

CHIMERIC RECEPTORS AND METHODS OF USE THEREOF

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of U.S. Provisional Patent Application No. 62/317,068, filed April 1, 2016, which is hereby incorporated by reference in its entirety.

5

SEQUENCE LISTING

[0002] The instant application contains a Sequence Listing which has been submitted electronically in ASCII format and is hereby incorporated by reference in its entirety. Said ASCII copy, created on March 31, 2017, is named K-1029_02_SL.txt and is 265,829 bytes in size.

10

BACKGROUND OF THE INVENTION

[0003] C-type lectin-like-1 (CLL-1, also known as CLEC-1, CLEC12A, MICL, Dendritic Cell-Associated Lectin-1 (DCAL-1), and DCAL-2) is a glycoprotein receptor and member of a family of C-type lectin-like receptors involved in the regulation of cell proliferation and immune regulation. CLL-1 is expressed in hematopoietic cells, primarily on innate immune cells including monocytes, granulocytes, dendritic cells, as well as myeloid progenitor cells. Van Rhenen *et al.*, Blood 2007:110(7). CLL-1 has been implicated in the regulation of myeloid cell proliferation and differentiation (Bakker *et al.*, Cancer Res. 64:8443-8450 (2004); Marshall *et al.*, J. Biol. Chem. 279:14792-14802 (2004)), and is present on acute myeloid (myelogenous) leukemia (AML) cells as well as on leukemic stem cells (Zhao *et al.*, Haematologica 2010, 95(1):71-78).

15

20

[0004] Accordingly, CLL-1 has been implicated in multiple diseases, including but not limited to, acute myeloid (myelogenous) leukemia (AML), chronic myeloid (myelogenous) leukemia (CML), chronic myelomonocytic leukemia (CMML), juvenile myelomonocytic leukemia, atypical chronic myeloid leukemia, acute promyelocytic leukemia (APL), acute monocytic leukemia, acute monoblastic leukemia, acute erythroid leukemia, acute megakaryoblastic leukemia, myelodysplastic syndrome (MDS), myeloproliferative disorder, myeloid neoplasm, myeloid sarcoma), Blastic Plasmacytoid Dendritic Cell Neoplasm (BPDCN), or combinations thereof.

25

30

[0005] CLL-1 may additionally play a role in inflammatory or autoimmune diseases such as rheumatoid arthritis, psoriasis, allergies, asthma, Crohn's disease, IBD, IBS, fibromyalgia, mastocytosis, and Celiac disease.

[0006] Human CLL-1 protein comprises a polypeptide of the following amino acid
5 sequence:

MSEEVTYADLQFQNSSEMEKIPEIGKFGEKAPPAPSHVWRPAALFLTLLCLL
LLIGLGVLASMFHVTLKIEMKKMNKLQNISEELQRNISLQLMSNMNISKIR
NLSTTLQTIATKLCRELYSKEQEHKCKPCRRWIWHKDSCYFLSDDVQWTWQ
ESKMACAAQNASLLKINNKNALFIKSQSRSDYWLGLSPEEDSTRGMRVD
10 NIINSSAWVIRNAPDLNNMYCGYINRLYVQYYHCTYKKRMICEKMANPVQ
LGSTYFREA (SEQ ID NO. 140).

[0007] Additional sequence information is contained in the CLL-1 Uniprot listing at: <http://www.uniprot.org/uniprot/Q5OGZ9>, as well as NCBI Reference Sequence NP_612210.4 (http://www.ncbi.nlm.nih.gov/protein/NP_612210.4).

15 [0008] When referring to CLL-1, it will be appreciated that reference thereto encompasses fragments thereof, as well as related polypeptides, which include, but are not limited to, allelic variants, splice variants, derivative variants, substitution variants, deletion variants, and/or insertion variants including the addition of an N-terminal methionine, fusion polypeptides, and interspecies homologs. In certain embodiments, a CLL-1 polypeptide
20 includes terminal residues, such as, but not limited to, leader sequence residues, targeting residues, amino terminal methionine residues, lysine residues, tag residues and/or fusion protein residues.

[0009] Certain antibodies to CLL-1 are described in U.S. Patent No. 8,536,310 and in U.S. Patent No. 9,163,090.

25 [0010] Engineered immune cells have been shown to possess desired qualities in therapeutic treatments, particularly in oncology. Two main types of engineered immune cells are those that contain chimeric antigen receptors (termed "CARs" or "CAR-Ts") and T-cell receptors ("TCRs"). These engineered cells are engineered to endow them with antigen specificity while retaining or enhancing their ability to recognize and kill a target cell.
30 Chimeric antigen receptors may comprise, for example, (i) an antigen-specific component ("antigen binding molecule"), (ii) an extracellular domain, (iii) one or more costimulatory

domains, and (iv) one or more activating domains. Each domain may be heterogeneous, that is, comprised of sequences derived from (or corresponding to) different protein chains. Chimeric antigen receptor-expressing immune cells (such as T cells) may be used in various therapies, including cancer therapies. It will be appreciated that costimulating domains may
5 be used to enhance the activation of CAR-expressing cells against target antigens, and therefore increase the potency of adoptive immunotherapy.

[0011] Certain CARs to CLL-1 have been described in, *e.g.*, U.S. Patent Application 20160051651 (PCT US2015/041337).

[0012] T cells can be engineered to possess specificity to one or more desired targets.
10 For example, T cells can be transduced with DNA or other genetic material encoding an antigen binding molecule, such as one or more single chain variable fragment ("scFv") of an antibody, in conjunction with one or more signaling molecules, and/or one or more activating domains, such as CD3 zeta.

[0013] In addition to the CAR-T cells' ability to recognize and destroy the targeted
15 cells, successful T cell therapy benefits from the CAR-T cells' ability to persist and maintain the ability to proliferate in response to antigen.

[0014] T cell receptors (TCRs) are molecules found on the surface of T cells that are responsible for recognizing antigen fragments as peptides bound to major histocompatibility complex (MHC) molecules. The TCR is comprised of two different protein chains - in
20 approximately 95% of human TCRs, the TCR consists of an alpha (α) and beta (β) chain. In approximately 5% of human T cells the TCR consists of gamma and delta (γ/δ) chains. Each chain is composed of two extracellular domains: a variable (V) region and a constant (C) region, both of the immunoglobulin superfamily. As in other immunoglobulins, the variable domains of the TCR α -chain and β -chain (or gamma and delta (γ/δ) chains) each have three
25 hypervariable or complementarity determining regions (CDRs). When the TCR engages with antigenic peptide and MHC (peptide/MHC), the T cell becomes activated, enabling it to attack and destroy the target cell.

[0015] However, current therapies have shown varying levels of effectiveness with undesired side effects. Therefore, a need exists to identify novel and improved therapies for
30 treating CLL-1 related diseases and disorders.

SUMMARY OF THE INVENTION

[0016] The invention relates to engineered immune cells (such as CARs or TCRs), antigen binding molecules (including but not limited to, antibodies, scFvs, heavy and/or light chains, and CDRs of these antigen binding molecules) with specificity to CLL-1.

5 [0017] The invention further relates to a novel CD28 extracellular (hinge) sequence useful as costimulatory domains in these cells.

[0018] Chimeric antigen receptors of the invention typically comprise: (i) a CLL-1 specific antigen binding molecule, (ii) an extracellular (which may comprise a hinge) domain, (iii) one or more costimulatory domain, and (iv) one or more activating domain. It will be
10 appreciated that each domain may be heterogeneous, thus comprised of sequences derived from (or corresponding to) different protein chains.

[0019] In some embodiments, the invention relates to a chimeric antigen receptor comprising an antigen binding molecule that specifically binds to CLL-1, wherein the antigen binding molecule comprises at least one of: a) a variable heavy chain CDR1 comprising an
15 amino acid sequence selected from the group consisting of SEQ ID NOs: 17, 51, 73, and 95, b) a variable heavy chain CDR2 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs 18, 52, 74, and 96, c) a variable heavy chain CDR3 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs 19, 53, 75, and 97, d) a variable light chain CDR1 comprising an amino acid sequence selected from the group
20 consisting of SEQ ID NOs: 22, 56, 78, and 100, e) a variable light chain CDR2 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs. 23, 57, 79, and 101, and f) a variable light chain CDR3 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs. 24, 58, 80, and 102. The chimeric antigen receptor can further comprise at least one costimulatory domain. The chimeric antigen receptor according
25 to claim 1 further comprising at least one activating domain.

[0020] In certain embodiments, the invention relates to chimeric antigen receptors having at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or 100% identical to the chimeric antigen receptors set forth herein.

30 [0021] Also encompassed by the invention are chimeric antigen receptors having no more than 8 amino acid substitutions thereto.

[0022] In certain embodiments the costimulatory domain comprises a signaling domain (or other suitable portion) of CD28, OX-40, 4-1BB/CD137, CD2, CD7, CD27, CD30, CD40, Programmed Death-1 (PD-1), inducible T cell costimulator (ICOS), lymphocyte function-associated antigen-1 (LFA-1, CD11a/CD18), CD3 gamma, CD3 delta, CD3 epsilon, CD247, CD276 (B7-H3), LIGHT, (TNFSF14), NKG2C, Ig alpha (CD79a), DAP-10, Fc gamma receptor, MHC class 1 molecule, TNF receptor proteins, an Immunoglobulin protein, 5 CD247, CD276 (B7-H3), LIGHT, (TNFSF14), NKG2C, Ig alpha (CD79a), DAP-10, Fc gamma receptor, MHC class 1 molecule, TNF receptor proteins, an Immunoglobulin protein, cytokine receptor, integrins, Signaling Lymphocytic Activation Molecules (SLAM proteins), activating NK cell receptors, BTLA, a Toll ligand receptor, ICAM-1, B7-H3, CDS, ICAM-1, GITR, BAFFR, LIGHT, HVEM (LIGHTR), KIRDS2, SLAMF7, NKp80 (KLRF1), 10 NKp44, NKp30, NKp46, CD19, CD4, CD8alpha, CD8beta, IL-2R beta, IL-2R gamma, IL-7R alpha, ITGA4, VLA1, CD49a, ITGA4, IA4, CD49D, ITGA6, VLA-6, CD49f, ITGAD, CD11d, ITGAE, CD103, ITGAL, CD11a, LFA-1, ITGAM, CD11b, ITGAX, CD11c, ITGB1, CD29, ITGB2, CD18, LFA-1, ITGB7, NKG2D, TNFR2, TRANCE/RANKL, DNAM1 (CD226), SLAMF4 (CD244, 2B4), CD84, CD96 (Tactile), CEACAM1, CRT AM, Ly9 15 (CD229), CD160 (BY55), PSGL1, CD100 (SEMA4D), CD69, SLAMF6 (NTB-A, Lyl08), SLAM (SLAMF1, CD150, IPO-3), BLAME (SLAMF8), SELPLG (CD162), LTBR, LAT, GADS, SLP-76, PAG/Cbp, CD19a, a ligand that specifically binds with CD83, or any combination thereof.

[0023] In some embodiments, the costimulatory domain can comprise all or a portion 20 of the 4-1BB nucleic acid sequence set forth in SEQ ID NO. 141, and the corresponding amino acid sequence as set forth in SEQ ID NO. 142. In other embodiments, the costimulatory domain can comprise all or a portion of the amino acid sequence of OX40 as set forth in SEQ ID NO. 143. See also Hombach *et al.*, *Oncoimmunology*. 2012 Jul. 1; 1(4): 458–466. In still other embodiments, the costimulatory domain can comprise all or a portion 25 of the ICOS molecule as described in Guedan *et al.*, August 14, 2014; *Blood*: 124 (7) and Shen *et al.*, *Journal of Hematology & Oncology* (2013) 6:33. In still other embodiments, the costimulatory domain can comprise all or a portion of CD27 as described in Song *et al.*, *Oncoimmunology*. 2012 Jul. 1; 1(4): 547–549.

[0024] Preferred embodiments include incorporation into the CARs of the invention 30 one or more of the following sequences: SEQ ID NO. 2, SEQ ID NO. 4, SEQ ID NO. 6, and SEQ ID NO. 8. Additional preferred embodiments include incorporation into the CARs of the invention the sequence set forth in SEQ ID NO. 14.

[0025] In further embodiments, the activating domain comprises CD3, preferably CD3 zeta, more preferably CD3 zeta having the sequence set forth in SEQ ID NO. 10.

[0026] In other embodiments, the invention relates to a chimeric antigen receptor comprising an antigen binding molecule further comprising SEQ ID NO. 2 and further
5 comprising SEQ ID NO. 10.

[0027] The invention further relates to isolated polynucleotides encoding the chimeric antigen receptors, and vectors comprising the polynucleotides. Any vector known in the art can be suitable for the present invention. In some embodiments, the vector is a viral vector. In some embodiments, the vector is a retroviral vector (such as pMSVG1), a DNA vector, a
10 murine leukemia virus vector, an SFG vector, a plasmid, a RNA vector, an adenoviral vector, a baculoviral vector, an Epstein Barr viral vector, a papovaviral vector, a vaccinia viral vector, a herpes simplex viral vector, an adenovirus associated vector (AAV), a lentiviral vector (such as pGAR), or any combination thereof. The pGAR sequence is as follows:

[0028] CTGACGCGCCCTGTAGCGGCGCATTAAGCGCGGCGGGTGTGGTGG
15 TTACGCGCAGCGTGACCGCTACACTTGCCAGCGCCCTAGCGCCCGCTCCTTT
CGCTTTTCTTCCCTTCCTTTCTCGCCACGTTTCGCCGGCTTTCCCCGTCAAGCTC
TAAATCGGGGGCTCCCTTTAGGGTTCCGATTTAGTGCTTTACGGCACCTCGA
CCCCAAAAAATTGATTAGGGTGATGGTTCACGTAGTGGGCCATCGCCCTGA
TAGACGGTTTTTCGCCCTTTGACGTTGGAGTCCACGTTCTTTAATAGTGGACT
20 CTTGTTCCAACTGGAACAACACTCAACCTATCTCGGTCTATTCTTTTGATT
TATAAGGGATTTTGCCGATTTTCGGCCTATTGGTTAAAAAATGAGCTGATTTA
ACAAAAATTTAACGCGAATTTTAAACAAAATATTAACGCTTACAATTTGCCAT
TCGCCATTCAGGCTGCGCAACTGTTGGGAAGGGCGATCGGTGCGGGCCTCTT
CGCTATTACGCCAGCTGGCGAAAGGGGGATGTGCTGCAAGGCGATTAAAGTT
25 GGGTAACGCCAGGGTTTTCCAGTCACGACGTTGTAAAACGACGGCCAGTG
AATTGTAATACGACTCACTATAGGGCGACCCGGGGATGGCGCGCCAGTAAT
CAATTACGGGGTCATTAGTTCATAGCCCATATATGGAGTTCCGCGTTACATA
ACTTACGGTAAATGGCCCGCCTGGCTGACCGCCCAACGACCCCCGCCCATTTG
ACGTCAATAATGACGTATGTTCCCATAGTAACGCCAATAGGGACTTTCCATT
30 GACGTCAATGGGTGGAGTATTTACGGTAAACTGCCCACTTGGCAGTACATCA
AGTGTATCATATGCCAAGTACGCCCCCTATTGACGTCAATGACGGTAAATGG
CCCGCCTGGCATTATGCCAGTACATGACCTTATGGGACTTTCTACTTGGC
AGTACATCTACGTATTAGTCATCGCTATTACCATGCTGATGCGGTTTTGGCA

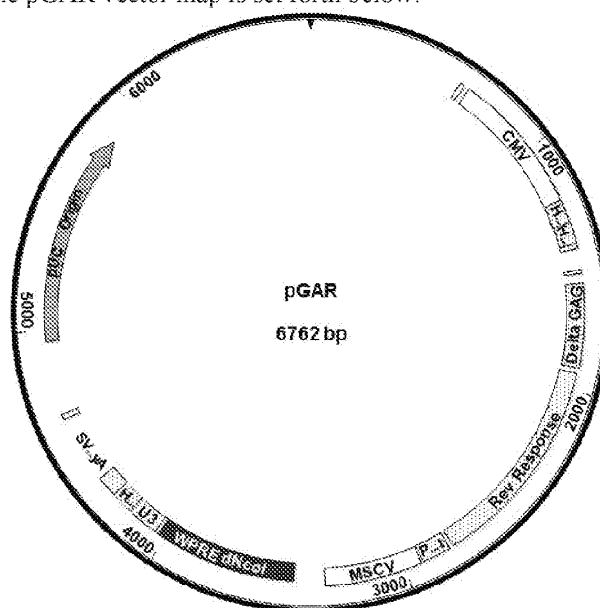
GTACATCAATGGGCGTGGATAGCGGTTTGACTCACGGGGATTTC AAGTCTC
CACCCCATTGACGTCAATGGGAGTTTGTTTTGGCACCAAAATCAACGGGACT
TTCCAAAATGTCGTAACAACCTCCGCCCCATTGACGCAAATGGGCGGTAGGC
GTGTACGGTGGGAGGTCTATATAAGCAGAGCTGGTTTAGTGAACCGGGGTC
5 TCTCTGGTTAGACCAGATCTGAGCCTGGGAGCTCTCTGGCTAACTAGGGAAC
CCACTGCTTAAGCCTCAATAAAGCTTGCCTTGAGTGCTTCAAGTAGTGTGTG
CCCGTCTGTTGTGTGACTCTGGTAACTAGAGATCCCTCAGACCCTTTTAGTC
AGTGTGGAAAATCTCTAGCAGTGGCGCCCGAACAGGGACTTGAAAGCGAAA
GGGAAACCAGAGGAGCTCTCTCGACGCAGGACTCGGCTTGCTGAAGCGCGC
10 ACGGCAAGAGGCGAGGGGCGGCGACTGGTGAGTACGCCAAAAATTTTGACT
AGCGGAGGCTAGAAGGAGAGAGATGGGTGCGAGAGCGTCAGTATTAAGCG
GGGGAGAATTAGATCGCGATGGGAAAAAATTCGGTTAAGGCCAGGGGGAA
AGAAAAAATATAAATTAACATATAGTATGGGCAAGCAGGGAGCTAGAA
CGATTCGCAGTTAATCCTGGCCTGTTAGAAACATCAGAAGGCTGTAGACAA
15 ATACTGGGACAGCTACAACCATCCCTTCAGACAGGATCAGAAGAACTTAGA
TCATTATATAATACAGTAGCAACCCTCTATTGTGTGCATCAAAGGATAGAGA
TAAAAGACACCAAGGAAGCTTTAGACAAGATAGAGGAAGAGCAAAACAAA
AGTAAGACCACCGCACAGCAAGCCGCCGCTGATCTTCAGACCTGGAGGAGG
AGATATGAGGGACAATTGGAGAAGTGAATTATATAAATATAAAGTAGTAAA
20 AATTGAACCATTAGGAGTAGCACCCACCAAGGCAAAGAGAAGAGTGGTGCA
GAGAGAAAAAAGAGCAGTGGGAATAGGAGCTTTGTTTCCTTGGGTTCTTGGG
AGCAGCAGGAAGCACTATGGGCGCAGCGTCAATGACGCTGACGGTACAGGC
CAGACAATTATTGTCTGGTATAGTGCAGCAGCAGAACAATTTGCTGAGGGCT
ATTGAGGCGCAACAGCATCTGTTGCAACTCACAGTCTGGGGCATCAAGCAG
25 CTCCAGGCAAGAATCCTGGCTGTGGAAAGATACCTAAAGGATCAACAGCTC
CTGGGGATTGTTGGGTTGCTCTGGAAAACCTCATTTGCACCACTGCTGTGCCTT
GGAATGCTAGTTGGAGTAATAAATCTCTGGAACAGATTTGGAATCACACGA
CCTGGATGGAGTGGGACAGAGAAATTAACAATTACACAAGCTTAATACACT
CCTTAATTGAAGAATCGCAAAACCAGCAAGAAAAGAATGAACAAGAATTAT
30 TGGAATTAGATAAATGGGCAAGTTTGTGGAATTGGTTTAACATAACAAATTG
GCTGTGGTATATAAAATTATTCATAATGATAGTAGGAGGCTTGGTAGGTTTA
AGAATAGTTTTTGCTGTACTTTCTATAGTGAATAGAGTTAGGCAGGGATATT
CACCATTATCGTTTCAGACCCACCTCCCAACCCCGAGGGGACCCGACAGGCC

CGAAGGAATAGAAGAAGAAGGTGGAGAGAGAGACAGAGACAGATCCATTC
GATTAGTGAACGGATCTCGACGGTATCGGTAACTTTTAAAAGAAAAGGGG
GGATTGGGGGGTACAGTGCAGGGGAAAGAATAGTAGACATAATAGCAACA
GACATACAACTAAAGAATTACAAAAACAAATTACAAAATTCAAAATTTTA
5 TCGCGATCGCGGAATGAAAGACCCACCTGTAGGTTTGGCAAGCTAGCTTA
AGTAACGCCATTTTGCAAGGCATGGAAAATACATAACTGAGAATAGAGAAG
TTCAGATCAAGGTTAGGAACAGAGAGACAGCAGAATATGGGCCAAACAGG
ATATCTGTGGTAAGCAGTTCCTGCCCCGGCTCAGGGCCAAGAACAGATGGT
CCCCAGATGCGGTCCCGCCCTCAGCAGTTTCTAGAGAACCATCAGATGTTTC
10 CAGGGTGCCCCAAGGACCTGAAAATGACCCTGTGCCTTATTTGAACTAACCA
ATCAGTTCGCTTCTCGCTTCTGTTTCGCGCGCTTCTGCTCCCCGAGCTCAATAA
AAGAGCCCACAACCCCTCACTCGGCGCGCCAGTCCTTCGAAGTAGATCTTTG
TCGATCCTACCATCCACTCGACACACCCGCCAGCGGCCGCTGCCAAGCTTCC
GAGCTCTCGAATTAATTACGGTACCCACCATGGCCTAGGGAGACTAGTCG
15 AATCGATATCAACCTCTGGATTACAAAATTTGTGAAAGATTGACTGGTATTC
TTAACTATGTTGCTCCTTTTACGCTATGTGGATACGCTGCTTTAATGCCTTTG
TATCATGCTATTGCTTCCCGTATGGCTTTTCATTTTCTCCTCCTTGTATAAATCC
TGGTTGCTGTCTCTTTATGAGGAGTTGTGGCCCGTTGTCAGGCAACGTGGCG
TGGTGTGCACTGTGTTTGCTGACGCAACCCCCACTGGTTGGGGCATTGCCAC
20 CACCTGTCAGCTCCTTTCCGGGACTTTTCGCTTTCCCCCTCCCTATTGCCACGG
CGGAACTCATCGCCGCCTGCCTTGCCCGCTGCTGGACAGGGGCTCGGCTGTT
GGGCACTGACAATTCCGTGGTGTGTCGGGGAAGCTGACGTCTTTTTCATGG
CTGCTCGCCTGTGTTGCCACCTGGATTCTGCGCGGGACGTCTTCTGCTACGT
CCCTTCGGCCCTCAATCCAGCGGACCTTCCTTCCCGCGGCCTGCTGCCGGCT
25 CTGCGGCCTCTTCCGCGTCTTCGCCTTCGCCCTCAGACGAGTCGGATCTCCCT
TTGGGGCCGCTCCCCGCCTGGTTAATTAAAGTACCTTTAAGACCAATGACTT
ACAAGGCAGCTGTAGATCTTAGCCACTTTTTTAAAAGAAAAGGGGGGACTGG
AAGGGCGAATTCACCTCCCAACGAAGACAAGATCTGCTTTTTTGCTTGTACTGG
GTCTCTCTGGTTAGACCAGATCTGAGCCTGGGAGCTCTCTGGCTAACTAGGG
30 AACCCACTGCTTAAGCCTCAATAAAGCTTGCCTTGAGTGCTTCAAGTAGTGT
GTGCCCCTGCTGTTGTGTGACTCTGGTAACTAGAGATCCCTCAGACCCTTTTA
GTCAGTGTGGAATCTCTAGCAGGCATGCCAGACATGATAAGATACATTG
ATGAGTTTGGACAAACCACAACCTAGAATGCAGTGAAAAAATGCTTTATTT

GTGAAATTTGTGATGCTATTGCTTTATTTGTAACCATTATAAGCTGCAATAA
ACAAGTTAACAACAACAATTGCATTCAATTTATGTTTCAGGTTTCAGGGGGAG
GTGTGGGAGGTTTTTTGGCGCGCCATCGTCGAGGTTCCCTTTAGTGAGGGTT
AATTGCGAGCTTGGCGTAATCATGGTCATAGCTGTTTCCTGTGTGAAATTGT
5 TATCCGCTCACAATTCCACACAACATACGAGCCGGAAGCATAAAGTGTA
GCCTGGGGTGCCTAATGAGTGAGCTAACTCACATTAATTGCGTTGCGCTCAC
TGCCCGCTTTCCAGTCGGGAAACCTGTCTGTCGCCAGCTGCATTAATGAATCGG
CCAACGCGCGGGGAGAGGGCGGTTTGGCGTATTGGGCGCTCTTCCGCTTCCTCG
CTCACTGACTCGCTGCGCTCGGTCTGTTTCGGCTGCGGCGAGCGGTATCAGCTC
10 ACTCAAAGGCGGTAATACGGTTATCCACAGAATCAGGGGATAACGCAGGAA
AGAACATGTGAGCAAAAGGCCAGCAAAAGGCCAGGAACCGTAAAAAGGCC
GCGTTGCTGGCGTTTTTCCATAGGCTCCGCCCCCTGACGAGCATCACAAAA
ATCGACGCTCAAGTCAGAGGTGGCGAAACCCGACAGGACTATAAAGATACC
AGGCGTTTTCCCCCTGGAAGCTCCCTCGTGCGCTCTCCTGTTCCGACCCTGCC
15 GCTTACCGGATACCTGTCCGCCTTTCTCCCTTCGGGAAGCGTGGCGCTTTCTC
ATAGCTCACGCTGTAGGTATCTCAGTTCGGTGTAGGTCGTTTCGCTCCAAGCT
GGGCTGTGTGCACGAACCCCCCGTTCAGCCCGACCGCTGCGCCTTATCCGGT
AACTATCGTCTTGAGTCCAACCCGGTAAGACACGACTTATCGCCACTGGCAG
CAGCCACTGGTAACAGGATTAGCAGAGCGAGGTATGTAGGCGGTGCTACAG
20 AGTTCTTGAAGTGGTGGCCTAACTACGGCTACACTAGAAGAACAGTATTTGG
TATCTGCGCTCTGCTGAAGCCAGTTACCTTCGGAAAAAGAGTTGGTAGCTCT
TGATCCGGCAAACAAACCACCGCTGGTAGCGGTGGTTTTTTTTGTTTGCAAGC
AGCAGATTACGCGCAGAAAAAAAGGATCTCAAGAAGATCCTTTGATCTTTT
CTACGGGGTCTGACGCTCAGTGGAACGAAAACCTCACGTTAAGGGATTTTGG
25 TCATGAGATTATCAAAAAGGATCTTCACCTAGATCCTTTTAAATTAATAATG
AAGTTTTAAATCAATCTAAAGTATATATGAGTAAACTTGGTCTGACAGTTAC
CAATGCTTAATCAGTGAGGCACCTATCTCAGCGATCTGTCTATTTTCGTTTCATC
CATAGTTGCCTGACTCCCCGTCGTGTAGATAACTACGATACGGGAGGGCTTA
CCATCTGGCCCCAGTGCTGCAATGATACCGCGAGACCCACGCTCACCGGCTC
30 CAGATTTATCAGCAATAAACCAGCCAGCCGGAAGGGCCGAGCGCAGAAGTG
GTCCTGCAACTTTATCCGCCTCCATCCAGTCTATTAATTGTTGCCGGGAAGCT
AGAGTAAGTAGTTCGCCAGTTAATAGTTTGGCGCAACGTTGTTGCCATTGCTA
CAGGCATCGTGGTGTACGCTCGTCGTTTGGTATGGCTTCATTCAGCTCCGG

TTCCAACGATCAAGGCGAGTTACATGATCCCCCATGTTGTGCAAAAAAGC
 GGTTAGCTCCTTCGGTCCTCCGATCGTTGTCAGAAGTAAGTTGGCCGCAGTG
 TTATCACTCATGGTTATGGCAGCACTGCATAATTCTCTTACTGTCATGCCATC
 CGTAAGATGCTTTTCTGTGACTGGTGAGTACTCAACCAAGTCATTCTGAGAA
 5 TAGTGTATGCGGCGACCGAGTTGCTCTTGCCCGGCGTCAATACGGGATAATA
 CCGCGCCACATAGCAGAACTTTAAAAGTGCTCATCATTGGAAAACGTTCTTC
 GGGGCGAAAACCTCTCAAGGATCTTACCGCTGTTGAGATCCAGTTCGATGTAA
 CCCACTCGTGCACCCAACTGATCTTCAGCATCTTTTACTTTTACCAGCGTTTC
 TGGGTGAGCAAAAACAGGAAGGCAAAATGCCGCAAAAAAGGGAATAAGGG
 10 CGACACGGAAATGTTGAATACTCATACTCTTCCTTTTCAATATTATTGAAG
 CATTTATCAGGGTTATTGTCTCATGAGCGGATACATATTTGAATGTATTTAG
 AAAAATAAACAAATAGGGGTTCGCGCACATTTCCCCGAAAAGTGCCAC

[0029] The pGAR vector map is set forth below:



15 [0030] Suitable additional exemplary vectors include e.g., pBABE-puro, pBABE-neo
 largeTcDNA, pBABE-hygro-hTERT, pMKO.1 GFP, MSCV-IRES-GFP, pMSCV PIG (Puro
 IRES GFP empty plasmid), pMSCV-loxp-dsRed-loxp-eGFP-Puro-WPRE, MSCV IRES
 Luciferase, pMIG, MDH1-PGK-GFP_2.0, TiRMPVIR, pMSCV-IRES-mCherry FP,
 pRetroX GFP T2A Cre, pRXTN, pLncEXP, and pLXIN-Luc.

20 [0031] Exemplary immune cells include, but are not limited to T cells, tumor
 infiltrating lymphocytes (TILs), NK cells, TCR-expressing cells, dendritic cells, or NK-T

cells. The T cells can be autologous, allogeneic, or heterologous. In other embodiments, the invention relates to pharmaceutical compositions comprising the immune cells of described herein.

[0032] In certain embodiments, the invention relates to antigen binding molecules
5 (and chimeric antigen receptors comprising these molecules) comprising at least one of:

(a) a VH region comprising the amino acid sequence of SEQ ID NO: 16 and a VL region comprising the amino acid sequence of SEQ ID NO: 21;

(b) a VH region comprising the amino acid sequence of SEQ ID NO: 50 and a VL region comprising the amino acid sequence of SEQ ID NO: 55;

10 (c) a VH region comprising the amino acid sequence of SEQ ID NO: 72 and a VL region comprising the amino acid sequence of SEQ ID NO: 77;

(d) a VH region comprising the amino acid sequence of SEQ ID NO: 94 and a VL region comprising the amino acid sequence of SEQ ID NO: 99;

and wherein the VH and VL region or regions are linked by at least one linker. Also
15 encompassed by the invention are chimeric antigen receptors and/or antigen binding molecules having no more than 8 amino acid substitutions thereto.

[0033] The linker may be, e.g., a poly-Gly linker such as GGGGSGGGGSGGGGS (SEQ ID NO. 130) or GGGGSGGGGSGGGGSGGGGS (SEQ ID NO. 145).

[0034] In other embodiments, the invention relates to antigen binding molecules (and
20 chimeric antigen receptors comprising these molecules) wherein the linker comprises at least one of SEQ ID NO. 130 and SEQ ID NO. 132.

[0035] In certain embodiments, the invention relates to antigen binding molecules and/or chimeric antigen receptors having at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least
25 about 98%, at least about 99%, or 100% identical to the antigen binding molecules and/or chimeric antigen receptors set forth herein.

[0036] In other embodiments, the invention relates to isolated polynucleotides comprising at least one of: SEQ ID NO. 27; SEQ ID NO. 31; SEQ ID NO. 35; SEQ ID NO. 39; SEQ ID NO. 43; SEQ ID NO. 47; SEQ ID NO. 61; SEQ ID NO. 65; SEQ ID NO. 69;
30 SEQ ID NO. 83; SEQ ID NO. 87; SEQ ID NO. 91; SEQ ID NO. 105; SEQ ID NO. 109; SEQ ID NO. 113; SEQ ID NO. 117; SEQ ID NO. 121; and SEQ ID NO. 125.

[0037] In certain embodiments, the invention relates to isolated polynucleotides having at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or 100% identical to the polynucleotides set forth herein.

5 [0038] The invention further relates to vectors comprising these polynucleotides, as well as cells transduced using these vectors.

[0039] In further embodiments, the invention relates to isolated polypeptides comprising the amino acid sequence set forth in at least one of: SEQ ID NO. 28; SEQ ID NO. 32; SEQ ID NO. 36; SEQ ID NO. 40; SEQ ID NO. 44; SEQ ID NO. 48; SEQ ID NO. 62;
10 SEQ ID NO. 66; SEQ ID NO. 70; SEQ ID NO. 84; SEQ ID NO. 88; SEQ ID NO. 92; SEQ ID NO. 106; SEQ ID NO. 110; SEQ ID NO. 114; SEQ ID NO. 118; SEQ ID NO. 122; and SEQ ID NO. 126. In other embodiments, the invention relates to vectors encoding these polypeptides, immune cells comprising these polypeptides. Preferred immune cells include
15 T cells, tumor infiltrating lymphocytes (TILs), NK cells, TCR-expressing cells, dendritic cells, or NK-T cells. The T cells may be autologous, allogeneic, or heterologous. Also encompassed by the invention are chimeric antigen receptors having no more than 8 amino acid substitutions thereto.

[0040] In other embodiments, the invention relates to isolated polynucleotides encoding a chimeric antigen receptor (CAR) or T cell receptor (TCR) comprising an antigen
20 binding molecule that specifically binds to CLL-1, wherein the antigen binding molecule comprises a variable heavy (V_H) chain CDR3 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 19, 53, 75, and 97. Also encompassed by the invention are chimeric antigen receptors having no more than 8 amino acid substitutions thereto. The polynucleotides may further comprising an activating domain. In preferred
25 embodiments, the activating domain is CD3, more preferably CD3 zeta, more preferably the amino acid sequence set forth in SEQ ID NO. 9.

[0041] In other embodiments, the invention includes a costimulatory domain comprising the signaling domain (or other suitable portion) of CD28, CD28T, OX40, 4-1BB/CD137, CD2, CD3 (alpha, beta, delta, epsilon, gamma, zeta), CD4, CD5, CD7, CD9,
30 CD16, CD22, CD27, CD30, CD 33, CD37, CD40, CD 45, CD64, CD80, CD86, CD134, CD137, CD154, PD-1, ICOS, lymphocyte function-associated antigen-1 (LFA-1 (CD11a/CD18), CD247, CD276 (B7-H3), LIGHT (tumor necrosis factor superfamily member 14;

TNFSF14), NKG2C, Ig alpha (CD79a), DAP-10, Fc gamma receptor, MHC class I molecule, TNF, TNFr, integrin, signaling lymphocytic activation molecule, BTLA, Toll ligand receptor, ICAM-1, B7-H3, CDS, ICAM-1, GITR, BAFFR, LIGHT, HVEM (LIGHTR), KIRDS2, SLAMF7, NKp80 (KLRF1), NKp44, NKp30, NKp46, CD19, CD4, CD8alpha, CD8beta, IL-2R beta, IL-2R gamma, IL-7R alpha, ITGA4, VLA1, CD49a, ITGA4, IA4, CD49D, ITGA6, VLA-6, CD49f, ITGAD, CD11d, ITGAE, CD103, ITGAL, CD11a, LFA-1, ITGAM, CD11b, ITGAX, CD11c, ITGB1, CD29, ITGB2, CD18, LFA-1, ITGB7, NKG2D, TNFR2, TRANCE/RANKL, DNAM1 (CD226), SLAMF4 (CD244, 2B4), CD84, CD96 (Tactile), CEACAM1, CRT AM, Ly9 (CD229), CD160 (BY55), PSGL1, CD100 (SEMA4D), CD69, SLAMF6 (NTB-A, Lyl08), SLAM (SLAMF1, CD150, IPO-3), BLAME (SLAMF8), SELPLG (CD162), LTBR, LAT, GADS, SLP-76, PAG/Cbp, CD19a, CD83 ligand, or fragments or combinations thereof. Preferred costimulatory domains are recited hereinbelow.

[0042] In further embodiments, the invention relates to isolated polynucleotides encoding a chimeric antigen receptor (CAR) or T cell receptor (TCR), wherein said CAR or TCR comprises an antigen binding molecule that specifically binds to CLL-1, and wherein the antigen binding molecule comprises a variable light (V_L) chain CDR3 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 24, 58, 80, and 102. The polynucleotide can further comprise an activating domain. The polynucleotide can further comprise a costimulatory domain.

[0043] In other embodiments, the invention relates to isolated polynucleotides encoding a chimeric antigen receptor (CAR) or T cell receptor (TCR) comprising an antigen binding molecule that specifically binds to CLL-1, wherein the antigen binding molecule heavy chain comprises CDR1 (SEQ ID NO. 17), CDR2 (SEQ ID NO. 18), and CDR3 (SEQ ID NO. 19) and the antigen binding molecule light chain comprises CDR1 (SEQ ID NO. 22), CDR2 (SEQ ID NO. 23), and CDR3 (SEQ ID NO. 24).

[0044] In certain embodiments, the invention relates to isolated polynucleotides having at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or 100% identical to the above sequences.

[0045] In other embodiments, the invention relates to isolated polynucleotides encoding a chimeric antigen receptor (CAR) or T cell receptor (TCR) comprising an antigen binding molecule that specifically binds to CLL-1, wherein the antigen binding molecule

heavy chain comprises CDR1 (SEQ ID NO. 51), CDR2 (SEQ ID NO. 52), and CDR3 (SEQ ID NO. 53) and the antigen binding molecule light chain comprises CDR1 (SEQ ID NO. 56), CDR2 (SEQ ID NO. 57), and CDR3 (SEQ ID NO. 58).

[0046] In certain embodiments, the invention relates to isolated polynucleotides
5 having at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or 100% identical to the above sequences.

[0047] In other embodiments, the invention relates to isolated polynucleotides encoding a chimeric antigen receptor (CAR) or T cell receptor (TCR) comprising an antigen
10 binding molecule that specifically binds to CLL-1, wherein the antigen binding molecule heavy chain comprises CDR1 (SEQ ID NO. 73), CDR2 (SEQ ID NO. 74), and CDR3 (SEQ ID NO. 75) and the antigen binding molecule light chain comprises CDR1 (SEQ ID NO. 78), CDR2 (SEQ ID NO. 79), and CDR3 (SEQ ID NO. 80).

[0048] In certain embodiments, the invention relates to isolated polynucleotides
15 having at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or 100% identical to the above sequences.

[0049] In other embodiments, the invention relates to isolated polynucleotides encoding a chimeric antigen receptor (CAR) or T cell receptor (TCR) comprising an antigen
20 binding molecule that specifically binds to CLL-1, wherein the antigen binding molecule heavy chain comprises CDR1 (SEQ ID NO. 95), CDR2 (SEQ ID NO. 96), and CDR3 (SEQ ID NO. 97) and the antigen binding molecule light chain comprises CDR1 (SEQ ID NO. 100), CDR2 (SEQ ID NO. 101), and CDR3 (SEQ ID NO. 102).

[0050] In certain embodiments, the invention relates to isolated polynucleotides
25 having at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or 100% identical to the above sequences.

[0051] In further embodiments, the invention relates to isolated polynucleotides encoding a chimeric antigen receptor (CAR) or T cell receptor (TCR) comprising an antigen
30 binding molecule that specifically binds to CLL-1, and wherein the antigen binding molecule comprises:

- (a) a heavy chain variable region (VH) complementarity determining region (CDR) 1 comprising the amino acid sequence GX₂X₃X₄X₅X₆X₇X₈X₉ (SEQ ID NO: 134), wherein X₂ is G, F, or Y; X₃ is S or T; X₄ is I, F, or L; X₅ is S or T; X₆ is not present or S; X₇ is not present or G; X₈ is not present or E or G; and X₉ is F, L, or Y;
- (b) a heavy chain variable region (VH) complementarity determining region (CDR) 2 comprising the amino acid sequence X₁X₂X₃X₄X₅X₆ (SEQ ID NO: 135), wherein X₁ is D, H, S, or Y; X₂ is H, P, or Y; X₃ is D, E, or S; X₄ is D or G; X₅ is G or S; and X₆ is not present of D or E;
- (c) a heavy chain variable region (VH) complementarity determining region (CDR) 3, comprising the amino acid sequence X₁X₂X₃X₄X₅X₆X₇X₈X₉X₁₀X₁₁X₁₂DY (SEQ ID NO: 136), wherein X₁ is E or L; X₂ is R, S, or V; X₃ is R or Y; X₄ is C, G, or S; X₅ is not present or G or I; X₆ is not present or G; X₇ is not present or D; X₈ is not present or C; X₉ is not present or W or Y; X₁₀ is not present or P or S; X₁₁ is not present or G or Y; and X₁₂ is F or R;
- (d) a light chain variable region (VL) CDR1 comprising the amino acid sequence X₁ASQX₅X₆X₇X₈X₉LX₁₁ (SEQ ID NO: 137), wherein X₁ is Q or R; X₅ is D or S; X₆ is I or V; X₇ is N or S; X₈ is N or S; X₉ is F, L, or Y; and X₁₁ is N or T;
- (e) a light chain variable region (VL) CDR2 comprising the amino acid sequence X₁ASX₄X₅X₆X₇ (SEQ ID NO: 138), wherein X₁ is D or G; X₄ is N, S, or T; X₅ is L or R; X₆ is A, E, or K; and X₇ is S or T; and/or
- (f) a light chain variable region (VL) CDR3 comprising the amino acid sequence QQX₃X₄X₅X₆PX₈T (SEQ ID NO: 139), wherein X₃ is S or Y; X₄ is D, G, or Y; X₅ is N, S, or T; X₆ is L, T, or Y; and X₈ is F or I.

[0052] The invention further relates to antigen binding molecules to CLL-1 comprising at least one variable heavy chain CDR3 or variable light chain CDR3 sequence as set forth herein. The invention further relates to antigen binding molecules to CLL-1 comprising at least one variable heavy chain CDR1, CDR2, and CDR3 sequences as described herein. The invention further relates to antigen binding molecules to CLL-1

comprising at least one variable light chain CDR1, CDR2, and CDR3 sequences as described herein. The invention further relates to antigen binding molecules to CLL-1 comprising both variable heavy chain CDR1, CDR2, CDR3, and variable light chain CDR1, CDR2, and CDR3 sequences as described herein.

- 5 **[0053]** The invention further relates to methods of treating a disease or disorder in a subject in need thereof comprising administering to the subject the antigen binding molecules, the CARs, TCRs, polynucleotides, vectors, cells, or compositions according to the invention. Suitable diseases for treatment include, but are not limited to, acute myeloid leukemia (AML), chronic myelogenous leukemia (CML), chronic myelomonocytic leukemia (CMML),
10 juvenile myelomonocytic leukemia, atypical chronic myeloid leukemia, acute promyelocytic leukemia (APL), acute monoblastic leukemia, acute erythroid leukemia, acute megakaryoblastic leukemia, myelodysplastic syndrome (MDS), myeloproliferative disorder, myeloid neoplasm, myeloid sarcoma), Blastic Plasmacytoid Dendritic Cell Neoplasm (BPDCN), or combinations thereof. Additional diseases include inflammatory and/or
15 autoimmune diseases such as rheumatoid arthritis, psoriasis, allergies, asthma, Crohn's disease, IBD, IBS, fibromyalgia, mastocytosis, and Celiac disease.

BRIEF DESCRIPTION OF THE FIGURES

[0054] FIGURE 1 shows CLL-1 expression in different cancer cell lines.

- [0055]** FIGURE 2 shows CLL-1 CAR expression determined by protein L 6 hours
20 post mRNA electroporation.

[0056] FIGURE 3 shows the results from a cytokine release assay from different CLL-1 CAR-T cell constructs 24 hours after mRNA electroporation.

[0057] FIGURE 4 shows cytolytic activity of different CLL-1 CAR-T cell constructs 24 hours after mRNA electroporation.

- 25 **[0058]** FIGURE 5 shows cytolytic activity of different CLL-1 CAR-T cell constructs 24 hours after mRNA electroporation.

[0059] FIGURE 6 shows CLL-1 CAR expression determined by protein L at day 12 after transduction.

- [0060]** FIGURE 7 shows cytokine release assay from CLL-1 CAR-T cells 16 hours
30 after co-culture with different target cell lines.

[0061] FIGURE 8 shows cytolytic activity from CLL-1 CAR-T cells 16 hours and 40 hours after co-culture with different target cell lines.

[0062] FIGURES 9A-9D set forth sequence alignments of the CLL-1 antigen binding molecules of the invention. CDRs are notated in boxes.

5 [0063] FIGURE 10 sets forth bioluminescence results on NSG mice treated with CARs according to the invention.

DETAILED DESCRIPTION OF THE INVENTION

[0064] It will be appreciated that chimeric antigen receptors (CARs or CAR-Ts) and T cell receptors (TCRs) are genetically engineered receptors. These engineered receptors can be readily inserted into and expressed by immune cells, including T cells in accordance with techniques known in the art. With a CAR, a single receptor can be programmed to both recognize a specific antigen and, when bound to that antigen, activate the immune cell to attack and destroy the cell bearing that antigen. When these antigens exist on tumor cells, an immune cell that expresses the CAR can target and kill the tumor cell.

15 [0065] CARs can be engineered to bind to an antigen (such as a cell-surface antigen) by incorporating an antigen binding molecule that interacts with that targeted antigen. Preferably, the antigen binding molecule is an antibody fragment thereof, and more preferably one or more single chain antibody fragment ("scFv"). An scFv is a single chain antibody fragment having the variable regions of the heavy and light chains of an antibody linked together. See U.S. Patent Nos. 7,741,465, and 6,319,494 as well as Eshhar *et al.*, Cancer Immunol Immunotherapy (1997) 45: 131-136. An scFv retains the parent antibody's ability to specifically interact with target antigen. scFvs are preferred for use in chimeric antigen receptors because they can be engineered to be expressed as part of a single chain along with the other CAR components. *Id.* See also Krause *et al.*, J. Exp. Med., Volume 188, No. 4, 20 1998 (619-626); Finney *et al.*, *Journal of Immunology*, 1998, 161: 2791-2797. It will be appreciated that the antigen binding molecule is typically contained within the extracellular portion of the CAR such that it is capable of recognizing and binding to the antigen of interest. Bispecific and multispecific CARs are contemplated within the scope of the invention, with specificity to more than one target of interest.

30 **Costimulatory Domains.**

[0066] Chimeric antigen receptors may incorporate costimulatory (signaling) domains to increase their potency. See U.S. Patent Nos. 7,741,465, and 6,319,494, as well as Krause *et al.* and Finney *et al.* (*supra*), Song *et al.*, Blood 119:696-706 (2012); Kalos *et al.*, Sci Transl. Med. 3:95 (2011); Porter *et al.*, N. Engl. J. Med. 365:725-33 (2011), and Gross *et al.*, Annu. Rev. Pharmacol. Toxicol. 56:59-83 (2016). For example, CD28 is a costimulatory protein found naturally on T-cells. A variety of costimulatory molecules are set forth herein, but it will be appreciated that additional costimulatory molecules are also included within the scope of this invention.

[0067] The complete native amino acid sequence of CD28 is described in NCBI Reference Sequence: NP_006130.1. The complete native CD28 nucleic acid sequence is described in NCBI Reference Sequence: NM_006139.1.

[0068] Certain CD28 domains have been used in chimeric antigen receptors. In accordance with the present invention, it has now been found that a novel CD28 extracellular (hinge) construct, termed "CD28T", unexpectedly provides certain benefits when utilized in a CAR construct. This construct demonstrates the ability to retain (and at times exceed) the properties of CD28-containing CARs, despite truncation (removal) of multiple amino acids from the extracellular CD28 sequence. These benefits include equivalent or superior cytokine production, equivalent or superior cytolytic activity, and/or equivalent or superior CAR expression levels.

[0069] The nucleotide sequence of the CD28T molecule, including the extracellular domain, and the CD28 transmembrane and intracellular domains is set forth in SEQ ID NO. 1:

CTTGATAATGAAAAGTCAAACGGAACAATCATTACGTGAAGGGCAAGC
ACCTCTGTCCGTCACCCCTTGTTCCCTGGTCCATCCAAGCCATTCTGGGTGT
TGGTCGTAGTGGGTGGAGTCCTCGCTTGTTACTCTCTGCTCGTCACCGTG
GCTTTTATAATCTTCTGGGTAGATCCAAAAGAAGCCGCCTGCTCCATAG
CGATTACATGAATATGACTCCACGCCGCCCTGGCCCCACAAGGAAACAC
TACCAGCCTTACGCACCACCTAGAGATTTCGCTGCCTATCGGAGC

[0070] The corresponding amino acid sequence is set forth in SEQ ID NO. 2:
LDNEKSNGTIIHVKGKHLCPSPFLPGPSKPFWVLVVGGVLACYSLLVTVAF
IIFWVRSK RSRLHSDYM NMTPRRPGPT RKHYQPYAPP RDFAAAYS

[0071] The nucleotide sequence of the extracellular portion of CD28T is set forth in SEQ ID NO. 3:

CTTGATAATGAAAAGTCAAACGGAACAATCATTACAGTGAAGGGCAAGC
ACCTCTGTCCGTCACCCTTGTTCCTGGTCCATCCAAGCCA

5 [0072] The corresponding amino acid sequence of the CD28T extracellular domain is set forth in SEQ ID NO. 4: LDNEKSNGTI IHVKGKHLCP SPLFPGPSKP

[0073] The nucleotide sequence of the CD28 transmembrane domain is set forth in SEQ ID NO. 5):

TTCTGGGTGTTGGTCGTAGTGGGTGGAGTCCTCGCTTGTTACTCTCTGCTC
10 GTCACCGTGGCTTTTATAATCTTCTGGGTT

[0074] The amino acid sequence of the CD28 transmembrane domain is set forth in SEQ ID NO. 6: FWVLVVVGGV LACYSLLVTV AFHFWV

[0075] The nucleotide sequence of the CD28 intracellular signaling domain is set forth in SEQ ID NO. 7:

15 AGATCCAAAAGAAGCCGCCTGCTCCATAGCGATTACATGAATATGACTC
CACGCCGCCCTGGCCCCACAAGGAAACACTACCAGCCTTACGCACCACC
TAGAGATTTCGCTGCCTATCGGAGC

[0076] The amino acid sequence of the CD28 intracellular signaling domain is set forth in SEQ ID NO. 8:

20 RSKRSRLLHSDYMNMTPRRPGPTRKHYPYAPPRDFAAYRS

[0077] Additional CD28 sequences suitable for use in the invention include the CD28 nucleotide sequence set forth in SEQ ID NO. 11:

ATTGAGGTGATGTATCCACCGCCTTACCTGGATAACGAAAAGAGTAACG
GTACCATCATTACAGTGAAAGGTAAACACCTGTGTCCTTCTCCCCCTCTTC
25 CCCGGGCCATCAAAGCCC

[0078] The corresponding amino acid sequence is set forth in SEQ ID NO. 12:

IEVMYPPPYLDNEKSNGTIIHVKGKHLCP SPLFPGPSKP

[0079] It will be appreciated that the invention relates to antigen binding molecules, CARs, TCRs, and the like comprising at least one isolated nucleic acid sequence of SEQ ID

NO. 1 or SEQ ID NO. 3. It will further be appreciated that the invention relates to antigen binding molecules, CARs, TCRs, and the like wherein the extracellular portion consists of at least one isolated nucleic acid sequence of SEQ ID NO. 1 or SEQ ID NO. 3. Additionally, it will be appreciated that the invention relates to antigen binding molecules, CARs, TCRs, and the like wherein the extracellular portion consists essentially of at least one isolated nucleic acid sequence of SEQ ID NO. 1 or SEQ ID NO. 3.

[0080] It will be appreciated that the invention relates to antigen binding molecules, CARs, TCRs, and the like comprising at least one amino acid sequence of SEQ ID NO. 2 or SEQ ID NO. 4. It will further be appreciated that the invention relates to antigen binding molecules, CARs, TCRs, and the like wherein the extracellular portion consists of at least one amino acid sequence of SEQ ID NO. 2 or SEQ ID NO. 4. It will also be appreciated that the invention relates to antigen binding molecules, CARs, TCRs, and the like wherein the extracellular portion consists essentially of at least one amino acid sequence of SEQ ID NO. 2 or SEQ ID NO. 4.

[0081] Another suitable source of extracellular and/or transmembrane domains can be derived from (or correspond to) some or all of CD8. The nucleotide sequence of a suitable CD8 extracellular and transmembrane domain is set forth in SEQ ID NO. 13:

GCTGCAGCATTGAGCAACTCAATAATGTATTTTAGTCACTTTGTACCAGT
GTTCTTGCCGGCTAAGCCTACTACCACACCCGCTCCACGGCCACCTACCC
CAGCTCCTACCATCGCTTCACAGCCTCTGTCCCTGCGCCCAGAGGCTTGC
CGACCGGCCGAGGGGGCGCTGTTTCATACCAGAGGACTGGATTTCGCCT
GCGATATCTATATCTGGGCACCCCTGGCCGGAACCTGCGGCGTACTCCTG
CTGTCCCTGGTCATCACGCTCTATTGTAATCACAGGAAC

[0082] The corresponding amino acid sequence is set forth in SEQ ID NO. 14:

AAALSNSIMYFSHFVPVFLPAKPTTTPAPRPPTPAPTIASQPLSLRPEACRPAA
GGAVHTRGLDFACDIYWAPLAGTCGVLLLSLVITLYCNHRN

[0083] It will be appreciated that suitable costimulatory domains within the scope of the invention can be derived from (or correspond to) for example, CD28, CD28T, OX40, 4-1BB/CD137, CD2, CD3 (alpha, beta, delta, epsilon, gamma, zeta), CD4, CD5, CD7, CD9, CD16, CD22, CD27, CD30, CD 33, CD37, CD40, CD 45, CD64, CD80, CD86, CD134, CD137, CD154, PD-1, ICOS, lymphocyte function-associated antigen-1 (LFA-1 (CD1

la/CD18), CD247, CD276 (B7-H3), LIGHT (tumor necrosis factor superfamily member 14; TNFSF14), NKG2C, Ig alpha (CD79a), DAP-10, Fc gamma receptor, MHC class I molecule, TNF, TNFr, integrin, signaling lymphocytic activation molecule, BTLA, Toll ligand receptor, ICAM-1, B7-H3, CDS, ICAM-1, GITR, BAFFR, LIGHT, HVEM (LIGHTR), KIRDS2, SLAMF7, NKp80 (KLRF1), NKp44, NKp30, NKp46, CD19, CD4, CD8alpha, CD8beta, IL-2R beta, IL-2R gamma, IL-7R alpha, ITGA4, VLA1, CD49a, ITGA4, IA4, CD49D, ITGA6, VLA-6, CD49f, ITGAD, CD11d, ITGAE, CD103, ITGAL, CD11a, LFA-1, ITGAM, CD11b, ITGAX, CD11c, ITGB1, CD29, ITGB2, CD18, LFA-1, ITGB7, NKG2D, TNFR2, TRANCE/RANKL, DNAM1 (CD226), SLAMF4 (CD244, 2B4), CD84, CD96 (Tactile), CEACAM1, CRT AM, Ly9 (CD229), CD160 (BY55), PSGL1, CD100 (SEMA4D), CD69, SLAMF6 (NTB-A, Lyl08), SLAM (SLAMF1, CD150, IPO-3), BLAME (SLAMF8), SELPLG (CD162), LTBR, LAT, GADS, SLP-76, PAG/Cbp, CD19a, CD83 ligand, or fragments or combinations thereof. It will be appreciated that additional costimulatory molecules, or fragments thereof, not listed above are within the scope of the invention.

15 **Activating Domains.**

[0084] CD3 is an element of the T cell receptor on native T cells, and has been shown to be an important intracellular activating element in CARs. In a preferred embodiment, the CD3 is CD3 zeta, the nucleotide sequence of which is set forth in SEQ ID NO. 9:

AGGGTGAAGTTTTCCAGATCTGCAGATGCACCAGCGTATCAGCAGGGCC
 20 AGAACCAACTGTATAACGAGCTCAACCTGGGACGCAGGGAAGAGTATG
 ACGTTTTGGACAAGCGCAGAGGACGGGACCCTGAGATGGGTGGCAAACC
 AAGACGAAAAAACCCCGAGGAGGGTCTCTATAATGAGCTGCAGAAGGA
 TAAGATGGCTGAAGCCTATTCTGAAATAGGCATGAAAGGAGAGCGGAG
 AAGGGGAAAAGGGCACGACGGTTTGTACCAGGGACTCAGCACTGCTACG
 25 AAGGATACTTATGACGCTCTCCACATGCAAGCCCTGCCACCTAGG

[0085] The corresponding amino acid of intracellular CD3 zeta is set forth in SEQ ID NO. 10:

RVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPGEMGGKPR
 RKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDT
 30 YDALHMQALPPR

Domain Orientation Relative to the Cell

[0086] Structurally, it will be appreciated that the domains described herein correspond to locations relative to an immune or other cell. These domains thus can be part of the (i) “hinge” or extracellular (EC) domain, (ii) the transmembrane (TM) domain, and/or (iii) the intracellular/cytoplasmic domain (IC). The intracellular component frequently comprises, in part, an activating domain such as a portion of a member of the CD3 family, preferably CD3 zeta. This domain is capable of activating the T cell upon binding of the antigen binding molecule to its target. It will be appreciated that the intracellular domain typically further comprises one or more costimulatory molecules as described herein.

[0087] “Activation” or “Stimulation” as used herein, refers to a primary response induced by binding of an activating molecule with its cognate ligand, wherein the binding mediates a signal transduction event.

[0088] An “activating molecule” or “stimulating molecule” refers to a molecule on a T cell, *e.g.*, the TCR/CD3 complex that specifically binds with a cognate stimulatory ligand present on an antigen present cell. Suitable activating molecules are described herein.

[0089] A “costimulatory molecule” as used herein refers to a molecule that provides a signal which mediates a T cell response, including, but not limited to, proliferation, activation, differentiation, and the like. Costimulatory molecules can provide a signal in addition to the primary signal provided by an activating molecule as described herein.

[0090] Suitable costimulatory molecules include, but are not limited to, all or portions of CD28, CD28T, OX40, 4-1BB/CD137, CD2, CD3 (alpha, beta, delta, epsilon, gamma, zeta), CD4, CD5, CD7, CD9, CD16, CD22, CD27, CD30, CD 33, CD37, CD40, CD 45, CD64, CD80, CD86, CD134, CD137, CD154, PD-1, ICOS, lymphocyte function-associated antigen-1 (LFA-1 (CD11a/CD18), CD247, CD276 (B7-H3), LIGHT (tumor necrosis factor superfamily member 14; TNFSF14), NKG2C, Ig alpha (CD79a), DAP-10, Fc gamma receptor, MHC class I molecule, TNF, TNFr, integrin, signaling lymphocytic activation molecule, BTLA, Toll ligand receptor, ICAM-1, B7-H3, CDS, ICAM-1, GITR, BAFR, LIGHT, HVEM (LIGHTR), KIRDS2, SLAMF7, NKp80 (KLRF1), NKp44, NKp30, NKp46, CD19, CD4, CD8alpha, CD8beta, IL2R beta, IL2R gamma, IL7R alpha, ITGA4, VLA1, CD49a, ITGA4, IA4, CD49D, ITGA6, VLA-6, CD49f, ITGAD, CD11d, ITGAE, CD103, ITGAL, CD11a, LFA-1, ITGAM, CD11b, ITGAX, CD11c, ITGB1, CD29, ITGB2, CD18, LFA-1, ITGB7, NKG2D, TNFR2, TRANCE/RANKL, DNAM1 (CD226), SLAMF4 (CD244, 2B4), CD84, CD96 (Tactile), CEACAM1, CRT AM, Ly9 (CD229), CD160 (BY55),

PSGL1, CD100 (SEMA4D), CD69, SLAMF6 (NTB-A, Lyl08), SLAM (SLAMF1, CD150, IPO-3), BLAME (SLAMF8), SELPLG (CD162), LTBR, LAT, GADS, SLP-76, PAG/Cbp, CD19a, CD83 ligand, or fragments or combinations thereof. It will be appreciated that the hinge region may contain some or all of a member of the immunoglobulin family such as

5 IgG1, IgG2, IgG3, IgG4, IgA, IgD, IgE, IgM, or fragment thereof.

[0091] In some embodiments, the extracellular domain is positioned between the antigen binding molecule and the transmembrane domain.

[0092] Exemplary CAR constructs in accordance with the invention are set forth in Table 1.

Table 1

Construct Name	scFv	Hinge Domain	Activating Domain
24C1 CD28T	24C1	CD28T	CD3 zeta
24C1 CD28	24C1	CD28	CD3 zeta
24C1 CD8	24C1	CD8	CD3 zeta
24C8 CD28T	24C8	CD28T	CD3 zeta
24C8 CD28	24C8	CD28	CD3 zeta
24C8 CD8	24C8	CD8	CD3 zeta
20C5.1 CD28T	20C5.1	CD28T	CD3 zeta
20C5.1 CD28	20C5.1	CD28	CD3 zeta
20C5.1 CD8	20C5.1	CD8	CD3 zeta
20C5.2 CD28T	20C5.2	CD28T	CD3 zeta
20C5.2 CD28	20C5.2	CD28	CD3 zeta
20C5.2 CD8	20C5.2	CD8	CD3 zeta

[0093] As noted, the engineered T cells of the invention comprise an antigen binding molecule (such as an scFv), an extracellular domain (which may comprise a “hinge” domain),
5 a transmembrane domain, and an intracellular domain. The intracellular domain can comprise at least in part an activating domain, preferably comprised of a CD3 family member such as CD3 zeta, CD3 epsilon, CD3 gamma, or portions thereof.

[0094] It will further be appreciated that the antigen binding molecule (*e.g.*, one or more scFvs) is engineered such that it is located in the extracellular portion of the
10 molecule/construct, such that it is capable of recognizing and binding to its target or targets.

[0095] **Extracellular Domain.** Extracellular domains of particular use in this invention may be derived from (*i.e.*, comprise) all or some of CD28, OX-40, 4-1BB/CD137, CD2, CD7, CD27, CD30, CD40, programmed death-1 (PD-1), inducible T cell costimulator (ICOS), lymphocyte function-associated antigen-1 (LFA-1, CD11a/CD18), CD3 gamma,
15 CD3 delta, CD3 epsilon, CD247, CD276 (B7-H3), LIGHT, (TNFSF14), NKG2C, Ig alpha

(CD79a), DAP-10, Fc gamma receptor, MHC class I molecule, TNF receptor proteins, an Immunoglobulin protein, cytokine receptor, integrins, Signaling Lymphocytic Activation Molecules (SLAM proteins), activating NK cell receptors, BTLA, a Toll ligand receptor, ICAM-1, B7-H3, CDS, ICAM-1, GITR, BAFFR, LIGHT, HVEM (LIGHTR), KIRDS2, SLAMF7, NKp80 (KLRF1), NKp44, NKp30, NKp46, CD19, CD4, CD8alpha, CD8beta, IL-2R beta, IL-2R gamma, IL-7R alpha, ITGA4, VLA1, CD49a, ITGA4, IA4, CD49D, ITGA6, VLA-6, CD49f, ITGAD, CD11d, ITGAE, CD103, ITGAL, CD11a, LFA-1, ITGAM, CD11b, ITGAX, CD11c, ITGB1, CD29, ITGB2, CD18, LFA-1, ITGB7, NKG2D, TNFR2, TRANCE/RANKL, DNAM1 (CD226), SLAMF4 (CD244, 2B4), CD84, CD96 (Tactile), CEACAM1, CRT AM, Ly9 (CD229), CD160 (BY55), PSGL1, CD100 (SEMA4D), CD69, SLAMF6 (NTB-A, Lyl08), SLAM (SLAMF1, CD150, IPO-3), BLAME (SLAMF8), SELPLG (CD162), LTBR, LAT, GADS, SLP-76, PAG/Cbp, CD19a, a ligand that specifically binds with CD83, or any combination thereof. The extracellular domain may be derived either from a natural or from a synthetic source.

[0096] Extracellular domains often comprise the hinge portion, sometimes referred to as the “spacer” region. A variety of hinges can be employed in accordance with the invention, including portions or derivatives of the molecules described herein.

[0097] In certain embodiments, the hinge region comprises an amino acid sequence that is at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or 100% identical to the extracellular domain amino acid sequences set forth herein.

[0098] In certain embodiments, the hinge region comprises an amino acid sequence that is at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or 100% identical to the extracellular nucleotide amino acid sequences set forth herein.

Transmembrane Domain.

[0099] The CAR can be designed with a transmembrane domain that is fused to the extracellular domain of the CAR. It can similarly be fused to the intracellular domain of the CAR. In some instances, the transmembrane domain can be selected or modified by amino acid substitution to avoid binding of such domains to the transmembrane domains of the same or different surface membrane proteins to minimize interactions with other members of the receptor complex. The transmembrane domain may be derived either from a natural or

from a synthetic source. Where the source is natural, the domain may be derived from any membrane-bound or transmembrane protein. Transmembrane regions of particular use in this invention may be derived from (comprise, or correspond to) CD28, CD28T, OX-40, 4-1BB/CD137, CD2, CD7, CD27, CD30, CD40, programmed death-1 (PD-1), inducible T cell costimulator (ICOS), lymphocyte function-associated antigen-1 (LFA-1, CD11a/CD18), CD3 gamma, CD3 delta, CD3 epsilon, CD247, CD276 (B7-H3), LIGHT, (TNFSF14), NKG2C, Ig alpha (CD79a), DAP-10, Fc gamma receptor, MHC class I molecule, TNF receptor proteins, an Immunoglobulin protein, cytokine receptor, integrins, Signaling Lymphocytic Activation Molecules (SLAM proteins), activating NK cell receptors, BTLA, a Toll ligand receptor, ICAM-1, B7-H3, CDS, ICAM-1, GITR, BAFFR, LIGHT, HVEM (LIGHTR), KIRDS2, SLAMF7, NKp80 (KLRF1), NKp44, NKp30, NKp46, CD19, CD4, CD8alpha, CD8beta, IL-2R beta, IL-2R gamma, IL-7R alpha, ITGA4, VLA1, CD49a, ITGA4, IA4, CD49D, ITGA6, VLA-6, CD49f, ITGAD, CD11d, ITGAE, CD103, ITGAL, CD11a, LFA-1, ITGAM, CD11b, ITGAX, CD11c, ITGB1, CD29, ITGB2, CD18, LFA-1, ITGB7, NKG2D, TNFR2, TRANCE/RANKL, DNAM1 (CD226), SLAMF4 (CD244, 2B4), CD84, CD96 (Tactile), CEACAM1, CRT AM, Ly9 (CD229), CD160 (BY55), PSGL1, CD100 (SEMA4D), CD69, SLAMF6 (NTB-A, Lyl08), SLAM (SLAMF1, CD150, IPO-3), BLAME (SLAMF8), SELPLG (CD162), LTBR, LAT, GADS, SLP-76, PAG/Cbp, CD19a, a ligand that specifically binds with CD83, or any combination thereof.

[0100] Optionally, short linkers may form linkages between any or some of the extracellular, transmembrane, and intracellular domains of the CAR.

[0101] In other embodiments, the transmembrane domain in the CAR of the invention is a CD8 transmembrane domain. In one embodiment, the CD8 transmembrane domain comprises the transmembrane portion of the nucleic acid sequence of SEQ ID NO: 13. In another embodiment, the CD8 transmembrane domain comprises the nucleic acid sequence that encodes the transmembrane amino acid sequence contained within SEQ ID NO: 14.

[0102] In certain embodiments, the transmembrane domain in the CAR of the invention is the CD28 transmembrane domain. In one embodiment, the CD28 transmembrane domain comprises the nucleic acid sequence of SEQ ID NO: 5. In one embodiment, the CD28 transmembrane domain comprises the nucleic acid sequence that encodes the amino acid sequence of SEQ ID NO: 6. In another embodiment, the CD28 transmembrane domain comprises the amino acid sequence of SEQ ID NO: 6.

[0103] Intracellular (Cytoplasmic) Domain. The intracellular (cytoplasmic) domain of the engineered T cells of the invention can provide activation of at least one of the normal effector functions of the immune cell. Effector function of a T cell, for example, may refer to cytolytic activity or helper activity, including the secretion of cytokines.

5 **[0104]** It will be appreciated that suitable intracellular molecules include (*i.e.*, comprise), but are not limited to signaling domains derived from (or corresponding to) CD28, CD28T, OX-40, 4-1BB/CD137, CD2, CD7, CD27, CD30, CD40, programmed death-1 (PD-1), inducible T cell costimulator (ICOS), lymphocyte function-associated antigen-1 (LFA-1, CD11a/CD18), CD3 gamma, CD3 delta, CD3 epsilon, CD247, CD276 (B7-H3), LIGHT, 10 (TNFSF14), NKG2C, Ig alpha (CD79a), DAP-10, Fc gamma receptor, MHC class 1 molecule, TNF receptor proteins, an Immunoglobulin protein, cytokine receptor, integrins, Signaling Lymphocytic Activation Molecules (SLAM proteins), activating NK cell receptors, BTLA, a Toll ligand receptor, ICAM-1, B7-H3, CDS, ICAM-1, GITR, BAFFR, LIGHT, HVEM (LIGHTR), KIRDS2, SLAMF7, NKp80 (KLRF1), NKp44, NKp30, NKp46, CD19, 15 CD4, CD8alpha, CD8beta, IL-2R beta, IL-2R gamma, IL-7R alpha, ITGA4, VLA1, CD49a, ITGA4, IA4, CD49D, ITGA6, VLA-6, CD49f, ITGAD, CD11d, ITGAE, CD103, ITGAL, CD11a, LFA-1, ITGAM, CD11b, ITGAX, CD11c, ITGB1, CD29, ITGB2, CD18, LFA-1, ITGB7, NKG2D, TNFR2, TRANCE/RANKL, DNAM1 (CD226), SLAMF4 (CD244, 2B4), CD84, CD96 (Tactile), CEACAM1, CRT AM, Ly9 (CD229), CD160 (BY55), PSGL1, 20 CD100 (SEMA4D), CD69, SLAMF6 (NTB-A, Lyl08), SLAM (SLAMF1, CD150, IPO-3), BLAME (SLAMF8), SELPLG (CD162), LTBR, LAT, GADS, SLP-76, PAG/Cbp, CD19a, a ligand that specifically binds with CD83, or any combination thereof.

[0105] In a preferred embodiment, the intracellular/cytoplasmic domain of the CAR can be designed to comprise the CD3 zeta domain by itself or combined with any other desired 25 intracellular domain(s) useful in the context of the CAR of the invention. For example, the intracellular domain of the CAR can comprise a CD3 zeta chain portion and a portion of a costimulatory signaling molecule. The intracellular signaling sequences within the intracellular signaling portion of the CAR of the invention may be linked to each other in a random or specified order.

30 **[0106]** In another preferred embodiment, the intracellular domain is designed to comprise the activating domain of CD3 zeta and a signaling domain of CD28. In another embodiment, the intracellular domain is designed to comprise the activating domain of CD3

zeta and a signaling domain of 4-1BB. In another embodiment, the intracellular domain in the CAR is designed to comprise a portion of CD28 and CD3 zeta, wherein the intracellular CD28 comprises the nucleic acid sequence set forth in SEQ ID NO: 7 and the amino acid sequence set forth in SEQ ID NO. 8. The CD3 zeta nucleic acid sequence is set forth in SEQ ID NO: 9, and the amino acid sequence is set forth in SEQ ID NO. 8.

[0107] It will be appreciated that one preferred orientation of the CARs in accordance with the invention comprises an antigen binding molecule (such as scFv) in tandem with an extracellular and/or hinge domain, a costimulatory domain, and an activating domain. It will be further appreciated that multiple domains can be utilized in tandem.

[0108] In some embodiments, isolated nucleic acids are provided comprising a promoter operably linked to a first polynucleotide encoding an antigen binding molecule, at least one costimulatory molecule, and an activating domain. In some embodiments, the nucleic acid construct is contained within a viral vector. In some embodiments, the viral vector is selected from the group consisting of retroviral vectors, murine leukemia virus vectors, SFG vectors, adenoviral vectors, lentiviral vectors, adeno-associated virus (AAV) vectors, Herpes virus vectors, and vaccinia virus vectors. In some embodiments, the nucleic acid is contained within a plasmid.

[0109] In some embodiments, the engineered immune cell is a T cell, tumor infiltrating lymphocyte (TIL), NK cell, TCR-expressing cell, dendritic cell, or NK-T cell. In some embodiments, the cell is obtained or prepared from peripheral blood. In some embodiments, the cell is obtained or prepared from peripheral blood mononuclear cells (PBMCs). In some embodiments, the cell is obtained or prepared from bone marrow. In some embodiments, the cell is obtained or prepared from umbilical cord blood. In some embodiments, the cell is a human cell. In some embodiments, the cell is transfected or transduced by the nucleic acid vector using a method selected from the group consisting of electroporation, sonoporation, biolistics (*e.g.*, Gene Gun), lipid transfection, polymer transfection, nanoparticles, or polyplexes.

[0110] In some embodiments, chimeric antigen receptors are expressed in the engineered immune cells that comprise the nucleic acids of the present application. These chimeric antigen receptors of the present application may comprise, in some embodiments, (i) an antigen binding molecule (such as an scFv), (ii) a transmembrane region, and (iii) a T cell activation molecule or region.

[0111] It is to be further understood that wherever aspects are described herein with the language “comprising,” otherwise analogous aspects described in terms of “consisting of” and/or “consisting essentially of” are also provided.

[0112] Additionally, the terms “about” or “comprising essentially of” refer to a value or composition that is within an acceptable error range for the particular value or composition as determined by one of ordinary skill in the art, which will depend in part on how the value or composition is measured or determined, *i.e.*, the limitations of the measurement system. For example, “about” or “comprising essentially of” can mean within 1 or more than 1 standard deviation per the practice in the art. Alternatively, “about” or “comprising essentially of” can mean a range of up to 10% (*i.e.*, $\pm 10\%$). For example, about 3mg can include any number between 2.7 mg and 3.3 mg (for 10%). Furthermore, particularly with respect to biological systems or processes, the terms can mean up to an order of magnitude or up to 5-fold of a value. When particular values or compositions are provided in the application and claims, unless otherwise stated, the meaning of “about” or “comprising essentially of” should be assumed to be within an acceptable error range for that particular value or composition.

Antigen Binding Molecules

[0113] Antigen binding molecules are within the scope of the invention. An “antigen binding molecule” as used herein means any protein that binds a specified target antigen. In the instant application, the specified target antigen is the CLL-1 protein or fragment thereof. Antigen binding molecules include, but are not limited to antibodies and binding parts thereof, such as immunologically functional fragments. Peptibodies (*i.e.*, Fc fusion molecules comprising peptide binding domains) are another example of suitable antigen binding molecules.

[0114] In certain embodiments, the invention is directed to an antigen binding molecule comprising:

- (a) a heavy chain variable region (VH) complementarity determining region (CDR) 1 comprising the amino acid sequence GX₂X₃X₄X₅X₆X₇X₈X₉ (SEQ ID NO: 134), wherein X₂ is G, F, or Y; X₃ is S or T; X₄ is I, F, or L; X₅ is S or T; X₆ is not present or S; X₇ is not present or G; X₈ is not present or E or G; and X₉ is F, L, or Y;

- (b) a heavy chain variable region (VH) complementarity determining region (CDR) 2 comprising the amino acid sequence $X_1X_2X_3X_4X_5X_6$ (SEQ ID NO: 135), wherein X_1 is D, H, S, or Y; X_2 is H, P, or Y; X_3 is D, E, or S; X_4 is D or G; X_5 is G or S; and X_6 is not present or D or E;
- 5 (c) a heavy chain variable region (VH) complementarity determining region (CDR) 3, comprising the amino acid sequence $X_1X_2X_3X_4X_5X_6X_7X_8X_9X_{10}X_{11}X_{12}DY$ (SEQ ID NO: 136), wherein X_1 is E or L; X_2 is R, S, or V; X_3 is R or Y; X_4 is C, G, or S; X_5 is not present or G or I; X_6 is not present or G; X_7 is not present or D; X_8 is not present or C; X_9 is not present or W or Y; X_{10} is not present or P or S; X_{11} is not present or G or Y; and X_{12} is F or R;
- 10 (d) a light chain variable region (VL) CDR1 comprising the amino acid sequence $X_1ASQX_5X_6X_7X_8X_9LX_{11}$ (SEQ ID NO: 137), wherein X_1 is Q or R; X_5 is D or S; X_6 is I or V; X_7 is N or S; X_8 is N or S; X_9 is F, L, or Y; and X_{11} is N or T;
- 15 (e) a light chain variable region (VL) CDR2 comprising the amino acid sequence $X_1ASX_4X_5X_6X_7$ (SEQ ID NO: 138), wherein X_1 is D or G; X_4 is N, S, or T; X_5 is L or R; X_6 is A, E, or K; and X_7 is S or T; and/or
- (f) a light chain variable region (VL) CDR3 comprising the amino acid sequence $QQX_3X_4X_5X_6PX_8T$ (SEQ ID NO: 139), wherein X_3 is S or Y; X_4 is D, G, or Y; X_5 is N, S, or T; X_6 is L, T, or Y; and X_8 is F or I.
- 20

[0115] In some embodiments, the invention relates to antigen binding molecules comprising at least one of: (a) a variable heavy chain CDR1 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 17, 51, 73, 95, 5, and 97; (b) a variable heavy chain CDR2 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs 18, 52, 74, 96; (c) a variable heavy chain CDR3 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs SEQ ID NO: 19, 53, 75, and 97; (d) a variable light chain CDR1 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 22, 56, 78, and 100; (e) a variable light chain CDR2 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs. 23, 57, 79, and 101; (f) a variable light chain CDR3 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs. 24, 58, 80, and 102.

25

30

[0116] In other embodiments, the invention relates to antigen binding molecules (and chimeric antigen receptors comprising these molecules) comprising at least one of: (a) a VH region comprising the amino acid sequence of SEQ ID NO: 16 and a VL region comprising the amino acid sequence of SEQ ID NO: 21; (b) a VH region comprising the amino acid sequence of SEQ ID NO: 50 and a VL region comprising the amino acid sequence of SEQ ID NO: 55; (c) a VH region comprising the amino acid sequence of SEQ ID NO: 72 and a VL region comprising the amino acid sequence of SEQ ID NO: 77; (d) a VH region comprising the amino acid sequence of SEQ ID NO: 94 and a VL region comprising the amino acid sequence of SEQ ID NO: 99; and wherein the VH and VL region or regions are linked by at least one linker. In other embodiments, the invention relates to antigen binding molecules (and chimeric antigen receptors comprising these molecules) wherein the linker comprises at least one of SEQ ID NO. 130 and SEQ ID NO. 132.

[0117] In further embodiments, the invention relates to antigen binding molecules comprising a variable light (VL) chain CDR3 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 24, 58, 80, and 102.

[0118] In other embodiments, the invention relates to isolated polynucleotides encoding an antigen binding molecule that specifically binds to CLL-1, wherein the antigen binding molecule heavy chain comprises CDR1 (SEQ ID NO. 17), CDR2 (SEQ ID NO. 18), and CDR3 (SEQ ID NO. 19) and the antigen binding molecule light chain comprises CDR1 (SEQ ID NO. 22), CDR2 (SEQ ID NO. 23), and CDR3 (SEQ ID NO. 24).

[0119] In other embodiments, the invention relates to an antigen binding molecule that specifically binds to CLL-1, wherein the antigen binding molecule heavy chain comprises CDR1 (SEQ ID NO. 51), CDR2 (SEQ ID NO. 52), and CDR3 (SEQ ID NO. 53) and the antigen binding molecule light chain comprises CDR1 (SEQ ID NO. 56), CDR2 (SEQ ID NO. 57), and CDR3 (SEQ ID NO. 58).

[0120] In other embodiments, the invention relates an antigen binding molecule that specifically binds to CLL-1, wherein the antigen binding molecule heavy chain comprises CDR1 (SEQ ID NO. 73), CDR2 (SEQ ID NO. 74), and CDR3 (SEQ ID NO. 75) and the antigen binding molecule light chain comprises CDR1 (SEQ ID NO. 78), CDR2 (SEQ ID NO. 79), and CDR3 (SEQ ID NO. 80).

[0121] In other embodiments, the invention relates to isolated polynucleotides encoding an antigen binding molecule that specifically binds to CLL-1, wherein the antigen

binding molecule heavy chain comprises CDR1 (SEQ ID NO. 95), CDR2 (SEQ ID NO. 96), and CDR3 (SEQ ID NO. 97) and the antigen binding molecule light chain comprises CDR1 (SEQ ID NO. 100), CDR2 (SEQ ID NO. 101), and CDR3 (SEQ ID NO. 102).

5 [0122] In certain embodiments, the present invention is directed to an isolated polynucleotide encoding an anti-CLL-1 antigen binding molecule which cross competes with one or more antibodies described herein or an antigen binding molecule thereof encoded by the polynucleotide. In one embodiment, the invention is directed to isolated polynucleotides encoding an anti-CLL-1 antigen binding molecule thereof which binds to the same epitope as one or more of the antigen binding molecules described herein.

10 [0123] In some embodiments, the antigen binding molecule binds to an antigen on a tumor cell. In some embodiments, the antigen binding molecule binds to an antigen on a cell involved in a hyperproliferative disease or to a viral or bacterial antigen. In further embodiments, the antigen binding molecule is an antibody or fragment thereof, including one or more of the complementarity determining regions (CDRs) thereof. In further
15 embodiments, the antigen binding molecule is a single chain variable fragment (scFv).

[0124] The term “immunologically functional fragment” (or “fragment”) of an antigen binding molecule is a species of antigen binding molecule comprising a portion (regardless of how that portion is obtained or synthesized) of an antibody that lacks at least some of the amino acids present in a full-length chain but which is still capable of specifically
20 binding to an antigen. Such fragments are biologically active in that they bind to the target antigen and can compete with other antigen binding molecules, including intact antibodies, for binding to a given epitope. In some embodiments, the fragments are neutralizing fragments. In some embodiments, the fragments can block or reduce the activity of CLL-1. In one aspect, such a fragment will retain at least one CDR present in the full-length light or
25 heavy chain, and in some embodiments will comprise a single heavy chain and/or light chain or portion thereof. These fragments can be produced by recombinant DNA techniques, or can be produced by enzymatic or chemical cleavage of antigen binding molecules, including intact antibodies.

[0125] Immunologically functional immunoglobulin fragments include, but are not
30 limited to, scFv fragments, Fab fragments (Fab', F(ab')₂, and the like), one or more CDR, a diabody (heavy chain variable domain on the same polypeptide as a light chain variable domain, connected via a short peptide linker that is too short to permit pairing between the

two domains on the same chain), domain antibodies, and single-chain antibodies. These fragments can be derived from any mammalian source, including but not limited to human, mouse, rat, camelid or rabbit. As will be appreciated by one of skill in the art, an antigen binding molecule can include non-protein components.

5 **[0126]** Variants of the antigen binding molecules are also within the scope of the invention, *e.g.*, variable light and/or variable heavy chains that each have at least 70-80%, 80-85%, 85-90%, 90-95%, 95-97%, 97-99%, or above 99% identity to the amino acid sequences of the sequences described herein. In some instances, such molecules include at least one heavy chain and one light chain, whereas in other instances the variant forms contain two
10 identical light chains and two identical heavy chains (or subparts thereof). A skilled artisan will be able to determine suitable variants of the antigen binding molecules as set forth herein using well-known techniques. In certain embodiments, one skilled in the art can identify suitable areas of the molecule that may be changed without destroying activity by targeting regions not believed to be important for activity.

15 **[0127]** In certain embodiments, the polypeptide structure of the antigen binding molecules is based on antibodies, including, but not limited to, monoclonal antibodies, bispecific antibodies, minibodies, domain antibodies, synthetic antibodies (sometimes referred to herein as “antibody mimetics”), chimeric antibodies, humanized antibodies, human antibodies, antibody fusions (sometimes referred to herein as “antibody conjugates”),
20 and fragments thereof, respectively. In some embodiments, the antigen binding molecule comprises or consists of avimers.

[0128] In some embodiments, an antigen binding molecule to CLL-1 is administered as part of a CAR, TCR, or other immune cell. In such immune cells, the antigen binding molecule to CLL-1 can be under the control of the same promoter region, or a separate
25 promoter. In certain embodiments, the genes encoding protein agents and/or an antigen binding molecule to CLL-1 can be in separate vectors.

[0129] The invention further provides for pharmaceutical compositions comprising an antigen binding molecule to CLL-1 together with a pharmaceutically acceptable diluent, carrier, solubilizer, emulsifier, preservative and/or adjuvant. In certain embodiments,
30 pharmaceutical compositions will include more than one different antigen binding molecule to CLL-1. In certain embodiments, pharmaceutical compositions will include more than one antigen binding molecule to CLL-1 wherein the antigen binding molecules to CLL-1 bind

more than one epitope. In some embodiments, the various antigen binding molecules will not compete with one another for binding to CLL-1.

[0130] In other embodiments, the pharmaceutical composition can be selected for parenteral delivery, for inhalation, or for delivery through the digestive tract, such as orally.

5 The preparation of such pharmaceutically acceptable compositions is within the ability of one skilled in the art. In certain embodiments, buffers are used to maintain the composition at physiological pH or at a slightly lower pH, typically within a pH range of from about 5 to about 8. In certain embodiments, when parenteral administration is contemplated, a therapeutic composition can be in the form of a pyrogen-free, parenterally acceptable aqueous
10 solution comprising a desired antigen binding molecule to CLL-1, with or without additional therapeutic agents, in a pharmaceutically acceptable vehicle. In certain embodiments, a vehicle for parenteral injection is sterile distilled water in which an antigen binding molecule to CLL-1, with or without at least one additional therapeutic agent, is formulated as a sterile, isotonic solution, properly preserved. In certain embodiments, the preparation can involve
15 the formulation of the desired molecule with polymeric compounds (such as polylactic acid or polyglycolic acid), beads or liposomes that can provide for the controlled or sustained release of the product which can then be delivered via a depot injection. In certain embodiments, implantable drug delivery devices can be used to introduce the desired molecule.

20 **[0131]** In some embodiments, the antigen binding molecule is used as a diagnostic or validation tool. The antigen binding molecule can be used to assay the amount of CLL-1 present in a sample and/or subject. In some embodiments, the diagnostic antigen binding molecule is not neutralizing. In some embodiments, the antigen binding molecules disclosed herein are used or provided in an assay kit and/or method for the detection of CLL-1 in
25 mammalian tissues or cells in order to screen/diagnose for a disease or disorder associated with changes in levels of CLL-1. The kit can comprise an antigen binding molecule that binds CLL-1, along with means for indicating the binding of the antigen binding molecule with CLL-1, if present, and optionally CLL-1 protein levels.

[0132] The antigen binding molecules will be further understood in view of the
30 definitions and descriptions below.

[0133] An “Fc” region comprises two heavy chain fragments comprising the CH1 and CH2 domains of an antibody. The two heavy chain fragments are held together by two or more disulfide bonds and by hydrophobic interactions of the CH3 domains.

[0134] A “Fab fragment” comprises one light chain and the CH1 and variable regions of one heavy chain. The heavy chain of a Fab molecule cannot form a disulfide bond with another heavy chain molecule. A “Fab’ fragment” comprises one light chain and a portion of one heavy chain that contains the VH domain and the CH1 domain and also the region between the CH1 and CH2 domains, such that an interchain disulfide bond can be formed between the two heavy chains of two Fab’ fragments to form an F(ab’)₂ molecule. An “F(ab’)₂ fragment” contains two light chains and two heavy chains containing a portion of the constant region between the CH1 and CH2 domains, such that an interchain disulfide bond is formed between the two heavy chains. An F(ab’)₂ fragment thus is composed of two Fab’ fragments that are held together by a disulfide bond between the two heavy chains.

[0135] The “Fv region” comprises the variable regions from both the heavy and light chains, but lacks the constant regions.

[0136] “Single chain variable fragment” (“scFv”, also termed “single-chain antibody”) refers to Fv molecules in which the heavy and light chain variable regions have been connected by a flexible linker to form a single polypeptide chain, which forms an antigen binding region. See PCT application WO88/01649 and U.S. Patent Nos. 4,946,778 and 5,260,203, the disclosures of which are incorporated by reference in their entirety.

[0137] A “bivalent antigen binding molecule” comprises two antigen binding sites. In some instances, the two binding sites have the same antigen specificities. Bivalent antigen binding molecules can be bispecific. A “multispecific antigen binding molecule” is one that targets more than one antigen or epitope. A “bispecific,” “dual-specific” or “bifunctional” antigen binding molecule is a hybrid antigen binding molecule or antibody, respectively, having two different antigen binding sites. The two binding sites of a bispecific antigen binding molecule will bind to two different epitopes, which can reside on the same or different protein targets.

[0138] An antigen binding molecule is said to “specifically bind” its target antigen when the dissociation constant (K_d) is $\sim 1 \times 10^{-7}$ M. The antigen binding molecule specifically binds antigen with “high affinity” when the K_d is $1-5 \times 10^{-9}$ M, and with “very high affinity” when the K_d is $1-5 \times 10^{-10}$ M. In one embodiment, the antigen binding molecule has a K_d of

10^{-9} M. In one embodiment, the off-rate is $<1 \times 10^{-5}$. In other embodiments, the antigen binding molecules will bind to human CLL-1 with a K_d of between about 10^{-7} M and 10^{-13} M, and in yet another embodiment the antigen binding molecules will bind with a K_d 1.0 - 5×10^{-10} .

[0139] In some embodiments, the antibody or antigen binding molecules of the present invention specifically bind CLL-1 (*e.g.*, hCLL-1). In certain embodiments, an anti-CLL-1 antibody or antigen binding molecule of the present invention binds human CLL-1 with a K_D of less than 1×10^{-6} M, less than 1×10^{-7} M, less than 1×10^{-8} M, or less than 1×10^{-9} M. In one particular embodiment, the anti-CLL-1 antibody or antigen binding molecules binds human CLL-1 with a K_D of less than 1×10^{-7} M. In another embodiment, the anti-CLL-1 antibody or antigen binding molecules binds human CLL-1 with a K_D of less than 1×10^{-8} M. In some embodiments, the anti-CLL-1 antibody or antigen binding molecules binds human CLL-1 with a K_D of about 1×10^{-7} M, about 2×10^{-7} M, about 3×10^{-7} M, about 4×10^{-7} M, about 5×10^{-7} M, about 6×10^{-7} M, about 7×10^{-7} M, about 8×10^{-7} M, about 9×10^{-7} M, about 1×10^{-8} M, about 2×10^{-8} M, about 3×10^{-8} M, about 4×10^{-8} M, about 5×10^{-8} M, about 6×10^{-8} M, about 7×10^{-8} M, about 8×10^{-8} M, about 9×10^{-8} M, about 1×10^{-9} M, about 2×10^{-9} M, about 3×10^{-9} M, about 4×10^{-9} M, about 5×10^{-9} M, about 6×10^{-9} M, about 7×10^{-9} M, about 8×10^{-9} M, about 9×10^{-9} M, about 1×10^{-10} M, or about 5×10^{-10} M. In certain embodiments, the K_D is calculated as the quotient of k_{off}/k_{on} , and the k_{on} and k_{off} are determined using a monovalent antibody, such as a Fab fragment, as measured by, *e.g.*, BIAcore® surface plasmon resonance technology. In other embodiments, the K_D is calculated as the quotient of k_{off}/k_{on} , and the k_{on} and k_{off} are determined using a bivalent antibody, such as a Fab fragment, as measured by, *e.g.*, BIAcore® surface plasmon resonance technology.

[0140] In other embodiments, the anti-CLL-1 antibody or antigen binding molecule binds human CLL-1-Fc with a K_D of less than 1×10^{-9} M, less than 3×10^{-9} M, less than 5×10^{-9} M, less than 1×10^{-10} M, less than 3×10^{-10} M, or less than 5×10^{-10} M. In other embodiments, the anti-CLL-1 antibody or antigen binding molecules binds cyno CLL-1-Fc with a K_D of less than 1×10^{-5} M, less than 1×10^{-6} M, less than 1×10^{-7} M, less than 1×10^{-8} M, less than 1×10^{-9} M, or less than 1×10^{-10} M.

[0141] In some embodiments, the anti-CLL-1 antibody or antigen binding molecule binds human CLL-1 with an association rate (k_{on}) of less than $1 \times 10^{-4} \text{ M}^{-1} \text{ s}^{-1}$, less than $2 \times 10^{-4} \text{ M}^{-1} \text{ s}^{-1}$, less than $3 \times 10^{-4} \text{ M}^{-1} \text{ s}^{-1}$, less than $4 \times 10^{-4} \text{ M}^{-1} \text{ s}^{-1}$, less than $5 \times 10^{-4} \text{ M}^{-1} \text{ s}^{-1}$, less than $6 \times 10^{-4} \text{ M}^{-1} \text{ s}^{-1}$, less than $7 \times 10^{-4} \text{ M}^{-1} \text{ s}^{-1}$, less than $8 \times 10^{-4} \text{ M}^{-1} \text{ s}^{-1}$, less than $9 \times 10^{-4} \text{ M}^{-1} \text{ s}^{-1}$.

¹ s⁻¹, less than 1 x 10⁻⁵ M⁻¹ s⁻¹, less than 2 x 10⁻⁵ M⁻¹ s⁻¹, less than 3 x 10⁻⁵ M⁻¹ s⁻¹, less than 4 x 10⁻⁵ M⁻¹ s⁻¹, less than 5 x 10⁻⁵ M⁻¹ s⁻¹, less than 6 x 10⁻⁵ M⁻¹ s⁻¹, less than 7 x 10⁻⁵ M⁻¹ s⁻¹, less than 8 x 10⁻⁵ M⁻¹ s⁻¹, less than 9 x 10⁻⁵ M⁻¹ s⁻¹, less than 1 x 10⁻⁶ M⁻¹ s⁻¹, less than 2 x 10⁻⁶ M⁻¹ s⁻¹, less than 3 x 10⁻⁶ M⁻¹ s⁻¹, less than 4 x 10⁻⁶ M⁻¹ s⁻¹, less than 5 x 10⁻⁶ M⁻¹ s⁻¹, less than 6 x 10⁻⁶ M⁻¹ s⁻¹, less than 7 x 10⁻⁶ M⁻¹ s⁻¹, less than 8 x 10⁻⁶ M⁻¹ s⁻¹, less than 9 x 10⁻⁶ M⁻¹ s⁻¹, or less than 1 x 10⁻⁷ M⁻¹ s⁻¹. In certain embodiments, the k_{on} is determined using a monovalent antibody, such as a Fab fragment, as measured by, *e.g.*, BIAcore® surface plasmon resonance technology. In other embodiments, the k_{on} is determined using a bivalent antibody as measured by, *e.g.*, BIAcore® surface plasmon resonance technology.

10 [0142] In some embodiments, the anti-CLL-1 antibody or antigen binding molecule binds human CLL-1 with an dissociation rate (k_{off}) of less than 1 x 10⁻² s⁻¹, less than 2 x 10⁻² s⁻¹, less than 3 x 10⁻² s⁻¹, less than 4 x 10⁻² s⁻¹, less than 5 x 10⁻² s⁻¹, less than 6 x 10⁻² s⁻¹, less than 7 x 10⁻² s⁻¹, less than 8 x 10⁻² s⁻¹, less than 9 x 10⁻² s⁻¹, less than 1 x 10⁻³ s⁻¹, less than 2 x 10⁻³ s⁻¹, less than 3 x 10⁻³ s⁻¹, less than 4 x 10⁻³ s⁻¹, less than 5 x 10⁻³ s⁻¹, less than 6 x 10⁻³ s⁻¹,
15 less than 7 x 10⁻³ s⁻¹, less than 8 x 10⁻³ s⁻¹, less than 9 x 10⁻³ s⁻¹, less than 1 x 10⁻⁴ s⁻¹, less than 2 x 10⁻⁴ s⁻¹, less than 3 x 10⁻⁴ s⁻¹, less than 4 x 10⁻⁴ s⁻¹, less than 5 x 10⁻⁴ s⁻¹, less than 6 x 10⁻⁴ s⁻¹, less than 7 x 10⁻⁴ s⁻¹, less than 8 x 10⁻⁴ s⁻¹, less than 9 x 10⁻⁴ s⁻¹, less than 1 x 10⁻⁴ s⁻¹, or less than 5 x 10⁻⁴ s⁻¹. In certain embodiments, the k_{off} is determined using a monovalent antibody, such as a Fab fragment, as measured by, *e.g.*, BIAcore® surface plasmon resonance technology. In other embodiments, the k_{off} is determined using a bivalent antibody as measured by, *e.g.*, BIAcore® surface plasmon resonance technology.

[0143] An antigen binding molecule is said to be “selective” when it binds to one target more tightly than it binds to a second target.

[0144] The term “antibody” refers to an intact immunoglobulin of any isotype, or a
25 fragment thereof that can compete with the intact antibody for specific binding to the target antigen, and includes, for instance, chimeric, humanized, fully human, and bispecific antibodies. An “antibody” is a species of an antigen binding molecule as defined herein. An intact antibody will generally comprise at least two full-length heavy chains and two full-length light chains, but in some instances can include fewer chains such as antibodies
30 naturally occurring in camelids which can comprise only heavy chains. Antibodies can be derived solely from a single source, or can be chimeric, that is, different portions of the antibody can be derived from two different antibodies as described further below. The

antigen binding molecules, antibodies, or binding fragments can be produced in hybridomas, by recombinant DNA techniques, or by enzymatic or chemical cleavage of intact antibodies. Unless otherwise indicated, the term “antibody” includes, in addition to antibodies comprising two full-length heavy chains and two full-length light chains, derivatives, 5 variants, fragments, and muteins thereof, examples of which are described below. Furthermore, unless explicitly excluded, antibodies include monoclonal antibodies, bispecific antibodies, minibodies, domain antibodies, synthetic antibodies (sometimes referred to herein as “antibody mimetics”), chimeric antibodies, humanized antibodies, human antibodies, antibody fusions (sometimes referred to herein as “antibody conjugates”) and fragments 10 thereof, respectively.

[0145] The variable regions typically exhibit the same general structure of relatively conserved framework regions (FR) joined by the 3 hypervariable regions (*i.e.*, “CDRs”). The CDRs from the two chains of each pair typically are aligned by the framework regions, which can enable binding to a specific epitope. From N-terminal to C-terminal, both light and heavy 15 chain variable regions typically comprise the domains FR1, CDR1, FR2, CDR2, FR3, CDR3 and FR4. By convention, CDR regions in the heavy chain are typically referred to as HC CDR1, CDR2, and CDR3. The CDR regions in the light chain are typically referred to as LC CDR1, CDR2, and CDR3. The assignment of amino acids to each domain is typically in accordance with the definitions of Kabat, Chothia, or the AbM definition.

20 **[0146]** The term “Kabat numbering” and like terms are recognized in the art and refer to a system of numbering amino acid residues in the heavy and light chain variable regions of an antibody, or an antigen-binding portion thereof. In certain aspects, the CDRs of an antibody can be determined according to the Kabat numbering system (*see, e.g.*, Kabat EA & Wu TT (1971) Ann NY Acad Sci 190: 382-391 and Kabat EA *et al.*, (1991) Sequences of 25 Proteins of Immunological Interest, Fifth Edition, U.S. Department of Health and Human Services, NIH Publication No. 91-3242). Using the Kabat numbering system, CDRs within an antibody heavy chain molecule are typically present at amino acid positions 31 to 35, which optionally can include one or two additional amino acids, following 35 (referred to in the Kabat numbering scheme as 35A and 35B) (CDR1), amino acid positions 50 to 65 (CDR2), and amino acid positions 95 to 102 (CDR3). Using the Kabat numbering system, 30 CDRs within an antibody light chain molecule are typically present at amino acid positions 24 to 34 (CDR1), amino acid positions 50 to 56 (CDR2), and amino acid positions 89 to 97

(CDR3). In a specific embodiment, the CDRs of the antibodies described herein have been determined according to the Kabat numbering scheme.

[0147] In certain aspects, the CDRs of an antibody can be determined according to the Chothia numbering scheme, which refers to the location of immunoglobulin structural loops (*see, e.g.*, Chothia C & Lesk AM, (1987), J Mol Biol 196: 901-917; Al-Lazikani B *et al.*, (1997) J Mol Biol 273: 927-948; Chothia C *et al.*, (1992) J Mol Biol 227: 799-817; Tramontano A *et al.*, (1990) J Mol Biol 215(1): 175-82; and U.S. Patent No. 7,709,226). Typically, when using the Kabat numbering convention, the Chothia CDR-H1 loop is present at heavy chain amino acids 26 to 32, 33, or 34, the Chothia CDR-H2 loop is present at heavy chain amino acids 52 to 56, and the Chothia CDR-H3 loop is present at heavy chain amino acids 95 to 102, while the Chothia CDR-L1 loop is present at light chain amino acids 24 to 34, the Chothia CDR-L2 loop is present at light chain amino acids 50 to 56, and the Chothia CDR-L3 loop is present at light chain amino acids 89 to 97. The end of the Chothia CDR-H1 loop when numbered using the Kabat numbering convention varies between H32 and H34 depending on the length of the loop (this is because the Kabat numbering scheme places the insertions at H35A and H35B; if neither 35A nor 35B is present, the loop ends at 32; if only 35A is present, the loop ends at 33; if both 35A and 35B are present, the loop ends at 34).

[0148] In a specific embodiment, the CDRs of the antibodies described herein have been determined according to the Chothia numbering scheme.

[0149] A number of definitions of the CDRs are commonly in use: Kabat numbering, Chothia numbering, AbM numbering, or contact numbering. The AbM definition is a compromise between the two used by Oxford Molecular's AbM antibody modelling software. The contact definition is based on an analysis of the available complex crystal structures.

Table 2: CDR Numbering

Loop	Kabat	AbM	Chothia	Contact
L1	L24--L34	L24--L34	L24--L34	L30--L36
L2	L50--L56	L50--L56	L50--L56	L46--L55
L3	L89--L97	L89--L97	L89--L97	L89--L96
H1	H31--H35B (Kabat Numbering)	H26--H35B	H26--H32..34	H30--H35B

H1	H31--H35 (Chothia Numbering)	H26--H35	H26--H32	H30--H35
H2	H50--H65	H50--H58	H52--H56	H47--H58
H3	H95--H102	H95--H102	H95--H102	H93--H101

[0150] As used herein, the term “heavy chain” when used in reference to an antibody can refer to any distinct type, *e.g.*, alpha (α), delta (δ), epsilon (ϵ), gamma (γ) and mu (μ), based on the amino acid sequence of the constant domain, which give rise to IgA, IgD, IgE, IgG and IgM classes of antibodies, respectively, including subclasses of IgG, *e.g.*, IgG₁, IgG₂, IgG₃ and IgG₄.

[0151] As used herein, the term “light chain” when used in reference to an antibody can refer to any distinct type, *e.g.*, kappa (κ) or lambda (λ) based on the amino acid sequence of the constant domains. Light chain amino acid sequences are well known in the art. In specific embodiments, the light chain is a human light chain.

[0152] The term “variable region” or “variable domain” refers to a portion of the light and/or heavy chains of an antibody, typically including approximately the amino-terminal 120 to 130 amino acids in the heavy chain and about 100 to 110 amino terminal amino acids in the light chain. The variable region of an antibody typically determines specificity of a particular antibody for its target.

[0153] Variability is not evenly distributed throughout the variable domains of antibodies or antigen binding molecules; it is concentrated in sub-domains of each of the heavy and light chain variable regions. These subdomains are called “hypervariable regions” or “complementarity determining regions” (CDRs) as further described herein. The more conserved (*i.e.*, non-hypervariable) portions of the variable domains are called the “framework” regions (FRM or FR) and provide a scaffold for the six CDRs in three dimensional space to form an antigen-binding surface. The variable domains of naturally occurring heavy and light chains each comprise four FRM regions (FR1, FR2, FR3, and FR4), largely adopting a β -sheet configuration, connected by three hypervariable regions, which form loops connecting, and in some cases forming part of, the β -sheet structure. The hypervariable regions in each chain are held together in close proximity by the FRM and, with the hypervariable regions from the other chain, contribute to the formation of the antigen-binding site (see Kabat *et al.*, described further herein).

[0154] Typically, CDRs form a loop structure that can be classified as a canonical structure. The term “canonical structure” refers to the main chain conformation that is adopted by the antigen binding (CDR) loops. From comparative structural studies, it has been found that five of the six antigen binding loops have only a limited repertoire of available conformations. Each canonical structure can be characterized by the torsion angles of the polypeptide backbone. Correspondent loops between antibodies may, therefore, have very similar three dimensional structures, despite high amino acid sequence variability in most parts of the loops (Chothia and Lesk, J. Mol. Biol., 1987, 196: 901; Chothia *et al.*, Nature, 1989, 342: 877; Martin and Thornton, J. Mol. Biol, 1996, 263: 800). Furthermore, there is a relationship between the adopted loop structure and the amino acid sequences surrounding it. The conformation of a particular canonical class is determined by the length of the loop and the amino acid residues residing at key positions within the loop, as well as within the conserved framework (i.e., outside of the loop). Assignment to a particular canonical class can therefore be made based on the presence of these key amino acid residues.

[0155] The term “canonical structure” may also include considerations as to the linear sequence of the antibody, for example, as catalogued by Kabat (Kabat *et al.*, herein). The Kabat numbering scheme (system) is a widely adopted standard for numbering the amino acid residues of an antibody variable domain in a consistent manner and is the preferred scheme applied in the present invention as also mentioned elsewhere herein. Additional structural considerations can also be used to determine the canonical structure of an antibody. For example, those differences not fully reflected by Kabat numbering can be described by the numbering system of Chothia *et al.* and/or revealed by other techniques, for example, crystallography and two- or three-dimensional computational modeling. Accordingly, a given antibody sequence may be placed into a canonical class which allows for, among other things, identifying appropriate chassis sequences (*e.g.*, based on a desire to include a variety of canonical structures in a library). Kabat numbering of antibody amino acid sequences and structural considerations as described by Chothia *et al.* (herein) and their implications for construing canonical aspects of antibody structure, are described in the literature. The subunit structures and three-dimensional configurations of different classes of immunoglobulins are well known in the art. For a review of the antibody structure, see Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory, eds. Harlow *et al.*, 1988.

[0156] The CDR3 of the light chain and, particularly, the CDR3 of the heavy chain may constitute the most important determinants in antigen binding within the light and heavy

chain variable regions. In some antibody constructs, the heavy chain CDR3 appears to constitute the major area of contact between the antigen and the antibody. In vitro selection schemes in which CDR3 alone is varied can be used to vary the binding properties of an antibody or determine which residues contribute to the binding of an antigen. Hence, CDR3
5 is typically the greatest source of molecular diversity within the antibody-binding site. H3, for example, can be as short as two amino acid residues or greater than 26 amino acids.

[0157] As used herein, the terms “constant region” and “constant domain” are interchangeable and have a meaning common in the art. The constant region is an antibody portion, *e.g.*, a carboxyl terminal portion of a light and/or heavy chain which is not directly
10 involved in binding of an antibody to antigen but which can exhibit various effector functions, such as interaction with the Fc receptor. The constant region of an immunoglobulin molecule generally has a more conserved amino acid sequence relative to an immunoglobulin variable domain.

[0158] “Binding affinity” generally refers to the strength of the sum total of non-covalent interactions between a single binding site of a molecule (*e.g.*, an antibody) and its
15 binding partner (*e.g.*, an antigen). Unless indicated otherwise, as used herein, “binding affinity” refers to intrinsic binding affinity which reflects a 1:1 interaction between members of a binding pair (*e.g.*, antibody and antigen). The affinity of a molecule X for its partner Y can generally be represented by the dissociation constant (K_D). Affinity can be measured
20 and/or expressed in a number of ways known in the art, including, but not limited to, equilibrium dissociation constant (K_D), and equilibrium association constant (K_A). The K_D is calculated from the quotient of k_{off}/k_{on} , whereas K_A is calculated from the quotient of k_{on}/k_{off} . k_{on} refers to the association rate constant of, *e.g.*, an antibody to an antigen, and k_{off} refers to the dissociation of, *e.g.*, an antibody to an antigen. The k_{on} and k_{off} can be determined by
25 techniques known to one of ordinary skill in the art, such as BIAcore® or KinExA.

[0159] The term “neutralizing” refers to an antigen binding molecule, scFv, or antibody, respectively, that binds to a ligand and prevents or reduces the biological effect of that ligand. This can be done, for example, by directly blocking a binding site on the ligand or by binding to the ligand and altering the ligand's ability to bind through indirect means
30 (such as structural or energetic alterations in the ligand). In some embodiments, the term can also denote an antigen binding molecule that prevents the protein to which it is bound from performing a biological function.

[0160] The term “target” or “antigen” refers to a molecule or a portion of a molecule capable of being bound by an antigen binding molecule. In certain embodiments, a target can have one or more epitopes.

[0161] The term “compete” when used in the context of antigen binding molecules that compete for the same epitope means competition between antigen binding molecules as determined by an assay in which the antigen binding molecule (*e.g.*, antibody or immunologically functional fragment thereof) being tested prevents or inhibits (*e.g.*, reduces) specific binding of a reference antigen binding molecule to an antigen. Numerous types of competitive binding assays can be used to determine if one antigen binding molecule competes with another, for example: solid phase direct or indirect radioimmunoassay (RIA), solid phase direct or indirect enzyme immunoassay (EIA), sandwich competition assay (Stahli *et al.*, 1983, *Methods in Enzymology* 9:242-253); solid phase direct biotin-avidin EIA (Kirkland *et al.*, 1986, *J. Immunol.* 137:3614-3619), solid phase direct labeled assay, solid phase direct labeled sandwich assay (Harlow and Lane, 1988, *Antibodies, A Laboratory Manual*, Cold Spring Harbor Press); solid phase direct label RIA using 1-125 label (Morel *et al.*, 1988, *Molec. Immunol.* 25:7-15); solid phase direct biotin-avidin EIA (Cheung, *et al.*, 1990, *Virology* 176:546-552); and direct labeled RIA (Moldenhauer *et al.*, 1990, *Scand. J. Immunol.* 32:77-82).

[0162] As used herein, the term “epitope” refers to a localized region of an antigen to which an antibody can specifically bind. An epitope can be, for example, contiguous amino acids of a polypeptide (linear or contiguous epitope) or an epitope can, for example, come together from two or more non-contiguous regions of a polypeptide or polypeptides (conformational, non-linear, discontinuous, or non-contiguous epitope). In certain embodiments, the epitope to which an antibody binds can be determined by, *e.g.*, NMR spectroscopy, X-ray diffraction crystallography studies, ELISA assays, hydrogen/deuterium exchange coupled with mass spectrometry (*e.g.*, liquid chromatography electrospray mass spectrometry), array-based oligo-peptide scanning assays, and/or mutagenesis mapping (*e.g.*, site-directed mutagenesis mapping). For X-ray crystallography, crystallization may be accomplished using any of the known methods in the art (*e.g.*, Giegé R. *et al.*, (1994) *Acta Crystallogr D Biol Crystallogr* 50(Pt 4): 339-350; McPherson A (1990) *Eur J Biochem* 189: 1-23; Chayen NE (1997) *Structure* 5: 1269-1274; McPherson A (1976) *J Biol Chem* 251: 6300-6303). Antibody:antigen crystals may be studied using well known X-ray diffraction techniques and may be refined using computer software such as X-PLOR (Yale University,

1992, distributed by Molecular Simulations, Inc.; see *e.g.* Meth Enzymol (1985) volumes 114 & 115, eds Wyckoff HW *et al.*,; U.S. 2004/0014194), and BUSTER (Bricogne G (1993) Acta Crystallogr D Biol Crystallogr 49(Pt 1): 37-60; Bricogne G (1997) Meth Enzymol 276A: 361-423, ed Carter CW; Roversi P *et al.*, (2000) Acta Crystallogr D Biol Crystallogr 56(Pt 10): 1316-1323). Mutagenesis mapping studies may be accomplished using any method known to one of skill in the art. See, *e.g.*, Champe M *et al.*, (1995) J Biol Chem 270: 1388-1394 and Cunningham BC & Wells JA (1989) Science 244: 1081-1085 for a description of mutagenesis techniques, including alanine scanning mutagenesis techniques.

[0163] As used herein, the terms “label” or “labeled” refers to incorporation of a detectable marker, *e.g.*, by incorporation of a radiolabeled amino acid or attachment to a polypeptide of biotin moieties that can be detected by marked avidin (*e.g.*, streptavidin containing a fluorescent marker or enzymatic activity that can be detected by optical or colorimetric methods). In certain embodiments, the label or marker can also be therapeutic. Various methods of labeling polypeptides and glycoproteins are known in the art and can be used.

METHODS OF TREATMENT

[0164] Using adoptive immunotherapy, native T cells can be (i) removed from a patient, (ii) genetically engineered to express a chimeric antigen receptor (CAR) that binds to at least one tumor antigen (iii) expanded *ex vivo* into a larger population of engineered T cells, and (iv) reintroduced into the patient. See *e.g.*, U.S. Patent Nos. 7,741,465, and 6,319,494, Eshhar *et al.* (Cancer Immunol, *supra*); Krause *et al.* (*supra*); Finney *et al.* (*supra*). After the engineered T cells are reintroduced into the patient, they mediate an immune response against cells expressing the tumor antigen. See *e.g.*, Krause *et al.*, J. Exp. Med., Volume 188, No. 4, 1998 (619–626). This immune response includes secretion of IL-2 and other cytokines by T cells, the clonal expansion of T cells recognizing the tumor antigen, and T cell-mediated specific killing of target-positive cells. See Hombach *et al.*, Journal of Immun. 167: 6123–6131 (2001).

[0165] The term “lymphocyte” as used herein includes natural killer (NK) cells, T cells, or B cells. NK cells are a type of cytotoxic (cell toxic) lymphocyte that represent a major component of the inherent immune system. NK cells reject tumors and cells infected by viruses. It works through the process of apoptosis or programmed cell death. They were

termed “natural killers” because they do not require activation in order to kill cells. T-cells play a major role in cell-mediated-immunity (no antibody involvement). Its T-cell receptors (TCR) differentiate themselves from other lymphocyte types. The thymus, a specialized organ of the immune system, is primarily responsible for the T cell’s maturation. There are
5 six types of T-cells, namely: Helper T-cells (*e.g.*, CD4+ cells), Cytotoxic T-cells (also known as TC, cytotoxic T lymphocyte, CTL, T-killer cell, cytolytic T cell, CD8+ T-cells or killer T cell), Memory T-cells ((i) stem memory T_{SCM} cells, like naive cells, are CD45RO[−], CCR7⁺, CD45RA⁺, CD62L⁺ (L-selectin), CD27⁺, CD28⁺ and IL-7Rα⁺, but they also express large amounts of CD95, IL-2Rβ, CXCR3, and LFA-1, and show numerous functional attributes
10 distinctive of memory cells); (ii) central memory T_{CM} cells express L-selectin and the CCR7, they secrete IL-2, but not IFNγ or IL-4, and (iii) effector memory T_{EM} cells, however, do not express L-selectin or CCR7 but produce effector cytokines like IFNγ and IL-4), Regulatory T-cells (Tregs, suppressor T cells, or CD4+CD25+ regulatory T cells), Natural Killer T-cells (NKT) and Gamma Delta T-cells. B-cells, on the other hand, play a principal role in humoral
15 immunity (with antibody involvement). It makes antibodies and antigens and performs the role of antigen-presenting cells (APCs) and turns into memory B-cells after activation by antigen interaction. In mammals, immature B-cells are formed in the bone marrow, where its name is derived from.

[0166] The term “genetically engineered” or “engineered” refers to a method of
20 modifying the genome of a cell, including, but not limited to, deleting a coding or non-coding region or a portion thereof or inserting a coding region or a portion thereof. In some embodiments, the cell that is modified is a lymphocyte, *e.g.*, a T cell, which can either be obtained from a patient or a donor. The cell can be modified to express an exogenous construct, such as, *e.g.*, a chimeric antigen receptor (CAR) or a T cell receptor (TCR), which
25 is incorporated into the cell's genome.

[0167] An “immune response” refers to the action of a cell of the immune system (for example, T lymphocytes, B lymphocytes, natural killer (NK) cells, macrophages, eosinophils, mast cells, dendritic cells and neutrophils) and soluble macromolecules produced by any of these cells or the liver (including Abs, cytokines, and complement) that results in selective
30 targeting, binding to, damage to, destruction of, and/or elimination from a vertebrate's body of invading pathogens, cells or tissues infected with pathogens, cancerous or other abnormal cells, or, in cases of autoimmunity or pathological inflammation, normal human cells or tissues.

[0168] The term “immunotherapy” refers to the treatment of a subject afflicted with, or at risk of contracting or suffering a recurrence of, a disease by a method comprising inducing, enhancing, suppressing or otherwise modifying an immune response. Examples of immunotherapy include, but are not limited to, T cell therapies. T cell therapy can include adoptive T cell therapy, tumor-infiltrating lymphocyte (TIL) immunotherapy, autologous cell therapy, engineered autologous cell therapy (eACT), and allogeneic T cell transplantation. However, one of skill in the art would recognize that the conditioning methods disclosed herein would enhance the effectiveness of any transplanted T cell therapy. Examples of T cell therapies are described in U.S. Patent Publication Nos. 2014/0154228 and 2002/0006409, U.S. Patent No. 5,728,388, and International Publication No. WO 2008/081035.

[0169] The T cells of the immunotherapy can come from any source known in the art. For example, T cells can be differentiated *in vitro* from a hematopoietic stem cell population, or T cells can be obtained from a subject. T cells can be obtained from, *e.g.*, peripheral blood mononuclear cells (PBMCs), bone marrow, lymph node tissue, cord blood, thymus tissue, tissue from a site of infection, ascites, pleural effusion, spleen tissue, and tumors. In addition, the T cells can be derived from one or more T cell lines available in the art. T cells can also be obtained from a unit of blood collected from a subject using any number of techniques known to the skilled artisan, such as FICOLL™ separation and/or apheresis. Additional methods of isolating T cells for a T cell therapy are disclosed in U.S. Patent Publication No. 2013/0287748, which is herein incorporated by references in its entirety.

[0170] The term “engineered Autologous Cell Therapy,” which can be abbreviated as “eACT™,” also known as adoptive cell transfer, is a process by which a patient's own T cells are collected and subsequently genetically altered to recognize and target one or more antigens expressed on the cell surface of one or more specific tumor cells or malignancies. T cells can be engineered to express, for example, chimeric antigen receptors (CAR) or T cell receptor (TCR). CAR positive (+) T cells are engineered to express an extracellular single chain variable fragment (scFv) with specificity for a particular tumor antigen linked to an intracellular signaling part comprising at least one costimulatory domain and at least one activating domain. The costimulatory domain can be derived from (or correspond to), *e.g.*, CD28, and the activating domain can be derived from (or correspond to) *e.g.*, CD3-zeta. In certain embodiments, the CAR is designed to have two, three, four, or more costimulatory domains.

[0171] The term “autologous” refers to any material derived from the same individual to which it is later to be re-introduced. For example, the engineered autologous cell therapy (eACT™) method described herein involves collection of lymphocytes from a patient, which are then engineered to express, *e.g.*, a CAR construct, and then administered back to the same patient.

[0172] The term “allogeneic” refers to any material derived from one individual which is then introduced to another individual of the same species, *e.g.*, allogeneic T cell transplantation.

[0173] In some aspects, the invention therefore comprises a method for treating or preventing a condition associated with undesired and/or elevated CLL-1 levels in a patient, comprising administering to a patient in need thereof an effective amount of at least one isolated antigen binding molecule, CAR, or TCR disclosed herein.

[0174] Methods are provided for treating diseases or disorders, including cancer. In some embodiments, the invention relates to creating a T cell-mediated immune response in a subject, comprising administering an effective amount of the engineered immune cells of the present application to the subject. In some embodiments, the T cell-mediated immune response is directed against a target cell or cells. In some embodiments, the engineered immune cell comprises a chimeric antigen receptor (CAR), or a T cell receptor (TCR). In some embodiments, the target cell is a tumor cell. In some aspects, the invention comprises a method for treating or preventing a malignancy, said method comprising administering to a subject in need thereof an effective amount of at least one isolated antigen binding molecule described herein. In some aspects, the invention comprises a method for treating or preventing a malignancy, said method comprising administering to a subject in need thereof an effective amount of at least one immune cell, wherein the immune cell comprises at least one chimeric antigen receptor, T cell receptor, and/or isolated antigen binding molecule as described herein.

[0175] In some aspects, the invention comprises a pharmaceutical composition comprising at least one antigen binding molecule as described herein and a pharmaceutically acceptable excipient. In some embodiments, the pharmaceutical composition further comprises an additional active agent.

[0176] The antigen binding molecules, CARs, TCRs, immune cells, and the like of the invention can be used to treat myeloid diseases including but not limited to acute myeloid

leukemia (AML), chronic myelogenous leukemia (CML), chronic myelomonocytic leukemia (CMML), juvenile myelomonocytic leukemia, atypical chronic myeloid leukemia, acute promyelocytic leukemia (APL), acute monoblastic leukemia, acute erythroid leukemia, acute megakaryoblastic leukemia, myelodysplastic syndrome (MDS), myeloproliferative disorder, 5 myeloid neoplasm, myeloid sarcoma), Blastic Plasmacytoid Dendritic Cell Neoplasm (BPDCN), or combinations thereof. Additional diseases include inflammatory and/or autoimmune diseases such as rheumatoid arthritis, psoriasis, allergies, asthma, Crohn's disease, IBD, IBS, fibromyalgia, mastocytosis, and Celiac disease.

[0177] It will be appreciated that target doses for CAR⁺/ CAR-T⁺/ TCR⁺ cells can range from 1x10⁶ - 2x10¹⁰ cells/kg, preferably 2x10⁶ cells/kg, more preferably. It will be appreciated that doses above and below this range may be appropriate for certain subjects, and appropriate dose levels can be determined by the healthcare provider as needed. Additionally, multiple doses of cells can be provided in accordance with the invention.

[0178] Also provided are methods for reducing the size of a tumor in a subject, 15 comprising administering to the subject an engineered cell of the present invention to the subject, wherein the cell comprises a chimeric antigen receptor, a T cell receptor, or a T cell receptor based chimeric antigen receptor comprising an antigen binding molecule binds to an antigen on the tumor. In some embodiments, the subject has a solid tumor, or a blood malignancy such as lymphoma or leukemia. In some embodiments, the engineered cell is 20 delivered to a tumor bed. In some embodiments, the cancer is present in the bone marrow of the subject. In some embodiments, the engineered cells are autologous T cells. In some embodiments, the engineered cells are allogeneic T cells. In some embodiments, the engineered cells are heterologous T cells. In some embodiments, the engineered cells of the present application are transfected or transduced *in vivo*. In other embodiments, the 25 engineered cells are transfected or transduced *ex vivo*. As used herein, the term "*in vitro* cell" refers to any cell which is cultured *ex vivo*. In particular, an *in vitro* cell can include a T cell.

[0179] The methods can further comprise administering one or more chemotherapeutic agent. In certain embodiments, the chemotherapeutic agent is a lymphodepleting (preconditioning) chemotherapeutic. Beneficial preconditioning treatment 30 regimens, along with correlative beneficial biomarkers are described in U.S. Provisional Patent Applications 62/262,143 and 62/167,750 which are hereby incorporated by reference in their entirety herein. These describe, *e.g.*, methods of conditioning a patient in need of a

T cell therapy comprising administering to the patient specified beneficial doses of cyclophosphamide (between 200 mg/m²/day and 2000 mg/m²/day) and specified doses of fludarabine (between 20 mg/m²/day and 900 mg/m²/day). A preferred dose regimen involves treating a patient comprising administering daily to the patient about 500 mg/m²/day of cyclophosphamide and about 60 mg/m²/day of fludarabine for three days prior to administration of a therapeutically effective amount of engineered T cells to the patient.

[0180] In other embodiments, the antigen binding molecule, transduced (or otherwise engineered) cells (such as CARs or TCRs), and the chemotherapeutic agent are administered each in an amount effective to treat the disease or condition in the subject.

[0181] In certain embodiments, compositions comprising CAR-expressing immune effector cells disclosed herein may be administered in conjunction with any number of chemotherapeutic agents. Examples of chemotherapeutic agents include alkylating agents such as thiotepa and cyclophosphamide (CYTOXANTM); alkyl sulfonates such as busulfan, improsulfan and piposulfan; aziridines such as benzodopa, carboquone, meturedopa, and uredopa; ethylenimines and methylamelamines including altretamine, triethylenemelamine, triethylenephosphoramidate, triethylenethiophosphoramidate and trimethylolomelamine resins; nitrogen mustards such as chlorambucil, chlornaphazine, chlorthalophamide, estramustine, ifosfamide, mechlorethamine, mechlorethamine oxide hydrochloride, melphalan, novembichin, phenesterine, prednimustine, trofosfamide, uracil mustard; nitrosureas such as carmustine, chlorozotocin, fotemustine, lomustine, nimustine, ranimustine; antibiotics such as aclacinomysins, actinomycin, anthramycin, azaserine, bleomycins, cactinomycin, calicheamicin, carabacin, carminomycin, carzinophilin, chromomycins, dactinomycin, daunorubicin, detorubicin, 6-diazo-5-oxo-L-norleucine, doxorubicin, epirubicin, esorubicin, idarubicin, marcellomycin, mitomycins, mycophenolic acid, nogalamycin, olivomycins, peplomycin, potfiromycin, puromycin, quelamycin, rodorubicin, streptonigrin, streptozocin, tubercidin, ubenimex, zinostatin, zorubicin; anti-metabolites such as methotrexate and 5-fluorouracil (5-FU); folic acid analogues such as denopterin, methotrexate, pteropterin, trimetrexate; purine analogs such as fludarabine, 6-mercaptopurine, thiamiprine, thioguanine; pyrimidine analogs such as ancitabine, azacitidine, 6-azauridine, carmofur, cytarabine, dideoxyuridine, doxifluridine, enocitabine, floxuridine, 5-FU; androgens such as calusterone, dromostanolone propionate, epitostanol, mepitostane, testolactone; anti-adrenals such as aminoglutethimide, mitotane, trilostane; folic acid replenisher such as frolinic acid; aceglatone; aldophosphamide glycoside; aminolevulinic acid; amsacrine; bestrabucil;

bisantrone; edatraxate; defofamine; demecolcine; diaziquone; elformithine; elliptinium acetate; etoglucid; gallium nitrate; hydroxyurea; lentinan; lonidamine; mitoguazone; mitoxantrone; mopidamol; nitracrine; pentostatin; phenamet; pirarubicin; podophyllinic acid; 2-ethylhydrazide; procarbazine; PSK[®]; razoxane; sizofiran; spirogermanium; tenuazonic acid; triaziquone; 2, 2',2''-trichlorotriethylamine; urethan; vindesine; dacarbazine; mannomustine; mitobronitol; mitolactol; pipobroman; gacytosine; arabinoside ("Ara-C"); cyclophosphamide; thiotepa; taxoids, *e.g.* paclitaxel (TAXOL[™], Bristol-Myers Squibb) and doxetaxel (TAXOTERE[®], Rhone-Poulenc Rorer); chlorambucil; gemcitabine; 6-thioguanine; mercaptopurine; methotrexate; platinum analogs such as cisplatin and carboplatin; vinblastine; platinum; etoposide (VP-16); ifosfamide; mitomycin C; mitoxantrone; vincristine; vinorelbine; navelbine; novantrone; teniposide; daunomycin; aminopterin; xeloda; ibandronate; CPT-11; topoisomerase inhibitor RFS2000; difluoromethylomithine (DMFO); retinoic acid derivatives such as Targretin[™] (bexarotene), Panretin[™], (alitretinoin); ONTAK[™] (denileukin diftitox); esperamicins; capecitabine; and pharmaceutically acceptable salts, acids or derivatives of any of the above. Also included in this definition are anti-hormonal agents that act to regulate or inhibit hormone action on tumors such as anti-estrogens including for example tamoxifen, raloxifene, aromatase inhibiting 4(5)-imidazoles, 4-hydroxytamoxifen, trioxifene, keoxifene, LY117018, onapristone, and toremifene (Fareston); and anti-androgens such as flutamide, nilutamide, bicalutamide, leuprolide, and goserelin; and pharmaceutically acceptable salts, acids or derivatives of any of the above. Combinations of chemotherapeutic agents are also administered where appropriate, including, but not limited to CHOP, *i.e.*, Cyclophosphamide (Cytoxan[®]), Doxorubicin (hydroxydoxorubicin), Vincristine (Oncovin[®]), and Prednisone.

[0182] In some embodiments, the chemotherapeutic agent is administered at the same time or within one week after the administration of the engineered cell, polypeptide, or nucleic acid. In other embodiments, the chemotherapeutic agent is administered from 1 to 4 weeks or from 1 week to 1 month, 1 week to 2 months, 1 week to 3 months, 1 week to 6 months, 1 week to 9 months, or 1 week to 12 months after the administration of the engineered cell, polypeptide, or nucleic acid. In other embodiments, the chemotherapeutic agent is administered at least 1 month before administering the cell, polypeptide, or nucleic acid. In some embodiments, the methods further comprise administering two or more chemotherapeutic agents.

[0183] A variety of additional therapeutic agents may be used in conjunction with the compositions described herein. For example, potentially useful additional therapeutic agents include PD-1 inhibitors such as nivolumab (Opdivo®), pembrolizumab (Keytruda®), pembrolizumab, pidilizumab, and atezolizumab.

5 **[0184]** Additional therapeutic agents suitable for use in combination with the invention include, but are not limited to, ibrutinib (Imbruvica®), ofatumumab (Arzerra®), rituximab (Rituxan®), bevacizumab (Avastin®), trastuzumab (Herceptin®), trastuzumab emtansine (KADCYLA®), imatinib (Gleevec®), cetuximab (Erbix®), panitumumab (Vectibix®), catumaxomab, ibritumomab, ofatumumab, tositumomab, brentuximab, 10 alemtuzumab, gemtuzumab, erlotinib, gefitinib, vandetanib, afatinib, lapatinib, neratinib, axitinib, masitinib, pazopanib, sunitinib, sorafenib, toceranib, lestaurtinib, axitinib, cediranib, lenvatinib, nintedanib, pazopanib, regorafenib, semaxanib, sorafenib, sunitinib, tivozanib, toceranib, vandetanib, entrectinib, cabozantinib, imatinib, dasatinib, nilotinib, ponatinib, radotinib, bosutinib, lestaurtinib, ruxolitinib, pacritinib, cobimetinib, selumetinib, trametinib, 15 binimetinib, alectinib, ceritinib, crizotinib, aflibercept, adipotide, denileukin diftitox, mTOR inhibitors such as Everolimus and Temsirolimus, hedgehog inhibitors such as sonidegib and vismodegib, CDK inhibitors such as CDK inhibitor (palbociclib).

[0185] In additional embodiments, the composition comprising CAR-containing immune can be administered with an anti-inflammatory agent. Anti-inflammatory agents or 20 drugs include, but are not limited to, steroids and glucocorticoids (including betamethasone, budesonide, dexamethasone, hydrocortisone acetate, hydrocortisone, hydrocortisone, methylprednisolone, prednisolone, prednisone, triamcinolone), nonsteroidal anti-inflammatory drugs (NSAIDs) including aspirin, ibuprofen, naproxen, methotrexate, sulfasalazine, leflunomide, anti-TNF medications, cyclophosphamide and 25 mycophenolate. Exemplary NSAIDs include ibuprofen, naproxen, naproxen sodium, Cox-2 inhibitors, and sialylates. Exemplary analgesics include acetaminophen, oxycodone, tramadol or propoxyphene hydrochloride. Exemplary glucocorticoids include cortisone, dexamethasone, hydrocortisone, methylprednisolone, prednisolone, or prednisone. Exemplary biological response modifiers include molecules directed against cell surface 30 markers (*e.g.*, CD4, CD5, etc.), cytokine inhibitors, such as the TNF antagonists, (*e.g.*, etanercept (ENBREL®), adalimumab (HUMIRA®) and infliximab (REMICADE®), chemokine inhibitors and adhesion molecule inhibitors. The biological response modifiers include monoclonal antibodies as well as recombinant forms of molecules. Exemplary

DMARDs include azathioprine, cyclophosphamide, cyclosporine, methotrexate, penicillamine, leflunomide, sulfasalazine, hydroxychloroquine, Gold (oral (auranofin) and intramuscular) and minocycline.

[0186] In certain embodiments, the compositions described herein are administered
5 in conjunction with a cytokine. "Cytokine" as used herein is meant to refer to proteins released by one cell population that act on another cell as intercellular mediators. Examples of 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;
10 insulin; proinsulin; relaxin; prorelaxin; glycoprotein hormones such as follicle stimulating hormone (FSH), thyroid stimulating hormone (TSH), and luteinizing hormone (LH); hepatic growth factor (HGF); fibroblast growth factor (FGF); prolactin; placental lactogen; mullerian-inhibiting substance; mouse gonadotropin-associated peptide; inhibin; activin; vascular endothelial growth factor; integrin; thrombopoietin (TPO); nerve growth factors
15 (NGFs) 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,
20 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.

25 **[0187]** In some aspects, the invention comprises an antigen binding molecule that binds to CLL-1 with a K_d that is smaller than 100 pM. In some embodiments, the antigen binding molecule binds with a K_d that is smaller than 10 pM. In other embodiments, the antigen binding molecule binds with a K_d that is less than 5 pM.

Methods of Making

[0188] A variety of known techniques can be utilized in making the polynucleotides, polypeptides, vectors, antigen binding molecules, immune cells, compositions, and the like according to the invention.

5 [0189] Prior to the *in vitro* manipulation or genetic modification of the immune cells described herein, the cells may be obtained from a subject. In some embodiments, the immune cells comprise T cells. T cells can be obtained from a number of sources, including peripheral blood mononuclear cells (PBMCs), bone marrow, lymph nodes tissue, cord blood, thymus tissue, tissue from a site of infection, ascites, pleural effusion, spleen tissue, and
10 tumors. In certain embodiments, T cells can be obtained from a unit of blood collected from the subject using any number of techniques known to the skilled person, such as FICOLL™ separation. Cells may preferably be obtained from the circulating blood of an individual by apheresis. The apheresis product typically contains lymphocytes, including T cells, monocytes, granulocytes, B cells, other nucleated white blood cells, red blood cells, and
15 platelets. In certain embodiments, the cells collected by apheresis may be washed to remove the plasma fraction, and placed in an appropriate buffer or media for subsequent processing. The cells may be washed with PBS. As will be appreciated, a washing step may be used, such as by using a semiautomated flowthrough centrifuge -- for example, the Cobe™ 2991 cell processor, the Baxter CytoMate™, or the like. After washing, the cells may be
20 resuspended in a variety of biocompatible buffers, or other saline solution with or without buffer. In certain embodiments, the undesired components of the apheresis sample may be removed.

[0190] In certain embodiments, T cells are isolated from PBMCs by lysing the red blood cells and depleting the monocytes, for example, using centrifugation through a
25 PERCOLL™ gradient. A specific subpopulation of T cells, such as CD28⁺, CD4⁺, CD8⁺, CD45RA⁺, and CD45RO⁺ T cells can be further isolated by positive or negative selection techniques known in the art. For example, enrichment of a T cell population by negative selection can be accomplished with a combination of antibodies directed to surface markers unique to the negatively selected cells. One method for use herein is cell sorting and/or
30 selection via negative magnetic immunoadherence or flow cytometry that uses a cocktail of monoclonal antibodies directed to cell surface markers present on the cells negatively selected. For example, to enrich for CD4⁺ cells by negative selection, a monoclonal antibody

cocktail typically includes antibodies to CD14, CD20, CD11b, CD16, HLA-DR, and CD8. Flow cytometry and cell sorting may also be used to isolate cell populations of interest for use in the present invention.

[0191] PBMCs may be used directly for genetic modification with the immune cells (such as CARs or TCRs) using methods as described herein. In certain embodiments, after isolating the PBMCs, T lymphocytes can be further isolated and both cytotoxic and helper T lymphocytes can be sorted into naive, memory, and effector T cell subpopulations either before or after genetic modification and/or expansion.

[0192] In some embodiments, CD8⁺ cells are further sorted into naive, central memory, and effector cells by identifying cell surface antigens that are associated with each of these types of CD8⁺ cells. In some embodiments, the expression of phenotypic markers of central memory T cells include CD45RO, CD62L, CCR7, CD28, CD3, and CD127 and are negative for granzyme B. In some embodiments, central memory T cells are CD45RO⁺, CD62L⁺, CD8⁺ T cells. In some embodiments, effector T cells are negative for CD62L, CCR7, CD28, and CD127, and positive for granzyme B and perforin. In certain embodiments, CD4⁺ T cells are further sorted into subpopulations. For example, CD4⁺ T helper cells can be sorted into naive, central memory, and effector cells by identifying cell populations that have cell surface antigens.

[0193] The immune cells, such as T cells, can be genetically modified following isolation using known methods, or the immune cells can be activated and expanded (or differentiated in the case of progenitors) *in vitro* prior to being genetically modified. In another embodiment, the immune cells, such as T cells, are genetically modified with the chimeric antigen receptors described herein (*e.g.*, transduced with a viral vector comprising one or more nucleotide sequences encoding a CAR) and then are activated and/or expanded *in vitro*. Methods for activating and expanding T cells are known in the art and are described, for example, in U.S. Patent No. 6,905,874; U.S. Patent No. 6,867,041; U.S. Patent No. 6,797,514; and PCT WO2012/079000, the contents of which are hereby incorporated by reference in their entirety. Generally, such methods include contacting PBMC or isolated T cells with a stimulatory molecule and a costimulatory molecule, such as anti-CD3 and anti-CD28 antibodies, generally attached to a bead or other surface, in a culture medium with appropriate cytokines, such as IL-2. Anti-CD3 and anti-CD28 antibodies attached to the same bead serve as a “surrogate” antigen presenting cell (APC). One example is The Dynabeads[®]

system, a CD3/CD28 activator/stimulator system for physiological activation of human T cells. In other embodiments, the T cells may be activated and stimulated to proliferate with feeder cells and appropriate antibodies and cytokines using methods such as those described in U.S. Patent No. 6,040,177; U.S. Patent No. 5,827,642; and
5 WO2012129514, the contents of which are hereby incorporated by reference in their entirety.

[0194] Certain methods for making the constructs and engineered immune cells of the invention are described in PCT application PCT/US15/14520, the contents of which are hereby incorporated by reference in their entirety. Additional methods of making the constructs and cells can be found in U.S. provisional patent application no. 62/244036 the
10 contents of which are hereby incorporated by reference in their entirety.

[0195] It will be appreciated that PBMCs can further include other cytotoxic lymphocytes such as NK cells or NKT cells. An expression vector carrying the coding sequence of a chimeric receptor as disclosed herein can be introduced into a population of human donor T cells, NK cells or NKT cells. Successfully transduced T cells that carry the
15 expression vector can be sorted using flow cytometry to isolate CD3 positive T cells and then further propagated to increase the number of these CAR expressing T cells in addition to cell activation using anti-CD3 antibodies and IL-2 or other methods known in the art as described elsewhere herein. Standard procedures are used for cryopreservation of T cells expressing the CAR for storage and/or preparation for use in a human subject. In one embodiment, the
20 *in vitro* transduction, culture and/or expansion of T cells are performed in the absence of non-human animal derived products such as fetal calf serum and fetal bovine serum.

[0196] For cloning of polynucleotides, the vector may be introduced into a host cell (an isolated host cell) to allow replication of the vector itself and thereby amplify the copies of the polynucleotide contained therein. The cloning vectors may contain sequence
25 components generally include, without limitation, an origin of replication, promoter sequences, transcription initiation sequences, enhancer sequences, and selectable markers. These elements may be selected as appropriate by a person of ordinary skill in the art. For example, the origin of replication may be selected to promote autonomous replication of the vector in the host cell.

[0197] In certain embodiments, the present disclosure provides isolated host cells
30 containing the vector provided herein. The host cells containing the vector may be useful in expression or cloning of the polynucleotide contained in the vector. Suitable host cells can

include, without limitation, prokaryotic cells, fungal cells, yeast cells, or higher eukaryotic cells such as mammalian cells. Suitable prokaryotic cells for this purpose include, without limitation, eubacteria, such as Gram-negative or Gram-positive organisms, for example, Enterobacterales such as *Escherichia*, *e.g.*, *E. coli*, *Enterobacter*, *Erwinia*, *Klebsiella*,
5 *Proteus*, *Salmonella*, *e.g.*, *Salmonella typhimurium*, *Serratia*, *e.g.*, *Serratia marcescens*, and *Shigella*, as well as Bacilli such as *B. subtilis* and *B. licheniformis*, *Pseudomonas* such as *P. aeruginosa*, and *Streptomyces*.

[0198] The vector can be introduced to the host cell using any suitable methods known in the art, including, without limitation, DEAE-dextran mediated delivery, calcium
10 phosphate precipitate method, cationic lipids mediated delivery, liposome mediated transfection, electroporation, microprojectile bombardment, receptor-mediated gene delivery, delivery mediated by polylysine, histone, chitosan, and peptides. Standard methods for transfection and transformation of cells for expression of a vector of interest are well known in the art. In a further embodiment, a mixture of different expression vectors can be
15 used in genetically modifying a donor population of immune effector cells wherein each vector encodes a different CAR as disclosed herein. The resulting transduced immune effector cells form a mixed population of engineered cells, with a proportion of the engineered cells expressing more than one different CARs.

[0199] In one embodiment, the invention provides a method of storing genetically
20 engineered cells expressing CARs or TCRs which target a CLL-1 protein. This involves cryopreserving the immune cells such that the cells remain viable upon thawing. A fraction of the immune cells expressing the CARs can be cryopreserved by methods known in the art to provide a permanent source of such cells for the future treatment of patients afflicted with a malignancy. When needed, the cryopreserved transformed immune cells can be thawed,
25 grown and expanded for more such cells.

[0200] As used herein, "cryopreserve" refers to the preservation of cells by cooling to sub-zero temperatures, such as (typically) 77 Kelvin or -196°C (the boiling point of liquid nitrogen). Cryoprotective agents are often used at sub-zero temperatures to prevent the cells being preserved from damage due to freezing at low temperatures or warming to room
30 temperature. Cryopreservative agents and optimal cooling rates can protect against cell injury. Cryoprotective agents which can be used in accordance with the invention include but are not limited to: dimethyl sulfoxide (DMSO) (Lovelock & Bishop, *Nature* (1959); 183:

1394-1395; Ashwood-Smith, Nature (1961); 190: 1204-1205), glycerol, polyvinylpyrrolidone (Rinfret, Ann. N.Y. Acad. Sci. (1960); 85: 576), and polyethylene glycol (Sloviter & Ravdin, Nature (1962); 196: 48). The preferred cooling rate is 1° - 3°C/minute.

[0201] The term, “substantially pure,” is used to indicate that a given component is present at a high level. The component is desirably the predominant component present in a composition. Preferably it is present at a level of more than 30%, of more than 50%, of more than 75%, of more than 90%, or even of more than 95%, said level being determined on a dry weight/dry weight basis with respect to the total composition under consideration. At very high levels (*e.g.* at levels of more than 90%, of more than 95% or of more than 99%) the component can be regarded as being in “pure form.” Biologically active substances of the present invention (CARs, TCRs, isolated polypeptides, isolated nucleic acid molecules, antigen binding molecules, moieties) can be provided in a form that is substantially free of one or more contaminants with which the substance might otherwise be associated. When a composition is substantially free of a given contaminant, the contaminant will be at a low level (*e.g.*, at a level of less than 10%, less than 5%, or less than 1% on the dry weight/dry weight basis set out above).

[0202] In some embodiments, the cells are formulated by first harvesting them from their culture medium, and then washing and concentrating the cells in a medium and container system suitable for administration (a “pharmaceutically acceptable” carrier) in a treatment-effective amount. Suitable infusion media can be any isotonic medium formulation, typically normal saline, Normosol™ R (Abbott) or Plasma-Lyte™ A (Baxter), but also 5% dextrose in water or Ringer's lactate can be utilized. The infusion medium can be supplemented with human serum albumin.

[0203] Desired treatment amounts of cells in the composition is generally at least 2 cells (for example, at least 1 CD8⁺ central memory T cell and at least 1 CD4⁺ helper T cell subset) or is more typically greater than 10² cells, and up to 10⁶, up to and including 10⁸ or 10⁹ cells and can be more than 10¹⁰ cells. The number of cells will depend upon the desired use for which the composition is intended, and the type of cells included therein. The density of the desired cells is typically greater than 10⁶ cells/ml and generally is greater than 10⁷ cells/ml, generally 10⁸ cells/ml or greater. The clinically relevant number of immune cells can be apportioned into multiple infusions that cumulatively equal or exceed 10⁵, 10⁶, 10⁷, 10⁸, 10⁹, 10¹⁰, 10¹¹, or 10¹² cells. In some aspects of the present invention, particularly since

all the infused cells will be redirected to a particular target antigen (CLL-1), lower numbers of cells, in the range of 10^6 /kilogram (10^6 - 10^{11} per patient) may be administered. CAR treatments may be administered multiple times at dosages within these ranges. The cells may be autologous, allogeneic, or heterologous to the patient undergoing therapy.

5 **[0204]** The CAR expressing cell populations of the present invention may be administered either alone, or as a pharmaceutical composition in combination with diluents and/or with other components such as IL-2 or other cytokines or cell populations. Pharmaceutical compositions of the present invention may comprise a CAR or TCR expressing cell population, such as T cells, as described herein, in combination with one or
10 more pharmaceutically or physiologically acceptable carriers, diluents or excipients. Such compositions may comprise buffers such as neutral buffered saline, phosphate buffered saline and the like; carbohydrates such as glucose, mannose, sucrose or dextrans, mannitol; proteins; polypeptides or amino acids such as glycine; antioxidants; chelating agents such as EDTA or glutathione; adjuvants (*e.g.*, aluminum hydroxide); and preservatives. Compositions of the
15 present invention are preferably formulated for intravenous administration.

[0205] The pharmaceutical compositions (solutions, suspensions or the like), may include one or more of the following: sterile diluents such as water for injection, saline solution, preferably physiological saline, Ringer's solution, isotonic sodium chloride, fixed oils such as synthetic mono- or diglycerides which may serve as the solvent or suspending
20 medium, polyethylene glycols, glycerin, propylene glycol or other solvents; antibacterial agents such as benzyl alcohol or methyl paraben; antioxidants such as ascorbic acid or sodium bisulfite; chelating agents such as ethylenediaminetetraacetic acid; buffers such as acetates, citrates or phosphates and agents for the adjustment of tonicity such as sodium chloride or dextrose. The parenteral preparation can be enclosed in ampoules, disposable syringes or
25 multiple dose vials made of glass or plastic. An injectable pharmaceutical composition is preferably sterile.

[0206] It will be appreciated that adverse events may be minimized by transducing the immune cells (containing one or more CARs or TCRs) with a suicide gene. It may also be desired to incorporate an inducible "on" or "accelerator" switch into the immune cells.
30 Suitable techniques include use of inducible caspase-9 (U.S. Appl. 2011/0286980) or a thymidine kinase, before, after or at the same time, as the cells are transduced with the CAR construct of the present invention. Additional methods for introducing suicide genes and/or

“on” switches include TALENS, zinc fingers, RNAi, siRNA, shRNA, antisense technology, and other techniques known in the art.

[0207] In accordance with the invention, additional on-off or other types of control switch techniques may be incorporated herein. These techniques may employ the use of dimerization domains and optional activators of such domain dimerization. These techniques include, e.g., those described by Wu et al., Science 2014 350 (6258) utilizing FKBP/Rapalog dimerization systems in certain cells, the contents of which are incorporated by reference herein in their entirety. Additional dimerization technology is described in, e.g., Fegan et al. Chem. Rev. 2010, 110, 3315–3336 as well as U.S. Patent Nos. 5,830,462; 5,834,266; 5,869,337; and 6,165,787, the contents of which are also incorporated by reference herein in their entirety. Additional dimerization pairs may include cyclosporine-A/cyclophilin, receptor, estrogen/estrogen receptor (optionally using tamoxifen), glucocorticoids/glucocorticoid receptor, tetracycline/tetracycline receptor, vitamin D/vitamin D receptor. Further examples of dimerization technology can be found in e.g., WO 2014/127261, WO 2015/090229, US 2014/0286987, US2015/0266973, US2016/0046700, U.S. Patent No. 8,486,693, US 2014/0171649, and US 2012/0130076, the contents of which are further incorporated by reference herein in their entirety.

[0208] It will be understood that descriptions herein are exemplary and explanatory only and are not restrictive of the invention as claimed. In this application, the use of the singular includes the plural unless specifically stated otherwise.

[0209] The section headings used herein are for organizational purposes only and are not to be construed as limiting the subject matter described. All documents, or portions of documents, cited in this application, including but not limited to patents, patent applications, articles, books, and treatises, are hereby expressly incorporated by reference in their entirety for any purpose. As utilized in accordance with the present disclosure, the following terms, unless otherwise indicated, shall be understood to have the following meanings:

[0210] In this application, the use of “or” means “and/or” unless stated otherwise. Furthermore, the use of the term “including”, as well as other forms, such as “includes” and “included”, is not limiting. Also, terms such as “element” or “component” encompass both elements and components comprising one unit and elements and components that comprise more than one subunit unless specifically stated otherwise.

[0211] The term “CLL-1 activity” includes any biological effect of CLL-1. In certain embodiments, CLL-1 activity includes the ability of CLL-1 to interact or bind to a substrate or receptor.

[0212] The term “polynucleotide,” “nucleotide,” or “nucleic acid” includes both
5 single-stranded and double-stranded nucleotide polymers. This preferably includes isolated polynucleotides, nucleotides or nucleic acids as defined herein. The nucleotides comprising the polynucleotide can be ribonucleotides or deoxyribonucleotides or a modified form of either type of nucleotide. Said modifications include base modifications such as bromouridine and inosine derivatives, ribose modifications such as 2',3'-dideoxyribose, and
10 internucleotide linkage modifications such as phosphorothioate, phosphorodithioate, phosphoroselenoate, phosphoro-diselenoate, phosphoro-anilothioate, phosphoraniladate and phosphoroamidate.

[0213] The term “oligonucleotide” refers to a polynucleotide comprising 200 or fewer nucleotides. Oligonucleotides can be single stranded or double stranded, *e.g.*, for use in the
15 construction of a mutant gene. Oligonucleotides can be sense or antisense oligonucleotides. An oligonucleotide can include a label, including a radiolabel, a fluorescent label, a hapten or an antigenic label, for detection assays. Oligonucleotides can be used, for example, as PCR primers, cloning primers or hybridization probes.

[0214] The term “control sequence” refers to a polynucleotide sequence that can
20 affect the expression and processing of coding sequences to which it is ligated. The nature of such control sequences can depend upon the host organism. In particular embodiments, control sequences for prokaryotes can include a promoter, a ribosomal binding site, and a transcription termination sequence. For example, control sequences for eukaryotes can include promoters comprising one or a plurality of recognition sites for transcription factors,
25 transcription enhancer sequences, and transcription termination sequence. “Control sequences” can include leader sequences (signal peptides) and/or fusion partner sequences.

[0215] In some embodiments, the polynucleotide of the present invention encodes a CAR or a TCR can further comprises a leader sequence or peptide (also referred to herein as a “signal peptide”). In certain embodiments, the leader peptide comprises an amino acid
30 sequence that is at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or 100% identical to the amino acid sequence MALPVTALLPLALLHAARP (SEQ

ID NO: 144). In some embodiments, the leader peptide comprises the amino acid sequence of SEQ ID NO: 144.

[0216] As used herein, “operably linked” means that the components to which the term is applied are in a relationship that allows them to carry out their inherent functions under suitable conditions.

[0217] The term “vector” means any molecule or entity (*e.g.*, nucleic acid, plasmid, bacteriophage or virus) used to transfer protein coding information into a host cell. The term “expression vector” or “expression construct” refers to a vector that is suitable for transformation of a host cell and contains nucleic acid sequences that direct and/or control (in conjunction with the host cell) expression of one or more heterologous coding regions operatively linked thereto. An expression construct can include, but is not limited to, sequences that affect or control transcription, translation, and, if introns are present, affect RNA splicing of a coding region operably linked thereto.

[0218] The term “host cell” refers to a cell that has been transformed, or is capable of being transformed, with a nucleic acid sequence and thereby expresses a gene of interest. The term includes the progeny of the parent cell, whether or not the progeny is identical in morphology or in genetic make-up to the original parent cell, so long as the gene of interest is present.

[0219] The term “transformation” refers to a change in a cell's genetic characteristics, and a cell has been transformed when it has been modified to contain new DNA or RNA. For example, a cell is transformed where it is genetically modified from its native state by introducing new genetic material via transfection, transduction, or other techniques. Following transfection or transduction, the transforming DNA can recombine with that of the cell by physically integrating into a chromosome of the cell, or can be maintained transiently as an episomal element without being replicated, or can replicate independently as a plasmid. A cell is considered to have been “stably transformed” when the transforming DNA is replicated with the division of the cell.

[0220] The term “transfection” refers to the uptake of foreign or exogenous DNA by a cell. A number of transfection techniques are well known in the art and are disclosed herein. See, *e.g.*, Graham *et al.*, 1973, *Virology* 52:456; Sambrook *et al.*, 2001, *Molecular Cloning: A Laboratory Manual*, *supra*; Davis *et al.*, 1986, *Basic Methods in Molecular Biology*, Elsevier; Chu *et al.*, 1981, *Gene* 13:197.

[0221] The term “transduction” refers to the process whereby foreign DNA is introduced into a cell via viral vector. See Jones *et al.*, (1998). Genetics: principles and analysis. Boston: Jones & Bartlett Publ.

[0222] The terms “polypeptide” or “protein” refer to a macromolecule having the amino acid sequence of a protein, including deletions from, additions to, and/or substitutions of one or more amino acids of the native sequence, and preferably no more than 8 amino acid substitutions therein. Preferably, the polypeptides or proteins are isolated as defined herein. The terms “polypeptide” and “protein” specifically encompass CLL-1 antigen binding molecules, antibodies, or sequences that have deletions from, additions to, and/or substitutions of one or more amino acid of antigen-binding protein, and preferably no more than 8 amino acid substitutions therein. The term “polypeptide fragment” refers to an isolated polypeptide that has an amino-terminal deletion, a carboxyl-terminal deletion, and/or an internal deletion as compared with the full-length native protein. Such fragments can also contain modified amino acids as compared with the native protein. Useful polypeptide fragments include immunologically functional fragments of antigen binding molecules. Useful fragments include but are not limited to one or more CDR regions, variable domains of a heavy and/or light chain, a portion of other portions of an antibody chain, and the like.

[0223] The term “isolated” means (i) free of at least some other proteins with which it would normally be found, (ii) is essentially free of other proteins from the same source, *e.g.*, from the same species, (iii) separated from at least about 50 percent of polynucleotides, lipids, carbohydrates, or other materials with which it is associated in nature, (iv) operably associated (by covalent or noncovalent interaction) with a polypeptide with which it is not associated in nature, or (v) does not occur in nature.

[0224] A “variant” of a polypeptide (*e.g.*, an antigen binding molecule, or an antibody) comprises an amino acid sequence wherein one or more amino acid residues are inserted into, deleted from and/or substituted into the amino acid sequence relative to another polypeptide sequence. Variants include fusion proteins.

[0225] The term “identity” refers to a relationship between the sequences of two or more polypeptide molecules or two or more nucleic acid molecules, as determined by aligning and comparing the sequences. “Percent identity” means the percent of identical residues between the amino acids or nucleotides in the compared molecules and is calculated based on the size of the smallest of the molecules being compared. For these calculations, gaps in

alignments (if any) are preferably addressed by a particular mathematical model or computer program (*i.e.*, an “algorithm”).

[0226] To calculate percent identity, the sequences being compared are typically aligned in a way that gives the largest match between the sequences. One example of a computer program that can be used to determine percent identity is the GCG program package, which includes GAP (Devereux *et al.*, 1984, Nucl. Acid Res. 12:387; Genetics Computer Group, University of Wisconsin, Madison, Wis.). The computer algorithm GAP is used to align the two polypeptides or polynucleotides for which the percent sequence identity is to be determined. The sequences are aligned for optimal matching of their respective amino acid or nucleotide (the “matched span”, as determined by the algorithm). In certain embodiments, a standard comparison matrix (see, Dayhoff *et al.*, 1978, Atlas of Protein Sequence and Structure 5:345-352 for the PAM 250 comparison matrix; Henikoff *et al.*, 1992, Proc. Natl. Acad. Sci. U.S.A. 89:10915-10919 for the BLOSUM 62 comparison matrix) is also used by the algorithm.

[0227] As used herein, the twenty conventional (*e.g.*, naturally occurring) amino acids and their abbreviations follow conventional usage. See Immunology - A Synthesis (2nd Edition, Golub and Gren, Eds., Sinauer Assoc., Sunderland, Mass. (1991)), which is incorporated herein by reference for any purpose. Stereoisomers (*e.g.*, D-amino acids) of the twenty conventional amino acids, unnatural amino acids such as alpha-, alpha-disubstituted amino acids, N-alkyl amino acids, lactic acid, and other unconventional amino acids can also be suitable components for polypeptides of the present invention. Examples of unconventional amino acids include: 4-hydroxyproline, gamma-carboxyglutamate, epsilon-N,N,N-trimethyllysine, e-N-acetyllysine, O-phosphoserine, N-acetylserine, N-formylmethionine, 3-methylhistidine, 5-hydroxylysine, sigma-N-methylarginine, and other similar amino acids and imino acids (*e.g.*, 4-hydroxyproline). In the polypeptide notation used herein, the left-hand direction is the amino terminal direction and the right-hand direction is the carboxy-terminal direction, in accordance with standard usage and convention.

[0228] Conservative amino acid substitutions can encompass non-naturally occurring amino acid residues, which are typically incorporated by chemical peptide synthesis rather than by synthesis in biological systems. These include peptidomimetics and other reversed

or inverted forms of amino acid moieties. Naturally occurring residues can be divided into classes based on common side chain properties:

- a) hydrophobic: norleucine, Met, Ala, Val, Leu, Ile;
- b) neutral hydrophilic: Cys, Ser, Thr, Asn, Gln;
- 5 c) acidic: Asp, Glu;
- d) basic: His, Lys, Arg;
- e) residues that influence chain orientation: Gly, Pro; and
- f) aromatic: Trp, Tyr, Phe.

[0229] For example, non-conservative substitutions can involve the exchange of a member of one of these classes for a member from another class. Such substituted residues can be introduced, for example, into regions of a human antibody that are homologous with non-human antibodies, or into the non-homologous regions of the molecule. Exemplary amino acid substitutions are set forth in Table 3.

15

Table 3

<u>Original Residues</u>	<u>Exemplary Substitutions</u>	<u>Preferred Substitutions</u>
Ala	Val, Leu, Ile	Val
Arg	Lys, Gln, Asn	Lys
Asn	Gln	Gln
Asp	Glu	Glu
Cys	Ser, Ala	Ser
Gln	Asn	Asn
Glu	Asp	Asp
Gly	Pro, Ala	Ala
His	Asn, Gln, Lys, Arg	Arg
Ile	Leu, Val, Met, Ala, Phe, Norleucine	Leu
Leu	Norleucine, Ile, Val, Met, Ala, Phe	Ile
Lys	Arg, 1,4 Diamino-butyric Acid, Gln, Asn	Arg
Met	Leu, Phe, Ile	Leu
Phe	Leu, Val, Ile, Ala,	Leu

	Tyr	
Pro	Ala	Gly
Ser	Thr, Ala, Cys	Thr
Thr	Ser	Ser
Trp	Tyr, Phe	Tyr
Tyr	Trp, Phe, Thr, Ser	Phe
Val	Ile, Met, Leu, Phe,	Leu
	Ala, Norleucine	

[0230] The term “derivative” refers to a molecule that includes a chemical modification other than an insertion, deletion, or substitution of amino acids (or nucleic acids). In certain embodiments, derivatives comprise covalent modifications, including, but not limited to, chemical bonding with polymers, lipids, or other organic or inorganic moieties. In certain embodiments, a chemically modified antigen binding molecule can have a greater circulating half-life than an antigen binding molecule that is not chemically modified. In some embodiments, a derivative antigen binding molecule is covalently modified to include one or more water soluble polymer attachments, including, but not limited to, polyethylene glycol, polyoxyethylene glycol, or polypropylene glycol.

[0231] Peptide analogs are commonly used in the pharmaceutical industry as non-peptide drugs with properties analogous to those of the template peptide. These types of non-peptide compound are termed “peptide mimetics” or “peptidomimetics.” Fauchere, J., *Adv. Drug Res.*, 15:29 (1986); Veber & Freidinger, *TINS*, p.392 (1985); and Evans *et al.*, *J. Med. Chem.*, 30:1229 (1987), which are incorporated herein by reference for any purpose.

[0232] A “therapeutically effective amount,” “effective dose,” “effective amount,” or “therapeutically effective dosage” of a therapeutic agent, *e.g.*, engineered CAR T cells, is any amount that, when used alone or in combination with another therapeutic agent, protects a subject against the onset of a disease or promotes disease regression evidenced by a decrease in severity of disease symptoms, an increase in frequency and duration of disease symptom-free periods, or a prevention of impairment or disability due to the disease affliction. The ability of a therapeutic agent to promote disease regression can be evaluated using a variety of methods known to the skilled practitioner, such as in human subjects during clinical trials, in animal model systems predictive of efficacy in humans, or by assaying the activity of the agent in *in vitro* assays.

[0233] The terms “patient” and “subject” are used interchangeably and include human and non-human animal subjects as well as those with formally diagnosed disorders, those without formally recognized disorders, those receiving medical attention, those at risk of developing the disorders, etc.

5 [0234] The term “treat” and “treatment” includes therapeutic treatments, prophylactic treatments, and applications in which one reduces the risk that a subject will develop a disorder or other risk factor. Treatment does not require the complete curing of a disorder and encompasses embodiments in which one reduces symptoms or underlying risk factors. The term “prevent” does not require the 100% elimination of the possibility of an
10 event. Rather, it denotes that the likelihood of the occurrence of the event has been reduced in the presence of the compound or method.

[0235] Standard techniques can be used for recombinant DNA, oligonucleotide synthesis, and tissue culture and transformation (*e.g.*, electroporation, lipofection). Enzymatic reactions and purification techniques can be performed according to
15 manufacturer's specifications or as commonly accomplished in the art or as described herein. The foregoing techniques and procedures can be generally performed according to conventional methods well known in the art and as described in various general and more specific references that are cited and discussed throughout the present specification. See, *e.g.*, Sambrook *et al.*, *Molecular Cloning: A Laboratory Manual* (2d ed., Cold Spring Harbor
20 Laboratory Press, Cold Spring Harbor, N.Y. (1989)), which is incorporated herein by reference for any purpose.

INCORPORATION BY REFERENCE

[0236] All publications, patents, and patent applications mentioned in this specification are herein incorporated by reference to the same extent as if each individual
25 publication, patent, or patent application was specifically and individually indicated to be incorporated by reference. However, the citation of a reference herein should not be construed as an acknowledgement that such reference is prior art to the present invention. To the extent that any of the definitions or terms provided in the references incorporated by reference differ from the terms and discussion provided herein, the present terms and definitions control.

30 [0237] The foregoing written specification is considered to be sufficient to enable one skilled in the art to practice the invention. The foregoing description and examples detail

certain preferred embodiments of the invention and describe the best mode contemplated by the inventors. It will be appreciated, however, that no matter how detailed the foregoing may appear in text, the invention may be practiced in many ways and the invention should be construed in accordance with the appended claims and any equivalents thereof.

- 5 **[0238]** The following examples, including the experiments conducted and results achieved, are provided for illustrative purposes only and are not to be construed as limiting the present invention.

EXAMPLE 1

- 10 **[0239]** Determining CLL-1 CAR activity by mRNA electroporation in human PBMCs. Plasmids encoding a T7 promoter, CAR construct and a beta globin stabilizing sequence were linearize by overnight digestion of 10ug of DNA with EcoRI and BamHI (NEB). DNA was then digested for 2 hours at 50°C with proteinase K (Thermo Fisher™, 600U/ml) purified with phenol/chloroform and precipitated by adding sodium acetate and two volumes of ethanol. Pellets were then dried, resuspended in RNase/DNase free water and
15 quantified. 1ug of the linear DNA was then use to generate the in vitro transcription using the mMESSAGE mMACHINE T7 Ultra (Thermo Fisher™) following the manufacturer instructions. RNA was further purified using the MEGAClear Kit (Thermo Fisher™) following the manufacturer instructions, and quantified using NanoDrop™. mRNA integrity was assessed by running an agarose gel.

- 20 **[0240]** Different cancer cell lines were evaluated for CLL-1 expression. Namalwa (ATCC), U937 (ATCC), HL-60 (ATCC), EoL-1 (Sigma), KG1a (ATCC) and MV4;11 (ATCC) cells were stained with anti-CLL-1 antibody conjugated to PE (BD Pharmingen™) in stain buffer (BD Pharmingen™) for 30 minutes at 4°C. Cells were then washed and resuspended in stain buffer with propidium iodide (BD Pharmingen™) prior to data
25 acquisition. Samples were then acquired by flow cytometry and data analyzed and plotted in histograms using FlowJo™. Results for the CLL-1 expression can be seen in FIGURE 1.

- 30 **[0241]** PBMCs were isolated from healthy donor leukopaks (Hemacare™) using ficoll-paque density centrifugation per manufacturer's instructions. PBMCs were stimulated using OKT3 (50ng/ml, Miltenyi Biotec™) in R10 media + IL-2 (300IU/ml, Proleukin®, Prometheus® Therapeutics and Diagnostics). Seven days after stimulation, T cells were washed twice in Opti-MEM™ (Thermo Fisher Scientific™) and resuspended at a final concentration of 2.5×10^7 cells/ml in Opti-MEM. 10µg of mRNA was used per

electroporation. Electroporation of cells was performed using a Gemini X2 system (Harvard Apparatus BTX™) set to deliver a single 400V pulse for 0.5ms in 2mm cuvettes (Harvard Apparatus BTX™). Cells were immediately transferred to R10 + IL-2 media and allowed to recover. Cells were maintained at $0.5\text{--}2.0 \times 10^6$ cells/ml prior to use in activity assays.

5 **[0242]** Six hours after mRNA electroporation, T cells were stained with biotinylated Protein L (Thermo Scientific™) in stain buffer (BD Pharmingen™) for 30 minutes at 4°C. Cells were then washed and stained with PE Streptavidin (BD Pharmingen™) in stain buffer for 30 minutes at 4°C. Cells were then washed and resuspended in stain buffer with propidium iodide (BD Pharmingen™) prior to data acquisition. Results for CAR detection
10 are shown in FIGURE 2.

[0243] Effector cells were cultured with target cells at a 1:1 E:T ratio in R10 media 6 hours after mRNA electroporation. Cell lines tested included Namalwa, U937, HL-60, EoL-1, KG1a and MV4;11. Sixteen hours post-coculture, supernatants were analyzed by Luminex (EMD Millipore) following manufacturer instructions and target cell viability was assessed
15 by flow cytometric analysis of propidium iodide (PI) uptake. Results corresponding to the cytokine release assay can be found in FIGURE 3. Results of the cytolytic activity assay can be found in FIGURE 4 and FIGURE 5.

EXAMPLE 2

[0244] Determining CLL-1 CAR activity by lentiviral transduction of human
20 PBMCs. A third generation lentiviral transfer vector containing the different CLL-1 CAR construct was used along with the ViraPower™ Lentiviral Packaging Mix (Life Technologies™) to generate the lentiviral supernatants. Briefly, a transfection mix was generated by mixing 15ug of DNA and 22.5ul of polyethileneimine (Polysciences™, 1mg/ml) in 600ul of OptiMEM™ media. The mix was incubated for 5 minutes at room temperature.
25 Simultaneously, 293T cells (ATCC) were trypsinized, counted and a total of 10×10^6 total cells were plated in a T75 flask along the transfection mix. Three days after the transfection, supernatants were collected and filtered through a 0.45um filter and stored at -80C until used.

[0245] PBMCs were isolated from healthy donor leukopaks (Hemacare™) using ficoll-paque density centrifugation per manufacturer's instructions. PBMCs were stimulated
30 using OKT3 (50ng/ml, Miltenyi Biotec™) in R10 media + IL-2 (300IU/ml, Proleukin®, Prometheus® Therapeutics and Diagnostics). Forty eight hours post-stimulation, cells were

transduced using lentivirus at a MOI=10. Cells were maintained at $0.5\text{--}2.0 \times 10^6$ cells/ml prior to use in activity assays.

[0246] At day 12 post stimulation, T cells were stained with biotinylated Protein L (Thermo Scientific™) in stain buffer (BD Pharmingen™) for 30 minutes at 4°C. Cells were then washed and stained with PE Streptavidin (BD Pharmingen™) in stain buffer for 30 minutes at 4°C. Cells were then washed and resuspended in stain buffer with propidium iodide (BD Pharmingen™) prior to data acquisition. Results for CAR detection are shown in FIGURE 6.

[0247] Effector cells were cultured with target cells at a 1:1 E:T ratio in R10 media 12 days after T cell stimulation. Cell lines tested included Namalwa, U937, HL-60, EoL-1, KG1a and MV4;11. 16 hours post-coculture, supernatants were analyzed by Luminex (EMD Millipore™) following manufacturer instructions and target cell viability was assessed by flow cytometric analysis of propidium iodide (PI) uptake. Results corresponding to the cytokine release assay can be found in FIGURE 7. Results of the cytolytic activity assay can be found in FIGURE 8.

EXAMPLE 3

[0248] Female Jackson NSG mice (NOD.Cg-Prkdc^{scid} Il2rg^{tm1Wjl}/SzJ) aged 5-6 weeks old were used in this study. The mice were fed an irradiated Harlan 2918.15 Rodent Diet and water *ad libitum*. The mice were housed in Innovive™ disposable ventilated caging with corn cob bedding inside Biobubble® Clean Rooms that provide H.E.P.A filtered air into the bubble environment at 100 complete air changes per hour. All treatments, body weight determinations, and tumor measurements were carried out in the bubble environment. The environment was controlled to a temperature range of $70^{\circ}\pm 2^{\circ}\text{F}$ and a humidity range of 30-70%. All procedures were conducted in compliance with all the laws, regulations and guidelines of the National Institutes of Health (NIH) and with the approval of Molecular Imaging, Inc.'s Animal Care and Use Committee.

Tumor Cell Preparation

[0249] U937-luc cells were obtained in Lifer® Preservation Solution. The cells were centrifuged at 200rcf for 8 minutes at 4°C, the supernatant was aspirated, and the pellet was re-suspended in cold Dulbecco's Phosphate Buffered Saline (DPBS) by pipetting. An aliquot of the homogeneous cell suspension was diluted in a trypan blue solution and counted using a Luna™ automated cell counter. The cell suspension was centrifuged at 200rcf for 8 minutes

at 4°C. The supernatant was aspirated and the cell pellet was re-suspended in cold serum-free medium to generate the final concentrations of trypan-excluding cells/ml. The cell suspension was maintained on wet ice during implantation. Test animals were implanted with 1.00E+06 cells intravenously via the lateral tail vein on Day 0 in 0.2ml using a 27-gauge
5 needle and syringe.

CAR T-Cell Preparation

[0250] T cells according to the invention were obtained, frozen on dry ice, and stored in liquid nitrogen. On the day of treatment, the provided cryovials were removed from cryostorage and thawed in a 37°C water bath. For each group, the provided T cells were
10 combined into a single 50ml conical tube with warm RPMI 1640 supplemented with 10% FBS. The cryovial tubes were rinsed with warm RPMI 1640 with 10% FBS to minimize loss of cells to reach a total volume of 50ml in each conical tube. Each 50ml conical tube was centrifuged at 200rcf for 8 minutes at 4°C. The supernatants were aspirated, and the cell pellets re-suspended in 10ml of room temperature DPBS. An aliquot of the homogeneous
15 cell suspension was diluted in a trypan blue solution and manually counted using a hemacytometer. The cell suspensions were again centrifuged at 200rcf for 8 minutes at 4°C. The supernatants were aspirated and the cell pellets were re-suspended in room temperature DPBS to generate the required final concentrations. The cell suspensions were maintained on wet ice during treatment administration.

Bioluminescence Imaging

[0251] *In vivo* bioluminescence imaging (BLI) was performed using an IVIS Spectrum (Perkin Elmer, Hopkinton, MA). Animals were imaged up to 5 at a time under ~1-2% isoflurane gas anesthesia. Each mouse was injected IP with 150mg/kg (15mg/ml) D-luciferin and imaged in the prone, then supine positions, 10 minutes following injection.
25 Large to small binning of the CCD chip was used, and exposure time adjusted (2 seconds to 2 minutes) to obtain at least several hundred counts per image, and further to avoid saturation of the CCD chip. BLI images were collected on Days 3, 11, 18, and 25. Images were analyzed using the Living Image version 4.5 (Perkin Elmer, Hopkinton, MA) software. Whole body fixed-volume ROIs were placed on prone and supine images for each individual
30 animal, and labeled based on animal identification. Total radiance expressed in photon/sec (p/s) was calculated and exported for all ROIs to facilitate analyses between groups. The prone and supine ROIs were summed together to estimate whole body tumor burden.

Treatment

[0252] All mice were sorted into study groups based on BLI-derived estimation of whole body tumor burden. The mice were distributed to ensure that the mean tumor burden for all groups was within 10% of the overall mean tumor burden for the study population.

- 5 Treatment with CAR T cells began on Day 3. All mice were dosed with a fixed volume of 0.2mL. The results are set forth in Figure 10.

Assessment of Side Effects

[0253] All animals were observed for clinical signs at least once daily. Animals were weighed on each day of treatment. Individual body weights were recorded 3 times weekly.

- 10 [0254] The following sequences will further exemplify the invention.

[0255] CD28T DNA Extracellular, transmembrane, intracellular

CTTGATAATGAAAAGTCAAACGGAACAATCATTCACGTGAAGGG
CAAGCACCTCTGTCCGTCACCCCTTGTTCCCTGGTCCATCCAAGCCA
TTCTGGGTGTTGGTCGTAGTGGGTGGAGTCCTCGCTTGTTACTCTC
15 TGCTCGTCACCGTGGCTTTTATAATCTTCTGGGTTAGATCCAAAAG
AAGCCGCCTGCTCCATAGCGATTACATGAATATGACTCCACGCCG
CCCTGGCCCCACAAGGAAACACTACCAGCCTTACGCACCACCTAG
AGATTTCGCTGCCTATCGGAGC (SEQ ID NO. 1)

[0256] CD28T Extracellular, transmembrane, intracellular AA

- 20 LDNEKSNGTIIHVKGKHLCPSPFPGPSKPFVVLVVVGGVLACYSL
VTVAFIIFWVRSKRSRLHSDYMNMTPRRPGPTRKHYPYAPPRDFA
AYRS (SEQ ID NO. 2)

[0257] CD28T DNA - Extracellular

- 25 CTTGATAATGAAAAGTCAAACGGAACAATCATTCACGTGAAGGG
CAAGCACCTCTGTCCGTCACCCCTTGTTCCCTGGTCCATCCAAGCCA
(SEQ ID NO. 3)

[0258] CD28T AA - Extracellular

LDNEKSNGTI IHVKGKHLCP SPLFPGPSKP (SEQ ID NO. 4)

[0259] CD28 DNA Transmembrane Domain

- 30 TTCTGGGTGTTGGTCGTAGTGGGTGGAGTCCTCGCTTGTTACTCTC
TGCTCGTCACCGTGGCTTTTATAATCTTCTGGGTT (SEQ ID NO. 5)

- [0260] CD28 AA Transmembrane Domain
FWVLVVVGGV LACYSLLVTV AFHFWV (SEQ ID NO. 6)
- [0261] CD28 DNA Intracellular Domain
AGATCCAAAAGAAGCCGCCTGCTCCATAGCGATTACATGAATATG
5 ACTCCACGCCGCCCTGGCCCCACAAGGAAACACTACCAGCCTTAC
GCACCACCTAGAGATTTTCGCTGCCTATCGGAGC (SEQ ID NO. 7)
- [0262] CD28 AA Intracellular Domain
RSKRSRLHSDYMNMTPRRPGPTRKHYPYAPPRDFAAYRS (SEQ ID
NO. 8)
- 10 [0263] CD3 zeta DNA
AGGGTGAAGTTTTCCAGATCTGCAGATGCACCAGCGTATCAGCAG
GGCCAGAACCAACTGTATAACGAGCTCAACCTGGGACGCAGGGA
AGAGTATGACGTTTTGGACAAGCGCAGAGGACGGGACCCTGAGA
TGGGTGGCAAACCAAGACGAAAAAACCCCAAGGAGGGTCTCTAT
15 AATGAGCTGCAGAAGGATAAGATGGCTGAAGCCTATTCTGAAAT
AGGCATGAAAGGAGAGCGGAGAAGGGGAAAAGGGCACGACGGT
TTGTACCAGGGACTCAGCACTGCTACGAAGGATACTTATGACGCT
CTCCACATGCAAGCCCTGCCACCTAGG (SEQ ID NO. 9)
- [0264] CD3 zeta AA
20 RVKFSRSADAPAYQGGQNQLYNELNLGRREEYDVLDKRRGRDPEM
GGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLY
QGLSTATKDTYDALHMQALPPR (SEQ ID NO. 10)
- [0265] CD3 zeta variant AA
RVKFSRSADAPAYKQGQNQLYNELNLGRREEYDVLDKRRGRDPEM
25 GGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLY
QGLSTATKDTYDALHMQALPPR (SEQ ID NO. 146)
- [0266] CD28 DNA
ATTGAGGTGATGTATCCACCGCCTTACCTGGATAACGAAAAGAGT
AACGGTACCATCATTCACGTGAAAGGTAAACACCTGTGTCCTTCT
30 CCCCTCTTCCCCGGGCCATCAAAGCCC (SEQ ID NO. 11)

[0267] CD28 AA
IEVMYPPPYLDNEKSNGTIIHVKGKHLCPSPFLPGPSKP (SEQ ID NO. 12)

[0268] CD8 DNA extracellular & transmembrane domain
5 GCTGCAGCATTGAGCAACTCAATAATGTATTTTAGTCACTTTGTAC
CAGTGTTCTTGCCGGCTAAGCCTACTACCACACCCGCTCCACGGC
CACCTACCCCAGCTCCTACCATCGCTTCACAGCCTCTGTCCCTGCG
CCCAGAGGCTTGCCGACCGGCCGCAGGGGGCGCTGTTTCATACCAG
AGGACTGGATTTTCGCTGCGATATCTATATCTGGGCACCCCTGGC
10 CGGAACCTGCGGCGTACTCCTGCTGTCCCTGGTCATCACGCTCTAT
TGTAATCACAGGAAC (SEQ ID NO. 13)

[0269] CD8 AA extracellular & transmembrane Domain
AAALSNSIMYFSHFVPVFLPAKPTTTPAPRPPTPAPTIASQPLSLRPEA
CRPAAGGAVHTRGLDFACDIYWAPLAGTCGVLLLSLVITLYCNHRN
15 (SEQ ID NO. 14)

[0270] Clone 24C1 HC DNA
CAGGTGCAGCTGCAGGAATCCGGACCGGGGCTGGTGAAGCCCAG
CGAGACTCTGAGTCTCACGTGTACAGTTTCTGGAGGTAGCATTAG
CTCCTACTATTGGTCATGGATAAGGCAGCCCCCGGGAAGGGATT
20 GGAATGGATCGGCTATATTTACTACAGTGGGAGCACCAATTACAA
CCCCTCACTGAAGTCTAGAGTTACAATCAGCGTTGACACCTCAA
GAATCAGTTCAGTTTGAAATTGTCTAGCGTCACAGCAGCTGATAC
AGCCGTCTATTATTGTGTTTCTCTGGTCTATTGCGGTGGGGATTGT
TACAGTGGCTTTGACTATTGGGGGCAGGGTACTCTGGTTACAGTT
25 TCTTCC (SEQ ID NO. 15)

[0271] Clone 24C1 HC AA (CDRs Underlined)
QVQLQESGPGLVKPSSETLSLTCTVSGGSISSYYWSWIRQPPGKGLEWI
GY
30 IYYSGSTNYNP SLKSRVTISVDTSKNQFSLKLSSVTAADTAVYYCVSL
VYCGGDCYSGFDYWGQGTLVTVSS (SEQ ID NO. 16)

[0272] Clone 24C1 HC AA CDR1: GGSISSY (SEQ ID NO. 17)

[0273] Clone 24C1 HC AA CDR2: YYS GS (SEQ ID NO. 18)

[0274] Clone 24C1 HC AA CDR3: LVYCGGDCYS GFDY (SEQ ID NO. 19)

[0275] Clone 24C1 LC DNA

5 GACATCCAGTTGACACAGAGCCCGAGTTCCTTGTCCGCCTCCGTC
GGGGATAGAGTGTCAATTTACCTGTCAGGCCTCTCAGGATATTAAT
AACTTTCTGAATTGGTATCAGCAAAAGCCCGGAAAGGCACCCAAG
CTGTTGATTTACGACGCCAGTAACCTGGAGACAGGCGTGCCCTCC
CGGTTTAGTGGTAGCGGAAGCGGTACGGATTTTACCTTTACTATC
10 AGCTCTCTCCAACCCGAAGACATTGCAACCTACTATTGTCAACAA
TATGGAAACCTGCCTTTTACATTTGGCGGCGGCACCAAGGTGGAG
ATTAAGCGG (SEQ ID NO. 20)

[0276] Clone 24C1 LC AA (CDRs Underlined)

DIQLTQSPSSLASVGDVRSFTCQASQDINNFLNWYQQKPGKAPKLLI
15 YDASNLETGVPSRFSGSGSGTDFTFTISSLQPEDATYYCQQYGNLPFT
FGGGTKVEIKR (SEQ ID NO. 21)

[0277] Clone 24C1 LC CDR1 AA: QASQDINNFLN (SEQ ID NO. 22)

[0278] Clone 24C1 LC CDR2 AA: DASNLET (SEQ ID NO. 23)

[0279] Clone 24C1 LC CDR3 AA: QQYGNLPFT (SEQ ID NO. 24)

20 [0280] Clone 24C1 CD28T CD3 zeta CAR DNA Heavy & Light Chains

ATGGCACTCCCCGTAAGTCTGCTGCTGCCGTTGGCATTGCTCC
TGACAGCCGCACGCCCGCAGGTCCAAGTCAAGAAAGCGGACCC
GGACTGGTGAAGCCTTCTGAGACACTTAGTCTGACGTGCACGGTC
AGTGCGGCTCCATCTCCTCCTATTATTGGTCATGGATACGACAA
25 CCCCCAGGTAAGGGCCTGGAATGGATTGGCTATATCTACTATTCA
GGAAGCACGAAGTACAATCCCAGCCTGAAGTCCCGAGTGACAATT
TCAGTAGATAACAGTAAAAACCAGTTCAGTCTTAAACTGTCAAGC
GTGACAGCTGCCGACACCGCTGTGTATTACTGCGTCTCACTGGTG
TATTGTGGAGGGGATTGTTATAGCGGGTTCGATTATTGGGGACAG
30 GGAACCCTGGTGAAGTGTATCTTCCGGCGGCGGCGGCTCAGGGGGT
GGCGGTAGTGGCGGTGGGGGTTCGATATTCAACTGACACAATCC

CCCAGCTCACTCAGCGCCAGCGTGGGGGACAGGGTTAGCTTTACC
 TGTC AAGCCTCTCAGGATATAAATAACTTTCTGAACTGGTATCAA
 CAGAAGCCTGGGAAGGCGCCCAAACCTCCTGATCTATGATGCGTCC
 AACCTGGAACTGGCGTGCCTTCACGCTTTAGCGGCTCTGGCAGT
 5 GGTACAGACTTCACTTTTACCATCTCTTCACTTCAGCCGGAGGACA
 TCGCCACATATTACTGTCAACAGTACGGAAACTTGCCCTTTACTTT
 TGGAGGCGGCACCAAAGTTGAAATCAAAAGGGCCGCTGCCCTGG
 ATAACGAAAAGAGCAATGGGACTATAATACATGTTAAAGGAAAA
 CACCTGTGTCCATCTCCCCTGTTCCCTGGACCGTCAAAGCCATTTT
 10 GGGTGCTCGTGGTTGTGCGGTGGCGTTCTCGCCTGTTATAGCTTGCT
 GGTGACAGTAGCCTTCATTATCTTTTGGGTGAGATCCAAAAGAAG
 CCGCCTGCTCCATAGCGATTACATGAATATGACTCCACGCCGCC
 TGGCCCCACAAGGAAACACTACCAGCCTTACGCACCACCTAGAGA
 TTTCGCTGCCTATCGGAGCAGGGTGAAGTTTCCAGATCTGCAGA
 15 TGCACCAGCGTATCAGCAGGGCCAGAACCAACTGTATAACGAGCT
 CAACCTGGGACGCAGGGAAGAGTATGACGTTTTGGACAAGCGCA
 GAGGACGGGACCCTGAGATGGGTGGCAAACCAAGACGAAAAAAC
 CCCCAGGAGGGTCTCTATAATGAGCTGCAGAAGGATAAGATGGCT
 GAAGCCTATTCTGAAATAGGCATGAAAGGAGAGCGGAGAAGGGG
 20 AAAAGGGCACGACGTTTGTACCAGGGACTCAGCACTGCTACGA
 AGGATACTTATGACGCTCTCCACATGCAAGCCCTGCCACCTAGGT
 AA (SEQ ID NO. 25)

[0281] Clone 24C1 CD28T CD3 zeta CAR AA Heavy & Light Chains

(Signal Peptide in **bold**)

25 **MALPVTALLPLALLHAARPQVQLQESGPGLVKPSETLSLTCTVS**
 GGSISYYWSWIRQPPGKGLEWIGYIYYSGSTNYPNPSLKSRTISVDT
 SKNQFSLKLSSVTAADTAVYYCVSLVYCGGDCYSGFDYWGQGLV
 TVSSGGGSGGGSGGGGSDIQLTQSPSSLSASVGDRVSFTCQASQDI
 NNFLNWFYQQKPGKAPKLLIYDASNLETGVPSRFSGSGSGTDFTFITSS
 30 LQPEDIATYYCQYGNLPFTFGGGTKVEIKRAAALDNEKSNGTIIHV
 KGKHLCPSPFPGPSKPFVVLVVVGGVLACYSLLVTVAFIIFWVRSK
 RSRLHSDYMNMTPRRPGPTRKHYPYAPPRDFAAYRSRVKFSRSA
 DAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKN

PQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATK
DTYDA LHMQUALPPR (SEQ ID NO. 26)

[0282] Clone 24C1 CD28T CD3 zeta CAR DNA Heavy & Light Chains

5 CAGGTCCAACCTGCAAGAAAGCGGACCCGGACTGGTGAAGCCTTCT
GAGACACTTAGTCTGACGTGCACGGTCAGTGGCGGCTCCATCTCC
TCCTATTATTGGTCATGGATACGACAACCCCCAGGTAAGGGCCTG
GAATGGATTGGCTATATCTACTATTCAGGAAGCACGAACACTACAAT
CCCAGCCTGAAGTCCCGAGTGACAATTTTCAGTAGATACCAGTAAA
AACCAGTTCAGTCTTAAACTGTCAAGCGTGACAGCTGCCGACACC
10 GCTGTGTATTACTGCGTCTCACTGGTGTATTGTGGAGGGGATTGTT
ATAGCGGGTTCGATTATTGGGGACAGGGAACCCCTGGTGACTGTAT
CTTCCGGCGGCGGCGGCTCAGGGGGTGGCGGTAGTGGCGGTGGG
GGTTCCGATATTCAACTGACACAATCCCCCAGCTCACTCAGCGCC
AGCGTGGGGGACAGGGTTAGCTTTACCTGTCAAGCCTCTCAGGAT
15 ATAAATAACTTTCTGAACTGGTATCAACAGAAGCCTGGGAAGGCG
CCCAAACCTCCTGATCTATGATGCGTCCAACCTGGAAACTGGCGTG
CCTTCACGCTTTAGCGGCTCTGGCAGTGGTACAGACTTCACCTTTA
CCATCTCTTCACTTCAGCCGGAGGACATCGCCACATATTACTGTCA
ACAGTACGGAAACTTGCCCTTTACTTTTGGAGGCGGCACCAAAGT
20 TGAAATCAAAAGGGCCGCTGCCCTGGATAACGAAAAGAGCAATG
GGACTATAATACATGTTAAAGGAAAACACCTGTGTCCATCTCCCC
TGTTCCCTGGACCGTCAAAGCCATTTTGGGTGCTCGTGGTTGTCGG
TGGCGTTCTCGCCTGTTATAGCTTGCTGGTGACAGTAGCCTTCATT
ATCTTTTGGGTGAGATCCAAAAGAAGCCGCCTGCTCCATAGCGAT
25 TACATGAATATGACTCCACGCCGCCCTGGCCCCACAAGGAAACAC
TACCAGCCTTACGCACCACCTAGAGATTTTCGCTGCCTATCGGAGC
AGGGTGAAGTTTTCCAGATCTGCAGATGCACCAGCGTATCAGCAG
GGCCAGAACCAACTGTATAACGAGCTCAACCTGGGACGCAGGGA
AGAGTATGACGTTTTTGGACAAGCGCAGAGGACGGGACCCTGAGA
30 TGGGTGGCAAACCAAGACGAAAAAACCCCCAGGAGGGTCTCTAT
AATGAGCTGCAGAAGGATAAGATGGCTGAAGCCTATTCTGAAAT
AGGCATGAAAGGAGAGCGGAGAAGGGGAAAAGGGCACGACGGT

TTGTACCAGGGACTCAGCACTGCTACGAAGGATACTTATGACGCT
CTCCACATGCAAGCCCTGCCACCTAGG (SEQ ID NO. 27)

[0283] Clone 24C1 CD28T CD3 zeta CAR AA Heavy & Light Chains

5 QVQLQESGPGLVKPSSETLSLTCTVSGGSISSYYWSWIRQPPGKGLEWI
GYIYYSGSTNYPNPSLKSRVTISVDTSKNQFSLKLSSVTAADTAVYYC
VSLVYCGGDCYSGFDYWQGGLTVTVSSGGGGSGGGGSGGGGSDIQ
LTQSPSSLSASVGDRVSFTCQASQDINNFLNWYQQKPGKAPKLLIYD
ASNLETGVPSRFSGSGSGTDFTFTISSLQPEDATYYCQQYGNLPFTFG
GGTKVEIKRAAALDNEKSNGTIIHVKGKHLCPSPFPGPSKPFVVLV
10 VVGGVLACYSLLVTVAFIIFWVRSKRSRLLHSDYMNMTPRRPGPTRK
HYQPYAPPRDFAAYRSRVKFSRSADAPAYQQGQNQLYNELNLGRRE
EYDVLDRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIG
MKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR (SEQ ID NO.
28)

15 [0284] Clone 24C1 CD28 CD3 zeta CAR DNA Heavy & Light Chains

ATGGCACTCCCCGTAAGTCTGCTGCTGCTGCCGTTGGCATTGCTCC
TGACGCGCCGACGCCCCGAGGTGCAGCTGCAGGAATCCGGACCG
GGGCTGGTGAAGCCCAGCGAGACTCTGAGTCTCACGTGTACAGTT
TCTGGAGGTAGCATTAGCTCCTACTATTGGTCATGGATAAGGCAG
20 CCCCCCGGAAGGGATTGGAATGGATCGGCTATATTTACTACAGT
GGGAGCACCAATTACAACCCCTCACTGAAGTCTAGAGTTACAATC
AGCGTTGACACCTCAAAGAATCAGTTCAGTTTGAAATTGTCTAGC
GTCACAGCAGCTGATACAGCCGTCTATTATTGTGTTTCTCTGGTCT
ATTGCGGTGGGGATTGTTACAGTGGCTTTGACTATTGGGGGCAGG
25 GTA CTCTGGTTACAGTTTCTTCCGGGGGGGGAGGCTCTGGGGGCG
GAGGCTCAGGTGGTGGAGGCAGCGACATCCAGTTGACACAGAGC
CCGAGTTCCTTGTCGCCCTCCGTCGGGGATAGAGTGTCAATTTACCT
GTCAGGCCTCTCAGGATATTAATAACTTTCTGAATTGGTATCAGC
AAAAGCCCCGAAAGGCACCCAAGCTGTTGATTACGACGCCAGT
30 AACCTGGAGACAGGCGTGCCCTCCCGGTTTAGTGGTAGCGGAAGC
GGTACGGATTTTACCTTTACTATCAGCTCTCTCCAACCCGAAGACA
TTGCAACCTACTATTGTCAACAATATGGAAACCTGCCTTTTACATT

TGGCGGCGGCACCAAGGTGGAGATTAAGCGGGCGGCAGCTATTG
 AGGTGATGTATCCACCGCCTTACCTGGATAACGAAAAGAGTAACG
 GTACCATCATTACGTGAAAGGTAAACACCTGTGTCCTTCTCCCCT
 CTTCCCCGGGCCATCAAAGCCCTTCTGGGTTCTTGTGGTCGTGGGA
 5 GGCGTGCTTGCTTGTTATTCTCTGCTCGTTACCGTGGCGTTTATCA
 TTTTTTGGGTTAGATCCAAAAGAAGCCGCTGCTCCATAGCGATT
 ACATGAATATGACTCCACGCCGCCCTGGCCCCACAAGGAAACACT
 ACCAGCCTTACGCACCACCTAGAGATTTTCGCTGCCTATCGGAGCA
 GGGTGAAGTTTTCCAGATCTGCAGATGCACCAGCGTATCAGCAGG
 10 GCCAGAACCAACTGTATAACGAGCTCAACCTGGGACGCAGGGAA
 GAGTATGACGTTTTGGACAAGCGCAGAGGACGGGACCCTGAGAT
 GGGTGGCAAACCAAGACGAAAAAACCCTCAGGAGGGTCTCTATA
 ATGAGCTGCAGAAGGATAAGATGGCTGAAGCCTATTCTGAAATA
 GGCATGAAAGGAGAGCGGAGAAGGGGAAAAGGGCACGACGGTT
 15 TGTACCAGGGACTCAGCACTGCTACGAAGGATACTTATGACGCTC
 TCCACATGCAAGCCCTGCCACCTAGGTAA (SEQ ID NO. 29)

[0285] Clone 24C1 CD28 CD3 zeta CAR AA Heavy & Light Chains

(Signal Peptide in **Bold**)

MALPVTALLLPLALLHAARPQVQLQESGPGLVKPSSETLSLTCTVS
 20 GGSISYYWSWIRQPPGKGLEWIGYIYSGSTNYPNPSLKSRTISVDT
 SKNQFSLKLSSVTAADTAVYYCVSLVYCGGDCYSGFDYWQGTLV
 TVSSGGGSGGGSGGGGSDIQLTQSPSSLSASVGDRVSFTCQASQDI
 NNFLNWFYQQKPGKAPKLLIYDASNLETGVPSRFSGSGSGTDFFTISS
 LQPEDIATYYCQYGNLPFTFGGGTKVEIKRAAAIEVMYPPPYLDNE
 25 KSNGTIIHVKGKHLCPSPFPGPSKPFVVLVVGGVLACYSLLVTVA
 FIIFWVRSKRSRLHSDYMNMTPRRPGPTRKHYPYAPPRDFAAYRS
 RVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEM
 GGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLY
 QGLSTATKDTYDALHMQALPPR (SEQ ID NO. 30)

30 **[0286]** Clone 24C1 CD28 CD3 zeta CAR DNA Heavy & Light Chains

CAGGTGCAGCTGCAGGAATCCGGACCGGGGCTGGTGAAGCCCAG
 CGAGACTCTGAGTCTCACGTGTACAGTTTCTGGAGGTAGCATTAG

CTCCTACTATTGGTCATGGATAAGGCAGCCCCCGGGAAGGGATT
GGAATGGATCGGCTATATTTACTACAGTGGGAGCACCAATTACAA
CCCCTCACTGAAGTCTAGAGTTACAATCAGCGTTGACACCTCAA
GAATCAGTTCAGTTTGAAATTGTCTAGCGTCACAGCAGCTGATAC
5 AGCCGTCTATTATTGTGTTTCTCTGGTCTATTGCGGTGGGGATTGT
TACAGTGGCTTTGACTATTGGGGGCAGGGTACTCTGGTTACAGTT
TCTTCCGGGGGGGAGGCTCTGGGGGCGGAGGCTCAGGTGGTGG
AGGCAGCGACATCCAGTTGACACAGAGCCCGAGTTCCTTGTCCGC
CTCCGTCGGGGATAGAGTGTCAATTTACCTGTCAGGCCTCTCAGGA
10 TATTAATAACTTTCTGAATTGGTATCAGCAAAAGCCCGGAAAGGC
ACCCAAGCTGTTGATTTACGACGCCAGTAACCTGGAGACAGGCGT
GCCCTCCCGGTTTAGTGGTAGCGGAAGCGGTACGGATTTTACCTT
TACTATCAGCTCTCTCCAACCCGAAGACATTGCAACCTACTATTGT
CAACAATATGGAAACCTGCCTTTTACATTTGGCGGGCGGCACCAAG
15 GTGGAGATTAAGCGGGCGGCAGCTATTGAGGTGATGTATCCACCG
CCTTACCTGGATAACGAAAAGAGTAACGGTACCATCATTCACGTG
AAAGGTAAACACCTGTGTCTTCTCCCCTCTTCCCCGGGCCATCAA
AGCCCTTCTGGGTTCTTGTGGTCGTGGGAGGCGTGCTTGCTTGTTA
TTCTCTGCTCGTTACCGTGGCGTTTATCATTTTTTTGGGTTAGATCC
20 AAAAGAAGCCGCTGCTCCATAGCGATTACATGAATATGACTCCA
CGCCGCCCTGGCCCCACAAGGAAACACTACCAGCCTTACGCACCA
CCTAGAGATTTGCTGCTATCGGAGCAGGGTGAAGTTTCCAGA
TCTGCAGATGCACCAGCGTATCAGCAGGGCCAGAACCAACTGTAT
AACGAGCTCAACCTGGGACGCAGGGAAGAGTATGACGTTTTGGA
25 CAAGCGCAGAGGACGGGACCCTGAGATGGGTGGCAAACCAAGAC
GAAAAAACCCCAAGGAGGGTCTCTATAATGAGCTGCAGAAGGAT
AAGATGGCTGAAGCCTATTCTGAAATAGGCATGAAAGGAGAGCG
GAGAAGGGGAAAAGGGCACGACGGTTTGTACCAGGGACTCAGCA
CTGCTACGAAGGATACTTATGACGCTCTCCACATGCAAGCCCTGC
30 CACCTAGG (SEQ ID NO. 31)

[0287] Clone 24C1 CD28 CD3 zeta CAR AA Heavy & Light Chains

QVQLQESGPGLVKPSSETLSLTCTVSGGSISSYYWSWIRQPPGKGLEWI
GYIYYSGSTNYPNPSLKSRVTISVDTSKNQFSLKLSSVTAADTAVYYC

VSLVYCGGDCYSGFDYWQGTLVTVSSGGGGSGGGGSGGGGSDIQ
LTQSPSSLSASVGDRVSFTCQASQDINNFLNWYQQKPGKAPKLLIYD
ASNLETGVPSRFSGSGSGTDFTFTISSLQPEDATYYCQQYGNLPFTFG
GGTKVEIKRAAAIEVMYPPPYLDNEKSNGTIIHVKGKHLCPSPFP
5 SKPFWVLVVVGGVLACYSLLVTVAFIIFWVRSKRSRLHSDYMNMT
PRRPGPTRKHYPYAPPRDFAAYRSRVKFSRSADAPAYQQGQNQLY
NELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDK
MAEAYSEIGMKGERRRGKGHDGLYQGLST
ATKDTYDALHMQALPPR (SEQ ID NO. 32)

10 [0288] Clone 24C1 CD8 CD3 zeta CAR DNA Heavy & Light Chains
ATGGCACTCCCCGTAACCTGCTCTGCTGCTGCCGTTGGCATTGCTCC
TGACGCGCCGACGCCCCGAGGTGCAATTGCAAGAGTCCGGCCCCG
GACTCGTTAAACCCAGTGAGACGCTTAGCCTGACCTGTACCGTCT
CAGGGGGCAGCATCTCCTCTTATTACTGGAGCTGGATCAGGCAGC
15 CTCCAGGAAAAGGCCTTGAATGGATTGGGTACATCTACTACTCTG
GCTCAACAAATTATAATCCATCCCTGAAGTCCCGCGTGACTATCT
CTGTGGACACCAGCAAGAATCAGTTTTCACTGAAGTTGTCTAGTG
TTACCGCGGCCGACACCGCCGTATACTACTGTGTGTCTCTTGTGTA
CTGTGGCGGCGACTGCTATTCCGGGTTGCGACTACTGGGGCCAAGG
20 GACTCTGGTAACCGTGTCTCAGGCGGCGGGGTCAGGAGGAG
GCGGCAGTGGAGGTGGCGGCTCCGACATCCAGCTGACACAATCA
CCATCTTCCCTTTCAGCTTCAGTCGGGGACAGAGTGTCTTCACAT
GCCAGGCCAGCCAGGATATCAATAACTTCCTGAACTGGTACCAAC
AGAAACCCGAAAGGCTCCAAAGCTCCTGATCTATGATGCTTCCA
25 ACCTGGAGACCGGCGTGCCCTCCAGGTTCACTGGTTCAGGATCAG
GCACTGACTTTACGTTACCATATCCAGTCTTCAGCCCGAAGACA
TTGCAACCTATTACTGCCAACAATACGGGAACCTTCCCTTTACATT
CGGAGGCGGCACCAAGGTGGAAATCAAAGGGCTGCAGCATTGA
GCAACTCAATAATGTATTTTAGTCACTTTGTACCAGTGTCTTGCC
30 GGCTAAGCCTACTACCACACCCGCTCCACGGCCACCTACCCAGC
TCCTACCATCGCTTCACAGCCTCTGTCCCTGCGCCCAGAGGCTTGC
CGACCGGCCGAGGGGGCGCTGTTTCATACCAGAGGACTGGATTTC
GCCTGCGATATCTATATCTGGGCACCCCTGGCCGGAACCTGCGGC

5 GTACTCCTGCTGTCCCTGGTCATCACGCTCTATTGTAATCACAGGA
 ACAGATCCAAAAGAAGCCGCTGCTCCATAGCGATTACATGAATA
 TGA TCCACGCCGCCCTGGCCCCACAAGGAAACACTACCAGCCTT
 ACGCACCACCTAGAGATTTTCGCTGCCTATCGGAGCAGGGTGAAGT
 TTTCCAGATCTGCAGATGCACCAGCGTATCAGCAGGGCCAGAACC
 AACTGTATAACGAGCTCAACCTGGGACGCAGGGAAGAGTATGAC
 GTTTTGGACAAGCGCAGAGGACGGGACCCTGAGATGGGTGGCAA
 ACCAAGACGAAAAAACCCCAAGGAGGGTCTCTATAATGAGCTGC
 AGAAGGATAAGATGGCTGAAGCCTATTCTGAAATAGGCATGAAA
 10 GGAGAGCGGAGAAGGGGAAAAGGGCACGACGGTTTGTACCAGGG
 ACTCAGCACTGCTACGAAGGATACTTATGACGCTCTCCACATGCA
 AGCCCTGCCACCTAGGTAA (SEQ ID NO. 33)

[0289] Clone 24C1 CD8 CD3 zeta CAR AA Heavy & Light Chains

(Signal peptide in **bold**)

15 **MALPVTALLLPLALLLHAARPQVQLQESGPGLVKPSSETLSLTCTVS**
 GGSISYYWSWIRQPPGKGLEWIGYIYYSGSTNYPNPSLKSRVTISVDT
 SKNQFSLKLSSVTAADTAVYYCVSLVYCGGDCYSGFDYWQGGLV
 TVSSGGGSGGGGSGGGGSDIQLTQSPSSLSASVGDRVSFTCQASQDI
 NNFLNWFYQQKPGKAPKLLIYDASNLETGVPSRFSGSGSGTDFFTISS
 20 LQPEDIATYYCQYGNLPFTFGGGTKVEIKRAAALSNSIMYFSHFVPV
 FLPAKPTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFA
 CDIYIWAPLAGTCGVLLLSLVITLYCNHRNRSKRSLHSDYMNMTPT
 RRPGPTRKHYQPYAPPRDFAAYRSRVKFSRSADAPAYQQGQNQLYN
 ELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKM
 25 AEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR
 (SEQ ID NO. 34)

[0290] Clone 24C1 CD8 CD3 zeta CAR DNA Heavy & Light Chains

30 CAGGTGCAATTGCAAGAGTCCGGCCCCGGACTCGTTAAACCCAGT
 GAGACGCTTAGCCTGACCTGTACCGTCTCAGGGGGCAGCATCTCC
 TCTTATTACTGGAGCTGGATCAGGCAGCCTCCAGGAAAAGGCCTT
 GAATGGATTGGGTACATCTACTACTCTGGCTCAACAAATTATAAT
 CCATCCCTGAAGTCCCGCGTGACTATCTCTGTGGACACCAGCAAG

AATCAGTTTTCACTGAAGTTGTCTAGTGTTACCGCGGCCGACACC
 GCCGTATACTACTGTGTGTCTCTTGTGTACTGTGGCGGCCGACTGCT
 ATTCCGGGTTTCGACTACTGGGGCCAAGGGACTCTGGTAACCGTGT
 CCTCAGGCGGGCGGGCGGGTCAGGAGGAGGCGGCAGTGGAGGTGGC
 5 GGCTCCGACATCCAGCTGACACAATCACCATCTTCCCTTTTCAGCTT
 CAGTCGGGGACAGAGTGTCTTCACATGCCAGGCCAGCCAGGATA
 TCAATAACTTCCTGAACTGGTACCAACAGAAACCCGGAAAGGCTC
 CAAAGCTCCTGATCTATGATGCTTCCAACCTGGAGACCGGCGTGC
 CCTCCAGGTTTCAGTGGTTCAGGATCAGGCACTGACTTTACGTTCA
 10 CCATATCCAGTCTTCAGCCCGAAGACATTGCAACCTATTACTGCC
 AACAAATACGGGAACCTTCCCTTTACATTTCGGAGGCGGCACCAAGG
 TGGAAATCAAAAGGGCTGCAGCATTGAGCAACTCAATAATGTATT
 TTAGTCACTTTGTACCAGTGTCTTGCCGGCTAAGCCTACTACCAC
 ACCCGCTCCACGGCCACCTACCCAGCTCCTACCATCGCTTCACA
 15 GCCTCTGTCCCTGCGCCCAGAGGCTTGCCGACCGGCCGCAGGGGG
 CGCTGTTTCATACCAGAGGACTGGATTTGCGCTGCGATATCTATATC
 TGGGCACCCCTGGCCGGAACCTGCGGCGTACTCCTGCTGTCCCTG
 GTCATCACGCTCTATTGTAATCACAGGAACAGATCCAAAAGAAGC
 CGCCTGCTCCATAGCGATTACATGAATATGACTCCACGCCGCCCT
 20 GGCCCCACAAGGAAACACTACCAGCCTTACGCACCACCTAGAGAT
 TTCGCTGCCTATCGGAGCAGGGTGAAGTTTTCCAGATCTGCAGAT
 GCACCAGCGTATCAGCAGGGCCAGAACCAACTGTATAACGAGCT
 CAACCTGGGACGCAGGGAAGAGTATGACGTTTTGGACAAGCGCA
 GAGGACGGGACCCTGAGATGGGTGGCAAACCAAGACGAAAAAAC
 25 CCCCAGGAGGGTCTCTATAATGAGCTGCAGAAGGATAAGATGGCT
 GAAGCCTATTCTGAAATAGGCATGAAAGGAGAGCGGAGAAGGGG
 AAAAGGGCACGACGTTTTGTACCAGGGACTCAGCACTGCTACGA
 AGGATACTTATGACGCTCTCCACATGCAAGCCCTGCCACCTAGG
 (SEQ ID NO. 35)

30 [0291] Clone 24C1 CD8 CD3 zeta CAR AA Heavy & Light Chains
 QVQLQESGPGLVKPSSETLSLTCTVSGGSISSYYWSWIRQPPGKGLEWI
 GYIYYSGSTNYPNPSLKSRVTISVDTSKNQFSLKLSSVTAADTAVYYC
 VSLVYCGGDCYSGFQDYWGQGLVTVSSGGGGSGGGGSGGGGSDIQ

LTQSPSSLSASVGDRVSFTTCQASQDINNFLNWYQQKPGKAPKLLIYD
 ASNLETGVPSRFSGSGSGTDFTFITISLQPEDATYYCQQYGNLPFTFG
 GGTKVEIKRAAALSNSIMYFSHFVPVFLPAKPTTTPAPRPPTPAPTAS
 QPLSLRPEACRPAAGGAVHTRGLDFACDIYWAPLAGTCGVLLLSLVI
 5 TLYCNHRNRSKRSRLHSDYMNMTPRRPGPTRKHYPYAPPRDFAA
 YRSRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDP
 EMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDG
 LYQGLSTATK DTYDALHMQALPPR (SEQ ID NO. 36)

[0292] Clone 24C1 CD28T CD3 zeta CAR DNA Heavy & Light Chains

10 ATGGCACTCCCCGTAAGTCTGCTGCTGCTGCCGTTGGCATTGCTCC
 TGCACGCCGCACGCCCGGATATCCAGCTCACGCAATCCCCCTCAA
 GCTTGAGTGCCTCCGTGGGCGACCGGGTGTCTTCACATGTCAGG
 CAAGCCAAGACATAAATAATTTCTGAATTGGTACCAACAAAAAC
 CCGGCAAGGCTCCCAAAGTCTGATTTATGATGCCTCCAATCTGG
 15 AGACCGGGGTCCCTTCTAGATTACAGCGGAAGTGGCAGCGGCACA
 GACTTTACATTTACTATCTCTTCTCTGCAACCAGAGGACATCGCCA
 CATACTATTGCCAGCAATACGGCAATCTGCCCTTCACCTTCGGAG
 GCGGAACCAAGGTAGAAATTAAAAGGGGCGGTGGAGGCTCCGGA
 GGGGGGGGCTCTGGCGGAGGGGGCTCCCAAGTACAATTGCAGGA
 20 GTCAGGGCCTGGACTCGTGAAGCCTTCAGAACTTTGTCACTGAC
 ATGTACAGTGTCCGGCGGAAGCATTTCAGTTACTATTGGTCCTG
 GATTAGACAGCCACCCGGCAAAGGACTGGAATGGATTGGATATA
 TCTACTACTCTGGATCTACAACTATAATCCCAGCCTCAAATCCA
 GGGTCACTATTAGTGTGGATACATCAAAGAATCAGTTCTCCTTGA
 25 AGCTGAGCTCAGTCACTGCTGCCGACACCGCAGTGTACTATTGTG
 TGAGCCTGGTCTACTGCGGCGGAGATTGCTACAGCGGTTTCGATT
 ACTGGGGCCAGGGCACCTGGTTACCGTTAGTTCCGCGGCTGCTC
 TTGATAACGAGAAGTCCAACGGTACGATTATCCACGTAAAGGGTA
 AGCACCTTTGCCCTAGCCCGCTGTTCCCAGGCCCCAGTAAGCCCTT
 30 TTGGGTCCTCGTTGTGGTAGGTGGGGTACTCGCCTGCTACTCCCTG
 CTCGTCACTGTGCGATTATCATCTTCTGGGTCAGATCCAAAAGA
 AGCCGCCTGCTCCATAGCGATTACATGAATATGACTCCACGCCGC
 CCTGGCCCCACAAGGAAACACTACCAGCCTTACGCACCACCTAGA

GATTTTCGCTGCCTATCGGAGCAGGGTGAAGTTTTCCAGATCTGCA
 GATGCACCAGCGTATCAGCAGGGCCAGAACCAACTGTATAACGA
 GCTCAACCTGGGACGCAGGGAAGAGTATGACGTTTTGGACAAGC
 GCAGAGGACGGGACCCTGAGATGGGTGGCAAACCAAGACGAAAA
 5 AACCCCCAGGAGGGTCTCTATAATGAGCTGCAGAAGGATAAGAT
 GGCTGAAGCCTATTCTGAAATAGGCATGAAAGGAGAGCGGAGAA
 GGGGAAAAGGGCACGACGGTTTGTACCAGGGACTCAGCACTGCT
 ACGAAGGATACTTATGACGCTCTCCACATGCAAGCCCTGCCACCT
 AGGTAA (SEQ ID NO. 37)

10 **[0293]** Clone 24C1 CD28T CD3 zeta CAR AA Heavy & Light Chains
 (Signal Peptide in **Bold**)
MALPVTALLPLALLHAARPDIQLTQSPSSLSASVGDRVSFTCQAS
 QDINNFLNWYQQKPGKAPKLLIYDASNLETGVPSRFSGSGSGTDFTF
 TISSLPEDIATYYCQQYGNLPFTFGGGTKVEIKRGGGSGGGGSGG
 15 GGSQVQLQESGPGLVKPSETLSLTCTVSGGSISSYYWSWIRQPPGKGL
 EWIGYIYYSGSTNYPNPSLKSRTISVDTSKNQFSLKLSSVTAADTAVY
 YCVSLVYCGGDCYSGFDYWGGTLVTVSSAAALDNEKSNGTIIHVK
 GKHLCPSPFLPGPSKPFWVLVVVGGVLACYSLLVTVAFIHFWVRSKRS
 RLLHSDYMNMTPRRPGPTRKHYQPYAPPRDFAAYRSRVKFSRSADA
 20 PAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPGEMGGKPRRKNPQ
 EGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDT
 YDA LHMQUALPPR (SEQ ID NO. 38)

[0294] Clone 24C1 CD28T CD3 zeta CAR DNA Heavy & Light Chains
 GATATCCAGCTCACGCAATCCCCCTCAAGCTTGAGTGCCTCCGTG
 25 GGCGACCGGGTGTCTTCACATGTCAGGCAAGCCAAGACATAAAT
 AATTTCTGAATTGGTACCAACAAAAACCCGGCAAGGCTCCCAA
 CTCCTGATTTATGATGCCTCCAATCTGGAGACCGGGGTCCCTTCTA
 GATTGAGCGGAAGTGGCAGCGGCACAGACTTTACATTTACTATCT
 CTTCTCTGCAACCAGAGGACATCGCCACATACTATTGCCAGCAAT
 30 ACGGCAATCTGCCCTTCACCTTCGGAGGCGGAACCAAGGTAGAAA
 TAAAAGGGGCGGTGGAGGCTCCGGAGGGGGGGGCTCTGGCGGA
 GGGGGCTCCCAAGTACAATTGCAGGAGTCAGGGCCTGGACTCGTG

5 AAGCCTTCAGAACTTTGTCACTGACATGTACAGTGTCCGGCGGA
AGCATTTCCAGTTACTATTGGTCCTGGATTAGACAGCCACCCGGC
AAAGGACTGGAATGGATTGGATATATCTACTACTCTGGATCTACA
AACTATAATCCCAGCCTCAAATCCAGGGTCACTATTAGTGTGGAT
ACATCAAAGAATCAGTTCTCCTTGAAGCTGAGCTCAGTCACTGCT
GCCGACACCGCAGTGTACTATTGTGTGAGCCTGGTCTACTGCGGC
GGAGATTGCTACAGCGGTTTCGATTACTGGGGCCAGGGCACCTG
GTTACCGTTAGTTCGCGGCTGCTCTTGATAACGAGAAGTCCAAC
GGTACGATTATCCACGTAAAGGGTAAGCACCTTTGCCCTAGCCCG
10 CTGTTCCCAGGCCCCAGTAAGCCCTTTTGGGTCCTCGTTGTGGTAG
GTGGGGTACTCGCCTGCTACTCCCTGCTCGTCACTGTCGCATTCAT
CATCTTCTGGGTCAGATCCAAAAGAAGCCGCCTGCTCCATAGCGA
TTACATGAATATGACTCCACGCCGCCCTGGCCCCACAAGGAAACA
CTACCAGCCTTACGCACCACCTAGAGATTTGCTGCCTATCGGAG
15 CAGGGTGAAGTTTTCCAGATCTGCAGATGCACCAGCGTATCAGCA
GGGCCAGAACCAACTGTATAACGAGCTCAACCTGGGACGCAGGG
AAGAGTATGACGTTTTGGACAAGCGCAGAGGACGGGACCCTGAG
ATGGGTGGCAAACCAAGACGAAAAAACCCCAGGAGGGTCTCTA
TAATGAGCTGCAGAAGGATAAGATGGCTGAAGCCTATTCTGAAAT
20 AGGCATGAAAGGAGAGCGGAGAAGGGGAAAAGGGCACGACGGT
TTGTACCAGGGACTCAGCACTGCTACGAAGGATACTTATGACGCT
CTCCACATGCAAGCCCTGCCACCTAGG (SEQ ID NO. 39)

[0295] Clone 24C1 CD28T CD3 zeta CAR AA Heavy & Light Chains

25 DIQLTQSPSSLASVGDVRSFTCSQSDINNFLNWFYQQKPGKAPKLLI
YDASNLETGVPSRFSGSGSGTDFTFTISLQPEDATYYCQYGNLPFT
FGGGTKVEIKRGGGSGGGGSGGGGSQVQLQESGPGLVKPSETLSLT
CTVSGGSISSYYWSWIRQPPGKLEWIGYIYSGSTNYPNPSLKSRVTI
SVDTSKNQFSLKLSSVTAADTAVYYCVSLVYCGGDCYSGFDYWGQ
30 GTLVTVSSAAALDNEKSNGTIIHVKGKHLCPSPFPGPSKPFVVLV
VGGVLACYSLLVTVAFIIFWVRSKRSRLHSDYMNMTPRRPGPTRKH
YQPYAPPRDFAAYRSRVKFSRSADAPAYQQGQNQLYNELNLGRREE
YDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGM

KGERRRGKGHDLGLYQGLSTATKDTYDALHMQALPPR (SEQ ID NO. 40)

[0296] Clone 24C1 CD28 CD3 zeta CAR DNA AA Heavy & Light Chains

5 ATGGCACTCCCCGTAAGTCTGCTGCTGCCGTTGGCATTGCTCC
TGCACGCCGCACGCCCGGATATCCAGCTGACCCAGTCTCCATCCT
CTTTGAGTGCCCTCCGTGGGTGACCGGTCTCTTTCACTTGCCAAGC
CAGCCAAGACATCAACAACCTTTCTGAATTGGTACCAGCAGAAACC
AGGCAAAGCACCAAAGCTCCTCATCTACGACGCCTCCAACCTGGA
AACCGGGGTGCCCAGCAGGTTTAGCGGGAGCGGTTCTGGCACGG
10 ATTTTACGTTACCATCTCCTCTCTGCAGCCCGAGGATATAGCTAC
TTATTACTGTCAGCAGTACGGGAATCTGCCATTTACTTTTGGGGGT
GGAACATAAGGTGGAAATCAAAAGGGGCGGGCGGGGAAGCGGGG
GCGGGGGCTCAGGTGGCGGAGGGAGCCAGGTGCAACTCCAGGAA
AGTGGCCCAGGATTGGTGAAGCCCAGCGAGACCCTTTCCCTTACT
15 TGTACTGTTAGCGGAGGCAGCATAAGCAGCTACTATTGGTCCTGG
ATCAGACAGCCACCAGGGAAAGGGCTTGAATGGATTGGCTACATT
TACTATTCGGGTCCACCAACTACAACCCATCCCTCAAGTCCCGC
GTGACAATTTCCGTGACACAAGCAAGAACCAGTTCTCCCTGAAA
CTTAGTAGCGTCACTGCTGCAGATACAGCAGTGTACTATTGTGTC
20 AGCCTTGTCTACTGTGGCGGCGACTGCTACAGTGGCTTTGATTACT
GGGGACAGGGCACGCTCGTGACAGTGTCCAGCGCTGCGGCTATCG
AGGTAATGTATCCGCCACCGTATCTGGACAACGAGAAGTCTAATG
GGACAATCATTACGTGAAGGGGAAGCACCTGTGTCCATCCCCC
TGTTTCCGGGTCCCAGTAAACCCTTCTGGGTGCTTGTGTGCGTTGG
25 CGGGGTGCTGGCCTGCTATTCCTGCTGGTGACCGTCGCGTTTATT
ATTTTCTGGGTAGATCCAAAAGAAGCCGCCTGCTCCATAGCGAT
TACATGAATATGACTCCACGCCGCCCTGGCCCCACAAGGAAACAC
TACCAGCCTTACGCACCACCTAGAGATTTGCTGCCTATCGGAGC
AGGGTGAAGTTTTCCAGATCTGCAGATGCACCAGCGTATCAGCAG
30 GGCCAGAACCAACTGTATAACGAGCTCAACCTGGGACGCAGGGA
AGAGTATGACGTTTTGGACAAGCGCAGAGGACGGGACCCTGAGA
TGGGTGGCAAACCAAGACGAAAAAACCCCAAGGAGGTCTCTAT
AATGAGCTGCAGAAGGATAAGATGGCTGAAGCCTATTCTGAAAT

AGGCATGAAAGGAGAGCGGAGAAGGGGAAAAGGGCACGACGGT
 TTGTACCAGGGACTCAGCACTGCTACGAAGGATACTTATGACGCT
 CTCCACATGCAAGCCCTGCCACCTAGGTAA (SEQ ID NO. 41)

[0297] Clone 24C1 CD28 CD3 zeta CAR AA Heavy & Light Chains

5 (Signal Peptide in **Bold**)

MALPVTALLPLALLLHAARPDIQLTQSPSSLSASVGDRVSFTCQAS
 QDINNFLNWYQQKPGKAPKLLIYDASNLETGVPSRFSGSGSGTDFTF
 TISSLQPEDIATYYCQQYGNLPFTFGGGTKVEIKRGGGSGGGGSGG
 GGSQVQLQESGPGLVKPSETLSLTCTVSGGSISSYYWSWIRQPPGKGL
 10 EWIGYIYYSGSTNYPNPSLKSRVTISVDTSKNQFSLKLSSVTAADTAVY
 YCVSLVYCGGDCYSGFDYWGGTLVTVSSAAAEVMPYPYLDNEK
 SNGTIIHVKGKHLCPSPFPGPSKPFVVLVVVGGVLACYSLLVTVAFI
 IFWVRSKRSRLLHSDYMNMTPRRPGPTRKHYQPYAPPRDFAAYRSR
 VKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDRRRGRDPEMG
 15 GKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQ
 GLSTATKDTYDALHMQALPPR (SEQ ID NO. 42)

[0298] Clone 24C1 CD28 CD3 zeta CAR DNA Heavy & Light Chains

GATATCCAGCTGACCCAGTCTCCATCCTCTTTGAGTGCCTCCGTGG
 GTGACCGCGTCTCTTTCACTTGCCAAGCCAGCCAAGACATCAACA
 20 ACTTTCTGAATTGGTACCAGCAGAAACCAGGCAAAGCACCAAAG
 CTCCTCATCTACGACGCCTCCAACCTGGAAACCGGGGTGCCCAGC
 AGGTTTAGCGGGAGCGGTTCTGGCACGGATTTTACGTTACCATC
 TCCTCTCTGCAGCCCGAGGATATAGCTACTTATTACTGTCAGCAGT
 ACGGGAATCTGCCATTTACTTTTGGGGGTGGAACAAAGGTGGAAA
 25 TCAAAAGGGGCGGCGGGGGAAGCGGGGGCGGGGGCTCAGGTGGC
 GGAGGGAGCCAGGTGCAACTCCAGGAAAGTGGCCCAGGATTGGT
 GAAGCCCAGCGAGACCCTTCCCTTACTTGTACTGTTAGCGGAGG
 CAGCATAAGCAGCTACTATTGGTCCTGGATCAGACAGCCACCAGG
 GAAAGGGCTTGAATGGATTGGCTACATTTACTATTCCGGGTCCAC
 30 CAACTACAACCCATCCCTCAAGTCCCGCGTGACAATTTCCGTCGA
 CACAAGCAAGAACCAGTTCTCCCTGAACTTAGTAGCGTCACTGC
 TGCAGATACAGCAGTGTACTATTGTGTCAGCCTTGTCTACTGTGGC

GGCGACTGCTACAGTGGCTTTGATTACTGGGGACAGGGCACGCTC
 GTGACAGTGTCCAGCGCTGCGGCTATCGAGGTAATGTATCCGCCA
 CCGTATCTGGACAACGAGAAGTCTAATGGGACAATCATTACGTG
 AAGGGGAAGCACCTGTGTCCATCCCCCTGTTTCCGGGTCCCAGT
 5 AAACCCTTCTGGGTGCTTGTTCGTTGGCGGGGTGCTGGCCTGCT
 ATTCCTGCTGGTGACCGTCGCGTTTATTATTTTCTGGGTAGATC
 CAAAAGAAGCCGCCTGCTCCATAGCGATTACATGAATATGACTCC
 ACGCCGCCCTGGCCCCACAAGGAAACACTACCAGCCTTACGCACC
 ACCTAGAGATTTTCGCTGCCTATCGGAGCAGGGTGAAGTTTTCCAG
 10 ATCTGCAGATGCACCAGCGTATCAGCAGGGCCAGAACCAACTGTA
 TAACGAGCTCAACCTGGGACGCAGGGAAGAGTATGACGTTTTGG
 ACAAGCGCAGAGGACGGGACCCTGAGATGGGTGGCAAACCAAGA
 CGAAAAAACCCCCAGGAGGGTCTCTATAATGAGCTGCAGAAGGA
 TAAGATGGCTGAAGCCTATTCTGAAATAGGCATGAAAGGAGAGC
 15 GGAGAAGGGGAAAAGGGCACGACGGTTTGTACCAGGGACTCAGC
 ACTGCTACGAAGGATACTTATGACGCTCTCCACATGCAAGCCCTG
 CCACCTAGG (SEQ ID NO. 43)

[0299] Clone 24C1 CD28 CD3 zeta CAR AA Heavy & Light Chains

DIQLTQSPSSLSASVGDRVSFTCQASQDINNFLNWFYQKPGKAPKLLI
 20 YDASNLETGVPSRFSGSGSGTDFTFTISSLQPEDIATYYCQYGNLPFT
 FGGGTKVEIKRGGGGSGGGSGGGGSQVQLQESGPGLVKPSSETLSLT
 CTVSGGSISSYYWSWIRQPPGKGLEWIGYIYYSGSTNYPNPSLKSRTVI
 SVDTSKNQFSLKLSSVTAADTAVYYCVSLVYCGGDCYSGFDYWGQ
 GTLVTVSSAAIEVMYPPPYLDNEKSNGTIIHVKGKHLCPSPFPGPS
 25 KPFWVLVVVGGVLACYSLLVTVAFIIFWVRSKRSRLHSDYMNMTF
 RRPGPTRKHYPYAPPRDFAAYRSRVKFSRSADAPAYQQGQNQLYN
 ELNLGRREEYDVLDRRGRDPGEMGGKPRRKNPQEGLYNELQKDKM
 AEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR
 (SEQ ID NO. 44)

30 **[0300]** Clone 24C1 CD8 CD3 zeta CAR DNA Heavy & Light Chains

ATGGCACTCCCCGTAACCTGCTCTGCTGCTGCCGTTGGCATTGCTCC
 TGCACGCCGCACGCCCGGACATTCAATTGACCCAGTCCCCTAGCA

GTCTCTCAGCAAGTGTGGGAGATAGGGTGTTCATTACCTGTCAGG
CTTCACAGGACATCAACAACCTTCCTCAATTGGTATCAGCAGAAGC
CAGGGAAGGCACCAAAGCTGCTCATATATGACGCTTCAAACCTTG
AAACCGGAGTACCTAGCCGCTTCAGCGGAAGCGGATCAGGGACT
5 GACTTCACTTTTACCATCTCTTCACTGCAGCCCGAAGACATCGCCA
CATACTACTGCCAGCAGTACGGAACTTGCCTTTTACATTTGGGG
GCGGCACCAAAGTGGAGATTAAGCGAGGGGGAGGCGGCTCAGGA
GGCGGTGGCTCCGGAGGCGGGGGTTCCCAGGTCCAGCTCCAGGA
ATCCGGCCCAGGTCTGGTTAAGCCCAGTGAACTTTGTCCCTCAC
10 GTGTACTGTGAGCGGTGGTTCAATCTCCTCATACTATTGGTCTTGG
ATACGGCAACCTCCTGGAAAGGGCCTCGAGTGGATCGGCTATATC
TACTATAGTGGCTCCACTAATTACAACCCTTCCCTCAAGTCCAGA
GTCACCATTTCCGTGGACACATCTAAGAACCAGTTCAGTCTGAAG
TTGTCCAGCGTTACAGCCGCAGACACAGCCGTTTATTACTGTGTGT
15 CTCTTGTTTACTGCGGGGGAGACTGTTATAGCGGCTTCGATTACTG
GGGCCAGGGCACCTTGGTACAGTCTCTTCCGCGGCCGCCCTCTC
TAACAGTATTATGTACTTTTCTCATTTTGTACCCGTGTTCTTCCCG
CTAAGCCAACTACTACCCCGGCCCCACGGCCGCCTACCCCTGCAC
CCACAATAGCCAGTCAGCCTTTGAGCCTGAGACCTGAGGCTTGTC
20 GGCCGGCTGCTGGGGGTGCAGTGCACACACGAGGTCTTGATTTTG
CTTGCGACATATACATCTGGGCCCCCTCTGGCCGGGACCTGTGGGG
TGCTGCTTCTGAGCTTGGTCATCACGCTCTATTGCAACCATCGCAA
CAGATCCAAAAGAAGCCGCCTGCTCCATAGCGATTACATGAATAT
GACTCCACGCCGCCCTGGCCCCACAAGGAAACACTACCAGCCTTA
25 CGCACCACTAGAGATTTTCGCTGCCTATCGGAGCAGGGTGAAGTT
TTCCAGATCTGCAGATGCACCAGCGTATCAGCAGGGCCAGAACCA
ACTGTATAACGAGCTCAACCTGGGACGCAGGGAAGAGTATGACG
TTTTGGACAAGCGCAGAGGACGGGACCCCTGAGATGGGTGGCAAA
CCAAGACGAAAAAACCCCCAGGAGGGTCTCTATAATGAGCTGCA
30 GAAGGATAAGATGGCTGAAGCCTATTCTGAAATAGGCATGAAAG
GAGAGCGGAGAAGGGGAAAAGGGCACGACGGTTTGTACCAGGGA
CTCAGCACTGCTACGAAGGATACTTATGACGCTCTCCACATGCAA
GCCCTGCCACCTAGGTAA (SEQ ID NO. 45)

[0301] Clone 24C1 CD8 CD3 zeta CAR AA Heavy & Light Chains(Signal Peptide in **Bold**)

MALPVTALLLPLALLLHAARPDIQLTQSPSSLSASVGDRVSFTCQAS
 QDINNFLNWYQQKPGKAPKLLIYDASNLETGVPSRFSGSGSGTDFTF
 5 TISSLQPEDIATYYCQQYGNLPFTFGGGTKVEIKRGGGSGGGGSGG
 GGSQVQLQESGPGLVKPSSETLSLTCTVSGGSISSYYWSWIRQPPGKGL
 EWIGYIYYSGSTNYPNPSLKSRVTISVDTSKNQFSLKLSSVTAADTAVY
 YCVSLVYCGGDCYSGFDYWGGTGLVTVSSAAALSNSIMYFSHFVPV
 FLPKPTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFA
 10 CDIYIWAPLAGTCGVLLSLVITLYCNHRNRSKRSRLHSDYMNMTF
 RRPGPTRKHYQPYAPPRDFAAYRSRVKFSRSADAPAYQQGQNQLYN
 ELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKM
 AEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR
 (SEQ ID NO. 46)

15 **[0302]** Clone 24C1 CD8 CD3 zeta CAR DNA Heavy & Light Chains

GACATTCAATTGACCCAGTCCCCTAGCAGTCTCTCAGCAAGTGTG
 GGAGATAGGGTGTCAATTCACCTGTCAGGCTTCACAGGACATCAAC
 AACTTCCTCAATTGGTATCAGCAGAAGCCAGGGAAGGCACCAAA
 GCTGCTCATATATGACGCTTCAAACCTTGAAACCGGAGTACCTAG
 20 CCGCTTCAGCGGAAGCGGATCAGGGACTGACTTCACTTTTACCAT
 CTCTTCACTGCAGCCCGAAGACATCGCCACATACTACTGCCAGCA
 GTACGGAAACTTGCCTTTTACATTTGGGGGCGGCACCAAAGTGGA
 GATTAAGCGAGGGGGAGGCGGCTCAGGAGGCGGTGGCTCCGGAG
 GCGGGGGTTCCAGGTCCAGCTCCAGGAATCCGGCCCAGGTCTGG
 25 TTAAGCCCAGTGAAACTTTGTCCCTCACGTGTACTGTGAGCGGTG
 GTTCAATCTCCTCATACTATTGGTCTTGGATACGGCAACCTCCTGG
 AAAGGGCCTCGAGTGGATCGGCTATATCTACTATAGTGGCTCCAC
 TAATTACAACCCTTCCCTCAAGTCCAGAGTCACCATTTCCGTGGAC
 ACATCTAAGAACCAGTTCAGTCTGAAGTTGTCCAGCGTTACAGCC
 30 GCAGACACAGCCGTTTATTACTGTGTGTCTCTTGTTTACTGCGGGG
 GAGACTGTTATAGCGGCTTCGATTACTGGGGCCAGGGCACCTTGG
 TCACAGTCTCTTCCGCGGCCGCCCTCTCTAACAGTATTATGTACTT
 TTCTCATTTTGTACCCGTGTTCCCTTCCCGCTAAGCCAACTACTACC

CCGGCCCCACGGCCGCCTACCCCTGCACCCACAATAGCCAGTCAG
 CCTTTGAGCCTGAGACCTGAGGCTTGTCGGCCGGCTGCTGGGGGT
 GCAGTGCACACACGAGGTCTTGATTTTGCTTGCGACATATACATC
 TGGGCCCCCTCTGGCCGGGACCTGTGGGGTGCTGCTTCTGAGCTTG
 5 GTCATCACGCTCTATTGCAACCATCGCAACAGATCCAAAAGAAGC
 CGCCTGCTCCATAGCGATTACATGAATATGACTCCACGCCGCCCT
 GGCCCCACAAGGAAACACTACCAGCCTTACGCACCACCTAGAGAT
 TTCGCTGCCTATCGGAGCAGGGTGAAGTTTTCCAGATCTGCAGAT
 GCACCAGCGTATCAGCAGGGCCAGAACCAACTGTATAACGAGCT
 10 CAACCTGGGACGCAGGGAAGAGTATGACGTTTTGGACAAGCGCA
 GAGGACGGGACCCTGAGATGGGTGGCAAACCAAGACGAAAAAAC
 CCCCAGGAGGGTCTCTATAATGAGCTGCAGAAGGATAAGATGGCT
 GAAGCCTATTCTGAAATAGGCATGAAAGGAGAGCGGAGAAGGGG
 AAAAGGGCACGACGGTTTGTACCAGGGACTCAGCACTGCTACGA
 15 AGGATACTTATGACGCTCTCCACATGCAAGCCCTGCCACCTAGG
 (SEQ ID NO. 47)

[0303] Clone 24C1 CD8 CD3 zeta CAR AA Heavy & Light Chains

DIQLTQSPSSLASVGDVRSFTCQASQDINNFLNWWYQQKPGKAPKLLI
 YDASNLETGVPSRFSGSGSGTDFTFITISLQPEDATYYCQYGNLPFT
 20 FGGGTKVEIKRGGGGSGGGSGGGGSQVQLQESGPGLVKPSETLSLT
 CTVSGGSISSYYWSWIRQPPGKLEWIGYIYYSGSTNYPNPSLKSRTI
 SVDTSKNQFSLKLSSVTADTAVYYCVSLVYCGGDCYSGFDYWGGQT
 LVTVSSAAALSNSIMYFSHFVPVFLPAKPTTTPAPRPPTPAPTIASQPLS
 LRPEACRPAAGGAVHTRGLDFACDIYWAPLAGTCGVLLLSLVITLY
 25 CNHRNRSKRSRLHSDYMNMTPRRPGPTRKHYPYAPPRDFAAYRS
 RVKFSRSADAPAYQQGQNQLYNELNLGEEYDVLDRGRDPEMG
 GKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQ
 GLSTATK DTYDALHMQA LPPR (SEQ ID NO. 48)

[0304] Clone 24C8 Heavy Chain (HC) DNA

30 CAGGTACAGCTGCAGGAATCTGGGCCCCGACTTGTCAGCCAAGT
 CAGACACTTTCTCTTACATGTACCGTGAGCGGCGGAAGTATAAGC
 AGTGGAGGCTTTTACTGGTCTTGATACGGCAGCACCCAGGCAAA

5 GGCTTGGAGTGGATTGGATACATTCATCATTGAGGATCTACACAC
 TATAATCCATCCCTTAAGTCCCGGGTCACCATTAGCATTGATACGT
 CTAAGAATCTGTTCAGTCTCAGGCTGTCCTCCGTCCTGCTGCCGA
 CACAGCCGTGTACTACTGCGCCTCCTTGGTTTACTGCGGAGGCCGA
 CTGTTATAGCGGCTTTGATTATTGGGGGCAGGGGACCCTCGTAAC
 CGTGAGCTCT (SEQ ID NO. 48)

[0305] Clone 24C8 AA HC (CDRs in Underline)
 QVQLQESGPGLVKPSQTLSTCTVSGGSISSGGFYWSWIRQHPGKGL
 EWIGYIHHSGSTHYNPSLKSRTISIDTSKNLFSRLSSVTAADTAVYY
 10 CASLVYCGGDCYSGFDYWGGQGLVTVSS (SEQ ID NO. 50)

[0306] Clone 24C8 HC CDR1 AA: GGSISSGGF (SEQ ID NO. 51)

[0307] Clone 24C8 HC CDR2 AA: HHSGS (SEQ ID NO. 52)

[0308] Clone 24C8 HC CDR3 AA: LVYCGGDCYS GFDY (SEQ ID NO. 53)

[0309] Clone 24C8 Light Chain (LC) DNA
 15 GATATCCAGCTCACTCAAAGCCCCTCTAGTCTCTCTGCCTCAGTGG
 GGGATCGGGTCAGTTTTACTTGTCAAGCTTCACAGGATATCAACA
 ACTTCCTTAATTGGTATCAGCAGAAGCCAGGAAAAGCACCCAAGC
 TGCTCATCTATGATGCCTCAAATTTGGAGACGGGTGTTCCAGTC
 GATTCTCTGGGTACGGGTCCGGGACCGACTTTACGTTTACGATCTC
 20 CTCTCTGCAGCCCGAAGACATCGCCACATACTATTGTCAACAGTA
 CGGCAACTTGCCTTTCACATTTGGGGGCGGGACTAAGGTTGAAAT
 CAAGAGG (SEQ ID NO. 54)

[0310] Clone 24C8 LC AA (CDRs in Underline)
 DIQLTQSPSSLSASVGDRVSFTCQASQDINNFLNHWYQQKPGKAPKLLI
 25 YDASNLETGVPSRFSGSGSGTDFTFITSSLPEDIATYYCQOYGNLPFT
 FGGGTKVEIKR (SEQ ID NO. 55)

[0311] Clone 24C8 LC CDR1 AA: QASQDINNFLN (SEQ ID NO. 56)

[0312] Clone 24C8 LC CDR2 AA: DASNLET (SEQ ID NO. 57)

[0313] Clone 24C8 LC CDR3 AA: QQYGNLPFT (SEQ ID NO. 58)

[0314] Clone 24C8 CD28T CD3 zeta CAR DNA Heavy & Light Chains

ATGGCACTCCCCGTAAGTCTGCTGCTGCCGTTGGCATTGCTCC
TGCACGCCGCACGCCCGCAGGTACAGCTGCAGGAATCTGGGCCCCG
5 GACTTGTCAAGCCAAGTCAGACACTTTCTCTTACATGTACCGTGA
GCGGCGGAAGTATAAGCAGTGGAGGCTTTTACTGGTCTTGGATAC
GGCAGCACCCAGGCAAAGGCTTGGAGTGGATTGGATACATTCATC
ATTCAGGATCTACACACTATAATCCATCCCTTAAGTCCCGGGTCA
CCATTAGCATTGATACGTCTAAGAATCTGTTTCAGTCTCAGGCTGTC
10 CTCCGTCAGTCTGCCGACACAGCCGTGTACTACTGCGCCTCCTTG
GTTTACTGCGGAGGCGACTGTTATAGCGGCTTTGATTATTGGGGG
CAGGGGACCCCTCGTAACCGTGAGCTCTGGAGGGGGTGGGAGCGG
GGGAGGAGGTTTCAGGGGGGGGCGGCTCCGATATCCAGCTCACTC
AAAGCCCCTCTAGTCTCTCTGCCTCAGTGGGGGATCGGGTCAGTT
15 TTACTTGTCAAGCTTCACAGGATATCAACAACCTTCCTTAATTGGTA
TCAGCAGAAGCCAGGAAAAGCACCCAAGCTGCTCATCTATGATGC
CTCAAATTTGGAGACGGGTGTTCCCAGTCGATTCTCTGGGTCAGG
GTCCGGGACCGACTTTACGTTTACGATCTCCTCTCTGCAGCCCGAA
GACATCGCCACATACTATTGTCAACAGTACGGCAACTTGCCTTTC
20 ACATTTGGGGGCGGGACTAAGGTTGAAATCAAGAGGGCCGCTGC
ACTGGACAATGAGAAGTCCAACGGCACCATCATCCACGTGAAGG
GCAAGCACCTGTGCCCTAGTCCTCTGTTCCCAGGCCCATCCAAAC
CTTTTTGGGTTCTTGTTGTGGTCGGGGGGGTGCTGGCCTGCTATTC
TCTGCTGGTCACGGTGGCCTTCATAATTTTCTGGGTTAGATCCAAA
25 AGAAGCCGCCTGCTCCATAGCGATTACATGAATATGACTCCACGC
CGCCCTGGCCCCACAAGGAAACACTACCAGCCTTACGCACCACCT
AGAGATTTGCTGCCTATCGGAGCAGGGTGAAGTTTTCCAGATCT
GCAGATGCACCAGCGTATCAGCAGGGCCAGAACCAACTGTATAA
CGAGCTCAACCTGGGACGCAGGGAAGAGTATGACGTTTTGGACA
30 AGCGCAGAGGACGGGACCCTGAGATGGGTGGCAAACCAAGACGA
AAAAACCCCCAGGAGGGTCTCTATAATGAGCTGCAGAAGGATAA
GATGGCTGAAGCCTATTCTGAAATAGGCATGAAAGGAGAGCGGA
GAAGGGGAAAAGGGCACGACGGTTTGTACCAGGGACTCAGCACT

GCTACGAAGGATACTTATGACGCTCTCCACATGCAAGCCCTGCCA
CCTAGGTAA (SEQ ID NO. 59)

[0315] Clone 24C8 CD28T CD3 zeta CAR AA Heavy & Light Chains
(Signal Peptide in **Bold**)

5 **MALPVTALLLPLALLLHAARPQVQLQESGPGLVKPSQTL**SLTCTVS
GGSISSGGFYWSWIRQHPGKGLEWIGYIHHSGSTHYNPSLKSRVTISI
DTSKNLFSRLSSVTAADTAVYYCASLVYCGGDCYSGFDYWQGQTL
VTVSSGGGGSGGGSGGGGSDIQLTQSPSSLASVGDVRSFTCQASQ
DINNFLNWWYQQKPGKAPKLLIYDASNLETGVPSRFSGSGSGTDFTFTI
10 SSLQPEDIATYYCQYGNLPFTFGGGTKVEIKRAAALDNEKSNGTIIH
VKGKHLCPSPLEPGPSKPFVVLVVVGGVLACYSLLVTVAFIIFWVRS
KRSRLHSDYMNMTPRRPGPTRKHYPYAPPRDFAAYRSRVKFSRS
ADAPAYQQGQNQLYNELNLGRREEYDVLDRRGRDPEMGGKPRRK
NPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTAT
15 KDTYDALHMQALPPR (SEQ ID NO. 60)

[0316] Clone 24C8 CD28T CD3 zeta CAR DNA Heavy & Light Chains

CAGGTACAGCTGCAGGAATCTGGGCCCGGACTTGTCAAGCCAAGT
CAGACACTTTCTCTTACATGTACCGTGAGCGGCGGAAGTATAAGC
AGTGGAGGCTTTTACTGGTCTTGGATACGGCAGCACCCAGGCAAA
20 GGCTTGGAGTGGATTGGATACATTCATCATTAGGATCTACACAC
TATAATCCATCCCTTAAGTCCCGGGTCACCATTAGCATTGATACGT
CTAAGAATCTGTTCAGTCTCAGGCTGTCCTCCGTCCTGCTGCCGA
CACAGCCGTGTACTACTGCGCCTCCTTGGTTTACTGCGGAGGCGA
CTGTTATAGCGGCTTTGATTATTGGGGGCAGGGGACCCTCGTAAC
25 CGTGAGCTCTGGAGGGGGTGGGAGCGGGGGAGGAGGTTCAAGGG
GGGGCGGCTCCGATATCCAGCTCACTCAAAGCCCCCTCTAGTCTCT
CTGCCTCAGTGGGGGATCGGGTCAGTTTTACTTGTCAAGCTTCAC
AGGATATCAACAACCTTCCTTAATTGGTATCAGCAGAAGCCAGGAA
AAGCACCCAAGCTGCTCATCTATGATGCCTCAAATTTGGAGACGG
30 GTGTTCCCAGTCGATTCTCTGGGTCAGGGTCCGGGACCGACTTTA
CGTTTACGATCTCCTCTCTGCAGCCCGAAGACATCGCCACATACT
ATTGTCAACAGTACGGCAACTTGCCTTTCACATTTGGGGGCGGGA

CTAAGGTTGAAATCAAGAGGGCCGCTGCACTGGACAATGAGAAG
TCCAACGGCACCATCATCCACGTGAAGGGCAAGCACCTGTGCCCT
AGTCCTCTGTTCCCAGGCCCATCCAAACCTTTTTGGGTTCTTGTTG
TGGTCGGGGGGGTGCTGGCCTGCTATTCTCTGCTGGTCACGGTGG
5 CCTTCATAATTTTCTGGGTTAGATCCAAAAGAAGCCGCCTGCTCC
ATAGCGATTACATGAATATGACTCCACGCCGCCCTGGCCCCACAA
GGAAACACTACCAGCCTTACGCACCACCTAGAGATTTTCGCTGCCT
ATCGGAGCAGGGTGAAGTTTTCCAGATCTGCAGATGCACCAGCGT
ATCAGCAGGGCCAGAACCAACTGTATAACGAGCTCAACCTGGGA
10 CGCAGGGAAGAGTATGACGTTTTGGACAAGCGCAGAGGACGGGA
CCCTGAGATGGGTGGCAAACCAAGACGAAAAAACCCCCAGGAGG
GTCTCTATAATGAGCTGCAGAAGGATAAGATGGCTGAAGCCTATT
CTGAAATAGGCATGAAAGGAGAGCGGAGAAGGGGAAAAGGGCA
CGACGTTTTGTACCAGGGACTCAGCACTGCTACGAAGGATACTTA
15 TGACGCTCTCCACATGCAAGCCCTGCCACCTAGG (SEQ ID NO. 61)

[0317] Clone 24C8 CD28T CD3 zeta CAR AA Heavy & Light Chains

QVQLQESGPGLVKPSQTLSTCTVSGGSISSGGFYWSWIRQHPGKGL
EWIGYIHHSSTHYNPSLKSRTISIDTSKNLFSRLSSVTAADTAVYY
CASLVYCGGDCYSGFDYWQGTLVTVSSGGGGSGGGGSGGGGSDI
20 QLTQSPSSLSASVGDRVSFTCSQASQDINNFLNWFYQQKPKAPKLLIY
DASNLETGVPSRFSGSGSGTDFTFITSSLPEDIATYYCQYGNLPFTF
GGGKVEIKRAAALDNEKSNGTHHVKGKHLCPSPFPGPSKPFVWL
VVVGVLACYSLLVTVAFHFWVRSKRSRLHSDYMNMTPRRPGPTR
KHYQPYAPPRDFAAYRSRVKFSRSADAPAYQQGQNQLYNELNLGRR
25 EEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEI
GMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR (SEQ ID
NO. 62)

[0318] Clone 24C8 CD28 CD3 zeta CAR DNA Heavy & Light Chains

ATGGCACTCCCCGTAAGTCTGCTGCTGCCGTTGGCATTGCTCC
TGCACGCCGCACGCCCGCAGGTGCAGCTGCAGGAAAGCGGTCCG
30 GGACTTGTCAAGCCGTCCCAAACGCTGAGTCTGACGTGTACTGTC
TCTGGTGGCTCTATTTCTTCCGGGGGCTTTTATTGGTCTTGATCA

GACAACACCCTGGCAAAGGGCTGGAGTGGATAGGGTATATTCAC
CACTCTGGGTCCACTCACTACAACCCATCATTGAAATCCAGAGTG
ACTATCTCAATCGACACATCCAAGAACCTTTTCAGCCTGAGGTTG
TCATCAGTTACCGCCGCTGACACCGCGGTGTATTATTGCGCCTCTC
5 TCGTGTACTGCGGTGGCGATTGTTATAGTGGCTTTGACTACTGGG
GGCAGGGGACATTGGTTACCGTTTCAAGTGGAGGCGGTGGGTCTG
GCGGGGGCGGTAGCGGAGGTGGGGGGAGCGACATACAGCTTACG
CAGAGCCCCCTCCAGCCTTTCAGCCTCCGTGGGGGATAGGGTGTCC
TTTACCTGCCAGGCTTCCCAGGACATAAACAACCTTCCTCAATTGGT
10 ATCAGCAAAAAGCCCGGGAAAGCACCAAAGCTGCTCATCTACGAT
GCCAGCAACCTGGAAACCGGAGTGCCGTCTCGCTTCTCTGGAAGT
GGCAGTGGGACCGATTTCACTTTTACAATCTCAAGTTTGCAGCCA
GAAGACATTGCAACATACTACTGTCAACAGTACGGCAATCTCCCC
TTTACATTTGGGGGGGGAACTAAAGTGGAGATTAAGCGCGCTGCA
15 GCCATTGAAGTTATGTATCCGCCCCCGTATCTGGATAACGAGAAA
TCTAATGGTACCATAATACATGTGAAGGGGAAGCACCTCTGTCCA
TCACCGCTGTTCCCCGGCCCTTCAAAACCTTTCTGGGTACTCGTTG
TCGTGGGTGGAGTTCTGGCCTGCTATAGTCTGCTGGTGACCGTGG
CGTTTATCATCTTCTGGGTAAGATCCAAAAGAAGCCGCCTGCTCC
20 ATAGCGATTACATGAATATGACTCCACGCCGCCCTGGCCCCACAA
GGAAACACTACCAGCCTTACGCACCACCTAGAGATTTTCGCTGCCT
ATCGGAGCAGGGTGAAGTTTTCCAGATCTGCAGATGCACCAGCGT
ATCAGCAGGGCCAGAACCAACTGTATAACGAGCTCAACCTGGGA
CGCAGGGAAGAGTATGACGTTTTTGGACAAGCGCAGAGGACGGGA
25 CCCTGAGATGGGTGGCAAACCAAGACGAAAAAACCCCCAGGAGG
GTCTCTATAATGAGCTGCAGAAGGATAAGATGGCTGAAGCCTATT
CTGAAATAGGCATGAAAGGAGAGCGGAGAAGGGGAAAAGGGCA
CGACGGTTTTGTACCAGGGACTCAGCACTGCTACGAAGGATACTTA
TGACGCTCTCCACATGCAAGCCCTGCCACCTAGGTAA (SEQ ID NO.
30 63)

[0319] Clone 24C8 CD28 CD3 zeta CAR AA Heavy & Light Chains(Signal Peptide in **Bold**)

MALPVTALLLPLALLLHAARPQVQLQESGPGLVKPSQTLSLTCTVS
 GGSISSGGFYWSWIRQHPGKGLEWIGYIHHSGSTHYNPSLKSRVTISI
 5 DTSKNLFSRLSSVTAADTAVYYCASLVYCGGDCYSGFDYWQGQTL
 VTVSSGGGGSGGGGSGGGGSDIQLTQSPSSLSASVGDRVSFTQCASQ
 DINNFLNWFYQQKPGKAPKLLIYDASNLETGVPSRFSGSGSGTDFFTI
 SSLQPEDIATYYCQQYGNLPFTFGGGTKVEIKRAAAIEVMYPPPYLD
 NEKSNGTIIHVKGKHLCPSPFPGPSKPFWVLVVVGVLACYSLLVT
 10 VAFIIFWVRSKRSRLHSDYMNMTPRRPGPTRKHYPYAPPRDFAAY
 RSRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPE
 MGGKPRRKNPQEGLYNELQDKMAEAYSEIGMKGERRRRGKGHDGL
 YQGLSTATKDTYDALHMQALPPR (SEQ ID NO. 64)

[0320] Clone 24C8 CD28 CD3 zeta CAR DNA Heavy & Light Chains

15 CAGGTGCAGCTGCAGGAAAGCGGTCCGGGACTTGTCAAGCCGTCC
 CAAACGCTGAGTCTGACGTGTACTGTCTCTGGTGGCTCTATTTCTT
 CCGGGGGCTTTTATTGGTCTTGGATCAGACAACACCCTGGCAAAG
 GGCTGGAGTGGATAGGGTATATTCACCACTCTGGGTCCACTCACT
 ACAACCCATCATTGAAATCCAGAGTGACTATCTCAATCGACACAT
 20 CCAAGAACCTTTTCAGCCTGAGGTTGTCATCAGTTACCGCCGCTG
 ACACCGCGGTGTATTATTGCGCCTCTCTCGTGTACTGCGGTGGCG
 ATTGTTATAGTGGCTTTGACTACTGGGGGCAGGGGACATTGGTTA
 CCGTTTCAAGTGGAGGCGGTGGGTCTGGCGGGGGCGGTAGCGGA
 GGTGGGGGGAGCGACATACAGCTTACGCAGAGCCCCCTCCAGCCTT
 25 TCAGCCTCCGTGGGGGATAGGGTGTCTTTACCTGCCAGGCTTCC
 CAGGACATAAAACAACCTTCTCAATTGGTATCAGCAAAAGCCCGGG
 AAAGCACCAAAGCTGCTCATCTACGATGCCAGCAACCTGGAAACC
 GGAGTGCCGTCTCGCTTCTCTGGAAGTGGCAGTGGGACCGATTTC
 ACTTTTACAATCTCAAGTTTGCAGCCAGAAGACATTGCAACATAC
 30 TACTGTCAACAGTACGGCAATCTCCCCTTTACATTTGGGGGGGGA
 ACTAAAGTGGAGATTAAGCGCGCTGCAGCCATTGAAGTTATGTAT
 CCGCCCCCGTATCTGGATAACGAGAAATCTAATGGTACCATAATA
 CATGTGAAGGGGAAGCACCTCTGTCCATCACCGCTGTTCCCCGGC

CCTTCAAAACCTTTCTGGGTACTCGTTGTCGTGGGTGGAGTTCTGG
 CCTGCTATAGTCTGCTGGTGACCGTGGCGTTTATCATCTTCTGGGT
 AAGATCCAAAAGAAGCCGCCTGCTCCATAGCGATTACATGAATAT
 GACTCCACGCCGCCCTGGCCCCACAAGGAAACACTACCAGCCTTA
 5 CGCACCACCTAGAGATTTTCGCTGCCTATCGGAGCAGGGTGAAGTT
 TTCCAGATCTGCAGATGCACCAGCGTATCAGCAGGGGCCAGAACCA
 ACTGTATAACGAGCTCAACCTGGGACGCAGGGAAGAGTATGACG
 TTTTGGACAAGCGCAGAGGACGGGACCCTGAGATGGGTGGCAAA
 CCAAGACGAAAAAACCCCCAGGAGGGTCTCTATAATGAGCTGCA
 10 GAAGGATAAGATGGCTGAAGCCTATTCTGAAATAGGCATGAAAG
 GAGAGCGGAGAAGGGGAAAAGGGCACGACGGTTTGTACCAGGGA
 CTCAGCACTGCTACGAAGGATACTTATGACGCTCTCCACATGCAA
 GCCCTGCCACCTAGG (SEQ ID NO. 65)

[0321] Clone 24C8 CD28 CD3 zeta CAR AA Heavy & Light Chains
 15 QVQLQESGPGLVKPSQTLSTCTVSGGSISSGGFYWSWIRQHPGKGL
 EWIGYIHHSSTHYNPSLKSRTISIDTSKNLFSRLSSVTAADTAVYY
 CASLVYCGGDCYSGFDYWQGTLVTVSSGGGGSGGGGSGGGGSDI
 QLTQSPSSLSASVGDRVSFTCSQASQDINNFLNWYQQKPGKAPKLLIY
 DASNLETGVPSRFSGSGSGTDFTFITISLQPEDATYYCQYGNLPFTF
 20 GGGTKVEIKRAAAIEVMYPPPYLDNEKSNGTHHVKGKHLCPSPFP
 PSKPFWVLVVVGGVLACYSLLVTVAFIIFWVRSKRSRLHSDYMN
 TPRRPGPTRKHYPYAPPRDFAAYRSRVKFSRSADAPAYQQGQNQL
 YNELNLGRREEYDVLDRRGRDPEMGGKPRRKNPQEGLYNELQKD
 KMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALP
 25 PR (SEQ ID NO. 66)

[0322] Clone 24C8 CD8 CD3 zeta CAR DNA Heavy & Light Chains
 ATGGCACTCCCCGTAAGTCTGCTGCTGCCGTTGGCATTGCTCC
 TGCACGCCGCACGCCGCAGGTGCAGTTGCAGGAAAGCGGGCCT
 GGCCTTGTGAAACCAAGCCAGACACTGAGCCTGACATGCACTGTG
 30 TCCGGCGGGTCCATATCTTCCGGGGGTTTTTATTGGTCTTGATAC
 GCCAGCATCCCGGGAAAGGACTTGAATGGATTGGATATATCCACC
 ATTCCGGAAGCACCCACTACAATCCAAGCCTTAAATCCCGGGTGA

CAATCTCCATCGACACCTCAAAGAATCTTTTTTCCCTGCGGTTGTC
 TTCAGTAACTGCCGCCGATACCGCTGTGTACTACTGTGCCAGCCTC
 GTCTATTGCGGCGGAGATTGTTATTCTGGGTTTCGATTATTGGGGTC
 AAGGCACACTGGTAACTGTCAGCAGCGGAGGCGGCGGTTCCGGG
 5 GGCGGGGGCAGTGGAGGGGGCGGATCTGACATTCAGCTTACGCA
 GTCCCCATCTTCACTTAGCGCCAGCGTTGGCGATCGGGTCAGCTTC
 ACGTGTCAAGCAAGTCAGGATATCAACAACCTTCTTAACTGGTAC
 CAGCAGAAGCCAGGCAAGGCACCCAAGTTGCTGATTTACGATGCT
 TCTAACCTCGAGACGGGAGTGCCTAGCCGCTTCTCCGGGAGCGGC
 10 AGCGGCACAGACTTTACCTTTACGATTTCCAGTCTGCAGCCAGAG
 GATATAGCAACTTATTACTGTCAGCAGTATGGCAACCTCCCTTTTA
 CCTTCGGTGGTGGCACAAAGGTCGAGATTAAGAGCCGCAGCG
 TTGTCCAACCTCCATAATGTATTTTTCTCATTTTGTGCCCGTCTTCT
 GCCTGCCAAACCTACCACCACCCCGCCCCACGACCACCTACTCC
 15 AGCCCCCACCATCGCCTCCCAGCCCCCTCAGCCTGAGGCCAGAGGC
 TTGTGCGCCTGCTGCGGGGGGCGCTGTCCATACCAGAGGACTCGA
 CTTCGCCTGCGATATTTATATATGGGCCCCCCTCGCCGGCACCTGC
 GGAGTCTTGCTCCTGAGCCTTGTGATCACGCTTTATTGTAACCATC
 GGAATAGATCCAAAAGAAGCCGCCTGCTCCATAGCGATTACATGA
 20 ATATGACTCCACGCCGCCCTGGCCCCACAAGGAAACACTACCAGC
 CTTACGCACCACCTAGAGATTTTCGCTGCCTATCGGAGCAGGGTGA
 AGTTTTCCAGATCTGCAGATGCACCAGCGTATCAGCAGGGCCAGA
 ACCAACTGTATAACGAGCTCAACCTGGGACGCAGGGAAGAGTAT
 GACGTTTTGGACAAGCGCAGAGGACGGGACCCTGAGATGGGTGG
 25 CAAACCAAGACGAAAAAACCCCCAGGAGGGTCTCTATAATGAGC
 TGCAGAAGGATAAGATGGCTGAAGCCTATTCTGAAATAGGCATG
 AAAGGAGAGCGGAGAAGGGGAAAAGGGCACGACGTTTGTACCA
 GGGACTCAGCACTGCTACGAAGGATACTTATGACGCTCTCCACAT
 GCAAGCCCTGCCACCTAGGTAA (SEQ ID NO. 67)

30 **[0323]** Clone 24C8 CD8 CD3 zeta CAR AA Heavy & Light Chains
(Signal Peptide in **Bold**)
MALPVTALLLPLALLLHAARPQVQLQESGPGLVKPSQTLSTCTVS
GGSISSGGFYWSWIRQHPGKGLEWIGYIHHSSTHYNPSLKSRTVSI

5 DTSKNLFSRLSSVTAADTAVYYCASLVYCGGDCYSGFDYWGQGTL
VTVSSGGGGSGGGSGGGGSDIQLTQSPSSLSASVGDRVSFTQCASQ
DINNFLNWFYQQKPGKAPKLLIYDASNLETGVPRFSGSGSGTDFTFTIS
SLQPEDIATYYCQYGNLPFTFGGGTKVEIKRAAALSNSIMYFSHFVPV
FLPAKPTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFA
CDIYIWAPLAGTCGVLLLSLVITLYCNHRNRSKRSRLHSDYMNMTF
RRPGPTRKHYQPYAPPRDFAAYRSRVKFSRSADAPAYQQGQNQLYN
ELNLGRREEYDVLDRRGRDPEMGGKPRRKNPQEGLYNELQKDKM
AEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR
10 (SEQ ID NO. 68)

[0324] Clone 24C8 CD8 CD3 zeta CAR DNA Heavy & Light Chains

CAGGTGCAGTTGCAGGAAAGCGGGCCTGGCCTTGTGAAACCAAG
CCAGACACTGAGCCTGACATGCACTGTGTCCGGCGGGTCCATATC
TTCCGGGGGTTTTTTATTGGTCCTGGATACGCCAGCATCCCGGGAA
15 AGGACTTGAATGGATTGGATATATCCACCATTCCGGAAGCACCCA
CTACAATCCAAGCCTTAAATCCCGGGTGACAATCTCCATCGACAC
CTCAAAGAATCTTTTTTCCCTGCGGTTGTCTTCAGTAACTGCCGCC
GATACCGCTGTGTACTACTGTGCCAGCCTCGTCTATTGCGGCGGA
GATTGTTATTCTGGGTTTCGATTATTGGGGTCAAGGCACACTGGTA
20 ACTGTCAGCAGCGGAGGCGGCGGTTCCGGGGGCGGGGGCAGTGG
AGGGGGCGGATCTGACATTCAGCTTACGCAGTCCCCATCTTCACT
TAGCGCCAGCGTTGGCGATCGGGTCAGCTTCACGTGTCAAGCAAG
TCAGGATATCAACAACCTTTCTTAACTGGTACCAGCAGAAGCCAGG
CAAGGCACCCAAGTTGCTGATTTACGATGCTTCTAACCTCGAGAC
25 GGGAGTGCCTAGCCGCTTCTCCGGGAGCGGCAGCGGCACAGACTT
TACCTTTACGATTTCCAGTCTGCAGCCAGAGGATATAGCAACTTA
TTACTGTCAGCAGTATGGCAACCTCCCTTTTACCTTCGGTGGTGGC
ACAAAGGTCGAGATTAAAAGAGCCGCGAGCGTTGTCCAACTCCATA
ATGTATTTTTCTCATTTTGTGCCCGTCTTTCTGCCTGCCAAACCTAC
30 CACCACCCCCGCCCCACGACCACCTACTCCAGCCCCCACCATCGC
CTCCCAGCCCCCTCAGCCTGAGGCCAGAGGCTTGTCGCCCTGCTGC
GGGGGGCGCTGTCCATAACCAGAGGACTCGACTTCGCCTGCGATAT
TTATATATGGGCCCCCTCGCCGGCACCTGCGGAGTCTTGCTCCTG

AGCCTTGTGATCACGCTTTATTGTAACCATCGGAATAGATCCAAA
AGAAGCCGCCTGCTCCATAGCGATTACATGAATATGACTCCACGC
CGCCCTGGCCCCACAAGGAAACACTACCAGCCTTACGCACCACCT
AGAGATTTTCGCTGCCTATCGGAGCAGGGTGAAGTTTTCCAGATCT
5 GCAGATGCACCAGCGTATCAGCAGGGCCAGAACCAACTGTATAA
CGAGCTCAACCTGGGACGCAGGGAAGAGTATGACGTTTTGGACA
AGCGCAGAGGACGGGACCCTGAGATGGGTGGCAAACCAAGACGA
AAAAACCCCCAGGAGGGTCTCTATAATGAGCTGCAGAAGGATAA
GATGGCTGAAGCCTATTCTGAAATAGGCATGAAAGGAGAGCGGA
10 GAAGGGGAAAAGGGCACGACGGTTTGTACCAGGGACTCAGCACT
GCTACGAAGGATACTTATGACGCTCTCCACATGCAAGCCCTGCCA
CCTAGG (SEQ ID NO. 69)

[0325] Clone 24C8 CD8 CD3 zeta CAR AA Heavy & Light Chains

QVQLQESGPGLVKPSQTLSTCTVSGGSISSGGFYWSWIRQHPGKGL
15 EWIGYIHHSSTHYNPSLKSRTISIDTSKNLFSRLSSVTAADTAVYY
CASLVYCGGDCYSGFDYWQGTLVTVSSGGGGSGGGGSGGGGSDI
QLTQSPSSLSASVGDRVSFTCSQASQDINNFLNWYQQKPGKAPKLLIY
DASNLETGVPSRFSGSGSGTDFTFTISSLPEDIATYYCQQYGNLPFTF
GGGTKVEIKRAAALSNSIMYFSHFVPVFLPAKPTTTPAPRPPTPAPTIA
20 SQPLSLRPEACRPAAGGAVHTRGLDFACDIYWAPLAGTCGVLLLSL
VITLYCNHRNRSKRSRLHSDYMNMTPRRPGPTRKHYPYAPPRDF
AAYRSRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRG
RDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKG
HDGLYQGLSTA TKDITYDALHM QALPPR (SEQ ID NO. 70)

25 [0326] Clone 20C5.1 HC DNA

CAGGTCCAACCTGGTGCAGTCCGGAGCCGAAGTCAAGAAACCAGG
TGCCTCCGTTAAAGTGAGTTGCAAAGTCTCTGGATACACTCTGAC
CGAGCTCTCTATGCACTGGGTCCGGCAGGCCCCCGGCAAGGGATT
GGAATGGATGGGCGGGTTCGATCCTGAGGACGGAGAGACTATCT
30 ACGCTCAAAAATTCCAGGGACGAGTGACTGTGACCGAAGACACT
AGTACCGACACTGCCTACATGGAACTTTCCTCTCTGCGATCAGAA
GATACCGCAGTGTACTACTGTGCTACTGAATCTAGGGGCATTGGA

TGGCCCTACTTCGATTACTGGGGTCAGGGAAGCTCTGGTGACTGTC
TCCAGC (SEQ ID NO. 71)

[0327] Clone 20C5.1 AA HC (CDRs in Underline)

5 QVQLVQSGAEVKKPGASVKVSCKVSGYTLTEL^{SMHWVRQAPGKGL}
EWMGGFD^{DPEDGE}ETIYAQKFQGRVTVTEDTSTD^{TAYMELSSLRSED}T
AVYYCATE^{ESRGIGWPYFDY}WGQGT^{LVTVSS} (SEQ ID NO. 72)

[0328] Clone 20C5.1 HC AA CDR1: GYTLTEL (SEQ ID NO. 73)

[0329] Clone 20C5.1 HC AA CDR2: DPEDGE (SEQ ID NO. 74)

[0330] Clone 20C5.1 HC AA CDR3: ESRGIGWPYFDY (SEQ ID NO. 75)

10 [0331] Clone 20C5.1 LC DNA

GATATTCAGATGACTCAATCTCCTTCTTCTCTGTCCGCTTCCGTGG
GCGATAGAGTGACCATTACTTGTAGGGCGTCCCAGTCAATCTCCA
GTTATTTGAATTGGTATCAGCAGAAGCCCGGGAAAGCACCTAAGC
TGTTGATCAGCGGGGCTTCTAGCCTGAAGAGTGGGGTACCTTCAC
15 GGTTTCAGCGGAAGCGGAAGCGGAACCGATTTACCCCTGACTATCA
GCAGCCTGCCACCTGAGGACTTTGCAACTTACTACTGCCAACAGT
CATAACAGCACTCCGATCACTTTCGGCCAGGGCACCCGGCTCGAAA
TCAAGCGC (SEQ ID NO. 76)

[0332] Clone 20C5.1 AA LC (CDRs in Underline)

20 DIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKLLI
SGASSLKSGVPSRFSGSGSGTDFTLTISSLPPEDFATYYCQ^{QSYSTPIT}F
GQGTRLEIKR (SEQ ID NO. 77)

[0333] Clone 20C5.1 AA LC CDR1: RASQSISSYLN (SEQ ID NO. 78)

[0334] Clone 20C5.1 AA LC CDR2: GASSLKS (SEQ ID NO. 79)

25 [0335] Clone 20C5.1 AA LC CDR3: QQSYSTPIT (SEQ ID NO. 80)

[0336] Clone 20C5.1 CD28T CD3 zeta CAR DNA Heavy & Light Chains

ATGGCACTCCCCGTAAGTCTGCTCTGCTGCTGCCGTTGGCATTGCTCC
TGACGCGCCGACGCCCCGAGGTCCAACTGGTGCAGTCCGGAGCCG
AAGTCAAGAAACCAGGTGCCTCCGTTAAAGTGAGTTGCAAAGTCT
CTGGATACACTCTGACCGAGCTCTCTATGCACTGGGTCCGGCAGG
5 CCCCCGGCAAGGGATTGGAATGGATGGGCGGGTTCGATCCTGAG
GACGGAGAGACTATCTACGCTCAAAAATTCCAGGGACGAGTGAC
TGTGACCGAAGACACTAGTACCGACACTGCCTACATGGAAC TTCT
CTCTCTGCGATCAGAAGATACCGCAGTGTACTACTGTGCTACTGA
ATCTAGGGGCATTGGATGGCCCTACTTCGATTACTGGGGTCAGGG
10 AACTCTGGTGACTGTCTCCAGCGGTGGAGGTGGCAGCGGTGGTGG
CGGAAGCGGGGGGGGGCGGCTCTGATATTCAGATGACTCAATCTCC
TTCTTCTCTGTCCGCTTCCGTGGGCGATAGAGTGACCATTACTTGT
AGGGCGTCCCAGTCAATCTCCAGTTATTTGAATTGGTATCAGCAG
AAGCCCGGGAAGACCTAAGCTGTTGATCAGCGGGGCTTCTAGC
15 CTGAAGAGTGGGGTACCTTCACGGTTCAGCGGAAGCGGAAGCGG
AACCGATTTACCCCTGACTATCAGCAGCCTGCCACCTGAGGACTT
TGCAACTTACTACTGCCAACAGTCATACAGCACTCCGATCACTTTC
GGCCAGGGCACCCGGCTCGAAATCAAGCGCGCTGCTGCTTTGGAC
AATGAGAAGTCAAACGGCACCATCATA CATGTAAAGGTAAACA
20 TCTGTGTCCCTCCCCGCTGTTCCCCGGCCCTTCCAAACCGTTCTGG
GTTCTGGTGGTGGTTCGGAGGCGTACTCGCTTGCTATAGTCTGCTG
GTAAGTGTGCGCTTCATCATCTTTTGGGTGAGATCCAAAAGAAGC
CGCCTGCTCCATAGCGATTACATGAATATGACTCCACGCCGCCCT
GGCCCCACAAGGAAACACTACCAGCCTTACGCACCACCTAGAGAT
25 TTCGCTGCCTATCGGAGCAGGGTGAAGTTTTCCAGATCTGCAGAT
GCACCAGCGTATCAGCAGGGCCAGAACCAACTGTATAACGAGCT
CAACCTGGGACGCAGGGAAGAGTATGACGTTTTTGGACAAGCGCA
GAGGACGGGACCCCTGAGATGGGTGGCAAACCAAGACGAAAAAAC
CCCCAGGAGGGTCTCTATAATGAGCTGCAGAAGGATAAGATGGCT
30 GAAGCCTATTCTGAAATAGGCATGAAAGGAGAGCGGAGAAGGGG
AAAAGGGCACGACGTTTTGTACCAGGGACTCAGCACTGCTACGA
AGGATACTTATGACGCTCTCCACATGCAAGCCCTGCCACCTAGGT
AA (SEQ ID NO. 81)

[0337] Clone 20C5.1 CD28T CD3 zeta CAR AA Heavy & Light Chains(Signal Peptide in **Bold**)

MALPVTALLLPLALLLHAARPQVQLVQSGAEVKKPGASVKVSCKV
 SGYTLTELSMHWVRQAPGKGLEWMGGFDPEDGETIYAQKFQGRVT
 5 VTEDTSTD TAYMELSSLRSED TAVYYCATESRGIGWPYFDYWGGQT
 LVTVSSGGGGSGGGGSGGGGSDIQMTQSPSSLSASVGDRTITCRAS
 QSISSYLNWYQQKPGKAPKLLISGASSLKSGVPSRFSGSGSGTDFTLTI
 SSLPPEDFATYYCQQSYSTPITFGQGTRLEIKRAAALDNEKSNGTIIHV
 KGKHLCPSPFPGPSKPFWVLVVVGGVLACYSLLVTVAFIIFWVRSK
 10 RSRLHSDYMNMTPRRPGPTRKHYPYAPPRDFAAYRSRVKFSRSA
 DAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKN
 PQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATK
 DTYDALHMQALPPR (SEQ ID NO. 82)

[0338] Clone 20C5.1 CD28T CD3 zeta CAR DNA Heavy & Light Chains

15 CAGGTCCAAC TGGTGCAGTCCGGAGCCGAAGTCAAGAAACCAGG
 TGCCTCCGTTAAAGTGAGTTGCAAAGTCTCTGGATACACTCTGAC
 CGAGCTCTCTATGCACTGGGTCCGGCAGGCCCCCGGCAAGGGATT
 GGAATGGATGGGCGGGTTCGATCCTGAGGACGGAGAGACTATCT
 ACGCTCAAAAATTCCAGGGACGAGTGACTGTGACCGAAGACACT
 20 AGTACCGACACTGCCTACATGGAACTTTCCTCTCTGCGATCAGAA
 GATACCGCAGTGTACTACTGTGCTACTGAATCTAGGGGCATTGGA
 TGGCCCTACTTCGATTACTGGGGTCAGGGAACCTCTGGTGACTGTC
 TCCAGCGGTGGAGGTGGCAGCGGTGGTGGCGGAAGCGGGGGGGG
 CGGCTCTGATATTCAGATGACTCAATCTCCTTCTTCTGTCCGCT
 25 TCCGTGGGCGATAGAGTGACCATTACTTGTAGGGCGTCCCAGTCA
 ATCTCCAGTTATTTGAATTGGTATCAGCAGAAGCCCGGGAAAGCA
 CCTAAGCTGTTGATCAGCGGGGCTTCTAGCCTGAAGAGTGGGGTA
 CCTTCACGGTTCAGCGGAAGCGGAAGCGGAACCGATTTACCCCTG
 ACTATCAGCAGCCTGCCACCTGAGGACTTTGCAACTTACTACTGC
 30 CAACAGTCATACAGCACTCCGATCACTTTCCGGCCAGGGCACCCGG
 CTCGAAATCAAGCGCGCTGCTGCTTTGGACAATGAGAAGTCAAAC
 GGCACCATCATACATGTTAAAGGTAAACATCTGTGTCCCTCCCCG
 CTGTTCCCCGGCCCTTCCAAACCGTTCTGGGTTCTGGTGGTGGTCG

GAGGCGTACTCGCTTGCTATAGTCTGCTGGTAACTGTCGCCTTCAT
CATCTTTTGGGTGAGATCCAAAAGAAGCCGCCTGCTCCATAGCGA
TTACATGAATATGACTCCACGCCGCCCTGGCCCCACAAGGAAACA
CTACCAGCCTTACGCACCACCTAGAGATTTTCGCTGCCTATCGGAG
5 CAGGGTGAAGTTTTCCAGATCTGCAGATGCACCAGCGTATCAGCA
GGGCCAGAACCAACTGTATAACGAGCTCAACCTGGGACGCAGGG
AAGAGTATGACGTTTTGGACAAGCGCAGAGGACGGGACCCTGAG
ATGGGTGGCAAACCAAGACGAAAAAACCCCAAGGAGGGTCTCTA
TAATGAGCTGCAGAAGGATAAGATGGCTGAAGCCTATTCTGAAAT
10 AGGCATGAAAGGAGAGCGGAGAAGGGGAAAAGGGCACGACGGT
TTGTACCAGGGACTCAGCACTGCTACGAAGGATACTTATGACGCT
CTCCACATGCAAGCCCTGCCACCTAGG (SEQ ID NO. 83)

[0339] Clone 20C5.1 CD28T CD3 zeta CAR AA Heavy & Light Chains

QVQLVQSGAEVKKPGASVKVSCKVSGYTLTELSMHWVRQAPGKGL
15 EWMGGFDPEDGETIYAQKFQGRVTVTEDTSTDYAYMELSSLRSEDY
AVYYCATESRGIGWPYFDYWQGQTLVTVSSGGGGSGGGGSGGGGS
DIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPKAPKLLI
SGASSLKSGVPSRFSGSGSGTDFTLTISSLPPEDFATYYCQQSYSTPITF
GQGTRLEIKRAAALDNEKSNGTIIHVKGKHLCPSPFPGPSKPFVWL
20 VVGGLVACYSLLVTVAFIIFWVRSKRSRLHSDYMNMTPRRPGPTRK
HYQPYAPPRDFAAYRSRVKFSRSADAPAYQQGQNQLYNELNLGRRE
EYDVLDRRGRDPGEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIG
MKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR (SEQ ID NO.
84)

25 **[0340]** Clone 20C5.1 CD28 CD3 zeta CAR DNA Heavy & Light Chains

ATGGCACTCCCCGTAAGTCTGCTGCTGCTGCCGTTGGCATTGCTCC
TGCACGCCGCACGCCCGCAGGTGCAGCTTGTGCAGAGCGGGGCC
GAGGTGAAGAAGCCCGGGGCCAGCGTCAAAGTGTCTGTAAAGT
CAGCGGTTACACCCTCACCAGCTGAGCATGCACTGGGTACGGCA
30 GGCTCCCGGCAAAGGTCTTGAGTGGATGGGTGGATTTGATCCAGA
AGATGGAGAGACTATCTACGCCCAGAAGTTCCAGGGCCGGGTCA
CCGTAAACAGAAGACACCTCAACTGACACCGCTTACATGGAGCTGA

GTTCACTGCGGTCCGAGGACACGGCCGTGTATTATTGTGCCACCG
 AGAGCCGCGGAATCGGATGGCCTTACTTCGACTACTGGGGACAGG
 GTACACTTGTTACAGTATCATCCGGGGGTGGCGGCTCTGGTGGGG
 GCGGCTCCGGAGGGGGGTGGATCAGATATCCAAATGACTCAAAGT
 5 CCAAGTTCCCTGTCTGCCTCAGTCGGAGATAGAGTCACCATAACC
 TGCAGGGCAAGTCAGTCCATCTCCTCCTATCTGAACTGGTACCAA
 CAGAAACCTGGAAAGGCGCCTAAGCTCCTGATCTCCGGAGCCTCA
 TCTTTGAAATCCGGTGTCCCATCTCGCTTCAGTGGCTCTGGAAGCG
 GTACAGATTTTACTTTGACCATTAGCAGCCTCCCACCGGAAGACT
 10 TTGCTACATATTACTGCCAGCAGTCTTACTCAACCCCAATCACCTT
 CGGGCAAGGCACCAGACTCGAAATAAAAAGAGCAGCTGCTATCG
 AGGTTATGTACCCACCGCCGTACTTGGATAACGAAAAAAGCAATG
 GGACCATCATTCATGTGAAGGGTAAGCACCTTTGCCCTAGCCCAC
 TGTTTTCTGGCCCGAGTAAACCCTTTTGGGTACTTGTGGTCGTCGG
 15 CGGCGTGCTGGCCTGCTACTCACTCCTGGTTACCGTCGCATTATC
 ATCTTTTGGGTGAGATCCAAAAGAAGCCGCCTGCTCCATAGCGAT
 TACATGAATATGACTCCACGCCGCCCTGGCCCCACAAGGAAACAC
 TACCAGCCTTACGCACCACCTAGAGATTTTCGCTGCCTATCGGAGC
 AGGGTGAAGTTTTCCAGATCTGCAGATGCACCAGCGTATCAGCAG
 20 GGCCAGAACCAACTGTATAACGAGCTCAACCTGGGACGCAGGGA
 AGAGTATGACGTTTTTGGACAAGCGCAGAGGACGGGACCCTGAGA
 TGGGTGGCAAACCAAGACGAAAAAACCCCCAGGAGGGTCTCTAT
 AATGAGCTGCAGAAGGATAAGATGGCTGAAGCCTATTCTGAAAT
 AGGCATGAAAGGAGAGCGGAGAAGGGGAAAAGGGCACGACGGT
 25 TTGTACCAGGGACTCAGCACTGCTACGAAGGATACTTATGACGCT
 CTCCACATGCAAGCCCTGCCACCTAGGTAA (SEQ ID NO. 85)

[0341] Clone 20C5.1 CD28 CD3 zeta CAR AA Heavy & Light Chains
(Signal Peptide in Bold)

MALPVTALLLPLALLLHAARPQVQLVQSGAEVKKPGASVKV
 30 **SGYTL**TELSMHWVRQAPGKGLEWMGGFDPEDGETIYAQKFQGRVT
 VTEDTSTD TAYMELSSLRSEDTAVYYCATESRGIGWPYFDYWGGT
 LTVVSSGGGSGGGGSGGGGSDIQMTQSPSSLSASVGDRTITCRAS
 QSISSYLNWYQQKPGKAPKLLISGASSLKSGVPSRFSGSGSGTDFTLTI

5 SSLPPEDFATYYCQQSYSTPITFGQGTRLEIKRAAAIEVMYPPPPYLDNE
KSNGTIIHVKGKHLCPSPLPFGPSKPFVWLVVVGGVLACYSLLVTV
FIIFWVRSKRSRLHSDYMNMTPRRPGPTRKHYPYAPPRDFAAYRS
RVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEM
GGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLY
QGLST ATKDTYDALH MQALPPR (SEQ ID NO. 86)

[0342] Clone 20C5.1 CD28 CD3 zeta CAR DNA Heavy & Light Chains

CAGGTGCAGCTTGTGCAGAGCGGGGCCGAGGTGAAGAAGCCCGG
GGCCAGCGTCAAAGTGTCTGTAAAGGTCAGCGGTTACACCCTCAC
10 CGAGCTGAGCATGCACTGGGTACGGCAGGCTCCCGGCAAAGGTCT
TGAGTGGATGGGTGGATTTGATCCAGAAGATGGAGAGACTATCTA
CGCCCAGAAGTTCCAGGGCCGGGTACCGTAACAGAAGACACCT
CAACTGACACCGCTTACATGGAGCTGAGTTCACTGCGGTCCGAGG
ACACGGCCGTGTATTATTGTGCCACCGAGAGCCGCGGAATCGGAT
15 GGCCTTACTTCGACTACTGGGGACAGGGTACACTTGTTACAGTAT
CATCCGGGGGTGGCGGCTCTGGTGGGGGCGGCTCCGGAGGGGGT
GGATCAGATATCCAAATGACTCAAAGTCCAAGTTCCTGTCTGCC
TCAGTCGGAGATAGAGTCACCATAACCTGCAGGGCAAGTCAGTCC
ATCTCCTCCTATCTGAACTGGTACCAACAGAAACCTGGAAAGGCG
20 CCTAAGCTCCTGATCTCCGGAGCCTCATCTTTGAAATCCGGTGTCC
CATCTCGCTTCAGTGGCTCTGGAAGCGGTACAGATTTTACTTTGAC
CATTAGCAGCCTCCCACCGGAAGACTTTGCTACATATTACTGCCA
GCAGTCTTACTCAACCCCAATCACCTTCGGGCAAGGCACCAGACT
CGAAATAAAAAGAGCAGCTGCTATCGAGGTTATGTACCCACCGCC
25 GTACTTGGATAACGAAAAAAGCAATGGGACCATCATTCATGTGAA
GGGTAAGCACCTTTGCCCTAGCCCACTGTTTCCTGGCCCCGAGTAA
ACCCTTTTGGGTACTTGTGGTCGTCGGCGGCGTGCTGGCCTGCTAC
TCACTCCTGGTTACCGTCGCATTCATCATCTTTTGGGTGAGATCCA
AAAGAAGCCGCCTGCTCCATAGCGATTACATGAATATGACTCCAC
30 GCCGCCCTGGCCCCACAAGGAAACACTACCAGCCTTACGCACCAC
CTAGAGATTTTCGCTGCCTATCGGAGCAGGGTGAAGTTTTCCAGAT
CTGCAGATGCACCAGCGTATCAGCAGGGCCAGAACCAACTGTATA
ACGAGCTCAACCTGGGACGCAGGGAAGAGTATGACGTTTTTGGAC

5 AAGCGCAGAGGACGGGACCCTGAGATGGGTGGCAAACCAAGACG
AAAAAACCCCCAGGAGGGTCTCTATAATGAGCTGCAGAAGGATA
AGATGGCTGAAGCCTATTCTGAAATAGGCATGAAAGGAGAGCGG
AGAAGGGGAAAAGGGCACGACGGTTTGTACCAGGGACTCAGCAC
TGCTACGAAGGATACTTATGACGCTCTCCACATGCAAGCCCTGCC
ACCTAGG (SEQ ID NO. 87)

[0343] Clone 20C5.1 CD28 CD3 zeta CAR AA Heavy & Light Chains
QVQLVQSGAEVKKPGASVKVSCKVSGYTLTELSMHWVRQAPGKGL
EWMGGFDPEDGETIYAQKFQGRVTVTEDTSTDATYMEISSLRSED
10 AVYYCATESRGIGWPFYFDYWGGTLVTVSSGGGGSGGGGSGGGGS
DIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKLLI
SGASSLKSGVPSRFSGSGSGTDFTLTISSLPPEDFATYYCQQSYSTPITF
GQGTRLEIKRAAAIEVMYPPPYLDNEKSNGTIIHVKGKHLCPSPLPFG
PSKPFVWLVVVGGVLACYSLLVTVAFIIFWVRSKRSRLHSDYMN
15 TPRRPGPTRKHYPYAPPRDFAAYRSRVKFSRSADAPAYQQGQNQL
YNELNLGRREEYDVLDKRRGRDPENGGKPRRKNPQEGLYNELQKD
KMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHM
QALPPR (SEQ ID NO. 88)

[0344] Clone 20C5.1 CD8 CD3 zeta CAR DNA Heavy & Light Chains
20 ATGGCACTCCCCGTAAGTCTGCTGCTGCTGCCGTTGGCATTGCTCC
TGCACGCCGCACGCCCGCAGGTGCAGTTGGTGCAAAGCGGCGCA
GAAGTTAAGAAACCTGGGGCGTCAGTTAAGGTGTCTTGCAAAGTA
TCTGGCTATACCCTCACTGAGCTGTCCATGCATTGGGTAAGGCAG
GCTCCTGGAAAGGGGCTCGAATGGATGGGAGGATTTGACCCTGA
25 AGACGGAGAGACCATCTACGCCAGAAATTCCAGGGTAGAGTAA
CAGTGACTGAGGACACTAGCACTGACACAGCGTACATGGAGCTG
AGTTCTCTGAGAAGTGAGGACACAGCCGTTTACTACTGCGCTACC
GAGTCCAGAGGTATTGGCTGGCCATACTTCGACTATTGGGGTCAG
GGCACCTGGTTACAGTGAGTTCAGGAGGCGGGGGCTCTGGGGG
30 GGGCGGTTCCGGAGGGGGGGGCTCAGATATACAGATGACGCAGA
GTCCATCAAGTCTCTCAGCCAGCGTGGGAGATCGCGTGACTATTA
CTTGCCGCGCCAGCCAGAGTATTAGCTCCTATCTGAATTGGTACC

AGCAAAAGCCCCGGGAAGGCCCTAAGCTTCTGATTTCTGGCGCCT
CCTCTTTGAAGTCAGGTGTGCCAAGCAGATTTAGCGGGTCTGGAA
GTGGCACTGACTTTACACTTACTATCTCCAGCCTGCCCCCAGAGG
ATTTTGCCACATATTACTGTTCAGCAAAGCTACTCTACTCCAATCAC
5 TTTCGGCCAGGGCACAAGATTGGAGATTAAGAGGGCTGCCGCACT
TTCAAATTCATCATGTATTTTCAGCCATTTTGTGCCTGTTTTCTTC
CGGCCAAACCTACAACCACTCCCGCCCCACGCCACCTACTCCCG
CCCCTACCATTGCCTCCAGCCTCTGTCTCTTAGACCTGAGGCTTG
TAGACCTGCTGCCGGCGGAGCCGTGCACACTCGCGGTCTGGACTT
10 CGCCTGCGACATCTATATCTGGGCCCCCTCTGGCCGGCACCTGCGG
CGTTCTCCTTCTCTCACTCGTAATCACACTCTATTGCAATCACAGG
AACAGATCCAAAAGAAGCCGCCTGCTCCATAGCGATTACATGAAT
ATGACTCCACGCCGCCCTGGCCCCACAAGGAAACACTACCAGCCT
TACGCACCACCTAGAGATTTTCGCTGCCTATCGGAGCAGGGTGAAG
15 TTTTCCAGATCTGCAGATGCACCAGCGTATCAGCAGGGCCAGAAC
CAACTGTATAACGAGCTCAACCTGGGACGCAGGGAAGAGTATGA
CGTTTTGGACAAGCGCAGAGGACGGGACCCTGAGATGGGTGGCA
AACCAAGACGAAAAAACCCCCAGGAGGGTCTCTATAATGAGCTG
CAGAAGGATAAGATGGCTGAAGCCTATTCTGAAATAGGCATGAA
20 AGGAGAGCGGAGAAGGGGAAAAGGGCACGACGGTTTGTACCAGG
GACTCAGCACTGCTACGAAGGATACTTATGACGCTCTCCACATGC
AAGCCCTGCCACCTAGGTAA (SEQ ID NO. 89)

[0345] Clone 20C5.1 CD8 CD3 zeta CAR AA Heavy & Light Chains
(Signal Peptide in **Bold**)

25 **MALPVTALLPLALLHAARPQVQLVQSGAEVKKPGASVKVSCKV**
SGYTLTELSMHWVRQAPGKGLEWMGGFDPEDGETIYAQKFQGRVT
VTEDTSTDYAYMELSSLRSEDTAVYYCATESRGIGWPYFDYWGQGT
LVTVSSGGGSGGGGSGGGGSDIQMTQSPSSLSASVGDRTITCRAS
QSISSYLNWYQQKPGKAPKLLISGASSLKSGVPSRFSGSGSGTDFTLTI
30 SSLPPEDFATYYCQQSYSTPITFGQGRLEIKRAAALSNSIMYFSHFVP
VFLPAKPTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDF
ACDIYIWAPLAGTCGVLLLSLVITLYCNHRNRSKRSRLHSDYMNMT
PRRPGPTRKHYQPYAPPRDFAAYRSRVKFSRSADAPAYQQGQNQLY

NELNLGRREEYDVLDKRRGRDPGEMGGKPRRKNPQEGLYNELQKDK
MAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPP
R (SEQ ID NO. 90)

[0346] Clone 20C5.1 CD8 CD3 zeta CAR DNA Heavy & Light Chains

5 CAGGTGCAGTTGGTGCAAAGCGGCGCAGAAGTTAAGAAACCTGG
GGCGTCAGTTAAGGTGTCTTGCAAAGTATCTGGCTATACCCCTCAC
TGAGCTGTCCATGCATTGGGTAAAGGCAGGCTCCTGGAAAGGGGCT
CGAATGGATGGGAGGATTTGACCCTGAAGACGGAGAGACCATCT
ACGCCCAGAAATTCCAGGGTAGAGTAACAGTGACTGAGGACACT
10 AGCACTGACACAGCGTACATGGAGCTGAGTTCTCTGAGAAGTGAG
GACACAGCCGTTTACTACTGCGCTACCGAGTCCAGAGGTATTGGC
TGGCCATACTTCGACTATTGGGGTTCAGGGCACCCCTGGTTACAGTG
AGTTCAGGAGGCGGGGGCTCTGGGGGGGGCGGTTCCGGAGGGGG
GGGCTCAGATATACAGATGACGCAGAGTCCATCAAGTCTCTCAGC
15 CAGCGTGGGAGATCGCGTGAATTTGCTGCGCGCCAGCCAGAG
TATTAGCTCCTATCTGAATTGGTACCAGCAAAAGCCCGGGAAGGC
CCCTAAGCTTCTGATTTCTGGCGCCTCCTCTTTGAAGTCAGGTGTG
CCAAGCAGATTTAGCGGGTCTGGAAGTGGCACTGACTTTACACTT
ACTATCTCCAGCCTGCCCCCAGAGGATTTTGCCACATATTACTGTC
20 AGCAAAGCTACTCTACTCCAATCACTTTCGGCCAGGGCACAAGAT
TGGAGATTAAGAGGGCTGCCGCACTTTCAAATTCCATCATGTATT
TCAGCCATTTTGTGCTGTTTTTTCTTCCGGCCAAACCTACAACCAC
TCCCGCCCCACGCCACCTACTCCCGCCCCTACCATTGCCTCCCAG
CCTCTGTCTCTTAGACCTGAGGCTTGTAGACCTGCTGCCGGCGGA
25 GCCGTGCACACTCGCGGTCTGGACTTCGCTGCGACATCTATATCT
GGGCCCTCTGGCCGGCACCTGCGGCGTTCTCCTTCTCTCACTCGT
AATCACACTCTATTGCAATCACAGGAACAGATCCAAAAGAAGCC
GCCTGCTCCATAGCGATTACATGAATATGACTCCACGCCGCCCTG
GCCCCACAAGGAAACACTACCAGCCTTACGCACCACCTAGAGATT
30 TCGCTGCCTATCGGAGCAGGGTGAAGTTTTCCAGATCTGCAGATG
CACCAGCGTATCAGCAGGGCCAGAACCAACTGTATAACGAGCTC
AACCTGGGACGCAGGGAAGAGTATGACGTTTTGGACAAGCGCAG
AGGACGGGACCCTGAGATGGGTGGCAAACCAAGACGAAAAAACC

CCCAGGAGGGTCTCTATAATGAGCTGCAGAAGGATAAGATGGCT
 GAAGCCTATTCTGAAATAGGCATGAAAGGAGAGCGGAGAAGGGG
 AAAAGGGCACGACGGTTTGTACCAGGGACTCAGCACTGCTACGA
 AGGATACTTATGACGCTCTCCACATGCAAGCCCTGCCACCTAGG
 5 (SEQ ID NO. 91)

[0347] Clone 20C5.1 CD8 CD3 zeta CAR AA Heavy & Light Chains

QVQLVQSGAEVKKPGASVKVSKVSGYTLTELSMHWVRQAPGKGL
 EWMGGFDPEDGETIYAQKFQGRVTVTEDTSTDtAYMELSSLRSEDt
 AVYYCATESRGIGWPYFDYWGQGTLVTVSSGGGGSGGGGSGGGGS
 10 DIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKLLI
 SGASSLKSGVPSRFSGSGSGTDFTLTISSLPPEDFATYYCQQSYSTPITF
 GQGTRLEIKRAAALSNSIMYFSHFVPVFLPAKPTTTPAPRPPTPAPTIA
 SQPLSLRPEACRPAAGGAVHTRGLDFACDIYIWAPLAGTCGVLLLSL
 VITLYCNHRNRSKRSRLHSDYMNMTPRRPGPTRKHYPYAPPRDF
 15 AAYRSRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRG
 RDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRRGKG
 HDGLYQGLSTATKDTYDALHMQALPPR (SEQ ID NO. 92)

[0348] Clone 20C5.2 HC DNA

CAGGTCCAGTTGGTCGAAAGTGGCGGTGGTGTAGTGCAGCCGGGC
 20 CGCAGTTTGAGGCTTTCTGTGCGGCTTCAGGCTTTACTTTTTCCA
 GCTATGGAATGCACTGGGTGCGGCAGGCCCCCGGCAAAGGACTT
 GAGTGGGTGGCCGTCATTTCTTATGACGGATCAGATAAGTACTAC
 GTGGACAGCGTCAAGGGCAGATTCACCATCTCTAGGGACAACAGT
 AAAAATAGACTCTACCTCCAGATGAATAGCCTCAGAGCTGAAGAC
 25 ACGGCCGTCTACTATTGTGCTCGGGAGCGGTATAGTGGCAGAGAC
 TACTGGGGGCAGGGCACACTCGTTACAGTGAGTAGC (SEQ ID NO.
 93)

[0349] Clone 20C5.2 AA HC (CDRs in Underline)

QVQLVESGGGVVQPGRSLRLSCAASGFTFSSYGMHWVRQAPGKGLE
 30 WVAVISYDGSDKYYVDSVKGRFTISRDN SKNRLYLQMNSLRAEDTA
 VYYCARERYSGRDYWGQGTLVTVSS (SEQ ID NO. 94)

[0350] Clone 20C5.2 HC AA CDR1: GFTFSSY (SEQ ID NO. 95)

[0351] Clone 20C5.2 HC AA CDR2: SYDGSD (SEQ ID NO. 96)

[0352] Clone 20C5.2 HC AA CDR3: ERYSGRDY (SEQ ID NO. 97)

[0353] Clone 20C5.2 LC DNA

5 GAGATTGTTATGACCCAGAGTCCTGCGACCCTCTCAGTCAGCCCC
GGGGAGCGCGCAACTTTGTCTTGCAGAGCTAGTCAGTCCGTGTCC
TCTCTTCTGACATGGTACCAGCAAAAGCCCCGGGCAGGCTCCGCGC
CTTTTGATCTTTGGGGCTTCAACAAGAGCCACTGGGATTCCCGCA
CGATTCTCTGGCTCCGGGAGCGGTACTGGTTTCACCTGACGATT
10 AGCAGTCTCCAGAGCGAGGACTTCGCCGTATACTACTGCCAGCAG
TACGATACGTGGCCATTCACTTTTGGACCAGGGACTAAAGTGGAT
TTTAAGCGC (SEQ ID NO. 98)

[0354] Clone 20C5.2 AA LC (CDRs in Underline)

15 EIVMTQSPATLSVSPGERATLSCRASQSVSSLLTWYQQKPGQAPRLLI
FGASTRATGIPARFSGSGSGTGFSLTISSLSQSEDFAVYYCQQYDTWPF
TFGPGTKVDFKR (SEQ ID NO. 99)

[0355] Clone 20C5.2 AA LC CDR1: RASQSVSSLLT (SEQ ID NO. 100)

[0356] Clone 20C5.2 AA LC CDR2: GASTRAT (SEQ ID NO. 101)

[0357] Clone 20C5.2 AA LC CDR3: QQYDTWPFT (SEQ ID NO. 102)

20 [0358] Clone 20C5.2 CD28T CD3 zeta CAR DNA Heavy & Light Chains

ATGGCACTCCCCGTAAGTCTGCTGCTGCCGTTGGCATTGCTCC
TGACGCGCCGACGCGCCGAGGTCCAGTTGGTCGAAAGTGGCGGTG
GTGTAGTGCAGCCGGGCCGAGTTTGAGGCTTTCCTGTGCGGCTT
CAGGCTTTACTTTTTCCAGCTATGGAATGCACTGGGTGCGGCAGG
25 CCCCCGGCAAAGGACTTGAGTGGGTGGCCGTCATTTCTTATGACG
GATCAGATAAGTACTACGTGGACAGCGTCAAGGGCAGATTCACC
ATCTCTAGGGACAACAGTAAAAATAGACTCTACCTCCAGATGAAT
AGCCTCAGAGCTGAAGACACGGCCGTCTACTATTGTGCTCGGGAG
CGGTATAGTGGCAGAGACTACTGGGGGCAGGGCACACTCGTTAC

AGTGAGTAGCGGCGGAGGAGGGAGTGGGGGCGGTGGCTCCGGTG
 GAGGAGGTTCTGAGATTGTTATGACCCAGAGTCCTGCGACCCTCT
 CAGTCAGCCCCGGGGAGCGCGCAACTTTGTCTTGCAGAGCTAGTC
 AGTCCGTGTCCTCTCTTCTGACATGGTACCAGCAAAAGCCCCGGC
 5 AGGCTCCGCGCCTTTTGATCTTTGGGGCTTCAACAAGAGCCACTG
 GGATTCCCGCACGATTCTCTGGCTCCGGGAGCGGTACTGGTTTCA
 CCCTGACGATTAGCAGTCTCCAGAGCGAGGACTTCGCCGTATACT
 ACTGCCAGCAGTACGATACGTGGCCATTCACTTTTGACCAGGGA
 CTAAAGTGGATTTTAAGCGCGCCGCGCTCTCGATAACGAAAAGT
 10 CAAATGGCACCATAATCCACGTCAAAGGCAAGCACCTGTGCCCTT
 CCCCCTCTTCCCCGGACCCAGTAAACCATTTTGGGTGCTGGTTGT
 TGTGGGGGGCGTGCTGGCCTGCTATAGCCTTTTGGTCACTGTAGC
 CTTCAATTATTTTTTGGGTCAGATCCAAAAGAAGCCGCCTGCTCCAT
 AGCGATTACATGAATATGACTCCACGCCGCCCTGGCCCCACAAGG
 15 AAACACTACCAGCCTTACGCACCACCTAGAGATTTTCGCTGCCTAT
 CGGAGCAGGGTGAAGTTTTCCAGATCTGCAGATGCACCAGCGTAT
 CAGCAGGGCCAGAACCAACTGTATAACGAGCTCAACCTGGGACG
 CAGGGAAGAGTATGACGTTTTGGACAAGCGCAGAGGACGGGACC
 CTGAGATGGGTGGCAAACCAAGACGAAAAAACCCCCAGGAGGGT
 20 CTCTATAATGAGCTGCAGAAGGATAAGATGGCTGAAGCCTATTCT
 GAAATAGGCATGAAAGGAGAGCGGAGAAGGGGAAAAGGGCACG
 ACGGTTTGTACCAGGGACTCAGCACTGCTACGAAGGATACTTATG
 ACGCTCTCCACATGCAAGCCCTGCCACCTAGGTAA (SEQ ID NO.
 103)

25 **[0359]** Clone 20C5.2 CD28T CD3 zeta CAR AA Heavy & Light Chains
(Signal Peptide in **Bold**)
MALPVTALLPLALLHAARPQVQLVESGGGVVQPGRSLRLSCAA
 SGFTFSSYGMHWVRQAPGKGLEWVAVISYDGS DKYYVDSVKGRFTI
 SRDNSKNRLYLQMNSLRAEDTAVYYCARERYSGRDYWGQGLVTV
 30 SSGGGSGGGSGGGGSEIVMTQSPATLSVSPGERATLSCRASQSVSS
 LLTWYQQKPGQAPRLIFGASTRATGIPARFSGSGSGTGFTLTISLQS
 EDFAVYYCQQYDTWPFTEGPGTKVDFKRAAALDNEKSNGTIIHVKG
 KHLCPSPLEPGPSKPFWVLVVGGVLACYSLLVTVAFIIFWVRSKRSR

LLHSDYMNMTPRRPGPTRKHYQPYAPPRDFAAYRSRVKFSRSADAP
AYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQE
GLYNELQKDKMAEAYSEIGMKGERRRRGKGHDGLYQGLSTATK
DTYDALHMQALPPR (SEQ ID NO. 104)

5 **[0360]** Clone 20C5.2 CD28T CD3 zeta CAR DNA Heavy & Light Chains
CAGGTCCAGTTGGTCGAAAGTGGCGGTGGTGTAGTGCAGCCGGGC
CGCAGTTTGAGGCTTTCCTGTGCGGCTTCAGGCTTTACTTTTTCCA
GCTATGGAATGCACTGGGTGCGGCAGGCCCCCGGCAAAGGACTT
GAGTGGGTGGCCGTCATTTCTTATGACGGATCAGATAAGTACTAC
10 GTGGACAGCGTCAAGGGCAGATTCACCATCTCTAGGGACAACAGT
AAAAATAGACTCTACCTCCAGATGAATAGCCTCAGAGCTGAAGAC
ACGGCCGTCTACTATTGTGCTCGGGAGCGGTATAGTGGCAGAGAC
TACTGGGGGCAGGGCACACTCGTTACAGTGAGTAGCGGCGGAGG
AGGGAGTGGGGGCGGTGGCTCCGGTGGAGGAGGTTCTGAGATTG
15 TTATGACCCAGAGTCCTGCGACCCTCTCAGTCAGCCCCGGGGAGC
GCGCAACTTTGTCTTGCAGAGCTAGTCAGTCCGTGTCCTCTCTTCT
GACATGGTACCAGCAAAAGCCCGGGCAGGCTCCGCGCCTTTTGAT
CTTTGGGGCTTCAACAAGAGCCACTGGGATTCCCGCACGATTCTC
TGGCTCCGGGAGCGGTACTGGTTTCACCCTGACGATTAGCAGTCT
20 CCAGAGCGAGGACTTCGCCGTATACTACTGCCAGCAGTACGATAC
GTGGCCATTCACTTTTGGACCAGGGACTAAAGTGGATTTTAAGCG
CGCCGCCGCTCTCGATAACGAAAAGTCAAATGGCACCATAATCCA
CGTCAAAGGCAAGCACCTGTGCCCTTCCCCGCTCTTCCCCGGACC
CAGTAAACCATTTTGGGTGCTGGTTGTTGTGGGGGGCGTGCTGGC
25 CTGCTATAGCCTTTTGGTCACTGTAGCCTTCATTATTTTTTGGGTC
AGATCCAAAAGAAGCCGCTGCTCCATAGCGATTACATGAATATG
ACTCCACGCCGCCCTGGCCCCACAAGGAAACACTACCAGCCTTAC
GCACCACCTAGAGATTTTCGCTGCCTATCGGAGCAGGGTGAAGTTT
TCCAGATCTGCAGATGCACCAGCGTATCAGCAGGGCCAGAACCA
30 ACTGTATAACGAGCTCAACCTGGGACGCAGGGAAGAGTATGACG
TTTTGGACAAGCGCAGAGGACGGGACCCTGAGATGGGTGGCAAA
CCAAGACGAAAAAACCCCCAGGAGGGTCTCTATAATGAGCTGCA
GAAGGATAAGATGGCTGAAGCCTATTCTGAAATAGGCATGAAAG

GAGAGCGGAGAAGGGGAAAAGGGCACGACGGTTTGTACCAGGGA
 CTCAGCACTGCTACGAAGGATACTTATGACGCTCTCCACATGCAA
 GCCCTGCCACCTAGG (SEQ ID NO. 105)

[0361] Clone 20C5.2 CD28T CD3 zeta CAR AA Heavy & Light Chains

5 QVQLVESGGGVVQPGRSLRLSCAASGFTFSSYGMHWVRQAPGKGLE
 WVAVISYDGS DKYYVDSVKGRFTISRDN SKNRLYLQMNSLRAEDTA
 VYYCARERYSGRDYWGQGLVTVSSGGGGSGGGGSGGGGSEIVMT
 QSPATLSVSPGERATLSCRASQSVSSLLTWYQQKPGQAPRLLIFGAST
 RATGIPARFSGSGSGTGTFTLTISSLQSEDFAVYYCQQYDTWPFTFGPG
 10 TKVDFKRAAALDNEKSNGTIIHVKGKHLCPSP LFPGPSKPFWVLVVV
 GGV LACYSLLVTVAFIIFWVR SKRSRL LHSDYMNMTPRRPGPTRKHY
 QPYAPPRDFAAYRSRVKFSRSADAPAYQQGQNQLYNELNLGRREEY
 DVL DKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMK
 GERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR (SEQ ID NO.
 15 106)

[0362] Clone 20C5.2 CD28 CD3 zeta CAR DNA Heavy & Light Chains

ATGGCACTCCCCGTA ACTGCTCTGCTGCTGCCGTTGGCATTGCTCC
 TGCACGCCGCACGCCGCAGGTGCAGCTCGTGGAGTCTGGCGGCGC
 GCGTGGTCCAGCCCGGCCG GTCCCTGCGCCTGTCTGCGCCGCCA
 20 GCGGGTTTACTTTTTCTCCTACGGCATGCACTGGGTGCGCCAGGC
 TCCCGGCAAGGGCCTCGAGTGGGTGCGCGTGATCTCATACGATGG
 GTCAGACAAATACTATGTCTGATTCTGTAAAGGGCGGTTTACCAT
 TTCAAGAGATAACTCTAAGAATAGGCTGTATTTGCAGATGAACAG
 CCTGAGGGCTGAAGATAACCGCAGTGTACTATTGCGCTAGGGAGCG
 25 GTATAGTGGCCGCGATTACTGGGGACAGGGTACACTGGTGACCGT
 GAGCTCTGGGGGTGGCGGAAGCGGGGGTGGCGGAAGCGGCGGAG
 GGGGTAGTGAAATTGTGATGACCCAGTCTCCGGCTACACTTTCAG
 TCTCCCTGGGGAGAGAGCTACACTGT CATGCAGAGCGTCCAGT
 CCGTCTCTTCTCTCCTTACCTGGTATCAGCAGAAGCCCGGCCAGGC
 30 TCCTCGACTGCTGATCTTCGGTGCCTCCACAAGGGCGACCGGGAT
 TCCAGCCCGCTTCTCAGGTTCTGGGAGCGGA ACTGGTTTCACTTTG
 ACAATCAGTTC ACTGCAGTCAGAGGATTTGCGCGTGTACTACTGC

5 CAGCAATACGACACATGGCCATTCACTTTTCGGACCCGGTACCAAA
GTCGATTTCAAGAGAGCCGCGGCCATCGAGGTTATGTACCCACCA
CCATATCTGGACAATGAAAAAAGCAATGGAACCATTATCCATGTG
AAGGGTAAACACCTCTGCCCTAGCCCACTTTTCCCTGGCCCATCA
AAGCCCTTCTGGGTCTTGGTGGTCGTGGGGGGTGTGCTGGCCTGT
TACAGCCTTCTGGTGACGGTTGCTTTCATTATCTTCTGGGTAGAT
CCAAAAGAAGCCGCCTGCTCCATAGCGATTACATGAATATGACTC
CACGCCGCCCTGGCCCCACAAGGAAACACTACCAGCCTTACGCAC
CACCTAGAGATTTCTGCTGCCTATCGGAGCAGGGTGAAGTTTTCCA
10 GATCTGCAGATGCACCAGCGTATCAGCAGGGCCAGAACCAACTGT
ATAACGAGCTCAACCTGGGACGCAGGGAAGAGTATGACGTTTTG
GACAAGCGCAGAGGACGGGACCCTGAGATGGGTGGCAAACCAAG
ACGAAAAAACCCCCAGGAGGGTCTCTATAATGAGCTGCAGAAGG
ATAAGATGGCTGAAGCCTATTCTGAAATAGGCATGAAAGGAGAG
15 CGGAGAAGGGGAAAAGGGCACGACGTTTGTACCAGGGACTCAG
CACTGCTACGAAGGATACTTATGACGCTCTCCACATGCAAGCCCT
GCCACCTAGGTAA (SEQ ID NO. 107)

[0363] Clone 20C5.2 CD28 CD3 zeta CAR AA Heavy & Light Chains
(Signal Peptide in **Bold**)

20 **MALPVTALLLPLALLLHAARPQVQLVESGGGVVQPGRSLRLSCAA**
SGFTFSSYGMHWVRQAPGKGLEWVAVISYDGSDKYYVDSVKGRFTI
SRDNSKNRLYLQMNSLRAEDTAVYYCARERYSGRDYWGQGTLVTV
SSGGGSGGGGSGGGGSEIVMTQSPATLSVSPGERATLSCRASQSVSS
LLTWYQQKPGQAPRLIFGASTRATGIPAREFSGSGSGTGFTLTISLQS
25 EDFAVYYCQQYDTWPFTFGPGTKVDFKRAAAIEVMYPPPYLDNEKS
NGTIIHVKGKHLCPSPLEPGPSKPFWVLVVVGGVLACYSLLVTVAFH
FWVRSKRSRLHSDYMNMTPRRPGPTRKHYQPYAPPRDFAAYRSRV
KFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGG
KPRRKNPQEGLYNELQDKMAEAYSEIGMKGERRRGKGHDGLYQG
30 LSTATKDTYDALHMQALPPR (SEQ ID NO. 108)

[0364] Clone 20C5.2 CD28 CD3 zeta CAR DNA Heavy & Light Chains

CAGGTGCAGCTCGTGGAGTCTGGCGGCGGCGTGGTCCAGCCCGGC
CGGTCCCTGCGCCTGTCCTGCGCCGCCAGCGGGTTTACTTTTTCT
CCTACGGCATGCACTGGGTGCGCCAGGCTCCCGGCAAGGGCCTCG
5 AGTGGGTCGCCGTGATCTCATACGATGGGTGAGACAAATACTATG
TCGATTCTGTTAAAGGGCGGTTTACCATTTCAGAGATAACTCTA
AGAATAGGCTGTATTTGCAGATGAACAGCCTGAGGGCTGAAGAT
ACCGCAGTGTACTATTGCGCTAGGGAGCGGTATAGTGGCCGCGAT
TACTGGGGACAGGGTACACTGGTGACCGTGAGCTCTGGGGGTGGC
10 GGAAGCGGGGGTGGCGGAAGCGGCGGAGGGGGTAGTGAAATTGT
GATGACCCAGTCTCCGGCTACACTTTCAGTCTCCCCTGGGGAGAG
AGCTACACTGTCATGCAGAGCGTCCCAGTCCGTCTCTTCTCTCCTT
ACCTGGTATCAGCAGAAGCCCGGCCAGGCTCCTCGACTGCTGATC
TTCGGTGCCTCCACAAGGGCGACCGGGATTCCAGCCCGCTTCTCA
15 GGTTCTGGGAGCGGAACCTGGTTTCACTTTGACAATCAGTTCCTG
CAGTCAGAGGATTTTCGCCGTGTACTACTGCCAGCAATACGACACA
TGGCCATTCACTTTCGGACCCGGTACCAAAGTCGATTTCAAGAGA
GCCGCGGCCATCGAGGTTATGTACCCACCACCATATCTGGACAAT
GAAAAAAGCAATGGAACCATTATCCATGTGAAGGGTAAACACCT
20 CTGCCCTAGCCCACTTTTCCCTGGCCCATCAAAGCCCTTCTGGGTG
TTGGTGGTTCGTGGGGGGTGTGCTGGCCTGTTACAGCCTTCTGGTG
ACGGTTGCTTTTCATTATCTTCTGGGTAGATCCAAAAGAAGCCGC
CTGCTCCATAGCGATTACATGAATATGACTCCACGCCGCCCTGGC
CCCACAAGGAAACACTACCAGCCTTACGCACCACCTAGAGATTTC
25 GCTGCCTATCGGAGCAGGGTGAAGTTTTCCAGATCTGCAGATGCA
CCAGCGTATCAGCAGGGCCAGAACCAACTGTATAACGAGCTCAA
CCTGGGACGCAGGGAAGAGTATGACGTTTTGGACAAGCGCAGAG
GACGGGACCCTGAGATGGGTGGCAAACCAAGACGAAAAAACCCC
CAGGAGGGTCTCTATAATGAGCTGCAGAAGGATAAGATGGCTGA
30 AGCCTATTCTGAAATAGGCATGAAAGGAGAGCGGAGAAGGGGAA
AAGGGCACGACGGTTTGTACCAGGGACTCAGCACTGCTACGAAG
GATACTTATGACGCTCTCCACATGCAAGCCCTGCCACCTAGG
(SEQ ID NO. 109)

[0365] Clone 20C5.2 CD28 CD3 zeta CAR AA Heavy & Light Chains

QVQLVESGGGVVQPGRSLRLSCAASGFTFSSYGMHWVRQAPGKGLE
WVAVISYDGS DKYYVDSVKGRFTISRDN SKNRLYLQMNSLRAEDTA
VYYCARERYSGRDYWGQGLVTVSSGGGGSGGGGSGGGGSEIVMT
5 QSPATLSVSPGERATLSCRASQSVSSLLTWYQKPGQAPRLLIFGAST
RATGIPARFSGSGSGTGTFTLTISSLQSEDFAVYYCQQYDTPFTFGPG
TKVDFKRAAAIEVMYPPPYLDNEKSNGTIIHVKGKHLCPSPFLPGPSK
PFWVLVVVGVLACYSLLVTVAFIIFWVRSKRSRLLHSDYMNMTPR
RPGPTRKHYQPYAPPRDFAAYRSRVKFSRSADAPAYQQGQNQLYNE
10 LNLGRREEYDVLDKRRGRDPGEMGGKPRRKNPQEGLYNELQKDKMA
EAYSEIGMKGERRRRGKGHDGLYQGLSTATKDTYDALHMQALPPR
(SEQ ID NO. 110)

[0366] Clone 20C5.2 CD8 CD3 zeta CAR DNA Heavy & Light Chains

ATGGCACTCCCCGTAAGTCTGCTGCTGCTGCCGTTGGCATTGCTCC
15 TGCACGCCGCACGCCCGCAGGTGCAGTTGGTTGAATCAGGAGGG
GGTGTGGTGCAACCCGGTCGGTCACTGCGCCTCAGTTGTGCTGCT
TCCGGGTTTACTTTCAGCTCATATGGGATGCACTGGGTACGGCAG
GCTCCAGGTAAAGGCTTGGAATGGGTGGCGGTGATCAGCTATGAC
GGCTCTGACAAATATTATGTGGACTCCGTGAAAGGCAGATTCACC
20 ATCAGTCGAGACAACTCAAAGAATAGACTCTACTTGAGATGAAT
AGCCTCCGGGCCGAAGATACTGCAGTCTATTATTGCGCCCGGGAG
CGCTACAGTGGAAGAGACTATTGGGGGCAAGGAACTCTTGTCACA
GTCTCATCTGGCGGCGGCGGCAGCGGTGGGGGCGGATCTGGCGG
GGGCGGCAGCGAAATCGTTATGACTCAGAGTCCTGCCACACTGAG
25 CGTTAGCCCTGGTGAGAGAGCAACACTTAGCTGCAGAGCTAGTCA
GAGTGTTTCCAGTCTTTTGACATGGTACCAACAGAAGCCCGGTCA
AGCTCCACGACTGCTCATCTTCGGTGCATCCACCCGCGCAACCGG
GATACCCGCCCGGTTTTCCGGTCTTGGAAGTGGCACAGGATTAC
GCTCACCATTCTTCTCTGCAGTCTGAAGACTTTGCCGTGTATTAC
30 TGCCAGCAGTACGATACCTGGCCCTTTACCTTTGGCCCAGGTACT
AAAGTGGATTTTAAACGAGCTGCTGCACTTTCCAATAGTATTATG
TACTTTTACATTTTGTGCCCGTGTTCCTGCCTGCGAAGCCTACGA
CAACCCAGCCCCTAGGCCGCCACACCGGCCCAACTATTGCCT

CCCAGCCATTGTCTCTGAGACCCGAAGCTTGCAGACCTGCTGCTG
 GAGGCGCCGTTACACCCGAGGATTGGATTTCGCATGTGACATTT
 ACATCTGGGCCCCCTTTGGCCGGAACCTGCGGTGTGCTGCTGCTGT
 CACTCGTGATTACACTTTACTGCAACCACCGAAACAGATCCAAAA
 5 GAAGCCGCCTGCTCCATAGCGATTACATGAATATGACTCCACGCC
 GCCCTGGCCCCACAAGGAAACACTACCAGCCTTACGCACCACCTA
 GAGATTTCGCTGCCTATCGGAGCAGGGTGAAGTTTTCCAGATCTG
 CAGATGCACCAGCGTATCAGCAGGGCCAGAACCAACTGTATAAC
 GAGCTCAACCTGGGACGCAGGGAAGAGTATGACGTTTTGGACAA
 10 GCGCAGAGGACGGGACCCTGAGATGGGTGGCAAACCAAGACGAA
 AAAACCCCCAGGAGGGTCTCTATAATGAGCTGCAGAAGGATAAG
 ATGGCTGAAGCCTATTCTGAAATAGGCATGAAAGGAGAGCGGAG
 AAGGGGAAAAGGGCACGACGTTTGTACCAGGGACTCAGCACTG
 CTACGAAGGATACTTATGACGCTCTCCACATGCAAGCCCTGCCAC
 15 CTAGGTAA (SEQ ID NO. 111)

[0367] Clone 20C5.2 CD8 CD3 zeta CAR AA Heavy & Light Chains

(Signal peptide in **Bold**)

MALPVTALLLPLALLHAARPQVQLVESGGGVVQPRSLRLSCAA
 20 SGFTFSSYGMHWVRQAPGKGLEWVAVISYDGSCKYYVDSVKGRFTI
 SRDNSKNRLYLQMNSLRAEDTAVYYCARERYSGRDYWGGLVTVS
 SGGGSGGGSGGGGSEIVMTQSPATLSVSPGERATLSCRASQSVSS
 LLTWYQQKPGQAPRLIFGASTRATGIPARFSGSGSGTGFTLTISLQS
 EDFAVYYCQQYDTWPFTFGPGTKVDFKRAAALNSIMYFSHFVPVFL
 25 PAKPTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFAC
 DIYIWAPLAGTCGVLLLSLVITLYCNHRNRSKRSRLHSDYMNMTPR
 RPGPTRKHYQPYAPPRDFAAYRSRVKFSRSADAPAYQQGQNQLYNE
 LNLGRREEYDVLDKRRGRDPGEMGGKPRRKNPQEGLYNELQKDKMA
 EAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR
 30 (SEQ ID NO. 112)

[0368] Clone 20C5.2 CD8 CD3 zeta CAR DNA Heavy & Light Chains

CAGGTGCAGTTGGTTGAATCAGGAGGGGGTGTGGTGCAACCCGGT
CGGTCACTGCGCCTCAGTTGTGCTGCTTCCGGGTTTACTTTCAGCT
CATATGGGATGCACTGGGTACGGCAGGCTCCAGGTAAAGGCTTGG
AATGGGTGGCGGTGATCAGCTATGACGGCTCTGACAAATATTATG
5 TGGACTCCGTGAAAGGCAGATTCACCATCAGTCGAGACAACTCAA
AGAATAGACTCTACTTGCAGATGAATAGCCTCCGGGCCGAAGATA
CTGCAGTCTATTATTGCGCCCGGGAGCGCTACAGTGGAAGAGACT
ATTGGGGGCAAGGAACTCTTGTACAGTCTCATCTGGCGGCGGCG
GCAGCGGTGGGGGCGGATCTGGCGGGGGCGGCAGCGAAATCGTT
10 ATGACTCAGAGTCCTGCCACACTGAGCGTTAGCCCTGGTGAGAGA
GCAACACTTAGCTGCAGAGCTAGTCAGAGTGTTTCCAGTCTTTTG
ACATGGTACCAACAGAAGCCCGGTCAAGCTCCACGACTGCTCATC
TTCGGTGCATCCACCCGCGCAACCGGGATACCCGCCCGGTTTTCC
GGTTCTGGAAGTGGCACAGGATTCACGCTCACCATTTCTTCTCTGC
15 AGTCTGAAGACTTTGCCGTGTATTACTGCCAGCAGTACGATACCT
GGCCCTTTACCTTTGGCCCAGGTACTAAAGTGGATTTTAAACGAG
CTGCTGCACTTTCCAATAGTATTATGTACTTTTACATTTTGTGCC
CGTGTTCCTGCCTGCGAAGCCTACGACAACCCAGCCCTAGGCC
GCCCACACCGGCCCACTATTGCCTCCCAGCCATTGTCTCTGAG
20 ACCCGAAGCTTGCAGACCTGCTGCTGGAGGCGCCGTTTACACCCG
AGGATTGGATTTTCGCATGTGACATTTACATCTGGGCCCCCTTTGGCC
GGAACCTGCGGTGTGCTGCTGCTGTCACTCGTGATTACACTTTACT
GCAACCACCGAAACAGATCCAAAAGAAGCCGCCTGCTCCATAGC
GATTACATGAATATGACTCCACGCCGCCCTGGCCCCACAAGGAAA
25 CACTACCAGCCTTACGCACCACCTAGAGATTTTCGCTGCCTATCGG
AGCAGGGTGAAGTTTTCCAGATCTGCAGATGCACCAGCGTATCAG
CAGGGCCAGAACCAACTGTATAACGAGCTCAACCTGGGACGCAG
GGAAGAGTATGACGTTTTGGACAAGCGCAGAGGACGGGACCCTG
AGATGGGTGGCAAACCAAGACGAAAAAACCCCAAGGAGGTCTC
30 TATAATGAGCTGCAGAAGGATAAGATGGCTGAAGCCTATTCTGAA
ATAGGCATGAAAGGAGAGCGGAGAAGGGGAAAAGGGCACGACG
GTTTGTACCAGGGACTCAGCACTGCTACGAAGGATACTTATGACG
CTCTCCACATGCAAGCCCTGCCACCTAGG (SEQ ID NO. 113)

[0369] Clone 20C5.2 CD8 CD3 zeta CAR AA Heavy & Light Chains

QVQLVESGGGVVQPGRSLRLSCAASGFTFSSYGMHWVRQAPGKGLE
WVAVISYDGS DKYYVDSVKGRFTISRDN SKNRLYLQMNSLRAEDTA
VYYCARERYSGRDYWGQGLVTVSSGGGGSGGGGSGGGGSEIVMT
5 QSPATLSVSPGERATLSCRASQSVSSLLTWYQKPGQAPRLLIFGAST
RATGIPARFSGSGSGTGTFTLTISSLQSEDFAVYYCQQYDTPFTFGPG
TKVDFKRAAALSNSIMYFSHFVPVFLPAKPTTTPAPRPPTPAPTIASQP
LSLRPEACRPAAGGAVHTRGLDFACDIYWAPLAGTCGVLLLSLVITL
YCNHRNRSKRSRLHSDYMNMTPRRPGPTRKHYPYAPPRDFAAYR
10 SRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPE
MGGKPRRKNPQEGLYNELQDKMAEAYSEIGMKGERRRGKGHDGL
YQGLSTATKDTYDALHMQALPPR (SEQ ID NO. 114)

[0370] Clone 20C5.2 CD28T CD3 zeta CAR DNA Heavy & Light Chains

ATGGCACTCCCCGTAAC TGTCTGCTGCTGCCGTTGGCATTGCTCC
15 TGCACGCCGCACGCCCGGAGATTGTGATGACCCAGTCCCCTGCTA
CCCTGTCCGTCAGTCCGGGCGAGAGAGCCACCTTGTCATGCCGGG
CCAGCCAGTCCGTCAGCAGTCTCCTGACTTGGTATCAGCAAAAC
CAGGGCAGGCACCGCGGCTTTTGATTTTTGGTGCAAGCACACGCG
CCACTGGCATTCCAGCTAGGTTTTCTGGAAGTGGATCTGGGACAG
20 GCTTCACTCTGACAATCAGTAGCCTGCAGAGTGAGGACTTTGCTG
TTTACTACTGTCAACAGTACGACACCTGGCCATTACATTCGGGC
CCGGCACCAAGGTCGACTTCAAGAGGGGCGGTGGAGGTTCAAGT
GGTGGCGGGTCAGGCGGCGGTGGGTCTCAGGTTCAACTGGTGGA
ATCAGGTGGCGGCGTTGTCCAACCGGGGCGATCACTTCGACTTTC
25 CTGTGCTGCCTCAGGCTTTACTTTTTCATCCTATGGGATGCACTGG
GTTCCGGCAGGCTCCCGGAAAAGGACTCGAGTGGGTTCAGTGATC
TCTTACGATGGCTCAGACAAGTATTATGTGGACTCAGTCAAGGGG
AGATTCACAATAAGCCGAGACAACCTCCAAAACCGGCTTTATCTC
CAGATGAACAGCCTTAGAGCGGAAGATAACCGCGGTATACTACTGT
30 GCCCGCGAGAGGTATTCGGGCAGAGACTACTGGGGACAGGGCAC
ACTGGTCACCGTGAGTTCTGCCGCAGCGCTCGATAACGAAAAGAG
CAACGGAACCATTATCCACGTTAAGGGCAAGCACCTGTGCCCCAG
TCCCCTCTTCCCAGGACCATCTAAACCCTTCTGGGTCTGGTAGTA

GTTGGAGGGGTCCTTGCATGTTACTCCCTTTTGGTCACCGTCGCCT
 TCATTATTTTCTGGGTGAGATCCAAAAGAAGCCGCCTGCTCCATA
 GCGATTACATGAATATGACTCCACGCCGCCCTGGCCCCACAAGGA
 AACACTACCAGCCTTACGCACCCACCTAGAGATTTTCGCTGCCTATC
 5 GGAGCAGGGTGAAGTTTTCCAGATCTGCAGATGCACCAGCGTATC
 AGCAGGGCCAGAACCAACTGTATAACGAGCTCAACCTGGGACGC
 AGGGAAGAGTATGACGTTTTGGACAAGCGCAGAGGACGGGACCC
 TGAGATGGGTGGCAAACCAAGACGAAAAAACCCCCAGGAGGGTC
 TCTATAATGAGCTGCAGAAGGATAAGATGGCTGAAGCCTATTCTG
 10 AAATAGGCATGAAAGGAGAGCGGAGAAGGGGAAAAGGGCACGA
 CGGTTTGTACCAGGGACTCAGCACTGCTACGAAGGATACTTATGA
 CGCTCTCCACATGCAAGCCCTGCCACCTAGGTAA (SEQ ID NO. 115)

[0371] Clone 20C5.2 CD28T CD3 zeta CAR AA Heavy & Light Chains

(Signal Peptide in **Bold**)

15 **MALPVTALLLPLALLLHAARPEIVMTQSPATLSVSPGERATLSCRA**
 SQSVSSLLTWYQQKPGQAPRLLIFGASTRATGIPARFSGSGSGTGFTL
 TISSLQSEDFAVYYCQQYDTWPFTFGPGTKVDFKRGSGSGSGSGSG
 GGGSQVQLVESGGGVVQPGRSLRLSCAASGFTFSSYGMHWVRQAPG
 KGLEWVAVISYDGSDKYYVDSVKGRFTISRDN SKNRLYLQMN SLRA
 20 EDTAVYYCARERYSGRDYWGQGLVTVSSAAALDNEKSNGTHHVK
 GKHLCPSP LFPGPSKPFWVLVVVGGVLACYSLLVTVAFIIFWVR SKRS
 RLLHSDYMNMTPRRPGPTRKHYPYAPPRDFAAYRSRVKFSRSADA
 PAYQQGQNQLYNELNLGRREEYDVL DKRRGRDP EMGGKPRRKNPQ
 EGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDT
 25 YDALHMQALPPR (SEQ ID NO. 116)

[0372] Clone 20C5.2 CD28T CD3 zeta CAR DNA Heavy & Light Chains

GAGATTGTGATGACCCAGTCCCCTGCTACCCTGTCCGTCAGTCCG
 GGCGAGAGAGCCACCTTGTCATGCCGGGCCAGCCAGTCCGTCAGC
 AGTCTCCTGACTTGGTATCAGCAAAAACCAGGGCAGGCACCGCGG
 30 CTTTTGATTTTTGGTGCAAGCACACGCGCCACTGGCATTCCAGCTA
 GGTTTTCTGGAAGTGGATCTGGGACAGGCTTCACTCTGACAATCA
 GTAGCCTGCAGAGTGAGGACTTTGCTGTTTACTACTGTCAACAGT

ACGACACCTGGCCATTCACATTCGGGCCCCGGCACCAAGGTCGACT
 TCAAGAGGGGGCGGTGGAGGTTTCAGGTGGTGGCGGGTCAGGCGGC
 GGTGGGTCTCAGGTTCAACTGGTGAATCAGGTGGCGGCGTTGTC
 CAACCGGGGCGATCACTTCGACTTTCCTGTGCTGCCTCAGGCTTTA
 5 CTTTTTCATCCTATGGGATGCACTGGGTTCGGCAGGCTCCCGGAA
 AAGGACTCGAGTGGGTTGCAGTGATCTCTTACGATGGCTCAGACA
 AGTATTATGTGGACTCAGTCAAGGGGAGATTCACAATAAGCCGAG
 ACAACTCCAAAAACCGGCTTTATCTCCAGATGAACAGCCTTAGAG
 CGGAAGATACCGCGGTATACTACTGTGCCCCGCGAGAGGTATTCCG
 10 GCAGAGACTACTGGGGACAGGGCACACTGGTCACCGTGAGTTCTG
 CCGCAGCGCTCGATAACGAAAAGAGCAACGGAACCATTATCCAC
 GTTAAGGGCAAGCACCTGTGCCCCAGTCCCCTCTTCCCAGGACCA
 TCTAAACCCTTCTGGGTTCTGGTAGTAGTTGGAGGGGTCTTG CAT
 GTTACTCCCTTTTGGTCACCGTCGCCTTCATTATTTTCTGGGTGAG
 15 ATCCAAAAGAAGCCGCCTGCTCCATAGCGATTACATGAATATGAC
 TCCACGCCGCCCTGGCCCCACAAGGAAACACTACCAGCCTTACGC
 ACCACCTAGAGATTTTCGCTGCCTATCGGAGCAGGGTGAAGTTTTTC
 CAGATCTGCAGATGCACCAGCGTATCAGCAGGGCCAGAACCAAC
 TGTATAACGAGCTCAACCTGGGACGCAGGGAAGAGTATGACGTTT
 20 TGGACAAGCGCAGAGGACGGGACCCTGAGATGGGTGGCAAACCA
 AGACGAAAAAACCCCCAGGAGGGTCTCTATAATGAGCTGCAGAA
 GGATAAGATGGCTGAAGCCTATTCTGAAATAGGCATGAAAGGAG
 AGCGGAGAAGGGGAAAAGGGCACGACGGTTTGTACCAGGGACTC
 AGCACTGCTACGAAGGATACTTATGACGCTCTCCACATGCAAGCC
 25 CTGCCACCTAGG (SEQ ID NO. 117)

[0373] Clone 20C5.2 CD28T CD3 zeta CAR AA Heavy & Light Chains

EIVMTQSPATLSVSPGERATLSCRASQSVSSLLTWYQQKPGQAPRLLI
 FGASTRATGIPARFSGSGSGTGFTLTISLQSEDFAVYYCQQYDTWPF
 TFGPGTKVDFKRGGGGSGGGGSGGGGSQVQLVESGGGVVQPGRSLR
 30 LSCAASGFTFSSYGMHWVRQAPGKGLEWVAVISYDGS DKYYVDSV
 KGRFTISRDN SKNRLYLQMNSLRAEDTAVYYCARERYSGRDYWGQ
 GTLVTVSSAAALDNEKSNGTIIHVKGKHLCPSP LFPGPSKPFVVLV
 VGGVLACYSLLVTVAFIIFWVR SKRSRL LHSDYMNMTPRRPGPTRKH

YQPYAPPRDFAAYRSRVKFSRSADAPAYQQGQNQLYNELNLGRREE
YDVLDKRRGRDPGEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGM
KGERRRGKGGHDGLYQGLSTATKDTYDALHMQALPPR (SEQ ID NO.
118)

5 **[0374]** Clone 20C5.2 CD28 CD3 zeta CAR DNA Heavy & Light Chains
ATGGCACTCCCCGTAACCTGCTCTGCTGCTGCCGTTGGCATTGCTCC
TGCACGCCGCACGCCCGGAGATCGTCATGACACAGAGTCCAGCTA
CCCTGAGCGTGTCCCCTGGAGAGAGAGCCACCCTGTCTGTAGGG
CTAGTCAGAGTGTGTCCAGCCTCCTCACCTGGTATCAACAGAAGC
10 CTGGTCAAGCTCCCCGGCTGCTTATCTTCGGGGCCAGCACGCGAG
CCACAGGCATCCCGGCCAGATTCTCTGGCTCTGGCAGTGGCACCG
GGTTCACCTCTCACGATCTCATCCCTGCAGTCAGAGGATTTGCTGT
GTATTACTGTCAGCAGTACGATACATGGCCCTTCACCTTCGGCCC
GGGCACAAAAGTAGATTTCAAGCGCGGCGGCGGGGGTAGTGGGG
15 GCGGGGGATCAGGAGGAGGGGGCTCCCAAGTACAGCTGGTTGAG
AGCGGCGGCGGGGTGGTTCAGCCCGGGCGCAGCCTCAGGCTGAG
TTGCGCAGCATCAGGATTCACATTCAGTTCTTATGGAATGCATTG
GGTCAGACAGGCTCCCGGGAAGGGCCTTGAATGGGTGGCAGTCA
TTAGCTACGACGGAAGCGATAAGTACTATGTGGACTCAGTTAAAG
20 GGAGATTTACTATCAGCCGCGACAATTCCAAAAACAGATTGTATT
TGCAGATGAACTCCCTCAGGGCGGAGGACACTGCTGTATATTACT
GCGCACGAGAGAGATACTCCGGCCGAGACTATTGGGGCCAAGGA
ACATTGGTAACTGTGAGCTCCGCCGAGCTATTGAGGTCATGTAC
CCCCACCTTATCTCGATAATGAGAAGAGTAATGGGACTATAATT
25 CACGTAAAGGGCAAACACCTGTGCCCTTCCCCGCTGTTTCCAGGT
CCAAGTAAGCCGTTCTGGGTCCTGGTTGTGGTGGGAGGGGTGCTG
GCCTGCTATTCTCTGTTGGTTACCGTGGCCTTTATCATTTTCTGGGT
GAGATCCAAAAGAAGCCGCCTGCTCCATAGCGATTACATGAATAT
GACTCCACGCCGCCCTGGCCCCACAAGGAAACACTACCAGCCTTA
30 CGCACCACCTAGAGATTTGCTGCCTATCGGAGCAGGGTGAAGTT
TTCCAGATCTGCAGATGCACCAGCGTATCAGCAGGGCCAGAACCA
ACTGTATAACGAGCTCAACCTGGGACGCAGGGAAGAGTATGACG
TTTTGGACAAGCGCAGAGGACGGGACCCTGAGATGGGTGGCAAA

CCAAGACGAAAAAACCCCCAGGAGGGTCTCTATAATGAGCTGCA
 GAAGGATAAGATGGCTGAAGCCTATTCTGAAATAGGCATGAAAG
 GAGAGCGGAGAAGGGGAAAAGGGCACGACGGTTTGTACCAGGGA
 CTCAGCACTGCTACGAAGGATACTTATGACGCTCTCCACATGCAA
 5 GCCCTGCCACCTAGGTAA (SEQ ID NO. 119)

[0375] Clone 20C5.2 CD28 CD3 zeta CAR AA Heavy & Light Chains
(Signal Peptide in **Bold**)

MALPVTALLPLALLLHAARPEIVMTQSPATLSVSPGERATLSCRA
 SQSVSSLLTWYQQKPGQAPRLLIFGASTRATGIPARFSGSGSGTGFTL
 10 TISSLQSEDFAVYYCQQYDTPFTFGPGTKVDFKRGGGSGGGGSG
 GGGSQVQLVESGGGVVQPGRSLRLSCAASGFTFSSYGMHWVRQAPG
 KGLEWVAVISYDGSDKYYVDSVKGRFTISRDNLSKNRLYLQMNSLRA
 EDTAVYYCARERYSGRDYWGQGTLLTVSSAAAEVMPYPYLDNEK
 SNGTIIHVKGKHLCPSPFPGPSKPFVWLTVVVGGLACYSLLVTVAFI
 15 IFWVRSKRSRLHSDYMNMTPRRPGPTRKHYQPYAPPRDFAAYRSR
 VKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDRGRDPEMG
 GKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQ
 GLSTATKD TYDALHMQALPPR (SEQ ID NO. 120)

[0376] Clone 20C5.2 CD28 CD3 zeta CAR DNA Heavy & Light Chains

GAGATCGTCATGACACAGAGTCCAGCTACCCTGAGCGTGTCCCCT
 20 GGAGAGAGAGCCACCCTGTCCTGTAGGGCTAGTCAGAGTGTGTCC
 AGCCTCCTCACCTGGTATCAACAGAAGCCTGGTCAAGCTCCCCGG
 CTGCTTATCTTCGGGGCCAGCACGCGAGCCACAGGCATCCCGGCC
 AGATTCTCTGGCTCTGGCAGTGGCACCGGGTTCCTCTCACGATCT
 25 CATCCCTGCAGTCAGAGGATTTCTGCTGTGTATTACTGTCAGCAGT
 ACGATACATGGCCCTTCACCTTCGGCCCGGGCACAAAAGTAGATT
 TCAAGCGCGGCGGGCGGGGGTAGTGGGGGCGGGGGATCAGGAGGA
 GGGGGCTCCCAAGTACAGCTGGTTGAGAGCGGCGGGGGTGGT
 TCAGCCCGGGCGCAGCCTCAGGCTGAGTTGCGCAGCATCAGGATT
 30 CACATTCAGTTCTTATGGAATGCATTGGGTCAGACAGGCTCCCGG
 GAAGGGCCTTGAATGGGTGGCAGTCATTAGCTACGACGGAAGCG
 ATAAGTACTATGTGGACTCAGTTAAAGGGAGATTTACTATCAGCC

GCGACAATTCCAAAAACAGATTGTATTTGCAGATGAACTCCCTCA
 GGGCGGAGGACACTGCTGTATATTACTGCGCACGAGAGAGATACT
 CCGGCCGAGACTATTGGGGCCAAGGAACATTGGTAACTGTGAGCT
 CCGCCGCAGCTATTGAGGTCATGTACCCCCACCTTATCTCGATA
 5 ATGAGAAGAGTAATGGGACTATAATTCACGTAAAGGGCAAACAC
 CTGTGCCCTTCCCCGCTGTTTCCAGGTCCAAGTAAGCCGTTCTGGG
 TCCTGGTTGTGGTGGGAGGGGTGCTGGCCTGCTATTCTCTGTTGGT
 TACCGTGGCCTTTATCATTTTCTGGGTGAGATCCAAAAGAAGCCG
 CCTGCTCCATAGCGATTACATGAATATGACTCCACGCCGCCCTGG
 10 CCCCACAAGGAAACACTACCAGCCTTACGCACCACCTAGAGATT
 CGCTGCCTATCGGAGCAGGGTGAAGTTTTCCAGATCTGCAGATGC
 ACCAGCGTATCAGCAGGGCCAGAACCAACTGTATAACGAGCTCA
 ACCTGGGACGCAGGGAAGAGTATGACGTTTTGGACAAGCGCAGA
 GGACGGGACCCTGAGATGGGTGGCAAACCAAGACGAAAAAACCC
 15 CCAGGAGGGTCTCTATAATGAGCTGCAGAAGGATAAGATGGCTG
 AAGCCTATTCTGAAATAGGCATGAAAGGAGAGCGGAGAAGGGGA
 AAAGGGCACGACGGTTTGTACCAGGGACTCAGCACTGCTACGAA
 GGATACTTATGACGCTCTCCACATGCAAGCCCTGCCACCTAGG
 (SEQ ID NO. 121)

20 [0377] Clone 20C5.2 CD28 CD3 zeta CAR AA Heavy & Light Chains
 EIVMTQSPATLSVSPGERATLSCRASQSVSSLLTWYQQKPGQAPRLLI
 FGASTRATGIPARFSGSGSGTGFSLTISSLSQSEDFAVYYCQQYDTWPF
 TFGPGTKVDFKRGSGSGSGSGSGSQVQLVESGGGVVQPGRSLR
 LSCAASGFTFSSYGMHWVRQAPGKGLEWVAVISYDGSCKYYVDSV
 25 KGRFTISRDNSKNRLYLQMNSLR AEDTAVYYCARERYSGRDYWGQ
 GTLVTVSSAAIEVMYPPPYLDNEKSNGTIIHVKGKHLCPSPFPGPS
 KPFWVLVVVGGVLACYSLLVTVAFIIFWVRSKRSRLHSDYMNMT
 RRPGPTRKHYPYAPPRDFAAYRSRVKFSRSADAPAYQQGQNQLYN
 ELNLGRREEYDVLDRGRDPEMGGKPRRKNPQEGLYNELQKDKM
 30 AEAYSEIGMKGERRRGK GHDGLYQGLSTATKDTYDALHMQALPPR
 (SEQ ID NO. 122)

[0378] Clone 20C5.2 CD8 CD3 zeta CAR DNA Heavy & Light Chains

ATGGCACTCCCCGTAAGTGTCTGTGCTGCCGTTGGCATTGCTCC
TGCACGCCGCACGCCCGGAAATAGTGATGACTCAGTCCCCGGCCA
CCCTCAGCGTGTCCCCCGGGGAGCGAGCGACCCTGTATGCAGGG
CTTCCCAGAGTGTCAGCTCCCTGCTCACTTGGTATCAGCAAAAGC
5 CGGGGCAGGCTCCCCGCCTCCTCATCTTCGGGGCATCAACTAGGG
CCACCGGCATTCCCTGCAAGATTTTCCGGGTCTGGCAGCGGCACCG
GCTTCACCCTTACCATTAGCTCTCTGCAGTCTGAGGACTTCGCCGT
TTACTATTGTCAGCAGTATGATACTTGGCCCTTTACCTTCGGTCCC
GGAATAAGGTGGACTTCAAGCGCGGGGGGGGTGGATCTGGAGG
10 TGGTGGCTCCGGGGGCGGTGGAAGCCAGGTCCAGTTGGTTGAGA
GCGGCGGCGGAGTGGTGCAGCCCGGAGGTCCTTGCGGCTGAGC
TGTGCAGCCTCCGGTTTTACTTTTTCTAGCTATGGAATGCATTGGG
TAAGACAGGCTCCCGGAAAAGGCCTCGAGTGGGTGGCGGTCATT
AGCTATGATGGATCTGATAAATACTATGTGGACTCAGTTAAGGGG
15 CGCTTCACAATCTCAAGAGACAATAGCAAAAATAGACTGTACCTG
CAGATGAATAGTCTGCGCGCCGAGGACACTGCCGTGTACTACTGC
GCCCCGCGAGAGATACAGCGGACGGGATTACTGGGGCCAGGGTAC
CCTCGTAACGGTGTCTCCGCTGCCGCCCTTAGCAACAGCATTAT
GTACTTTTCTCATTTTCGTGCCAGTCTTTCTCCCAGCAAAGCCCACC
20 ACTACCCCGGCCCCAGGCCGCTACTCCTGCCCCACTATCGCG
TCTCAGCCTCTCTCCTTGCGGCCCCGAGGCCTGCCGGCCAGCCGCA
GGGGGCGCCGTACATACTCGGGGTTTGGATTTTCGCTTGCGACATA
TATATTTGGGCCCCCCTCGCCGGCACATGTGGAGTGCTGCTCCTG
AGTCTCGTTATAACCCTCTATTGCAACCATAGAAACAGATCCAAA
25 AGAAGCCGCCTGCTCCATAGCGATTACATGAATATGACTCCACGC
CGCCCTGGCCCCACAAGGAAACACTACCAGCCTTACGCACCACCT
AGAGATTTTCGCTGCCTATCGGAGCAGGGTGAAGTTTTCCAGATCT
GCAGATGCACCAGCGTATCAGCAGGGCCAGAACCAACTGTATAA
CGAGCTCAACCTGGGACGCAGGGAAGAGTATGACGTTTTGGACA
30 AGCGCAGAGGACGGGACCCTGAGATGGGTGGCAAACCAAGACGA
AAAAACCCCCAGGAGGGTCTCTATAATGAGCTGCAGAAGGATAA
GATGGCTGAAGCCTATTCTGAAATAGGCATGAAAGGAGAGCGGA
GAAGGGGAAAAGGGCACGACGGTTTGTACCAGGGACTCAGCACT

GCTACGAAGGATACTTATGACGCTCTCCACATGCAAGCCCTGCCA
CCTAGGTAA (SEQ ID NO. 123)

[0379] Clone 20C5.2 CD8 CD3 zeta CAR AA Heavy & Light Chains

(Signal Peptide in **Bold**)

5 **MALPVTALLLPLALLLHAARPEIVMTQSPATLSVSPGERATLSCRA**
SQSVSSLLTWYQQKPGQAPRLIFGASTRATGIPARFSGSGSGTGTFTL
TISSLQSEDFAVYYCQQYDTWPFTFGPGTKVDFKRGGGGSGGGGSG
GGGSQVQLVESGGGVVQPGRSLRLSCAASGFTFSSYGMHWVRQAPG
KGLEWVAVISYDGSDKYYVDSVKGRFTISRDN SKNRLYLQMNSLR
10 EDTAVYYCARERYSGRDYWGQGT LVTVSSAAALSNSIMYFSHFVPV
FLPAKPTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFA
CDIYIWAPLAGTCGVLLLSLVITLYCNHRNRSKRSRLHSDYMNMT
RRPGPTRKHYQPYAPPRDFAAYRSRVKFSRSADAPAYQQGQNQLYN
ELNLGRREEYDVLDRGRDPEMGGKPRRKNPQEGLYNELQKDKM
15 AEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR
(SEQ ID NO. 124)

[0380] Clone 20C5.2 CD8 CD3 zeta CAR DNA Heavy & Light Chains

GAAATAGTGATGACTCAGTCCCCGGCCACCCTCAGCGTGTCCCC
GGGAGCGAGCGACCCTGTCATGCAGGGCTTCCCAGAGTGT CAGC
20 TCCCTGCTCACTTGGTATCAGCAAAAGCCGGGGCAGGCTCCCCGC
CTCCTCATCTTCGGGGCATCAACTAGGGCCACCGGCATTCTTGCA
AGATTTTCCGGGTCTGGCAGCGGCACCGGCTTCACCCTTACCATT
AGCTCTCTGCAGTCTGAGGACTTCGCCGTTTACTATTGTCAGCAGT
ATGATACTTGGCCCTTTACCTTCGGTCCCGGA ACTAAGGTGGACTT
25 CAAGCGCGGGGGGGGTGGATCTGGAGGTGGTGGCTCCGGGGGCG
GTGGAAGCCAGGTCCAGTTGGTTGAGAGCGGCGGCGAGTGGTG
CAGCCCGGGAGGTCCTTGCGGCTGAGCTGTGCAGCCTCCGGTTTT
ACTTTTTCTAGCTATGGAATGCATTGGGTAAGACAGGCTCCCGGA
AAAGGCCTCGAGTGGGTGGCGGTCATTAGCTATGATGGATCTGAT
30 AAATACTATGTGGACTCAGTTAAGGGGCGCTTCACAATCTCAAGA
GACAATAGCAAAAATAGACTGTACCTGCAGATGAATAGTCTGCGC
GCCGAGGACACTGCCGTGTACTACTGCGCCCGCGAGAGATACAGC

GGACGGGATTACTGGGGCCAGGGTACCCTCGTAACGGTGTCTCTCC
 GCTGCCGCCCTTAGCAACAGCATTATGTACTTTTCTCATTTTCGTGC
 CAGTCTTTCTCCCAGCAAAGCCCACCACTACCCCGGCCCCCAGGC
 CGCCTACTCCTGCCCCCACTATCGCGTCTCAGCCTCTCTCCTTGCG
 5 GCCCGAGGCCTGCCGGCCAGCCGCAGGGGGCGCCGTACATACTC
 GGGGTTTGGATTTCGCTTGCGACATATATATTTGGGCCCCCCTCGC
 CGGCACATGTGGAGTGCTGCTCCTGAGTCTCGTTATAACCCTCTAT
 TGCAACCATAGAAACAGATCCAAAAGAAGCCGCCTGCTCCATAG
 CGATTACATGAATATGACTCCACGCCGCCCTGGCCCCACAAGGAA
 10 AACTACCAGCCTTACGCACCACCTAGAGATTTTCGCTGCCTATCG
 GAGCAGGGTGAAGTTTTCCAGATCTGCAGATGCACCAGCGTATCA
 GCAGGGCCAGAACCAACTGTATAACGAGCTCAACCTGGGACGCA
 GGGAAGAGTATGACGTTTTGGACAAGCGCAGAGGACGGGACCCT
 GAGATGGGTGGCAAACCAAGACGAAAAAACCCCAAGGAGGGTCT
 15 CTATAATGAGCTGCAGAAGGATAAGATGGCTGAAGCCTATTCTGA
 AATAGGCATGAAAGGAGAGCGGAGAAGGGGAAAAGGGCACGAC
 GGTTTGTACCAGGGACTCAGCACTGCTACGAAGGATACTTATGAC
 GCTCTCCACATGCAAGCCCTGCCACCTAGG (SEQ ID NO. 125)

[0381] Clone 20C5.2 CD8 CD3 zeta CAR AA Heavy & Light Chains

20 EIVMTQSPATLSVSPGERATLSCRASQSVSSLLTWYQQKPGQAPRLLI
 FGASTRATGIPARFSGSGSGTGTFTLTISLQSEDFAVYYCQYDTWPF
 TFGPGTKVDFKRGGGSGGGSGGGGSQVQLVESGGGVVQPGRSLR
 LSCAASGFTFSSYGMHWVRQAPGKGLEWVAVISYDGS DKYYVDSV
 KGRFTISRDN SKNRLYLQMNSLR AEDTAVYYCARERYSGRDYWGQ
 25 GTLVTVSSAAALSNSIMYFSHFVPVFLPAKPTTTPAPRPPTPAPTASQ
 PLSLRPEACRPAAGGAVHTRGLDFACDIYIWAPLAGTCGVLLLSLVIT
 LYCNHRNRSKRSRLLHSDYMNMTPRRPGPTRKHYQPYAPPRDFAAY
 RSRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVL DKRRGRDPE
 30 MGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGL
 YQGLSTATKDTYDALHMQALPPR (SEQ ID NO. 126)

[0382] CAR Signal Peptide DNA

ATGGCACTCCCCGTAAGTCTGCTGCTGCCGTTGGCATTGCTCC
TGCACGCCGCACGCCCG (SEQ ID NO. 127)

[0383] CAR Signal Peptide: MALPVTALLPLALLHAARP (SEQ ID NO. 128)

- [0384] scFv G4S linker DNA
GGCGGTGGAGGCTCCGGAGGGGGGGGCTCTGGCGGAGGGGGCTC
C (SEQ ID NO. 129)
- [0385] scFv G4s linker: GGGGSGGGGSGGGGS (SEQ ID NO. 130)
- 5 [0386] Additional G4S linker: GGGGSGGGGSGGGGSGGGGS (SEQ ID NO. 145)
- [0387] scFv Whitlow linker DNA
GGGTCTACATCCGGCTCCGGGAAGCCCGGAAGTGGCGAAGGTAG
TACAAAGGGG (SEQ ID NO. 131)
- [0388] scFv Whitlow linker: GSTSGSGKPGSGEGSTKG (SEQ ID NO. 132)
- 10 [0389] CD28 AA Extracellular Domain
MLRLLLALNLFPSIQVTGNKILVKQSPMLVAYDNAVNLSCKYSYNLF
SREFRASLHKGLDSAVEVCVVYGNYSQQQLQVYSKTGFNC DGKLGNE
SVTFY LQNLYVNQTDIYFCKIEVMYPPPYLDNEKSNGTIIHVKGKHL C
PSPLFPGPSKP (SEQ ID NO. 133)
- 15 [0390] $GX_2X_3X_4X_5X_6X_7X_8X_9$ (SEQ ID NO: 134)
- [0391] $X_1X_2X_3X_4X_5X_6$ (SEQ ID NO: 135)
- [0392] $X_1X_2X_3X_4X_5X_6X_7X_8X_9X_{10}X_{11}X_{12}DY$ (SEQ ID NO: 136)
- [0393] $X_1ASQX_5X_6X_7X_8X_9LX_{11}$ (SEQ ID NO: 137)
- [0394] $X_1ASX_4X_5X_6X_7$ (SEQ ID NO: 138)
- 20 [0395] $QQX_3X_4X_5X_6PX_8T$ (SEQ ID NO: 139)
- [0396] CLL-1 AA (a.k.a., CLEC12A)
MSEEVTYADLQFQNSSEMEKIP EIGKFGEKAPPAPSHVWRPAALFLT
LLCLLLLIGLGVLASMFHVT LK IEMKKMNKLQNISEELQRNISLQLMS
NMNISNKIRNLSTTLQTIATKL CRELYSKEQEHKCKPCPRRWIHKD
25 SCYFLSDDVQTWQESKMACAAQNASLLKINNKNLEFIKSQSRSYD

YWLGLSPEEDSTRGMRVDNIINSSAWVIRNAPDLNNMYCGYINRLY
VQYYHCTYKKRMICEKMANPVQLGSTYFREA (SEQ ID NO. 140)

[0397] 4-1BB Nucleic Acid Sequence (intracellular domain)

5 AAGCGCGGCAGGAAGAAGCTCCTCTACATTTTAAAGCAGCCTTTT
ATGAGGCCCGTACAGACAACACAGGAGGAAGATGGCTGTAGCTG
CAGATTTCCCGAGGAGGAGGAAGGTGGGTGCGAGCTG (SEQ ID
NO. 141)

[0398] 4-1BB AA (intracellular domain)

10 KRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCEL (SEQ ID
NO. 142)

[0399] OX40 AA

RRDQRLPPDAHKPPGGGSFRTPIQEEQADAHSTLAKI (SEQ ID NO.
143)

[0400] Leader Sequence AA

15 MALPVTALLLPLALLLHAARP (SEQ ID NO: 144)

CLAIMS

What is Claimed:

1. A chimeric antigen receptor comprising an antigen binding molecule that specifically binds to CLL-1, wherein the antigen binding molecule comprises at least one of:
 - 5 a) a variable heavy chain CDR1 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 17, 51, 73, and 95,
 - b) a variable heavy chain CDR2 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs 18, 52, 74, and 96,
 - c) a variable heavy chain CDR3 comprising an amino acid sequence selected from
 - 10 the group consisting of SEQ ID NOs 19, 53, 75, and 97,
 - d) a variable light chain CDR1 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 22, 56, 78, and 100,
 - e) a variable light chain CDR2 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs. 23, 57, 79, and 101,
 - 15 f) a variable light chain CDR3 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs. 24, 58, 80, and 102.
2. The chimeric antigen receptor according to claim 1 having no more than 8 amino acid substitutions.
3. The chimeric antigen receptor according to claim 1 further comprising at least one
- 20 costimulatory domain.
4. The chimeric antigen receptor according to claim 1 further comprising at least one activating domain.
5. The chimeric antigen receptor according to claim 3 wherein the costimulatory domain is a signaling region (or other suitable portion) of CD28, OX-40, 4-1BB/CD137, CD2, CD7,
- 25 CD27, CD30, CD40, programmed death-1 (PD-1), inducible T cell costimulator (ICOS), lymphocyte function-associated antigen-1 (LFA-1 (CD11a/CD18), CD3 gamma, CD3 delta, CD3 epsilon, CD247, CD276 (B7-H3), LIGHT, (TNFSF14), NKG2C, Ig alpha (CD79a), DAP-10, Fc gamma receptor, MHC class I molecule, TNF receptor proteins, an Immunoglobulin protein, cytokine receptor, integrins, Signaling Lymphocytic Activation
- 30 Molecules (SLAM proteins), activating NK cell receptors, BTLA, a Toll ligand receptor, ICAM-1, B7-H3, CD28, ICAM-1, GITR, BAFTR, LIGHT, HVEM (LIGHTR), KIRDS2, SLAMF7, NKp80 (KLRP1), NKp44, NKp30, NKp46, CD19, CD4, CD8alpha, CD8beta, IL-

- 2R beta, IL-2R gamma, IL-7R alpha, ITGA4, VLA1, CD49a, ITGA4, IA4, CD49D, ITGA6, VLA-6, CD49f, ITGAD, CD11d, ITGAE, CD103, ITGAL, CD11a, LFA-1, ITGAM, CD11b, ITGAX, CD11c, ITGB1, CD29, ITGB2, CD18, LFA-1, ITGB7, NKG2D, TNFR2, TRANCE/RANKL, DNAM1 (CD226), SLAMF4 (CD244, 2B4), CD84, CD96 (Tactile),
- 5 CEACAM1, CRT AM, Ly9 (CD229), CD160 (BY55), PSGL1, CD100 (SEMA4D), CD69, SLAMF6 (NTB-A, Lyl08), SLAM (SLAMF1, CD150, IPO-3), BLAME (SLAMF8), SELPLG (CD162), LTBR, LAT, GADS, SLP-76, PAG/Cbp, CD19a, a ligand that specifically binds with CD83, or any combination thereof.
6. The chimeric antigen receptor according to claim 5 wherein the costimulatory domain
- 10 comprises CD28.
7. The chimeric antigen receptor according to claim 6 wherein the CD28 costimulatory domain comprises a sequence selected from the group consisting of SEQ ID NO. 2, SEQ ID NO. 4, SEQ ID NO. 6, and SEQ ID NO. 8.
8. The chimeric antigen receptor according to claim 5 wherein the CD8 costimulatory
- 15 domain comprises SEQ ID NO. 14.
9. The chimeric antigen receptor according to claim 4 wherein the activating domain comprises CD3.
10. The chimeric antigen receptor according to claim 9 wherein the CD3 comprises CD3 zeta.
- 20 11. The chimeric antigen receptor according to claim 10 wherein the CD3 zeta comprises SEQ ID NO. 10.
12. The chimeric antigen receptor according to claim 1 further comprising SEQ ID NO. 2 and SEQ ID NO. 10.
13. An isolated polynucleotide encoding the chimeric antigen receptor of claim 1.
- 25 14. A vector comprising the polynucleotide of claim 13.
15. The vector according to claim 14 which is a retroviral vector, a DNA vector, a plasmid, a RNA vector, an adenoviral vector, an adenovirus associated vector, a lentiviral vector, or any combination thereof.
16. An immune cell comprising the vector of claim 14.

17. The immune cell according to claim 16, wherein the immune cell is a T cell, tumor infiltrating lymphocyte (TIL), NK cell, TCR-expressing cell, dendritic cell, or NK-T cell.
18. The immune cell according to claim 17, wherein the cell is an autologous T cell.
19. The immune cell according to claim 17, wherein the cell is an allogeneic T cell.
- 5 20. A chimeric antigen receptor having at least 90% identity to the sequence of the antigen binding molecule of claim 1.
21. A chimeric antigen receptor having at least 95% identity to the sequence of the antigen binding molecule of claim 1.
22. A pharmaceutical composition comprising the T cell of claim 17, 18, or 19.
- 10 23. A chimeric antigen receptor comprising at least one of:
- (a) a VH region comprising the amino acid sequence of SEQ ID NO: 16 and a VL region comprising the amino acid sequence of SEQ ID NO: 21;
- (b) a VH region comprising the amino acid sequence of SEQ ID NO: 50 and a VL region comprising the amino acid sequence of SEQ ID NO: 55;
- 15 (c) a VH region comprising the amino acid sequence of SEQ ID NO: 72 and a VL region comprising the amino acid sequence of SEQ ID NO: 77;
- (d) a VH region comprising the amino acid sequence of SEQ ID NO: 94 and a VL region comprising the amino acid sequence of SEQ ID NO: 99;
- wherein the VH and VL region is linked by at least one linker.
- 20 24. The chimeric antigen receptor according to claim 23 having no more than 8 amino acid substitutions.
25. The chimeric antigen receptor according to claim 23, wherein the linker comprises at least one of SEQ ID NO. 130 and SEQ ID NO. 132.
26. The chimeric antigen receptor according to claim 23, further comprising at least one
- 25 costimulatory domain.
27. The chimeric antigen receptor according to claim 23, further comprising at least one activating domain.
28. The chimeric antigen receptor according to claim 26 wherein the costimulatory domain is a signaling region of CD28, OX-40, 4-1BB/CD137, CD2, CD7, CD27, CD30,

CD40, programmed death-1 (PD-1), inducible T cell costimulator (ICOS), lymphocyte function-associated antigen-1 (LFA-1 (CD11a/CD18), CD3 gamma, CD3 delta, CD3 epsilon, CD247, CD276 (B7-H3), LIGHT, (TNFSF14), NKG2C, Ig alpha (CD79a), DAP-10, Fc gamma receptor, MHC class I molecule, TNF receptor proteins, an Immunoglobulin protein, cytokine receptor, integrins, Signaling Lymphocytic Activation Molecules (SLAM proteins), activating NK cell receptors, BTLA, a Toll ligand receptor, ICAM-1, B7-H3, CDS, ICAM-1, GITR, BAFFR, LIGHT, HVEM (LIGHTR), KIRDS2, SLAMF7, NKp80 (KLRF1), NKp44, NKp30, NKp46, CD19, CD4, CD8alpha, CD8beta, IL-2R beta, IL-2R gamma, IL-7R alpha, ITGA4, VLA1, CD49a, ITGA4, IA4, CD49D, ITGA6, VLA-6, CD49f, ITGAD, CD11d, ITGAE, CD103, ITGAL, CD11a, LFA-1, ITGAM, CD11b, ITGAX, CD11c, ITGB1, CD29, ITGB2, CD18, LFA-1, ITGB7, NKG2D, TNFR2, TRANCE/RANKL, DNAM1 (CD226), SLAMF4 (CD244, 2B4), CD84, CD96 (Tactile), CEACAM1, CRT AM, Ly9 (CD229), CD160 (BY55), PSGL1, CD100 (SEMA4D), CD69, SLAMF6 (NTB-A, Lyl08), SLAM (SLAMF1, CD150, IPO-3), BLAME (SLAMF8), SELPLG (CD162), LTBR, LAT, GADS, SLP-76, PAG/Cbp, CD19a, a ligand that specifically binds with CD83, or any combination thereof.

29. An immune cell comprising the chimeric antigen receptor of claim 23.

30. The immune cell according to claim 29, wherein the immune cell is a T cell, tumor infiltrating lymphocyte (TIL), NK cell, TCR-expressing cell, dendritic cell, or NK-T cell.

31. The T cell of claim 30 that is an autologous T cell.

32. The T cell of claim 30 that is an allogeneic T cell.

33. A pharmaceutical composition comprising the immune cell of claim 29.

34. An isolated polynucleotide comprising at least one of:

SEQ ID NO. 27; SEQ ID NO. 31; SEQ ID NO. 35; SEQ ID NO. 39; SEQ ID NO. 43; SEQ ID NO. 47; SEQ ID NO. 61; SEQ ID NO. 65; SEQ ID NO. 69; SEQ ID NO. 83; SEQ ID NO. 87; SEQ ID NO. 91; SEQ ID NO. 105; SEQ ID NO. 109; SEQ ID NO. 113; SEQ ID NO. 117; SEQ ID NO. 121; and SEQ ID NO. 125.

35. A vector comprising the polynucleotide according to claim 34.

36. An immune cell comprising the vector of claim 33.

37. The immune cell according to claim 36, wherein the immune cell is a T cell, tumor infiltrating lymphocyte (TIL), NK cell, TCR-expressing cell, dendritic cell, or NK-T cell.
38. The T cell of claim 37 that is an autologous T cell.
39. The T cell of claim 37 that is an allogeneic T cell.
- 5 40. An isolated polypeptide comprising the amino acid sequence set forth in at least one of:
- SEQ ID NO. 28; SEQ ID NO. 32; SEQ ID NO. 36; SEQ ID NO. 40; SEQ ID NO. 44;
SEQ ID NO. 48; SEQ ID NO. 62; SEQ ID NO. 66; SEQ ID NO. 70; SEQ ID NO. 84;
SEQ ID NO. 88; SEQ ID NO. 92; SEQ ID NO. 106; SEQ ID NO. 110; SEQ ID NO. 114;
10 SEQ ID NO. 118; SEQ ID NO. 122; and SEQ ID NO. 126.
41. The isolated polypeptide according to claim 40 having no more than 8 amino acid substitutions.
42. A vector encoding the polypeptide of claim 40.
43. An immune cell comprising the vector of claim 42.
- 15 44. The immune cell according to claim 43, wherein the immune cell is a T cell, tumor infiltrating lymphocyte (TIL), NK cell, TCR-expressing cell, dendritic cell, or NK-T cell.
45. The T cell of claim 44 that is an autologous T cell or an allogeneic T cell.
46. An isolated polynucleotide encoding a chimeric antigen receptor (CAR) or T cell receptor (TCR) comprising an antigen binding molecule that specifically binds to CLL-1,
20 wherein the antigen binding molecule comprises a variable heavy chain CDR3 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 19, 53, 75, and 97.
47. The polynucleotide according to claim 46 further comprising an activating domain.
48. The polynucleotide according to claim 47 wherein the activating domain is CD3.
49. The polynucleotide according to claim 48 wherein the CD3 is CD3 zeta.
- 25 50. The polynucleotide according to claim 49 wherein the CD3 zeta comprises the amino acid sequence set forth in SEQ ID NO. 9.
51. The polynucleotide according to claim 46 further comprising a costimulatory domain.
52. The polynucleotide according to claim 51 wherein the costimulatory domain is a signaling region of CD28, OX-40, 4-1BB/CD137, CD2, CD7, CD27, CD30, CD40,

programmed death-1 (PD-1), inducible T cell costimulator (ICOS), lymphocyte function-associated antigen-1 (LFA-1 (CD11a/CD18), CD3 gamma, CD3 delta, CD3 epsilon, CD247, CD276 (B7-H3), LIGHT, (TNFSF14), NKG2C, Ig alpha (CD79a), DAP-10, Fc gamma receptor, MHC class I molecule, TNF receptor proteins, an Immunoglobulin protein, cytokine
 5 receptor, integrins, Signaling Lymphocytic Activation Molecules (SLAM proteins), activating NK cell receptors, BTLA, a Toll ligand receptor, ICAM-1, B7-H3, CDS, ICAM-1, GITR, BAFR, LIGHT, HVEM (LIGHTR), KIRDS2, SLAMF7, NKp80 (KLRF1), NKp44, NKp30, NKp46, CD19, CD4, CD8alpha, CD8beta, IL-2R beta, IL-2R gamma, IL-7R alpha, ITGA4, VLA1, CD49a, ITGA4, IA4, CD49D, ITGA6, VLA-6, CD49f, ITGAD,
 10 CD11d, ITGAE, CD103, ITGAL, CD11a, LFA-1, ITGAM, CD11b, ITGAX, CD11c, ITGB1, CD29, ITGB2, CD18, LFA-1, ITGB7, NKG2D, TNFR2, TRANCE/RANKL, DNAM1 (CD226), SLAMF4 (CD244, 2B4), CD84, CD96 (Tactile), CEACAM1, CRT AM, Ly9 (CD229), CD160 (BY55), PSGL1, CD100 (SEMA4D), CD69, SLAMF6 (NTB-A, Lyl08), SLAM (SLAMF1, CD150, IPO-3), BLAME (SLAMF8), SELPLG (CD162), LTBR, LAT,
 15 GADS, SLP-76, PAG/Cbp, CD19a, a ligand that specifically binds with CD83, or any combination thereof.

53. The polynucleotide according to claim 52 wherein the CD28 costimulatory domain encodes the amino acid sequence set forth in SEQ ID NO 2.

54. A vector comprising the polynucleotide of claim 46.

20 55. An immune cell comprising the vector of claim 54.

56. The immune cell of claim 50, wherein the immune cell is a T cell, tumor infiltrating lymphocyte (TIL), NK cell, TCR-expressing cell, dendritic cell, or NK-T cell.

57. The T cell of claim 51 that is an autologous T cell, or an allogeneic T cell.

58. An isolated polynucleotide encoding a chimeric antigen receptor (CAR) or T cell
 25 receptor (TCR), said CAR or TCR comprising an antigen binding molecule that specifically binds to CLL-1, wherein the antigen binding molecule comprises a variable light chain CDR3 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 24, 58, 80, and 102.

59. The polynucleotide according to claim 58 further comprising an activating domain.

30 60. The polynucleotide according to claim 59 wherein the activating domain is CD3.

61. The polynucleotide according to claim 60 wherein the CD3 is CD3 zeta.

62. The polynucleotide according to claim 61 wherein the CD3 zeta comprises the amino acid sequence set forth in SEQ ID NO. 9.

63. The polynucleotide according to claim 58 further comprising a costimulatory domain.

64. The polynucleotide according to claim 63 wherein the costimulatory domain is a
 5 signaling region of CD28, OX-40, 4-1BB/CD137, CD2, CD7, CD27, CD30, CD40, programmed death-1 (PD-1), inducible T cell costimulator (ICOS), lymphocyte function-associated antigen-1 (LFA-1 (CD11a/CD18), CD3 gamma, CD3 delta, CD3 epsilon, CD247, CD276 (B7-H3), LIGHT, (TNFSF14), NKG2C, Ig alpha (CD79a), DAP-10, Fc gamma receptor, MHC class I molecule, TNF receptor proteins, an Immunoglobulin protein, cytokine
 10 receptor, integrins, Signaling Lymphocytic Activation Molecules (SLAM proteins), activating NK cell receptors, BTLA, a Toll ligand receptor, ICAM-1, B7-H3, CDS, ICAM-1, GITR, BAFR, LIGHT, HVEM (LIGHTR), KIRDS2, SLAMF7, NKp80 (KLRF1), NKp44, NKp30, NKp46, CD19, CD4, CD8alpha, CD8beta, IL-2R beta, IL-2R gamma, IL-7R alpha, ITGA4, VLA1, CD49a, ITGA4, IA4, CD49D, ITGA6, VLA-6, CD49f, ITGAD,
 15 CD11d, ITGAE, CD103, ITGAL, CD11a, LFA-1, ITGAM, CD11b, ITGAX, CD11c, ITGB1, CD29, ITGB2, CD18, LFA-1, ITGB7, NKG2D, TNFR2, TRANCE/RANKL, DNAM1 (CD226), SLAMF4 (CD244, 2B4), CD84, CD96 (Tactile), CEACAM1, CRT AM, Ly9 (CD229), CD160 (BY55), PSGL1, CD100 (SEMA4D), CD69, SLAMF6 (NTB-A, Lyl08), SLAM (SLAMF1, CD150, IPO-3), BLAME (SLAMF8), SELPLG (CD162), LTBR, LAT,
 20 GADS, SLP-76, PAG/Cbp, CD19a, a ligand that specifically binds with CD83, or any combination thereof.

65. The polynucleotide according to claim 64 wherein the CD28 costimulatory domain comprises the nucleotide sequence set forth in SEQ ID NO 3 or in SEQ ID NO 1.

66. An isolated polynucleotide encoding a chimeric antigen receptor (CAR) or T cell
 25 receptor (TCR) comprising an antigen binding molecule that specifically binds to CLL-1, wherein the antigen binding molecule heavy chain comprises CDR1 (SEQ ID NO. 17), CDR2 (SEQ ID NO. 18), and CDR3 (SEQ ID NO. 19) and the antigen binding molecule light chain comprises CDR1 (SEQ ID NO. 22), CDR2 (SEQ ID NO. 23), and CDR3 (SEQ ID NO. 24).

67. An isolated polynucleotide encoding a chimeric antigen receptor (CAR) or T cell
 30 receptor (TCR) comprising an antigen binding molecule that specifically binds to CLL-1, wherein the antigen binding molecule heavy chain comprises CDR1 (SEQ ID NO. 51), CDR2

(SEQ ID NO. 52), and CDR3 (SEQ ID NO. 53) and the antigen binding molecule light chain comprises CDR1 (SEQ ID NO. 56), CDR2 (SEQ ID NO. 57), and CDR3 (SEQ ID NO. 58).

68. An isolated polynucleotide encoding a chimeric antigen receptor (CAR) or T cell receptor (TCR) comprising an antigen binding molecule that specifically binds to CLL-1,
 5 wherein the antigen binding molecule heavy chain comprises CDR1 (SEQ ID NO. 73), CDR2 (SEQ ID NO. 74), and CDR3 (SEQ ID NO. 75) and the antigen binding molecule light chain comprises CDR1 (SEQ ID NO. 78), CDR2 (SEQ ID NO. 79), and CDR3 (SEQ ID NO. 80).

69. An isolated polynucleotide encoding a chimeric antigen receptor (CAR) or T cell receptor (TCR) comprising an antigen binding molecule that specifically binds to CLL-1,
 10 wherein the antigen binding molecule heavy chain comprises CDR1 (SEQ ID NO. 95), CDR2 (SEQ ID NO. 96), and CDR3 (SEQ ID NO. 97) and the antigen binding molecule light chain comprises CDR1 (SEQ ID NO. 100), CDR2 (SEQ ID NO. 101), and CDR3 (SEQ ID NO. 102).

70. An isolated polynucleotide encoding a chimeric antigen receptor (CAR) or T cell
 15 receptor (TCR) comprising an antigen binding molecule that specifically binds to CLL-1, wherein the antigen binding molecule comprises:

- (a) a heavy chain variable region (VH) complementarity determining region (CDR) 1 comprising the amino acid sequence GX₂X₃X₄X₅X₆X₇X₈X₉ (SEQ ID NO: 134), wherein:

20 X₂ is G, F, or Y;
 X₃ is S or T;
 X₄ is I, F, or L;
 X₅ is S or T;
 X₆ is not present or S;
 25 X₇ is not present or G;
 X₈ is not present or E or G; and
 X₉ is F, L, or Y;

- (b) a heavy chain variable region (VH) complementarity determining region (CDR) 2 comprising the amino acid sequence X₁X₂X₃X₄X₅X₆ (SEQ ID NO: 135), wherein:

30 X₁ is D, H, S, or Y;
 X₂ is H, P, or Y;

X₃ is D, E, or S;
 X₄ is D or G;
 X₅ is G or S; and
 X₆ is not present of D or E;

- 5 (c) a heavy chain variable region (VH) complementarity determining region (CDR) 3, comprising the amino acid sequence X₁X₂X₃X₄X₅X₆X₇X₈X₉X₁₀X₁₁X₁₂DY (SEQ ID NO: 136), wherein:

X₁ is E or L;
 X₂ is R, S, or V;
 10 X₃ is R or Y;
 X₄ is C, G, or S;
 X₅ is not present or G or I;
 X₆ is not present or G;
 X₇ is not present or D;
 15 X₈ is not present or C;
 X₉ is not present or W or Y;
 X₁₀ is not present or P or S;
 X₁₁ is not present or G or Y; and
 X₁₂ is F or R;

- 20 (d) a light chain variable region (VL) CDR1 comprising the amino acid sequence X₁ASQX₅X₆X₇X₈X₉LX₁₁ (SEQ ID NO: 137), wherein

X₁ is Q or R;
 X₅ is D or S;
 X₆ is I or V;
 25 X₇ is N or S;
 X₈ is N or S;
 X₉ is F, L, or Y; and
 X₁₁ is N or T;

- (e) a light chain variable region (VL) CDR2 comprising the amino acid sequence
 30 X₁ASX₄X₅X₆X₇ (SEQ ID NO: 138), wherein

X₁ is D or G;
 X₄ is N, S, or T;

X₅ is L or R;
X₆ is A, E, or K; and
X₇ is S or T; and/or

- (f) a light chain variable region (VL) CDR3 comprising the amino acid sequence
5 QQX₃X₄X₅X₆PX₈T (SEQ ID NO: 139), wherein
 X₃ is S or Y;
 X₄ is D, G, or Y;
 X₅ is N, S, or T;
 X₆ is L, T, or Y; and
10 X₈ is F or I.

71. A method of treating a disease or disorder in a subject in need thereof comprising administering to the subject the polynucleotide according to any of claims 46, 58, 66, 67, 68, 69, or 70.

72. A method of treating a disease or disorder in a subject in need thereof comprising
15 administering to the subject the polypeptide according to 40.

73. A method of treating a disease or disorder in a subject in need thereof comprising administering to the subject the chimeric antigen receptor according to any of claims 1, 20, 21, and 23.

74. A method of treating a disease or disorder in a subject in need thereof comprising
20 administering to the subject the cells according any of claims 16, 29, 36, 43, and 55.

75. A method of treating a disease or disorder in a subject in need thereof comprising administering to the subject the composition according to claim 22 and 33.

76. The method according to any of claims 71-75 wherein the disease or disorder is cancer.

25 77. The method according to claim 76 wherein the cancer is leukemia, lymphoma, or myeloma.

78. The method according to any of claims 71-75 wherein the disease or disorder is at least one of acute myeloid leukemia (AML), chronic myelogenous leukemia (CML), chronic myelomonocytic leukemia (CMML), juvenile myelomonocytic leukemia, atypical chronic
30 myeloid leukemia, acute promyelocytic leukemia (APL), acute monoblastic leukemia, acute erythroid leukemia, acute megakaryoblastic leukemia, myelodysplastic syndrome (MDS),

myeloproliferative disorder, myeloid neoplasm, myeloid sarcoma), Blastic Plasmacytoid Dendritic Cell Neoplasm (BPDCN), and inflammatory/autoimmune disease.

79. The method according to claim 78 wherein the inflammatory/autoimmune disease is at least one of rheumatoid arthritis, psoriasis, allergies, asthma, Crohn's disease, IBD, IBS,
5 fibromyalgia, mastocytosis, and Celiac disease.

80. The lentiviral vector according to claim 15 that comprises pGAR, or a derivative thereof.