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(54) **Title**: CHIMERIC RECEPTORS AND METHODS OF USE THEREOF

(57) **Abstract**: Antigen binding molecules, chimeric receptors, and engineered immune cells are disclosed in accordance with the invention. The invention further relates to vectors, compositions, and methods of treatment and/or detection using the antigen binding molecules and engineered immune cells.

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.

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.

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).

[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.

[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, fibromyalga, mastocytosis, and Celiac disease.

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

MSEEVTYADLQFQNSSEMEKIPEIGKFGEKAPPAPSHVWRPAALFLTLLCLL
LLIGLGVLASMFHVTLKIEMKKMNKLQNISEELQRNISLQLMSNMNISNKIR
NLSTTLQTIATKLCRELYSKEQEHKCKPCPRRWIWHKDSCYFLSDDVQTWQ
ESKMACAAQNASLLKINNKNALEFIKSQSRSYDYWLGLSPEEDSTRGMRVD
NIINSSAWVIRNAPDLNNMYCGYINRLYVQYYHCTYKKRMICEKMANPVQ
LGSTYFREA (SEQ ID NO. 140).

[0007] Additional sequence information is contained in the CLL-1 Uniprot listing at: http://www.uniprot.org/uniprot/Q5QGZ9, as well as NCBI Reference Sequence NP_612210.4 (http://www.ncbi.nlm.nih.gov/protein/NP_612210.4).

[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 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.

[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. 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 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. 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 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 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 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 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.

[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 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 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 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 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.

[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, CDl-la/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, 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, CDl ld, ITGAE, CD103, ITGAL, CDl la, LFA-1, ITGAM, CDl lb, ITGAX, CDl lc, ITGBl, 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.

[0023] In some embodiments, the costimulatory domain can comprise all or a portion 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 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 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 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 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
TTACGCGCAGCGTGACCGCTACACTTGCCAGCGCCCTAGCGCCCGCTCCTTT
CGCTTTCTTCCCTTCCTTTCTCGCCACGTTCGCCGGCTTTCCCCGTCAAGCTC
TAAATCGGGGGCTCCCTTTAGGGTTCCGATTTAGTGCTTTACGGCACCTCGA
CCCCAAAAAACTTGATTAGGGTGATGGTTCACGTAGTGGGCCATCGCCCTGA
TAGACGGTTTTTCGCCCTTTGACGTTGGAGTCCACGTTCTTTAATAGTGGACT
CTTGTTCCAAACTGGAACAACACTCAACCCTATCTCGGTCTATTCTTTTGATT
TATAAGGGATTTTGCCGATTTCGGCCTATTGGTTAAAAAATGAGCTGATTTA
ACAAAAATTTAACGCGAATTTTAACAAAATATTAACGCTTACAATTTGCCAT
TCGCCATTCAGGCTGCGCAACTGTTGGGAAGGGCGATCGGTGCGGGCCTCTT
CGCTATTACGCCAGCTGGCGAAAGGGGGATGTGCTGCAAGGCGATTAAGTT
GGGTAACGCCAGGGTTTTCCCAGTCACGACGTTGTAAAACGACGGCCAGTG
AATTGTAATACGACTCACTATAGGGCGACCCGGGGATGGCGCGCCAGTAAT
CAATTACGGGGTCATTAGTTCATAGCCCATATATGGAGTTCCGCGTTACATA
ACTTACGGTAAATGGCCCGCCTGGCTGACCGCCCAACGACCCCCGCCCATTG
ACGTCAATAATGACGTATGTTCCCATAGTAACGCCAATAGGGACTTTCCATT
GACGTCAATGGGTGGAGTATTTACGGTAAACTGCCCACTTGGCAGTACATCA
AGTGTATCATATGCCAAGTACGCCCCCTATTGACGTCAATGACGGTAAATGG
CCCGCCTGGCATTATGCCCAGTACATGACCTTATGGGACTTTCCTACTTGGC
AGTACATCTACGTATTAGTCATCGCTATTACCATGCTGATGCGGTTTTGGCA

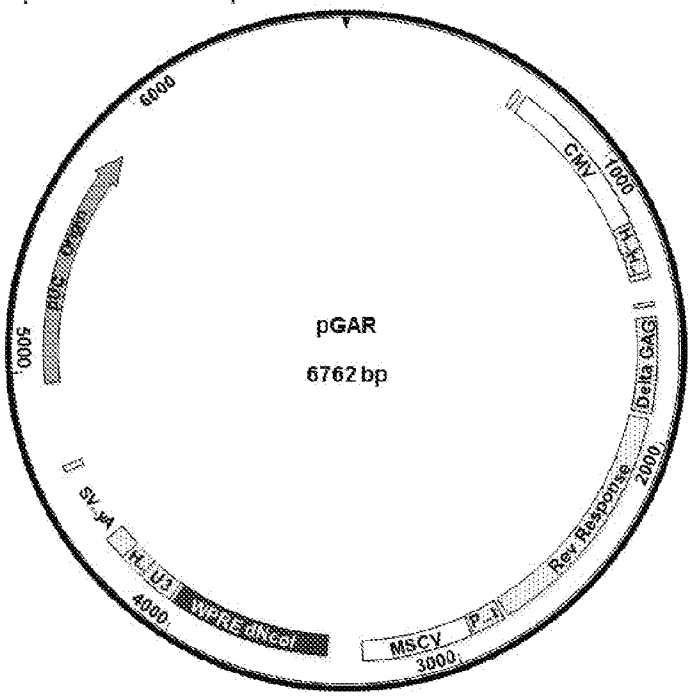
GTACATCAATGGGCGTGGATAGCGGTTTGACTCACGGGGATTTCCAAGTCTC
CACCCCATTGACGTCAATGGGAGTTTGTTTTGGCACCAAAATCAACGGGACT
TTCCAAAATGTCGTAACAACTCCGCCCCATTGACGCAAATGGGCGGTAGGC
GTGTACGGTGGGAGGTCTATATAAGCAGAGCTGGTTTAGTGAACCGGGGTC
TCTCTGGTTAGACCAGATCTGAGCCTGGGAGCTCTCTGGCTAACTAGGGAAC
CCACTGCTTAAGCCTCAATAAAGCTTGCCTTGAGTGCTTCAAGTAGTGTGTG
CCCGTCTGTTGTGTGACTCTGGTAACTAGAGATCCCTCAGACCCTTTTAGTC
AGTGTGGAAAATCTCTAGCAGTGGCGCCCGAACAGGGACTTGAAAGCGAAA
GGGAAACCAGAGGAGCTCTCTCGACGCAGGACTCGGCTTGCTGAAGCGCGC
ACGGCAAGAGGCGAGGGGCGGCGACTGGTGAGTACGCCAAAAATTTTGACT
AGCGGAGGCTAGAAGGAGAGAGATGGGTGCGAGAGCGTCAGTATTAAGCG
GGGGAGAATTAGATCGCGATGGGAAAAAATTCGGTTAAGGCCAGGGGGAA
AGAAAAAATATAAATTAAAACATATAGTATGGGCAAGCAGGGAGCTAGAA
CGATTCGCAGTTAATCCTGGCCTGTTAGAAACATCAGAAGGCTGTAGACAA
ATACTGGGACAGCTACAACCATCCCTTCAGACAGGATCAGAAGAACTTAGA
TCATTATATAATACAGTAGCAACCCTCTATTGTGTGCATCAAAGGATAGAGA
TAAAAGACACCAAGGAAGCTTTAGACAAGATAGAGGAAGAGCAAAACAAA
AGTAAGACCACCGCACAGCAAGCCGCCGCTGATCTTCAGACCTGGAGGAGG
AGATATGAGGGACAATTGGAGAAGTGAATTATATAAATATAAAGTAGTAAA
AATTGAACCATTAGGAGTAGCACCCACCAAGGCAAAGAGAAGAGTGGTGCA
GAGAGAAAAAAGAGCAGTGGGAATAGGAGCTTTGTTCCTTGGGTTCTTGGG
AGCAGCAGGAAGCACTATGGGCGCAGCGTCAATGACGCTGACGGTACAGGC
CAGACAATTATTGTCTGGTATAGTGCAGCAGCAGAACAATTTGCTGAGGGCT
ATTGAGGCGCAACAGCATCTGTTGCAACTCACAGTCTGGGGCATCAAGCAG
CTCCAGGCAAGAATCCTGGCTGTGGAAAGATACCTAAAGGATCAACAGCTC
CTGGGGATTTGGGGTTGCTCTGGAAAACTCATTTGCACCACTGCTGTGCCTT
GGAATGCTAGTTGGAGTAATAAATCTCTGGAACAGATTTGGAATCACACGA
CCTGGATGGAGTGGGACAGAGAAATTAACAATTACACAAGCTTAATACACT
CCTTAATTGAAGAATCGCAAAACCAGCAAGAAAAGAATGAACAAGAATTAT
TGGAATTAGATAAATGGGCAAGTTTGTGGAATTGGTTTAACATAACAAATTG
GCTGTGGTATATAAAATTATTCATAATGATAGTAGGAGGCTTGGTAGGTTTA
AGAATAGTTTTTGCTGTACTTTCTATAGTGAATAGAGTTAGGCAGGGATATT
CACCATTATCGTTTCAGACCCACCTCCCAACCCCGAGGGGACCCGACAGGCC

CGAAGGAATAGAAGAAGAAGGTGGAGAGAGAGACAGAGACAGATCCATTC
GATTAGTGAACGGATCTCGACGGTATCGGTTAACTTTTAAAAGAAAAGGGG
GGATTGGGGGGTACAGTGCAGGGGAAAGAATAGTAGACATAATAGCAACA
GACATACAAACTAAAGAATTACAAAAACAAATTACAAAATTCAAAATTTTA
TCGCGATCGCGGAATGAAAGACCCCACCTGTAGGTTTGGCAAGCTAGCTTA
AGTAACGCCATTTTGCAAGGCATGGAAAATACATAACTGAGAATAGAGAAG
TTCAGATCAAGGTTAGGAACAGAGAGACAGCAGAATATGGGCCAAACAGG
ATATCTGTGGTAAGCAGTTCCTGCCCCGGCTCAGGGCCAAGAACAGATGGT
CCCCAGATGCGGTCCCGCCCTCAGCAGTTTCTAGAGAACCATCAGATGTTTC
CAGGGTGCCCCAAGGACCTGAAAATGACCCTGTGCCTTATTTGAACTAACCA
ATCAGTTCGCTTCTCGCTTCTGTTCGCGCGCTTCTGCTCCCCGAGCTCAATAA
AAGAGCCCACAACCCCTCACTCGGCGCGCCAGTCCTTCGAAGTAGATCTTTG
TCGATCCTACCATCCACTCGACACACCCGCCAGCGGCCGCTGCCAAGCTTCC
GAGCTCTCGAATTAATTCACGGTACCCACCATGGCCTAGGGAGACTAGTCG
AATCGATATCAACCTCTGGATTACAAAATTTGTGAAAGATTGACTGGTATTC
TTAACTATGTTGCTCCTTTTACGCTATGTGGATACGCTGCTTTAATGCCTTTG
TATCATGCTATTGCTTCCCGTATGGCTTTCATTTTCTCCTCCTTGTATAAATCC
TGGTTGCTGTCTCTTTATGAGGAGTTGTGGCCCGTTGTCAGGCAACGTGGCG
TGGTGTGCACTGTGTTTGCTGACGCAACCCCCACTGGTTGGGGCATTGCCAC
CACCTGTCAGCTCCTTTCCGGGACTTTCGCTTTCCCCCTCCCTATTGCCACGG
CGGAACTCATCGCCGCCTGCCTTGCCCGCTGCTGGACAGGGGCTCGGCTGTT
GGGCACTGACAATTCCGTGGTGTTGTCGGGGAAGCTGACGTCCTTTTCATGG
CTGCTCGCCTGTGTTGCCACCTGGATTCTGCGCGGGACGTCCTTCTGCTACGT
CCCTTCGGCCCTCAATCCAGCGGACCTTCCTTCCCGCGGCCTGCTGCCGGCT
CTGCGGCCTCTTCCGCGTCTTCGCCTTCGCCCTCAGACGAGTCGGATCTCCCT
TTGGGCCGCCTCCCCGCCTGGTTAATTAAAGTACCTTTAAGACCAATGACTT
ACAAGGCAGCTGTAGATCTTAGCCACTTTTTAAAAGAAAAGGGGGGACTGG
AAGGGCGAATTCACTCCCAACGAAGACAAGATCTGCTTTTTGCTTGTACTGG
GTCTCTCTGGTTAGACCAGATCTGAGCCTGGGAGCTCTCTGGCTAACTAGGG
AACCCACTGCTTAAGCCTCAATAAAGCTTGCCTTGAGTGCTTCAAGTAGTGT
GTGCCCGTCTGTTGTGTGACTCTGGTAACTAGAGATCCCTCAGACCCTTTTA
GTCAGTGTGGAAAATCTCTAGCAGGCATGCCAGACATGATAAGATACATTG
ATGAGTTTGGACAAACCACAACTAGAATGCAGTGAAAAAAATGCTTTATTT

GTGAAATTTGTGATGCTATTGCTTTATTTGTAACCATTATAAGCTGCAATAA
ACAAGTTAACAACAACAATTGCATTCATTTTATGTTTCAGGTTCAGGGGGAG
GTGTGGGAGGTTTTTTGGCGCGCCATCGTCGAGGTTCCCTTTAGTGAGGGTT
AATTGCGAGCTTGGCGTAATCATGGTCATAGCTGTTTCCTGTGTGAAATTGT
TATCCGCTCACAATTCCACACAACATACGAGCCGGAAGCATAAAGTGTAAA
GCCTGGGGTGCCTAATGAGTGAGCTAACTCACATTAATTGCGTTGCGCTCAC
TGCCCGCTTTCCAGTCGGGAAACCTGTCGTGCCAGCTGCATTAATGAATCGG
CCAACGCGCGGGGAGAGGCGGTTTGCGTATTGGGCGCTCTTCCGCTTCCTCG
CTCACTGACTCGCTGCGCTCGGTCGTTCGGCTGCGGCGAGCGGTATCAGCTC
ACTCAAAGGCGGTAATACGGTTATCCACAGAATCAGGGGATAACGCAGGAA
AGAACATGTGAGCAAAAGGCCAGCAAAAGGCCAGGAACCGTAAAAAGGCC
GCGTTGCTGGCGTTTTTCCATAGGCTCCGCCCCCCTGACGAGCATCACAAAA
ATCGACGCTCAAGTCAGAGGTGGCGAAACCCGACAGGACTATAAAGATACC
AGGCGTTTCCCCCTGGAAGCTCCCTCGTGCGCTCTCCTGTTCCGACCCTGCC
GCTTACCGGATACCTGTCCGCCTTTCTCCCTTCGGGAAGCGTGGCGCTTTCTC
ATAGCTCACGCTGTAGGTATCTCAGTTCGGTGTAGGTCGTTCGCTCCAAGCT
GGGCTGTGTGCACGAACCCCCCGTTCAGCCCGACCGCTGCGCCTTATCCGGT
AACTATCGTCTTGAGTCCAACCCGGTAAGACACGACTTATCGCCACTGGCAG
CAGCCACTGGTAACAGGATTAGCAGAGCGAGGTATGTAGGCGGTGCTACAG
AGTTCTTGAAGTGGTGGCCTAACTACGGCTACACTAGAAGAACAGTATTTGG
TATCTGCGCTCTGCTGAAGCCAGTTACCTTCGGAAAAAGAGTTGGTAGCTCT
TGATCCGGCAAACAAACCACCGCTGGTAGCGGTGGTTTTTTTGTTTGCAAGC
AGCAGATTACGCGCAGAAAAAAAGGATCTCAAGAAGATCCTTTGATCTTTT
CTACGGGGTCTGACGCTCAGTGGAACGAAAACTCACGTTAAGGGATTTTGG
TCATGAGATTATCAAAAAGGATCTTCACCTAGATCCTTTTAAATTAAAAATG
AAGTTTTAAATCAATCTAAAGTATATATGAGTAAACTTGGTCTGACAGTTAC
CAATGCTTAATCAGTGAGGCACCTATCTCAGCGATCTGTCTATTTCGTTCATC
CATAGTTGCCTGACTCCCCGTCGTGTAGATAACTACGATACGGGAGGGCTTA
CCATCTGGCCCCAGTGCTGCAATGATACCGCGAGACCCACGCTCACCGGCTC
CAGATTTATCAGCAATAAACCAGCCAGCCGGAAGGGCCGAGCGCAGAAGTG
GTCCTGCAACTTTATCCGCCTCCATCCAGTCTATTAATTGTTGCCGGGAAGCT
AGAGTAAGTAGTTCGCCAGTTAATAGTTTGCGCAACGTTGTTGCCATTGCTA
CAGGCATCGTGGTGTCACGCTCGTCGTTTGGTATGGCTTCATTCAGCTCCGG

TTCCCAACGATCAAGGCGAGTTACATGATCCCCCATGTTGTGCAAAAAAGC
GGTTAGCTCCTTCGGTCCTCCGATCGTTGTCAGAAGTAAGTTGGCCGCAGTG
TTATCACTCATGGTTATGGCAGCACTGCATAATTCTCTTACTGTCATGCCATC
CGTAAGATGCTTTTCTGTGACTGGTGAGTACTCAACCAAGTCATTCTGAGAA
TAGTGTATGCGGCGACCGAGTTGCTCTTGCCCGGCGTCAATACGGGATAATA
CCGCGCCACATAGCAGAACTTTAAAAGTGCTCATCATTGGAAAACGTTCTTC
GGGGCGAAAACTCTCAAGGATCTTACCGCTGTTGAGATCCAGTTCGATGTAA
CCCACTCGTGCACCCAACTGATCTTCAGCATCTTTTACTTTCACCAGCGTTTC
TGGGTGAGCAAAAACAGGAAGGCAAAATGCCGCAAAAAAGGGAATAAGGG
CGACACGGAAATGTTGAATACTCATACTCTTCCTTTTTCAATATTATTGAAG
CATTTATCAGGGTTATTGTCTCATGAGCGGATACATATTTGAATGTATTTAG
AAAAATAAACAAATAGGGGTTCCGCGCACATTTCCCCGAAAAGTGCCAC

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



[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, TtRMPVIR, pMSCV-IRES-mCherry FP, pRetroX GFP T2A Cre, pRXTN, pLncEXP, and pLXIN-Luc.

[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 (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. Also 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 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 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; 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.

[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; 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 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 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 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, 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 (CDl 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, CDl-ld, ITGAE, CD103, ITGAL, CDl-la, LFA-1, ITGAM, CDl-lb, ITGAX, CDl-lc, ITGBl, 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 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 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 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 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 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 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 $GX_2X_3X_4X_5X_6X_7X_8X_9$ (SEQ ID NO: 134), wherein X_2 is G, F, or Y; X_3 is S or T; X_4 is I, F, or L; X_5 is S or T; X_6 is not present or S; X_7 is not present or G; X_8 is not present or E or G; and X_9 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 of D or E;

(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;

(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;

(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.

[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.

[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), 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 autoimmune diseases such as rheumatoid arthritis, psoriasis, allergies, asthma, Crohn's disease, IBD, IBS, fibromyalga, 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 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.

[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 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.

[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.

[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, 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.

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:

CTTGATAATGAAAAGTCAAACGGAACAATCATTCACGTGAAGGGCAAGC
ACCTCTGTCCGTCACCCTTGTTCCCTGGTCCATCCAAGCCATTCTGGGTGT
TGGTCGTAGTGGGTGGAGTCCTCGCTTGTTACTCTCTGCTCGTCACCGTG
GCTTTTATAATCTTCTGGGTTAGATCCAAAAGAAGCCGCCTGCTCCATAG
CGATTACATGAATATGACTCCACGCCGCCCTGGCCCCACAAGGAAACAC
TACCAGCCTTACGCACCACCTAGAGATTTCGCTGCCTATCGGAGC

[0070] The corresponding amino acid sequence is set forth in SEQ ID NO. 2:

LDNEKSNGTIIHVKGKHLCPSPLFPGPSKPFWVLVVVGGVLACYSLLVTVAF
IIFWVRSK RSRLLHSDYM NMTPRRPGPT RKHYQPYAPP RDFAAYRS

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

CTTGATAATGAAAAGTCAAACGGAACAATCATTCACGTGAAGGGCAAGC
ACCTCTGTCCGTCACCCTTGTTCCCTGGTCCATCCAAGCCA

[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
GTCACCGTGGCTTTTATAATCTTCTGGGTT

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

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

AGATCCAAAAGAAGCCGCCTGCTCCATAGCGATTACATGAATATGACTC
CACGCCGCCCTGGCCCCACAAGGAAACACTACCAGCCTTACGCACCACC
TAGAGATTTCGCTGCCTATCGGAGC

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

RSKRSRLLHSDYMNMTPRRPGPTRKHYQPYAPPRDFAAYRS

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

ATTGAGGTGATGTATCCACCGCCTTACCTGGATAACGAAAAGAGTAACG
GTACCATCATTCACGTGAAAGGTAAACACCTGTGTCCTTCTCCCCTCTTC
CCCGGGCCATCAAAGCCC

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

IEVMYPPPYLDNEKSNGTIIHVKGKHLCPSPLFPGPSKP

[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
CGACCGGCCGCAGGGGGCGCTGTTCATACCAGAGGACTGGATTTCGCCT
GCGATATCTATATCTGGGCACCCCTGGCCGGAACCTGCGGCGTACTCCTG
CTGTCCCTGGTCATCACGCTCTATTGTAATCACAGGAAC

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

AAALSNSIMYFSHFVPVFLPAKPTTTPAPRPPTPAPTIASQPLSLRPEACRPAA
GGAVHTRGLDFACDIYIWAPLAGTCGVLLLSLVITLYCNHRN

[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 (CDl

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, CDl-ld, ITGAE, CD103, ITGAL, CDl-la, LFA-1, ITGAM, CDl-lb, ITGAX, CDl-lc, ITGBl, 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.

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
AGAACCAACTGTATAACGAGCTCAACCTGGGACGCAGGGAAGAGTATG
ACGTTTTGGACAAGCGCAGAGGACGGGACCCTGAGATGGGTGGCAAACC
AAGACGAAAAAACCCCCAGGAGGGTCTCTATAATGAGCTGCAGAAGGA
TAAGATGGCTGAAGCCTATTCTGAAATAGGCATGAAAGGAGAGCGGAG
AAGGGGAAAAGGGCACGACGGTTTGTACCAGGGACTCAGCACTGCTACG
AAGGATACTTATGACGCTCTCCACATGCAAGCCCTGCCACCTAGG

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

RVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPR
RKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDT
YDALHMQALPPR

Domain Orientation Relative to the Cell

[0086] Structurally, it will 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 (CDl 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, IL2R beta, IL2R gamma, IL7R alpha, ITGA4, VLA1, CD49a, ITGA4, IA4, CD49D, ITGA6, VLA-6, CD49f, ITGAD, CDl-ld, ITGAE, CD103, ITGAL, CDl-la, LFA-1, ITGAM, CDl-lb, ITGAX, CDl-lc, ITGBl, 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, Ly108), 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 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), 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 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, CDl-la/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, 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, CDl ld, ITGAE, CD103, ITGAL, CDl la, LFA-1, ITGAM, CDl lb, ITGAX, CDl lc, ITGBl, 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, CDl-la/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, 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, CDl ld, ITGAE, CD103, ITGAL, CDl la, LFA-1, ITGAM, CDl lb, ITGAX, CDl lc, ITGBl, 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.

[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, CDl-la/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, 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, CDl ld, ITGAE, CD103, ITGAL, CDl la, LFA-1, ITGAM, CDl lb, ITGAX, CDl lc, ITGBl, 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.

[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 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.

[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.*, ±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 $GX_2X_3X_4X_5X_6X_7X_8X_9$ (SEQ ID NO: 134), wherein X_2 is G, F, or Y; X_3 is S or T; X_4 is I, F, or L; X_5 is S or T; X_6 is not present or S; X_7 is not present or G; X_8 is not present or E or G; and X_9 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 of D or E;

(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;

(d) a light chain variable region (VL) CDR1 comprising the amino acid sequence X_1ASQ$X_5X_6X_7X_8X_9$LX_{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;

(e) a light chain variable region (VL) CDR2 comprising the amino acid sequence X_1AS$X_4X_5X_6X_7$ (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 QQ$X_3X_4X_5X_6$PX_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.

[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.

[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 (V_L) 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).

[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.

[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 of fragment thereof, including one or more of the complementarity determining regions (CDRs) thereof. In further 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 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 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 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.

[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 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.

[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"), 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 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, 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. 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 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 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.

[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 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 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 $1\text{-}5 \times 10^{-9}$ M, and with "very high affinity" when the K_d is $1\text{-}5 \times 10^{-10}$ M. In one embodiment, the antigen binding molecule has a K_d of

10^{-9} M. In one embodiment, the off-rate is $<1x10^{-5}$. 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-$5x10^{-10}$.

[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 x 10^{-6} M, less than 1 x 10^{-7} M, less than 1 x 10^{-8} M, or less than 1 x 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 x 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 x 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 x 10^{-7} M, about 2 x 10^{-7} M, about 3 x 10^{-7} M, about 4 x 10^{-7} M, about 5 x 10^{-7} M, about 6 x 10^{-7} M, about 7 x 10^{-7} M, about 8 x 10^{-7} M, about 9 x 10^{-7} M, about 1 x 10^{-8} M, about 2 x 10^{-8} M, about 3 x 10^{-8} M, about 4 x 10^{-8} M, about 5 x 10^{-8} M, about 6 x 10^{-8} M, about 7 x 10^{-8} M, about 8 x 10^{-8} M, about 9 x 10^{-8} M, about 1 x 10^{-9} M, about 2 x 10^{-9} M, about 3 x 10^{-9} M, about 4 x 10^{-9} M, about 5 x 10^{-9} M, about 6 x 10^{-9} M, about 7 x 10^{-9} M, about 8 x 10^{-9} M, about 9 x 10^{-9} M, about 1 x 10^{-10} M, or about 5 x 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 x 10^{-9} M, less than 3 x 10^{-9} M, less than 5 x 10^{-9} M, less than 1 x 10^{-10} M, less than 3 x 10^{-10} M, or less than 5 x 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 x 10^{-5} M, less than 1 x 10^{-6} M, less than 1 x 10^{-7} M, less than 1 x 10^{-8} M, less than 1 x 10^{-9} M, or less than 1 x 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 x 10^{-4} M^{-1} s^{-1}, less than 2 x 10^{-4} M^{-1} s^{-1}, less than 3 x 10^{-4} M^{-1} s^{-1}, less than 4 x 10^{-4} M^{-1} s^{-1}, less than 5 x 10^{-4} M^{-1} s^{-1}, less than 6 x 10^{-4} M^{-1} s^{-1}, less than 7 x 10^{-4} M^{-1} s^{-1}, less than 8 x 10^{-4} M^{-1} s^{-1}, less than 9 x 10^{-4} M^{-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.

[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}, 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 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 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, 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 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 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.

[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 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, 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-HI 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.*, IgG_1, IgG_2, IgG_3 and IgG_4.

[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 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 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 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 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 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 (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 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 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 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 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 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 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, 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, fibromyalga, mastocytosis, and Celiac disease.

[0177] It will be appreciated that target doses for CAR^+/ $CAR\text{-}T^+$/ TCR^+ cells can range from $1x10^6$ - $2x10^{10}$ cells/kg, preferably $2x10^6$ 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, 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 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 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 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/m^2/day and 2000 mg/m^2/day) and specified doses of fludarabine (between 20 mg/m^2/day and 900 mg/m^2/day). A preferred dose regimen involves treating a patient comprising administering daily to the patient about 500 mg/m^2/day of cyclophosphamide and about 60 mg/m^2/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 (CYTOXAN™); alkyl sulfonates such as busulfan, improsulfan and piposulfan; aziridines such as benzodopa, carboquone, meturedopa, and uredopa; ethylenimines and methylamelamines including altretamine, triethylenemelamine, trietylenephosphoramide, triethylenethiophosphaoramide and trimethylolomelamine resume; nitrogen mustards such as chlorambucil, chlornaphazine, cholophosphamide, 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, authramycin, azaserine, bleomycins, cactinomycin, calicheamicin, carabicin, 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, epitiostanol, mepitiostane, testolactone; anti-adrenals such as aminoglutethimide, mitotane, trilostane; folic acid replenisher such as frolinic acid; aceglatone; aldophosphamide glycoside; aminolevulinic acid; amsacrine; bestrabucil;

bisantrene; 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.

[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 (Erbitux®), panitumumab (Vectibix®), catumaxomab, ibritumomab, ofatumumab, tositumomab, brentuximab, 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, 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 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 mycophenolate. Exemplary NSAIDs include ibuprofen, naproxen, naproxen sodium, Cox-2 inhibitors, and sialylates. Exemplary analgesics include acetaminophen, oxycodone, tramadol of proporxyphene hydrochloride. Exemplary glucocorticoids include cortisone, dexamethasone, hydrocortisone, methylprednisolone, prednisolone, or prednisone. Exemplary biological response modifiers include molecules directed against cell surface 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 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; 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 (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, 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.

[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.

[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 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 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 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 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 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 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 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 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 *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 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 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, Enterobactehaceae such as Escherichia, *e.g.*, E. coli, Enterobacter, Erwinia, Klebsiella, Proteus, Salmonella, *e.g.*, Salmonella typhimurium, Serratia, *e.g.*, Serratia marcescans, 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 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 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 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, 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 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, polyvinylpyrrolidine (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 10^2 cells, and up to 10^6, up to and including 10^8 or 10^9 cells and can be more than 10^{10} 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 10^6 cells/ml and generally is greater than 10^7 cells/ml, generally 10^8 cells/ml or greater. The clinically relevant number of immune cells can be apportioned into multiple infusions that cumulatively equal or exceed 10^5, 10^6, 10^7, 10^8, 10^9, 10^{10}, 10^{11}, or 10^{12} 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.

[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 more pharmaceutically or physiologically acceptable carriers, diluents or excipients. Such compositions may comprise buffers such as neutral buffered saline, phosphate buffered saline and the like; carbohydrates such as glucose, mannose, sucrose or dextrans, mannitol; proteins; polypeptides or amino acids such as glycine; antioxidants; chelating agents such as EDTA or glutathione; adjuvants (*e.g.*, aluminum hydroxide); and preservatives. Compositions of the present invention are 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 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 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. 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 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 internucleotide linkage modifications such as phosphorothioate, phosphorodithioate, phosphoroselenoate, phosphoro-diselenoate, phosphoro-anilothioate, phoshoraniladate 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 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 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, 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 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 MALPVTALLLPLALLLHAARP (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;
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.

Table 3

Original Residues	Exemplary Substitutions	Preferred Substitutions
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	Arg
	Acid, Gln, Asn	
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.

[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 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 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 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 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.

[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.

[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

[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 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.

[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 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.

[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 $2.5x10^7$ 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 0.5-2.0 x 10^6 cells/ml prior to use in activity assays.

[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 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 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 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. Simultaneously, 293T cells (ATCC) were trypsinized, counted and a total of 10x10^6 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 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 0.5-2.0 x 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°±2°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 Lifor® 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 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 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 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. 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 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. 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.

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

[0255] CD28T DNA Extracellular, transmembrane, intracellular

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

[0256] CD28T Extracellular, transmembrane, intracellular AA

LDNEKSNGTIIHVKGKHLCPSPLFPGPSKPFWVLVVVGGVLACYSLL VTVAFIIFWVRSKRSRLLHSDYMNMTPRRPGPTRKHYQPYAPPRDFA AYRS (SEQ ID NO. 2)

[0257] CD28T DNA - Extracellular

CTTGATAATGAAAAGTCAAACGGAACAATCATTCACGTGAAGGG CAAGCACCTCTGTCCGTCACCCTTGTTCCCTGGTCCATCCAAGCCA (SEQ ID NO. 3)

[0258] CD28T AA - Extracellular

LDNEKSNGTI IHVKGKHLCP SPLFPGPSKP (SEQ ID NO. 4)

[0259] CD28 DNA Transmembrane Domain

TTCTGGGTGTTGGTCGTAGTGGGTGGAGTCCTCGCTTGTTACTCTC TGCTCGTCACCGTGGCTTTTATAATCTTCTGGGTT (SEQ ID NO. 5)

[0260] CD28 AA Transmembrane Domain

FWVLVVVGGV LACYSLLVTV AFIIFWV (SEQ ID NO. 6)

[0261] CD28 DNA Intracellular Domain

AGATCCAAAAGAAGCCGCCTGCTCCATAGCGATTACATGAATATG
ACTCCACGCCGCCCTGGCCCCACAAGGAAACACTACCAGCCTTAC
GCACCACCTAGAGATTTCGCTGCCTATCGGAGC (SEQ ID NO. 7)

[0262] CD28 AA Intracellular Domain

RSKRSRLLHSDYMNMTPRRPGPTRKHYQPYAPPRDFAAYRS (SEQ ID NO. 8)

[0263] CD3 zeta DNA

AGGGTGAAGTTTTCCAGATCTGCAGATGCACCAGCGTATCAGCAG
GGCCAGAACCAACTGTATAACGAGCTCAACCTGGGACGCAGGGA
AGAGTATGACGTTTTGGACAAGCGCAGAGGACGGGACCCTGAGA
TGGGTGGCAAACCAAGACGAAAAAACCCCCAGGAGGGTCTCTAT
AATGAGCTGCAGAAGGATAAGATGGCTGAAGCCTATTCTGAAAT
AGGCATGAAAGGAGAGCGGAGAAGGGGAAAAGGGCACGACGGT
TTGTACCAGGGACTCAGCACTGCTACGAAGGATACTTATGACGCT
CTCCACATGCAAGCCCTGCCACCTAGG (SEQ ID NO. 9)

[0264] CD3 zeta AA

RVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEM
GGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLY
QGLSTATKDTYDALHMQALPPR (SEQ ID NO. 10)

[0265] CD3 zeta variant AA

RVKFSRSADAPAYKQGQNQLYNELNLGRREEYDVLDKRRGRDPEM
GGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLY
QGLSTATKDTYDALHMQALPPR (SEQ ID NO. 146)

[0266] CD28 DNA

ATTGAGGTGATGTATCCACCGCCTTACCTGGATAACGAAAAGAGT
AACGGTACCATCATTCACGTGAAAGGTAAACACCTGTGTCCTTCT
CCCCTCTTCCCCGGGCCATCAAAGCCC (SEQ ID NO. 11)

[0267] CD28 AA

IEVMYPPPYLDNEKSNGTIIHVKGKHLCPSPLFPGPSKP (SEQ ID NO. 12)

[0268] CD8 DNA extracellular & transmembrane domain

GCTGCAGCATTGAGCAACTCAATAATGTATTTTAGTCACTTTGTAC CAGTGTTCTTGCCGGCTAAGCCTACTACCACACCCGCTCCACGGC CACCTACCCCAGCTCCTACCATCGCTTCACAGCCTCTGTCCCTGCG CCCAGAGGCTTGCCGACCGGCCGCAGGGGGCGCTGTTCATACCAG AGGACTGGATTTCGCCTGCGATATCTATATCTGGGCACCCCTGGC CGGAACCTGCGGCGTACTCCTGCTGTCCCTGGTCATCACGCTCTAT TGTAATCACAGGAAC (SEQ ID NO. 13)

[0269] CD8 AA extracellular & transmembrane Domain

AAALSNSIMYFSHFVPVFLPAKPTTTPAPRPPTPAPTIASQPLSLRPEA CRPAAGGAVHTRGLDFACDIYIWAPLAGTCGVLLLSLVITLYCNHRN (SEQ ID NO. 14)

[0270] Clone 24C1 HC DNA

CAGGTGCAGCTGCAGGAATCCGGACCGGGGCTGGTGAAGCCCAG CGAGACTCTGAGTCTCACGTGTACAGTTTCTGGAGGTAGCATTAG CTCCTACTATTGGTCATGGATAAGGCAGCCCCCCGGGAAGGGATT GGAATGGATCGGCTATATTTACTACAGTGGGAGCACCAATTACAA CCCCTCACTGAAGTCTAGAGTTACAATCAGCGTTGACACCTCAAA GAATCAGTTCAGTTTGAAATTGTCTAGCGTCACAGCAGCTGATAC AGCCGTCTATTATTGTGTTTCTCTGGTCTATTGCGGTGGGGATTGT TACAGTGGCTTTGACTATTGGGGGCAGGGTACTCTGGTTACAGTT TCTTCC (SEQ ID NO. 15)

[0271] Clone 24C1 HC AA (CDRs Underlined)

QVQLQESGPGLVKPSETLSLTCTVSGGSISSYYWSWIRQPPGKGLEWI GY

IYYSGSTNYNPSLKSRVTISVDTSKNQFSLKLSSVTAADTAVYYCVSL VYCGGDCYSGFDYWGQGTLVTVSS (SEQ ID NO. 16)

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

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

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

[0275] Clone 24C1 LC DNA

GACATCCAGTTGACACAGAGCCCGAGTTCCTTGTCCGCCTCCGTC
GGGGATAGAGTGTCATTTACCTGTCAGGCCTCTCAGGATATTAAT
AACTTTCTGAATTGGTATCAGCAAAAGCCCGGAAAGGCACCCAAG
CTGTTGATTTACGACGCCAGTAACCTGGAGACAGGCGTGCCCTCC
CGGTTTAGTGGTAGCGGAAGCGGTACGGATTTTACCTTTACTATC
AGCTCTCTCCAACCCGAAGACATTGCAACCTACTATTGTCAACAA
TATGGAAACCTGCCTTTTACATTTGGCGGCGGCACCAAGGTGGAG
ATTAAGCGG (SEQ ID NO. 20)

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

DIQLTQSPSSLSASVGDRVSFTCQASQDINNFLNWYQQKPGKAPKLLI
YDASNLETGVPSRFSGSGSGTDFTFTISSLQPEDIATYYCQQYGNLPFT
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)

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

ATGGCACTCCCCGTAACTGCTCTGCTGCTGCCGTTGGCATTGCTCC
TGCACGCCGCACGCCCGCAGGTCCAACTGCAAGAAAGCGGACCC
GGACTGGTGAAGCCTTCTGAGACACTTAGTCTGACGTGCACGGTC
AGTGGCGGCTCCATCTCCTCCTATTATTGGTCATGGATACGACAA
CCCCCAGGTAAGGGCCTGGAATGGATTGGCTATATCTACTATTCA
GGAAGCACGAACTACAATCCCAGCCTGAAGTCCCGAGTGACAATT
TCAGTAGATACCAGTAAAAACCAGTTCAGTCTTAAACTGTCAAGC
GTGACAGCTGCCGACACCGCTGTGTATTACTGCGTCTCACTGGTG
TATTGTGGAGGGGATTGTTATAGCGGGTTCGATTATTGGGGACAG
GGAACCCTGGTGACTGTATCTTCCGGCGGCGGCGGCTCAGGGGGT
GGCGGTAGTGGCGGTGGGGGTTCCGATATTCAACTGACACAATCC

CCCAGCTCACTCAGCGCCAGCGTGGGGGACAGGGTTAGCTTTACC
TGTCAAGCCTCTCAGGATATAAATAACTTTCTGAACTGGTATCAA
CAGAAGCCTGGGAAGGCGCCCAAACTCCTGATCTATGATGCGTCC
AACCTGGAAACTGGCGTGCCTTCACGCTTTAGCGGCTCTGGCAGT
GGTACAGACTTCACTTTTACCATCTCTTCACTTCAGCCGGAGGACA
TCGCCACATATTACTGTCAACAGTACGGAAACTTGCCCTTTACTTT
TGGAGGCGGCACCAAAGTTGAAATCAAAAGGGCCGCTGCCCTGG
ATAACGAAAAGAGCAATGGGACTATAATACATGTTAAAGGAAAA
CACCTGTGTCCATCTCCCCTGTTCCCTGGACCGTCAAAGCCATTTT
GGGTGCTCGTGGTTGTCGGTGGCGTTCTCGCCTGTTATAGCTTGCT
GGTGACAGTAGCCTTCATTATCTTTTGGGTGAGATCCAAAAGAAG
CCGCCTGCTCCATAGCGATTACATGAATATGACTCCACGCCGCCC
TGGCCCCACAAGGAAACACTACCAGCCTTACGCACCACCTAGAGA
TTTCGCTGCCTATCGGAGCAGGGTGAAGTTTTCCAGATCTGCAGA
TGCACCAGCGTATCAGCAGGGCCAGAACCAACTGTATAACGAGCT
CAACCTGGGACGCAGGGAAGAGTATGACGTTTTGGACAAGCGCA
GAGGACGGGACCCTGAGATGGGTGGCAAACCAAGACGAAAAAAC
CCCCAGGAGGGTCTCTATAATGAGCTGCAGAAGGATAAGATGGCT
GAAGCCTATTCTGAAATAGGCATGAAAGGAGAGCGGAGAAGGGG
AAAAGGGCACGACGGTTTGTACCAGGGACTCAGCACTGCTACGA
AGGATACTTATGACGCTCTCCACATGCAAGCCCTGCCACCTAGGT
AA (SEQ ID NO. 25)

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

(Signal Peptide in **bold**)

MALPVTALLLPLALLLHAARPQVQLQESGPGLVKPSETLSLTCTVS
GGSISSYYWSWIRQPPGKGLEWIGYIYYSGSTNYNPSLKSRVTISVDT
SKNQFSLKLSSVTAADTAVYYCVSLVYCGGDCYSGFDYWGQGTLV
TVSSGGGGSGGGGSGGGGSDIQLTQSPSSLSASVGDRVSFTCQASQDI
NNFLNWYQQKPGKAPKLLIYDASNLETGVPSRFSGSGSGTDFTFTISS
LQPEDIATYYCQQYGNLPFTFGGGTKVEIKRAAALDNEKSNGTIIHV
KGKHLCPSPLFPGPSKPFWVLVVVGGVLACYSLLVTVAFIIFWVRSK
RSRLLHSDYMNMTPRRPGPTRKHYQPYAPPRDFAAYRSRVKFSRSA
DAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKN

PQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATK
DTYDA LHMQALPPR (SEQ ID NO. 26)

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

CAGGTCCAACTGCAAGAAAGCGGACCCGGACTGGTGAAGCCTTCT
GAGACACTTAGTCTGACGTGCACGGTCAGTGGCGGCTCCATCTCC
TCCTATTATTGGTCATGGATACGACAACCCCCAGGTAAGGGCCTG
GAATGGATTGGCTATATCTACTATTCAGGAAGCACGAACTACAAT
CCCAGCCTGAAGTCCCGAGTGACAATTTCAGTAGATACCAGTAAA
AACCAGTTCAGTCTTAAACTGTCAAGCGTGACAGCTGCCGACACC
GCTGTGTATTACTGCGTCTCACTGGTGTATTGTGGAGGGGATTGTT
ATAGCGGGTTCGATTATTGGGGACAGGGAACCCTGGTGACTGTAT
CTTCCGGCGGCGGCGGCTCAGGGGGTGGCGGTAGTGGCGGTGGG
GGTTCCGATATTCAACTGACACAATCCCCCAGCTCACTCAGCGCC
AGCGTGGGGGACAGGGTTAGCTTTACCTGTCAAGCCTCTCAGGAT
ATAAATAACTTTCTGAACTGGTATCAACAGAAGCCTGGGAAGGCG
CCCAAACTCCTGATCTATGATGCGTCCAACCTGGAAACTGGCGTG
CCTTCACGCTTTAGCGGCTCTGGCAGTGGTACAGACTTCACTTTTA
CCATCTCTTCACTTCAGCCGGAGGACATCGCCACATATTACTGTCA
ACAGTACGGAAACTTGCCCTTTACTTTTGGAGGCGGCACCAAAGT
TGAAATCAAAAGGGCCGCTGCCCTGGATAACGAAAAGAGCAATG
GGACTATAATACATGTTAAAGGAAAACACCTGTGTCCATCTCCCC
TGTTCCCTGGACCGTCAAAGCCATTTTGGGTGCTCGTGGTTGTCGG
TGGCGTTCTCGCCTGTTATAGCTTGCTGGTGACAGTAGCCTTCATT
ATCTTTTGGGTGAGATCCAAAAGAAGCCGCCTGCTCCATAGCGAT
TACATGAATATGACTCCACGCCGCCCTGGCCCCACAAGGAAACAC
TACCAGCCTTACGCACCACCTAGAGATTTCGCTGCCTATCGGAGC
AGGGTGAAGTTTTCCAGATCTGCAGATGCACCAGCGTATCAGCAG
GGCCAGAACCAACTGTATAACGAGCTCAACCTGGGACGCAGGGA
AGAGTATGACGTTTTGGACAAGCGCAGAGGACGGGACCCTGAGA
TGGGTGGCAAACCAAGACGAAAAAACCCCCAGGAGGGTCTCTAT
AATGAGCTGCAGAAGGATAAGATGGCTGAAGCCTATTCTGAAAT
AGGCATGAAAGGAGAGCGGAGAAGGGGAAAAGGGCACGACGGT

TTGTACCAGGGACTCAGCACTGCTACGAAGGATACTTATGACGCT
CTCCACATGCAAGCCCTGCCACCTAGG (SEQ ID NO. 27)

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

QVQLQESGPGLVKPSETLSLTCTVSGGSISSYYWSWIRQPPGKGLEWI
GYIYYSGSTNYNPSLKSRVTISVDTSKNQFSLKLSSVTAADTAVYYC
VSLVYCGGDCYSGFDYWGQGTLVTVSSGGGGSGGGGSGGGGSDIQ
LTQSPSSLSASVGDRVSFTCQASQDINNFLNWYQQKPGKAPKLLIYD
ASNLETGVPSRFSGSGSGTDFTFTISSLQPEDIATYYCQQYGNLPFTFG
GGTKVEIKRAAALDNEKSNGTIIHVKGKHLCPSPLFPGPSKPFWVLV
VVGGVLACYSLLVTVAFIIFWVRSKRSRLLHSDYMNMTPRRPGPTRK
HYQPYAPPRDFAAYRSRVKFSRSADAPAYQQGQNQLYNELNLGRRE
EYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIG
MKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR (SEQ ID NO.
28)

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

ATGGCACTCCCCGTAACTGCTCTGCTGCTGCCGTTGGCATTGCTCC
TGCACGCCGCACGCCCGCAGGTGCAGCTGCAGGAATCCGGACCG
GGGCTGGTGAAGCCCAGCGAGACTCTGAGTCTCACGTGTACAGTT
TCTGGAGGTAGCATTAGCTCCTACTATTGGTCATGGATAAGGCAG
CCCCCCGGGAAGGGATTGGAATGGATCGGCTATATTTACTACAGT
GGGAGCACCAATTACAACCCCTCACTGAAGTCTAGAGTTACAATC
AGCGTTGACACCTCAAAGAATCAGTTCAGTTTGAAATTGTCTAGC
GTCACAGCAGCTGATACAGCCGTCTATTATTGTGTTTCTCTGGTCT
ATTGCGGTGGGGATTGTTACAGTGGCTTTGACTATTGGGGGCAGG
GTACTCTGGTTACAGTTTCTTCCGGGGGGGGAGGCTCTGGGGGCG
GAGGCTCAGGTGGTGGAGGCAGCGACATCCAGTTGACACAGAGC
CCGAGTTCCTTGTCCGCCTCCGTCGGGGATAGAGTGTCATTTACCT
GTCAGGCCTCTCAGGATATTAATAACTTTCTGAATTGGTATCAGC
AAAAGCCCGGAAAGGCACCCAAGCTGTTGATTTACGACGCCAGT
AACCTGGAGACAGGCGTGCCCTCCCGGTTTAGTGGTAGCGGAAGC
GGTACGGATTTTACCTTTACTATCAGCTCTCTCCAACCCGAAGACA
TTGCAACCTACTATTGTCAACAATATGGAAACCTGCCTTTTACATT

TGGCGGCGGCACCAAGGTGGAGATTAAGCGGGCGGCAGCTATTG
AGGTGATGTATCCACCGCCTTACCTGGATAACGAAAAGAGTAACG
GTACCATCATTCACGTGAAAGGTAAACACCTGTGTCCTTCTCCCCT
CTTCCCCGGGCCATCAAAGCCCTTCTGGGTTCTTGTGGTCGTGGGA
GGCGTGCTTGCTTGTTATTCTCTGCTCGTTACCGTGGCGTTTATCA
TTTTTTGGGTTAGATCCAAAAGAAGCCGCCTGCTCCATAGCGATT
ACATGAATATGACTCCACGCCGCCCTGGCCCCACAAGGAAACACT
ACCAGCCTTACGCACCACCTAGAGATTTCGCTGCCTATCGGAGCA
GGGTGAAGTTTTCCAGATCTGCAGATGCACCAGCGTATCAGCAGG
GCCAGAACCAACTGTATAACGAGCTCAACCTGGGACGCAGGGAA
GAGTATGACGTTTTGGACAAGCGCAGAGGACGGGACCCTGAGAT
GGGTGGCAAACCAAGACGAAAAAACCCCCAGGAGGGTCTCTATA
ATGAGCTGCAGAAGGATAAGATGGCTGAAGCCTATTCTGAAATA
GGCATGAAAGGAGAGCGGAGAAGGGGAAAAGGGCACGACGGTT
TGTACCAGGGACTCAGCACTGCTACGAAGGATACTTATGACGCTC
TCCACATGCAAGCCCTGCCACCTAGGTAA (SEQ ID NO. 29)

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

(Signal Peptide in **Bold**)

MALPVTALLLPLALLLHAARPQVQLQESGPGLVKPSETLSLTCTVS
GGSISSYYWSWIRQPPGKGLEWIGYIYYSGSTNYNPSLKSRVTISVDT
SKNQFSLKLSSVTAADTAVYYCVSLVYCGGDCYSGFDYWGQGTLV
TVSSGGGGSGGGGSGGGGSDIQLTQSPSSLSASVGDRVSFTCQASQDI
NNFLNWYQQKPGKAPKLLIYDASNLETGVPSRFSGSGSGTDFTFTISS
LQPEDIATYYCQQYGNLPFTFGGGTKVEIKRAAAIEVMYPPPYLDNE
KSNGTIIHVKGKHLCPSPLFPGPSKPFWVLVVVGGVLACYSLLVTVA
FIIFWVRSKRSRLLHSDYMNMTPRRPGPTRKHYQPYAPPRDFAAYRS
RVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEM
GGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLY
QGLSTATKDTYDALHMQALPPR (SEQ ID NO. 30)

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

CAGGTGCAGCTGCAGGAATCCGGACCGGGGCTGGTGAAGCCCAG
CGAGACTCTGAGTCTCACGTGTACAGTTTCTGGAGGTAGCATTAG

CTCCTACTATTGGTCATGGATAAGGCAGCCCCCCGGGAAGGGATT
GGAATGGATCGGCTATATTTACTACAGTGGGAGCACCAATTACAA
CCCCTCACTGAAGTCTAGAGTTACAATCAGCGTTGACACCTCAAA
GAATCAGTTCAGTTTGAAATTGTCTAGCGTCACAGCAGCTGATAC
AGCCGTCTATTATTGTGTTTCTCTGGTCTATTGCGGTGGGGATTGT
TACAGTGGCTTTGACTATTGGGGGCAGGGTACTCTGGTTACAGTT
TCTTCCGGGGGGGGAGGCTCTGGGGGCGGAGGCTCAGGTGGTGG
AGGCAGCGACATCCAGTTGACACAGAGCCCGAGTTCCTTGTCCGC
CTCCGTCGGGGATAGAGTGTCATTTACCTGTCAGGCCTCTCAGGA
TATTAATAACTTTCTGAATTGGTATCAGCAAAAGCCCGGAAAGGC
ACCCAAGCTGTTGATTTACGACGCCAGTAACCTGGAGACAGGCGT
GCCCTCCCGGTTTAGTGGTAGCGGAAGCGGTACGGATTTTACCTT
TACTATCAGCTCTCTCCAACCCGAAGACATTGCAACCTACTATTGT
CAACAATATGGAAACCTGCCTTTTACATTTGGCGGCGGCACCAAG
GTGGAGATTAAGCGGGCGGCAGCTATTGAGGTGATGTATCCACCG
CCTTACCTGGATAACGAAAAGAGTAACGGTACCATCATTCACGTG
AAAGGTAAACACCTGTGTCCTTCTCCCCTCTTCCCCGGGCCATCAA
AGCCCTTCTGGGTTCTTGTGGTCGTGGGAGGCGTGCTTGCTTGTTA
TTCTCTGCTCGTTACCGTGGCGTTTATCATTTTTTGGGTTAGATCC
AAAAGAAGCCGCCTGCTCCATAGCGATTACATGAATATGACTCCA
CGCCGCCCTGGCCCCACAAGGAAACACTACCAGCCTTACGCACCA
CCTAGAGATTTCGCTGCCTATCGGAGCAGGGTGAAGTTTTCCAGA
TCTGCAGATGCACCAGCGTATCAGCAGGGCCAGAACCAACTGTAT
AACGAGCTCAACCTGGGACGCAGGGAAGAGTATGACGTTTTGGA
CAAGCGCAGAGGACGGGACCCTGAGATGGGTGGCAAACCAAGAC
GAAAAAACCCCCAGGAGGGTCTCTATAATGAGCTGCAGAAGGAT
AAGATGGCTGAAGCCTATTCTGAAATAGGCATGAAAGGAGAGCG
GAGAAGGGGAAAAGGGCACGACGGTTTGTACCAGGGACTCAGCA
CTGCTACGAAGGATACTTATGACGCTCTCCACATGCAAGCCCTGC
CACCTAGG (SEQ ID NO. 31)

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

QVQLQESGPGLVKPSETLSLTCTVSGGSISSYYWSWIRQPPGKGLEWI
GYIYYSGSTNYNPSLKSRVTISVDTSKNQFSLKLSSVTAADTAVYYC

VSLVYCGGDCYSGFDYWGQGTLVTVSSGGGGSGGGGSGGGGSDIQ
LTQSPSSLSASVGDRVSFTCQASQDINNFLNWYQQKPGKAPKLLIYD
ASNLETGVPSRFSGSGSGTDFTFTISSLQPEDIATYYCQQYGNLPFTFG
GGTKVEIKRAAAIEVMYPPPYLDNEKSNGTIIHVKGKHLCPSPLFPGP
SKPFWVLVVVGGVLACYSLLVTVAFIIFWVRSKRSRLLHSDYMNMT
PRRPGPTRKHYQPYAPPRDFAAYRSRVKFSRSADAPAYQQGQNQLY
NELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDK
MAEAYSEIGMKGERRRGKGHDGLYQGLST
ATKDTYDALHMQALPPR (SEQ ID NO. 32)

[0288] Clone 24C1 CD8 CD3 zeta CAR DNA Heavy & Light Chains

ATGGCACTCCCCGTAACTGCTCTGCTGCTGCCGTTGGCATTGCTCC
TGCACGCCGCACGCCCGCAGGTGCAATTGCAAGAGTCCGGCCCCG
GACTCGTTAAACCCAGTGAGACGCTTAGCCTGACCTGTACCGTCT
CAGGGGGCAGCATCTCCTCTTATTACTGGAGCTGGATCAGGCAGC
CTCCAGGAAAAGGCCTTGAATGGATTGGGTACATCTACTACTCTG
GCTCAACAAATTATAATCCATCCCTGAAGTCCCGCGTGACTATCT
CTGTGGACACCAGCAAGAATCAGTTTTCACTGAAGTTGTCTAGTG
TTACCGCGGCCGACACCGCCGTATACTACTGTGTGTCTCTTGTGTA
CTGTGGCGGCGACTGCTATTCCGGGTTCGACTACTGGGGCCAAGG
GACTCTGGTAACCGTGTCCTCAGGCGGCGGCGGGTCAGGAGGAG
GCGGCAGTGGAGGTGGCGGCTCCGACATCCAGCTGACACAATCA
CCATCTTCCCTTTCAGCTTCAGTCGGGGACAGAGTGTCCTTCACAT
GCCAGGCCAGCCAGGATATCAATAACTTCCTGAACTGGTACCAAC
AGAAACCCGGAAAGGCTCCAAAGCTCCTGATCTATGATGCTTCCA
ACCTGGAGACCGGCGTGCCCTCCAGGTTCAGTGGTTCAGGATCAG
GCACTGACTTTACGTTCACCATATCCAGTCTTCAGCCCGAAGACA
TTGCAACCTATTACTGCCAACAATACGGGAACCTTCCCTTTACATT
CGGAGGCGGCACCAAGGTGGAAATCAAAAGGGCTGCAGCATTGA
GCAACTCAATAATGTATTTTAGTCACTTTGTACCAGTGTTCTTGCC
GGCTAAGCCTACTACCACACCCGCTCCACGGCCACCTACCCCAGC
TCCTACCATCGCTTCACAGCCTCTGTCCCTGCGCCCAGAGGCTTGC
CGACCGGCCGCAGGGGGCGCTGTTCATACCAGAGGACTGGATTTC
GCCTGCGATATCTATATCTGGGCACCCCTGGCCGGAACCTGCGGC

GTACTCCTGCTGTCCCTGGTCATCACGCTCTATTGTAATCACAGGA
ACAGATCCAAAAGAAGCCGCCTGCTCCATAGCGATTACATGAATA
TGACTCCACGCCGCCCTGGCCCCACAAGGAAACACTACCAGCCTT
ACGCACCACCTAGAGATTTCGCTGCCTATCGGAGCAGGGTGAAGT
TTTCCAGATCTGCAGATGCACCAGCGTATCAGCAGGGCCAGAACC
AACTGTATAACGAGCTCAACCTGGGACGCAGGGAAGAGTATGAC
GTTTTGGACAAGCGCAGAGGACGGGACCCTGAGATGGGTGGCAA
ACCAAGACGAAAAAACCCCCAGGAGGGTCTCTATAATGAGCTGC
AGAAGGATAAGATGGCTGAAGCCTATTCTGAAATAGGCATGAAA
GGAGAGCGGAGAAGGGGAAAAGGGCACGACGGTTTGTACCAGGG
ACTCAGCACTGCTACGAAGGATACTTATGACGCTCTCCACATGCA
AGCCCTGCCACCTAGGTAA (SEQ ID NO. 33)

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

(Signal peptide in **bold**)

MALPVTALLLPLALLLHAARPQVQLQESGPGLVKPSETLSLTCTVS
GGSISSYYWSWIRQPPGKGLEWIGYIYYSGSTNYNPSLKSRVTISVDT
SKNQFSLKLSSVTAADTAVYYCVSLVYCGGDCYSGFDYWGQGTLV
TVSSGGGGSGGGGSGGGGSDIQLTQSPSSLSASVGDRVSFTCQASQDI
NNFLNWYQQKPGKAPKLLIYDASNLETGVPSRFSGSGSGTDFTFTISS
LQPEDIATYYCQQYGNLPFTFGGGTKVEIKRAAALSNSIMYFSHFVPV
FLPAKPTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFA
CDIYIWAPLAGTCGVLLLSLVITLYCNHRNRSKRSRLLHSDYMNMTP
RRPGPTRKHYQPYAPPRDFAAYRSRVKFSRSADAPAYQQGQNQLYN
ELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKM
AEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR
(SEQ ID NO. 34)

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

CAGGTGCAATTGCAAGAGTCCGGCCCCGGACTCGTTAAACCCAGT
GAGACGCTTAGCCTGACCTGTACCGTCTCAGGGGGCAGCATCTCC
TCTTATTACTGGAGCTGGATCAGGCAGCCTCCAGGAAAAGGCCTT
GAATGGATTGGGTACATCTACTACTCTGGCTCAACAAATTATAAT
CCATCCCTGAAGTCCCGCGTGACTATCTCTGTGGACACCAGCAAG

AATCAGTTTTCACTGAAGTTGTCTAGTGTTACCGCGGCCGACACC
GCCGTATACTACTGTGTGTCTCTTGTGTACTGTGGCGGCGACTGCT
ATTCCGGGTTCGACTACTGGGGCCAAGGGACTCTGGTAACCGTGT
CCTCAGGCGGCGGCGGGTCAGGAGGAGGCGGCAGTGGAGGTGGC
GGCTCCGACATCCAGCTGACACAATCACCATCTTCCCTTTCAGCTT
CAGTCGGGGACAGAGTGTCCTTCACATGCCAGGCCAGCCAGGATA
TCAATAACTTCCTGAACTGGTACCAACAGAAACCCGGAAAGGCTC
CAAAGCTCCTGATCTATGATGCTTCCAACCTGGAGACCGGCGTGC
CCTCCAGGTTCAGTGGTTCAGGATCAGGCACTGACTTTACGTTCA
CCATATCCAGTCTTCAGCCCGAAGACATTGCAACCTATTACTGCC
AACAATACGGGAACCTTCCCTTTACATTCGGAGGCGGCACCAAGG
TGGAAATCAAAAGGGCTGCAGCATTGAGCAACTCAATAATGTATT
TTAGTCACTTTGTACCAGTGTTCTTGCCGGCTAAGCCTACTACCAC
ACCCGCTCCACGGCCACCTACCCCAGCTCCTACCATCGCTTCACA
GCCTCTGTCCCTGCGCCCAGAGGCTTGCCGACCGGCCGCAGGGGG
CGCTGTTCATACCAGAGGACTGGATTTCGCCTGCGATATCTATATC
TGGGCACCCCTGGCCGGAACCTGCGGCGTACTCCTGCTGTCCCTG
GTCATCACGCTCTATTGTAATCACAGGAACAGATCCAAAAGAAGC
CGCCTGCTCCATAGCGATTACATGAATATGACTCCACGCCGCCCT
GGCCCCACAAGGAAACACTACCAGCCTTACGCACCACCTAGAGAT
TTCGCTGCCTATCGGAGCAGGGTGAAGTTTTCCAGATCTGCAGAT
GCACCAGCGTATCAGCAGGGCCAGAACCAACTGTATAACGAGCT
CAACCTGGGACGCAGGGAAGAGTATGACGTTTTGGACAAGCGCA
GAGGACGGGACCCTGAGATGGGTGGCAAACCAAGACGAAAAAAC
CCCCAGGAGGGTCTCTATAATGAGCTGCAGAAGGATAAGATGGCT
GAAGCCTATTCTGAAATAGGCATGAAAGGAGAGCGGAGAAGGGG
AAAAGGGCACGACGGTTTGTACCAGGGACTCAGCACTGCTACGA
AGGATACTTATGACGCTCTCCACATGCAAGCCCTGCCACCTAGG
(SEQ ID NO. 35)

[0291] Clone 24C1 CD8 CD3 zeta CAR AA Heavy & Light Chains

QVQLQESGPGLVKPSETLSLTCTVSGGSISSYYWSWIRQPPGKGLEWI
GYIYYSGSTNYNPSLKSRVTISVDTSKNQFSLKLSSVTAADTAVYYC
VSLVYCGGDCYSGFDYWGQGTLVTVSSGGGGSGGGGSGGGGSDIQ

LTQSPSSLSASVGDRVSFTCQASQDINNFLNWYQQKPGKAPKLLIYD
ASNLETGVPSRFSGSGSGTDFTFTISSLQPEDIATYYCQQYGNLPFTFG
GGTKVEIKRAAALSNSIMYFSHFVPVFLPAKPTTTPAPRPPTPAPTIAS
QPLSLRPEACRPAAGGAVHTRGLDFACDIYIWAPLAGTCGVLLLSLVI
TLYCNHRNRSKRSRLLHSDYMNMTPRRPGPTRKHYQPYAPPRDFAA
YRSRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDP
EMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDG
LYQGLSTATK DTYDALHMQALPPR (SEQ ID NO. 36)

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

ATGGCACTCCCCGTAACTGCTCTGCTGCTGCCGTTGGCATTGCTCC
TGCACGCCGCACGCCCGGATATCCAGCTCACGCAATCCCCCTCAA
GCTTGAGTGCCTCCGTGGGCGACCGGGTGTCCTTCACATGTCAGG
CAAGCCAAGACATAAATAATTTCCTGAATTGGTACCAACAAAAAC
CCGGCAAGGCTCCCAAACTCCTGATTTATGATGCCTCCAATCTGG
AGACCGGGGTCCCTTCTAGATTCAGCGGAAGTGGCAGCGGCACA
GACTTTACATTTACTATCTCTTCTCTGCAACCAGAGGACATCGCCA
CATACTATTGCCAGCAATACGGCAATCTGCCCTTCACCTTCGGAG
GCGGAACCAAGGTAGAAATTAAAAGGGGCGGTGGAGGCTCCGGA
GGGGGGGGCTCTGGCGGAGGGGGCTCCCAAGTACAATTGCAGGA
GTCAGGGCCTGGACTCGTGAAGCCTTCAGAAACTTTGTCACTGAC
ATGTACAGTGTCCGGCGGAAGCATTTCCAGTTACTATTGGTCCTG
GATTAGACAGCCACCCGGCAAAGGACTGGAATGGATTGGATATA
TCTACTACTCTGGATCTACAAACTATAATCCCAGCCTCAAATCCA
GGGTCACTATTAGTGTGGATACATCAAAGAATCAGTTCTCCTTGA
AGCTGAGCTCAGTCACTGCTGCCGACACCGCAGTGTACTATTGTG
TGAGCCTGGTCTACTGCGGCGGAGATTGCTACAGCGGTTTCGATT
ACTGGGGCCAGGGCACCCTGGTTACCGTTAGTTCCGCGGCTGCTC
TTGATAACGAGAAGTCCAACGGTACGATTATCCACGTTAAGGGTA
AGCACCTTTGCCCTAGCCCGCTGTTCCCAGGCCCCAGTAAGCCCTT
TTGGGTCCTCGTTGTGGTAGGTGGGGTACTCGCCTGCTACTCCCTG
CTCGTCACTGTCGCATTCATCATCTTCTGGGTCAGATCCAAAAGA
AGCCGCCTGCTCCATAGCGATTACATGAATATGACTCCACGCCGC
CCTGGCCCCACAAGGAAACACTACCAGCCTTACGCACCACCTAGA

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

[0293] Clone 24C1 CD28T CD3 zeta CAR AA Heavy & Light Chains
(Signal Peptide in **Bold**)
MALPVTALLLPLALLLHAARPDIQLTQSPSSLSASVGDRVSFTCQAS
QDINNFLNWYQQKPGKAPKLLIYDASNLETGVPSRFSGSGSGTDFTF
TISSLQPEDIATYYCQQYGNLPFTFGGGTKVEIKRGGGGSGGGGSGG
GGSQVQLQESGPGLVKPSETLSLTCTVSGGSISSYYWSWIRQPPGKGL
EWIGYIYYSGSTNYNPSLKSRVTISVDTSKNQFSLKLSSVTAADTAVY
YCVSLVYCGGDCYSGFDYWGQGTLVTVSSAAALDNEKSNGTIIHVK
GKHLCPSPLFPGPSKPFWVLVVVGGVLACYSLLVTVAFIIFWVRSKRS
RLLHSDYMNMTPRRPGPTRKHYQPYAPPRDFAAYRSRVKFSRSADA
PAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQ
EGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDT
YDA LHMQALPPR (SEQ ID NO. 38)

[0294] Clone 24C1 CD28T CD3 zeta CAR DNA Heavy & Light Chains
GATATCCAGCTCACGCAATCCCCCTCAAGCTTGAGTGCCTCCGTG
GGCGACCGGGTGTCCTTCACATGTCAGGCAAGCCAAGACATAAAT
AATTTCCTGAATTGGTACCAACAAAAACCCGGCAAGGCTCCCAAA
CTCCTGATTTATGATGCCTCCAATCTGGAGACCGGGGTCCCTTCTA
GATTCAGCGGAAGTGGCAGCGGCACAGACTTTACATTTACTATCT
CTTCTCTGCAACCAGAGGACATCGCCACATACTATTGCCAGCAAT
ACGGCAATCTGCCCTTCACCTTCGGAGGCGGAACCAAGGTAGAAA
TTAAAAGGGGCGGTGGAGGCTCCGGAGGGGGGGGCTCTGGCGGA
GGGGGCTCCCAAGTACAATTGCAGGAGTCAGGGCCTGGACTCGTG

AAGCCTTCAGAAACTTTGTCACTGACATGTACAGTGTCCGGCGGA
AGCATTTCCAGTTACTATTGGTCCTGGATTAGACAGCCACCCGGC
AAAGGACTGGAATGGATTGGATATATCTACTACTCTGGATCTACA
AACTATAATCCCAGCCTCAAATCCAGGGTCACTATTAGTGTGGAT
ACATCAAAGAATCAGTTCTCCTTGAAGCTGAGCTCAGTCACTGCT
GCCGACACCGCAGTGTACTATTGTGTGAGCCTGGTCTACTGCGGC
GGAGATTGCTACAGCGGTTTCGATTACTGGGGCCAGGGCACCCTG
GTTACCGTTAGTTCCGCGGCTGCTCTTGATAACGAGAAGTCCAAC
GGTACGATTATCCACGTTAAGGGTAAGCACCTTTGCCCTAGCCCG
CTGTTCCCAGGCCCCAGTAAGCCCTTTTGGGTCCTCGTTGTGGTAG
GTGGGGTACTCGCCTGCTACTCCCTGCTCGTCACTGTCGCATTCAT
CATCTTCTGGGTCAGATCCAAAAGAAGCCGCCTGCTCCATAGCGA
TTACATGAATATGACTCCACGCCGCCCTGGCCCCACAAGGAAACA
CTACCAGCCTTACGCACCACCTAGAGATTTCGCTGCCTATCGGAG
CAGGGTGAAGTTTTCCAGATCTGCAGATGCACCAGCGTATCAGCA
GGGCCAGAACCAACTGTATAACGAGCTCAACCTGGGACGCAGGG
AAGAGTATGACGTTTTGGACAAGCGCAGAGGACGGGACCCTGAG
ATGGGTGGCAAACCAAGACGAAAAAACCCCCAGGAGGGTCTCTA
TAATGAGCTGCAGAAGGATAAGATGGCTGAAGCCTATTCTGAAAT
AGGCATGAAAGGAGAGCGGAGAAGGGGAAAAGGGCACGACGGT
TTGTACCAGGGACTCAGCACTGCTACGAAGGATACTTATGACGCT
CTCCACATGCAAGCCCTGCCACCTAGG (SEQ ID NO. 39)

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

DIQLTQSPSSLSASVGDRVSFTCQASQDINNFLNWYQQKPGKAPKLLI
YDASNLETGVPSRFSGSGSGTDFTFTISSLQPEDIATYYCQQYGNLPFT
FGGGTKVEIKRGGGGSGGGGSGGGGSQVQLQESGPGLVKPSETLSLT
CTVSGGSISSYYWSWIRQPPGKGLEWIGYIYYSGSTNYNPSLKSRVTI
SVDTSKNQFSLKLSSVTAADTAVYYCVSLVYCGGDCYSGFDYWGQ
GTLVTVSSAAALDNEKSNGTIIHVKGKHLCPSPLFPGPSKPFWVLVV
VGGVLACYSLLVTVAFIIFWVRSKRSRLLHSDYMNMTPRRPGPTRKH
YQPYAPPRDFAAYRSRVKFSRSADAPAYQQGQNQLYNELNLGRREE
YDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGM

KGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR (SEQ ID NO. 40)

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

ATGGCACTCCCCGTAACTGCTCTGCTGCTGCCGTTGGCATTGCTCC
TGCACGCCGCACGCCCGGATATCCAGCTGACCCAGTCTCCATCCT
CTTTGAGTGCCTCCGTGGGTGACCGCGTCTCTTTCACTTGCCAAGC
CAGCCAAGACATCAACAACTTTCTGAATTGGTACCAGCAGAAACC
AGGCAAAGCACCAAAGCTCCTCATCTACGACGCCTCCAACCTGGA
AACCGGGGTGCCCAGCAGGTTTAGCGGGAGCGGTTCTGGCACGG
ATTTTACGTTCACCATCTCCTCTCTGCAGCCCGAGGATATAGCTAC
TTATTACTGTCAGCAGTACGGGAATCTGCCATTTACTTTTGGGGGT
GGAACTAAGGTGGAAATCAAAAGGGGCGGCGGGGGAAGCGGGG
GCGGGGGCTCAGGTGGCGGAGGGAGCCAGGTGCAACTCCAGGAA
AGTGGCCCAGGATTGGTGAAGCCCAGCGAGACCCTTTCCCTTACT
TGTACTGTTAGCGGAGGCAGCATAAGCAGCTACTATTGGTCCTGG
ATCAGACAGCCACCAGGGAAAGGGCTTGAATGGATTGGCTACATT
TACTATTCCGGGTCCACCAACTACAACCCATCCCTCAAGTCCCGC
GTGACAATTTCCGTCGACACAAGCAAGAACCAGTTCTCCCTGAAA
CTTAGTAGCGTCACTGCTGCAGATACAGCAGTGTACTATTGTGTC
AGCCTTGTCTACTGTGGCGGCGACTGCTACAGTGGCTTTGATTACT
GGGGACAGGGCACGCTCGTGACAGTGTCCAGCGCTGCGGCTATCG
AGGTAATGTATCCGCCACCGTATCTGGACAACGAGAAGTCTAATG
GGACAATCATTCACGTGAAGGGGAAGCACCTGTGTCCATCCCCCC
TGTTTCCGGGTCCCAGTAAACCCTTCTGGGTGCTTGTTGTCGTTGG
CGGGGTGCTGGCCTGCTATTCCCTGCTGGTGACCGTCGCGTTTATT
ATTTTCTGGGTTAGATCCAAAAGAAGCCGCCTGCTCCATAGCGAT
TACATGAATATGACTCCACGCCGCCCTGGCCCCACAAGGAAACAC
TACCAGCCTTACGCACCACCTAGAGATTTCGCTGCCTATCGGAGC
AGGGTGAAGTTTTCCAGATCTGCAGATGCACCAGCGTATCAGCAG
GGCCAGAACCAACTGTATAACGAGCTCAACCTGGGACGCAGGGA
AGAGTATGACGTTTTGGACAAGCGCAGAGGACGGGACCCTGAGA
TGGGTGGCAAACCAAGACGAAAAAACCCCCAGGAGGGTCTCTAT
AATGAGCTGCAGAAGGATAAGATGGCTGAAGCCTATTCTGAAAT

AGGCATGAAAGGAGAGCGGAGAAGGGGAAAAGGGCACGACGGT
TTGTACCAGGGACTCAGCACTGCTACGAAGGATACTTATGACGCT
CTCCACATGCAAGCCCTGCCACCTAGGTAA (SEQ ID NO. 41)

[0297] Clone 24C1 CD28 CD3 zeta CAR AA Heavy & Light Chains
(Signal Peptide in **Bold**)
MALPVTALLLPLALLLHAARPDIQLTQSPSSLSASVGDRVSFTCQAS
QDINNFLNWYQQKPGKAPKLLIYDASNLETGVPSRFSGSGSGTDFTF
TISSLQPEDIATYYCQQYGNLPFTFGGGTKVEIKRGGGGSGGGGSGG
GGSQVQLQESGPGLVKPSETLSLTCTVSGGSISSYYWSWIRQPPGKGL
EWIGYIYYSGSTNYNPSLKSRVTISVDTSKNQFSLKLSSVTAADTAVY
YCVSLVYCGGDCYSGFDYWGQGTLVTVSSAAAIEVMYPPPYLDNEK
SNGTIIHVKGKHLCPSPLFPGPSKPFWVLVVVGGVLACYSLLVTVAFI
IFWVRSKRSRLLHSDYMNMTPRRPGPTRKHYQPYAPPRDFAAYRSR
VKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMG
GKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQ
GLSTATKDTYDALHMQALPPR (SEQ ID NO. 42)

[0298] Clone 24C1 CD28 CD3 zeta CAR DNA Heavy & Light Chains
GATATCCAGCTGACCCAGTCTCCATCCTCTTTGAGTGCCTCCGTGG
GTGACCGCGTCTCTTTCACTTGCCAAGCCAGCCAAGACATCAACA
ACTTTCTGAATTGGTACCAGCAGAAACCAGGCAAAGCACCAAAG
CTCCTCATCTACGACGCCTCCAACCTGGAAACCGGGGTGCCCAGC
AGGTTTAGCGGGAGCGGTTCTGGCACGGATTTTACGTTCACCATC
TCCTCTCTGCAGCCCGAGGATATAGCTACTTATTACTGTCAGCAGT
ACGGGAATCTGCCATTTACTTTTGGGGGTGGAACTAAGGTGGAAA
TCAAAAGGGGCGGCGGGGGAAGCGGGGGCGGGGGCTCAGGTGGC
GGAGGGAGCCAGGTGCAACTCCAGGAAAGTGGCCCAGGATTGGT
GAAGCCCAGCGAGACCCTTTCCCTTACTTGTACTGTTAGCGGAGG
CAGCATAAGCAGCTACTATTGGTCCTGGATCAGACAGCCACCAGG
GAAAGGGCTTGAATGGATTGGCTACATTTACTATTCCGGGTCCAC
CAACTACAACCCATCCCTCAAGTCCCGCGTGACAATTTCCGTCGA
CACAAGCAAGAACCAGTTCTCCCTGAAACTTAGTAGCGTCACTGC
TGCAGATACAGCAGTGTACTATTGTGTCAGCCTTGTCTACTGTGGC

GGCGACTGCTACAGTGGCTTTGATTACTGGGGACAGGGCACGCTC
GTGACAGTGTCCAGCGCTGCGGCTATCGAGGTAATGTATCCGCCA
CCGTATCTGGACAACGAGAAGTCTAATGGGACAATCATTCACGTG
AAGGGGAAGCACCTGTGTCCATCCCCCCTGTTTCCGGGTCCCAGT
AAACCCTTCTGGGTGCTTGTTGTCGTTGGCGGGGTGCTGGCCTGCT
ATTCCCTGCTGGTGACCGTCGCGTTTATTATTTTCTGGGTTAGATC
CAAAAGAAGCCGCCTGCTCCATAGCGATTACATGAATATGACTCC
ACGCCGCCCTGGCCCCACAAGGAAACACTACCAGCCTTACGCACC
ACCTAGAGATTTCGCTGCCTATCGGAGCAGGGTGAAGTTTTCCAG
ATCTGCAGATGCACCAGCGTATCAGCAGGGCCAGAACCAACTGTA
TAACGAGCTCAACCTGGGACGCAGGGAAGAGTATGACGTTTTGG
ACAAGCGCAGAGGACGGGACCCTGAGATGGGTGGCAAACCAAGA
CGAAAAAACCCCCAGGAGGGTCTCTATAATGAGCTGCAGAAGGA
TAAGATGGCTGAAGCCTATTCTGAAATAGGCATGAAAGGAGAGC
GGAGAAGGGGAAAAGGGCACGACGGTTTGTACCAGGGACTCAGC
ACTGCTACGAAGGATACTTATGACGCTCTCCACATGCAAGCCCTG
CCACCTAGG (SEQ ID NO. 43)

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

DIQLTQSPSSLSASVGDRVSFTCQASQDINNFLNWYQQKPGKAPKLLI
YDASNLETGVPSRFSGSGSGTDFTFTISSLQPEDIATYYCQQYGNLPFT
FGGGTKVEIKRGGGGSGGGGSGGGGSQVQLQESGPGLVKPSETLSLT
CTVSGGSISSYYWSWIRQPPGKGLEWIGYIYYSGSTNYNPSLKSRVTI
SVDTSKNQFSLKLSSVTAADTAVYYCVSLVYCGGDCYSGFDYWGQ
GTLVTVSSAAAIEVMYPPPYLDNEKSNGTIIHVKGKHLCPSPLFPGPS
KPFWVLVVVGGVLACYSLLVTVAFIIFWVRSKRSRLLHSDYMNMTP
RRPGPTRKHYQPYAPPRDFAAYRSRVKFSRSADAPAYQQGQNQLYN
ELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKM
AEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR
(SEQ ID NO. 44)

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

ATGGCACTCCCCGTAACTGCTCTGCTGCTGCCGTTGGCATTGCTCC
TGCACGCCGCACGCCCGGACATTCAATTGACCCAGTCCCCTAGCA

GTCTCTCAGCAAGTGTGGGAGATAGGGTGTCATTCACCTGTCAGG
CTTCACAGGACATCAACAACTTCCTCAATTGGTATCAGCAGAAGC
CAGGGAAGGCACCAAAGCTGCTCATATATGACGCTTCAAACCTTG
AAACCGGAGTACCTAGCCGCTTCAGCGGAAGCGGATCAGGGACT
GACTTCACTTTTACCATCTCTTCACTGCAGCCCGAAGACATCGCCA
CATACTACTGCCAGCAGTACGGAAACTTGCCTTTTACATTTGGGG
GCGGCACCAAAGTGGAGATTAAGCGAGGGGGAGGCGGCTCAGGA
GGCGGTGGCTCCGGAGGCGGGGGTTCCCAGGTCCAGCTCCAGGA
ATCCGGCCCAGGTCTGGTTAAGCCCAGTGAAACTTTGTCCCTCAC
GTGTACTGTGAGCGGTGGTTCAATCTCCTCATACTATTGGTCTTGG
ATACGGCAACCTCCTGGAAAGGGCCTCGAGTGGATCGGCTATATC
TACTATAGTGGCTCCACTAATTACAACCCTTCCCTCAAGTCCAGA
GTCACCATTTCCGTGGACACATCTAAGAACCAGTTCAGTCTGAAG
TTGTCCAGCGTTACAGCCGCAGACACAGCCGTTTATTACTGTGTGT
CTCTTGTTTACTGCGGGGGAGACTGTTATAGCGGCTTCGATTACTG
GGGCCAGGGCACCTTGGTCACAGTCTCTTCCGCGGCCGCCCTCTC
TAACAGTATTATGTACTTTTCTCATTTTGTACCCGTGTTCCTTCCCG
CTAAGCCAACTACTACCCCGGCCCCACGGCCGCCTACCCCTGCAC
CCACAATAGCCAGTCAGCCTTTGAGCCTGAGACCTGAGGCTTGTC
GGCCGGCTGCTGGGGGTGCAGTGCACACACGAGGTCTTGATTTTG
CTTGCGACATATACATCTGGGCCCCTCTGGCCGGGACCTGTGGGG
TGCTGCTTCTGAGCTTGGTCATCACGCTCTATTGCAACCATCGCAA
CAGATCCAAAAGAAGCCGCCTGCTCCATAGCGATTACATGAATAT
GACTCCACGCCGCCCTGGCCCCACAAGGAAACACTACCAGCCTTA
CGCACCACCTAGAGATTTCGCTGCCTATCGGAGCAGGGTGAAGTT
TTCCAGATCTGCAGATGCACCAGCGTATCAGCAGGGCCAGAACCA
ACTGTATAACGAGCTCAACCTGGGACGCAGGGAAGAGTATGACG
TTTTGGACAAGCGCAGAGGACGGGACCCTGAGATGGGTGGCAAA
CCAAGACGAAAAAACCCCCAGGAGGGTCTCTATAATGAGCTGCA
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
TISSLQPEDIATYYCQQYGNLPFTFGGGTKVEIKRGGGGSGGGGSGG
GGSQVQLQESGPGLVKPSETLSLTCTVSGGSISSYYWSWIRQPPGKGL
EWIGYIYYSGSTNYNPSLKSRVTISVDTSKNQFSLKLSSVTAADTAVY
YCVSLVYCGGDCYSGFDYWGQGTLVTVSSAAALSNSIMYFSHFVPV
FLPAKPTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFA
CDIYIWAPLAGTCGVLLLSLVITLYCNHRNRSKRSRLLHSDYMNMTP
RRPGPTRKHYQPYAPPRDFAAYRSRVKFSRSADAPAYQQGQNQLYN
ELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKM
AEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR
(SEQ ID NO. 46)

[0302] Clone 24C1 CD8 CD3 zeta CAR DNA Heavy & Light Chains
GACATTCAATTGACCCAGTCCCCTAGCAGTCTCTCAGCAAGTGTG
GGAGATAGGGTGTCATTCACCTGTCAGGCTTCACAGGACATCAAC
AACTTCCTCAATTGGTATCAGCAGAAGCCAGGGAAGGCACCAAA
GCTGCTCATATATGACGCTTCAAACCTTGAAACCGGAGTACCTAG
CCGCTTCAGCGGAAGCGGATCAGGGACTGACTTCACTTTTACCAT
CTCTTCACTGCAGCCCGAAGACATCGCCACATACTACTGCCAGCA
GTACGGAAACTTGCCTTTTACATTTGGGGGCGGCACCAAAGTGGA
GATTAAGCGAGGGGGAGGCGGCTCAGGAGGCGGTGGCTCCGGAG
GCGGGGGTTCCCAGGTCCAGCTCCAGGAATCCGGCCCAGGTCTGG
TTAAGCCCAGTGAAACTTTGTCCCTCACGTGTACTGTGAGCGGTG
GTTCAATCTCCTCATACTATTGGTCTTGGATACGGCAACCTCCTGG
AAAGGGCCTCGAGTGGATCGGCTATATCTACTATAGTGGCTCCAC
TAATTACAACCCTTCCCTCAAGTCCAGAGTCACCATTTCCGTGGAC
ACATCTAAGAACCAGTTCAGTCTGAAGTTGTCCAGCGTTACAGCC
GCAGACACAGCCGTTTATTACTGTGTGTCTCTTGTTTACTGCGGGG
GAGACTGTTATAGCGGCTTCGATTACTGGGGCCAGGGCACCTTGG
TCACAGTCTCTTCCGCGGCCGCCCTCTCTAACAGTATTATGTACTT
TTCTCATTTTGTACCCGTGTTCCTTCCCGCTAAGCCAACTACTACC

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

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

DIQLTQSPSSLSASVGDRVSFTCQASQDINNFLNWYQQKPGKAPKLLI
YDASNLETGVPSRFSGSGSGTDFTFTISSLQPEDIATYYCQQYGNLPFT
FGGGTKVEIKRGGGGSGGGGSGGGGSQVQLQESGPGLVKPSETLSLT
CTVSGGSISSYYWSWIRQPPGKGLEWIGYIYYSGSTNYNPSLKSRVTI
SVDTSKNQFSLKLSSVTADTAVYYCVSLVYCGGDCYSGFDYWGQGT
LVTVSSAAALSNSIMYFSHFVPVFLPAKPTTTPAPRPPTPAPTIASQPLS
LRPEACRPAAGGAVHTRGLDFACDIYIWAPLAGTCGVLLLSLVITLY
CNHRNRSKRSRLLHSDYMNMTPRRPGPTRKHYQPYAPPRDFAAYRS
RVKFSRSADAPAYQQGQNQLYNELNLGREEYDVLDKRRGRDPEMG
GKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQ
GLSTATK DTYDALHMQA LPPR (SEQ ID NO. 48)

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

CAGGTACAGCTGCAGGAATCTGGGCCCGGACTTGTCAAGCCAAGT
CAGACACTTTCTCTTACATGTACCGTGAGCGGCGGAAGTATAAGC
AGTGGAGGCTTTTACTGGTCTTGGATACGGCAGCACCCAGGCAAA

GGCTTGGAGTGGATTGGATACATTCATCATTCAGGATCTACACAC
TATAATCCATCCCTTAAGTCCCGGGTCACCATTAGCATTGATACGT
CTAAGAATCTGTTCAGTCTCAGGCTGTCCTCCGTCACTGCTGCCGA
CACAGCCGTGTACTACTGCGCCTCCTTGGTTTACTGCGGAGGCGA
CTGTTATAGCGGCTTTGATTATTGGGGGCAGGGGACCCTCGTAAC
CGTGAGCTCT (SEQ ID NO. 48)

[0305] Clone 24C8 AA HC (CDRs in Underline)
QVQLQESGPGLVKPSQTLSLTCTVSGGSISSGGFYWSWIRQHPGKGL
EWIGYIHHSGSTHYNPSLKSRVTISIDTSKNLFSLRLSSVTAADTAVYY
CASLVYCGGDCYSGFDYWGQGTLVTVSS (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
GATATCCAGCTCACTCAAAGCCCCTCTAGTCTCTCTGCCTCAGTGG
GGGATCGGGTCAGTTTTACTTGTCAAGCTTCACAGGATATCAACA
ACTTCCTTAATTGGTATCAGCAGAAGCCAGGAAAAGCACCCAAGC
TGCTCATCTATGATGCCTCAAATTTGGAGACGGGTGTTCCCAGTC
GATTCTCTGGGTCAGGGTCCGGGACCGACTTTACGTTTACGATCTC
CTCTCTGCAGCCCGAAGACATCGCCACATACTATTGTCAACAGTA
CGGCAACTTGCCTTTCACATTTGGGGGCGGGACTAAGGTTGAAAT
CAAGAGG (SEQ ID NO. 54)

[0310] Clone 24C8 LC AA (CDRs in Underline)
DIQLTQSPSSLSASVGDRVSFTCQASQDINNFLNWYQQKPGKAPKLLI
YDASNLETGVPSRFSGSGSGTDFTFTISSLQPEDIATYYCQQYGNLPFT
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

ATGGCACTCCCCGTAACTGCTCTGCTGCTGCCGTTGGCATTGCTCC
TGCACGCCGCACGCCCGCAGGTACAGCTGCAGGAATCTGGGCCCG
GACTTGTCAAGCCAAGTCAGACACTTTCTCTTACATGTACCGTGA
GCGGCGGAAGTATAAGCAGTGGAGGCTTTTACTGGTCTTGGATAC
GGCAGCACCCAGGCAAAGGCTTGGAGTGGATTGGATACATTCATC
ATTCAGGATCTACACACTATAATCCATCCCTTAAGTCCCGGGTCA
CCATTAGCATTGATACGTCTAAGAATCTGTTCAGTCTCAGGCTGTC
CTCCGTCACTGCTGCCGACACAGCCGTGTACTACTGCGCCTCCTTG
GTTTACTGCGGAGGCGACTGTTATAGCGGCTTTGATTATTGGGGG
CAGGGGACCCTCGTAACCGTGAGCTCTGGAGGGGGTGGGAGCGG
GGGAGGAGGTTCAGGGGGGGGCGGCTCCGATATCCAGCTCACTC
AAAGCCCCTCTAGTCTCTCTGCCTCAGTGGGGGATCGGGTCAGTT
TTACTTGTCAAGCTTCACAGGATATCAACAACTTCCTTAATTGGTA
TCAGCAGAAGCCAGGAAAAGCACCCAAGCTGCTCATCTATGATGC
CTCAAATTTGGAGACGGGTGTTCCCAGTCGATTCTCTGGGTCAGG
GTCCGGGACCGACTTTACGTTTACGATCTCCTCTCTGCAGCCCGAA
GACATCGCCACATACTATTGTCAACAGTACGGCAACTTGCCTTTC
ACATTTGGGGGCGGGACTAAGGTTGAAATCAAGAGGGCCGCTGC
ACTGGACAATGAGAAGTCCAACGGCACCATCATCCACGTGAAGG
GCAAGCACCTGTGCCCTAGTCCTCTGTTCCCAGGCCCATCCAAAC
CTTTTTGGGTTCTTGTTGTGGTCGGGGGGGTGCTGGCCTGCTATTC
TCTGCTGGTCACGGTGGCCTTCATAATTTTCTGGGTTAGATCCAAA
AGAAGCCGCCTGCTCCATAGCGATTACATGAATATGACTCCACGC
CGCCCTGGCCCCACAAGGAAACACTACCAGCCTTACGCACCACCT
AGAGATTTCGCTGCCTATCGGAGCAGGGTGAAGTTTTCCAGATCT
GCAGATGCACCAGCGTATCAGCAGGGCCAGAACCAACTGTATAA
CGAGCTCAACCTGGGACGCAGGGAAGAGTATGACGTTTTGGACA
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**)

MALPVTALLLPLALLLHAARPQVQLQESGPGLVKPSQTLSLTCTVS
GGSISSGGFYWSWIRQHPGKGLEWIGYIHHSGSTHYNPSLKSRVTISI
DTSKNLFSLRLSSVTAADTAVYYCASLVYCGGDCYSGFDYWGQGTL
VTVSSGGGGSGGGGSGGGGSDIQLTQSPSSLSASVGDRVSFTCQASQ
DINNFLNWYQQKPGKAPKLLIYDASNLETGVPSRFSGSGSGTDFTFTI
SSLQPEDIATYYCQQYGNLPFTFGGGTKVEIKRAAALDNEKSNGTIIH
VKGKHLCPSPLFPGPSKPFWVLVVVGGVLACYSLLVTVAFIIFWVRS
KRSRLLHSDYMNMTPRRPGPTRKHYQPYAPPRDFAAYRSRVKFSRS
ADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRK
NPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTAT
KDTYDALHMQALPPR (SEQ ID NO. 60)

[0316] Clone 24C8 CD28T CD3 zeta CAR DNA Heavy & Light Chains
CAGGTACAGCTGCAGGAATCTGGGCCCGGACTTGTCAAGCCAAGT
CAGACACTTTCTCTTACATGTACCGTGAGCGGCGGAAGTATAAGC
AGTGGAGGCTTTTACTGGTCTTGGATACGGCAGCACCCAGGCAAA
GGCTTGGAGTGGATTGGATACATTCATCATTCAGGATCTACACAC
TATAATCCATCCCTTAAGTCCCGGGTCACCATTAGCATTGATACGT
CTAAGAATCTGTTCAGTCTCAGGCTGTCCTCCGTCACTGCTGCCGA
CACAGCCGTGTACTACTGCGCCTCCTTGGTTTACTGCGGAGGCGA
CTGTTATAGCGGCTTTGATTATTGGGGGCAGGGGACCCTCGTAAC
CGTGAGCTCTGGAGGGGGTGGGAGCGGGGGAGGAGGTTCAGGGG
GGGGCGGCTCCGATATCCAGCTCACTCAAAGCCCCTCTAGTCTCT
CTGCCTCAGTGGGGGATCGGGTCAGTTTTACTTGTCAAGCTTCAC
AGGATATCAACAACTTCCTTAATTGGTATCAGCAGAAGCCAGGAA
AAGCACCCAAGCTGCTCATCTATGATGCCTCAAATTTGGAGACGG
GTGTTCCCAGTCGATTCTCTGGGTCAGGGTCCGGGACCGACTTTA
CGTTTACGATCTCCTCTCTGCAGCCCGAAGACATCGCCACATACT
ATTGTCAACAGTACGGCAACTTGCCTTTCACATTTGGGGGCGGGA

CTAAGGTTGAAATCAAGAGGGCCGCTGCACTGGACAATGAGAAG
TCCAACGGCACCATCATCCACGTGAAGGGCAAGCACCTGTGCCCT
AGTCCTCTGTTCCCAGGCCCATCCAAACCTTTTTGGGTTCTTGTTG
TGGTCGGGGGGGTGCTGGCCTGCTATTCTCTGCTGGTCACGGTGG
CCTTCATAATTTTCTGGGTTAGATCCAAAAGAAGCCGCCTGCTCC
ATAGCGATTACATGAATATGACTCCACGCCGCCCTGGCCCCACAA
GGAAACACTACCAGCCTTACGCACCACCTAGAGATTTCGCTGCCT
ATCGGAGCAGGGTGAAGTTTTCCAGATCTGCAGATGCACCAGCGT
ATCAGCAGGGCCAGAACCAACTGTATAACGAGCTCAACCTGGGA
CGCAGGGAAGAGTATGACGTTTTGGACAAGCGCAGAGGACGGGA
CCCTGAGATGGGTGGCAAACCAAGACGAAAAAACCCCCAGGAGG
GTCTCTATAATGAGCTGCAGAAGGATAAGATGGCTGAAGCCTATT
CTGAAATAGGCATGAAAGGAGAGCGGAGAAGGGGAAAAGGGCA
CGACGGTTTGTACCAGGGACTCAGCACTGCTACGAAGGATACTTA
TGACGCTCTCCACATGCAAGCCCTGCCACCTAGG (SEQ ID NO. 61)

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

QVQLQESGPGLVKPSQTLSLTCTVSGGSISSGGFYWSWIRQHPGKGL
EWIGYIHHSGSTHYNPSLKSRVTISIDTSKNLFSLRLSSVTAADTAVYY
CASLVYCGGDCYSGFDYWGQGTLVTVSSGGGGSGGGGSGGGGSDI
QLTQSPSSLSASVGDRVSFTCQASQDINNFLNWYQQKPGKAPKLLIY
DASNLETGVPSRFSGSGSGTDFTFTISSLQPEDIATYYCQQYGNLPFTF
GGGTKVEIKRAAALDNEKSNGTIIHVKGKHLCPSPLFPGPSKPFWVL
VVVGGVLACYSLLVTVAFIIFWVRSKRSRLLHSDYMNMTPRRPGPTR
KHYQPYAPPRDFAAYRSRVKFSRSADAPAYQQGQNQLYNELNLGRR
EEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEI
GMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR (SEQ ID NO. 62)

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

ATGGCACTCCCCGTAACTGCTCTGCTGCTGCCGTTGGCATTGCTCC
TGCACGCCGCACGCCCGCAGGTGCAGCTGCAGGAAAGCGGTCCG
GGACTTGTCAAGCCGTCCCAAACGCTGAGTCTGACGTGTACTGTC
TCTGGTGGCTCTATTTCTTCCGGGGGCTTTTATTGGTCTTGGATCA

GACAACACCCTGGCAAAGGGCTGGAGTGGATAGGGTATATTCAC
CACTCTGGGTCCACTCACTACAACCCATCATTGAAATCCAGAGTG
ACTATCTCAATCGACACATCCAAGAACCTTTTCAGCCTGAGGTTG
TCATCAGTTACCGCCGCTGACACCGCGGTGTATTATTGCGCCTCTC
TCGTGTACTGCGGTGGCGATTGTTATAGTGGCTTTGACTACTGGG
GGCAGGGGACATTGGTTACCGTTTCAAGTGGAGGCGGTGGGTCTG
GCGGGGGCGGTAGCGGAGGTGGGGGGAGCGACATACAGCTTACG
CAGAGCCCCTCCAGCCTTTCAGCCTCCGTGGGGGATAGGGTGTCC
TTTACCTGCCAGGCTTCCCAGGACATAAACAACTTCCTCAATTGGT
ATCAGCAAAAGCCCGGGAAAGCACCAAAGCTGCTCATCTACGAT
GCCAGCAACCTGGAAACCGGAGTGCCGTCTCGCTTCTCTGGAAGT
GGCAGTGGGACCGATTTCACTTTTACAATCTCAAGTTTGCAGCCA
GAAGACATTGCAACATACTACTGTCAACAGTACGGCAATCTCCCC
TTTACATTTGGGGGGGGAACTAAAGTGGAGATTAAGCGCGCTGCA
GCCATTGAAGTTATGTATCCGCCCCCGTATCTGGATAACGAGAAA
TCTAATGGTACCATAATACATGTGAAGGGGAAGCACCTCTGTCCA
TCACCGCTGTTCCCCGGCCCTTCAAAACCTTTCTGGGTACTCGTTG
TCGTGGGTGGAGTTCTGGCCTGCTATAGTCTGCTGGTGACCGTGG
CGTTTATCATCTTCTGGGTAAGATCCAAAAGAAGCCGCCTGCTCC
ATAGCGATTACATGAATATGACTCCACGCCGCCCTGGCCCCACAA
GGAAACACTACCAGCCTTACGCACCACCTAGAGATTTCGCTGCCT
ATCGGAGCAGGGTGAAGTTTTCCAGATCTGCAGATGCACCAGCGT
ATCAGCAGGGCCAGAACCAACTGTATAACGAGCTCAACCTGGGA
CGCAGGGAAGAGTATGACGTTTTGGACAAGCGCAGAGGACGGGA
CCCTGAGATGGGTGGCAAACCAAGACGAAAAAACCCCCAGGAGG
GTCTCTATAATGAGCTGCAGAAGGATAAGATGGCTGAAGCCTATT
CTGAAATAGGCATGAAAGGAGAGCGGAGAAGGGGAAAAGGGCA
CGACGGTTTGTACCAGGGACTCAGCACTGCTACGAAGGATACTTA
TGACGCTCTCCACATGCAAGCCCTGCCACCTAGGTAA (SEQ ID NO. 63)

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

MALPVTALLLPLALLLHAARPQVQLQESGPGLVKPSQTLSLTCTVS
GGSISSGGFYWSWIRQHPGKGLEWIGYIHHSGSTHYNPSLKSRVTISI
DTSKNLFSLRLSSVTAADTAVYYCASLVYCGGDCYSGFDYWGQGTL
VTVSSGGGGSGGGGSGGGGSDIQLTQSPSSLSASVGDRVSFTCQASQ
DINNFLNWYQQKPGKAPKLLIYDASNLETGVPSRFSGSGSGTDFTFTI
SSLQPEDIATYYCQQYGNLPFTFGGGTKVEIKRAAAIEVMYPPPYLD
NEKSNGTIIHVKGKHLCPSPLFPGPSKPFWVLVVVGGVLACYSLLVT
VAFIIFWVRSKRSRLLHSDYMNMTPRRPGPTRKHYQPYAPPRDFAAY
RSRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPE
MGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGL
YQGLSTATKDTYDALHMQALPPR (SEQ ID NO. 64)

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

CAGGTGCAGCTGCAGGAAAGCGGTCCGGGACTTGTCAAGCCGTCC
CAAACGCTGAGTCTGACGTGTACTGTCTCTGGTGGCTCTATTTCTT
CCGGGGGCTTTTATTGGTCTTGGATCAGACAACACCCTGGCAAAG
GGCTGGAGTGGATAGGGTATATTCACCACTCTGGGTCCACTCACT
ACAACCCATCATTGAAATCCAGAGTGACTATCTCAATCGACACAT
CCAAGAACCTTTTCAGCCTGAGGTTGTCATCAGTTACCGCCGCTG
ACACCGCGGTGTATTATTGCGCCTCTCTCGTGTACTGCGGTGGCG
ATTGTTATAGTGGCTTTGACTACTGGGGGCAGGGGACATTGGTTA
CCGTTTCAAGTGGAGGCGGTGGGTCTGGCGGGGGCGGTAGCGGA
GGTGGGGGGAGCGACATACAGCTTACGCAGAGCCCCTCCAGCCTT
TCAGCCTCCGTGGGGGATAGGGTGTCCTTTACCTGCCAGGCTTCC
CAGGACATAAACAACTTCCTCAATTGGTATCAGCAAAAGCCCGGG
AAAGCACCAAAGCTGCTCATCTACGATGCCAGCAACCTGGAAACC
GGAGTGCCGTCTCGCTTCTCTGGAAGTGGCAGTGGGACCGATTTC
ACTTTTACAATCTCAAGTTTGCAGCCAGAAGACATTGCAACATAC
TACTGTCAACAGTACGGCAATCTCCCCTTTACATTTGGGGGGGGA
ACTAAAGTGGAGATTAAGCGCGCTGCAGCCATTGAAGTTATGTAT
CCGCCCCCGTATCTGGATAACGAGAAATCTAATGGTACCATAATA
CATGTGAAGGGGAAGCACCTCTGTCCATCACCGCTGTTCCCCGGC

CCTTCAAAACCTTTCTGGGTACTCGTTGTCGTGGGTGGAGTTCTGG
CCTGCTATAGTCTGCTGGTGACCGTGGCGTTTATCATCTTCTGGGT
AAGATCCAAAAGAAGCCGCCTGCTCCATAGCGATTACATGAATAT
GACTCCACGCCGCCCTGGCCCCACAAGGAAACACTACCAGCCTTA
CGCACCACCTAGAGATTTCGCTGCCTATCGGAGCAGGGTGAAGTT
TTCCAGATCTGCAGATGCACCAGCGTATCAGCAGGGCCAGAACCA
ACTGTATAACGAGCTCAACCTGGGACGCAGGGAAGAGTATGACG
TTTTGGACAAGCGCAGAGGACGGGACCCTGAGATGGGTGGCAAA
CCAAGACGAAAAAACCCCCAGGAGGGTCTCTATAATGAGCTGCA
GAAGGATAAGATGGCTGAAGCCTATTCTGAAATAGGCATGAAAG
GAGAGCGGAGAAGGGGAAAAGGGCACGACGGTTTGTACCAGGGA
CTCAGCACTGCTACGAAGGATACTTATGACGCTCTCCACATGCAA
GCCCTGCCACCTAGG (SEQ ID NO. 65)

[0321] Clone 24C8 CD28 CD3 zeta CAR AA Heavy & Light Chains

QVQLQESGPGLVKPSQTLSLTCTVSGGSISSGGFYWSWIRQHPGKGL
EWIGYIHHSGSTHYNPSLKSRVTISIDTSKNLFSLRLSSVTAADTAVYY
CASLVYCGGDCYSGFDYWGQGTLVTVSSGGGGSGGGGSGGGGSDI
QLTQSPSSLSASVGDRVSFTCQASQDINNFLNWYQQKPGKAPKLLIY
DASNLETGVPSRFSGSGSGTDFTFTISSLQPEDIATYYCQQYGNLPFTF
GGGTKVEIKRAAAIEVMYPPPYLDNEKSNGTIIHVKGKHLCPSPLFPG
PSKPFWVLVVVGGVLACYSLLVTVAFIIFWVRSKRSRLLHSDYMNM
TPRRPGPTRKHYQPYAPPRDFAAYRSRVKFSRSADAPAYQQGQNQL
YNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKD
KMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALP
PR(SEQ ID NO. 66)

[0322] Clone 24C8 CD8 CD3 zeta CAR DNA Heavy & Light Chains

ATGGCACTCCCCGTAACTGCTCTGCTGCTGCCGTTGGCATTGCTCC
TGCACGCCGCACGCCCGCAGGTGCAGTTGCAGGAAAGCGGGCCT
GGCCTTGTGAAACCAAGCCAGACACTGAGCCTGACATGCACTGTG
TCCGGCGGGTCCATATCTTCCGGGGGTTTTTATTGGTCCTGGATAC
GCCAGCATCCCGGGAAAGGACTTGAATGGATTGGATATATCCACC
ATTCCGGAAGCACCCACTACAATCCAAGCCTTAAATCCCGGGTGA

CAATCTCCATCGACACCTCAAAGAATCTTTTTTCCCTGCGGTTGTC
TTCAGTAACTGCCGCCGATACCGCTGTGTACTACTGTGCCAGCCTC
GTCTATTGCGGCGGAGATTGTTATTCTGGGTTCGATTATTGGGGTC
AAGGCACACTGGTAACTGTCAGCAGCGGAGGCGGCGGTTCCGGG
GGCGGGGGCAGTGGAGGGGGCGGATCTGACATTCAGCTTACGCA
GTCCCCATCTTCACTTAGCGCCAGCGTTGGCGATCGGGTCAGCTTC
ACGTGTCAAGCAAGTCAGGATATCAACAACTTTCTTAACTGGTAC
CAGCAGAAGCCAGGCAAGGCACCCAAGTTGCTGATTTACGATGCT
TCTAACCTCGAGACGGGAGTGCCTAGCCGCTTCTCCGGGAGCGGC
AGCGGCACAGACTTTACCTTTACGATTTCCAGTCTGCAGCCAGAG
GATATAGCAACTTATTACTGTCAGCAGTATGGCAACCTCCCTTTTA
CCTTCGGTGGTGGCACAAAGGTCGAGATTAAAAGAGCCGCAGCG
TTGTCCAACTCCATAATGTATTTTTCTCATTTTGTGCCCGTCTTTCT
GCCTGCCAAACCTACCACCACCCCCGCCCCACGACCACCTACTCC
AGCCCCCACCATCGCCTCCCAGCCCCTCAGCCTGAGGCCAGAGGC
TTGTCGCCCTGCTGCGGGGGGCGCTGTCCATACCAGAGGACTCGA
CTTCGCCTGCGATATTTATATATGGGCCCCCCTCGCCGGCACCTGC
GGAGTCTTGCTCCTGAGCCTTGTGATCACGCTTTATTGTAACCATC
GGAATAGATCCAAAAGAAGCCGCCTGCTCCATAGCGATTACATGA
ATATGACTCCACGCCGCCCTGGCCCCACAAGGAAACACTACCAGC
CTTACGCACCACCTAGAGATTTCGCTGCCTATCGGAGCAGGGTGA
AGTTTTCCAGATCTGCAGATGCACCAGCGTATCAGCAGGGCCAGA
ACCAACTGTATAACGAGCTCAACCTGGGACGCAGGGAAGAGTAT
GACGTTTTGGACAAGCGCAGAGGACGGGACCCTGAGATGGGTGG
CAAACCAAGACGAAAAAACCCCCAGGAGGGTCTCTATAATGAGC
TGCAGAAGGATAAGATGGCTGAAGCCTATTCTGAAATAGGCATG
AAAGGAGAGCGGAGAAGGGGAAAAGGGCACGACGGTTTGTACCA
GGGACTCAGCACTGCTACGAAGGATACTTATGACGCTCTCCACAT
GCAAGCCCTGCCACCTAGGTAA (SEQ ID NO. 67)

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

DTSKNLFSLRLSSVTAADTAVYYCASLVYCGGDCYSGFDYWGQGTL
VTVSSGGGGSGGGGSGGGGSDIQLTQSPSSLSASVGDRVSFTCQASQ
DINNFLNWYQQKPGKAPKLLIYDASNLETGVPRFSGSGSGTDFTFTIS
SLQPEDIATYYCQYGNLPFTFGGGTKVEIKRAAALSNSIMYFSHFVPV
FLPAKPTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFA
CDIYIWAPLAGTCGVLLLSLVITLYCNHRNRSKRSRLLHSDYMNMTP
RRPGPTRKHYQPYAPPRDFAAYRSRVKFSRSADAPAYQQGQNQLYN
ELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKM
AEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR
(SEQ ID NO. 68)

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

CAGGTGCAGTTGCAGGAAAGCGGGCCTGGCCTTGTGAAACCAAG
CCAGACACTGAGCCTGACATGCACTGTGTCCGGCGGGTCCATATC
TTCCGGGGGTTTTTATTGGTCCTGGATACGCCAGCATCCCGGGAA
AGGACTTGAATGGATTGGATATATCCACCATTCCGGAAGCACCCA
CTACAATCCAAGCCTTAAATCCCGGGTGACAATCTCCATCGACAC
CTCAAAGAATCTTTTTTCCCTGCGGTTGTCTTCAGTAACTGCCGCC
GATACCGCTGTGTACTACTGTGCCAGCCTCGTCTATTGCGGCGGA
GATTGTTATTCTGGGTTCGATTATTGGGGTCAAGGCACACTGGTA
ACTGTCAGCAGCGGAGGCGGCGGTTCCGGGGGCGGGGGCAGTGG
AGGGGGCGGATCTGACATTCAGCTTACGCAGTCCCCATCTTCACT
TAGCGCCAGCGTTGGCGATCGGGTCAGCTTCACGTGTCAAGCAAG
TCAGGATATCAACAACTTTCTTAACTGGTACCAGCAGAAGCCAGG
CAAGGCACCCAAGTTGCTGATTTACGATGCTTCTAACCTCGAGAC
GGGAGTGCCTAGCCGCTTCTCCGGGAGCGGCAGCGGCACAGACTT
TACCTTTACGATTTCCAGTCTGCAGCCAGAGGATATAGCAACTTA
TTACTGTCAGCAGTATGGCAACCTCCCTTTTACCTTCGGTGGTGGC
ACAAAGGTCGAGATTAAAAGAGCCGCAGCGTTGTCCAACTCCATA
ATGTATTTTTCTCATTTTGTGCCCGTCTTTCTGCCTGCCAAACCTAC
CACCACCCCCGCCCCACGACCACCTACTCCAGCCCCCACCATCGC
CTCCCAGCCCCTCAGCCTGAGGCCAGAGGCTTGTCGCCCTGCTGC
GGGGGGCGCTGTCCATACCAGAGGACTCGACTTCGCCTGCGATAT
TTATATATGGGCCCCCCTCGCCGGCACCTGCGGAGTCTTGCTCCTG

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

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

QVQLQESGPGLVKPSQTLSLTCTVSGGSISSGGFYWSWIRQHPGKGL
EWIGYIHHSGSTHYNPSLKSRVTISIDTSKNLFSLRLSSVTAADTAVYY
CASLVYCGGDCYSGFDYWGQGTLVTVSSGGGGSGGGGSGGGGSDI
QLTQSPSSLSASVGDRVSFTCQASQDINNFLNWYQQKPGKAPKLLIY
DASNLETGVPSRFSGSGSGTDFTFTISSLQPEDIATYYCQQYGNLPFTF
GGGTKVEIKRAAALSNSIMYFSHFVPVFLPAKPTTTPAPRPPTPAPTIA
SQPLSLRPEACRPAAGGAVHTRGLDFACDIYIWAPLAGTCGVLLLSL
VITLYCNHRNRSKRSRLLHSDYMNMTPRRPGPTRKHYQPYAPPRDF
AAYRSRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRG
RDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKG
HDGLYQGLSTA TKDTYDALHM QALPPR (SEQ ID NO. 70)

[0326] Clone 20C5.1 HC DNA

CAGGTCCAACTGGTGCAGTCCGGAGCCGAAGTCAAGAAACCAGG
TGCCTCCGTTAAAGTGAGTTGCAAAGTCTCTGGATACACTCTGAC
CGAGCTCTCTATGCACTGGGTCCGGCAGGCCCCCGGCAAGGGATT
GGAATGGATGGGCGGGTTCGATCCTGAGGACGGAGAGACTATCT
ACGCTCAAAAATTCCAGGGACGAGTGACTGTGACCGAAGACACT
AGTACCGACACTGCCTACATGGAACTTTCCTCTCTGCGATCAGAA
GATACCGCAGTGTACTACTGTGCTACTGAATCTAGGGGCATTGGA

TGGCCCTACTTCGATTACTGGGGTCAGGGAACTCTGGTGACTGTC
TCCAGC (SEQ ID NO. 71)

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

[0331] Clone 20C5.1 LC DNA
GATATTCAGATGACTCAATCTCCTTCTTCTCTGTCCGCTTCCGTGG
GCGATAGAGTGACCATTACTTGTAGGGCGTCCCAGTCAATCTCCA
GTTATTTGAATTGGTATCAGCAGAAGCCCGGGAAAGCACCTAAGC
TGTTGATCAGCGGGGCTTCTAGCCTGAAGAGTGGGGTACCTTCAC
GGTTCAGCGGAAGCGGAAGCGGAACCGATTTCACCCTGACTATCA
GCAGCCTGCCACCTGAGGACTTTGCAACTTACTACTGCCAACAGT
CATACAGCACTCCGATCACTTTCGGCCAGGGCACCCGGCTCGAAA
TCAAGCGC (SEQ ID NO. 76)

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

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

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

ATGGCACTCCCCGTAACTGCTCTGCTGCTGCCGTTGGCATTGCTCC
TGCACGCCGCACGCCCGCAGGTCCAACTGGTGCAGTCCGGAGCCG
AAGTCAAGAAACCAGGTGCCTCCGTTAAAGTGAGTTGCAAAGTCT
CTGGATACACTCTGACCGAGCTCTCTATGCACTGGGTCCGGCAGG
CCCCCGGCAAGGGATTGGAATGGATGGGCGGGTTCGATCCTGAG
GACGGAGAGACTATCTACGCTCAAAAATTCCAGGGACGAGTGAC
TGTGACCGAAGACACTAGTACCGACACTGCCTACATGGAACTTTC
CTCTCTGCGATCAGAAGATACCGCAGTGTACTACTGTGCTACTGA
ATCTAGGGGCATTGGATGGCCCTACTTCGATTACTGGGGTCAGGG
AACTCTGGTGACTGTCTCCAGCGGTGGAGGTGGCAGCGGTGGTGG
CGGAAGCGGGGGGGGCGGCTCTGATATTCAGATGACTCAATCTCC
TTCTTCTCTGTCCGCTTCCGTGGGCGATAGAGTGACCATTACTTGT
AGGGCGTCCCAGTCAATCTCCAGTTATTTGAATTGGTATCAGCAG
AAGCCCGGGAAAGCACCTAAGCTGTTGATCAGCGGGGCTTCTAGC
CTGAAGAGTGGGGTACCTTCACGGTTCAGCGGAAGCGGAAGCGG
AACCGATTTCACCCTGACTATCAGCAGCCTGCCACCTGAGGACTT
TGCAACTTACTACTGCCAACAGTCATACAGCACTCCGATCACTTTC
GGCCAGGGCACCCGGCTCGAAATCAAGCGCGCTGCTGCTTTGGAC
AATGAGAAGTCAAACGGCACCATCATACATGTTAAAGGTAAACA
TCTGTGTCCCTCCCCGCTGTTCCCCGGCCCTTCCAAACCGTTCTGG
GTTCTGGTGGTGGTCGGAGGCGTACTCGCTTGCTATAGTCTGCTG
GTAACTGTCGCCTTCATCATCTTTTGGGTGAGATCCAAAAGAAGC
CGCCTGCTCCATAGCGATTACATGAATATGACTCCACGCCGCCCT
GGCCCCACAAGGAAACACTACCAGCCTTACGCACCACCTAGAGAT
TTCGCTGCCTATCGGAGCAGGGTGAAGTTTTCCAGATCTGCAGAT
GCACCAGCGTATCAGCAGGGCCAGAACCAACTGTATAACGAGCT
CAACCTGGGACGCAGGGAAGAGTATGACGTTTTGGACAAGCGCA
GAGGACGGGACCCTGAGATGGGTGGCAAACCAAGACGAAAAAAC
CCCCAGGAGGGTCTCTATAATGAGCTGCAGAAGGATAAGATGGCT
GAAGCCTATTCTGAAATAGGCATGAAAGGAGAGCGGAGAAGGGG
AAAAGGGCACGACGGTTTGTACCAGGGACTCAGCACTGCTACGA
AGGATACTTATGACGCTCTCCACATGCAAGCCCTGCCACCTAGGT
AA (SEQ ID NO. 81)

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

MALPVTALLLPLALLLHAARPQVQLVQSGAEVKKPGASVKVSCKV
SGYTLTELSMHWVRQAPGKGLEWMGGFDPEDGETIYAQKFQGRVT
VTEDTSTDTAYMELSSLRSEDTAVYYCATESRGIGWPYFDYWGQGT
LVTVSSGGGGSGGGGSGGGGSDIQMTQSPSSLSASVGDRVTITCRAS
QSISSYLNWYQQKPGKAPKLLISGASSLKSGVPSRFSGSGSGTDFTLTI
SSLPPEDFATYYCQQSYSTPITFGQGTRLEIKRAAALDNEKSNGTIIHV
KGKHLCPSPLFPGPSKPFWVLVVVGGVLACYSLLVTVAFIIFWVRSK
RSRLLHSDYMNMTPRRPGPTRKHYQPYAPPRDFAAYRSRVKFSRSA
DAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKN
PQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATK
DTYDALHMQALPPR (SEQ ID NO. 82)

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

CAGGTCCAACTGGTGCAGTCCGGAGCCGAAGTCAAGAAACCAGG
TGCCTCCGTTAAAGTGAGTTGCAAAGTCTCTGGATACACTCTGAC
CGAGCTCTCTATGCACTGGGTCCGGCAGGCCCCCGGCAAGGGATT
GGAATGGATGGGCGGGTTCGATCCTGAGGACGGAGAGACTATCT
ACGCTCAAAAATTCCAGGGACGAGTGACTGTGACCGAAGACACT
AGTACCGACACTGCCTACATGGAACTTTCCTCTCTGCGATCAGAA
GATACCGCAGTGTACTACTGTGCTACTGAATCTAGGGGCATTGGA
TGGCCCTACTTCGATTACTGGGGTCAGGGAACTCTGGTGACTGTC
TCCAGCGGTGGAGGTGGCAGCGGTGGTGGCGGAAGCGGGGGGGG
CGGCTCTGATATTCAGATGACTCAATCTCCTTCTTCTCTGTCCGCT
TCCGTGGGCGATAGAGTGACCATTACTTGTAGGGCGTCCCAGTCA
ATCTCCAGTTATTTGAATTGGTATCAGCAGAAGCCCGGGAAAGCA
CCTAAGCTGTTGATCAGCGGGGCTTCTAGCCTGAAGAGTGGGGTA
CCTTCACGGTTCAGCGGAAGCGGAAGCGGAACCGATTTCACCCTG
ACTATCAGCAGCCTGCCACCTGAGGACTTTGCAACTTACTACTGC
CAACAGTCATACAGCACTCCGATCACTTTCGGCCAGGGCACCCGG
CTCGAAATCAAGCGCGCTGCTGCTTTGGACAATGAGAAGTCAAAC
GGCACCATCATACATGTTAAAGGTAAACATCTGTGTCCCTCCCCG
CTGTTCCCCGGCCCTTCCAAACCGTTCTGGGTTCTGGTGGTGGTCG

GAGGCGTACTCGCTTGCTATAGTCTGCTGGTAACTGTCGCCTTCAT
CATCTTTTGGGTGAGATCCAAAAGAAGCCGCCTGCTCCATAGCGA
TTACATGAATATGACTCCACGCCGCCCTGGCCCCACAAGGAAACA
CTACCAGCCTTACGCACCACCTAGAGATTTCGCTGCCTATCGGAG
CAGGGTGAAGTTTTCCAGATCTGCAGATGCACCAGCGTATCAGCA
GGGCCAGAACCAACTGTATAACGAGCTCAACCTGGGACGCAGGG
AAGAGTATGACGTTTTGGACAAGCGCAGAGGACGGGACCCTGAG
ATGGGTGGCAAACCAAGACGAAAAAACCCCCAGGAGGGTCTCTA
TAATGAGCTGCAGAAGGATAAGATGGCTGAAGCCTATTCTGAAAT
AGGCATGAAAGGAGAGCGGAGAAGGGGAAAAGGGCACGACGGT
TTGTACCAGGGACTCAGCACTGCTACGAAGGATACTTATGACGCT
CTCCACATGCAAGCCCTGCCACCTAGG (SEQ ID NO. 83)

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

QVQLVQSGAEVKKPGASVKVSCKVSGYTLTELSMHWVRQAPGKGL
EWMGGFDPEDGETIYAQKFQGRVTVTEDTSTDTAYMELSSLRSEDT
AVYYCATESRGIGWPYFDYWGQGTLVTVSSGGGGSGGGGSGGGGS
DIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKLLI
SGASSLKSGVPSRFSGSGSGTDFTLTISSLPPEDFATYYCQQSYSTPITF
GQGTRLEIKRAAALDNEKSNGTIIHVKGKHLCPSPLFPGPSKPFWVLV
VVGGVLACYSLLVTVAFIIFWVRSKRSRLLHSDYMNMTPRRPGPTRK
HYQPYAPPRDFAAYRSRVKFSRSADAPAYQQGQNQLYNELNLGRRE
EYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIG
MKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR (SEQ ID NO. 84)

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

ATGGCACTCCCCGTAACTGCTCTGCTGCTGCCGTTGGCATTGCTCC
TGCACGCCGCACGCCCGCAGGTGCAGCTTGTGCAGAGCGGGGCC
GAGGTGAAGAAGCCCGGGGCCAGCGTCAAAGTGTCCTGTAAGGT
CAGCGGTTACACCCTCACCGAGCTGAGCATGCACTGGGTACGGCA
GGCTCCCGGCAAAGGTCTTGAGTGGATGGGTGGATTTGATCCAGA
AGATGGAGAGACTATCTACGCCCAGAAGTTCCAGGGCCGGGTCA
CCGTAACAGAAGACACCTCAACTGACACCGCTTACATGGAGCTGA

GTTCACTGCGGTCCGAGGACACGGCCGTGTATTATTGTGCCACCG
AGAGCCGCGGAATCGGATGGCCTTACTTCGACTACTGGGGACAGG
GTACACTTGTTACAGTATCATCCGGGGGTGGCGGCTCTGGTGGGG
GCGGCTCCGGAGGGGGTGGATCAGATATCCAAATGACTCAAAGT
CCAAGTTCCCTGTCTGCCTCAGTCGGAGATAGAGTCACCATAACC
TGCAGGGCAAGTCAGTCCATCTCCTCCTATCTGAACTGGTACCAA
CAGAAACCTGGAAAGGCGCCTAAGCTCCTGATCTCCGGAGCCTCA
TCTTTGAAATCCGGTGTCCCATCTCGCTTCAGTGGCTCTGGAAGCG
GTACAGATTTTACTTTGACCATTAGCAGCCTCCCACCGGAAGACT
TTGCTACATATTACTGCCAGCAGTCTTACTCAACCCCAATCACCTT
CGGGCAAGGCACCAGACTCGAAATAAAAAGAGCAGCTGCTATCG
AGGTTATGTACCCACCGCCGTACTTGGATAACGAAAAAAGCAATG
GGACCATCATTCATGTGAAGGGTAAGCACCTTTGCCCTAGCCCAC
TGTTTCCTGGCCCGAGTAAACCCTTTTGGGTACTTGTGGTCGTCGG
CGGCGTGCTGGCCTGCTACTCACTCCTGGTTACCGTCGCATTCATC
ATCTTTTGGGTGAGATCCAAAAGAAGCCGCCTGCTCCATAGCGAT
TACATGAATATGACTCCACGCCGCCCTGGCCCCACAAGGAAACAC
TACCAGCCTTACGCACCACCTAGAGATTTCGCTGCCTATCGGAGC
AGGGTGAAGTTTTCCAGATCTGCAGATGCACCAGCGTATCAGCAG
GGCCAGAACCAACTGTATAACGAGCTCAACCTGGGACGCAGGGA
AGAGTATGACGTTTTGGACAAGCGCAGAGGACGGGACCCTGAGA
TGGGTGGCAAACCAAGACGAAAAAACCCCCAGGAGGGTCTCTAT
AATGAGCTGCAGAAGGATAAGATGGCTGAAGCCTATTCTGAAAT
AGGCATGAAAGGAGAGCGGAGAAGGGGAAAAGGGCACGACGGT
TTGTACCAGGGACTCAGCACTGCTACGAAGGATACTTATGACGCT
CTCCACATGCAAGCCCTGCCACCTAGGTAA (SEQ ID NO. 85)

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

MALPVTALLLPLALLLHAARPQVQLVQSGAEVKKPGASVKVSCKV
SGYTLTELSMHWVRQAPGKGLEWMGGFDPEDGETIYAQKFQGRVT
VTEDTSTDTAYMELSSLRSEDTAVYYCATESRGIGWPYFDYWGQGT
LVTVSSGGGGSGGGGSGGGGSDIQMTQSPSSLSASVGDRVTITCRAS
QSISSYLNWYQQKPGKAPKLLISGASSLKSGVPSRFSGSGSGTDFTLTI

SSLPPEDFATYYCQQSYSTPITFGQGTRLEIKRAAAIEVMYPPPYLDNE
KSNGTIIHVKGKHLCPSPLFPGPSKPFWVLVVVGGVLACYSLLVTVA
FIIFWVRSKRSRLLHSDYMNMTPRRPGPTRKHYQPYAPPRDFAAYRS
RVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEM
GGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLY
QGLST ATKDTYDALH MQALPPR (SEQ ID NO. 86)

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

CAGGTGCAGCTTGTGCAGAGCGGGGCCGAGGTGAAGAAGCCCGG
GGCCAGCGTCAAAGTGTCCTGTAAGGTCAGCGGTTACACCCTCAC
CGAGCTGAGCATGCACTGGGTACGGCAGGCTCCCGGCAAAGGTCT
TGAGTGGATGGGTGGATTTGATCCAGAAGATGGAGAGACTATCTA
CGCCCAGAAGTTCCAGGGCCGGGTCACCGTAACAGAAGACACCT
CAACTGACACCGCTTACATGGAGCTGAGTTCACTGCGGTCCGAGG
ACACGGCCGTGTATTATTGTGCCACCGAGAGCCGCGGAATCGGAT
GGCCTTACTTCGACTACTGGGGACAGGGTACACTTGTTACAGTAT
CATCCGGGGGTGGCGGCTCTGGTGGGGGCGGCTCCGGAGGGGGT
GGATCAGATATCCAAATGACTCAAAGTCCAAGTTCCCTGTCTGCC
TCAGTCGGAGATAGAGTCACCATAACCTGCAGGGCAAGTCAGTCC
ATCTCCTCCTATCTGAACTGGTACCAACAGAAACCTGGAAAGGCG
CCTAAGCTCCTGATCTCCGGAGCCTCATCTTTGAAATCCGGTGTCC
CATCTCGCTTCAGTGGCTCTGGAAGCGGTACAGATTTTACTTTGAC
CATTAGCAGCCTCCCACCGGAAGACTTTGCTACATATTACTGCCA
GCAGTCTTACTCAACCCCAATCACCTTCGGGCAAGGCACCAGACT
CGAAATAAAAAGAGCAGCTGCTATCGAGGTTATGTACCCACCGCC
GTACTTGGATAACGAAAAAAGCAATGGGACCATCATTCATGTGAA
GGGTAAGCACCTTTGCCCTAGCCCACTGTTTCCTGGCCCGAGTAA
ACCCTTTTGGGTACTTGTGGTCGTCGGCGGCGTGCTGGCCTGCTAC
TCACTCCTGGTTACCGTCGCATTCATCATCTTTTGGGTGAGATCCA
AAAGAAGCCGCCTGCTCCATAGCGATTACATGAATATGACTCCAC
GCCGCCCTGGCCCCACAAGGAAACACTACCAGCCTTACGCACCAC
CTAGAGATTTCGCTGCCTATCGGAGCAGGGTGAAGTTTTCCAGAT
CTGCAGATGCACCAGCGTATCAGCAGGGCCAGAACCAACTGTATA
ACGAGCTCAACCTGGGACGCAGGGAAGAGTATGACGTTTTGGAC

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

[0343] Clone 20C5.1 CD28 CD3 zeta CAR AA Heavy & Light Chains

QVQLVQSGAEVKKPGASVKVSCKVSGYTLTELSMHWVRQAPGKGL
EWMGGFDPEDGETIYAQKFQGRVTVTEDTSTDTAYMELSSLRSEDT
AVYYCATESRGIGWPYFDYWGQGTLVTVSSGGGGSGGGGSGGGGS
DIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKLLI
SGASSLKSGVPSRFSGSGSGTDFTLTISSLPPEDFATYYCQQSYSTPITF
GQGTRLEIKRAAAIEVMYPPPYLDNEKSNGTIIHVKGKHLCPSPLFPG
PSKPFWVLVVVGGVLACYSLLVTVAFIIFWVRSKRSRLLHSDYMNM
TPRRPGPTRKHYQPYAPPRDFAAYRSRVKFSRSADAPAYQQGQNQL
YNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKD
KMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHM
QALPPR (SEQ ID NO. 88)

[0344] Clone 20C5.1 CD8 CD3 zeta CAR DNA Heavy & Light Chains

ATGGCACTCCCCGTAACTGCTCTGCTGCTGCCGTTGGCATTGCTCC
TGCACGCCGCACGCCCGCAGGTGCAGTTGGTGCAAAGCGGCGCA
GAAGTTAAGAAACCTGGGGCGTCAGTTAAGGTGTCTTGCAAAGTA
TCTGGCTATACCCTCACTGAGCTGTCCATGCATTGGGTAAGGCAG
GCTCCTGGAAAGGGGCTCGAATGGATGGGAGGATTTGACCCTGA
AGACGGAGAGACCATCTACGCCCAGAAATTCCAGGGTAGAGTAA
CAGTGACTGAGGACACTAGCACTGACACAGCGTACATGGAGCTG
AGTTCTCTGAGAAGTGAGGACACAGCCGTTTACTACTGCGCTACC
GAGTCCAGAGGTATTGGCTGGCCATACTTCGACTATTGGGGTCAG
GGCACCCTGGTTACAGTGAGTTCAGGAGGCGGGGGCTCTGGGGG
GGGCGGTTCCGGAGGGGGGGGCTCAGATATACAGATGACGCAGA
GTCCATCAAGTCTCTCAGCCAGCGTGGGAGATCGCGTGACTATTA
CTTGCCGCGCCAGCCAGAGTATTAGCTCCTATCTGAATTGGTACC

AGCAAAAGCCCGGGAAGGCCCCTAAGCTTCTGATTTCTGGCGCCT
CCTCTTTGAAGTCAGGTGTGCCAAGCAGATTTAGCGGGTCTGGAA
GTGGCACTGACTTTACACTTACTATCTCCAGCCTGCCCCCAGAGG
ATTTTGCCACATATTACTGTCAGCAAAGCTACTCTACTCCAATCAC
TTTCGGCCAGGGCACAAGATTGGAGATTAAGAGGGCTGCCGCACT
TTCAAATTCCATCATGTATTTCAGCCATTTTGTGCCTGTTTTTCTTC
CGGCCAAACCTACAACCACTCCCGCCCCACGCCCACCTACTCCCG
CCCCTACCATTGCCTCCCAGCCTCTGTCTCTTAGACCTGAGGCTTG
TAGACCTGCTGCCGGCGGAGCCGTGCACACTCGCGGTCTGGACTT
CGCCTGCGACATCTATATCTGGGCCCCTCTGGCCGGCACCTGCGG
CGTTCTCCTTCTCTCACTCGTAATCACACTCTATTGCAATCACAGG
AACAGATCCAAAAGAAGCCGCCTGCTCCATAGCGATTACATGAAT
ATGACTCCACGCCGCCCTGGCCCCACAAGGAAACACTACCAGCCT
TACGCACCACCTAGAGATTTCGCTGCCTATCGGAGCAGGGTGAAG
TTTTCCAGATCTGCAGATGCACCAGCGTATCAGCAGGGCCAGAAC
CAACTGTATAACGAGCTCAACCTGGGACGCAGGGAAGAGTATGA
CGTTTTGGACAAGCGCAGAGGACGGGACCCTGAGATGGGTGGCA
AACCAAGACGAAAAAACCCCCAGGAGGGTCTCTATAATGAGCTG
CAGAAGGATAAGATGGCTGAAGCCTATTCTGAAATAGGCATGAA
AGGAGAGCGGAGAAGGGGAAAAGGGCACGACGGTTTGTACCAGG
GACTCAGCACTGCTACGAAGGATACTTATGACGCTCTCCACATGC
AAGCCCTGCCACCTAGGTAA (SEQ ID NO. 89)

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

MALPVTALLLPLALLLHAARPQVQLVQSGAEVKKPGASVKVSCKV
SGYTLTELSMHWVRQAPGKGLEWMGGFDPEDGETIYAQKFQGRVT
VTEDTSTDTAYMELSSLRSEDTAVYYCATESRGIGWPYFDYWGQGT
LVTVSSGGGGSGGGGSGGGGSDIQMTQSPSSLSASVGDRVTITCRAS
QSISSYLNWYQQKPGKAPKLLISGASSLKSGVPSRFSGSGSGTDFTLTI
SSLPPEDFATYYCQQSYSTPITFGQGTRLEIKRAAALSNSIMYFSHFVP
VFLPAKPTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDF
ACDIYIWAPLAGTCGVLLLSLVITLYCNHRNRSKRSRLLHSDYMNMT
PRRPGPTRKHYQPYAPPRDFAAYRSRVKFSRSADAPAYQQGQNQLY

NELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDK
MAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPP
R (SEQ ID NO. 90)

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

CAGGTGCAGTTGGTGCAAAGCGGCGCAGAAGTTAAGAAACCTGG
GGCGTCAGTTAAGGTGTCTTGCAAAGTATCTGGCTATACCCTCAC
TGAGCTGTCCATGCATTGGGTAAGGCAGGCTCCTGGAAAGGGGCT
CGAATGGATGGGAGGATTTGACCCTGAAGACGGAGAGACCATCT
ACGCCCAGAAATTCCAGGGTAGAGTAACAGTGACTGAGGACACT
AGCACTGACACAGCGTACATGGAGCTGAGTTCTCTGAGAAGTGAG
GACACAGCCGTTTACTACTGCGCTACCGAGTCCAGAGGTATTGGC
TGGCCATACTTCGACTATTGGGGTCAGGGCACCCTGGTTACAGTG
AGTTCAGGAGGCGGGGGCTCTGGGGGGGGCGGTTCCGGAGGGGG
GGGCTCAGATATACAGATGACGCAGAGTCCATCAAGTCTCTCAGC
CAGCGTGGGAGATCGCGTGACTATTACTTGCCGCGCCAGCCAGAG
TATTAGCTCCTATCTGAATTGGTACCAGCAAAAGCCCGGGAAGGC
CCCTAAGCTTCTGATTTCTGGCGCCTCCTCTTTGAAGTCAGGTGTG
CCAAGCAGATTTAGCGGGTCTGGAAGTGGCACTGACTTTACACTT
ACTATCTCCAGCCTGCCCCCAGAGGATTTTGCCACATATTACTGTC
AGCAAAGCTACTCTACTCCAATCACTTTCGGCCAGGGCACAAGAT
TGGAGATTAAGAGGGCTGCCGCACTTTCAAATTCCATCATGTATT
TCAGCCATTTTGTGCCTGTTTTTCTTCCGGCCAAACCTACAACCAC
TCCCGCCCCACGCCCACCTACTCCCGCCCCTACCATTGCCTCCCAG
CCTCTGTCTCTTAGACCTGAGGCTTGTAGACCTGCTGCCGGCGGA
GCCGTGCACACTCGCGGTCTGGACTTCGCCTGCGACATCTATATCT
GGGCCCCTCTGGCCGGCACCTGCGGCGTTCTCCTTCTCTCACTCGT
AATCACACTCTATTGCAATCACAGGAACAGATCCAAAAGAAGCC
GCCTGCTCCATAGCGATTACATGAATATGACTCCACGCCGCCCTG
GCCCCACAAGGAAACACTACCAGCCTTACGCACCACCTAGAGATT
TCGCTGCCTATCGGAGCAGGGTGAAGTTTTCCAGATCTGCAGATG
CACCAGCGTATCAGCAGGGCCAGAACCAACTGTATAACGAGCTC
AACCTGGGACGCAGGGAAGAGTATGACGTTTTGGACAAGCGCAG
AGGACGGGACCCTGAGATGGGTGGCAAACCAAGACGAAAAAACC

CCCAGGAGGGTCTCTATAATGAGCTGCAGAAGGATAAGATGGCT
GAAGCCTATTCTGAAATAGGCATGAAAGGAGAGCGGAGAAGGGG
AAAAGGGCACGACGGTTTGTACCAGGGACTCAGCACTGCTACGA
AGGATACTTATGACGCTCTCCACATGCAAGCCCTGCCACCTAGG
(SEQ ID NO. 91)

[0347] Clone 20C5.1 CD8 CD3 zeta CAR AA Heavy & Light Chains
QVQLVQSGAEVKKPGASVKVSCKVSGYTLTELSMHWVRQAPGKGL
EWMGGFDPEDGETIYAQKFQGRVTVTEDTSTDTAYMELSSLRSEDT
AVYYCATESRGIGWPYFDYWGQGTLVTVSSGGGGSGGGGSGGGGS
DIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKLLI
SGASSLKSGVPSRFSGSGSGTDFTLTISSLPPEDFATYYCQQSYSTPITF
GQGTRLEIKRAAALSNSIMYFSHFVPVFLPAKPTTTPAPRPPTPAPTIA
SQPLSLRPEACRPAAGGAVHTRGLDFACDIYIWAPLAGTCGVLLLSL
VITLYCNHRNRSKRSRLLHSDYMNMTPRRPGPTRKHYQPYAPPRDF
AAYRSRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRG
RDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKG
HDGLYQGLSTATKDTYDALHMQALPPR (SEQ ID NO. 92)

[0348] Clone 20C5.2 HC DNA
CAGGTCCAGTTGGTCGAAAGTGGCGGTGGTGTAGTGCAGCCGGGC
CGCAGTTTGAGGCTTTCCTGTGCGGCTTCAGGCTTTACTTTTTCCA
GCTATGGAATGCACTGGGTGCGGCAGGCCCCCGGCAAAGGACTT
GAGTGGGTGGCCGTCATTTCTTATGACGGATCAGATAAGTACTAC
GTGGACAGCGTCAAGGGCAGATTCACCATCTCTAGGGACAACAGT
AAAAATAGACTCTACCTCCAGATGAATAGCCTCAGAGCTGAAGAC
ACGGCCGTCTACTATTGTGCTCGGGAGCGGTATAGTGGCAGAGAC
TACTGGGGGCAGGGCACACTCGTTACAGTGAGTAGC (SEQ ID NO.
93)

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

GAGATTGTTATGACCCAGAGTCCTGCGACCCTCTCAGTCAGCCCC
GGGGAGCGCGCAACTTTGTCTTGCAGAGCTAGTCAGTCCGTGTCC
TCTCTTCTGACATGGTACCAGCAAAAGCCCGGGCAGGCTCCGCGC
CTTTTGATCTTTGGGGCTTCAACAAGAGCCACTGGGATTCCCGCA
CGATTCTCTGGCTCCGGGAGCGGTACTGGTTTCACCCTGACGATT
AGCAGTCTCCAGAGCGAGGACTTCGCCGTATACTACTGCCAGCAG
TACGATACGTGGCCATTCACTTTTGGACCAGGGACTAAAGTGGAT
TTTAAGCGC (SEQ ID NO. 98)

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

EIVMTQSPATLSVSPGERATLSCRASQSVSSLLTWYQQKPGQAPRLLI
FGASTRATGIPARFSGSGSGTGFTLTISSLQSEDFAVYYCQQYDTWPF
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)

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

ATGGCACTCCCCGTAACTGCTCTGCTGCTGCCGTTGGCATTGCTCC
TGCACGCCGCACGCCCGCAGGTCCAGTTGGTCGAAAGTGGCGGTG
GTGTAGTGCAGCCGGGCCGCAGTTTGAGGCTTTCCTGTGCGGCTT
CAGGCTTTACTTTTTCCAGCTATGGAATGCACTGGGTGCGGCAGG
CCCCCGGCAAAGGACTTGAGTGGGTGGCCGTCATTTCTTATGACG
GATCAGATAAGTACTACGTGGACAGCGTCAAGGGCAGATTCACC
ATCTCTAGGGACAACAGTAAAAATAGACTCTACCTCCAGATGAAT
AGCCTCAGAGCTGAAGACACGGCCGTCTACTATTGTGCTCGGGAG
CGGTATAGTGGCAGAGACTACTGGGGGCAGGGCACACTCGTTAC

AGTGAGTAGCGGCGGAGGAGGGAGTGGGGGCGGTGGCTCCGGTG
GAGGAGGTTCTGAGATTGTTATGACCCAGAGTCCTGCGACCCTCT
CAGTCAGCCCCGGGGAGCGCGCAACTTTGTCTTGCAGAGCTAGTC
AGTCCGTGTCCTCTCTTCTGACATGGTACCAGCAAAAGCCCGGGC
AGGCTCCGCGCCTTTTGATCTTTGGGGCTTCAACAAGAGCCACTG
GGATTCCCGCACGATTCTCTGGCTCCGGGAGCGGTACTGGTTTCA
CCCTGACGATTAGCAGTCTCCAGAGCGAGGACTTCGCCGTATACT
ACTGCCAGCAGTACGATACGTGGCCATTCACTTTTGGACCAGGGA
CTAAAGTGGATTTTAAGCGCGCCGCCGCTCTCGATAACGAAAAGT
CAAATGGCACCATAATCCACGTCAAAGGCAAGCACCTGTGCCCTT
CCCCGCTCTTCCCCGGACCCAGTAAACCATTTTGGGTGCTGGTTGT
TGTGGGGGGCGTGCTGGCCTGCTATAGCCTTTTGGTCACTGTAGC
CTTCATTATTTTTTGGGTCAGATCCAAAAGAAGCCGCCTGCTCCAT
AGCGATTACATGAATATGACTCCACGCCGCCCTGGCCCCACAAGG
AAACACTACCAGCCTTACGCACCACCTAGAGATTTCGCTGCCTAT
CGGAGCAGGGTGAAGTTTTCCAGATCTGCAGATGCACCAGCGTAT
CAGCAGGGCCAGAACCAACTGTATAACGAGCTCAACCTGGGACG
CAGGGAAGAGTATGACGTTTTGGACAAGCGCAGAGGACGGGACC
CTGAGATGGGTGGCAAACCAAGACGAAAAAACCCCCAGGAGGGT
CTCTATAATGAGCTGCAGAAGGATAAGATGGCTGAAGCCTATTCT
GAAATAGGCATGAAAGGAGAGCGGAGAAGGGGAAAAGGGCACG
ACGGTTTGTACCAGGGACTCAGCACTGCTACGAAGGATACTTATG
ACGCTCTCCACATGCAAGCCCTGCCACCTAGGTAA (SEQ ID NO. 103)

[0359] Clone 20C5.2 CD28T CD3 zeta CAR AA Heavy & Light Chains
(Signal Peptide in **Bold**)

MALPVTALLLPLALLLHAARPQVQLVESGGGVVQPGRSLRLSCAA
SGFTFSSYGMHWVRQAPGKGLEWVAVISYDGSDKYYVDSVKGRFTI
SRDNSKNRLYLQMNSLRAEDTAVYYCARERYSGRDYWGQGTLVTV
SSGGGGSGGGGSGGGGSEIVMTQSPATLSVSPGERATLSCRASQSVSS
LLTWYQQKPGQAPRLLIFGASTRATGIPARFSGSGSGTGFTLTISSLQS
EDFAVYYCQQYDTWPFTFGPGTKVDFKRAAALDNEKSNGTIIHVKG
KHLCPSPLFPGPSKPFWVLVVVGGVLACYSLLVTVAFIIFWVRSKRSR

LLHSDYMNMTPRRPGPTRKHYQPYAPPRDFAAYRSRVKFSRSADAP
AYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQE
GLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATK
DTYDALHMQALPPR (SEQ ID NO. 104)

[0360] Clone 20C5.2 CD28T CD3 zeta CAR DNA Heavy & Light Chains

CAGGTCCAGTTGGTCGAAAGTGGCGGTGGTGTAGTGCAGCCGGGC
CGCAGTTTGAGGCTTTCCTGTGCGGCTTCAGGCTTTACTTTTTCCA
GCTATGGAATGCACTGGGTGCGGCAGGCCCCCGGCAAAGGACTT
GAGTGGGTGGCCGTCATTTCTTATGACGGATCAGATAAGTACTAC
GTGGACAGCGTCAAGGGCAGATTCACCATCTCTAGGGACAACAGT
AAAAATAGACTCTACCTCCAGATGAATAGCCTCAGAGCTGAAGAC
ACGGCCGTCTACTATTGTGCTCGGGAGCGGTATAGTGGCAGAGAC
TACTGGGGGCAGGGCACACTCGTTACAGTGAGTAGCGGCGGAGG
AGGGAGTGGGGGCGGTGGCTCCGGTGGAGGAGGTTCTGAGATTG
TTATGACCCAGAGTCCTGCGACCCTCTCAGTCAGCCCCGGGGAGC
GCGCAACTTTGTCTTGCAGAGCTAGTCAGTCCGTGTCCTCTCTTCT
GACATGGTACCAGCAAAAGCCCGGGCAGGCTCCGCGCCTTTTGAT
CTTTGGGGCTTCAACAAGAGCCACTGGGATTCCCGCACGATTCTC
TGGCTCCGGGAGCGGTACTGGTTTCACCCTGACGATTAGCAGTCT
CCAGAGCGAGGACTTCGCCGTATACTACTGCCAGCAGTACGATAC
GTGGCCATTCACTTTTGGACCAGGGACTAAAGTGGATTTTAAGCG
CGCCGCCGCTCTCGATAACGAAAAGTCAAATGGCACCATAATCCA
CGTCAAAGGCAAGCACCTGTGCCCTTCCCCGCTCTTCCCCGGACC
CAGTAAACCATTTTGGGTGCTGGTTGTTGTGGGGGGCGTGCTGGC
CTGCTATAGCCTTTTGGTCACTGTAGCCTTCATTATTTTTTGGGTC
AGATCCAAAAGAAGCCGCCTGCTCCATAGCGATTACATGAATATG
ACTCCACGCCGCCCTGGCCCCACAAGGAAACACTACCAGCCTTAC
GCACCACCTAGAGATTTCGCTGCCTATCGGAGCAGGGTGAAGTTT
TCCAGATCTGCAGATGCACCAGCGTATCAGCAGGGCCAGAACCA
ACTGTATAACGAGCTCAACCTGGGACGCAGGGAAGAGTATGACG
TTTTGGACAAGCGCAGAGGACGGGACCCTGAGATGGGTGGCAAA
CCAAGACGAAAAAACCCCCAGGAGGGTCTCTATAATGAGCTGCA
GAAGGATAAGATGGCTGAAGCCTATTCTGAAATAGGCATGAAAG

GAGAGCGGAGAAGGGGAAAAGGGCACGACGGTTTGTACCAGGGA
CTCAGCACTGCTACGAAGGATACTTATGACGCTCTCCACATGCAA
GCCCTGCCACCTAGG (SEQ ID NO. 105)

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

QVQLVESGGGVVQPGRSLRLSCAASGFTFSSYGMHWVRQAPGKGLE
WVAVISYDGSDKYYVDSVKGRFTISRDNSKNRLYLQMNSLRAEDTA
VYYCARERYSGRDYWGQGTLVTVSSGGGGSGGGGSGGGGSEIVMT
QSPATLSVSPGERATLSCRASQSVSSLLTWYQQKPGQAPRLLIFGAST
RATGIPARFSGSGSGTGFTLTISSLQSEDFAVYYCQQYDTWPFTFGPG
TKVDFKRAAALDNEKSNGTIIHVKGKHLCPSPLFPGPSKPFWVLVVV
GGVLACYSLLVTVAFIIFWVRSKRSRLLHSDYMNMTPRRPGPTRKHY
QPYAPPRDFAAYRSRVKFSRSADAPAYQQGQNQLYNELNLGRREEY
DVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMK
GERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR (SEQ ID NO.
106)

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

ATGGCACTCCCCGTAACTGCTCTGCTGCTGCCGTTGGCATTGCTCC
TGCACGCCGCACGCCCGCAGGTGCAGCTCGTGGAGTCTGGCGGCG
GCGTGGTCCAGCCCGGCCGGTCCCTGCGCCTGTCCTGCGCCGCCA
GCGGGTTTACTTTTTCCTCCTACGGCATGCACTGGGTGCGCCAGGC
TCCCGGCAAGGGCCTCGAGTGGGTCGCCGTGATCTCATACGATGG
GTCAGACAAATACTATGTCGATTCTGTTAAAGGGCGGTTTACCAT
TTCAAGAGATAACTCTAAGAATAGGCTGTATTTGCAGATGAACAG
CCTGAGGGCTGAAGATACCGCAGTGTACTATTGCGCTAGGGAGCG
GTATAGTGGCCGCGATTACTGGGGACAGGGTACACTGGTGACCGT
GAGCTCTGGGGGTGGCGGAAGCGGGGGTGGCGGAAGCGGCGGAG
GGGGTAGTGAAATTGTGATGACCCAGTCTCCGGCTACACTTTCAG
TCTCCCCTGGGGAGAGAGCTACACTGTCATGCAGAGCGTCCCAGT
CCGTCTCTTCTCTCCTTACCTGGTATCAGCAGAAGCCCGGCCAGGC
TCCTCGACTGCTGATCTTCGGTGCCTCCACAAGGGCGACCGGGAT
TCCAGCCCGCTTCTCAGGTTCTGGGAGCGGAACTGGTTTCACTTTG
ACAATCAGTTCACTGCAGTCAGAGGATTTCGCCGTGTACTACTGC

CAGCAATACGACACATGGCCATTCACTTTCGGACCCGGTACCAAA
GTCGATTTCAAGAGAGCCGCGGCCATCGAGGTTATGTACCCACCA
CCATATCTGGACAATGAAAAAAGCAATGGAACCATTATCCATGTG
AAGGGTAAACACCTCTGCCCTAGCCCACTTTTCCCTGGCCCATCA
AAGCCCTTCTGGGTCTTGGTGGTCGTGGGGGGTGTGCTGGCCTGT
TACAGCCTTCTGGTGACGGTTGCTTTCATTATCTTCTGGGTTAGAT
CCAAAAGAAGCCGCCTGCTCCATAGCGATTACATGAATATGACTC
CACGCCGCCCTGGCCCCACAAGGAAACACTACCAGCCTTACGCAC
CACCTAGAGATTTCGCTGCCTATCGGAGCAGGGTGAAGTTTTCCA
GATCTGCAGATGCACCAGCGTATCAGCAGGGCCAGAACCAACTGT
ATAACGAGCTCAACCTGGGACGCAGGGAAGAGTATGACGTTTTG
GACAAGCGCAGAGGACGGGACCCTGAGATGGGTGGCAAACCAAG
ACGAAAAAACCCCCAGGAGGGTCTCTATAATGAGCTGCAGAAGG
ATAAGATGGCTGAAGCCTATTCTGAAATAGGCATGAAAGGAGAG
CGGAGAAGGGGAAAAGGGCACGACGGTTTGTACCAGGGACTCAG
CACTGCTACGAAGGATACTTATGACGCTCTCCACATGCAAGCCCT
GCCACCTAGGTAA (SEQ ID NO. 107)

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

MALPVTALLLPLALLLHAARPQVQLVESGGGVVQPGRSLRLSCAA
SGFTFSSYGMHWVRQAPGKGLEWVAVISYDGSDKYYVDSVKGRFTI
SRDNSKNRLYLQMNSLRAEDTAVYYCARERYSGRDYWGQGTLVTV
SSGGGGSGGGGSGGGGSEIVMTQSPATLSVSPGERATLSCRASQSVSS
LLTWYQQKPGQAPRLLIFGASTRATGIPARFSGSGSGTGFTLTISSLQS
EDFAVYYCQQYDTWPFTFGPGTKVDFKRAAAIEVMYPPPYLDNEKS
NGTIIHVKGKHLCPSPLFPGPSKPFWVLVVVGGVLACYSLLVTVAFII
FWVRSKRSRLLHSDYMNMTPRRPGPTRKHYQPYAPPRDFAAYRSRV
KFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGG
KPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQG
LSTATKDTYDALHMQALPPR (SEQ ID NO. 108)

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

CAGGTGCAGCTCGTGGAGTCTGGCGGCGGCGTGGTCCAGCCCGGC
CGGTCCCTGCGCCTGTCCTGCGCCGCCAGCGGGTTTACTTTTTCCT
CCTACGGCATGCACTGGGTGCGCCAGGCTCCCGGCAAGGGCCTCG
AGTGGGTCGCCGTGATCTCATACGATGGGTCAGACAAATACTATG
TCGATTCTGTTAAAGGGCGGTTTACCATTTCAAGAGATAACTCTA
AGAATAGGCTGTATTTGCAGATGAACAGCCTGAGGGCTGAAGAT
ACCGCAGTGTACTATTGCGCTAGGGAGCGGTATAGTGGCCGCGAT
TACTGGGGACAGGGTACACTGGTGACCGTGAGCTCTGGGGGTGGC
GGAAGCGGGGGTGGCGGAAGCGGCGGAGGGGGTAGTGAAATTGT
GATGACCCAGTCTCCGGCTACACTTTCAGTCTCCCCTGGGGAGAG
AGCTACACTGTCATGCAGAGCGTCCCAGTCCGTCTCTTCTCTCCTT
ACCTGGTATCAGCAGAAGCCCGGCCAGGCTCCTCGACTGCTGATC
TTCGGTGCCTCCACAAGGGCGACCGGGATTCCAGCCCGCTTCTCA
GGTTCTGGGAGCGGAACTGGTTTCACTTTGACAATCAGTTCACTG
CAGTCAGAGGATTTCGCCGTGTACTACTGCCAGCAATACGACACA
TGGCCATTCACTTTCGGACCCGGTACCAAAGTCGATTTCAAGAGA
GCCGCGGCCATCGAGGTTATGTACCCACCACCATATCTGGACAAT
GAAAAAAGCAATGGAACCATTATCCATGTGAAGGGTAAACACCT
CTGCCCTAGCCCACTTTTCCCTGGCCCATCAAAGCCCTTCTGGGTC
TTGGTGGTCGTGGGGGGTGTGCTGGCCTGTTACAGCCTTCTGGTG
ACGGTTGCTTTCATTATCTTCTGGGTTAGATCCAAAAGAAGCCGC
CTGCTCCATAGCGATTACATGAATATGACTCCACGCCGCCCTGGC
CCCACAAGGAAACACTACCAGCCTTACGCACCACCTAGAGATTTC
GCTGCCTATCGGAGCAGGGTGAAGTTTTCCAGATCTGCAGATGCA
CCAGCGTATCAGCAGGGCCAGAACCAACTGTATAACGAGCTCAA
CCTGGGACGCAGGGAAGAGTATGACGTTTTGGACAAGCGCAGAG
GACGGGACCCTGAGATGGGTGGCAAACCAAGACGAAAAAACCCC
CAGGAGGGTCTCTATAATGAGCTGCAGAAGGATAAGATGGCTGA
AGCCTATTCTGAAATAGGCATGAAAGGAGAGCGGAGAAGGGGAA
AAGGGCACGACGGTTTGTACCAGGGACTCAGCACTGCTACGAAG
GATACTTATGACGCTCTCCACATGCAAGCCCTGCCACCTAGG
(SEQ ID NO. 109)

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

QVQLVESGGGVVQPGRSLRLSCAASGFTFSSYGMHWVRQAPGKGLE
WVAVISYDGSDKYYVDSVKGRFTISRDNSKNRLYLQMNSLRAEDTA
VYYCARERYSGRDYWGQGTLVTVSSGGGGSGGGGSGGGGSEIVMT
QSPATLSVSPGERATLSCRASQSVSSLLTWYQQKPGQAPRLLIFGAST
RATGIPARFSGSGSGTGFTLTISSLQSEDFAVYYCQQYDTWPFTFGPG
TKVDFKRAAAIEVMYPPPYLDNEKSNGTIIHVKGKHLCPSPLFPGPSK
PFWVLVVVGGVLACYSLLVTVAFIIFWVRSKRSRLLHSDYMNMTPR
RPGPTRKHYQPYAPPRDFAAYRSRVKFSRSADAPAYQQGQNQLYNE
LNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMA
EAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR
(SEQ ID NO. 110)

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

ATGGCACTCCCCGTAACTGCTCTGCTGCTGCCGTTGGCATTGCTCC
TGCACGCCGCACGCCCGCAGGTGCAGTTGGTTGAATCAGGAGGG
GGTGTGGTGCAACCCGGTCGGTCACTGCGCCTCAGTTGTGCTGCT
TCCGGGTTTACTTTCAGCTCATATGGGATGCACTGGGTACGGCAG
GCTCCAGGTAAAGGCTTGGAATGGGTGGCGGTGATCAGCTATGAC
GGCTCTGACAAATATTATGTGGACTCCGTGAAAGGCAGATTCACC
ATCAGTCGAGACAACTCAAAGAATAGACTCTACTTGCAGATGAAT
AGCCTCCGGGCCGAAGATACTGCAGTCTATTATTGCGCCCGGGAG
CGCTACAGTGGAAGAGACTATTGGGGGCAAGGAACTCTTGTCACA
GTCTCATCTGGCGGCGGCGGCAGCGGTGGGGGCGGATCTGGCGG
GGGCGGCAGCGAAATCGTTATGACTCAGAGTCCTGCCACACTGAG
CGTTAGCCCTGGTGAGAGAGCAACACTTAGCTGCAGAGCTAGTCA
GAGTGTTTCCAGTCTTTTGACATGGTACCAACAGAAGCCCGGTCA
AGCTCCACGACTGCTCATCTTCGGTGCATCCACCCGCGCAACCGG
GATACCCGCCCGGTTTTCCGGTTCTGGAAGTGGCACAGGATTCAC
GCTCACCATTTCTTCTCTGCAGTCTGAAGACTTTGCCGTGTATTAC
TGCCAGCAGTACGATACCTGGCCCTTTACCTTTGGCCCAGGTACT
AAAGTGGATTTTAAACGAGCTGCTGCACTTTCCAATAGTATTATG
TACTTTTCACATTTTGTGCCCGTGTTCCTGCCTGCGAAGCCTACGA
CAACCCCAGCCCCTAGGCCGCCCACACCGGCCCCAACTATTGCCT

CCCAGCCATTGTCTCTGAGACCCGAAGCTTGCAGACCTGCTGCTG
GAGGCGCCGTTCACACCCGAGGATTGGATTTCGCATGTGACATTT
ACATCTGGGCCCCTTTGGCCGGAACCTGCGGTGTGCTGCTGCTGT
CACTCGTGATTACACTTTACTGCAACCACCGAAACAGATCCAAAA
GAAGCCGCCTGCTCCATAGCGATTACATGAATATGACTCCACGCC
GCCCTGGCCCCACAAGGAAACACTACCAGCCTTACGCACCACCTA
GAGATTTCGCTGCCTATCGGAGCAGGGTGAAGTTTTCCAGATCTG
CAGATGCACCAGCGTATCAGCAGGGCCAGAACCAACTGTATAAC
GAGCTCAACCTGGGACGCAGGGAAGAGTATGACGTTTTGGACAA
GCGCAGAGGACGGGACCCTGAGATGGGTGGCAAACCAAGACGAA
AAAACCCCCAGGAGGGTCTCTATAATGAGCTGCAGAAGGATAAG
ATGGCTGAAGCCTATTCTGAAATAGGCATGAAAGGAGAGCGGAG
AAGGGGAAAAGGGCACGACGGTTTGTACCAGGGACTCAGCACTG
CTACGAAGGATACTTATGACGCTCTCCACATGCAAGCCCTGCCAC
CTAGGTAA (SEQ ID NO. 111)

[0367] Clone 20C5.2 CD8 CD3 zeta CAR AA Heavy & Light Chains
(Signal peptide in **Bold**)

MALPVTALLLPLALLLHAARPQVQLVESGGGVVQPGRSLRLSCAA
SGFTFSSYGMHWVRQAPGKGLEWVAVISYDGSDKYYVDSVKGRFTI
SRDNSKNRLYLQMNSLRAEDTAVYYCARERYSGRDYWGGTLVTVS
SGGGGSGGGGSGGGGSEIVMTQSPATLSVSPGERATLSCRASQSVSS
LLTWYQQKPGQAPRLLIFGASTRATGIPARFSGSGSGTGFTLTISSLQS
EDFAVYYCQQYDTWPFTFGPGTKVDFKRAAALSNSIMYFSHFVPVFL
PAKPTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFAC
DIYIWAPLAGTCGVLLLSLVITLYCNHRNRSKRSRLLHSDYMNMTPR
RPGPTRKHYQPYAPPRDFAAYRSRVKFSRSADAPAYQQGQNQLYNE
LNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMA
EAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR
(SEQ ID NO. 112)

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

CAGGTGCAGTTGGTTGAATCAGGAGGGGGTGTGGTGCAACCCGGT
CGGTCACTGCGCCTCAGTTGTGCTGCTTCCGGGTTTACTTTCAGCT
CATATGGGATGCACTGGGTACGGCAGGCTCCAGGTAAAGGCTTGG
AATGGGTGGCGGTGATCAGCTATGACGGCTCTGACAAATATTATG
TGGACTCCGTGAAAGGCAGATTCACCATCAGTCGAGACAACTCAA
AGAATAGACTCTACTTGCAGATGAATAGCCTCCGGGCCGAAGATA
CTGCAGTCTATTATTGCGCCCGGGAGCGCTACAGTGGAAGAGACT
ATTGGGGGCAAGGAACTCTTGTCACAGTCTCATCTGGCGGCGGCG
GCAGCGGTGGGGGCGGATCTGGCGGGGGCGGCAGCGAAATCGTT
ATGACTCAGAGTCCTGCCACACTGAGCGTTAGCCCTGGTGAGAGA
GCAACACTTAGCTGCAGAGCTAGTCAGAGTGTTTCCAGTCTTTTG
ACATGGTACCAACAGAAGCCCGGTCAAGCTCCACGACTGCTCATC
TTCGGTGCATCCACCCGCGCAACCGGGATACCCGCCCGGTTTTCC
GGTTCTGGAAGTGGCACAGGATTCACGCTCACCATTTCTTCTCTGC
AGTCTGAAGACTTTGCCGTGTATTACTGCCAGCAGTACGATACCT
GGCCCTTTACCTTTGGCCCAGGTACTAAAGTGGATTTTAAACGAG
CTGCTGCACTTTCCAATAGTATTATGTACTTTTCACATTTTGTGCC
CGTGTTCCTGCCTGCGAAGCCTACGACAACCCCAGCCCCTAGGCC
GCCCACACCGGCCCCAACTATTGCCTCCCAGCCATTGTCTCTGAG
ACCCGAAGCTTGCAGACCTGCTGCTGGAGGCGCCGTTCACACCCG
AGGATTGGATTTCGCATGTGACATTTACATCTGGGCCCCTTTGGCC
GGAACCTGCGGTGTGCTGCTGCTGTCACTCGTGATTACACTTTACT
GCAACCACCGAAACAGATCCAAAAGAAGCCGCCTGCTCCATAGC
GATTACATGAATATGACTCCACGCCGCCCTGGCCCCACAAGGAAA
CACTACCAGCCTTACGCACCACCTAGAGATTTCGCTGCCTATCGG
AGCAGGGTGAAGTTTTCCAGATCTGCAGATGCACCAGCGTATCAG
CAGGGCCAGAACCAACTGTATAACGAGCTCAACCTGGGACGCAG
GGAAGAGTATGACGTTTTGGACAAGCGCAGAGGACGGGACCCTG
AGATGGGTGGCAAACCAAGACGAAAAAACCCCCAGGAGGGTCTC
TATAATGAGCTGCAGAAGGATAAGATGGCTGAAGCCTATTCTGAA
ATAGGCATGAAAGGAGAGCGGAGAAGGGGAAAAGGGCACGACG
GTTTGTACCAGGGACTCAGCACTGCTACGAAGGATACTTATGACG
CTCTCCACATGCAAGCCCTGCCACCTAGG (SEQ ID NO. 113)

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

QVQLVESGGGVVQPGRSLRLSCAASGFTFSSYGMHWVRQAPGKGLE
WVAVISYDGSDKYYVDSVKGRFTISRDNSKNRLYLQMNSLRAEDTA
VYYCARERYSGRDYWGQGTLVTVSSGGGGSGGGGSGGGGSEIVMT
QSPATLSVSPGERATLSCRASQSVSSLLTWYQQKPGQAPRLLIFGAST
RATGIPARFSGSGSGTGFTLTISSLQSEDFAVYYCQQYDTWPFTFGPG
TKVDFKRAAALSNSIMYFSHFVPVFLPAKPTTTPAPRPPTPAPTIASQP
LSLRPEACRPAAGGAVHTRGLDFACDIYIWAPLAGTCGVLLLSLVITL
YCNHRNRSKRSRLLHSDYMNMTPRRPGPTRKHYQPYAPPRDFAAYR
SRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPE
MGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGL
YQGLSTATKDTYDALHMQALPPR (SEQ ID NO. 114)

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

ATGGCACTCCCCGTAACTGCTCTGCTGCTGCCGTTGGCATTGCTCC
TGCACGCCGCACGCCCGGAGATTGTGATGACCCAGTCCCCTGCTA
CCCTGTCCGTCAGTCCGGGCGAGAGAGCCACCTTGTCATGCCGGG
CCAGCCAGTCCGTCAGCAGTCTCCTGACTTGGTATCAGCAAAAAC
CAGGGCAGGCACCGCGGCTTTTGATTTTTGGTGCAAGCACACGCG
CCACTGGCATTCCAGCTAGGTTTTCTGGAAGTGGATCTGGGACAG
GCTTCACTCTGACAATCAGTAGCCTGCAGAGTGAGGACTTTGCTG
TTTACTACTGTCAACAGTACGACACCTGGCCATTCACATTCGGGC
CCGGCACCAAGGTCGACTTCAAGAGGGGCGGTGGAGGTTCAGGT
GGTGGCGGGTCAGGCGGCGGTGGGTCTCAGGTTCAACTGGTGGA
ATCAGGTGGCGGCGTTGTCCAACCGGGGCGATCACTTCGACTTTC
CTGTGCTGCCTCAGGCTTTACTTTTTCATCCTATGGGATGCACTGG
GTTCGGCAGGCTCCCGGAAAAGGACTCGAGTGGGTTGCAGTGATC
TCTTACGATGGCTCAGACAAGTATTATGTGGACTCAGTCAAGGGG
AGATTCACAATAAGCCGAGACAACTCCAAAAACCGGCTTTATCTC
CAGATGAACAGCCTTAGAGCGGAAGATACCGCGGTATACTACTGT
GCCCGCGAGAGGTATTCCGGCAGAGACTACTGGGGACAGGGCAC
ACTGGTCACCGTGAGTTCTGCCGCAGCGCTCGATAACGAAAAGAG
CAACGGAACCATTATCCACGTTAAGGGCAAGCACCTGTGCCCCAG
TCCCCTCTTCCCAGGACCATCTAAACCCTTCTGGGTTCTGGTAGTA

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

[0371] Clone 20C5.2 CD28T CD3 zeta CAR AA Heavy & Light Chains (Signal Peptide in **Bold**)

MALPVTALLLPLALLLHAARPEIVMTQSPATLSVSPGERATLSCRA
SQSVSSLLTWYQQKPGQAPRLLIFGASTRATGIPARFSGSGSGTGFTL
TISSLQSEDFAVYYCQQYDTWPFTFGPGTKVDFKRGGGGSGGGGSG
GGGSQVQLVESGGGVVQPGRSLRLSCAASGFTFSSYGMHWVRQAPG
KGLEWVAVISYDGSDKYYVDSVKGRFTISRDNSKNRLYLQMNSLRA
EDTAVYYCARERYSGRDYWGQGTLVTVSSAAALDNEKSNGTIIHVK
GKHLCPSPLFPGPSKPFWVLVVVGGVLACYSLLVTVAFIIFWVRSKRS
RLLHSDYMNMTPRRPGPTRKHYQPYAPPRDFAAYRSRVKFSRSADA
PAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQ
EGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDT
YDALHMQALPPR (SEQ ID NO. 116)

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

GAGATTGTGATGACCCAGTCCCCTGCTACCCTGTCCGTCAGTCCG
GGCGAGAGAGCCACCTTGTCATGCCGGGCCAGCCAGTCCGTCAGC
AGTCTCCTGACTTGGTATCAGCAAAAACCAGGGCAGGCACCGCGG
CTTTTGATTTTTGGTGCAAGCACACGCGCCACTGGCATTCCAGCTA
GGTTTTCTGGAAGTGGATCTGGGACAGGCTTCACTCTGACAATCA
GTAGCCTGCAGAGTGAGGACTTTGCTGTTTACTACTGTCAACAGT

ACGACACCTGGCCATTCACATTCGGGCCCGGCACCAAGGTCGACT
TCAAGAGGGGCGGTGGAGGTTCAGGTGGTGGCGGGTCAGGCGGC
GGTGGGTCTCAGGTTCAACTGGTGGAATCAGGTGGCGGCGTTGTC
CAACCGGGGCGATCACTTCGACTTTCCTGTGCTGCCTCAGGCTTTA
CTTTTTCATCCTATGGGATGCACTGGGTTCGGCAGGCTCCCGGAA
AAGGACTCGAGTGGGTTGCAGTGATCTCTTACGATGGCTCAGACA
AGTATTATGTGGACTCAGTCAAGGGGAGATTCACAATAAGCCGAG
ACAACTCCAAAAACCGGCTTTATCTCCAGATGAACAGCCTTAGAG
CGGAAGATACCGCGGTATACTACTGTGCCCGCGAGAGGTATTCCG
GCAGAGACTACTGGGGACAGGGCACACTGGTCACCGTGAGTTCTG
CCGCAGCGCTCGATAACGAAAAGAGCAACGGAACCATTATCCAC
GTTAAGGGCAAGCACCTGTGCCCCAGTCCCCTCTTCCCAGGACCA
TCTAAACCCTTCTGGGTTCTGGTAGTAGTTGGAGGGGTCCTTGCAT
GTTACTCCCTTTTGGTCACCGTCGCCTTCATTATTTTCTGGGTGAG
ATCCAAAAGAAGCCGCCTGCTCCATAGCGATTACATGAATATGAC
TCCACGCCGCCCTGGCCCCACAAGGAAACACTACCAGCCTTACGC
ACCACCTAGAGATTTCGCTGCCTATCGGAGCAGGGTGAAGTTTTC
CAGATCTGCAGATGCACCAGCGTATCAGCAGGGCCAGAACCAAC
TGTATAACGAGCTCAACCTGGGACGCAGGGAAGAGTATGACGTTT
TGGACAAGCGCAGAGGACGGGACCCTGAGATGGGTGGCAAACCA
AGACGAAAAAACCCCCAGGAGGGTCTCTATAATGAGCTGCAGAA
GGATAAGATGGCTGAAGCCTATTCTGAAATAGGCATGAAAGGAG
AGCGGAGAAGGGGAAAAGGGCACGACGGTTTGTACCAGGGACTC
AGCACTGCTACGAAGGATACTTATGACGCTCTCCACATGCAAGCC
CTGCCACCTAGG (SEQ ID NO. 117)

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

EIVMTQSPATLSVSPGERATLSCRASQSVSSLLTWYQQKPGQAPRLLI
FGASTRATGIPARFSGSGSGTGFTLTISSLQSEDFAVYYCQQYDTWPF
TFGPGTKVDFKRGGGGSGGGGSGGGGSQVQLVESGGGVVQPGRSLR
LSCAASGFTFSSYGMHWVRQAPGKGLEWVAVISYDGSDKYYVDSV
KGRFTISRDNSKNRLYLQMNSLRAEDTAVYYCARERYSGRDYWGQ
GTLVTVSSAAALDNEKSNGTIIHVKGKHLCPSPLFPGPSKPFWVLVV
VGGVLACYSLLVTVAFIIFWVRSKRSRLLHSDYMNMTPRRPGPTRKH

YQPYAPPRDFAAYRSRVKFSRSADAPAYQQGQNQLYNELNLGRREE YDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGM KGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR (SEQ ID NO. 118)

[0374] Clone 20C5.2 CD28 CD3 zeta CAR DNA Heavy & Light Chains

ATGGCACTCCCCGTAACTGCTCTGCTGCTGCCGTTGGCATTGCTCC
TGCACGCCGCACGCCCGGAGATCGTCATGACACAGAGTCCAGCTA
CCCTGAGCGTGTCCCCTGGAGAGAGAGCCACCCTGTCCTGTAGGG
CTAGTCAGAGTGTGTCCAGCCTCCTCACCTGGTATCAACAGAAGC
CTGGTCAAGCTCCCCGGCTGCTTATCTTCGGGGCCAGCACGCGAG
CCACAGGCATCCCGGCCAGATTCTCTGGCTCTGGCAGTGGCACCG
GGTTCACTCTCACGATCTCATCCCTGCAGTCAGAGGATTTCGCTGT
GTATTACTGTCAGCAGTACGATACATGGCCCTTCACCTTCGGCCC
GGGCACAAAAGTAGATTTCAAGCGCGGCGGCGGGGGTAGTGGGG
GCGGGGGATCAGGAGGAGGGGGCTCCCAAGTACAGCTGGTTGAG
AGCGGCGGCGGGGTGGTTCAGCCCGGGCGCAGCCTCAGGCTGAG
TTGCGCAGCATCAGGATTCACATTCAGTTCTTATGGAATGCATTG
GGTCAGACAGGCTCCCGGGAAGGGCCTTGAATGGGTGGCAGTCA
TTAGCTACGACGGAAGCGATAAGTACTATGTGGACTCAGTTAAAG
GGAGATTTACTATCAGCCGCGACAATTCCAAAAACAGATTGTATT
TGCAGATGAACTCCCTCAGGGCGGAGGACACTGCTGTATATTACT
GCGCACGAGAGAGATACTCCGGCCGAGACTATTGGGGCCAAGGA
ACATTGGTAACTGTGAGCTCCGCCGCAGCTATTGAGGTCATGTAC
CCCCCACCTTATCTCGATAATGAGAAGAGTAATGGGACTATAATT
CACGTAAAGGGCAAACACCTGTGCCCTTCCCCGCTGTTTCCAGGT
CCAAGTAAGCCGTTCTGGGTCCTGGTTGTGGTGGGAGGGGTGCTG
GCCTGCTATTCTCTGTTGGTTACCGTGGCCTTTATCATTTTCTGGGT
GAGATCCAAAAGAAGCCGCCTGCTCCATAGCGATTACATGAATAT
GACTCCACGCCGCCCTGGCCCCACAAGGAAACACTACCAGCCTTA
CGCACCACCTAGAGATTTCGCTGCCTATCGGAGCAGGGTGAAGTT
TTCCAGATCTGCAGATGCACCAGCGTATCAGCAGGGCCAGAACCA
ACTGTATAACGAGCTCAACCTGGGACGCAGGGAAGAGTATGACG
TTTTGGACAAGCGCAGAGGACGGGACCCTGAGATGGGTGGCAAA

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

[0375] Clone 20C5.2 CD28 CD3 zeta CAR AA Heavy & Light Chains
(Signal Peptide in **Bold**)
MALPVTALLLPLALLLHAARPEIVMTQSPATLSVSPGERATLSCRA
SQSVSSLLTWYQQKPGQAPRLLIFGASTRATGIPARFSGSGSGTGFTL
TISSLQSEDFAVYYCQQYDTWPFTFGPGTKVDFKRGGGGSGGGGSG
GGGSQVQLVESGGGVVQPGRSLRLSCAASGFTFSSYGMHWVRQAPG
KGLEWVAVISYDGSDKYYVDSVKGRFTISRDNSKNRLYLQMNSLRA
EDTAVYYCARERYSGRDYWGQGTLVTVSSAAAIEVMYPPPYLDNEK
SNGTIIHVKGKHLCPSPLFPGPSKPFWVLVVVGGVLACYSLLVTVAFI
IFWVRSKRSRLLHSDYMNMTPRRPGPTRKHYQPYAPPRDFAAYRSR
VKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMG
GKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQ
GLSTATKD TYDALHMQALPPR (SEQ ID NO. 120)

[0376] Clone 20C5.2 CD28 CD3 zeta CAR DNA Heavy & Light Chains
GAGATCGTCATGACACAGAGTCCAGCTACCCTGAGCGTGTCCCCT
GGAGAGAGAGCCACCCTGTCCTGTAGGGCTAGTCAGAGTGTGTCC
AGCCTCCTCACCTGGTATCAACAGAAGCCTGGTCAAGCTCCCCGG
CTGCTTATCTTCGGGGCCAGCACGCGAGCCACAGGCATCCCGGCC
AGATTCTCTGGCTCTGGCAGTGGCACCGGGTTCACTCTCACGATCT
CATCCCTGCAGTCAGAGGATTTCGCTGTGTATTACTGTCAGCAGT
ACGATACATGGCCCTTCACCTTCGGCCCGGGCACAAAAGTAGATT
TCAAGCGCGGCGGCGGGGGTAGTGGGGGCGGGGGATCAGGAGGA
GGGGGCTCCCAAGTACAGCTGGTTGAGAGCGGCGGCGGGGTGGT
TCAGCCCGGGCGCAGCCTCAGGCTGAGTTGCGCAGCATCAGGATT
CACATTCAGTTCTTATGGAATGCATTGGGTCAGACAGGCTCCCGG
GAAGGGCCTTGAATGGGTGGCAGTCATTAGCTACGACGGAAGCG
ATAAGTACTATGTGGACTCAGTTAAAGGGAGATTTACTATCAGCC

GCGACAATTCCAAAAACAGATTGTATTTGCAGATGAACTCCCTCA
GGGCGGAGGACACTGCTGTATATTACTGCGCACGAGAGAGATACT
CCGGCCGAGACTATTGGGGCCAAGGAACATTGGTAACTGTGAGCT
CCGCCGCAGCTATTGAGGTCATGTACCCCCCACCTTATCTCGATA
ATGAGAAGAGTAATGGGACTATAATTCACGTAAAGGGCAAACAC
CTGTGCCCTTCCCCGCTGTTTCCAGGTCCAAGTAAGCCGTTCTGGG
TCCTGGTTGTGGTGGGAGGGGTGCTGGCCTGCTATTCTCTGTTGGT
TACCGTGGCCTTTATCATTTTCTGGGTGAGATCCAAAAGAAGCCG
CCTGCTCCATAGCGATTACATGAATATGACTCCACGCCGCCCTGG
CCCCACAAGGAAACACTACCAGCCTTACGCACCACCTAGAGATTT
CGCTGCCTATCGGAGCAGGGTGAAGTTTTCCAGATCTGCAGATGC
ACCAGCGTATCAGCAGGGCCAGAACCAACTGTATAACGAGCTCA
ACCTGGGACGCAGGGAAGAGTATGACGTTTTGGACAAGCGCAGA
GGACGGGACCCTGAGATGGGTGGCAAACCAAGACGAAAAAACCC
CCAGGAGGGTCTCTATAATGAGCTGCAGAAGGATAAGATGGCTG
AAGCCTATTCTGAAATAGGCATGAAAGGAGAGCGGAGAAGGGGA
AAAGGGCACGACGGTTTGTACCAGGGACTCAGCACTGCTACGAA
GGATACTTATGACGCTCTCCACATGCAAGCCCTGCCACCTAGG
(SEQ ID NO. 121)

[0377] Clone 20C5.2 CD28 CD3 zeta CAR AA Heavy & Light Chains

EIVMTQSPATLSVSPGERATLSCRASQSVSSLLTWYQQKPGQAPRLLI
FGASTRATGIPARFSGSGSGTGFTLTISSLQSEDFAVYYCQQYDTWPF
TFGPGTKVDFKRGGGGSGGGGSGGGGSQVQLVESGGGVVQPGRSLR
LSCAASGFTFSSYGMHWVRQAPGKGLEWVAVISYDGSDKYYVDSV
KGRFTISRDNSKNRLYLQMNSLRAEDTAVYYCARERYSGRDYWGQ
GTLVTVSSAAAIEVMYPPPYLDNEKSNGTIIHVKGKHLCPSPLFPGPS
KPFWVLVVVGGVLACYSLLVTVAFIIFWVRSKRSRLLHSDYMNMTP
RRPGPTRKHYQPYAPPRDFAAYRSRVKFSRSADAPAYQQGQNQLYN
ELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKM
AEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR
(SEQ ID NO. 122)

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

ATGGCACTCCCCGTAACTGCTCTGCTGCTGCCGTTGGCATTGCTCC
TGCACGCCGCACGCCCGGAAATAGTGATGACTCAGTCCCCGGCCA
CCCTCAGCGTGTCCCCCGGGGAGCGAGCGACCCTGTCATGCAGGG
CTTCCCAGAGTGTCAGCTCCCTGCTCACTTGGTATCAGCAAAAGC
CGGGGCAGGCTCCCCGCCTCCTCATCTTCGGGGCATCAACTAGGG
CCACCGGCATTCCTGCAAGATTTTCCGGGTCTGGCAGCGGCACCG
GCTTCACCCTTACCATTAGCTCTCTGCAGTCTGAGGACTTCGCCGT
TTACTATTGTCAGCAGTATGATACTTGGCCCTTTACCTTCGGTCCC
GGAACTAAGGTGGACTTCAAGCGCGGGGGGGGTGGATCTGGAGG
TGGTGGCTCCGGGGGCGGTGGAAGCCAGGTCCAGTTGGTTGAGA
GCGGCGGCGGAGTGGTGCAGCCCGGGAGGTCCTTGCGGCTGAGC
TGTGCAGCCTCCGGTTTTACTTTTTCTAGCTATGGAATGCATTGGG
TAAGACAGGCTCCCGGAAAAGGCCTCGAGTGGGTGGCGGTCATT
AGCTATGATGGATCTGATAAATACTATGTGGACTCAGTTAAGGGG
CGCTTCACAATCTCAAGAGACAATAGCAAAAATAGACTGTACCTG
CAGATGAATAGTCTGCGCGCCGAGGACACTGCCGTGTACTACTGC
GCCCGCGAGAGATACAGCGGACGGGATTACTGGGGCCAGGGTAC
CCTCGTAACGGTGTCCTCCGCTGCCGCCCTTAGCAACAGCATTAT
GTACTTTTCTCATTTCGTGCCAGTCTTTCTCCCAGCAAAGCCCACC
ACTACCCCGGCCCCCAGGCCGCCTACTCCTGCCCCCACTATCGCG
TCTCAGCCTCTCTCCTTGCGGCCCGAGGCCTGCCGGCCAGCCGCA
GGGGGCGCCGTACATACTCGGGGTTTGGATTTCGCTTGCGACATA
TATATTTGGGCCCCCCTCGCCGGCACATGTGGAGTGCTGCTCCTG
AGTCTCGTTATAACCCTCTATTGCAACCATAGAAACAGATCCAAA
AGAAGCCGCCTGCTCCATAGCGATTACATGAATATGACTCCACGC
CGCCCTGGCCCCACAAGGAAACACTACCAGCCTTACGCACCACCT
AGAGATTTCGCTGCCTATCGGAGCAGGGTGAAGTTTTCCAGATCT
GCAGATGCACCAGCGTATCAGCAGGGCCAGAACCAACTGTATAA
CGAGCTCAACCTGGGACGCAGGGAAGAGTATGACGTTTTGGACA
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**)

MALPVTALLLPLALLLHAARPEIVMTQSPATLSVSPGERATLSCRA
SQSVSSLLTWYQQKPGQAPRLLIFGASTRATGIPARFSGSGSGTGFTL
TISSLQSEDFAVYYCQQYDTWPFTFGPGTKVDFKRGGGGSGGGGSG
GGGSQVQLVESGGGVVQPGRSLRLSCAASGFTFSSYGMHWVRQAPG
KGLEWVAVISYDGSDKYYVDSVKGRFTISRDNSKNRLYLQMNSLRA
EDTAVYYCARERYSGRDYWGQGTLVTVSSAAALSNSIMYFSHFVPV
FLPAKPTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFA
CDIYIWAPLAGTCGVLLLSLVITLYCNHRNRSKRSRLLHSDYMNMTP
RRPGPTRKHYQPYAPPRDFAAYRSRVKFSRSADAPAYQQGQNQLYN
ELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKM
AEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR
(SEQ ID NO. 124)

[0380] Clone 20C5.2 CD8 CD3 zeta CAR DNA Heavy & Light Chains
GAAATAGTGATGACTCAGTCCCCGGCCACCCTCAGCGTGTCCCCC
GGGGAGCGAGCGACCCTGTCATGCAGGGCTTCCCAGAGTGTCAGC
TCCCTGCTCACTTGGTATCAGCAAAAGCCGGGGCAGGCTCCCCGC
CTCCTCATCTTCGGGGCATCAACTAGGGCCACCGGCATTCCTGCA
AGATTTTCCGGGTCTGGCAGCGGCACCGGCTTCACCCTTACCATT
AGCTCTCTGCAGTCTGAGGACTTCGCCGTTTACTATTGTCAGCAGT
ATGATACTTGGCCCTTTACCTTCGGTCCCGGAACTAAGGTGGACTT
CAAGCGCGGGGGGGGTGGATCTGGAGGTGGTGGCTCCGGGGGCG
GTGGAAGCCAGGTCCAGTTGGTTGAGAGCGGCGGCGGAGTGGTG
CAGCCCGGGAGGTCCTTGCGGCTGAGCTGTGCAGCCTCCGGTTTT
ACTTTTTCTAGCTATGGAATGCATTGGGTAAGACAGGCTCCCGGA
AAAGGCCTCGAGTGGGTGGCGGTCATTAGCTATGATGGATCTGAT
AAATACTATGTGGACTCAGTTAAGGGGCGCTTCACAATCTCAAGA
GACAATAGCAAAAATAGACTGTACCTGCAGATGAATAGTCTGCGC
GCCGAGGACACTGCCGTGTACTACTGCGCCCGCGAGAGATACAGC

GGACGGGATTACTGGGGCCAGGGTACCCTCGTAACGGTGTCCTCC
GCTGCCGCCCTTAGCAACAGCATTATGTACTTTTCTCATTTCGTGC
CAGTCTTTCTCCCAGCAAAGCCCACCACTACCCCGGCCCCCAGGC
CGCCTACTCCTGCCCCCACTATCGCGTCTCAGCCTCTCTCCTTGCG
GCCCGAGGCCTGCCGGCCAGCCGCAGGGGGCGCCGTACATACTC
GGGGTTTGGATTTCGCTTGCGACATATATATTTGGGCCCCCCTCGC
CGGCACATGTGGAGTGCTGCTCCTGAGTCTCGTTATAACCCTCTAT
TGCAACCATAGAAACAGATCCAAAAGAAGCCGCCTGCTCCATAG
CGATTACATGAATATGACTCCACGCCGCCCTGGCCCCACAAGGAA
ACACTACCAGCCTTACGCACCACCTAGAGATTTCGCTGCCTATCG
GAGCAGGGTGAAGTTTTCCAGATCTGCAGATGCACCAGCGTATCA
GCAGGGCCAGAACCAACTGTATAACGAGCTCAACCTGGGACGCA
GGGAAGAGTATGACGTTTTGGACAAGCGCAGAGGACGGGACCCT
GAGATGGGTGGCAAACCAAGACGAAAAAACCCCCAGGAGGGTCT
CTATAATGAGCTGCAGAAGGATAAGATGGCTGAAGCCTATTCTGA
AATAGGCATGAAAGGAGAGCGGAGAAGGGGAAAAGGGCACGAC
GGTTTGTACCAGGGACTCAGCACTGCTACGAAGGATACTTATGAC
GCTCTCCACATGCAAGCCCTGCCACCTAGG (SEQ ID NO. 125)

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

EIVMTQSPATLSVSPGERATLSCRASQSVSSLLTWYQQKPGQAPRLLI
FGASTRATGIPARFSGSGSGTGFTLTISSLQSEDFAVYYCQQYDTWPF
TFGPGTKVDFKRGGGGSGGGGSGGGGSQVQLVESGGGVVQPGRSLR
LSCAASGFTFSSYGMHWVRQAPGKGLEWVAVISYDGSDKYYVDSV
KGRFTISRDNSKNRLYLQMNSLRAEDTAVYYCARERYSGRDYWGQ
GTLVTVSSAAALSNSIMYFSHFVPVFLPAKPTTTPAPRPPTPAPTIASQ
PLSLRPEACRPAAGGAVHTRGLDFACDIYIWAPLAGTCGVLLLSLVIT
LYCNHRNRSKRSRLLHSDYMNMTPRRPGPTRKHYQPYAPPRDFAAY
RSRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPE
MGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGL
YQGLSTATKDTYDALHMQALPPR (SEQ ID NO. 126)

[0382] CAR Signal Peptide DNA

ATGGCACTCCCCGTAACTGCTCTGCTGCTGCCGTTGGCATTGCTCC
TGCACGCCGCACGCCCG (SEQ ID NO. 127)

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

[0384] scFv G4S linker DNA
GGCGGTGGAGGCTCCGGAGGGGGGGGCTCTGGCGGAGGGGGCTC
C (SEQ ID NO. 129)

[0385] scFv G4s linker: GGGGSGGGGSGGGGS (SEQ ID NO. 130)

[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)

[0389] CD28 AA Extracellular Domain
MLRLLLALNLFPSIQVTGNKILVKQSPMLVAYDNAVNLSCKYSYNLF
SREFRASLHKGLDSAVEVCVVYGNYSQQLQVYSKTGFNCDGKLGNE
SVTFYLQNLYVNQTDIYFCKIEVMYPPPYLDNEKSNGTIIHVKGKHLC
PSPLFPGPSKP (SEQ ID NO. 133)

[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)

[0395] $QQX_3X_4X_5X_6PX_8T$ (SEQ ID NO: 139)

[0396] CLL-1 AA (a.k.a., CLEC12A)
MSEEVTYADLQFQNSSEMEKIPEIGKFGEKAPPAPSHVWRPAALFLT
LLCLLLLIGLGVLASMFHVTLKIEMKKMNKLQNISEELQRNISLQLMS
NMNISNKIRNLSTTLQTIATKLCRELYSKEQEHKCKPCPRRWIWHKD
SCYFLSDDVQTWQESKMACAAQNASLLKINNKNALEFIKSQSRSYD

YWLGLSPEEDSTRGMRVDNIINSSAWVIRNAPDLNNMYCGYINRLY VQYYHCTYKKRMICEKMANPVQLGSTYFREA (SEQ ID NO. 140)

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

AAGCGCGGCAGGAAGAAGCTCCTCTACATTTTTAAGCAGCCTTTT ATGAGGCCCGTACAGACAACACAGGAGGAAGATGGCTGTAGCTG CAGATTTCCCGAGGAGGAGGAAGGTGGGTGCGAGCTG (SEQ ID NO. 141)

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

KRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCEL (SEQ ID NO. 142)

[0399] OX40 AA

RRDQRLPPDAHKPPGGGSFRTPIQEEQADAHSTLAKI (SEQ ID NO. 143)

[0400] Leader Sequence AA

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:

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 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,

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 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, CD27, CD30, CD40, programmed death-1 (PD-1), inducible T cell costimulator (ICOS), lymphocyte function-associated antigen-1 (LFA-1 (CDl la/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, CDl ld, ITGAE, CD103, ITGAL, CDl la, LFA-1, ITGAM, CDl lb, ITGAX, CDl lc, ITGBl, 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.

6. The chimeric antigen receptor according to claim 5 wherein the costimulatory domain 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 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.

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.

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.

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.

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;

(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.

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 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 (CDl la/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, CDl ld, ITGAE, CD103, ITGAL, CDl la, LFA-1, ITGAM, CDl lb, ITGAX, CDl lc, ITGBl, 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.

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; 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.

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, 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.

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 (CDl la/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, CDl ld, ITGAE, CD103, ITGAL, CDl la, LFA-1, ITGAM, CDl lb, ITGAX, CDl lc, ITGBl, 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.

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.

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 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.

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 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 (CDl la/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, CDl ld, ITGAE, CD103, ITGAL, CDl la, LFA-1, ITGAM, CDl lb, ITGAX, CDl lc, ITGBl, 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.

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 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 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, 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, 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 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 $GX_2X_3X_4X_5X_6X_7X_8X_9$ (SEQ ID NO: 134), wherein:

X_2 is G, F, or Y;

X_3 is S or T;

X_4 is I, F, or L;

X_5 is S or T;

X_6 is not present or S;

X_7 is not present or G;

X_8 is not present or E or G; and

X_9 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 of D or E;

(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;

(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;

(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.

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 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 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.

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 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, fibromyalga, mastocytosis, and Celiac disease.

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

CLL1 expression in different cancer cell lines

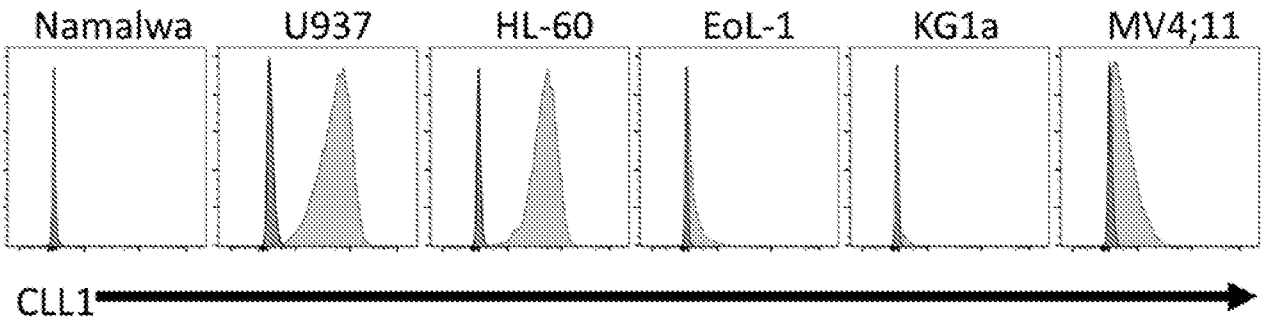


FIGURE 1

CLL1 CAR expression determined by protein L 6h post mRNA electroporation

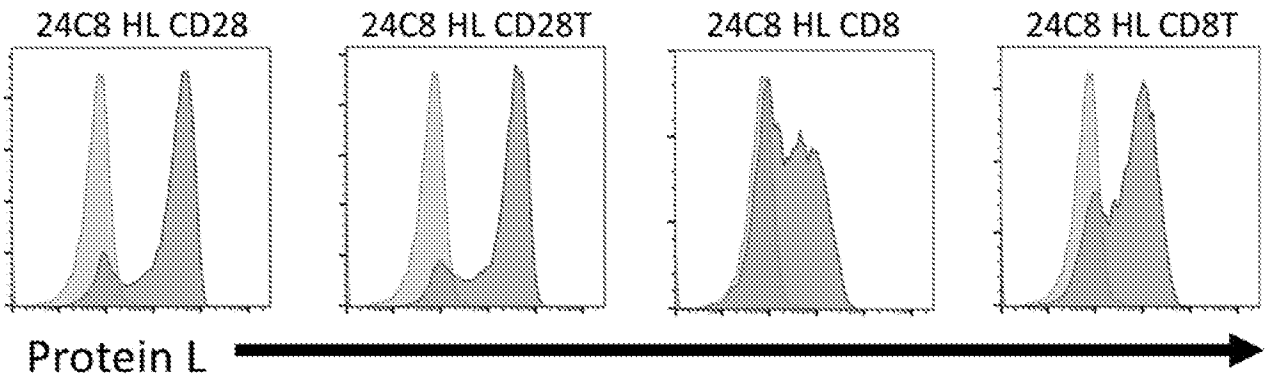


FIGURE 2

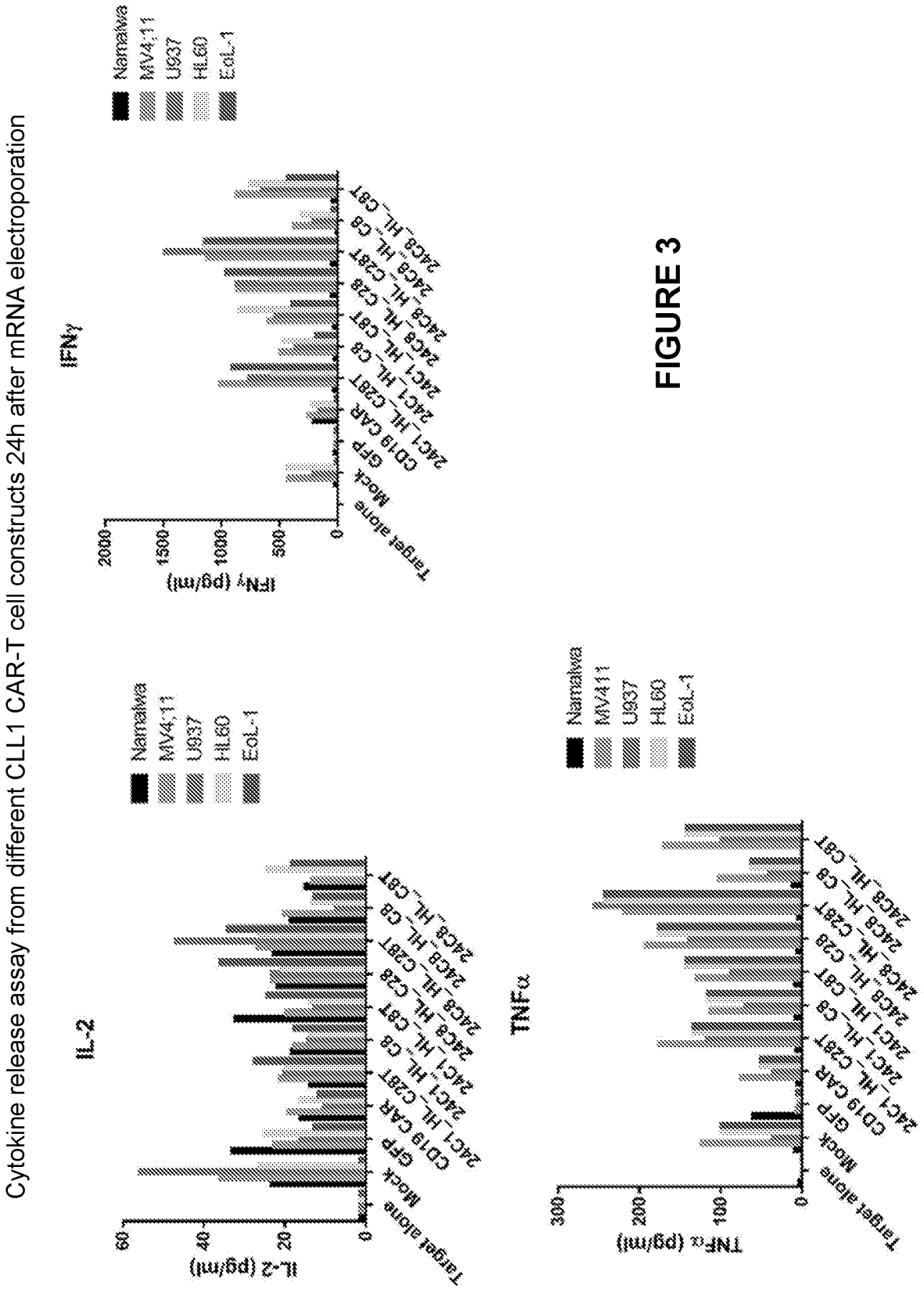

Cytokine release assay from different CLL1 CAR-T cell constructs 24h after mRNA electroporation
IFNγ
IFNγ (pg/ml)
2000
1500
1000
500
0
Target alone
Mock
GFP
CD19 CAR
24C1_HL_C28T
24C1_HL_C8
24C1_HL_C3T
24C8_HL_C28
24C8_HL_C28T
24C8_HL_C8
24C8_HL_C3T
Namalwa
MV4;11
U937
HL60
EoL-1
IL-2
IL-2 (pg/ml)
60
40
20
0
TNFα
TNFα (pg/ml)
300
200
100
0
MV411

FIGURE 3

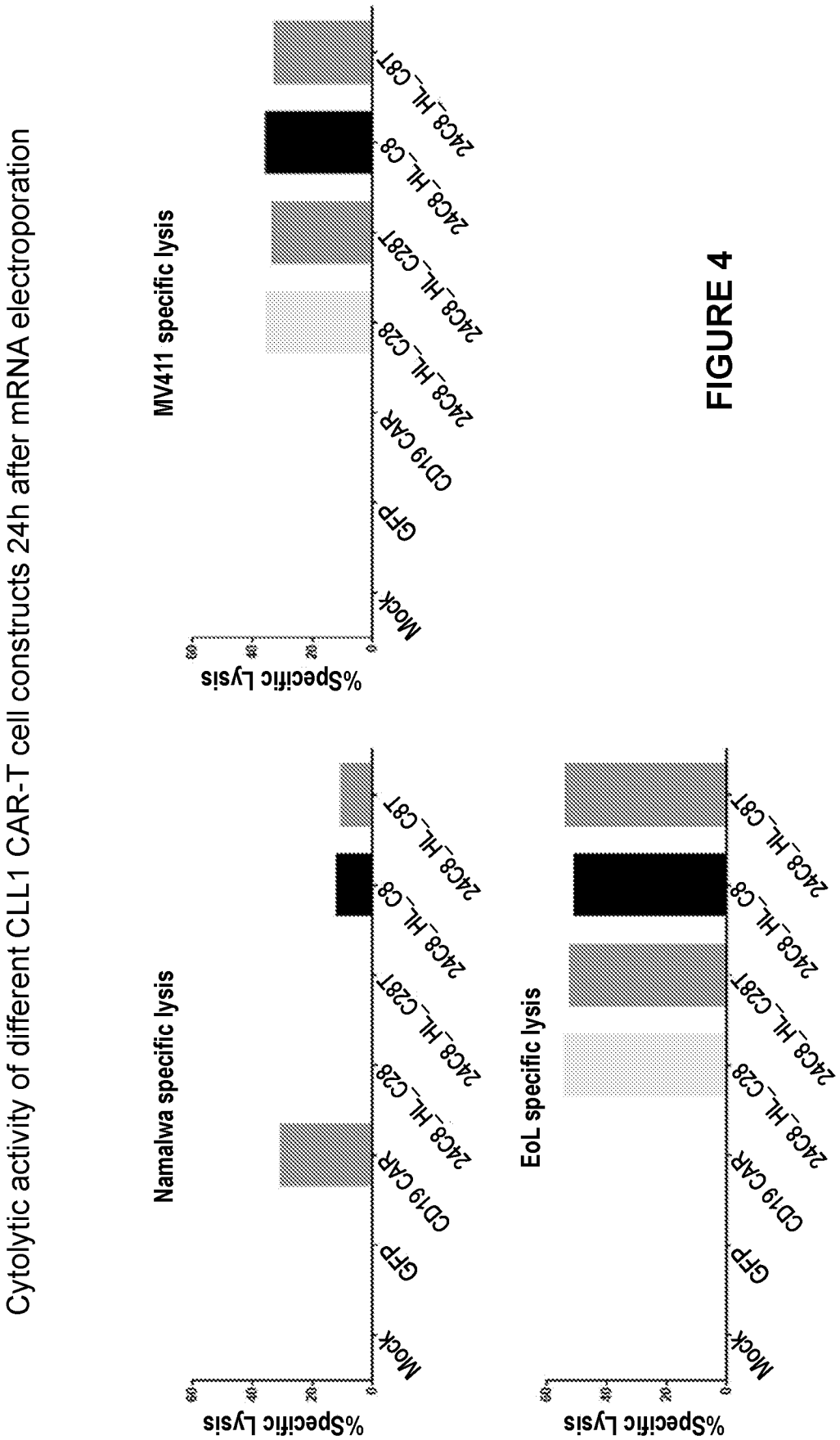

Cytolytic activity of different CLL1 CAR-T cell constructs 24h after mRNA electroporation
MV411 specific lysis
Namalwa specific lysis
EoL specific lysis
%Specific Lysis
0
20
40
60
Mock
GFP
CD19 CAR
24C8_HL_C28
24C8_HL_C28T
24C8_HL_C8
24C8_HL_C8T

FIGURE 4

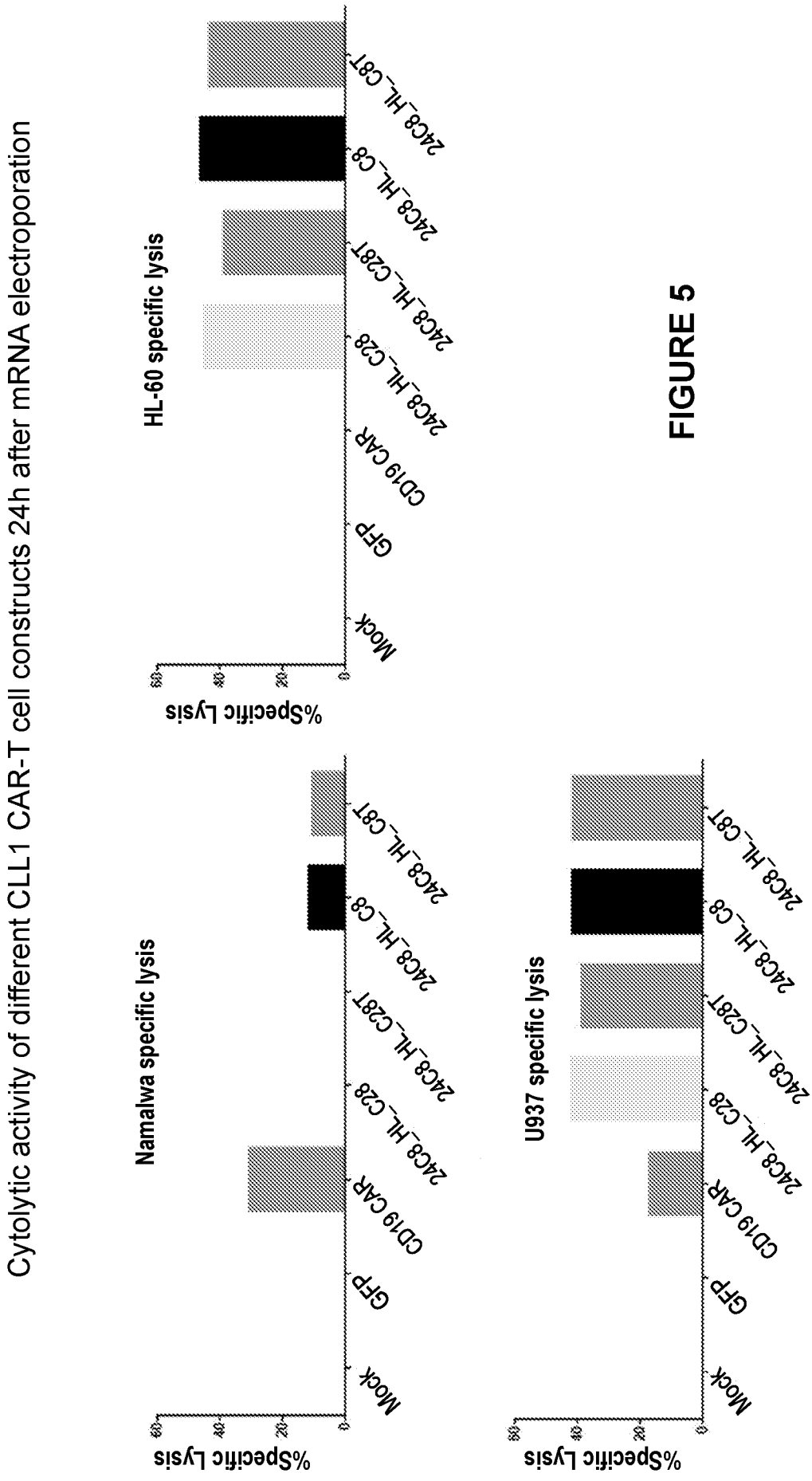

Cytolytic activity of different CLL1 CAR-T cell constructs 24h after mRNA electroporation
HL-60 specific lysis
Namalwa specific lysis
U937 specific lysis
%Specific Lysis
0
20
40
60
Mock
GFP
CD19 CAR
24C8_HL_C28
24C8_HL_C28T
24C8_HL_C8
24C8_HL_C8T

FIGURE 5

CLL1 expression determined by protein L at day 12 after transduction

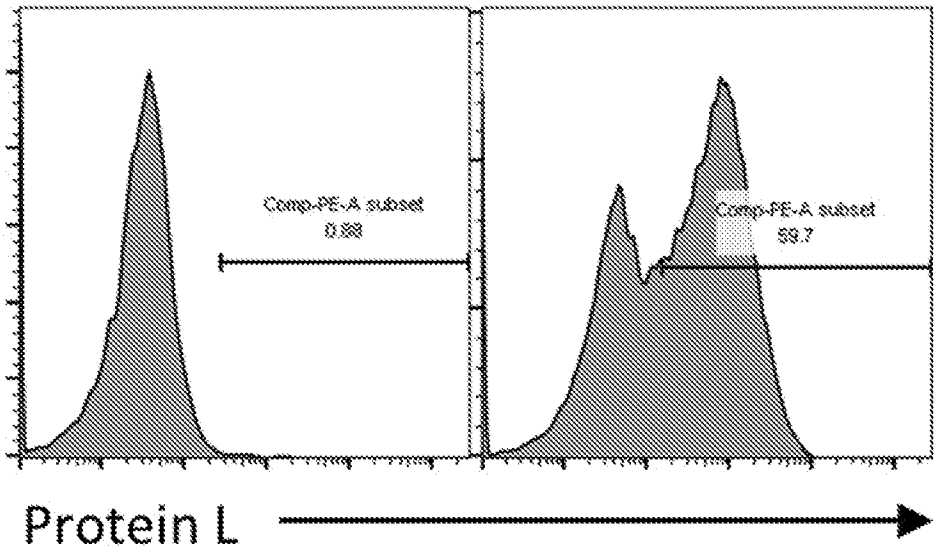


FIGURE 6

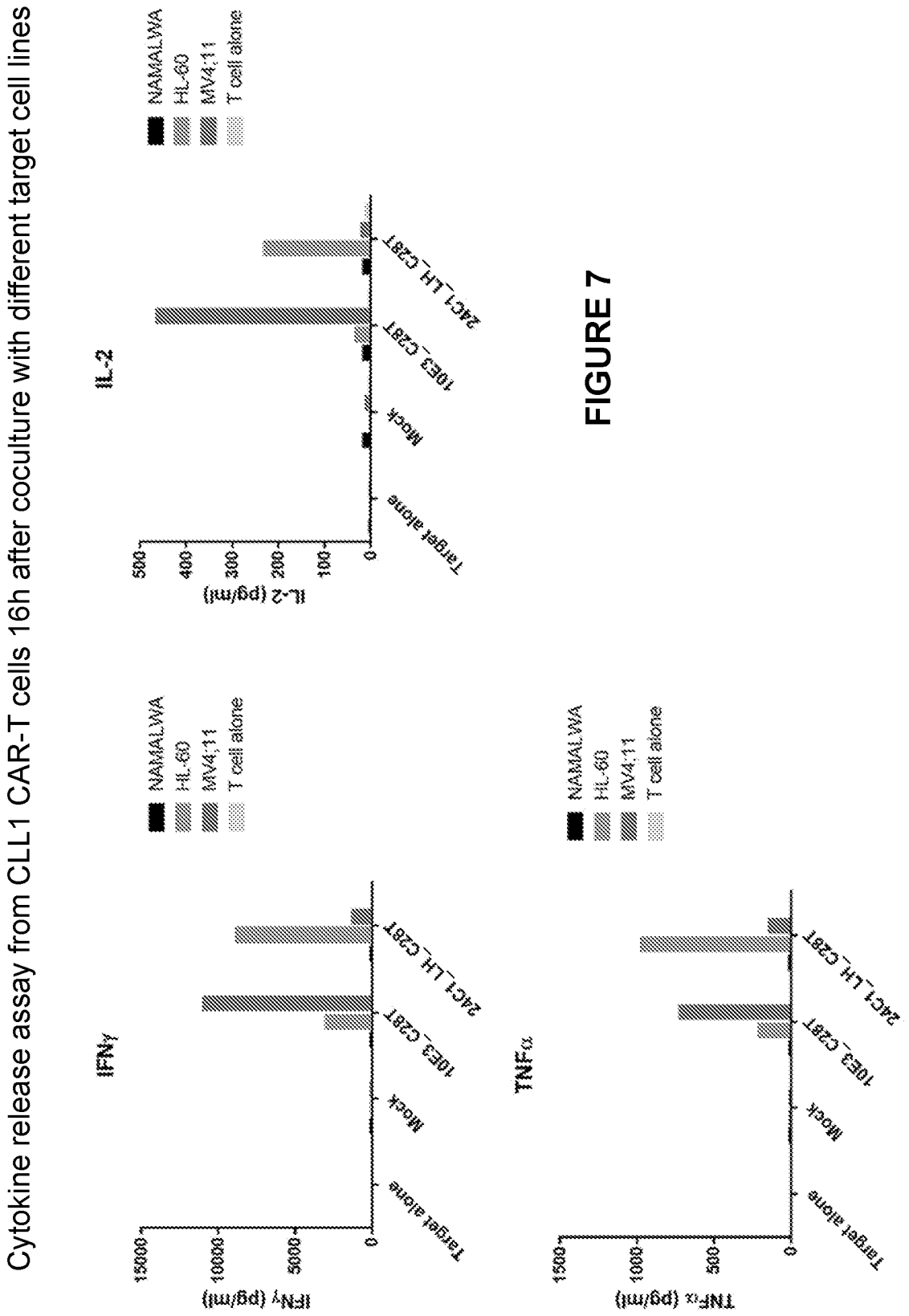

Cytokine release assay from CLL1 CAR-T cells 16h after coculture with different target cell lines
IL-2
IL-2 (pg/ml)
500
400
300
200
100
0
NAMALWA
HL-60
MV4;11
T cell alone
Target alone
Mock
10E3_C28T
24C1_LH_C28T
IFNγ
IFNγ (pg/ml)
15000
10000
5000
0
TNFα
TNFα (pg/ml)
1500
1000
500
0

FIGURE 7

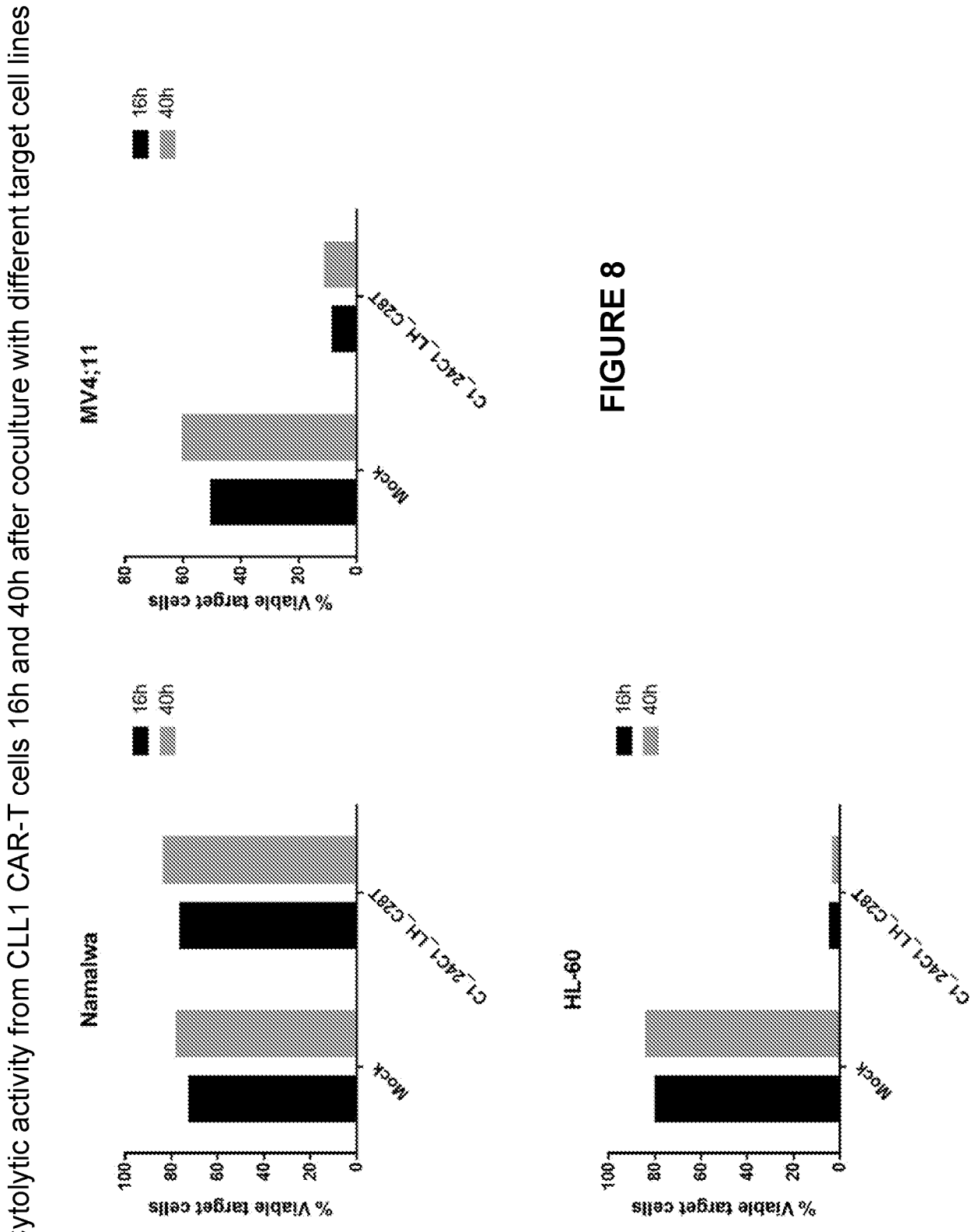

Cytolytic activity from CLL1 CAR-T cells 16h and 40h after coculture with different target cell lines
MV4;11
Namalwa
HL-60
16h
40h
% Viable target cells
Mock
C1_24C1_LH_C28T
0
20
40
60
80
100

FIGURE 8

FIGURE 9A– Anti-CLL-1 Binding Molecules

```
                    FR1                      CDR1         FR2              CDR2
24C1_VH      QVQLQESGPGLVKPSETLSLTCTVSGGSISS--YYWSWIRQPPGKGLEWIGYIYYSGS-T
24C8_VH      QVQLQESGPGLVKPSQTLSLTCTVSGGSISSGGYYWSWIRQHPGKGLEWIGYIHHSGS-T
20C5.1_VH    QVQLVQSGAEVKKPGASVKVSCKVSGYTLT--ELSMHWVRQAPGKGLEWMGGFDPEDGET
20C5.2_VH    QVQLVESGGGVVQPGRSLRLSCAASGFTFSS--YGMHWVRQAPGKGLEWVAVISYDGSDK
             **** :**  : :*. :: ::* .** :::         *:** *******:. :  ... .

                         FR3                             CDR3         FR4
24C1_VH      NYNPSLKSRVTISVDTSKNQFSLKLSSVTAADTAVYYCVSLVYCGGDCYSGFDYWGQGTL
24C8_VH      HYNPSLKSRVTISIDTSKNLFSLRLSSVTAADTAVYYCASLVYCGGDCYSGFDYWGQGTL
20C5.1_VH    IYAQKFQGRVTVTEDTSTDTAYMELSSLRSEDTAVYYCATESRGIG--WPYFDYWGQGTL
20C5.2_VH    YYVDSVKGRFTISRDNSKNRLYLQMNSLRAEDTAVYYCARERYSG------RDYWGQGTL
              *  ..:.*.*:: *.*.:    :.:.*: : *******.           ********

             FR4
24C1_VH      VTVSS
24C8_VH      VTVSS
20C5.1_VH    VTVSS
20C5.2_VH    VTVSS
             *****
```

FIGURE 9B

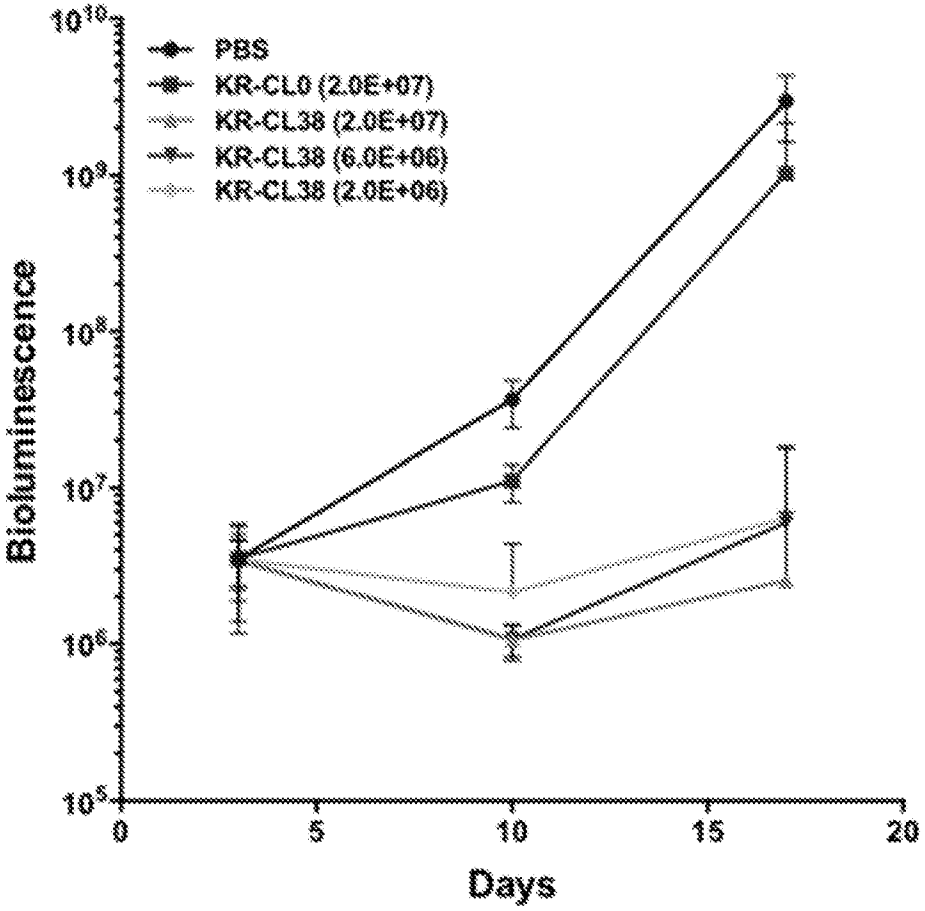
	SEQ ID NO:			
	VH	CDR1	CDR2	CDR3
24C1_VH	16	17	18	19
24C8_VH	50	51	52	53
20C5.1_VH	72	73	74	75
20C5.2_VH	94	95	96	97

FIGURE 9C– Anti-CLL-1 Binding Molecules

FIGURE 9D

	SEQ ID NO:			
	VL	CDR1	CDR2	CDR3
24C1_VL	21	22	23	24
24C8_VL	55	56	57	58
20C5.1_VL	77	78	79	80
20C5.2_VL	99	100	101	102

FIGURE 10


PBS
KR-CL0 (2.0E+07)
KR-CL38 (2.0E+07)
KR-CL38 (6.0E+06)
KR-CL38 (2.0E+06)
Bioluminescence
10^{10}
10^{9}
10^{8}
10^{7}
10^{6}
10^{5}
0
5
10
15
20
Days

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2017/025573

A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - A61K 31/7068; A61K 35/17; A61K 38/17; A61K 39/00; A61K 39/395; A61K 45/06 (2017.01)

CPC - A61K 31/7068; A61K 35/17; A61K 39/0011; A61K 45/06; A61K 2039/5156; C07K 14/7051; C07K 14/70578; C07K 16/2851; C07K 2317/622; C07K 2319/02; C07K 2319/03 (2017.02)

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

See Search History document

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

USPC - 424/185.1; 435/455; 435/320.1; 435/372.3; 530/387.3; 536/23.4 (keyword delimited)

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

See Search History document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 2005/000894 A2 (CRUCELL HOLLAND B.V. et al) 06 January 2005 (06.01.2005) entire document	1, 3-7, 9-19, 22, 23, 25-49, 51-55, 57-61, 63-66, 70-74, 80
A	LU et al. "Targeting Human C-Type Lectin-like Molecule-1 (CLL1) with a Bispecific Antibody for Immunotherapy of Acute Myeloid Leukemia," Angew Chem Int Ed Engl, 08 September 2014 (08.09.2014), Vol. 53, Iss. 37, Pgs. 9841-9845. entire document	1, 3-7, 9-19, 22, 23, 25-49, 51-55, 57-61, 63-66, 70-74, 80
A	US 2016/0051651 A1 (THE TRUSTEES OF THE UNIVERSITY OF PENNSYLVANIA et al) 25 February 2016 (25.02.2016) entire document	1, 3-7, 9-19, 22, 23, 25-49, 51-55, 57-61, 63-66, 70-74, 80
A	ZHAO et al. "Targeting C-type lectin-like molecule-1 for antibody-mediated immunotherapy in acute myeloid leukemia," Haematologica, 31 July 2009 (31.07.2009), Vol. 95, Pgs. 71-78. entire document	1, 3-7, 9-19, 22, 23, 25-49, 51-55, 57-61, 63-66, 70-74, 80
A	WO 2015/142675 A2 (NOVARTIS AG et al) 24 September 2015 (24.09.2015) entire document	1, 3-7, 9-19, 22, 23, 25-49, 51-55, 57-61, 63-66, 70-74, 80
T	TASHIRO et al. "Treatment of Acute Myeloid Leukemia with T Cells Expressing Chimeric Antigen Receptors Directed to C-type Lectin-like Molecule 1," Molecular Therapy, 01 July 2017 (01.07.2017), Pgs. . entire document	1, 3-7, 9-19, 22, 23, 25-49, 51-55, 57-61, 63-66, 70-74, 80

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
27 July 2017	11 AUG 2017
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, VA 22313-1450 Facsimile No. 571-273-8300	Authorized officer Blaine R. Copenheaver PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2017/025573

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☒ Claims Nos.: 75-79
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:
See extra sheet(s).

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
1, 3-7, 9-19, 22, 23, 25-49, 51-55, 57-61, 63-66, 70-74, and 80

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2017/025573

Continued from Box No. III Observations where unity of invention is lacking

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees need to be paid.

Group I+: claims 1-74, and 80 are drawn to chimeric antigen receptors, and polynucleotides and polypeptides comprising the same.

The first invention of Group I+ is restricted to a chimeric antigen receptor, polynucleotides and polypeptides comprising the same, wherein the chimeric antigen receptor is selected to be SEQ ID NO:28 (encoded by SEQ ID NO:27), wherein the chimeric antigen receptor comprises: an antigen binding molecule, wherein the antigen binding molecule comprises a heavy chain variable region (VH), wherein in the heavy chain variable region is selected to be SEQ ID NO:16, encoded by SEQ ID NO:15, the heavy chain further comprising heavy chain complementary determining regions CDR1, CDR2, and CDR3, where CDR1 is selected to be SEQ ID NO:17, CDR2 is selected to be SEQ ID NO:18, and CDR3 is selected to be SEQ ID NO:19; and a light chain variable region (VL), wherein the light chain variable region is selected to be SEQ ID NO:21, encoded by SEQ ID NO:20, the light chain further comprising light chain complementary determining regions CDR1, CDR2, and CDR3, where CDR1 is selected to be SEQ ID NO:22, CDR2 is selected to be SEQ ID NO:23, and CDR3 is selected to be SEQ ID NO:24; wherein the VH and VL are linked by a linker, wherein the linker is selected to be SEQ ID NO:130; a CD28 costimulatory domain, wherein the CD28 costimulatory domain is selected to be SEQ ID NO:2 (encoded by SEQ ID NO:1); an activating domain, wherein the activating domain is selected to be CD3ζ of SEQ ID NO:10. It is believed that claims 1, 3-7, 9-19, 22, 23, 25-49, 51-55, 57-61, 63-66, 70-74, and 80 read on this first named invention and thus these claims will be searched without fee to the extent that they read on a chimeric antigen receptor of SEQ ID NO: 28.

Applicant is invited to elect additional chimeric antigen receptors, each with a specified SEQ ID NO to be searched in a specific combination by paying an additional fee for each set of election. An exemplary election would be a chimeric antigen receptor, polynucleotides and polypeptides comprising the same, wherein the chimeric antigen receptor is selected to be SEQ ID NO:32 (encoded by SEQ ID NO:31). Additional chimeric antigen receptors will be searched upon the payment of additional fees. Applicants must specify the claims that read on any additional elected inventions. Applicants must further indicate, if applicable, the claims which read on the first named invention if different than what was indicated above for this group. Failure to clearly identify how any paid additional invention fees are to be applied to the "+" group(s) will result in only the first claimed invention to be searched/examined.

The inventions listed in Groups I+ do not relate to a single general inventive concept under PCT Rule 13.1, because under PCT Rule 13.2 they lack the same or corresponding special technical features for the following reasons:

The Groups I+ formulas do not share a significant structural element responsible for binding CLL-1, requiring the selection of alternatives for the heavy and light chain variable domains of the antigen binding molecules, the costimulatory domains, the activating domains, and the linker, where "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; X11i 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" and "the costimulatory domain is a signaling region (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 (CD1 1a/CD18), CD3 gamma, CD3 delta, CD3 epsilon, CD247, CD276 (137-1-13), LIGHT, (TNFSFI4), 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, NKp8O (KLRF1), NKp44, NKp3O, NKp46, CD19, CD4, CD8alpha, CD8beta, IL-2R beta, IL-2R gamma, IL-7R alpha, ITGA4, VLA1, C[)49a, ITGA4, IA4, C [)49D, ITGA6, VLA-6, CD49f, ITGAD, CDI Id, ITGAE, CD103, ITGAL, CD1 Ia, LFA-1, ITGAM, CD11b, ITGAX, CD11c, ITGB1, CD29, ITGB2, CD18, LFA-1, ITGB7, NKG2D, TNFR2, TRANCE/RANKL, DNAMI (CD226), SLAMVIF4 (CD244, 2B4), CD84, CD96 (Tactile), CEACAM1, CRT AM, Ly9 (CD229), CD160 (BY55), PSGLi, CD100 (SEMA4D), CD69, SLAMF6 (NTB-A, Ly108), SLAM (SLAMFI, CD150, 1IPO-3), BLAME (SLAMF8), SELPLG (CD162), LTBR, LAT, GADS, SLP-76, PAG/Cbp, CD19a, a ligand that specifically binds with CD83, or any combination thereof" and "the CD3 zeta comprises the amino acid sequence set forth in SEQ ID NO:9; the CD33 zeta comprises SEQ ID NO:10" and "the linker comprises at least one of SEQ ID NO:130 and SEQ ID NO:132".

The Groups I+ share the technical features of a chimeric antigen receptor comprising an antigen binding molecule that specifically binds to CLL- 1, chimeric antigen receptor; an isolated polynucleotide; an isolated polypeptide; 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, wherein the antigen binding molecule comprises a variable heavy chain CDR3; an isolated polynucleotide encoding a chimeric antigen receptor (CAR) or T cell receptor (TCR), said CAR or TCR comprising an antigen binding molecule that specifically binds to CLIL-1, wherein the antigen binding molecule comprises a variable light chain CDR3; 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, wherein the antigen binding molecule heavy chain comprises CDR1, CDR2, and CDR3 and the antigen binding molecule light chain comprises CDR1, CDR2, and CDR3; 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, wherein the antigen binding molecule comprises a heavy chain variable region (VH) complementarity

determining region (CDR) 1; a heavy chain variable region (VH) complementarity determining region (CDR) 2; a heavy chain variable region (VH) complementarity determining region (CDR) 3; a light chain variable region (VL) CDR1; a light chain variable region (VL) CDR2, a light chain variable region (VL) CDR3. However, these shared technical features do not represent a contribution over the prior art.

Specifically, US 2016/0051651 A1 to Pennsylvania et al. discloses a chimeric antigen receptor (The invention also relates to chimeric antigen receptor (CAR) specific to CLL-1, Abstract) comprising an antigen binding molecule that specifically binds to CLL- 1 (In a first aspect, the invention features an isolated nucleic acid molecule encoding a chimeric antigen receptor (CAR), wherein the CAR comprises an antibody or antibody fragment which includes a human anti-CLL-1 binding domain, Para. [0005]), chimeric antigen receptor (a chimeric antigen receptor (CAR), Para. [0005]); an isolated polynucleotide (an isolated nucleic acid molecule encoding a chimeric antigen receptor, Para. [0005]); an isolated polypeptide (the isolated polypeptide molecule of a CAR of the invention, Para. [0074]); an isolated polynucleotide encoding a chimeric antigen receptor (CAR) or T cell receptor (TCR) (the invention features an isolated nucleic acid molecule encoding a chimeric antigen receptor (CAR), Para. [0005]) comprising an antigen binding molecule that specifically binds to CLL-1 (wherein the CAR comprises an antibody or antibody fragment which includes a human anti-CLL-1 binding domain, Para. [0005]), wherein the antigen binding molecule comprises a variable heavy chain CDR3 (a heavy chain complementary determining region 3 (HC CDR3), Para. [0006]); an isolated polynucleotide encoding a chimeric antigen receptor (CAR) or T cell receptor (TCR) (the invention features an isolated nucleic acid molecule encoding a chimeric antigen receptor (CAR), Para. [0005]), said CAR or TCR comprising an antigen binding molecule that specifically binds to CLL-1 (In a first aspect, the invention features an isolated nucleic acid molecule encoding a chimeric antigen receptor (CAR), wherein the CAR comprises an antibody or antibody fragment which includes a human anti-CLL-1 binding domain, Para. [0005]), wherein the antigen binding molecule comprises a variable light chain CDR3 (a light chain complementary determining region 3 (LC CDR3), Para. [0006]); an isolated polynucleotide encoding a chimeric antigen receptor (CAR) or T cell receptor (TCR) (the invention features an isolated nucleic acid molecule encoding a chimeric antigen receptor (CAR), Para. [0005]) comprising an antigen binding molecule that specifically binds to CLL-1 (In a first aspect, the invention features an isolated nucleic acid molecule encoding a chimeric antigen receptor (CAR), wherein the CAR comprises an antibody or antibody fragment which includes a human anti-CLL-1 binding domain, Para. [0005]), wherein the antigen binding molecule heavy chain comprises CDR1, CDR2, and CDR3 (the encoded CLL-1 binding domain comprises a HC CDR1, a HC CDR2, and a HC CDR3 of any CLL-1 heavy chain binding domain, Para. [0010]) and the antigen binding molecule light chain comprises CDR1, CDR2, and CDR3 (light chain complementary determining region 1 (LC CDR1), light chain complementary determining region 2 (LC CDR2), and light chain complementary determining region 3 (LC CDR3) of a human anti-CLL-1 binding domain, Para. [0008]); 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 (In a first aspect, the invention features an isolated nucleic acid molecule encoding a chimeric antigen receptor (CAR), wherein the CAR comprises an antibody or antibody fragment which includes a human anti-CLL-1 binding domain, Para. [0005]), wherein the antigen binding molecule comprises a heavy chain variable region (VH) complementarity determining region (CDR) 1 (the CLL-1 binding domain comprises a HC CDR1, Para. [0031]); a heavy chain variable region (VH) complementarity determining region (CDR) 2 (the CLL-1 binding domain comprises a HC CDR1, a HC CDR2, Para. [0031]); a heavy chain variable region (VH) complementarity determining region (CDR) 3 (the CLL-1 binding domain comprises a HC CDR1, a HC CDR2, and a HC CDR3, Para. [0031]); a light chain variable region (VL) CDR1; a light chain variable region (VL) CDR2 (), a light chain variable region (VL) CDR3 (light chain complementary determining region 1 (LC CDR1), light chain complementary determining region 2 (LC CDR2), and light chain complementary determining region 3 (LC CDR3) of an anti-CLL-1 binding domain described herein, Para. [0030]).

The inventions listed in Groups I+ therefore lack unity under Rule 13 because they do not share a same or corresponding special technical features.