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Innovation, Science and Economic Development Canada
Canadian Intellectual Property Office

CA 3082010 A1 2019/05/09

(21) 3 082 010

(12) DEMANDE DE BREVET CANADIEN
CANADIAN PATENT APPLICATION

(13) A1

(86) Date de dépôt PCT/PCT Filing Date: 2018/11/01
(87) Date publication PCT/PCT Publication Date: 2019/05/09
(85) Entrée phase nationale/National Entry: 2020/04/28
(86) N° demande PCT/PCT Application No.: US 2018/058811
(87) N° publication PCT/PCT Publication No.: 2019/090003
(30) Priorités/Priorities: 2017/11/01 (US62/580,445); 2017/11/01 (US62/580,439); 2017/11/07 (US62/582,938); 2017/11/07 (US62/582,932); 2017/12/08 (US62/596,765); 2017/12/08 (US62/596,763); 2018/01/08 (US62/614,963); 2018/01/08 (US62/614,960); 2018/05/01 (US62/665,447); 2018/05/01 (US62/665,442)

(51) Cl.Int./Int.Cl. *A61K 39/395* (2006.01), *A61K 35/17* (2015.01), *A61P 35/00* (2006.01), *A61P 39/02* (2006.01), *C07K 14/725* (2006.01), *C07K 16/28* (2006.01)

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(54) Titre : RECEPTEURS D'ANTIGENES CHIMERIQUES SPECIFIQUES DE L'ANTIGENE DE MATURATION DES CELLULES B (BCMA)
(54) Title: CHIMERIC ANTIGEN RECEPTORS SPECIFIC FOR B-CELL MATURATION ANTIGEN (BCMA)

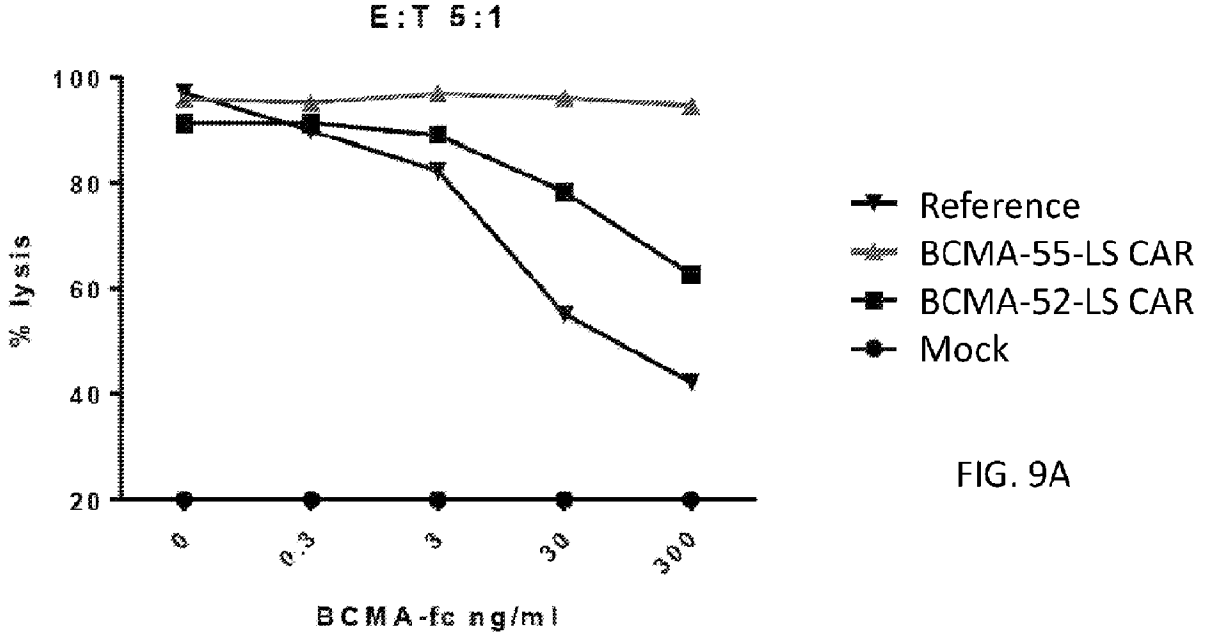

FIG. 9A

(57) Abrégé/Abstract:
Provided herein are chimeric antigen receptors (CARs), comprising an extracellular BCMA-binding domain, in particular a scFv. The CAR also comprises a spacer of at least 125 amino acids in length, a transmembrane domain, and an intracellular signaling region. It may also include an intracellular costimulatory domain. Also provided are genetically engineered cells expressing the CARs and uses thereof such as in adoptive cell therapy.

(21) 3 082 010

(13) A1

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(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

REVISED VERSION

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
09 May 2019 (09.05.2019)

WIPO | PCT

(10) International Publication Number
WO 2019/090003 A9

(51) International Patent Classification:
A61K 39/395 (2006.01) *A61P 35/00* (2006.01)
C07K 16/28 (2006.01) *C07K 14/725* (2006.01)
A61P 39/02 (2006.01) *A61K 35/17* (2015.01)

(21) International Application Number:
PCT/US2018/058811

(22) International Filing Date:
01 November 2018 (01.11.2018)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:

62/580,439	01 November 2017 (01.11.2017)	US
62/580,445	01 November 2017 (01.11.2017)	US
62/582,932	07 November 2017 (07.11.2017)	US
62/582,938	07 November 2017 (07.11.2017)	US
62/596,763	08 December 2017 (08.12.2017)	US
62/596,765	08 December 2017 (08.12.2017)	US
62/614,960	08 January 2018 (08.01.2018)	US
62/614,963	08 January 2018 (08.01.2018)	US
62/665,442	01 May 2018 (01.05.2018)	US
62/665,447	01 May 2018 (01.05.2018)	US

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(54) Title: CHIMERIC ANTIGEN RECEPTORS SPECIFIC FOR B-CELL MATURATION ANTIGEN (BCMA)

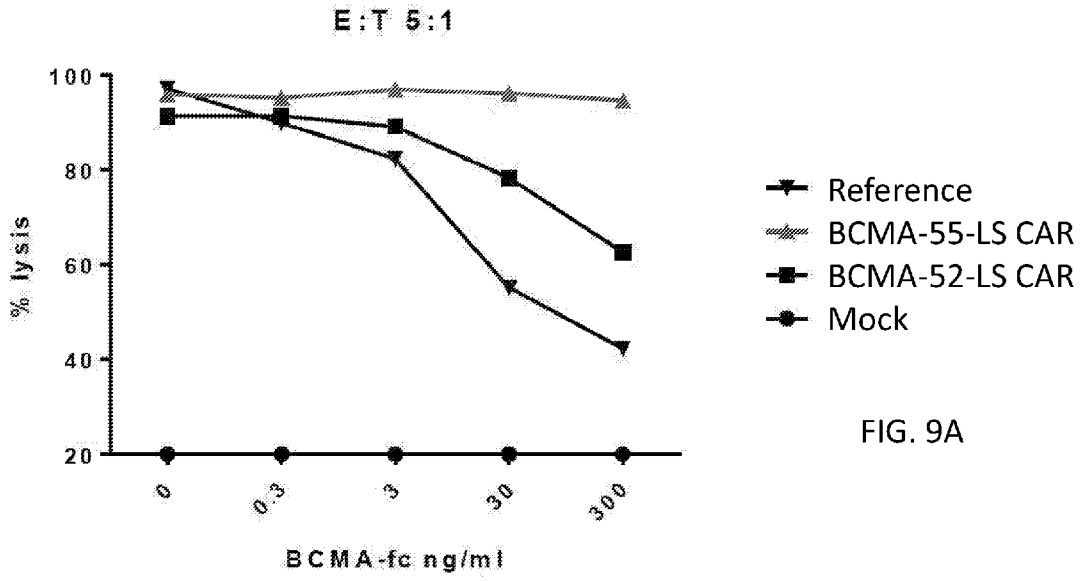

FIG. 9A

(57) Abstract: Provided herein are chimeric antigen receptors (CARs), comprising an extracellular BCMA-binding domain, in particular a scFv. The CAR also comprises a spacer of at least 125 amino acids in length, a transmembrane domain, and an intracellular signaling region. It may also include an intracellular costimulatory domain. Also provided are genetically engineered cells expressing the CARs and uses thereof such as in adoptive cell therapy.

[Continued on next page]

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(81) **Designated States** *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DJ, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JO, JP, KE, KG, KH, KN, KP, KR, KW, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) **Designated States** *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Declarations under Rule 4.17:

— *as to the applicant's entitlement to claim the priority of the earlier application (Rule 4.17(iii))*

Published:

— *with international search report (Art. 21(3))*

— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(h))*

— *with sequence listing part of description (Rule 5.2(a))*

(88) **Date of publication of the revised international search report:**

19 September 2019 (19.09.2019)

(15) **Information about Correction:**

see Notice of 19 September 2019 (19.09.2019)

Previous Correction:

see Notice of 11 July 2019 (11.07.2019)

DEMANDE OU BREVET VOLUMINEUX

LA PRÉSENTE PARTIE DE CETTE DEMANDE OU CE BREVET COMPREND PLUS D'UN TOME.

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CONTENANT LES PAGES 1 À 247

NOTE : Pour les tomes additionels, veuillez contacter le Bureau canadien des brevets

JUMBO APPLICATIONS/PATENTS

THIS SECTION OF THE APPLICATION/PATENT CONTAINS MORE THAN ONE VOLUME

THIS IS VOLUME 1 OF 2
CONTAINING PAGES 1 TO 247

NOTE: For additional volumes, please contact the Canadian Patent Office

NOM DU FICHIER / FILE NAME :

NOTE POUR LE TOME / VOLUME NOTE:

CHIMERIC ANTIGEN RECEPTORS SPECIFIC FOR B-CELL MATURATION ANTIGEN (BCMA)

Cross-Reference to Related Applications

[0001] This application claims priority from U.S. provisional application 62/580,439, filed November 1, 2017, entitled "CHIMERIC ANTIGEN RECEPTORS SPECIFIC FOR B-CELL MATURATION ANTIGEN AND ENCODING POLYNUCLEOTIDES," U.S. provisional application No. 62/580,445, filed November 1, 2017, entitled "CHIMERIC ANTIGEN RECEPTORS SPECIFIC FOR B-CELL MATURATION ANTIGEN AND ENCODING POLYNUCLEOTIDES," U.S. provisional application No. 62/582,932, filed November 7, 2017, entitled "CHIMERIC ANTIGEN RECEPTORS SPECIFIC FOR B-CELL MATURATION ANTIGEN AND ENCODING POLYNUCLEOTIDES," U.S. provisional application No. 62/582,938, filed November 7, 2017, entitled "CHIMERIC ANTIGEN RECEPTORS SPECIFIC FOR B-CELL MATURATION ANTIGEN AND ENCODING POLYNUCLEOTIDES," U.S. provisional application No. 62/596,765, filed December 8, 2017, entitled "CHIMERIC ANTIGEN RECEPTORS SPECIFIC FOR B-CELL MATURATION ANTIGEN AND ENCODING POLYNUCLEOTIDES," U.S. provisional application No. 62/596,763, filed December 8, 2017, entitled "CHIMERIC ANTIGEN RECEPTORS SPECIFIC FOR B-CELL MATURATION ANTIGEN AND ENCODING POLYNUCLEOTIDES," U.S. provisional application No. 62/614,960, filed January 8, 2018, entitled "CHIMERIC ANTIGEN RECEPTORS SPECIFIC FOR B-CELL MATURATION ANTIGEN AND ENCODING POLYNUCLEOTIDES," U.S. provisional application No. 62/614,963, filed January 8, 2018, entitled "CHIMERIC ANTIGEN RECEPTORS SPECIFIC FOR B-CELL MATURATION ANTIGEN AND ENCODING POLYNUCLEOTIDES," U.S. provisional application No. 62/665,442, filed May 1, 2018, entitled "CHIMERIC ANTIGEN RECEPTORS SPECIFIC FOR B-CELL MATURATION ANTIGEN AND ENCODING POLYNUCLEOTIDES," and U.S. provisional application No. 62/665,447, filed May 1, 2018, entitled "METHOD OF ASSESSING ACTIVITY OF RECOMBINANT ANTIGEN RECEPTORS," the contents of which are incorporated by reference in their entirety.

Incorporation by Reference of Sequence Listing

[0002] The present application is being filed along with a Sequence Listing in electronic format. The Sequence Listing is provided as a file entitled 735042009940SeqList.txt, created

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 embodiments, the spacer includes a sequence of a hinge region, a C_H2 and a C_H3 region. In some embodiments, one of 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 aspects, 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 embodiments, 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 embodiments, the encoded spacer is or includes the sequence set forth in SEQ ID NO: 649.

[0006] In some of any embodiments, the spacer has a length of 125 to 300 amino acids in length, 125 to 250 amino acids in length, 125 to 230 amino acids in length, 125 to 200 amino acids in length, 125 to 180 amino acids in length, 125 to 150 amino acids in length, 150 to 300 amino acids in length, 150 to 250 amino acids in length, 150 to 230 amino acids in length, 150 to 200 amino acids in length, 150 to 180 amino acids in length, 180 to 300 amino acids in length, 180 to 250 amino acids in length, 180 to 230 amino acids in length, 180 to 200 amino acids in length, 200 to 300 amino acids in length, 200 to 250 amino acids in length, 200 to 230 amino acids in length, 230 to 300 amino acids in length, 230 to 250 amino acids in length or 250 to 300 amino acids in length. In some embodiments, the spacer is at least or at least about or is or is about 130, 140, 150, 160, 170, 180, 190, 200, 210, 220, 221, 222, 223, 224, 225, 226, 227, 228 or 229 amino acids in length, or a length between any of the foregoing.

[0007] In some embodiments 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 embodiments 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

aagtttctttctgtattccaggctgaccgtggataaatctc (SEQ ID NO:742) and/or gggcaacgtgttctcttgcagtgtcatgcacgaagccctgc (SEQ ID NO:743). In some embodiments, 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 embodiments, the reference splice donor site contains the sequence tcaactggtacgtgg (SEQ ID NO:706); and/or the reference splice acceptor site contains the sequence aagtttctttctgtattccaggctgaccgtggataaatctc (SEQ ID NO:742).

[0009] In some embodiments 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 aspects, 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 embodiments, 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 cagtttcttcctgtatagtagactcaccgtggataaatcaa (SEQ ID NO:672) gggcaacgtgttcagctgcagcgtgatgcacgaggccctgc (SEQ ID NO: 673) and/or aagtttctttctgtattccagactgaccgtggataaatctc (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 cagtttcttcctgtatagtagactcaccgtggataaatcaa (SEQ ID NO:672). In some of any such embodiments, 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.

[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 embodiments, following expression of the polynucleotide in a cell, the transcribed RNA, optionally messenger RNA (mRNA), from the polynucleotide, exhibits at least 70%, 75%, 80%, 85%, 90%, or 95% RNA homogeneity. In some embodiments, 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 embodiments, 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 embodiments, the polynucleotide is codon-optimized.

[0013] In some embodiments 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 embodiments, 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 embodiments, 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 aspects, 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 embodiments 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 embodiments, 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 embodiments, 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 embodiments 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 embodiments 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 embodiments 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 embodiments 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 aspects, 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 embodiments, 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 embodiments, the V_H region is or includes the amino acid sequence set forth in SEQ ID NO: 617.

[0020] In some embodiments 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-

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:383, 403, and 419, respectively; a CDR-L1, CDR-L2, and CDR-L3 containing the amino acid sequence of SEQ ID NOs:384, 39, and 54, respectively; a CDR-L1, CDR-L2, and CDR-L3 containing the amino acid sequence of SEQ ID NOs:385, 180, and 58, 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:386, 404, and 420, respectively; a CDR-L1, CDR-L2, and CDR-L3 containing the amino acid sequence of SEQ ID NOs:387, 405, and 422, respectively; a CDR-L1, CDR-L2, and CDR-L3 containing the amino acid sequence of SEQ ID NOs:388, 406, and 423, respectively; a CDR-L1, CDR-L2, and CDR-L3 containing the amino acid sequence of SEQ ID NOs:388, 407, and 424, respectively; a CDR-L1, CDR-L2, and CDR-L3 containing the amino acid sequence of SEQ ID NOs:389, 408, and 425, respectively; a CDR-L1, CDR-L2, and CDR-L3 containing the amino acid sequence of SEQ ID NOs:390, 183, and 193, respectively; a CDR-L1, CDR-L2, and CDR-L3 containing the amino acid sequence of SEQ ID NOs:391, 409, and 426, respectively; a CDR-L1, CDR-L2, and CDR-L3 containing the amino acid sequence of SEQ ID NOs:392, 40, and 427, respectively; a CDR-L1, CDR-L2, and CDR-L3 containing the amino acid sequence of SEQ ID NOs:394, 39, and 429, respectively; a CDR-L1, CDR-L2, and CDR-L3 containing the amino acid sequence of SEQ ID NOs:395, 411, and 430, respectively; a CDR-L1, CDR-L2, and CDR-L3 containing the amino acid sequence of SEQ ID NOs:396, 412, and 431, respectively; a CDR-L1, CDR-L2, and CDR-L3 containing the

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 embodiments 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 embodiments, 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 aspects, 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 embodiments 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 embodiments, 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 embodiments, 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 embodiments, 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 embodiments, 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

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 forth in SEQ ID NOs:114 and 126, respectively, or a sequence of amino acids that has at least 90% identity to SEQ ID NO:114 and 126, respectively; the V_H region and the V_L regions contain the amino acid sequence set forth in SEQ ID NOs:115 and 127, respectively, or a sequence of amino acids that has at least 90% identity to SEQ ID NO:115 and 127, respectively; the V_H region and the V_L regions contain the amino acid sequence set forth in SEQ ID NOs:247 and 257, respectively, or a sequence of amino acids that has at least 90% identity to SEQ ID NO:247 and 257, respectively; the V_H region and the V_L regions contain the amino acid sequence set forth in SEQ ID NOs:248 and 258, respectively, or a sequence of amino acids that has at least 90% identity to SEQ ID NO:248 and 258, respectively; the V_H region and the V_L regions contain the amino acid sequence set forth in SEQ ID NOs:249 and 259, respectively, or a sequence of amino acids that has at least 90% identity to SEQ ID NO:249 and 259, respectively; the V_H region and the V_L regions contain the amino acid sequence set forth in SEQ ID NOs:250 and 260, respectively, or a sequence of amino acids that has at least 90% identity to SEQ ID NO:250 and 260, respectively; the V_H region and the V_L regions contain the amino acid sequence set forth in SEQ ID NOs:251 and 261, respectively, or a sequence of amino acids that has at least 90% identity to SEQ ID NO:251 and 261, respectively; the V_H region and the V_L regions contain the amino acid sequence set forth in SEQ ID NOs:252 and 262, respectively, or a sequence of amino acids that has at least 90% identity to SEQ ID NO:252 and 262, respectively; the V_H region and the V_L regions contain the amino acid sequence set forth in SEQ ID NOs:253 and 263, respectively, or a sequence of amino acids that has at least 90% identity to SEQ ID NO:253 and 263, respectively; the V_H region and the V_L regions contain the amino acid sequence set forth in SEQ ID NOs:254 and 264, respectively, or a sequence of amino acids that has at least 90% identity to SEQ ID NO:254 and 264, respectively; the V_H region and the V_L regions contain the amino acid sequence set forth in SEQ ID NOs:255 and 265, respectively, or a sequence of amino acids that has at least 90% identity to SEQ ID NO:255 and 265, respectively; the V_H region and the V_L regions contain the amino acid sequence set forth in SEQ ID NOs:256 and 266, respectively, or a sequence of amino acids that has at least 90% identity to SEQ ID NO:256 and 266, respectively; the V_H region and the V_L regions contain the amino acid sequence set forth in SEQ ID NOs:256 and 267,

respectively, or a sequence of amino acids that has at least 90% identity to SEQ ID NO:256 and 267, respectively; the V_H region and the V_L regions contain the amino acid sequence set forth in SEQ ID NOs:518 and 534, respectively, or a sequence of amino acids that has at least 90% identity to SEQ ID NO:518 and 534, respectively; the V_H region and the V_L regions contain the amino acid sequence set forth in SEQ ID NOs:519 and 535, respectively, or a sequence of amino acids that has at least 90% identity to SEQ ID NO:519 and 535, respectively; the V_H region and the V_L regions contain the amino acid sequence set forth in SEQ ID NOs:115 and 536, respectively, or a sequence of amino acids that has at least 90% identity to SEQ ID NO:115 and 536, respectively; the V_H region and the V_L regions contain the amino acid sequence set forth in SEQ ID NOs:520 and 264, respectively, or a sequence of amino acids that has at least 90% identity to SEQ ID NO:520 and 264, respectively; the V_H region and the V_L regions contain the amino acid sequence set forth in SEQ ID NOs:521 and 537, respectively, or a sequence of amino acids that has at least 90% identity to SEQ ID NO:521 and 537, respectively; the V_H region and the V_L regions contain the amino acid sequence set forth in SEQ ID NOs:522 and 538, respectively, or a sequence of amino acids that has at least 90% identity to SEQ ID NO:522 and 538, respectively; the V_H region and the V_L regions contain the amino acid sequence set forth in SEQ ID NOs:523 and 539, respectively, or a sequence of amino acids that has at least 90% identity to SEQ ID NO:523 and 539, respectively; the V_H region and the V_L regions contain the amino acid sequence set forth in SEQ ID NOs:519 and 540, respectively, or a sequence of amino acids that has at least 90% identity to SEQ ID NO:519 and 540, respectively; the V_H region and the V_L regions contain the amino acid sequence set forth in SEQ ID NOs:524 and 541, respectively, or a sequence of amino acids that has at least 90% identity to SEQ ID NO:524 and 541, respectively; the V_H region and the V_L regions contain the amino acid sequence set forth in SEQ ID NOs:525 and 261, respectively, or a sequence of amino acids that has at least 90% identity to SEQ ID NO:525 and 261, respectively; the V_H region and the V_L regions contain the amino acid sequence set forth in SEQ ID NOs:526 and 542, respectively, or a sequence of amino acids that has at least 90% identity to SEQ ID NO:526 and 542, respectively; the V_H region and the V_L regions contain the amino acid sequence set forth in SEQ ID NOs:527 and 543, respectively, or a sequence of amino acids that has at least 90% identity to SEQ ID NO:527 and 543, respectively; the V_H region and the V_L regions contain the amino acid sequence set forth in SEQ ID NOs:528 and 544, respectively, or a sequence of amino acids that has at least 90% identity to SEQ ID NO:528 and 544, respectively; the V_H region and the V_L regions contain the amino acid sequence set forth in SEQ ID NOs:529 and 545, respectively, or a sequence of amino

acids that has at least 90% identity to SEQ ID NO:529 and 545, respectively; the V_H region and the V_L regions contain the amino acid sequence set forth in SEQ ID NOs:528 and 546, respectively, or a sequence of amino acids that has at least 90% identity to SEQ ID NO:528 and 546, respectively; the V_H region and the V_L regions contain the amino acid sequence set forth in SEQ ID NOs:522 and 547, respectively, or a sequence of amino acids that has at least 90% identity to SEQ ID NO:522 and 547, respectively; the V_H region and the V_L regions contain the amino acid sequence set forth in SEQ ID NOs:256 and 548, respectively, or a sequence of amino acids that has at least 90% identity to SEQ ID NO:256 and 548, respectively; the V_H region and the V_L regions contain the amino acid sequence set forth in SEQ ID NOs:530 and 549, respectively, or a sequence of amino acids that has at least 90% identity to SEQ ID NO:530 and 549, respectively; the V_H region and the V_L regions contain the amino acid sequence set forth in SEQ ID NOs:531 and 550, respectively, or a sequence of amino acids that has at least 90% identity to SEQ ID NO:531 and 550, respectively; the V_H region and the V_L regions contain the amino acid sequence set forth in SEQ ID NOs:519 and 552, respectively, or a sequence of amino acids that has at least 90% identity to SEQ ID NO:519 and 552, respectively; the V_H region and the V_L regions contain the amino acid sequence set forth in SEQ ID NOs:110 and 553, respectively, or a sequence of amino acids that has at least 90% identity to SEQ ID NO:110 and 553, 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:533 and 554, respectively, or a sequence of amino acids that has at least 90% identity to SEQ ID NO:533 and 554, respectively; the V_H region and the V_L regions contain the amino acid sequence set forth in SEQ ID NOs:115 and 555, respectively, or a sequence of amino acids that has at least 90% identity to 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; or the V_H region and the V_L regions contain the amino acid sequence set forth in SEQ ID NOs:772 and 775, respectively, or a sequence of amino acids that has at least 90% identity to SEQ ID NO:772 and 775, respectively; or the V_H region and the V_L regions contain the amino acid sequence set forth in SEQ ID NOs:773 and 776, respectively, or a sequence of amino acids that has at least 90% identity to SEQ ID NO:773 and 776, respectively; or the V_H region and the V_L regions contain the amino acid sequence set forth in SEQ ID NOs:774 and 777, respectively, or a sequence of amino acids that has at least 90% identity to SEQ ID NO:774 and 777, respectively; or the V_H region and the V_L regions contain the amino acid sequence set forth in SEQ ID NOs:815 and 833, respectively, or a sequence of amino acids that has at least 90% identity to SEQ ID NO:815 and 833, respectively; or the V_H region and the V_L regions contain the amino acid sequence set forth in SEQ ID NOs:816 and 834, respectively, or a sequence of amino acids that has at least 90% identity to SEQ ID NO:816 and 834, respectively; or the V_H region and the V_L regions contain the amino acid sequence set forth in SEQ ID NOs:817 and 835, respectively, or a sequence of amino acids that has at least 90% identity to SEQ ID NO:817 and 835, respectively; or the V_H region and the V_L regions contain the amino acid sequence set forth in SEQ ID NOs:818 and 836, respectively, or a sequence of amino acids that has at least 90% identity to SEQ ID NO:818 and 836, respectively; or the V_H region and the V_L regions contain the amino acid sequence set forth in SEQ ID NOs:819 and 837, respectively, or a sequence of amino acids that has at least 90% identity to SEQ ID NO:819 and 837, respectively; or the V_H region and the V_L regions contain the amino acid sequence set forth in SEQ ID 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.

[0028] In some embodiments 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

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 forth in SEQ ID NOs:248 and 258, respectively, or a sequence of amino acids that has at least 90% identity to SEQ ID NO:248 and 258, respectively; the V_H region and the V_L regions contain the amino acid sequence set forth in SEQ ID NOs:252 and 262, respectively, or a sequence of amino acids that has at least 90% identity to SEQ ID NO:252 and 262, respectively; the V_H region and the V_L regions contain the amino acid sequence set forth in SEQ ID NOs:253 and 263, respectively, or a sequence of amino acids that has at least 90% identity to SEQ ID NO:253 and 263, respectively; the V_H region and the V_L regions contain the amino acid sequence set forth in SEQ ID NOs:254 and 264, respectively, or a sequence of amino acids that has at least 90% identity to SEQ ID NO:254 and 264, respectively; the V_H region and the V_L regions contain the amino acid sequence set forth in SEQ ID NOs:255 and 265, respectively, or a sequence of amino acids that has at least 90% identity to SEQ ID NO:255 and 265, respectively; the V_H region and the V_L regions contain the amino acid sequence set forth in SEQ ID NOs:256 and 266, respectively, or a sequence of amino acids that has at least 90% identity to SEQ ID NO:256 and 266, respectively; the V_H region and the V_L regions contain the amino acid sequence set forth in SEQ ID NOs:256 and 267, respectively, or a sequence of amino acids that has at least 90% identity to SEQ ID NO:256 and 267, respectively; the V_H region and the V_L regions contain the amino acid sequence set forth in SEQ ID NOs:518 and 534, respectively, or a sequence of amino acids that has at least 90% identity to SEQ ID NO:518 and 534, respectively; the V_H region and the V_L regions contain the amino acid sequence set forth in SEQ ID NOs:519 and 535, respectively, or a sequence of amino acids that has at least 90% identity to SEQ ID NO:519 and 535, respectively; the V_H region and the V_L regions contain the amino acid sequence set forth in SEQ ID NOs:115 and 536, respectively, or a sequence of amino acids that has at least 90% identity to SEQ ID NO:115 and 536, respectively; the V_H region and the V_L regions contain the amino acid sequence set forth in SEQ ID NOs:520 and 264, respectively, or a sequence of amino acids that has at least 90% identity to SEQ ID NO:520 and 264, respectively; the V_H region and the V_L regions contain the amino acid sequence set forth in

SEQ ID NOs:521 and 537, respectively, or a sequence of amino acids that has at least 90% identity to SEQ ID NO:521 and 537, respectively; the V_H region and the V_L regions contain the amino acid sequence set forth in SEQ ID NOs:522 and 538, respectively, or a sequence of amino acids that has at least 90% identity to SEQ ID NO:522 and 538, 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; the V_H region and the V_L regions contain the amino acid sequence set forth in SEQ ID NOs:772 and 775, respectively, or a sequence of amino acids that has at least 90% identity to SEQ ID NO:772 and 775, respectively; the V_H region and the V_L regions contain the amino acid sequence set forth in SEQ ID NOs:773 and 776, respectively, or a sequence of amino acids that has at least 90% identity to SEQ ID NO:773 and 776, respectively; or the V_H region and the V_L regions contain the amino acid sequence set forth in SEQ ID NOs:774 and 777, respectively, or a sequence of amino acids that has at least 90% identity to SEQ ID NO:774 and 777, respectively; or the V_H region and the V_L regions contain the amino acid sequence set forth in SEQ ID NOs:815 and 833, respectively, or a sequence of amino acids that has at least 90% identity to SEQ ID NO:815 and 833, respectively; or the V_H region and the V_L regions contain the amino acid sequence set forth in SEQ ID NOs:816 and 834, respectively, or a sequence of amino acids that has at least 90% identity to SEQ ID NO:816 and 834, respectively; or the V_H region and the V_L regions contain the amino acid sequence set forth in SEQ ID NOs:817 and 835, respectively, or a sequence of amino acids that has at least 90% identity to SEQ ID NO:817 and 835, respectively; or the V_H region and the V_L regions contain the amino acid sequence set forth in SEQ ID NOs:818 and 836, respectively, or a sequence of amino acids that has at least 90% identity to SEQ ID NO:818 and 836, respectively; or the V_H region and the V_L regions contain the amino acid sequence set forth in SEQ ID NOs:819 and 837, respectively, or a sequence of amino acids that has at least 90% identity to SEQ ID NO:819 and 837, respectively; or the V_H region and the V_L regions contain the amino acid sequence set forth in SEQ ID

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

[0031] In some embodiments 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 embodiments, 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 embodiments 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 embodiments, 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 embodiments, the nucleic acid encoding the antigen-binding domain is codon-optimized. In some embodiments, 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 embodiments, the nucleic acid encoding the antigen-binding domain includes the sequence of nucleotides set forth in SEQ ID NO:460.

[0033] In some embodiments of any of the polynucleotides described herein, the V_H region is carboxy-terminal to the V_L region. In some embodiments, 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 (CD3ζ) 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 embodiments, the V_H region comprises a CDR-H1, CDR-H2 and CDR-H3 contained within the V_H region sequence of SEQ ID NO: 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.

[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 (CD3ζ) 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 embodiments, 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 embodiments, 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 VH/VL or CDR sequences, described herein including in any of the foregoing embodiments. In some embodiments, the binding domain recognizes an epitope comprising a portion of one or more amino acid sequences within a BCMA polypeptide. In some aspects, 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 aspects, such one or more amino acid sequences are or comprise: MLMAG (SEQ ID NO:640), YFDSLL (SEQ ID NO:641), and QLRCSSNTPPL (SEQ ID NO:642). In some aspects, such one or more amino acid sequences are or comprise:: MLMAG (SEQ ID NO:640), QNEYFDSLL (SEQ ID NO:780), and QLRCSSNTPPL (SEQ ID NO:642). In some aspects, 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 aspects, 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 embodiments of any of the polynucleotides described herein, the intracellular signaling region includes an activating cytoplasmic signaling domain. In some embodiments, 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 embodiments, the activating cytoplasmic signaling domain is or includes a cytoplasmic signaling domain of a zeta chain of a CD3-zeta (CD3ζ) chain or a functional variant or signaling portion thereof. In some embodiments, the activating cytoplasmic domain is human or is derived from a human protein. In some embodiments, 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 embodiments 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 embodiments, the nucleic acid encoding the activating cytoplasmic signaling domain is or includes the sequence set forth in SEQ ID NO:652.

[0039] In some embodiments of any of the polynucleotides described herein, the intracellular signaling region further includes a costimulatory signaling region. In some embodiments, the costimulatory signaling region includes an intracellular signaling domain of a T cell costimulatory molecule or a signaling portion thereof. In some embodiments, the costimulatory signaling region includes an intracellular signaling domain of a CD28, a 4-1BB or an ICOS or a signaling portion thereof. In some embodiments, the costimulatory signaling region includes an intracellular signaling domain of 4-1BB. In some embodiments, the costimulatory signaling region is human or is derived from a human protein. In other embodiments, the costimulatory signaling region is or includes the sequence set forth in SEQ ID

NO: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 embodiments 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 embodiments, the nucleic acid encoding the costimulatory signaling region includes the sequence set forth in SEQ ID NO:681. In some embodiments, the costimulatory signaling region is between the transmembrane domain and the intracellular signaling region. In some embodiments, the transmembrane domain is or includes a transmembrane domain derived from CD4, CD28, or CD8. In some embodiments, the transmembrane domain is or includes a transmembrane domain derived from a CD28. In some embodiments, the transmembrane domain is human or is derived from a human protein. In other embodiments, the transmembrane domain is or includes the sequence set forth in SEQ ID NO:624 or a sequence of amino acids that exhibits at least 90% sequence identity to SEQ ID NO:624.

[0041] In some embodiments 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 embodiments, the nucleic acid encoding the transmembrane domain includes the sequence set forth in SEQ ID NO:688. In some embodiments 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 embodiments, 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 embodiments, 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 embodiments, 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 embodiments, 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 embodiments, the polynucleotide further encodes a truncated receptor

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

[0045] Provided in some aspects are chimeric antigen receptors encoded a polynucleotide of any of the embodiments described herein. In some embodiments, 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.

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

[0047] In some embodiments 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 embodiments, the encoded spacer is or includes the sequence set forth in SEQ ID NO: 649.

[0048] Provided in other aspects 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 embodiments 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 embodiments 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 embodiments 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 embodiments 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 embodiments 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 embodiments 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 embodiments 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 embodiments 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: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;

[0055] In some embodiments 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 embodiments 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 embodiments 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 embodiments, 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 embodiments, the V_H region is or includes the amino acid sequence set forth in SEQ ID NO:617.

[0057] In some embodiments 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

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:383, 403, and 419, respectively; a CDR-L1, CDR-L2, and CDR-L3 containing the amino acid sequence of SEQ ID NOs:384, 39, and 54, respectively; a CDR-L1, CDR-L2, and CDR-L3 containing the amino acid sequence of SEQ ID NOs:385, 180, and 58, 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:386, 404, and 420, respectively; a CDR-L1, CDR-L2, and CDR-L3 containing the amino acid sequence of SEQ ID NOs:387, 405, and 422, respectively; a CDR-L1, CDR-L2, and CDR-L3 containing the amino acid sequence of SEQ ID NOs:388, 406, and 423, respectively; a CDR-L1, CDR-L2, and CDR-L3 containing the amino acid sequence of SEQ ID NOs:388, 407, and 424, respectively; a CDR-L1, CDR-L2, and CDR-L3 containing the amino acid sequence of SEQ ID NOs:389, 408, and 425, respectively; a CDR-L1, CDR-L2, and CDR-L3 containing the amino acid sequence of SEQ ID NOs:390, 183, and 193, respectively; a CDR-L1, CDR-L2, and CDR-L3 containing the amino acid sequence of SEQ ID NOs:391, 409, and 426, respectively; a CDR-L1, CDR-L2, and CDR-L3 containing the amino acid sequence of SEQ ID NOs:392, 40, and 427, respectively; a CDR-L1, CDR-L2, and CDR-L3 containing the amino acid sequence of SEQ ID NOs:394, 39, and 429, respectively; a CDR-L1, CDR-L2, and CDR-L3 containing the amino acid sequence of SEQ ID NOs:395, 411, and 430, respectively; a CDR-L1, CDR-L2, and CDR-L3 containing the amino acid sequence of SEQ ID NOs:396, 412, and 431, respectively; a CDR-L1, CDR-L2, and CDR-L3 containing the 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.

[0058] In some embodiments 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 embodiments 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 embodiments 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 embodiments 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 embodiments 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 embodiments 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

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 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 forth in SEQ ID NOs:114 and 126, respectively, or a sequence of amino acids that has at least 90% identity to SEQ ID NO:114 and 126, respectively; the V_H region and the V_L regions contain the amino acid sequence set forth in SEQ ID NOs:115 and 127, respectively, or a sequence of amino acids that has at least 90% identity to SEQ ID NO:115 and 127, respectively; the V_H region and the V_L regions contain the amino acid sequence set forth in SEQ ID NOs:247 and 257, respectively, or a sequence of amino acids that has at least 90% identity to SEQ ID NO:247 and 257, respectively; the V_H region and the V_L regions contain the amino acid sequence set forth in SEQ ID NOs:248 and 258, respectively, or a sequence of amino acids that has at least 90% identity to SEQ ID NO:248 and 258, respectively; the V_H region and the V_L regions contain the amino acid sequence set forth in SEQ ID NOs:249 and 259, respectively, or a sequence of amino acids that has at least 90% identity to SEQ ID NO:249 and 259, respectively; the V_H region and the V_L regions contain the amino acid sequence set forth in SEQ ID NOs:250 and 260, respectively, or a sequence of amino acids that has at least 90% identity to SEQ ID NO:250 and 260, respectively; the V_H region and the V_L regions contain the amino acid sequence set forth in SEQ ID NOs:251 and 261, respectively, or a sequence of amino acids that has at least 90% identity to SEQ ID NO:251 and 261, respectively; the V_H region and the V_L regions contain the amino acid sequence set forth in SEQ ID NOs:252 and 262, respectively, or a sequence of amino acids that has at least 90% identity to SEQ ID NO:252 and 262, respectively; the V_H region and the V_L regions contain the amino acid sequence set forth in SEQ ID NOs:253 and 263, respectively, or

a sequence of amino acids that has at least 90% identity to SEQ ID NO:253 and 263, respectively; the V_H region and the V_L regions contain the amino acid sequence set forth in SEQ ID NOs:254 and 264, respectively, or a sequence of amino acids that has at least 90% identity to SEQ ID NO:254 and 264, respectively; the V_H region and the V_L regions contain the amino acid sequence set forth in SEQ ID NOs:255 and 265, respectively, or a sequence of amino acids that has at least 90% identity to SEQ ID NO:255 and 265, respectively; the V_H region and the V_L regions contain the amino acid sequence set forth in SEQ ID NOs:256 and 266, respectively, or a sequence of amino acids that has at least 90% identity to SEQ ID NO:256 and 266, respectively; the V_H region and the V_L regions contain the amino acid sequence set forth in SEQ ID NOs:256 and 267, respectively, or a sequence of amino acids that has at least 90% identity to SEQ ID NO:256 and 267, respectively; the V_H region and the V_L regions contain the amino acid sequence set forth in SEQ ID NOs:518 and 534, respectively, or a sequence of amino acids that has at least 90% identity to SEQ ID NO:518 and 534, respectively; the V_H region and the V_L regions contain the amino acid sequence set forth in SEQ ID NOs:519 and 535, respectively, or a sequence of amino acids that has at least 90% identity to SEQ ID NO:519 and 535, respectively; the V_H region and the V_L regions contain the amino acid sequence set forth in SEQ ID NOs:115 and 536, respectively, or a sequence of amino acids that has at least 90% identity to SEQ ID NO:115 and 536, respectively; the V_H region and the V_L regions contain the amino acid sequence set forth in SEQ ID NOs:520 and 264, respectively, or a sequence of amino acids that has at least 90% identity to SEQ ID NO:520 and 264, respectively; the V_H region and the V_L regions contain the amino acid sequence set forth in SEQ ID NOs:521 and 537, respectively, or a sequence of amino acids that has at least 90% identity to SEQ ID NO:521 and 537, respectively; the V_H region and the V_L regions contain the amino acid sequence set forth in SEQ ID NOs:522 and 538, respectively, or a sequence of amino acids that has at least 90% identity to SEQ ID NO:522 and 538, respectively; the V_H region and the V_L regions contain the amino acid sequence set forth in SEQ ID NOs:523 and 539, respectively, or a sequence of amino acids that has at least 90% identity to SEQ ID NO:523 and 539, respectively; the V_H region and the V_L regions contain the amino acid sequence set forth in SEQ ID NOs:519 and 540, respectively, or a sequence of amino acids that has at least 90% identity to SEQ ID NO:519 and 540, respectively; the V_H region and the V_L regions contain the amino acid sequence set forth in SEQ ID NOs:524 and 541, respectively, or a sequence of amino acids that has at least 90% identity to SEQ ID NO:524 and 541, respectively; the V_H region and the V_L regions contain the amino acid sequence set forth in SEQ ID NOs:525 and 261, respectively, or a sequence of amino acids that

has at least 90% identity to SEQ ID NO:525 and 261, respectively; the V_H region and the V_L regions contain the amino acid sequence set forth in SEQ ID NOs:526 and 542, respectively, or a sequence of amino acids that has at least 90% identity to SEQ ID NO:526 and 542, respectively; the V_H region and the V_L regions contain the amino acid sequence set forth in SEQ ID NOs:527 and 543, respectively, or a sequence of amino acids that has at least 90% identity to SEQ ID NO:527 and 543, respectively; the V_H region and the V_L regions contain the amino acid sequence set forth in SEQ ID NOs:528 and 544, respectively, or a sequence of amino acids that has at least 90% identity to SEQ ID NO:528 and 544, respectively; the V_H region and the V_L regions contain the amino acid sequence set forth in SEQ ID NOs:529 and 545, respectively, or a sequence of amino acids that has at least 90% identity to SEQ ID NO:529 and 545, respectively; the V_H region and the V_L regions contain the amino acid sequence set forth in SEQ ID NOs:528 and 546, respectively, or a sequence of amino acids that has at least 90% identity to SEQ ID NO:528 and 546, respectively; the V_H region and the V_L regions contain the amino acid sequence set forth in SEQ ID NOs:522 and 547, respectively, or a sequence of amino acids that has at least 90% identity to SEQ ID NO:522 and 547, respectively; the V_H region and the V_L regions contain the amino acid sequence set forth in SEQ ID NOs:256 and 548, respectively, or a sequence of amino acids that has at least 90% identity to SEQ ID NO:256 and 548, respectively; the V_H region and the V_L regions contain the amino acid sequence set forth in SEQ ID NOs:530 and 549, respectively, or a sequence of amino acids that has at least 90% identity to SEQ ID NO:530 and 549, respectively; the V_H region and the V_L regions contain the amino acid sequence set forth in SEQ ID NOs:531 and 550, respectively, or a sequence of amino acids that has at least 90% identity to SEQ ID NO:531 and 550, respectively; the V_H region and the V_L regions contain the amino acid sequence set forth in SEQ ID NOs:519 and 552, respectively, or a sequence of amino acids that has at least 90% identity to SEQ ID NO:519 and 552, respectively; the V_H region and the V_L regions contain the amino acid sequence set forth in SEQ ID NOs:110 and 553, respectively, or a sequence of amino acids that has at least 90% identity to SEQ ID NO:110 and 553, 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:533 and 554, respectively, or a sequence of amino acids that has at least 90% identity to SEQ ID NO:533 and 554, respectively; the V_H region and the V_L regions contain the amino acid sequence set forth in SEQ ID NOs:115 and 555, respectively, or a sequence of amino acids that has at least 90% identity to

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 embodiments 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

forth in SEQ ID NOs:248 and 258, respectively, or a sequence of amino acids that has at least 90% identity to SEQ ID NO:248 and 258, respectively; the V_H region and the V_L regions contain the amino acid sequence set forth in SEQ ID NOs:252 and 262, respectively, or a sequence of amino acids that has at least 90% identity to SEQ ID NO:252 and 262, respectively; the V_H region and the V_L regions contain the amino acid sequence set forth in SEQ ID NOs:253 and 263, respectively, or a sequence of amino acids that has at least 90% identity to SEQ ID NO:253 and 263, respectively; the V_H region and the V_L regions contain the amino acid sequence set forth in SEQ ID NOs:254 and 264, respectively, or a sequence of amino acids that has at least 90% identity to SEQ ID NO:254 and 264, respectively; the V_H region and the V_L regions contain the amino acid sequence set forth in SEQ ID NOs:255 and 265, respectively, or a sequence of amino acids that has at least 90% identity to SEQ ID NO:255 and 265, respectively; the V_H region and the V_L regions contain the amino acid sequence set forth in SEQ ID NOs:256 and 266, respectively, or a sequence of amino acids that has at least 90% identity to SEQ ID NO:256 and 266, respectively; the V_H region and the V_L regions contain the amino acid sequence set forth in SEQ ID NOs:256 and 267, respectively, or a sequence of amino acids that has at least 90% identity to SEQ ID NO:256 and 267, respectively; the V_H region and the V_L regions contain the amino acid sequence set forth in SEQ ID NOs:518 and 534, respectively, or a sequence of amino acids that has at least 90% identity to SEQ ID NO:518 and 534, respectively; the V_H region and the V_L regions contain the amino acid sequence set forth in SEQ ID NOs:519 and 535, respectively, or a sequence of amino acids that has at least 90% identity to SEQ ID NO:519 and 535, respectively; the V_H region and the V_L regions contain the amino acid sequence set forth in SEQ ID NOs:115 and 536, respectively, or a sequence of amino acids that has at least 90% identity to SEQ ID NO:115 and 536, respectively; the V_H region and the V_L regions contain the amino acid sequence set forth in SEQ ID NOs:520 and 264, respectively, or a sequence of amino acids that has at least 90% identity to SEQ ID NO:520 and 264, respectively; the V_H region and the V_L regions contain the amino acid sequence set forth in SEQ ID NOs:521 and 537, respectively, or a sequence of amino acids that has at least 90% identity to SEQ ID NO:521 and 537, respectively; the V_H region and the V_L regions contain the amino acid sequence set forth in SEQ ID NOs:522 and 538, respectively, or a sequence of amino acids that has at least 90% identity to SEQ ID NO:522 and 538, 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.

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

[0064] In some embodiments 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 embodiments, 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 embodiments of any of the chimeric antigen receptors described herein, the V_H region is carboxy-terminal to the V_L region. In some embodiments, 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 embodiments of any of the chimeric antigen receptors described herein, the intracellular signaling region includes an activating cytoplasmic signaling domain. In some embodiments 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 embodiments, the activating cytoplasmic signaling domain is or includes

a cytoplasmic signaling domain of a zeta chain of a CD3-zeta (CD3ζ) chain or a functional variant or signaling portion thereof. In some embodiments, the activating cytoplasmic domain is human or is derived from a human protein. In some embodiments, 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 embodiments of any of the chimeric antigen receptors described herein, the intracellular signaling region further includes a costimulatory signaling region. In some embodiments, the costimulatory signaling region includes an intracellular signaling domain of a T cell costimulatory molecule or a signaling portion thereof. In some embodiments, the costimulatory signaling region includes an intracellular signaling domain of a CD28, a 4-1BB or an ICOS or a signaling portion thereof. In some embodiments, the costimulatory signaling region includes an intracellular signaling domain of 4-1BB. In some embodiments, the costimulatory signaling region is human or is derived from a human protein. In some embodiments, 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 embodiments, the costimulatory signaling region is between the transmembrane domain and the intracellular signaling region. In some embodiments, the transmembrane domain is or includes a transmembrane domain derived from CD4, CD28, or CD8. In some embodiments, the transmembrane domain is or includes a transmembrane domain derived from a CD28. In some embodiments, the transmembrane domain is human or is derived from a human protein. In some embodiments 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 embodiments 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 embodiments, 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 embodiments, 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 embodiments, 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 embodiments, the chimeric antigen receptor is encoded by a polynucleotide sequence comprising the sequence set forth in SEQ ID NO: 755.

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

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

[0072] In some embodiments 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 embodiments, 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 embodiments, the RNA heterogeneity is reduced by greater than or greater than about 10%, 15%, 20%, 25%, 30%, 40%, 50% or more. In some embodiments, 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 embodiments, 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 embodiments 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 embodiments, 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 embodiments described herein, a chimeric antigen receptor of any of the embodiments described herein, or a engineered cell of any of the embodiments described herein. In some embodiments, the composition further contains a pharmaceutically acceptable excipient. In some of any of these embodiments, the composition is sterile.

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

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

[0080] In some of any embodiments, the dose of engineered T cells comprises between at or about 1 x 10^7 CAR-expressing T cells and at or about 2 x 10^9 CAR-expressing T cells. In some of any embodiments, the dose of engineered T cells comprise between at or about 2.5 x 10^7 CAR-expressing T cells and at or about 1.2 x 10^9 CAR-expressing T cells, between at or about 5.0 x 10^7 CAR-expressing T cells and at or about 4.5 x 10^8 CAR-expressing T cells, or between at or about 1.5 x 10^8 CAR-expressing T cells and at or about 3.0 x 10^8 CAR-expressing T cells. In some of any embodiments, the dose of engineered T cells comprise at or about 2.5 x 10^7, at or about 5.0 x 10^7, at or about 1.5 x 10^8, at or about 3.0 x 10^8, at or about 4.5 x 10^8, at or about 8.0 x 10^8 or at or about 1.2 x 10^9 CAR-expressing T cells. In some of any embodiments, the dose of engineered T cells comprise at or about 5.0 x 10^7, at or about 1.5 x 10^8, at or about 3.0 x 10^8 or at or about 4.5 x 10^8 CAR-expressing T cells. In some of any embodiments, 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 embodiments, less than about 25%, 20%, 15%, 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2% or 1% of the CAR-expressing T cells in the dose of engineered T cells express a marker of apoptosis, optionally Annexin V or active Caspase 3. In some of any embodiments, less than 5%, 4%, 3%, 2% or 1% of the CAR-expressing T cells in the dose of engineered T cells express Annexin V or active Caspase 3.

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

[0083] In some of any embodiments, the subject has received a lymphodepleting therapy comprising the administration of fludarabine at or about 30 mg/m^2 body surface area of the subject, daily, and cyclophosphamide at or about 300 mg/m^2 body surface area of the subject, daily, for 3 days.

[0084] In some of any embodiments, 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 embodiments, the immunomodulatory agent is selected from among thalidomide, lenalidomide or pomalidomide. In some of any embodiments, the proteasome inhibitor is selected from among bortezomib, carfilzomib or ixazomib. In some of any embodiments, the anti-CD38 antibody is or comprises daratumumab.

[0086] In some of any embodiments, 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 embodiments, 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 embodiments, 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 embodiments, 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 embodiments, 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 embodiments, the dose of engineered T cells comprise at or about 5.0 x 10^7, at or about 1.5 x 10^8, at or about 3.0 x 10^8 or at or about 4.5 x 10^8 CAR-expressing T cells. In some of any embodiments, the dose of the engineered T cells comprise at or about 5.0 x 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.

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

[0091] In some embodiments, 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 embodiments, the 5′ primer is specific to sequence transcribed from the promoter region of the transcribed nucleic acid. In some embodiments, 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 embodiments, 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 embodiments, 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 embodiments, 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 embodiments, the parallel or subsequent amplification reactions amplify overlapping portions of the transcript.

[0093] In some embodiments, 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 embodiments, 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 embodiments, 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.

[0096] In some of any such embodiments, the method also involves d) assessing the transgene candidacy for the removal of splice sites as in step a). In some of any such embodiments, 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 embodiments, 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 embodiments, splice donor sites and splice acceptor sites are identified independently. In some of any such embodiments, 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 embodiments, the transgene is a chimeric antigen receptor or a portion of a chimeric antigen receptor. In some of any such embodiments, 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 embodiments, 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 embodiments, the encoded amino acid sequence of the transgene is unchanged following modification of the polynucleotide. In some of any such embodiments, 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 embodiments, the cell is a human cell. In some of any such embodiments, the cell is a T-cell.

[0102] In some of any such embodiments, 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.

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).

[0105] FIG. 2 depicts results of an assay assessing levels of BCMA-LS CAR expression on the surface of transduced T cells before (Non-SSE) and after (O/SSE) optimization and splice site elimination of the coding sequence.

[0106] FIG. 3 depicts the comparison of transduction efficiency of lentiviral vectors encoding BMCA-LS CAR constructs and lentiviral vectors encoding BCMA-LS CAR constructs that have been codon optimized and modified to eliminate predicted splice sites (O/SSE).

[0107] FIG. 4A depicts results of an assay assessing the cytolytic activity of BMCA-LS CAR-expressing T cells against cell lines that express high (K562/BCMA) or low (RPMI 8226) levels of BCMA at several effector:target cell (E:T) ratios. **FIG. 4B** depicts the cytolytic activity of several BMCA-LS CAR-expressing T cells against RPMI-8226 cells at an E:T ratio of 3:1. **FIG. 4C** and **FIG. 4D** depict the cytolytic activity of non-optimized BCMA-LS CAR-expressing T cells and optimized (O/SSE) BCMA-LS CAR-expressing T cells on various BCMA-expressing cell lines.

[0108] FIG. 5A depicts results of an assay assessing IFNγ, IL-2, and TNFα cytokine release of BMCA-LS CAR-expressing T cells in response to incubation with cell lines that express high (K562/BCMA) or low (RPMI 8226) levels of BCMA at several effector:target cell (E:T) ratios (5:1, 2.5:1, 1.25:1 and 0.6:1 indicated as a, b, c and d, respectively, in the figure). **FIG. 5B** depicts the IFNγ, IL-2, and TNFα cytokine release of non-optimized BMCA-LS CAR-expressing T cells and optimized (O/SSE) BCMA-LS CAR-expressing T cells in response to incubation with BCMA-expressing K562/BCMA and RPMI 8226 cells at different E:T ratios (3:1, 1.5:1, 0.75:1 and 0.375:1 indicated as a, b, c and d, respectively, in the figure).

[0109] FIG. 6 depicts results of an assay assessing cytolytic activity following incubation of BCMA-55-LS-O/SSE CAR-expressing T cells, from two donors, with BCMA-expressing cells that express varying levels of BCMA.

[0110] FIG. 7 depicts results of an assay assessing IFNγ release following incubation of BCMA-55-LS CAR O/SSE-expressing T cells, from two donors, with BCMA-expressing cells that express varying levels of BCMA.

[0111] FIG. 8 depicts results of an assay assessing cytolytic activity of anti-BCMA-expressing CAR T cells that express CARs containing different spacer regions, on OPM2 target cells.

[0112] FIG. 9A and **9B** depict results of an assay assessing cytolytic activity of anti-BCMA CAR-expressing T cells following incubation of anti-BCMA CAR-expressing T cells with OPM2 target cells in the presence of soluble BCMA-Fc.

[0113] FIG. 10A depicts results of an assay assessing cytolytic activity of optimized (O/SSE) anti-BCMA CAR-expressing T cells in the presence of supernatant from the H929 multiple myeloma cell line. **FIG. 10B** depicts results of an assay assessing cytolytic activity of optimize (O/SSE) anti-BCMA CAR-expressing T cells in the presence of recombinant B-cell activating factor (BAFF).

[0114] FIG. 11A and **11B** depict results of an assay assessing IFNγ, IL-2, and TNFα cytokine release following incubation of anti-BCMA CAR-expressing T cells with OPM2 target cells in the presence of soluble BCMA-Fc (**FIG. 11A**) or supernatant from a multiple myeloma cell line H929 (**FIG. 11B**) at different concentrations (0 ng/mL, 111 ng/mL, 333 ng/mL and 1000 ng/mL indicated as a, b, c and d, respectively, in the figures).

[0115] FIG. 12A depicts results of an assay assessing tumor growth in an OPM2 human multiple myeloma xenograft mouse model, following a single intravenous injection of CAR T cells expressing optimized (O/SSE) anti-BCMA CARs. **FIG. 12B** depicts results of an assay assessing survival in an OPM2 human multiple myeloma xenograft mouse model, following a single intravenous injection of CAR T cells expressing optimized (O/SSE) anti-BCMA CARs.

[0116] FIG. 13A depicts results of an assay assessing tumor growth in an RPMI-8226 (subcutaneous) xenograft mouse model, following a single intravenous injection of CAR T cells expressing optimized (O/SSE) anti-BCMA CARs. **FIG. 13B** depicts survival in an RPMI-8226 (subcutaneous) xenograft mouse model, following a single intravenous injection of CAR T cells expressing optimized (O/SSE) anti-BCMA CARs.

[0117] FIG. 14A and **14B** depict results of an assay assessing the number of CD4+ (**FIG. 14A**) and CD8+ (**FIG. 14B**) CAR-positive T cells in the blood from RPMI-8226 (subcutaneous) xenograft mice treated with optimized (O/SSE) anti-BCMA CAR T cells derived from a single donor (Donor 2).

[0118] FIG. 15A and **15B** depict results of an assay assessing the number of CD4+ (**FIG. 15A**) and CD8+ (**FIG. 15B**) CAR-positive T cells in the blood from RPMI-8226 (subcutaneous) xenograft mice treated with optimized (O/SSE) anti-BCMA CAR T cells derived from a single donor (Donor 1).

[0119] FIG. 16A depicts results of an assay assessing expression level of tdTomato and a truncated receptor (surrogate marker for CAR expression), as detected by flow cytometry, in

BCMA-55-LS-O/SSE CAR-expressing cells, incubated for 6 hours in 96-well cell culture plates coated overnight with (0.008 µg/mL, 0.04 µg/mL, 0.2 µg/mL, 1 µg/mL and 5 µg/mL) of BCMA-Fc (soluble human BCMA fused at its C-terminus to an Fc region of IgG) fusion polypeptide. A recombinant Fc polypeptide was used as a control (Fc Control).

[0120] FIG. 16B depicts results of an assay assessing percentage of tdTomato+ cells among cells expressing the truncated receptor, in reporter cells expressing BCMA-55-LS-O/SSE CAR, BCMA-26-LS-O/SSE CAR, BCMA-23-LS-O/SSE CAR, and BCMA-25-LS-O/SSE CAR, incubated with ten (10) 2-fold serial dilution of BCMA-Fc. Cells expressing a CAR specific for a different antigen (anti-CD19 CAR) was used as control.

[0121] FIG. 17 depicts the percentage of tdTomato+ cells among reporter cells expressing BCMA-55-LS-O/SSE CAR or BCMA-55-SS CAR, following co-cultured with human BCMA-expressing K562 target cells (BCMA.K562) target cells at various E:T ratios.

[0122] FIG. 18 depicts the expression level of tdTomato and GFP (surrogate marker for CAR expression), as detected by flow cytometry, in reporter cells expressing an anti-CD19 CAR, BCMA-55-LS-O/SSE CAR, BCMA-26-LS-O/SSE CAR, BCMA-23-LS-O/SSE CAR, or BCMA-52-LS-O/SSE CAR, incubated without antigen stimulation to assess the degree of antigen-independent (tonic) signaling for 3 days.

[0123] FIGS. 19A and **19B** depict the expression level of tdTomato and truncated receptor (surrogate marker for CAR expression), as detected by flow cytometry, in reporter cells expressing an anti-CD19 CAR, BCMA-55-LS-O/SSE CAR, BCMA-26-LS-O/SSE CAR, BCMA-23-LS-O/SSE CAR, or BCMA-52-LS-O/SSE CAR that contain intracellular domains derived from 4-1BB or CD28 incubated without antigen stimulation to assess the degree of antigen-independent (tonic) signaling.

[0124] FIG. 20A depicts the percentage of tdTomato+ cells, as assessed by flow cytometry, among the Nur77-tdTomato reporter cells engineered to express BCMA-55-LS-O/SSE CAR, specific for human BCMA, co-cultured with K562 human myelogenous leukemia cells expressing human BCMA (huBCMA), murine BCMA (muBCMA) or cynomolgus monkey BCMA (cynoBCMA), at an E:T ratio of 2:1 or 5:1. **FIG. 20B** and **20C** depict the percentage (**FIG. 20B**) and mean fluorescence intensity (MFI; **FIG. 20C**) of tdTomato+ cells, as assessed by flow cytometry, among reporter cells expressing BCMA-55-LS-O/SSE CAR, incubated with increasing concentrations (0, 0.1, 0.25, 1, 2.5, 10, 25 and 100 µg/mL) of huBCMA and cynoBCMA coated on 96-well flat-bottom plates.

[0125] **FIG. 21A** depicts an exemplary amplification strategy for a transcript and predicted amplified product. **FIG. 21B** depicts exemplary amplified products resulting from amplification of a transcript known and unknown (cryptic) splice sites. **FIG. 21C** depicts exemplary sliding window amplification of a transcript using nested primer pairs.

Detailed Description

[0126] Among the provided embodiments are compositions, articles of manufacture, compounds, methods and uses including those targeting or directed to BCMA and BCMA-expressing cells and diseases. It is observed that BCMA is expressed, e.g., heterogeneously expressed, on certain diseases and conditions such as malignancies or tissues or cells thereof, e.g., on malignant plasma cells such as from all relapsed or newly diagnosed myeloma patients, for example, with little expression on normal tissues. Among the provided embodiments are approaches useful in the treatment of such diseases and conditions and/or for targeting such cell types, including nucleic acid molecules that encode BCMA-binding receptors, including chimeric antigen receptors (CARs), and the encoded receptors such as the encoded CARs, and compositions and articles of manufacture comprising the same. The receptors generally can contain antibodies (including antigen-binding antibody fragments, such as heavy chain variable (V_H) regions, single domain antibody fragments and single chain fragments, including scFvs) specific for BCMA. Also provided are cells, such as engineered or recombinant cells expressing such BCMA-binding receptors, *e.g.*, anti-BCMA CARs and/or containing nucleic acids encoding such receptors, and compositions and articles of manufacture and therapeutic doses containing such cells. Also provided are methods of evaluating, optimizing, making and using nucleic acid sequence(s), for example, nucleic acid sequences encoding recombinant BCMA-binding receptors. Also provided are methods of making and using (such as in the treatment or amelioration of BCMA-expressing diseases and conditions) cells (*e.g.*, engineered cells) expressing or containing the recombinant BCMA-binding receptors and recombinant BCMA-binding receptor-encoding polynucleotides or compositions containing such cells.

[0127] Adoptive cell therapies (including those involving the administration of cells expressing chimeric receptors specific for a disease or disorder of interest, such as chimeric antigen receptors (CARs) and/or other recombinant antigen receptors, as well as other adoptive immune cell and adoptive T cell therapies) can be effective in the treatment of cancer and other diseases and disorders. In certain contexts, available approaches to adoptive cell therapy may not always be entirely satisfactory. In some aspects, the ability of the administered cells to

recognize and bind to a target, *e.g.*, target antigen such as BCMA, to traffic, localize to and successfully enter appropriate sites within the subject, tumors, and environments thereof, to become activated, expand, to exert various effector functions, including cytotoxic killing and secretion of various factors such as cytokines, to persist, including long-term, to differentiate, transition or engage in reprogramming into certain phenotypic states to provide effective and robust recall responses following clearance and re-exposure to target ligand or antigen, and avoid or reduce exhaustion, anergy, terminal differentiation, and/or differentiation into a suppressive state.

[0128] In some contexts, optimal response to therapy can depend on the ability of the engineered recombinant receptors such as CARs, to be consistently and reliably expressed on the surface of the cells and/or bind the target antigen. For example, in some cases, heterogeneity of the transcribed RNA from an introduced transgene (e.g., encoding the recombinant receptor) can affect the expression and/or activity of the recombinant receptor, in some cases when expressed in a cell, such as a human T cell, used in cell therapy. In some contexts, the length and type of spacer in the recombinant receptor, such as a CAR, can affect the expression, activity and/or function of the receptor.

[0129] Also, in some contexts, certain recombinant receptors can exhibit antigen-independent activity or signaling (also known as "tonic signaling"), which could lead to undesirable effects, such as due to increased differentiation and/or exhaustion of T cells that express the recombinant receptor. In some aspects, such activities may limit the T cell's activity, effect or potency. In some cases, during engineering and ex vivo expansion of the cells for recombinant receptor expression, the cells may exhibit phenotypes indicative of exhaustion, due to tonic signaling through the recombinant receptor.

[0130] In some contexts, properties of particular target antigens that the recombinant receptors specifically bind, recognize or target, can that affect the activity of the receptor. In some contexts, B-cell maturation antigen (BCMA), is typically expressed on malignant plasma cells and is an attractive therapeutic target for cell therapy. In some cases, BCMA is can be cleaved by gamma secretase, generating a soluble BCMA (sBCMA), or "shed" form of BCMA, reducing the BCMA expressed on the surface of target cells. In some cases, the activity of the BCMA-binding molecules, such as anti-BCMA chimeric antigen receptors, can be blocked or inhibited by the presence of soluble BCMA. Improved strategies are needed for optimal responses to cell therapies, in particular, for recombinant receptors that specifically bind, recognize or target BCMA.

[0131] The provided embodiments, in some contexts, are based on the observation that particular spacers and optimization of the nucleic acid sequences can lead to consistent and robust expression of the recombinant receptor. The provided BCMA-binding recombinant receptors offer advantages over available approaches for cell therapies, in particular, BCMA-targeting cell therapy. In some embodiments, provided BCMA-binding recombinant receptors are observed to exhibit reduced antigen-independent, tonic signaling and lack of inhibition by soluble BCMA. In various aspects, the provided BCMA-binding recombinant receptors, polynucleotides encoding such receptors, engineered cells and cell compositions, exhibit certain desired properties that can overcome or counteract certain limitations that can reduce optimal responses to cell therapy, for example, cell therapy with engineered cells expressing a BCMA-binding recombinant receptor. In some aspects, compositions containing engineered cells expressing an exemplary BCMA-binding recombinant receptor provided herein was observed to exhibit consistency of cell health of the engineered cells, and was associated with clinical response. In some contexts, the provided embodiments, including the recombinant receptors, polynucleotides encoding such receptors, engineered cells and cell compositions, can provide various advantages over available therapies targeting BCMA, to improve the activity of the recombinant receptors and response to BCMA-targeting cell therapies.

I. BCMA-BINDING RECEPTORS AND ENCODING POLYNUCLEOTIDES

[0132] Provided in some aspects are BCMA-binding agents, such as cell surface proteins, such as recombinant receptors or chimeric antigen receptors that bind or recognize BCMA molecules and polynucleotides encoding BCMA-binding cell surface proteins, such as recombinant receptors (e.g, CARs), and cells expressing such receptors. The BCMA-binding cell surface proteins generally contain antibodies (*e.g.*, antigen-binding antibody fragments), and/or other binding peptides that specifically recognize, such as specifically bind to BCMA, such as to BCMA proteins, such as human BCMA protein. In some aspects, the agents bind to an extracellular portion of BCMA.

[0133] In some embodiments, the polynucleotides are optimized, or contain certain features designed for optimization, such as for codon usage, to reduce RNA heterogeneity and/or to modify, e.g., increase or render more consistent among cell product lots, expression, such as surface expression, of the encoded receptor. In some embodiments, polynucleotides, encoding BCMA-binding cell surface proteins, are modified as compared to a reference polynucleotide, such as to remove cryptic or hidden splice sites, to reduce RNA heterogeneity. In some

embodiments, polynucleotides, encoding BCMA-binding cell surface proteins, are codon optimized, such as for expression in a mammalian, e.g., human, cell such as in a human T cell. In some aspects, the modified polynucleotides result in in improved, e.g., increased or more uniform or more consistent level of, expression, e.g., surface expression, when expressed in a cell. Such polynucleotides can be utilized in constructs for generation of engineered cells that express the encoded BCMA-binding cell surface protein. Thus, also provided are cells expressing the recombinant receptors encoded by the polynucleotides provided herein and uses thereof in adoptive cell therapy, such as treatment of diseases and disorders associated with BCMA expression.

[0134] Among the provided polynucleotides are those that encode recombinant receptors, such as antigen receptors, that specifically recognize, such as specifically bind,BCMA. In some aspects, the encoded receptors, such as those containing BCMA-binding polypeptides, and compositions and articles of manufacture and uses of the same, also are provided.

[0135] Among the BCMA-binding polypeptides are antibodies, such as single-chain antibodies (e.g., antigen binding antibody fragments), or portions thereof. In some examples, the recombinant receptors are chimeric antigen receptors, such as those containing anti-BCMA antibodies or antigen-binding fragments thereof. In any of the embodiments, an antibody or antigen binding fragment, in the provided CARs, that specifically recognizes an antigen, e.g. BCMA, specifically binds to the antigen. The provided polynucleotides can be incorporated into constructs, such as deoxyribonucleic acid (DNA) or RNA constructs, such as those that can be introduced into cells for expression of the encoded recombinant BCMA-binding receptors.

[0136] In some cases, the polynucleotide encoding the BCMA-binding receptor contains a signal sequence that encodes a signal peptide, in some cases encoded upstream of the nucleic acid sequences encoding the BCMA-binding receptor, or joined at the 5' terminus of the nucleic acid sequences encoding the antigen-binding domain. In some cases, the polynucleotide containing nucleic acid sequences encoding the BCMA-binding receptor, e.g., chimeric antigen receptor (CAR), contains a signal sequence that encodes a signal peptide. In some aspects, the signal sequence may encode a signal peptide derived from a native polypeptide. In other aspects, the signal sequence may encode a heterologous or non-native signal peptide. In some aspects, non-limiting exemplary signal peptide include a signal peptide of the IgG kappa chain set forth in SEQ ID NO: 620, or encoded by the nucleotide sequence set forth in SEQ ID NO: 619 or 682-685; a GMCSFR alpha chain set forth in SEQ ID NO:851 and encoded by the nucleotide sequence set forth in SEQ ID NO:850; a CD8 alpha signal peptide set forth in SEQ

ID NO:852; or a CD33 signal peptide set forth in SEQ ID NO:853. In some cases, the polynucleotide encoding the BCMA-binding receptor can contain nucleic acid sequence encoding additional molecules, such as a surrogate marker or other markers, or can contain additional components, such as promoters, regulatory elements and/or multicistronic elements. In some embodiments, the nucleic acid sequence encoding the BCMA-binding receptor can be operably linked to any of the additional components.

A. Components of Encoded Recombinant BCMA-Binding Receptors

[0137] The provided BCMA-binding receptors generally contain an extracellular binding molecule and an intracellular signaling domain. Among the provided binding molecules are polypeptides containing antibodies, including single chain cell surface proteins, *e.g.*, recombinant receptors such as chimeric antigen receptors, containing such antibodies.

[0138] Among the provided binding molecules (*e.g.*, BCMA-binding molecules) are single chain cell surface proteins, such as recombinant receptors (*e.g.*, antigen receptors), that include one of the provided antibodies or fragment thereof (*e.g.*, BCMA-binding fragment). The recombinant receptors include antigen receptors that specifically bind to or specifically recognize BCMA, such as antigen receptors containing the provided anti-BCMA antibodies, *e.g.*, antigen-binding fragments. Among the antigen receptors are functional non-TCR antigen receptors, such as chimeric antigen receptors (CARs). Also provided are cells expressing the recombinant receptors and uses thereof in adoptive cell therapy, such as treatment of diseases and disorders associated with BCMA expression.

[0139] Exemplary antigen receptors, including CARs, and methods for engineering and introducing such antigen receptors into cells, include those described, for example, in international patent application publication Nos. WO200014257, WO2013126726, WO2012/129514, WO2014031687, WO2013166321, WO2013071154, WO2013123061 U.S. patent application publication Nos. US2002131960, US2013287748, US20130149337, U.S. Patent Nos. 6,451,995, 7,446,190, 8,252,592, 8,339,645, 8,398,282, 7,446,179, 6,410,319, 7,070,995, 7,265,209, 7,354,762, 7,446,191, 8,324,353, and 8,479,118, and European patent application No. EP2537416, and/or those described by Sadelain *et al.*, Cancer Discov. 2013 April; 3(4): 388–398; Davila *et al.* (2013) PLoS ONE 8(4): e61338; Turtle *et al.*, Curr. Opin. Immunol., 2012 October; 24(5): 633-39; Wu *et al.*, Cancer, 2012 March 18(2): 160-75. In some aspects, the antigen receptors include a CAR as described in U.S. Patent No. 7,446,190, and those described in International Patent Application Publication No. WO2014055668.

Exemplary CARs include CARs as disclosed in any of the aforementioned publications, such as WO2014031687, US 8,339,645, US 7,446,179, US 2013/0149337, US 7,446,190, and US 8,389,282, and in which the antigen-binding portion, *e.g.*, scFv, is replaced by an antibody or an antigen-binding fragment thereof, as provided herein.

[0140] In some embodiments, the provided CAR has an amino acid sequence selected from among SEQ ID NOs: 757-762, or exhibits at least or about at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% sequence identity to the amino acid sequence set forth in any of SEQ ID NOs 757-762. In some embodiments, the provided CAR is encoded by a polynucleotide, such as an polynucleotide with the nucleic acid sequence set forth in any of SEQ ID NOs 751-756, or a sequences that exhibits at least or at least about 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% sequence identity to the nucleic acid sequence set forth in any of SEQ ID NOs: 751-756.

[0141] In some embodiments, the provided CAR is encoded by a polynucleotide, such as an polynucleotide with the nucleic acid sequence set forth in any of SEQ ID NOs:755 and 756, or a sequences that exhibits at least or at least about 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% sequence identity to the nucleic acid sequence set forth in any of SEQ ID NOs: 755 and 756.

[0142] In some embodiments, the provided CAR is encoded by a polynucleotide, such as an polynucleotide with the nucleic acid sequence set forth in SEQ ID 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. In some embodiments, the provided CAR is encoded by a polynucleotide, such as an polynucleotide with the nucleic acid sequence set forth in SEQ ID NOs:755.

[0143] In some embodiments, the nucleic acid encoding the antigen-binding domain comprises (a) the sequence of nucleotides set forth in any of SEQ ID NOS: 648, 330-352, 647, 716, or 718; (b) a sequence of nucleotides that has at least 90% sequence identity to any of SEQ ID NOS: 648, 330-352, 647, 716, or 718; or (c) a degenerate sequence of (a) or (b).

1. Antigen-binding domain

[0144] Among the chimeric receptors are chimeric antigen receptors (CARs). The chimeric receptors, such as CARs, generally include an extracellular antigen binding domain that includes, is, or is comprised within or comprises, one of the provided anti-BCMA antibodies. Thus, the chimeric receptors, *e.g.*, CARs, typically include in their extracellular portions one or

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

[0145] The term "antibody" herein is used in the broadest sense and includes polyclonal and monoclonal antibodies, including intact antibodies and functional (antigen-binding) antibody fragments, including fragment antigen binding (Fab) fragments, $F(ab')_2$ fragments, Fab' fragments, Fv fragments, recombinant IgG (rIgG) fragments, heavy chain variable (V_H) regions capable of specifically binding the antigen, single chain antibody fragments, including single chain variable fragments (scFv), and single domain antibodies (*e.g.*, sdAb, sdFv, nanobody) fragments. The term encompasses genetically engineered and/or otherwise modified forms of immunoglobulins, such as intrabodies, peptibodies, chimeric antibodies, fully human antibodies, humanized antibodies, and heteroconjugate antibodies, multispecific, *e.g.*, bispecific or trispecific, antibodies, diabodies, triabodies, and tetrabodies, tandem di-scFv, tandem tri-scFv. Unless otherwise stated, the term "antibody" should be understood to encompass functional antibody fragments thereof also referred to herein as "antigen-binding fragments." The term also encompasses intact or full-length antibodies, including antibodies of any class or sub-class, including IgG and sub-classes thereof, IgM, IgE, IgA, and IgD.

[0146] The terms "complementarity determining region," and "CDR," synonymous with "hypervariable region" or "HVR," are known in the art to refer to non-contiguous sequences of amino acids within antibody variable regions, which confer antigen specificity and/or binding affinity. In general, there are three CDRs in each heavy chain variable region (CDR-H1, CDR-H2, CDR-H3) and three CDRs in each light chain variable region (CDR-L1, CDR-L2, CDR-L3). "Framework regions" and "FR" are known in the art to refer to the non-CDR portions of the variable regions of the heavy and light chains. In general, there are four FRs in each full-length heavy chain variable region (FR-H1, FR-H2, FR-H3, and FR-H4), and four FRs in each full-length light chain variable region (FR-L1, FR-L2, FR-L3, and FR-L4).

[0147] The precise amino acid sequence boundaries of a given CDR or FR can be readily determined using any of a number of well-known schemes, including those described by Kabat *et al.* (1991), "Sequences of Proteins of Immunological Interest," 5th Ed. Public Health Service, National Institutes of Health, Bethesda, MD ("Kabat" numbering scheme); Al-Lazikani *et al.*, (1997) JMB 273,927-948 ("Chothia" numbering scheme); MacCallum *et al.*, J. Mol. Biol. 262:732-745 (1996), "Antibody-antigen interactions: Contact analysis and binding site topography," J. Mol. Biol. 262, 732-745." ("Contact" numbering scheme); Lefranc MP *et al.*,

"IMGT unique numbering for immunoglobulin and T cell receptor variable domains and Ig superfamily V-like domains," Dev Comp Immunol, 2003 Jan;27(1):55-77 ("IMGT" numbering scheme); Honegger A and Plückthun A, "Yet another numbering scheme for immunoglobulin variable domains: an automatic modeling and analysis tool," J Mol Biol, 2001 Jun 8;309(3):657-70, ("Aho" numbering scheme); and Martin *et al.*, "Modeling antibody hypervariable loops: a combined algorithm," PNAS, 1989, 86(23):9268-9272, ("AbM" numbering scheme).

[0148] The boundaries of a given CDR or FR may vary depending on the scheme used for identification. For example, the Kabat scheme is based on structural alignments, while the Chothia scheme is based on structural information. Numbering for both the Kabat and Chothia schemes is based upon the most common antibody region sequence lengths, with insertions accommodated by insertion letters, for example, "30a," and deletions appearing in some antibodies. The two schemes place certain insertions and deletions ("indels") at different positions, resulting in differential numbering. The Contact scheme is based on analysis of complex crystal structures and is similar in many respects to the Chothia numbering scheme. The AbM scheme is a compromise between Kabat and Chothia definitions based on that used by Oxford Molecular's AbM antibody modeling software.

[0149] **Table 1**, below, lists exemplary position boundaries of CDR-L1, CDR-L2, CDR-L3 and CDR-H1, CDR-H2, CDR-H3 as identified by Kabat, Chothia, AbM, and Contact schemes, respectively. For CDR-H1, residue numbering is listed using both the Kabat and Chothia numbering schemes. FRs are located between CDRs, for example, with FR-L1 located before CDR-L1, FR-L2 located between CDR-L1 and CDR-L2, FR-L3 located between CDR-L2 and CDR-L3 and so forth. It is noted that because the shown Kabat numbering scheme places insertions at H35A and H35B, the end of the Chothia CDR-H1 loop when numbered using the shown Kabat numbering convention varies between H32 and H34, depending on the length of the loop.

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

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

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

[0150] Thus, unless otherwise specified, a "CDR" or "complementary determining region," or individual specified CDRs (*e.g.*, CDR-H1, CDR-H2, CDR-H3), of a given antibody or region thereof, such as a variable region thereof, should be understood to encompass a (or the specific) complementary determining region as defined by any of the aforementioned schemes, or other known schemes. For example, where it is stated that a particular CDR (*e.g.*, a CDR-H3) contains the amino acid sequence of a corresponding CDR in a given V_H or V_L region amino acid sequence, it is understood that such a CDR has a sequence of the corresponding CDR (*e.g.*, CDR-H3) within the variable region, as defined by any of the aforementioned schemes, or other known schemes. In some embodiments, specific CDR sequences are specified. Exemplary CDR sequences of provided antibodies are described using various numbering schemes, although it is understood that a provided antibody can include CDRs as described according to any of the other aforementioned numbering schemes or other numbering schemes known to a skilled artisan.

[0151] Likewise, unless otherwise specified, a FR or individual specified FR(s) (*e.g.*, FR-H1, FR-H2, FR-H3, FR-H4), of a given antibody or region thereof, such as a variable region thereof, should be understood to encompass a (or the specific) framework region as defined by any of the known schemes. In some instances, the scheme for identification of a particular CDR, FR, or FRs or CDRs is specified, such as the CDR as defined by the Kabat, Chothia, AbM or Contact method, or other known schemes. In other cases, the particular amino acid sequence of a CDR or FR is given.

[0152] The term "variable region" or "variable domain" refers to the domain of an antibody heavy or light chain that is involved in binding the antibody to antigen. The variable regions of the heavy chain and light chain (V_H and V_L, respectively) of a native antibody generally have similar structures, with each domain comprising four conserved framework regions (FRs) and three CDRs. (See, *e.g.*, Kindt *et al.* Kuby Immunology, 6th ed., W.H. Freeman and Co., page 91 (2007). A single V_H or V_L domain may be sufficient to confer antigen-binding specificity. Furthermore, antibodies that bind a particular antigen may be isolated using a V_H or V_L domain from an antibody that binds the antigen to screen a library of complementary V_L or V_H domains, respectively. See, *e.g.*, Portolano *et al.*, J. Immunol. 150:880-887 (1993); Clarkson *et al.*, Nature 352:624-628 (1991).

[0153] Among the antibodies included in the provided CARs are antibody fragments. An "antibody fragment" or "antigen-binding fragment" refers to a molecule other than an intact antibody that comprises a portion of an intact antibody that binds the antigen to which the intact antibody binds. Examples of antibody fragments include but are not limited to Fv, Fab, Fab', Fab'-SH, $F(ab')_2$; diabodies; linear antibodies; heavy chain variable (V_H) regions, single-chain antibody molecules such as scFvs and single-domain antibodies comprising only the V_H region; and multispecific antibodies formed from antibody fragments. In some embodiments, the antigen-binding domain in the provided CARs is or comprises an antibody fragment comprising a variable heavy chain (V_H) and a variable light chain (V_L) region. In particular embodiments, the antibodies are single-chain antibody fragments comprising a heavy chain variable (V_H) region and/or a light chain variable (V_L) region, such as scFvs.

[0154] Single-domain antibodies (sdAbs) are antibody fragments comprising all or a portion of the heavy chain variable region or all or a portion of the light chain variable region of an antibody. In certain embodiments, a single-domain antibody is a human single-domain antibody.

[0155] Antibody fragments can be made by various techniques, including but not limited to proteolytic digestion of an intact antibody as well as production by recombinant host cells. In some embodiments, the antibodies are recombinantly-produced fragments, such as fragments comprising arrangements that do not occur naturally, such as those with two or more antibody regions or chains joined by synthetic linkers, *e.g.*, peptide linkers, and/or that are may not be produced by enzyme digestion of a naturally-occurring intact antibody. In some aspects, the antibody fragments are scFvs.

[0156] A "humanized" antibody is an antibody in which all or substantially all CDR amino acid residues are derived from non-human CDRs and all or substantially all FR amino acid residues are derived from human FRs. A humanized antibody optionally may include at least a portion of an antibody constant region derived from a human antibody. A "humanized form" of a non-human antibody, refers to a variant of the non-human antibody that has undergone humanization, typically to reduce immunogenicity to humans, while retaining the specificity and affinity of the parental non-human antibody. In some embodiments, some FR residues in a humanized antibody are substituted with corresponding residues from a non-human antibody (*e.g.*, the antibody from which the CDR residues are derived), *e.g.*, to restore or improve antibody specificity or affinity.

[0157] Among the anti-BCMA antibodies included in the provided CARs are human antibodies. A "human antibody" is an antibody with an amino acid sequence corresponding to that of an antibody produced by a human or a human cell, or non-human source that utilizes human antibody repertoires or other human antibody-encoding sequences, including human antibody libraries. The term excludes humanized forms of non-human antibodies comprising non-human antigen-binding regions, such as those in which all or substantially all CDRs are non-human. The term includes antigen-binding fragments of human antibodies.

[0158] Human antibodies may be prepared by administering an immunogen to a transgenic animal that has been modified to produce intact human antibodies or intact antibodies with human variable regions in response to antigenic challenge. Such animals typically contain all or a portion of the human immunoglobulin loci, which replace the endogenous immunoglobulin loci, or which are present extrachromosomally or integrated randomly into the animal's chromosomes. In such transgenic animals, the endogenous immunoglobulin loci have generally been inactivated. Human antibodies also may be derived from human antibody libraries, including phage display and cell-free libraries, containing antibody-encoding sequences derived from a human repertoire.

[0159] Among the antibodies included in the provided CARs are those that are monoclonal antibodies, including monoclonal antibody fragments. The term "monoclonal antibody" as used herein refers to an antibody obtained from or within a population of substantially homogeneous antibodies, *i.e.*, the individual antibodies comprising the population are identical, except for possible variants containing naturally occurring mutations or arising during production of a monoclonal antibody preparation, such variants generally being present in minor amounts. In contrast to polyclonal antibody preparations, which typically include different antibodies directed against different epitopes, each monoclonal antibody of a monoclonal antibody preparation is directed against a single epitope on an antigen. The term is not to be construed as requiring production of the antibody by any particular method. A monoclonal antibody may be made by a variety of techniques, including but not limited to generation from a hybridoma, recombinant DNA methods, phage-display and other antibody display methods.

[0160] In some embodiments, the CAR includes a BCMA-binding portion or portions of the antibody molecule, such as a heavy chain variable (V_H) region and/or light chain variable (V_L) region of the antibody, *e.g.*, an scFv antibody fragment. In some embodiments, the provided BCMA-binding CARs contain an antibody, such as an anti-BCMA antibody, or an antigen-binding fragment thereof that confers the BCMA-binding properties of the provided CAR. In

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

[0161] In some embodiments, the antibody, *e.g.*, the anti-BCMA antibody or antigen-binding fragment, contains a heavy and/or light chain variable (V_H or V_L) region sequence as described, or a sufficient antigen-binding portion thereof. In some embodiments, the anti-BCMA antibody, *e.g.*, antigen-binding fragment, contains a V_H region sequence or sufficient antigen-binding portion thereof that contains a CDR-H1, CDR-H2 and/or CDR-H3 as described. In some embodiments, the anti-BCMA antibody, *e.g.*, antigen-binding fragment, contains a V_L region sequence or sufficient antigen-binding portion that contains a CDR-L1, CDR-L2 and/or CDR-L3 as described. In some embodiments, the anti-BCMA antibody, *e.g.*, antigen-binding fragment, contains a V_H region sequence that contains a CDR-H1, CDR-H2 and/or CDR-H3 as described and contains a V_L region sequence that contains a CDR-L1, CDR-L2 and/or CDR-L3 as described. Also among the antibodies are those having sequences at least at or about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, or about 99% identical to such a sequence.

[0162] In some embodiments, the antibody, *e.g.*, antigen-binding fragment thereof, in the provided CAR, has a heavy chain variable (V_H) region having the amino acid sequence selected from any one of SEQ ID NOs:110-115, 247-256, 324, 325, 518-531, 533, 609, 617, and 772-774, and 814-832, or an amino acid sequence that has at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to the V_H region amino acid selected from any one of SEQ ID NOs:110-115, 247-256, 324, 325, 518-531, 533, 609, 617, 772-774, and 814-832, or contains a CDR-H1, CDR-H2, and/or CDR-H3 present in such a V_H sequence. In some embodiments, the antibody or antibody fragment, in the provided CAR, has a V_H region of any of the antibodies or antibody binding fragments described in WO 2016090327, WO 2016090320, or WO 2017173256.

[0163] In some embodiments, the V_H region of the anti-BCMA antibody is one that includes a heavy chain complementarity determining region 3 (CDR-H3) comprising the amino acid sequence $X_1X_2X_3X_4X_5X_6X_7X_8X_9X_{10}X_{11}X_{12}X_{13}X_{14}$ (SEQ ID NO:355), wherein X_1 is A, D, E, G,

L, V or W; X_2 is A, D, G, L, P, Q or S; X_3 is A, D, G, L or Y; X_4 is D, G, P, R, S, V, Y or null; X_5 is D, I, P, S, T, Y or null; X_6 is A, G, I, S, T, V, Y or null; X_7 is A, D, E, F, L, P, S, Y or null; X_8 is P, Q, T, Y or null; X_9 is D, G, R, Y or null; X_{10} is A, F, Y or null; X_{11} is D, F or null; X_{12} is F or null; X_{13} is D, T or Y; and X_{14} is I, L, N, V or Y. In some such embodiments, in said CDR-H3, X_1 is V; X_2 is D; X_3 is G; X_4 is D; X_5 is Y; X_6 is V; X_7 is D; X_8 is null; X_9 is null; X_{10} is null; X_{11} is null; X_{12} is null; X_{13} is D; and X_{14} is Y.

[0164] In some embodiments, the antibody or antigen-binding fragment thereof comprises a CDR-H3 comprising the amino acid sequence selected from any one of SEQ ID NOs:7-11, 149-157, 279-287, 292, 293, 376-378, 517, 595, according to Kabat numbering. In some embodiments, the V_H region of an antibody or antigen-binding fragment thereof contains a CDR-H3 having the amino acid sequence comprising the amino acid sequence selected from any one of SEQ ID NOs:7-11, 149-157, 279-287, 292, 293, 376-378, 517, and 595 according to Chothia numbering or AbM numbering. In some embodiments, the VH region of an antibody or antigen-binding fragment thereof contains a CDR-H3 having the amino acid sequence comprising the amino acid sequence selected from SEQ ID NOs: 606 and 613. In some embodiments, the antibody or antigen-binding fragment thereof contains a CDR-H3 having the amino acid sequence of SEQ ID NO: 517, 595, 606, or 613. In any of such examples, the antibody or antigen-binding fragment thereof can contain a V_H region sequence selected from any one of SEQ ID NOs:110-115, 247-256, 324, 325, 518-531, 533, 609, 617, 772-774, and 814-832 in which the corresponding CDR-H3 sequence contained therein (*e.g.* corresponding to amino acid residues H95 to H102 by Kabat numbering) is replaced by the CDR-H3 sequence selected from any one of SEQ ID NOs:7-11, 149-157, 279-287, 292, 293, 376-378, 517, and 595 according to Kabat numbering, any one of SEQ ID NOs:7-11, 149-157, 279-287, 292, 293, 376-378, 517, and 595 according to Chothia numbering or AbM numbering, or any one of SEQ ID NOs: 606 and 613.

[0165] In some embodiments, the V_H region of an antibody or antigen-binding fragment thereof comprises a 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, and 814-832.

[0166] In some embodiments, the V_H region of the antibody or antigen-binding fragment thereof is one that includes a heavy chain complementarity determining region 1 (CDR-H1) comprising the amino acid sequence of $X_1X_2X_3MX_4$ (SEQ ID NO:353) X_1 is D or S; X_2 is Y or

S; X_3 is A, G, W, or Y; and X_4 is H, Q, or S. In some embodiments, in said CDR-H1, X_1 is D; X_2 is Y; X_3 is Y; and X_4 is S.

[0167] In some embodiments, the V_H region of an antibody or antigen-binding fragment thereof contains a CDR-H1 having the amino acid sequence comprising the amino acid sequence selected from any one of SEQ ID NOs:1-3, 140-144, 288, 289, 507, and 593 according to Kabat numbering. In some embodiments, the V_H region of an antibody or antigen-binding fragment thereof contains a CDR-H1 having the amino acid sequence comprising the amino acid sequence selected from any one of SEQ ID NOs:12-15, 158-160, 294, 295, 532, and 596 according to Chothia numbering. In some embodiments, the V_H region of an antibody or antigen-binding fragment thereof contains a CDR-H1 having the amino acid sequence comprising the amino acid sequence selected from any one of SEQ ID NOs:19-22, 165-169, 298, 299, 509, 577, and 598 according to AbM numbering. In some embodiments, the V_H region of an antibody or antigen-binding fragment thereof contains a CDR-H1 having the amino acid sequence comprising the amino acid sequence selected from any one of SEQ ID NOs 604, and 611. In some embodiments, the V_H region of an antibody or antigen-binding fragment thereof contains a CDR-H1 having the amino acid sequence of SEQ ID NO:507, 532, 577, 593, 596, 598, 604, and 611. In any of such examples, the antibody or antigen-binding fragment thereof can contain a V_H region sequence selected from any one of SEQ ID NOs:110-115, 247-256, 324, 325, 518-531, 533, 609, 617, 772-774, and 814-832 in which the corresponding CDR-H1 sequence contained therein (*e.g.* corresponding to amino acid residues H31 to H35 by Kabat numbering) is replaced by the CDR-H1 sequence selected from any one of SEQ ID NOs:1-3, 140-144, 288, 289, 507, and 593 according to Kabat numbering, any one of SEQ ID NOs:12-15, 158-160, 294, 295, 532, and 596 according to Chothia numbering, any one of SEQ ID NOs:19-22, 165-169, 509, 298, 299, 509, 577, and 598 according to AbM numbering, or any one of SEQ ID NOs: 604 and 611.

[0168] In some embodiments, the V_H region of an antibody or antigen-binding fragment thereof contains a CDR-H1 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, and 814-832.

[0169] In some embodiments, the V_H region of an antibody or antigen-binding fragment thereof is one that includes a heavy chain complementarity determining region 2 (CDR-H2) comprising the amino acid sequence of $X_1IX_2X_3X_4X_5X_6X_7X_8X_9X_{10}X_{11}YX_{12}$ X_{13} X_{14} X_{15} X_{16} X_{17} (SEQ ID NO:354), wherein X_1 is F, G, H, V, W or Y; X_2 is N, R, S or V; X_3 is P, Q, S, V, W

or Y; X_4 is K or null; X_5 is A or null; X_6 is D, G, N, S, or Y; X_7 is G or S; X_8 is G or S; X_9 is E, G, N, T or S; X_{10} is I, K, or T; X_{11} is E, G, N or Y; X_{12} is A or V; X_{13} is A, D or Q; X_{14} is K or S; X_{15} is F or V; X_{16} is K or Q; and X_{17} is E or G. In some embodiments in said CDR-H2, X_1 is Y; X_2 is S, X_3 is S; X_4 is null; X_5 is null; X_6 is S; X_7 is G; X_8 is S; X_9 is T; X_{10} is I; X_{11} is Y; X_{12} is A; X_{13} is D; X_{14} is S; X_{15} is V; X_{16} is K; and X_{17} is G.

[0170] In some embodiments, the V_H region of an antibody or antigen-binding fragment thereof contains a CDR-H2 comprising the amino acid sequence selected from any one of SEQ ID NOs: 4-6, 145-148, 290, 291, 372-374, 513, and 594 according to Kabat numbering. In some embodiments, the V_H region of an antibody or antigen-binding fragment thereof contains a CDR-H2 comprising the amino acid sequence selected from any one of SEQ ID NOs:16-18, 161-164, 296, 297, 514-516, 551, 597 according to Chothia numbering. In some embodiments, the V_H region of an antibody or antigen-binding fragment thereof contains a CDR-H2 comprising the amino acid sequence selected from any one of SEQ ID NOs: 23-25, 170-173, 300, 301, 510-512, 587, and 599 according to AbM numbering. In some embodiments, the V_H region of an antibody or antigen-binding fragment thereof contains a CDR-H2 comprising the amino acid sequence selected from any one of SEQ ID NOs: 605 and 612. In some embodiments, the V_H region of an antibody or antigen-binding fragment thereof contains a CDR-H2 having the amino acid sequence of any of SEQ ID NOs: 513, 551, 587, 594, 597, 599, 605, or 612. In any of such examples, the antibody or antigen-binding fragment thereof can contain a V_H region sequence selected from any one of SEQ ID NOs:110-115, 247-256, 324, 325, 518-531, 533, 609, 617, 772-774, and 814-832 in which the corresponding CDR-H2 sequence contained therein (*e.g.* corresponding to amino acid residues H50 to H65 by Kabat numbering) is replaced by the CDR-H2 sequence selected from any one of SEQ ID NOs: 4-6, 145-148, 290, 291, 372-374, 513, and 594 according to Kabat numbering, any one of SEQ ID NOs: 16-18, 161-164, 296, 297, 514-516, 551, 597 according to Chothia numbering, any one of SEQ ID NOs: 23-25, 170-173, 300, 301, 510-512, 587, and 599 according to AbM numbering, or any one of SEQ ID NOs 605 or 612.

[0171] In some embodiments, the V_H region of an antibody or antigen-binding fragment thereof contains a CDR-H2 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, and 814-832.

[0172] In some embodiments, the antibody or antigen-binding fragment thereof contains a CDR-H1 that is or comprises the amino acid sequence selected from any one of SEQ ID NOs:1-

3, 140-144, 288, 289, 507, and 593 according to Kabat numbering; a CDR-H2 that is or comprises the amino acid sequence selected from any one of SEQ ID NOs: 4-6, 145-148, 290, 291, 372-374, 513, and 594 according to Kabat numbering; and a CDR-H3 that is or comprises the amino acid sequence selected from any one of SEQ ID NOs: 7-11, 149-157, 279-287, 292, 293, 376-378, 517, and 595 according to Kabat numbering. In some embodiments, the antibody or antigen-binding fragment thereof contains a CDR-H1 that is or comprises the amino acid sequence selected from any one of SEQ ID NOs:12-15, 158-160, 294, 295, 532, and 596 according to Chothia numbering; a CDR-H2 that is or comprises the amino acid sequence selected from any one of SEQ ID NOs: 16-18, 161-164, 296, 297, 514-516, 551, 597 according to Chothia numbering; and a CDR-H3 that is or comprises the amino acid sequence selected from any one of SEQ ID NOs: 7-11, 149-157, 279-287, 292, 293, 376-378, 517, and 595 according to Chothia numbering. In some embodiments, the antibody or antigen-binding fragment thereof contains a CDR-H1 that is or comprises the amino acid sequence selected from any one of SEQ ID NO:19-22, 165-169, 509, 298, 299, 509, 577, and 598 according to AbM numbering; a CDR-H2 that is or comprises the amino acid sequence selected from any one of SEQ ID NOs:23-25, 170-173, 300, 201, 510-512, 587, and 599 according to AbM numbering; and a CDR-H3 that is or comprises 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, and 613 according to AbM numbering. In some embodiments, the antibody or antigen-binding fragment thereof contains a CDR-H1 that is or comprises the amino acid sequence selected from any one of SEQ ID NO:604 and 611; a CDR-H2 that is or comprises the amino acid sequence selected from any one of SEQ ID NOs:605 and 612; and a CDR-H3 that is or comprises the amino acid sequence selected from any one of SEQ ID NOs:606 and 613.

[0173] In some embodiments, the V_H region of an antibody or antigen-binding fragment thereof comprises a CDR-H1, CDR-H2, and/or CDR-H3 according to Kabat numbering. In some embodiments, the V_H region of an antibody or antigen-binding fragment thereof comprises a CDR-H1, CDR-H2, and/or CDR-H3 according to Chothia numbering. In some embodiments, the V_H region of an antibody or antigen-binding fragment thereof comprises a CDR-H1, CDR-H2, and/or CDR-H3 according to AbM numbering.

[0174] In some embodiments, the antibody or antigen-binding fragment thereof comprises an V_H region comprising a CDR-H1, CDR-H2, and CDR-H3 selected from the group consisting of: a CDR-H1, CDR-H2, and CDR-H3 comprising the amino acid sequence of SEQ ID NOs:1, 4, and 7, respectively; a CDR-H1, CDR-H2, and CDR-H3 comprising the amino acid sequence

of SEQ ID NOs:2, 5, and 8, respectively; a CDR-H1, CDR-H2, and CDR-H3 comprising the amino acid sequence of SEQ ID NOs:2, 5, and 9, respectively; a CDR-H1, CDR-H2, and CDR-H3 comprising the amino acid sequence of SEQ ID NOs:2, 5, and 10, respectively; a CDR-H1, CDR-H2, and CDR-H3 comprising the amino acid sequence of SEQ ID NOs:3, 6, and 11, respectively; a CDR-H1, CDR-H2, and CDR-H3 comprising the amino acid sequence of SEQ ID NOs:140, 145, and 149, respectively; a CDR-H1, CDR-H2, and CDR-H3 comprising the amino acid sequence of SEQ ID NOs:141, 145, and 149, respectively; a CDR-H1, CDR-H2, and CDR-H3 comprising the amino acid sequence of SEQ ID NOs:141, 145, and 150, respectively; a CDR-H1, CDR-H2, and CDR-H3 comprising the amino acid sequence of SEQ ID NOs:142, 146, and 151, respectively; a CDR-H1, CDR-H2, and CDR-H3 comprising the amino acid sequence of SEQ ID NOs:2, 5, and 152, respectively; a CDR-H1, CDR-H2, and CDR-H3 comprising the amino acid sequence of SEQ ID NOs:143, 147, and 153, respectively; a CDR-H1, CDR-H2, and CDR-H3 comprising the amino acid sequence of SEQ ID NOs:144, 148, and 154, respectively; a CDR-H1, CDR-H2, and CDR-H3 comprising the amino acid sequence of SEQ ID NOs:3, 6, and 155, respectively; a CDR-H1, CDR-H2, and CDR-H3 comprising the amino acid sequence of SEQ ID NOs:2, 5, and 156, respectively; a CDR-H1, CDR-H2, and CDR-H3 comprising the amino acid sequence of SEQ ID NOs:2, 5, and 157, respectively; a CDR-H1, CDR-H2, and CDR-H3 comprising the amino acid sequence of SEQ ID NOs:2, 6, and 376, respectively; a CDR-H1, CDR-H2, and CDR-H3 comprising the amino acid sequence of SEQ ID NOs:3, 372, and 376, respectively; a CDR-H1, CDR-H2, and CDR-H3 comprising the amino acid sequence of SEQ ID NOs:3, 6, and 376, respectively; a CDR-H1, CDR-H2, and CDR-H3 comprising the amino acid sequence of SEQ ID NOs:3, 6, and 377, respectively; a CDR-H1, CDR-H2, and CDR-H3 comprising the amino acid sequence of SEQ ID NOs:2, 373, and 152, respectively; a CDR-H1, CDR-H2, and CDR-H3 comprising the amino acid sequence of SEQ ID NOs:2, 5, and 378, respectively; a CDR-H1, CDR-H2, and CDR-H3 comprising the amino acid sequence of SEQ ID NOs:2, 374, and 9; a CDR-H1, CDR-H2, and CDR-H3 comprising the amino acid sequence of SEQ ID NOs:288, 290, and 292; a CDR-H1, CDR-H2, and CDR-H3 comprising the amino acid sequence of SEQ ID NOs:289, 291, 293; a CDR-H1, CDR-H2, and CDR-H3 comprising the amino acid sequence of SEQ ID NOs:507, 513, and 517; a CDR-H1, CDR-H2, and CDR-H3 comprising the amino acid sequence of SEQ ID NOs:593, 594, and 595, respectively, according to Kabat numbering.

[0175] For example, the antibody or antigen-binding fragment thereof provided herein comprises a V_H region comprising a CDR-H1, CDR-H2, and CDR-H3 comprising the amino

acid sequence selected from among: SEQ ID NOs:1, 4, and 7; SEQ ID NOs:2, 5, and 8; SEQ ID NOs:2, 5, and 9; SEQ ID NOs:2, 5, and 10; SEQ ID NOs:3, 6, and 11; SEQ ID NOs:140, 145, and 149; SEQ ID NOs:141, 145, and 149; SEQ ID NOs:141, 145, and 150; SEQ ID NOs:142, 146, and 151; SEQ ID NOs:2, 5, and 152; SEQ ID NOs:143, 147, and 153; SEQ ID NOs:144, 148, and 154; SEQ ID NOs:3, 6, and 155; SEQ ID NOs:2, 5, and 156; SEQ ID NOs:2, 5, and 157; SEQ ID NOs:2, 6, and 376; SEQ ID NOs:3, 372, and 376; SEQ ID NOs:3, 6, and 376; SEQ ID NOs:3, 6, and 377; SEQ ID NOs:2, 373, and 152; SEQ ID NOs:2, 5, and 378; SEQ ID NOs:2, 374, and 9, SEQ ID NOs:288, 290, and 292; SEQ ID NOs:289, 291, 293; SEQ ID NOs:507, 513, and 517; and SEQ ID NOs:593, 594, and 595, respectively, according to Kabat numbering.

[0176] In some embodiments, the antibody or antigen-binding fragment thereof comprises a CDR-H1, CDR-H2 and CDR-H3, respectively, comprising the amino acid sequence of a CDR-H1, a CDR-H2, and a 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, and 814-832. In some embodiments, the antibody or antigen-binding fragment thereof comprises a CDR-H1, CDR-H2 and CDR-H3, respectively, comprising the amino acid sequence of a CDR-H1, a CDR-H2, and a CDR-H3 contained within the V_H region amino acid sequence of SEQ ID NO:609 or SEQ ID NO: 617. In some embodiments, the antibody or antigen-binding fragment thereof comprises a V_H region that comprises a CDR-H1, CDR-H2, and CDR-H3 comprising the amino acid sequence of SEQ ID NOS:593, 594, and 595, respectively; SEQ ID NOS: 596, 597, and 595, respectively; SEQ ID NOS: 598, 599, and 595, respectively; or SEQ ID NOS: 611, 612, and 613, respectively.

[0177] In some embodiments of the antibody or antigen-binding fragment thereof provided herein, the V_H region comprises any of the CDR-H1, CDR-H2 and CDR-H3 as described and comprises a framework region 1 (FR1), a FR2, a FR3 and/or a FR4 having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity, respectively, to a FR1, a FR2, a FR3 and/or a FR4 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, and 814-832. For example, the anti-BCMA antibody or antigen-binding fragment thereof can comprise a CDR-H1, CDR-H2 and CDR-H3, respectively, 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, and 814-832, and a framework region (*e.g.*, a FR1, a FR2, a FR3 and/or a FR4) that contains at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% sequence

identity to a framework region (*e.g.*, a FR1, a FR2, a FR3 and/or a FR4) 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, and 814-832. In some embodiments, the V_H region comprises a FR1, a FR2, a FR3 and/or a FR4 selected from a FR1 comprising the amino acid sequence selected from any one of SEQ ID NOs:59-63, 195-203, 308, 309, and 434-439; a FR2 comprising the amino acid sequence selected from any one of SEQ ID NOs:64-66, 204-209, 310, and 311; a FR3 comprising the amino acid sequence selected from any one of SEQ ID NOs:67-69, 210-216, 312, 313, 441 and 443; and/or a FR4 comprising the amino acid sequence selected from any one of SEQ ID NOs:70-71, 217-220, 314, 315, 444 and 445. In some embodiments, the V_H region comprises a FR1 comprising the amino acid sequence of SEQ ID NO:61, a FR2 comprising the amino acid sequence of SEQ ID NO:65, a FR3 comprising the amino acid sequence of SEQ ID NO:69, and/or a FR4 comprising the amino acid of SEQ ID NO:70.

[0178] In some embodiments, the antibody or antigen-binding fragment thereof comprises a V_H region comprising the amino acid sequence selected from any one of SEQ ID NOs: 110-115, 247-256, 324, 325, 518-531, 533, 609, 617, 772-774, and 814-832.

[0179] Also provided are antibodies and antigen-binding fragments thereof having sequences at least at or about at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to such sequences. For example, provided herein is an antibody or antigen-binding fragment comprising a V_H region comprising an amino acid sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to a 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, and 814-832.

[0180] In some embodiments, the antibody is a single domain antibody (sdAb) comprising only a V_H region sequence or a sufficient antigen-binding portion thereof, such as any of the above described V_H sequences (*e.g.*, a CDR-H1, a CDR-H2, a CDR-H3 and/or a CDR-H4).

[0181] In some embodiments, an antibody provided herein (*e.g.*, an anti-BCMA antibody) or antigen-binding fragment thereof comprising a V_H region further comprises a light chain or a sufficient antigen binding portion thereof. For example, in some embodiments, the antibody or antigen-binding fragment thereof contains a V_H region and a V_L region, or a sufficient antigen-binding portion of a V_H and V_L region. In such embodiments, a V_H region sequence can be any of the above described V_H sequence. In some such embodiments, the antibody is an antigen-

binding fragment, such as a Fab or an scFv. In some such embodiments, the antibody is a full-length antibody that also contains a constant region.

[0182] In some embodiments, the antibody, *e.g.*, antigen-binding fragment thereof, has a light chain variable (V_L) region having the 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, and 833-849, or an amino acid sequence that has at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to a 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, and 833-849. In some embodiments, the antibody or antigen-binding fragment has a VL region described in any of WO 2016090327, WO 2016090320, or WO 2017173256

[0183] In some embodiments, the V_L region of the antibody described herein (*e.g.*, an anti-BCMA antibody) or antigen-binding fragment thereof is one that includes a light chain complementarity determining region 3 (CDR-L3) comprising the amino acid sequence $X_1X_2X_3X_4X_5X_6X_7X_8X_9X_{10}X_{11}X_{12}$, (SEQ ID NO:358), wherein X_1 is A, C, G, H, I, Q or S; X_2 is A, Q, S or V; X_3 is S, W or Y; X_4 is D, F, G, H or Y; X_5 is D, G, M, R, S or T; X_6 is A, G, H, L, R, S, T or Y; X_7 is L, P, R, S or null; X_8 is D, G, N, R, S, T or null; X_9 is A, G, H, L, P or null; X_{10} is F, S or null; X_{11} is L, P, W or Y; and X_{12} is S, T or V. In some embodiments, in said CDR-L3, X_1 is H; X_2 is V; X_3 is W; X_4 is D; X_5 is R; X_6 is S; X_7 is R; X_8 is D; X_9 is H; X_{10} is null; X_{11} is Y; and X_{12} is V.

[0184] In some embodiments, the antibody or antigen-binding fragment thereof contains a CDR-L3 comprising the amino acid sequence selected from any one of SEQ ID NOs:47-58, 184-194, 306, 307, 415-427, 429-433, 591 and 603 according to Kabat numbering, Chothia numbering or AbM numbering. In some embodiments, the antibody or antigen-binding fragment thereof contains a CDR-L3 having the amino acid sequence of SEQ ID NO:591 or 603 according to Kabat numbering, Chothia numbering or AbM numbering. In any of such examples, the antibody or antigen-binding fragment thereof can contain a V_L region sequence selected from any one of SEQ ID NOs: 116-127, 257-267, 326, 327, 534-550, 552-557, 610, 618, 775-777, and 833-849 in which the corresponding CDR-L3 sequence contained therein (*e.g.* corresponding to amino acid residues L89 to L97 by Kabat numbering) is replaced by the CDR-L3 sequence selected from any one of SEQ ID NOs: 47-58, 184-194, 306, 307, 415-427, 429-433, 591 and 603 according to Kabat numbering, Chothia numbering or AbM numbering.

[0185] In some embodiments, the V_L region of an antibody or antigen-binding fragment thereof comprises a 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, and 833-849. In some embodiments, the V_L region of an antibody or antigen-binding fragment thereof comprises a CDR-L3 contained within the V_L region amino acid sequence of SEQ ID NO:610 or SEQ ID NO: 618.

[0186] In some embodiments, the V_L region of the antibody described herein (*e.g.*, an anti-BCMA antibody) or antigen-binding fragment thereof is one that includes a light chain complementarity determining region 1 (CDR-L1) that contains the amino acid sequence: $X_1X_2X_3X_4X_5X_6X_7X_8X_9X_{10}X_{11}X_{12}X_{13}X_{14}X_{15}X_{16}X_{17}$ (SEQ ID NO:356), wherein X_1 is G, K, R, S or T; X_2 is A, G or S; X_3 is G, N, S or T; X_4 is G, K, N, Q, R or S; X_5 is S or null; X_6 is D, N, V or null; X_7 is L, V or null; X_8 is H, S, Y or null; X_9 is S, T or null; X_{10} is S or null; X_{11} is D, G, I, N, S or null; X_{12} is D, E, G, K, I, N or null; X_{13} is F, G, K, N, R, S, Y or null; X_{14} is D, K, N, T or null; X_{15} is A, D, G, L, N, S, T or Y; X_{16} is L or V; X_{17} is A, H, N, Q or S. In some embodiments, X_1 is G; X_2 is A; X_3 is N; X_4 is N; X_5 is null; X_6 is null; X_7 is null; X_8 is null; X_9 is null; X_{10} is null; X_{11} is I; X_{12} is G; X_{13} is S; X_{14} is K; X_{15} is S; X_{16} is V; X_{17} is H.

[0187] In some embodiments, the antibody or antigen-binding fragment thereof contains a CDR-L1 comprising the amino acid sequence selected from any one of SEQ ID NOs: 26-36, 174-178, 302, 303, 380-392, 394-398, 589 or 601 according to Kabat numbering, Chothia numbering or AbM numbering. In some embodiments, the antibody or antigen-binding fragment thereof contains a CDR-L1 comprising the amino acid sequence selected from any one of SEQ ID NOs: 607 and 614. In some embodiments, the antibody or antigen-binding fragment thereof contains a CDR-L1 having the amino acid sequence of SEQ ID NO:589 or 601 according to Kabat numbering, Chothia numbering or AbM numbering. In any of such examples, the antibody or antigen-binding fragment thereof can contain a V_L region sequence selected from any one of SEQ ID NOs: 116-127, 257-267, 326, 327, 534-550, 552-557, 610, 618, 775-777, and 833-849 in which the corresponding CDR-L1 sequence contained therein (*e.g.* corresponding to amino acid residues L24 to L34 by Kabat numbering) is replaced by the CDR-L1 sequence selected from any one of SEQ ID NOs: 26-36, 174-178, 302, 303, 380-392, 394-398, 589 or 601 according to Kabat numbering, Chothia numbering or AbM numbering.

[0188] In some embodiments, the V_L region of an antibody or antigen-binding fragment thereof comprises a CDR-L1 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, and 833-849. In some embodiments, the V_L region of an antibody or antigen-binding fragment

thereof comprises a CDR-L1 contained within the V_L region amino acid sequence of SEQ ID NO:589, 601, 607 or 614.

[0189] In some embodiments, the V_L region of the antibody provided herein (*e.g.*, an anti-BCMA antibody) or antigen-binding fragment thereof is one that includes a light chain complementarity determining region 2 (CDR-L2) that contains the amino acid sequence of $X_1X_2X_3X_4X_5X_6X_7$ (SEQ ID NO:357), wherein X_1 is A, D, E, N, S, V or W; X_2 is A, D, N, S or V; X_3 is A, D, H, I, N or S; X_4 is D, K, N, Q, R or T; X_5 is L, R or V; X_6 is A, E, P or Q; and X_7 is A, D, S or T. In some embodiments, X_1 is D; X_2 is D; X_3 is D; X_4 is D; X_5 is R; X_6 is P; and X_7 is S.

[0190] In some embodiments, the antibody or antigen-binding fragment thereof contains a CDR-L2 comprising the amino acid sequence selected from any one of SEQ ID NOs:37-46, 179-183, 304, 305, 399-409, 411-414, 590 and 602 according to Kabat numbering, Chothia numbering or AbM numbering. In some embodiments, the antibody or antigen-binding fragment thereof contains a CDR-L2 comprising the amino acid sequence selected from any one of SEQ ID NOs: 608 and 615. In some embodiments, the antibody or antigen-binding fragment thereof contains a CDR-L2 having the amino acid sequence of SEQ ID NO:590 or SEQ ID NO: 602 according to Kabat numbering, Chothia numbering or AbM numbering. In any of such examples, the antibody or antigen-binding fragment thereof can contain a V_L region sequence selected from any one of SEQ ID NOs: 116-127, 257-267, 326, 327, 534-550, 552-557, 610, 618, 775-777, and 833-849 in which the corresponding CDR-L2 sequence contained therein (*e.g.* corresponding to amino acid residues L50 to L56 by Kabat numbering) is replaced by the CDR-L2 sequence selected from any one of SEQ ID NOs: 37-46, 179-183, 304, 305, 399-409, 411-414, 590 and 602 according to Kabat numbering, Chothia numbering or AbM numbering, or with any of SEQ ID NOs: 608 and 615.

[0191] In some embodiments, the V_L region of an antibody or antigen-binding fragment thereof comprises a CDR-L2 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, and 833-849. In some embodiments, the V_L region of an antibody or antigen-binding fragment thereof comprises a CDR-L2 contained within the V_L region amino acid sequence of SEQ ID NO: 589, 601, 607 or 614.

[0192] In some embodiments, the antibody or antigen-binding fragment thereof contains a CDR-L1 that is or comprises the amino acid sequence selected from any one of SEQ ID NOs: 26-36, 174-178, 302, 303, 380-392, 394-398, 589 or 601 according to Kabat numbering, Chothia

numbering or AbM numbering; a CDR-L2 that is or comprises the amino acid sequence selected from any one of SEQ ID NOs: 37-46, 179-183, 304, 305, 399-409, 411-414, 590 and 602 according to Kabat numbering, Chothia numbering or AbM numbering; and a CDR-L3 that is or comprises the amino acid sequence selected from any one of SEQ ID NOs: 47-58, 184-194, 306, 307, 415-427, 429-433, 591 and 603 according to Kabat numbering, Chothia numbering or AbM numbering.

[0193] In some embodiments, the V_L region of an antibody or antigen-binding fragment thereof comprises a CDR-L1, CDR-L2, and/or CDR-L3 according to Kabat numbering. In some embodiments, the V_L region of an antibody or antigen-binding fragment thereof comprises a CDR-L1, CDR-L2, and/or CDR-L3 according to Chothia numbering. In some embodiments, the V_L region of an antibody or antigen-binding fragment thereof comprises a CDR-L1, CDR-L2, and/or CDR-L3 according to AbM numbering.

[0194] In some embodiments of the antibody or antigen-binding fragment thereof provided herein, the V_L region comprises a CDR-L1, a CDR-L2, and a CDR-L3 selected from among: a CDR-L1, CDR-L2, and CDR-L3 comprising the amino acid sequence of SEQ ID NOs:26, 37, and 47, respectively; a CDR-L1, CDR-L2, and CDR-L3 comprising the amino acid sequence of SEQ ID NOs:27, 38, and 48, respectively; a CDR-L1, CDR-L2, and CDR-L3 comprising the amino acid sequence of SEQ ID NOs:28, 39, and 49, respectively; a CDR-L1, CDR-L2, and CDR-L3 comprising the amino acid sequence of SEQ ID NOs:29, 40, and 50, respectively; a CDR-L1, CDR-L2, and CDR-L3 comprising the amino acid sequence of SEQ ID NOs:30, 39, and 51, respectively; a CDR-L1, CDR-L2, and CDR-L3 comprising the amino acid sequence of SEQ ID NOs:31, 41, and 52, respectively; a CDR-L1, CDR-L2, and CDR-L3 comprising the amino acid sequence of SEQ ID NOs:32, 42, and 53, respectively; a CDR-L1, CDR-L2, and CDR-L3 comprising the amino acid sequence of SEQ ID NOs:30, 39, and 54, respectively; a CDR-L1, CDR-L2, and CDR-L3 comprising the amino acid sequence of SEQ ID NOs:33, 43, and 55, respectively; a CDR-L1, CDR-L2, and CDR-L3 comprising the amino acid sequence of SEQ ID NOs:34, 44, and 56, respectively; a CDR-L1, CDR-L2, and CDR-L3 comprising the amino acid sequence of SEQ ID NOs:35, 45, and 57, respectively; a CDR-L1, CDR-L2, and CDR-L3 comprising the amino acid sequence of SEQ ID NOs:36, 46, and 58, respectively; a CDR-L1, CDR-L2, and CDR-L3 comprising the amino acid sequence of SEQ ID NOs:174, 179, and 184, respectively; a CDR-L1, CDR-L2, and CDR-L3 comprising the amino acid sequence of SEQ ID NOs:174, 179, and 185, respectively; a CDR-L1, CDR-L2, and CDR-L3 comprising the amino acid sequence of SEQ ID NOs:174, 179, and 186, respectively; a CDR-L1, CDR-L2,

and CDR-L3 comprising the amino acid sequence of SEQ ID NOs:174, 179, and 187, respectively; a CDR-L1, CDR-L2, and CDR-L3 comprising the amino acid sequence of SEQ ID NOs:175, 180, and 188, respectively; a CDR-L1, CDR-L2, and CDR-L3 comprising the amino acid sequence of SEQ ID NOs:174, 179, and 189, respectively; a CDR-L1, CDR-L2, and CDR-L3 comprising the amino acid sequence of SEQ ID NOs:176, 181, and 190, respectively; a CDR-L1, CDR-L2, and CDR-L3 comprising the amino acid sequence of SEQ ID NOs:177, 182, and 191, respectively; a CDR-L1, CDR-L2, and CDR-L3 comprising the amino acid sequence of SEQ ID NOs:174, 179, and 192, respectively; a CDR-L1, CDR-L2, and CDR-L3 comprising the amino acid sequence of SEQ ID NOs:178, 183, and 193, respectively; a CDR-L1, CDR-L2, and CDR-L3 comprising the amino acid sequence of SEQ ID NOs:178, 183, and 194, respectively; a CDR-L1, CDR-L2, and CDR-L3 comprising the amino acid sequence of SEQ ID NOs:30, 399, and 415, respectively; a CDR-L1, CDR-L2, and CDR-L3 comprising the amino acid sequence of SEQ ID NOs:380, 400, and 416, respectively; a CDR-L1, CDR-L2, and CDR-L3 comprising the amino acid sequence of SEQ ID NOs:33, 43, and 421, respectively; a CDR-L1, CDR-L2, and CDR-L3 comprising the amino acid sequence of SEQ ID NOs:381, 401, and 417, respectively; a CDR-L1, CDR-L2, and CDR-L3 comprising the amino acid sequence of SEQ ID NOs:382, 402, and 418, respectively; a CDR-L1, CDR-L2, and CDR-L3 comprising the amino acid sequence of SEQ ID NOs:383, 403, and 419, respectively; a CDR-L1, CDR-L2, and CDR-L3 comprising the amino acid sequence of SEQ ID NOs:384, 39, and 54, respectively; a CDR-L1, CDR-L2, and CDR-L3 comprising the amino acid sequence of SEQ ID NOs:385, 180, and 58, respectively; a CDR-L1, CDR-L2, and CDR-L3 comprising the amino acid sequence of SEQ ID NOs:175, 180, and 188, respectively; a CDR-L1, CDR-L2, and CDR-L3 comprising the amino acid sequence of SEQ ID NOs:386, 404, and 420, respectively; a CDR-L1, CDR-L2, and CDR-L3 comprising the amino acid sequence of SEQ ID NOs:387, 405, and 422, respectively; a CDR-L1, CDR-L2, and CDR-L3 comprising the amino acid sequence of SEQ ID NOs:388, 406, and 423, respectively; a CDR-L1, CDR-L2, and CDR-L3 comprising the amino acid sequence of SEQ ID NOs:388, 407, and 424, respectively; a CDR-L1, CDR-L2, and CDR-L3 comprising the amino acid sequence of SEQ ID NOs:389, 408, and 425, respectively; a CDR-L1, CDR-L2, and CDR-L3 comprising the amino acid sequence of SEQ ID NOs:390, 183, and 193, respectively; a CDR-L1, CDR-L2, and CDR-L3 comprising the amino acid sequence of SEQ ID NOs:391, 409, and 426, respectively; a CDR-L1, CDR-L2, and CDR-L3 comprising the amino acid sequence of SEQ ID NOs:392, 40, and 427, respectively; a CDR-L1, CDR-L2, and CDR-L3 comprising the amino acid sequence of SEQ ID NOs:394, 39, and 429, respectively; a CDR-

L1, CDR-L2, and CDR-L3 comprising the amino acid sequence of SEQ ID NOs:395, 411, and 430, respectively; a CDR-L1, CDR-L2, and CDR-L3 comprising the amino acid sequence of SEQ ID NOs:396, 412, and 431, respectively; a CDR-L1, CDR-L2, and CDR-L3 comprising the amino acid sequence of SEQ ID NOs:396, 412, and 58, respectively; a CDR-L1, CDR-L2, and CDR-L3 comprising the amino acid sequence of SEQ ID NOs:397, 413, and 432, respectively; a CDR-L1, CDR-L2, and CDR-L3 comprising the amino acid sequence of SEQ ID NOs:398, 414, and 433, respectively; a CDR-L1, CDR-L2, and CDR-L3 comprising the amino acid sequence of SEQ ID NOs:302, 304, and 306, respectively; a CDR-L1, CDR-L2, and CDR-L3 comprising the amino acid sequence of SEQ ID NOs:303, 305, and 307, respectively; a CDR-L1, CDR-L2, and CDR-L3 comprising the amino acid sequence of SEQ ID NOs:589, 590, and 591, respectively; a CDR-L1, CDR-L2, and CDR-L3 comprising the amino acid sequence of SEQ ID NOs:607, 608, and 591, respectively; a CDR-L1, CDR-L2, and CDR-L3 comprising the amino acid sequence of SEQ ID NOs: 601, 602, and 603, respectively; a CDR-L1, CDR-L2, and CDR-L3 comprising the amino acid sequence of SEQ ID NOs:614, 615, and 603, respectively. In some embodiments of the antibody or antigen-binding fragment thereof provided herein, the V_L region comprises a CDR-L1, CDR-L2, and CDR-L3 comprising the amino acid sequences of SEQ ID NOs:589, 590, and 591, respectively; SEQ ID NOs:607, 608, and 591, respectively; SEQ ID NOs: 601, 602, and 603, respectively; or SEQ ID NOs:614, 615, and 603, respectively.

[0195] For example, the antibody or antigen-binding fragment thereof provided herein comprises an V_L region comprising a CDR-L1, CDR-L2, and CDR-L3 comprising the amino acid sequence selected from among: SEQ ID NOs:26, 37, and 47; SEQ ID NOs:27, 38, and 48; SEQ ID NOs:28, 39, and 49; SEQ ID NOs:29, 40, and 50; SEQ ID NOs:30, 39, and 51; SEQ ID NOs:31, 41, and 52; SEQ ID NOs:32, 42, and 53; SEQ ID NOs:30, 39, and 54; SEQ ID NOs:33, 43, and 55; SEQ ID NOs:34, 44, and 56; SEQ ID NOs:35, 45, and 57; SEQ ID NOs:36, 46, and 58; SEQ ID NOs:174, 179, and 184; SEQ ID NOs:174, 179, and 185; SEQ ID NOs:174, 179, and 186; SEQ ID NOs:174, 179, and 187; SEQ ID NOs:175, 180, and 188; SEQ ID NOs:174, 179, and 189; SEQ ID NOs:176, 181, and 190; SEQ ID NOs:177, 182, and 191; SEQ ID NOs:174, 179, and 192; SEQ ID NOs:178, 183, and 193; SEQ ID NOs:178, 183, and 194; SEQ ID NOs:30, 399, and 415; SEQ ID NOs:380, 400, and 416; SEQ ID NOs:33, 43, and 421; SEQ ID NOs:381, 401, and 417; SEQ ID NOs:382, 402, and 418; SEQ ID NOs:383, 403, and 419; SEQ ID NOs:384, 39, and 54; SEQ ID NOs:385, 180, and 58; SEQ ID NOs:175, 180, and 188; SEQ ID NOs:386, 404, and 420; SEQ ID NOs:387, 405, and 422; SEQ ID NOs:388, 406, and 423; SEQ ID NOs:388, 407, and 424; SEQ ID NOs:389, 408, and 425; SEQ ID NOs:390, 183,

and 193; SEQ ID NOs:391, 409, and 426; SEQ ID NOs:392, 40, and 427; SEQ ID NOs:394, 39, and 429; SEQ ID NOs:395, 411, and 430; SEQ ID NOs:396, 412, and 431; SEQ ID NOs:396, 412, and 58; SEQ ID NOs:397, 413, and 432; SEQ ID NOs:398, 414, and 433; SEQ ID NOs:589, 590, and 591; SEQ ID NOs:607, 608, and 591; SEQ ID NOs: 601, 602, and 603; or SEQ ID NOs:614, 615, and 603, respectively.

[0196] In some embodiments, the antibody or antigen-binding fragment thereof contains a CDR-L1, CDR-L2, and CDR-L3, respectively, 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, and 833-849. In some embodiments, the antibody contains a CDR-L1, CDR-L2, and CDR-L3, respectively, contained within the V_L region amino acid sequence selected of SEQ ID NO: 610 or SEQ ID NO: 618.

[0197] In some embodiments, the antibody or antigen-binding fragment thereof comprises a V_L region that comprises a CDR-L1, CDR-L2, and CDR-L3 comprising the amino acid sequence of SEQ ID NOS:601, 602, and 603, respectively; or SEQ ID NOS: 614, 615, and 603, respectively.

[0198] In some embodiments of the antibody or antigen-binding fragment thereof provided herein, the V_L region comprises any of the CDR-L1, CDR-L2 and CDR-L3 as described and comprises a framework region 1 (FR1), a FR2, a FR3 and/or a FR4 having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity, respectively, to a FR1, a FR2, a FR3 and/or a FR4 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, and 833-849. For example, the anti-BCMA antibody or antigen-binding fragment thereof can comprise a CDR-L1, CDR-L2 and CDR-L3, respectively, 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, and 833-849, and a framework region (*e.g.*, a FR1, a FR2, a FR3 and/or a FR4) that contains at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% sequence identity to a framework region (*e.g.*, a FR1, a FR2, a FR3 and/or a FR4) 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, and 833-849. In some embodiments, the V_L region comprises a FR1, a FR2, a FR3 and/or a FR4 selected from a FR1 comprising the amino acid sequence selected from any one of SEQ ID NOs:72-82, 221-227, 316, 317, 446-459 and 461-466; a FR2 comprising the amino acid sequence selected from any one of SEQ ID NOs:83-92, 228-232, 318, 319, 467-477 and 479-482; a FR3 comprising the amino acid

sequence selected from any one of SEQ ID NOs:93-101, 233-242, 320, 321, 483-495 and 497-501; and/or a FR4 comprising the amino acid sequence selected from any one of SEQ ID NOs:102-109, 243-246, 322, 323, 502-506 and 508. In some embodiments, the V_L region comprises a FR1 comprising the amino acid sequence of SEQ ID NO:79, a FR2 comprising the amino acid sequence of SEQ ID NO:89, a FR3 comprising the amino acid sequence of SEQ ID NO:98, and/or a FR4 comprising the amino acid sequence of SEQ ID NO:108.

[0199] In some embodiments, the antibody or antigen-binding fragment thereof comprises a V_L region comprising an 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, and 833-849. In some embodiments, the antibody or antigen-binding fragment thereof contains a V_L region comprises the amino acid sequence of SEQ ID NO: 610 or SEQ ID NO: 618.

[0200] Also provided are antibodies having sequences at least at or about at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to such sequences.

[0201] In some embodiments, the V_H region of the antibody or fragment comprises the amino acid sequence selected from any one of SEQ ID NOs: 110-115, 247-256, 324, 325, 518-531, 533, 609, 617, 772-774, and 814-832 and the V_L region of the antibody or fragment comprises the 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, and 833-849.

[0202] Also provided are antibodies and antigen-binding fragments thereof having sequences at least at or about at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to such sequences. For example, provided herein is an antibody or antigen-binding fragment containing a 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 a 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, and 833-849 and/or comprising an amino acid sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to a 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, and 814-832. In some embodiments, the antibody or antigen-binding fragment contains a V_L region comprising the 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, and 833-849 and a V_H region the amino acid sequence selected from any one of SEQ ID NOs: 110-115, 247-256, 324, 325, 518-531, 533, 609, 617, 772-774, and 814-832.

[0203] In some embodiments, 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.

[0204] In some embodiments, the V_H region and the V_L regions comprise the sequence of SEQ ID NOs:617 and 618, respectively, or a sequence of amino acids having at least 90% identity thereto; the V_H region and the V_L regions comprise the sequence of SEQ ID NOs:110 and 116, respectively, or a sequence of amino acids having at least 90% identity thereto; the V_H region and the V_L regions comprise the sequence of SEQ ID NOs:111 and 117, respectively, or a sequence of amino acids having at least 90% identity thereto; the V_H region and the V_L regions comprise the sequence of SEQ ID NOs:110 and 118, respectively, or a sequence of amino acids having at least 90% identity thereto; the V_H region and the V_L regions comprise the sequence of SEQ ID NOs:110 and 119, respectively, or a sequence of amino acids having at least 90% identity thereto; the V_H region and the V_L regions comprise the sequence of SEQ ID NOs:110 and 120, respectively, or a sequence of amino acids having at least 90% identity thereto; the V_H region and the V_L regions comprise the sequence of SEQ ID NOs:110 and 121, respectively, or a sequence of amino acids having at least 90% identity thereto; the V_H region and the V_L regions comprise the sequence of SEQ ID NOs:110 and 122, respectively, or a sequence of amino acids having at least 90% identity thereto; the V_H region and the V_L regions comprise the sequence of SEQ ID NOs:110 and 123, respectively, or a sequence of amino acids having at least 90% identity thereto; the V_H region and the V_L regions comprise the sequence of SEQ ID NOs:112 and 124, respectively, or a sequence of amino acids having at least 90% identity thereto; the V_H region and the V_L regions comprise the sequence of SEQ ID NOs:113 and 125, respectively, or a sequence of amino acids having at least 90% identity thereto; the V_H region and the V_L regions comprise the sequence of SEQ ID NOs:114 and 126, respectively, or a sequence of amino acids having at least 90% identity thereto; the V_H region and the V_L regions comprise the sequence of SEQ ID NOs:115 and 127, respectively, or a sequence of amino acids having at least 90% identity thereto; the V_H region and the V_L regions comprise the sequence of SEQ ID NOs:247 and 257, respectively, or a sequence of amino acids having at least 90% identity thereto; the V_H region and the V_L regions comprise the sequence of SEQ ID NOs:248 and 258, respectively, or a

sequence of amino acids having at least 90% identity thereto; the V_H region and the V_L regions comprise the sequence of SEQ ID NOs:249 and 259, respectively, or a sequence of amino acids having at least 90% identity thereto; the V_H region and the V_L regions comprise the sequence of SEQ ID NOs:250 and 260, respectively, or a sequence of amino acids having at least 90% identity thereto; the V_H region and the V_L regions comprise the sequence of SEQ ID NOs:251 and 261, respectively, or a sequence of amino acids having at least 90% identity thereto; the V_H region and the V_L regions comprise the sequence of SEQ ID NOs:252 and 262, respectively, or a sequence of amino acids having at least 90% identity thereto; the V_H region and the V_L regions comprise the sequence of SEQ ID NOs:253 and 263, respectively, or a sequence of amino acids having at least 90% identity thereto; the V_H region and the V_L regions comprise the sequence of SEQ ID NOs:254 and 264, respectively, or a sequence of amino acids having at least 90% identity thereto; the V_H region and the V_L regions comprise the sequence of SEQ ID NOs:255 and 265, respectively, or a sequence of amino acids having at least 90% identity thereto; the V_H region and the V_L regions comprise the sequence of SEQ ID NOs:256 and 266, respectively, or a sequence of amino acids having at least 90% identity thereto; the V_H region and the V_L regions comprise the sequence of SEQ ID NOs:256 and 267, respectively, or a sequence of amino acids having at least 90% identity thereto; the V_H region and the V_L regions comprise the sequence of SEQ ID NOs:518 and 534, respectively, or a sequence of amino acids having at least 90% identity thereto; the V_H region and the V_L regions comprise the sequence of SEQ ID NOs:519 and 535, respectively, or a sequence of amino acids having at least 90% identity thereto; the V_H region and the V_L regions comprise the sequence of SEQ ID NOs:115 and 536, respectively, or a sequence of amino acids having at least 90% identity thereto; the V_H region and the V_L regions comprise the sequence of SEQ ID NOs:520 and 264, respectively, or a sequence of amino acids having at least 90% identity thereto; the V_H region and the V_L regions comprise the sequence of SEQ ID NOs:521 and 537, respectively, or a sequence of amino acids having at least 90% identity thereto; the V_H region and the V_L regions comprise the sequence of SEQ ID NOs:522 and 538, respectively, or a sequence of amino acids having at least 90% identity thereto; the V_H region and the V_L regions comprise the sequence of SEQ ID NOs:523 and 539, respectively, or a sequence of amino acids having at least 90% identity thereto; the V_H region and the V_L regions comprise the sequence of SEQ ID NOs:519 and 540, respectively, or a sequence of amino acids having at least 90% identity thereto; the V_H region and the V_L regions comprise the sequence of SEQ ID NOs:524 and 541, respectively, or a sequence of amino acids having at least 90% identity thereto; the V_H region and the V_L regions comprise the sequence of SEQ ID NOs:525

and 261, respectively, or a sequence of amino acids having at least 90% identity thereto; the V_H region and the V_L regions comprise the sequence of SEQ ID NOs:526 and 542, respectively, or a sequence of amino acids having at least 90% identity thereto; the V_H region and the V_L regions comprise the sequence of SEQ ID NOs:527 and 543, respectively, or a sequence of amino acids having at least 90% identity thereto; the V_H region and the V_L regions comprise the sequence of SEQ ID NOs:528 and 544, respectively, or a sequence of amino acids having at least 90% identity thereto; the V_H region and the V_L regions comprise the sequence of SEQ ID NOs:529 and 545, respectively, or a sequence of amino acids having at least 90% identity thereto; the V_H region and the V_L regions comprise the sequence of SEQ ID NOs:528 and 546, respectively, or a sequence of amino acids having at least 90% identity thereto; the V_H region and the V_L regions comprise the sequence of SEQ ID NOs:522 and 547, respectively, or a sequence of amino acids having at least 90% identity thereto; the V_H region and the V_L regions comprise the sequence of SEQ ID NOs:256 and 548, respectively, or a sequence of amino acids having at least 90% identity thereto; the V_H region and the V_L regions comprise the sequence of SEQ ID NOs:530 and 549, respectively, or a sequence of amino acids having at least 90% identity thereto; the V_H region and the V_L regions comprise the sequence of SEQ ID NOs:531 and 550, respectively, or a sequence of amino acids having at least 90% identity thereto; the V_H region and the V_L regions comprise the sequence of SEQ ID NOs:519 and 552, respectively, or a sequence of amino acids having at least 90% identity thereto; the V_H region and the V_L regions comprise the sequence of SEQ ID NOs:110 and 553, respectively, or a sequence of amino acids having at least 90% identity thereto; the V_H region and the V_L regions comprise the sequence of SEQ ID NOs:533 and 554, respectively, or a sequence of amino acids having at least 90% identity thereto; the V_H region and the V_L regions comprise the sequence of SEQ ID NOs:115 and 555, respectively, or a sequence of amino acids having at least 90% identity thereto; the V_H region and the V_L regions comprise the sequence of SEQ ID NOs:524 and 556, respectively, or a sequence of amino acids having at least 90% identity thereto; the V_H region and the V_L regions comprise the sequence of SEQ ID NOs:519 and 557, respectively, or a sequence of amino acids having at least 90% identity thereto; the V_H region and the V_L regions comprise the sequence of SEQ ID NOs:324 and 326, respectively, or a sequence of amino acids having at least 90% identity thereto; the V_H region and the V_L regions comprise the sequence of SEQ ID NOs:325 and 327, respectively, or a sequence of amino acids having at least 90% identity thereto; the V_H region and the V_L regions comprise the sequence of SEQ ID NOs:609 and 610, respectively, or a sequence of amino acids having at least 90% identity thereto; the V_H region and the V_L regions comprise the sequence of

SEQ ID NOs:772 and 775, respectively, or a sequence of amino acids having at least 90% identity thereto; the V_H region and the V_L regions comprise the sequence of SEQ ID NOs:773 and 776, respectively, or a sequence of amino acids having at least 90% identity thereto; the V_H region and the V_L regions comprise the sequence of SEQ ID NOs:774 and 777, respectively, or a sequence of amino acids having at least 90% identity thereto; the V_H region and the V_L regions comprise the sequence of SEQ ID NOs:815 and 833, respectively, or a sequence of amino acids having at least 90% identity thereto; the V_H region and the V_L regions comprise the sequence of SEQ ID NOs:816 and 834, respectively, or a sequence of amino acids having at least 90% identity thereto; the V_H region and the V_L regions comprise the sequence of SEQ ID NOs:817 and 835, respectively, or a sequence of amino acids having at least 90% identity thereto; the V_H region and the V_L regions comprise the sequence of SEQ ID NOs:818 and 836, respectively, or a sequence of amino acids having at least 90% identity thereto; the V_H region and the V_L regions comprise the sequence of SEQ ID NOs:819 and 837, respectively, or a sequence of amino acids having at least 90% identity thereto; the V_H region and the V_L regions comprise the sequence of SEQ ID NOs:820 and 838, respectively, or a sequence of amino acids having at least 90% identity thereto; the V_H region and the V_L regions comprise the sequence of SEQ ID NOs:821 and 839, respectively, or a sequence of amino acids having at least 90% identity thereto; the V_H region and the V_L regions comprise the sequence of SEQ ID NOs:822 and 840, respectively, or a sequence of amino acids having at least 90% identity thereto; the V_H region and the V_L regions comprise the sequence of SEQ ID NOs:823 and 841, respectively, or a sequence of amino acids having at least 90% identity thereto; the V_H region and the V_L regions comprise the sequence of SEQ ID NOs:824 and 842, respectively, or a sequence of amino acids having at least 90% identity thereto; the V_H region and the V_L regions comprise the sequence of SEQ ID NOs:825 and 843, respectively, or a sequence of amino acids having at least 90% identity thereto; the V_H region and the V_L regions comprise the sequence of SEQ ID NOs:826 and 844, respectively, or a sequence of amino acids having at least 90% identity thereto; the V_H region and the V_L regions comprise the sequence of SEQ ID NOs:827 and 845, respectively, or a sequence of amino acids having at least 90% identity thereto; the V_H region and the V_L regions comprise the sequence of SEQ ID NOs:828 and 846, respectively, or a sequence of amino acids having at least 90% identity thereto; the V_H region and the V_L regions comprise the sequence of SEQ ID NOs:829 and 847, respectively, or a sequence of amino acids having at least 90% identity thereto; the V_H region and the V_L regions comprise the sequence of SEQ ID NOs:830 and 847, respectively, or a sequence of amino acids having at least 90% identity thereto; the V_H region and the V_L regions

comprise the sequence of SEQ ID NOs:831 and 848, respectively, or a sequence of amino acids having at least 90% identity thereto; or the V_H region and the V_L regions comprise the sequence of SEQ ID NOs:832 and 849, respectively, or a sequence of amino acids having at least 90% identity thereto.

[0205] In some embodiments, the V_H region of the antibody or antigen-binding fragment thereof comprises a CDR-H1, a CDR-H2, a CDR-H3, respectively, comprising the amino acid sequences of 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, and 814-832; and comprises a CDR-L1, a CDR-L2, a CDR-L3, respectively, comprising the amino acid sequences of CDR-L1, CDR-L2, and CDR-L3, respectively 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, and 833-849.

[0206] In some of any embodiments, the V_H is or comprises a CDR-H1, CDR-H2 and CDR-H3 contained within the V_H sequence of SEQ ID NO: 617; and the V_L is or comprises a CDR-L1, CDR-L2 and CDR-L3 contained within the V_L sequence of SEQ ID NO: 618; the V_H is or comprises a CDR-H1, CDR-H2 and CDR-H3 contained within the V_H sequence of SEQ ID NO: 256; and the V_L is or comprises a CDR-L1, CDR-L2 and CDR-L3 contained within the V_L sequence of SEQ ID NO: 267; the V_H is or comprises a CDR-H1, CDR-H2 and CDR-H3 contained within the V_H sequence of SEQ ID NO: 519; and the V_L is or comprises a CDR-L1, CDR-L2 and CDR-L3 contained within the V_L sequence of SEQ ID NO: 535; the V_H is or comprises a CDR-H1, CDR-H2 and CDR-H3 contained within the V_H sequence of SEQ ID NO:115; and the V_L is or comprises a CDR-L1, CDR-L2 and CDR-L3 contained within the V_L sequence of SEQ ID NO: 536; or the V_H is or comprises a CDR-H1, CDR-H2 and CDR-H3 contained within the V_H sequence of SEQ ID NO: 609; and the V_L is or comprises a CDR-L1, CDR-L2 and CDR-L3 contained within the V_L sequence of SEQ ID NO: 610. In some of any embodiments, 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; 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: 256; 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: 267; 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: 519; 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: 535; 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:115; 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: 536; or 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: 609; 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: 610.

[0207] In some embodiments, 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.

[0208] In some embodiments, 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:1, 4, and 7, respectively, and the V_L region comprises a CDR-L1, CDR-L2, and CDR-L3 comprising the sequence of SEQ ID NOS:26, 37, and 47, respectively; the V_H region comprises a CDR-H1, CDR-H2, and CDR-H3 comprising the sequence of SEQ ID NOS:2, 5, and 8, respectively, and the V_L region comprises a CDR-L1, CDR-L2, and CDR-L3 comprising the sequence of SEQ ID NOS:27, 38, and 48, respectively; the V_H region comprises a CDR-H1, CDR-H2, and CDR-H3 comprising the 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 sequence of SEQ ID NOS:28, 39, and 49, respectively; the V_H region comprises a CDR-H1, CDR-H2, and CDR-H3 comprising the 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 sequence of SEQ ID NOS:29, 40, and 50, respectively; the V_H region comprises a CDR-H1, CDR-H2, and CDR-H3 comprising the 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 sequence of SEQ ID NOS:30, 39, and 51, respectively; the V_H region comprises a CDR-H1, CDR-H2, and CDR-H3 comprising the 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 sequence of SEQ ID NOS:31, 41, and 52, respectively; the V_H region comprises a CDR-H1, CDR-H2, and CDR-H3 comprising the 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 sequence of SEQ ID NOS:32, 42, and 53, respectively; the V_H region comprises a CDR-H1, CDR-H2, and CDR-H3 comprising the 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 sequence of SEQ ID NOS:30, 39, and 54, respectively; the V_H region comprises a CDR-H1, CDR-H2, and CDR-H3 comprising the sequence of SEQ ID NOS:2, 5, and 9, respectively, and the V_L region comprises a CDR-L1, CDR-L2, and CDR-L3 comprising the sequence of SEQ ID NOS:33, 43, and 55, respectively; the V_H region comprises a CDR-H1, CDR-H2, and CDR-H3 comprising the 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 sequence of SEQ ID NOS:34, 44, and 56, respectively; the V_H region comprises a CDR-H1, CDR-H2, and CDR-H3 comprising the sequence of SEQ ID NOS:3, 6, and 11, respectively, and the V_L region comprises a CDR-L1, CDR-L2, and CDR-L3 comprising the sequence of SEQ ID NOS:35, 45, and 57, respectively; the V_H region comprises a CDR-H1, CDR-H2, and CDR-H3 comprising the 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 sequence of SEQ ID NOS:36, 46, and 58, respectively; the V_H region comprises a CDR-H1, CDR-H2, and CDR-H3 comprising the sequence of SEQ ID NOS:140, 145, and 149, respectively, and the V_L region comprises a CDR-L1, CDR-L2, and CDR-L3 comprising the sequence of SEQ ID NOS:174, 179, and 184, respectively; the V_H region comprises a CDR-H1, CDR-H2, and CDR-H3 comprising the sequence of SEQ ID NOS:141, 145, and 149, respectively, and the V_L region comprises a CDR-L1, CDR-L2, and CDR-L3 comprising the sequence of SEQ ID NOS:174, 179, and 185, respectively; the V_H region comprises a CDR-H1, CDR-H2, and CDR-H3 comprising the sequence of SEQ ID NOS:141, 145, and 150, respectively, and the V_L region comprises a CDR-L1, CDR-L2, and CDR-L3 comprising the sequence of SEQ ID NOS:174, 179, and 186, respectively; the V_H region comprises a CDR-H1, CDR-H2, and CDR-H3 comprising the sequence of SEQ ID

NOS:142, 146, and 151, respectively, and the V_L region comprises a CDR-L1, CDR-L2, and CDR-L3 comprising the sequence of SEQ ID NOS:174, 179, and 187, respectively; the V_H region comprises a CDR-H1, CDR-H2, and CDR-H3 comprising the sequence of SEQ ID NOS:2, 5, and 152, respectively, and the V_L region comprises a CDR-L1, CDR-L2, and CDR-L3 comprising the sequence of SEQ ID NOS:175, 180, and 188, respectively; the V_H region comprises a CDR-H1, CDR-H2, and CDR-H3 comprising the sequence of SEQ ID NOS:143, 147, and 153, respectively, and the V_L region comprises a CDR-L1, CDR-L2, and CDR-L3 comprising the sequence of SEQ ID NOS:174, 179, and 189, respectively; the V_H region comprises a CDR-H1, CDR-H2, and CDR-H3 comprising the sequence of SEQ ID NOS:144, 148, and 154, respectively, and the V_L region comprises a CDR-L1, CDR-L2, and CDR-L3 comprising the sequence of SEQ ID NOS:176, 181, and 190, respectively; the V_H region comprises a CDR-H1, CDR-H2, and CDR-H3 comprising the sequence of SEQ ID NOS:3, 6, and 155, respectively, and the V_L region comprises a CDR-L1, CDR-L2, and CDR-L3 comprising the sequence of SEQ ID NOS:177, 182, and 191, respectively; the V_H region comprises a CDR-H1, CDR-H2, and CDR-H3 comprising the sequence of SEQ ID NOS:2, 5, and 156, respectively, and the V_L region comprises a CDR-L1, CDR-L2, and CDR-L3 comprising the sequence of SEQ ID NOS:174, 179, and 192, respectively; the V_H region comprises a CDR-H1, CDR-H2, and CDR-H3 comprising the 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 sequence of SEQ ID NOS:178, 183, and 193, respectively; the V_H region comprises a CDR-H1, CDR-H2, and CDR-H3 comprising the 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 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 sequence of SEQ ID NOS:2, 6, and 376, respectively, and the V_L region comprises a CDR-L1, CDR-L2, and CDR-L3 comprising the sequence of SEQ ID NOS:30, 399, and 415, respectively; the V_H region comprises a CDR-H1, CDR-H2, and CDR-H3 comprising the 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 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 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 sequence of SEQ ID NOS:33, 43, and 421, respectively; the V_H region comprises a CDR-H1, CDR-H2, and CDR-H3 comprising the sequence of SEQ ID NOS:3, 6, and 155, respectively, and the V_L

region comprises a CDR-L1, CDR-L2, and CDR-L3 comprising the sequence of SEQ ID NOS:177, 182, and 191, respectively; the V_H region comprises a CDR-H1, CDR-H2, and CDR-H3 comprising the sequence of SEQ ID NOS:3, 372, and 376, respectively, and the V_L region comprises a CDR-L1, CDR-L2, and CDR-L3 comprising the sequence of SEQ ID NOS:381, 401, and 417, respectively; the V_H region comprises a CDR-H1, CDR-H2, and CDR-H3 comprising the sequence of SEQ ID NOS:3, 6, and 376, respectively, and the V_L region comprises a CDR-L1, CDR-L2, and CDR-L3 comprising the sequence of SEQ ID NOS:382, 402, and 418, respectively; the V_H region comprises a CDR-H1, CDR-H2, and CDR-H3 comprising the sequence of SEQ ID NOS:3, 6, and 377, respectively, and the V_L region comprises a CDR-L1, CDR-L2, and CDR-L3 comprising the sequence of SEQ ID NOS:383, 403, and 419, respectively; the V_H region comprises a CDR-H1, CDR-H2, and CDR-H3 comprising the 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 sequence of SEQ ID NOS:384, 39, and 54, respectively; the V_H region comprises a CDR-H1, CDR-H2, and CDR-H3 comprising the 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 sequence of SEQ ID NOS:385, 180, and 58, respectively; the V_H region comprises a CDR-H1, CDR-H2, and CDR-H3 comprising the sequence of SEQ ID NOS:2, 373, and 152, respectively, and the V_L region comprises a CDR-L1, CDR-L2, and CDR-L3 comprising the sequence of SEQ ID NOS:175, 180, and 188, respectively; the V_H region comprises a CDR-H1, CDR-H2, and CDR-H3 comprising the sequence of SEQ ID NOS:3, 6, and 11, respectively, and the V_L region comprises a CDR-L1, CDR-L2, and CDR-L3 comprising the sequence of SEQ ID NOS:386, 404, and 420, respectively; the V_H region comprises a CDR-H1, CDR-H2, and CDR-H3 comprising the sequence of SEQ ID NOS:2, 5, and 378, respectively, and the V_L region comprises a CDR-L1, CDR-L2, and CDR-L3 comprising the sequence of SEQ ID NOS:33, 43, and 421, respectively; the V_H region comprises a CDR-H1, CDR-H2, and CDR-H3 comprising the sequence of SEQ ID NOS:2, 5, and 9, respectively, and the V_L region comprises a CDR-L1, CDR-L2, and CDR-L3 comprising the sequence of SEQ ID NOS:387, 405, and 422, respectively; the V_H region comprises a CDR-H1, CDR-H2, and CDR-H3 comprising the sequence of SEQ ID NOS:2, 5, and 9, respectively, and the V_L region comprises a CDR-L1, CDR-L2, and CDR-L3 comprising the sequence of SEQ ID NOS:388, 406, and 423, respectively; the V_H region comprises a CDR-H1, CDR-H2, and CDR-H3 comprising the sequence of SEQ ID NOS:2, 5, and 9, respectively, and the V_L region comprises a CDR-L1, CDR-L2, and CDR-L3 comprising the sequence of SEQ ID NOS:388,

407, and 424, respectively; the V_H region comprises a CDR-H1, CDR-H2, and CDR-H3 comprising the sequence of SEQ ID NOS:3, 6, and 376, respectively, and the V_L region comprises a CDR-L1, CDR-L2, and CDR-L3 comprising the sequence of SEQ ID NOS:389, 408, and 425, respectively; the V_H region comprises a CDR-H1, CDR-H2, and CDR-H3 comprising the 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 sequence of SEQ ID NOS:390, 183, and 193, respectively; the V_H region comprises a CDR-H1, CDR-H2, and CDR-H3 comprising the sequence of SEQ ID NOS:2, 374, and 9, respectively, and the V_L region comprises a CDR-L1, CDR-L2, and CDR-L3 comprising the sequence of SEQ ID NOS:391, 409, and 426, respectively; the V_H region comprises a CDR-H1, CDR-H2, and CDR-H3 comprising the 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 sequence of SEQ ID NOS:392, 40, and 427, respectively; the V_H region comprises a CDR-H1, CDR-H2, and CDR-H3 comprising the 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 sequence of SEQ ID NOS:394, 39, and 429, respectively; the V_H region comprises a CDR-H1, CDR-H2, and CDR-H3 comprising the 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 sequence of SEQ ID NOS:395, 411, and 430, respectively; the V_H region comprises a CDR-H1, CDR-H2, and CDR-H3 comprising the 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 sequence of SEQ ID NOS:28, 39, and 49, respectively; the V_H region comprises a CDR-H1, CDR-H2, and CDR-H3 comprising the 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 sequence of SEQ ID NOS:396, 412, and 431, respectively; the V_H region comprises a CDR-H1, CDR-H2, and CDR-H3 comprising the 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 sequence of SEQ ID NOS:396, 412, and 58, respectively; the V_H region comprises a CDR-H1, CDR-H2, and CDR-H3 comprising the 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 sequence of SEQ ID NOS:397, 413, and 432, respectively; the V_H region comprises a CDR-H1, CDR-H2, and CDR-H3 comprising the 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 sequence of SEQ ID NOS:398, 414, and 433, respectively; the V_H region comprises a CDR-H1, CDR-H2, and CDR-H3 comprising the

sequence of SEQ ID NOS:288, 290, and 292, respectively, and the V_L region comprises a CDR-L1, CDR-L2, and CDR-L3 comprising the sequence of SEQ ID NOS:302, 304, and 306, respectively; the V_H region comprises a CDR-H1, CDR-H2, and CDR-H3 comprising the sequence of SEQ ID NOS:288, 290, and 292, respectively, and the V_L region comprises a CDR-L1, CDR-L2, and CDR-L3 comprising the sequence of SEQ ID NOS:302, 304, and 306, respectively; the V_H region comprises a CDR-H1, CDR-H2, and CDR-H3 comprising the sequence of SEQ ID NOS:289, 291, and 293, respectively, and the V_L region comprises a CDR-L1, CDR-L2, and CDR-L3 comprising the sequence of SEQ ID NOS:303, 305, and 307, respectively; the V_H region comprises a CDR-H1, CDR-H2, and CDR-H3 comprising the sequence of SEQ ID NOS:289, 291, and 293, respectively, and the V_L region comprises a CDR-L1, CDR-L2, and CDR-L3 comprising the sequence of SEQ ID NOS:303, 305, and 307, respectively; or the V_H region comprises a CDR-H1, CDR-H2, and CDR-H3 comprising the 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 sequence of SEQ ID NOS:589, 590, and 591, respectively.

[0209] In some embodiments, 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. In some embodiments, 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. In some embodiments, 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.

[0210] In some embodiments, 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.

[0211] In some embodiments, the V_H and V_L regions of the antibody or antigen-binding fragment thereof comprise the amino acid sequences of SEQ ID NOs:110 and 116, respectively; the V_H and V_L regions of the antibody or antigen-binding fragment thereof comprise the amino acid sequences of SEQ ID NOs:111 and 117, respectively; the V_H and V_L regions of the

antibody or antigen-binding fragment thereof comprise the amino acid sequences of SEQ ID NOs:110 and 118, respectively; the V_H and V_L regions of the antibody or antigen-binding fragment thereof comprise the amino acid sequences of SEQ ID NOs:110 and 119, respectively; the V_H and V_L regions of the antibody or antigen-binding fragment thereof comprise the amino acid sequences of SEQ ID NOs:110 and 120, respectively; the V_H and V_L regions of the antibody or antigen-binding fragment thereof comprise the amino acid sequences of SEQ ID NOs:110 and 121, respectively; the V_H and V_L regions of the antibody or antigen-binding fragment thereof comprise the amino acid sequences of SEQ ID NOs:110 and 122, respectively; the V_H and V_L regions of the antibody or antigen-binding fragment thereof comprise the amino acid sequences of SEQ ID NOs:110 and 123, respectively; the V_H and V_L regions of the antibody or antigen-binding fragment thereof comprise the amino acid sequences of SEQ ID NOs:112 and 124, respectively; the V_H and V_L regions of the antibody or antigen-binding fragment thereof comprise the amino acid sequences of SEQ ID NOs:113 and 125, respectively; the V_H and V_L regions of the antibody or antigen-binding fragment thereof comprise the amino acid sequences of SEQ ID NOs:114 and 126, respectively; the V_H and V_L regions of the antibody or antigen-binding fragment thereof comprise the amino acid sequences of SEQ ID NOs:115 and 127, respectively; the V_H and V_L regions of the antibody or antigen-binding fragment thereof comprise the amino acid sequences of SEQ ID NOs:247 and 257, respectively; the V_H and V_L regions of the antibody or antigen-binding fragment thereof comprise the amino acid sequences of SEQ ID NOs:248 and 258, respectively; the V_H and V_L regions of the antibody or antigen-binding fragment thereof comprise the amino acid sequences of SEQ ID NOs:249 and 259, respectively; the V_H and V_L regions of the antibody or antigen-binding fragment thereof comprise the amino acid sequences of SEQ ID NOs:250 and 260, respectively; the V_H and V_L regions of the antibody or antigen-binding fragment thereof comprise the amino acid sequences of SEQ ID NOs:251 and 261, respectively; the V_H and V_L regions of the antibody or antigen-binding fragment thereof comprise the amino acid sequences of SEQ ID NOs:252 and 262, respectively; the V_H and V_L regions of the antibody or antigen-binding fragment thereof comprise the amino acid sequences of SEQ ID NOs:253 and 263, respectively; the V_H and V_L regions of the antibody or antigen-binding fragment thereof comprise the amino acid sequences of SEQ ID NOs:254 and 264, respectively; the V_H and V_L regions of the antibody or antigen-binding fragment thereof comprise the amino acid sequences of SEQ ID NOs:255 and 265, respectively; the V_H and V_L regions of the antibody or antigen-binding fragment thereof comprise the amino acid sequences of SEQ ID NOs:256 and 266, respectively;

the V_H and V_L regions of the antibody or antigen-binding fragment thereof comprise the amino acid sequences of SEQ ID NOs:256 and 267, respectively; the V_H and V_L regions of the antibody or antigen-binding fragment thereof comprise the amino acid sequences of SEQ ID NOs:518 and 534, respectively; the V_H and V_L regions of the antibody or antigen-binding fragment thereof comprise the amino acid sequences of SEQ ID NOs:519 and 535, respectively; the V_H and V_L regions of the antibody or antigen-binding fragment thereof comprise the amino acid sequences of SEQ ID NOs:115 and 536, respectively; the V_H and V_L regions of the antibody or antigen-binding fragment thereof comprise the amino acid sequences of SEQ ID NOs:520 and 264, respectively; the V_H and V_L regions of the antibody or antigen-binding fragment thereof comprise the amino acid sequences of SEQ ID NOs:521 and 537, respectively; the V_H and V_L regions of the antibody or antigen-binding fragment thereof comprise the amino acid sequences of SEQ ID NOs:522 and 538, respectively; the V_H and V_L regions of the antibody or antigen-binding fragment thereof comprise the amino acid sequences of SEQ ID NOs:523 and 539, respectively; the V_H and V_L regions of the antibody or antigen-binding fragment thereof comprise the amino acid sequences of SEQ ID NOs:519 and 540, respectively; the V_H and V_L regions of the antibody or antigen-binding fragment thereof comprise the amino acid sequences of SEQ ID NOs:524 and 541, respectively; the V_H and V_L regions of the antibody or antigen-binding fragment thereof comprise the amino acid sequences of SEQ ID NOs:525 and 261, respectively; the V_H and V_L regions of the antibody or antigen-binding fragment thereof comprise the amino acid sequences of SEQ ID NOs:526 and 542, respectively; the V_H and V_L regions of the antibody or antigen-binding fragment thereof comprise the amino acid sequences of SEQ ID NOs:527 and 543, respectively; the V_H and V_L regions of the antibody or antigen-binding fragment thereof comprise the amino acid sequences of SEQ ID NOs:528 and 544, respectively; the V_H and V_L regions of the antibody or antigen-binding fragment thereof comprise the amino acid sequences of SEQ ID NOs:529 and 545, respectively; the V_H and V_L regions of the antibody or antigen-binding fragment thereof comprise the amino acid sequences of SEQ ID NOs:528 and 546, respectively; the V_H and V_L regions of the antibody or antigen-binding fragment thereof comprise the amino acid sequences of SEQ ID NOs:522 and 547, respectively; the V_H and V_L regions of the antibody or antigen-binding fragment thereof comprise the amino acid sequences of SEQ ID NOs:256 and 548, respectively; the V_H and V_L regions of the antibody or antigen-binding fragment thereof comprise the amino acid sequences of SEQ ID NOs:530 and 549, respectively; the V_H and V_L regions of the antibody or antigen-binding fragment thereof comprise the amino acid sequences of SEQ ID NOs:531 and 550,

respectively; the V_H and V_L regions of the antibody or antigen-binding fragment thereof comprise the amino acid sequences of SEQ ID NOs:519 and 552, respectively; the V_H and V_L regions of the antibody or antigen-binding fragment thereof comprise the amino acid sequences of SEQ ID NOs:110 and 553, respectively; the V_H and V_L regions of the antibody or antigen-binding fragment thereof comprise the amino acid sequences of SEQ ID NOs:110 and 118, respectively; the V_H and V_L regions of the antibody or antigen-binding fragment thereof comprise the amino acid sequences of SEQ ID NOs:533 and 554, respectively; the V_H and V_L regions of the antibody or antigen-binding fragment thereof comprise the amino acid sequences of SEQ ID NOs:115 and 555, respectively; the V_H and V_L regions of the antibody or antigen-binding fragment thereof comprise the amino acid sequences of SEQ ID NOs:524 and 556, respectively; the V_H and V_L regions of the antibody or antigen-binding fragment thereof comprise the amino acid sequences of SEQ ID NOs:519 and 557, respectively; the V_H and V_L regions of the antibody or antigen-binding fragment thereof comprise the amino acid sequences of SEQ ID NOs:324 and 326, respectively; the V_H and V_L regions of the antibody or antigen-binding fragment thereof comprise the amino acid sequences of SEQ ID NOs:325 and 327, respectively; the V_H and V_L regions of the antibody or antigen-binding fragment thereof comprise the amino acid sequences of SEQ ID NOs:609 and 610, respectively; the V_H and V_L regions of the antibody or antigen-binding fragment thereof comprise the amino acid sequences of SEQ ID NOs:617 and 618, respectively; the V_H and V_L regions of the antibody or antigen-binding fragment thereof comprise the amino acid sequences of SEQ ID NOs:772 and 775, respectively; the V_H and V_L regions of the antibody or antigen-binding fragment thereof comprise the amino acid sequences of SEQ ID NOs:773 and 776, respectively; the V_H and V_L regions of the antibody or antigen-binding fragment thereof comprise the amino acid sequences of SEQ ID NOs:774 and 777, respectively; the V_H and V_L regions of the antibody or antigen-binding fragment thereof comprise the amino acid sequences of SEQ ID NOs:815 and 833, respectively; the V_H and V_L regions of the antibody or antigen-binding fragment thereof comprise the amino acid sequences of SEQ ID NOs:816 and 834, respectively; the V_H and V_L regions of the antibody or antigen-binding fragment thereof comprise the amino acid sequences of SEQ ID NO:817 and 835, respectively; the V_H and V_L regions of the antibody or antigen-binding fragment thereof comprise the amino acid sequences of SEQ ID NO:818 and 836, respectively; the V_H and V_L regions of the antibody or antigen-binding fragment thereof comprise the amino acid sequences of SEQ ID NO:819 and 837, respectively; the V_H and V_L regions of the antibody or antigen-binding fragment thereof comprise the amino acid sequences

of SEQ ID NO:820 and 838, respectively; the V_H and V_L regions of the antibody or antigen-binding fragment thereof comprise the amino acid sequences of SEQ ID NO:821 and 839, respectively; the V_H and V_L regions of the antibody or antigen-binding fragment thereof comprise the amino acid sequences of SEQ ID NO:822 and 840, respectively; the V_H and V_L regions of the antibody or antigen-binding fragment thereof comprise the amino acid sequences of SEQ ID NO:823 and 841, respectively; the V_H and V_L regions of the antibody or antigen-binding fragment thereof comprise the amino acid sequences of SEQ ID NO:824 and 842, respectively; the V_H and V_L regions of the antibody or antigen-binding fragment thereof comprise the amino acid sequences of SEQ ID NO:825 and 843, respectively; the V_H and V_L regions of the antibody or antigen-binding fragment thereof comprise the amino acid sequences of SEQ ID NO:826 and 844, respectively; the V_H and V_L regions of the antibody or antigen-binding fragment thereof comprise the amino acid sequences of SEQ ID NO:827 and 845, respectively; the V_H and V_L regions of the antibody or antigen-binding fragment thereof comprise the amino acid sequences of SEQ ID NO:828 and 846, respectively; the V_H and V_L regions of the antibody or antigen-binding fragment thereof comprise the amino acid sequences of SEQ ID NO:829 and 847, respectively; the V_H and V_L regions of the antibody or antigen-binding fragment thereof comprise the amino acid sequences of SEQ ID NO:830 and 847, respectively; the V_H and V_L regions of the antibody or antigen-binding fragment thereof comprise the amino acid sequences of SEQ ID NO:831 and 848, respectively; the V_H and V_L regions of the antibody or antigen-binding fragment thereof comprise the amino acid sequences of SEQ ID NO:832 and 849, respectively, or any antibody or antigen-binding fragment thereof that has at least 90% sequence identity to any of the above V_H and V_L, such as at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity thereto.

[0212] For example, the V_H and V_L regions of the antibody or antigen-binding fragment thereof provided therein comprise the amino acid sequences selected from: SEQ ID NOs:110 and 116; SEQ ID NOs:111 and 117; SEQ ID NOs:110 and 118; SEQ ID NOs:110 and 119; SEQ ID NOs:110 and 120; SEQ ID NOs:110 and 121; SEQ ID NOs:110 and 122; SEQ ID NOs:110 and 123; SEQ ID NOs:112 and 124; SEQ ID NOs:113 and 125; SEQ ID NOs:114 and 126; SEQ ID NOs:115 and 127; SEQ ID NOs:247 and 257; SEQ ID NOs:248 and 258; SEQ ID NOs:249 and 259; SEQ ID NOs:250 and 260; SEQ ID NOs:251 and 261; SEQ ID NOs:252 and 262; SEQ ID NOs:253 and 263; SEQ ID NOs:254 and 264; SEQ ID NOs:255 and 265; SEQ ID NOs:256 and 266; SEQ ID NOs:256 and 267; SEQ ID NOs:518 and 534; SEQ ID NOs:519 and 535; SEQ ID NOs:115 and 536; SEQ ID NOs:520 and 264; SEQ ID NOs:521 and 537; SEQ ID NOs:522

and 538; SEQ ID NOs:523 and 539; SEQ ID NOs:519 and 540; SEQ ID NOs:524 and 541; SEQ ID NOs:525 and 261; SEQ ID NOs:526 and 542; SEQ ID NOs:527 and 543; SEQ ID NOs:528 and 544; SEQ ID NOs:529 and 545; SEQ ID NOs:528 and 546; SEQ ID NOs:522 and 547; SEQ ID NOs:256 and 548; SEQ ID NOs:530 and 549; SEQ ID NOs:531 and 550; SEQ ID NOs:519 and 552; SEQ ID NOs:110 and 553; SEQ ID NOs:110 and 118; SEQ ID NOs:533 and 554; SEQ ID NOs:115 and 555; SEQ ID NOs:524 and 556; SEQ ID NOs:519 and 557, SEQ ID NOs:324 and 326, SEQ ID NOs:325 and 327, SEQ ID NOs:609 and 610; SEQ ID NOs:617 and 618; SEQ ID NOs:772 and 775; SEQ ID NOs:773 and 776; SEQ ID NOs:774 and 777; SEQ ID NOs:815 and 833; SEQ ID NOs:816 and 834; SEQ ID NO:817 and 835; SEQ ID NO:818 and 836; SEQ ID NO:819 and 837; SEQ ID NO:820 and 838; SEQ ID NO:821 and 839; NO:822 and 840; SEQ ID NO:823 and 841; SEQ ID NO:824 and 842; SEQ ID NO:825 and 843; SEQ ID NO:826 and 844; SEQ ID NO:827 and 845; SEQ ID NO:828 and 846; SEQ ID NO:829 and 847; SEQ ID NO:830 and 847; SEQ ID NO:831 and 848; and SEQ ID NO:832 and 849, respectively, or any antibody or antigen-binding fragment thereof that has at least 90% sequence identity to any of the above V_H and V_L, such as at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity thereto, or any antibody or antigen-binding fragment thereof that comprises a CDR-H1, CDR-H2 and CDR-H3 contained within the V_H region and a CDR-L1, CDR-L2 and CDR-L3 contained within the V_L region of any of the above V_H and V_L.

[0213] In some embodiments, the V_H and V_L regions of the antibody or antigen-binding fragment thereof provided therein comprise the amino acid sequences selected from: SEQ ID NOs:617 and 618; SEQ ID NOs:256 and 267; SEQ ID NOs:519 and 535; SEQ ID NOs:115 and 536; or SEQ ID NOs:609 and 610; respectively, or any antibody or antigen-binding fragment thereof that has at least 90% sequence identity to any of the above V_H and V_L, such as at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity thereto, or any antibody or antigen-binding fragment thereof that comprises a CDR-H1, CDR-H2 and CDR-H3 contained within the V_H region and a CDR-L1, CDR-L2 and CDR-L3 contained within the V_L region of any of the above V_H and V_L.

[0214] In some embodiments, the V_H and V_L regions of the antibody or antigen-binding fragment thereof provided therein comprise the amino acid sequences selected from: SEQ ID NOs:617 and 618, or any antibody or antigen-binding fragment thereof that has at least 90% sequence identity to any of the above V_H and V_L, such as at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity thereto, or any antibody or antigen-binding fragment thereof that comprises a CDR-H1, CDR-H2 and CDR-H3 contained within the V_H

region and a CDR-L1, CDR-L2 and CDR-L3 contained within the V_L region of any of the above V_H and V_L.

[0215] In some embodiments, the antibody or antigen-binding fragment thereof is a single-chain antibody fragment, such as a single chain variable fragment (scFv) or a diabody or a single domain antibody (sdAb). In some embodiments, the antibody or antigen-binding fragment is a single domain antibody comprising only the V_H region. In some embodiments, the antibody or antigen binding fragment is an scFv comprising a heavy chain variable (V_H) region and a light chain variable (V_L) region. In some embodiments, the single-chain antibody fragment (*e.g.* scFv) includes one or more linkers joining two antibody domains or regions, such as a heavy chain variable (V_H) region and a light chain variable (V_L) region. The linker typically is a peptide linker, *e.g.*, a flexible and/or soluble peptide linker. Among the linkers are those rich in glycine and serine and/or in some cases threonine. In some embodiments, the linkers further include charged residues such as lysine and/or glutamate, which can improve solubility. In some embodiments, the linkers further include one or more proline.

[0216] Accordingly, the provided anti-BCMA antibodies include single-chain antibody fragments, such as scFvs and diabodies, particularly human single-chain antibody fragments, typically comprising linker(s) joining two antibody domains or regions, such V_H and V_L regions. The linker typically is a peptide linker, *e.g.*, a flexible and/or soluble peptide linker, such as one rich in glycine and serine.

[0217] In some aspects, the linkers rich in glycine and serine (and/or threonine) include at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% such amino acid(s). In some embodiments, they include at least at or about 50%, 55%, 60%, 70%, or 75%, glycine, serine, and/or threonine. In some embodiments, the linker is comprised substantially entirely of glycine, serine, and/or threonine. The linkers generally are between about 5 and about 50 amino acids in length, typically between at or about 10 and at or about 30, *e.g.*, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30, and in some examples between 10 and 25 amino acids in length. Exemplary linkers include linkers having various numbers of repeats of the sequence GGGGS (4GS; SEQ ID NO:359) or GGGS (3GS; SEQ ID NO:360), such as between 2, 3, 4, and 5 repeats of such a sequence. Exemplary linkers include those having or consisting of an sequence set forth in SEQ ID NO:361 (GGGGSGGGGSGGGGS). Exemplary linkers further include those having or consisting of the sequence set forth in SEQ ID NO:362 (GSTSGSGKPGSGEGSTKG). Exemplary linkers further include those having or consisting of the sequence set forth in SEQ ID NO:778 (SRGGGGSGGGGSGGGGSLEMA).

[0218] Accordingly, in some embodiments, the provided embodiments include single-chain antibody fragments, *e.g.*, scFvs, comprising one or more of the aforementioned linkers, such as glycine/serine rich linkers, including linkers having repeats of GGGS (SEQ ID NO: 360) or GGGGS (SEQ ID NO: 359), such as the linker set forth in SEQ ID NO:361.

[0219] In some embodiments, the linker has an amino acid sequence containing the sequence set forth in SEQ ID NO:361. The fragment, *e.g.*, scFv, may include a V_H region or portion thereof, followed by the linker, followed by a V_L region or portion thereof. The fragment, *e.g.*, the scFv, may include the V_L region or portion thereof, followed by the linker, followed by the V_H region or portion thereof.

[0220] In some embodiments, the antigen-binding domain comprises the 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 sequence selected from any one of SEQ ID NOs: 478, 128-139, 268-278, 329, 442, 558-576, 578-583, 585, or 769-771.

[0221] In some aspects, an scFv provided herein comprises the amino acid sequence selected from any one of SEQ ID NOs:128-139, 268-278, 328, 329, 442, 478, 558-576, 578-583, 585, 586, and 769-771, or has an amino acid sequence having at least at or about 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, 328, 329, 442, 478, 558-576, 578-583, 585, 586, and 769-771.

[0222] For example, the scFv provided herein comprises the amino acid sequence selected from any of SEQ ID NOS:128, 129, 130, 132, 133, 136, 137, 269, 273, 274, 275, 276, 277, 278, 328, 329, 442, 478, 558, 559, 560, 561, 562, 563, 564, 565, 566, 567, 568, 569, 570, 571, 572, 573, 574, 575, 576, 577, 578, 579, 580, 581, 582, 583 585, 586, 769, 770, 771, 781, 782, 783, 784, 785, 786, 787, 788, 789, 790, 791, 792, 793, 794, 795, 796, 797, 798, 799, 800, 801, 802, 803, 804, 805, 806, 807, 808, 809, 810, 811, 812, or 813 or has an amino acid sequence having at least at or about 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, 129, 130, 132, 133, 136, 137, 269, 273, 274, 275, 276, 277, 278, 328, 329, 442, 478, 558, 559, 560, 561, 562, 563, 564, 565, 566, 567, 568, 569, 570, 571, 572, 573, 574, 575, 576, 577, 578, 579, 580, 581, 582, 583 585, 586, 769, 770, 771, 781, 782, 783, 784, 785, 786, 787, 788, 789, 790, 791, 792, 793, 794, 795, 796, 797, 798, 799, 800, 801, 802, 803, 804, 805, 806, 807, 808, 809, 810, 811, 812, or 813.

[0223] **Table 2** provides the SEQ ID NOS: of exemplary antigen-binding domains, such as antibodies or antigen-binding fragments, that can be comprised in the provided BCMA-binding receptors, such as anti-BCMA chimeric antigen receptors (CARs). In some embodiments, the BCMA-binding receptor contains a BCMA-binding antibody or fragment thereof, comprising a V_H region that comprises the CDR-H1, CDR-H2, and CDR-H3 sequence and a V_L region that comprises the CDR-L1, CDR-L2 and CDR-L3 sequence set forth in the SEQ ID NOS: listed in each row of **Table 2** below (by Kabat numbering). In some embodiments, the BCMA-binding receptor contains a BCMA-binding antibody or fragment thereof, comprising a V_H region sequence and a V_L region sequence set forth in the SEQ ID NOS: listed in each row of **Table 2** below, or an antibody comprising a V_H and V_L region amino acid sequence that has at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to the V_H region sequence and the V_L region sequence set forth in the SEQ ID NOS: listed in each row of **Table 2** below. In some embodiments, the BCMA-binding receptor contains a BCMA-binding antibody or fragment thereof, comprising a V_H region sequence and a V_L region sequence set forth in the SEQ ID NOS: listed in each row of **Table 2** below. In some embodiments, the BCMA-binding receptor contains a BCMA-binding antibody or fragment thereof, comprising an scFv sequence set forth in the SEQ ID NOS: listed in each row of **Table 2** below, or an antibody comprising an scFv amino acid sequence that has at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to the scFv sequence set forth in the SEQ ID NOS: listed in each row of **Table 2** below. In some embodiments, the BCMA-binding receptor contains a BCMA-binding antibody or fragment thereof, comprising an scFv sequence set forth in the SEQ ID NOS: listed in each row of **Table 2** below.

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

Antigen-binding domain	CDR-H1	CDR-H2	CDR-H3	CDR-L1	CDR-L2	CDR-L3	V_H	V_L	scFv
BCMA-1	1	4	7	26	37	47	110	116	128
BCMA-2	2	5	8	27	38	48	111	117	129
BCMA-3	1	4	7	28	39	49	110	118	130
BCMA-4	1	4	7	29	40	50	110	119	131
BCMA-5	1	4	7	30	39	51	110	120	132
BCMA-6	1	4	7	31	41	52	110	121	133
BCMA-7	1	4	7	32	42	53	110	122	134
BCMA-8	1	4	7	30	39	54	110	123	135
BCMA-9	2	5	9	33	43	55	112	124	136
BCMA-10	2	5	10	34	44	56	113	125	137
BCMA-11	3	6	11	35	45	57	114	126	138
BCMA-12	2	5	10	36	46	58	115	127	139
BCMA-13	140	145	149	174	179	184	247	257	268

Table 2.Sequence identifier (SEQ ID NO) for Exemplary Antigen-binding Domains									
Antigen-binding domain	**CDR-H1**	**CDR-H2**	**CDR-H3**	**CDR-L1**	**CDR-L2**	**CDR-L3**	**V_H**	**V_L**	**scFv**
BCMA-14	141	145	149	174	179	185	248	258	269
BCMA-15	141	145	150	174	179	186	249	259	270
BCMA-16	142	146	151	174	179	187	250	260	271
BCMA-17	2	5	152	175	180	188	251	261	272
BCMA-18	143	147	153	174	179	189	252	262	273
BCMA-19	144	148	154	176	181	190	253	263	274
BCMA-20	3	6	155	177	182	191	254	264	275
BCMA-21	2	5	156	174	179	192	255	265	276
BCMA-22	2	5	157	178	183	193	256	266	277
BCMA-23	2	5	157	178	183	194	256	267	278
BCMA-24	2	6	376	30	399	415	518	534	558
BCMA-25	1	4	7	380	400	416	519	535	559
BCMA-26	2	5	10	33	43	421	115	536	560
BCMA-27	3	6	155	177	182	191	520	264	561
BCMA-28	3	372	376	381	401	417	521	537	562
BCMA-29	3	6	376	382	402	418	522	538	563
BCMA-30	3	6	377	383	403	419	523	539	564
BCMA-31	1	4	7	384	39	54	519	540	565
BCMA-32	2	5	10	385	180	58	524	541	566
BCMA-33	2	373	152	175	180	188	525	261	567
BCMA-34	3	6	11	386	404	420	526	542	568
BCMA-35	2	5	378	33	43	421	527	543	569
BCMA-36	2	5	9	387	405	422	528	544	570
BCMA-37	2	5	9	388	406	423	529	545	571
BCMA-38	2	5	9	388	407	424	528	546	572
BCMA-39	3	6	376	389	408	425	522	547	573
BCMA-40	2	5	157	390	183	193	256	548	574
BCMA-41	2	374	9	391	409	426	530	549	575
BCMA-42	1	4	7	392	40	427	531	550	576
BCMA-44	1	4	7	394	39	429	519	552	578
BCMA-45	1	4	7	395	411	430	110	553	579
BCMA-46	1	4	7	28	39	49	110	118	130
BCMA-47	2	5	10	396	412	431	533	554	580
BCMA-48	2	5	10	396	412	58	115	555	581
BCMA-49	2	5	10	397	413	432	524	556	582
BCMA-51	1	4	7	398	414	433	519	557	583
BCMA-52	507	513	517	589	590	591	609	610	442
BCMA-55	593	594	595	601	602	603	617	618	478
BCMA-C1, VH-VL	288	290	292	302	304	306	324	326	585
BCMA-C1, VL-VH	288	290	292	302	304	306	324	326	328
BCMA-C2, VH-VL	289	291	293	303	305	307	325	327	329
BCMA-C2, VL-VH	289	291	293	303	305	307	325	327	586
BCMA-D1							772	775	769
BCMA-D2							773	776	770

Table 2.Sequence identifier (SEQ ID NO) for Exemplary Antigen-binding Domains									
Antigen-binding domain	**CDR-H1**	**CDR-H2**	**CDR-H3**	**CDR-L1**	**CDR-L2**	**CDR-L3**	**V_H**	**V_L**	**scFv**
BCMA-D3							774	777	771
BCMA-D4							814		
BCMA-D5							815	833	781
BCMA-D6							816	834	782
BCMA-D7							816	834	783
BCMA-D8							817	835	784
BCMA-D9							817	835	785
BCMA-D10							818	836	786
BCMA-D11							818	836	787
BCMA-D12							819	837	788
BCMA-D13							819	837	789
BCMA-D14							820	838	790
BCMA-D15							820	838	791
BCMA-D16							821	839	792
BCMA-D17							821	839	793
BCMA-D18							822	840	794
BCMA-D19							822	840	795
BCMA-D20							823	841	796
BCMA-D21							823	841	797
BCMA-D22							824	842	798
BCMA-D23							824	842	799
BCMA-D24							824	842	800
BCMA-D25							825	843	801
BCMA-D26							826	844	802
BCMA-D27							827	845	803
BCMA-D28							828	846	804
BCMA-D29									805
BCMA-D30							829	847	806
BCMA-D31							830	847	807
BCMA-D32							831	848	808
BCMA-D33							832	849	809
BCMA-D34									810
BCMA-D35							832	849	811
BCMA-D36							831	848	812
BCMA-D37									813

[0224] Among the antibodies, *e.g.* antigen-binding fragments, in the provided CARs, are human antibodies. In some embodiments of a provided human anti-BCMA antibody, *e.g.*, antigen-binding fragments, the human antibody contains a V_H region that comprises a portion having at least 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to an amino acid sequence encoded by a germline nucleotide human heavy chain V segment, a portion having at least 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to an amino acid sequence encoded by a germline nucleotide human heavy chain D segment, and/or a portion having at least 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to an amino acid sequence encoded by a

germline nucleotide human heavy chain J segment; and/or contains a V_L region that comprises a portion having at least 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to an amino acid sequence encoded by a germline nucleotide human kappa or lambda chain V segment, and/or a portion having at least 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to an amino acid sequence encoded by a germline nucleotide human kappa or lambda chain J segment. In some embodiments, the portion of the V_H region corresponds to the CDR-H1, CDR-H2 and/or CDR-H3. In some embodiments, the portion of the V_H region corresponds to the framework region 1 (FR1), FR2, FR2 and/or FR4. In some embodiments, the portion of the V_L region corresponds to the CDR-L1, CDR-L2 and/or CDR-L3. In some embodiments, the portion of the V_L region corresponds to the FR1, FR2, FR2 and/or FR4.

[0225] In some embodiments, the human antibody, *e.g.*, antigen-binding fragment, contains a CDR-H1 having at least 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to an amino acid sequence of the corresponding CDR-H1 region within a sequence encoded by a germline nucleotide human heavy chain V segment. For example, the human antibody in some embodiments contains a CDR-H1 having a sequence that is 100% identical or with no more than one, two or three amino acid differences as compared to the corresponding CDR-H1 region within a sequence encoded by a germline nucleotide human heavy chain V segment.

[0226] In some embodiments, the human antibody, *e.g.*, antigen-binding fragment, contains a CDR-H2 having at least 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to an amino acid sequence of the corresponding CDR-H2 region within a sequence encoded by a germline nucleotide human heavy chain V segment. For example, the human antibody in some embodiments contains a CDR-H2 having a sequence that is 100% identical or with no more than one, two or three amino acid difference as compared to the corresponding CDR-H2 region within a sequence encoded by a germline nucleotide human heavy chain V segment.

[0227] In some embodiments, the human antibody, *e.g.*, antigen-binding fragment, contains a CDR-H3 having at least 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to an amino acid sequence of the corresponding CDR-H3 region within a sequence encoded by a germline nucleotide human heavy chain V segment, D segment and J segment. For example, the human antibody in some embodiments contains a CDR-H3 having a sequence that is 100% identical or with no more than one, two or three amino acid differences as compared to the corresponding CDR-H3 region within a sequence encoded by a germline nucleotide human heavy chain V segment, D segment and J segment.

[0228] In some embodiments, the human antibody, *e.g.*, antigen-binding fragment, contains a CDR-L1 having at least 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to an amino acid sequence of the corresponding CDR-L1 region within a sequence encoded by a germline nucleotide human light chain V segment. For example, the human antibody in some embodiments contains a CDR-L1 having a sequence that is 100% identical or with no more than one, two or three amino acid differences as compared to the corresponding CDR-L1 region within a sequence encoded by a germline nucleotide human light chain V segment.

[0229] In some embodiments, the human antibody, *e.g.*, antigen-binding fragment, contains a CDR-L2 having at least 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to an amino acid sequence of the corresponding CDR-L2 region within a sequence encoded by a germline nucleotide human light chain V segment. For example, the human antibody in some embodiments contains a CDR-L2 having a sequence that is 100% identical or with no more than one, two or three amino acid difference as compared to the corresponding CDR-L2 region within a sequence encoded by a germline nucleotide human light chain V segment.

[0230] In some embodiments, the human antibody, *e.g.*, antigen-binding fragment, contains a CDR-L3 having at least 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to an amino acid sequence of the corresponding CDR-L3 region within a sequence encoded by a germline nucleotide human light chain V segment and J segment. For example, the human antibody in some embodiments contains a CDR-L3 having a sequence that is 100% identical or with no more than one, two or three amino acid differences as compared to the corresponding CDR-L3 region within a sequence encoded by a germline nucleotide human light chain V segment and J segment.

[0231] In some embodiments, the human antibody, *e.g.*, antigen-binding fragment, contains a framework region that contains human germline gene segment sequences. For example, in some embodiments, the human antibody contains a V_H region in which the framework region, *e.g.* FR1, FR2, FR3 and FR4, has at least 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to a framework region encoded by a human germline antibody segment, such as a V segment and/or J segment. In some embodiments, the human antibody contains a V_L region in which the framework region *e.g.* FR1, FR2, FR3 and FR4, has at least 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to a framework region encoded by a human germline antibody segment, such as a V segment and/or J segment. For example, in some such embodiments, the framework region sequence contained within the V_H region and/or V_L region differs by no more

than 10 amino acids, such as no more than 9, 8, 7, 6, 5, 4, 3, 2 or 1 amino acid, compared to the framework region sequence encoded by a human germline antibody segment.

[0232] In some embodiments, the reference antibody can be a mouse anti-BCMA scFv described in International Patent App. Pub. No. WO 2010/104949.

[0233] The antibody, *e.g.*, antigen-binding fragment, may contain at least a portion of an immunoglobulin constant region, such as one or more constant region domain. In some embodiments, the constant regions include a light chain constant region and/or a heavy chain constant region 1 (C_H1). In some embodiments, the antibody includes a C_H2 and/or C_H3 domain, such as an Fc region. In some embodiments, the Fc region is an Fc region of a human IgG, such as an IgG1 or IgG4.

2. Spacer

[0234] In some embodiments, the recombinant receptor such as a CAR comprising an antibody (*e.g.*, antigen-binding fragment) provided herein, further includes a spacer or spacer region. The spacer typically is a polypeptide spacer and in general is located within the CAR between the antigen binding domain and the transmembrane domain of the CAR. In some aspects, the spacer may be or include at least a portion of an immunoglobulin constant region or variant or modified version thereof, such as a hinge region of an immunoglobulin, such as an IgG hinge region, *e.g.*, an IgG4 or IgG4-derived hinge region, and/or a C_H1/CL and/or Fc region. In some embodiments, the constant region or one or more of the portion(s) thereof is of a human IgG, such as of a human IgG4 or IgG1 or IgG2. In general, the spacer, such as the portion of the constant region, serves as a spacer region between the antigen-recognition component (*e.g.*, scFv) and transmembrane domain. In some embodiments, the length and/or composition of the spacer is designed to optimize or promote certain features of the interaction between the CAR and its target; in some aspects, it is designed to optimize the biophysical synapse distance between the CAR-expressing celland the cell expressing the target of the CAR during or upon or following binding of the CAR to its target on the target-expressing cell; in some aspects, the target expressing cell is a BCMA-expressing tumor cell. In some embodiments, The CAR is expressed by a T-cell, and the length of the spacer is of a length that is compatible for T-cell activation or to optimize CAR T-cell performance. In some embodiments, the spacer is a spacer region, located between the ligand-binding domain and the transmembrane domain, of the recombinant receptor, e.g., CAR. In some embodiments, the

spacer region is a region located between the ligand-binding domain and the transmembrane domain, of the recombinant receptor, e.g., CAR.

[0235] In some embodiments, the spacer can be of a length that provides for increased responsiveness of the cell following antigen binding, as compared to in the absence of the spacer and/or in the presence of a different spacer, such as one different only in length. In some embodiments, the spacer is at least 100 amino acids in length, such as at least 110, 125, 130, 135, 140, 145, 150, 160, 170, 180, 190, 200, 210, 220, 230, 240, or 250 amino acids in length. In some examples, the spacer is at or about 12 amino acids in length or is no more than 12 amino acids in length. Exemplary spacers include those having at least about 10 to 300 amino acids, about 10 to 200 amino acids, about 50 to 175 amino acids, about 50 to 150 amino acids, about 10 to 125 amino acids, about 50 to 100 amino acids, about 100 to 300 amino acids, about 100 to 250 amino acids, about 125 to 250 amino acids, or about 200 to 250 amino acids, and including any integer between the endpoints of any of the listed ranges. In some embodiments, aspacer or spacer region is at least about 12 amino acids, at least about 119 amino acids or less, at least about 125 amino acids, at least about 200 amino acids, or at least about 220 amino acids, or at least about 225 amino acids in length.

[0236] In some embodiments, the spacer has a length of 125 to 300 amino acids in length, 125 to 250 amino acids in length, 125 to 230 amino acids in length, 125 to 200 amino acids in length, 125 to 180 amino acids in length, 125 to 150 amino acids in length, 150 to 300 amino acids in length, 150 to 250 amino acids in length, 150 to 230 amino acids in length, 150 to 200 amino acids in length, 150 to 180 amino acids in length, 180 to 300 amino acids in length, 180 to 250 amino acids in length, 180 to 230 amino acids in length, 180 to 200 amino acids in length, 200 to 300 amino acids in length, 200 to 250 amino acids in length, 200 to 230 amino acids in length, 230 to 300 amino acids in length, 230 to 250 amino acids in length or 250 to 300 amino acids in length. In some embodiments, the spacer is at least or at least about or is or is about 130, 140, 150, 160, 170, 180, 190, 200, 210, 220, 221, 222, 223, 224, 225, 226, 227, 228 or 229 amino acids in length, or a length between any of the foregoing.

[0237] Exemplary spacers include those containing portion(s) of an immunoglobulin constant region such as those containing an Ig hinge, such as an IgG hinge domain. In some aspects, the spacer includes an IgG hinge alone, an IgG hinge linked to one or more of a C_H2 and C_H3 domain, or IgG hinge linked to the C_H3 domain. In some embodiments, the IgG hinge, C_H2 and/or C_H3 can be derived all or in part from IgG4 or IgG2. In some embodiments, the spacer can be a chimeric polypeptide containing one or more of a hinge, C_H2 and/or C_H3

sequence(s) derived from IgG4, IgG2, and/or IgG2 and IgG4. In some embodiments, the hinge region comprises all or a portion of an IgG4 hinge region and/or of an IgG2 hinge region, wherein the IgG4 hinge region is optionally a human IgG4 hinge region and the IgG2 hinge region is optionally a human IgG2 hinge region; the C_H2 region comprises all or a portion of an IgG4 C_H2 region and/or of an IgG2 C_H2 region, wherein the IgG4 C_H2 region is optionally a human IgG4 C_H2 region and the IgG2 C_H2 region is optionally a human IgG2 C_H2 region; and/or the C_H3 region comprises all or a portion of an IgG4 C_H3 region and/or of an IgG2 C_H3 region, wherein the IgG4 C_H3 region is optionally a human IgG4 C_H3 region and the IgG2 C_H3 region is optionally a human IgG2 C_H3 region. In some embodiments, the hinge, C_H2 and C_H3 comprises all or a portion of each of a hinge region, C_H2 and C_H3 from IgG4. In some embodiments, the hinge region is chimeric and comprises a hinge region from human IgG4 and human IgG2; the C_H2 region is chimeric and comprises a C_H2 region from human IgG4 and human IgG2; and/or the C_H3 region is chimeric and comprises a C_H3 region from human IgG4 and human IgG2. In some embodiments, the spacer comprises an IgG4/2 chimeric hinge or a modified IgG4 hinge comprising at least one amino acid replacement compared to human IgG4 hinge region; an human IgG2/4 chimeric C_H2 region; and a human IgG4 C_H3 region.

[0238] In some embodiments, the spacer can be derived all or in part from IgG4 and/or IgG2 and can contain mutations, such as one or more single amino acid mutations in one or more domains. In some examples, the amino acid modification is a substitution of a proline (P) for a serine (S) in the hinge region of an IgG4. In some embodiments, the amino acid modification is a substitution of a glutamine (Q) for an asparagine (N) to reduce glycosylation heterogeneity, such as an N177Q mutation at position 177, in the C_H2 region, of the full-length IgG4 Fc sequence set forth in SEQ ID NO: 750 or an N176Q. at position 176, in the C_H2 region, of the full-length IgG2 Fc sequence set forth in SEQ ID NO: 749. In some embodiments, the spacer is or comprises an IgG4/2 chimeric hinge or a modified IgG4 hinge; an IgG2/4 chimeric C_H2 region; and an IgG4 C_H3 region and optionally is about 228 amino acids in length; or a spacer set forth in SEQ ID NO: 649. In some embodiments, the spacer comprises the amino acid sequence

ESKYGPPCPPCPAPPVAGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDG
VEVHNAKTKPREEQFQSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQP
REPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFF
LYSRLTVDKSRWQEGNVFSCSVMHEALHNHYTQKSLSLSLGK (SEQ ID NO: 649)

encoded by a polynucleotide that has been optimized for codon expression and/or to eliminate splice sites such as cryptic splice sites. In some embodiments, the coding sequence for the spacer comprises the nucleic acid sequence set forth in SEQ ID NO: 622. In some embodiments, the coding sequence for the spacer comprises the nucleic acid sequence set forth in SEQ ID NO: 855 or 856.

[0239] Additional exemplary spacers include, but are not limited to, those described in Hudecek *et al.* (2013) *Clin. Cancer Res.*, 19:3153, Hudecek *et al.* (2015) *Cancer Immunol. Res.*, 3(2):125-135, or international patent application publication number WO2014031687. In some embodiments, the nucleotide sequence of the spacer is optimized to reduce RNA heterogeneity following expression. In some embodiments, the nucleotide sequence of the spacer is optimized to reduce cryptic splice sites or reduce the likelihood of a splice event at a splice site.

[0240] In some embodiments, the spacer has the amino acid sequence set forth in SEQ ID NO:363, and is encoded by the polynucleotide sequence set forth in SEQ ID NO:364. In some embodiments, the spacer has the amino acid sequence set forth in SEQ ID NO:365. In some embodiments, the spacer has the amino acid sequence set forth in SEQ ID NO:366. In some embodiments, the spacer has the amino acid sequence set forth in SEQ ID NO: 630, and is encoded by the polynucleotide sequence set forth in SEQ ID NO: 629. In some embodiments, the spacer has an amino acid sequence set forth in SEQ ID NO: 649, encoded by the polynucleotide sequence set forth in SEQ ID NO: 621,622, 855 or 856 or a polynucleotide that exhibits at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to SEQ ID NO: 621, 622, 855 or 856. In some embodiments, the spacer has an amino acid sequence that exhibits at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to SEQ ID NO: 649, encoded by a polynucleotide that has been optionally optimized for codon usage and/or to reduce RNA heterogeneity.

[0241] In some embodiments, the spacer is or comprises an amino acid sequence encoded by the nucleotide sequence set forth in SEQ ID NO:622.

3. Transmembrane domain and intracellular signaling components

[0242] The antigen-recognition component generally is linked to one or more intracellular signaling regions containing signaling components, such as signaling components that mimic stimulation and/or activation through an antigen receptor complex, such as a TCR complex, in the case of a CAR, and/or signal via another cell surface receptor. Thus, in some embodiments,

the BCMA-binding molecule (*e.g.*, antibody or antigen binding fragment thereof) is linked to one or more transmembrane domains such as those described herein and intracellular signaling regions or domains comprising one or more intracellular components such as those described herein. In some embodiments, the transmembrane domain is fused to the extracellular domain. In one embodiment, a transmembrane domain that naturally is associated with one of the domains in the receptor, *e.g.*, CAR, is used. In some instances, the transmembrane domain is selected or modified by amino acid substitution to avoid binding of such domains to the transmembrane domains of the same or different surface membrane proteins to minimize interactions with other members of the receptor complex.

[0243] The transmembrane domain in some embodiments is derived either from a natural or from a synthetic source. Where the source is natural, the domain in some aspects is derived from any membrane-bound or transmembrane protein. Transmembrane domains include those derived from (*i.e.* comprise at least the transmembrane domain(s) of) the alpha, beta or zeta chain of the T-cell receptor, CD3 epsilon, CD4, CD5, CD8, CD9, CD16, CD22, CD28, CD33, CD37, CD45, CD64, CD80, CD86, CD134, CD137, and/or CD154. For example, the transmembrane domain can be a CD28 transmembrane domain that comprises the sequence of amino acids set forth in SEQ ID NO: 624, encoded by the nucleic acid sequence set forth in SEQ ID NO: 623 or SEQ ID NO:688. Alternatively the transmembrane domain in some embodiments is synthetic. In some aspects, the synthetic transmembrane domain comprises predominantly hydrophobic residues such as leucine and valine. In some aspects, a triplet of phenylalanine, tryptophan and valine will be found at each end of a synthetic transmembrane domain. In some embodiments, the linkage is by linkers, spacers, and/or transmembrane domain(s).

[0244] Among the intracellular signaling regions or domains are those that mimic or approximate a signal through a natural antigen receptor, a signal through such a receptor in combination with a costimulatory receptor, and/or a signal through a costimulatory receptor alone. In some embodiments, a short oligo- or polypeptide linker, for example, a linker of between 2 and 10 amino acids in length, such as one containing glycines and serines, *e.g.*, glycine-serine doublet, is present and forms a linkage between the transmembrane domain and the intracellular signaling domain of the CAR.

[0245] The receptor, *e.g.*, the CAR, generally includes an intracellular signaling region comprising at least one intracellular signaling component or components. In some embodiments, the receptor includes an intracellular component or signaling domain of a TCR complex, such as a TCR CD3 chain that mediates T-cell activation and cytotoxicity, *e.g.*, CD3

zeta chain. Thus, in some aspects, the BCMA-binding antibody is linked to one or more cell signaling modules. In some embodiments, cell signaling modules include CD3 transmembrane domain, CD3 intracellular signaling domains, and/or other CD transmembrane domains. In some embodiments, the receptor, *e.g.*, CAR, further includes a portion of one or more additional molecules such as Fc receptor γ, CD8, CD4, CD25, or CD16. For example, in some aspects, the CAR includes a chimeric molecule between CD3-zeta (CD3-ζ) or Fc receptor γ and CD8, CD4, CD25 or CD16.

[0246] In some embodiments, upon or following ligation of the CAR, the cytoplasmic domain or intracellular signaling domain of the CAR stimulates and/or activates at least one of the normal effector functions or responses of the immune cell, *e.g.*, T cell engineered to express the CAR. For example, in some contexts, the CAR induces a function of a T cell such as cytolytic activity or T-helper activity, such as secretion of cytokines or other factors. In some embodiments, a truncated portion of an intracellular signaling domain of an antigen receptor component or costimulatory molecule is used in place of an intact immunostimulatory chain, for example, if it transduces the effector function signal. In some embodiments, the intracellular signaling domain or domains include the cytoplasmic sequences of the T cell receptor (TCR), and in some aspects also those of co-receptors that in the natural context act in concert with such receptor to initiate signal transduction following antigen receptor engagement, and/or any derivative or variant of such molecules, and/or any synthetic sequence that has the same functional capability.

[0247] In the context of a natural TCR, full activation generally requires not only signaling through the TCR, but also a costimulatory signal. Thus, in some embodiments, to promote full activation, a component for generating secondary or co-stimulatory signal is also included in the CAR. In other embodiments, the CAR does not include a component for generating a costimulatory signal. In some aspects, an additional CAR is expressed in the same cell and provides the component for generating the secondary or costimulatory signal.

[0248] T cell activation is in some aspects described as being mediated by two classes of cytoplasmic signaling sequences: those that initiate antigen-dependent primary activation through the TCR (primary cytoplasmic signaling sequences), and those that act in an antigen-independent manner to provide a secondary or co-stimulatory signal (secondary cytoplasmic signaling sequences). In some aspects, the CAR includes one or both of such classes of cytoplasmic signaling sequences.

[0249] In some aspects, the CAR includes a primary cytoplasmic signaling sequence that regulates primary stimulation and/or activation of the TCR complex. Primary cytoplasmic signaling sequences that act in a stimulatory manner may contain signaling motifs which are known as immunoreceptor tyrosine-based activation motifs or ITAMs. Examples of ITAM containing primary cytoplasmic signaling sequences include those derived from TCR or CD3 zeta, FcR gamma, CD3 gamma, CD3 delta and CD3 epsilon. In some embodiments, the intracellular signaling region or domain in the CAR contain(s) a cytoplasmic signaling domain, portion thereof, or sequence derived from CD3 zeta. In some embodiments the CD3 zeta comprises the sequence of amino acids set forth in SEQ ID NO: 628, encoded by the nucleic acid sequence set forth in SEQ ID NO: 627 or SEQ ID NO: 652.

[0250] In some embodiments, the CAR includes a signaling domain (*e.g.*, an intracellular or cytoplasmic signaling domain) and/or transmembrane portion of a costimulatory molecule, such as a T cell costimulatory molecule. Exemplary costimulatory molecules include CD28, 4-1BB, OX40, DAP10, and ICOS. For example, a costimulatory molecule can be derived from 4-1BB and can comprise the amino acid sequence set forth in SEQ ID NO: 626, encoded by the nucleotide sequence set forth in SEQ ID NO: 625 or SEQ ID NO: 681. In some aspects, the same CAR includes both the stimulatory or activating components (*e.g.*, cytoplasmic signaling sequence) and costimulatory components.

[0251] In some embodiments, the stimulatory or activating components are included within one CAR, whereas the costimulatory component is provided by another CAR recognizing another antigen. In some embodiments, the CARs include activating or stimulatory CARs, and costimulatory CARs, both expressed on the same cell (see WO2014/055668). In some aspects, the BCMA-targeting CAR is the stimulatory or activating CAR; in other aspects, it is the costimulatory CAR. In some embodiments, the cells further include inhibitory CARs (iCARs, see Fedorov *et al.*, Sci. Transl. Medicine, 5(215) (December, 2013), such as a CAR recognizing an antigen other than BCMA, whereby a stimulatory or an activating signal delivered through the BCMA-targeting CAR is diminished or inhibited by binding of the inhibitory CAR to its ligand, *e.g.*, to reduce off-target effects.

[0252] In certain embodiments, the intracellular signaling region comprises a CD28 transmembrane and signaling domain linked to a CD3 (*e.g.*, CD3-zeta) intracellular domain. In some embodiments, the intracellular signaling domain comprises a chimeric CD28 and CD137 (4-1BB, TNFRSF9) co-stimulatory domains, linked to a CD3 zeta intracellular domain.

[0253] In some embodiments, the CAR encompasses one or more, *e.g.*, two or more, costimulatory domains and a stimulatory or activation domain, *e.g.*, primary activation domain, in the cytoplasmic portion. Exemplary CARs include intracellular components of CD3-zeta, CD28, and 4-1BB.

[0254] In some embodiments, the provided chimeric antigen receptor comprises: (a) an extracellular antigen-binding domain that specifically recognizes B cell maturation antigen (BCMA), such as any any antigen-binding domain described herein; (b) a spacer of at least 125 amino acids in length; (c) a transmembrane domain; and (d) an intracellular signaling region. In some embodiments, the antigen-binding domain of such receptor, comprising a V_H region and a V_L region comprising the amino acid sequence of SEQ ID NOs:617 and 618, respectively, or a sequence of amino acids having at least 90% identity to SEQ ID NOS:617 and 618, respectively. In some embodiments, the antigen-binding domain of such receptor, comprising a V_H region that is or comprises a CDR-H1, CDR-H2 and CDR-H3 contained within the V_H region amino acid sequence of SEQ ID NO: 617; and a V_L region that is or comprises a CDR-L1, CDR-L2 and CDR-L3 contained within the V_L region amino acid sequence of SEQ ID NO: 618. In some embodiments, the antigen-binding domain of such receptor, comprising a V_H region comprising a CDR-H1, CDR-H2, and CDR-H3 comprising SEQ ID NOS:593, 594, and 595, respectively, and a V_L region comprising a CDR-L1, CDR-L2, and CDR-L3 comprising SEQ ID NOS:601, 602, and 603, respectively. In some embodiments, the antigen-binding domain of such receptor, comprising a V_H region comprising a CDR-H1, CDR-H2, and CDR-H3 comprising SEQ ID NOS:596, 597, and 595, respectively, and a V_L region comprising a CDR-L1, CDR-L2, and CDR-L3 comprising SEQ ID NOS:601, 602, and 603, respectively. In some embodiments, the antigen-binding domain of such receptor, comprising a V_H region comprising a CDR-H1, CDR-H2, and CDR-H3 comprising SEQ ID NOS: 598, 599, and 595, respectively, and a V_L region comprising a CDR-L1, CDR-L2, and CDR-L3 comprising SEQ ID NOS:601, 602, and 603, respectively. In some embodiments, the antigen-binding domain of such receptor, comprising a V_H region comprising a CDR-H1, CDR-H2, and CDR-H3 comprising SEQ ID NOS: 611, 612, and 613, respectively, and a V_L region comprising a CDR-L1, CDR-L2, and CDR-L3 comprising SEQ ID NOS: 614, 615, and 603, respectively. In some embodiments, the antigen-binding domain of such receptor, comprising a V_H region that is or comprises the amino acid sequence of SEQ ID NO: 617; and a V_L region that is or comprises the amino acid sequence of SEQ ID NO: 618. In some embodiments, the antigen-binding domain of such receptor, comprising the amino acid sequence of SEQ ID NO: 478. In some embodiments, the

intracellular signaling region includes an stimulating cytoplasmic signaling domain. In some embodiments, the stimulating cytoplasmic signaling domain is capable of inducing a primary activation signal in a T cell, is a T cell receptor (TCR) component and/or includes an immunoreceptor tyrosine-based activation motif (ITAM). In some embodiments, the stimulating cytoplasmic signaling domain is or includes a cytoplasmic signaling domain of a CD3-zeta (CD3ζ) chain or a functional variant or signaling portion thereof. In some embodiments, the stimulating cytoplasmic domain is human or is derived from a human protein. In some embodiments, the stimulating cytoplasmic domain is or includes the sequence set forth in SEQ ID NO:628 or a sequence of amino acids that has at least 90% sequence identity to SEQ ID NO:628. In some embodiments, the nucleic acid encoding the stimulating 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 embodiments, the nucleic acid encoding the stimulating cytoplasmic signaling domain is or includes the sequence set forth in SEQ ID NO:652. In some embodiments, the intracellular signaling region further includes a costimulatory signaling region. In some embodiments, the costimulatory signaling region includes an intracellular signaling domain of a T cell costimulatory molecule or a signaling portion thereof. In some embodiments, the costimulatory signaling region includes an intracellular signaling domain of a CD28, a 4-1BB or an ICOS or a signaling portion thereof. In some embodiments, the costimulatory signaling region includes an intracellular signaling domain of 4-1BB. In some embodiments, the costimulatory signaling region is human or is derived from a human protein. In other embodiments, the costimulatory signaling region is or includes the sequence set forth in SEQ ID NO: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 embodiments, 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 embodiments, the nucleic acid encoding the costimulatory signaling region includes the sequence set forth in SEQ ID NO:681. In some embodiments, the costimulatory signaling region is between the transmembrane domain and the intracellular signaling region. In some embodiments, the transmembrane domain is or includes a transmembrane domain derived from CD4, CD28, or CD8. In some embodiments, the transmembrane domain is or includes a transmembrane domain derived from a CD28. In some embodiments, the transmembrane domain is human or is derived from a human protein. In other embodiments, the transmembrane domain is or includes the sequence set forth in SEQ ID

NO:624 or a sequence of amino acids that exhibits at least 90% sequence identity to SEQ ID NO:624.

[0255] 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 (CD3ζ) chain and an intracellular signaling domain of a T cell costimulatory molecule. Also provided are polynucleotides encoding such a chimeric antigen receptor.

[0256] In some embodiments, the V_H region comprises a CDR-H1, CDR-H2 and CDR-H3 contained within the V_H region sequence of SEQ ID NO: 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.

[0257] 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 (CD3ζ) chain and an intracellular signaling domain of a T cell costimulatory molecule, optionally from a human 4-1BB or a human CD28. Also provided are polynucleotides encoding such a chimeric antigen receptor. In some embodiments, the extracellular antigen-binding domain comprises the V_H region sequence of SEQ ID NO:617 and the V_L region sequence of SEQ ID NO:618. In some embodiments, the antigen-binding domain of such receptor, comprising the amino acid sequence of SEQ ID NO: 478. In some embodimnents, other domains, regions, or components of the chimeric antigen receptor includes any domains, regions, or components described herein.

4. Surrogate marker

[0258] In some embodiments, the CAR further includes a surrogate marker, such as a cell surface marker (e.g., a truncated cell surface marker), which may be used to confirm transduction or engineering of the cell to express the receptor. For example, in some aspects,

extrinsic marker genes are utilized in connection with engineered cell therapies to permit detection or selection of cells and, in some cases, also to promote cell suicide by ADCC. Exemplary marker genes include truncated epidermal growth factor receptor (EGFRt), which can be co-expressed with a transgene of interest (e.g., a CAR or TCR) in transduced cells (see, e.g., U.S. Patent No. 8,802,374). EGFRt contains an epitope recognized by the antibody cetuximab (Erbitux®). For this reason, Erbitux® can be used to identify or select cells that have been engineered with the EGFRt construct, including in cells also co-engineered with another recombinant receptor, such as a chimeric antigen receptor (CAR). Additionally, EGFRt is commonly used as a suicide mechanism in connection with cell therapies. In some aspects, when EGFRt is co-expressed in cells with a transgene of interest (e.g. CAR or TCR), it can be targeted by the cetuximab monoclonal antibody to reduce or deplete the transferred gene-modified cells via ADCC (see U.S. Patent No. 8,802,374 and Liu et al., Nature Biotech. 2016 April; 34(4): 430–434). Importantly, the suicide killing approach using tEGFR requires availability of the antibody epitope. Another example of such a marker gene is prostate-specific membrane antigen (PSMA) or a modified form thereof. PSMA or modified forms thereof may comprise a sequence of amino acids bound by or recognized by a PSMA-targeting molecule, such as an antibody or an antigen-binding fragment thereof. PSMA-targeting molecules can be used to identify or select cells that have been engineered with a PSMA or modified construct, including in cells also co-engineered with another recombinant receptor, such as a chimeric antigen receptor (CAR) provided herein. In some aspects, the marker includes all or part (*e.g.*, truncated form) of CD34, a nerve growth factor receptor (NGFR), epidermal growth factor receptor (*e.g.*, EGFR), or PSMA.

[0259] Exemplary surrogate markers can include truncated forms of cell surface polypeptides, such as truncated forms that are non-functional and to not transduce or are not capable of transducing a signal or a signal ordinarily transduced by the full-length form of the cell surface polypeptide, and/or do not or are not capable of internalizing. Exemplary truncated cell surface polypeptides including truncated forms of growth factors or other receptors such as a truncated human epidermal growth factor receptor 2 (tHER2), a truncated epidermal growth factor receptor (tEGFR, exemplary tEGFR sequence set forth in SEQ ID NO:11 or 76) or a prostate-specific membrane antigen (PSMA) or modified form thereof. tEGFR may contain an epitope recognized by the antibody cetuximab (Erbitux®) or other therapeutic anti-EGFR antibody or binding molecule, which can be used to identify or select cells that have been engineered with the tEGFR construct and an encoded exogenous protein, and/or to eliminate or

separate cells expressing the encoded exogenous protein. See U.S. Patent No. 8,802,374 and Liu et al., Nature Biotech. 2016 April; 34(4): 430–434). In some aspects, the marker, *e.g.* surrogate marker, includes all or part (*e.g.*, truncated form) of CD34, a NGFR, a CD19 or a truncated CD19, e.g., a truncated non-human CD19, or epidermal growth factor receptor (*e.g.*, tEGFR). In some embodiments, the marker is or comprises a fluorescent protein, such as green fluorescent protein (GFP), enhanced green fluorescent protein (EGFP), such as super-fold GFP (sfGFP), red fluorescent protein (RFP), such as tdTomato, mCherry, mStrawberry, AsRed2, DsRed or DsRed2, cyan fluorescent protein (CFP), blue green fluorescent protein (BFP), enhanced blue fluorescent protein (EBFP), and yellow fluorescent protein (YFP), and variants thereof, including species variants, monomeric variants, and codon-optimized and/or enhanced variants of the fluorescent proteins. In some embodiments, the marker is or comprises an enzyme, such as a luciferase, the lacZ gene from *E. coli*, alkaline phosphatase, secreted embryonic alkaline phosphatase (SEAP), chloramphenicol acetyl transferase (CAT). Exemplary light-emitting reporter genes include luciferase (luc), β-galactosidase, chloramphenicol acetyltransferase (CAT), β-glucuronidase (GUS) or variants thereof.

[0260] In some embodiments, the marker is a selection marker. In some embodiments, the selection marker is or comprises a polypeptide that confers resistance to exogenous agents or drugs. In some embodiments, the selection marker is an antibiotic resistance gene. In some embodiments, the selection marker is an antibiotic resistance gene confers antibiotic resistance to a mammalian cell. In some embodiments, the selection marker is or comprises a Puromycin resistance gene, a Hygromycin resistance gene, a Blasticidin resistance gene, a Neomycin resistance gene, a Geneticin resistance gene or a Zeocin resistance gene or a modified form thereof.

[0261] In some embodiments, the nucleic acid encoding the marker is operably linked to a polynucleotide encoding for a linker sequence, such as a cleavable linker sequence, *e.g.*, T2A. See WO2014031687. In some embodiments, introduction of a construct encoding the CAR and surrogate marker, separated by a T2A ribosome switch, can express two proteins from the same construct, such that the surrogate marker can be used as a marker to detect cells expressing such construct. In some embodiments, the surrogate marker, and optionally a linker sequence, can be any as disclosed in international publication no. WO2014031687. For example, the marker can be a truncated EGFR (tEGFR) or PSMA that is, optionally, linked to a linker sequence, such as a 2A cleavable linker sequence (e.g., a T2A, P2A, E2A or F2A cleavable linker, described elsewhere herein). An exemplary polypeptide for a truncated EGFR surrogate marker comprises

the sequence of amino acids set forth in SEQ ID NO: 634 or a sequence of amino acids that exhibits at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to SEQ ID NO: 634. In some embodiments, the spacer is or comprises a glycine-serine rich sequence or other flexible linker such as known flexible linkers.

[0262] In some embodiments, the marker is a molecule, *e.g.*, cell surface protein, not naturally found on T cells or not naturally found on the surface of T cells, or a portion thereof.

[0263] In some embodiments, the molecule is a non-self molecule, *e.g.*, non-self protein, *i.e.*, one that is not recognized as "self" by the immune system of the host into which the cells will be adoptively transferred.

[0264] In some embodiments, the marker serves no therapeutic function and/or produces no effect other than to be used as a marker for genetic engineering, *e.g.*, for selecting cells successfully engineered. In other embodiments, the marker may be a therapeutic molecule or molecule otherwise exerting some desired effect, such as a ligand for a cell to be encountered *in vivo*, such as a costimulatory or immune checkpoint molecule to enhance and/or dampen responses of the cells following adoptive transfer and encounter with ligand.

[0265] In some cases, CARs are referred to as first, second, and/or third generation CARs. In some aspects, a first generation CAR is one that solely provides a CD3-chain induced signal upon or in response to antigen binding; in some aspects, a second-generation CARs is one that provides such a signal and costimulatory signal, such as one including an intracellular signaling domain from a costimulatory receptor such as CD28 or CD137; in some aspects, a third generation CAR in some aspects is one that includes multiple costimulatory domains of different costimulatory receptors.

[0266] In some embodiments, the chimeric antigen receptor includes an extracellular portion containing the antibody or fragment described herein. In some aspects, the chimeric antigen receptor includes an extracellular portion containing the antibody or fragment described herein and an intracellular signaling domain. In some embodiments, the antibody or fragment includes an scFv or a single-domain antibody comprising only the V_H region and the intracellular signaling domain contains an ITAM. In some aspects, the intracellular signaling domain includes a signaling domain of a zeta chain of a CD3-zeta (CD3ζ) chain. In some embodiments, the chimeric antigen receptor includes a transmembrane domain linking the extracellular domain and the intracellular signaling domain. In some aspects, the transmembrane domain contains a transmembrane portion of CD28. The extracellular domain and transmembrane can be linked

directly or indirectly. In some embodiments, the extracellular domain and transmembrane are linked by a spacer, such as any described herein. In some embodiments, the chimeric antigen receptor contains an intracellular domain of a co-stimulatory molecule (*e.g.*, T cell costimulatory molecule), such as between the transmembrane domain and intracellular signaling domain. In some aspects, the T cell costimulatory molecule is CD28 or 4-1BB.

[0267] In some embodiments, the transmembrane domain of the receptor (*e.g.*, CAR) is a transmembrane domain of human CD28 or variant thereof, *e.g.*, a 27-amino acid transmembrane domain of a human CD28 (Accession No.: P10747.1). In some embodiments, the intracellular signaling domain comprises an intracellular costimulatory signaling domain of human CD28 or functional variant thereof, such as a 41 amino acid domain thereof and/or such a domain with an LL to GG substitution at positions 186-187 of a native CD28 protein. In some embodiments, the intracellular domain comprises an intracellular costimulatory signaling domain of 4-1BB or functional variant thereof, such as a 42-amino acid cytoplasmic domain of a human 4-1BB (Accession No. Q07011.1). In some embodiments, the intracellular signaling domain comprises a human CD3 zeta stimulatory signaling domain or functional variant thereof, such as an 112 AA cytoplasmic domain of isoform 3 of human CD3ζ (Accession No.: P20963.2) or a CD3 zeta signaling domain as described in U.S. Patent No.: 7,446,190.

[0268] For example, in some embodiments, the CAR includes a BCMA antibody or fragment, such as any of the human BCMA antibodies, including sdAbs and scFvs, described herein, a spacer such as any of the Ig-hinge containing spacers, a CD28 transmembrane domain, a CD28 intracellular signaling domain, and a CD3 zeta signaling domain. In some embodiments, the CAR includes the BCMA antibody or fragment, such as any of the human BCMA antibodies, including sdAbs and scFvs described herein, a spacer such as any of the Ig-hinge containing spacers, a CD28 transmembrane domain, a 4-1BB intracellular signaling domain, and a CD3 zeta signaling domain. In some embodiments, such CAR constructs further includes a T2A ribosomal skip element and/or a tEGFR sequence, *e.g.*, downstream of the CAR.

[0269] In certain embodiments, multispecific binding molecules, e.g., multispecific chimeric receptors, such as multispecific CARs, can contain any of the multispecific antibodies, including, *e.g.* bispecific antibodies, multispecific single-chain antibodies, *e.g.*, diabodies, triabodies, and tetrabodies, tandem di-scFvs, and tandem tri-scFvs, such as any described above in Section I.A.

B. Exemplary features

[0270] In some aspects, the antibodies or antigen-binding fragments thereof, in the provided CARs, have one or more specified functional features, such as binding properties, including recognizing or binding to particular epitopes, such as to epitopes that are similar to or overlap with those specifically bound by other antibodies such as reference antibodies, or epitopes that are different from those specifically bound by other antibodies such as reference antibodies, the ability to compete for binding with other antibodies such as reference antibodies, and/or particular binding affinities. In other embodiments, the antibodies or antigen-binding fragments thereof, in the provided CARs, recognize, such as specifically recognize, or bind, *e.g.*, specifically bind, to epitopes that are different from, or do not overlap with those specifically bound by other antibodies such as reference antibodies. For example, the epitopes specifically bound by the antibodies, in the provided CARs, are different from those specifically bound by other antibodies such as reference antibodies. In some embodiments, the antibodies and antigen binding fragments thereof do not directly compete for, or compete to a lower degree, with binding with other antibodies such as reference antibodies.

[0271] In some embodiments, the antibodies or antigen-binding fragments thereof specifically recognize or specifically bind to BCMA protein. In any of the embodiments, an antibody or antigen binding fragment, in the provided CARs, that specifically recognize BCMA, specifically binds BCMA. In some embodiments provided herein, BCMA protein refers to human BCMA, a mouse BCMA protein, or a non-human primate (*e.g.*, cynomolgus monkey) BCMA protein. In some embodiments of any of the embodiments herein, BCMA protein refers to human BCMA protein. The observation that an antibody or other binding molecule binds to BCMA protein or specifically binds to BCMA protein does not necessarily mean that it binds to a BCMA protein of every species. For example, in some embodiments, features of binding to BCMA protein, such as the ability to specifically bind thereto and/or to compete for binding thereto with a reference antibody, and/or to bind with a particular affinity or compete to a particular degree, in some embodiments, refers to the ability with respect to a human BCMA protein and the antibody may not have this feature with respect to a BCMA protein of another species, such as mouse.

[0272] In some embodiments, the antibody or antigen-binding fragment binds to a mammalian BCMA protein, including to naturally occurring variants of BCMA, such as certain splice variants or allelic variants.

[0273] In some embodiments, the antibodies specifically bind to human BCMA protein, such as to an epitope or region of human BCMA protein, such as the human BCMA protein comprising the amino acid sequence of SEQ ID NO:367 (GenBank No. BAB60895.1), or SEQ ID NO:368 (NCBI No. NP_001183.2) or an allelic variant or splice variant thereof. In one embodiment, the human BCMA protein is encoded by a transcript variant or is an isoform that has the sequence of amino acids forth in SEQ ID NO:369. In some embodiments, the antibodies bind to cynomolgus monkey BCMA protein, such as the cynomolgus monkey BCMA protein set forth in SEQ ID NO:371 (GenBank No. EHH60172.1). In some embodiments, the antibodies bind to human BCMA but do not bind to or bind in a lower level or degree or affinity to cynomolgus monkey BCMA protein, such as the cynomolgus monkey BCMA protein set forth in SEQ ID NO:371 (GenBank No. EHH60172.1). In some embodiments, the antibodies do not bind to or bind in a lower level or degree or affinity to mouse BCMA protein, such as the mouse BCMA protein set forth in SEQ ID NO:370 (NCBI No. NP_035738.1). In some embodiments, the antibodies bind to mouse BCMA protein, such as the mouse BCMA protein set forth in SEQ ID NO:370 (NCBI No. NP_035738.1). In some embodiments, the antibodies bind to mouse BCMA protein, with lower affinity than its binding to a human BCMA protein and/or a cynomolgus monkey BCMA protein. In some embodiments, the antibodies bind to mouse BCMA protein and/or a cynomolgus monkey BCMA protein with lower affinity than its binding to a human BCMA protein. In some embodiments, the antibodies bind to mouse BCMA protein and/or a cynomolgus monkey BCMA protein with similar binding affinity compared to its binding to a human BCMA protein.

[0274] In some embodiments, the provided antigen-binding domain or CAR exhibits preferential binding to membrane-bound BCMA as compared to soluble BCMA. In some embodiments, the provided antigen-binding domain or CAR exhibits greater binding affinity for, membrane-bound BCMA compared to soluble BCMA.

[0275] In one embodiment, the extent of binding of an anti-BCMA antibody or antigen-binding domain or CAR to an unrelated, non-BCMA protein, such as a non-human BCMA protein or other non-BCMA protein, is less than at or about 10% of the binding of the antibody or antigen-binding domain or CAR to human BCMA protein or human membrane-bound BCMA as measured, *e.g.*, by a radioimmunoassay (RIA). In some embodiments, among the antibodies or antigen-binding domains in the provided CARs, are antibodies or antigen-binding domains or CARs in which binding to mouse BCMA protein is less than or at or about 10% of the binding of the antibody to human BCMA protein. In some embodiments, among the

antibodies or antigen-binding domains in the provided CARs, are antibodies in which binding to cynomolgus monkey BCMA protein is less than or at or about 10% of the binding of the antibody to human BCMA protein. In some embodiments, among the antibodies or antigen-binding domains in the provided CARs, are antibodies in which binding to cynomolgus monkey BCMA protein and/or a mouse BCMA protein is similar to or about the same as the binding of the antibody to human BCMA protein. In some embodiments, among the antibodies or antigen-binding domains in the provided CARs, are antibodies or antigen-binding domains or CARs in which binding to solubleBCMA protein is less than or at or about 10% of the binding of the antibody to membrane-bound BCMA protein.

[0276] In some embodiments, the antibody specifically binds to, and/or competes for binding thereto with a reference antibody, and/or binds with a particular affinity or competes to a particular degree, to a BCMA protein, *e.g.*, human BCMA, a mouse BCMA protein, or a non-human primate (*e.g.*, cynomolgus monkey) BCMA protein.

[0277] In some embodiments, the antibodies, in the provided CARs, are capable of binding BCMA protein, such as human BCMA protein, with at least a certain affinity, as measured by any of a number of known methods. In some embodiments, the affinity is represented by an equilibrium dissociation constant (K_D); in some embodiments, the affinity is represented by EC_{50}.

[0278] A variety of assays are known for assessing binding affinity and/or determining whether a binding molecule (*e.g.*, an antibody or fragment thereof) specifically binds to a particular ligand (*e.g.*, an antigen, such as a BCMA protein). It is within the level of a skilled artisan to determine the binding affinity of a binding molecule, *e.g.*, an antibody, for an antigen, *e.g.*, BCMA, such as human BCMA or cynomolgus BCMA or mouse BCMA, such as by using any of a number of binding assays that are well known in the art. For example, in some embodiments, a BIAcore® instrument can be used to determine the binding kinetics and constants of a complex between two proteins (*e.g.*, an antibody or fragment thereof, and an antigen, such as a BCMA protein), using surface plasmon resonance (SPR) analysis (*see*, *e.g.*, Scatchard *et al.*, *Ann. N.Y. Acad. Sci. 51*:660, 1949; Wilson, *Science 295*:2103, 2002; Wolff *et al.*, *Cancer Res. 53*:2560, 1993; and U.S. Patent Nos. 5,283,173, 5,468,614, or the equivalent).

[0279] SPR measures changes in the concentration of molecules at a sensor surface as molecules bind to or dissociate from the surface. The change in the SPR signal is directly proportional to the change in mass concentration close to the surface, thereby allowing measurement of binding kinetics between two molecules. The dissociation constant for the

complex can be determined by monitoring changes in the refractive index with respect to time as buffer is passed over the chip. Other suitable assays for measuring the binding of one protein to another include, for example, immunoassays such as enzyme linked immunosorbent assays (ELISA) and radioimmunoassays (RIA), or determination of binding by monitoring the change in the spectroscopic or optical properties of the proteins through fluorescence, UV absorption, circular dichroism, or nuclear magnetic resonance (NMR). Other exemplary assays include, but are not limited to, Western blot, ELISA, analytical ultracentrifugation, spectroscopy, flow cytometry, sequencing and other methods for detection of expressed polynucleotides or binding of proteins.

[0280] In some embodiments, the binding molecule, *e.g.*, antibody or fragment thereof or antigen-binding domain of a CAR, binds, such as specifically binds, to an antigen, *e.g.*, a BCMA protein or an epitope therein, with an affinity or K_A (*i.e.*, an equilibrium association constant of a particular binding interaction with units of 1/M; equal to the ratio of the on-rate [k_{on} or k_a] to the off-rate [k_{off} or k_d] for this association reaction, assuming bimolecular interaction) equal to or greater than 10^5 M^{-1}. In some embodiments, the antibody or fragment thereof or antigen-binding domain of a CAR exhibits a binding affinity for the peptide epitope with a K_D (*i.e.*, an equilibrium dissociation constant of a particular binding interaction with units of M; equal to the ratio of the off-rate [k_{off} or k_d] to the on-rate [k_{on} or k_a] for this association reaction, assuming bimolecular interaction) of equal to or less than 10^{-5} M. For example, the equilibrium dissociation constant K_D ranges from 10^{-5} M to 10^{-13} M, such as 10^{-7} M to 10^{-11} M, 10^{-8} M to 10^{-10} M, or 10^{-9} M to 10^{-10} M. The on-rate (association rate constant; k_{on} or k_a; units of 1/Ms) and the off-rate (dissociation rate constant; k_{off} or k_d; units of 1/s) can be determined using any of the assay methods known in the art, for example, surface plasmon resonance (SPR).

[0281] In some embodiments, the binding affinity (EC_{50}) and/or the dissociation constant of the antibody (*e.g.* antigen-binding fragment) or antigen-binding domain of a CAR to about BCMA protein, such as human BCMA protein, is from or from about 0.01 nM to about 500 nM, from or from about 0.01 nM to about 400 nM, from or from about 0.01 nM to about 100 nM, from or from about 0.01 nM to about 50 nM, from or from about 0.01 nM to about 10 nM, from or from about 0.01 nM to about 1 nM, from or from about 0.01 nM to about 0.1 nM, is from or from about 0.1 nM to about 500 nM, from or from about 0.1 nM to about 400 nM, from or from about 0.1 nM to about 100 nM, from or from about 0.1 nM to about 50 nM, from or from about 0.1 nM to about 10 nM, from or from about 0.1 nM to about 1 nM, from or from about 0.5 nM to about 200 nM, from or from about 1 nM to about 500 nM, from or from about 1 nM to about

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

[0282] In some embodiments, the binding affinity may be classified as high affinity or as low affinity. In some cases, the binding molecule (*e.g.* antibody or fragment thereof) or antigen-binding domain of a CAR that exhibits low to moderate affinity binding exhibits a K_A of up to 10^7 M^{-1}, up to 10^6 M^{-1}, up to 10^5 M^{-1}. In some cases, a binding molecule (*e.g.* antibody or fragment thereof) that exhibits high affinity binding to a particular epitope interacts with such epitope with a K_A of at least 10^7 M^{-1}, at least 10^8 M^{-1}, at least 10^9 M^{-1}, at least 10^{10} M^{-1}, at least 10^{11} M^{-1}, at least 10^{12} M^{-1}, or at least 10^{13} M^{-1}. In some embodiments, the binding affinity (EC_{50}) and/or the equilibrium dissociation constant, K_D, of the binding molecule, *e.g.*, anti-BCMA antibody or fragment thereof or antigen-binding domain of a CAR, to a BCMA protein, is from or from about 0.01 nM to about 1 μM, 0.1 nM to 1 μM, 1 nM to 1 μM, 1 nM to 500 nM, 1 nM to 100 nM, 1 nM to 50 nM, 1 nM to 10 nM, 10 nM to 500 nM, 10 nM to 100 nM, 10 nM to 50 nM, 50 nM to 500 nM, 50 nM to 100 nM or 100 nM to 500 nM. In certain embodiments, the binding affinity (EC_{50}) and/or the dissociation constant of the equilibrium dissociation constant, K_D, of the binding molecule, *e.g.*, anti-BCMA antibody or fragment thereof or antigen-binding domain of a CAR, to a BCMA protein, is at or about or less than at or about 1 μM, 500 nM, 100 nM, 50 nM, 40 nM, 30 nM, 25 nM, 20 nM, 19 nM, 18 nM, 17 nM, 16 nM, 15 nM, 14 nM, 13 nM, 12 nM, 11 nM, 10 nM, 9 nM, 8 nM, 7 nM, 6 nM, 5 nM, 4 nM, 3 nM, 2 nM, or 1 nM or less. The degree of affinity of a particular antibody can be compared with the affinity of a known antibody, such as a reference antibody.

[0283] In some embodiments, the binding affinity of a binding molecule, such as an anti-BCMA antibody or antigen-binding domain of a CAR, for different antigens, *e.g.*, BCMA proteins from different species can be compared to determine the species cross-reactivity. For example, species cross-reactivity can be classified as high cross reactivity or low cross reactivity. In some embodiments, the equilibrium dissociation constant, K_D, for different antigens, *e.g.*, BCMA proteins from different species such as human, cynomolgus monkey or mouse, can be compared to determine species cross-reactivity. In some embodiments, the species cross-reactivity of an anti-BCMA antibody or antigen-binding domain of a CAR can be high, *e.g.*, the anti-BCMA antibody binds to human BCMA and a species variant BCMA to a similar degree, *e.g.*, the ratio of K_D for human BCMA and K_D for the species variant BCMA is or is about 1. In some embodiments, the species cross-reactivity of an anti-BCMA antibody or antigen-binding domain of a CAR can be low, *e.g.*, the anti-BCMA antibody has a high affinity for human BCMA but a low affinity for a species variant BCMA, or vice versa. For example, the ratio of K_D for the species variant BCMA and K_D for the human BCMA is more than 10, 15, 20, 25, 30, 40, 50, 60, 70, 80, 90, 100, 200, 500, 1000, 2000 or more, and the anti-BCMA antibody has low species cross-reactivity. The degree of species cross-reactivity can be compared with the species cross-reactivity of a known antibody, such as a reference antibody.

[0284] In some embodiments, the binding affinity of the anti-BCMA antibody or antigen-binding domain of a CAR, for different form or topological type of antigens, *e.g.*, soluble BCMA protein compared to the binding affinity to a membrane-bound BCMA, to determine the preferential binding or relative affinity for a particular form or topological type. For example, in some aspects, the provided anti-BCMA antibodies or antigen-binding domains can exhibit preferential binding to membrane-bound BCMA as compared to soluble BCMA and/or exhibit greater binding affinity for, membrane-bound BCMA compared to soluble BCMA. In some embodiments, the equilibrium dissociation constant, K_D, for different form or topological type of BCMA proteins, can be compared to determine preferential binding or relative binding affinity. In some embodiments, the preferential binding or relative affinity to a membrane-bound BCMA compared to soluble BCMA can be high. For example, in some cases, the ratio of K_D for soluble BCMA and the K_D for membrane-bound BCMA is more than 10, 15, 20, 25, 30, 40, 50, 60, 70, 80, 90, 100, 200, 500, 1000, 2000 or more and the antibody or antigen-binding domain preferentially binds or has higher binding affinity for membrane-bound BCMA. In some cases, the ratio of K_A for membrane-bound BCMA and the K_A for soluble BCMA is more than 10, 15, 20, 25, 30, 40, 50, 60, 70, 80, 90, 100, 200, 500, 1000, 2000 or more and the antibody or

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

[0285] In some embodiments, the antibodies or antigen binding fragments thereof, in the provided CARs, bind to a similar degree to a human BCMA protein and a non-human BCMA protein or other non-BCMA proteins. For example, in some embodiments, the antibodies or antigen binding fragments thereof or antigen-binding domain of a CAR bind to a human BCMA protein, such as the human BCMA protein comprising the amino acid sequence of SEQ ID NO:367 (GenBank No. BAB60895.1), or SEQ ID NO:368 (NCBI No. NP_001183.2) or an allelic variant or splice variant thereof, with an equilibrium dissociation constant (K_D), and to a non-human BCMA, such as a cynomolgus monkey BCMA, such as the cynomolgus monkey BCMA protein set forth in SEQ ID NO:371 (GenBank No. EHH60172.1), with a K_D that is similar, or about the same, or less than 2-fold different, or less than 5-fold different.

[0286] In some embodiments, the antibodies or antigen binding fragments thereof, in the provided CARs, bind to a similar degree to a soluble BCMA protein and a membrane-bound BCMA protein, with an equilibrium dissociation constant (K_D) that is similar, or about the same, or less than 2-fold different, or less than 5-fold different.

[0287] For example, in some embodiments, the antibodies, in the provided CARs, or antigen binding fragments thereof bind to a human BCMA with a K_D of about or less than at or about 1 μM, 500 nM, 100 nM, 50 nM, 40 nM, 30 nM, 25 nM, 20 nM, 19 nM, 18 nM, 17 nM, 16 nM, 15 nM, 14 nM, 13 nM, 12 nM, 11 nM, 10 nM, 9 nM, 8 nM, 7 nM, 6 nM, 5 nM, 4 nM, 3 nM, 2 nM, or 1 nM or less, and binds to a cynomolgus monkey BCMA with a K_D of about or less than at or about 1 μM, 500 nM, 100 nM, 50 nM, 40 nM, 30 nM, 25 nM, 20 nM, 19 nM, 18 nM, 17 nM, 16 nM, 15 nM, 14 nM, 13 nM, 12 nM, 11 nM, 10 nM, 9 nM, 8 nM, 7 nM, 6 nM, 5 nM, 4 nM, 3 nM, 2 nM, or 1 nM or less. In some embodiments, the antibodies or antigen binding fragments thereof bind to a mouse BCMA protein with a K_D of about or less than at or about 1 μM, 500 nM, 100 nM, 50 nM, 40 nM, 30 nM, 25 nM, 20 nM, 19 nM, 18 nM, 17 nM, 16 nM, 15 nM, 14 nM, 13 nM, 12 nM, 11 nM, 10 nM, 9 nM, 8 nM, 7 nM, 6 nM, 5 nM, 4 nM, 3 nM, 2 nM, or 1 nM

or less. In some embodiments, the antibodies or antigen binding fragments thereof, in the provided CARs, bind to a human BCMA, a cynomolgus monkey BCMA and a mouse BCMA with high affinity. In some embodiments, the antibodies or antigen binding fragments thereof bind to a human BCMA and cynomolgus monkey BCMA with a high affinity, and to a mouse BCMA with low affinity. In some embodiments, the antibodies or antigen binding fragments thereof bind to a human BCMA and BCMA from other species, or other variants of the BCMA protein, with high affinity.

[0288] In some embodiments, the total binding capacity (R_{max}), as measured using particular surface plasmon resonance (SPR) conditions, is used to determine the ability or capacity of binding of the antibody or antigen binding fragment thereof, to the antigen, *e.g.*, a BCMA protein, such as a human BCMA protein. For SPR analysis, the "ligand" is the immobilized target molecule on the surface of the sensor, for example, a BCMA protein, and the "analyte" is the tested molecule, *e.g.*, antibody, for binding to the "ligand". For example, the "analyte" can be any of the antibodies, or antigen binding fragments thereof, that binds to a BCMA protein. For a particular ligand and analyte pair in SPR, the R_{max} can be determined assuming a 1:1 binding stoichiometry model, for a particular condition. Binding capacity (R_{max}) was determined using the following formula: R_{max} (RU) = (analyte molecular weight)/(ligand molecular weight) × immobilized ligand level (RU). For example, in a particular SPR conditions, the R_{max} of binding between any of the antibody or antigen binding fragment thereof and a BCMA protein, such as a human BCMA or a cynomolgus BCMA, is at least or at least about 50 resonance units (RU), such as about 25 RU, 20 RU, 15 RU, 10 RU, 5 RU or 1 RU.

[0289] In some embodiments, the antibodies, such as the human antibodies, in the provided CAR, specifically bind to a particular epitope or region of BCMA protein, such as generally an extracellular epitope or region. BCMA protein is a type III membrane 184 amino acid protein that contains an extracellular domain, a transmembrane domain, and a cytoplasmic domain. With reference to a human BCMA amino acid sequence set forth in SEQ ID NO:367, the extracellular domain corresponds to amino acids 1-54, amino acids 55-77 correspond to the transmembrane domain, and amino acids 78-184 correspond to the cytoplasmic domain.

[0290] Among the provided CARs are CARs that exhibit antigen-dependent activity or signaling, i.e. signaling activity that is measurably absent or at background levels in the absence of antigen, e.g. BCMA. Thus, in some aspects, provided CARs do not exhibit, or exhibit no more than background or a tolerable or low level of, tonic signaling or antigen-independent activity or signaling in the absence of antigen, e.g. BCMA, being present. In some

embodiments, the provided anti-BCMA CAR-expressing cells exhibit biological activity or function, including cytotoxic activity, cytokine production, and ability to proliferate.

[0291] In some embodiments, biological activity or functional activity of a chimeric receptor, such as cytotoxic activity, can be measured using any of a number of known methods. The activity can be assessed or determined either in vitro or in vivo. In some embodiments, activity can be assessed once the cells are administered to the subject (e.g., human). Parameters to assess include specific binding of an engineered or natural T cell or other immune cell to antigen, e.g., in vivo, e.g., by imaging, or ex vivo, e.g., by ELISA or flow cytometry. In certain embodiments, the ability of the engineered cells to destroy target cells can be measured using any suitable method known in the art, such as cytotoxicity assays described in, for example, Kochenderfer et al., J. Immunotherapy, 32(7): 689-702 (2009), and Herman et al. J. Immunological Methods, 285(1): 25-40 (2004). In certain embodiments, the biological activity of the cells also can be measured by assaying expression and/or secretion of certain cytokines, such as interlekukin-2 (IL-2), interferon-gamma (IFNγ), interleukin-4 (IL-4), TNF-alpha (TNFα), interleukin-6 (IL-6), interleukin-10 (IL-10), interleukin-12 (IL-12), granulocyte-macrophage colony-stimulating factor (GM-CSF), CD107a, and/or TGF-beta (TGFβ). Assays to measure cytokines are well known in the art, and include but are not limited to, ELISA, intracellular cytokine staining, cytometric bead array, RT-PCR, ELISPOT, flow cytometry and bio-assays in which cells responsive to the relevant cytokine are tested for responsiveness (*e.g.* proliferation) in the presence of a test sample. In some aspects the biological activity is measured by assessing clinical outcome, such as reduction in tumor burden or load.

[0292] In some aspects, a reporter cell line can be employed to monitor antigen-independent activity and/or tonic signaling through anti-BCMA CAR-expressing cells. In some embodiments, a T cell line, such as a Jurkat cell line, contains a reporter molecule, such as a fluorescent protein or other detectable molecule, such as a red fluorescent protein, expressed under the control of the endogenous Nur77 transcriptional regulatory elements. In some embodiments, the Nur77 reporter expression is cell intrinsic and dependent upon signaling through a recombinant reporter containing a primary activation signal in a T cell, a signaling domain of a T cell receptor (TCR) component, and/or a signaling domain comprising an immunoreceptor tyrosine-based activation motif (ITAM), such as a CD3ζ chain. Nur77 expression is generally not affected by other signaling pathways such as cytokine signaling or toll-like receptor (TLR) signaling, which may act in a cell extrinsic manner and may not depend on signaling through the recombinant receptor. Thus, only cells that express the exogenous

recombinant receptor, e.g. anti-BCMA CAR, containing the appropriate signaling regions is capable of expressing Nur77 upon stimulation (e.g., binding of the specific antigen). In some cases, Nur77 expression also can show a dose-dependent response to the amount of stimulation (e.g., antigen).

[0293] In some embodiments, the provided anti-BCMA CARs exhibit improved expression on the surface of cells, such as compared to an alternative CAR that has an identical amino acid sequence but that is encoded by non-splice site eliminated and/or a codon-optimized nucleotide sequence. In some embodiments, the expression of the recombinant receptor on the surface of the cell can be assessed. Approaches for determining expression of the recombinant receptor on the surface of the cell may include use of chimeric antigen receptor (CAR)-specific antibodies (e.g., Brentjens et al., Sci. Transl. Med. 2013 Mar; 5(177): 177ra38), Protein L (Zheng et al., J. Transl. Med. 2012 Feb; 10:29), epitope tags, and monoclonal antibodies that specifically bind to a CAR polypeptide (see international patent application Pub. No. WO2014190273). In some embodiments, the expression of the recombinant receptor on the surface of the cell, e.g., primary T cell, can be assessed, for example, by flow cytometry, using binding molecules that can bind to the recombinant receptor or a portion thereof that can be detected. In some embodiments, the binding molecules used for detecting expression of the recombinant receptor an anti-idiotypic antibody, e.g., an anti-idiotypic agonist antibody specific for a binding domain, e.g., scFv, or a portion thereof. In some embodiments, the binding molecule is or comprises an isolated or purified antigen, e.g., recombinantly expressed antigen.

C. Multispecific antibodies

[0294] In certain embodiments, the BCMA-binding molecules, *e.g.*, antibodies or polypeptides, such as chimeric receptors containing the same, are multispecific. Among the multispecific binding molecules are multispecific antibodies, including, *e.g.* bispecific antibodies. Multispecific binding partners, *e.g.*, antibodies, have binding specificities for at least two different sites, which may be in the same or different antigens. In certain embodiments, one of the binding specificities is for BCMA and the other is for another antigen. In some embodiments, additional binding molecules bind to and/or recognize a third, or more antigens. In certain embodiments, bispecific antibodies may bind to two different epitopes of BCMA. Bispecific antibodies may also be used to localize cytotoxic agents to cells which express BCMA. Bispecific antibodies can be prepared as full length antibodies or antibody fragments. Among the multispecific antibodies are multispecific single-chain antibodies, *e.g.*, diabodies,

triabodies, and tetrabodies, tandem di-scFvs, and tandem tri-scFvs. Also provided are multispecific chimeric receptors, such as multispecific CARs, containing the antibodies (*e.g.*, antigen-binding fragments). Also provided are multispecific cells containing the antibodies or polypeptides including the same, such as cells containing a cell surface protein including the anti-BCMA antibody and an additional cell surface protein, such as an additional chimeric receptor, which binds to a different antigen or a different epitope on BCMA.

[0295] Exemplary antigens include B cell specific antigens, other tumor-specific antigens, such as antigens expressed specifically on or associated with a leukemia (*e.g.*, B cell leukemia), lymphoma (*e.g.*, Hodgkin's lymphoma, non-Hodgkin's lymphoma, etc.), or a myeloma, *e.g.*, a multiple myeloma (MM), a plasma cell malignancy (*e.g.*, plasmacytoma). For example, antigens include those expressed specifically on or associated with B cell chronic lymphocytic leukemia (CLL), a diffuse large B-cell lymphoma (DLBCL), acute myeloid leukemia (AML), acute lymphocytic leukemia (ALL), Burkitt's lymphoma (*e.g.*, endemic Burkitt's lymphoma or sporadic Burkitt's lymphoma), mantle cell lymphoma (MCL), non-small cell lung cancer (NSCLC), chronic myeloid (or myelogenous) leukemia (CML), hairy cell leukemia (HCL), small lymphocytic lymphoma (SLL), Marginal zone lymphoma, Hodgkin lymphoma (HL), non-Hodgkin lymphoma (NHL), Anaplastic large cell lymphoma (ALCL), refractory follicular lymphoma, Waldenstrom macroglobulinemia, follicular lymphoma, small non-cleaved cell lymphoma, mucosa-associated lymphatic tissue lymphoma (MALT), marginal zone lymphoma, nodal monocytoid B cell lymphoma, immunoblastic lymphoma, large cell lymphoma, diffuse mixed cell lymphoma, pulmonary B cell angiocentric lymphoma, small lymphocytic lymphoma, primary mediastinal B cell lymphoma, lymphoplasmacytic lymphoma (LPL), neuroblastoma, renal cell carcinoma, colon cancer, colorectal cancer, breast cancer, epithelial squamous cell cancer, melanoma, myeloma such as multiple myeloma (*e.g.*, non-secretory multiple myeloma, smoldering multiple myeloma), stomach cancer, esophageal cancer, brain cancer, lung cancer (*e.g.*, small-cell lung cancer), pancreatic cancer, cervical cancer, ovarian cancer, liver cancer (*e.g.*, hepatic carcinoma, hepatoma, etc.), bladder cancer, prostate cancer, testicular cancer, thyroid cancer, uterine cancer, spleen cancer (*e.g.*, splenic lymphoma), adrenal cancer and/or head and neck cancer, and antigens expressed on T cells.

[0296] In some embodiments, among the second or additional antigens for multi-targeting strategies includes those in which at least one of the antigens is a universal tumor antigen, or a family member thereof. In some embodiments, the second or additional antigen is an antigen expressed on a tumor. In some embodiments, the BCMA-binding molecules provided herein

target an antigen on the same tumor type as the second or additional antigen. In some embodiments, the second or additional antigen may also be a universal tumor antigen or may be a tumor antigen specific to a tumor type.

[0297] Exemplary second or additional antigens include CD4, CD5, CD8, CD14, CD15, CD19, CD20, CD21, CD22, CD23, CD25, CD33, CD37, CD38, CD40, CD40L, CD46, CD52, CD54, CD74, CD80, CD126, CD138, B7, MUC-1, Ia, HM1.24, HLA-DR, tenascin, an angiogenesis factor, VEGF, PIGF, ED-B fibronectin, an oncogene, an oncogene product, CD66a-d, necrosis antigens, Ii, IL-2, T101, TAC, IL-6, ROR1, TRAIL-R1 (DR4), TRAIL-R2 (DR5), , tEGFR, Her2, L1-CAM, mesothelin, CEA, hepatitis B surface antigen, anti-folate receptor, CD24, CD30, CD44, EGFR, EGP-2, EGP-4, EPHa2, ErbB2, ErbB3, ErbB4, erbB dimers, EGFR vIII, FBP, FCRL5, FCRH5, fetal acetylcholine receptor, GD2, GD3, G protein-coupled receptor class C group 5 member D (GPRC5D), HMW-MAA, IL-22R-alpha, IL-13R-alpha2, kdr, kappa light chain, Lewis Y, L1-cell adhesion molecule (L1-CAM), Melanoma-associated antigen (MAGE)-A1, MAGE-A3, MAGE-A6, Preferentially expressed antigen of melanoma (PRAME), survivin, EGP2, EGP40, TAG72, B7-H6, IL-13 receptor a2 (IL-13Ra2), CA9, CD171, G250/CAIX, HLA-AI MAGE Al, HLA-A2 NY-ESO-1, PSCA, folate receptor-a, CD44v6, CD44v7/8, avb6 integrin, 8H9, NCAM, VEGF receptors, 5T4, Foetal AchR, NKG2D ligands, dual antigen, an antigen associated with a universal tag, a cancer-testes antigen, MUC1, MUC16, NY-ESO-1, MART-1, gp100, oncofetal antigen, VEGF-R2, carcinoembryonic antigen (CEA), prostate specific antigen, PSMA, Her2/neu, estrogen receptor, progesterone receptor, ephrinB2, CD123, c-Met, GD-2, O-acetylated GD2 (OGD2), CE7, Wilms Tumor 1 (WT-1), a cyclin, cyclin A2, CCL-1, hTERT, MDM2, CYP1B, WT1, livin, AFP, p53, cyclin (D1), CS-1, BAFF-R, TACI, CD56, TIM-3, CD123, L1-cell adhesion molecule, MAGE-A1, MAGE A3, a cyclin, such as cyclin A1 (CCNA1) and/or a pathogen-specific antigen, biotinylated molecules, molecules expressed by HIV, HCV, HBV and/or other pathogens, and/or in some aspects, neoepitopes or neoantigens thereof. In some embodiments, the antigen is associated with or is a universal tag.

[0298] In some aspects, the antigen, e.g., the second or additional antigen, such as the disease-specific antigen and/or related antigen, is expressed on multiple myeloma, such as G protein-coupled receptor class C group 5 member D (GPRC5D), CD38 (cyclic ADP ribose hydrolase), CD138 (syndecan-1, syndecan, SYN-1), CS-1 (CS1, CD2 subset 1, CRACC, SLAMF7, CD319, and 19A24), BAFF-R, TACI and/or FcRH5. Other exemplary multiple myeloma antigens include CD56, TIM-3, CD33, CD123, CD44, CD20, CD40, CD74, CD200,

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

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

[0299] Provided herein are methods for optimizing polynucleotides for expression and/or therapeutic use, and polynucleotides optimized, e.g., according to the methods. In some embodiments, the provided methods or optimizations reduce heterogeneity and/or increase homogeneity of transcribed RNA, such as messenger RNA (mRNA), for example, when the polynucleotide is expressed in a cell, such as in a particular cell type, such as in a mammalian, e.g., human cell type such as a human T cell such as a primary human T cell or T cell line. In some embodiments, the methods for optimizing polynucleotides include methods to identify and remove or alter the sequence of one or more cryptic splice site, such as one or both of a donor splice site or an acceptor splice site. In some embodiments, the methods can additionally or further include codon optimization. In some embodiments, codon optimization can be performed prior to and/or after methods of reducing heterogeneity of transcribed RNA (e.g., mRNA), such as by removal or elimination of predicted splice sites. In some embodiments, codon optimization is integrated in any one or more steps of the method of reducing heterogeneity of transcribed RNAs. In some embodiments, methods of reducing heterogeneity, such as by removal or elimination of predicted splice sites, can be performed after codon optimization.In some embodiments, provided are methods in which a polynucleotide encoding a transgene, including a polynucleotide encoding any of the provided anti-BCMA CAR

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

[0300] Genomic nucleic acid sequences generally, in nature, in a mammalian cell, undergo processing co-transcriptionally or immediately following transcription, wherein a nascent precursor messenger ribonucleic acid (pre-mRNA), transcribed from a genomic deoxyribonucleic acid (DNA) sequence, is in some cases edited by way of splicing, to remove introns, followed by ligation of the exons in eukaryotic cells. Consensus sequences for splice sites are known, but in some aspects, specific nucleotide information defining a splice site may be complex and may not be readily apparent based on available methods. Cryptic splice sites are splice sites that are not predicted based on the standard consensus sequences and are variably activated. Hence, variable splicing of pre-mRNA at cryptic splice sites leads to heterogeneity in the transcribed mRNA products following expression in eukaryotic cells.

[0301] Polynucleotides generated for the expression of transgenes are typically constructed from nucleic acid sequences, such as complementary DNA (cDNA), or portions thereof, that do not contain introns. Thus, splicing of such sequences is not expected to occur. However, the presence of cryptic splice sites within the cDNA sequence can lead to unintended or undesired splicing reactions and heterogeneity in the transcribed mRNA. Such heterogeneity results in translation of unintended protein products, such as truncated protein products with variable amino acid sequences that exhibit modified expression and/or activity.

[0302] Also provided are methods and approaches for determining the heterogeneity of a transcribed nucleic acid such as one encoding or containing a transgene or encoding a recombinant protein. In some embodiments, the methods include determining the heterogeneity of a transcribed nucleic acid sequence that includes all or a portion of the 5′ untranslated region (5′ UTR), and/or all or a portion of the 3′ untranslated region (3′ UTR), of the transcribed nucleic acid. Also provided herein are methods of identifying the presence of splice sites, such as cryptic splice sites, based on the heterogeneity of the transcribed nucleic acid. Also provided are methods of identifying a transgene candidate for the removal of splice sites, such as cryptic splice sites, using the provided methods of determining the heterogeneity of the transcribed nucleic acid of the transgene. Also provided are methods of reducing the heterogeneity of an expressed transgene transcript.

[0303] Also provided herein are methods of identifying a transgene or recombinant protein or nucleic acid candidate for the removal or modification of one or more splice sites, such as cryptic splice sites, such as based on the determined heterogeneity of the transcribed nucleic acid, e.g., of the transgene.

[0304] Also provided are methods and approaches for reducing the heterogeneity of a transcribed nucleic acid (e.g., transcript) of a transgene (e.g., an expressed transgene transcript) or other nucleic acid. Such methods and approaches can include identifying a transgene candidate for the removal of splice sites (such as cryptic splice sites) according to the provided methods and identifying one or more potential splice donor and/or splice acceptor sites within the transgene. In embodiments of the provided methods the splice donor and/or splice acceptor sites can be in the translated and/or untranslated regions of the transcribed nucleic acid (e.g., transcript).

[0305] In some embodiments, eliminating splice sites, such as cryptic splice sites, can improve or optimize expression of a transgene product, such as a polypeptide translated from the transgene, such as an anti-BCMA CAR polypeptide. Splicing at cryptic splice sites of an encoded transgene, such as an encoded BMCA CAR molecule, can lead to reduced protein expression, e.g., expression on cell surfaces, and/or reduced function, e.g., reduced intracellular signaling. Provided herein are polynucleotides, encoding anti-BMCA CAR proteins that have been optimized to reduce or eliminate cryptic splice sites. Also provided herein are polynucleotides encoding anti-BCMA CAR proteins that have been optimized for codon expression and/or in which one or more sequence, such as one identified by the methods or observations herein regarding splice sites, is present, and/or in which an identified splice site,

such as any of the identified splice sites herein, is not present. Among the provided polynucleotides are those exhibiting below a certain degree of RNA heterogeneity or splice forms when expressed under certain conditions and/or introduced into a specified cell type, such as a human T cell, such as a primary human T cell, and cells and compositions and articles of manufacture containing such polypeptides and/or exhibiting such properties.

[0306] In some embodiments, reducing RNA heterogeneity or removing potential splice site comprises modifying a polynucleotide. In some embodiments, the modification includes one or more nucleotide modifications, such as a replacement or substitution, compared to a reference polynucleotideu such as an unmodified polynucleotide that encodes the same polypeptide. In some embodiments, the reference polynucleotide is one in which the transcribed RNA (e.g. mRNA), when expressed in a cell, exhibits greater than or greater than about 10%, 15%, 20%, 25%, 30%, 40%, 50% or more RNA heterogeneity. In some embodiments, the provided methods can result in polynucleotides in which RNA heterogeneity of transcribed RNA is reduced by greater than or greater than about 10%, 15%, 20%, 25%, 30%, 40%, 50% or more. In some embodiments, the provided methods produce polynucleotides in which RNA homogeneity of transcribed RNA is at least 70%, 75%, 80%, 85%, 90%, or 95% or greater.

A. Methods of Measuring and Reducing RNA Heterogeneity

[0307] Provided herein are methods, approaches, and strategies for measuring, evaluating and/or reducing RNA heterogeneity of a nucleic acid, such as of a transcribed RNA, e.g., when expressed in a particular cell type or context, as well as polynucleotides exhibiting reduction in such heterogeneity and/or risk thereof, as compared to a reference polynucleotide. In some embodiments, a reference polynucleotide can be assessed for RNA heterogeneity, such as by methods as described in this Section. In some embodiments, the provided approaches involve identifying RNA (e.g., mRNA) heterogeneity or likelihood thereof, such as in a particular cell or context, such as due to cryptic splice sites. In some aspects, such heterogeneity is identified by amplifying RNA transcripts using a first primer specific to the 5′ untranslated region (5′ UTR), corresponding to a portion of an element located upstream of the transgene in the transcribed RNA, such as a promoter, and a second primer specific to a 3′ untranslated region (3′ UTR), located downstream of the expressed transgene in the transcribed RNA sequence or specific to a sequence within the transgene. In some embodiments, the methods involve amplifying a transcribed nucleic acid using at least one 5′ and 3′ primer pair, wherein at least one pair comprises a 5′ primer that is complementary to a nucleic acid sequence within the 5′

untranslated region (5′ UTR) of the transcribed nucleic acid and a 3′ primer that is complementary to a nucleic acid sequence within the 3′ untranslated region (3′ UTR) of the transcribed nucleic acid to generate one or more amplified products. In some embodiments, the methods involve detecting the amplified products, wherein the presence of two or more amplified products from at least one 5′ and 3′ primer pair indicates heterogeneity in the amplified products. In some embodiments, the detected difference in transcripts are different lengths of the amplified transcript. In some embodiments, the detected difference in transcripts are differences in chromatographic profiles. Exemplary methods for identifying a polynucleotide with RNA heterogeneity are described below. In some embodiments, the methods comprise evaluating RNA heterogeneity for the need of being modified to reduce heterogeneity. In some embodiments, polynucleotides that exhibit RNA heterogeneity greater than or greater than about 10%, 15%, 20%, 25%, 30%, 40%, 50% or more are selected for nucleotide modification to remove one or more splice sites, such as one or more cryptic splice sites.

1. Measuring RNA Heterogeneity

[0308] RNA heterogeneity can be determined by any of a number of methods provided herein or described or known. In some embodiments, RNA heterogeneity of a transcribed nucleic acid is determined by amplifying the transcribed nucleic acid, such as by reverse transcriptase polymerase chain reaction (RT-PCR) followed by detecting one or more differences, such as differences in size, in the one or more amplified products. In some embodiments, the RNA heterogeneity is determined based on the number of differently sized amplified products, or the proportion of various differently sized amplified products. For example, in some embodiments, RNA heterogeneity is quantified by determining the number, amount or proportion of differently sized amplified product compared to the number or amount of total amplified products. In some cases, all or substantially all of a particular transcript is determined to be equal in size, and in this case, the RNA heterogeneity is low. In some cases, a variety of differently sized transcripts are present, or a large proportion of a particular transcript is of a different size compared to the predicted size of the amplified product without cryptic or undesired splicing events. In some embodiments, RNA heterogeneity can be calculated by dividing the total number or amount of all of amplified products that are of a different size compared to the predicted size of the amplified product by the total number or amount of all amplified products. In some embodiments, the predicted size of the transcript or amplified

product is from an RNA that does not contain or is not predicted to contain a cryptic splice site. In some embodiments, the predicted size of the transcript or amplified product takes into account one or more splice sites that are desired or intentionally placed.

[0309] In some embodiments, RNA, such as total RNA or cytoplasmic polyadenylated RNA, is harvested from cells, expressing the transgene to be optimized, and amplified by reverse transcriptase polymerase chain reaction (RT-PCR) using a primer specific to the 5′ untranslated region (5′ UTR), in some cases corresponding to a portion of the promoter sequence in the expression vector, located upstream of the transgene in the transcribed RNA, and a primer specific to the 3′ untranslated region (3′ UTR), located downstream of the expressed transgene in the transcribed RNA sequence or a primer specific to a sequence within the transgene. In particular embodiments, at least one primer complementary to a sequence in the 5′ untranslated region (UTR) and at least one primer complementary to a sequence in the 3′ untranslated region (UTR) are employed to amplify the transgene. An exemplary depiction of the amplification of a transcript and resulting product using a forward primer specific to the 5′ UTR and a primer specific to a nucleotide sequence in the 3′ UTR and a predicted amplified product, where no splice events have occurred, is provided in **FIG. 21A**. An exemplary depiction of exemplary multiple amplified products (i.e., heterogeneity) resulting from amplification of a transcript that has a 5′ UTR, with a transcribed promoter sequence that contains a known splice donor site (P-SD) and a known splice acceptor site (P-SD), a transcribed transgene containing an unknown (cryptic) splice donor site (T-SD) and two unknown (cryptic) splice acceptor sites (T-SA) and a 3′ UTR, using primers specific to regions of the 5′ UTR and 3′ UTR, is shown in **FIG. 21B**.

[0310] Exemplary primers specific for the 5′ untranslated region (UTR) include primers directed to sequences within the promoter of the transgene. In some examples, a primer specific to an EF1a/HTLV promoter. An exemplary forward primer, specific to an EF1a-HTLV promoter is set forth in SEQ ID NO: 763.

[0311] Exemplary primers specific for the 3′ untranslated region (UTR) include primers directed to 3′ posttranscriptional regulatory elements located downstream of the transgene. Exemplary 3′ posttranscriptional regulatory elements include the woodchuck hepatitis virus (WHP) posttranscriptional regulatory element (WPRE), set forth in SEQ ID NO: 636. An exemplary forward primer, specific to a WPRE is set forth in SEQ ID NO: 764.

[0312] In some embodiments, multiple primer pairs can be used to amplify the transgene, such as for long transgenes. In some embodiments, sequential or nested pairs of forward and reverse primers, to crease a sliding window of amplified products, can be used to gain full and

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

[0313] The amplified nucleic acid sequence is then analyzed for heterogeneity in terms of amplified transcript lengths. In some examples, heterogeneity is determined by the number and intensity of the bands for the expressed sequence. In some embodiments, RNA sequences having splice events upon expression generate multiple bands with different mobilities. In some embodiments, a major band is detected at the predicted mobility for a sequence not having any unpredicted splice events, and 1 or more additional bands of varying intensities and mobilities indicate the occurrence of one or more cryptic splice events within the transgene sequence.

[0314] The skilled artisan can resolve RNA, such as messenger RNA, and analyze the heterogeneity thereof by several methods. Non-limiting, exemplary methods include agarose gel electrophoresis, chip-based capillary electrophoresis, analytical centrifugation, field flow fractionation, and chromatography, such as size exclusion chromatography or liquid chromatography.

[0315] One or more steps of the above techniques can be performed under denaturing conditions, partially denaturing conditions, or non-denaturing conditions. The denaturing conditions can include conditions that cause denaturing of the nucleic acid transcript (e.g., mRNA) due to temperature, chaotropic agents (including salts), organic agents, among other mechanisms for denaturing. With thermal denaturing conditions, an elevated temperature can be applied. The elevated temperature can be one that is sufficient to denature intramolecular hydrogen bonds, to cause a change in or loss of secondary or tertiary structure, and so forth. For example, the temperature or thermal denaturing conditions can include a temperature of 25 degrees Celsius to 95 degrees Celsius, 35 to 85 degrees Celsius, 55 to 75 degrees Celsius, or of another range within those ranges. Similarly, higher or lower temperatures can be used as appropriate to cause the desired level of denaturing. The temperature or thermal denaturing conditions can also be dependent on the identity of the nucleic acid transcript, such that different temperatures are used for different nucleic acid transcripts or types of nucleic acid transcripts. The denaturing conditions can also include using chaotropic agents, such as lithium perchlorate and other perchlorate salts, guanidinium chloride and other guanidinium salts, urea, butanol, ethanol, lithium acetate, magnesium chloride, phenol, propanol, sodium dodecyl sulfate, thiourea, or others. The denaturing conditions can further include organic denaturing agents, such as dimethyl sulfoxide (DMSO), acetonitrile, and glyoxal. In addition, the denaturing

conditions can include a combination of two or more of these types of denaturing conditions. Any one or more of the steps of the RNA heterogeneity determining techniques can be performed at an elevated temperature or at ambient temperature, with or without chaotropic or organic agents.

a) Gel Electrophoresis

[0316] In some embodiments, RNA transcript topology and apparent (hydrodynamic) size can be analyzed by gele electrophoresis, such as agarose gel electrophoresis. In some examples, RNA transcript can be resolved on a 0.05% to 2% agarose gel, such as a 1.2% agarose gel, and visualized by staining or using probes that are specific to a particular sequence. In some embodiments, RNA transcripts can be directly assessed by gel electrophoresis, or can be assessed after amplification, such as quantitative amplification methods. Nucleic acid stains for visualizing nucleic acid on agarose gel are well known. Exemplary stains include BlueView™ Nucleic Acid Stain (Millipore Sigma), SYBR® Gold Nucleic Acid Stain (ThermoFisher), SYBR® Green Nucleic Acid Stain (Millipore Sigma), SYBR® Green II (ThermoFisher), PicoGreen® nucleic acid stain (Invitrogen), and ethidium bromide: 0.5 μg/mL prepared in distilled water, or incorporated into the gel. In some examples, the nucleic acid is stained using Quant-iT™ PicoGreen ® binding followed by fluorescence detection and quantitation of the amplified products. The agarose gel method gives a more quantitative, but less resolving, measure of size distribution. In some embodiments, the nucleic acid fragments, resolved by agarose gel electrophoresis can be visualized by Northern blot for RNA or Southern blot for amplified reverse transcriptase-polymerase chain reaction (RT-PCR) products.

b) Chip-based Capillary Electrophoresis

[0317] Chip-based capillary electrophoresis (e.g., with the AGILENT 2100 BIOANALYZER™) can be used a rapid and routine method for monitoring RNA transcript integrity and its size distribution. The separation is based on hydrodynamic size and charge, and is affected by the nucleotide length and folded structure of the RNA transcript. In one embodiment, the method includes delivering the sample into a channel of a chip with an electrolyte medium and applying an electric field to the chip that causes the RNA transcript and the impurities migrate through the channel. The RNA transcript has a different electrophoretic mobility than the impurities such that the RNA transcript migrates through the channel at rate that is different from a rate at which the impurities migrate through the channel. The electrophoretic mobility of the RNA transcript is proportional to an ionic charge the RNA

transcript and inversely proportional to frictional forces in the electrolyte medium. The method also includes collecting from the chip the sample comprising the RNA transcript and one or more separate portions of the sample comprising the impurities. In addition, the method includes characterizing an aspect of at least one of the portion of the sample comprising the RNA transcript and the one or more separate portions of the sample comprising the impurities. The characterizing can include, for example, quantifying charge variants.

c) Analytical Ultracentrifugation (AUC)

[0318] Analytical ultracentrifugation (AUC) is a solution phase method for measuring molecular weight distribution, without the potential artifacts that could be introduced by matrix (resin or gel) interaction in the SEC, agarose, or other methods. Both equilibrium AUC and sedimentation ultracentrifugation are used, and the latter provides sedimentation coefficients that are related to both size and shape of the RNA transcript. A BECKMAN™ analytical ultracentrifuge equipped with a scanning UV/visible optics is used for analysis of the RNA transcript.

d) Field Flow Fractionation (FFF)

[0319] Another solution phase method for assessing hydrodynamic size distribution is field flow fractionation (FFF). FFF is a separation technique where a field is applied to a fluid suspension or solution pumped through a long and narrow channel, perpendicular to the direction of flow, to cause separation of the polynucleotides (RNA transcripts) present in the fluid, under the force exerted by the field. The field can be asymmetrical flow through a semi-permeable membrane, gravitational, centrifugal, thermal-gradient, electrical, magnetic etc.

e) Chromatography

[0320] Chromatography also can be used to detect heterogeneity of RNA transcript lengths. Methods of size exclusion chromatography and liquid chromatography for determining mRNA heterogeneity are described in WO2014144711 which is incorporated herein by reference.

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

[0321] In some embodiments, the provided methods include optimizing and/or modifying the polynucleotide, for example, to reduce RNA heterogeneity and/or removing or eliminating cryptic or undesired splice sites. In some aspects, provided are methods of of reducing the heterogeneity of an expressed transgene transcript that involves identifying a transgene candidate for the removal of splice sites, such as by the methods described above in Section I.A.;

identifying one or more potential splice donor and/or splice acceptor sites; and modifying the nucleic acid sequence at or near the one or more identified splice donor sites that were identified, thereby generating a modified polynucleotide. In some aspects, the methods also involve assessing the transgene candidacy for the removal of splice sites. In some embodiments, the methods also include repeating one or more steps above until the heterogeneity of the transcript is reduced compared to the initial heterogeneity of the transcript as determined (such as before modification).

[0322] In some embodiments, methods of reducing heterogeneity, such as by removal or elimination of predicted splice sites, can be performed after codon optimization, or on non codon-optimized RNA. In some aspects, the methods involve identifying splice sites, such as one or more potential splice donor and/or acceptor sites, and modifying or change the RNA sequence (e.g., by replacing or substituting one or more nucleotides at or near the splice site.In some embodiments, codon optimization can be performed prior to and/or after methods of reducing heterogeneity of transcribed RNA (e.g., mRNA), such as by removal or elimination of predicted splice sites. In some embodiments, whether a transcript is a candidate for reducing RNA heterogeneity is determined based on the method of measuring RNA heterogeneity, e.g., as described in Section II.A herein. In some aspects, a transcribed nucleic acid that is detected as having heterogeneity is identified as a transgene candidate for removal of one or more splice site. In some embodiments, a transgene sequence can be a candidate for reducing heterogeneity when the transcribed nucleic acid of the transgene candidate exhibits at least or at least about 5%, 10%, 15%, 20%, 25%, 30%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75% or more heterogeneity following expression in a cell. In some embodiments, following transcription and processing of the polynucleotide in a human cell, optionally a human T cell, the messenger RNA (mRNA) from the polynucleotide, exhibits at least 70%, 75%, 80%, 85%, 90%, or 95% RNA homogeneity.

1. Methods of Reducing RNA Heterogeneity

[0323] Provided are methods of reducing heterogeneity of an expressed transgene transcript. In some embodiments, the methods involve identifying one or more potential splice donor and/or splice acceptor sites and modifying the nucleic acid sequence at or near the one or more of the identified splice donor sites. In some embodiments, the methods also involve assessing the transgene candidacy for removal of splice sites. In some aspects, one or more steps

described herein can be repeated, for example, until the potential RNA heterogeneity is reduced compared to the starting or unmodified transcript.

a) Splice Site Identification

[0324] In some aspects, the presence of potential cryptic splice sites (splice donor and/or acceptor sites that are present in a transcript, such as a transgene transcript, can result in RNA heterogeneity of the transcript following expression in a cell. In some embodiments, the methods involve identifying one or more potential splice sites that can be present in the transgene transcript, that are not desired and/or that may be created in a transgene transcript from various underlying sequences, following codon optimization of a transcript and/or by mutation or mistake or error in transcription. In some aspects of the provided embodiments, the splice donor sites and splice acceptor sites are identified independently. In some embodiments, the splice acceptor and/or donor site(s) is/are canonical, non-canonical, and/or cryptic splice acceptor and/or donor site(s).

[0325] In some embodiments, the provided methods include identifying one or more potential splice site (e.g., canonical, non-canonical, and/or cryptic splice acceptor and/or donor site(s) or branch sites) in a polynucleotide, such as a polynucleotide encoding a transgene, such as a recombinant receptor, that may exhibit RNA heterogeneity or contain undesired. Also provided are polypeptides having reduced numbers of such splice sites as compared to such reference polynucleotides.

[0326] In some aspects, identification of the one or more splice sites in a nucleic acid sequence is an iterative process. In some embodiments, splice sites can be identified using a splice site and/or codon optimization prediction tool, such as by submitting the starting or reference sequence encoding the transgene, such as a BCMA-binding receptor, e.g., anti-BCMA CAR, to a database, a gene synthesis vendor or other source able to computationally or algorithmically compare the starting or reference sequence to identify or predict splice sites and/or for codon optimization and/or splice site removal. In some embodiments, after modifying the sequence for codon optimization and/or splice site removal, one or more further assessment of a sequence, such as a revised or modified nucleic acid sequence, is carried out to further evaluate for splice site removal, such as cryptic splice sites, using one or more other or additional splice site prediction tool(s).

[0327] In some aspects, RNA heterogeneity can be a result of the activity of the spliceosome present in a eukaryotic cell. In some aspects, splicing is typically carried out in a series of

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

[0328] In some embodiments, splice sites, including potential cryptic splice sites can be identified by comparing sequences to known splice site sequences, such as those in a sequence database. In some embodiments, splice sites can be identified by computationally by submitting nucleotide sequences for analysis by splice site prediction tools, such as Human Splice Finder (Desmet et al., Nucl. Acids Res. 37(9):e67 (2009)), a neural network splice site prediction tool, NNSplice (Reese et al., *J. Comput. Biol.*, 4(4):311 (1997)), GeneSplicer (Pertea et al., Nucleic Acids Res. 2001 29(5): 1185–1190) or NetUTR (Eden and Brunak, Nucleic Acids Res. 32(3):1131 (2004)), which identify potential splice sites and the probability of a splicing event at such sites. Additional splice prediction tools include RegRNA, ESEfinder, and MIT splice predictor. Splice site prediction tools such as GeneSplicer has been trained and/or tested successfully on databases for different species, such as human, Drosophila melanogaster, Plasmodium falciparum, Arabidopsis thaliana, and rice. In some embodiments, different prediction tools may be adapted for different extents on different database and/or for different species. In some embodiments, the one or more prediction tools are selected based upon their utility in certain database and/or for certain species. *See*, e.g.,Saxonov et al., (2000) Nucleic Acids Res., 28, 185-190.

[0329] In some embodiments, one or more splice site prediction tools are selected for use in the determination of potential splice donor and/or acceptor sites. In some embodiments, splice site prediction tools that can be run locally;that can be retrained with a set of data at the user site; that can use databases for particular species (such as human), that can be compiled for multiple platforms, that allow real-time predictions for sequence selections, and/or that is an OSI certified open source software such that particular tools or plugins can be modified, can be employed. Exemplary tools that can be employed include NNSplice, GeneSplicer or both. .

[0330] In some aspects, the splice site prediction tools be used to identify a list of potential splice donor and/or splice acceptor sites in a sequence such as a polynucleotide sequence containing transgene sequences. In some aspects, the prediction tools also can generate one or more prediction scores for one or more sequences in the polynucleotide, that can indicate the likelihoods of the one or more sequences being a splice donor or acceptor site sequence.

[0331] In some embodiments, the method involves comparing the prediction score for a particular splice site with a threshold score or reference score to determine or identify a particular splice sites that are candidate for elimination or removal. For example, in some embodiments, the predicted splice site is identified as a potential splice site when the prediction score is greater or no less than the threshold score or reference score. In some aspects, considerations for eliminating or removing a particular splice site include the prediction score as compared to a reference score or a threshold score; and whether a particular splice site is desired or intentional (for example, when the splicing event is more advantageous or is required for regulation of transcription and/or translation). In some aspects, the likelihood that the resulting splice variant loses the desired function or has compromised function can also be considered when determining particular donor and/or acceptor sites for elimination or removal. In some aspects, the one or more potential splice donor and/or splice acceptor sites exhibit a score about or at least about 0.7, 0.75, 0.8, 0.85, 0.9, 0.95, or 1.0 (e.g., on a scale with a maximum of 1.0) of a splice event or probability of a splice event, and the site can be a candidate for splice site elimination or removal. In some aspects, the score, e.g., used by GeneSplicer, at the one or more potential splice donor and/or splice site is based on the difference between the log-odds score returned for that sequence by the true Markov model and the score is computed by the false Markov model.In particular embodiments, the splice donor sites and splice acceptor sites are evaluated independently, or individually. In some embodiments, splice donor sites and splice acceptor sites are evaluated as a splice donor/acceptor pair.

b) Splice Site Elimination

[0332] In some embodiments, the provided methods involve eliminating or eliminating one or more splice splice donor and/or splice acceptor site(s), such as the potential splice donor and/or acceptor sites that may be involved in a cryptic splicing event that is not desired or that results in undesired RNA heterogeneity. In some embodiments, eliminating one or more splice sites comprises modifying one or more nucleotides (e.g., by substitution or replacement) in at, containing or near the splice donor and/or acceptor sites that are candidates for removal. In some

aspects, a particular nucleotide within a codon that is at, contains or is near the splice site is modified (e.g., substituted or replaced). In some aspects, the modification (such as substitution or replacement) retains or preserves the amino acid encoded by the particular codon at the site, at the same time removing the potential splice donor and/or acceptor sites.

[0333] In some embodiments, the codon at or near the splice site for modification comprises one or more codons that involve one or both of the two nucleotides at the potential splice site (in some cases referred to as "splice site codon"). When the potential splicing is predicted to occur between two nucleotides in a codon, the codon is the only splice site codon for this splice site. If the potential splicing is predicted to occur between two adjacent codons, for example, between the last nucleotide of the first codon and the first nucleotide of the next codon, the two codons are splice site codons. For example, for splice sites that are predicted to be at boundaries of two codons, the two adjacent codons can be candidates for nucleotide modification. In some embodiments, the one or more codons comprise one splice site codon. In some embodiments, the one or more codons comprise both splice site codons. In some embodiments, the method involves eliminating potential potential splice donor site by modifying one or both splice site codons. In some embodiments, the method involves eliminating a potential splice acceptor donor site by modifying one or both splice site codons. In some embodiments, the one or both codons at the splice site is not modified, for example, when there are no synonymous codon for the splice site codon. In some embodiments, if there are no synonymous codons available for the particular splice site codon, one or more nucleotides in a nearby codon can be modified. In some embodiments, one or more codons that are modified include a splice site codon, wherein the modification comprises changing one or both nucleotides at the splice site to a different nucleotide or different nucleotides. In some embodiments, In some embodiments, the method involves eliminating the splice donor site by modifying one or both splice site codons., wherein the modification does not change one or two of the nucleotidesof the at the splice site to a different nucleotide, but a nearby nucleotide, e.g., a part of a codon adjacent to the splice site, is modified. In some embodiments, the nearby or adjacent nucleotides that can be modified include modification of a nucleotide that is a part of a nearby or adjacent codon, such as a codon that is within one, two, three, four, five, six, seven, eight, nine or ten codons upstream or downstream of the splice site codon.

[0334] In some cases, manual modification of the polynucleotides can be employed, while preserving the encoded amino acid sequence, to reduce the probability of a predicted splice site. In some embodiments, one or more of the predicted splice sites having at least 80%, 85%, 90%,

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

[0335] In some embodiments, the method involves eliminating one or more potential donor splice site by modifying one or two splice site codons or one or more nearby or adjacent codons (for example, if a synonymous codon is not available for the splice site codon). In some embodiments, the method involves eliminating one or more potential acceptor splice site by modifying one or two splice site codons or one or more nearby or adjacent codons (for example, if a synonymous codon is not available for the splice site codon). In some embodiments, the nearby or adjacent codon that is subject to modification include a codon that is within one, two, three, four, five, six, seven, eight, nine or ten codons upstream or downstream of the splice site codon, such as a codon that is within one, two or three codons from the splice site. In some embodiments, the methods can include removal or elimination of a potential branch site for splicing. In some aspects, a nucleotide within the codon at or near the branch site can be modified, e.g., substituted or replaced, to eliminiate cryptic splicing and/or reduce RNA heterogeneity. In some embodiments, the modification of the one or more nucleotides can involve a substitution or replacement of one of the nucleotides that may be involved in splicing (such as at the splice donor site, splice acceptor site or splice branch site), such that the amino acid encoded by the codon is preserved, and the nucleotide substitution or replacement does not change the polypeptide sequence that is encoded by the polynucleotide. In some cases, the third position in the codon is more degenerate than the other two positions. Thus, various synonymous codons can encode a particular amino acid (see, e.g., Section II.B.2 below). In some embodiments, the modification includes replacing the codon with a synonymous codon

used in the species of the cell into which the polynucleotide is introduced (e.g., human). In some embodiments, the species is human. In some embodiments, the one or more codon is replaced with a corresponding synonymous codons that the most frequently used in the species or synonymous codons that have a similar frequency of usage (e.g., most closest frequency of usage) as the corresponding codon (see, e.g., Section II.B.2 below).

[0336] In some embodiments, the methods also involve assessing the transgene candidacy for the removal of splice sites, after initial proposed modification. In some aspects, the proposed modification can be evaluated again, to assess the proposed modification and identify any further potential splice sites after modification and/or codon optimization. In some aspects, after modifying the sequence for codon optimization and/or splice site removal, one or more further assessment of a sequence, such as a revised or modified nucleic acid sequence, is carried out to further evaluate for splice site removal, such as cryptic splice sites, using the same or one or more other or additional splice site prediction tool(s). In some aspects, proposed modifications are considered for subsequent steps, and iterative optimization can be used. In some aspects, the methods also include repeating any of the identification and/or modification step, for example, until heterogeneity of the transcript is reduced compared to the heterogeneity of the transcript as initially determined. In some embodiments, a further or a different modification, such as with a different nucleotide replacement at the same codon or a modification at a different position or codon, can be done after an interative evaluation and assessment. In some embodiments, corresponding different synonymous codon can be used, such as the second most frequently used in the particular species or a codon that has a similar frequency of usage (e.g., the next closest frequency of usage) as the corresponding codon (see, e.g., Section II.B.2 below).

[0337] In some aspects, a proposed modification can be further evaluated, for example, to assess whether the modification generates an undesired or additional restriction site in the polynucleotide. In some aspects, an additional restriction site may not be desired, and a further or a different modification (e.g., with a different nucleotide replacement at the same codon or a modification at a different position or codon) can be considered. In some aspects, particular restriction site, such as a designated restriction site, is avoided. In some aspects, if the modification does not substantially reduce or,the splice site prediction score, an additional or alternative modification can be proposed. In some embodiments, the splice site prediction score can be is reduced or lowered by at least about 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70% or 75%, after one or more iteration of the methods.

[0338] In some embodiments of any of the methods provided herein, a computer system can be used to execute one or more steps, tools, functions, processes or scripts. In certain embodiments, methods provided herein are computer implemented methods and/or are performed with the aid of a computer. In some embodiments, the splice site prediction, evaluation and modification for elimination or removal of a splice site can be performed by computer implemented methods and/or by methods which include steps that are computer implemented steps. In some embodiments, comparison of the sequences to a known database, calculating a splice site prediction score, determining potential nucleotide modifications, codon optimization and/or any one of the iterative steps can be implemented by a computer or using a computer-implemented steps, tools, functions, processes or scripts. In particular embodiments, a computer system comprising a processor and memory is provided, wherein the memory contains instructions operable to cause the processor to carry out any one or more of steps of the methods provided herein. In some embodiments, the methods include steps, functions, processes or scripts that are performed computationally, e.g., performed using one or more computer programs and/or via the use of computational algorithms.

[0339] Exemplary steps, functions, processes or scripts of the provided methods for identifying and/or removing possible splice sites include one or more steps of: selecting sequence, writing FASTA format sequences, loading codon table (e.g., from www.kazusa.or.jp/codon, running GeneSplicer, loading predictions, parsing codons, determining overlaps in prediction, identifying next highest usage synonymous codon, reviewing for restriction site, creating annotations or assessing other codons. Particular steps can assess both forward and reverse strands. In some aspects, previously annotated splice site modifications can also be considered, to allow for iterative optimization. In some embodiments, any one or more of the steps, functions, processes or scripts can be repeated.

[0340] In certain embodiments, methods provided herein may be practiced, at least in part, with computer system configurations, including single-processor or multi-processor computer systems, minicomputers, mainframe computers, as well as personal computers, hand-held computing devices, microprocessor-based and/or programmable consumer electronics and the like, each of which may operatively communicate with one or more associated devices. In particular embodiments, the methods provided herein may be practiced, at least in part, in distributed computing environments such that certain tasks are performed by remote processing devices that are linked through a communications network. In a distributed computing environment, program modules may be located in local and/or remote memory storage devices.

In particular embodiments, some or all steps of the methods provided herein may be practiced on stand-alone computers.

[0341] In particular embodiments, some or all of the steps of the methods provided herein can operate in the general context of computer-executable instructions, such as program modules, plugins and/or scripts executed by one or more components. Generally, program modules include routines, programs, objects, data structures and/or scripts, that perform particular tasks or implement particular abstract data types. Typically, the functionality of the program modules may be combined or distributed as desired. In certain embodiments, instructions operable to cause the processor to carry out any one or more steps of the methods provided herein can be embodied on a computer-readable medium having computer-executable instructions and transmitted as signals manufactured to transmit such instructions as well as the results of performing the instructions, for instance, on a network. In some embodiments, also provided are computer systems, computer readable instructions, software, systems, networks and/or devices for carrying out or performing one or more steps of the methods provided herein.

2. Codon optimization

[0342] In some embodiments the polynucleotides are modified by optimization of the codons for expression in humans. In some aspects, codon optimization can be considered before and/or after the steps for splice site identification and/or splice site elimination, and/or at each of the iterative steps for reducing RNA heterogeneity. Codon optimization generally involves balancing the percentages of codons selected with the abundance, e.g., published abundance, of human transfer RNAs, for example, so that none is overloaded or limiting. In some cases, such balancing is necessary or useful because most amino acids are encoded by more than one codon, and codon usage generally varies from organism to organism. Differences in codon usage between transfected or transduced genes or nucleic acids and host cells can have effects on protein expression from the nucleic acid molecule. **Table 3** below sets forth an exemplary human codon usage frequency table. In some embodiments, to generate codon-optimized nucleic acid sequences, codons are chosen to select for those codons that are in balance with human usage frequency. The redundancy of the codons for amino acids is such that different codons code for one amino acid, such as depicted in **Table 3**. In selecting a codon for replacement, it is desired that the resulting mutation is a silent mutation such that the codon change does not affect the amino acid sequence. Generally, the last nucleotide of the codon (e.g., at the third position) can remain unchanged without affecting the amino acid sequence.

Table 3. Human Codon Usage Frequency

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

[0343] For example, the codons TCT, TCC, TCA, TCG, AGT and AGC all code for Serine (note that T in the DNA equivalent to the U in RNA). From a human codon usage frequency, such as set forth in **Table 3** above, the corresponding usage frequencies for these codons are 15.2, 17.7, 12.2, 4.4, 12.1, and 19.5, respectively. Since TCG corresponds to 4.4%, if this codon were commonly used in a gene synthesis, the tRNA for this codon would be limiting. In codon optimization, the goal is to balance the usage of each codon with the normal frequency of usage in the species of animal in which the transgene is intended to be expressed.

C. Optimized Anti-BCMA CAR

[0344] In some embodiments, a starting or reference sequence encoding a transgene, such as a BCMA-binding receptor, e.g., anti-BCMA CAR, is assessed for codon optimization and/or splice site removal.

[0345] In some embodiments, the methods are carried out on an anti-BCMA CAR, such as a CAR containing an scFv antigen-binding domain specific to BCMA, a spacer, such as a spacer set forth in SEQ ID NO:649, a costimulatory signaling region, such as a costimulatory signaling domain from 4-1BB and a CD3 zeta signaling region. Exemplary identified splice donor sites and splice acceptor sites, and their corresponding scores, are listed in Tables 3 and 4 below for exemplary anti-BCMA CARs.

Table 4. Predicted Splice Donor Sites

	STARTING SEQUENCE			O/SSE SEQUENCE		
Region of Construct	**splice donor site**	**SEQ ID NO**	**Splice score**	**optimized splice donor site**	**SEQ ID NO**	**Splice score**
promoter	cgtctag**gt**aagttt	689	1	no change		<0.7
scFv-encoding						
BCMA-23	gaccaag**gt**gaccgt	690	N/A	caccaag**gt**gaccgt	698	0.54
BCMA-26	tgcactg**gt**accagc	691	0.55	no change		
BCMA-52	taaactg**gt**accagc	692	0.76	tgaactg**gt**atcagc	699	<0.7
BCMA-52	atctcct**gt**aagggt	693	0.79	atctctt**ga**aatggt	700	<0.7
BCMA-52	ggtcaag**gt**actctg	694	0.85	ggccagg**gc**acactg	701	<0.7
BCMA-55	gaggaca**gt**aagcgg	695	0.66	gaggaca**gc**aagagg	702	<0.5
BCMA-55	ggtcaag**gt**actctg	696	0.85	ggccagg**ga**accctg	703	<0.5
BCMA-55	tgcctcc**gt**gtctgc	697	<0.50	tgccagc**gt**tagtgc	704	0.60
Spacer-encoding						
	aatctaa**gt**acggac	705	0.65	agtctaa**at**acggac	661	<0.7
	tcaactg**gt**acgtgg	706	0.96	tcaactg**gt**atgtgg	662	<0.7
	tcaattg**gt**acgtgg	616	0.97	tcaactg**gt**atgtgg	662	<0.7
	acaatta**gt**aaggca	707	0.43	accatct**cc**aaggcc	663	<0.7
	accacag**gt**gtatac	708	0.42	gccccag**gt**tacac	664	<0.7
CD3zeta signaling region-encoding	tttccag**gt**ccgccg	709	0.74	tcagcag**at**ccgccg	665	<0.7
Truncated receptor surrogate marker - encoding						
	ctgctct**gt**gagtta	710	0.56	ctcctgt**gt**gaactc	666	<0.7
	acgcaaa**gt**gtgtaa	711	0.5	tcggaaa**gt**gtgcaa	667	<0.7
	caacatg**gt**cagttt	712	0.71	cagcacg**gc**cagttt	668	<0.7
	aacagag**gt**gaaaac	713	0.42	aaccggg**gc**gagaac	669	<0.7
	ctggagg**gt**gagcca	714	0.82	ctggaag**gc**gagccc	670	<0.7
	tcttcat**gt**gagcgg	720	0.84	tgttcat**gt**gagcgg	671	<0.7

Table 4. Predicted Splice Acceptor Sites

	STARTING SEQUENCE			O/SSE SEQUENCE		
Region of Construct	**splice acceptor site**	**SEQ ID NO**	**splice score**	**optimized splice acceptor site**	**SEQ ID NO**	**Splice score**
Promoter						
	tggctccgccttttttcccg**ag** ggtgggggagaaccgtatat	721	0.50	no change		
	tgaactgcgtccgccgtct**ag** gtaagtttaaagctcaggtc	722	0.71	no change		
	ttctgttctgcgccgttac**ag** atccaagctgtgaccggcgc	723	0.89	no change		
scFv-encoding						
BCMA-23	ctactacatgagctggatc**cg** ccaggctccagggaaggggc	724	N/A	ctactatatgtcctggatc**ag** acaggcacctggcaagggcc	735	0.46
BCMA-23	ggctgattattattgtagc**tc** atatggaggtagtaggtctt	725	N/A	ggcagattactattgttct**ag** ctacggcggcagcagatcct	736	0.55
BCMA-25	ctatgccatgtcctggttc**ag** gcaggcaccaggcaagggcc	726	0.95	ctatgccatgtcctggttc**aa** gcaggcaccaggcaagggcc	737	<0.7
BCMA-25	gtccgcctctgtgggcgat**ag** ggtgaccgtgacatgtcgcg	727	0.50	no change		
BCMA-25	gtgggctttatccgctcta**ag** gcctacggcggcaccacaga	728	0.55	no change		
BCMA-25	gtgacatgtcgcgcctccc**ag** ggcatctctaactacctggc	729	0.67	no change		
BCMA-25	tacagcgcctccaccctgc**ag** agcggagtgccctccggtt	730	0.66	no change		
BCMA-52	ctggccatcagtggcctcc**ag** tctgaggatgaggctgatta	731	<0.50	ctggctatttctggactgc**ag** agcgaggacgaggccgacta	738	0.62
BCMA-52	agatacagcccgtccttcc**aa** ggccacgtcaccatctcagc	732	<0.50	agatacagccctagctttc**ag** ggccacgtgaccatcagcgc	739	0.67
BCMA-55	cgaggctgattattactgc**ag** ctcaaatacaagaagcagca	733	0.79	cgaggccgattactactgc**ag** cagcaacacccggtccagca	740	<0.40
BCMA-55	gccctcaggggtttctaat**cg** cttctctggctccaagtctg	734	<0.50	gcccagcggcgtgtccaat**ag** attcagcggcagcaagagcg	741	0.40

Table 4. Predicted Splice Acceptor Sites

	STARTING SEQUENCE			O/SSE SEQUENCE		
Region of Construct	**splice acceptor site**	**SEQ ID NO**	**splice score**	**optimized splice acceptor site**	**SEQ ID NO**	**Splice score**
Spacer-encoding						
	cgccttgtcctccttgtcc**ag** ctcctcctgttgccggacct	765	0.84	cgccttgtcctccttgtcc**cg** ctcctcctgttgccggacct	766	<0.7
	aagtttctttctgtattcc**ag** gctgaccgtggataaatctc	742	0.97	cagtttcttcctgtatagt**ag** actcaccgtggataaatcaa	672	<0.7
	aagtttctttctgtattcc**ag** gctgaccgtggataaatctc	742	0.97	aagtttctttctgtattcc**ag** actgaccgtggataaatctc	854	
	gggcaacgtgttctcttgc**ag** tgtcatgcacgaagccctgc	743	0.55	gggcaacgtgttcagctgc**ag** cgtgatgcacgaggccctgc	673	<0.7
	cagtttcttcctgtatagt**ag** actcaccgtggataaatcaa	767	0.74	No change		
CD28 TM - encoding	aggggtgctggcctgttac**ag** cctgctggtgacagtcgctt	744	0.4	cggagtgctggcctgttac**ag** cctgctggttaccgtggcct	674	0.75
4-1BB/ CD3zeta signaling region-encoding	gctgagagtcaagttttcc**ag** gtccgccgacgctccagcct	745	0.55	gctgagagtgaagttcagc**ag** atccgccgacgctccagcct	675	<0.7
Truncated Receptor Surrogate Marker-encoding						
	actcctcctctggatccac**ag** gaactggatattctgaaaac	746	0.74	acacctccactggatcccc**aa** gagctggatatcctgaaaac	676	<0.7
	acagggtttttgctgattc**ag** gcttggcctgaaaacaggac	747	0.73	accggattcctcctgatcc**aa** gcctggccagagaacagaac	677	<0.7
	accggattcctcctgattc**ag** gcctggccagagaacagaac	768	0.82	accggattcctcctgatcc**aa** gcctggccagagaacagaac	677	<0.7
	atggtcagttttctcttgc**ag** tcgtcagcctgaacataaca	748	0.89	acggccagtttagcctggc**tg** tggtgtctctgaacatcacc	678	<0.7

[0346] In some embodiments, the resulting modified nucleic acid sequence(s) is/are then synthesized and used to transduce cells to test for splicing as indicated by RNA heterogeneity. Exemplary methods are as follows and described in the Examples. Briefly, RNA is harvested from the expressing cells, amplified by reverse transcriptase polymerase chain reaction (RT-PCR) and resolved by agarose gel electrophoresis to determine the heterogeneity of the RNA, compared to the starting sequence. In some cases, improved sequences can be resubmitted to the gene synthesis vendor for further codon optimization and splice site removal, followed by further cryptic splice site evaluation, modification, synthesis and testing, until the RNA on the agarose gel exhibits minimal RNA heterogeneity.

[0347] In some embodiments, the provided methods for optimizing a coding nucleic acid sequence encoding a transgene, such as an anti-BCMA CAR provided herein, or a construct provided herein, is to both reduce or eliminate cryptic splice sites (see, e.g., SEQ ID NO: 622 for an exemplary codon optimized and splice site eliminated spacer sequence) and optimize human codon usage (see, e.g., SEQ ID NO: 855 for an exemplary codon optimized and spacer sequence). An exemplary optimization strategy is described in the Examples.

[0348] In some embodiments, provided are polynucleotides encoding a chimeric antigen receptor, comprising nucleic acid encoding: (a) an extracellular antigen-binding domain that specifically recognizes BCMA, including any of the antigen-binding domains described below; (b) a spacer of at least 125 amino acids in length; (c) a transmembrane domain; and (d) an intracellular signaling region, wherein following expression of the polynucleotide in a cell, the transcribed RNA, optionally messenger RNA (mRNA), from the polynucleotide, exhibits at least 70%, 75%, 80%, 85%, 90%, or 95% RNA homogeneity. In some embodiments the antigen-binding domain comprises a V_H region and a V_L region comprising the amino acid sequence set forth in SEQ ID NOs:617 and 618, respectively, or a sequence of amino acids having at least 90% identity to SEQ ID NOS:617 and 618, respectively. In some embodiments, the antigen-binding domain comprises a V_H region that is or comprises a CDR-H1, CDR-H2 and CDR-H3 contained within the V_H region amino acid sequence selected from SEQ ID NO: 617; and a V_L region that is or comprises a CDR-L1, CDR-L2 and CDR-L3 contained within the V_L region amino acid sequence selected from SEQ ID NO: 618. In some embodiments, In some embodiments, the antigen-binding domain comprises a V_H region comprising a CDR-H1, CDR-H2, and CDR-H3 comprising the amino acid sequence of SEQ ID NOS:593, 594, and 595, respectively, and a V_L region comprising a CDR-L1, CDR-L2, and CDR-L3 comprising the

amino acid sequence of SEQ ID NOS:601, 602, and 603, respectively; or a V_H region comprising a CDR-H1, CDR-H2, and CDR-H3 comprising the amino acid sequence of SEQ ID NOS:596, 597, and 595, respectively, and a V_L region comprising a CDR-L1, CDR-L2, and CDR-L3 comprising the amino acid sequence of SEQ ID NOS:601, 602, and 603, respectively; or a V_H region comprising a CDR-H1, CDR-H2, and CDR-H3 comprising the amino acid sequence of SEQ ID NOS: 598, 599, and 595, respectively, and a V_L region comprising a CDR-L1, CDR-L2, and CDR-L3 comprising the amino acid sequence of SEQ ID NOS:601, 602, and 603, respectively; or a V_H region comprising a CDR-H1, CDR-H2, and CDR-H3 comprising the amino acid sequence of SEQ ID NOS: 611, 612, and 613, respectively, and a V_L region comprising a CDR-L1, CDR-L2, and CDR-L3 comprising the amino acid sequence of SEQ ID NOS: 614, 615, and 603, respectively; or a V_H region that is or comprises the amino acid sequence set forth in SEQ ID NO: 617; and a V_L region that is or comprises the amino acid sequence set forth in SEQ ID NO: 618. In some embodiments, exemplary antigen-binding domain in the chimeric antigen receptor encoded by the polynucleotide include those described in each row of **Table 2** herein. In any of such embodiments, the transmembrane domain of the CAR is or comprises a transmembrane domain derived from a CD28; the intracellular signaling region comprises a cytoplasmic signaling domain of a CD3-zeta (CD3ζ) chain or a functional variant or signaling portion thereof and a costimulatory signaling region comprises an intracellular signaling domain of 4-1BB.

[0349] In some embodiments, provided are polynucleotides encoding a chimeric antigen receptor, comprising nucleic acid encoding: (a) an extracellular antigen-binding domain that specifically recognizes BCMA, including any of the antigen-binding domains described below; (b) (b) a spacer, wherein the encoding nucleic acid is or comprises, or consists or consists essentially of, the sequence set forth in SEQ ID NO: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. In some embodiments the antigen-binding domain comprises a V_H region and a V_L region comprising the amino acid sequence set forth in SEQ ID NOs:617 and 618, respectively, or a sequence of amino acids having at least 90% identity to SEQ ID NOS:617 and 618, respectively. In some embodiments, the antigen-binding domain comprises a V_H region that is or comprises a CDR-H1, CDR-H2 and CDR-H3 contained within the V_H region amino acid sequence selected from SEQ ID NO: 617; and a V_L region that is or comprises a CDR-L1, CDR-L2 and CDR-L3 contained within the V_L region amino acid sequence selected from SEQ ID

NO: 618. In some embodiments, In some embodiments, the antigen-binding domain comprises a V_H region comprising a CDR-H1, CDR-H2, and CDR-H3 comprising the amino acid sequence of SEQ ID NOS:593, 594, and 595, respectively, and a V_L region comprising a CDR-L1, CDR-L2, and CDR-L3 comprising the amino acid sequence of SEQ ID NOS:601, 602, and 603, respectively; or a V_H region comprising a CDR-H1, CDR-H2, and CDR-H3 comprising the amino acid sequence of SEQ ID NOS:596, 597, and 595, respectively, and a V_L region comprising a CDR-L1, CDR-L2, and CDR-L3 comprising the amino acid sequence of SEQ ID NOS:601, 602, and 603, respectively; or a V_H region comprising a CDR-H1, CDR-H2, and CDR-H3 comprising the amino acid sequence of SEQ ID NOS: 598, 599, and 595, respectively, and a V_L region comprising a CDR-L1, CDR-L2, and CDR-L3 comprising the amino acid sequence of SEQ ID NOS:601, 602, and 603, respectively; or a V_H region comprising a CDR-H1, CDR-H2, and CDR-H3 comprising the amino acid sequence of SEQ ID NOS: 611, 612, and 613, respectively, and a V_L region comprising a CDR-L1, CDR-L2, and CDR-L3 comprising the amino acid sequence of SEQ ID NOS: 614, 615, and 603, respectively; or a V_H region that is or comprises the amino acid sequence set forth in SEQ ID NO: 617; and a V_L region that is or comprises the amino acid sequence set forth in SEQ ID NO: 618. In some embodiments, exemplary antigen-binding domain in the chimeric antigen receptor encoded by the polynucleotide include those described in each row of **Table 2** herein. In any of such embodiments, the transmembrane domain of the CAR is or comprises a transmembrane domain derived from a CD28; the intracellular signaling region comprises a cytoplasmic signaling domain of a CD3-zeta (CD3ζ) chain or a functional variant or signaling portion thereof and a costimulatory signaling region comprises an intracellular signaling domain of 4-1BB.

[0350] Also provided herein are exemplary modified polynucleotides, including polynucleotides that were modified for codon optimization (O) and/or splice site elimination (SSE). Examples of such polynucleotides are set forth in **Table 5**, wherein exemplary nucleotide (nt) sequences for the components of the exemplary CAR constructs prior to splice site elimination and codon optimization (non-opt), nucleic acid (nt) sequences for the components of the CAR constructs following splice site elimination and optimization (O/SSE), and the corresponding amino acid (aa) sequences encoded by the nucleic acid sequences are provided. The components include the IgG-kappa signaling sequence (ss), the anti-BCMA scFv, spacer region, transmembrane (tm) domain, co-signaling sequence (4-1BB co-sig or CD28 co-sig), CD3-ζ signaling domain (CD3-ζ), T2A ribosomal skip element (T2A) and truncated EGF

receptor (EGFRt) sequence. Polynucleotide sequences of exemplary CAR constructs are set forth in SEQ ID NOs: 751-756, encoding the amino acid sequences set forth in SEQ ID NOs: 757-762.

Table 5. Exemplary BCMA CAR components (SEQ ID NOs)

Construct	Sequence	ss	scFv	spacer	TM	4-1BB co-stim	CD3-ζ
BCMA-23-L CAR	non-opt (nt)	619	352	621	623	625	627
BCMA-23-L CAR CO/SSE	O/SSE (nt)	684	715	622 or 856	688	681	652
both	aa	620	278	649	624	626	628
BCMA-25-L CAR	non-opt (nt)	619	716	621	623	625	627
BCMA-25-L CAR CO/SSE	O/SSE (nt)	682	717	622 or 856	688	681	652
both	Aa	620	559	649	624	626	628
BCMA-26-L CAR	non-opt (nt)	619	718	621	623	625	627
BCMA-26-L CAR CO/SSE	O/SSE (nt)	685	719	622 or 856	688	681	652
both	aa	620	560	649	624	626	628
BCMA-52-L CAR	non-opt (nt)	619	647	621	623	625	627
BCMA-52-L CAR CO/SSE	O/SSE (nt)	682	440	622 or 856	688	681	652
both	Aa	620	442	649	624	626	628
BCMA-55-L CAR	non-opt (nt)	619	648	621	623	625	627
BCMA-55-L CAR CO/SSE	O/SSE (nt)	683	460	622 or 856	688	681	652
both	aa	620	478	649	624	626	628
Construct	**Sequence**	**ss**	**scFv**	**spacer**	**TM**	**CD28 co-stim**	**CD3-ζ**
BCMA-55-L-CD28 CAR	non-opt (nt)	619	648	621	623	679	627
BCMA-55-L-CD28 CAR CO/SSE	O/SSE (nt)	683	460	622	688	679	652
both	aa	620	478	649	624	680	628

III. ENGINEERED CELLS

[0351] Also provided are cells such as engineered cells that contain a recombinant receptor (*e.g.*, a chimeric antigen receptor) such as one that contains an extracellular domain including an anti-BCMA antibody or fragment as described herein. Also provided are populations of such cells, compositions containing such cells and/or enriched for such cells, such as in which cells expressing the BCMA-binding molecule make up at least 50, 60, 70, 80, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, or more percent of the total cells in the composition or cells of a certain type such as T cells or CD8+ or CD4+ cells. Among the compositions are pharmaceutical compositions and formulations for administration, such as for adoptive cell therapy. Also provided are therapeutic methods for administering the cells and compositions to subjects, *e.g.*, patients.

[0352] Thus also provided are genetically engineered cells expressing the recombinant receptors containing the antibodies, *e.g.*, cells containing the CARs. The cells generally are eukaryotic cells, such as mammalian cells, and typically are human cells. In some embodiments, the cells are derived from the blood, bone marrow, lymph, or lymphoid organs, are cells of the immune system, such as cells of the innate or adaptive immunity, *e.g.*, myeloid or lymphoid cells, including lymphocytes, typically T cells and/or NK cells. Other exemplary cells include stem cells, such as multipotent and pluripotent stem cells, including induced pluripotent stem cells (iPSCs). The cells typically are primary cells, such as those isolated directly from a subject and/or isolated from a subject and frozen. In some embodiments, the cells include one or more subsets of T cells or other cell types, such as whole T cell populations, CD4+ cells, CD8+ cells, and subpopulations thereof, such as those defined by function, activation state, maturity, potential for differentiation, expansion, recirculation, localization, and/or persistence capacities, antigen-specificity, type of antigen receptor, presence in a particular organ or compartment, marker or cytokine secretion profile, and/or degree of differentiation. With reference to the subject to be treated, the cells may be allogeneic and/or autologous. Among the methods include off-the-shelf methods. In some aspects, such as for off-the-shelf technologies, the cells are pluripotent and/or multipotent, such as stem cells, such as induced pluripotent stem cells (iPSCs). In some embodiments, the methods include isolating cells from the subject, preparing, processing, culturing, and/or engineering them, as described herein, and re-introducing them into the same patient, before or after cryopreservation.

[0353] Among the sub-types and subpopulations of T cells and/or of CD4+ and/or of CD8+ T cells are naïve T (T_N) cells, effector T cells (T_{EFF}), memory T cells and sub-types thereof, such as stem cell memory T (T_{SCM}), central memory T (T_{CM}), effector memory T (T_{EM}), or terminally differentiated effector memory T cells, tumor-infiltrating lymphocytes (TIL), immature T cells, mature T cells, helper T cells, cytotoxic T cells, mucosa-associated invariant T (MAIT) cells, naturally occurring and adaptive regulatory T (Treg) cells, helper T cells, such as TH1 cells, TH2 cells, TH3 cells, TH17 cells, TH9 cells, TH22 cells, follicular helper T cells, alpha/beta T cells, and delta/gamma T cells.

[0354] In some embodiments, the cells are natural killer (NK) cells. In some embodiments, the cells are monocytes or granulocytes, *e.g.*, myeloid cells, macrophages, neutrophils, dendritic cells, mast cells, eosinophils, and/or basophils.

[0355] In some embodiments, the cells include one or more polynucleotides introduced via genetic engineering, and thereby express recombinant or genetically engineered products of such polynucleotides. In some embodiments, the polynucleotides are heterologous, *i.e.*, normally not present in a cell or sample obtained from the cell, such as one obtained from another organism or cell, which for example, is not ordinarily found in the cell being engineered and/or an organism from which such cell is derived. In some embodiments, the polynucleotides are not naturally occurring, such as a polynucleotide not found in nature, including one comprising chimeric combinations of polynucleotides encoding various domains from multiple different cell types. In some embodiments, the cells (*e.g.*, engineered cells) comprise a vector (*e.g.*, a viral vector, expression vector, etc.) as described herein such as a vector comprising a nucleic acid encoding a recombinant receptor described herein.

A. Vectors and Methods for Genetic Engineering

[0356] Also provided are methods, polynucleotides, compositions, and kits, for expressing the binding molecules (*e.g.*, anti-BCMA binding molecules), including recombinant receptors (*e.g.*, CARs) comprising the binding molecules, and for producing the genetically engineered cells expressing such binding molecules. In some embodiments, one or more binding molecules, including recombinant receptors (e.g., CARs) can be genetically engineered into cells or plurality of cells. The genetic engineering generally involves introduction of a nucleic acid encoding the recombinant or engineered component into the cell, such as by retroviral transduction, transfection, or transformation.

[0357] Also provided are polynucleotides encoding the chimeric antigen receptors and/or portions, *e.g.*, chains, thereof. Among the provided polynucleotides are those encoding the anti-BCMA chimeric antigen receptors (*e.g.*, antigen-binding fragment) described herein. Also provided are polynucleotides encoding one or more antibodies and/or portions thereof, e.g., those encoding one or more of the anti-BCMA antibodies (*e.g.*, antigen-binding fragment) described herein and/or other antibodies and/or portions thereof, e.g., antibodies and/or portions thereof that binds other target antigens. The polynucleotides may include those encompassing natural and/or non-naturally occurring nucleotides and bases, *e.g.*, including those with backbone modifications. The terms "nucleic acid molecule", "nucleic acid" and "polynucleotide" may be used interchangeably, and refer to a polymer of nucleotides. Such polymers of nucleotides may contain natural and/or non-natural nucleotides, and include, but are

not limited to, DNA, RNA, and PNA. "Nucleic acid sequence" refers to the linear sequence of nucleotides that comprise the nucleic acid molecule or polynucleotide.

[0358] Also provided are polynucleotides that have been optimized for codon usage and/or to eliminate splice sites, such as cryptic splice sites. Also provided are methods of optimizing and producing the coding sequences of chimeric antigen receptors, such as any of the chimeric antigen receptors described herein. Such methods are described in Section II herein.

[0359] Also provided are vectors containing the polynucleotides, such as any of the polynucleotides described herein, and host cells containing the vectors, *e.g.*, for producing the antibodies or antigen-binding fragments thereof. In some embodiments, the vector is a viral vector. In some embodiments, the vector is a retroviral vector, or a lentiviral vector. Also provided are methods for producing the antibodies or antigen-binding fragments thereof. The nucleic acid may encode an amino acid sequence comprising the V_L region and/or an amino acid sequence comprising the V_H region of the antibody (*e.g.*, the light and/or heavy chains of the antibody). The nucleic acid may encode one or more amino acid sequence comprising the V_L region and/or an amino acid sequence comprising the V_H region of the antibody (*e.g.*, the light and/or heavy chains of the antibody). In a further embodiment, one or more vectors (*e.g.*, expression vectors) comprising such polynucleotides are provided. In a further embodiment, a host cell comprising such polynucleotides is provided. In one such embodiment, a host cell comprises (*e.g.*, has been transformed with) a vector comprising a nucleic acid that encodes an amino acid sequence comprising the V_H region of the antibody. In another such embodiment, a host cell comprises (*e.g.*, has been transformed with) (1) a vector comprising a nucleic acid that encodes an amino acid sequence comprising the V_L region of the antibody and an amino acid sequence comprising the V_H region of the antibody, or (2) a first vector comprising a nucleic acid that encodes an amino acid sequence comprising the V_L region of the antibody and a second vector comprising a nucleic acid that encodes an amino acid sequence comprising the V_H region of the antibody. In some embodiments, a host cell comprises (*e.g.*, has been transformed with) one or more vectors comprising one or more nucleic acid that encodes one or more an amino acid sequence comprising one or more antibodies and/or portions thereof, e.g., antigen-binding fragments thereof. In some embodiments, one or more such host cells are provided. In some embodiments, a composition containing one or more such host cells are provided. In some embodiments, the one or more host cells can express different antibodies, or the same antibody. In some embodiments, each of the host cells can express more than one antibody.

[0360] Also provided are methods of making the anti-BCMA chimeric antigen receptors. For recombinant production of the chimeric receptors, a nucleic acid sequence encoding a chimeric receptor antibody, *e.g.*, as described herein, may be isolated and inserted into one or more vectors for further cloning and/or expression in a host cell. Such nucleic acid sequences may be readily isolated and sequenced using conventional procedures (*e.g.*, by using oligonucleotide probes that are capable of binding specifically to genes encoding the heavy and light chains of the antibody). In some embodiments, a method of making the anti-BCMA chimeric antigen receptor is provided, wherein the method comprises culturing a host cell comprising a nucleic acid sequence encoding the antibody, as provided above, under conditions suitable for expression of the receptor.

[0361] In some aspects, for production of isolated or secreted polypeptides, in addition to prokaryotes, eukaryotic microbes such as filamentous fungi or yeast are suitable cloning or expression hosts for antibody-encoding vectors, including fungi and yeast strains whose glycosylation pathways have been modified to mimic or approximate those in human cells, resulting in the production of an antibody with a partially or fully human glycosylation pattern. See Gerngross, Nat. Biotech. 22:1409-1414 (2004), and Li *et al.*, Nat. Biotech. 24:210-215 (2006).

[0362] Exemplary eukaryotic cells that may be used to express polypeptides, including isolated or secreted polypeptides, include, but are not limited to, COS cells, including COS 7 cells; 293 cells, including 293-6E cells; CHO cells, including CHO-S, DG44. Lec13 CHO cells, and FUT8 CHO cells; PER.C6® cells; and NSO cells. In some embodiments, the antibody heavy chains and/or light chains (*e.g.*, V_H region and/or V_L region) may be expressed in yeast. See, *e.g.*, U.S. Publication No. US 2006/0270045 A1. In some embodiments, a particular eukaryotic host cell is selected based on its ability to make desired post-translational modifications to the heavy chains and/or light chains (*e.g.*, V_H region and/or V_L region). For example, in some embodiments, CHO cells produce polypeptides that have a higher level of sialylation than the same polypeptide produced in 293 cells.

[0363] In particular examples immune cells, such as human immune cells are used to express the provided polypeptides encoding chimeric antigen receptors. In some examples, the immune cells are T cells, such as CD4+ and/or CD8+ immune cells, including primary cels, such as primary CD4+ and CD8+ cells.

[0364] In some embodiments, gene transfer is accomplished by first stimulating the cell, such as by combining it with a stimulus that induces a response such as proliferation, survival, and/or activation, *e.g.*, as measured by expression of a cytokine or activation marker, followed by transduction of the activated cells, and expansion in culture to numbers sufficient for clinical applications.

[0365] In some contexts, overexpression of a stimulatory factor (for example, a lymphokine or a cytokine) may be toxic to a subject. Thus, in some contexts, the engineered cells include gene segments that cause the cells to be susceptible to negative selection *in vivo*, such as following administration in adoptive immunotherapy. For example in some aspects, the cells are engineered so that they can be eliminated as a result of a change in the *in vivo* condition of the patient to which they are administered. The negative selectable phenotype may result from the insertion of a gene that confers sensitivity to an administered agent, for example, a compound. Negative selectable genes include the Herpes simplex virus type I thymidine kinase (HSV-I TK) gene (Wigler *et al.*, Cell 2:223, 1977) which confers ganciclovir sensitivity; the cellular hypoxanthine phosphoribosyltransferase (HPRT) gene, the cellular adenine phosphoribosyltransferase (APRT) gene, bacterial cytosine deaminase, (Mullen *et al.*, Proc. Natl. Acad. Sci. USA. 89:33 (1992)).

[0366] In some aspects, the cells further are engineered to promote expression of cytokines or other factors. Various methods for the introduction of genetically engineered components, *e.g.*, antigen receptors, *e.g.*, CARs, are well known and may be used with the provided methods and compositions. Exemplary methods include those for transfer of polynucleotides encoding the receptors, including via viral, *e.g.*, retroviral or lentiviral, transduction, transposons, and electroporation.

[0367] In some embodiments, recombinant polynucleotides are transferred into cells using recombinant infectious virus particles, such as, *e.g.*, vectors derived from simian virus 40 (SV40), adenoviruses, adeno-associated virus (AAV). In some embodiments, recombinant polynucleotides are transferred into T cells using recombinant lentiviral vectors or retroviral vectors, such as gamma-retroviral vectors (see, *e.g.*, Koste *et al.* (2014) Gene Therapy 2014 Apr 3. doi: 10.1038/gt.2014.25; Carlens *et al.* (2000) Exp Hematol 28(10): 1137-46; Alonso-Camino *et al.* (2013) Mol Ther Nucl Acids 2, e93; Park *et al.*, Trends Biotechnol. 2011 November 29(11): 550–557).

[0368] In some embodiments, the retroviral vector has a long terminal repeat sequence (LTR), *e.g.*, a retroviral vector derived from the Moloney murine leukemia virus (MoMLV), myeloproliferative sarcoma virus (MPSV), murine embryonic stem cell virus (MESV), murine stem cell virus (MSCV), spleen focus forming virus (SFFV), or human immunodeficiency virus type 1 (HIV-1). Most retroviral vectors are derived from murine retroviruses. In some embodiments, the retroviruses include those derived from any avian or mammalian cell source. The retroviruses typically are amphotropic, meaning that they are capable of infecting host cells of several species, including humans. In one embodiment, the gene to be expressed replaces the retroviral gag, pol and/or env sequences. A number of illustrative retroviral systems have been described (*e.g.*, U.S. Pat. Nos. 5,219,740; 6,207,453; 5,219,740; Miller and Rosman (1989) BioTechniques 7:980-990; Miller, A. D. (1990) Human Gene Therapy 1:5-14; Scarpa *et al.* (1991) Virology 180:849-852; Burns *et al.* (1993) Proc. Natl. Acad. Sci. USA 90:8033-8037; and Boris-Lawrie and Temin (1993) Cur. Opin. Genet. Develop. 3:102-109.

[0369] Methods of lentiviral transduction are known. Exemplary methods are described in, *e.g.*, Wang *et al.* (2012) J. Immunother. 35(9): 689-701; Cooper *et al.* (2003) Blood. 101:1637–1644; Verhoeyen *et al.* (2009) Methods Mol Biol. 506: 97-114; and Cavalieri *et al.* (2003) Blood. 102(2): 497-505.

[0370] In some embodiments, recombinant polynucleotides are transferred into T cells via electroporation (see, *e.g.*, Chicaybam et al, (2013) PLoS ONE 8(3): e60298 and Van Tedeloo *et al.* (2000) Gene Therapy 7(16): 1431-1437). In some embodiments, recombinant polynucleotides are transferred into T cells via transposition (see, *e.g.*, Manuri *et al.* (2010) Hum Gene Ther 21(4): 427-437; Sharma *et al.* (2013) Molec Ther Nucl Acids 2, e74; and Huang *et al.* (2009) Methods Mol Biol 506: 115-126). Other methods of introducing and expressing genetic material in immune cells include calcium phosphate transfection (*e.g.*, as described in Current Protocols in Molecular Biology, John Wiley & Sons, New York. N.Y.), protoplast fusion, cationic liposome-mediated transfection; tungsten particle-facilitated microparticle bombardment (Johnston, Nature, 346: 776-777 (1990)); and strontium phosphate DNA co-precipitation (Brash *et al.*, Mol. Cell Biol., 7: 2031-2034 (1987)).

[0371] Other approaches and vectors for transfer of the polynucleotides encoding the recombinant products are those described, *e.g.*, in international patent application, Publication No.: WO2014055668, and U.S. Patent No. 7,446,190.

[0372] Among additional polynucleotides, *e.g.*, genes for introduction are those to improve the efficacy of therapy, such as by promoting viability and/or function of transferred cells; genes to provide a genetic marker for selection and/or evaluation of the cells, such as to assess *in vivo* survival or localization; genes to improve safety, for example, by making the cell susceptible to negative selection *in vivo* as described by Lupton S. D. *et al.*, Mol. and Cell Biol., 11:6 (1991); and Riddell *et al.*, Human Gene Therapy 3:319-338 (1992); see also the publications of PCT/US91/08442 and PCT/US94/05601 by Lupton *et al.* describing the use of bifunctional selectable fusion genes derived from fusing a dominant positive selectable marker with a negative selectable marker. See, *e.g.*, Riddell *et al.*, US Patent No. 6,040,177, at columns 14-17.

[0373] In some embodiments, one or more binding molecules, including antibodies and/or recombinant receptors (e.g., CARs), can be genetically engineered to be expressed in cells or plurality of cells. In some embodiments, a first recombinant receptor and a second binding molecule, e.g., recombinant receptor, are encoded by the same or separate nucleic acid molecules. In some embodiments, additional binding molecules are engineered to be expressed in cells or a plurality of cells.

[0374] In some cases, the polynucleotide containing nucleic acid sequences encoding the BCMA-binding receptor, e.g., chimeric antigen receptor (CAR), contains a signal sequence that encodes a signal peptide. In some aspects, the signal sequence may encode a signal peptide derived from a native polypeptide. In other aspects, the signal sequence may encode a heterologous or non-native signal peptide. In some aspects, non-limiting exemplary signal peptide include a signal peptide of the IgG kappa chain set forth in SEQ ID NO: 620, or encoded by the nucleotide sequence set forth in SEQ ID NO: 619 or 682-685; a GMCSFR alpha chain set forth in SEQ ID NO:851 and encoded by the nucleotide sequence set forth in SEQ ID NO:850; a CD8 alpha signal peptide set forth in SEQ ID NO:852; or a CD33 signal peptide set forth in SEQ ID NO:853.

[0375] In some embodiments the vector or construct can contain promoter and/or enhancer or regulatory elements to regulate expression of the encoded recombinant receptor. In some examples the promoter and/or enhancer or regulatory elements can be condition-dependent promoters, enhancers, and/or regulatory elements. In some examples these elements drive expression of the transgene. In some examples, the CAR transgene can be operatively linked to a promoter, such as an EF1alpha promoter with an HTLV1 enhancer (SEQ ID NO: 635). In some examples, the CAR transgene is operatively linked to a Woodchuck Hepatitis Virus (WHP)

Posttranscriptional Regulatory Element (WPRE; SEQ ID NO: 636), located downstream of the transgene.

[0376] In some embodiments, the vector or construct can contain a single promoter that drives the expression of one or more nucleic acid molecules. In some embodiments, such nucleic acid molecules, *e.g.*, transcripts, can be multicistronic (bicistronic or tricistronic, see *e.g.*, U.S. Patent No. 6,060,273). For example, in some embodiments, transcription units can be engineered as a bicistronic unit containing an IRES (internal ribosome entry site), which allows coexpression of gene products (*e.g.* encoding a first and second chimeric receptor) by a message from a single promoter. Alternatively, in some cases, a single promoter may direct expression of an RNA that contains, in a single open reading frame (ORF), two or three genes (*e.g.* encoding a first and second binding molecules, e.g., antibody recombinant receptor) separated from one another by sequences encoding a self-cleavage peptide (*e.g.*, 2A cleavage sequences) or a protease recognition site (*e.g.*, furin). The ORF thus encodes a single polypeptide, which, either during (in the case of T2A) or after translation, is cleaved into the individual proteins. In some cases, the peptide, such as T2A, can cause the ribosome to skip (ribosome skipping) synthesis of a peptide bond at the C-terminus of a 2A element, leading to separation between the end of the 2A sequence and the next peptide downstream (*see*, for example, de Felipe. *Genetic Vaccines and Ther.* 2:13 (2004) and deFelipe *et al. Traffic* 5:616-626 (2004)). Many 2A elements are known. Examples of 2A sequences that can be used in the methods and polynucleotides disclosed herein, without limitation, 2A sequences from the foot-and-mouth disease virus (F2A, *e.g.*, SEQ ID NO: 659 or 660), equine rhinitis A virus (E2A, *e.g.*, SEQ ID NO: 657 or 658), Thosea asigna virus (T2A, *e.g.*, SEQ ID NO: 631, 653, or 654), and porcine teschovirus-1 (P2A, *e.g.*, SEQ ID NO: 655 or 656) as described in U.S. Patent Publication No. 20070116690. In some embodiments, the one or more different or separate promoters drive the expression of one or more nucleic acid molecules encoding the one or more binding molecules, e.g., recombinant receptors.

[0377] Any of the binding molecules, e.g., antibodies and/or recombinant receptors provided herein, e.g., BCMA-binding molecules and/or the additional recombinant receptors, can be encoded by polynucleotides containing one or more nucleic acid molecules encoding the receptors, in any combinations or arrangements. For example, one, two, three or more polynucleotides can encode one, two, three or more different receptors or domains. In some embodiments, one vector or construct contains nucleic acid molecules encoding one or more

binding molecules, e.g., antibody and/or recombinant receptor, and a separate vector or construct contains nucleic acid molecules encoding an additional binding molecule, e.g., antibody and/or recombinant receptor. Each of the nucleic acid molecules can also encode one or more marker(s), such as a surface marker, e.g., truncated EGFR (tEGFR).

[0378] Also provided are compositions containing one or more of the nucleic acid molecules, vectors or constructs, such as any described above. In some embodiments, the nucleic acid molecules, vectors, constructs or compositions can be used to engineer cells, such as T cells, to express any of the binding molecules, e.g., antibody or recombinant receptor, and/or the additional binding molecules.

B. Preparation of Cells for Engineering

[0379] In some embodiments, preparation of the engineered cells includes one or more culture and/or preparation steps. The cells for introduction of the recombinant receptor (*e.g.*, CAR) may be isolated from a sample, such as a biological sample, *e.g.*, one obtained from or derived from a subject. In some embodiments, the subject from which the cell is isolated is one having the disease or condition or in need of a cell therapy or to which cell therapy will be administered. The subject in some embodiments is a human in need of a particular therapeutic intervention, such as the adoptive cell therapy for which cells are being isolated, processed, and/or engineered.

[0380] Accordingly, the cells in some embodiments are primary cells, *e.g.*, primary human cells. The samples include tissue, fluid, and other samples taken directly from the subject, as well as samples resulting from one or more processing steps, such as separation, centrifugation, genetic engineering (*e.g.* transduction with viral vector), washing, and/or incubation. The biological sample can be a sample obtained directly from a biological source or a sample that is processed. Biological samples include, but are not limited to, body fluids, such as blood, plasma, serum, cerebrospinal fluid, synovial fluid, urine and sweat, tissue and organ samples, including processed samples derived therefrom.

[0381] In some aspects, the sample from which the cells are derived or isolated is blood or a blood-derived sample, or is or is derived from an apheresis or leukapheresis product. Exemplary samples include whole blood, peripheral blood mononuclear cells (PBMCs), leukocytes, bone marrow, thymus, tissue biopsy, tumor, leukemia, lymphoma, lymph node, gut associated lymphoid tissue, mucosa associated lymphoid tissue, spleen, other lymphoid tissues, liver, lung, stomach, intestine, colon, kidney, pancreas, breast, bone, prostate, cervix, testes, ovaries, tonsil,

or other organ, and/or cells derived therefrom. Samples include, in the context of cell therapy, *e.g.*, adoptive cell therapy, samples from autologous and allogeneic sources.

[0382] In some embodiments, the cells are derived from cell lines, *e.g.*, T cell lines. The cells in some embodiments are obtained from a xenogeneic source, for example, from mouse, rat, non-human primate, or pig.

[0383] In some embodiments, isolation of the cells includes one or more preparation and/or non-affinity based cell separation steps. In some examples, cells are washed, centrifuged, and/or incubated in the presence of one or more reagents, for example, to remove unwanted components, enrich for desired components, lyse or remove cells sensitive to particular reagents. In some examples, cells are separated based on one or more property, such as density, adherent properties, size, sensitivity and/or resistance to particular components.

[0384] In some examples, cells from the circulating blood of a subject are obtained, *e.g.*, by apheresis or leukapheresis. The samples, in some aspects, contain lymphocytes, including T cells, monocytes, granulocytes, B cells, other nucleated white blood cells, red blood cells, and/or platelets, and in some aspects contain cells other than red blood cells and platelets.

[0385] In some embodiments, the blood cells collected from the subject are washed, *e.g.*, to remove the plasma fraction and to place the cells in an appropriate buffer or media for subsequent processing steps. In some embodiments, the cells are washed with phosphate buffered saline (PBS). In some embodiments, the wash solution lacks calcium and/or magnesium and/or many or all divalent cations. In some aspects, a washing step is accomplished a semi-automated "flow-through" centrifuge (for example, the Cobe 2991 cell processor, Baxter) according to the manufacturer's instructions. In some aspects, a washing step is accomplished by tangential flow filtration (TFF) according to the manufacturer's instructions. In some embodiments, the cells are resuspended in a variety of biocompatible buffers after washing, such as, for example, Ca^{++}/Mg^{++} free PBS. In certain embodiments, components of a blood cell sample are removed and the cells directly resuspended in culture media.

[0386] In some embodiments, the methods include density-based cell separation methods, such as the preparation of white blood cells from peripheral blood by lysing the red blood cells and centrifugation through a Percoll or Ficoll gradient.

[0387] In some embodiments, the isolation methods include the separation of different cell types based on the expression or presence in the cell of one or more specific molecules, such as surface markers, *e.g.*, surface proteins, intracellular markers, or nucleic acid. In some

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

[0388] Such separation steps can be based on positive selection, in which the cells having bound the reagents are retained for further use, and/or negative selection, in which the cells having not bound to the antibody or binding partner are retained. In some examples, both fractions are retained for further use. In some aspects, negative selection can be particularly useful where no antibody is available that specifically identifies a cell type in a heterogeneous population, such that separation is best carried out based on markers expressed by cells other than the desired population.

[0389] The separation need not result in 100% enrichment or removal of a particular cell population or cells expressing a particular marker. For example, positive selection of or enrichment for cells of a particular type, such as those expressing a marker, refers to increasing the number or percentage of such cells, but need not result in a complete absence of cells not expressing the marker. Likewise, negative selection, removal, or depletion of cells of a particular type, such as those expressing a marker, refers to decreasing the number or percentage of such cells, but need not result in a complete removal of all such cells.

[0390] In some examples, multiple rounds of separation steps are carried out, where the positively or negatively selected fraction from one step is subjected to another separation step, such as a subsequent positive or negative selection. In some examples, a single separation step can deplete cells expressing multiple markers simultaneously, such as by incubating cells with a plurality of antibodies or binding partners, each specific for a marker targeted for negative selection. Likewise, multiple cell types can simultaneously be positively selected by incubating cells with a plurality of antibodies or binding partners expressed on the various cell types.

[0391] For example, in some aspects, specific subpopulations of T cells, such as cells positive or expressing high levels of one or more surface markers, *e.g.*, CD28+, CD62L+, CCR7+, CD27+, CD127+, CD4+, CD8+, CD45RA+, and/or CD45RO+ T cells, are isolated by positive or negative selection techniques.

[0392] For example, CD3+, CD28+ T cells can be positively selected using CD3/CD28 conjugated magnetic beads (*e.g.*, DYNABEADS® M-450 CD3/CD28 T Cell Expander, MACSiBeads™, etc.).

[0393] In some embodiments, isolation is carried out by enrichment for a particular cell population by positive selection, or depletion of a particular cell population, by negative selection. In some embodiments, positive or negative selection is accomplished by incubating cells with one or more antibodies or other binding agent that specifically bind to one or more surface markers expressed or expressed (marker^{+}) at a relatively higher level (marker^{high}) on the positively or negatively selected cells, respectively.

[0394] In some embodiments, T cells are separated from a PBMC sample by negative selection of markers expressed on non-T cells, such as B cells, monocytes, or other white blood cells, such as CD14. In some aspects, CD4+ and/or CD8+ selection steps are used to separate CD4+ helper and CD8+ cytotoxic T cells from a composition, such as from a PBMC composition such as one obtained via leukapheresis. Such CD4+ and CD8+ populations, in some aspects, can be further sorted into sub-populations by positive or negative selection for markers expressed or expressed to a relatively higher degree on one or more naive, memory, and/or effector T cell subpopulations. In some embodiments, CD4+ and CD8+ cells are mixed at a desired ratio

[0395] In some embodiments, CD8+ cells are further enriched for or depleted of naive, central memory, effector memory, and/or central memory stem cells, such as by positive or negative selection based on surface antigens associated with the respective subpopulation. In some embodiments, enrichment for central memory T (T_{CM}) cells is carried out to increase efficacy, such as to improve long-term survival, expansion, and/or engraftment following administration, which in some aspects is particularly robust in such sub-populations. See Terakura *et al.* (2012) Blood.1:72–82; Wang *et al.* (2012) J Immunother. 35(9):689-701. In some embodiments, combining T_{CM}-enriched CD8+ T cells and CD4+ T cells further enhances efficacy.

[0396] In embodiments, memory T cells are present in both CD62L+ and CD62L- subsets of CD8+ peripheral blood lymphocytes. PBMC can be enriched for or depleted of CD62L-CD8+ and/or CD62L+CD8+ fractions, such as using anti-CD8 and anti-CD62L antibodies.

[0397] In some embodiments, the enrichment for central memory T (T_{CM}) cells is based on positive or high surface expression of CD45RO, CD62L, CCR7, CD28, CD3, and/or CD 127; in

some aspects, it is based on negative selection for cells expressing or highly expressing CD45RA and/or granzyme B. In some aspects, isolation of a CD8+ population enriched for T_{CM} cells is carried out by depletion of cells expressing CD4, CD14, CD45RA, and positive selection or enrichment for cells expressing CD62L. In one aspect, enrichment for central memory T (T_{CM}) cells is carried out starting with a negative fraction of cells selected based on CD4 expression, which is subjected to a negative selection based on expression of CD14 and CD45RA, and a positive selection based on CD62L. Such selections in some aspects are carried out simultaneously and in other aspects are carried out sequentially, in either order. In some aspects, the same CD4 expression-based selection step used in preparing the CD8+ cell population or subpopulation, also is used to generate the CD4+ cell population or sub-population, such that both the positive and negative fractions from the CD4-based separation are retained and used in subsequent steps of the methods, optionally following one or more further positive or negative selection steps.

[0398] In a particular example, a sample of PBMCs or other white blood cell sample is subjected to selection of CD4+ cells, where both the negative and positive fractions are retained. The negative fraction then is subjected to negative selection based on expression of CD14 and CD45RA, and positive selection based on a marker characteristic of central memory T cells, such as CD62L or CCR7, where the positive and negative selections are carried out in either order.

[0399] CD4+ T helper cells are sorted into naïve, central memory, and effector cells by identifying cell populations that have cell surface antigens. CD4+ lymphocytes can be obtained by standard methods. In some embodiments, naive CD4+ T lymphocytes are CD45RO-, CD45RA+, CD62L+, CD4+ T cells. In some embodiments, central memory CD4+ cells are CD62L+ and CD45RO+. In some embodiments, effector CD4+ cells are CD62L- and CD45RO-.

[0400] In one example, to enrich for CD4+ cells by negative selection, a monoclonal antibody cocktail typically includes antibodies to CD14, CD20, CD11b, CD16, HLA-DR, and CD8. In some embodiments, the antibody or binding partner is bound to a solid support or matrix, such as a magnetic bead or paramagnetic bead, to allow for separation of cells for positive and/or negative selection. For example, in some embodiments, the cells and cell populations are separated or isolated using immunomagnetic (or affinitymagnetic) separation techniques (reviewed in Methods in Molecular Medicine, vol. 58: Metastasis Research

Protocols, Vol. 2: Cell Behavior *In vitro* and *In vivo*, p 17-25 Edited by: S. A. Brooks and U. Schumacher © Humana Press Inc., Totowa, NJ).

[0401] In some aspects, the sample or composition of cells to be separated is incubated with small, magnetizable or magnetically responsive material, such as magnetically responsive particles or microparticles, such as paramagnetic beads (*e.g.*, such as Dynabeads® or MACS® beads). The magnetically responsive material, *e.g.*, particle, generally is directly or indirectly attached to a binding partner, *e.g.*, an antibody, that specifically binds to a molecule, *e.g.*, surface marker, present on the cell, cells, or population of cells that it is desired to separate, *e.g.*, that it is desired to negatively or positively select.

[0402] In some embodiments, the magnetic particle or bead comprises a magnetically responsive material bound to a specific binding member, such as an antibody or other binding partner. There are many well-known magnetically responsive materials used in magnetic separation methods. Suitable magnetic particles include those described in Molday, U.S. Pat. No. 4,452,773, and in European Patent Specification EP 452342 B, which are hereby incorporated by reference. Colloidal sized particles, such as those described in Owen U.S. Pat. No. 4,795,698, and Liberti *et al.*, U.S. Pat. No. 5,200,084, are other examples.

[0403] The incubation generally is carried out under conditions whereby the antibodies or binding partners, or molecules, such as secondary antibodies or other reagents, which specifically bind to such antibodies or binding partners, which are attached to the magnetic particle or bead, specifically bind to cell surface molecules if present on cells within the sample.

[0404] In some aspects, the sample is placed in a magnetic field, and those cells having magnetically responsive or magnetizable particles attached thereto will be attracted to the magnet and separated from the unlabeled cells. For positive selection, cells that are attracted to the magnet are retained; for negative selection, cells that are not attracted (unlabeled cells) are retained. In some aspects, a combination of positive and negative selection is performed during the same selection step, where the positive and negative fractions are retained and further processed or subject to further separation steps.

[0405] In certain embodiments, the magnetically responsive particles are coated in primary antibodies or other binding partners, secondary antibodies, lectins, enzymes, or streptavidin. In certain embodiments, the magnetic particles are attached to cells via a coating of primary antibodies specific for one or more markers. In certain embodiments, the cells, rather than the beads, are labeled with a primary antibody or binding partner, and then cell-type specific

secondary antibody- or other binding partner (*e.g.*, streptavidin)-coated magnetic particles, are added. In certain embodiments, streptavidin-coated magnetic particles are used in conjunction with biotinylated primary or secondary antibodies.

[0406] In some embodiments, the magnetically responsive particles are left attached to the cells that are to be subsequently incubated, cultured and/or engineered; in some aspects, the particles are left attached to the cells for administration to a patient. In some embodiments, the magnetizable or magnetically responsive particles are removed from the cells. Methods for removing magnetizable particles from cells are known and include, *e.g.*, the use of competing non-labeled antibodies, magnetizable particles or antibodies conjugated to cleavable linkers, etc. In some embodiments, the magnetizable particles are biodegradable.

[0407] In some embodiments, the affinity-based selection is via magnetic-activated cell sorting (MACS®) (Miltenyi Biotec, Auburn, CA). Magnetic Activated Cell Sorting (MACS®) systems are capable of high-purity selection of cells having magnetized particles attached thereto. In certain embodiments, MACS® operates in a mode wherein the non-target and target species are sequentially eluted after the application of the external magnetic field. That is, the cells attached to magnetized particles are held in place while the unattached species are eluted. Then, after this first elution step is completed, the species that were trapped in the magnetic field and were prevented from being eluted are freed in some manner such that they can be eluted and recovered. In certain embodiments, the non-target cells are labelled and depleted from the heterogeneous population of cells.

[0408] In certain embodiments, the isolation or separation is carried out using a system, device, or apparatus that carries out one or more of the isolation, cell preparation, separation, processing, incubation, culture, and/or formulation steps of the methods. In some aspects, the system is used to carry out each of these steps in a closed or sterile environment, for example, to minimize error, user handling and/or contamination. In one example, the system is a system as described in International Patent Application, Publication Number WO2009/072003, or US 20110003380 A1.

[0409] In some embodiments, the system or apparatus carries out one or more, *e.g.*, all, of the isolation, processing, engineering, and formulation steps in an integrated or self-contained system, and/or in an automated or programmable fashion. In some aspects, the system or apparatus includes a computer and/or computer program in communication with the system or

apparatus, which allows a user to program, control, assess the outcome of, and/or adjust various aspects of the processing, isolation, engineering, and formulation steps.

[0410] In some aspects, the separation and/or other steps is carried out using CliniMACS® system (Miltenyi Biotec), for example, for automated separation of cells on a clinical-scale level in a closed and sterile system. Components can include an integrated microcomputer, magnetic separation unit, peristaltic pump, and various pinch valves. The integrated computer in some aspects controls all components of the instrument and directs the system to perform repeated procedures in a standardized sequence. The magnetic separation unit in some aspects includes a movable permanent magnet and a holder for the selection column. The peristaltic pump controls the flow rate throughout the tubing set and, together with the pinch valves, ensures the controlled flow of buffer through the system and continual suspension of cells.

[0411] The CliniMACS® system in some aspects uses antibody-coupled magnetizable particles that are supplied in a sterile, non-pyrogenic solution. In some embodiments, after labelling of cells with magnetic particles the cells are washed to remove excess particles. A cell preparation bag is then connected to the tubing set, which in turn is connected to a bag containing buffer and a cell collection bag. The tubing set consists of pre-assembled sterile tubing, including a pre-column and a separation column, and are for single use only. After initiation of the separation program, the system automatically applies the cell sample onto the separation column. Labelled cells are retained within the column, while unlabeled cells are removed by a series of washing steps. In some embodiments, the cell populations for use with the methods described herein are unlabeled and are not retained in the column. In some embodiments, the cell populations for use with the methods described herein are labeled and are retained in the column. In some embodiments, the cell populations for use with the methods described herein are eluted from the column after removal of the magnetic field, and are collected within the cell collection bag.

[0412] In certain embodiments, separation and/or other steps are carried out using the CliniMACS Prodigy® system (Miltenyi Biotec). The CliniMACS Prodigy® system in some aspects is equipped with a cell processing unity that permits automated washing and fractionation of cells by centrifugation. The CliniMACS Prodigy® system can also include an onboard camera and image recognition software that determines the optimal cell fractionation endpoint by discerning the macroscopic layers of the source cell product. For example, peripheral blood may be automatically separated into erythrocytes, white blood cells and plasma

layers. The CliniMACS Prodigy® system can also include an integrated cell cultivation chamber which accomplishes cell culture protocols such as, *e.g.*, cell differentiation and expansion, antigen loading, and long-term cell culture. Input ports can allow for the sterile removal and replenishment of media and cells can be monitored using an integrated microscope. See, *e.g.*, Klebanoff *et al.* (2012) J Immunother. 35(9): 651–660, Terakura *et al.* (2012) Blood.1:72–82, and Wang *et al.* (2012) J Immunother. 35(9):689-701.

[0413] In some embodiments, a cell population described herein is collected and enriched (or depleted) via flow cytometry, in which cells stained for multiple cell surface markers are carried in a fluidic stream. In some embodiments, a cell population described herein is collected and enriched (or depleted) via preparative scale (FACS)-sorting. In certain embodiments, a cell population described herein is collected and enriched (or depleted) by use of microelectromechanical systems (MEMS) chips in combination with a FACS-based detection system (see, *e.g.*, WO 2010/033140, Cho *et al.* (2010) Lab Chip 10, 1567-1573; and Godin *et al.* (2008) J Biophoton. 1(5):355–376. In both cases, cells can be labeled with multiple markers, allowing for the isolation of well-defined T cell subsets at high purity.

[0414] In some embodiments, the antibodies or binding partners are labeled with one or more detectable marker, to facilitate separation for positive and/or negative selection. For example, separation may be based on binding to fluorescently labeled antibodies. In some examples, separation of cells based on binding of antibodies or other binding partners specific for one or more cell surface markers are carried in a fluidic stream, such as by fluorescence-activated cell sorting (FACS), including preparative scale (FACS) and/or microelectromechanical systems (MEMS) chips, *e.g.*, in combination with a flow-cytometric detection system. Such methods allow for positive and negative selection based on multiple markers simultaneously.

[0415] In some embodiments, the preparation methods include steps for freezing, *e.g.*, cryopreserving, the cells, either before or after isolation, incubation, and/or engineering. In some embodiments, the freeze and subsequent thaw step removes granulocytes and, to some extent, monocytes in the cell population. In some embodiments, the cells are suspended in a freezing solution, *e.g.*, following a washing step to remove plasma and platelets. Any of a variety of known freezing solutions and parameters in some aspects may be used. One example involves using PBS containing 20% DMSO and 8% human serum albumin (HSA), or other suitable cell freezing media. This is then diluted 1:1 with media so that the final concentration of

DMSO and HSA are 10% and 4%, respectively. The cells are then frozen to −80° C. at a rate of 1° per minute and stored in the vapor phase of a liquid nitrogen storage tank.

[0416] In some embodiments, the provided methods include cultivation, incubation, culture, and/or genetic engineering steps. For example, in some embodiments, provided are methods for incubating and/or engineering the depleted cell populations and culture-initiating compositions.

[0417] Thus, in some embodiments, the cell populations are incubated in a culture-initiating composition. The incubation and/or engineering may be carried out in a culture vessel, such as a unit, chamber, well, column, tube, tubing set, valve, vial, culture dish, bag, or other container for culture or cultivating cells.

[0418] In some embodiments, the cells are incubated and/or cultured prior to or in connection with genetic engineering. The incubation steps can include culture, cultivation, stimulation, activation, and/or propagation. In some embodiments, the compositions or cells are incubated in the presence of stimulating conditions or a stimulatory agent. Such conditions include those designed to induce proliferation, expansion, activation, and/or survival of cells in the population, to mimic antigen exposure, and/or to prime the cells for genetic engineering, such as for the introduction of a recombinant antigen receptor.

[0419] The conditions can include one or more of particular media, temperature, oxygen content, carbon dioxide content, time, agents, *e.g.*, nutrients, amino acids, antibiotics, ions, and/or stimulatory factors, such as cytokines, chemokines, antigens, binding partners, fusion proteins, recombinant soluble receptors, and any other agents designed to activate the cells.

[0420] In some embodiments, the stimulating conditions or agents include one or more agent, e.g., ligand, which is capable of stimulating or activating an intracellular signaling domain of a TCR complex. In some aspects, the agent turns on or initiates TCR/CD3 intracellular signaling cascade in a T cell. Such agents can include antibodies, such as those specific for a TCR, e.g. anti-CD3. In some embodiments, the stimulating conditions include one or more agent, e.g. ligand, which is capable of stimulating a costimulatory receptor, e.g., anti-CD28. In some embodiments, such agents and/or ligands may be, bound to solid support such as a bead, and/or one or more cytokines. Optionally, the expansion method may further comprise the step of adding anti-CD3 and/or anti CD28 antibody to the culture medium (e.g., at a concentration of at least about 0.5 ng/ml). In some embodiments, the stimulating agents include IL-2, IL-15 and/or IL-7. In some aspects, the IL-2 concentration is at least about 10 units/mL.

[0421] In some aspects, incubation is carried out in accordance with techniques such as those described in US Patent No. 6,040,177 to Riddell *et al.*, Klebanoff *et al.* (2012) J Immunother. 35(9): 651–660, Terakura *et al.* (2012) Blood.1:72–82, and/or Wang *et al.* (2012) J Immunother. 35(9):689-701.

[0422] In some embodiments, the T cells are expanded by adding to the culture-initiating composition feeder cells, such as non-dividing peripheral blood mononuclear cells (PBMC), (*e.g.*, such that the resulting population of cells contains at least about 5, 10, 20, or 40 or more PBMC feeder cells for each T lymphocyte in the initial population to be expanded); and incubating the culture (*e.g.* for a time sufficient to expand the numbers of T cells). In some aspects, the non-dividing feeder cells can comprise gamma-irradiated PBMC feeder cells. In some embodiments, the PBMC are irradiated with gamma rays in the range of about 3000 to 3600 rads to prevent cell division. In some aspects, the feeder cells are added to culture medium prior to the addition of the populations of T cells.

[0423] In some embodiments, the stimulating conditions include temperature suitable for the growth of human T lymphocytes, for example, at least about 25 degrees Celsius, generally at least about 30 degrees, and generally at or about 37 degrees Celsius. Optionally, the incubation may further comprise adding non-dividing EBV-transformed lymphoblastoid cells (LCL) as feeder cells. LCL can be irradiated with gamma rays in the range of about 6000 to 10,000 rads. The LCL feeder cells in some aspects is provided in any suitable amount, such as a ratio of LCL feeder cells to initial T lymphocytes of at least about 10:1.

[0424] In embodiments, antigen-specific T cells, such as antigen-specific CD4+ and/or CD8+ T cells, are obtained by stimulating naive or antigen specific T lymphocytes with antigen. For example, antigen-specific T cell lines or clones can be generated to cytomegalovirus antigens by isolating T cells from infected subjects and stimulating the cells *in vitro* with the same antigen.

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

[0425] Also provided are cells such as engineered cells that can bind to and/or target multiple antigens. In some embodiments, improved selectivity and specificity is achieved through strategies targeting multiple antigens. Such strategies generally involve multiple antigen-binding domains, which typically are present on distinct genetically engineered antigen receptors and specifically bind to distinct antigens. In some embodiments, the cells are engineered with the ability to bind more than one antigen. For example, in some embodiments,

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

[0426] Also provided are multispecific cells containing any of the binding molecules described herein, such as cells containing a cell surface protein including the anti-BCMA antibody and an additional cell surface protein, such as an additional chimeric receptor, which binds to a different antigen or a different epitope on BCMA. In some embodiments, provided are compositions of cells that express recombinant receptors, wherein one or more of the binding molecules, multispecific binding molecules and/or recombinant receptors bind and/or target BCMA. In some embodiments, the multispecific binding molecules and/or recombinant receptors target one or more different epitopes on BCMA.

[0427] In some embodiments, provided are composition of cells, wherein each type of cell expresses one or more binding molecules, e.g., recombinant receptors. In some embodiments, the cell comprises (*e.g.*, has been transformed with) one or more vectors comprising one or more nucleic acid that encodes one or more an amino acid sequence comprising one or more antibodies and/or portions thereof, e.g., antigen-binding fragments thereof. In some embodiments, one or more such cells are provided. In some embodiments, a composition containing one or more such cells is provided. In some embodiments, the one or more cells can express different antibodies, or the same antibody. In some embodiments, each of the cells expresses one or more antibodies, such as more than one antibody. In some embodiments, each of the cells expresses a multispecific binding molecule, e.g., a multispecific receptor, e.g., CAR.

[0428] In some embodiments, the cells include multi-targeting strategies that target BCMA and a second or additional antigen associated with a particular disease or condition. In some embodiments, the second or additional antigen is targeted by a multispecific binding molecule and/or multiple binding molecules and/or a plurality of cells, e.g., one or more cells, each engineered to express one or more recombinant receptors. In some embodiments, a recombinant receptor targeting a second or additional antigen is expressed on the same cell as a BCMA binding molecule, or on a different cell.

[0429] In some embodiments, among the second or additional antigens for multi-targeting strategies includes those in which at least one of the antigens is a universal tumor antigen, or a family member thereof. In some embodiments, the second or additional antigen is an antigen expressed on a tumor. In some embodiments, the BCMA-binding molecules provided herein target an antigen on the same tumor type as the second or additional antigen. In some embodiments, the second or additional antigen may also be a universal tumor antigen or may be a tumor antigen specific to a tumor type. In some embodiments, the cell further comprises an additional genetically engineered antigen receptor that recognizes a second or additional antigen expressed on a disease or condition to be treated and induces a stimulatory or activating signal.

[0430] Exemplary antigens include CD4, CD5, CD8, CD14, CD15, CD19, CD20, CD21, CD22, CD23, CD25, CD33, CD37, CD38, CD40, CD40L, CD46, CD52, CD54, CD74, CD80, CD126, CD138, B7, MUC-1, Ia, HM1.24, HLA-DR, tenascin, an angiogenesis factor, VEGF, PIGF, ED-B fibronectin, an oncogene, an oncogene product, CD66a-d, necrosis antigens, Ii, IL-2, T101, TAC, IL-6, ROR1, TRAIL-R1 (DR4), TRAIL-R2 (DR5), B cell maturation antigen (BCMA), tEGFR, Her2, L1-CAM, mesothelin, CEA, hepatitis B surface antigen, anti-folate receptor, CD24, CD30, CD44, EGFR, EGP-2, EGP-4, EPHa2, ErbB2, ErbB3, ErbB4, erbB dimers, EGFR vIII, FBP, FCRL5, FCRH5, fetal acetylcholine receptor, GD2, GD3, G protein-coupled receptor class C group 5 member D (GPRC5D), HMW-MAA, IL-22R-alpha, IL-13R-alpha2, kdr, kappa light chain, Lewis Y, L1-cell adhesion molecule (L1-CAM), Melanoma-associated antigen (MAGE)-A1, MAGE-A3, MAGE-A6, Preferentially expressed antigen of melanoma (PRAME), survivin, EGP2, EGP40, TAG72, B7-H6, IL-13 receptor a2 (IL-13Ra2), CA9, CD171, G250/CAIX, HLA-AI MAGE Al, HLA-A2 NY-ESO-1, PSCA, folate receptor-a, CD44v6, CD44v7/8, avb6 integrin, 8H9, NCAM, VEGF receptors, 5T4, Foetal AchR, NKG2D ligands, dual antigen, an antigen associated with a universal tag, a cancer-testes antigen, MUC1, MUC16, NY-ESO-1, MART-1, gp100, oncofetal antigen, VEGF-R2, carcinoembryonic antigen

(CEA), prostate specific antigen, PSMA, Her2/neu, estrogen receptor, progesterone receptor, ephrinB2, CD123, c-Met, GD-2, O-acetylated GD2 (OGD2), CE7, Wilms Tumor 1 (WT-1), a cyclin, cyclin A2, CCL-1, hTERT, MDM2, CYP1B, WT1, livin, AFP, p53, cyclin (D1), CS-1, BCMA, BAFF-R, TACI, CD56, TIM-3, CD123, L1-cell adhesion molecule, MAGE-A1, MAGE A3, a cyclin, such as cyclin A1 (CCNA1) and/or a pathogen-specific antigen, biotinylated molecules, molecules expressed by HIV, HCV, HBV and/or other pathogens, and/or in some aspects, neoepitopes or neoantigens thereof. In some embodiments, the antigen is associated with or is a universal tag.

[0431] In some embodiments, the plurality of antigens, e.g., the first antigen, e.g., BCMA, and the second or additional antigens, are expressed on the cell, tissue, or disease or condition being targeted, such as on the cancer cell. In some aspects, the cell, tissue, disease or condition is multiple myeloma or a multiple myeloma cell. One or more of the plurality of antigens generally also is expressed on a cell which it is not desired to target with the cell therapy, such as a normal or non-diseased cell or tissue, and/or the engineered cells themselves. In such embodiments, by requiring ligation of multiple receptors to achieve a response of the cell, specificity and/or efficacy is achieved.

[0432] In some aspects, the antigen, e.g., the second or additional antigen, such as the disease-specific antigen and/or related antigen, is expressed on multiple myeloma, such as G protein-coupled receptor class C group 5 member D (GPRC5D), CD38 (cyclic ADP ribose hydrolase), CD138 (syndecan-1, syndecan, SYN-1), CS-1 (CS1, CD2 subset 1, CRACC, SLAMF7, CD319, and 19A24), BAFF-R, TACI and/or FcRH5. Other exemplary multiple myeloma antigens include CD56, TIM-3, CD33, CD123, CD44, CD20, CD40, CD74, CD200, EGFR, β2-Microglobulin, HM1.24, IGF-1R, IL-6R, TRAIL-R1, and the activin receptor type IIA (ActRIIA). See Benson and Byrd, J. Clin. Oncol. (2012) 30(16): 2013-15; Tao and Anderson, Bone Marrow Research (2011):924058; Chu et al., Leukemia (2013) 28(4):917-27; Garfall et al., Discov Med. (2014) 17(91):37-46. In some embodiments, the antigens include those present on lymphoid tumors, myeloma, AIDS-associated lymphoma, and/or post-transplant lymphoproliferations, such as CD38. Antibodies or antigen-binding fragments directed against such antigens are known and include, for example, those described in U.S. Patent No. 8,153,765; 8,603477, 8,008,450; U.S. Pub. No. US20120189622 or US20100260748; and/or International PCT Publication Nos. WO2006099875, WO2009080829 or WO2012092612 or WO2014210064. In some embodiments, such antibodies or antigen-binding

fragments thereof (e.g. scFv) are contained in multispecific antibodies, multispecific chimeric receptors, such as multispecific CARs, and/or multispecific cells.

[0433] In some embodiments, the cells and methods include multi-targeting strategies, such as expression of two or more genetically engineered receptors on the cell, each recognizing a different antigen and typically each including a different intracellular signaling component. Such multi-targeting strategies are described, for example, in International Patent Application, Publication No.: WO 2014055668 A1 (describing combinations of a stimulatory or activating and costimulatory CARs, e.g., targeting two different antigens present individually on off-target, e.g., normal cells, but present together only on cells of the disease or condition to be treated) and Fedorov et al., *Sci. Transl. Medicine*, 5(215) (December, 2013) (describing cells expressing a stimulatory or an activating and an inhibitory CAR, such as those in which the stimulatory or activating CAR binds to one antigen expressed on both normal or non-diseased cells and cells of the disease or condition to be treated, and the inhibitory CAR binds to another antigen expressed only on the normal cells or cells which it is not desired to treat).

[0434] In some embodiments, a plurality of cells, each engineered to express one or more recombinant receptors, are provided. For example, in some embodiments, one cell is engineered to express a binding molecule that binds and/or targets BCMA, and another cell is engineered to express a binding molecule that binds and/or targets an additional or second antigen. In some embodiments, the cells can each express a multispecific binding molecule, e.g., a multispecific recombinant receptor, where one or more of the target antigen is BCMA. In some of such embodiments, the plurality of cells can be administered together or separately. In some embodiments, the plurality of cells are administered simultaneously or concurrently with the cells, e.g., administered on the same day, and/or sequentially with or intermittently with, in any order, another engineered cell in the plurality. For example, in some embodiments, an engineered cell expressing a BCMA-binding molecule, e.g., CAR, is administered simultaneously with or sequentially with, in any order, another engineered cell expressing a binding molecule that binds a different target antigen or a different epitope on BCMA. In some embodiments, the plurality of cells can be in the same composition. Exemplary compositions of the cells include compositions described in Section II below.

IV. PHARMACEUTICAL COMPOSITIONS

[0435] Also provided are compositions including the BCMA-binding molecules, immunoconjugates, recombinant receptors, and engineered cells, including pharmaceutical

compositions and formulations. Among such compositions are those that include engineered cells, such as a plurality of engineered cells, expressing the provided anti-BCMA recombinant receptors (e.g CARs).

[0436] Provided are pharmaceutical formulations comprising a BCMA-binding recombinant chimeric antigen receptors or engineered cells expressing said receptors, a plurality of engineered cells expressing said receptors and/or additional agents for combination treatment or therapy. The pharmaceutical compositions and formulations generally include one or more optional pharmaceutically acceptable carrier(s) or excipient(s). In some embodiments, the composition includes at least one additional therapeutic agent.

[0437] The term "pharmaceutical formulation" refers to a preparation which is in such form as to permit the biological activity of an active ingredient contained therein to be effective, and which contains no additional components which are unacceptably toxic to a subject to which the formulation would be administered.

[0438] A "pharmaceutically acceptable carrier" refers to an ingredient in a pharmaceutical formulation, other than an active ingredient, which is nontoxic to a subject. A pharmaceutically acceptable carrier includes, but is not limited to, a buffer, excipient, stabilizer, or preservative.

[0439] In some aspects, the choice of carrier is determined in part by the particular cell, binding molecule, and/or antibody, and/or by the method of administration. Accordingly, there are a variety of suitable formulations. For example, the pharmaceutical composition can contain preservatives. Suitable preservatives may include, for example, methylparaben, propylparaben, sodium benzoate, and benzalkonium chloride. In some aspects, a mixture of two or more preservatives is used. The preservative or mixtures thereof are typically present in an amount of about 0.0001% to about 2% by weight of the total composition. Carriers are described, *e.g.*, by Remington's Pharmaceutical Sciences 16th edition, Osol, A. Ed. (1980). Pharmaceutically acceptable carriers are generally nontoxic to recipients at the dosages and concentrations employed, and include, but are not limited to: buffers such as phosphate, citrate, and other organic acids; antioxidants including ascorbic acid and methionine; preservatives (such as octadecyldimethylbenzyl ammonium chloride; hexamethonium chloride; benzalkonium chloride; benzethonium chloride; phenol, butyl or benzyl alcohol; alkyl parabens such as methyl or propyl paraben; catechol; resorcinol; cyclohexanol; 3-pentanol; and m-cresol); low molecular weight (less than about 10 residues) polypeptides; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids such as

glycine, glutamine, asparagine, histidine, arginine, or lysine; monosaccharides, disaccharides, and other carbohydrates including glucose, mannose, or dextrins; chelating agents such as EDTA; sugars such as sucrose, mannitol, trehalose or sorbitol; salt-forming counter-ions such as sodium; metal complexes (*e.g.* Zn-protein complexes); and/or non-ionic surfactants such as polyethylene glycol (PEG).

[0440] Buffering agents in some aspects are included in the compositions. Suitable buffering agents include, for example, citric acid, sodium citrate, phosphoric acid, potassium phosphate, and various other acids and salts. In some aspects, a mixture of two or more buffering agents is used. The buffering agent or mixtures thereof are typically present in an amount of about 0.001% to about 4% by weight of the total composition. Methods for preparing administrable pharmaceutical compositions are known. Exemplary methods are described in more detail in, for example, Remington: The Science and Practice of Pharmacy, Lippincott Williams & Wilkins; 21st ed. (May 1, 2005).

[0441] Formulations of the antibodies described herein can include lyophilized formulations and aqueous solutions.

[0442] The formulation or composition may also contain more than one active ingredient useful for the particular indication, disease, or condition being treated with the binding molecules or cells, preferably those with activities complementary to the binding molecule or cell, where the respective activities do not adversely affect one another. Such active ingredients are suitably present in combination in amounts that are effective for the purpose intended. Thus, in some embodiments, the pharmaceutical composition further includes other pharmaceutically active agents or drugs, such as chemotherapeutic agents, *e.g.*, asparaginase, busulfan, carboplatin, cisplatin, daunorubicin, doxorubicin, fluorouracil, gemcitabine, hydroxyurea, methotrexate, paclitaxel, rituximab, vinblastine, vincristine, etc. In some embodiments, the cells or antibodies are administered in the form of a salt, *e.g.*, a pharmaceutically acceptable salt. Suitable pharmaceutically acceptable acid addition salts include those derived from mineral acids, such as hydrochloric, hydrobromic, phosphoric, metaphosphoric, nitric, and sulphuric acids, and organic acids, such as tartaric, acetic, citric, malic, lactic, fumaric, benzoic, glycolic, gluconic, succinic, and arylsulphonic acids, for example, p-toluenesulphonic acid.

[0443] Active ingredients may be entrapped in microcapsules, in colloidal drug delivery systems (for example, liposomes, albumin microspheres, microemulsions, nano-particles and nanocapsules) or in macroemulsions. In certain embodiments, the pharmaceutical composition

is formulated as an inclusion complex, such as cyclodextrin inclusion complex, or as a liposome. Liposomes can serve to target the host cells (*e.g.*, T-cells or NK cells) to a particular tissue. Many methods are available for preparing liposomes, such as those described in, for example, Szoka *et al.*, Ann. Rev. Biophys. Bioeng., 9: 467 (1980), and U.S. Patents 4,235,871, 4,501,728, 4,837,028, and 5,019,369.

[0444] The pharmaceutical composition in some aspects can employ time-released, delayed release, and sustained release delivery systems such that the delivery of the composition occurs prior to, and with sufficient time to cause, sensitization of the site to be treated. Many types of release delivery systems are available and known. Such systems can avoid repeated administrations of the composition, thereby increasing convenience to the subject and the physician.

[0445] The pharmaceutical composition in some embodiments contains the binding molecules and/or cells in amounts effective to treat or prevent the disease or condition, such as a therapeutically effective or prophylactically effective amount. Therapeutic or prophylactic efficacy in some embodiments is monitored by periodic assessment of treated subjects. For repeated administrations over several days or longer, depending on the condition, the treatment is repeated until a desired suppression of disease symptoms occurs. However, other dosage regimens may be useful and can be determined. The desired dosage can be delivered by a single bolus administration of the composition, by multiple bolus administrations of the composition, or by continuous infusion administration of the composition.

[0446] In certain embodiments, in the context of genetically engineered cells containing the binding molecules, e.g., CAR, a subject is administered the range of about one million to about 100 billion cells, such as, *e.g.*, 1 million to about 50 billion cells (*e.g.*, about 5 million cells, about 25 million cells, about 500 million cells, about 1 billion cells, about 5 billion cells, about 20 billion cells, about 30 billion cells, about 40 billion cells, or a range defined by any two of the foregoing values), such as about 10 million to about 100 billion cells (*e.g.*, about 20 million cells, about 30 million cells, about 40 million cells, about 60 million cells, about 70 million cells, about 80 million cells, about 90 million cells, about 10 billion cells, about 25 billion cells, about 50 billion cells, about 75 billion cells, about 90 billion cells, or a range defined by any two of the foregoing values), and in some cases about 100 million cells to about 50 billion cells (*e.g.*, about 120 million cells, about 250 million cells, about 350 million cells, about 450 million cells, about 650 million cells, about 800 million cells, about 900 million cells, about 3 billion cells,

about 30 billion cells, about 45 billion cells) or any value in between these ranges, and/or such a number of cells per kilogram of body weight of the subject. In some aspects, in the context of genetically engineered cells expressing the binding molecules, e.g., CAR, a composition can contain at least the number of cells for administration for a dose of cell therapy, such as about or at least a number of cells described herein for administration, e.g., in Section V.A.

[0447] The may be administered using standard administration techniques, formulations, and/or devices. Provided are formulations and devices, such as syringes and vials, for storage and administration of the compositions. Administration of the cells can be autologous or heterologous. For example, immunoresponsive cells or progenitors can be obtained from one subject, and administered to the same subject or a different, compatible subject. Peripheral blood derived immunoresponsive cells or their progeny (*e.g.*, *in vivo*, ex vivo or *in vitro* derived) can be administered via localized injection, including catheter administration, systemic injection, localized injection, intravenous injection, or parenteral administration. When administering a therapeutic composition (*e.g.*, a pharmaceutical composition containing a genetically modified immunoresponsive cell), it will generally be formulated in a unit dosage injectable form (solution, suspension, emulsion).

[0448] Formulations include those for oral, intravenous, intraperitoneal, subcutaneous, pulmonary, transdermal, intramuscular, intranasal, buccal, sublingual, or suppository administration. In some embodiments, the cell populations are administered parenterally. The term "parenteral," as used herein, includes intravenous, intramuscular, subcutaneous, rectal, vaginal, intracranial, intrathoracic, and intraperitoneal administration. In some embodiments, the cell populations are administered to a subject using peripheral systemic delivery by intravenous, intraperitoneal, or subcutaneous injection.

[0449] Compositions in some embodiments are provided as sterile liquid preparations, *e.g.*, isotonic aqueous solutions, suspensions, emulsions, dispersions, or viscous compositions, which may in some aspects be buffered to a selected pH. Liquid preparations are normally easier to prepare than gels, other viscous compositions, and solid compositions. Additionally, liquid compositions are somewhat more convenient to administer, especially by injection. Viscous compositions, on the other hand, can be formulated within the appropriate viscosity range to provide longer contact periods with specific tissues. Liquid or viscous compositions can comprise carriers, which can be a solvent or dispersing medium containing, for example, water,

saline, phosphate buffered saline, polyol (for example, glycerol, propylene glycol, liquid polyethylene glycol) and suitable mixtures thereof.

[0450] Sterile injectable solutions can be prepared by incorporating the binding molecule in a solvent, such as in admixture with a suitable carrier, diluent, or excipient such as sterile water, physiological saline, glucose, dextrose, or the like. The compositions can also be lyophilized. The compositions can contain auxiliary substances such as wetting, dispersing, or emulsifying agents (*e.g.*, methylcellulose), pH buffering agents, gelling or viscosity enhancing additives, preservatives, flavoring agents, colors, and the like, depending upon the route of administration and the preparation desired. Standard texts may in some aspects be consulted to prepare suitable preparations.

[0451] Various additives which enhance the stability and sterility of the compositions, including antimicrobial preservatives, antioxidants, chelating agents, and buffers, can be added. Prevention of the action of microorganisms can be ensured by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, sorbic acid, and the like. Prolonged absorption of the injectable pharmaceutical form can be brought about by the use of agents delaying absorption, for example, aluminum monostearate and gelatin.

[0452] Sustained-release preparations may be prepared. Suitable examples of sustained-release preparations include semipermeable matrices of solid hydrophobic polymers containing the antibody, which matrices are in the form of shaped articles, *e.g.* films, or microcapsules.

[0453] The formulations to be used for *in vivo* administration are generally sterile. Sterility may be readily accomplished, *e.g.*, by filtration through sterile filtration membranes.

[0454] Also provided are pharmaceutical compositions for combination therapy. Any of the additional agents for combination therapy described herein, such as agents described in Section III.B, can be prepared and administered as one or more pharmaceutical compositions, with the BCMA-binding molecule (*e.g.*, antibody), immunoconjugate, recombinant receptor (*e.g.*, chimeric antigen receptor) and/or engineered cells expressing said molecules (*e.g.*, recombinant receptor) described herein. The combination therapy can be administered in one or more pharmaceutical compositions, e.g., where the binding molecules, recombinant receptors and/or cells are in the same pharmaceutical composition as the additional agent, or in separate pharmaceutical compositions. For example, in some embodiments, the additional agent is an additional engineered cell, e.g., cell engineered to express a different recombinant receptor, and is administered in the same composition or in a separate composition. In some embodiments,

each of the pharmaceutical composition is formulated in a suitable formulation according to the particular binding molecule, recombinant receptor, cell, e.g., engineered cell, and/or additional agent, and the particular dosage regimen and/or method of delivery.

V. METHODS AND USES

[0455] Also provided methods of using and uses of the BCMA-binding molecules, immunoconjugates, recombinant receptors, engineered cells, and pharmaceutical compositions and formulations thereof, such as in the treatment of diseases, conditions, and disorders in which BCMA is expressed, and/or detection, diagnostic, and prognostic methods. Among such methods, such as methods of treatment, and usesare those that involve administering to a subject engineered cells, such as a plurality of engineered cells, expressing the provided anti-BCMA recombinant receptors (e.g CARs). Also provided are methods of combination therapy and/or treatment.

A. Therapeutic and prophylactic methods and uses

[0456] Also provided are methods of administering and uses, such as therapeutic and prophylactic uses, of the BCMA-binding molecules, including the anti-BCMA recombinant receptors (*e.g.*, CARs), engineered cells expressing the recombinant receptors (*e.g.*, CARs), plurality of engineered cells expressing the receptors, and/or compositions comprising the same. Such methods and uses include therapeutic methods and uses, for example, involving administration of the molecules (*e.g.*, recombinant receptors), cells (*e.g.*, engineered cells), or compositions containing the same, to a subject having a disease, condition, or disorder associated with BCMA such as a disease, condition, or disorder associated with BCMA expression, and/or in which cells or tissues express, *e.g.*, specifically express, BCMA. In some embodiments, the molecule, cell, and/or composition is/are administered in an effective amount to effect treatment of the disease or disorder. Provided herein are uses of the recombinant receptors (*e.g.*, CARs), and cells (*e.g.*, engineered cells) in such methods and treatments, and in the preparation of a medicament in order to carry out such therapeutic methods. In some embodiments, the methods are carried out by administering the binding molecules or cells, or compositions comprising the same, to the subject having, having had, or suspected of having the disease or condition. In some embodiments, the methods thereby treat the disease or condition or disorder in the subject. Also provided herein are of use of any of the compositions, such as pharmaceutical compositions provided herein, for the treatment of a disease or disorder associated with BCMA, such as use in a treatment regimen.

[0457] As used herein, "treatment" (and grammatical variations thereof such as "treat" or "treating") refers to complete or partial amelioration or reduction of a disease or condition or disorder, or a symptom, adverse effect or outcome, or phenotype associated therewith. Desirable effects of treatment include, but are not limited to, preventing occurrence or recurrence of disease, alleviation of symptoms, diminishment of any direct or indirect pathological consequences of the disease, preventing metastasis, decreasing the rate of disease progression, amelioration or palliation of the disease state, and remission or improved prognosis. The terms do not imply complete curing of a disease or complete elimination of any symptom or effect(s) on all symptoms or outcomes.

[0458] As used herein, "delaying development of a disease" means to defer, hinder, slow, retard, stabilize, suppress and/or postpone development of the disease (such as cancer). This delay can be of varying lengths of time, depending on the history of the disease and/or subject being treated. As sufficient or significant delay can, in effect, encompass prevention, in that the subject does not develop the disease. For example, a late stage cancer, such as development of metastasis, may be delayed.

[0459] "Preventing," as used herein, includes providing prophylaxis with respect to the occurrence or recurrence of a disease in a subject that may be predisposed to the disease but has not yet been diagnosed with the disease. In some embodiments, the provided molecules and compositions are used to delay development of a disease or to slow the progression of a disease.

[0460] As used herein, to "suppress" a function or activity is to reduce the function or activity when compared to otherwise same conditions except for a condition or parameter of interest, or alternatively, as compared to another condition. For example, an antibody or composition or cell which suppresses tumor growth reduces the rate of growth of the tumor compared to the rate of growth of the tumor in the absence of the antibody or composition or cell.

[0461] An "effective amount" of an agent, *e.g.*, a pharmaceutical formulation, binding molecule, antibody, cells, or composition, in the context of administration, refers to an amount effective, at dosages/amounts and for periods of time necessary, to achieve a desired result, such as a therapeutic or prophylactic result.

[0462] A "therapeutically effective amount" of an agent, *e.g.*, a pharmaceutical formulation, binding molecule, antibody, cells, or composition refers to an amount effective, at dosages and for periods of time necessary, to achieve a desired therapeutic result, such as for treatment of a

disease, condition, or disorder, and/or pharmacokinetic or pharmacodynamic effect of the treatment. The therapeutically effective amount may vary according to factors such as the disease state, age, sex, and weight of the subject, and the populations of cells administered. In some embodiments, the provided methods involve administering the molecules, antibodies, cells, and/or compositions at effective amounts, *e.g.*, therapeutically effective amounts.

[0463] A "prophylactically effective amount" refers to an amount effective, at dosages and for periods of time necessary, to achieve the desired prophylactic result. Typically but not necessarily, since a prophylactic dose is used in subjects prior to or at an earlier stage of disease, the prophylactically effective amount will be less than the therapeutically effective amount.

[0464] As used herein, a "subject" or an "individual" is a mammal. In some embodiments, a "mammal" includes humans, non-human primates, domestic and farm animals, and zoo, sports, or pet animals, such as dogs, horses, rabbits, cattle, pigs, hamsters, gerbils, mice, ferrets, rats, cats, monkeys, *etc.* In some embodiments, the subject is human.

[0465] Methods for administration of cells for adoptive cell therapy are known and may be used in connection with the provided methods and compositions. For example, adoptive T cell therapy methods are described, e.g., in US Pat. App. Pub. No. 2003/0170238 to Gruenberg et al; US Patent No. 4,690,915 to Rosenberg; Rosenberg (2011) Nat Rev Clin Oncol. 8(10):577-85). See, e.g., Themeli et al. (2013) Nat Biotechnol. 31(10): 928-933; Tsukahara et al. (2013) Biochem Biophys Res Commun 438(1): 84-9; Davila et al. (2013) PLoS ONE 8(4): e61338.

[0466] Among the diseases to be treated is any disease or disorder associated with BCMA or any disease or disorder in which BCMA is specifically expressed and/or in which BCMA has been targeted for treatment (also referred to herein interchangeably as a "BCMA-associated disease or disorder"). Cancers associated with BCMA expression include hematologic malignancies such as multiple myeloma, Waldenstrom macroglobulinemia, as well as both Hodgkin's and non-Hodgkin's lymphomas. *See* Coquery *et al.*, *Crit Rev Immunol.*, 2012, 32(4):287-305 for a review of BCMA. Since BCMA has been implicated in mediating tumor cell survival, it is a potential target for cancer therapy. Chimeric antigen receptors containing mouse anti-human BCMA antibodies and cells expressing such chimeric receptors have been previously described. *See* Carpenter *et al.*, *Clin Cancer Res.*, 2013, 19(8):2048-2060.

[0467] In some embodiments, the disease or disorder associated with BCMA is a B cell-related disorder. In some embodiments, the disease or disorder associated with BCMA is one or more diseases or conditions from among glioblastoma, lymphomatoid granulomatosis, post-

transplant lymphoproliferative disorder, an immunoregulatory disorder, heavy-chain disease, primary or immunocyte-associated amyloidosis, or monoclonal gammopathy of undetermined significance.

[0468] In some embodiments, the disease or disorder associated with BCMA is an autoimmune disease or disorder. Such autoimmune diseases or disorder include, but are not limited to, systemic lupus erythematosus (SLE), lupus nephritis, inflammatory bowel disease, rheumatoid arthritis (*e.g.*, juvenile rheumatoid arthritis), ANCA associated vasculitis, idiopathic thrombocytopenia purpura (ITP), thrombotic thrombocytopenia purpura (TTP), autoimmune thrombocytopenia, Chagas' disease, Grave's disease, Wegener's granulomatosis, polyarteritis nodosa, Sjogren's syndrome, pemphigus vulgaris, scleroderma, multiple sclerosis, psoriasis, IgA nephropathy, IgM polyneuropathies, vasculitis, diabetes mellitus, Reynaud's syndrome, anti-phospholipid syndrome, Goodpasture's disease, Kawasaki disease, autoimmune hemolytic anemia, myasthenia gravis, or progressive glomerulonephritis.

[0469] In certain diseases and conditions, BCMA is expressed on malignant cells and cancers. In some embodiments, the cancer (*e.g.*, a BCMA-expressing cancer) is a B cell malignancy. In some embodiments, the cancer (*e.g.*, a BCMA-expressing cancer) is a lymphoma, a leukemia, or a plasma cell malignancy. Lymphomas contemplated herein include, but are not limited to, Burkitt lymphoma (*e.g.*, endemic Burkitt's lymphoma or sporadic Burkitt's lymphoma), non-Hodgkin's lymphoma (NHL), Hodgkin's lymphoma, Waldenstrom macroglobulinemia, follicular lymphoma, small non-cleaved cell lymphoma, mucosa-associated lymphatic tissue lymphoma (MALT), marginal zone lymphoma, splenic lymphoma, nodal monocytoid B cell lymphoma, immunoblastic lymphoma, large cell lymphoma, diffuse mixed cell lymphoma, pulmonary B cell angiocentric lymphoma, small lymphocytic lymphoma, primary mediastinal B cell lymphoma, lymphoplasmacytic lymphoma (LPL), or mantle cell lymphoma (MCL). Leukemias contemplated here, include, but are not limited to, chronic lymphocytic leukemia (CLL), plasma cell leukemia or acute lymphocytic leukemia (ALL). Also contemplated herein are plasma cell malignancies including, but not limited to, multiple myeloma (*e.g.*, non-secretory multiple myeloma, smoldering multiple myeloma) or plasmacytoma. In some embodiments the disease or condition is multiple myeloma (MM), such as relapsed and/or refractory multiple myeloma (R/R MM). Among the diseases, disorders or conditions associated with BCMA (*e.g.*, a BCMA-expressing cancer) that can be treated include, but are not limited to, neuroblastoma, renal cell carcinoma, colon cancer, colorectal cancer,

breast cancer, epithelial squamous cell cancer, melanoma, myeloma (*e.g.*, multiple myeloma), stomach cancer, brain cancer, lung cancer, pancreatic cancer, cervical cancer, ovarian cancer, liver cancer, bladder cancer, prostate cancer, testicular cancer, thyroid cancer, uterine cancer, adrenal cancer and head and neck cancer.

[0470] In some embodiments, the methods may identify a subject who has, is suspected to have, or is at risk for developing a BCMA-associated disease or disorder. Hence, provided are methods for identifying subjects with diseases or disorders associated with elevated BCMA expression and selecting them for treatment with a provided BCMA-binding e recombinant receptors (*e.g.*, CARs), and/or engineered cells expressing the recombinant receptors.

[0471] For example, a subject may be screened for the presence of a disease or disorder associated with elevated BCMA expression, such as a BCMA-expressing cancer. In some embodiments, the methods include screening for or detecting the presence of a BCMA-associated disease, *e.g.* a tumor. Thus, in some aspects, a sample may be obtained from a patient suspected of having a disease or disorder associated with elevated BCMA expression and assayed for the expression level of BCMA. In some aspects, a subject who tests positive for a BCMA-associated disease or disorder may be selected for treatment by the present methods, and may be administered a therapeutically effective amount of a recombinant receptor (*e.g.*, CAR) comprising a BCMA-binding molecule, cells containing a recombinant receptor or a pharmaceutical composition thereof as described herein.

[0472] In some embodiments, the subject has persistent or relapsed disease, *e.g.*, following treatment with another BCMA-specific antibody and/or cells expressing a BCMA-targeting chimeric receptor and/or other therapy, including chemotherapy, radiation, and/or hematopoietic stem cell transplantation (HSCT), *e.g.*, allogeneic HSCT or autologous HSCT. In some embodiments, the administration effectively treats the subject despite the subject having become resistant to another BCMA-targeted therapy. In some embodiments, the subject has not relapsed but is determined to be at risk for relapse, such as at a high risk of relapse, and thus the compound or composition is administered prophylactically, *e.g.*, to reduce the likelihood of or prevent relapse.

[0473] In some embodiments, the subject is one that is eligible for a transplant, such as is eligible for a hematopoietic stem cell transplantation (HSCT), *e.g.*, allogeneic HSCT or autologous HSCT. In some such embodiments, the subject has not previously received a transplant, despite being eligible, prior to administration of the BCMA-binding molecules,

including the anti-BCMA recombinant receptors (*e.g.*, CARs), engineered cells expressing the recombinant receptors (*e.g.*, CARs), plurality of engineered cells expressing the receptors, and/or compositions comprising the same, as provided herein.

[0474] In some embodiments, the subject is one that is not eligible for a transplant, such as is not eligible for a hematopoietic stem cell transplantation (HSCT), *e.g.*, allogenic HSCT or autologous HSCT. In some such embodiments, such a subject is administered the BCMA-binding molecules, including the anti-BCMA recombinant receptors (*e.g.*, CARs), engineered cells expressing the recombinant receptors (*e.g.*, CARs), plurality of engineered cells expressing the receptors, and/or compositions comprising the same, according to the provided embodiments herein.

[0475] In some embodiments, prior to the initiation of administration of the engineered cells, the subject has received one or more prior therapies. In some embodiments, the subject has received at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20 or more prior therapies. In some embodiments, the subject has received at least 3, 4, 5, 6, 7, 8, 9, 10 or more prior therapies.

[0476] In some aspects, the subject has relapsed or has been refractory to the one or more prior therapies. In some aspects, the prior therapies include treatment with autologous stem cell transplant (ASCT); an immunomodulatory agent; a proteasome inhibitor; and an anti-CD38 antibody; unless the subject was not a candidate for or was contraindicated for one or more of the therapies. In some embodiments, the immunomodulatory agent is selected from among thalidomide, lenalidomide or pomalidomide. In some embodiments, the proteasome inhibitor is selected from among bortezomib, carfilzomib or ixazomib. In some embodiments, the anti-CD38 antibody is or comprises daratumumab. In some embodiments, the subject must have undergone at least 2 consecutive cycles of treatment for each regimen unless progressive disease was the best response to the regimen.

[0477] In some embodiments, the method can involve including or excluding particular subjects for therapy with the provided anti-BCMA antibodies, recombinant receptors and/or cells comprising such receptors, based on particular criteria, diagnosis or indication. In some embodiments, at the time of administration of the dose of cells or pre-treatment lymphodepleting chemotherapy, the subject has not had active or history of plasma cell leukemia (PCL). In some embodiments, if the subject had active or a history of PCL at the time of administration, the subject can be excluded from being treated according to the provided methods. In some

embodiments, if the subject develops a PCL, such as secondary PCL, at the time of administration, the subject can be excluded from being treated according to the provided methods. In some embodiments, the assessment for the criteria, diagnosis or indication can be performed at the time of screening the subjects for eligibility or suitability of treatment according to the provided methods, at various steps of the treatment regimen, at the time of receiving lymphodepleting therapy, and/or at or immediately prior to the initiation of administration of the engineered cells or composition thereof.

[0478] In some embodiments, the treatment does not induce an immune response by the subject to the therapy, and/or does not induce such a response to a degree that prevents effective treatment of the disease or condition. In some aspects, the degree of immunogenicity and/or graft versus host response is less than that observed with a different but comparable treatment. For example, in the case of adoptive cell therapy using cells expressing CARs including the provided anti-BCMA antibodies, the degree of immunogenicity in some embodiments is reduced compared to CARs including a different antibody that binds to a similar, *e.g.*, overlapping epitope and/or that competes for binding to BCMA with the antibody, such as a mouse or monkey or rabbit or humanized antibody.

[0479] In some embodiments, the methods include adoptive cell therapy, whereby genetically engineered cells expressing the provided recombinant receptors comprising a BCMA-binding molecule (*e.g.*, CARs comprising anti-BCMA antibody or antigen-binding fragment thereof) are administered to subjects. Such administration can promote activation of the cells (*e.g.*, T cell activation) in a BCMA-targeted manner, such that the cells of the disease or disorder are targeted for destruction.

[0480] Thus, the provided methods and uses include methods and uses for adoptive cell therapy. In some embodiments, the methods include administration of the cells or a composition containing the cells to a subject, tissue, or cell, such as one having, at risk for, or suspected of having the disease, condition or disorder. In some embodiments, the cells, populations, and compositions are administered to a subject having the particular disease or condition to be treated, *e.g.*, via adoptive cell therapy, such as adoptive T cell therapy. In some embodiments, the cells or compositions are administered to the subject, such as a subject having or at risk for the disease or condition. In some aspects, the methods thereby treat, *e.g.*, ameliorate one or more symptom of the disease or condition, such as by lessening tumor burden in a BCMA-expressing cancer.

[0481] Methods for administration of cells for adoptive cell therapy are known and may be used in connection with the provided methods and compositions. For example, adoptive T cell therapy methods are described, *e.g.*, in US Patent Application Publication No. 2003/0170238 to Gruenberg et al; US Patent No. 4,690,915 to Rosenberg; Rosenberg (2011) Nat Rev Clin Oncol. 8(10):577-85). See, *e.g.*, Themeli *et al.* (2013) Nat Biotechnol. 31(10): 928-933; Tsukahara *et al.* (2013) Biochem Biophys Res Commun 438(1): 84-9; Davila *et al.* (2013) PLoS ONE 8(4): e61338.

[0482] In some embodiments, the cell therapy, *e.g.*, adoptive cell therapy, *e.g.*, adoptive T cell therapy, is carried out by autologous transfer, in which the cells are isolated and/or otherwise prepared from the subject who is to receive the cell therapy, or from a sample derived from such a subject. Thus, in some aspects, the cells are derived from a subject, *e.g.*, patient, in need of a treatment and the cells, following isolation and processing are administered to the same subject.

[0483] In some embodiments, the cell therapy, *e.g.*, adoptive cell therapy, *e.g.*, adoptive T cell therapy, is carried out by allogeneic transfer, in which the cells are isolated and/or otherwise prepared from a subject other than a subject who is to receive or who ultimately receives the cell therapy, *e.g.*, a first subject. In such embodiments, the cells then are administered to a different subject, *e.g.*, a second subject, of the same species. In some embodiments, the first and second subjects are genetically identical. In some embodiments, the first and second subjects are genetically similar. In some embodiments, the second subject expresses the same HLA class or supertype as the first subject.

[0484] In some embodiments, the subject, to whom the cells, cell populations, or compositions are administered, is a primate, such as a human. In some embodiments, the subject, to whom the cells, cell populations, or compositions are administered, is a non-human primate. In some embodiments, the non-human primate is a monkey (*e.g.*, cynomolgus monkey) or an ape. The subject can be male or female and can be any suitable age, including infant, juvenile, adolescent, adult, and geriatric subjects. In some embodiments, the subject is a non-primate mammal, such as a rodent (*e.g.*, mouse, rat, etc.). In some examples, the patient or subject is a validated animal model for disease, adoptive cell therapy, and/or for assessing toxic outcomes such as cytokine release syndrome (CRS).

[0485] The BCMA-binding molecules such as recombinant receptors (*e.g.*, CARs) and cells expressing the same, can be administered by any suitable means, for example, by injection, *e.g.*,

intravenous or subcutaneous injections, intraocular injection, periocular injection, subretinal injection, intravitreal injection, trans-septal injection, subscleral injection, intrachoroidal injection, intracameral injection, subconjunctival injection, subconjunctival injection, sub-Tenon's injection, retrobulbar injection, peribulbar injection, or posterior juxtascleral delivery. In some embodiments, they are administered by parenteral, intrapulmonary, and intranasal, and, if desired for local treatment, intralesional administration. Parenteral infusions include intramuscular, intravenous, intraarterial, intraperitoneal, intracranial, intrathoracic, or subcutaneous administration. Dosing and administration may depend in part on whether the administration is brief or chronic. Various dosing schedules include but are not limited to single or multiple administrations over various time-points, bolus administration, and pulse infusion.

[0486] For the prevention or treatment of disease, the appropriate dosage of the binding molecule, recombinant receptor or cell may depend on the type of disease to be treated, the type of binding molecule or recombinant receptor, the severity and course of the disease, whether the binding molecule or recombinant receptor is administered for preventive or therapeutic purposes, previous therapy, the patient's clinical history and response to the recombinant receptor or cell, and the discretion of the attending physician. The compositions and molecules and cells are in some embodiments suitably administered to the patient at one time or over a series of treatments.

[0487] In some embodiments, the dose and/or frequency of administration is determined based on efficacy and/or response. In some embodiments, efficacy is determined by evaluating disease status. Exemplary methods for assessing disease status include: measurement of M protein in biological fluids, such as blood and/or urine, by electrophoresis and immunofixation; quantification of sFLC (κ and λ) in blood; skeletal survey; and imaging by positron emission tomography (PET)/computed tomography (CT) in subjects with extramedullary disease. In some embodiments, disease status can be evaluated by bone marrow examination. In some examples, dose and/or frequency of administration is determined by the expansion and persistence of the recombinant receptor or cell in the blood and/or bone marrow. In some embodiments, dose and/or frequency of administration is determined based on the antitumor activity of the recombinant receptor or engineered cell. In some embodiments antitumor activity is determined by the overall response rate (ORR) and/or International Myeloma Working Group (IMWG) Uniform Response Criteria (see Kumar et al. (2016) Lancet Oncol 17(8):e328-346). In some embodiments, response is evaluated using minimal residual disease (MRD) assessment. In some

embodiments, MRD can be assessed by methods such as flow cytometry and high-throughput sequencing, e.g., deep sequencing. In some embodiments, response is evaluated based on the duration of response following administration of the recombinant receptor or cells. In some examples, dose and/or frequency of administration can be based on toxicity. In some embodiments, dose and/or frequency can be determined based on health-related quality of life (HRQoL) of the subject to which the recombinant receptor and/or cells is/are administered. In some embodiments, dose and/or frequency of administration can be changed, i.e., increased or decreased, based on any of the above criteria.

[0488] In some embodiments, the disease or disorder to be treated is multiple myeloma. In some embodiments, measurable disease criteria for multiple myeloma can include (1) serum M-protein 1 g/dL or greater; (2) Urine M-protein 200 mg or greater/24 hour; (3) involved serum free light chain (sFLC) level 10 mg/dL or greater, with abnormal κ to λ ratio. In some cases, light chain disease is acceptable only for subjects without measurable disease in the serum or urine.

[0489] In some embodiments, the Eastern Cooperative Oncology Group (ECOG) performance status indicator can be used to assess or select subjects for treatment, e.g., subjects who have had poor performance from prior therapies (see, e.g., Oken et al. (1982) Am J Clin Oncol. 5:649-655). The ECOG Scale of Performance Status describes a patient's level of functioning in terms of their ability to care for themselves, daily activity, and physical ability (e.g., walking, working, etc.). In some embodiments, an ECOG performance status of 0 indicates that a subject can perform normal activity. In some aspects, subjects with an ECOG performance status of 1 exhibit some restriction in physical activity but the subject is fully ambulatory. In some aspects, patients with an ECOG performance status of 2 is more than 50% ambulatory. In some cases, the subject with an ECOG performance status of 2 may also be capable of selfcare; see e.g., Sørensen et al., (1993) Br J Cancer 67(4) 773-775. In some embodiments, the subject that are to be administered according to the methods or treatment regimen provided herein include those with an ECOG performance status of 0 or 1.

[0490] In some embodiments, the administration can treat the subject despite the subject having become resistant to another therapy. In some embodiments, when administered to subjects according to the embodiments described herein, the dose or the composition is capable of achieving objective response (OR), in at least 50%, 60%, 70%, 80%, 90%, or 95% of subjects that were administered. In some embodiments, OR includes subjects who achieve stringent

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

[0491] In some embodiments, toxicity and/or side-effects of treatment can be monitored and used to adjust dose and/or frequency of administration of the recombinant receptor, e.g., CAR, cells, and or compositions. For example, adverse events and laboratory abnormalities can be monitored and used to adjust dose and/or frequency of administration. Adverse events include infusion reactions, cytokine release syndrome (CRS), neurotoxicity, macrophage activation syndrome, and tumor lysis syndrome (TLS). Any of such events can establish dose-limiting toxicities and warrant decrease in dose and/or a termination of treatment. Other side effects or adverse events which can be used as a guideline for establishing dose and/or frequency of administration include non-hematologic adverse events, which include but are not limited to fatigue, fever or febrile neutropenia, increase in transaminases for a set duration (e.g., less than or equal to 2 weeks or less than or equal to 7 days), headache, bone pain, hypotension, hypoxia, chills, diarrhea, nausea/vomiting, neurotoxicity (e.g., confusion, aphasia, seizures, convulsions, lethargy, and/or altered mental status), disseminated intravascular coagulation, other asymptomatic non-hematological clinical laboratory abnormalities, such as electrolyte abnormalities. Other side effects or adverse events which can be used as a guideline for establishing dose and/or frequency of administration include hematologic adverse events, which include but are not limited to neutropenia, leukopenia, thrombocytopenia, animal, and/or B-cell aplasia and hypogammaglobinemia.

[0492] In some embodiments, treatment according to the provided methods can result in a lower rate and/or lower degree of toxicity, toxic outcome or symptom, toxicity-promoting profile, factor, or property, such as a symptom or outcome associated with or indicative of cytokine release syndrome (CRS) or neurotoxicity, such as severe CRS or severe neurotoxicity, for example, compared to administration of other therapies.

[0493] In certain embodiments, in the context of genetically engineered cells containing the binding molecules or recombinant receptors, a subject is administered the range of about one million to about 100 billion cells and/or that amount of cells per kilogram of body weight, such as, *e.g.*, about 1 million to about 50 billion cells (*e.g.*, about 5 million cells, about 25 million cells, about 500 million cells, about 1 billion cells, about 5 billion cells, about 20 billion cells, about 30 billion cells, about 40 billion cells, or a range defined by any two of the foregoing values), such as about 10 million to about 100 billion cells (*e.g.*, about 20 million cells, about 25 million cells, about 30 million cells, about 40 million cells, about 50 million cells, about 60 million cells, about 70 million cells, about 80 million cells, about 90 million cells, about 10 billion cells, about 25 billion cells, about 50 billion cells, about 75 billion cells, about 90 billion cells, or a range defined by any two of the foregoing values), and in some cases about 100 million cells to about 50 billion cells (*e.g.*, about 120 million cells, about 150 million cells, about 250 million cells, about 300 million cells, about 350 million cells, about 450 million cells, about 500 million cells, about 600 million cells, about 650 million cells, about 800 million cells, about 900 million cells, about 1 billion cells, about 1.2 billion cells, about 3 billion cells, about 30 billion cells, about 45 billion cells, or about 50 billion cells.) or any value in between these ranges and/or per kilogram of body weight. Again, dosages may vary depending on attributes particular to the disease or disorder and/or patient and/or other treatments.

[0494] In some embodiments, the methods comprises administering a dose of the engineered cells or a composition comprising a dose of the engineered cells. In some embodiments, the engineered cells or compositions containing engineered cells can be used in a treatment regimen, wherein the treatment regimen comprises administering a dose of the engineered cells or a composition comprising a dose of the engineered cells. In some embodiments, the dose can contain, for example, a particular number or range of recombinant receptor-expressing T cells, total T cells, or total peripheral blood mononuclear cells (PBMCs), such as any number of such cells described herein. In some embodiments, a composition containing a dose of the cells can be administered. In some aspects, the number, amount or proportion of CAR-expressing cells in

a cell population or a cell composition can be assessed by detection of a surrogate marker, e.g., by flow cytometry or other means, or by detecting binding of a labelled molecule, such as a labelled antigen, that can specifically bind to the binding molecules or receptors provided herein.

[0495] In some embodiments, for example, where the subject is a human, the dose includes more than about 1 x 10^6 total recombinant receptor (e.g., CAR)-expressing cells, T cells, or peripheral blood mononuclear cells (PBMCs) and fewer than about 2 x 10^9 total recombinant receptor (e.g., CAR)-expressing cells, T cells, or peripheral blood mononuclear cells (PBMCs), e.g., in the range of about 2.5 x 10^7 to about 1.2 x 10^9 such cells, such as 2.5 x 10^7, 5 x 10^7, 1.5 x 10^8, 3 x 10^8, 4.5 x 10^8, 8 x 10^8,or 1.2 x 10^9 total such cells, or the range between any two of the foregoing values.

[0496] In some embodiments, the dose of genetically engineered cells comprises between at or about 2.5 x 10^7 CAR-expressing T cells, total T cells, or total peripheral blood mononuclear cells (PBMCs), and at or about 1.2 x 10^9 CAR-expressing T cells, total T cells, or total PBMCs, between at or about 5.0 x 10^7 CAR-expressing T cells and at or about 4.5 x 10^8 CAR-expressing T cells, total T cells, or total peripheral blood mononuclear cells (PBMCs), between at or about 1.5 x 10^8 CAR-expressing T cells and at or about 3.0 x 10^8 CAR-expressing T cells, total T cells, or total PBMCs, each inclusive.. In some embodiments, the number is with reference to the total number of CD3+ or CD8+, in some cases also CAR-expressing (e.g. CAR+) cells. In some embodiments, the dose comprises a number of cell from or from about 2.5 x 10^7 to or to about 1.2 x 10^9 CD3+ or CD8+ total T cells or CD3+ or CD8+ CAR-expressing cells, from or from about 5.0 x 10^7 to or to about 4.5 x 10^8 CD3+ or CD8+ total T cells or CD3+ or CD8+ CAR-expressing cells, or from or from about 1.5 x 10^8 to or to about 3.0 x 10^8 CD3+ or CD8+ total T cells or CD3+ or CD8+CAR-expressing cells, each inclusive.

[0497] In some embodiments, the T cells of the dose include CD4+ T cells, CD8+ T cells or CD4+ and CD8+ T cells.

[0498] In some embodiments, for example, where the subject is human, the CD8+ T cells of the dose, including in a dose including CD4+ and CD8+ T cells, includes between at or about 1 x 10^6 and at or about 2 x 10^9 total recombinant receptor (e.g., CAR)-expressing CD8+cells, e.g., in the range of at or about 5 x 10^7 to at or about 4.5 x 10^8 such cells, such as at or about 2.5 x 10^7, at or about 5 x 10^7, at or about 1.5 x 10^8, at or about 3 x 10^8, at or about 4.5 x 10^8, at or about 8 x 10^8, or at or about 1.2 x 10^9 total such cells, or the range between any two of the foregoing values.

[0499] In some embodiments, the dose of cells, e.g., recombinant receptor-expressing T cells, is administered to the subject as a single dose or is administered only one time within a period of two weeks, one month, three months, six months, 1 year or more. In some embodiments, the patient is administered multiple doses, and each of the doses or the total dose can be within any of the foregoing values.In some embodiments, the engineered cells for administration or composition of engineered cells for administration,exhibits properties indicative of or consistent with cell health. In some embodiments, at or about or at least at or about 70, 75, 80, 85, or 90% CAR+ cells of such dose exhibit one or more properties or phenotypes indicative of cell health or biologically active CAR cell, such as absence expression of an apoptotic marker.

[0500] In particular embodiments, the phenotype is or includes an absence of apoptosis and/or an indication the cell is undergoing the apoptotic process. Apoptosis is a process of programmed cell death that includes a series of stereotyped morphological and biochemical events that lead to characteristic cell changes and death, including blebbing, cell shrinkage, nuclear fragmentation, chromatin condensation, chromosomal DNA fragmentation, and global mRNA decay. In some aspects, early stages of apoptosis can be indicated by activation of certain caspases, e.g., 2, 8, 9, and 10. In some aspects, middle to late stages of apoptosis are characterized by further loss of membrane integrity, chromatin condensation and DNA fragmentation, include biochemical events such as activation of caspases 3, 6, and 7.

[0501] In particular embodiments, the phenotype is negative expression of one or more factors associated with programmed cell death, for example pro-apoptotic factors known to initiate apoptosis, e.g., members of the death receptor pathway, activated members of the mitochondrial (intrinsic) pathway, such as Bcl-2 family members, e.g., Bax, Bad, and Bid, and caspases. In certain embodiments, the phenotype is the absence of an indicator, e.g., an Annexin V molecule or by TUNEL staining, that will preferentially bind to cells undergoing apoptosis when incubated with or contacted to a cell composition. In some embodiments, the phenotype is or includes the expression of one or more markers that are indicative of an apoptotic state in the cell. In some embodiments, the phenotype is lack of expression and/or activation of a caspase, such as caspase 3. In some aspects, activation of caspase-3 is indicative of an increase or revival of apoptosis. In certain embodiments, caspase activation can be detected by known methods. In some embodiments, an antibody that binds specifically to an activated caspase (i.e., binds specifically to the cleaved polypeptide) can be used to detect caspase activation. In

particular embodiments, the phenotype is or includes active caspase 3-. In some embodiments, the marker of apoptosis is a reagent that detects a feature in a cell that is associated with apoptosis. In certain embodiments, the reagent is an annexin V molecule.

[0502] In some embodiments, the compositions containing the engineered cells for administration contain a certain number or amount of cells that exhibit phenotypes indicative of or consistent with cell health. In some of any embodiments, less than about 25%, 20%, 15%, 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2% or 1% of the CAR-expressing T cells in the dose of engineered T cells express a marker of apoptosis, optionally Annexin V or active Caspase 3. In some of any embodiments, less than 5%, 4%, 3%, 2% or 1% of the CAR-expressing T cells in the dose of engineered T cells express Annexin V or active Caspase 3.

[0503] In some embodiments the cells administered are immune cells engineered to express the BCMA-binding recombinant receptor, e.g., CAR. In some embodiments the immune cells are T cells. In some embodiments, the administered cells are CD4+ T cells. In some embodiments the administered cells are CD8+ T cells. In some embodiments, the administered cells are a combination of CD4+ and CD8+ T cells, such as a combination of CD4+ CAR T cells and CD8+ CAR T cells, which in some aspects are within the same vessel or cell composition or suspsension. In some examples the ratio of CD4+ cells to CD8+ cells (CD4:CD8) administered, such as ratio within the suspension or composition or vessel, is 1:10, 1:9, 1:8, 1:7, 1:6, 1:5, 1:4, 1:3, 1:2, 1:1, 2:1, 3:1, 4:1, 5:1, 6:1, 7:1, 8:1, 9:1, 10:1. In some embodiments, the ratio is between 1:3 and 3:1 or is between at or about 1:4 to at or about 4:1, or between at or about 1:3 to at or about 3:1, or between at or about 1:2 to at or about 2:1, or any of such ratios, within a tolerated error rate. In some aspects, among subjects receiving the therapy and/or among subjects from whom samples are taken and processed to produce the cell compositions, the ratio of CD4+ CAR-T cells to CD8+ CAR-T cells or ratio of CD4+ to CD8+ cells is within a desired range, such as between at or about 1:4 to at or about 4:1, or between at or about 1:3 to at or about 3:1, or between at or about 1:2 to at or about 2:1, or is within such desired ratio for a given percentage of such subjects, such as for at least 65 %, at least 70 %, at least 75 % or at least 80 % or at least 85 % or at least 90 % or at least 95 %, of such subjects.

[0504] In some embodiments, the cells, binding molecules, or recombinant receptors are administered as part of a combination treatment, such as simultaneously with or sequentially with, in any order, another therapeutic intervention, such as another antibody or engineered cell or receptor or agent, such as a cytotoxic or therapeutic agent.

[0505] The cells, binding molecules and/or recombinant receptors in some embodiments are co-administered with one or more additional therapeutic agents or in connection with another therapeutic intervention, either simultaneously or sequentially in any order. In some contexts, the cells are co-administered with another therapy sufficiently close in time such that the cell populations enhance the effect of one or more additional therapeutic agents, or vice versa. In some embodiments, the cells, binding molecules and/or recombinant receptors are administered prior to the one or more additional therapeutic agents. In some embodiments, the cells, binding molecules and/or recombinant receptors are administered after to the one or more additional therapeutic agents.

[0506] In some embodiments, the subject may receive a bridging therapy after leukapheresis and before lymphodepleting chemotherapy. A treating physician can determine if bridging therapy is necessary, for example for disease control, during manufacturing of the provided composition or cells. In some embodiments, bridging therapies do not include biological agents, such as antibodies (e.g., Daratumumab). In some embodiments, bridging therapies are discontinued prior to initiation of lymphodepletion. In some embodiments, bridging therapies are discontinued 1 day, 2 days 3 days, 4 days, 5 days, 7 days, 10 days, 14 days, 21 days, 28 days, 45 days, or 60 days before lymphodepletion.

[0507] Once the cells are administered to a mammal (*e.g.*, a human), the biological activity of the engineered cell populations and/or antibodies in some aspects is measured by any of a number of known methods. Parameters to assess include specific binding of an engineered or natural T cell or other immune cell to antigen, *in vivo*, *e.g.*, by imaging, or ex vivo, *e.g.*, by ELISA or flow cytometry. In certain embodiments, the ability of the engineered cells to destroy target cells can be measured using any suitable method known in the art, such as cytotoxicity assays described in, for example, Kochenderfer *et al.*, *J. Immunotherapy*, 32(7): 689-702 (2009), and Herman *et al. J. Immunological Methods*, 285(1): 25-40 (2004). In certain embodiments, the biological activity of the cells also can be measured by assaying expression and/or secretion of certain cytokines, such as CD 107a, IFNγ, IL-2, and TNF. In some aspects the biological activity is measured by assessing clinical outcome, such as reduction in tumor burden or load.

[0508] In certain embodiments, engineered cells are modified in any number of ways, such that their therapeutic or prophylactic efficacy is increased. For example, the engineered CAR or TCR expressed by the population in some embodiments are conjugated either directly or indirectly through a linker to a targeting moiety. The practice of conjugating compounds, *e.g.*,

the CAR or TCR, to targeting moieties is known in the art. See, for instance, Wadwa *et al.*, *J. Drug Targeting,* 3(2):111 (1995), and U.S. Patent 5,087,616.

B. Combination Therapy

[0509] Also provided are methods of combination therapy that includes administering and uses, such as therapeutic and prophylactic uses, of the BCMA-binding recombinant receptors (*e.g.*, CARs), engineered cells expressing the recombinant receptors (*e.g.*, CARs), plurality of engineered cells expressing the receptors, and/or compositions comprising the same.

[0510] In some embodiments, the BCMA-binding recombinant receptor (*e.g.*, chimeric antigen receptor) and/or engineered cells expressing said molecules (*e.g.*, recombinant receptor) described herein are administered as part of a combination treatment or combination therapy, such as simultaneously with, sequentially with or intermittently with, in any order, one or more additional therapeutic intervention. In some embodiments, the one or more additional therapeutic intervention includes, for example, an antibody, an engineered cell, a receptor and/or an agent, such as a cell expressing a recombinant receptor, and/or cytotoxic or therapeutic agent, e.g., a chemotherapeutic agent. In some embodiments, the combination therapy includes administration of one or more additional agents, therapies and/or treatments, e.g., any of the additional agents, therapy and/or treatments described herein. In some embodiments, the combination therapy includes administration of one or more additional agents for treatment or therapy, such as an immunomodulatory agent, immune checkpoint inhibitor, adenosine pathway or adenosine receptor antagonist or agonist and kinase inhibitors. In some embodiments, the combination treatment or combination therapy includes an additional treatment, such as a surgical treatment, transplant, and/or radiation therapy. Also provided are methods of combination treatment or combination therapy that includes BCMA-binding recombinant receptors (e.g., CARs), cells and/or compositions described herein and one or more additional therapeutic interventions.

[0511] In some embodiments, the additional agent for combination treatment or combination therapy enhances, boosts and/or promotes the efficacy and/or safety of the therapeutic effect of binding molecules, recombinant receptors, cells and/or compositions. In some embodiments, the additional agent enhances or improves the efficacy, survival or persistence of the administered cells, e.g., cells expressing the binding molecule or a recombinant receptor. In some embodiments, the additional agent is selected from among a protein phosphatase inhibitor, a kinase inhibitor, a cytokine, an immunomodulator, or an agent that decreases the level or activity

of a regulatory T (Treg) cell. In some embodiments, the additional agent enhances safety, by virtue of reducing or ameliorating adverse effects of the administered binding molecules, recombinant receptors, cells and/or compositions. In some embodiments, the additional agent can treat the same disease, condition or a comorbidity. In some embodiments, the additional agent can ameliorate, reduce or eliminate one or more toxicities, adverse effects or side effects that are associated with administration of the recombinant receptors, cells and/or compositions, e.g., CAR-expressing cells.

[0512] In some embodiments, pain management medication such as acetaminophen, or antihistamine, such as diphenhydramine can be administered prior to, during or after administration of the recombinant receptor, cell or composition provided herein, to ameliorate or reduce or eliminate minor side effects associated with treatment. In some examples, red blood cell and platelet transfusions, and/or colony-stimulating factors can be administered reduce or eliminate one or more toxicities, adverse effects or side effects that are associated with administration of the recombinant receptors, cells and/or compositions, e.g., CAR-expressing cells. In some embodiments, prophylactic or empiric anti-infective agents (e.g., trimethoprim/sulfamethoxazole for pneumocystis pneumonia [PCP] prophylaxis, broad spectrum antibiotics, antifungals, or antiviral agents for febrile neutropenia) can be administered to treat side-effects resulting from treatment. In some examples, when necessary, prophylaxis may be provided to treat lymphopenia and/or neutropenia occurring as a result of treatment.

[0513] In some embodiments, the additional therapy, treatment or agent includes chemotherapy, radiation therapy, surgery, transplantation, adoptive cell therapy, antibodies, cytotoxic agents, chemotherapeutic agents, cytokines, growth inhibitory agents, anti-hormonal agents, kinase inhibitors, anti-angiogenic agents, cardioprotectants, immunostimulatory agents, immunosuppressive agents, immune checkpoint inhibitors, antibiotics, angiogenesis inhibitors, metabolic modulators or other therapeutic agents or any combination thereof. In some embodiments, the additional agent is a protein, a peptide, a nucleic acid, a small molecule agent, a cell, a toxin, a lipid, a carbohydrate or combinations thereof, or any other type of therapeutic agent, e.g. radiation. In some embodiments, the additional therapy, agent or treatment includes surgery, chemotherapy, radiation therapy, transplantation, administration of cells expressing a recombinant receptor, e.g., CAR, kinase inhibitor, immune checkpoint inhibitor, mTOR pathway inhibitor, immunosuppressive agents, immunomodulators, antibodies, immunoablative agents, antibodies and/or antigen binding fragments thereof, antibody conjugates, other antibody

therapies, cytotoxins, steroids, cytokines, peptide vaccines, hormone therapy, antimetabolites, metabolic modulators, drugs that inhibit either the calcium dependent phosphatase calcineurin or the p70S6 kinase FK506) or inhibit the p70S6 kinase, alkylating agents, anthracyclines, vinca alkaloids, proteasome inhibitors, GITR agonists, protein tyrosine phosphatase inhibitors, protein kinase inhibitors, an oncolytic virus, and/or other types of immunotherapy. In some embodiments, the additional agent or treatment is bone marrow transplantation, T cell ablative therapy using chemotherapy agents such as, fludarabine, external-beam radiation therapy (XRT), cyclophosphamide, and/or antibody therapy.

[0514] In some embodiments, the cells, BCMA-binding recombinant receptors and/or compositions, e.g., CAR-expressing cells, are administered in combination with other engineered cells, e.g., other CAR-expressing cells. In some embodiments, the additional agent is a kinase inhibitor, e.g., an inhibitor of Bruton's tyrosine kinase (Btk), e.g., ibrutinib. In some embodiments, the additional agent is an adenosine pathway or adenosine receptor antagonist or agonist. In some embodiments, the additional agent is an immunomodulator such as thalidomide or a thalidomide derivative (e.g., lenalidomide). In some embodiments, the additional agent is a gamma secretase inhibitor, such as a gamma secretase inhibitor that inhibits or reduces intramembrane cleavage of a target of a gamma secretase, e.g. BCMA, on a cell (such as a tumor/cancer cell). In some embodiments, the additional therapy, agent or treatment is a cytotoxic or chemotherapy agent, a biologic therapy (e.g., antibody, e.g., monoclonal antibody, or cellular therapy), or an inhibitor (e.g., kinase inhibitor).

[0515] In some embodiments, the additional agent is a chemotherapeutic agent. Exemplary chemotherapeutic agents include an anthracycline (e.g., doxorubicin, such as liposomal doxorubicin); a vinca alkaloid (e.g., vinblastine, vincristine, vindesine, vinorelbine); an alkylating agent (e.g., cyclophosphamide, decarbazine, melphalan, ifosfamide, temozolomide); an immune cell antibody (e.g., alemtuzumab, gemtuzumab, rituximab, tositumomab); an antimetabolite (including, e.g., folic acid antagonists, pyrimidine analogs, purine analogs and adenosine deaminase inhibitors such as fludarabine); a TNFR glucocorticoid induced TNFR related protein (GITR) agonist; a proteasome inhibitor (e.g., aclacinomycin A, gliotoxin or bortezomib); an immunomodulatory such as thalidomide or a thalidomide derivative (e.g., lenalidomide).

[0516] In some embodiments, the additional therapy or treatment is cell therapy, e.g., adoptive cell therapy. In some embodiments, the additional therapy includes administration of

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

[0517] In some embodiments, the configurations of one or more of the CAR molecules comprise a primary intracellular signaling domain and two or more, e.g., 2, 3, 4, or 5 or more, costimulatory signaling domains. In some embodiments, the one or more of the CAR molecules may have the same or a different primary intracellular signaling domain, the same or different costimulatory signaling domains, or the same number or a different number of costimulatory signaling domains. In some embodiments, the one or more of the CAR molecules can be configured as a split CAR, in which one of the CAR molecules comprises an antigen binding domain and a costimulatory domain (e.g., 4-1BB), while the other CAR molecule comprises an antigen binding domain and a primary intracellular signaling domain (e.g., CD3 zeta).

[0518] In some embodiments, the additional agent is any of the cells engineered to express one or more of the anti-BCMA binding molecules and/or cells engineered to express additional binding molecules, e.g., recombinant receptors, e.g., CAR, that target a different antigen. In some embodiments, the additional agent includes any of the cells or plurality of cells described herein, e.g., in Section I.C. In some embodiments, the additional agent is a cell engineered to express a recombinant receptor, e.g., CAR, targeting a different epitope and/or antigen, e.g., a different antigen associated with a disease or condition. In some embodiments, the additional agent is a cell engineered to express a recombinant receptor, e.g., CAR, targeting a second or

additional antigen expressed in multiple myeloma, e.g., CD38, CD138, CS-1, BAFF-R, TACI and/or FcRH5.

[0519] In some embodiments, the additional agent is an immunomodulatory agent. In some embodiments, the combination therapy includes an immunomodulatory agent that can stimulate, amplify and/or otherwise enhance an anti-tumor immune response, e.g. anti-tumor immune response from the administered engineered cells, such as by inhibiting immunosuppressive signaling or enhancing immunostimulant signaling. In some embodiments, the immunomodulatory agent is a peptide, protein or is a small molecule. In some embodiments, the protein can be a fusion protein or a recombinant protein. In some embodiments, the immunomodulatory agent binds to an immunologic target, such as a cell surface receptor expressed on immune cells, such a T cells, B cells or antigen-presenting cells. For example, in some embodiments, the immunomodulatory agent is an antibody or antigen-binding antibody fragment, a fusion protein, a small molecule or a polypeptide. In some embodiments, the recombinant receptors, cells and/or compositions are administered in combination with an additional agent that is an antibody or an antigen-binding fragment thereof, such as a monoclonal antibody.

[0520] In some embodiments, the immunomodulatory agent blocks, inhibits or counteracts a component of the immune checkpoint pathway. The immune system has multiple inhibitory pathways that are involved in maintaining self-tolerance and for modulating immune responses. Tumors can use certain immune-checkpoint pathways as a major mechanism of immune resistance, particularly against T cells that are specific for tumor antigens (Pardoll (2012) Nature Reviews Cancer 12:252-264), e.g., engineered cells such as CAR-expressing cells. Because many such immune checkpoints are initiated by ligand-receptor interactions, they can be readily blocked by antibodies against the ligands and/or their receptors.

[0521] Therefore, therapy with antagonistic molecules blocking an immune checkpoint pathway, such as small molecules, nucleic acid inhibitors (*e.g.*, RNAi) or antibody molecules, are becoming promising avenues of immunotherapy for cancer and other diseases. In contrast to the majority of anti-cancer agents, checkpoint inhibitors do not necessarily target tumor cells directly, but rather target lymphocyte receptors or their ligands in order to enhance the endogenous antitumor activity of the immune system.

[0522] As used herein, the term "immune checkpoint inhibitor" refers to molecules that totally or partially reduce, inhibit, interfere with or modulate one or more checkpoint proteins.

Checkpoint proteins regulate T-cell activation or function. These proteins are responsible for co-stimulatory or inhibitory interactions of T-cell responses. Immune checkpoint proteins regulate and maintain self-tolerance and the duration and amplitude of physiological immune responses. In some embodiments, the subject can be administered an additional agent that can enhance or boost the immune response, e.g., immune response effected by the BCMA-binding recombinant receptors, cells and/or compositions provided herein, against a disease or condition, e.g., a cancer, such as any described herein.

[0523] Immune checkpoint inhibitors include any agent that blocks or inhibits in a statistically significant manner, the inhibitory pathways of the immune system. Such inhibitors may include small molecule inhibitors or may include antibodies, or antigen binding fragments thereof, that bind to and block or inhibit immune checkpoint receptors, ligands and/or receptor-ligand interaction. In some embodiments, modulation, enhancement and/or stimulation of particular receptors can overcome immune checkpoint pathway components. Illustrative immune checkpoint molecules that may be targeted for blocking, inhibition, modulation, enhancement and/or stimulation include, but are not limited to, PD-1 (CD279), PD-L1 (CD274, B7-H1), PDL2 (CD273, B7-DC), CTLA-4, LAG-3 (CD223), TIM-3, 4-1BB (CD137), 4-1BBL (CD137L), GITR (TNFRSF18, AITR), CD40, OX40 (CD134, TNFRSF4), CXCR2, tumor associated antigens (TAA), B7-H3, B7-H4, BTLA, HVEM, GAL9, B7H3, B7H4, VISTA, KIR, 2B4 (belongs to the CD2 family of molecules and is expressed on all NK, γδ, and memory CD8+ (αβ) T cells), CD160 (also referred to as BY55), CGEN-15049, CEACAM (e.g., CEACAM-1, CEACAM-3 and/or CEACAM-5), TIGIT, LAIR1, CD160, 2B4, CD80, CD86, B7-H3 (CD276), B7-H4 (VTCN1), HVEM (TNFRSF14 or CD270), KIR, A2aR, MHC class I, MHC class II, GAL9, adenosine, and a transforming growth factor receptor (TGFR; e.g., TGFR beta). Immune checkpoint inhibitors include antibodies, or antigen binding fragments thereof, or other binding proteins, that bind to and block or inhibit and/or enhance or stimulate the activity of one or more of any of the said molecules.

[0524] Exemplary immune checkpoint inhibitors include Tremelimumab (CTLA-4 blocking antibody, also known as ticilimumab, CP-675,206), anti-OX40, PD-L1 monoclonal antibody (Anti-B7-H1; MEDI4736), MK-3475 (PD-1 blocker), nivolumab (anti-PD-1 antibody), CT-011 (anti-PD-1 antibody), BY55 monoclonal antibody, AMP224 (anti-PD-L1 antibody), BMS-936559 (anti-PD-L1 antibody), MPLDL3280A (anti-PD-L1 antibody), MSB0010718C (anti-PD-L1 antibody) and ipilimumab (anti-CTLA-4 antibody, also known as Yervoy®, MDX-010

and MDX-101). Exemplary immunomodulatory antibodies include, but are not limited to, Daclizumab (Zenapax), Bevacizumab (Avastin ®), Basiliximab, Ipilimumab, Nivolumab, pembrolizumab, MPDL3280A, Pidilizumab (CT-011), MK-3475, BMS-936559, MPDL3280A (Atezolizumab), tremelimumab, IMP321, BMS-986016, LAG525, urelumab, PF-05082566, TRX518, MK-4166, dacetuzumab (SGN-40), lucatumumab (HCD122), SEA-CD40, CP-870, CP-893, MEDI6469, MEDI6383, MOXR0916, AMP-224, MSB0010718C (Avelumab), MEDI4736, PDR001, rHIgM12B7, Ulocuplumab, BKT140, Varlilumab (CDX-1127), ARGX-110, MGA271, lirilumab (BMS-986015, IPH2101), IPH2201, ARGX-115, Emactuzumab, CC-90002 and MNRP1685A or an antibody-binding fragment thereof. Other exemplary immunomodulators include, e.g., afutuzumab (available from Roche®); pegfilgrastim (Neulasta®); lenalidomide (CC-5013, Revlimid®); thalidomide (Thalomid®), actimid (CC4047); and IRX-2 (mixture of human cytokines including interleukin 1, interleukin 2, and interferon gamma, CAS 951209-71-5, available from IRX Therapeutics).

[0525] Programmed cell death 1 (PD-1) is an immune checkpoint protein that is expressed in B cells, NK cells, and T cells (Shinohara et al., 1995, Genomics 23:704-6; Blank et al., 2007, Cancer Immunol Immunother 56:739-45; Finger et al., 1997, Gene 197:177-87; Pardoll (2012) Nature Reviews Cancer 12:252-264). The major role of PD-1 is to limit the activity of T cells in peripheral tissues during inflammation in response to infection, as well as to limit autoimmunity. PD-1 expression is induced in activated T cells and binding of PD-1 to one of its endogenous ligands acts to inhibit T-cell activation by inhibiting stimulatory kinases. PD-1 also acts to inhibit the TCR "stop signal". PD-1 is highly expressed on Treg cells and may increase their proliferation in the presence of ligand (Pardoll (2012) Nature Reviews Cancer 12:252-264). Anti-PD 1 antibodies have been used for treatment of melanoma, non-small-cell lung cancer, bladder cancer, prostate cancer, colorectal cancer, head and neck cancer, triple-negative breast cancer, leukemia, lymphoma and renal cell cancer (Topalian et al., 2012, N Engl J Med 366:2443-54; Lipson et al., 2013, Clin Cancer Res 19:462-8; Berger et al., 2008, Clin Cancer Res 14:3044-51; Gildener-Leapman et al., 2013, Oral Oncol 49:1089-96; Menzies & Long, 2013, Ther Adv Med Oncol 5:278-85). Exemplary anti-PD-1 antibodies include nivolumab (Opdivo by BMS), pembrolizumab (Keytruda by Merck), pidilizumab (CT-011 by Cure Tech), lambrolizumab (MK-3475 by Merck), and AMP-224 (Merck), nivolumab (also referred to as Opdivo, BMS-936558 or MDX1106; Bristol-Myers Squibb) is a fully human IgG4 monoclonal antibody which specifically blocks PD-1. Nivolumab (clone 5C4) and other human monoclonal

antibodies that specifically bind to PD-1 are described in US 8,008,449 and WO2006/121168. Pidilizumab (CT-011; Cure Tech) is a humanized IgG1k monoclonal antibody that binds to PD-1. Pidilizumab and other humanized anti-PD-1 monoclonal antibodies are described in WO2009/101611. Pembrolizumab (formerly known as lambrolizumab, and also referred to as Keytruda, MK03475; Merck) is a humanized IgG4 monoclonal antibody that binds to PD-1. Pembrolizumab and other humanized anti-PD-1 antibodies are described in US 8,354,509 and WO2009/114335. Other anti-PD-1 antibodies include AMP 514 (Amplimmune), among others, e.g., anti-PD-1 antibodies described in US 8,609,089, US 2010028330, US 20120114649 and/or US 20150210769. AMP-224 (B7-DCIg; Amplimmune; e.g., described in WO2010/027827 and WO2011/066342), is a PD-L2 Fc fusion soluble receptor that blocks the interaction between PD-1 and B7-H1.

[0526] PD-L1 (also known as CD274 and B7-H1) and PD-L2 (also known as CD273 and B7-DC) are ligands for PD-1, found on activated T cells, B cells, myeloid cells, macrophages, and some types of tumor cells. Anti-tumor therapies have focused on anti-PD-L1 antibodies. The complex of PD-1 and PD-L1 inhibits proliferation of CD8+ T cells and reduces the immune response (Topalian et al., 2012, N Engl J Med 366:2443-54; Brahmer et al., 2012, N Eng J Med 366:2455-65). Anti-PD-L1 antibodies have been used for treatment of non-small cell lung cancer, melanoma, colorectal cancer, renal-cell cancer, pancreatic cancer, gastric cancer, ovarian cancer, breast cancer, and hematologic malignancies (Brahmer et al., 2012, N Eng J Med 366:2455-65; Ott et al., 2013, Clin Cancer Res 19:5300-9; Radvanyi et al., 2013, Clin Cancer Res 19:5541; Menzies & Long, 2013, Ther Adv Med Oncol 5:278-85; Berger et al., 2008, Clin Cancer Res 14:13044-51). Exemplary anti-PD-L1 antibodies include MDX-1105 (Medarex), MEDI4736 (Medimmune) MPDL3280A (Genentech), BMS-935559 (Bristol-Myers Squibb) and MSB0010718C. MEDI4736 (Medimmune) is a human monoclonal antibody that binds to PD-L1, and inhibits interaction of the ligand with PD-1. MDPL3280A (Genentech/Roche) is a human Fc optimized IgG1 monoclonal antibody that binds to PD-L1. MDPL3280A and other human monoclonal antibodies to PD-L1 are described in U.S. Patent No. 7,943,743 and U.S Publication No. 20120039906. Other anti-PD-L1 binding agents include YW243.55.S70 (see WO2010/077634) and MDX-1105 (also referred to as BMS-936559, and, e.g., anti-PD-L1 binding agents described in WO2007/005874).

[0527] Cytotoxic T-lymphocyte-associated antigen (CTLA-4), also known as CD152, is a co-inhibitory molecule that functions to regulate T-cell activation. CTLA-4 is a member of the

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

[0528] Lymphocyte activation gene-3 (LAG-3), also known as CD223, is another immune checkpoint protein. LAG-3 has been associated with the inhibition of lymphocyte activity and in some cases the induction of lymphocyte anergy. LAG-3 is expressed on various cells in the immune system including B cells, NK cells, and dendritic cells. LAG-3 is a natural ligand for the MHC class II receptor, which is substantially expressed on melanoma-infiltrating T cells including those endowed with potent immune-suppressive activity. Exemplary anti-LAG-3 antibodies include BMS-986016 (Bristol-Myers Squib), which is a monoclonal antibody that targets LAG-3. IMP701 (Immutep) is an antagonist LAG-3 antibody and IMP731 (Immutep and GlaxoSmithKline) is a depleting LAG-3 antibody. Other LAG-3 inhibitors include IMP321 (Immutep), which is a recombinant fusion protein of a soluble portion of LAG-3 and Ig that binds to MHC class II molecules and activates antigen presenting cells (APC). Other antibodies are described, e.g., in WO2010/019570 and US 2015/0259420

[0529] T-cell immunoglobulin domain and mucin domain-3 (TIM-3), initially identified on activated Th1 cells, has been shown to be a negative regulator of the immune response. Blockade of TIM-3 promotes T-cell mediated anti-tumor immunity and has anti-tumor activity in a range of mouse tumor models. Combinations of TIM-3 blockade with other

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

[0530] In some embodiments, the additional agent is a CEACAM inhibitor (e.g., CEACAM-1, CEACAM-3, and/or CEACAM-5 inhibitor). In some embodiments, the inhibitor of CEACAM is an anti-CEACAM antibody molecule. Exemplary anti-CEACAM-1 antibodies are described in WO 2010/125571, WO 2013/082366 WO 2014/059251 and WO 2014/022332, e.g., a monoclonal antibody 34B1, 26H7, and 5F4; or a recombinant form thereof, as described in, e.g., US 2004/0047858, US 7,132,255 and WO 99/052552. In some embodiments, the anti-CEACAM antibody binds to CEACAM-5 as described in, e.g., Zheng et al. PLoS One. (2011) 6(6): e21146), or cross reacts with CEACAM-1 and CEACAM-5 as described in, e.g., WO 2013/054331 and US 2014/0271618.

[0531] 4-1BB, also known as CD137, is transmembrane glycoprotein belonging to the TNFR superfamily. 4-1BB receptors are present on activated T cells and B cells and monocytes. An exemplary anti-4-1BB antibody is urelumab (BMS-663513), which has potential immunostimulatory and antineoplastic activities.

[0532] Tumor necrosis factor receptor superfamily, member 4 (TNFRSF4), also known as OX40 and CD134, is another member of the TNFR superfamily. OX40 is not constitutively expressed on resting naïve T cells and acts as a secondary co-stimulatory immune checkpoint

molecule. Exemplary anti-OX40 antibodies are MEDI6469 and MOXR0916 (RG7888, Genentech).

[0533] In some embodiments, the additional agent includes a molecule that decreases the regulatory T cell (Treg) population. Methods that decrease the number of (e.g., deplete) Treg cells are known in the art and include, e.g., CD25 depletion, cyclophosphamide administration, and modulating Glucocorticoid-induced TNFR family related gene (GITR) function. GITR is a member of the TNFR superfamily that is upregulated on activated T cells, which enhances the immune system. Reducing the number of Treg cells in a subject prior to apheresis or prior to administration of engineered cells, e.g., CAR-expressing cells, can reduce the number of unwanted immune cells (e.g., Tregs) in the tumor microenvironment and reduces the subject's risk of relapse. In some embodiments, the additional agent includes a molecule targeting GITR and/or modulating GITR functions, such as a GITR agonist and/or a GITR antibody that depletes regulatory T cells (Tregs). In some embodiments, the additional agent includes cyclophosphamide. In some embodiments, the GITR binding molecule and/or molecule modulating GITR function (e.g., GITR agonist and/or Treg depleting GITR antibodies) is administered prior to the engineered cells, e.g., CAR-expressing cells. For example, in some embodiments, the GITR agonist can be administered prior to apheresis of the cells. In some embodiments, cyclophosphamide is administered to the subject prior to administration (e.g., infusion or re-infusion) of the engineered cells, e.g., CAR-expressing cells or prior to apheresis of the cells. In some embodiments, cyclophosphamide and an anti-GITR antibody are administered to the subject prior to administration (e.g., infusion or re-infusion) of the engineered cells, e.g., CAR-expressing cells or prior to apheresis of the cells.

[0534] In some embodiments, the additional agent is a GITR agonist. Exemplary GITR agonists include, e.g., GITR fusion proteins and anti-GITR antibodies (e.g., bivalent anti-GITR antibodies) such as, e.g., a GITR fusion protein described in U.S. Patent No. 6,111,090, European Patent No. 090505B 1, U.S Patent No. 8,586,023, PCT Publication Nos.: WO 2010/003118 and 2011/090754, or an anti-GITR antibody described, e.g., in U.S. Patent No. 7,025,962, European Patent No. 1947183B 1, U.S. Patent No. 7,812,135, U.S. Patent No. 8,388,967, U.S. Patent No. 8,591,886, European Patent No. EP 1866339, PCT Publication No. WO 2011/028683, PCT Publication No. WO 2013/039954, PCT Publication No. WO2005/007190, PCT Publication No. WO 2007/133822, PCT Publication No. WO2005/055808, PCT Publication No. WO 99/40196, PCT Publication No. WO 2001/03720,

PCT Publication No. WO99/20758, PCT Publication No. WO2006/083289, PCT Publication No. WO 2005/115451, U.S. Patent No. 7,618,632, and PCT Publication No. WO 2011/051726. An exemplary anti-GITR antibody is TRX518.

[0535] In some embodiments, the additional agent enhances tumor infiltration or transmigration of the administered cells, e.g., CAR-expressing cells. For example, in some embodiments, the additional agent stimulates CD40, such as CD40L, e.g., recombinant human CD40L. Cluster of differentiation 40 (CD40) is also a member of the TNFR superfamily. CD40 is a costimulatory protein found on antigen-presenting cells and mediates a broad variety of immune and inflammatory responses. CD40 is also expressed on some malignancies, where it promotes proliferation. Exemplary anti-CD40 antibodies are dacetuzumab (SGN-40), lucatumumab (Novartis, antagonist), SEA-CD40 (Seattle Genetics), and CP-870,893. In some embodiments, the additional agent that enhances tumor infiltration includes tyrosine kinase inhibitor sunitnib, heparanase, and/or chemokine receptors such as CCR2, CCR4, and CCR7.

[0536] In some embodiments, the additional agent includes thalidomide drugs or analogs thereof and/or derivatives thereof, such as lenalidomide, pomalidomide or apremilast. See, e.g., Bertilaccio et al., Blood (2013) 122:4171, Otahal et al., Oncoimmunology (2016) 5(4):e1115940; Fecteau et al., Blood (2014) 124(10):1637-1644 and Kuramitsu et al., Cancer Gene Therapy (2015) 22:487-495). Lenalidomide ((RS)-3-(4-Amino-1-oxo-1,3-dihydro-2H-isoindol-2-yl)piperidine-2,6-dione; also known as Revlimid) is a synthetic derivative of thalidomide, and has multiple immunomodulatory effects, including enforcement of immune synapse formation between T cell and antigen presenting cells (APCs). For example, in some cases, lenalidomide modulates T cell responses and results in increased interleukin (IL)-2 production in CD4+ and CD8+ T cells, induces the shift of T helper (Th) responses from Th2 to Th1, inhibits expansion of regulatory subset of T cells (Tregs), and improves functioning of immunological synapses in follicular lymphoma and chronic lymphocytic leukemia (CLL) (Otahal et al., Oncoimmunology (2016) 5(4):e1115940). Lenalidomide also has direct tumoricidal activity in patients with multiple myeloma (MM) and directly and indirectly modulates survival of CLL tumor cells by affecting supportive cells, such as nurse-like cells found in the microenvironment of lymphoid tissues. Lenalidomide also can enhance T-cell proliferation and interferon-γ production in response to activation of T cells via CD3 ligation or dendritic cell-mediated activation. Lenalidomide can also induce malignant B cells to express higher levels of immunostimulatory molecules such as CD80, CD86, HLA-DR, CD95, and

CD40 (Fecteau et al., Blood (2014) 124(10):1637-1644). In some embodiments, lenalidomide is administered at a dosage of from about 1 mg to about 20 mg daily, e.g., from about 1 mg to about 10 mg, from about 2.5 mg to about 7.5 mg, from about 5 mg to about 15 mg, such as about 5 mg, 10 mg, 15 mg or 20 mg daily. In some embodiments, lenalidomide is administered at a dose of from about 10 μg/kg to 5 mg/kg, e.g., about 100 μg/kg to about 2 mg/kg, about 200 μg/kg to about 1 mg/kg, about 400 μg/kg to about 600 μg/kg, such as about 500 μg/kg. In some embodiments, rituximab is administered at a dosage of about 350-550 mg/m^2 (e.g., 350-375, 375-400, 400-425, 425-450, 450-475, or 475-500 mg/m^2), e.g., intravenously. In some embodiments, lenalidomide is administered at a low dose.

[0537] In some embodiments, the additional agent is a B-cell inhibitor. In some embodiments, the additional agent is one or more B-cell inhibitors selected from among inhibitors of CD10, CD19, CD20, CD22, CD34, CD123, CD79a, CD79b, CD179b, FLT-3, or ROR1, or a combination thereof. In some embodiments, the B-cell inhibitor is an antibody (e.g., a mono- or bispecific antibody) or an antigen binding fragment thereof. In some embodiments, the additional agent is an engineered cell expressing recombinant receptors that target B-cell targets, e.g., CD10, CD19, CD20, CD22, CD34, CD123, CD79a, CD79b, CD179b, FLT-3, or ROR1.

[0538] In some embodiments, the additional agent is a CD20 inhibitor, e.g., an anti-CD20 antibody (e.g., an anti-CD20 mono- or bi-specific antibody) or a fragment thereof. Exemplary anti-CD20 antibodies include but are not limited to rituximab, ofatumumab, ocrelizumab (also known as GA101 or RO5072759), veltuzumab, obinutuzumab, TRU-015 (Trubion Pharmaceuticals), ocaratuzumab (also known as AME-133v or ocaratuzumab), and Pro131921 (Genentech). See, e.g., Lim et al. Haematologica. (2010) 95(1):135-43. In some embodiments, the anti-CD20 antibody comprises rituximab. Rituximab is a chimeric mouse/human monoclonal antibody IgG1 kappa that binds to CD20 and causes cytolysis of a CD20 expressing cell. In some embodiments, the additional agent includes rituximab. In some embodiments, the CD20 inhibitor is a small molecule.

[0539] In some embodiments, the additional agent is a CD22 inhibitor, e.g., an anti-CD22 antibody (e.g., an anti-CD22 mono- or bi-specific antibody) or a fragment thereof. Exemplary anti-CD22 antibodies include epratuzumab and RFB4. In some embodiments, the CD22 inhibitor is a small molecule. In some embodiments, the antibody is a monospecific antibody, optionally conjugated to a second agent such as a chemotherapeutic agent. For instance, in some

embodiments, the antibody is an anti-CD22 monoclonal antibody-MMAE conjugate (e.g., DCDT2980S). In some embodiments, the antibody is an scFv of an anti-CD22 antibody, e.g., an scFv of antibody RFB4. In some embodiments, the scFv is fused to all of or a fragment of Pseudomonas exotoxin-A (e.g., BL22). In some embodiments, the scFv is fused to all of or a fragment of (e.g., a 38 kDa fragment of) Pseudomonas exotoxin-A (e.g., moxetumomab pasudotox). In some embodiments, the anti-CD22 antibody is an anti-CD19/CD22 bispecific antibody, optionally conjugated to a toxin. For instance, in some embodiments, the anti-CD22 antibody comprises an anti-CD19/CD22 bispecific portion, (e.g., two scFv ligands, recognizing human CD19 and CD22) optionally linked to all of or a portion of diphtheria toxin (DT), e.g., first 389 amino acids of diphtheria toxin (DT), DT 390, e.g., a ligand-directed toxin such as DT2219ARL). In some embodiments, the bispecific portion (e.g., anti-CD 19/anti-CD22) is linked to a toxin such as deglycosylated ricin A chain (e.g., Combotox).

[0540] In some embodiments, the immunomodulatory agent is a cytokine. In some embodiments, the immunomodulatory agent is a cytokine or is an agent that induces increased expression of a cytokine in the tumor microenvironment. Cytokines have important functions related to T cell expansion, differentiation, survival, and homeostasis. Cytokines that can be administered to the subject receiving the BCMA-binding recombinant receptors, cells and/or compositions provided herein include one or more of IL-2, IL-4, IL-7, IL-9, IL-15, IL-18, and IL-21. In some embodiments, the cytokine administered is IL-7, IL-15, or IL-21, or a combination thereof. In some embodiments, administration of the cytokine to the subject that has sub-optimal response to the administration of the engineered cells, e.g., CAR-expressing cells improves efficacy and/or anti-tumor activity of the administered cells, e.g., CAR-expressing cells.

[0541] By "cytokine" is meant a generic term for proteins released by one cell population that act on another cell as intercellular mediators. Examples of such cytokines are lymphokines, monokines, and traditional polypeptide hormones. Included among the cytokines are growth hormones such as human growth hormone, N-methionyl human growth hormone, and bovine growth hormone; parathyroid hormone; thyroxine; insulin; proinsulin; relaxin; prorelaxin; glycoprotein hormones such as follicle stimulating hormone (FSH), thyroid stimulating hormone (TSH), and luteinizing hormone (LH); hepatic growth factor; fibroblast growth factor; prolactin; placental lactogen; tumor necrosis factor-alpha and -beta; mullerian-inhibiting substance; mouse gonadotropin-associated peptide; inhibin; activin; vascular endothelial growth factor; integrin;

thrombopoietin (TPO); nerve growth factors such as NGF-beta; platelet-growth factor; transforming growth factors (TGFs) such as TGF-alpha and TGF-beta; insulin-like growth factor-I and -II; erythropoietin (EPO); osteoinductive factors; interferons such as interferon-alpha, beta, and -gamma; colony stimulating factors (CSFs) such as macrophage-CSF (M-CSF); granulocyte-macrophage-CSF (GM-CSF); and granulocyte-CSF (G-CSF); interleukins (ILs) such as IL-1, IL-1alpha, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-11, IL-12; IL-15, a tumor necrosis factor such as TNF-alpha or TNF-beta; and other polypeptide factors including LIF and kit ligand (KL). As used herein, the term cytokine includes proteins from natural sources or from recombinant cell culture, and biologically active equivalents of the native sequence cytokines. For example, the immunomodulatory agent is a cytokine and the cytokine is IL-4, TNF-α, GM-CSF or IL-2.

[0542] In some embodiments, the additional agent includes an interleukin-15 (IL-15) polypeptide, an interleukin-15 receptor alpha (IL-15Rα) polypeptide, or combination thereof, e.g., hetIL-15 (Admune Therapeutics, LLC). hetIL-15 is a heterodimeric non-covalent complex of IL-15 and IL-15Rα. hetIL-15 is described in, e.g., U.S. 8,124,084, U.S. 2012/0177598, U.S. 2009/0082299, U.S. 2012/0141413, and U.S. 2011/0081311. In some embodiments, the immunomodulatory agent can contain one or more cytokines. For example, the interleukin can include leukocyte interleukin injection (Multikine), which is a combination of natural cytokines. In some embodiments, the immunomodulatory agent is a Toll-like receptor (TLR) agonist, an adjuvant or a cytokine.

[0543] In some embodiments, the additional agent is an agent that ameliorates or neutralizes one or more toxicities or side effects associated with the cell therapy. In some embodiments, the additional agent is selected from among a steroid (e.g., corticosteroid), an inhibitor of TNFα, and an inhibitor of IL-6. An example of a TNFα inhibitor is an anti- TNFα antibody molecule such as, infliximab, adalimumab, certolizumab pegol, and golimumab. Another example of a TNFα inhibitor is a fusion protein such as entanercept. Small molecule inhibitors of TNFα include, but are not limited to, xanthine derivatives (e.g. pentoxifylline) and bupropion. An example of an IL-6 inhibitor is an anti-IL-6 antibody molecule such as tocilizumab, sarilumab, elsilimomab, CNTO 328, ALD518/BMS-945429, CNTO 136, CPSI-2364, CDP6038, VX30, ARGX-109, FE301, and FM101. In some embodiments, the anti-IL-6 antibody molecule is tocilizumab. In some embodiments, the additional agent is an IL-1R inhibitor, such as anakinra.

[0544] In some embodiments, the additional agent is a modulator of adenosine levels and/or an adenosine pathway component. Adenosine can function as an immunomodulatory agent in the body. For example, adenosine and some adenosine analogs that non-selectively activate adenosine receptor subtypes decrease neutrophil production of inflammatory oxidative products (Cronstein et al., Ann. N.Y. Acad. Sci. 451:291, 1985; Roberts et al., Biochem. J., 227:669, 1985; Schrier et al., J. Immunol. 137:3284, 1986; Cronstein et al., Clinical Immunol. Immunopath. 42:76, 1987). In some cases, concentration of extracellular adenosine or adenosine analogs can increase in specific environments, e.g., tumor microenvironment (TME). In some cases, adenosine or adenosine analog signaling depends on hypoxia or factors involved in hypoxia or its regulation, e.g., hypoxia inducible factor (HIF). In some embodiments, increase in adenosine signaling can increase in intracellular cAMP and cAMP-dependent protein kinase that results in inhibition of proinflammatory cytokine production, and can lead to the synthesis of immunosuppressive molecules and development of Tregs (Sitkovsky et al., Cancer Immunol Res (2014) 2(7):598-605). In some embodiments, the additional agent can reduce or reverse immunosuppressive effects of adenosine, adenosine analogs and/or adenosine signaling. In some embodiments, the additional agent can reduce or reverse hypoxia-driven A2-adenosinergic T cell immunosuppression. In some embodiments, the additional agent is selected from among antagonists of adenosine receptors, extracellular adenosine-degrading agents, inhibitors of adenosine generation by CD39/CD73 ectoenzymes, and inhibitors of hypoxia-HIF-1α signaling. In some embodiments, the additional agent is an adenosine receptor antagonist or agonist.

[0545] Inhibition or reduction of extracellular adenosine or the adenosine receptor by virtue of an inhibitor of extracellular adenosine (such as an agent that prevents the formation of, degrades, renders inactive, and/or decreases extracellular adenosine), and/or an adenosine receptor inhibitor (such as an adenosine receptor antagonist) can enhance immune response, such as a macrophage, neutrophil, granulocyte, dendritic cell, T- and/or B cell-mediated response. In addition, inhibitors of the Gs protein mediated cAMP dependent intracellular pathway and inhibitors of the adenosine receptor-triggered Gi protein mediated intracellular pathways, can also increase acute and chronic inflammation.

[0546] In some embodiments, the additional agent is an adenosine receptor antagonist or agonist, e.g., an antagonist or agonist of one or more of the adenosine receptors A2a, A2b, A1, and A3. A1 and A3 inhibit, and A2a and A2b stimulate, respectively, adenylate cyclase activity.

Certain adenosine receptors, such as A2a, A2b, and A3, can suppress or reduce the immune response during inflammation. Thus, antagonizing immunosuppressive adenosine receptors can augment, boost or enhance immune response, e.g., immune response from administered cells, e.g., CAR-expressing T cells. In some embodiments, the additional agent inhibits the production of extracellular adenosine and adenosine-triggered signaling through adenosine receptors. For example, enhancement of an immune response, local tissue inflammation, and targeted tissue destruction can be enhanced by inhibiting or reducing the adenosine-producing local tissue hypoxia; by degrading (or rendering inactive) accumulated extracellular adenosine; by preventing or decreasing expression of adenosine receptors on immune cells; and/or by inhibiting/antagonizing signaling by adenosine ligands through adenosine receptors.

[0547] An antagonist is any substance that tends to nullify the action of another, as an agent that binds to a cell receptor without eliciting a biological response. In some embodiments, the antagonist is a chemical compound that is an antagonist for an adenosine receptor, such as the A2a, A2b, or A3 receptor. In some embodiments, the antagonist is a peptide, or a pepidomimetic, that binds the adenosine receptor but does not trigger a Gi protein dependent intracellular pathway. Exemplary antagonists are described in U.S. Pat. Nos. 5,565,566; 5,545, 627, 5,981,524; 5,861,405; 6,066,642; 6,326,390; 5,670,501; 6,117,998; 6,232,297; 5,786,360; 5,424,297; 6,313,131, 5,504,090; and 6,322,771.

[0548] In some embodiments, the additional agent is an A2 receptor (A2R) antagonist, such as an A2a antagonist. Exemplary A2R antagonists include KW6002 (istradefyline), SCH58261, caffeine, paraxanthine, 3,7-dimethyl-1-propargylxanthine (DMPX), 8-(m-chlorostyryl) caffeine (CSC), MSX-2, MSX-3, MSX-4, CGS-15943, ZM-241385, SCH-442416, preladenant, vipadenant (BII014), V2006, ST-1535, SYN-115, PSB-1115, ZM241365, FSPTP, and an inhibitory nucleic acid targeting A2R expression, e.g., siRNA or shRNA, or any antibodies or antigen-binding fragment thereof that targets an A2R. In some embodiments, the additional agent is an A2R antagonist described in, e.g., Ohta et al., Proc Natl Acad Sci U S A (2006) 103:13132-13137; Jin et al., Cancer Res. (2010) 70(6):2245-2255; Leone et al., Computational and Structural Biotechnology Journal (2015) 13:265-272; Beavis et al., Proc Natl Acad Sci U S A (2013) 110:14711–14716; and Pinna, A., Expert Opin Investig Drugs (2009) 18:1619-1631; Sitkovsky et al., Cancer Immunol Res (2014) 2(7):598-605; US 8,080,554; US 8,716,301; US 20140056922; WO2008/147482; US 8,883,500; US 20140377240; WO02/055083; US 7,141,575; US 7,405,219; US 8,883,500; US 8,450,329 and US 8,987,279).

[0549] In some embodiments, the antagonist is an antisense molecule, inhibitory nucleic acid molecule (e.g., small inhibitory RNA (siRNA)) or catalytic nucleic acid molecule (e.g. a ribozyme) that specifically binds mRNA encoding an adenosine receptor. In some embodiments, the antisense molecule, inhibitory nucleic acid molecule or catalytic nucleic acid molecule binds nucleic acids encoding A2a, A2b, or A3. In some embodiments, an antisense molecule, inhibitory nucleic acid molecule or catalytic nucleic acid targets biochemical pathways downstream of the adenosine receptor. For example, the antisense molecule or catalytic nucleic acid can inhibit an enzyme involved in the Gs protein- or Gi protein-dependent intracellular pathway. In some embodiments, the additional agent includes dominant negative mutant form of an adenosine receptor, such as A2a, A2b, or A3.

[0550] In some embodiments, the additional agent that inhibits extracellular adenosine includes agents that render extracellular adenosine non-functional (or decrease such function), such as a substance that modifies the structure of adenosine to inhibit the ability of adenosine to signal through adenosine receptors. In some embodiments, the additional agent is an extracellular adenosine-generating or adenosine-degrading enzyme, a modified form thereof or a modulator thereof. For example, in some embodiments, the additional agent is an enzyme (e.g. adenosine deaminase) or another catalytic molecule that selectively binds and destroys the adenosine, thereby abolishing or significantly decreasing the ability of endogenously formed adenosine to signal through adenosine receptors and terminate inflammation.

[0551] In some embodiments, the additional agent is an adenosine deaminase (ADA) or a modified form thereof, e.g., recombinant ADA and/or polyethylene glycol-modified ADA (ADA-PEG), which can inhibit local tissue accumulation of extracellular adenosine. ADA-PEG has been used in treatment of patients with ADA SCID (Hershfield (1995) Hum Mutat. 5:107). In some embodiments, an agent that inhibits extracellular adenosine includes agents that prevent or decrease formation of extracellular adenosine, and/or prevent or decrease the accumulation of extracellular adenosine, thereby abolishing, or substantially decreasing, the immunosuppressive effects of adenosine. In some embodiments, the additional agent specifically inhibits enzymes and proteins that are involved in regulation of synthesis and/or secretion of pro-inflammatory molecules, including modulators of nuclear transcription factors. Suppression of adenosine receptor expression or expression of the Gs protein- or Gi protein-dependent intracellular pathway, or the cAMP dependent intracellular pathway, can result in an increase/enhancement of immune response.

[0552] In some embodiments, the additional agent can target ectoenzymes that generate or produce extracellular adenosine. In some embodiments, the additional agent targets CD39 and CD73 ectoenzymes, which function in tandem to generate extracellular adenosine. CD39 (also called ectonucleoside triphosphate diphosphohydrolase) converts extracellular ATP (or ADP) to 5′AMP. Subsequently, CD73 (also called 5′nucleotidase) converts 5′AMP to adenosine. The activity of CD39 is reversible by the actions of NDP kinase and adenylate kinase, whereas the activity of CD73 is irreversible. CD39 and CD73 are expressed on tumor stromal cells, including endothelial cells and Tregs, and also on many cancer cells. For example, the expression of CD39 and CD73 on endothelial cells is increased under the hypoxic conditions of the tumor microenvironment. Tumor hypoxia can result from inadequate blood supply and disorganized tumor vasculature, impairing delivery of oxygen (Carroll and Ashcroft (2005), Expert. Rev. Mol. Med. 7(6):1-16). Hypoxia also inhibits adenylate kinase (AK), which converts adenosine to AMP, leading to very high extracellular adenosine concentration. Thus, adenosine is released at high concentrations in response to hypoxia, which is a condition that frequently occurs the tumor microenvironment (TME), in or around solid tumors. In some embodiments, the additional agent is one or more of anti-CD39 antibody or antigen binding fragment thereof, anti-CD73 antibody or antigen binding fragment thereof, e.g., MEDI9447 or TY/23, α-β-methylene-adenosine diphosphate (ADP), ARL 67156, POM-3, IPH52 (see, e.g., Allard et al. Clin Cancer Res (2013) 19(20):5626-5635; Hausler et al., Am J Transl Res (2014) 6(2):129-139; Zhang, B., Cancer Res. (2010) 70(16):6407-6411).

[0553] In some embodiments, the additional agent is an inhibitor of hypoxia inducible factor 1 alpha (HIF-1α) signaling. Exemplary inhibitors of HIF-1α include digoxin, acriflavine, sirtuin-7 and ganetespib.

[0554] In some embodiments, the additional agent includes a protein tyrosine phosphatase inhibitor, e.g., a protein tyrosine phosphatase inhibitor described herein. In some embodiments, the protein tyrosine phosphatase inhibitor is an SHP-1 inhibitor, e.g., an SHP-1 inhibitor described herein, such as, e.g., sodium stibogluconate. In some embodiments, the protein tyrosine phosphatase inhibitor is an SHP-2 inhibitor, e.g., an SHP-2 inhibitor described herein.

[0555] In some embodiments, the additional agent is a kinase inhibitor. Kinase inhibitors, such as a CDK4 kinase inhibitor, a BTK kinase inhibitor, a MNK kinase inhibitor, or a DGK kinase inhibitor, can regulate the constitutively active survival pathways that exist in tumor cells and/or modulate the function of immune cells. In some embodiments, the kinase inhibitor is a

Bruton's tyrosine kinase (BTK) inhibitor, e.g., ibrutinib. In some embodiments, the kinase inhibitor is a phosphatidylinositol-4,5-bisphosphate 3-kinase (PI3K) inhibitor. In some embodiments, the kinase inhibitor is a CDK4 inhibitor, e.g., a CDK4/6 inhibitor. In some embodiments, the kinase inhibitor is an mTOR inhibitor, such as, e.g., rapamycin, a rapamycin analog, OSI-027. The mTOR inhibitor can be, e.g., an mTORC1 inhibitor and/or an mTORC2 inhibitor, e.g., an mTORC1 inhibitor and/or mTORC2 inhibitor. In some embodiments, the kinase inhibitor is an MNK inhibitor, or a dual PI3K/mTOR inhibitor. In some embodiments, other exemplary kinase inhibitors include the AKT inhibitor perifosine, the mTOR inhibitor temsirolimus, the Src kinase inhibitors dasatinib and fostamatinib, the JAK2 inhibitors pacritinib and ruxolitinib, the PKCβ inhibitors enzastaurin and bryostatin, and the AAK inhibitor alisertib.

[0556] In some embodiments, the kinase inhibitor is a BTK inhibitor selected from ibrutinib (PCI- 32765); GDC-0834; RN-486; CGI-560; CGI-1764; HM-71224; CC-292; ONO-4059; CNX-774; and LFM-A13. In some embodiments, the BTK inhibitor does not reduce or inhibit the kinase activity of interleukin-2-inducible kinase (ITK), and is selected from GDC-0834; RN-486; CGI-560; CGI-1764; HM-71224; CC-292; ONO-4059; CNX-774; and LFM-A13.

[0557] In some embodiments, the kinase inhibitor is a BTK inhibitor, e.g., ibrutinib (1-[(3R)-3-[4-Amino-3-(4-phenoxyphenyl)-1H-pyrazolo[3,4-d]pyrimidin-1-yl]piperidin-1-yl]prop-2-en-1-one; also known as PCI-32765). In some embodiments, the kinase inhibitor is a BTK inhibitor, e.g., ibrutinib (PCI-32765), and the ibrutinib is administered at a dose of about 250 mg, 300 mg, 350 mg, 400 mg, 420 mg, 440 mg, 460 mg, 480 mg, 500 mg, 520 mg, 540 mg, 560 mg, 580 mg, 600 mg (e.g., 250 mg, 420 mg or 560 mg) daily for a period of time, e.g., daily for 21 day cycle, or daily for 28 day cycle. In some embodiments, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12 or more cycles of ibrutinib are administered. In some embodiments, the BTK inhibitor is a BTK inhibitor described in International Application WO 2015/079417.

[0558] In some embodiments, the kinase inhibitor is a PI3K inhibitor. PI3K is central to the PI3K/Akt/mTOR pathway involved in cell cycle regulation and lymphoma survival. Exemplary PI3K inhibitor includes idelalisib (PI3Kδ inhibitor). In some embodiments, the additional agent is idelalisib and rituximab.

[0559] In some embodiments, the additional agent is an inhibitor of mammalian target of rapamycin (mTOR). In some embodiments, the kinase inhibitor is an mTOR inhibitor selected from temsirolimus; ridaforolimus (also known as AP23573 and MK8669); everolimus (RAD001); rapamycin (AY22989); simapimod; AZD8055; PF04691502; SF1126; and XL765.

In some embodiments, the additional agent is an inhibitor of mitogen-activated protein kinase (MAPK), such as vemurafenib, dabrafenib, and trametinib.

[0560] In some embodiments, the additional agent is an agent that regulates pro- or anti-apoptotic proteins. In some embodiments, the additional agent includes a B-cell lymphoma 2 (BCL-2) inhibitor (e.g., venetoclax, also called ABT-199 or GDC-0199; or ABT-737). Venetoclax is a small molecule (4-(4-{[2-(4-Chlorophenyl)-4,4-dimethyl-1-cyclohexen-1-yl]methyl}-1-piperazinyl)-N-({3-nitro-4-[(tetrahydro-2H-pyran-4-ylmethyl)amino]phenyl}sulfonyl)-2-(1H-pyrrolo[2,3-b]pyridin-5-yloxy)benzamide) that inhibits the anti-apoptotic protein, BCL-2. Other agents that modulate pro- or anti-apoptotic protein include BCL-2 inhibitor ABT-737, navitoclax (ABT-263); Mcl-1 siRNA or Mcl-1 inhibitor retinoid N-(4-hydroxyphenyl) retinamide (4-HPR) for maximal efficacy. In some embodiments, the additional agent provides a pro-apoptotic stimuli, such as recombinant tumor necrosis factor-related apoptosis-inducing ligand (TRAIL), which can activate the apoptosis pathway by binding to TRAIL death receptors DR-4 and DR-5 on tumor cell surface, or TRAIL-R2 agonistic antibodies.

[0561] In some embodiments, the additional agent includes an indoleamine 2,3-dioxygenase (IDO) inhibitor. IDO is an enzyme that catalyzes the degradation of the amino acid, L-tryptophan, to kynurenine. Many cancers overexpress IDO, e.g., prostatic, colorectal, pancreatic, cervical, gastric, ovarian, head, and lung cancer. Plasmacytoid dendritic cells (pDCs), macrophages, and dendritic cells (DCs) can express IDO. In some aspects, a decrease in L-tryptophan (e.g., catalyzed by IDO) results in an immunosuppressive milieu by inducing T-cell anergy and apoptosis. Thus, in some aspects, an IDO inhibitor can enhance the efficacy of the BCMA-binding recombinant receptors, cells and/or compositions described herein, e.g., by decreasing the suppression or death of the administered CAR-expressing cell. Exemplary inhibitors of IDO include but are not limited to 1-methyl-tryptophan, indoximod (New Link Genetics) (see, e.g., Clinical Trial Identifier Nos. NCT01191216; NCT01792050), and INCB024360 (Incyte Corp.) (see, e.g., Clinical Trial Identifier Nos. NCT01604889; NCT01685255).

[0562] In some embodiments, the additional agent includes a cytotoxic agent, e.g., CPX-351 (Celator Pharmaceuticals), cytarabine, daunorubicin, vosaroxin (Sunesis Pharmaceuticals), sapacitabine (Cyclacel Pharmaceuticals), idarubicin, or mitoxantrone. In some embodiments,

the additional agent includes a hypomethylating agent, e.g., a DNA methyltransferase inhibitor, e.g., azacitidine or decitabine.

[0563] In another embodiment, the additional therapy is transplantation, e.g., an allogeneic stem cell transplant.

[0564] In some embodiments, the additional therapy is a lymphodepleting therapy. Lymphodepleting chemotherapy is thought to improve engraftment and activity of recombinant receptor-expressing cells, such as CAR T cells. In some embodiments, lymphodepleting chemotherapy may enhance adoptively transferred tumor-specific T cells to proliferate in vivo through homeostatic proliferation (Grossman 2004, Stachel 2004). In some embodiments, chemotherapy may reduce or eliminate CD4+CD25+ regulatory T cells, which can suppress the function of tumor-targeted adoptively transferred T cells (Turk 2004). In some embodiments, lymphodepleting chemotherapy prior to adoptive T-cell therapy may enhance the expression of stromal cell-derived factor 1 (SDF-1) in the bone marrow, enhancing the homing of modified T cells to the primary tumor site through binding of SDF-1 with CXCR-4 expressed on the T-cell surface (Pinthus 2004). In some embodiments, lymphodepleting chemotherapy may further reduce the subject's tumor burden and potentially lower the risk and severity of CRS.

[0565] In some embodiments, lymphodepletion is performed on a subject, e.g., prior to administering engineered cells, e.g., CAR-expressing cells. In some embodiments, the lymphodepletion comprises administering one or more of melphalan, Cytoxan, cyclophosphamide, and/or fludarabine. In some embodiments, a lymphodepleting chemotherapy is administered to the subject prior to, concurrently with, or after administration (e.g., infusion) of engineered cells, e.g., CAR-expressing cells. In an example, the lymphodepleting chemotherapy is administered to the subject prior to administration of engineered cells, e.g., CAR-expressing cells. In some embodiments the lymphodepleting chemotherapy is administered 1 to 10 days prior to administration of engineered cells, such as 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 days prior to the initiation of administration of engineered cells, or at least 2 days prior, such as at least 3, 4, 5, 6, or 7 days prior, to the initiation of administration of engineered cell.. In some embodiments, the subject is administered a preconditioning agent no more than 7 days prior, such as no more than 6, 5, 4, 3, or 2 days prior, to the initiation of administration of engineered cell.The number of days after lymphodepleting chemotherapy that the engineered ells are administered can be determined based on clinical or logistical circumstances. In some examples, dose adjustments or other changes to the lymphodepleting chemotherapy regimen can

implemented due to a subject's health, such as the subject's underlying organ function, as determined by the treating physician.

[0566] In some embodiments, lymphodepleting chemotherapy comprises administration of a lymphodepleting agent, such as cyclophosphamide, fludarabine, or combinations thereof, In some embodiments, the subject is administered cyclophosphamide at a dose between or between about 20 mg/kg and 100 mg/kg body weight of the subject, such as between or between about 40 mg/kg and 80 mg/kg. In some aspects, the subject is administered about 60 mg/kg of cyclophosphamide. In some embodiments, the cyclophosphamide is administered once daily for one or two days. In some embodiments, where the lymphodepleting agent comprises cyclophosphamide, the subject is administered cyclophosphamide at a dose between or between about 100 mg/m^2 and 500 mg/m^2 body surface area of the subject, such as between or between about 200 mg/m^2 and 400 mg/m^2, or 250 mg/m^2 and 350 mg/m^2, inclusive. In some instances, the subject is administered about 300 mg/m^2 of cyclophosphamide. In some embodiments, the cyclophosphamide can be administered in a single dose or can be administered in a plurality of doses, such as given daily, every other day or every three days. In some embodiments, cyclophosphamide is administered daily, such as for 1-5 days, for example, for 2 to 4 days. In some instances, the subject is administered about 300 mg/m^2 body surface area of the subject, of cyclophosphamide, daily for 3 days, prior to initiation of the cell therapy.

[0567] In some embodiments, where the lymphodepleting agent comprises fludarabine, the subject is administered fludarabine at a dose between or between about 1 mg/m^2 and 100 mg/m^2 body surface area of the subject, such as between or between about 10 mg/m^2 and 75 mg/m^2, 15 mg/m^2 and 50 mg/m^2, 20 mg/m^2 and 40 mg/m^2, or 24 mg/m^2 and 35 mg/m^2, inclusive. In some instances, the subject is administered about 30 mg/m^2 of fludarabine. In some embodiments, the fludarabine can be administered in a single dose or can be administered in a plurality of doses, such as given daily, every other day or every three days. In some embodiments, fludarabine is administered daily, such as for 1-5 days, for example, for 2 to 4 days. In some instances, the subject is administered about 30 mg/m^2 body surface area of the subject, of fludarabine, daily for 3 days, prior to initiation of the cell therapy.

[0568] In some embodiments, the lymphodepleting agent comprises a combination of agents, such as a combination of cyclophosphamide and fludarabine. Thus, the combination of agents may include cyclophosphamide at any dose or administration schedule, such as those described above, and fludarabine at any dose or administration schedule, such as those described

above. For example, in some aspects, the subject is administered fludarabine at or about 30 mg/m^2 body surface area of the subject, daily, and cyclophosphamide at or about 300 mg/m^2 body surface area of the subject, daily, for 3 days.

[0569] In some embodiments, antiemetic therapy, except dexamethasone or other steroids, may be given prior to lymphodepleting chemotherapy. In some embodiments, Mesna may be used for subjects with a history of hemorrhagic cystitis.

[0570] In some embodiments, the additional agent is an oncolytic virus. In some embodiments, oncolytic viruses are capable of selectively replicating in and triggering the death of or slowing the growth of a cancer cell. In some cases, oncolytic viruses have no effect or a minimal effect on non-cancer cells. An oncolytic virus includes but is not limited to an oncolytic adenovirus, oncolytic Herpes Simplex Viruses, oncolytic retrovirus, oncolytic parvovirus, oncolytic vaccinia virus, oncolytic Sinbis virus, oncolytic influenza virus, or oncolytic RNA virus (e.g., oncolytic reovirus, oncolytic Newcastle Disease Virus (NDV), oncolytic measles virus, or oncolytic vesicular stomatitis virus (VSV)).

[0571] Other exemplary combination therapy, treatment and/or agents include anti-allergenic agents, anti-emetics, analgesics and adjunct therapies. In some embodiments, the additional agent includes cytoprotective agents, such as neuroprotectants, free-radical scavengers, cardioprotectors, anthracycline extravasation neutralizers and nutrients.

[0572] In some embodiments, an antibody used as an additional agent is conjugated or otherwise bound to a therapeutic agent, e.g., a chemotherapeutic agent (e.g., Cytoxan, fludarabine, histone deacetylase inhibitor, demethylating agent, peptide vaccine, anti-tumor antibiotic, tyrosine kinase inhibitor, alkylating agent, anti-microtubule or anti-mitotic agent), anti-allergic agent, anti-nausea agent (or anti-emetic), pain reliever, or cytoprotective agent described herein. In some embodiments, the additional agent is an antibody-drug conjugate.

[0573] In some embodiments, the additional agent can modulate, inhibit or stimulate particular factors at the DNA, RNA or protein levels, to enhance or boost the efficacy of the BCMA-binding recombinant receptors, cells and/or compositions provided herein. In some embodiments, the additional agent can modulate the factors at the nucleic acid level, e.g., DNA or RNA, within the administered cells, e.g., cells engineered to express recombinant receptors, e.g., CAR. In some embodiments, an inhibitory nucleic acid, e.g., an inhibitory nucleic acid, e.g., a dsRNA, e.g., an siRNA or shRNA, or a clustered regularly interspaced short palindromic repeats (CRISPR), a transcription-activator like effector nuclease (TALEN), or a zinc finger

endonuclease (ZFN), can be used to inhibit expression of an inhibitory molecule in the engineered cell, e.g., CAR-expressing cell. In some embodiments the inhibitor is an shRNA. In some embodiments, the inhibitory molecule is inhibited within the engineered cell, e.g., CAR-expressing cell. In some embodiments, a nucleic acid molecule that encodes a dsRNA molecule that inhibits expression of the molecule that modulates or regulates, e.g., inhibits, T-cell function is operably linked to a promoter, e.g., a HI- or a U6-derived promoter such that the dsRNA molecule that inhibits expression of the inhibitory molecule is expressed within the engineered cell, e.g., CAR-expressing cell. See, e.g., Brummelkamp TR, et al. (2002) Science 296: 550-553; Miyagishi M, et al. (2002) Nat. Biotechnol. 19: 497-500.

[0574] In some embodiments, the additional agent is capable of disrupting the gene encoding an inhibitory molecule, such as any immune checkpoint inhibitors described herein. In some embodiments, disruption is by deletion, e.g., deletion of an entire gene, exon, or region, and/or replacement with an exogenous sequence, and/or by mutation, *e.g.*, frameshift or missense mutation, within the gene, typically within an exon of the gene. In some embodiments, the disruption results in a premature stop codon being incorporated into the gene, such that the inhibitory molecule is not expressed or is not expressed in a form that is capable of being expressed on the cells surface and/or capable of mediating cell signaling. The disruption is generally carried out at the DNA level. The disruption generally is permanent, irreversible, or not transient.

[0575] In some aspects, the disruption is carried out by gene editing, such as using a DNA binding protein or DNA-binding nucleic acid, which specifically binds to or hybridizes to the gene at a region targeted for disruption. In some aspects, the protein or nucleic acid is coupled to or complexed with a nuclease, such as in a chimeric or fusion protein. For example, in some embodiments, the disruption is effected using a fusion comprising a DNA-targeting protein and a nuclease, such as a Zinc Finger Nuclease (ZFN) or TAL-effector nuclease (TALEN), or an RNA-guided nuclease such as a clustered regularly interspersed short palindromic nucleic acid (CRISPR)-Cas system, such as CRISPR-Cas9 system, specific for the gene being disrupted. In some embodiments, methods of producing or generating genetically engineered cells, e.g., CAR-expressing cells, include introducing into a population of cells nucleic acid molecules encoding a genetically engineered antigen receptor (e.g. CAR) and nucleic acid molecules encoding an agent targeting an inhibitory molecule that is a gene editing nuclease, such as a fusion of a

DNA-targeting protein and a nuclease such as a ZFN or a TALEN, or an RNA-guided nuclease such as of the CRISPR-Cas9 system, specific for an inhibitory molecule.

[0576] Any of the additional agents described herein can be prepared and administered as combination therapy with the BCMA-binding recombinant receptor (*e.g.*, chimeric antigen receptor) and/or engineered cells expressing said molecules (*e.g.*, recombinant receptor) described herein, such as in pharmaceutical compositions comprising one or more agents of the combination therapy and a pharmaceutically acceptable carrier, such as any described herein. In some embodiments, the BCMA-binding recombinant receptor (*e.g.*, chimeric antigen receptor), engineered cells expressing said molecules (*e.g.*, recombinant receptor), plurality of engineered cells expressing said molecules (e.g., recombinant receptor) can be administered simultaneously, concurrently or sequentially, in any order with the additional agents, therapy or treatment, wherein such administration provides therapeutically effective levels each of the agents in the body of the subject. In some embodiments, the additional agent can be co-administered with the BCMA-binding recombinant receptors, cells and/or compositions described herein, for example, as part of the same pharmaceutical composition or using the same method of delivery. In some embodiments, the additional agent is administered simultaneously with the BCMA-binding recombinant receptors, cells and/or compositions described herein, but in separate compositions. In some embodiments, the additional agent is an additional engineered cell, e.g., cell engineered to express a different recombinant receptor, and is administered in the same composition or in a separate composition. In some embodiments, the additional agent is incubated with the engineered cell, e.g., CAR-expressing cells, prior to administration of the cells.

[0577] In some examples, the one or more additional agents are administered subsequent to or prior to the administration of the BCMA-binding recombinant receptors, cells and/or compositions described herein, separated by a selected time period. In some examples, the time period is 1 day, 2 days, 3 days, 4 days, 5 days, 6 days, 1 week, 2 weeks, 3 weeks, 1 month, 2 months, or 3 months. In some examples, the one or more additional agents are administered multiple times and/or the BCMA-binding recombinant receptors, cells and/or compositions described herein, is administered multiple times. For example, in some embodiments, the additional agent is administered prior to the BCMA-binding recombinant receptors, cells and/or compositions described herein, e.g., two weeks, 12 days, 10 days, 8 days, one week, 6 days, 5 days, 4 days, 3 days, 2 days or 1 day before the administration. For example, in some embodiments, the additional agent is administered after the BCMA-binding recombinant

receptors, cells and/or compositions described herein, e.g., two weeks, 12 days, 10 days, 8 days, one week, 6 days, 5 days, 4 days, 3 days, 2 days or 1 day after the administration.

[0578] The dose of the additional agent can be any therapeutically effective amount, e.g., any dose amount described herein, and the appropriate dosage of the additional agent may depend on the type of disease to be treated, the type, dose and/or frequency of the recombinant receptor, cell and/or composition administered, the severity and course of the disease, whether the recombinant receptor, cell and/or composition is administered for preventive or therapeutic purposes, previous therapy, the patient's clinical history and response to the recombinant receptor, cell and/or composition, and the discretion of the attending physician. The recombinant receptor, cell and/or composition and/or the additional agent and/or therapy can be administered to the patient at one time, repeated or administered over a series of treatments.

VI. ARTICLES OF MANUFACTURE OR KITS

[0579] Also provided are articles of manufacture or kit containing the provided recombinant receptors (*e.g.*, CARs), genetically engineered cells, and/or compositions comprising the same. The articles of manufacture may include a container and a label or package insert on or associated with the container. Suitable containers include, for example, bottles, vials, syringes, test tubes, IV solution bags, etc. The containers may be formed from a variety of materials such as glass or plastic. In some embodiments, the container has a sterile access port. Exemplary containers include an intravenous solution bags, vials, including those with stoppers pierceable by a needle for injection. The article of manufacture or kit may further include a package insert indicating that the compositions can be used to treat a particular condition such as a condition described herein (*e.g.*, multiple myeloma). Alternatively, or additionally, the article of manufacture or kit may further include another or the same container comprising a pharmaceutically-acceptable buffer. It may further include other materials such as other buffers, diluents, filters, needles, and/or syringes.

[0580] The label or package insert may indicate that the composition is used for treating the BCMA-expressing or BCMA-associated disease, disorder or condition in an individual. The label or a package insert, which is on or associated with the container, may indicate directions for reconstitution and/or use of the formulation. The label or package insert may further indicate that the formulation is useful or intended for subcutaneous, intravenous, or other modes of administration for treating or preventing a BCMA-expressing or BCMA-associated disease, disorder or condition in an individual.

[0581] The container in some embodiments holds a composition which is by itself or combined with another composition effective for treating, preventing and/or diagnosing the condition. The article of manufacture or kit may include (a) a first container with a composition contained therein (*i.e.*, first medicament), wherein the composition includes the antibody (*e.g.*, anti-BCMA antibody) or antigen-binding fragment thereof or recombinant receptor (*e.g.*, CAR); and (b) a second container with a composition contained therein (*i.e.*, second medicament), wherein the composition includes a further agent, such as a cytotoxic or otherwise therapeutic agent, and which article or kit further comprises instructions on the label or package insert for treating the subject with the second medicament, in an effective amount.

VII. DEFINITIONS

[0582] As used herein, reference to a "corresponding form" of an antibody means that when comparing a property or activity of two antibodies, the property is compared using the same form of the antibody. For example, if it is stated that an antibody has greater activity compared to the activity of the corresponding form of a first antibody, that means that a particular form, such as an scFv of that antibody, has greater activity compared to the scFv form of the first antibody.

[0583] The term "Fc region" herein is used to define a C-terminal region of an immunoglobulin heavy chain that contains at least a portion of the constant region. The term includes native sequence Fc regions and variant Fc regions. In one embodiment, a human IgG heavy chain Fc region extends from Cys226, or from Pro230, to the carboxyl-terminus of the heavy chain. However, the C-terminal lysine (Lys447) of the Fc region may or may not be present. Unless otherwise specified herein, numbering of amino acid residues in the Fc region or constant region is according to the EU numbering system, also called the EU index, as described in Kabat *et al.*, Sequences of Proteins of Immunological Interest, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, MD, 1991.

[0584] The terms "full length antibody," "intact antibody," and "whole antibody" are used herein interchangeably to refer to an antibody having a structure substantially similar to a native antibody structure or having heavy chains that contain an Fc region as defined herein.

[0585] An "isolated" antibody is one which has been separated from a component of its natural environment. In some embodiments, an antibody is purified to greater than 95% or 99% purity as determined by, for example, electrophoretic (*e.g.*, SDS-PAGE, isoelectric focusing (IEF), capillary electrophoresis) or chromatographic (*e.g.*, ion exchange or reverse phase

HPLC). For review of methods for assessment of antibody purity, see, *e.g.*, Flatman *et al.*, J. Chromatogr. B 848:79-87 (2007).

[0586] An "isolated" nucleic acid refers to a nucleic acid molecule that has been separated from a component of its natural environment. An isolated nucleic acid includes a nucleic acid molecule contained in cells that ordinarily contain the nucleic acid molecule, but the nucleic acid molecule is present extrachromosomally or at a chromosomal location that is different from its natural chromosomal location.

[0587] "Isolated nucleic acid encoding an anti-BCMA antibody" refers to one or more nucleic acid molecules encoding antibody heavy and light chains (or fragments thereof), including such nucleic acid molecule(s) in a single vector or separate vectors, and such nucleic acid molecule(s) present at one or more locations in a host cell.

[0588] The terms "host cell," "host cell line," and "host cell culture" are used interchangeably and refer to cells into which exogenous nucleic acid has been introduced, including the progeny of such cells. Host cells include "transformants" and "transformed cells," which include the primary transformed cell and progeny derived therefrom without regard to the number of passages. Progeny may not be completely identical in nucleic acid content to a parent cell, but may contain mutations. Mutant progeny that have the same function or biological activity as screened or selected for in the originally transformed cell are included herein.

[0589] The terms "polypeptide" and "protein" are used interchangeably to refer to a polymer of amino acid residues, and are not limited to a minimum length. Polypeptides, including the antibodies and antibody chains and other peptides, *e.g.*, linkers and BCMA-binding peptides, may include amino acid residues including natural and/or non-natural amino acid residues. The terms also include post-expression modifications of the polypeptide, for example, glycosylation, sialylation, acetylation, phosphorylation, and the like. In some aspects, the polypeptides may contain modifications with respect to a native or natural sequence, as long as the protein maintains the desired activity. These modifications may be deliberate, as through site-directed mutagenesis, or may be accidental, such as through mutations of hosts which produce the proteins or errors due to PCR amplification.

[0590] As used herein, "percent (%) amino acid sequence identity" and "percent identity" and "sequence identity" when used with respect to an amino acid sequence (reference polypeptide sequence) is defined as the percentage of amino acid residues in a candidate sequence (*e.g.*, the subject antibody or fragment) that are identical with the amino acid residues

in the reference polypeptide sequence, after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent sequence identity, and not considering any conservative substitutions as part of the sequence identity. Alignment for purposes of determining percent amino acid sequence identity can be achieved in various ways that are within the skill in the art, for instance, using publicly available computer software such as BLAST, BLAST-2, ALIGN or Megalign (DNASTAR) software. Those skilled in the art can determine appropriate parameters for aligning sequences, including any algorithms needed to achieve maximal alignment over the full length of the sequences being compared.

[0591] An amino acid substitution may include replacement of one amino acid in a polypeptide with another amino acid. Amino acid substitutions may be introduced into a binding molecule, *e.g.*, antibody, of interest and the products screened for a desired activity, *e.g.*, retained/improved antigen binding, or decreased immunogenicity.

[0592] Amino acids generally can be grouped according to the following common side-chain properties:

(1) hydrophobic: Norleucine, Met, Ala, Val, Leu, Ile;

(2) neutral hydrophilic: Cys, Ser, Thr, Asn, Gln;

(3) acidic: Asp, Glu;

(4) basic: His, Lys, Arg;

(5) residues that influence chain orientation: Gly, Pro;

(6) aromatic: Trp, Tyr, Phe.

[0593] Non-conservative amino acid substitutions will involve exchanging a member of one of these classes for another class.

[0594] The term "vector," as used herein, refers to a nucleic acid molecule capable of propagating another nucleic acid to which it is linked. The term includes the vector as a self-replicating nucleic acid structure as well as the vector incorporated into the genome of a host cell into which it has been introduced. Certain vectors are capable of directing the expression of nucleic acids to which they are operatively linked. Such vectors are referred to herein as "expression vectors."

[0595] The term "package insert" is used to refer to instructions customarily included in commercial packages of therapeutic products, that contain information about the indications, usage, dosage, administration, combination therapy, contraindications and/or warnings concerning the use of such therapeutic products.

[0596] As used herein, the singular forms "a," "an," and "the" include plural referents unless the context clearly dictates otherwise. For example, "a" or "an" means "at least one" or "one or more." It is understood that aspects, embodiments, and variations described herein include "comprising," "consisting," and/or "consisting essentially of" aspects, embodiments and variations.

[0597] Throughout this disclosure, various aspects of the claimed subject matter are presented in a range format. It should be understood that the description in range format is merely for convenience and brevity and should not be construed as an inflexible limitation on the scope of the claimed subject matter. Accordingly, the description of a range should be considered to have specifically disclosed all the possible sub-ranges as well as individual numerical values within that range. For example, where a range of values is provided, it is understood that each intervening value, between the upper and lower limit of that range and any other stated or intervening value in that stated range is encompassed within the claimed subject matter. The upper and lower limits of these smaller ranges may independently be included in the smaller ranges, and are also encompassed within the claimed subject matter, subject to any specifically excluded limit in the stated range. Where the stated range includes one or both of the limits, ranges excluding either or both of those included limits are also included in the claimed subject matter. This applies regardless of the breadth of the range.

[0598] The term "about" as used herein refers to the usual error range for the respective value readily known to the skilled person in this technical field. Reference to "about" a value or parameter herein includes (and describes) embodiments that are directed to that value or parameter per se. For example, description referring to "about X" includes description of "X".

[0599] As used herein, a "composition" refers to any mixture of two or more products, substances, or compounds, including cells. It may be a solution, a suspension, liquid, powder, a paste, aqueous, non-aqueous or any combination thereof.

[0600] As used herein, a statement that a cell or population of cells is "positive" for a particular marker refers to the detectable presence on or in the cell of a particular marker, typically a surface marker. When referring to a surface marker, the term refers to the presence of surface expression as detected by flow cytometry, for example, by staining with an antibody that specifically binds to the marker and detecting said antibody, wherein the staining is detectable by flow cytometry at a level substantially above the staining detected carrying out the same procedure with an isotype-matched control under otherwise identical conditions and/or at a

level substantially similar to that for cell known to be positive for the marker, and/or at a level substantially higher than that for a cell known to be negative for the marker.

[0601] As used herein, a statement that a cell or population of cells is "negative" for a particular marker refers to the absence of substantial detectable presence on or in the cell of a particular marker, typically a surface marker. When referring to a surface marker, the term refers to the absence of surface expression as detected by flow cytometry, for example, by staining with an antibody that specifically binds to the marker and detecting said antibody, wherein the staining is not detected by flow cytometry at a level substantially above the staining detected carrying out the same procedure with an isotype-matched control under otherwise identical conditions, and/or at a level substantially lower than that for cell known to be positive for the marker, and/or at a level substantially similar as compared to that for a cell known to be negative for the marker.

[0602] Unless defined otherwise, all terms of art, notations and other technical and scientific terms or terminology used herein are intended to have the same meaning as is commonly understood by one of ordinary skill in the art to which the claimed subject matter pertains. In some cases, terms with commonly understood meanings are defined herein for clarity and/or for ready reference, and the inclusion of such definitions herein should not necessarily be construed to represent a substantial difference over what is generally understood in the art.

[0603] All publications, including patent documents, scientific articles and databases, referred to in this application are incorporated by reference in their entirety for all purposes to the same extent as if each individual publication were individually incorporated by reference. If a definition set forth herein is contrary to or otherwise inconsistent with a definition set forth in the patents, applications, published applications and other publications that are herein incorporated by reference, the definition set forth herein prevails over the definition that is incorporated herein by reference.

[0604] The section headings used herein are for organizational purposes only and are not to be construed as limiting the subject matter described.

VIII. EXEMPLARY EMBODIMENTS

[0605] Among the embodiments provided herein are:

1. A polynucleotide encoding a chimeric antigen receptor, comprising 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.

2. The polynucleotide of embodiment 1, wherein the spacer is derived from an immunoglobulin.

3. The polynucleotide of embodiment 1 or embodiment 2, wherein the spacer comprises a sequence of a hinge region, a C_H2 and C_H3 region.

4. The polynucleotide of any of embodiments 1-3, 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.

5. The polynucleotide of any of embodiments 1-4, 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.

6. The polynucleotide of embodiment 5, wherein the one or more nucleotide modifications comprise an insertion, deletion, substitution or combinations thereof.

7. The polynucleotide of embodiment 5 or embodiment 6, wherein the reference splice acceptor and/or reference splice donor sites are canonical, non-canonical, or cryptic splice sites.

8. The polynucleotide of any of embodiment 5-7, 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; 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%.

9. The polynucleotide of any of embodiments 5-8, wherein:

the reference splice donor site comprises 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 comprises the sequence aagtttctttctgtattccaggctgaccgtggataaatctc (SEQ ID NO:742) and/or gggcaacgtgttctcttgcagtgtcatgcacgaagccctgc (SEQ ID NO:743).

10. The polynucleotide of any of embodiment 5-8, wherein:

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%.

11. The polynucleotide of any of embodiments 5-8 and 10, wherein:

the reference splice donor site comprises the sequence tcaactggtacgtgg (SEQ ID NO:706); and/or

the reference splice acceptor site comprises the sequence aagtttctttctgtattccaggctgaccgtggataaatctc (SEQ ID NO:742).

12. The polynucleotide of any of embodiments 5-11, wherein 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.

13. The polynucleotide of any of embodiments 5-12, 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.

14. The polynucleotide of any of embodiments 5-9 and 12-13, 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 cagtttcttcctgtatagtagactcaccgtggataaatcaa (SEQ ID NO:672), gggcaacgtgttcagctgcagcgtgatgcacgaggccctgc (SEQ ID NO: 673) and/or cgccttgtcctccttgtcccgctcctcctgttgccggacct (SEQ ID NO:766).

15. The polynucleotide of any of embodiments 5-14, wherein the modified splice donor site is set forth in tcaactggtatgtgg (SEQ ID NO:662) and/or the modified acceptor site is set forth in cagtttcttcctgtatagtagactcaccgtggataaatcaa (SEQ ID NO:672) and/or cgccttgtcctccttgtcccgctcctcctgttgccggacct (SEQ ID NO:766).

16. The polynucleotide of any of embodiments 1-15, wherein the spacer is encoded by a sequence of nucleotide set forth in SEQ ID NO:622 or a portion thereof.

17. A polynucleotide encoding a chimeric antigen receptor, wherein the polynucleotide comprises nucleic acid encoding: (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.

18. A polynucleotide encoding a chimeric antigen receptor, wherein the polynucleotide comprises nucleic acid encoding: (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.

19. The polynucleotide of embodiment 17 or embodiment 18, 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.

20. The polynucleotide of any of embodiments 1-19, wherein, 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 comprises one or more nucleotide modifications compared to the polynucleotide.

21. The polynucleotide of embodiment 20, wherein the RNA heterogeneity is reduced by greater than or greater than about 10%, 15%, 20%, 25%, 30%, 40%, 50% or more.

22. The polynucleotide of embodiment 20 or embodiment 21, wherein 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.

23. The polynucleotide of any of embodiments 1-22, wherein the RNA homogeneity and/or heterogeneity is determined by agarose gel electrophoresis, chip-based capillary

electrophoresis, analytical ultracentrifugation, field flow fractionation, or liquid chromatography.

24. The polynucleotide of any of embodiments 1-23, wherein the polynucleotide is codon-optimized.

25. The polynucleotide of any of embodiments 1-24, wherein 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.

26. The polynucleotide of any of embodiments 1-25, wherein the disease or condition is a cancer.

27. The polynucleotide of any of embodiments 1-26, wherein the disease or condition is a myeloma, leukemia or lymphoma.

28. The polynucleotide of any of embodiments 1-27, wherein 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.

29. The polynucleotide of embodiment 28, wherein the antigen is B cell maturation antigen (BCMA).

30. The polynucleotide of any of embodiments 1-29, wherein the antigen-binding domain is an antibody fragment comprising a variable heavy chain (V_H) and a variable light chain (V_L) region.

31. The polynucleotide of embodiment 30, wherein:

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 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 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 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.

32. The polynucleotide of embodiment 30 or embodiment 31, wherein:

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 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; and/or

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 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 or 618.

33. The polynucleotide of embodiment 30 or embodiment 31, wherein:

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:110-115, 247-256, 324, 325, 518-531, 533, 609, 617, 772-774, or 814-832; and/or

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:116-127, 257-267, 326, 327, 534-550, 552-557, 610, 618, 775-777, or 833-849.

34. The polynucleotide of any of embodiments 30-33, wherein:

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: 110, 111, 112, 113, 115, 248, 252, 253, 254, 255, 256, 324, 325, 518, 519, 520, 521, 522, 609 or 617; and/or

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: 116, 117, 118, 120, 121, 124, 125, 258, 262, 263, 264, 265, 266, 267, 326, 327, 534, 535, 536, 537, 538, 610 or 618.

35 The polynucleotide of any of embodiments 30-34, wherein:

the V_H region is or comprises (a) a heavy chain complementarity determining region 1 (CDR-H1) comprising 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) comprising 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) comprising 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 comprises (a) a light chain complementarity determining region 1 (CDR-L1) comprising 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) comprising 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) comprising 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.

36. The polynucleotide of any of embodiments 30-35, wherein:

the V_H region is or comprises (a) a heavy chain complementarity determining region 1 (CDR-H1) comprising 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) comprising 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) comprising 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 comprises (a) a light chain complementarity determining region 1 (CDR-L1) comprising 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) comprising 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) comprising 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.

37. The polynucleotide of any of embodiments 30-36, wherein the V_H region comprises a CDR-H1, CDR-H2, and CDR-H3, selected from:

a CDR-H1, CDR-H2, and CDR-H3 comprising the amino acid sequence of SEQ ID NOs:1, 4, and 7, respectively;

a CDR-H1, CDR-H2, and CDR-H3 comprising the amino acid sequence of SEQ ID NOs:2, 5, and 8, respectively;

a CDR-H1, CDR-H2, and CDR-H3 comprising the amino acid sequence of SEQ ID NOs:2, 5, and 9, respectively;

a CDR-H1, CDR-H2, and CDR-H3 comprising the amino acid sequence of SEQ ID NOs:2, 5, and 10, respectively;

a CDR-H1, CDR-H2, and CDR-H3 comprising the amino acid sequence of SEQ ID NOs:3, 6, and 11, respectively;

a CDR-H1, CDR-H2, and CDR-H3 comprising the amino acid sequence of SEQ ID NOs:140, 145, and 149, respectively;

a CDR-H1, CDR-H2, and CDR-H3 comprising the amino acid sequence of SEQ ID NOs:141, 145, and 149, respectively;

a CDR-H1, CDR-H2, and CDR-H3 comprising the amino acid sequence of SEQ ID NOs:141, 145, and 150, respectively;

a CDR-H1, CDR-H2, and CDR-H3 comprising the amino acid sequence of SEQ ID NOs:142, 146, and 151, respectively;

a CDR-H1, CDR-H2, and CDR-H3 comprising the amino acid sequence of SEQ ID NOs:2, 5, and 152, respectively;

a CDR-H1, CDR-H2, and CDR-H3 comprising the amino acid sequence of SEQ ID NOs:143, 147, and 153, respectively;

a CDR-H1, CDR-H2, and CDR-H3 comprising the amino acid sequence of SEQ ID NOs:144, 148, and 154, respectively;

a CDR-H1, CDR-H2, and CDR-H3 comprising the amino acid sequence of SEQ ID NOs:3, 6, and 155, respectively;

a CDR-H1, CDR-H2, and CDR-H3 comprising the amino acid sequence of SEQ ID NOs:2, 5, and 156, respectively;

a CDR-H1, CDR-H2, and CDR-H3 comprising the amino acid sequence of SEQ ID NOs:2, 5, and 157, respectively;

a CDR-H1, CDR-H2, and CDR-H3 comprising the amino acid sequence of SEQ ID NOs:2, 6, and 376, respectively;

a CDR-H1, CDR-H2, and CDR-H3 comprising the amino acid sequence of SEQ ID NOs:3, 6, and 155, respectively;

a CDR-H1, CDR-H2, and CDR-H3 comprising the amino acid sequence of SEQ ID NOs:3, 372, and 376, respectively;

a CDR-H1, CDR-H2, and CDR-H3 comprising the amino acid sequence of SEQ ID NOs:3, 6, and 376, respectively;

a CDR-H1, CDR-H2, and CDR-H3 comprising the amino acid sequence of SEQ ID NOs:3, 6, and 377, respectively;

a CDR-H1, CDR-H2, and CDR-H3 comprising the amino acid sequence of SEQ ID NOs:2, 373, and 152, respectively;

a CDR-H1, CDR-H2, and CDR-H3 comprising the amino acid sequence of SEQ ID NOs:2, 5, and 378, respectively;

a CDR-H1, CDR-H2, and CDR-H3 comprising the amino acid sequence of SEQ ID NOs:2, 374, and 9, respectively;

a CDR-H1, CDR-H2, and CDR-H3 comprising the amino acid sequence of SEQ ID NOs:593, 594, and 595, respectively;

a CDR-H1, CDR-H2, and CDR-H3 comprising the amino acid sequence of SEQ ID NOs:611, 612, and 613, respectively;

a CDR-H1, CDR-H2, and CDR-H3 comprising the amino acid sequence of SEQ ID NOs:507, 513, and 517, respectively;

a CDR-H1, CDR-H2, and CDR-H3 comprising the amino acid sequence of SEQ ID NOs:604, 605, and 606, respectively;

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

a CDR-H1, CDR-H2, and CDR-H3 comprising the amino acid sequence of SEQ ID NOs:289, 291, and 293, respectively.

38. The polynucleotide of any of embodiments 30-37, wherein the V_H region comprises a CDR-H1, CDR-H2, and CDR-H3, selected from:

a CDR-H1, CDR-H2, and CDR-H3 comprising the amino acid sequence of SEQ ID NOs:1, 4, and 7, respectively;

a CDR-H1, CDR-H2, and CDR-H3 comprising the amino acid sequence of SEQ ID NOs:2, 5, and 8, respectively;

a CDR-H1, CDR-H2, and CDR-H3 comprising the amino acid sequence of SEQ ID NOs:2, 5, and 9, respectively;

a CDR-H1, CDR-H2, and CDR-H3 comprising the amino acid sequence of SEQ ID NOs:2, 5, and 10, respectively;

a CDR-H1, CDR-H2, and CDR-H3 comprising the amino acid sequence of SEQ ID NOs:141, 145, and 149, respectively;

a CDR-H1, CDR-H2, and CDR-H3 comprising the amino acid sequence of SEQ ID NOs:143, 147, and 153, respectively;

a CDR-H1, CDR-H2, and CDR-H3 comprising the amino acid sequence of SEQ ID NOs:144, 148, and 154, respectively;

a CDR-H1, CDR-H2, and CDR-H3 comprising the amino acid sequence of SEQ ID NOs:3, 6, and 155, respectively;

a CDR-H1, CDR-H2, and CDR-H3 comprising the amino acid sequence of SEQ ID NOs:2, 5, and 156, respectively;

a CDR-H1, CDR-H2, and CDR-H3 comprising the amino acid sequence of SEQ ID NOs:2, 5, and 157, respectively;

a CDR-H1, CDR-H2, and CDR-H3 comprising the amino acid sequence of SEQ ID NOs:2, 6, and 376, respectively;

a CDR-H1, CDR-H2, and CDR-H3 comprising the amino acid sequence of SEQ ID NOs:3, 6, and 155, respectively;

a CDR-H1, CDR-H2, and CDR-H3 comprising the amino acid sequence of SEQ ID NOs:3, 372, and 376, respectively;

a CDR-H1, CDR-H2, and CDR-H3 comprising the amino acid sequence of SEQ ID NOs:3, 6, and 376, respectively;

a CDR-H1, CDR-H2, and CDR-H3 comprising the amino acid sequence of SEQ ID NOs:593, 594, and 595, respectively;

a CDR-H1, CDR-H2, and CDR-H3 comprising the amino acid sequence of SEQ ID NOs:611, 612, and 613, respectively;

a CDR-H1, CDR-H2, and CDR-H3 comprising the amino acid sequence of SEQ ID NOs:507, 513, and 517, respectively;

a CDR-H1, CDR-H2, and CDR-H3 comprising the amino acid sequence of SEQ ID NOs:604, 605, and 606, respectively;

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

a CDR-H1, CDR-H2, and CDR-H3 comprising the amino acid sequence of SEQ ID NOs:289, 291, and 293, respectively.

39. The polynucleotide of any of embodiments 30-38, wherein the V_H region is or comprises 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.

40. The polynucleotide of any of embodiments 30-39, wherein the V_H region is or comprises 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.

41. The polynucleotide of any of embodiments 30-40, 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; or

the V_H region comprises a CDR-H1, CDR-H2, and CDR-H3 comprising the amino acid sequence of SEQ ID NOs:611, 612, and 613, respectively.

42. The polynucleotide of any of embodiments 30-41, wherein the V_H region is or comprises the amino acid sequence set forth in SEQ ID NO:617.

43. The polynucleotide of any one of embodiments 30-42, wherein the V_L region comprises a CDR-L1, CDR-L2, and CDR-L3 selected from:

a CDR-L1, CDR-L2, and CDR-L3 comprising the amino acid sequence of SEQ ID NOs:26, 37, and 47, respectively;

a CDR-L1, CDR-L2, and CDR-L3 comprising the amino acid sequence of SEQ ID NOs:27, 38, and 48, respectively;

a CDR-L1, CDR-L2, and CDR-L3 comprising the amino acid sequence of SEQ ID NOs:28, 39, and 49, respectively;

a CDR-L1, CDR-L2, and CDR-L3 comprising the amino acid sequence of SEQ ID NOs:29, 40, and 50, respectively;

a CDR-L1, CDR-L2, and CDR-L3 comprising the amino acid sequence of SEQ ID NOs:30, 39, and 51, respectively;

a CDR-L1, CDR-L2, and CDR-L3 comprising the amino acid sequence of SEQ ID NOs:31, 41, and 52, respectively;

a CDR-L1, CDR-L2, and CDR-L3 comprising the amino acid sequence of SEQ ID NOs:32, 42, and 53, respectively;

a CDR-L1, CDR-L2, and CDR-L3 comprising the amino acid sequence of SEQ ID NOs:30, 39, and 54, respectively;

a CDR-L1, CDR-L2, and CDR-L3 comprising the amino acid sequence of SEQ ID NOs:33, 43, and 55, respectively;

a CDR-L1, CDR-L2, and CDR-L3 comprising the amino acid sequence of SEQ ID NOs:34, 44, and 56, respectively;

a CDR-L1, CDR-L2, and CDR-L3 comprising the amino acid sequence of SEQ ID NOs:35, 45, and 57, respectively;

a CDR-L1, CDR-L2, and CDR-L3 comprising the amino acid sequence of SEQ ID NOs:36, 46, and 58, respectively;

a CDR-L1, CDR-L2, and CDR-L3 comprising the amino acid sequence of SEQ ID NOs:174, 179, and 184, respectively;

a CDR-L1, CDR-L2, and CDR-L3 comprising the amino acid sequence of SEQ ID NOs:174, 179, and 185, respectively;

a CDR-L1, CDR-L2, and CDR-L3 comprising the amino acid sequence of SEQ ID NOs:174, 179, and 186, respectively;

a CDR-L1, CDR-L2, and CDR-L3 comprising the amino acid sequence of SEQ ID NOs:174, 179, and 187, respectively;

a CDR-L1, CDR-L2, and CDR-L3 comprising the amino acid sequence of SEQ ID NOs:175, 180, and 188, respectively;

a CDR-L1, CDR-L2, and CDR-L3 comprising the amino acid sequence of SEQ ID NOs:174, 179, and 189, respectively;

a CDR-L1, CDR-L2, and CDR-L3 comprising the amino acid sequence of SEQ ID NOs:176, 181, and 190, respectively;

a CDR-L1, CDR-L2, and CDR-L3 comprising the amino acid sequence of SEQ ID NOs:177, 182, and 191, respectively;

a CDR-L1, CDR-L2, and CDR-L3 comprising the amino acid sequence of SEQ ID NOs:174, 179, and 192, respectively;

a CDR-L1, CDR-L2, and CDR-L3 comprising the amino acid sequence of SEQ ID NOs:178, 183, and 193, respectively;

a CDR-L1, CDR-L2, and CDR-L3 comprising the amino acid sequence of SEQ ID NOs:178, 183, and 194, respectively;

a CDR-L1, CDR-L2, and CDR-L3 comprising the amino acid sequence of SEQ ID NOs:30, 399, and 415, respectively;

a CDR-L1, CDR-L2, and CDR-L3 comprising the amino acid sequence of SEQ ID NOs:380, 400, and 416, respectively;

a CDR-L1, CDR-L2, and CDR-L3 comprising the amino acid sequence of SEQ ID NOs:33, 43, and 421, respectively;

a CDR-L1, CDR-L2, and CDR-L3 comprising the amino acid sequence of SEQ ID NOs:381, 401, and 417, respectively;

a CDR-L1, CDR-L2, and CDR-L3 comprising the amino acid sequence of SEQ ID NOs:382, 402, and 418, respectively;

a CDR-L1, CDR-L2, and CDR-L3 comprising the amino acid sequence of SEQ ID NOs:383, 403, and 419, respectively;

a CDR-L1, CDR-L2, and CDR-L3 comprising the amino acid sequence of SEQ ID NOs:384, 39, and 54, respectively;

a CDR-L1, CDR-L2, and CDR-L3 comprising the amino acid sequence of SEQ ID NOs:385, 180, and 58, respectively;

a CDR-L1, CDR-L2, and CDR-L3 comprising the amino acid sequence of SEQ ID NOs:175, 180, and 188, respectively;

a CDR-L1, CDR-L2, and CDR-L3 comprising the amino acid sequence of SEQ ID NOs:386, 404, and 420, respectively;

a CDR-L1, CDR-L2, and CDR-L3 comprising the amino acid sequence of SEQ ID NOs:387, 405, and 422, respectively;

a CDR-L1, CDR-L2, and CDR-L3 comprising the amino acid sequence of SEQ ID NOs:388, 406, and 423, respectively;

a CDR-L1, CDR-L2, and CDR-L3 comprising the amino acid sequence of SEQ ID NOs:388, 407, and 424, respectively;

a CDR-L1, CDR-L2, and CDR-L3 comprising the amino acid sequence of SEQ ID NOs:389, 408, and 425, respectively;

a CDR-L1, CDR-L2, and CDR-L3 comprising the amino acid sequence of SEQ ID NOs:390, 183, and 193, respectively;

a CDR-L1, CDR-L2, and CDR-L3 comprising the amino acid sequence of SEQ ID NOs:391, 409, and 426, respectively;

a CDR-L1, CDR-L2, and CDR-L3 comprising the amino acid sequence of SEQ ID NOs:392, 40, and 427, respectively;

a CDR-L1, CDR-L2, and CDR-L3 comprising the amino acid sequence of SEQ ID NOs:394, 39, and 429, respectively;

a CDR-L1, CDR-L2, and CDR-L3 comprising the amino acid sequence of SEQ ID NOs:395, 411, and 430, respectively;

a CDR-L1, CDR-L2, and CDR-L3 comprising the amino acid sequence of SEQ ID NOs:396, 412, and 431, respectively;

a CDR-L1, CDR-L2, and CDR-L3 comprising the amino acid sequence of SEQ ID NOs:396, 412, and 58, respectively;

a CDR-L1, CDR-L2, and CDR-L3 comprising the amino acid sequence of SEQ ID NOs:397, 413, and 432, respectively;

a CDR-L1, CDR-L2, and CDR-L3 comprising the amino acid sequence of SEQ ID NOs:398, 414, and 433, respectively;

a CDR-L1, CDR-L2, and CDR-L3 comprising the amino acid sequence of SEQ ID NOs:601, 602, and 603, respectively;

a CDR-L1, CDR-L2, and CDR-L3 comprising the amino acid sequence of SEQ ID NOs:614, 615, and 603, respectively;

a CDR-L1, CDR-L2, and CDR-L3 comprising the amino acid sequence of SEQ ID NOs:589, 590, and 591, respectively;

a CDR-L1, CDR-L2, and CDR-L3 comprising the amino acid sequence of SEQ ID NOs:607, 608, and 591, respectively;

a CDR-L1, CDR-L2, and CDR-L3 comprising the amino acid sequence of SEQ ID NOs: 302, 304, and 306, respectively; or

a CDR-L1, CDR-L2, and CDR-L3 comprising the amino acid sequence of SEQ ID NOs:303, 305, and 307, respectively.

44. The polynucleotide of any one of embodiments 30-43, wherein the V_L region comprises a CDR-L1, CDR-L2, and CDR-L3 selected from:

a CDR-L1, CDR-L2, and CDR-L3 comprising the amino acid sequence of SEQ ID NOs:26, 37, and 47, respectively;

a CDR-L1, CDR-L2, and CDR-L3 comprising the amino acid sequence of SEQ ID NOs:27, 38, and 48, respectively;

a CDR-L1, CDR-L2, and CDR-L3 comprising the amino acid sequence of SEQ ID NOs:28, 39, and 49, respectively;

a CDR-L1, CDR-L2, and CDR-L3 comprising the amino acid sequence of SEQ ID NOs:30, 39, and 51, respectively;

a CDR-L1, CDR-L2, and CDR-L3 comprising the amino acid sequence of SEQ ID NOs:31, 41, and 52, respectively;

a CDR-L1, CDR-L2, and CDR-L3 comprising the amino acid sequence of SEQ ID NOs:33, 43, and 55, respectively;

a CDR-L1, CDR-L2, and CDR-L3 comprising the amino acid sequence of SEQ ID NOs:34, 44, and 56, respectively;

a CDR-L1, CDR-L2, and CDR-L3 comprising the amino acid sequence of SEQ ID NOs:174, 179, and 185, respectively;

a CDR-L1, CDR-L2, and CDR-L3 comprising the amino acid sequence of SEQ ID NOs:174, 179, and 189, respectively;

a CDR-L1, CDR-L2, and CDR-L3 comprising the amino acid sequence of SEQ ID NOs:176, 181, and 190, respectively;

a CDR-L1, CDR-L2, and CDR-L3 comprising the amino acid sequence of SEQ ID NOs:177, 182, and 191, respectively;

a CDR-L1, CDR-L2, and CDR-L3 comprising the amino acid sequence of SEQ ID NOs:174, 179, and 192, respectively;

a CDR-L1, CDR-L2, and CDR-L3 comprising the amino acid sequence of SEQ ID NOs:178, 183, and 193, respectively;

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JUMBO APPLICATIONS/PATENTS

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CLAIMS

WHAT IS CLAIMED:

1. 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.

2. The chimeric antigen receptor of claim 1, wherein the spacer comprises a portion of an immunoglobulin constant region.

3. The chimeric antigen receptor of claim 1 or claim 2, wherein the spacer comprises a sequence of a hinge region, a C_H2 region and a C_H3 region.

4. The chimeric antigen receptor of claim 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 chimeric antigen receptor of claim 3or claim 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 chimeric antigen receptor of claim 3 or claim 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 chimeric antigen receptor 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 chimeric antigen receptor of any of claims 1-4, 6 and 7, 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.

9. The chimeric antigen receptor of any of claims 1-3 and 6-8, wherein the spacer is or comprises the sequence set forth in SEQ ID NO: 649.

10. A chimeric antigen receptor comprising: (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.

11. The chimeric antigen receptor of any of claims 1-10, wherein the antigen-binding domain is an antibody fragment comprising a variable heavy chain (V_H) and a variable light chain (V_L) region.

12. The chimeric antigen receptor of claim 11, wherein:

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 amino acid sequence set forth in any of SEQ ID NOs: 617, 115, 256, 519, or 609; 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 amino acid sequence set forth in any of SEQ ID NOs: 618, 267, 535, 536, or 610.

13. The chimeric antigen receptor of claim 11 or claim 12, 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.

14. The chimeric antigen receptor of any of claims 11-13, 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.

15. The chimeric antigen receptor of any of claims 11-13, 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.

16. The chimeric antigen receptor of any of claims 11-15, 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.

17. The chimeric antigen receptor of any of claims 11-13 and 15, wherein:

the V_H region comprises (a) a CDR-H1 comprising the amino acid sequence selected from any one of SEQ ID NOs:1, 2, 507 or 593; (b) a CDR-H2 comprising the amino acid sequence selected from any one of SEQ ID NOs: 4, 5, 513 or 594; and (c) a CDR-H3 comprising the amino acid sequence selected from any one of SEQ ID NOs:7, 10, 157, 517 or 595; and

the V_L region comprises (a) a CDR-L1 comprising the amino acid sequence selected from any one of SEQ ID NOs:33, 178, 380, 589 or 601; (b) a CDR-L2 comprising the amino acid sequence selected from any one of SEQ ID NOs: 43, 183, 400, 590 or 602; and (c) a CDR-L3 comprising the amino acid sequence selected from any one of SEQ ID NOs:194, 416, 421, 591 or 603.

18. The chimeric antigen receptor of any of claims 11-13, 15 and 17, 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;

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;

the V_H region comprises a CDR-H1, CDR-H2, and CDR-H3 comprising the amino acid 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 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:598, 599, 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; or

the V_H region comprises a CDR-H1, CDR-H2, and CDR-H3 comprising the amino acid 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 amino acid sequence of SEQ ID NOS:614, 615, and 603, respectively.

19. The chimeric antigen receptor of any of claims 11-18, 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.

20. The chimeric antigen receptor of any of claims 11-13, 15, 17 and 18, wherein the V_H region is or comprises the amino acid sequence set forth in any of SEQ ID NOs: 617, 115,

256, 519, or 609; and the V_L region is or comprises the amino acid sequence set forth in any of SEQ ID NOs: 618, 267, 535, 536, or 610.

21. The chimeric antigen receptor of any of claims 11-13, 15, 17, 18 and 20, 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.

22. The chimeric antigen receptor of any of claims 11-21, 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; or the V_H region comprises a CDR-H1, CDR-H2, and CDR-H3 comprising the amino acid 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 amino acid sequence of SEQ ID NOs:601, 602, and 603, respectively; or the V_L region comprises a CDR-L1, CDR-L2, and CDR-L3 comprising the amino acid sequence of SEQ ID NOs:614, 615, and 603, respectively.

23. The chimeric antigen receptor of any of claims 11-22, 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.

24. The chimeric antigen receptor of any of claims 11-23, wherein the fragment comprises an scFv.

25. The chimeric antigen receptor of any of claims 11-24, when the V_H region and the V_L region are joined by a flexible linker.

26. The chimeric antigen receptor of claim 25, wherein the scFv comprises a linker comprising the amino acid sequence GGGGSGGGGSGGGGS (SEQ ID NO:361).

27. The chimeric antigen receptor of any of claims 11-26, wherein the V_H region is amino-terminal to the V_L region.

28. The chimeric antigen receptor of any of claims 11-27, 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.

29. The chimeric antigen receptor of any of claims 1-28, 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.

30. The chimeric antigen receptor of any of claims 1-29, 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.

31. The chimeric antigen receptor of any of claims 1-30, wherein the antigen-binding domain comprises the amino acid sequence set forth in SEQ ID NO: 478.

32. The chimeric antigen receptor of any of claims 1-31, wherein a nucleic acid 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).

33. The chimeric antigen receptor of any of claims 1-32, wherein the nucleic acid encoding the antigen-binding domain comprises the sequence of nucleotides set forth in any of SEQ ID NO: 460, 440, 715, 717 or 719.

34. The chimeric antigen receptor of any of claims 1-33, wherein the nucleic acid encoding the antigen-binding domain comprises the sequence of nucleotides set forth in SEQ ID NO:460.

35. The chimeric antigen receptor of any of claims 11-26, wherein the V_H region is carboxy-terminal to the V_L region.

36. 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 (CD3ζ) chain and an intracellular signaling domain of a T cell costimulatory molecule.

37. The chimeric antigen receptor of claim 36, 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;

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:596, 597, 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:598, 599, 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; or

the V_H region comprises a CDR-H1, CDR-H2, and CDR-H3 comprising the amino acid 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 amino acid sequence of SEQ ID NOS:614, 615, and 603, respectively.

38. 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:

a variable heavy (V_H) region comprising 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 a variable light (V_L) region comprising 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

a V_H region comprising a CDR-H1, CDR-H2, and CDR-H3 comprising the amino acid sequence of SEQ ID NOS:593, 594, and 595, respectively, and a V_L region comprising a CDR-

L1, CDR-L2, and CDR-L3 comprising the amino acid sequence of SEQ ID NOS:601, 602, and 603, respectively;

a V_H region comprising a CDR-H1, CDR-H2, and CDR-H3 comprising the amino acid sequence of SEQ ID NOS:596, 597, and 595, respectively, and a V_L region comprising a CDR-L1, CDR-L2, and CDR-L3 comprising the amino acid sequence of SEQ ID NOS:601, 602, and 603, respectively;

a V_H region comprising a CDR-H1, CDR-H2, and CDR-H3 comprising the amino acid sequence of SEQ ID NOS:598, 599, and 595, respectively, and a V_L region comprising a CDR-L1, CDR-L2, and CDR-L3 comprising the amino acid sequence of SEQ ID NOS:601, 602, and 603, respectively; or

a V_H region comprising a CDR-H1, CDR-H2, and CDR-H3 comprising the amino acid sequence of SEQ ID NOS:611, 612, and 613, respectively, and a V_L region comprising a CDR-L1, CDR-L2, and CDR-L3 comprising the amino acid sequence of SEQ ID NOS:614, 615, and 603, respectively;

(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.

39. The chimeric antigen receptor of any of claims 36-38, 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.

40. The chimeric antigen receptor of any of claims 1-35, wherein the intracellular signaling region comprises an activating cytoplasmic signaling domain.

41. The chimeric antigen receptor of claim 40, wherein 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 comprises an immunoreceptor tyrosine-based activation motif (ITAM).

42. The chimeric antigen receptor of claim 40 or claim 41, 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.

43. The chimeric antigen receptor of any of claims 40-42, wherein the activating cytoplasmic domain is human or is from a human protein.

44. The chimeric antigen receptor of any of claims 36-39, 42 and 43, wherein thecytoplasmic signaling domain is or comprises 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.

45. The chimeric antigen receptor of any of claims 40-44, wherein the intracellular signaling region further comprises a costimulatory signaling region.

46. The chimeric antigen receptor of claim 45, wherein the costimulatory signaling region comprises an intracellular signaling domain of a T cell costimulatory molecule or a signaling portion thereof.

47. The chimeric antigen receptor of any of claims 36-39, 45 and 46, wherein the costimulatory signaling region comprises an intracellular signaling domain of a CD28, a 4-1BB or an ICOS or a signaling portion thereof.

48. The chimeric antigen receptor of any of claims 36-39 and 45-47, wherein the costimulatory signaling region comprises an intracellular signaling domain of 4-1BB.

49. The chimeric antigen receptor of any of claims 36-39 and 45-48, wherein the costimulatory signaling region is human or is from a human protein.

50. The chimeric antigen receptor of any of claims 36-39 and 45-49, wherein the costimulatory signaling region is or comprises 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.

51. The chimeric antigen receptor of any of claims 36-50, wherein the costimulatory signaling region is between the transmembrane domain and the intracellular signaling region.

52. The chimeric antigen receptor of any of claims 1-51, wherein the transmembrane domain is or comprises a transmembrane domain from CD4, CD28, or CD8.

53. The chimeric antigen receptor of claim 52, wherein the transmembrane domain is or comprises a transmembrane domain from a CD28.

54. The chimeric antigen receptor of any of claims 1-53, wherein the transmembrane domain is human or is from a human protein.

55. The chimeric antigen receptor of any of claims 1-54, wherein the transmembrane domain is or comprises 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.

56. The chimeric antigen receptor of any of claims 1-55, wherein the chimeric antigen receptor comprises from its N to C terminus in order: the antigen-binding domain, the spacer, the transmembrane domain and the intracellular signaling domain.

57. The chimeric antigen receptor of any of claims 1-56, wherein (a) the ability ofthe 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.

58. The chimeric antigen receptor of claim 57, wherein the concentration or amount of the soluble or shed form of the BCMA:

is a concentration or amount present in serum or blood or plasma of the subject or of a multiple myeloma patient, or an average concentration or amount present in serum, blood or plasma of patients within a patient population having multiple myeloma or a subtype or subpopulation thereof, or

is a concentration or amount at which the binding or measure is reduced or blocked, or is substantially reduced or blocked, for a reference anti-BCMA recombinant receptor, optionally a reference anti-BCMA CAR, under the same or substantially the same conditions.

59. The chimeric antigen receptor of any of claims 1-58, wherein the chimeric antigen receptor is encoded by a polynucleotide sequence comprising the sequence set forth in any of SEQ ID NOS: 751-756 or by 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.

60. The chimeric antigen receptor of any of claims 1-59, wherein 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 by 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.

61. The chimeric antigen receptor of any of claims 1-60, wherein the chimeric antigen receptor is encoded by a polynucleotide sequence comprising the sequence set forth in SEQ ID NO: 755 or by 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.

62. The chimeric antigen receptor of any of claims 1-61, wherein the chimeric antigen receptor is encoded by a polynucleotide sequence comprising the sequence set forth in SEQ ID NO: 755.

63. A polynucleotide encoding the chimeric antigen receptor of any of claims 1-62.

64. The polynucleotide of claim 63, wherein following expression of the polynucleotide in a human cell, optionally a human T cell, the RNA, optionally the messenger RNA (mRNA), from the polynucleotide, exhibits at least 70%, 75%, 80%, 85%, 90%, or 95% RNA homogeneity.

65. The polynucleotide of claim 63 or claim 64, wherein the encoded chimeric antigen receptor comprises 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 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.

66. A polynucleotide encoding a chimeric antigen receptor, comprising 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 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.

67. The polynucleotide of any of claims 63-66, wherein the encoded spacer comprises a portion of an immunoglobulin.

68. The polynucleotide of any of claims 63-67, wherein the encoded spacer comprises a sequence of a hinge region, a C_H2 region and a C_H3 region.

69. The polynucleotide of any of claims 63, 64 and 66-68, 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.

70. The polynucleotide of any of claims 63, 64 and 66-69, 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.

71. The polynucleotide of any of claims 63, 64 and 66-69, 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

72. The polynucleotide of claim any of claims 63, 64 and 66-71, 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.

73. The polynucleotide of any of claims 63, 64 and 66-72, 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.

74. The polynucleotide of any of claims 63-73, wherein the encoded spacer is or comprises the sequence set forth in SEQ ID NO: 649.

75. The polynucleotide of any of claims 63-74, 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.

76. The polynucleotide of claim 75, wherein the one or more nucleotide modifications comprise a nucleotide substitution.

77. The polynucleotide of claim 75 or claim 76, wherein the reference splice donor and/or reference splice acceptor sites are canonical, non-canonical, or cryptic splice sites.

78. The polynucleotide of any of claim 75-77, 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; 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%.

79. The polynucleotide of any of claims 75-78, wherein:

the reference splice donor site comprises 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 comprises the sequence aagtttctttctgtattccaggctgaccgtggataaatctc (SEQ ID NO:742) and/or gggcaacgtgttctcttgcagtgtcatgcacgaagccctgc (SEQ ID NO:743).

80. The polynucleotide of any of claims 75-78, wherein:

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%.

81. The polynucleotide of any of claims 75-78 and 80, wherein:

the reference splice donor site comprises the sequence tcaactggtacgtgg (SEQ ID NO:706); and/or

the reference splice acceptor site comprises the sequence aagtttctttctgtattccaggctgaccgtggataaatctc (SEQ ID NO:742).

82. The polynucleotide of any of claims 75-81, wherein 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.

83. The polynucleotide of any of claims 75-82, 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.

84. The polynucleotide of any of claims 75-83, 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 cagtttcttcctgtatagtagactcaccgtggataaatcaa (SEQ ID NO:672), gggcaacgtgttcagctgcagcgtgatgcacgaggccctgc (SEQ ID NO: 673) and/or cgccttgtcctccttgtcccgctcctcctgttgccggacct (SEQ ID NO:766).

85. The polynucleotide of any of claims 75-84, wherein the modified splice donor site is set forth in tcaactggtatgtgg (SEQ ID NO:662) and/or the modified acceptor site is set forth in cagtttcttcctgtatagtagactcaccgtggataaatcaa (SEQ ID NO:672) and/or cgccttgtcctccttgtcccgctcctcctgttgccggacct (SEQ ID NO:766).

86. The polynucleotide of any of claims 63-85, wherein the spacer is encoded bythe nucleotide sequence set forth in SEQ ID NO:622 or a portion thereof.

87. A polynucleotide encoding a chimeric antigen receptor, wherein the polynucleotide comprises nucleic acid encoding: (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.

88. A polynucleotide encoding a chimeric antigen receptor, wherein the polynucleotide comprises nucleic acid encoding: (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.

89. The polynucleotide of claim 87 or claim 88, 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.

90. The polynucleotide of any of claims 63-89, 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.

91. The polynucleotide of claim 90, wherein the RNA heterogeneity is reduced by greater than or greater than about 10%, 15%, 20%, 25%, 30%, 40%, 50% or more.

92. The polynucleotide of claim 90 or claim 91, wherein 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.

93. The polynucleotide of any of claims 63-92, wherein the RNA homogeneity and/or heterogeneity is determined by agarose gel electrophoresis, chip-based capillary electrophoresis, analytical ultracentrifugation, field flow fractionation, or liquid chromatography.

94. The polynucleotide of any of claims 63-93, wherein the polynucleotide is codon-optimized for expression in a human cell.

95. The polynucleotide of any of claims 63-94, 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.

96. The polynucleotide of any of claims 63-95, wherein the disease or condition is a cancer.

97. The polynucleotide of any of claims 63-96, wherein the disease or condition is a myeloma, leukemia or lymphoma.

98. The polynucleotide of any of claims 63-97, 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

(GPRC5D), 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 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-9, O-acetylated GD2 (OGD2), CE7, Wilms Tumor 1 (WT-1), a cyclin, cyclin A2, CCL-1, CD138, a pathogen-specific antigen.

99. The polynucleotide of claim 98, wherein the antigen is B cell maturation antigen (BCMA).

100. The polynucleotide of any of claims 63-99, 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.

101. The polynucleotide of claim 100, wherein:

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 amino acid sequence set forth in any of SEQ ID NOs: 617, 115, 256, 519, or 609; 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 amino acid sequence set forth in any of SEQ ID NOs: 618, 267, 535, 536, or 610.

102. The polynucleotide of claim 100 or claim 101, 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.

103. The polynucleotide of any of claims 100-102, 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.

104. The polynucleotide of any of claims 100-102, 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.

105. The polynucleotide of any of claims 100-104, 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.

106. The polynucleotide of any of claims 100-102 and 104, wherein:

the V_H region comprises (a) a CDR-H1 comprising the amino acid sequence selected from any one of SEQ ID NOs:1, 2, 507 or 593; (b) a CDR-H2 comprising the amino acid sequence selected from any one of SEQ ID NOs: 4, 5, 513 or 594; and (c) a CDR-H3 comprising the amino acid sequence selected from any one of SEQ ID NOs:7, 10, 157, 517 or 595; and

the V_L region comprises (a) a CDR-L1 comprising the amino acid sequence selected from any one of SEQ ID NOs:33, 178, 380, 589 or 601; (b) a CDR-L2 comprising the amino acid sequence selected from any one of SEQ ID NOs: 43, 183, 400, 590 or 602; and (c) a CDR-L3 comprising the amino acid sequence selected from any one of SEQ ID NOs:194, 416, 421, 591 or 603.

107. The polynucleotide of any of claims 100-102, 104 and 106, 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;

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;

the V_H region comprises a CDR-H1, CDR-H2, and CDR-H3 comprising the amino acid 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 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:598, 599, 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; or

the V_H region comprises a CDR-H1, CDR-H2, and CDR-H3 comprising the amino acid 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 amino acid sequence of SEQ ID NOS:614, 615, and 603, respectively.

108. The polynucleotide of any of claims 100-107, 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.

109. The polynucleotide of any of claims 100-102, 104, 106 and 107, wherein the V_H region is or comprises the amino acid sequence set forth in any of SEQ ID NOs: 617, 115, 256, 519, or 609; and the V_L region is or comprises the amino acid sequence set forth in any of SEQ ID NOs: 618, 267, 535, 536, or 610.

110. The polynucleotide of any of claims 100-102, 104, 106, 107 and 109, 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.

111. The polynucleotide of any of claims 100-110, 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; or the V_H region comprises a CDR-H1, CDR-H2, and CDR-H3 comprising the amino acid 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 amino acid sequence of SEQ ID NOs:601, 602, and 603, respectively; or the V_L region comprises a CDR-L1, CDR-L2, and CDR-L3 comprising the amino acid sequence of SEQ ID NOs:614, 615, and 603, respectively.

112. The polynucleotide of any of claims 110-111, 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.

113. The polynucleotide of any of claims 100-112, wherein the fragment comprises an scFv.

114. The polynucleotide of any of claims 100-113, when the V_H region and the V_L region are joined by a flexible linker.

115. The polynucleotide of claim 114, wherein the scFv comprises a linker comprising the amino acid sequence GGGGSGGGGSGGGGS (SEQ ID NO:361).

116. The polynucleotide of any of claims 100-115, wherein the V_H region is amino-terminal to the V_L region.

117. The polynucleotide of any of claims 100-116, 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.

118. The polynucleotide of any of claims 100-117, 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.

119. The polynucleotide of any of claims 100-118, wherein the antigen-binding domain comprises the amino acid sequence set forth in SEQ ID NO: 478.

120. The polynucleotide of any of claims 100-119, wherein the nucleic acid 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).

121. The polynucleotide of any of claims 100-120, wherein the nucleic acid encoding the antigen-binding domain comprises the sequence of nucleotides set forth in any of SEQ ID NO: 460, 440, 715, 717 or 719.

122. The polynucleotide of any of claims 100-121, wherein the nucleic acid encoding the antigen-binding domain comprises the sequence of nucleotides set forth in SEQ ID NO:460.

123. The polynucleotide of any of claims 100-116, wherein the V_H region is carboxy-terminal to the V_L region.

124. 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.

125. The polynucleotide of claim 124, 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;

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:596, 597, 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:598, 599, 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; or

the V_H region comprises a CDR-H1, CDR-H2, and CDR-H3 comprising the amino acid 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 amino acid sequence of SEQ ID NOS:614, 615, and 603, respectively.

126. 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:

a variable heavy (V_H) region comprising 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 a variable light (V_L) region comprising 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

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

a V_H region comprising a CDR-H1, CDR-H2, and CDR-H3 comprising the amino acid sequence of SEQ ID NOS:596, 597, and 595, respectively, and a V_L region comprising a CDR-L1, CDR-L2, and CDR-L3 comprising the amino acid sequence of SEQ ID NOS:601, 602, and 603, respectively;

a V_H region comprising a CDR-H1, CDR-H2, and CDR-H3 comprising the amino acid sequence of SEQ ID NOS:598, 599, and 595, respectively, and a V_L region comprising a CDR-L1, CDR-L2, and CDR-L3 comprising the amino acid sequence of SEQ ID NOS:601, 602, and 603, respectively; or

a V_H region comprising a CDR-H1, CDR-H2, and CDR-H3 comprising the amino acid sequence of SEQ ID NOS:611, 612, and 613, respectively, and a V_L region comprising a CDR-

L1, CDR-L2, and CDR-L3 comprising the amino acid sequence of SEQ ID NOS:614, 615, and 603, respectively;

(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.

127. The polynucleotide of any of claims 124-126, 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.

128. The polynucleotide of any of claims 63-123, wherein the intracellular signaling region comprises an activating cytoplasmic signaling domain.

129. The polynucleotide of claim 128, wherein 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 comprises an immunoreceptor tyrosine-based activation motif (ITAM).

130. The polynucleotide of claim 128 or claim 129, 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.

131. The polynucleotide of any of claims 128-130, wherein the activating cytoplasmic domain is human or is from a human protein.

132. The polynucleotide of any of claims 124-127, 130 and 131, wherein the cytoplasmic signaling domain is or comprises 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.

133. The polynucleotide of any of claims 124-127 and 130-132, wherein the nucleic acid encoding the cytoplasmic signaling domain is or comprises the sequence set forth in SEQ ID NO:627 or is a codon-optimized sequence and/or degenerate sequence thereof.

134. The polynucleotide of any of claims 124-127 and 130-133, wherein the nucleic acid encoding the cytoplasmic signaling domain is or comprises the sequence set forth in SEQ ID NO:652.

135. The polynucleotide of any of claims 128-134, wherein the intracellular signaling region further comprises a costimulatory signaling region.

136. The polynucleotide of claim 135, wherein the costimulatory signaling region comprises an intracellular signaling domain of a T cell costimulatory molecule or a signaling portion thereof.

137. The polynucleotide of claim 124-127, 135 and 136, wherein the costimulatory signaling region comprises an intracellular signaling domain of a CD28, a 4-1BB or an ICOS or a signaling portion thereof.

138. The polynucleotide of any of claims 124-127 and 135-137, wherein the costimulatory signaling region comprises an intracellular signaling domain of 4-1BB.

139. The polynucleotide of any of claims 124-127 and 135-138, wherein the costimulatory signaling region is human or is from a human protein.

140. The polynucleotide of any of claims 124-127 and 135-139, wherein the costimulatory signaling region is or comprises 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.

141. The polynucleotide of any of claims 124-127 and 135-140, wherein the nucleic acid encoding the costimulatory region is or comprises the sequence set forth in SEQ ID NO:625 or is a codon-optimized sequence and/or degenerate sequence thereof.

142. The polynucleotide of any of claims 124-127 and 135-141, wherein the nucleic acid encoding the costimulatory signaling region comprises the sequence set forth in SEQ ID NO:681.

143. The polynucleotide of any of claims 63-139, wherein the intracellular signaling region comprises the sequence set forth in SEQ ID NO:628 or a sequence of amino acids that has at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to SEQ ID NO:628 and the sequence set forth in SEQ ID NO:626 or a sequence of amino acids that exhibits at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to the sequence set forth in SEQ ID NO: 626.

144. The polynucleotide of any of claims 63-139 and 143, wherein the intracellular signaling region is or comprises the sequences set forth in SEQ ID NO:628 and SEQ ID NO:626.

145. The polynucleotide of any of claims 124-127, 135-137, 139 and 144, wherein the costimulatory signaling region comprises an intracellular signaling domain of CD28.

146. The polynucleotide of any of claims 63-139 and 145, wherein the intracellular signaling region comprises the sequence set forth in SEQ ID NO:628 or a sequence of amino acids that has at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to SEQ ID NO:628 and the sequence set forth in SEQ ID NO:680 or a sequence of amino acids that exhibits at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to the sequence set forth in SEQ ID NO: 680.

147. The polynucleotide of any of claims 63-139, 145 and 146, wherein the intracellular signaling region is or comprises the sequences set forth in SEQ ID NO:628 and SEQ ID NO:680.

148. The polynucleotide of any of claims 135-147, wherein the costimulatory signaling region is between the transmembrane domain and the intracellular signaling region.

149. The polynucleotide of any of claims 63-148, wherein the transmembrane domain is or comprises a transmembrane domain from CD4, CD28, or CD8.

150. The polynucleotide of claim 149, wherein the transmembrane domain is or comprises a transmembrane domain from a CD28.

151. The polynucleotide of any of claims 63-150, wherein the transmembrane domain is human or is from a human protein.

152. The polynucleotide of any of claims 63-151, wherein the transmembrane domain is or comprises 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.

153. The polynucleotide of any of claims 63-152, wherein the nucleic acid encoding the transmembrane domain is or comprises the sequence set forth in SEQ ID NO:623 or is a codon-optimized sequence and/or degenerate sequence thereof.

154. The polynucleotide of claim 153, wherein the nucleic acid encoding the transmembrane domain comprises the sequence set forth in SEQ ID NO:688.

155. The polynucleotide of any of claims 63-154, wherein the encoded chimeric antigen receptor comprises from its N to C terminus in order: the antigen-binding domain, the spacer, the transmembrane domain and the intracellular signaling region.

156. The polynucleotide of any of claims 63-155, wherein the polynucleotide further encodes a truncated receptor.

157. The polynucleotide of any of claims 63-156, 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.

158. The polynucleotide of claim 157, wherein the concentration or amount of the soluble or shed form of the BCMA corresponds to a concentration or amount present in serum or blood or plasma of the subject or of a multiple myeloma patient, or on average in a patient population for the disease or disorder, or at a concentration or amount of the soluble or shed BCMA at which the binding or measure is reduced or blocked, or is substantially reduced or blocked, for cells expressing a reference anti-BCMA recombinant receptor, optionally a reference anti-BCMA CAR, in the same assay.

159. The polynucleotide of any of claims 63-158, 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.

160. The polynucleotide of any of claims 63-159, wherein 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.

161. The polynucleotide of any of claims 63-160, wherein 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.

162. The polynucleotide of any of claims 63-161, 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.

163. A vector comprising the polynucleotide of any of claims 63-162.

164. The vector of claim 163, wherein the vector is a viral vector.

165. The vector of claim 164, wherein the viral vector is a retroviral vector.

166. The vector of claim 164 or claim 165, wherein the viral vector is a lentiviral vector.

167. A chimeric antigen receptor encoded by the polynucleotide of any of claims 63-162.

168. An engineered cell, comprising the chimeric antigen receptor of any of claims 1-62 and 167.

169. An engineered cell, comprising the polynucleotide of any of claims 63-162 or the vector of any of claims 163-166.

170. The engineered cell of claim 168 or claim 169, wherein the cell is an immune cell.

171. The engineered cell of any of claims 168-170- wherein the immune cell is a primary cell obtained from a subject.

172. The engineered cell of claim 170 or claim 171, wherein the immune cell is an NK cell or a T cell.

173. The engineered cell of any of claims 170-172, wherein the immune cell is a T cell and the T cell is a CD4+ and/or CD8+ T cell.

174. The engineered cell of any of claims 168-173, 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.

175. The engineered cell of any of claims 168-174, wherein the cell comprises 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 comprising the same amino acid sequence as the chimeric antigen receptor but encoded by a different polynucleotide sequence comprising 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 comprising one or more splice donor site and/or one or more splice acceptor site in the nucleic acid encoding the spacer.

176. The engineered cell of claim 175, wherein the RNA heterogeneity is reduced by greater than or greater than about 10%, 15%, 20%, 25%, 30%, 40%, 50% or more.

177. The engineered cell of claim 175 or claim 176, wherein the polynucleotide encoding the reference CAR comprises 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.

178. The engineered cell of any of claims 174-177, wherein the RNA homogeneity and/or heterogeneity is determined by agarose gel electrophoresis, chip-based capillary electrophoresis, analytical ultracentrifugation, field flow fractionation, or liquid chromatography.

179. The engineered cell of any of claims 168-178, wherein, 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 comprise a chimeric antigen receptor that exhibits tonic signaling and/or antigen independent activity or signaling.

180. A composition comprising the chimeric antigen receptor of any one of claims 1-62 and 167, the polynucleotide of any of claims 63-162, or the vector of any of claims 163-166 .

181. A composition comprising the engineered cell of any one of claims 168-179.

182. The composition of claim 181, 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.

183. The composition of any of claims 180-182, further comprising a pharmaceutically acceptable excipient.

184. A method of treatment, comprising administering the engineered cell of any of claims 168-179 or the composition of any of claims 180-183 to a subject having a disease or disorder.

185. The method of claim 184, wherein the method comprises administering a dose of the engineered cells or a composition comprising a dose of the engineered cells.

186. Use of the engineered cell of any of claims 168-179 or the composition of any of claims 180-183 for the manufacture of a medicament for the treatment of a disease or disorder.

187. Use of the engineered cells of any of claims 168-179 or the composition of any of claims 180-183 for treating a disease or disorder.

188. The use of claim 186 or claim 187, wherein 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.

189. The method of claim 184 or claim 185 or the use of any of claims 186-188, wherein the disease or disorder is associated with expression of B cell maturation antigen (BCMA), optionally a B cell-related disorder.

190. The method or the use of any of claims 184-189, wherein the disease or disorder associated with BCMA is an autoimmune disease or disorder.

191. The method or the use of any one of claims 185-190, wherein the disease or disorder associated with BCMA is a cancer.

192. The method or the use of claim 191, wherein the cancer is a BCMA-expressing cancer.

193. The method or the use of claim 191 or claim 192, wherein the cancer is a B cell malignancy.

194. The method or the use of any one of claims 191-193, wherein the cancer is a lymphoma, a leukemia, or a plasma cell malignancy.

195. The method or the use of claim 194, wherein 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).

196. The method or the use of claim 195, wherein the cancer is a leukemia and the leukemia is chronic lymphocytic leukemia (CLL), plasma cell leukemia or acute lymphocytic leukemia (ALL).

197. The method or the use of claim 194, wherein the cancer is a plasma cell malignancy and the plasma cell malignancy is multiple myeloma (MM) or plasmacytoma.

198. The method or the use of any of claims 191-194 and 197, wherein the cancer is multiple myeloma (MM).

199. The method or the use of any of claims 185 and 188-198, wherein the dose of engineered T cells comprises between at or about 1 x 10^7 CAR-expressing T cells and at or about 2 x 10^9 CAR-expressing T cells or between at or about .

200. The method or the use of any of claims 185 and 188-199, wherein the dose of engineered T cells comprise between at or about 2.5 x 10^7 CAR-expressing T cells and at or about 1.2 x 10^9 CAR-expressing T cells, between at or about 5.0 x 10^7 CAR-expressing T cells and at or about 4.5 x 10^8 CAR-expressing T cells, or between at or about 1.5 x 10^8 CAR-expressing T cells and at or about 3.0 x 10^8 CAR-expressing T cells.

201. The method or the use of any of claims 185 and 188-200, wherein the dose of engineered T cells comprise at or about 2.5 x 10^7, at or about 5.0 x 10^7, at or about 1.5 x 10^8, at or about 3.0 x 10^8, at or about 4.5 x 10^8, at or about 8.0 x 10^8 or at or about 1.2 x 10^9 CAR-expressing T cells.

202. The method or the use of any of claims 185 and 188-201-, wherein the dose of engineered T cells comprise at or about 5.0 x 10^7, at or about 1.5 x 10^8, at or about 3.0 x 10^8 or at or about 4.5 x 10^8 CAR-expressing T cells.

203. The method or the use of any of claims 185 and 188-202, wherein the dose of engineered T cells comprises a combination of $CD4^+$ T cells and $CD8^+$ T cells, at a ratio of $CD4^+$ CAR-expressing T cells to $CD8^+$ CAR-expressing T cells and/or of $CD4^+$ T cells to $CD8^+$ T cells, that is or is approximately 1:1 or is between approximately 1:3 and approximately 3:1.

204. The method or the use of any of claims 185 and 188-203, wherein 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.

205. The method or the use of any of claims 185 and 188-204 , wherein 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.

206. The method or the use of any of claims 184, 185, and 188-205, wherein prior to the administration, the subject has received a lymphodepleting therapy comprising the administration of fludarabine at or about 20-40 mg/m^2 body surface area of the subject, optionally at or about 30 mg/m^2, daily, for 2-4 days, and/or cyclophosphamide at or about 200-400 mg/m^2 body surface area of the subject, optionally at or about 300 mg/m^2, daily, for 2-4 days.

207. The method or the use of any of claims 184, 185, and 188-206 , wherein the subject has received a lymphodepleting therapy comprising the administration of fludarabine at or about 30 mg/m^2 body surface area of the subject, daily, and cyclophosphamide at or about 300 mg/m^2 body surface area of the subject, daily, for 3 days.

208. The method or the use of any of claims 184, 185, and 188-207 , wherein at or prior to the administration of the dose of cells, the subject has received three or more prior therapies for the disease or disorder, optionally four or more prior therapies, optionally selected from among:

autologous stem cell transplant (ASCT);
an immunomodulatory agent;
a proteasome inhibitor; and
an anti-CD38 antibody.

209. The method or the use of any of claim 208, wherein the immunomodulatory agent is selected from among thalidomide, lenalidomide and pomalidomide.

210. The method or the use of claim 208 or claim 209, wherein the proteasome inhibitor is selected from among bortezomib, carfilzomib and ixazomib.

211. The method or the use of any of claims 208-210, wherein the anti-CD38 antibody is or comprises daratumumab.

212. The method or the use of any of claims 184, 185, and 188-211, wherein at the time of the administration of the dose of cells, and/or at the time of lymphodepleting chemotherapy or leukapheresis, the subject has not had active or history of plasma cell leukemia (PCL).

213. The method or the use of any of claims 184, 185, and 188-212, wherein at the time of the administration of the dose of cells the subject has developed secondary plasma cell leukemia (PCL).

214. The method or use of any of claims 184, 185, and 188-213, wherein, at the time of administration, the subject:

has relapsed or been refractory following at least 3 or at least 4 prior therapies for multiple myeloma;

is an adult subject or is 25 or 35 years of age or older;

has a time from diagnosis of multiple myeloma of approximately 4 years or between 2 and 15 or 2 and 12 years;

has received about 10 or between 3 and 15 or between 4 and 15 prior regimens for multiple myeloma;

has been refractory to or not responded to bortezomib, carfilzomib, lenalidomide, pomalidomide and/or an anti-CD38 monoclonal antibody;

has had prior autologous stem cell transplant or has not had prior autologous stem cell transplant; and/or

has IMWG high risk cytogenetics.

215. The method or the use of any of claims 184-214, wherein the method is capable of achieving a specified response or outcome , optionally at a designated timepoint following

initiation of the administration, in at least one or in at least 10 %, at least 20 %, at least 30 %, at least 40 %, at least 50%, 60%, 70%, 80%, 90%, or 95% of subjects in a cohort of subjects having the disease or disorder of the subject, optionally wherein the cohort of subjects has at least the same number of prior therapies, prognosis or prognostic factor, sub-type, secondary involvement or other specified patient characteristic or characteristics, as the subject treated by the method, wherein:

the response is selected from the group consisting of objective response (OR), complete response (CR), stringent complete response (sCR), very good partial response (VGPR), partial response (PR) and minimal response (MR);

the response or outcome is or comprises an OR

the response or outcome is or comprises a CR.

216. The method or the use of claim 215, wherein the response or outcome is an OR and is achieved in at least 40 %, at least 50 %, at least 60 %, at least 70 %, or at least 80 % of subjects of the cohort.

217. The method or the use of claim 215, wherein the response or outcome is a CR or sCR and is achieved in at least 20 %, 30 %, or 40 % of subjects of the cohort.

218. The method or use of any of claims 215-217, wherein the dose of cells is less than 1.5 x 10^8 cells or less than 1.5 x 10^8 CAR+ T cells or less than 3 x 10^8 CAR+ T cells or less than 4.5 x 10^8 CAR+ T cells.

219. The method or use of any of claims 215-217, wherein the dose of cells is at or less than 1.5 x 10^8 cells or less than 1.5 x 10^8 CAR+ T cells.

220. The method or use of any one of claims 215-219, wherein the dose of cells at or about 5 x 10^7 cells or CAR+ T cells.

221. The method or use of any one of claims 215-219, wherein the dose of cells at or about 1.5 x 10^8 cells or CAR+ T cells.

222. The method or use of any one of claims 215-219, wherein the dose of cells at or about 3 x 10^8 cells or CAR+ T cells.

223. The method or use of any one of claims 215-219, wherein the dose of cells at or about 4.5 x 10^8 cells or CAR+ T cells.

224. The method or use of any one of claims 215-223, wherein the response or outcome comprises or further comprises the absence of grade 3 or higher, or grade 4 or higher, neurotoxicity, the absence of grade 3 or higher, or grade 4 or higher, cytokine release syndrome.

225. The method or the use of any of claims 215-224, wherein the dose of engineered T cells comprise at or about 5.0 x 10^7, at or about 1.5 x 10^8, at or about 3.0 x 10^8 or at or about 4.5 x 10^8 CAR-expressing T cells.

226. The method or the use of any of claims 215-225, wherein the dose of the engineered T cells comprise at or about 5.0 x 10^7 CAR-expressing T cells.

227. The method or the use of any of claims 215-225, wherein the dose of the engineered T cells comprise at or about 1.5 x 10^8 CAR-expressing T cells.

228. The method or the use of any of claims 215-225, wherein the dose of the engineered T cells comprise at or about 3 x 10^8 CAR-expressing T cells.

229. The method or the use of any of claims 215-225, wherein the dose of the engineered T cells comprise at or about 4.5 x 10^8 CAR-expressing T cells.

230. The cell of any of claims 168-179 or composition of any of claims 180-183, wherein the cell or composition, following administration at a dose of CAR+ cells is capable of achieving, optionally at a designated time following initiation of the administration, a specified response or outcome in at least one of, or in at least 10 %, at least 20 %, at least 30 %, at least 40 %, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, or at least 95% of subjects

within a cohort of subjects or evaluable subjects thereof, wherein the cohort of subjects is a cohort having multiple myeloma.

231. The cell or composition of claim 230, wherein the achievement of the response or outcome is at the designated time following initiation of administration, which is at 1, 2, 3, 6, 9, or 12 months following said initiation.

232. The cell or composition of claim 231, wherein the achievement of the response or outcome is at the designated time following initiation of administration, which is at 1 or 2 or 3 months following said initiation.

233. The cell or composition of claim 230, wherein:

the cohort of subjects is subjects having relapsed or refractory multiple myeloma;

the cohort of subjects is subjects having relapsed or refractory multiple myeloma having been adminiseterd, and relapsed or been refractory following, at least 3 prior therapies for multiple myeloma, said prior therapies optionally including an immunomodulatory agent; a proteasome inhibitor; and/or an anti-CD38 antibody;

the cohort of subjects is subjects having relapsed or refractory multiple myeloma having been adminiseterd, and relapsed or been refractory following, at least 3 prior therapies for multiple myeloma, said prior therapies optionally including an immunomodulatory agent; a proteasome inhibitor; and/or an anti-CD38 antibody and/or an autologous stem cell transplant; and/or

the cohort of subjects is subjects has no active plasma cell leukemia (PCL) or no history of PCL at the time of said administration;

the cohort of subjects is subjects has developed secondary plasma cell leukemia (PCL) prior to administration of the cells

the cohort of subjects is or includes subjects having relapsed or refractory multiple myeloma having been adminiseterd, and relapsed or been refractory following, at least 4 or an average of at least 10 prior therapies for multiple myeloma;

the cohort of subjects consists of or includes adult subjects;

the cohort of subjects has a median time from diagnosis of 4 years and/or a range of time from diagnostis from 2 to 12 years;

the cohort of subjects has received a median of 10 prior regimens or between 3 and 15 or 4 and 15 prior therapies for multiple myeloma;

the cohort of subjects includes subjects refractory to bortezomib, carfilzomib, lenalidomide, pomalidomide and an anti-CD38 monoclonal antibody;

the cohort of subjects includes subjects having had prior autologous stem cell transplant; and/or

the cohort of subjects includes subjects having IMWG high risk cytogenetics.

234. The cell or composition of claim 233, wherein the immunomodulatory agent is selected from among thalidomide, lenalidomide and pomalidomide, the proteasome inhibitor is selected from among bortezomib, carfilzomib and ixazomib, and/or the anti-CD38 antibody is or comprises daratumumab.

235. The cell or composition of any one of claims 230 to 234, wherein

the response or outcome is selected from the group consisting of objective response (OR), complete response (CR), stringent complete response (sCR), very good partial response (VGPR), partial response (PR) and minimal response (MR) , optionally based on the International Myeloma Working Group (IMWG) uniform response criteria;

the response or outcome is or comprises an OR, optionally based on the International Myeloma Working Group (IMWG) uniform response criteria; or

the response or outcome is or comprises a CR, optionally based on the International Myeloma Working Group (IMWG) uniform response criteria.

236. The cell or composition of any one of claims 230 to 235, wherein the response or outcome is or comprises an OR.

237. The cell or composition of any one of claims 230 to 236, wherein the dose is capable of achieving the response or outcome in at least 40 %, at least 50 %, at least 60 %, at least 70 %, or at least 80 % of subjects of the cohort.

238. The cell or composition of any of claims 230 to 235, wherein the response or outcome is or comprises a CR or sCR.

239. The cell or composition of any one of claims 230 to 238, wherein the dose is capable of achieving the response or outcome in at least 20 %, 30 %, or 40 % of subjects of the cohort.

240. The cell or composition of any one of claims 230 to 239, wherein

the dose capable of achieving said response or outcome is less than 1.5 x 10^8 cells

the dose capable of achieving said response or outcome is less than 1.5 x 10^8 CAR+ T cells.

241. The cell or composition of any one of claims 230 to 240, wherein

the dose capable of achieving said response or outcome is less than 1.5 x 10^8 cells

the dose capable of achieving said response or outcome is less than 1.5 x 10^8 CAR+ T cellsthe dose capable of achieving said response or outcome is less than 3 x 10^8 CAR+ T cells; or

the dose capable of achieving said response or outcome is less than or less than 4.5 x 10^8 CAR+ T cells.

242. The cell or composition of any one of claims 230 to 241, wherein

the dose capable of achieving said response or outcome is less than 1 x 10^8 cells

the dose capable of achieving said response or outcome is less than 1 x 10^8 CAR+ T cells.

243. The cell or composition of any one of claims 230 to 242, wherein the dose capable of achieving said response or outcome is at or about 5 x 10^7 cells or at or about 5 x 10^7 CAR+ T cells.

244. The cell or composition of any one of claims 230 to 243, wherein the dose capable of achieving said response or outcome is at or about 1.5 x 10^8 cells or CAR+ T cells.

245. The cell or composition of any one of claims 230 to 244, wherein the dose capable of achieving said response or outcome is at or about 3 x 10^8 cells or CAR+ T cells.

246. The cell or composition of any one of claims 230 to 245, wherein the dose capable of achieving said response or outcome is at or about 4.5 x 10^8 cells or CAR+ T cells.

247. The cell or composition of any one of claims 230 to 246, wherein the response or outcome comprises or further comprises the absence of grade 3 or higher, or grade 4 or higher, neurotoxicity, the absence of grade 3 or higher, or grade 4 or higher, cytokine release syndrome.

248. 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.

249. The method of claim 248 wherein the detected differences in b) are different lengths of the amplified transcripts.

250. The method of claim 248 wherein the differences in b) are differences in chromatographic profiles of the amplified transcripts.

251. The method of any of claims 248-250, wherein the differences in the amplified products are determined by agarose gel electrophoresis, chip-based capillary electrophoresis, analytical ultracentrifugation, field flow fractionation, or chromatography.

252. The method of any of claims 248-251, wherein the 5′ primer is specific to sequence transcribed from the promoter region of the transcribed nucleic acid.

253. The method of any of claims 248-252, wherein 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.

254. The method of any of claims 248-253, wherein the 3 primer is specific to the polyadenylation sequence or enhancer region of the 3′ untranslated region of the transcribed pre-mRNA.

255. The method of any of claims 248-254, wherein 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).

256. The method of any of claims 248-255, wherein 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.

257. The method of claim 256, wherein the parallel or subsequent amplification reactions amplify overlapping portions of the transcript.

258. The method of any of claims 248-257, wherein 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.

259. The method of any of claims 248-258, 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.

260. The method of claim 259, 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.

261. 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 259 or claim 260;

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.

262. The method of claim 261, further comprising: d) assessing the transgene candidacy for the removal of splice sites as in step a).

263. The method of claim 262, further comprising 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).

264. The method of any of claims 261-263, 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%.

265. The method of any of claims 261-264, wherein splice donor sites and splice acceptor sites are identified independently.

266. The method of any of claims 261-265, wherein the splice acceptor and/or donor site(s) is/are canonical, non-canonical, and/or cryptic splice acceptor and/or donor site(s).

267. The method of any of claims 261-266, wherein the transgene is a chimeric antigen receptor or a portion of a chimeric antigen receptor.

268. The method of claim 267, 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.

269. The method of claim 267 or claim 268, wherein the modified polynucleotide is not modified within the coding sequence for the antigen-binding domain of the encoded CAR polypeptide.

270. The method of any of claims 261-269, wherein the encoded amino acid sequence of the transgene is unchanged following modification of the polynucleotide.

271. The method of any of claims 261-270, 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.

272. The method of any of claims 248-271, wherein the cell is a human cell.

273. The method of any of claims 248-272, wherein the cell is a T-cell.

274. The method of any of claims 248-273, wherein 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.

275. 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 248-274.

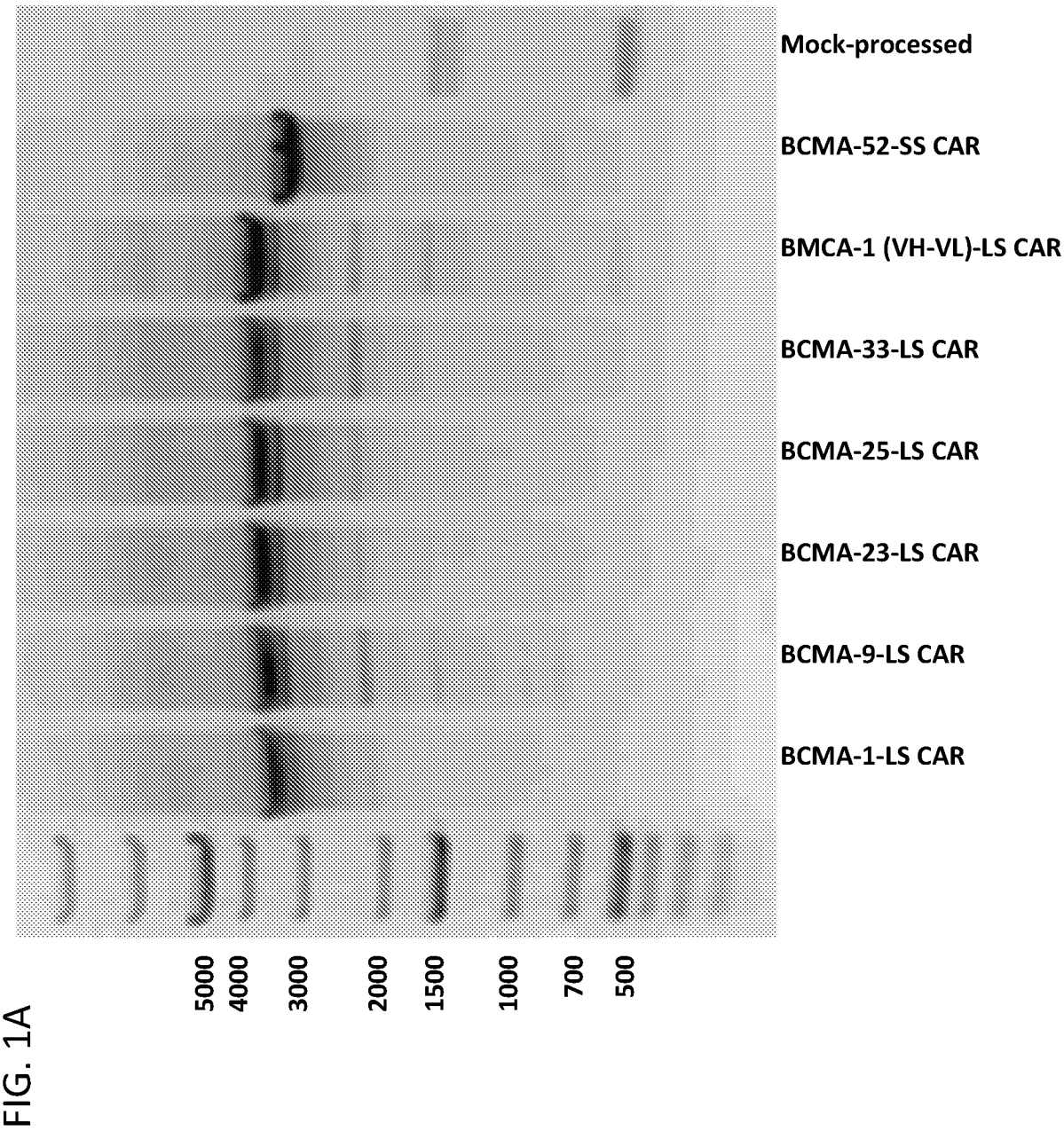

Mock-processed
BCMA-52-SS CAR
BMCA-1 (VH-VL)-LS CAR
BCMA-33-LS CAR
BCMA-25-LS CAR
BCMA-23-LS CAR
BCMA-9-LS CAR
BCMA-1-LS CAR
5000
4000
3000
2000
1500
1000
700
500

FIG. 1A

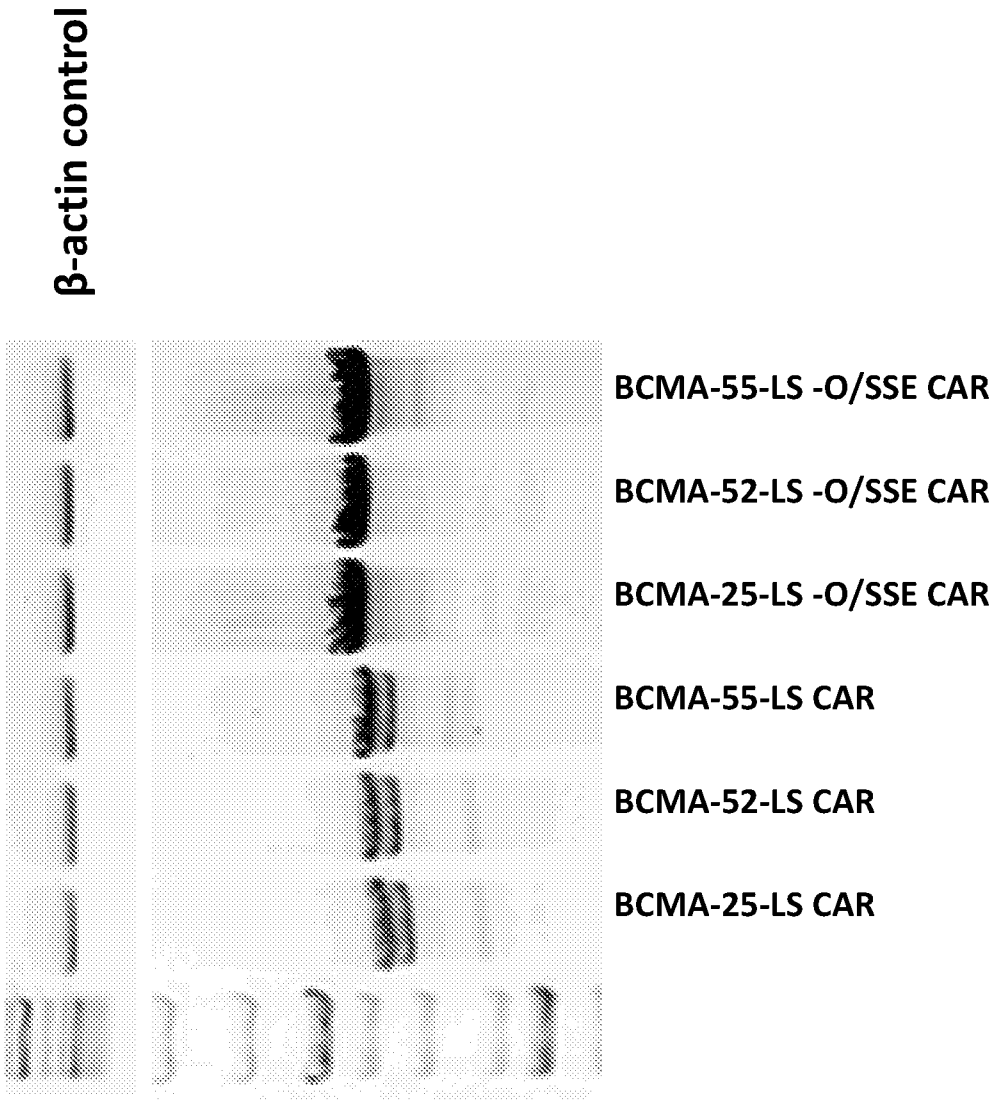

β-actin control
BCMA-55-LS -O/SSE CAR
BCMA-52-LS -O/SSE CAR
BCMA-25-LS -O/SSE CAR
BCMA-55-LS CAR
BCMA-52-LS CAR
BCMA-25-LS CAR

FIG. 1B

FIG. 2

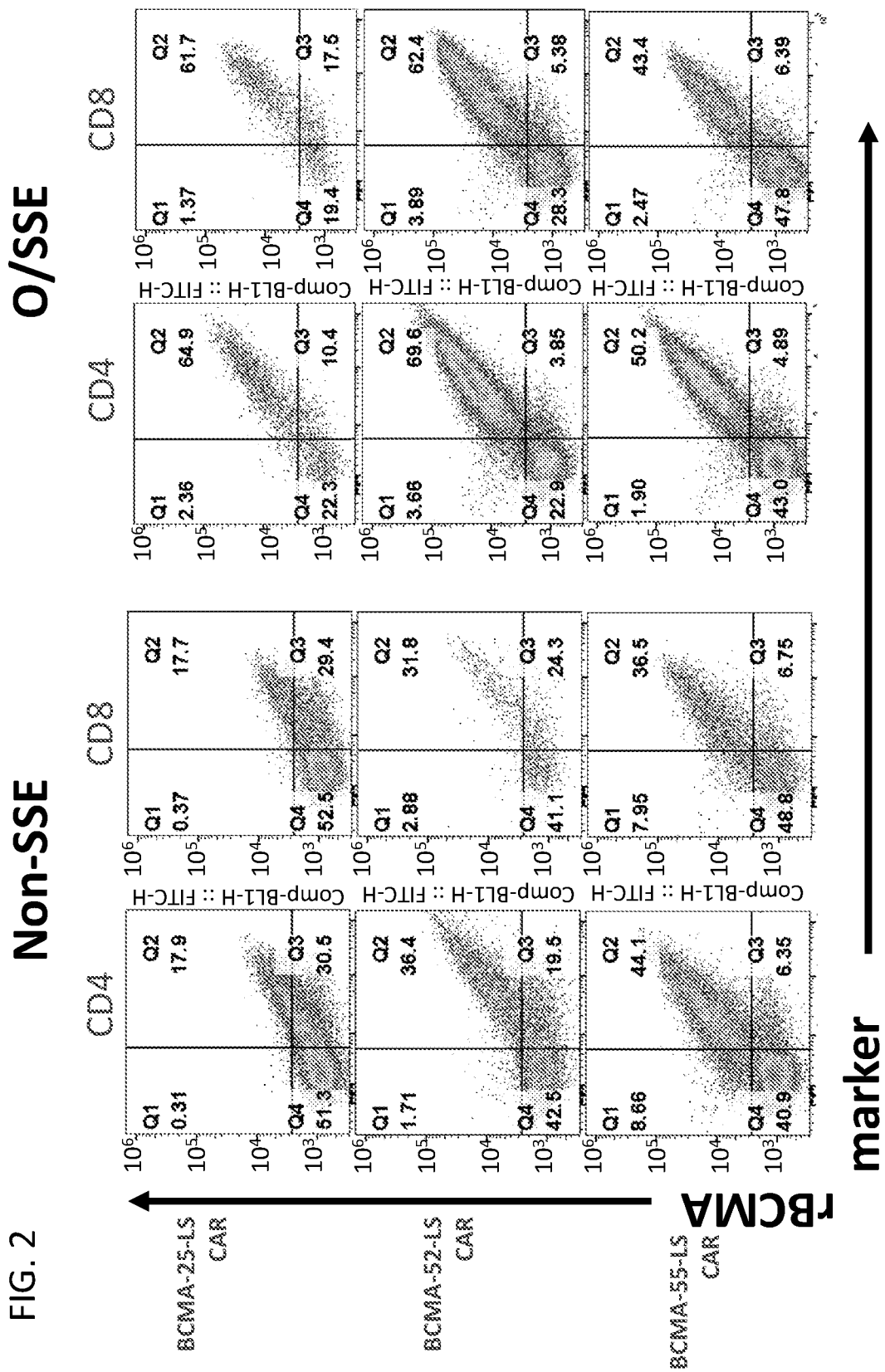

Non-SSE
O/SSE
CD4
CD8
BCMA-25-LS CAR
BCMA-52-LS CAR
BCMA-55-LS CAR
rBCMA marker
Comp-BL1-H :: FITC-H
Q1 0.31
Q2 17.9
Q3 30.5
Q4 51.3
Q1 0.37
Q2 17.7
Q3 29.4
Q4 52.5
Q1 1.71
Q2 36.4
Q3 19.5
Q4 42.5
Q1 2.88
Q2 31.8
Q3 24.3
Q4 41.1
Q1 8.66
Q2 44.1
Q3 6.35
Q4 40.9
Q1 7.95
Q2 36.5
Q3 6.75
Q4 48.8
Q1 2.36
Q2 64.9
Q3 10.4
Q4 22.3
Q1 1.37
Q2 61.7
Q3 17.5
Q4 19.4
Q1 3.66
Q2 69.6
Q3 3.85
Q4 22.9
Q1 3.89
Q2 62.4
Q3 5.38
Q4 28.3
Q1 1.90
Q2 50.2
Q3 4.89
Q4 43.0
Q1 2.47
Q2 43.4
Q3 6.39
Q4 47.8

FIG. 3

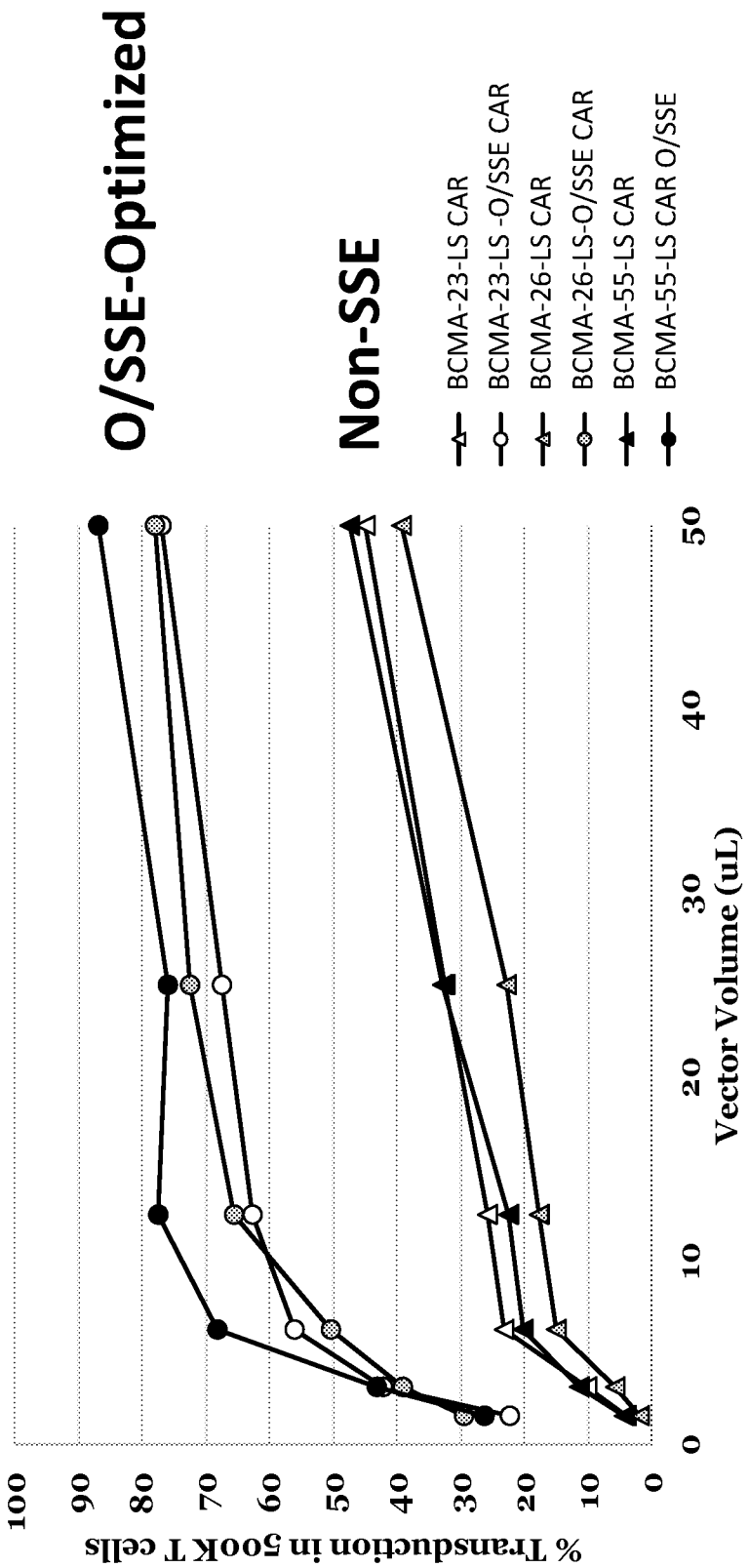
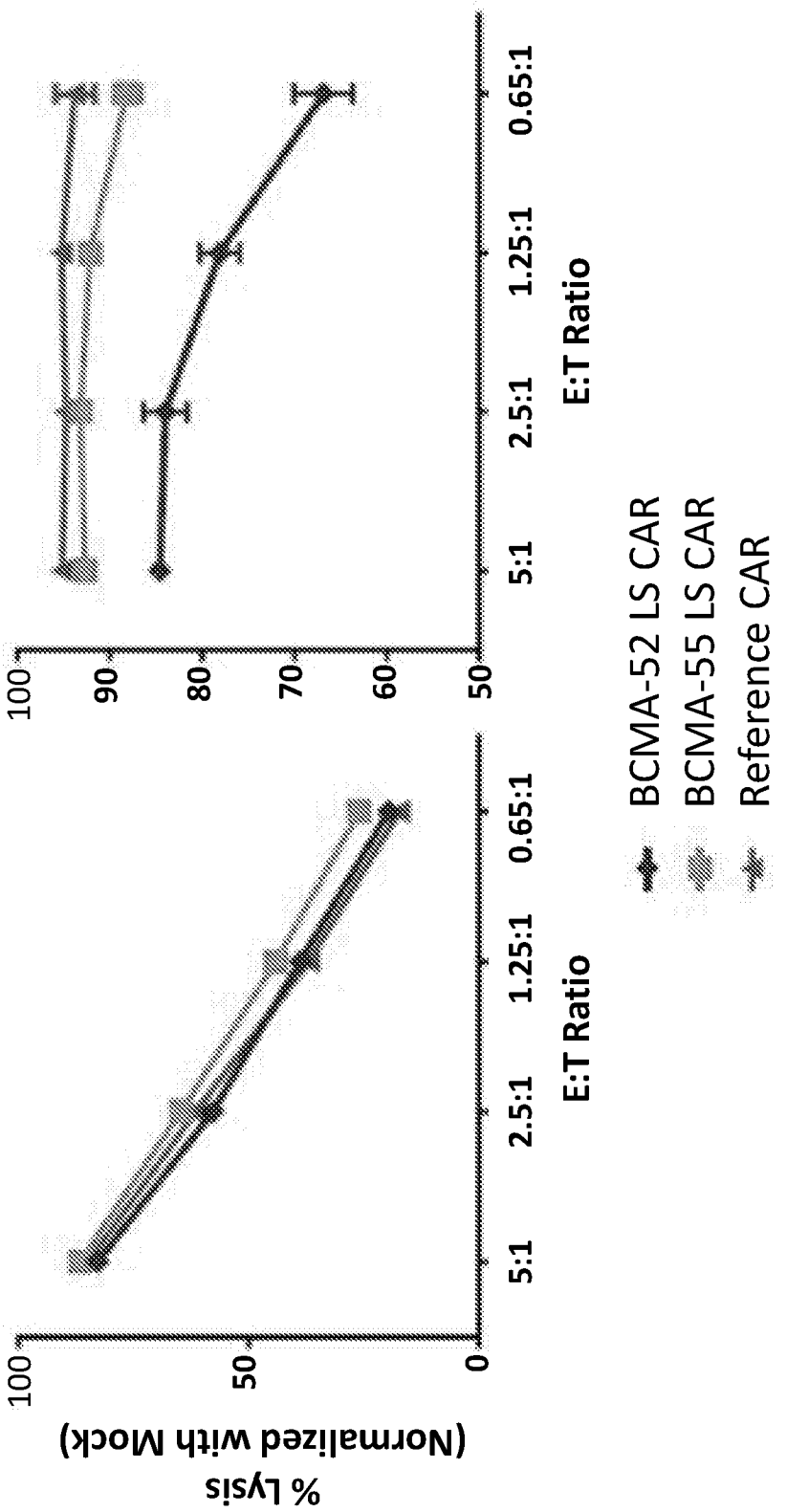

O/SSE-Optimized
Non-SSE
BCMA-23-LS CAR
BCMA-23-LS -O/SSE CAR
BCMA-26-LS CAR
BCMA-26-LS-O/SSE CAR
BCMA-55-LS CAR
BCMA-55-LS CAR O/SSE
% Transduction in 500K T cells
Vector Volume (uL)
0
10
20
30
40
50
0
10
20
30
40
50
60
70
80
90
100

FIG. 4A


CART + K562-BCMA
CART + RPMI-8226
% Lysis
(Normalized with Mock)
100
50
0
100
90
80
70
60
50
5:1
2.5:1
1.25:1
0.65:1
E:T Ratio
BCMA-52 LS CAR
BCMA-55 LS CAR
Reference CAR

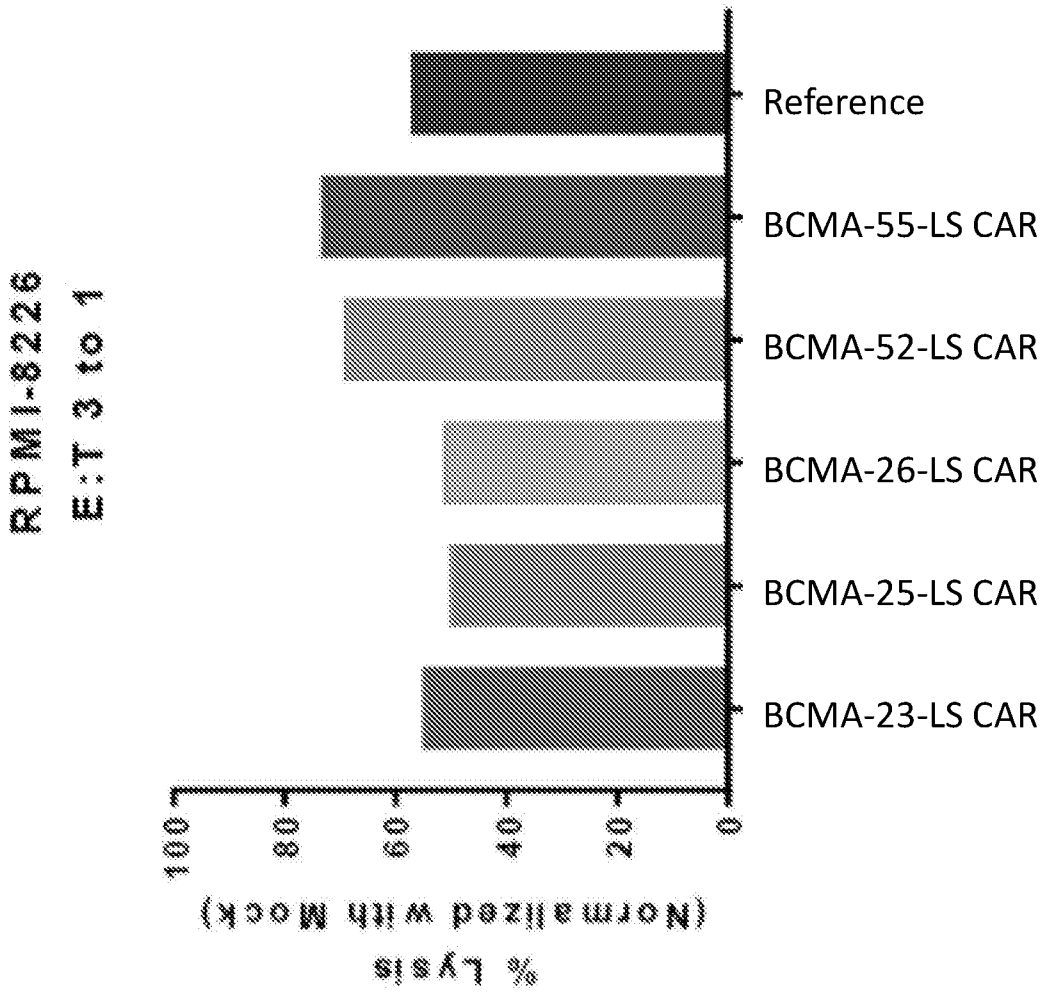

RPMI-8226
E:T 3 to 1
% Lysis
(Normalized with Mock)
100
80
60
40
20
0
Reference
BCMA-55-LS CAR
BCMA-52-LS CAR
BCMA-26-LS CAR
BCMA-25-LS CAR
BCMA-23-LS CAR

FIG. 4B

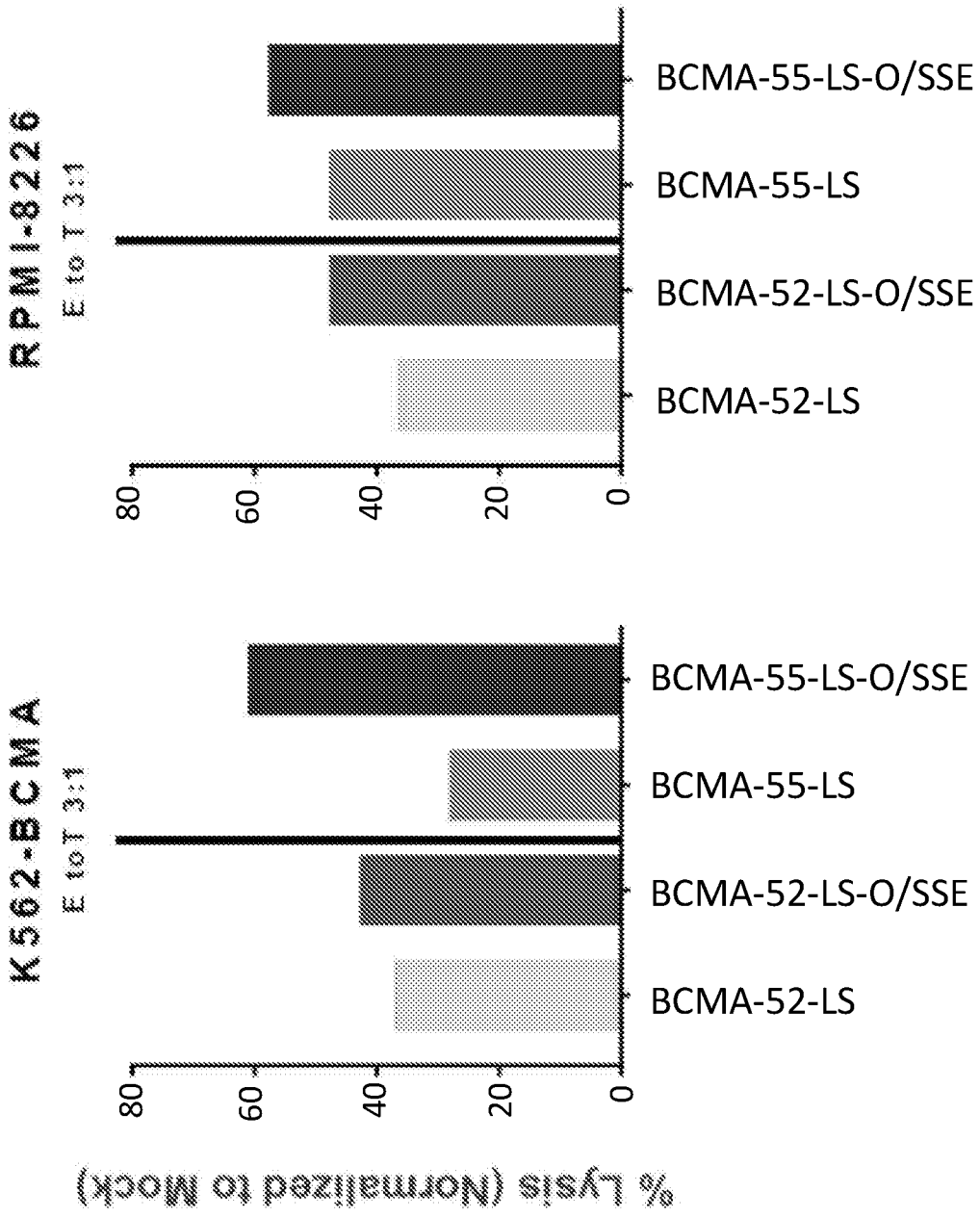

RPMI-8226
E to T 3:1
BCMA-55-LS-O/SSE
BCMA-55-LS
BCMA-52-LS-O/SSE
BCMA-52-LS
80
60
40
20
0
K562-BCMA
E to T 3:1
BCMA-55-LS-O/SSE
BCMA-55-LS
BCMA-52-LS-O/SSE
BCMA-52-LS
80
60
40
20
0
% Lysis (Normalized to Mock)

FIG. 4C

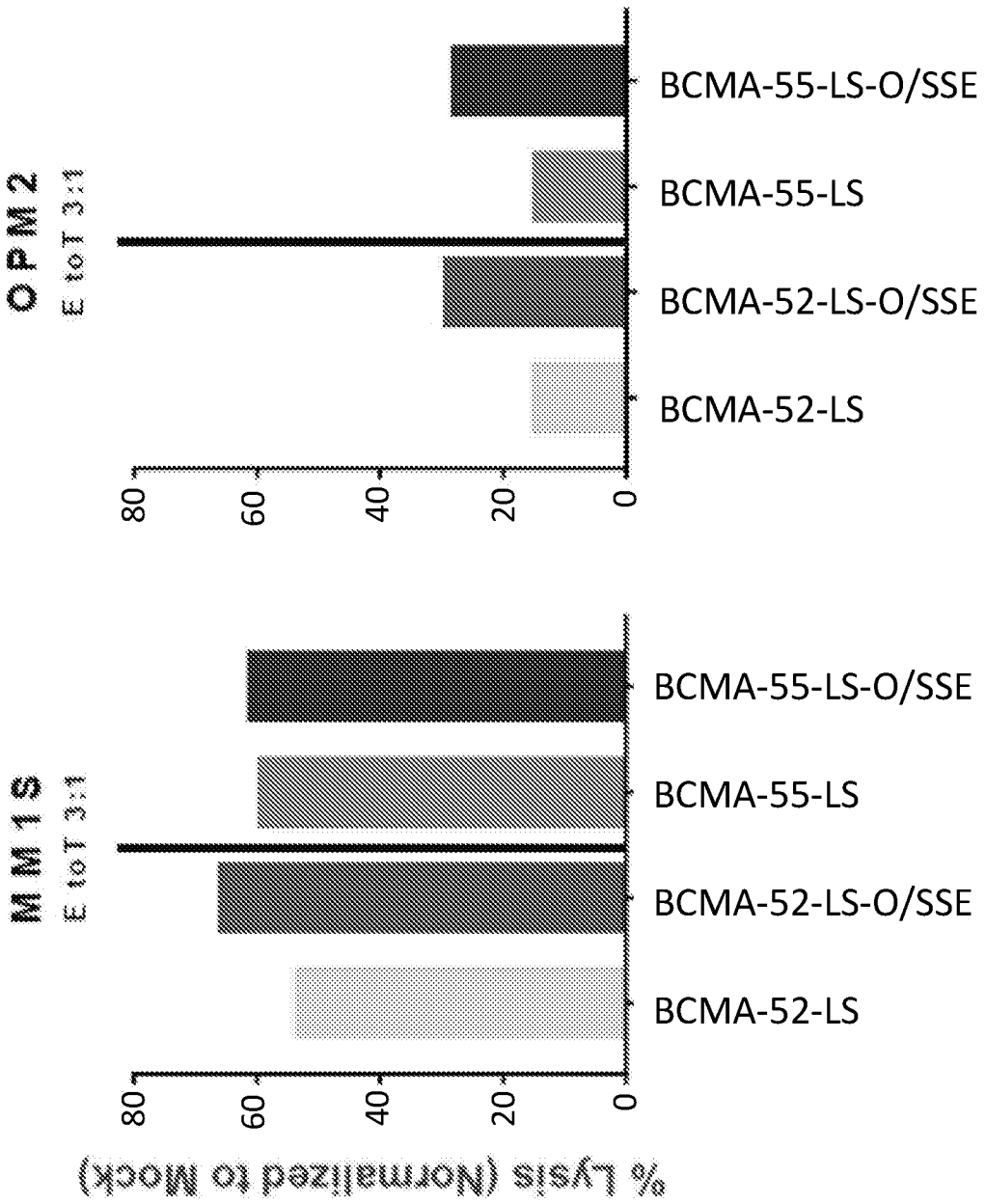

OPM2
E toT 3:1
BCMA-55-LS-O/SSE
BCMA-55-LS
BCMA-52-LS-O/SSE
BCMA-52-LS
80
60
40
20
0
MM1S
E toT 3:1
BCMA-55-LS-O/SSE
BCMA-55-LS
BCMA-52-LS-O/SSE
BCMA-52-LS
80
60
40
20
0
% Lysis (Normalized to Mock)

FIG. 4D

FIG. 5A

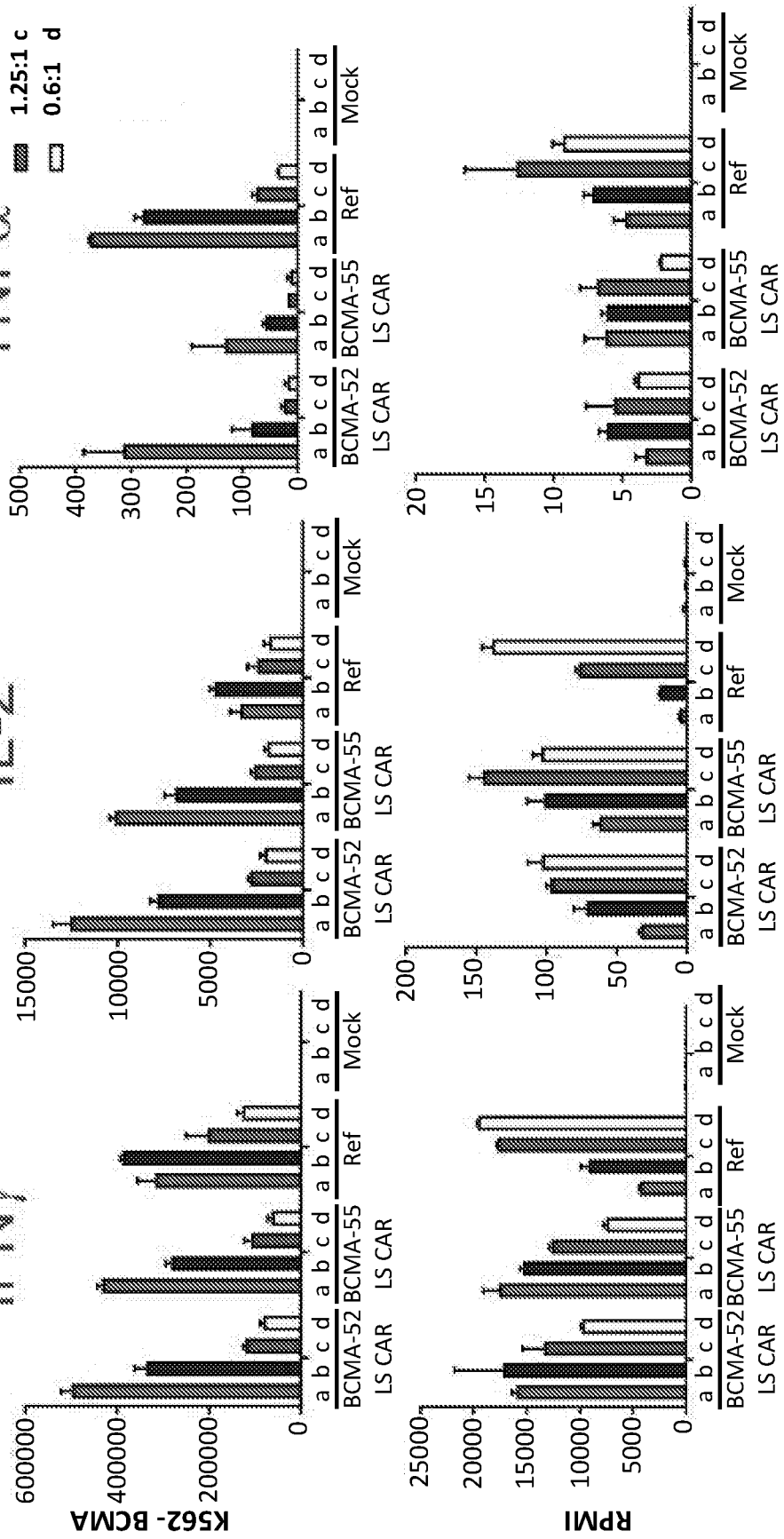
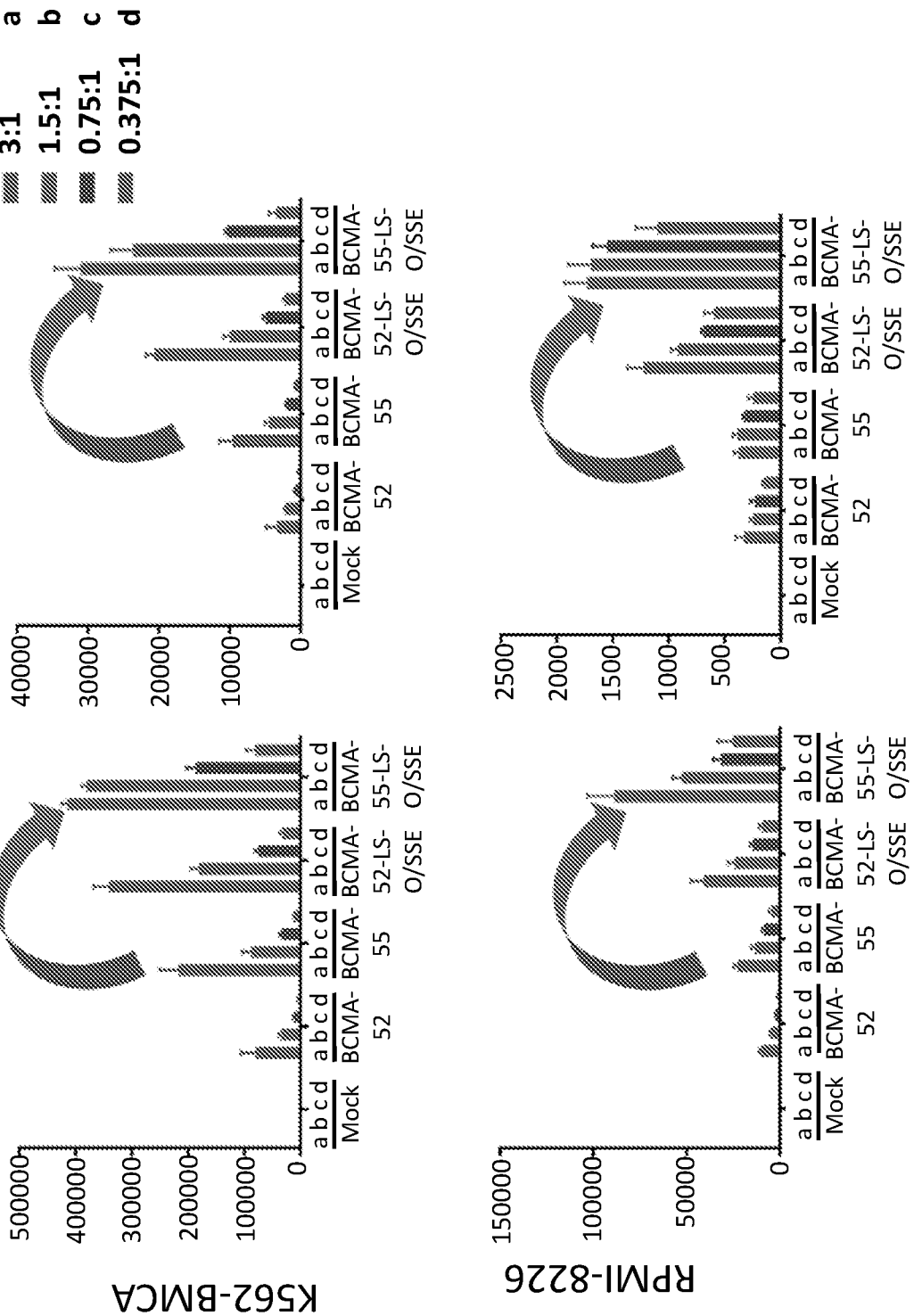

IFNγ
IL-2
TNFα
5:1 a
2.5:1 b
1.25:1 c
0.6:1 d
K562- BCMA
RPMI
a b c d
BCMA-52
BCMA-55
Ref
Mock
LS CAR

FIG. 5B


IFNγ
IL-2
3:1 a
1.5:1 b
0.75:1 c
0.375:1 d
K562-BMCA
RPMI-8226
Mock
BCMA-
52
55
52-LS-
55-LS-
O/SSE

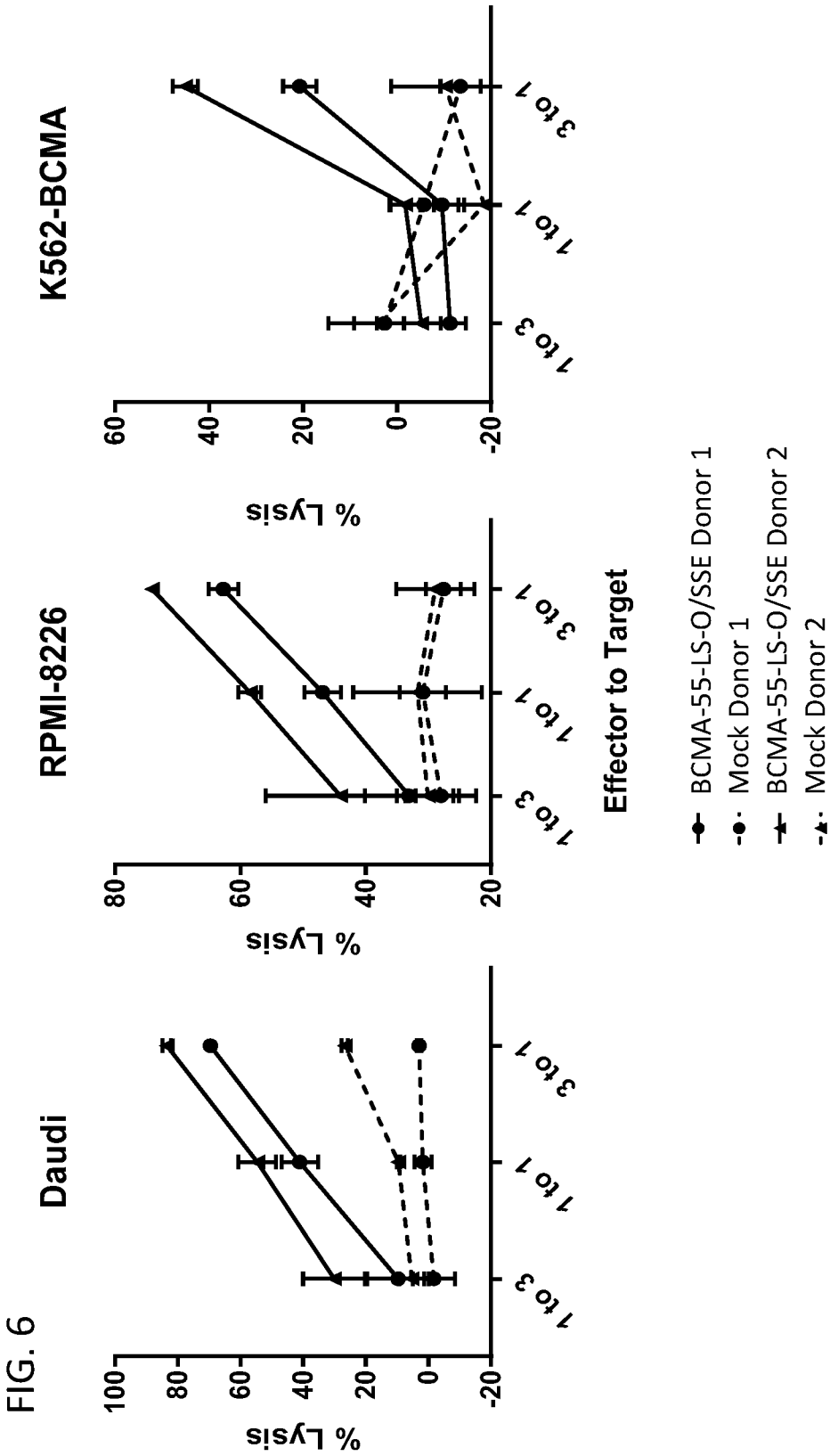

Daudi
RPMI-8226
K562-BCMA
% Lysis
Effector to Target
1 to 3
1 to 1
3 to 1
BCMA-55-LS-O/SSE Donor 1
Mock Donor 1
BCMA-55-LS-O/SSE Donor 2
Mock Donor 2

FIG. 6

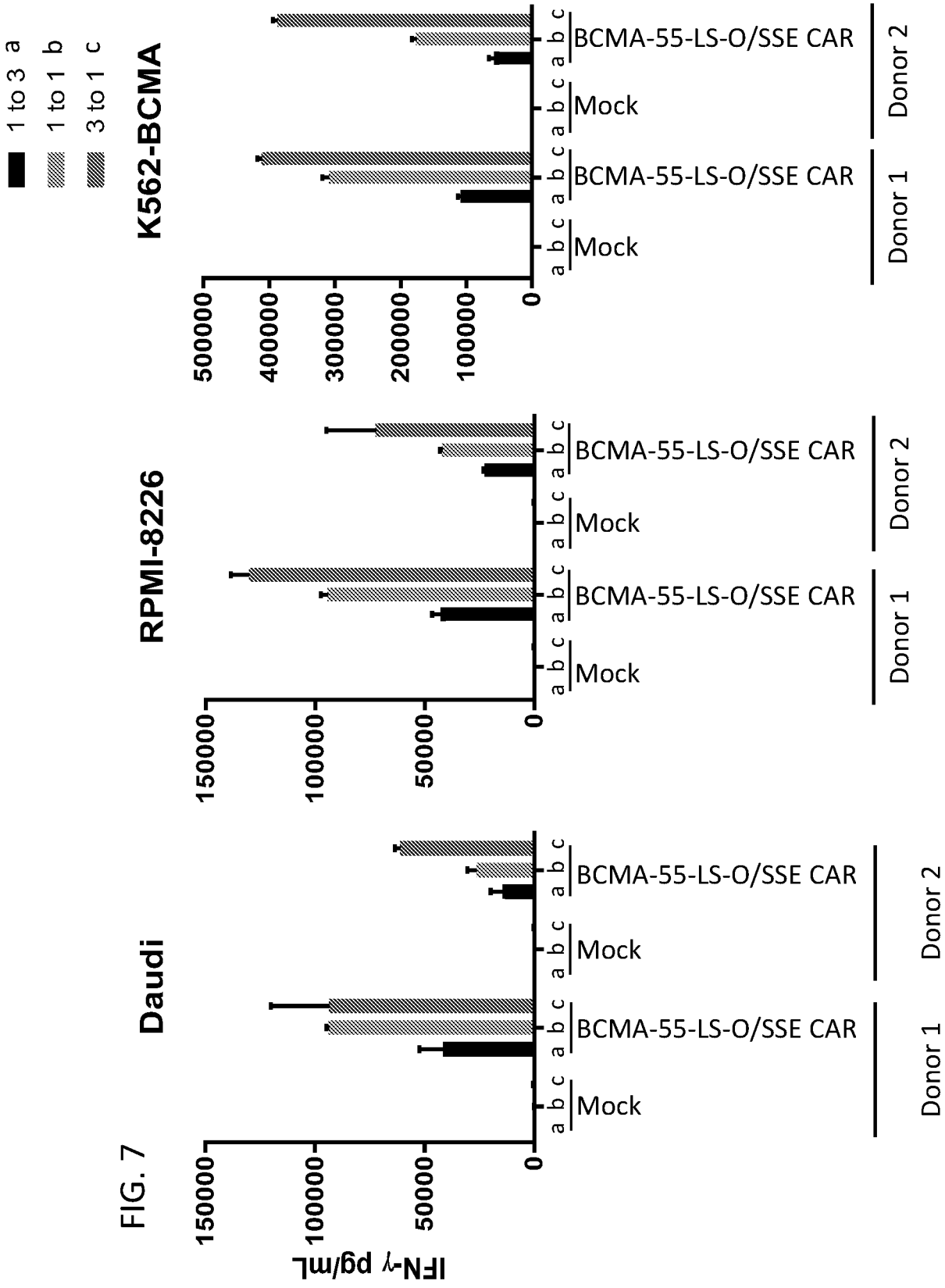

1 to 3 a
1 to 1 b
3 to 1 c
K562-BCMA
RPMI-8226
Daudi
IFN-γ pg/mL
0
50000
100000
150000
200000
300000
400000
500000
a b c
BCMA-55-LS-O/SSE CAR
Mock
Donor 1
Donor 2

FIG. 7

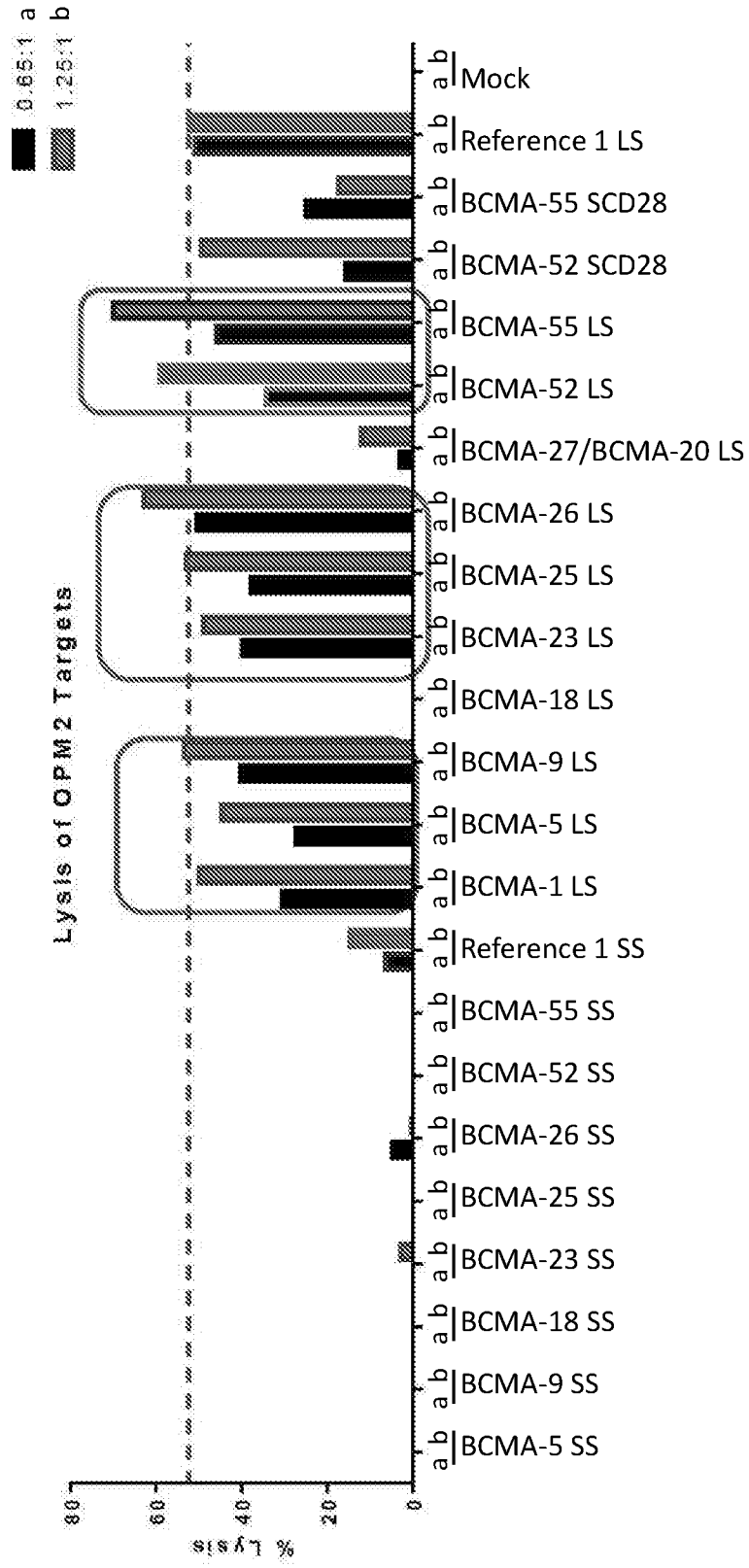

0.65:1 a
1.25:1 b
Lysis of OPM2 Targets
% Lysis
0
20
40
60
80
a b Mock
a b Reference 1 LS
a b BCMA-55 SCD28
a b BCMA-52 SCD28
a b BCMA-55 LS
a b BCMA-52 LS
a b BCMA-27/BCMA-20 LS
a b BCMA-26 LS
a b BCMA-25 LS
a b BCMA-23 LS
a b BCMA-18 LS
a b BCMA-9 LS
a b BCMA-5 LS
a b BCMA-1 LS
a b Reference 1 SS
a b BCMA-55 SS
a b BCMA-52 SS
a b BCMA-26 SS
a b BCMA-25 SS
a b BCMA-23 SS
a b BCMA-18 SS
a b BCMA-9 SS
a b BCMA-5 SS

FIG. 8

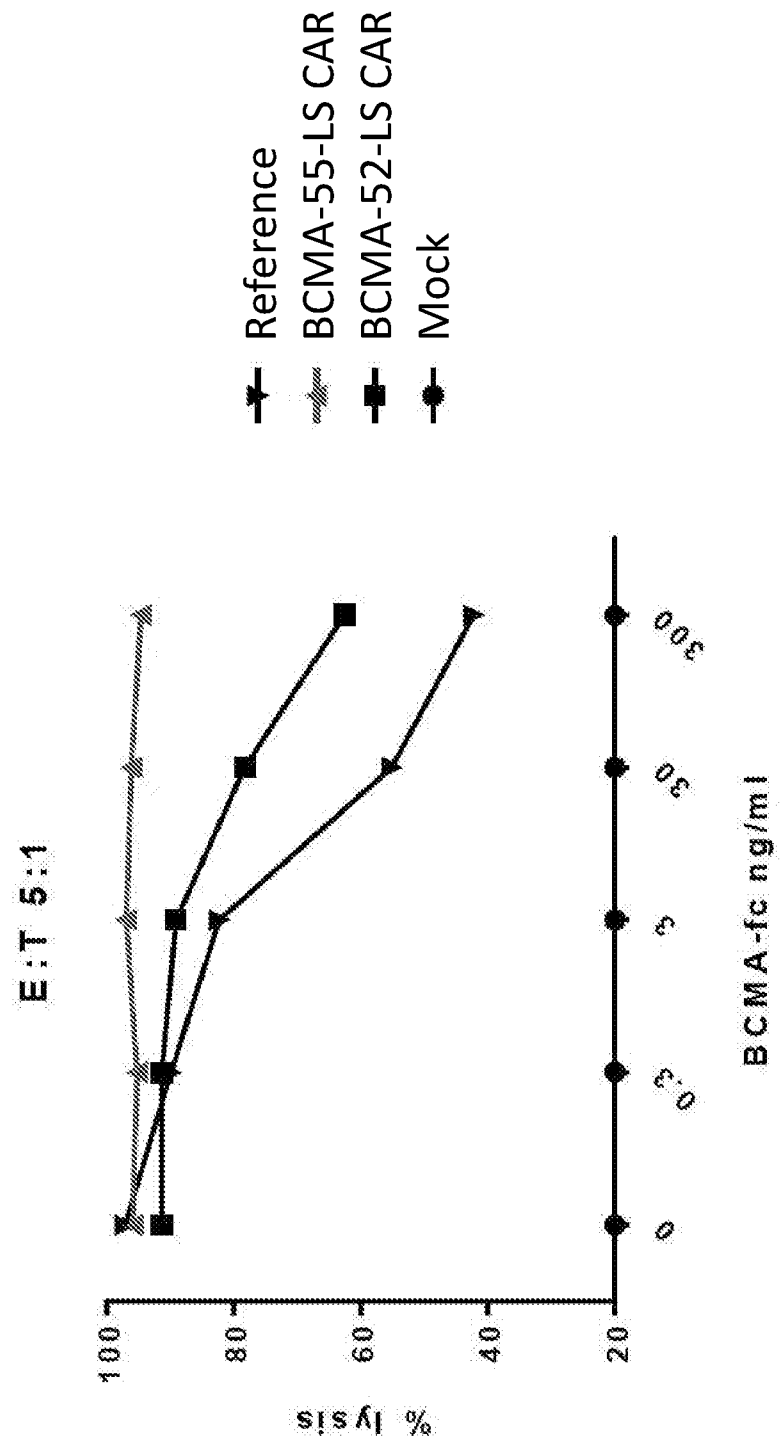

Reference
BCMA-55-LS CAR
BCMA-52-LS CAR
Mock
E:T 5:1
% lysis
100
80
60
40
20
0
0.3
3
30
300
BCMA-fc ng/ml

FIG. 9A

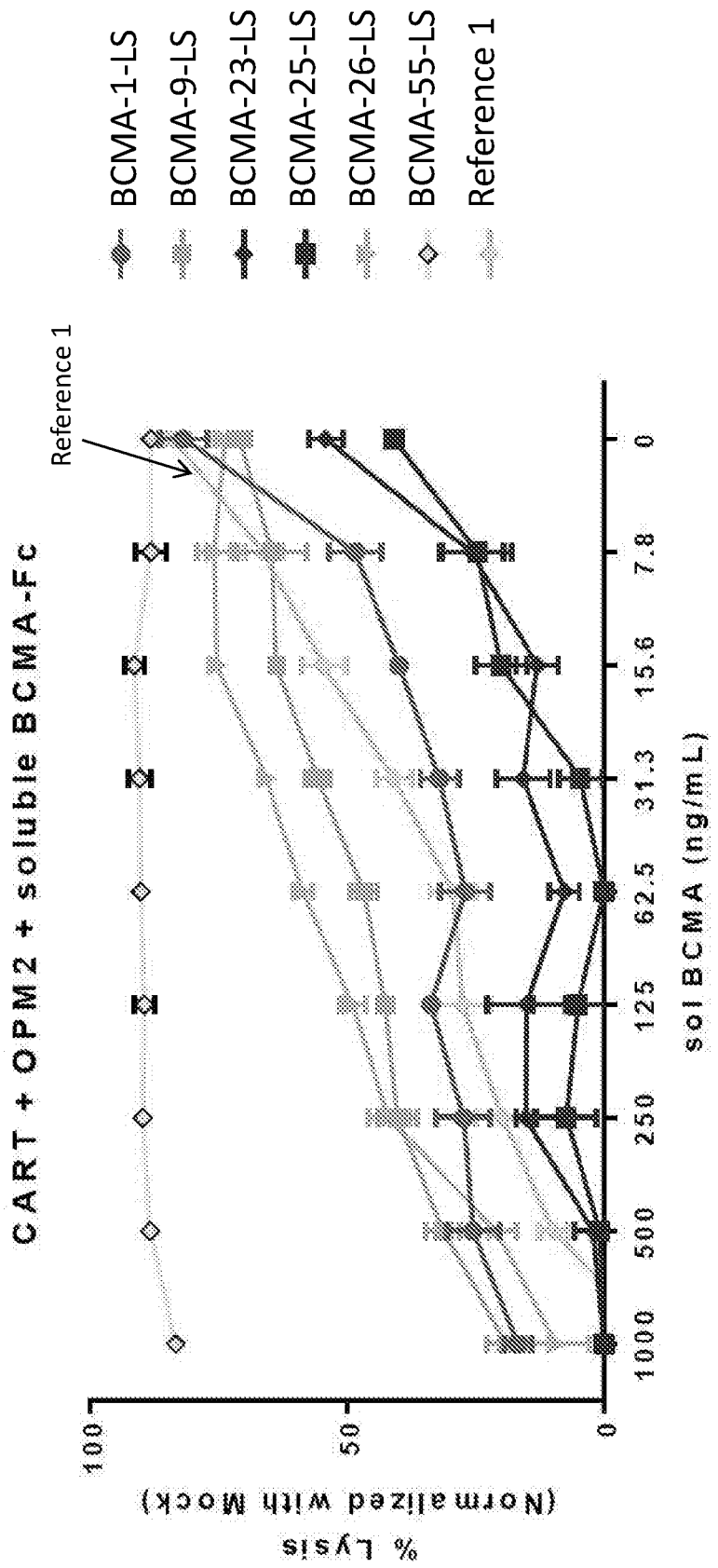

BCMA-1-LS
BCMA-9-LS
BCMA-23-LS
BCMA-25-LS
BCMA-26-LS
BCMA-55-LS
Reference 1
CART + OPM2 + soluble BCMA-Fc
% Lysis
(Normalized with Mock)
100
50
0
1000
500
250
125
62.5
31.3
15.6
7.8
0
solBCMA (ng/mL)

FIG. 9B

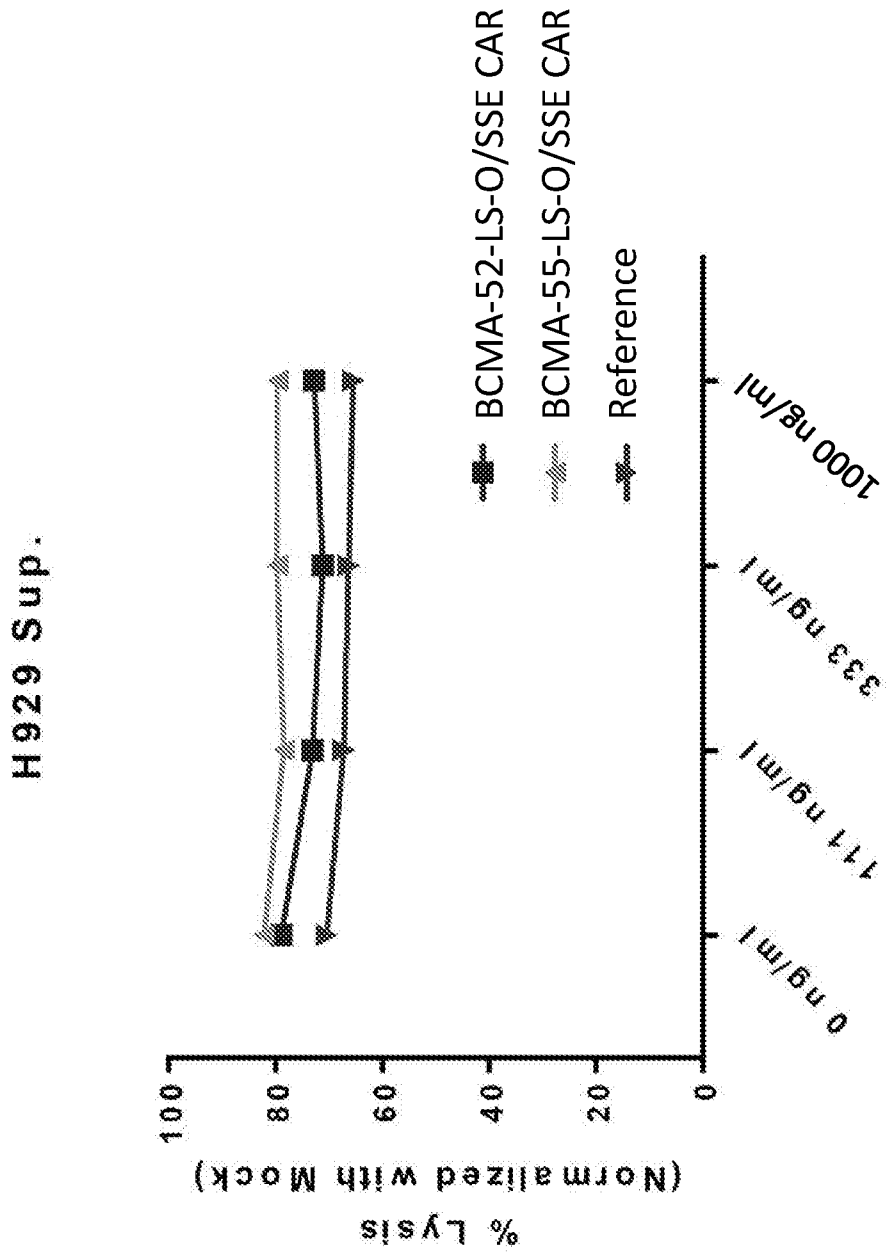

H929 Sup.
% Lysis
(Normalized with Mock)
100
80
60
40
20
0
0 ng/ml
111 ng/ml
333 ng/ml
1000 ng/ml
BCMA-52-LS-O/SSE CAR
BCMA-55-LS-O/SSE CAR
Reference

FIG. 10A

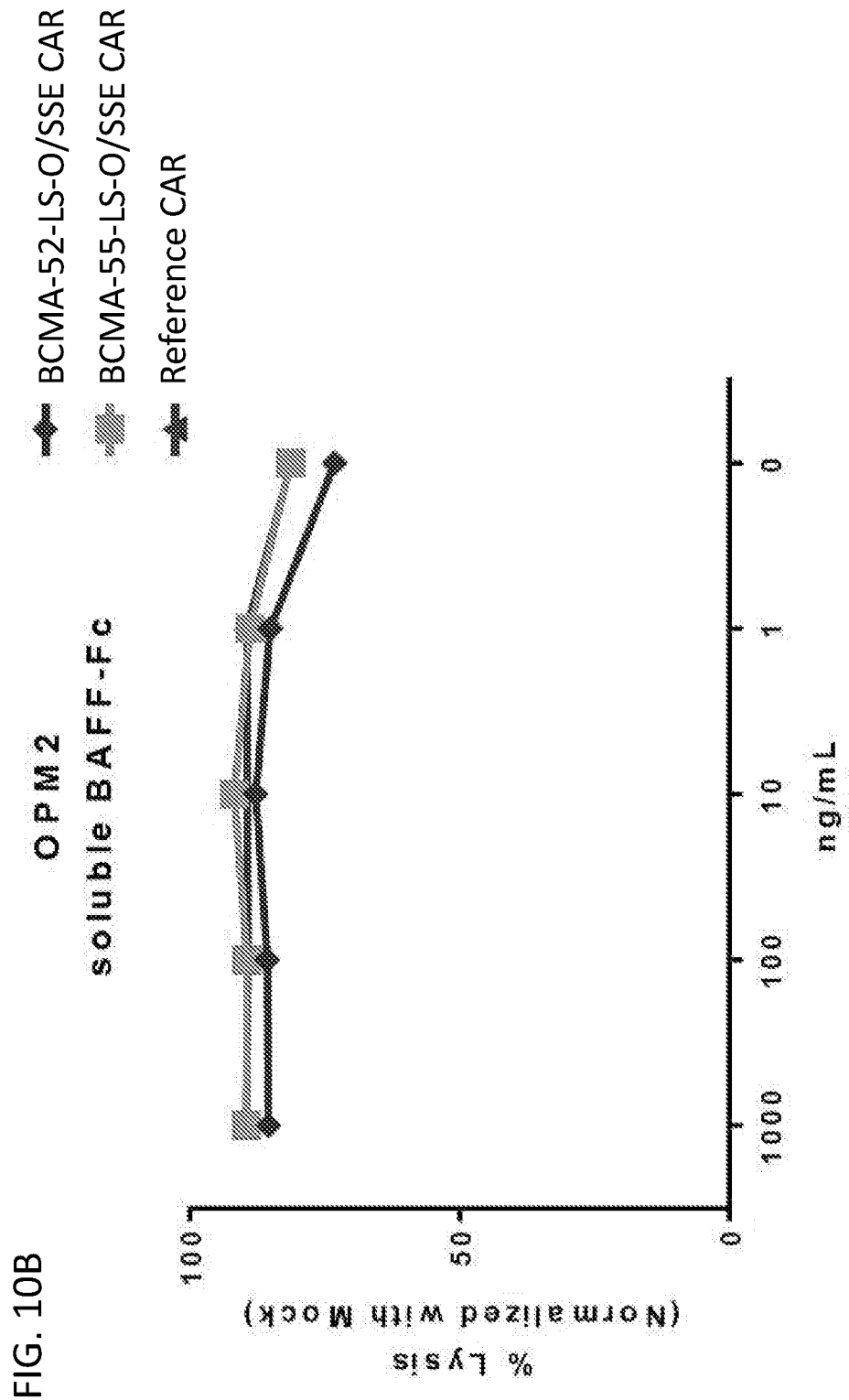

BCMA-52-LS-O/SSE CAR
BCMA-55-LS-O/SSE CAR
Reference CAR
OPM2
soluble BAFF-Fc
% Lysis
(Normalized with Mock)
100
50
0
1000
100
10
1
0
ng/mL

FIG. 10B

FIG. 11A

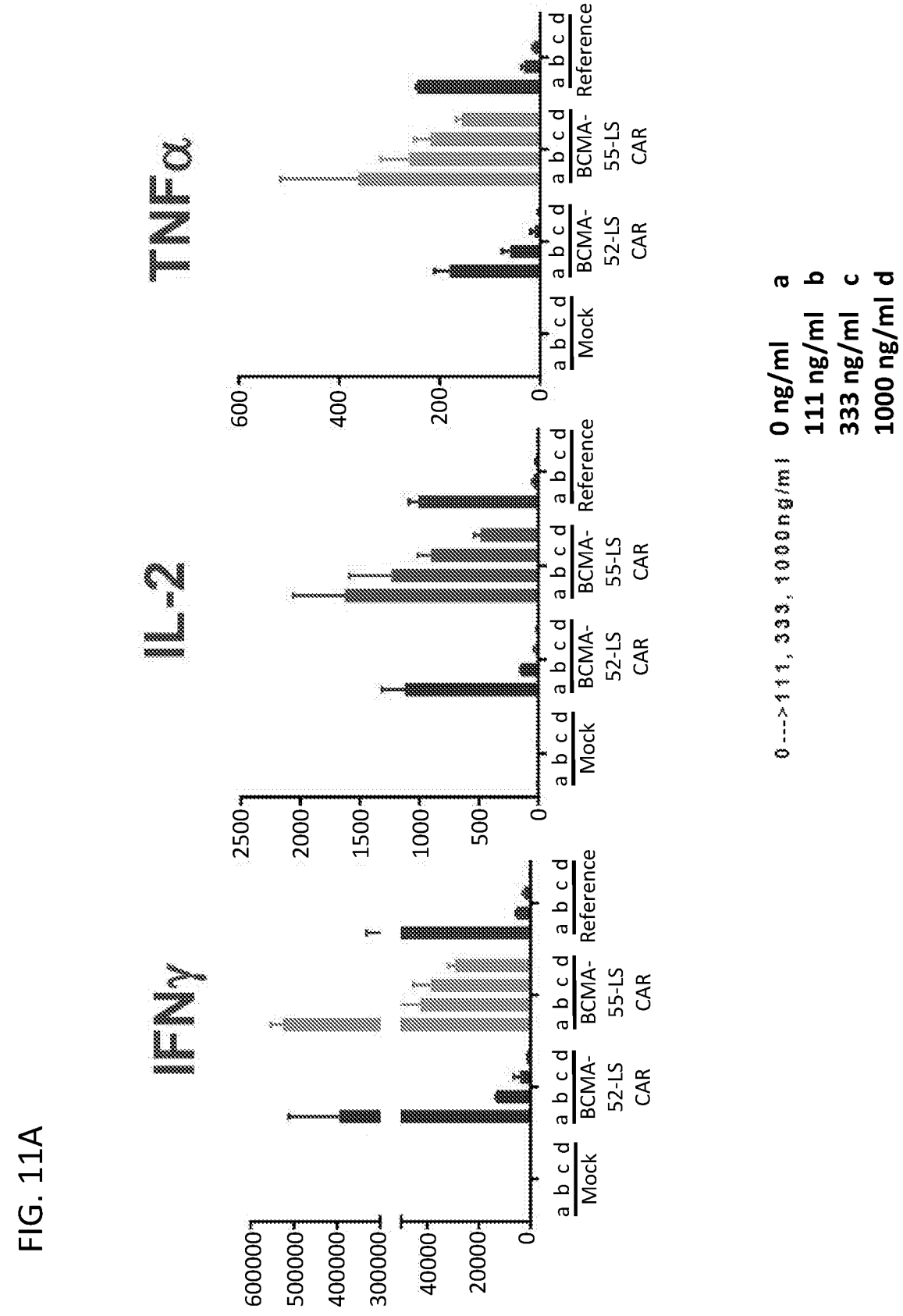

IFNγ
IL-2
TNFα
600000
500000
400000
300000
40000
20000
0
2500
2000
1500
1000
500
600
400
200
a b c d
Mock
BCMA-52-LS CAR
BCMA-55-LS CAR
Reference
0--->111, 333, 1000ng/ml
0 ng/ml a
111 ng/ml b
333 ng/ml c
1000 ng/ml d

FIG. 11B

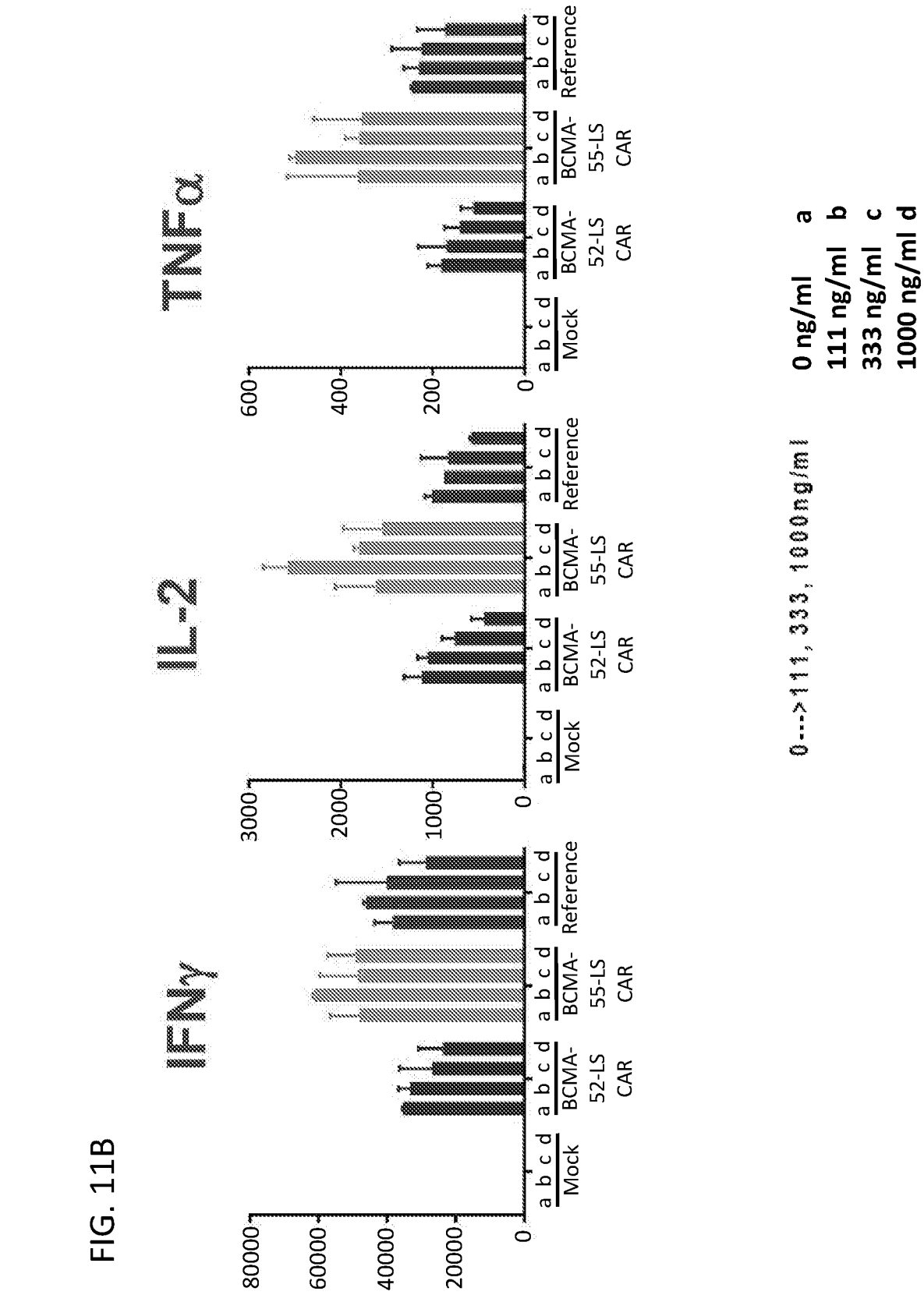

IFNγ
IL-2
TNFα
80000
60000
40000
20000
0
3000
2000
1000
600
400
200
a b c d
Mock
BCMA-52-LS CAR
BCMA-55-LS CAR
Reference
0--->111, 333, 1000ng/ml
0 ng/ml a
111 ng/ml b
333 ng/ml c
1000 ng/ml d

FIG. 12A

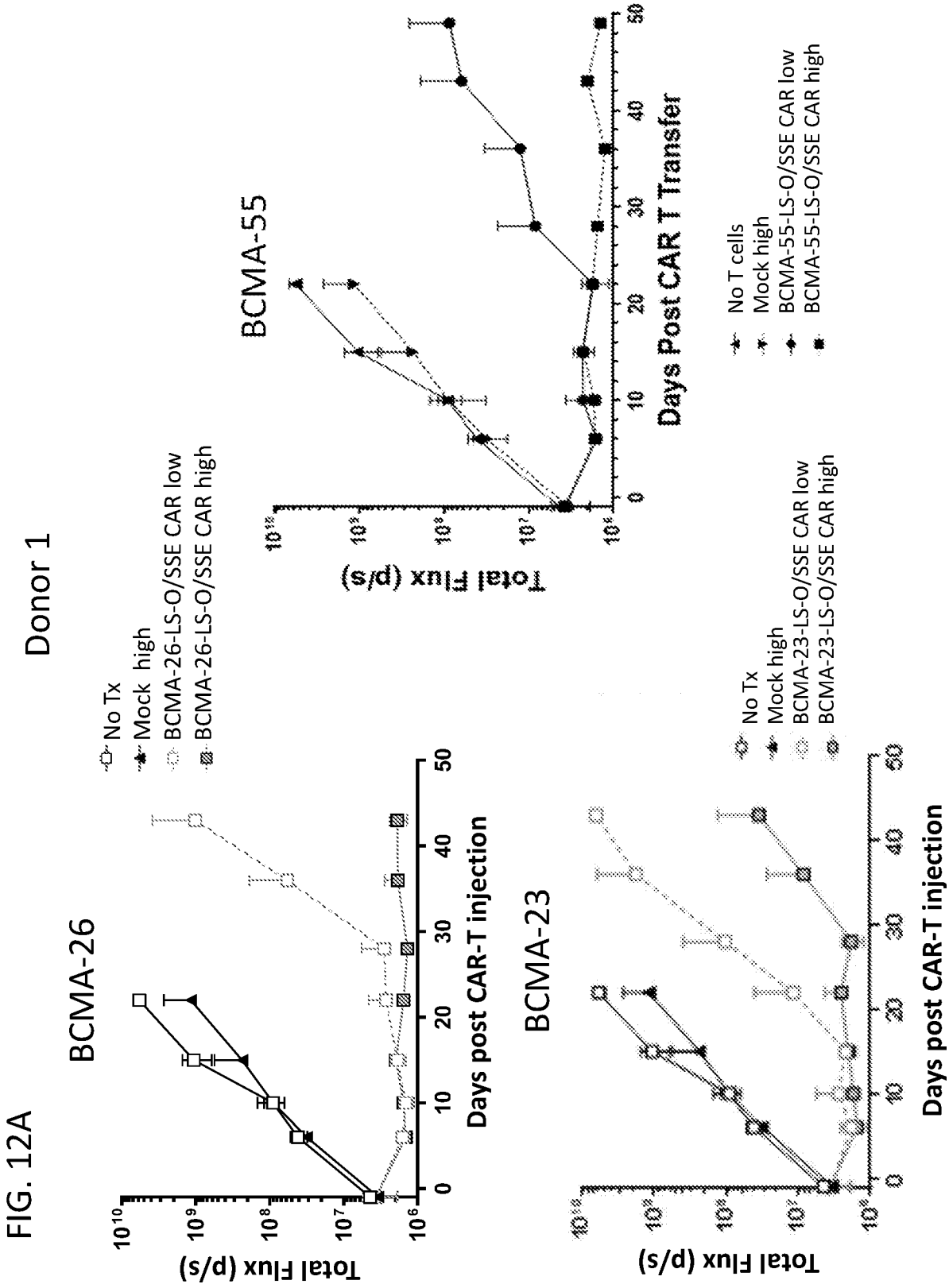

Donor 1
BCMA-26
No Tx
Mock high
BCMA-26-LS-O/SSE CAR low
BCMA-26-LS-O/SSE CAR high
Total Flux (p/s)
Days post CAR-T injection
BCMA-55
Total Flux (p/s)
Days Post CAR T Transfer
No T cells
Mock high
BCMA-55-LS-O/SSE CAR low
BCMA-55-LS-O/SSE CAR high
BCMA-23
No Tx
Mock high
BCMA-23-LS-O/SSE CAR low
BCMA-23-LS-O/SSE CAR high
Total Flux (p/s)
Days post CAR-T injection

FIG. 12B

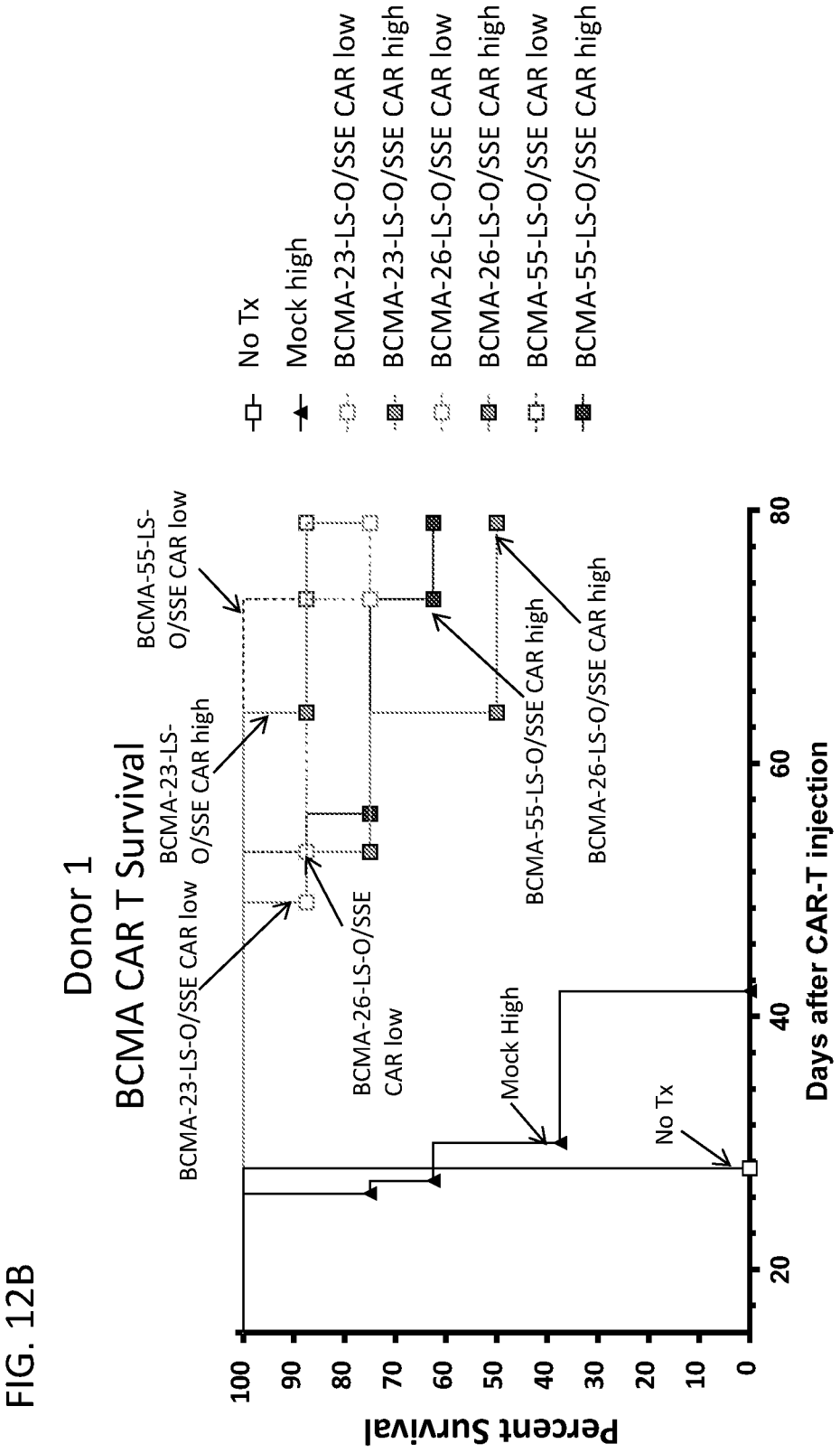

Donor 1
BCMA CAR T Survival
Percent Survival
Days after CAR-T injection
0
10
20
30
40
50
60
70
80
90
100
20
40
60
80
No Tx
Mock high
BCMA-23-LS-O/SSE CAR low
BCMA-23-LS-O/SSE CAR high
BCMA-26-LS-O/SSE CAR low
BCMA-26-LS-O/SSE CAR high
BCMA-55-LS-O/SSE CAR low
BCMA-55-LS-O/SSE CAR high
BCMA-55-LS-O/SSE CAR low
BCMA-23-LS-O/SSE CAR high
BCMA-26-LS-O/SSE CAR low
Mock High
No Tx
BCMA-55-LS-O/SSE CAR high
BCMA-26-LS-O/SSE CAR high

FIG. 13A

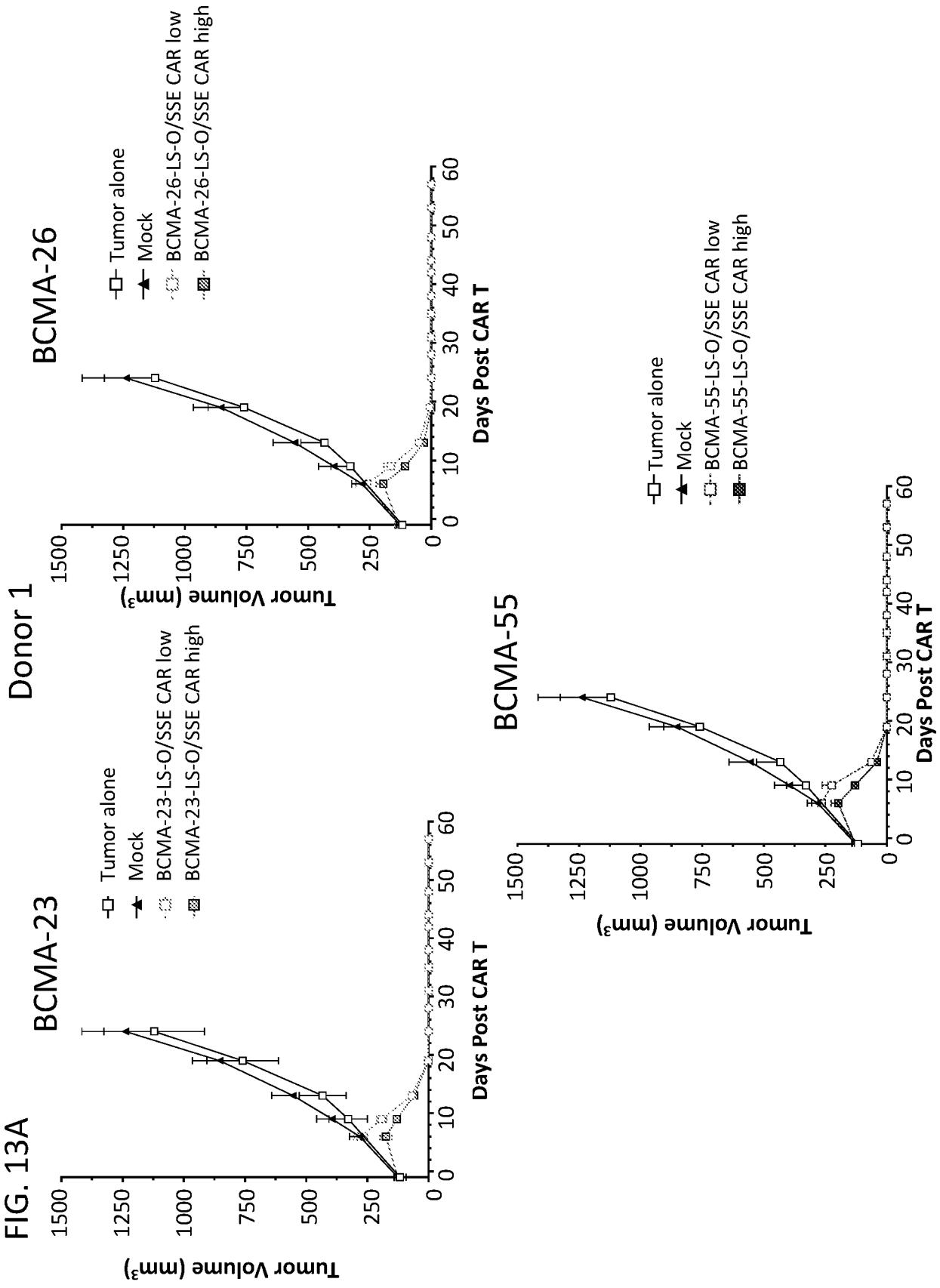

Donor 1
BCMA-23
BCMA-26
BCMA-55
Tumor alone
Mock
BCMA-23-LS-O/SSE CAR low
BCMA-23-LS-O/SSE CAR high
BCMA-26-LS-O/SSE CAR low
BCMA-26-LS-O/SSE CAR high
BCMA-55-LS-O/SSE CAR low
BCMA-55-LS-O/SSE CAR high
Tumor Volume (mm3)
Days Post CAR T
0
250
500
750
1000
1250
1500
0
10
20
30
40
50
60

FIG. 13B

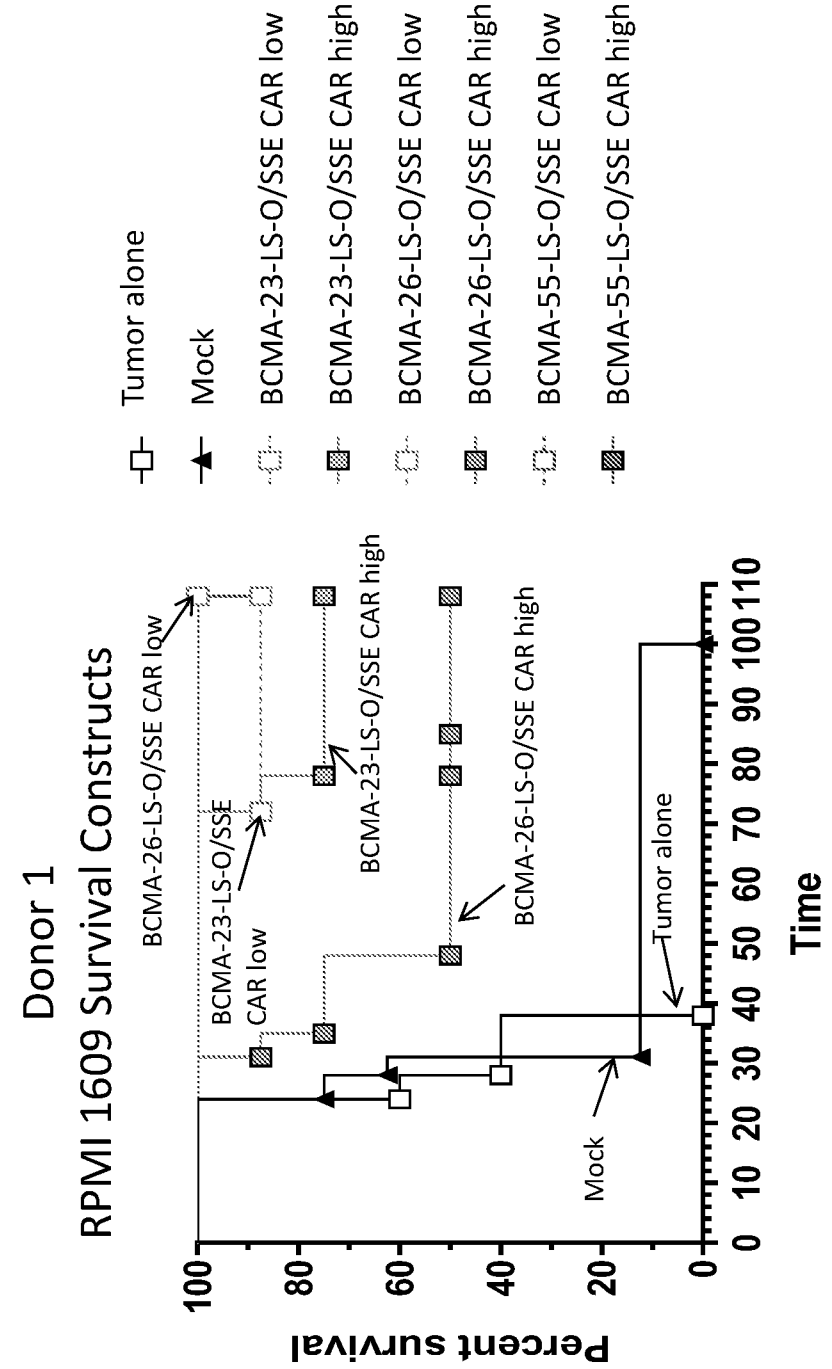

Donor 1
RPMI 1609 Survival Constructs
Tumor alone
Mock
BCMA-23-LS-O/SSE CAR low
BCMA-23-LS-O/SSE CAR high
BCMA-26-LS-O/SSE CAR low
BCMA-26-LS-O/SSE CAR high
BCMA-55-LS-O/SSE CAR low
BCMA-55-LS-O/SSE CAR high
Percent survival
Time
0
20
40
60
80
100
0 10 20 30 40 50 60 70 80 90 100 110
BCMA-26-LS-O/SSE CAR low
BCMA-23-LS-O/SSE CAR low
BCMA-23-LS-O/SSE CAR high
BCMA-26-LS-O/SSE CAR high
Mock
Tumor alone

FIG. 14A

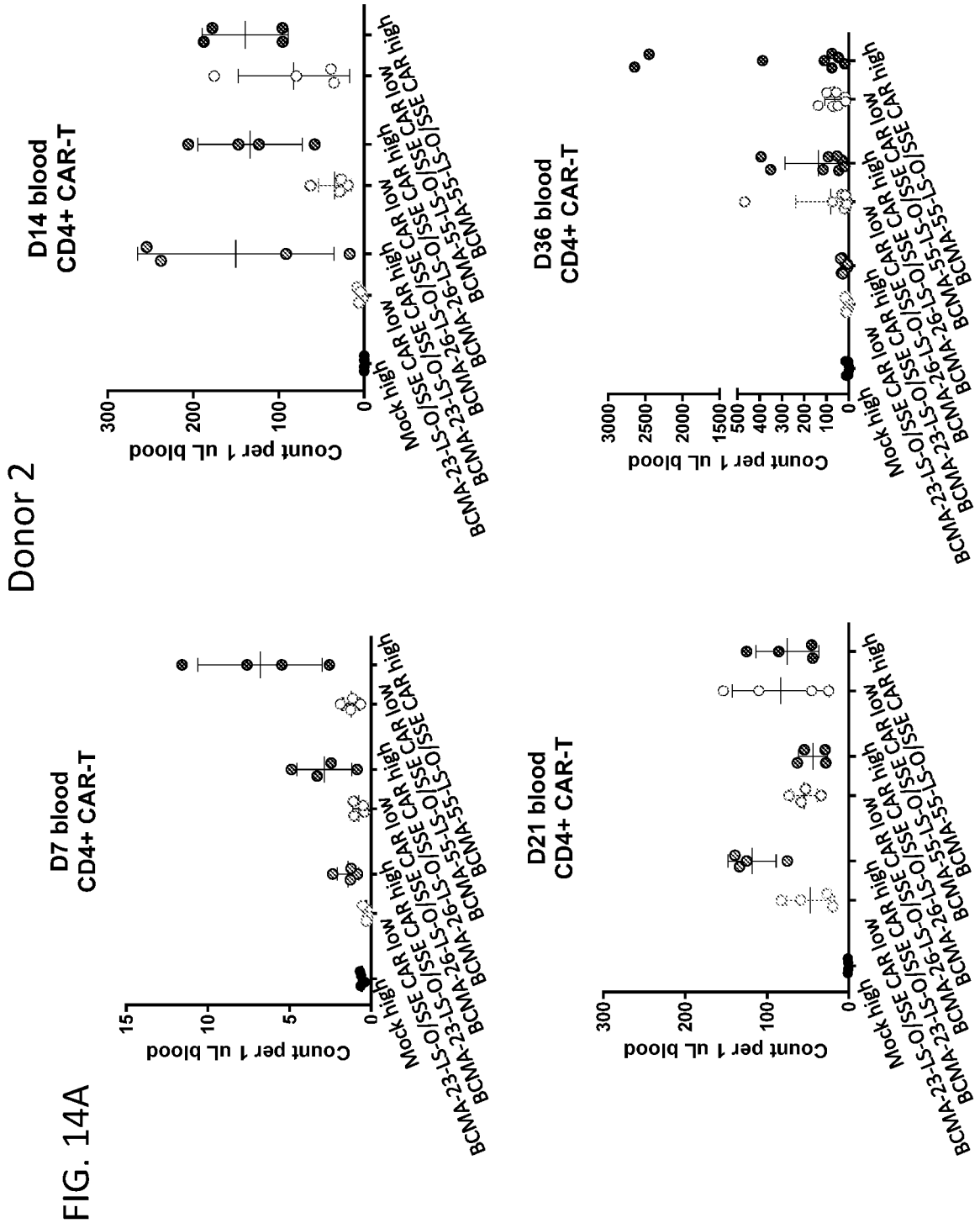

Donor 2
D7 blood
CD4+ CAR-T
D14 blood
CD4+ CAR-T
D21 blood
CD4+ CAR-T
D36 blood
CD4+ CAR-T
Count per 1 uL blood
Mock high
BCMA-23-LS-O|SSE CAR low high
BCMA-23-LS-O|SSE CAR high low
BCMA-26-LS-O|SSE CAR low high
BCMA-26-LS-O|SSE CAR high low
BCMA-55-LS-O|SSE CAR low high
BCMA-55-LS-O|SSE CAR high low

FIG. 14B

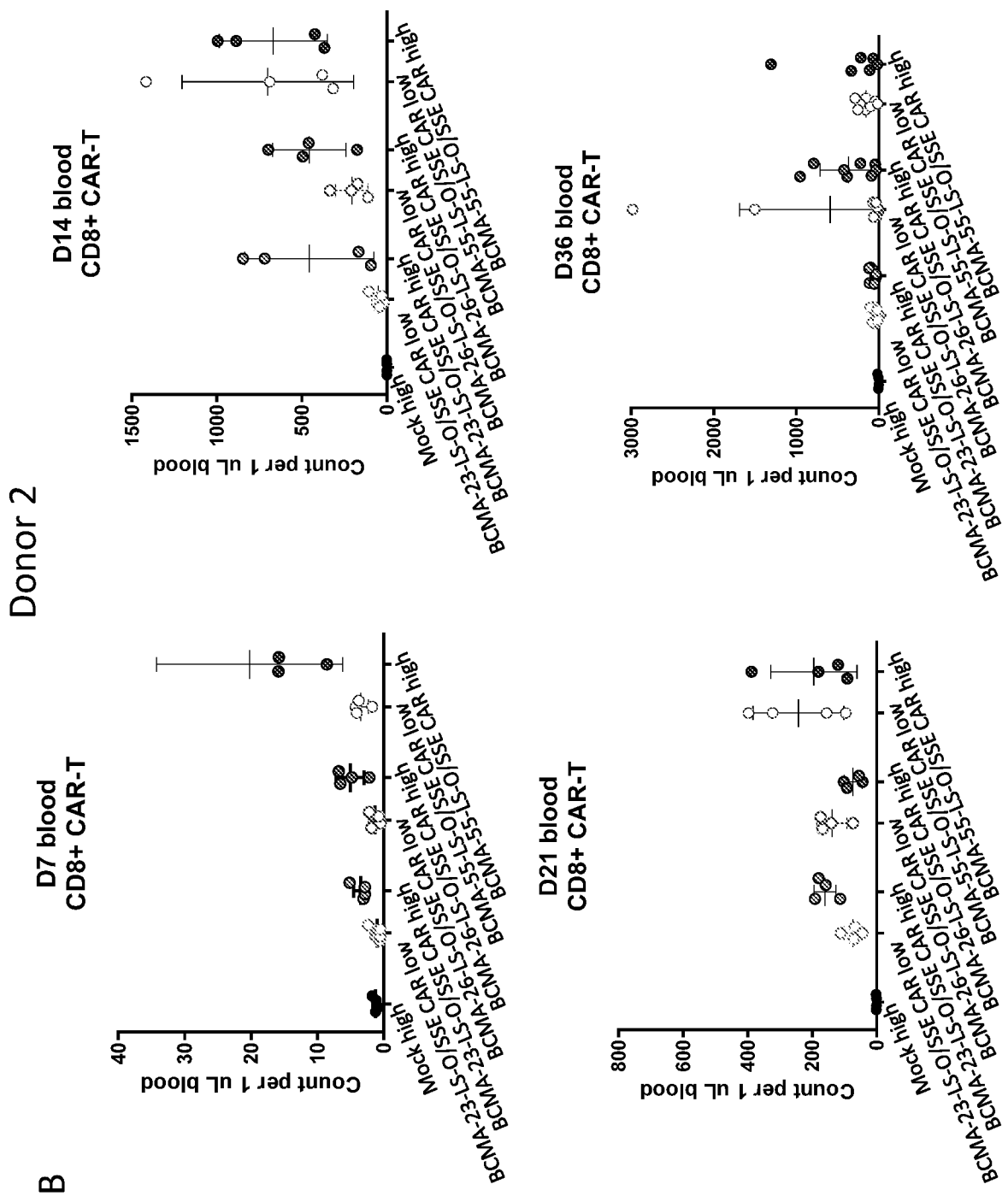

Donor 2
D7 blood
CD8+ CAR-T
D14 blood
CD8+ CAR-T
D21 blood
CD8+ CAR-T
D36 blood
CD8+ CAR-T
Count per 1 uL blood
Mock high
BCMA-23-LS-O|SSE CAR low
BCMA-23-LS-O|SSE CAR high
BCMA-26-LS-O|SSE CAR low
BCMA-26-LS-O|SSE CAR high
BCMA-55-LS-O|SSE CAR low
BCMA-55-LS-O|SSE CAR high

FIG. 15A

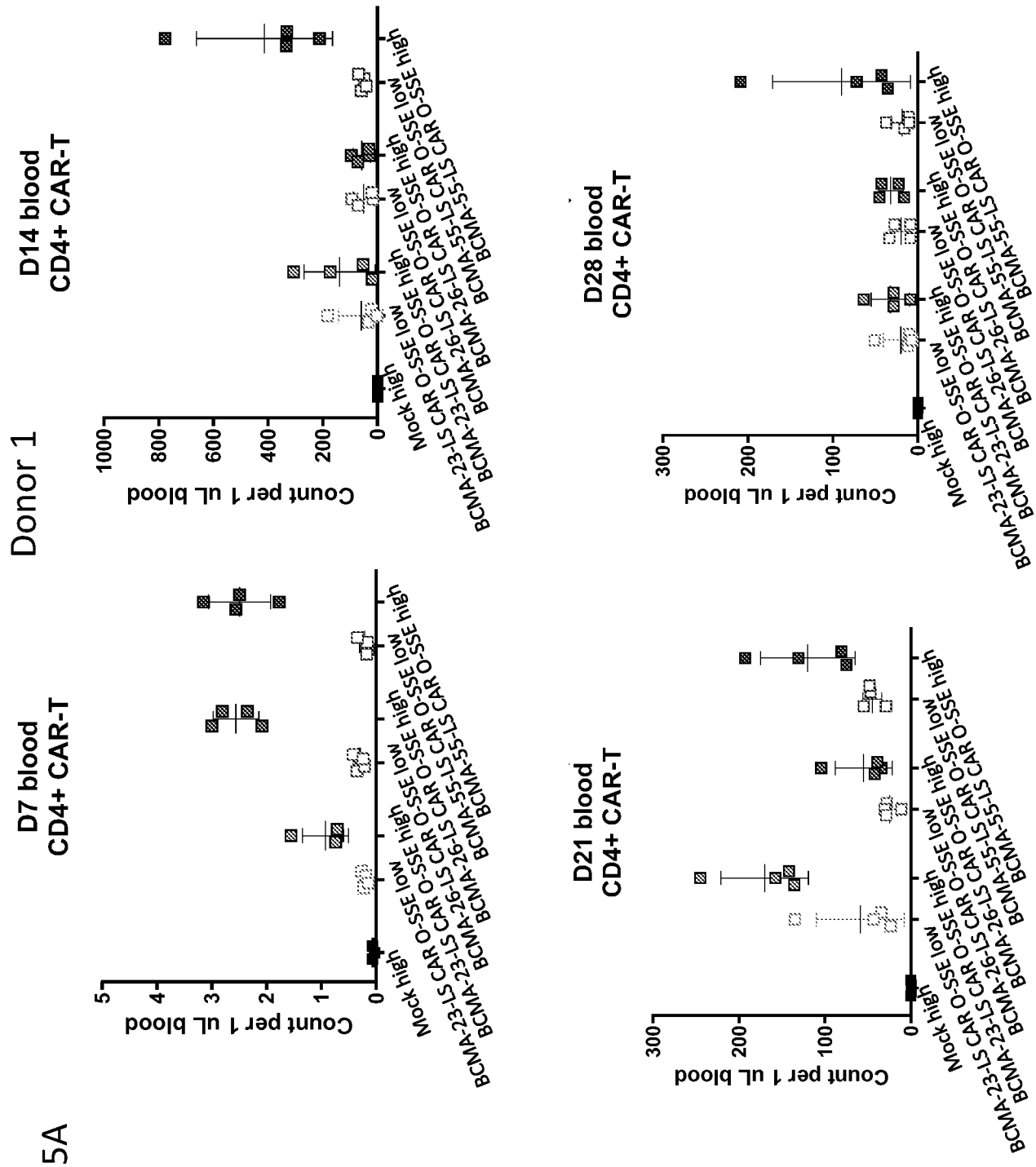
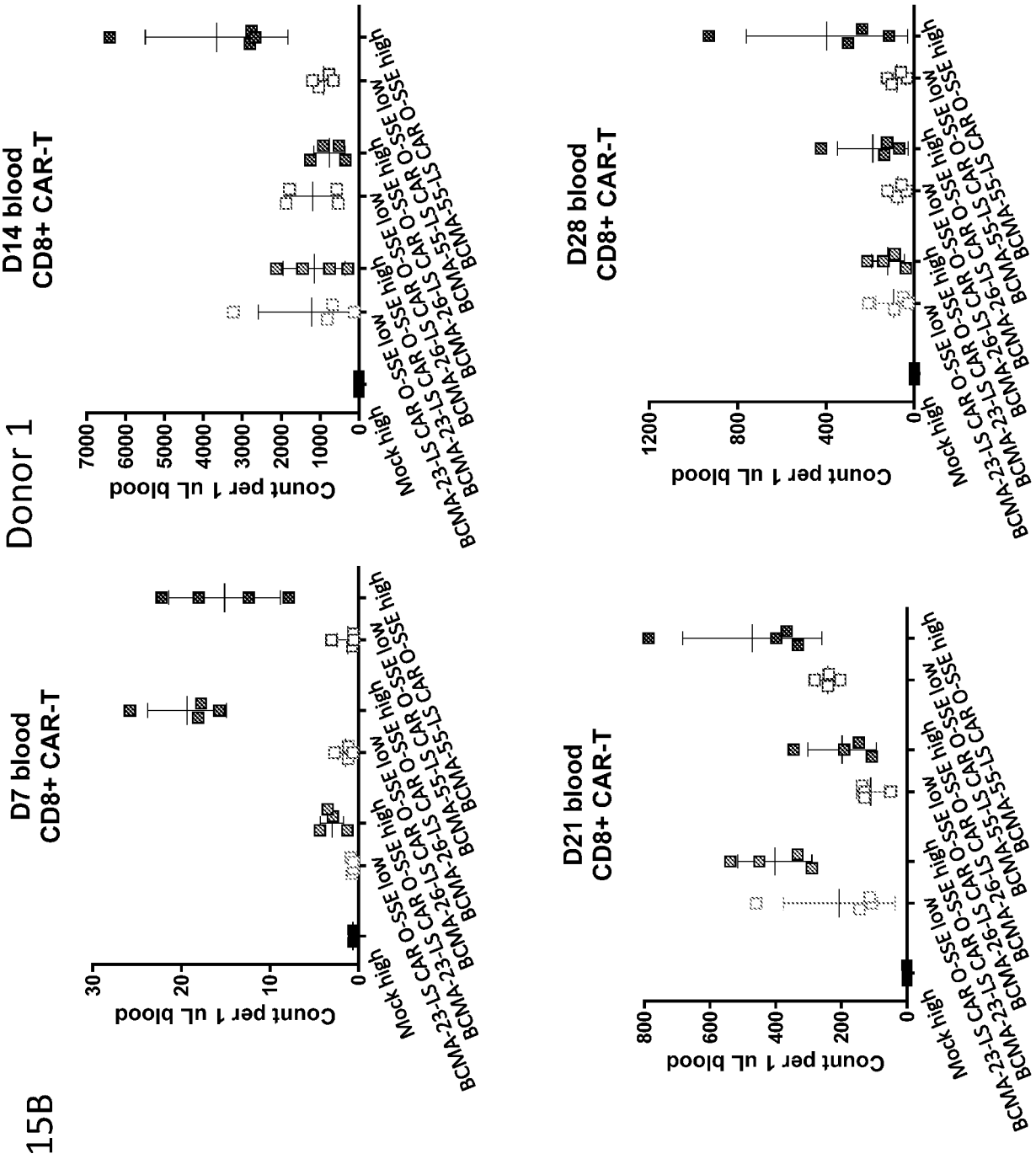

Donor 1
D14 blood
CD4+ CAR-T
D7 blood
CD4+ CAR-T
D28 blood
CD4+ CAR-T
D21 blood
CD4+ CAR-T
Count per 1 uL blood
Mock high
BCMA-23-LS CAR O-SSE low
BCMA-23-LS CAR O-SSE high
BCMA-26-LS CAR O-SSE low
BCMA-26-LS CAR O-SSE high
BCMA-55-LS CAR O-SSE low
BCMA-55-LS CAR O-SSE high

FIG. 15B


Donor 1
D14 blood
CD8+ CAR-T
D7 blood
CD8+ CAR-T
D28 blood
CD8+ CAR-T
D21 blood
CD8+ CAR-T
Count per 1 uL blood
Mock high
BCMA-23-LS CAR O-SSE low high
BCMA-23-LS CAR O-SSE high low
BCMA-26-LS CAR O-SSE low high
BCMA-26-LS CAR O-SSE high low
BCMA-55-LS CAR O-SSE low high
BCMA-55-LS CAR O-SSE high low

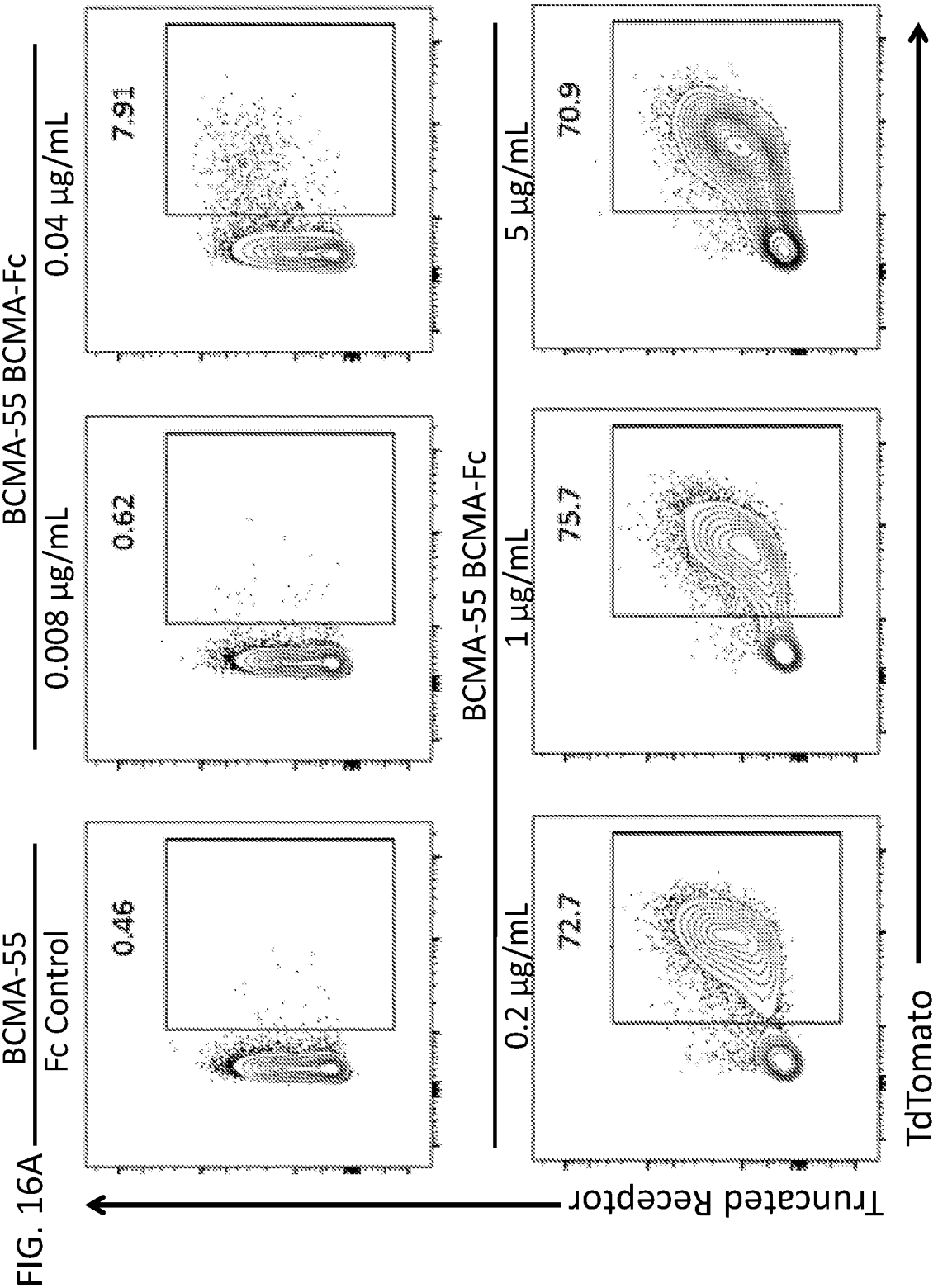

BCMA-55
Fc Control
BCMA-55 BCMA-Fc
0.008 µg/mL
0.04 µg/mL
BCMA-55 BCMA-Fc
0.2 µg/mL
1 µg/mL
5 µg/mL
0.46
0.62
7.91
72.7
75.7
70.9
Truncated Receptor
TdTomato

FIG. 16A

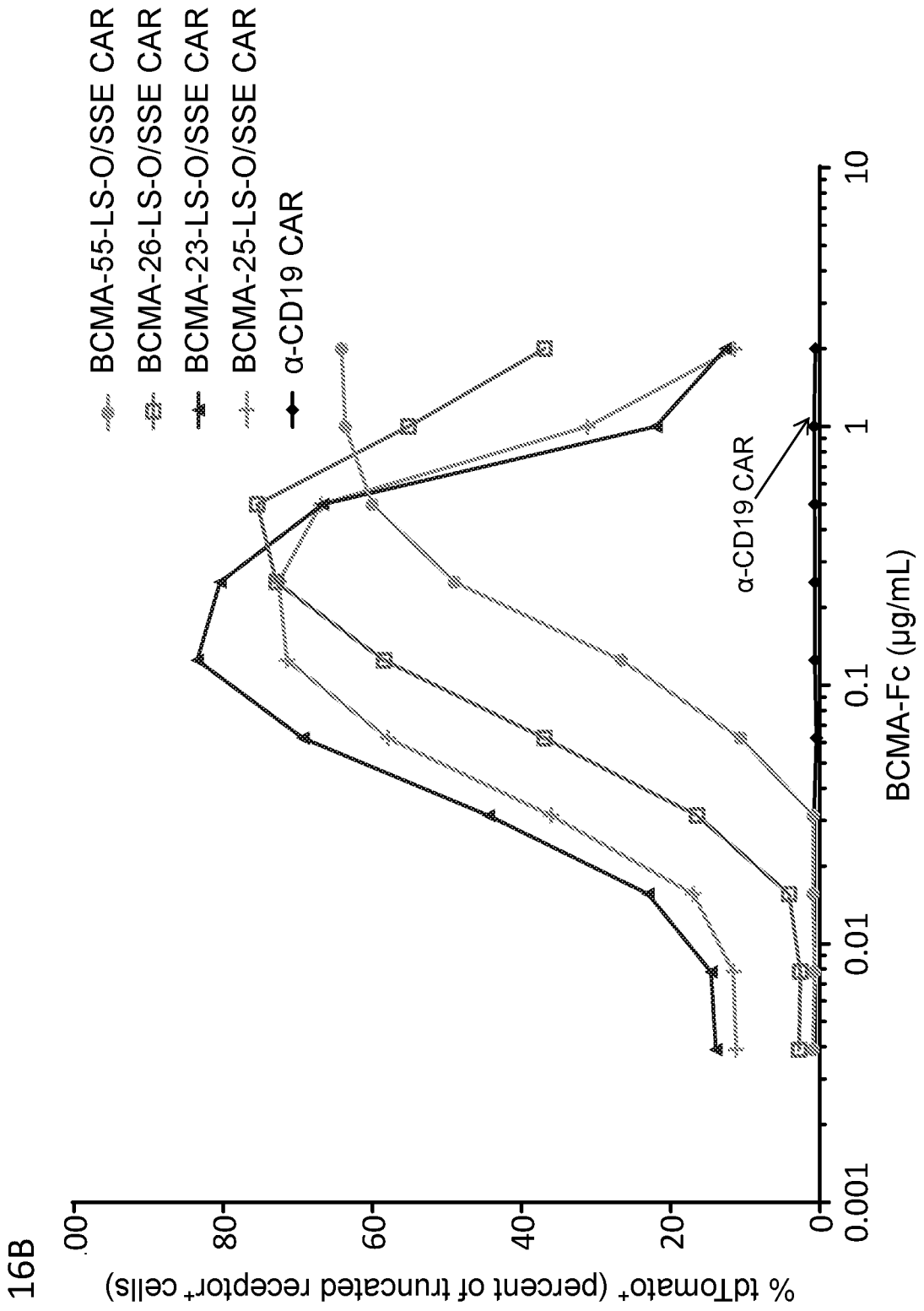

BCMA-55-LS-O/SSE CAR
BCMA-26-LS-O/SSE CAR
BCMA-23-LS-O/SSE CAR
BCMA-25-LS-O/SSE CAR
α-CD19 CAR
α-CD19 CAR
% tdTomato+ (percent of truncated receptor+ cells)
0
20
40
60
80
100
0.001
0.01
0.1
1
10
BCMA-Fc (μg/mL)

FIG. 16B

FIG. 17

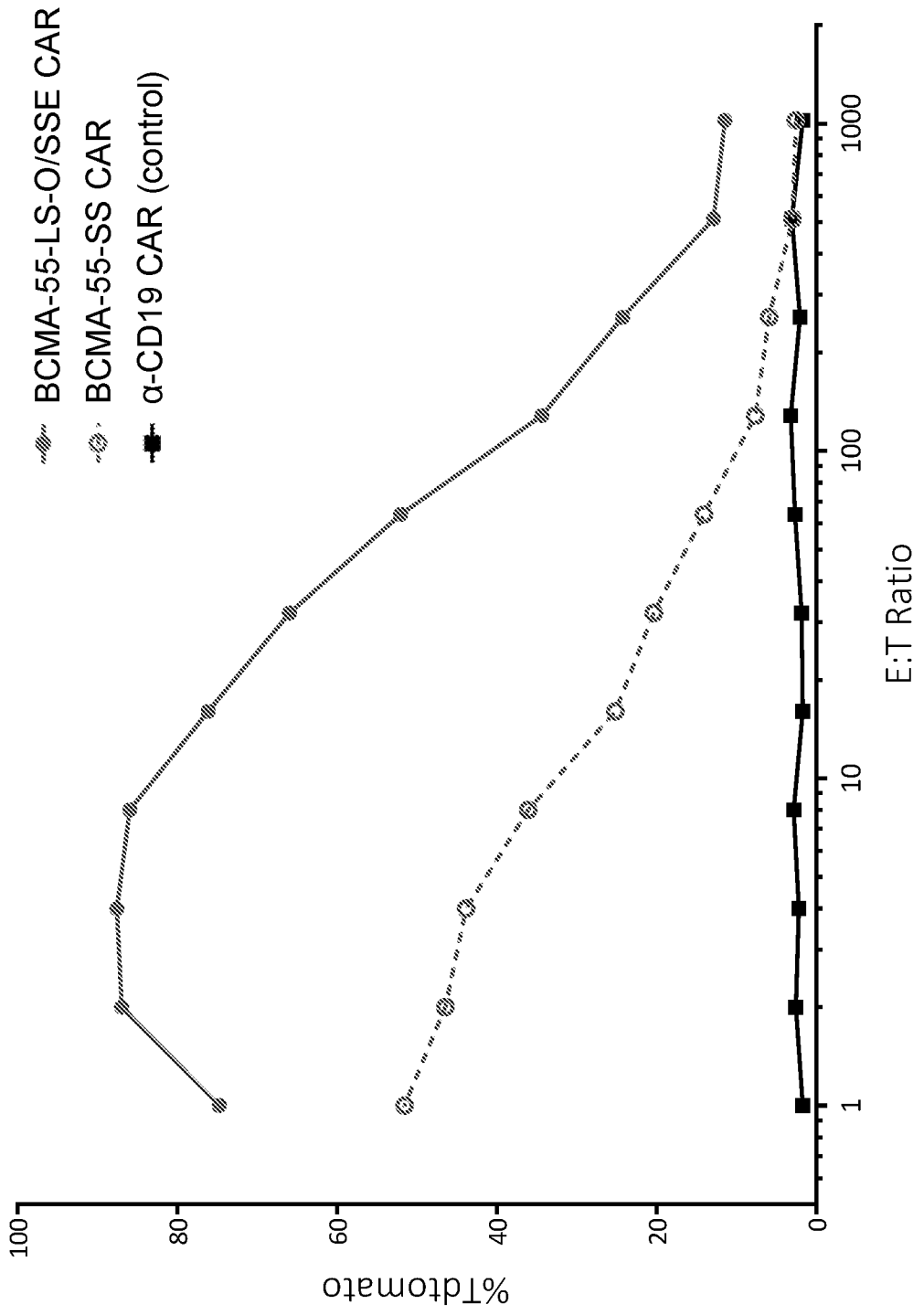
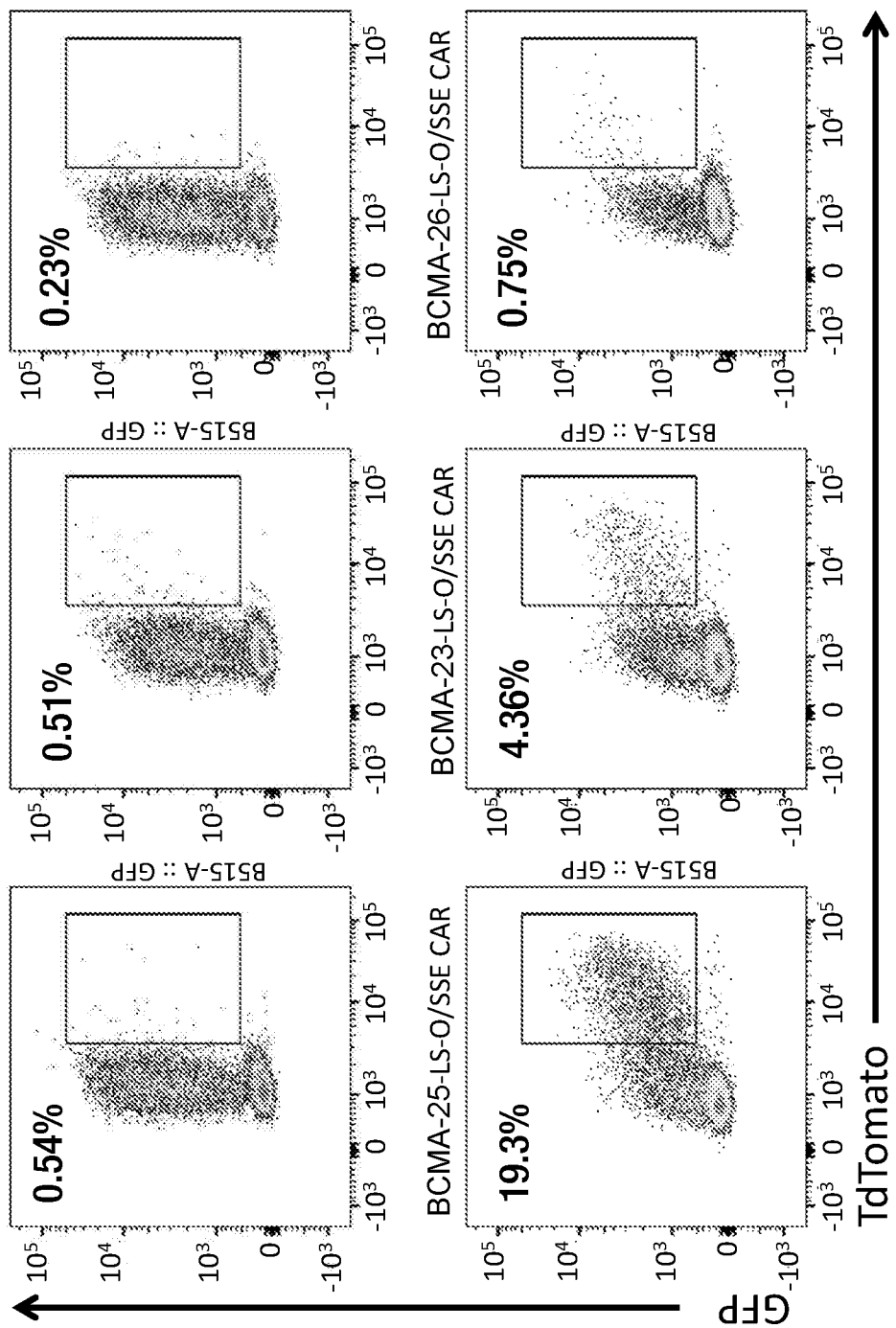

Co-culture of anti-BCMA CAR-expressing Nur77-tdTomato reporter cells with BCMA.K562 target cells
BCMA-55-LS-O/SSE CAR
BCMA-55-SS CAR
α-CD19 CAR (control)
%Tdtomato
100
80
60
40
20
0
1
10
100
1000
E:T Ratio

FIG. 18


α-CD19 CAR
0.54%
BCMA-52-LS-O/SSE CAR
0.51%
BCMA-55-LS-O/SSE CAR
0.23%
BCMA-25-LS-O/SSE CAR
19.3%
BCMA-23-LS-O/SSE CAR
4.36%
BCMA-26-LS-O/SSE CAR
0.75%
B515-A :: GFP
GFP
TdTomato

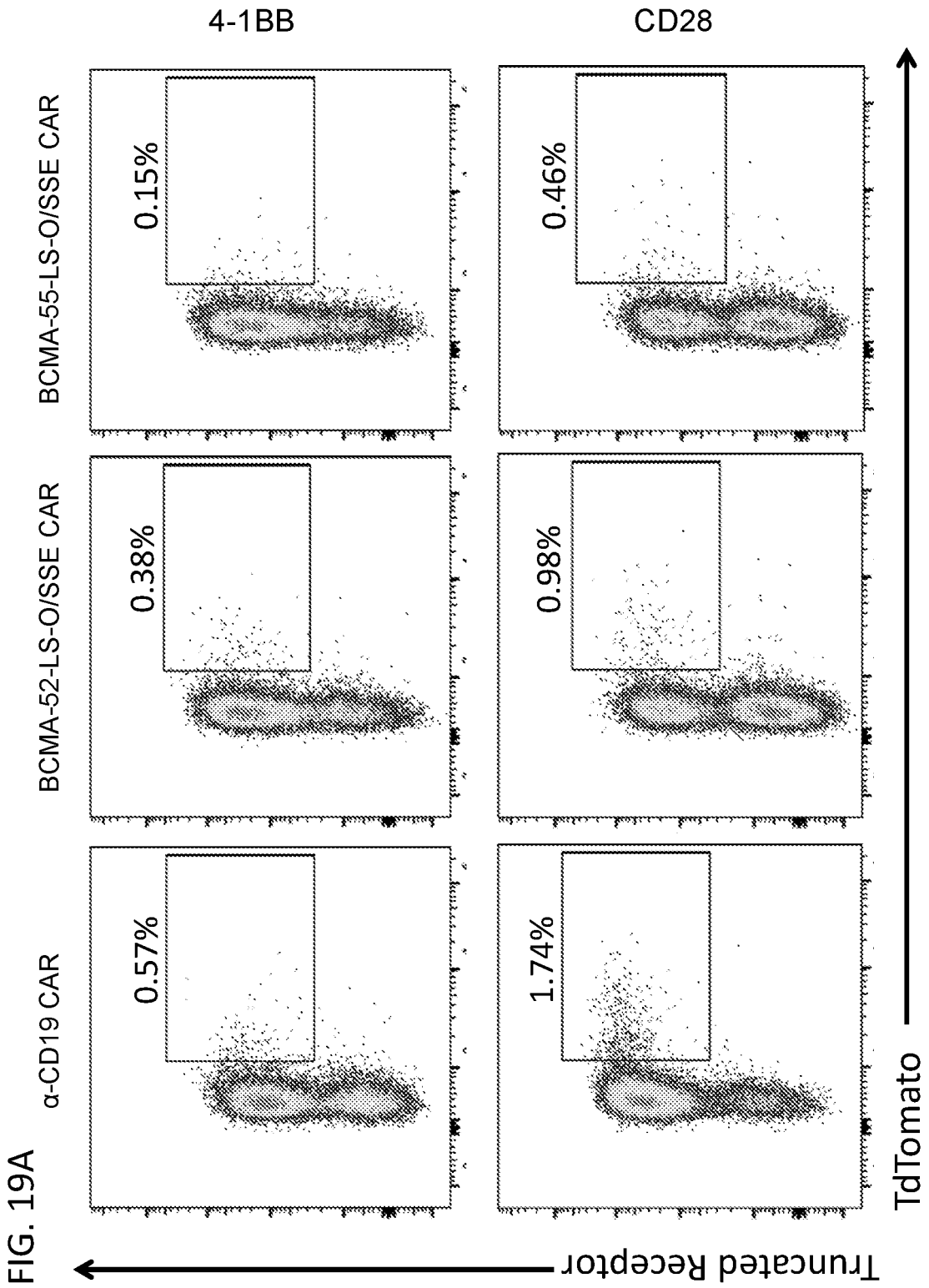

4-1BB
CD28
BCMA-55-LS-O/SSE CAR
BCMA-52-LS-O/SSE CAR
α-CD19 CAR
0.15%
0.46%
0.38%
0.98%
0.57%
1.74%
Truncated Receptor
TdTomato

FIG. 19A

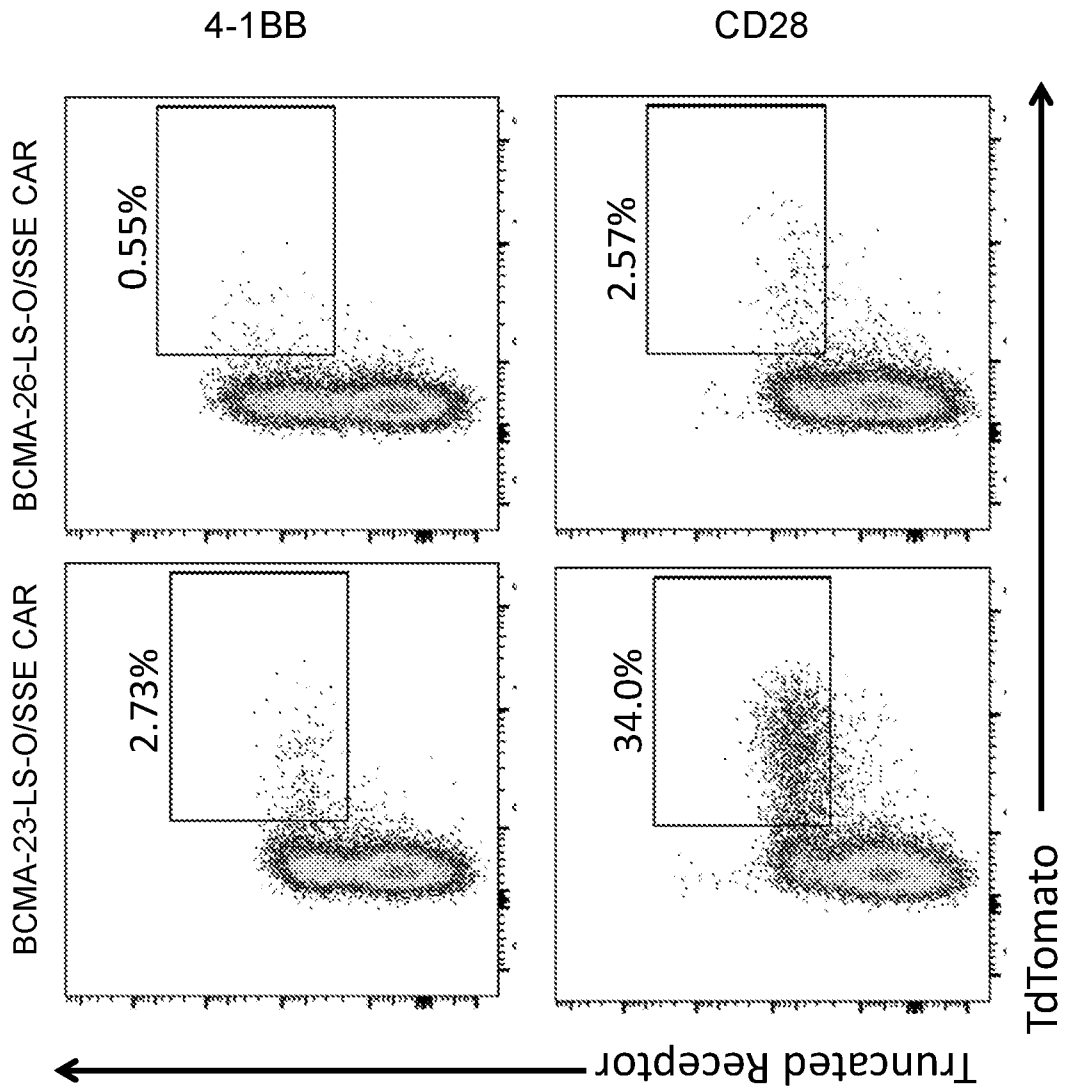

4-1BB
CD28
BCMA-26-LS-O/SSE CAR
BCMA-23-LS-O/SSE CAR
0.55%
2.57%
2.73%
34.0%
Truncated Receptor
TdTomato

FIG. 19B

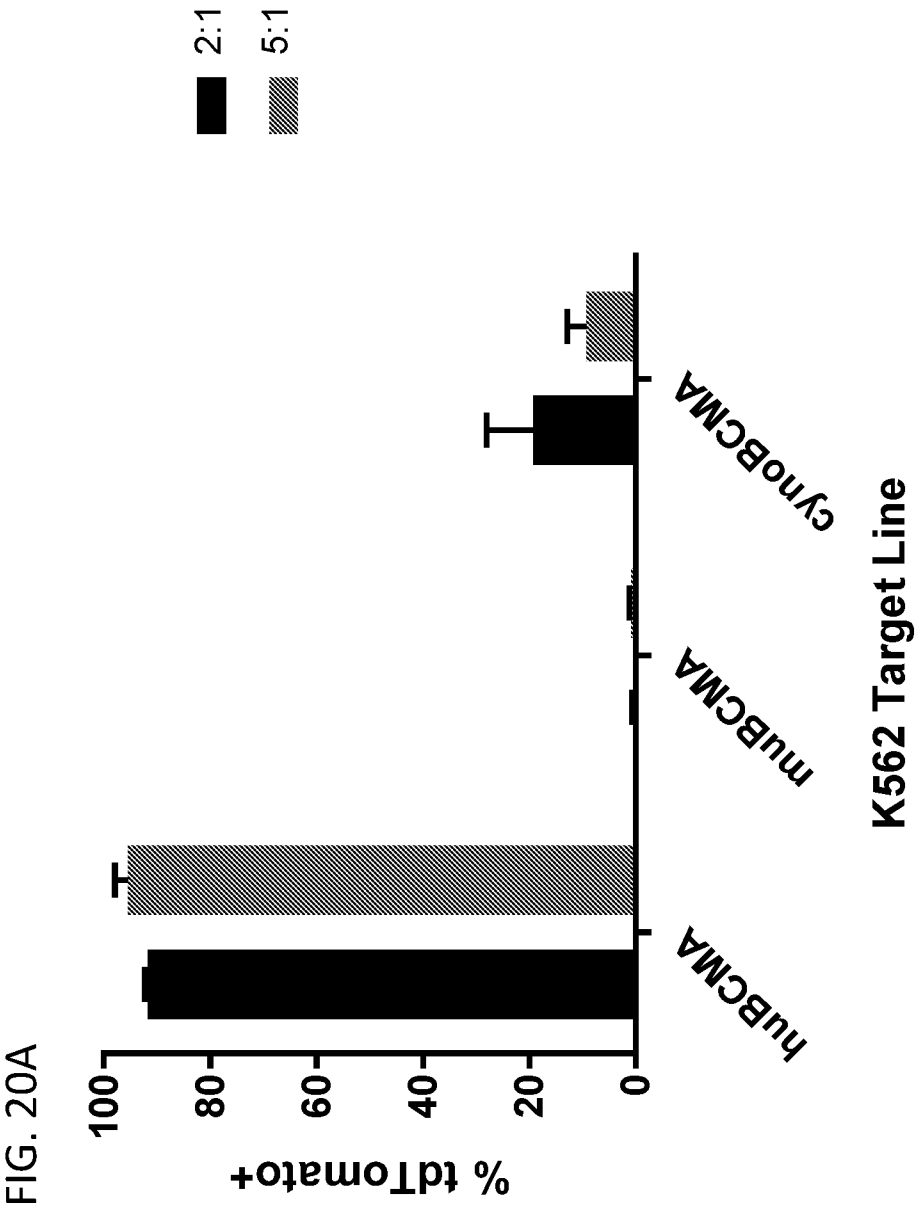

2:1
5:1
% tdTomato+
100
80
60
40
20
0
huBCMA
muBCMA
cynoBCMA
K562 Target Line

FIG. 20A

FIG. 20C

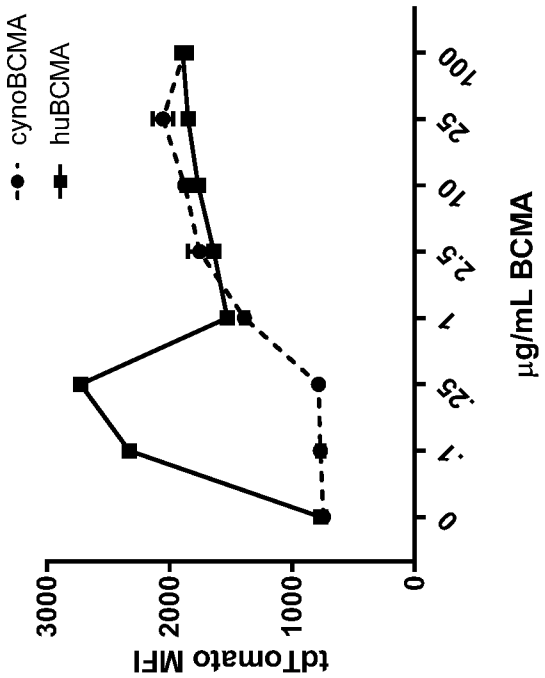


FIG. 20B

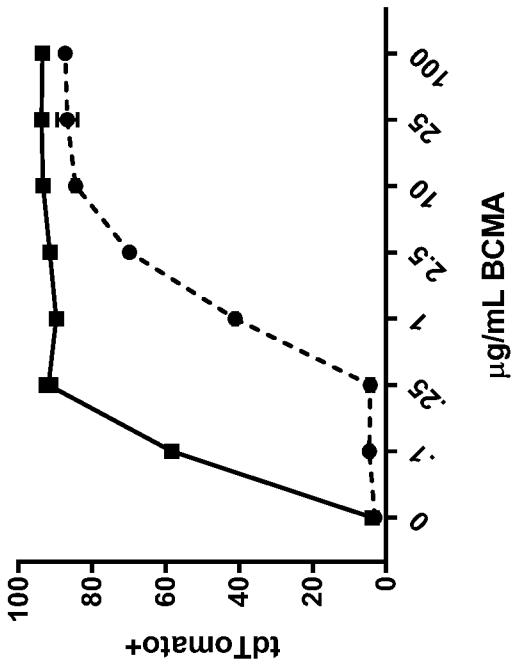


FIG. 21A

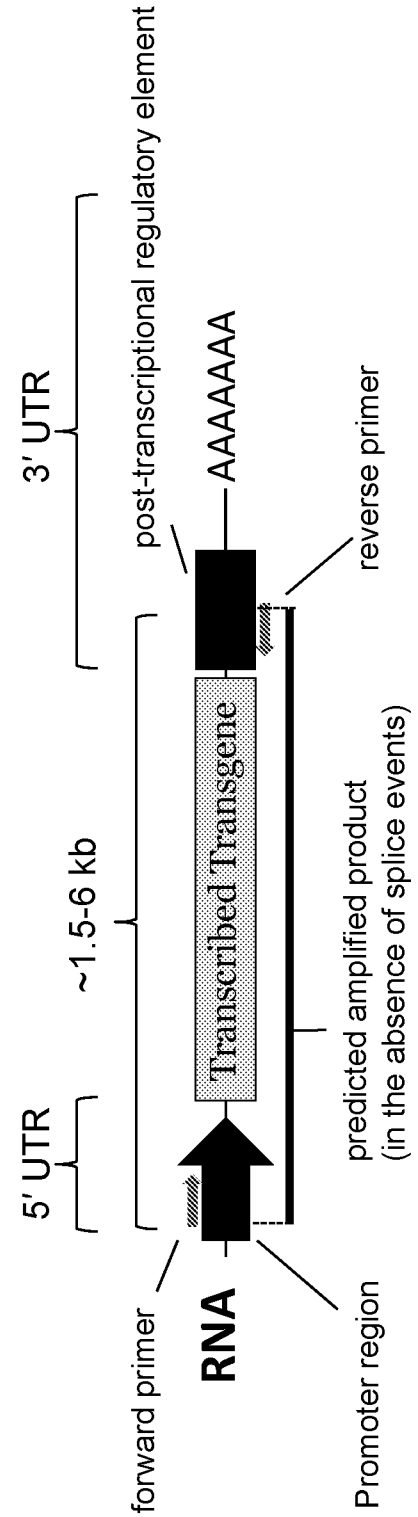


FIG. 21B

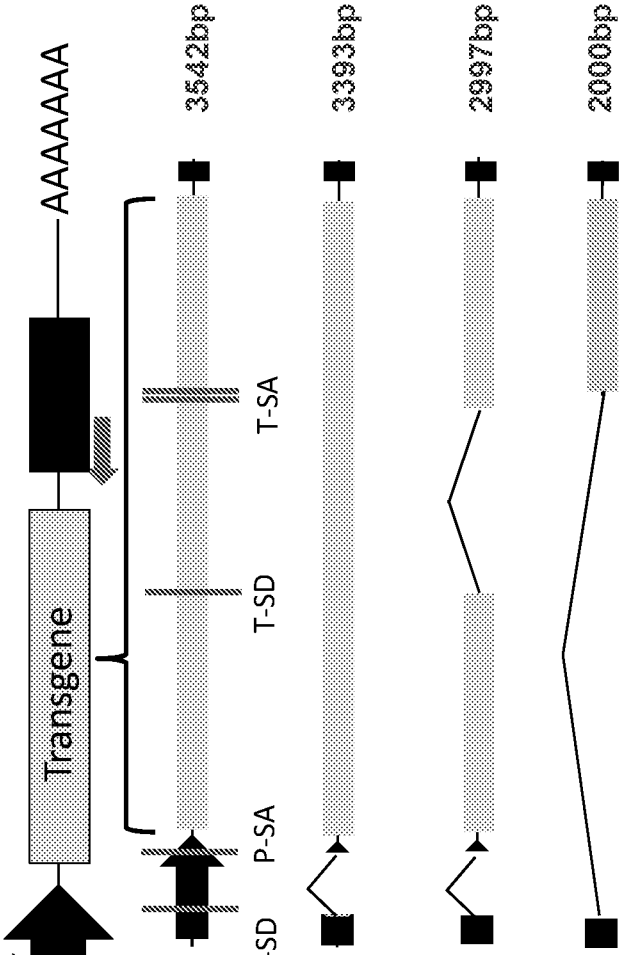
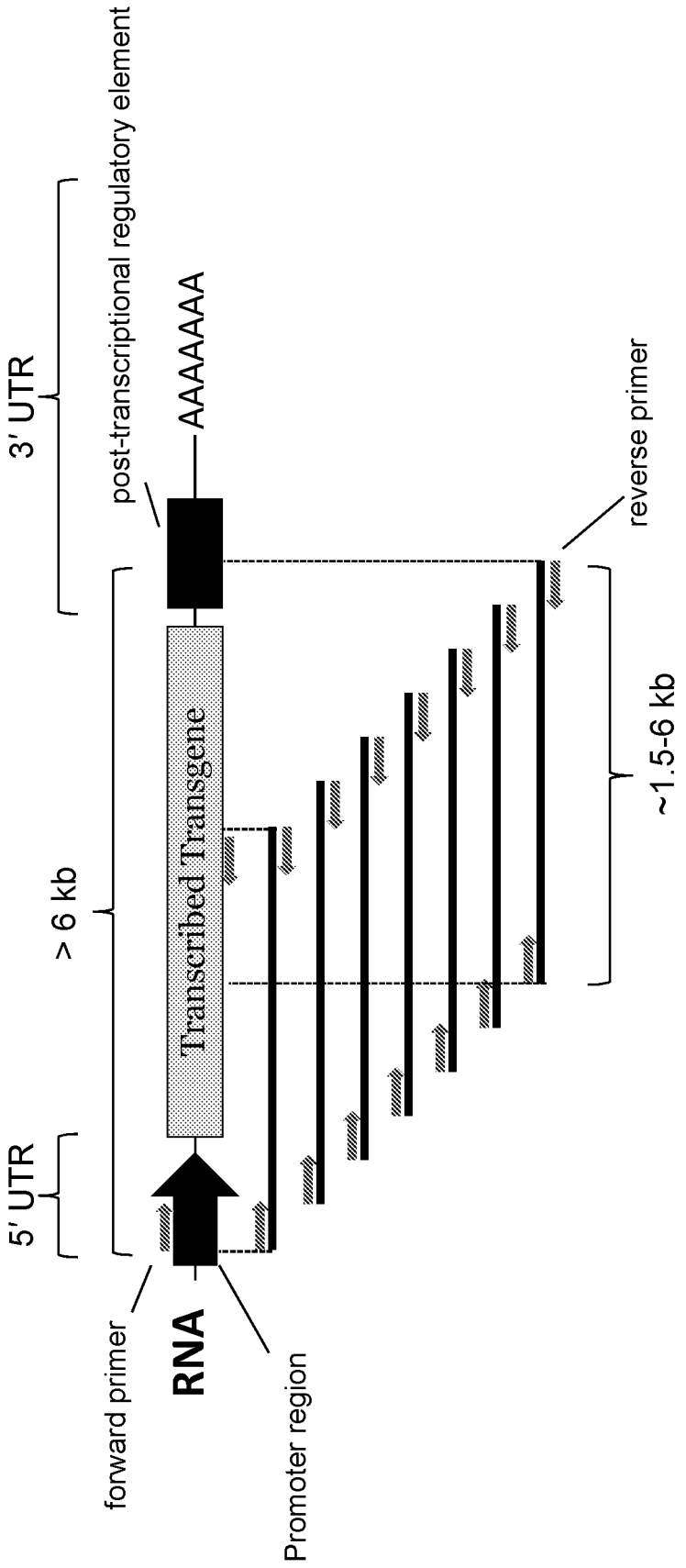


FIG. 21C


3' UTR
post-transcriptional regulatory element
AAAAAAA
reverse primer
> 6 kb
Transcribed Transgene
~1.5-6 kb
5' UTR
forward primer
RNA
Promoter region

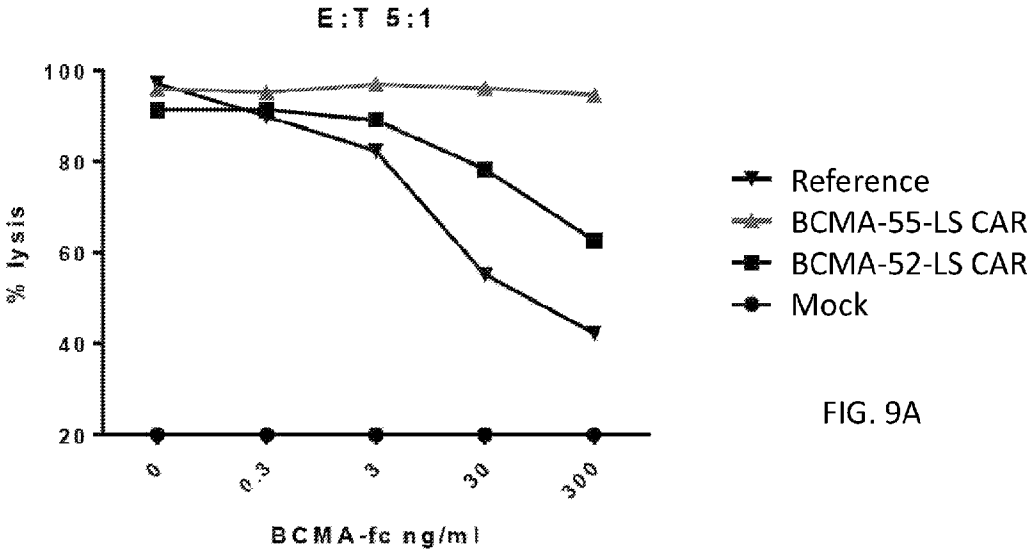

E:T 5:1
100
80
60
40
20
% lysis
0
0.3
3
30
300
BCMA-fc ng/ml
Reference
BCMA-55-LS CAR
BCMA-52-LS CAR
Mock

FIG. 9A