region. In some examples, the transmembrane domain is or includes a transmembrane domain derived from CD4, CD28, or CD8. In some examples, the transmembrane domain is or includes a transmembrane domain derived from a CD28. In some examples, the transmembrane domain is human or is derived from a human protein. In other examples, the transmembrane domain is or includes the sequence set forth in SEQ ID NO:624 or a sequence of amino acids that exhibits at least 90% sequence identity to SEQ ID NO:624.

[0041] In some examples of any of the polynucleotides described herein, the nucleic acid encoding the transmembrane domain is or includes the sequence set forth in SEQ ID NO:623 or is a codon-optimized sequence and/or degenerate sequence thereof. In some examples, the nucleic acid encoding the transmembrane domain includes the sequence set forth in SEQ ID NO:688. In some examples of any of the polynucleotides described herein, the encoded chimeric antigen receptor includes from its N to C terminus in order: the antigen-binding domain, the spacer, the transmembrane domain and the intracellular signaling domain.

[0042] In some of any of the examples, the polynucleotide comprises the sequence set forth in any of SEQ ID NOS: 751-756 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 to the sequence set forth in any of SEQ ID NOS: 751-756 and the encoded receptor retains the function to bind to BCMA and retains the reduced RNA heterogeneity. In some of any of the examples, the polynucleotide comprises the sequence set forth in any of SEQ ID NOS: 755 and 756 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 to the sequence set forth in any of SEQ ID NOS: 755 and 756 and the encoded receptor retains the function to bind to BCMA and retains the reduced RNA heterogeneity. In some of any of the examples, the polynucleotide comprises the sequence set forth in SEQ ID NOS:755 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

and the encoded receptor retains the function to bind to BCMA and retains the reduced RNA heterogeneity. In some of any of the examples, the polynucleotide comprises the sequence set forth in SEQ ID NOs:755 and the encoded receptor retains the function to bind to BCMA and retains the reduced RNA heterogeneity.

[0043] In some examples, the polynucleotide further encodes a truncated receptor.

[0044] Also provided are vectors comprising any of the polynucleotides described herein. In some of any examples, the vector is a viral vector. In some of any examples, the viral vector is a retroviral vector. In some of any examples, the viral vector is a lentiviral vector.

[0044a] In another aspect, the present disclosure refers to a vector comprising the polynucleotide as disclosed herein.

[0045] Provided in some examples are chimeric antigen receptors encoded a polynucleotide of any of the examples described herein. In some examples, the chimeric antigen receptor includes: (a) an extracellular antigen-binding domain that specifically recognizes B cell maturation antigen (BCMA); (b) a spacer of at least 125 amino acids in length; (c) a transmembrane domain; and (d) an intracellular signaling region.

[0045a] In another aspect, the present disclosure refers to a chimeric antigen receptor encoded by the polynucleotide as disclosed herein.

[0045b] In another aspect, the present disclosure refers to a chimeric antigen receptor comprising: (a) an extracellular antigen-binding domain that specifically recognizes B cell maturation antigen (BCMA); (b) a spacer of at least 125 amino acids in length; (c) a transmembrane domain; and (d) an intracellular signaling region.

[0046] In some examples of any of the chimeric antigen receptors described herein, the spacer is derived from an immunoglobulin. In some examples, the spacer includes a sequence of a hinge region, a C_H2 and C_H3 region. In some examples of any of the chimeric antigen receptors described herein, one of more of the hinge, C_H2 and C_H3 is derived all or in part from IgG4 or IgG2. In some examples, the hinge, C_H2 and C_H3 is derived from IgG4. In some examples, one or more of the hinge, C_H2 and C_H3 is chimeric and includes sequence derived from IgG4 and IgG2. In some examples, the spacer includes an IgG4/2 chimeric hinge, an IgG2/4 C_H2, and an IgG4 C_H3 region.

[0047] In some examples of any of the chimeric antigen receptors described herein, the spacer is or includes (i) the sequence set forth in SEQ ID NO: 649; (ii) a functional variant of SEQ ID NO:649 that has at least 95%, 96%, 97%, 98% or 99% sequence identity to SEQ ID NO:649; or (iii) a contiguous portion of (i) or (ii) that is at least 125 amino acids in length. In some examples, the encoded spacer is or includes the sequence set forth in SEQ ID NO: 649.

[0048] Provided in other examples are chimeric antigen receptors that include (a) an extracellular antigen-binding domain that specifically recognizes B cell maturation antigen (BCMA); (b) a spacer set forth in SEQ ID NO:649; (c) a transmembrane domain; and (d) an intracellular signaling region. In some examples of any of the chimeric antigen receptors described herein, the antigen-binding domain is an antibody fragment containing a variable heavy chain (V_H) and a variable light chain (V_L) region.

[0049] In some examples of any of the chimeric antigen receptors described herein, the V_H region is or includes 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 amino acid sequence set forth in any of SEQ ID NOs:110-115, 247-256, 324, 325, 518-531, 533, 609, 617, 772-774, or 814-832; and/or the V_L region is or includes 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 amino acid sequence set forth in any of SEQ ID NOs:116-127, 257-267, 326, 327, 534-550, 552-557, 610, 618, 775-777, or 833-849.

[0050] In some examples of any of the chimeric antigen receptors described herein, the V_H region is or includes 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 amino acid sequence set forth in any of SEQ ID NOs: 110, 111, 112, 113, 115, 248, 252, 253, 254, 255, 256, 324, 325, 518, 519, 520, 521, 522, 609, 617, 772-774, or 814-832; and/or the V_L region is or includes 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 amino acid sequence set forth in any of SEQ ID NOs: 116, 117, 118, 120, 121, 124, 125, 258, 262, 263, 264, 265, 266, 267, 326, 327, 534, 535, 536, 537, 538, 610, 618, 775-777, or 833-849.

[0051] In some examples of any of the chimeric antigen receptors described herein, the V_H region is or includes a CDR-H1, CDR-H2 and CDR-H3 contained within the V_H region amino acid sequence selected from any one of SEQ ID NOs:110-115, 247-256, 324, 325, 518-531, 533, 609, 617, 772-774, or 814-832; and/or the V_L region is or includes a CDR-L1, CDR-L2 and CDR-L3 contained within the V_L region amino acid sequence selected from any one of SEQ ID NOs:116-127, 257-267, 326, 327, 534-550, 552-557, 610, 618, 775-777, or 833-849.

[0052] In some examples of any of the chimeric antigen receptors described herein, the V_H region is or includes a CDR-H1, CDR-H2 and CDR-H3 contained within the V_H region amino acid sequence selected from any one of SEQ ID NOs: 110, 111, 112, 113, 115, 248, 252, 253, 254, 255, 256, 324, 325, 518, 519, 520, 521, 522, 609, 617, 772-774, or 814-832; and/or the V_L region is or includes a CDR-L1, CDR-L2 and CDR-L3 contained within the V_L region amino acid sequence selected from any one of SEQ ID NOs: 116, 117, 118, 120, 121, 124, 125, 258, 262, 263, 264, 265, 266, 267, 326, 327, 534, 535, 536, 537, 538, 610, 618, 775-777, or 833-849.

[0053] In some examples of any of the chimeric antigen receptors described herein, the V_H region is or includes (a) a heavy chain complementarity determining region 1 (CDR-H1) containing the amino acid sequence selected from any one of SEQ ID NOs:1-3, 140-144, 288,

289, 294, 295, 507, 532, 593, 596, 604, 611; and/or (b) a heavy chain complementarity determining region 2 (CDR-H2) containing the amino acid sequence selected from any one of SEQ ID NOs:4-6, 145-148, 290, 291, 296, 297, 372-374, 513, 551, 594, 597, 605, 612; and (c) a heavy chain complementarity determining region 3 (CDR-H3) containing the amino acid sequence selected from any one of SEQ ID NOs:7-11, 149-157, 279-287, 292, 293, 376-378, 517, 595, 606, 613; and/or the V_L region is or includes (a) a light chain complementarity determining region 1 (CDR-L1) containing the amino acid sequence selected from any one of SEQ ID NOs:26-36, 174-178, 302, 303, 380-392, 394-398, 589, 601, 607 or 614; (b) a light chain complementarity determining region 2 (CDR-L2) containing the amino acid sequence selected from any one of SEQ ID NOs:37-46, 179-183, 304, 305, 399-409, 411-414, 590, 602, 608 or 615; and (c) a light chain complementarity determining region 3 (CDR-L3) containing the amino acid sequence selected from any one of SEQ ID NOs:47-58, 184-194, 306, 307, 415-427, 429-433, 591, or 603.

[0054] In some examples of any of the chimeric antigen receptors described herein, the V_H region is or includes (a) a heavy chain complementarity determining region 1 (CDR-H1) containing the amino acid sequence selected from any one of SEQ ID NOs: 1, 2, 3, 141, 143, 144, 288, 289, 507, 593, 604, 611; and/or (b) a heavy chain complementarity determining region 2 (CDR-H2) containing the amino acid sequence selected from any one of SEQ ID NOs: 4, 5, 6, 145, 147, 148, 290, 291, 372, 513, 594, 605 or 612; and (c) a heavy chain complementarity determining region 3 (CDR-H3) containing the amino acid sequence selected from any one of SEQ ID NOs: 7, 8, 9, 10, 149, 153, 154, 155, 156, 157, 292, 293, 376, 517, 595, 606 or 613; and/or the V_L region is or includes (a) a light chain complementarity determining region 1 (CDR-L1) containing the amino acid sequence selected from any one of SEQ ID NOs: 26, 27, 28, 30, 31, 33, 34, 174, 176, 177, 178, 302, 303, 380, 381, 382, 589, 601, 607 or 614; (b) a light chain complementarity determining region 2 (CDR-L2) containing the amino acid sequence selected from any one of SEQ ID NOs: 37, 38, 39, 41, 43, 44, 179, 181, 182, 183, 304, 305, 399, 400, 401, 402, 590, 602, 608 or 615; and (c) a light chain complementarity determining region 3 (CDR-L3) containing the amino acid sequence selected from any one of SEQ ID NOs: 47, 48, 49, 51, 52, 55, 56, 185, 189, 190, 191, 192, 193, 194, 306, 307, 415, 417, 418, 421, 591, or 603. In some examples of any of the chimeric antigen receptors described herein, the V_H region includes a CDR-H1, CDR-H2, and CDR-H3, selected from: a CDR-H1, CDR-H2, and CDR-H3 containing the amino acid sequence of SEQ ID NOs:1, 4, and 7, respectively; a CDR-H1, CDR-H2, and CDR-H3 containing the amino acid sequence of SEQ ID NOs:2, 5, and 8, respectively;

of SEQ ID NOs:288, 290, and 292, respectively; or a CDR-H1, CDR-H2, and CDR-H3 containing the amino acid sequence of SEQ ID NOs:289, 291, and 293, respectively;

[0055] In some examples of any of the chimeric antigen receptors described herein, the V_H region includes a CDR-H1, CDR-H2, and CDR-H3, selected from: a CDR-H1, CDR-H2, and CDR-H3 containing the amino acid sequence of SEQ ID NOs:1, 4, and 7, respectively; a CDR-H1, CDR-H2, and CDR-H3 containing the amino acid sequence of SEQ ID NOs:2, 5, and 8, respectively; a CDR-H1, CDR-H2, and CDR-H3 containing the amino acid sequence of SEQ ID NOs:2, 5, and 9, respectively; a CDR-H1, CDR-H2, and CDR-H3 containing the amino acid sequence of SEQ ID NOs:2, 5, and 10, respectively; a CDR-H1, CDR-H2, and CDR-H3 containing the amino acid sequence of SEQ ID NOs:141, 145, and 149, respectively; a CDR-H1, CDR-H2, and CDR-H3 containing the amino acid sequence of SEQ ID NOs:143, 147, and 153, respectively; a CDR-H1, CDR-H2, and CDR-H3 containing the amino acid sequence of SEQ ID NOs:144, 148, and 154, respectively; a CDR-H1, CDR-H2, and CDR-H3 containing the amino acid sequence of SEQ ID NOs:3, 6, and 155, respectively; a CDR-H1, CDR-H2, and CDR-H3 containing the amino acid sequence of SEQ ID NOs:2, 5, and 156, respectively; a CDR-H1, CDR-H2, and CDR-H3 containing the amino acid sequence of SEQ ID NOs:2, 5, and 157, respectively; a CDR-H1, CDR-H2, and CDR-H3 containing the amino acid sequence of SEQ ID NOs:2, 6, and 376, respectively; a CDR-H1, CDR-H2, and CDR-H3 containing the amino acid sequence of SEQ ID NOs:3, 6, and 155, respectively; a CDR-H1, CDR-H2, and CDR-H3 containing the amino acid sequence of SEQ ID NOs:3, 372, and 376, respectively; a CDR-H1, CDR-H2, and CDR-H3 containing the amino acid sequence of SEQ ID NOs:3, 6, and 376, respectively; a CDR-H1, CDR-H2, and CDR-H3 containing the amino acid sequence of SEQ ID NOs:593, 594, and 595, respectively; a CDR-H1, CDR-H2, and CDR-H3 containing the amino acid sequence of SEQ ID NOs:611, 612, and 613, respectively; a CDR-H1, CDR-H2, and CDR-H3 containing the amino acid sequence of SEQ ID NOs:507, 513, and 517, respectively; a CDR-H1, CDR-H2, and CDR-H3 containing the amino acid sequence of SEQ ID NOs:604, 605, and 606, respectively; a CDR-H1, CDR-H2, and CDR-H3 containing the amino acid sequence of SEQ ID NOs:288, 290, and 292, respectively; or a CDR-H1, CDR-H2, and CDR-H3 containing the amino acid sequence of SEQ ID NOs:289, 291, and 293, respectively;

[0056] In some examples of any of the chimeric antigen receptors described herein, the V_H region is or includes the amino acid sequence set forth in any of SEQ ID NOs: 110-115, 247-256, 324, 325, 518-531, 533, 609, 617, 772-774, or 814-832. In some examples of any of the chimeric antigen receptors described herein, the V_H region is or includes the amino acid

sequence set forth in any of SEQ ID NOs: 110, 111, 112, 113, 115, 248, 252, 253, 254, 255, 256, 324, 325, 518, 519, 520, 521, 522, 609, 617, 772-774, or 814-832. In some examples, the V_H region includes a CDR-H1, CDR-H2, and CDR-H3 containing the amino acid sequence of SEQ ID NOs:593, 594, and 595, respectively; or the V_H region includes a CDR-H1, CDR-H2, and CDR-H3 containing the amino acid sequence of SEQ ID NOs:611, 612, and 613, respectively. In some examples, the V_H region is or includes the amino acid sequence set forth in SEQ ID NO:617.

[0057] In some examples of any of the chimeric antigen receptors described herein, the V_L region includes a CDR-L1, CDR-L2, and CDR-L3 selected from: a CDR-L1, CDR-L2, and CDR-L3 containing the amino acid sequence of SEQ ID NOs:26, 37, and 47, respectively; a CDR-L1, CDR-L2, and CDR-L3 containing the amino acid sequence of SEQ ID NOs:27, 38, and 48, respectively; a CDR-L1, CDR-L2, and CDR-L3 containing the amino acid sequence of SEQ ID NOs:28, 39, and 49, respectively; a CDR-L1, CDR-L2, and CDR-L3 containing the amino acid sequence of SEQ ID NOs:29, 40, and 50, respectively; a CDR-L1, CDR-L2, and CDR-L3 containing the amino acid sequence of SEQ ID NOs:30, 39, and 51, respectively; a CDR-L1, CDR-L2, and CDR-L3 containing the amino acid sequence of SEQ ID NOs:31, 41, and 52, respectively; a CDR-L1, CDR-L2, and CDR-L3 containing the amino acid sequence of SEQ ID NOs:32, 42, and 53, respectively; a CDR-L1, CDR-L2, and CDR-L3 containing the amino acid sequence of SEQ ID NOs:30, 39, and 54, respectively; a CDR-L1, CDR-L2, and CDR-L3 containing the amino acid sequence of SEQ ID NOs:33, 43, and 55, respectively; a CDR-L1, CDR-L2, and CDR-L3 containing the amino acid sequence of SEQ ID NOs:34, 44, and 56, respectively; a CDR-L1, CDR-L2, and CDR-L3 containing the amino acid sequence of SEQ ID NOs:35, 45, and 57, respectively; a CDR-L1, CDR-L2, and CDR-L3 containing the amino acid sequence of SEQ ID NOs:36, 46, and 58, respectively; a CDR-L1, CDR-L2, and CDR-L3 containing the amino acid sequence of SEQ ID NOs:174, 179, and 184, respectively; a CDR-L1, CDR-L2, and CDR-L3 containing the amino acid sequence of SEQ ID NOs:174, 179, and 185, respectively; a CDR-L1, CDR-L2, and CDR-L3 containing the amino acid sequence of SEQ ID NOs:174, 179, and 186, respectively; a CDR-L1, CDR-L2, and CDR-L3 containing the amino acid sequence of SEQ ID NOs:174, 179, and 187, respectively; a CDR-L1, CDR-L2, and CDR-L3 containing the amino acid sequence of SEQ ID NOs:175, 180, and 188, respectively; a CDR-L1, CDR-L2, and CDR-L3 containing the amino acid sequence of SEQ ID NOs:174, 179, and 189, respectively; a CDR-L1, CDR-L2, and CDR-L3 containing the amino acid sequence of SEQ ID NOs:176, 181, and 190, respectively; a CDR-L1, CDR-L2, and CDR-L3 containing the

and 433, respectively; a CDR-L1, CDR-L2, and CDR-L3 containing the amino acid sequence of SEQ ID NOs:601, 602, and 603, respectively; a CDR-L1, CDR-L2, and CDR-L3 containing the amino acid sequence of SEQ ID NOs:614, 615, and 603, respectively; a CDR-L1, CDR-L2, and CDR-L3 containing the amino acid sequence of SEQ ID NOs:589, 590, and 591, respectively; a CDR-L1, CDR-L2, and CDR-L3 containing the amino acid sequence of SEQ ID NOs:607, 608, and 591, respectively; a CDR-L1, CDR-L2, and CDR-L3 containing the amino acid sequence of SEQ ID NOs: 302, 304, and 306, respectively; or a CDR-L1, CDR-L2, and CDR-L3 containing the amino acid sequence of SEQ ID NOs:303, 305, and 307, respectively.

[0058] In some examples of any of the chimeric antigen receptors described herein, the V_L region includes a CDR-L1, CDR-L2, and CDR-L3 selected from: a CDR-L1, CDR-L2, and CDR-L3 containing the amino acid sequence of SEQ ID NOs:26, 37, and 47, respectively; a CDR-L1, CDR-L2, and CDR-L3 containing the amino acid sequence of SEQ ID NOs:27, 38, and 48, respectively; a CDR-L1, CDR-L2, and CDR-L3 containing the amino acid sequence of SEQ ID NOs:28, 39, and 49, respectively; a CDR-L1, CDR-L2, and CDR-L3 containing the amino acid sequence of SEQ ID NOs:30, 39, and 51, respectively; a CDR-L1, CDR-L2, and CDR-L3 containing the amino acid sequence of SEQ ID NOs:31, 41, and 52, respectively; a CDR-L1, CDR-L2, and CDR-L3 containing the amino acid sequence of SEQ ID NOs:33, 43, and 55, respectively; a CDR-L1, CDR-L2, and CDR-L3 containing the amino acid sequence of SEQ ID NOs:34, 44, and 56, respectively; a CDR-L1, CDR-L2, and CDR-L3 containing the amino acid sequence of SEQ ID NOs:174, 179, and 185, respectively; a CDR-L1, CDR-L2, and CDR-L3 containing the amino acid sequence of SEQ ID NOs:174, 179, and 189, respectively; a CDR-L1, CDR-L2, and CDR-L3 containing the amino acid sequence of SEQ ID NOs:176, 181, and 190, respectively; a CDR-L1, CDR-L2, and CDR-L3 containing the amino acid sequence of SEQ ID NOs:177, 182, and 191, respectively; a CDR-L1, CDR-L2, and CDR-L3 containing the amino acid sequence of SEQ ID NOs:174, 179, and 192, respectively; a CDR-L1, CDR-L2, and CDR-L3 containing the amino acid sequence of SEQ ID NOs:178, 183, and 193, respectively; a CDR-L1, CDR-L2, and CDR-L3 containing the amino acid sequence of SEQ ID NOs:178, 183, and 194, respectively; a CDR-L1, CDR-L2, and CDR-L3 containing the amino acid sequence of SEQ ID NOs:30, 399, and 415, respectively; a CDR-L1, CDR-L2, and CDR-L3 containing the amino acid sequence of SEQ ID NOs:380, 400, and 416, respectively; a CDR-L1, CDR-L2, and CDR-L3 containing the amino acid sequence of SEQ ID NOs:33, 43, and 421, respectively; a CDR-L1, CDR-L2, and CDR-L3 containing the amino acid sequence of SEQ ID NOs:381, 401, and 417, respectively; a CDR-L1, CDR-L2, and CDR-L3 containing the amino acid sequence of

SEQ ID NOs:382, 402, and 418, respectively; a CDR-L1, CDR-L2, and CDR-L3 containing the amino acid sequence of SEQ ID NOs:601, 602, and 603, respectively; a CDR-L1, CDR-L2, and CDR-L3 containing the amino acid sequence of SEQ ID NOs:614, 615, and 603, respectively; a CDR-L1, CDR-L2, and CDR-L3 containing the amino acid sequence of SEQ ID NOs:589, 590, and 591, respectively; a CDR-L1, CDR-L2, and CDR-L3 containing the amino acid sequence of SEQ ID NOs:607, 608, and 591, respectively; a CDR-L1, CDR-L2, and CDR-L3 containing the amino acid sequence of SEQ ID NOs: 302, 304, and 306, respectively; or a CDR-L1, CDR-L2, and CDR-L3 containing the amino acid sequence of SEQ ID NOs:303, 305, and 307, respectively.

[0059] In some examples of any of the chimeric antigen receptors described herein, the V_L region is or includes the amino acid sequence set forth in any of SEQ ID NOs: 116-127, 257-267, 326, 327, 534-550, 552-557, 610, 618, 775-777, or 833-849. In some examples of any of the chimeric antigen receptors described herein, the V_L region is or includes the amino acid sequence set forth in any of SEQ ID NOs: 116, 117, 118, 120, 121, 124, 125, 258, 262, 263, 264, 265, 266, 267, 326, 327, 534, 535, 536, 537, 538, 610, 618, 775-777, or 833-849.

[0060] In some examples of any of the chimeric antigen receptors described herein, the V_L region includes a CDR-L1, CDR-L2, and CDR-L3 containing the amino acid sequence of SEQ ID NOs:601, 602, and 603, respectively; or the V_L region includes a CDR-L1, CDR-L2, and CDR-L3 containing the amino acid sequence of SEQ ID NOs:614, 615, and 603, respectively;

[0061] In some examples of any of the chimeric antigen receptors described herein, the V_L region is or includes the amino acid sequence set forth in SEQ ID NO:618. In some examples of any of the chimeric antigen receptors described herein, the V_H region and the V_L regions contain the amino acid sequence set forth in SEQ ID NOs:110 and 116, respectively, or a sequence of amino acids that has at least 90% identity to SEQ ID NO:110 and 116, respectively; the V_H region and the V_L regions contain the amino acid sequence set forth in SEQ ID NOs:111 and 117, respectively, or a sequence of amino acids that has at least 90% identity to SEQ ID NO:111 and 117, respectively; the V_H region and the V_L regions contain the amino acid sequence set forth in SEQ ID NOs:110 and 118, respectively, or a sequence of amino acids that has at least 90% identity to SEQ ID NO:110 and 118, respectively; the V_H region and the V_L regions contain the amino acid sequence set forth in SEQ ID NOs:110 and 119, respectively, or a sequence of amino acids that has at least 90% identity to SEQ ID NO:110 and 119, respectively; the V_H region and the V_L regions contain the amino acid sequence set forth in SEQ

SEQ ID NO:115 and 555, respectively; the V_H region and the V_L regions contain the amino acid sequence set forth in SEQ ID NOs:524 and 556, respectively, or a sequence of amino acids that has at least 90% identity to SEQ ID NO:524 and 556, respectively; the V_H region and the V_L regions contain the amino acid sequence set forth in SEQ ID NOs:519 and 557, respectively, or a sequence of amino acids that has at least 90% identity to SEQ ID NO:519 and 557, respectively; the V_H region and the V_L regions contain the amino acid sequence set forth in SEQ ID NOs:609 and 610, respectively, or a sequence of amino acids that has at least 90% identity to SEQ ID NO:609 and 610, respectively; the V_H region and the V_L regions contain the amino acid sequence set forth in SEQ ID NOs:617 and 618, respectively, or a sequence of amino acids that has at least 90% identity to SEQ ID NO:617 and 618, respectively; the V_H region and the V_L regions contain the amino acid sequence set forth in SEQ ID NOs:324 and 326, respectively, or a sequence of amino acids that has at least 90% identity to SEQ ID NO:324 and 326, respectively; or the V_H region and the V_L regions contain the amino acid sequence set forth in SEQ ID NOs:325 and 327, respectively, or a sequence of amino acids that has at least 90% identity to SEQ ID NO:325 and 327, respectively.

[0062] In some examples of any of the chimeric antigen receptors described herein, the V_H region and the V_L regions contain the amino acid sequence set forth in SEQ ID NOs:110 and 116, respectively, or a sequence of amino acids that has at least 90% identity to SEQ ID NO:110 and 116, respectively; the V_H region and the V_L regions contain the amino acid sequence set forth in SEQ ID NOs:111 and 117, respectively, or a sequence of amino acids that has at least 90% identity to SEQ ID NO:111 and 117, respectively; the V_H region and the V_L regions contain the amino acid sequence set forth in SEQ ID NOs:110 and 118, respectively, or a sequence of amino acids that has at least 90% identity to SEQ ID NO:110 and 118, respectively; the V_H region and the V_L regions contain the amino acid sequence set forth in SEQ ID NOs:110 and 120, respectively, or a sequence of amino acids that has at least 90% identity to SEQ ID NO:110 and 120, respectively; the V_H region and the V_L regions contain the amino acid sequence set forth in SEQ ID NOs:110 and 121, respectively, or a sequence of amino acids that has at least 90% identity to SEQ ID NO:110 and 121, respectively; the V_H region and the V_L regions contain the amino acid sequence set forth in SEQ ID NOs:112 and 124, respectively, or a sequence of amino acids that has at least 90% identity to SEQ ID NO:112 and 124, respectively; the V_H region and the V_L regions contain the amino acid sequence set forth in SEQ ID NOs:113 and 125, respectively, or a sequence of amino acids that has at least 90% identity to SEQ ID NO:113 and 125, respectively; the V_H region and the V_L regions contain the amino acid sequence set

618, respectively, or a sequence of amino acids that has at least 90% identity to SEQ ID NO:617 and 618, respectively; the V_H region and the V_L regions contain the amino acid sequence set forth in SEQ ID NOs:324 and 326, respectively, or a sequence of amino acids that has at least 90% identity to SEQ ID NO:324 and 326, respectively; or the V_H region and the V_L regions contain the amino acid sequence set forth in SEQ ID NOs:325 and 327, respectively, or a sequence of amino acids that has at least 90% identity to SEQ ID NO:325 and 327, respectively.

[0063] In some examples of any of the chimeric antigen receptors described herein, the fragment includes an scFv. In some examples, the V_H region and the V_L region are joined by a flexible linker. In some examples, the scFv includes a linker containing the amino acid sequence GGGGSGGGGSGGGGS (SEQ ID NO: 361). In some examples, the V_H region is amino-terminal to the V_L region.

[0064] In some examples of any of the chimeric antigen receptors described herein, the antigen-binding domain includes the amino acid sequence selected from any one of SEQ ID NOs:128-139, 268-278, 329, 442, 478, 558-576, 578-583, 585, or 769-771 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 selected from any one of SEQ ID NOs: 128-139, 268-278, 329, 442, 478, 558-576, 578-583, 585, or 769-771. In some examples, the antigen-binding domain includes the amino acid sequence selected from any one of SEQ ID NOs:128-130, 132, 133, 136, 137, 269, 273-278, 329, 442, 478, 558-563 or 585 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 selected from any one of SEQ ID NOs: 128-130, 132, 133, 136, 137, 269, 273-278, 329, 442, 478, 558-563 or 585.

[0065] In some examples of any of the chimeric antigen receptors described herein, the V_H region is carboxy-terminal to the V_L region. In some examples, the scFv includes the amino acid sequence set forth in SEQ ID NOs: 328 or 586, 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 set forth in SEQ ID NO: 328 or 586.

[0066] In some examples of any of the chimeric antigen receptors described herein, the intracellular signaling region includes an activating cytoplasmic signaling domain. In some examples of any of the chimeric antigen receptors described herein, the activating 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 examples, the activating cytoplasmic signaling domain is or includes

a cytoplasmic signaling domain of a zeta chain of a CD3-zeta (CD3 ζ) chain or a functional variant or signaling portion thereof. In some examples, the activating cytoplasmic domain is human or is derived from a human protein. In some examples, the activating cytoplasmic domain is or includes the sequence set forth in SEQ ID NO:628 or a sequence of amino acids that has at least 90% sequence identity to SEQ ID NO:628.

[0067] In some examples of any of the chimeric antigen receptors described herein, the intracellular signaling region further includes a costimulatory signaling region. In some examples, the costimulatory signaling region includes an intracellular signaling domain of a T cell costimulatory molecule or a signaling portion thereof. In some examples, 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 examples, the costimulatory signaling region includes an intracellular signaling domain of 4-1BB. In some examples, the costimulatory signaling region is human or is derived from a human protein. In some examples, the costimulatory signaling region is or includes the sequence set forth in SEQ ID NO:626 or a sequence of amino acids that exhibits at least 90% sequence identity to the sequence set forth in SEQ ID NO: 626. In some examples, the costimulatory signaling region is between the transmembrane domain and the intracellular signaling region. In some examples, the transmembrane domain is or includes a transmembrane domain derived from CD4, CD28, or CD8. In some examples, the transmembrane domain is or includes a transmembrane domain derived from a CD28. In some examples, the transmembrane domain is human or is derived from a human protein. In some examples of any of the chimeric antigen receptors described herein, the transmembrane domain is or includes the sequence set forth in SEQ ID NO:624 or a sequence of amino acids that exhibits at least 90% sequence identity to SEQ ID NO:624.

[0068] In some examples of any of the chimeric antigen receptors described herein, the encoded chimeric antigen receptor includes from its N to C terminus in order: the antigen-binding domain, the spacer, the transmembrane domain and the intracellular signaling domain.

[0069] In some of any of the examples, the chimeric antigen receptor is encoded by a polynucleotide sequence comprising the sequence set forth in any of SEQ ID NOS: 751-756 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 to the sequence set forth in any of SEQ ID NOS: 751-756. In some of any of the examples, the chimeric antigen receptor is encoded by a polynucleotide sequence comprising the sequence set forth in any of SEQ ID NOS: 755 and 756

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 to the sequence set forth in any of SEQ ID NOS: 755 and 756. In some of any of the examples, the chimeric antigen receptor is encoded by a polynucleotide sequence comprising the sequence set forth in SEQ ID NO: 755 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 of the examples, the chimeric antigen receptor is encoded by a polynucleotide sequence comprising the sequence set forth in SEQ ID NO: 755.

[0070] Provided in some examples are engineered cells that contain a polynucleotide of any of the examples described herein. In some examples of any of the engineered cells described herein, the engineered cell contains the chimeric antigen receptor of any of the examples described herein.

[0070a] In another aspect, the present disclosure refers to an engineered cell, comprising the chimeric antigen receptor as disclosed herein, the polynucleotide as disclosed herein, or the vector as disclosed herein.

[0071] In some examples of any of the engineered cells described herein, the cell is an immune cell. In some examples, the immune cell is a primary cell obtained from a subject. In some examples, the immune cell is an NK cell or a T cell. In some examples, the immune cell is a T cell and the T cell is a CD4+ and/or CD8+ T cell.

[0072] In some examples of any of the engineered cells described herein, the cell contains transcribed RNA encoding the chimeric antigen receptor, optionally messenger RNA (mRNA), that exhibits at least 70%, 75%, 80%, 85%, 90%, or 95% RNA homogeneity. In some examples, the cell contains transcribed RNA encoding the chimeric antigen receptor, optionally messenger RNA (mRNA), that exhibits reduced heterogeneity compared to the heterogeneity of transcribed mRNA in a cell encoding a reference chimeric antigen receptor, said reference chimeric antigen receptor containing the same amino acid sequence as the chimeric antigen receptor but encoded by a different polynucleotide sequence containing one or more nucleotide differences in the polynucleotide encoding the CARs and/or in which the reference chimeric antigen receptor is encoded by a polynucleotide containing one or more splice donor site and/or one or more splice acceptor site in the nucleic acid encoding the spacer. In some examples, the RNA heterogeneity is reduced by greater than or greater than about 10%, 15%, 20%, 25%, 30%, 40%, 50% or more. In some examples, the cell encoding the reference CAR includes transcribed RNA encoding the reference CAR, optionally messenger RNA (mRNA), that exhibits greater than or greater than about 10%, 15%, 20%, 25%, 30%, 40%, 50% or more RNA heterogeneity. In some examples, the RNA homogeneity and/or

heterogeneity is determined by agarose gel electrophoresis, chip-based capillary electrophoresis, analytical ultracentrifugation, field flow fractionation, or liquid chromatography.

[0073] In some examples of any of the engineered cells described herein, among a plurality of the engineered cells, less than or less than about 10%, 9%, 8%, 7%, 5%, 4%, 3%, 2% or 1% of the cells in the plurality contain a chimeric antigen receptor that exhibits tonic signaling and/or antigen independent activity or signaling.

[0074] Also provided are compositions comprising any of the engineered cells provided herein. In some of any such examples, the composition comprises CD4+ and CD8+ T cells and the ratio of CD4+ to CD8+ T cells is from or from about 1:3 to 3:1.

[0075] Also provided herein are compositions containing a polynucleotide of any of the examples described herein, a chimeric antigen receptor of any of the examples described herein, or a engineered cell of any of the examples described herein. In some examples, the composition further contains a pharmaceutically acceptable excipient. In some of any of these examples, the composition is sterile.

[0075a] In another aspect, the present disclosure refers to a composition comprising the chimeric antigen receptor as disclosed herein, the polynucleotide as disclosed herein, the vector as disclosed herein.

[0075b] In another aspect, the present disclosure refers to a composition comprising the engineered cell as disclosed herein.

[0076] Provided in other examples are methods of treatment that involve administering the engineered cells of any of the examples described herein or the composition of any of the examples described herein to a subject having a disease or disorder. In some of any examples, the method comprises administering a dose of the engineered cells or a composition comprising a dose of the engineered cells.

[0076a] In another aspect, the present disclosure refers to the engineered cells as disclosed herein or the composition as disclosed herein for use in treating a disease or disorder.

[0076b] In another aspect, the present disclosure refers to use of the engineered cells as disclosed herein or the composition as disclosed herein in the manufacture of a medicament for the treatment of a disease or disorder.

[0077] Also provided are uses any of the engineered cells or the compositions described herein for the manufacture of a medicament for the treatment of a disease or disorder. Also provided are uses any of the engineered cells or the compositions described herein for treating a disease or disorder. In some of any such examples, the engineered cells or the composition are for use 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.

[0078] In some examples of any of the methods described herein, the disease or disorder is associated with expression of B cell maturation antigen (BCMA). In some examples, the disease or disorder associated with BCMA is a B cell-related disorder. In some examples, the disease or disorder associated with BCMA is an autoimmune disease or disorder. In some examples, the autoimmune disease or disorder is systemic lupus erythematosus (SLE), lupus nephritis, inflammatory bowel disease, rheumatoid arthritis, ANCA associated vasculitis, idiopathic thrombocytopenia purpura (ITP), thrombotic thrombocytopenia purpura (TTP), autoimmune thrombocytopenia, Chagas' disease, Grave's disease, Wegener's granulomatosis,

poly-arteritis 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.

[0079] In some examples of any of the methods described herein, the disease or disorder associated with BCMA is a cancer. In some examples, the cancer is a BCMA-expressing cancer. In some examples, the cancer is a B cell malignancy. In some examples, the cancer is a lymphoma, a leukemia, or a plasma cell malignancy. In some examples, the cancer is a lymphoma and the lymphoma is 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). In some examples, the cancer is a leukemia and the leukemia is chronic lymphocytic leukemia (CLL), plasma cell leukemia or acute lymphocytic leukemia (ALL). In some examples, the cancer is a plasma cell malignancy and the plasma cell malignancy is multiple myeloma (MM) or plasmacytoma. In some examples, the cancer is multiple myeloma (MM).

[0080] In some of any examples, the dose of engineered T cells comprises between at or about 1×10^7 CAR-expressing T cells and at or about 2×10^9 CAR-expressing T cells. In some of any examples, the dose of engineered T cells comprise between at or about 2.5×10^7 CAR-expressing T cells and at or about 1.2×10^9 CAR-expressing T cells, between at or about 5.0×10^7 CAR-expressing T cells and at or about 4.5×10^8 CAR-expressing T cells, or between at or about 1.5×10^8 CAR-expressing T cells and at or about 3.0×10^8 CAR-expressing T cells. In some of any examples, the dose of engineered T cells comprise at or about 2.5×10^7 , at or about 5.0×10^7 , at or about 1.5×10^8 , at or about 3.0×10^8 , at or about 4.5×10^8 , at or about 8.0×10^8 or at or about 1.2×10^9 CAR-expressing T cells. In some of any examples, 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 examples, the dose of engineered T cells comprises 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.

[0081] In some of any examples, 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 examples, 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.

[0082] In some of any examples, prior to the administration, the subject has received a lymphodepleting therapy comprising the administration of fludarabine at or about 20-40 mg/m² body surface area of the subject, optionally at or about 30 mg/m², daily, for 2-4 days, and/or cyclophosphamide at or about 200-400 mg/m² body surface area of the subject, optionally at or about 300 mg/m², daily, for 2-4 days.

[0083] In some of any examples, the subject has received a lymphodepleting therapy comprising the administration of fludarabine at or about 30 mg/m² body surface area of the subject, daily, and cyclophosphamide at or about 300 mg/m² body surface area of the subject, daily, for 3 days.

[0084] In some of any examples, at or prior to the administration of the dose of 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.

[0085] In some of any examples, the immunomodulatory agent is selected from among thalidomide, lenalidomide or pomalidomide. In some of any examples, the proteasome inhibitor is selected from among bortezomib, carfilzomib or ixazomib. In some of any examples, the anti-CD38 antibody is or comprises daratumumab.

[0086] In some of any examples, at the administration of the dose of cells, the subject has not had active or history of plasma cell leukemia (PCL).

[0087] In some of any examples, when administered to subjects, the dose or the composition is capable of achieving objective response (OR), in at least 50%, 60%, 70%, 80%, 90%, or 95% of subjects that were administered. In some of any examples, the 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 of any examples, when administered to subjects, 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 of any examples, when administered to subjects, 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.

[0088] In some of any examples, 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 examples, the dose of the engineered T cells comprise at or about 5.0×10^7 CAR-expressing T cells.

[0089] Also provided herein are methods of determining the heterogeneity of a transcribed nucleic acid of a transgene, the method comprising: a) 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; and b) 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.

[0089a] In another aspect, the present disclosure refers to a method of determining the heterogeneity of a transcribed nucleic acid of a transgene, the method comprising:

a) 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; and

b) 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.

[0090] In some examples of the method, the detected differences in b) are different lengths of the amplified transcripts. In some examples, the differences in b) are differences in chromatographic profiles of the amplified transcripts.

[0091] In some examples, the differences in the amplified products are determined by agarose gel electrophoresis, chip-based capillary electrophoresis, analytical ultracentrifugation, field flow fractionation, or chromatography. In some examples, the 5' primer is specific to sequence transcribed from the promoter region of the transcribed nucleic acid. In some examples, the transcribed nucleic acid is amplified using a 3' primer specific to a sequence within the amino acid-coding sequence of the polynucleotide, and/or the 3' untranslated region, on of the transcribed pre-mRNA. In some examples, the 3 primer is specific to the polyadenylation sequence or enhancer region of the 3' untranslated region of the transcribed pre-mRNA.

[0092] In some examples, step a) is effected by a single amplification reaction, using a single 5' and 3' primer pair comprising 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). In some examples, step a) is effected by parallel or subsequent amplification reactions using a first 5' and 3' primer pair, a second 5' and 3' primer pair, and optionally

additional 5' and 3' primer pairs, wherein: the first 5' and 3' primer pair contains a 5' primer that is complementary to a nucleic acid sequence within the 5' UTR of the transcribed nucleic acid and a 3' primer that is complementary to a nucleic acid sequence within the 3' UTR of the transcribed nucleic acid; the second 5' and 3' primer pair contains a 5' primer whose sequence is complementary to a portion of the translated sequence of the nucleic acid transcript and a 3' primer whose sequence is complementary to a nucleic acid sequence within the 3' UTR of the transcript; and the optionally additional 5' and 3' primer pairs each contain sequences complementary to sequences within the translated region of the transcript. In some examples, the parallel or subsequent amplification reactions amplify overlapping portions of the transcript.

[0093] In some examples, the amplified products are predicted to be about 1.5 kilobases, 2 kilobases, 2.5 kilobases, 3 kilobases, 3.5 kilobases, 4 kilobases, 4.5 kilobases, 5 kilobases, 5.5 kilobases, 6 kilobases, 7 kilobases, or 8 kilobases in length.

[0094] In some of any examples, 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 of any examples, 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.

[0095] Also provided are methods of reducing the heterogeneity of an expressed transgene transcript, the method comprising: a) identifying a transgene candidate for the removal of splice sites according to any of the methods for determining the heterogeneity of a transcribed nucleic acid provided herein; b) identifying one or more potential splice donor and/or splice acceptor sites; and c) modifying the nucleic acid sequence at or near the one or more potential splice donor and/or splice acceptor sites identified in b), thereby generating a modified polynucleotide.

[0095a] In another aspect, the present disclosure refers to a method of reducing the heterogeneity of an expressed transgene transcript, the method comprising:

- a) identifying a transgene candidate for the removal of splice sites according to the method as disclosed herein;
- b) identifying one or more potential splice donor and/or splice acceptor sites; and
- c) modifying the nucleic acid sequence at or near the one or more potential splice donor and/or splice acceptor sites identified in b), thereby generating a modified polynucleotide.

[0096] In some of any such examples, the method also involves d) assessing the transgene candidacy for the removal of splice sites as in step a). In some of any such examples, the method also involves e) repeating steps b)-d) until the heterogeneity of the transcript in step d) is reduced compared to the heterogeneity of the transcript as determined in step a).

[0097] In some of any such examples, 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 of a splice event, and/or is/are predicted to be involved in a splice event with a probability of at least 70%, 75%, 80%, 85%, 90%, 95%, 99%, or 100%.

[0098] In some of any such examples, splice donor sites and splice acceptor sites are identified independently. In some of any such examples, the splice acceptor and/or donor site(s) is/are canonical, non-canonical, and/or cryptic splice acceptor and/or donor site(s).

[0099] In some of any such examples, the transgene is a chimeric antigen receptor or a portion of a chimeric antigen receptor. In some of any such examples, the CAR polypeptide comprises an antigen-binding domain comprising an antibody fragment, optionally a single chain antibody fragment (scFv), comprising a variable heavy chain (V_H) and a variable light chain (V_L), a spacer (e.g., a spacer region located between the ligand-binding domain and the transmembrane domain, of the recombinant receptor), a transmembrane region, and an intracellular signaling region.

[0100] In some of any such examples, the modified polynucleotide is not modified within the coding sequence for the antigen-binding domain of the encoded CAR polypeptide. In some of any such examples, the encoded amino acid sequence of the transgene is unchanged following modification of the polynucleotide. In some of any such examples, the RNA transcribed from the modified polynucleotide exhibits at least or at least about 70%, 75%, 80%, 85%, 90%, or 95% homogeneity following expression of the unmodified polynucleotide in a cell.

[0101] In some of any such examples, the cell is a human cell. In some of any such examples, the cell is a T-cell.

[0102] In some of any such examples, the method is a computer implemented method, and wherein one or more steps a)-c) occur at an electronic device comprising one or more processors and memory.

[0103] Also provided are computer systems comprising a processor and memory, the memory comprising instructions operable to cause the processor to carry out any one or more of steps of the methods of reducing the heterogeneity of an expressed transgene transcript.

[0103a] In another aspect, the present disclosure refers to a computer system comprising a processor and memory, the memory comprising instructions operable to cause the processor to carry out any one or more of steps of the methods as disclosed herein.

Brief Description of the Drawings

[0104] **FIG. 1A** and **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).

CLAIMS

WHAT IS CLAIMED:

1. A polynucleotide encoding a chimeric antigen receptor, the polynucleotide comprising a nucleic acid sequence encoding: (a) an extracellular antigen-binding domain that specifically recognizes an antigen; (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 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.
2. The polynucleotide of claim 1, wherein the encoded spacer comprises a portion of an immunoglobulin.
3. The polynucleotide of claim 1 or claim 2, wherein the encoded spacer comprises a sequence of a hinge region, a C_H2 region and a C_H3 region.
4. The polynucleotide of any of claims 1-3, wherein
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 CH2 region comprises all or a portion of an IgG4 CH2 region and/or of an IgG2 CH2 region, wherein the IgG4 CH2 region is optionally a human IgG4 CH2 region and the IgG2 CH2 region is optionally a human IgG2 CH2 region; and/or
the CH3 region comprises all or a portion of an IgG4 CH3 region and/or of an IgG2 CH3 region, wherein the IgG4 CH3 region is optionally a human IgG4 CH3 region and the IgG2 CH3 region is optionally a human IgG2 CH3 region.
5. The polynucleotide of any of claims 1-4, wherein 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.
6. The polynucleotide of any of claims 1-4, wherein:

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

7. The polynucleotide of claim any of claims 1-6, wherein 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.

8. The polynucleotide of any of claims 1-7, wherein the encoded spacer is or comprises (i) the sequence set forth in SEQ ID NO: 649; (ii) a functional variant of SEQ ID NO:649 that has at least 95%, 96%, 97%, 98% or 99% sequence identity to SEQ ID NO:649; or (iii) a contiguous portion of (i) or (ii) that is at least 125 amino acids in length.

9. The polynucleotide of any of claims 1-8, wherein the encoded spacer is or comprises the sequence set forth in SEQ ID NO: 649.

10. The polynucleotide of any of claims 1-9, wherein the nucleic acid encoding the spacer comprises at least one modified splice donor and/or splice acceptor site, said modified splice donor and/or acceptor site comprising one or more nucleotide modifications corresponding to a reference splice donor site and/or reference splice acceptor site contained in the sequence set forth in SEQ ID NO:621, optionally wherein:

the one or more nucleotide modifications comprises a nucleotide substitution; and/or

at least one of the one or more nucleotide modifications are within 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 residues of the splice site junction of the reference splice acceptor and/or reference splice donor site.

11. A polynucleotide encoding a chimeric antigen receptor, the polynucleotide comprising a nucleic acid sequence encoding: (a) an extracellular antigen-binding domain that

specifically recognizes an antigen; (b) a spacer of at least 125 amino acids in length; (c) a transmembrane domain; and (d) an intracellular signaling region, wherein the nucleic acid sequence encoding the spacer comprises at least one modified splice donor and/or splice acceptor site, the modified splice donor and/or splice acceptor site comprising one or more nucleotide modifications corresponding to a reference splice donor site and/or splice acceptor site contained in the sequence set forth in SEQ ID NO:621.

12. The polynucleotide of claim 10 or claim 11, wherein the reference splice donor and/or reference splice acceptor sites are canonical, non-canonical, or cryptic splice sites.

13. The polynucleotide of any of claim 10-12, wherein:
the reference splice donor and/or reference splice acceptor site(s) has a splice site prediction score of at least or about 0.4, 0.5, 0.6, 0.70, 0.75, 0.80, 0.85, 0.90, 0.95, 0.99, or 1.0;

the reference splice donor and/or reference splice acceptor site(s) is/are predicted to be involved in a splice event with a probability of at least 40%, 50%, 60%, 70%, 75%, 80%, 85%, 90%, 95%, 99%, or 100%;

the reference splice donor and/or reference splice acceptor site(s) has a splice site prediction score of at least or about 0.70, 0.75, 0.80, 0.85, 0.90, 0.95, 0.99, or 1.0; and/or

the reference splice donor and/or reference splice acceptor site(s) is/are predicted to be involved in a splice event with a probability of at least 70%, 75%, 80%, 85%, 90%, 95%, 99%, or 100%.

14. The polynucleotide of any of claims 10-13, wherein:
the reference splice donor site comprises the sequence aatctaagtagcgac (SEQ ID NO: 705), tcaactggtacgtgg (SEQ ID NO:706), acaattagtaaggca (SEQ ID NO:707) and/or accacaggtgtatac (SEQ ID NO:708); and/or

the reference splice acceptor site comprises the sequence
aagtttctttctgtattccaggtgaccgtggataaatctc (SEQ ID NO:742) and/or
gggcaacgtgttctcttgcagtgcatgcacgaagccctgc (SEQ ID NO:743).

15. The polynucleotide of any of claims 10-14, wherein the one or more nucleotide modifications is silent and/or results in a degenerate codon compared to SEQ ID NO:621 and/or does not change the amino acid sequence of the encoded spacer.

16. The polynucleotide of any of claims 10-15, wherein:
the modified splice donor site is set forth in agtctaaatacggac (SEQ ID NO:661), tcaactggtatgtgg (SEQ ID NO:662), accatctccaaggcc (SEQ ID NO:663) and/or gccccaggtttacac (SEQ ID NO:664); and/or

the modified splice acceptor site is set forth in cagtttctctgtatagtagactcacctggataaatcaa (SEQ ID NO:672), gggcaacgtgttcagctgcagcgtgatgcacaggccctgc (SEQ ID NO: 673) and/or cgcttgctctcctgtcccgcctcctctgttgccggacct (SEQ ID NO:766).

17. The polynucleotide of any of claims 10-16, wherein:
the reference splice donor site comprises the sequence tcaactggtacgtgg (SEQ ID NO:706) and/or the reference splice acceptor site comprises the sequence aagtttcttctgtattccaggtgaccgtggataaatctc (SEQ ID NO:742); and/or

the modified splice donor site is set forth in tcaactggtatgtgg (SEQ ID NO:662) and/or the modified acceptor site is set forth in cagtttctctgtatagtagactcacctggataaatcaa (SEQ ID NO:672).

18. The polynucleotide of any of claims 1-17, wherein the spacer is encoded by the nucleotide sequence set forth in SEQ ID NO:622 or a portion thereof.

19. A polynucleotide encoding a chimeric antigen receptor, wherein the polynucleotide comprises a nucleic acid sequence encoding:

(i) (a) an extracellular antigen-binding domain that specifically recognizes an antigen; (b) a spacer, wherein the encoding nucleic acid is or comprises the sequence set forth in SEQ ID NO:622 or encodes a sequence of amino acids set forth in SEQ ID NO:649; (c) a transmembrane domain; and (d) an intracellular signaling region; or

(ii) (a) an extracellular antigen-binding domain that specifically recognizes an antigen; (b) a spacer, wherein the encoding nucleic acid consists or consists essentially of the sequence

set forth in SEQ ID NO:622 or encodes a sequence of amino acids set forth in SEQ ID NO:649; (c) a transmembrane domain; and (d) an intracellular signaling region.

20. The polynucleotide of claim 19, 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.

21. The polynucleotide of any of claims 1-20, wherein, following expression in a human cell, optionally a human T cell, the transcribed RNA, optionally messenger RNA (mRNA), from the polynucleotide exhibits reduced heterogeneity compared to the heterogeneity of the mRNA transcribed from a reference polynucleotide, said reference polynucleotide encoding the same amino acid sequence as the polynucleotide, wherein the reference polynucleotide differs by the presence of one or more splice donor site and/or one or more splice acceptor site in the nucleic acid encoding the spacer and/or comprises one or more nucleotide modifications compared to the polynucleotide and/or comprises the sequence set forth in SEQ ID NO:621.

22. The polynucleotide of any of claims 1-21, wherein the polynucleotide is codon-optimized for expression in a human cell.

23. The polynucleotide of any of claims 1-22, wherein the antigen is associated with a disease or condition or is expressed in cells of the environment of a lesion associated with the disease or condition, optionally wherein the disease or condition is a cancer.

24. The polynucleotide of any of claims 1-23, wherein the antigen is B cell maturation antigen (BCMA), ROR1, carbonic anhydrase 9 (CAIX), tEGFR, Her2/neu (receptor tyrosine kinase erbB2), L1-CAM, CD19, CD20, CD22, mesothelin, CEA, and hepatitis B surface antigen, anti-folate receptor, CD23, CD24, CD30, CD33, CD38, CD44, EGFR, epithelial glycoprotein 2 (EPG-9), epithelial glycoprotein 40 (EPG-40), EPHA2, erb-B2, erb-B3, erb-B4, erbB dimers, EGFR vIII, folate binding protein (FBP), FCRL5, FCRH5, fetal acetylcholine receptor, GD2, GD3, G protein-coupled receptor class C group 5 member D (GPC5D), HMW-MAA, IL-92R-alpha, IL-13R-alpha2, kinase insert domain receptor (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, TAG72, B7-H6, IL-13 receptor alpha 2 (IL-13Ra2), CA9, GD3, HMW-MAA, CD171, G250/CAIX, HLA-AI MAGE A1, HLA-A2 NY-ESO-1, PSCA, folate receptor-a, CD44v6, CD44v7/8, avb6 integrin, 8H9, NCAM, VEGF receptors, 5T4, Foetal AchR, NKG2D ligands, CD44v6, dual antigen, a cancer-testes antigen, mesothelin, murine CMV, mucin 1 (MUC1), MUC16, PSCA, NKG2D, NY-ESO-1, MART-1, gp100, oncofetal antigen, ROR1, TAG72, VEGF-R2, carcinoembryonic antigen (CEA), Her2/neu, estrogen receptor, progesterone receptor, ephrinB2, CD123, c-Met, GD-9, O-acetylated GD2 (OGD2), CE7, Wilms Tumor 1 (WT-1), a cyclin, cyclin A2, CCL-1, CD138, a pathogen-specific antigen.

25. The polynucleotide of any of claims 1-24, wherein the antigen is B cell maturation antigen (BCMA).

26. The polynucleotide of any of claims 1-25, wherein the encoded antigen-binding domain is an antibody fragment comprising a variable heavy chain (V_H) and a variable light chain (V_L) region.

27. The polynucleotide of claim 26, wherein:

the V_H region and the V_L regions comprise the amino acid sequence set forth in SEQ ID NOs:617 and 618, respectively, or a sequence of amino acids having at least 90% identity to SEQ ID NOS:617 and 618, respectively;

the V_H region and the V_L regions comprise the amino acid sequence set forth in SEQ ID NOs:256 and 267, respectively, or a sequence of amino acids having at least 90% identity to SEQ ID NOS:256 and 267, respectively;

the V_H region and the V_L regions comprise the amino acid sequence set forth in SEQ ID NOs:519 and 535, respectively, or a sequence of amino acids having at least 90% identity to SEQ ID NOS:519 and 535, respectively;

the V_H region and the V_L regions comprise the amino acid sequence set forth in SEQ ID NOs:115 and 536, respectively, or a sequence of amino acids having at least 90% identity to SEQ ID NOS:115 and 536, respectively; or

the V_H region and the V_L regions comprise the amino acid sequence set forth in SEQ ID NOS:609 and 610, respectively, or a sequence of amino acids having at least 90% identity to SEQ ID NOS:609 and 610, respectively.

28. The polynucleotide of claim 26 or claim 27, wherein:

the V_H region comprises a CDR-H1, CDR-H2, and CDR-H3 comprising the amino acid sequence of SEQ ID NOS:593, 594, and 595, respectively, and the V_L region comprises a CDR-L1, CDR-L2, and CDR-L3 comprising the amino acid sequence of SEQ ID NOS:601, 602, and 603, respectively;

the V_H region comprises a CDR-H1, CDR-H2, and CDR-H3 comprising the amino acid sequence of SEQ ID NOS:2, 5, and 157, respectively, and the V_L region comprises a CDR-L1, CDR-L2, and CDR-L3 comprising the amino acid sequence of SEQ ID NOS:178, 183, and 194, respectively;

the V_H region comprises a CDR-H1, CDR-H2, and CDR-H3 comprising the amino acid sequence of SEQ ID NOS:1, 4, and 7, respectively, and the V_L region comprises a CDR-L1, CDR-L2, and CDR-L3 comprising the amino acid sequence of SEQ ID NOS:380, 400, and 416, respectively;

the V_H region comprises a CDR-H1, CDR-H2, and CDR-H3 comprising the amino acid sequence of SEQ ID NOS:2, 5, and 10, respectively, and the V_L region comprises a CDR-L1, CDR-L2, and CDR-L3 comprising the amino acid sequence of SEQ ID NOS:33, 43, and 421, respectively; or

the V_H region comprises a CDR-H1, CDR-H2, and CDR-H3 comprising the amino acid sequence of SEQ ID NOS:507, 513, and 517, respectively, and the V_L region comprises a CDR-L1, CDR-L2, and CDR-L3 comprising the amino acid sequence of SEQ ID NOS:589, 590, and 591, respectively.

29. The polynucleotide of any of claims 26-28, wherein:

the V_H region is or comprises the amino acid sequence set forth in SEQ ID NO: 617; and the V_L region is or comprises the amino acid sequence set forth in SEQ ID NO:618

the V_H region is or comprises the amino acid sequence set forth in SEQ ID NO: 256; and the V_L region is or comprises the amino acid sequence set forth in SEQ ID NO:267;

the V_H region is or comprises the amino acid sequence set forth in SEQ ID NO: 519; and the V_L region is or comprises the amino acid sequence set forth in SEQ ID NO:535;

the V_H region is or comprises the amino acid sequence set forth in SEQ ID NO: 115; and the V_L region is or comprises the amino acid sequence set forth in SEQ ID NO:536; or

the V_H region is or comprises the amino acid sequence set forth in SEQ ID NO: 609; and the V_L region is or comprises the amino acid sequence set forth in SEQ ID NO:610.

30. The polynucleotide of any of claims 26-29, wherein the antibody fragment is an scFv.

31. The polynucleotide of any of claims 26-30, wherein: the antigen-binding domain comprises the amino acid sequence selected from any one of SEQ ID NOs: 478, 278, 559, 560, or 442 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 selected from any one of SEQ ID NOs: 478, 278, 559, 560, or 442;

the nucleic acid sequence encoding the antigen-binding domain comprises (a) the sequence of nucleotides set forth in any of SEQ ID NOS: 648, 352, 647, 716, or 718; (b) a sequence of nucleotides that has at least 90% sequence identity to any of SEQ ID NOS: 648, 352, 647, 716, or 718; or (c) a degenerate sequence of (a) or (b); and/or the nucleic acid sequence encoding the antigen-binding domain comprises the sequence of nucleotides set forth in any of SEQ ID NO: 460, 440, 715, 717 or 719.

32. A polynucleotide encoding a chimeric antigen receptor, comprising a nucleic acid encoding:

(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 amino acid sequence set forth in SEQ ID NO: 617; 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 amino acid sequence set forth in any of SEQ ID NO: 618;

(2) 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, optionally that is about 228 amino acids in length; or a spacer set forth in SEQ ID NO: 649;

(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.

33. The polynucleotide of any of claims 26-32, wherein:

the V_H region comprises a CDR-H1, CDR-H2 and CDR-H3 contained within the V_H region amino acid sequence set forth in SEQ ID NO: 617; and the V_L region comprises a CDR-L1, CDR-L2 and CDR-L3 contained within the V_L region amino acid sequence set forth in SEQ ID NO: 618; and/or

the V_H region comprises a CDR-H1, CDR-H2, and CDR-H3 comprising the amino acid sequence of SEQ ID NOS:593, 594, and 595, respectively, and the V_L region comprises a CDR-L1, CDR-L2, and CDR-L3 comprising the amino acid sequence of SEQ ID NOS:601, 602, and 603, respectively.

34. The polynucleotide of any of claims 1-33, wherein the extracellular antigen-binding domain comprises the V_H region amino acid sequence set forth in SEQ ID NO:617 and the V_L region amino acid sequence set forth in SEQ ID NO:618.

35. The polynucleotide of any of claims 1-31, wherein the intracellular signaling region comprises an activating cytoplasmic signaling domain, optionally wherein the activating cytoplasmic signaling domain is or comprises a cytoplasmic signaling domain of a CD3-zeta (CD3ζ) chain or a functional variant or signaling portion thereof.

36. The polynucleotide of claim 35, wherein the intracellular signaling region further comprises a costimulatory signaling region, optionally wherein the costimulatory signaling region comprises an intracellular signaling domain of a CD28, a 4-1BB or an ICOS or a signaling portion thereof.

37. The polynucleotide of claim 36, wherein the costimulatory signaling region comprises an intracellular signaling domain of a CD28, a 4-1BB or an ICOS or a signaling portion thereof.

38. The polynucleotide of claim 36 or claim 37, wherein the costimulatory signaling region comprises an intracellular signaling domain of 4-1BB.

39. The polynucleotide of any of claims 1-38, wherein the transmembrane domain is or comprises a transmembrane domain from CD4, CD28, or CD8.

40. The polynucleotide of any of claims 1-39, wherein the transmembrane domain is or comprises a transmembrane domain from a CD28.

41. The polynucleotide of any of claims 1-40, wherein the nucleic acid encoding the transmembrane domain comprises the sequence set forth in SEQ ID NO:688.

42. The polynucleotide of any of claims 1-41, wherein the binding of the encoded antigen-binding domain and/or the encoded chimeric antigen receptor, or a measure indicative of function or activity of the encoded chimeric antigen receptor 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.

43. The polynucleotide of any of claims 1-42, wherein the polynucleotide comprises the sequence set forth in any of SEQ ID NOS: 751-756 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 to the sequence set forth in any of SEQ ID NOS: 751-756 and the encoded receptor retains the function to bind to BCMA and retains the reduced RNA heterogeneity.

44. The polynucleotide of any of claims 1-43, wherein the polynucleotide comprises the sequence set forth in SEQ ID NOS:755 and the encoded receptor retains the function to bind to BCMA and retains the reduced RNA heterogeneity.

45. A chimeric antigen receptor encoded by the polynucleotide of any of claims 1-44.
46. A chimeric antigen receptor comprising: (a) an extracellular antigen-binding domain that specifically recognizes B cell maturation antigen (BCMA); (b) a spacer of at least 125 amino acids in length; (c) a transmembrane domain; and (d) an intracellular signaling region.
47. The chimeric antigen receptor of claim 46, wherein the spacer comprises a sequence of a hinge region, a C_H2 region and a C_H3 region.
48. The chimeric antigen receptor of claim 47, wherein:
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 CH2 region comprises all or a portion of an IgG4 CH2 region and/or of an IgG2 CH2 region, wherein the IgG4 CH2 region is optionally a human IgG4 CH2 region and the IgG2 CH2 region is optionally a human IgG2 CH2 region; and/or
the CH3 region comprises all or a portion of an IgG4 CH3 region and/or of an IgG2 CH3 region, wherein the IgG4 CH3 region is optionally a human IgG4 CH3 region and the IgG2 CH3 region is optionally a human IgG2 CH3 region.
49. The chimeric antigen receptor of claim 47 or claim 48, wherein:
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.
50. The chimeric antigen receptor of claim any of claims 46-49, wherein 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.

51. The chimeric antigen receptor of any of claims 46-50, wherein the spacer is or comprises (i) the sequence set forth in SEQ ID NO: 649; (ii) a functional variant of SEQ ID NO:649 that has at least 95%, 96%, 97%, 98% or 99% sequence identity to SEQ ID NO:649; or (iii) a contiguous portion of (i) or (ii) that is at least 125 amino acids in length.

52. The chimeric antigen receptor of any of claims 46, 47, and 49-51, wherein the spacer is or comprises the sequence set forth in SEQ ID NO: 649.

53. The chimeric antigen receptor of any of claims 46-52, wherein the antigen-binding domain is an antibody fragment comprising a variable heavy chain (V_H) and a variable light chain (V_L) region.

54. The chimeric antigen receptor of claim 53, wherein:
the V_H region and the V_L regions comprise the amino acid sequence set forth in SEQ ID NOS:617 and 618, respectively, or a sequence of amino acids having at least 90% identity to SEQ ID NOS:617 and 618, respectively;

the V_H region and the V_L regions comprise the amino acid sequence set forth in SEQ ID NOS:256 and 267, respectively, or a sequence of amino acids having at least 90% identity to SEQ ID NOS:256 and 267, respectively;

the V_H region and the V_L regions comprise the amino acid sequence set forth in SEQ ID NOS:519 and 535, respectively, or a sequence of amino acids having at least 90% identity to SEQ ID NOS:519 and 535, respectively;

the V_H region and the V_L regions comprise the amino acid sequence set forth in SEQ ID NOS:115 and 536, respectively, or a sequence of amino acids having at least 90% identity to SEQ ID NOS:115 and 536, respectively; or

the V_H region and the V_L regions comprise the amino acid sequence set forth in SEQ ID NOS:609 and 610, respectively, or a sequence of amino acids having at least 90% identity to SEQ ID NOS:609 and 610, respectively.

55. The chimeric antigen receptor of claim 53 or claim 54, wherein:

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:617, 115, 256, 519, or 609; 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:618, 267, 535, 536, or 610.

56. The chimeric antigen receptor of any of claims 53-55, wherein:

the V_H region comprises a CDR-H1, CDR-H2 and CDR-H3 contained within the V_H region amino acid sequence set forth in SEQ ID NO:617; and the V_L region comprises a CDR-L1, CDR-L2 and CDR-L3 contained within the V_L region amino acid sequence set forth in SEQ ID NO:618.

57. The chimeric antigen receptor of any of claims 53-55, wherein:

the V_H region comprises a CDR-H1, CDR-H2, and CDR-H3 comprising the amino acid sequence of SEQ ID NOS:593, 594, and 595, respectively, and the V_L region comprises a CDR-L1, CDR-L2, and CDR-L3 comprising the amino acid sequence of SEQ ID NOS:601, 602, and 603, respectively;

the V_H region comprises a CDR-H1, CDR-H2, and CDR-H3 comprising the amino acid sequence of SEQ ID NOS:2, 5, and 157, respectively, and the V_L region comprises a CDR-L1, CDR-L2, and CDR-L3 comprising the amino acid sequence of SEQ ID NOS:178, 183, and 194, respectively;

the V_H region comprises a CDR-H1, CDR-H2, and CDR-H3 comprising the amino acid sequence of SEQ ID NOS:1, 4, and 7, respectively, and the V_L region comprises a CDR-L1, CDR-L2, and CDR-L3 comprising the amino acid sequence of SEQ ID NOS:380, 400, and 416, respectively;

the V_H region comprises a CDR-H1, CDR-H2, and CDR-H3 comprising the amino acid sequence of SEQ ID NOS:2, 5, and 10, respectively, and the V_L region comprises a CDR-L1, CDR-L2, and CDR-L3 comprising the amino acid sequence of SEQ ID NOS:33, 43, and 421, respectively; or

the V_H region comprises a CDR-H1, CDR-H2, and CDR-H3 comprising the amino acid sequence of SEQ ID NOS:507, 513, and 517, respectively, and the V_L region comprises a CDR-L1, CDR-L2, and CDR-L3 comprising the amino acid sequence of SEQ ID NOS:589, 590, and 591, respectively.

58. The chimeric antigen receptor of any of claims 53-57, wherein the V_H region comprises a CDR-H1, CDR-H2, and CDR-H3 comprising the amino acid sequence of SEQ ID NOS:593, 594, and 595, respectively, and the V_L region comprises a CDR-L1, CDR-L2, and CDR-L3 comprising the amino acid sequence of SEQ ID NOS:601, 602, and 603, respectively.

59. The chimeric antigen receptor of any of claims 53-55 and 57, wherein:
the V_H region is or comprises the amino acid sequence set forth in SEQ ID NO:617; and
the V_L region is or comprises the amino acid sequence set forth in SEQ ID NO:618
the V_H region is or comprises the amino acid sequence set forth in SEQ ID NO:256; and
the V_L region is or comprises the amino acid sequence set forth in SEQ ID NO:267;
the V_H region is or comprises the amino acid sequence set forth in SEQ ID NO:519; and
the V_L region is or comprises the amino acid sequence set forth in SEQ ID NO:535;
the V_H region is or comprises the amino acid sequence set forth in SEQ ID NO:115; and
the V_L region is or comprises the amino acid sequence set forth in SEQ ID NO:536; or
the V_H region is or comprises the amino acid sequence set forth in SEQ ID NO:609; and
the V_L region is or comprises the amino acid sequence set forth in SEQ ID NO:610.

60. The chimeric antigen receptor of any of claims 53-59, wherein the V_H region is or comprises the amino acid sequence set forth in SEQ ID NO:617; and the V_L region is or comprises the amino acid sequence set forth in SEQ ID NO:618.

61. The chimeric antigen receptor of any of claims 53-60, wherein the antibody fragment is an scFv.

62. The chimeric antigen receptor of any of claims 53-61, wherein the V_H region is amino-terminal to the V_L region or the V_H region is carboxy-terminal to the V_L region.

63. The chimeric antigen receptor of any of claims 53-62, wherein the antigen-binding domain comprises the amino acid sequence selected from any one of SEQ ID NOs:478, 128-139, 268-278, 329, 442, 558-576, 578-583, 585, or 769-771 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 selected from any one of SEQ ID NOs:478, 128-139, 268-278, 329, 442, 558-576, 578-583, 585, or 769-771.

64. The chimeric antigen receptor of any of claims 46-63, wherein the antigen-binding domain comprises the amino acid sequence set forth in SEQ ID NO:478 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 set forth in SEQ ID NO:478.

65. A chimeric antigen receptor, 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 amino acid sequence set forth in SEQ ID NO:617; 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 amino acid sequence set forth in any of SEQ ID NO:618;

(2) 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, optionally that is about 228 amino acids in length; or a spacer set forth in SEQ ID NO:649;

(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 ($CD3\zeta$) chain and an intracellular signaling domain of a T cell costimulatory molecule.

66. The chimeric antigen receptor of claim 65, wherein

the V_H region comprises a CDR-H1, CDR-H2 and CDR-H3 contained within the V_H region amino acid sequence set forth in SEQ ID NO:617; and the V_L region comprises a CDR-L1, CDR-L2 and CDR-L3 contained within the V_L region amino acid sequence set forth in SEQ ID NO:618; or

the V_H region comprises a CDR-H1, CDR-H2, and CDR-H3 comprising the amino acid sequence of SEQ ID NOS:593, 594, and 595, respectively, and the V_L region comprises a CDR-L1, CDR-L2, and CDR-L3 comprising the amino acid sequence of SEQ ID NOS:601, 602, and 603, respectively.

67. The chimeric antigen receptor of claim 65 or claim 66, wherein the extracellular antigen-binding domain comprises the V_H region amino acid sequence set forth in SEQ ID NO:617 and the V_L region amino acid sequence set forth in SEQ ID NO:618.

68. The chimeric antigen receptor of any of claims 46-64, wherein the intracellular signaling region comprises an activating cytoplasmic signaling domain, optionally wherein the activating cytoplasmic signaling domain is or comprises a cytoplasmic signaling domain of a CD3-zeta (CD3ζ) chain or a functional variant or signaling portion thereof.

69. The chimeric antigen receptor of any of claims 46-68, wherein the intracellular signaling region further comprises a costimulatory signaling region, optionally wherein the costimulatory signaling region comprises an intracellular signaling domain of a CD28, a 4-1BB or an ICOS or a signaling portion thereof.

70. The chimeric antigen receptor of any of claims 46-69, wherein the transmembrane domain is or comprises a transmembrane domain from CD4, CD28, or CD8.

71. The chimeric antigen receptor of any of claims 46-70, wherein (a) the ability of the antigen binding domain or of the chimeric antigen receptor to bind to BCMA expressed on the surface of a target cell, or (b) a measure indicative of function or activity of the chimeric antigen receptor following exposure of cells expressing the chimeric antigen receptor to cells expressing surface BCMA, is not reduced or blocked or is not substantially reduced or blocked in the presence of a concentration or amount of a soluble or shed form of BCMA, wherein the

concentration or amount is a concentration or amount capable of blocking or reducing or substantially blocking or reducing binding or a measure of function or activity associated with a reference anti-BCMA recombinant receptor or a reference anti-BCMA binding domain, under the same or substantially the same conditions, or is a concentration or amount present in a biological sample.

72. A polynucleotide encoding the chimeric antigen receptor of any of claims 46-71.

73. A vector comprising the polynucleotide of any of claims 1-44.

74. The vector of claim 73, wherein the vector is a viral vector, optionally wherein the viral vector is a retroviral vector, optionally a lentiviral vector.

75. An engineered cell, comprising the chimeric antigen receptor of any of claims 45-71, the polynucleotide of any of claims 1-44, or the vector of claim 73 or claim 74.

76. The engineered cell of claim 75, wherein the cell is an immune cell, optionally wherein the immune cell is an NK cell or a T cell, optionally a CD4+ and/or CD8+ T cell.

77. The engineered cell of claim 75 or claim 76, wherein the immune cell is a primary cell obtained from a subject.

78. The engineered cell of any of claims 75-77, wherein the cell comprises transcribed RNA encoding the chimeric antigen receptor, optionally messenger RNA (mRNA), that exhibits at least 70%, 75%, 80%, 85%, 90%, or 95% RNA homogeneity.

79. A composition comprising the chimeric antigen receptor of any one of claims 45-71, the polynucleotide of any of claims 1-44, the vector of claim 73 or claim 74.

80. A composition comprising the engineered cell of any one of claims 75-78.

81. The composition of claim 80, wherein the composition comprises CD4+ and CD8+ T cells and the ratio of CD4+ to CD8+ T cells is from or from about 1:3 to 3:1.

82. The engineered cells of any of claims 75-78 or the composition of any of claims 79-81 for use in treating a disease or disorder.

83. Use of the engineered cells of any of claims 75-78 or the composition of any of claims 79-81 in the manufacture of a medicament for the treatment of a disease or disorder.

84. The engineered cells or the composition for use of claim 82 or the use of claim 83, wherein the disease or disorder is associated with expression of B cell maturation antigen (BCMA), optionally a B cell-related disorder.

85. The engineered cells or the composition for use of claim 82 or claim 84, or the use of claim 83 or claim 84, wherein the disease or disorder associated with BCMA is an autoimmune disease or disorder or a cancer, optionally a lymphoma, a leukemia, or a plasma cell malignancy.

86. The engineered cells or the composition for use or the use of claim 85, wherein the cancer is multiple myeloma (MM).

87. The engineered cells or the composition for use of any one of claims 82 and 84-86 or the use of any of claims 83-86, wherein a dose of engineered T cell or a composition comprising a dose of the engineered T cells is to be administered, and the dose of engineered T cells comprises between at or about 2.5×10^7 CAR-expressing T cells and at or about 1.2×10^9 CAR-expressing T cells.

88. The engineered cells or the composition for use of any one of claims 82 and 84-87 or the use of any of claims 83-87, wherein a dose of the engineered cells or a composition comprising a dose of the engineered cells is to be administered, and 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 approximately 1:3 and approximately 3:1.

89. The engineered cells or the composition for use of any one of claims 82 and 84-88 or the use of any of claims 83-88, wherein a dose of the engineered cells or a composition comprising a dose of the engineered cells is to be administered, and prior to the administration, the subject has received a lymphodepleting therapy.

90. The engineered cells or the composition for use of any one of claims 82 and 84-89 or the use of any of claims 83-89, wherein a dose of the engineered cells or a composition comprising a dose of the engineered cells is to be administered, and 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;

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.

91. A method of determining the heterogeneity of a transcribed nucleic acid of a transgene, the method comprising:

a) 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; and

b) 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.

92. The method of claim 91 wherein the detected differences in b) are different lengths of the amplified transcripts and/or differences in chromatographic profiles of the amplified transcripts.

93. The method of claim 91 or claim 92, wherein:

the 5' primer is specific to sequence transcribed from the promoter region of the transcribed nucleic acid;

the transcribed nucleic acid is amplified using a 3' primer specific to a sequence within the amino acid-coding sequence of the polynucleotide, and/or the 3' untranslated region of the transcribed pre-mRNA; and/or

the 3' primer is specific to the polyadenylation sequence or enhancer region of the 3' untranslated region of the transcribed pre-mRNA.

94. The method of any of claims 91-93, wherein a transcribed nucleic acid that is detected as having heterogeneity is identified as a transgene candidate for removal of one or more splice site, optionally wherein 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.

95. A method of reducing the heterogeneity of an expressed transgene transcript, the method comprising:

a) identifying a transgene candidate for the removal of splice sites according to the method of claim 109;

b) identifying one or more potential splice donor and/or splice acceptor sites; and

c) modifying the nucleic acid sequence at or near the one or more potential splice donor and/or splice acceptor sites identified in b), thereby generating a modified polynucleotide.

96. The method of claim 95, wherein 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 of a splice event, and/or is/are predicted to be involved in a splice event with a probability of at least 70%, 75%, 80%, 85%, 90%, 95%, 99%, or 100%.

97. The method of claim 95 or claim 96, wherein the transgene is a chimeric antigen receptor or a portion of a chimeric antigen receptor, optionally wherein the CAR polypeptide comprises an antigen-binding domain comprising an antibody fragment, optionally a single chain antibody fragment (scFv), comprising a variable heavy chain (V_H) and a variable light chain (V_L), a spacer, a transmembrane region, and an intracellular signaling region.

98. The method of any of claims 95-97, wherein the encoded amino acid sequence of the transgene is unchanged following modification of the polynucleotide.

99. The method of any of claims 95-98, wherein the RNA transcribed from the modified polynucleotide exhibits at least or at least about 70%, 75%, 80%, 85%, 90%, or 95% homogeneity following expression of the unmodified polynucleotide in a cell.

100. A computer system comprising a processor and memory, the memory comprising instructions operable to cause the processor to carry out any one or more of steps of the methods of any of claims 91-99.