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(54) Title: GENETICALLY ENGINEERED CELLS HAVING ANTI-CD19 / ANTI-CD22 CHIMERIC ANTIGEN RECEPTORS, AND USES THEREOF

(57) Abstract: Provided are genetically engineered induced pluripotent stem cells (iPSCs) and derivative cells thereof expressing a chimeric antigen receptor (CAR) and methods of using the same. Also provided are compositions, polypeptides, vectors, and methods of manufacturing.

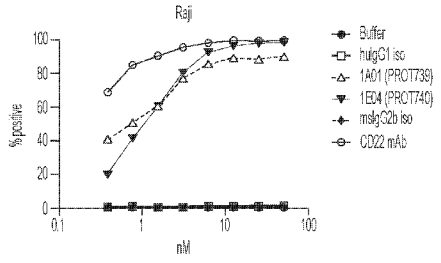


FIG. 1A

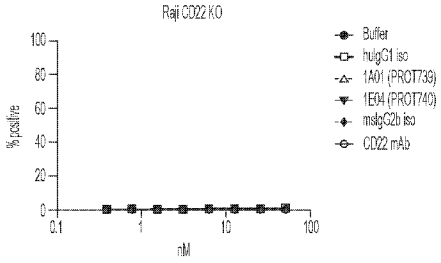


FIG. 1B

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GENETICALLY ENGINEERED CELLS HAVING ANTI-CD19 / ANTI-CD22 CHIMERIC ANTIGEN RECEPTORS, AND USES THEREOF

CROSS REFERENCE TO RELATED APPLICATIONS

This application claims the benefit of U.S. Provisional Patent Application No. 63/294,618 filed December 29, 2021, and U.S. Provisional Patent Application No. 63/350,156 filed June 8, 2022, each of which is incorporated by reference herein in its entirety.

TECHNICAL FIELD

This application provides genetically engineered induced pluripotent stem cells (iPSCs) and derivative cells thereof. Also provided are uses of the iPSCs or derivative cells thereof to express a chimeric antigen receptor for allogenic cell therapy. Also provided are related vectors, polynucleotides, and pharmaceutical compositions.

REFERENCE TO SEQUENCE LISTING SUBMITTED ELECTRONICALLY

This application contains a sequence listing, which is submitted electronically via EFS-Web as an ASCII formatted sequence listing with a file name "SequenceListing_ST26.xml" and a creation date of December 23, 2022 having a size of 381 kb. The sequence listing submitted via EFS-Web is part of the specification and is herein incorporated by reference in its entirety.

INCORPORATION BY REFERENCE

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.

BACKGROUND

Chimeric antigen receptors (CARs) have shown remarkable activity in the treatment of acute lymphocytic leukemia by enhancing anti-tumor activity of immune

effector cells. Autologous, patient-specific CAR-T therapy has emerged as a powerful and potentially curative therapy for cancer, especially for CD19- and CD22- associated malignancies. In particular, CAR-T cells with dual-antigen targeting (e.g., using two CARs by co-administration or co-transduction into an immune cell) have shown potential for overcoming the antigen downregulation in ALL that is a common cause for treatment failure. However, the autologous T cells must be generated on a custom-made basis, which remains a significant limiting factor for large-scale clinical application due to the production costs and the risk of production failure. The development of CAR-T technology and its wider application is also limited due to a number of other key shortcomings, including, e.g., a) an inefficient anti-tumor response in solid tumors, b) limited penetration and susceptibility of adoptively transferred CAR T cells to an immunosuppressive tumor microenvironment (TME), c) poor persistence of CAR-T cells *in vivo*, d) serious adverse events in the patients including cytokine release syndrome (CRS) and graft-versus-host disease (GVHD) mediated by the CAR-T, and e) the time required for manufacturing.

Therefore, there is an unmet need for therapeutically sufficient and functional antigen-specific immune cells for effective use in immunotherapy.

BRIEF SUMMARY

In some aspects, the present disclosure provides an induced pluripotent stem cell (iPSC) or a derivative cell thereof comprising: one or more exogenous polynucleotides encoding a chimeric antigen receptor (CAR) comprising an antigen binding domain targeting a CD22 antigen; and optionally, at least one of: (i) a CD19 antigen-binding domain encoded by the one or more exogenous polynucleotides; (ii) a deletion or reduced expression of one or more of B2M, TAP 1, TAP 2, Tapasin, RFXANK, CIITA, RFX5, RFXAP genes; (iii) an exogenous polynucleotide encoding a human leukocyte antigen E (HLA-E) and/or human leukocyte antigen G (HLA-G); (iv) an exogenous polynucleotide encoding an NK cell receptor immunoglobulin gamma Fc region receptor III (FcγRIII, cluster of differentiation 16 (CD16) and/or an NKG2D protein; (v) a deletion or reduced expression of one or more of NKG2A or CD70 genes (vi) an exogeneous polynucleotide encoding a cytokine; (vii) an exogenous polynucleotide

encoding a safety switch; and (viii) an exogeneous polynucleotide encoding a PSMA cell tracer. In some embodiments, the CD19 antigen-binding domain, wherein: (i) the CAR is a bispecific CAR comprising the CD19 antigen-binding domain, or (ii) the one or more exogenous polynucleotides encode a additional CAR comprising the CD19 antigen-binding domain. In some embodiments, the CAR comprises an anti-CD22 VHH domain, and/or wherein the CD19 antigen-binding domain comprises an anti-CD19 VHH domain. In some embodiments, the cytokine comprises an IL-15 protein. In some embodiments, the IL-15 protein comprises an inactivated cell surface receptor that comprises a monoclonal antibody-specific epitope and an interleukin 15 (IL-15), and wherein the inactivated cell surface receptor and the IL-15 are operably linked by an autoprotease peptide. In some embodiments, the IL-15 protein comprises a fusion polypeptide comprising an IL-15 and an IL-15 receptor alpha (IL-15Rα). In some embodiments, the IL-15 comprises an amino acid sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to SEQ ID NO: 72. In some embodiments, the iPSC or the derivative cell comprises the deletion or reduced expression of one or more of B2M, TAP 1, TAP 2, Tapasin, RFXANK, CIITA, RFX5 and RFXAP genes. In some embodiments, the iPSC or the derivative cell comprises an exogenous polynucleotide encoding a human leukocyte antigen E (HLA-E) and/or human leukocyte antigen G (HLA-G). In some embodiments, wherein the CD16 is a CD16 variant protein. In some embodiments, the CD16 variant protein is a high affinity CD16 variant. In some embodiments, the CD16 variant protein is a non-cleavable CD16 variant. In some embodiments, the CD16 variant protein comprises one or more amino acid substitutions selected from the group consisting of F158V, F176V, S197P, D205A, S219A, T220A. In some embodiments, the CD16 variant protein comprises an amino acid sequence having at least 90% sequence identity to any one of SEQ ID NOS:181 and 182. In some embodiments, the iPSC or the derivative cell comprises an exogenous polynucleotide encoding the CD16 protein and the NKG2D protein, wherein the CD16 protein and the NKG2D protein are operably linked by an autoprotease peptide. In some embodiments, the NKG2D protein is a wildtype NKG2D protein. In some embodiments, the NKG2D protein comprises an amino acid sequence having at least 90% sequence identity to SEQ ID NO: 184. In some embodiments, the autoprotease peptide is selected

from the group consisting of a porcine tesehovirus-1 2A (P2A) peptide, a foot-and-mouth disease virus 2A (F2A) peptide, an Equine Rhinitis A Virus (ERAV) 2A (E2A) peptide, a Thosea asigna virus 2A (T2A) peptide, a cytoplasmic polyhedrosis virus 2A (BmCPV2A) peptide, and a Flacherie Virus 2A (BmIFV2A) peptide. In some embodiments, the autoprotease peptide is a P2A peptide comprising an amino acid sequence having at least 90% sequence identity to SEQ ID NO: 186. In some embodiments, the exogenous polynucleotide encoding the CD16 protein and the NKG2D protein comprises a nucleic acid sequence having at least 90% sequence identity to SEQ ID NO: 186. In some embodiments, one or more of the exogenous polynucleotides are integrated at one or more loci on the chromosome of the cell selected from the group consisting of AAVS1, CCR5, ROSA26, collagen, HTRP, Hl l, GAPDH, RUNX1, B2M, TAPI, TAP2, Tapasin, NLRC5, RFXANK, CIITA, RFX5, RFXAP, TCR a or b constant region, NKG2A, NKG2D, CD38, CIS, CBL-B, SOCS2, PD1, CTLA4, LAG3, TIM3, and TIGIT genes, provided at least one of the exogenous polynucleotides is integrated at a locus of a gene selected from the group consisting of AAVS1, B2M, TAP 1, TAP 2, Tapasin, RFXANK, CIITA, RFX5 and RFXAP genes to thereby result in a deletion or reduced expression of the gene. In some embodiments, one or more of the exogenous polynucleotides are integrated at the loci of the AAVS1 and B2M genes. In some embodiments, the iPSC or the derivative cell comprises a deletion or reduced expression of one or more of B2M or CIITA genes. In some embodiments, the iPSC or the derivative cell thereof comprises the deletion or reduced expression of B2M and CIITA genes. In some embodiments, the iPSC is reprogrammed from whole peripheral blood mononuclear cells (PBMCs). In some embodiments, the iPSC is derived from a re-programmed T-cell. In some embodiments, the CAR comprises: (i) a signal peptide; (ii) a extracellular domain comprising the antigen binding domain targeting the CD22 antigen; (iii) a hinge region; (iv) a transmembrane domain, (v) an intracellular signaling domain; and (vi) a co-stimulatory domain. In some embodiments, the extracellular domain comprises a VHH single domain antibody that specifically binds the CD22 antigen. In some embodiments, the extracellular domain comprises an amino acid sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to one of SEQ ID NOs: 96-98, 152, and 155. In some embodiments, the extracellular domain comprises

a polynucleotide sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to one of SEQ ID NOs: 99-101, 153, and 156. In some embodiments, the additional CAR comprises: (i) a signal peptide; (ii) an additional extracellular domain comprising a binding domain that specifically binds the CD19 antigen; (iii) a hinge region; (iv) a transmembrane domain, (v) an intracellular signaling domain; and (vi) a co-stimulatory domain. In some embodiments, the additional extracellular domain comprises an scFv derived from an antibody that specifically binds the CD19 antigen. In some embodiments, the additional extracellular domain comprises (i) an amino acid sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to one or more of SEQ ID NOs: 2, 4, and 7, or (ii) is encoded by a polynucleotide sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to one or more of SEQ ID NOs: 145 and 147. In some embodiments, the signal peptide comprises a GMCSFR signal peptide. In some embodiments, the hinge region for each of the CAR and the additional CAR are independently selected from the group consisting of a CD28 hinge region, an IgG4 hinge region, and a CD8 hinge region. In some embodiments, the transmembrane domain for each of the CAR and the additional CAR are independently selected from the group consisting of a CD28 transmembrane domain and a Cd8 transmembrane domain. In some embodiments, the intracellular signaling domain comprises a CD3ζ intracellular domain. In some embodiments, the co-stimulatory domain for each of the CAR and the additional CAR are independently selected from the group consisting of a CD28 signaling domain, a 41BB signaling domain, a DAP10 signaling domain, an IL18R1 signaling domain, and an IL18RAP signaling domain. In some embodiments, the additional CAR comprises: (i) the signal peptide comprising an amino acid sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to SEQ ID NOs: 1, 103, or 144; (ii) the additional extracellular domain comprising an amino acid sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to SEQ ID NOs: 2, 4, or 7, or the additional extracellular domain encoded by a polynucleotide sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to SEQ ID NOs: 145 or 147; (iii) the hinge region comprising an amino acid sequence

having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to SEQ ID NO: 22; (iv) the transmembrane domain comprising an amino acid sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to SEQ ID NO: 24; (v) the intracellular signaling domain comprising an amino acid sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to SEQ ID NO: 6, or the intracellular signaling domain encoded by a polynucleotide sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to SEQ ID NO: 149; and (vi) the co-stimulatory domain comprising an amino acid sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to SEQ ID NO: 20. In some embodiments, the additional CAR comprises: (i) the signal peptide comprising the amino acid sequence of SEQ ID NOs: 1, 103, or 144; (ii) the additional extracellular domain (i) comprising the amino acid sequence of SEQ ID NO: 2, 4, and 7, or (ii) encoded by the polynucleotide sequence of SEQ ID NOs: 145 and 147; (iii) the hinge region comprising the amino acid sequence of SEQ ID NO: 22; (iv) the transmembrane domain comprising the amino acid sequence of SEQ ID NO: 24; (v) the intracellular signaling domain comprising the amino acid sequence of SEQ ID NO: 6, or the intracellular signaling domain encoded by the polynucleotide sequence of SEQ ID NO: 149; and (vi) the co-stimulatory domain comprising the amino acid sequence of SEQ ID NO: 20. In some embodiments, the CAR comprises: (i) the signal peptide comprising an amino acid sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to SEQ ID NOs: 1, 103, or 144; (ii) the extracellular domain comprising an amino acid sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to one of SEQ ID NOs: 96-98, 152, and 155; (iii) the hinge region comprising an amino acid sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to SEQ ID NO: 21 or 102; (iv) the transmembrane domain comprising an amino acid sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to SEQ ID NO: 23 or 24; (v) the intracellular signaling domain comprising an amino acid sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to SEQ ID NO: 6,

198, or 199, or the intracellular signaling domain encoded by a polynucleotide sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to SEQ ID NO: 149; and (vi) the co-stimulatory domain comprising an amino acid sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to SEQ ID NO: 8, 17, 198, or 199. In some embodiments, the CAR comprises: (i) the signal peptide comprising the amino acid sequence of SEQ ID NO: 1, 103, or 144; (ii) the extracellular domain comprising the amino acid sequence of one of SEQ ID NOs: 96-98, 152, and 155; (iii) the hinge region comprising the amino acid sequence of SEQ ID NO: 21 or 102; (iv) the transmembrane domain comprising the amino acid sequence of SEQ ID NO: 23 or 24; (v) the intracellular signaling domain comprising the amino acid sequence of SEQ ID NO: 6, 198, or 199, or the intracellular signaling domain encoded by the polynucleotide sequence of SEQ ID NO: 149; and (vi) the co-stimulatory domain comprising the amino acid sequence of SEQ ID NO: 8, 17, 198, or 199. In some embodiments, the iPSC or the derivative cell comprises an exogenous polynucleotide encoding a safety switch. In some embodiments, the safety switch comprises an exogenous polynucleotide encoding an inactivated cell surface receptor that comprises a monoclonal antibody-specific epitope. In some embodiments, the inactivated cell surface protein is selected from the group of monoclonal antibody specific epitopes selected from epitopes specifically recognized by ibritumomab, tiuxetan, muromonab-CD3, tositumomab, abciximab, basiliximab, brentuximab vedotin, cetuximab, infliximab, rituximab, alemtuzumab, bevacizumab, certolizumab pegol, daclizumab, eculizumab, efalizumab, gemtuzumab, natalizumab, omalizumab, palivizumab, polatuzumab vedotin, ranibizumab, tocilizumab, trastuzumab, vedolizumab, adalimumab, belimumab, canakinumab, denosumab, golimumab, ipilimumab, ofatumumab, panitumumab, and ustekinumab. In some embodiments, the inactivated cell surface protein is a truncated epithelial growth factor (tEGFR) variant. In some embodiments, the tEGFR variant consists of an amino acid sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to SEQ ID NO: 71. In some embodiments, the safety switch comprises (i) an intracellular domain having a herpes simplex virus thymidine kinase (HSV-TK) or (ii) an inducible Caspase 9 (iCasp9). In some embodiments, the iPSC or the derivative cell comprises the

exogeneous polynucleotide encoding the PSMA cell tracer, wherein the PSMA cell tracer comprises an extracellular domain comprising a PSMA extracellular domain or fragment thereof. In some embodiments, the iPSC or the derivative cell comprises a combined artificial cell death/reporter system polypeptide comprising an intracellular domain having a herpes simplex virus thymidine kinase (HSV-TK) and a linker, a transmembrane region, and an extracellular domain comprising the PSMA extracellular domain or fragment thereof. In some embodiments, (i) the HSV-TK comprises an amino acid sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to SEQ ID NO: 187 or 188, or (ii) the iCasp9 comprises an amino acid sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to SEQ ID NO: 200 or 201. In some embodiments, the combined artificial cell death/reporter system polypeptide comprises the HSV-TK fused to a truncated variant PSMA polypeptide via the linker. In some embodiments, the truncated variant PSMA polypeptide comprises an amino acid sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to SEQ ID NO: 189. In some embodiments, the linker comprises an autoprotease peptide sequence selected from the group consisting of P2A peptide sequence, T2A peptide sequence, E2A peptide sequence, and F2A peptide sequence. In some embodiments, the artificial cell death/reporter system polypeptide comprises an amino acid sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to a sequence selected from the group consisting of SEQ ID NO: 190. In some embodiments, the artificial cell death/reporter system polypeptide comprises an amino acid sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to a sequence selected from the group consisting of SEQ ID NOS: 191-193. In some embodiments, the artificial cell death/reporter system polypeptide comprises nucleic acid sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to a sequence selected from the group consisting of SEQ ID NOS: 194-196. In some embodiments, the HLA-E comprises an amino acid sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to SEQ ID NO: 66 or the HLA-G comprises an amino acid sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%,

99% or 100% sequence identity to SEQ ID NO: 69. In some embodiments, (i) the one or more exogenous polynucleotides encoding a chimeric antigen receptor (CAR) comprising an antigen binding domain targeting a CD22 and/or CD19 antigen comprises a sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to one or more polynucleotide sequences selected from the group consisting of SEQ ID NOs: 62, 99-101, 112-119, 132-143, 153, 156, 158, 160, 162, 164, 168-170, 172, and 176-178; (ii) the exogenous polynucleotide encoding a human leukocyte antigen E (HLA-E) and/or human leukocyte antigen G (HLA-G) comprises the polynucleotide sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to one or more of SEQ ID NOs: 67 and 70; (iii) the exogenous polynucleotide encoding an NK cell receptor immunoglobulin gamma Fc region receptor III (FcyRIII, cluster of differentiation 16 (CD16)) and/or an NKG2D protein comprises the polynucleotide sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to one or more of SEQ ID NOs: 179, 183, and 185; (iv) the exogeneous polynucleotide encoding a cytokine comprises the polynucleotide sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to SEQ ID NO: 197; (v) the exogenous polynucleotide encoding a safety switch comprises the polynucleotide sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to one or more of SEQ ID NO: 194-196; and/or (vi) the exogeneous polynucleotide encoding a PSMA cell tracer comprising the amino acid sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to SEQ ID NO: 189. In some embodiments, (i) the one or more exogenous polynucleotides encoding a chimeric antigen receptor (CAR) comprising an antigen binding domain targeting a CD22 and/or CD19 antigen comprises one or more sequences selected from the group consisting of SEQ ID NOs: 62, 99-101, 112-119, 132-143, 153, 156, 158, 160, 162, 164, 168-170, 172, and 176-178; (ii) the exogenous polynucleotide encoding a human leukocyte antigen E (HLA-E) and/or human leukocyte antigen G (HLA-G) comprises the polynucleotide sequence having the sequence SEQ ID NO: 67 or 70; (iii) the exogenous polynucleotide encoding an NK cell receptor immunoglobulin gamma Fc region receptor III (FcyRIII, cluster of differentiation 16 (CD16)) and/or an NKG2D protein comprises

the polynucleotide sequence of SEQ ID NO: 179, 183, or 185; (iv) the exogeneous polynucleotide encoding the cytokine comprises the polynucleotide sequence of SEQ ID NO: 197; and/or (v) the exogenous polynucleotide encoding the safety switch comprises the polynucleotide sequence having the sequence of one of SEQ ID NOs: 194-196. In some embodiments, the exogenous polynucleotides are integrated into a gene locus independently selected from the group consisting of an AAVS1 locus, a B2M locus, a CIITA locus, a CCR5 locus, a CD70 locus, a CLYBL locus, an NKG2A locus, an NKG2D locus, a CD33 locus, a CD38 locus, a TRAC locus, a TRBC1 locus, a ROSA26 locus, an HTRP locus, a GAPDH locus, a RUNX1 locus, a TAP1 locus, a TAP2 locus, a TAPBP locus, an NLRC5 locus, a RFXANK locus, a RFX5 locus, a RFXAP locus, a CISH locus, a CBLB locus, a SOCS2 locus, a PD1 locus, a CTLA4 locus, a LAG3 locus, a TIM3 locus, and a TIGIT locus. In some embodiments, (i) the one or more exogenous polynucleotides encoding a chimeric antigen receptor (CAR) comprising one or more antigen binding domains targeting CD22 and/or CD19 antigens is integrated at a locus of the AAVS1 gene; (ii) the exogenous polynucleotide encoding a human leukocyte antigen E (HLA-E) and/or human leukocyte antigen G (HLA-G) is integrated at a locus of the B2M gene; (iii) the exogenous polynucleotide encoding an NK cell receptor immunoglobulin gamma Fc region receptor III (FcγRIII, cluster of differentiation 16 (CD16)) and/or an NKG2D is integrated at a locus of the CD70 gene; (iv) the exogeneous polynucleotide encoding the cytokine is integrated at the locus of the NKG2A gene; (v) there is a deletion or reduced expression of the CIITA gene; and (vi) optionally, there a safety switch or PSMA is integrated at the locus of the CIITA gene. In some embodiments, the CAR is a bispecific CAR comprising a CD22/CD19 loop. In some embodiments, the bispecific CAR comprises one or more amino acid sequences having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to a sequence selected from the group consisting of SEQ ID NOs: 61, 96-98, 104-111, 120-131, 152, 155, 157, 159, 161, 163, 165-167, 171, and 173-175. In some embodiments, the bispecific CAR comprises one or more polynucleotide sequences having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to a sequence selected from the group consisting of SEQ ID NOs: 62, 99-101, 112-119, 132-143, 153, 156, 158, 160, 162, 164, 168-170, 172, and 176-178. In

some embodiments, the derivative cell is a natural killer (NK) cell or a T cell. In some embodiments, the derivative cell is a natural killer (NK) cell. In some embodiments, the derivative cell is a T cell. In some embodiments, the T cell is a gamma delta T cell. In some embodiments, the T cell is a gamma delta Vγ9/Vδ1 T cell.

In some aspects, the present disclosure provides a composition comprising any of the iPSC or derivative cells of the present disclosure. In some embodiments, the composition further comprises or is used in combination with one or more therapeutic agents selected from the group consisting of a peptide, a cytokine, a checkpoint inhibitor, a mitogen, a growth factor, a small RNA, a dsRNA (double stranded RNA), siRNA, oligonucleotide, mononuclear blood cells, a vector comprising one or more polynucleic acids of interest, an antibody, a chemotherapeutic agent or a radioactive moiety, or an immunomodulatory drug (IMiD).

In some aspects, the present disclosure provides an induced pluripotent stem cell (iPSC) or a derivative cell thereof comprising: one or more exogenous polynucleotides encoding a chimeric antigen receptor (CAR) targeting a CD22 antigen and a CD19 antigen; and at least one of: (i) a deletion or reduced expression of one or more of B2M, TAP 1, TAP 2, Tapasin, RFXANK, CIITA, RFX5, RFXAP genes; (ii) an exogenous polynucleotide encoding a human leukocyte antigen E (HLA-E) and/or human leukocyte antigen G (HLA-G); (iii) an exogenous polynucleotide encoding an NK cell receptor immunoglobulin gamma Fc region receptor III (FcyRIII, cluster of differentiation 16 (CD16) and/or an NKG2D protein; (iv) a deletion or reduced expression of one or more of NKG2A or CD70 genes; (v) an exogeneous polynucleotide encoding a cytokine; (vi) an exogenous polynucleotide encoding a safety switch; and (vi) an exogeneous polynucleotide encoding a PSMA cell tracer. In some embodiments, the CAR is a bispecific CAR comprising a CD22/CD19 loop. In some embodiments, the CAR comprises an anti-CD22 VHH domain. In some embodiments, the one or more exogenous polynucleotides each comprise a sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to one or more sequences independently selected from the group consisting of SEQ ID NOs: 62, 99-101, 112-119, 132-143, 153, 156, 158, 160, 162, 164, 168-170, 172, and 176-178.

In some aspects, the present disclosure provides a CD34+ hematopoietic progenitor cell (HPC) derived from an induced pluripotent stem cell (iPSC) comprising: one or more exogenous polynucleotides encoding a chimeric antigen receptor (CAR) comprising an antigen binding domain targeting a CD22 antigen; and optionally, at least one of: (i) a CD19 antigen-binding domain encoded by the one or more exogenous polynucleotides; (ii) a deletion or reduced expression of one or more of B2M, TAP 1, TAP 2, Tapasin, RFXANK, CIITA, RFX5, and RFXAP genes; (iii) an exogenous polynucleotide encoding a human leukocyte antigen E (HLA-E) and/or human leukocyte antigen G (HLA-G); (iv) an exogenous polynucleotide encoding an NK cell receptor immunoglobulin gamma Fc region receptor III (FcyRIII, cluster of differentiation 16 (CD16) and/or an NKG2D protein; (v) a deletion or reduced expression of one or more of NKG2A or CD70 genes; (vi) an exogeneous polynucleotide encoding a cytokine; (vii) an exogenous polynucleotide encoding a safety switch; and (viii) an exogeneous polynucleotide encoding a PSMA cell tracer. In some embodiments, the CD34+ HPC comprises the CD19 antigen-binding domain, wherein: (i) the CAR is a bispecific CAR comprising the CD19 antigen-binding domain, or (ii) the one or more exogenous polynucleotides encode an additional CAR comprising the CD19 antigen-binding domain. In some embodiments, the CAR comprises an anti-CD22 VHH domain, and/or wherein the CD19 antigen-binding domain comprises an anti-CD19 VHH domain. In some embodiments, the cytokine comprises an IL-15 protein. In some embodiments, the IL-15 protein comprises an inactivated cell surface receptor that comprises a monoclonal antibody-specific epitope and an interleukin 15 (IL-15), and wherein the inactivated cell surface receptor and the IL-15 are operably linked by an autoprotease peptide. In some embodiments, the IL-15 protein comprises a fusion polypeptide comprising an IL-15 and an IL-15 receptor alpha (IL-15Rα). In some embodiments, the IL-15 comprises an amino acid sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to SEQ ID NO: 72. In some embodiments, the deletion or reduced expression of one or more of B2M, TAP 1, TAP 2, Tapasin, RFXANK, CIITA, RFX5 and RFXAP genes. In some embodiments, the exogenous polynucleotide encoding a human leukocyte antigen E (HLA-E) and/or human leukocyte antigen G (HLA-G). In some embodiments, one or more of the exogenous polynucleotides are integrated at one

or more loci on the chromosome of the cell independently selected from the group consisting of AAVS1, CCR5, ROSA26, collagen, HTRP, Hl l, GAPDH, RUNX1, B2M, TAPI, TAP2, Tapasin, NLRC5, RFXANK, CIITA, RFX5, RFXAP, TCR a or b constant region, NKG2A, NKG2D, CD38, CIS, CBL-B, SOCS2, PD1, CTLA4, LAG3, TIM3, and TIGIT genes, provided at least one of the exogenous polynucleotides is integrated at a locus of a gene selected from the group consisting of AAVS1, B2M, TAP 1, TAP 2, Tapasin, RFXANK, CIITA, RFX5 and RFXAP genes to thereby result in a deletion or reduced expression of the gene. In some embodiments, one or more of the exogenous polynucleotides are integrated at the loci of the CIITA, AAVS1 and B2M genes. In some embodiments, the CD34+ HPC comprises a deletion or reduced expression of one or more of B2M or CIITA genes. In some embodiments, the CAR comprises: (i) a signal peptide; (ii) a extracellular domain comprising a binding domain that specifically binds the CD22 antigen and, optionally, a binding domain that specifically binds the CD19 antigen; (iii) a hinge region; (iv) a transmembrane domain; (v) an intracellular signaling domain; and (vi) a co-stimulatory domain. In some embodiments, the extracellular domain comprises a VHH single domain antibody that specifically binds the CD22 antigen. In some embodiments, the extracellular domain comprises an amino acid sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to one of SEQ ID NOs: 96-98, 152, and 155. In some embodiments, the extracellular domain comprises a polynucleotide sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to one of SEQ ID NOs: 99-101, 153, and 156. In some embodiments, the bispecific CAR, wherein the bispecific CAR comprises one or more amino acid sequences having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to a sequence selected from the group consisting of SEQ ID NOs: 61, 96-98, 104-111, 120-131, 152, 155, 157, 159, 161, 163, 165-167, 171, and 173-175. In some embodiments, the bispecific CAR, wherein the bispecific CAR comprises an amino acid sequence encoded by one or more polynucleotide sequences having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to a sequence selected from the group consisting of SEQ ID NOs: 62, 99-101, 112-119, 132-143, 153, 156, 158, 160, 162, 164, 168-170, 172, and 176-178. In some embodiments, the additional CAR

comprises: (i) a signal peptide; (ii) an additional extracellular domain comprising a binding domain that specifically binds the CD19 antigen; (iii) a hinge region; (iv) a transmembrane domain; (v) an intracellular signaling domain; and (vi) a co-stimulatory domain. In some embodiments, the additional extracellular domain comprises an scFv derived from an antibody that specifically binds the CD19 antigen. In some embodiments, the additional extracellular domain comprises (i) an amino acid sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to one or more of SEQ ID NOs: 2, 4, and 7, or (ii) is encoded by a polynucleotide sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to one or more of SEQ ID NOs: 145 and 147.

In some aspects, the present disclosure provides a chimeric antigen receptor (CAR) polypeptide comprising an extracellular domain that specifically binds to CD22 having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to SEQ ID NOs: 62, 99-101, 112-119, 132-143, 153, 156, 158, 160, 162, 164, 168-170, 172, and 176-178.

In some aspects, the present disclosure provides a method of treating cancer in a subject in need thereof, comprising administering any of the derivative cells of the present disclosure, or any of the compositions of the present disclosure to a subject in need thereof. In some embodiments, the cancer is selected from the group consisting of leukemia, such as AML, CML, acute lymphocytic leukemia (ALL), B-cell acute lymphocytic leukemia (B-ALL), and chronic lymphocytic leukemia (CLL), lymphomas, such as Hodgkin lymphoma, non-Hodgkin lymphoma, multiple myeloma, and follicular lymphoma, and solid cancers such as sarcomas, skin cancer, melanoma, bladder cancer, brain cancer, breast cancer, uterus cancer, ovary cancer, prostate cancer, lung cancer, colorectal cancer, cervical cancer, liver cancer, head and neck cancer, esophageal cancer, pancreatic cancer, renal cancer, adrenal cancer, stomach cancer, testicular cancer, cancer of the gall bladder and biliary tracts, thyroid cancer, thymus cancer, cancer of bone, and cerebral cancer, as well as cancer of unknown primary (CUP). In some embodiments, the cancer is a B-cell malignancy, acute lymphocytic leukemia (ALL), B-cell acute lymphocytic leukemia (B-ALL), chronic lymphocytic leukemia (CLL), or non-Hodgkin lymphoma, follicular lymphoma. In some embodiments, the subject has minimal residual

disease (MRD) after an initial cancer treatment. In some embodiments, the subject has no minimal residual disease (MRD) after one or more cancer treatments or repeated dosing.

In some aspects, the present disclosure provides a method of manufacturing any of the derivative cells of the present disclosure, the method comprising differentiating the iPSC cell under conditions for cell differentiation to thereby obtain the derivative cell. In some embodiments, the iPSC is obtained by genomic engineering an unmodified iPSC, wherein the genomic engineering comprises targeted editing. In some embodiments, the targeted editing comprises deletion, insertion, or in/del carried out by CRISPR, ZFN, TALEN, homing nuclease, homology recombination, or any other functional variation of these methods.

In some aspects, the present disclosure provides a method of differentiating an induced pluripotent stem cell (iPSC) into an NK cell, comprising subjecting the iPSCs to a differentiation protocol including culturing the cells in a medium containing a recombinant human IL-12 for the final 24 hours of culturing under the differentiation protocol. In some embodiments, the recombinant IL-12 comprises IL12p70.

BRIEF DESCRIPTION OF THE DRAWINGS

The foregoing summary, as well as the following detailed description of preferred embodiments of the present application, will be better understood when read in conjunction with the appended drawings. It should be understood, however, that the application is not limited to the precise embodiments shown in the drawings.

FIGs 1A-C show results of VHH-Fc fusion proteins that were examined by flow cytometry for CD22 cellular binding in Raji cells. A) PROT739 and PROT740 from VHH phage libraries show binding to Raji cells with EC50 values of approximately 2nM. No binding was seen to Raji CD22 knock-out cells. B) PROT265 and PROT810 (an affinity matured variant of PROT265) bind to Raji cells, with PROT810 showing improved binding relative to PROT265.

FIG. 2 shows schematics of VHH-Fc fusion proteins that were examined for binding to full length CD22, CD22Δ2-3 and CD22ΔV-1. PROT265 (D04) bound to all proteins tested, suggesting that it binds to the IgC domains 4-6. PROT739 (A01) bound

to the full length CD22 and had weak binding to the CD22Δ2-3 protein with no binding to the CD22ΔV-1 protein, suggesting that it binds to the IgC1-2 domains. PROT740 (E04) bound to the full length CD22 and the CD22Δ2-3 protein, but not the CD22ΔV-1 protein, suggesting that it binds to the IgV-IgC1 domains.

FIGs. 3A-B show VHH-CAR constructs that were transduced into Jurkat cells. Cells were tested for activation by co-culture with cells expressing the CD22 target protein at an effector:target (E:T) ratio of approximately 4:1. Cells were stained for CD3 and CD69 expression using BV421-anti-human CD69 antibody (Biolegend) and PE-anti-CD3 clone OKT3 (Biolegend). A) Jurkat CAR-T cells ($CD3^+$) bearing clones A01 (P952) and E04 (P953) show activation of CD69 expression following 48 hours of co-incubation with various $CD22^+$ or $CD22^-$ cell lines. B) Jurkat CAR-T cells bearing clone D04 (D04.P262) show activation of CD69 expression following 48 hours of co-incubation with various $CD22^+$ or $CD22^-$ cell lines.

FIGs. 4A-B show cytotoxicity of VHH-CAR expressing cells towards Raji cells. VHH-CAR lentivirus was transduced into primary T-cells. Cells were grown for 14 days, then co-cultured with CD22+ Raji cells at E:T ratios of 2:1 and 5:1 for 48 hours. Raji cell killing was assessed by flow cytometry. A) P403 (D04), P1278 (D04_AM_D11) and P456 (a positive control scFv-CAR) were transduced into T-cells and assessed for cytotoxicity towards Raji cells. The P403 construct (CD22_D04) showed modest Raji cell killing over background (untransduced cells; UTD). The affinity improved variant P1278 (CD22_D04_AM_D11) had significantly better cytotoxicity, up to 80% at the 5:1 E:T ratio. B) The P952 (CD22_CNTY_VHH1_A01) and P953 (CD22_CNTY_VHH1_E04) constructs showed modest cytotoxicity above background.

FIGs. 5A-B show cytotoxicity of tandem VHH-CAR expressing cells towards (A) Raji cells and (B) K-562 cells. VHH-CAR lentivirus was transduced into primary T-cells. Cells were grown for 14 days, then co-cultured with $CD22^+$ Raji cells at E:T ratios of 1:2, 1:1 and 2:1 for 48 hours. Target cell killing was assessed by flow cytometry. The single VHH (D04_AM_D11) CAR (P1729) demonstrated minimal killing of Raji cells. The tandem VHH of E04 - D04_AM_D11 (P1738) showed slightly improved Raji cell killing, up to 20% at the 2:1 E:T ratio. However, when put in the other orientation,

D04_AM_D11 - E04 (P1730), cytotoxicity improved to 40% at 2:1, similar to the positive control scFv. The D04_AM_D11 – A01 CARs, in either orientation (P1732 and P1733) showed robust cytotoxicity up to 90% at the 2:1 E:T ratio.

FIGs. 6A-R show various bispecific CAR constructs (targeting CD19 and CD22 antigens) which are used in various embodiments of the present disclosure. CAR ectodomains were engineered with select CD22 binders in combination with FMC63. Selected CARs were expressed and tested for cytotoxicity activity in T-cells along with mono-specific CAR-T cells.

FIGs. 7A-B show (A) CD19 dependent killing of Cho-CD19 cells and Raji-CD22 knockout cells using therapeutic cells expressing the mono- and bispecific CAR constructs of the present disclosure at a 1.25:1 E:T ratio. All of the bispecific CAR-T cells tested exhibited CD19-targeted cytotoxicity activity equivalent to the FMC63 CAR (e.g., P1209). The bispecific CARS showed comparable CD22-directed cytotoxicity as the mono-specific tandem CARs (e.g., P1631, P1633, P1702 and P1734). (B) CD22-dependent killing of Cho-CD22 cells, Raji-CD19 knockout cells, and Nalm6-CD19 knockout cells using therapeutic cells expressing the mono- and bispecific CAR constructs of the present disclosure at a 1.25:1 E:T ratio. Bispecific CAR T cells showed CD22-dependent activity that was comparable to CD22 mono-specific CARs. P2015 and p2016 had reduced activity on CHO-CD22, the highest activity on Raji-CD19 knockout cells and Nalm6-CD19 knockout cells.

FIG. 8 shows CD19/CD22 double-positive killing of Reh cells, Raji cells, Jeko cells, and Nalm6 cells using therapeutic cells expressing the mono- and bispecific CAR constructs of the present disclosure at a 1.25:1 E:T ratio.

FIG. 9 shows the percent expression of various mono- and bispecific CAR constructs of the present disclosure in T cells.

FIGs. 10A-B show (A) various mono-, bi- and tri-specific CAR constructs of the present disclosure, and (B) the percent expression of the various CAR constructs in T cells.

FIGs. 11A-B show (A) various bi- and tri-specific CAR constructs of the present disclosure, and (B) the target-dependent cytotoxicity of therapeutic cells expressing the various CAR constructs against Cho cells.

FIGs. 12A-G show results of a cytotoxicity analysis of therapeutic cells expressing various mono-, bi- and tri-specific CAR constructs of the present disclosure at E:T ratios between 0 and 10:1 in (A) Cho cells (CD22-/CD19-), (B) Cho-CD22 cells (CD22+/CD19-), (C) Cho-CD19 cells (CD22-/CD19+), (D) Raji cells (CD22+/CD19+), (E) Raji-CD22KO cells (CD22-/CD19+), (F) Raji-CD19KO cells (CD22+/CD19-), and (G) Jeko cells (CD22+/CD19+).

FIG. 13 shows CD19-, CD22-, and CD19/CD22-dependent killing of Reh cells, Jeko cells, Cho-CD22 cells, Cho-CD19 cells, Cho cells, Raji-CD22 KO cells, Raji-CD19 KO cells, Raji cells, Nalm6-CD19 KO cells, and Nalm6 cells using therapeutics cells expressing various mono- and bispecific CARs of the present disclosure.

FIGs. 14A-J shows results of cumulative cytotoxicity analysis of therapeutic cells expressing various mono- and bispecific CAR constructs of the present disclosure at E:T ratios between 0 and 5:1 in (A) Cho cells (CD22-/CD19-), (B) Cho-CD22 cells (CD22+/CD19-), (C) Cho-CD19 cells (CD22-/CD19+), (D) Raji cells (CD22+/CD19+), (E) Raji-CD19KO cells (CD22+/CD19-), (F) Raji-CD22KO cells (CD22-/CD19+), (G) Nalm6 cells (CD22+/CD19+), (H) Nalm6-CD19KO cells (CD22+/CD19-), (I) Reh cells (CD22+/CD19+), and (J) Jeko cells (CD22+/CD19+).

FIGs. 15A-C show results of a cytotoxicity analysis of therapeutic cells expressing various mono- and bispecific CAR constructs of the present disclosure at E:T ratios between 0 and 5:1 in (A) Cho cells (CD22-/CD19-), (B) Cho-CD22 cells (CD22+/CD19-), and (C) Cho-CD19 cells (CD22-/CD19+).

FIG. 16 shows the target-dependent cytotoxicity of therapeutic cells expressing mono- and bispecific CAR constructs against Cho cells, Cho-CD19 cells, and Cho-CD22 cells.

FIGs. 17A-B show results of a cytotoxicity analysis of therapeutic cells expressing various mono- and bispecific CAR constructs of the present disclosure at E:T ratios between 0 and 5:1 in (A) Nalm6 cells (CD22+/CD19+) and (B) Nalm6-CD19KO cells (CD22+/CD19-).

FIG. 18 shows the target-dependent cytotoxicity of therapeutic cells expressing mono- and bispecific CAR constructs against Nalm6 cells, and Nalm6-CD19 cells.

FIGs. 19A-C show results of a cytotoxicity analysis of therapeutic cells expressing various mono- and bispecific CAR constructs of the present disclosure at E:T ratios between 0 and 5:1 in (A) Raji cells (CD22+/CD19+), (B) Raji-CD22KO cells (CD22-/CD19+), and (C) Raji-CD19KO cells (CD22+/CD19-).

FIG. 20 shows the target-dependent cytotoxicity of therapeutic cells expressing mono- and bispecific CAR constructs against Raji cells, Raji-CD19KO cells, and Raji-CD22 KO cells.

FIG. 21 shows a diagram of an exemplary cell of the present disclosure, which expresses an anti-CD22/CD19 loop CAR on the cell surface.

FIG. 22 shows CAR expression in cells of the present disclosure are engineered to express a bispecific CAR that recognizes both CD19 and CD22. Initially, iPSCs were genetically modified to express a transgene for the CD19/CD22 CAR or a control CD19 CAR (or cells were not engineered for the CAR-less control). Those iPSCs were differentiated into T cells (iT cells) and CAR expression was evaluated by staining with either anti-idiotype antibody that binds to the CD19 recognition domain of the CAR (left) or anti-VHH antibody that binds to the CD22 recognition domain of the CAR (right). Staining of cells was evaluated using flow cytometry. CAR-less iT cells were not stained with either antibody. The CD19/CD22 CAR-iT cells were stained with antibodies for both the CD19 recognition domain and the CD22 recognition domain. The CD19 CAR-iT cells were stained with the antibody for the CD19 recognition domain but not the CD22 recognition domain. These results demonstrate that the CD19/CD22 bispecific CAR-iT cells express a CAR molecule that has the potential to bind to either CD19 antigen or CD22 antigen on target cells.

FIG. 23 shows antigen specific cytolysis of tumor cells by CD19/CD22 bispecific CAR-iT cells. CAR-iT cells with a bispecific CAR eliminate tumor cells through recognition of either CD19 or CD22. Either CD19 CAR-iT cells or CD19/CD22 CAR-iT cells were co-cultured with tumor cells at a 1:1 effector to target ratio for 24 hours. Cytolysis (%) was calculated based on the number of tumor cells present after 24 hours of co-culture with iT cells compared to the number of tumor cells present when cultured alone. Data was collected using an IncuCyte live cell imaging platform and red fluorescent labeled tumor cells. (A) When both CD19 and CD22 were present on the

surface of tumor cells (Daudi B cell lymphoma), both the CD19 CAR-iT cells and CD19/CD22 CAR-iT cells mediated tumor killing. (B) When tumor cells expressed CD22 but not CD19 (Daudi CD19-knockout cells), only the bispecific CD19/CD22 CAR-iT cells mediated tumor killing. (C) When tumor cells expressed CD19 but not CD22 (Daudi CD22-knockout cells), both CD19 CAR-iT cells and CD19/CD22 CAR-iT cells mediated tumor killing. Thus, a CD19/CD22 bispecific CAR-iT cell can target B cell malignancies that have lost expression of CD19 but targeting CD22.

DETAILED DESCRIPTION

Various publications, articles and patents are cited or described in the background and throughout the specification; each of these references is herein incorporated by reference in its entirety. Discussion of documents, acts, materials, devices, articles or the like which has been included in the present specification is for the purpose of providing context for the invention. Such discussion is not an admission that any or all of these matters form part of the prior art with respect to any inventions disclosed or claimed.

Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood to one of ordinary skill in the art to which this application pertains. Otherwise, certain terms used herein have the meanings as set forth in the specification.

It must be noted that as used herein and in the appended claims, the singular forms “a,” “an,” and “the” include plural reference unless the context clearly dictates otherwise.

Unless otherwise stated, any numerical values, such as a concentration or a concentration range described herein, are to be understood as being modified in all instances by the term “about.” Thus, a numerical value typically includes ± 10% of the recited value. For example, a concentration of 1 mg/mL includes 0.9 mg/mL to 1.1 mg/mL. Likewise, a concentration range of 1% to 10% (w/v) includes 0.9% (w/v) to 11% (w/v). As used herein, the use of a numerical range expressly includes all possible subranges, all individual numerical values within that range, including integers within such ranges and fractions of the values unless the context clearly indicates otherwise.

Unless otherwise indicated, the term "at least" preceding a series of elements is to be understood to refer to every element in the series. Those skilled in the art will recognize or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments of the application described herein. Such equivalents are intended to be encompassed by the application.

As used herein, the terms "comprises," "comprising," "includes," "including," "has," "having," "contains" or "containing," or any other variation thereof, will be understood to imply the inclusion of a stated integer or group of integers but not the exclusion of any other integer or group of integers and are intended to be non-exclusive or open-ended. For example, a composition, a mixture, a process, a method, an article, or an apparatus that comprises a list of elements is not necessarily limited to only those elements but can include other elements not expressly listed or inherent to such composition, mixture, process, method, article, or apparatus. Further, unless expressly stated to the contrary, "or" refers to an inclusive or and not to an exclusive or. For example, a condition A or B is satisfied by any one of the following: A is true (or present) and B is false (or not present), A is false (or not present) and B is true (or present), and both A and B are true (or present).

As used herein, the conjunctive term "and/or" between multiple recited elements is understood as encompassing both individual and combined options. For instance, where two elements are conjoined by "and/or," a first option refers to the applicability of the first element without the second. A second option refers to the applicability of the second element without the first. A third option refers to the applicability of the first and second elements together. Any one of these options is understood to fall within the meaning, and therefore satisfy the requirement of the term "and/or" as used herein. Concurrent applicability of more than one of the options is also understood to fall within the meaning, and therefore satisfy the requirement of the term "and/or."

As used herein, the term "consists of," or variations such as "consist of" or "consisting of," as used throughout the specification and claims, indicate the inclusion of any recited integer or group of integers, but that no additional integer or group of integers can be added to the specified method, structure, or composition.

As used herein, the term "consists essentially of," or variations such as "consist essentially of" or "consisting essentially of," as used throughout the specification and claims, indicate the inclusion of any recited integer or group of integers, and the optional inclusion of any recited integer or group of integers that do not materially change the basic or novel properties of the specified method, structure or composition. See M.P.E.P. § 2111.03.

As used herein, "subject" means any animal, preferably a mammal, most preferably a human. The term "mammal" as used herein, encompasses any mammal. Examples of mammals include, but are not limited to, cows, horses, sheep, pigs, cats, dogs, mice, rats, rabbits, guinea pigs, monkeys, humans, etc., more preferably a human.

It should also be understood that the terms "about," "approximately," "generally," "substantially," and like terms, used herein when referring to a dimension or characteristic of a component of the preferred invention, indicate that the described dimension/characteristic is not a strict boundary or parameter and does not exclude minor variations therefrom that are functionally the same or similar, as would be understood by one having ordinary skill in the art. At a minimum, such references that include a numerical parameter would include variations that, using mathematical and industrial principles accepted in the art (e.g., rounding, measurement or other systematic errors, manufacturing tolerances, etc.), would not vary the least significant digit.

The terms "identical" or percent "identity," in the context of two or more nucleic acids or polypeptide sequences (*e.g.*, CAR polypeptides and the CAR polynucleotides that encode them), refer to two or more sequences or subsequences that are the same or have a specified percentage of amino acid residues or nucleotides that are the same, when compared and aligned for maximum correspondence, as measured using one of the following sequence comparison algorithms or by visual inspection.

For sequence comparison, typically one sequence acts as a reference sequence, to which test sequences are compared. When using a sequence comparison algorithm, test and reference sequences are input into a computer, subsequence coordinates are designated, if necessary, and sequence algorithm program parameters are designated. The sequence comparison algorithm then calculates the percent sequence identity for the

test sequence(s) relative to the reference sequence, based on the designated program parameters.

Optimal alignment of sequences for comparison can be conducted, *e.g.*, by the local homology algorithm of Smith & Waterman, *Adv. Appl. Math.* 2:482 (1981), by the homology alignment algorithm of Needleman & Wunsch, *J. Mol. Biol.* 48:443 (1970), by the search for similarity method of Pearson & Lipman, *Proc. Nat'l. Acad. Sci. USA* 85:2444 (1988), by computerized implementations of these algorithms (GAP, BESTFIT, FASTA, and TFASTA in the Wisconsin Genetics Software Package, Genetics Computer Group, 575 Science Dr., Madison, WI), or by visual inspection (*see generally*, *Current Protocols in Molecular Biology*, F.M. Ausubel *et al.*, eds., Current Protocols, a joint venture between Greene Publishing Associates, Inc. and John Wiley & Sons, Inc., (1995 Supplement) (Ausubel)).

Examples of algorithms that are suitable for determining percent sequence identity and sequence similarity are the BLAST and BLAST 2.0 algorithms, which are described in Altschul *et al.* (1990) *J. Mol. Biol.* 215: 403-410 and Altschul *et al.* (1997) *Nucleic Acids Res.* 25: 3389-3402, respectively. Software for performing BLAST analyses is publicly available through the National Center for Biotechnology Information. This algorithm involves first identifying high scoring sequence pairs (HSPs) by identifying short words of length W in the query sequence, which either match or satisfy some positive-valued threshold score T when aligned with a word of the same length in a database sequence. T is referred to as the neighborhood word score threshold (Altschul *et al.*, *supra*). These initial neighborhood word hits act as seeds for initiating searches to find longer HSPs containing them. The word hits are then extended in both directions along each sequence for as far as the cumulative alignment score can be increased.

Cumulative scores are calculated using, for nucleotide sequences, the parameters M (reward score for a pair of matching residues; always > 0) and N (penalty score for mismatching residues; always < 0). For amino acid sequences, a scoring matrix is used to calculate the cumulative score. Extension of the word hits in each direction are halted when: the cumulative alignment score falls off by the quantity X from its maximum achieved value; the cumulative score goes to zero or below, due to the accumulation of

one or more negative-scoring residue alignments; or the end of either sequence is reached. The BLAST algorithm parameters W, T, and X determine the sensitivity and speed of the alignment. The BLASTN program (for nucleotide sequences) uses as defaults a wordlength (W) of 11, an expectation (E) of 10, M=5, N= -4, and a comparison of both strands. For amino acid sequences, the BLASTP program uses as defaults a wordlength (W) of 3, an expectation (E) of 10, and the BLOSUM62 scoring matrix (*see* Henikoff & Henikoff, *Proc. Natl. Acad. Sci. USA* 89:10915 (1989)).

In addition to calculating percent sequence identity, the BLAST algorithm also performs a statistical analysis of the similarity between two sequences (*see, e.g.*, Karlin & Altschul, *Proc. Nat'l. Acad. Sci. USA* 90:5873-5787 (1993)). One measure of similarity provided by the BLAST algorithm is the smallest sum probability (P(N)), which provides an indication of the probability by which a match between two nucleotide or amino acid sequences would occur by chance. For example, a nucleic acid is considered similar to a reference sequence if the smallest sum probability in a comparison of the test nucleic acid to the reference nucleic acid is less than about 0.1, more preferably less than about 0.01, and most preferably less than about 0.001.

A further indication that two nucleic acid sequences or polypeptides are substantially identical is that the polypeptide encoded by the first nucleic acid is immunologically cross reactive with the polypeptide encoded by the second nucleic acid, as described below. Thus, a polypeptide is typically substantially identical to a second polypeptide, for example, where the two peptides differ only by conservative substitutions. Another indication that two nucleic acid sequences are substantially identical is that the two molecules hybridize to each other under stringent conditions.

As used herein, the term "isolated" means a biological component (such as a nucleic acid, peptide, protein, or cell) has been substantially separated, produced apart from, or purified away from other biological components of the organism in which the component naturally occurs, e.g., other chromosomal and extrachromosomal DNA and RNA, proteins, cells, and tissues. Nucleic acids, peptides, proteins, and cells that have been "isolated" thus include nucleic acids, peptides, proteins, and cells purified by standard purification methods and purification methods described herein. "Isolated" nucleic acids, peptides, proteins, and cells can be part of a composition and still be

isolated if the composition is not part of the native environment of the nucleic acid, peptide, protein, or cell. The term also embraces nucleic acids, peptides and proteins prepared by recombinant expression in a host cell as well as chemically synthesized nucleic acids.

As used herein, the term "polynucleotide," synonymously referred to as "nucleic acid molecule," "nucleotides" or "nucleic acids," refers to any polyribonucleotide or polydeoxyribonucleotide, which can be unmodified RNA or DNA or modified RNA or DNA. "Polynucleotides" include, without limitation single- and double-stranded DNA, DNA that is a mixture of single- and double-stranded regions, single- and double-stranded RNA, and RNA that is mixture of single- and double-stranded regions, hybrid molecules comprising DNA and RNA that can be single-stranded or, more typically, double-stranded or a mixture of single- and double-stranded regions. In addition, "polynucleotide" refers to triple-stranded regions comprising RNA or DNA or both RNA and DNA. The term polynucleotide also includes DNAs or RNAs containing one or more modified bases and DNAs or RNAs with backbones modified for stability or for other reasons. "Modified" bases include, for example, tritylated bases and unusual bases such as inosine. A variety of modifications can be made to DNA and RNA; thus, "polynucleotide" embraces chemically, enzymatically or metabolically modified forms of polynucleotides as typically found in nature, as well as the chemical forms of DNA and RNA characteristic of viruses and cells. "Polynucleotide" also embraces relatively short nucleic acid chains, often referred to as oligonucleotides.

A "construct" refers to a macromolecule or complex of molecules comprising a polynucleotide to be delivered to a host cell, either in vitro or in vivo. A "vector," as used herein refers to any nucleic acid construct capable of directing the delivery or transfer of a foreign genetic material to target cells, where it can be replicated and/or expressed. The term "vector" as used herein comprises the construct to be delivered. A vector can be a linear or a circular molecule. A vector can be integrating or non-integrating. The major types of vectors include, but are not limited to, plasmids, episomal vector, viral vectors, cosmids, and artificial chromosomes. Viral vectors include, but are not limited to, adenovirus vector, adeno-associated virus vector, retrovirus vector, lentivirus vector, Sendai virus vector, and the like.

By "integration" it is meant that one or more nucleotides of a construct is stably inserted into the cellular genome, e.g., covalently linked to the nucleic acid sequence within the cell's chromosomal DNA. By "targeted integration" it is meant that the nucleotide(s) of a construct is inserted into the cell's chromosomal or mitochondrial DNA at a pre-selected site or "integration site". The term "integration" as used herein further refers to a process involving insertion of one or more exogenous sequences or nucleotides of the construct, with or without deletion of an endogenous sequence or nucleotide at the integration site. In the case, where there is a deletion at the insertion site, "integration" can further comprise replacement of the endogenous sequence or a nucleotide that is deleted with the one or more inserted nucleotides.

As used herein, the term "exogenous" is intended to mean that the referenced molecule or the referenced activity is introduced into, or non-native to, the host cell. The molecule can be introduced, for example, by introduction of an encoding nucleic acid into the host genetic material such as by integration into a host chromosome or as non-chromosomal genetic material such as a plasmid. Therefore, the term as it is used in reference to expression of an encoding nucleic acid refers to introduction of the encoding nucleic acid in an expressible form into the cell. The term "endogenous" refers to a referenced molecule or activity that is present in the host cell in its native form. Similarly, the term when used in reference to expression of an encoding nucleic acid refers to expression of an encoding nucleic acid natively contained within the cell and not exogenously introduced.

As used herein, a "gene of interest" or "a polynucleotide sequence of interest" is a DNA sequence that is transcribed into RNA and in some instances translated into a polypeptide in vivo when placed under the control of appropriate regulatory sequences. A gene or polynucleotide of interest can include, but is not limited to, prokaryotic sequences, cDNA from eukaryotic mRNA, genomic DNA sequences from eukaryotic (e.g., mammalian) DNA, and synthetic DNA sequences. For example, a gene of interest may encode an miRNA, an shRNA, a native polypeptide (e.g., a polypeptide found in nature) or fragment thereof; a variant polypeptide (e.g., a mutant of the native polypeptide having less than 100% sequence identity with the native polypeptide) or

fragment thereof; an engineered polypeptide or peptide fragment, a therapeutic peptide or polypeptide, an imaging marker, a selectable marker, and the like.

"Operably-linked" refers to the association of nucleic acid sequences on a single nucleic acid fragment so that the function of one is affected by the other. For example, a promoter is operably-linked with a coding sequence or functional RNA when it is capable of affecting the expression of that coding sequence or functional RNA (e.g., the coding sequence or functional RNA is under the transcriptional control of the promoter). Coding sequences can be operably-linked to regulatory sequences in sense or antisense orientation.

The term "expression" as used herein, refers to the biosynthesis of a gene product. The term encompasses the transcription of a gene into RNA. The term also encompasses translation of RNA into one or more polypeptides, and further encompasses all naturally occurring post-transcriptional and post-translational modifications. The expressed CAR can be within the cytoplasm of a host cell, into the extracellular milieu such as the growth medium of a cell culture or anchored to the cell membrane.

As used herein, the terms "peptide," "polypeptide," or "protein" can refer to a molecule comprised of amino acids and can be recognized as a protein by those of skill in the art. The conventional one-letter or three-letter code for amino acid residues is used herein. The terms "peptide," "polypeptide," and "protein" can be used interchangeably herein to refer to polymers of amino acids of any length. The polymer can be linear or branched, it can comprise modified amino acids, and it can be interrupted by non-amino acids. The terms also encompass an amino acid polymer that has been modified naturally or by intervention; for example, disulfide bond formation, glycosylation, lipidation, acetylation, phosphorylation, or any other manipulation or modification, such as conjugation with a labeling component. Also included within the definition are, for example, polypeptides containing one or more analogs of an amino acid (including, for example, unnatural amino acids, etc.), as well as other modifications known in the art.

The peptide sequences described herein are written according to the usual convention whereby the N-terminal region of the peptide is on the left and the C-terminal region is on the right. Although isomeric forms of the amino acids are known, it is the L-form of the amino acid that is represented unless otherwise expressly indicated.

As used herein, the term “engineered immune cell” refers to an immune cell, also referred to as an immune effector cell, that has been genetically modified by the addition of exogenous genetic material in the form of DNA or RNA to the total genetic material of the cell.

Induced Pluripotent Stem Cells (IPSCs) And Immune Effector Cells

IPSCs have unlimited self-renewing capacity. Use of iPSCs enables cellular engineering to produce a controlled cell bank of modified cells that can be expanded and differentiated into desired immune effector cells, supplying large amounts of homogeneous allogeneic therapeutic products.

Provided herein are genetically engineered IPSCs and derivative cells thereof. The selected genomic modifications provided herein enhance the therapeutic properties of the derivative cells. The derivative cells are functionally improved and suitable for allogenic off-the-shelf cell therapies following a combination of selective modalities being introduced to the cells at the level of iPSC through genomic engineering. This approach can help to reduce the side effects mediated by CRS/GVHD and prevent long-term autoimmunity while providing excellent efficacy.

As used herein, the term "differentiation" is the process by which an unspecialized ("uncommitted") or less specialized cell acquires the features of a specialized cell. Specialized cells include, for example, a blood cell or a muscle cell. A differentiated or differentiation- induced cell is one that has taken on a more specialized ("committed") position within the lineage of a cell. The term "committed", when applied to the process of differentiation, refers to a cell that has proceeded in the differentiation pathway to a point where, under normal circumstances, it will continue to differentiate into a specific cell type or subset of cell types, and cannot, under normal circumstances, differentiate into a different cell type or revert to a less differentiated cell type. As used herein, the term "pluripotent" refers to the ability of a cell to form all lineages of the body or soma or the embryo proper. For example, embryonic stem cells are a type of pluripotent stem cells that are able to form cells from each of the three germs layers, the ectoderm, the mesoderm, and the endoderm. Pluripotency is a continuum of developmental potencies ranging from the incompletely or partially pluripotent cell (e.g.,

an epiblast stem cell or EpiSC), which is unable to give rise to a complete organism to the more primitive, more pluripotent cell, which is able to give rise to a complete organism (e.g., an embryonic stem cell).

As used herein, the terms "reprogramming" or "dedifferentiation" refers to a method of increasing the potency of a cell or dedifferentiating the cell to a less differentiated state. For example, a cell that has an increased cell potency has more developmental plasticity (e.g., can differentiate into more cell types) compared to the same cell in the non-reprogrammed state. In other words, a reprogrammed cell is one that is in a less differentiated state than the same cell in a non-reprogrammed state.

As used herein, the term "induced pluripotent stem cells" or, iPSCs, means that the stem cells are produced from differentiated adult, neonatal or fetal cells that have been induced or changed or reprogrammed into cells capable of differentiating into tissues of all three germ or dermal layers: mesoderm, endoderm, and ectoderm. The iPSCs produced do not refer to cells as they are found in nature.

The term “hematopoietic stem and progenitor cells,” “hematopoietic stem cells,” “hematopoietic progenitor cells,” or “hematopoietic precursor cells” or “HPCs” refers to cells which are committed to a hematopoietic lineage but are capable of further hematopoietic differentiation. Hematopoietic stem cells include, for example, multipotent hematopoietic stem cells (hematoblasts), myeloid progenitors, megakaryocyte progenitors, erythrocyte progenitors, and lymphoid progenitors. Hematopoietic stem and progenitor cells (HSCs) are multipotent stem cells that give rise to all the blood cell types including myeloid (monocytes and macrophages, neutrophils, basophils, eosinophils, erythrocytes, megakaryocytes/platelets, dendritic cells), and lymphoid lineages (T cells, B cells, NK cells). As used herein, “CD34+ hematopoietic progenitor cell” refers to an HPC that expresses CD34 on its surface.

As used herein, the term “immune cell” or “immune effector cell” refers to a cell that is involved in an immune response. Immune response includes, for example, the promotion of an immune effector response. Examples of immune cells include T cells, B cells, natural killer (NK) cells, mast cells, and myeloid-derived phagocytes.

As used herein, the terms “T lymphocyte” and “T cell” are used interchangeably and refer to a type of white blood cell that completes maturation in the thymus and that

has various roles in the immune system. A T cell can have the roles including, e.g., the identification of specific foreign antigens in the body and the activation and deactivation of other immune cells. A T cell can be any T cell, such as a cultured T cell, e.g., a primary T cell, or a T cell from a cultured T cell line, e.g., Jurkat, SupTl, etc., or a T cell obtained from a mammal. The T cell can be CD3+ cells. The T cell can be any type of T cell and can be of any developmental stage, including but not limited to, CD4+/CD8+ double positive T cells, CD4+ helper T cells (e.g., Thl and Th2 cells), CD8+ T cells (e.g., cytotoxic T cells), peripheral blood mononuclear cells (PBMCs), peripheral blood leukocytes (PBLs), tumor infiltrating lymphocytes (TILs), memory T cells, naive T cells, regulator T cells, gamma delta T cells (gd T cells), and the like. Additional types of helper T cells include cells such as Th3 (Treg), Thl7, Th9, or Tfh cells. Additional types of memory T cells include cells such as central memory T cells (Tcm cells), effector memory T cells (Tern cells and TEMRA cells). The T cell can also refer to a genetically engineered T cell, such as a T cell modified to express a T cell receptor (TCR) or a chimeric antigen receptor (CAR). The T cell can also be differentiated from a stem cell or progenitor cell.

"CD4+ T cells" refers to a subset of T cells that express CD4 on their surface and are associated with cell-mediated immune response. They are characterized by the secretion profiles following stimulation, which may include secretion of cytokines such as IFN-gamma, TNF-alpha, IL2, IL4 and IL10. "CD4" are 55-kD glycoproteins originally defined as differentiation antigens on T-lymphocytes, but also found on other cells including monocytes/macrophages. CD4 antigens are members of the immunoglobulin supergene family and are implicated as associative recognition elements in MHC (major histocompatibility complex) class II-restricted immune responses. On T-lymphocytes they define the helper/inducer subset.

"CD8+ T cells" refers to a subset of T cells which express CD8 on their surface, are MHC class I-restricted, and function as cytotoxic T cells. "CD8" molecules are differentiation antigens found on thymocytes and on cytotoxic and suppressor T-lymphocytes. CD8 antigens are members of the immunoglobulin supergene family and are associative recognition elements in major histocompatibility complex class I-restricted interactions.

As used herein, the term "NK cell" or "Natural Killer cell" refers to a subset of peripheral blood lymphocytes defined by the expression of CD56 and CD45 and the absence of the T cell receptor (TCR chains). The NK cell can also refer to a genetically engineered NK cell, such as a NK cell modified to express a chimeric antigen receptor (CAR). The NK cell can also be differentiated from a stem cell or progenitor cell.

As used herein, the term "genetic imprint" refers to genetic or epigenetic information that contributes to preferential therapeutic attributes in a source cell or an iPSC, and is retainable in the source cell derived iPSCs, and/or the iPSC-derived hematopoietic lineage cells. As used herein, "a source cell" is a non-pluripotent cell that may be used for generating iPSCs through reprogramming, and the source cell derived iPSCs may be further differentiated to specific cell types including any hematopoietic lineage cells. The source cell derived iPSCs, and differentiated cells therefrom are sometimes collectively called "derived" or "derivative" cells depending on the context. For example, derivative effector cells, or derivative NK or "iNK" cells or derivative T or "iT" cells, as used throughout this application are cells differentiated from an iPSC, as compared to their primary counterpart obtained from natural/native sources such as peripheral blood, umbilical cord blood, or other donor tissues. As used herein, the genetic imprint(s) conferring a preferential therapeutic attribute is incorporated into the iPSCs either through reprogramming a selected source cell that is donor-, disease-, or treatment response- specific, or through introducing genetically modified modalities to iPSC using genomic editing.

The induced pluripotent stem cell (iPSC) parental cell lines may be generated from peripheral blood mononuclear cells (PBMCs) or T-cells using any known method for introducing re-programming factors into non-pluripotent cells such as the episomal plasmid-based process as previously described in U.S. Pat. Nos. 8,546,140; 9,644,184; 9,328,332; and 8,765,470, the complete disclosures of which are incorporated herein by reference. The reprogramming factors may be in a form of polynucleotides, and thus are introduced to the non-pluripotent cells by vectors such as a retrovirus, a Sendai virus, an adenovirus, an episome, and a mini-circle. In particular embodiments, the one or more polynucleotides encoding at least one reprogramming factor are introduced by a lentiviral vector. In some embodiments, the one or more polynucleotides introduced by an

episomal vector. In various other embodiments, the one or more polynucleotides are introduced by a Sendai viral vector. In some embodiments, the iPSC's are clonal iPSC's or are obtained from a pool of iPSCs and the genome edits are introduced by making one or more targeted integration and/or in/del at one or more selected sites. In another embodiment, the iPSC's are obtained from human T cells having antigen specificity and a reconstituted TCR gene (hereinafter, also refer to as "T-iPS" cells) as described in US Pat. Nos. 9206394, and 10787642 hereby incorporated by reference into the present application.

According to a particular aspect, the application relates to an induced pluripotent stem cell (iPSC) cell or a derivative cell thereof comprising: (i) an exogenous polynucleotide encoding a chimeric antigen receptor (CAR); (ii) a deletion or reduced expression of B2M and CIITA genes; and optionally (iii) an exogenous polynucleotide encoding a chimeric IL-15RA and an interleukin 15 (IL-15), wherein the IL-15RA and IL-15 are operably linked.

I. Chimeric Antigen Receptor (CAR) Expression

According to embodiments of the application, an iPSC cell or a derivative cell thereof comprises one or more first exogenous polynucleotides encoding a first and a second chimeric antigen receptor (CAR), such as a CAR targeting one or more tumor antigens. In one embodiment, the CAR targets a CD19 antigen, and the second CAR targets a CD22 antigen. In another embodiment, the CAR targets a CD19 antigen, and the second CAR targets a CD22 antigen, and the targeting regions (e.g., the extracellular domains) of one or both of the CARs comprises an antibody fragment (e.g, a VHH domain). In other embodiments, the CAR can be a bispecific CAR targeting two or more antigens (e.g., a CD19/CD22 CAR). In one example, a therapeutic cell of the present disclosure can express a bispecific CAR targeting CD19 and CD22. The targeting domains of the CAR (e.g., the antigen binding regions) can comprise VHH and/or scFv domains. In some embodiments, the CAR can be a bispecific CAR in a loop configuration.

As used herein, the term "chimeric antigen receptor" (CAR) refers to a recombinant polypeptide comprising at least an extracellular domain that binds

specifically to an antigen or a target, a transmembrane domain and an intracellular signaling domain. Engagement of the extracellular domain of the CAR with the target antigen on the surface of a target cell results in clustering of the CAR and delivers an activation stimulus to the CAR-containing cell. CARs redirect the specificity of immune effector cells and trigger proliferation, cytokine production, phagocytosis and/or production of molecules that can mediate cell death of the target antigen-expressing cell in a major histocompatibility (MHC)-independent manner.

As used herein, the term "signal peptide" refers to a leader sequence at the amino-terminus (N-terminus) of a nascent CAR protein, which co-translationally or post-translationally directs the nascent protein to the endoplasmic reticulum and subsequent surface expression.

As used herein, the term "extracellular antigen-binding domain," "extracellular domain," or "extracellular ligand binding domain" refers to the part of a CAR that is located outside of the cell membrane and is capable of binding to an antigen, target or ligand.

As used herein, the term "hinge region" or "hinge domain" refers to the part of a CAR that connects two adjacent domains of the CAR protein, e.g., the extracellular domain and the transmembrane domain of the CAR protein.

As used herein, the term "transmembrane domain" refers to the portion of a CAR that extends across the cell membrane and anchors the CAR to cell membrane.

As used herein, the term "intracellular signaling domain," "cytoplasmic signaling domain," or "intracellular signaling domain" refers to the part of a CAR that is located inside of the cell membrane and is capable of transducing an effector signal.

As used herein, the term "stimulatory molecule" refers to a molecule expressed by an immune cell (e.g., NK cell or T cell) that provides the primary cytoplasmic signaling sequence(s) that regulate primary activation of receptors in a stimulatory way for at least some aspect of the immune cell signaling pathway. Stimulatory molecules comprise two distinct classes of cytoplasmic signaling sequence, those that initiate antigen-dependent primary activation (referred to as "primary signaling domains"), and those that act in an antigen-independent manner to provide a secondary of co-stimulatory signal (referred to as "co-stimulatory signaling domains").

In certain embodiments, the extracellular domain comprises an antigen-binding domain and/or an antigen-binding fragment. The antigen-binding fragment can, for example, be an antibody or antigen-binding fragment thereof that specifically binds a tumor antigen. The antigen-binding fragments of the application possess one or more desirable functional properties, including but not limited to high-affinity binding to a tumor antigen, high specificity to a tumor antigen, the ability to stimulate complement-dependent cytotoxicity (CDC), antibody-dependent phagocytosis (ADPC), and/or antibody-dependent cellular-mediated cytotoxicity (ADCC) against cells expressing a tumor antigen, and the ability to inhibit tumor growth in subjects in need thereof and in animal models when administered alone or in combination with other anti-cancer therapies.

As used herein, the term "antibody" is used in a broad sense and includes immunoglobulin or antibody molecules including human, humanized, composite and chimeric antibodies and antibody fragments that are monoclonal or polyclonal. In general, antibodies are proteins or peptide chains that exhibit binding specificity to a specific antigen. Antibody structures are well known. Immunoglobulins can be assigned to five major classes (e.g., IgA, IgD, IgE, IgG and IgM), depending on the heavy chain constant domain amino acid sequence. IgA and IgG are further sub-classified as the isotypes IgA1, IgA2, IgG1, IgG2, IgG3 and IgG4. Accordingly, the antibodies of the application can be of any of the five major classes or corresponding sub-classes. Preferably, the antibodies of the application are IgG1, IgG2, IgG3 or IgG4. Antibody light chains of vertebrate species can be assigned to one of two clearly distinct types, namely kappa and lambda, based on the amino acid sequences of their constant domains. Accordingly, the antibodies of the application can contain a kappa or lambda light chain constant domain. According to particular embodiments, the antibodies of the application include heavy and/or light chain constant regions from rat or human antibodies. In addition to the heavy and light constant domains, antibodies contain an antigen-binding region that is made up of a light chain variable region and a heavy chain variable region, each of which contains three domains (e.g., complementarity determining regions 1-3; CDR1, CDR2, and CDR3). The light chain variable region domains are alternatively

referred to as LCDR1, LCDR2, and LCDR3, and the heavy chain variable region domains are alternatively referred to as HCDR1, HCDR2, and HCDR3.

As used herein, the term an "isolated antibody" refers to an antibody which is substantially free of other antibodies having different antigenic specificities (e.g., an isolated antibody that specifically binds to the specific tumor antigen is substantially free of antibodies that do not bind to the tumor antigen). In addition, an isolated antibody is substantially free of other cellular material and/or chemicals.

As used herein, the term "monoclonal antibody" refers to an antibody obtained from a population of substantially homogeneous antibodies, e.g., the individual antibodies comprising the population are identical except for possible naturally occurring mutations that can be present in minor amounts. The monoclonal antibodies of the application can be made by the hybridoma method, phage display technology, single lymphocyte gene cloning technology, or by recombinant DNA methods. For example, the monoclonal antibodies can be produced by a hybridoma which includes a B cell obtained from a transgenic nonhuman animal, such as a transgenic mouse or rat, having a genome comprising a human heavy chain transgene and a light chain transgene.

As used herein, the term "antigen-binding fragment" refers to an antibody fragment such as, for example, a diabody, a Fab, a Fab', a F(ab')2, an Fv fragment, a disulfide stabilized Fv fragment (dsFv), a $(dsFv)_2$, a bispecific dsFv (dsFv-dsFv'), a disulfide stabilized diabody (ds diabody), a single-chain antibody molecule (scFv), a single domain antibody (sdAb), a scFv dimer (bivalent diabody), a multispecific antibody formed from a portion of an antibody comprising one or more CDRs, a camelized single domain antibody, a minibody, a nanobody, a domain antibody, a bivalent domain antibody, a light chain variable domain (VL), a variable domain (V_HH) of a camelid antibody, or any other antibody fragment that binds to an antigen but does not comprise a complete antibody structure. An antigen-binding fragment is capable of binding to the same antigen to which the parent antibody or a parent antibody fragment binds.

As used herein, the term "single-chain antibody" refers to a conventional single-chain antibody in the field, which comprises a heavy chain variable region and a light chain variable region connected by a short peptide of about 15 to about 20 amino acids (e.g., a linker peptide).

As used herein, the term "single domain antibody" refers to a conventional single domain antibody in the field, which comprises a heavy chain variable region and a heavy chain constant region or which comprises only a heavy chain variable region.

As used herein, the term "human antibody" refers to an antibody produced by a human or an antibody having an amino acid sequence corresponding to an antibody produced by a human made using any technique known in the art. This definition of a human antibody includes intact or full-length antibodies, fragments thereof, and/or antibodies comprising at least one human heavy and/or light chain polypeptide.

As used herein, the term "humanized antibody" refers to a non-human antibody that is modified to increase the sequence homology to that of a human antibody, such that the antigen-binding properties of the antibody are retained, but its antigenicity in the human body is reduced.

As used herein, the term "chimeric antibody" refers to an antibody wherein the amino acid sequence of the immunoglobulin molecule is derived from two or more species. The variable region of both the light and heavy chains often corresponds to the variable region of an antibody derived from one species of mammal (e.g., mouse, rat, rabbit, etc.) having the desired specificity, affinity, and capability, while the constant regions correspond to the sequences of an antibody derived from another species of mammal (e.g., human) to avoid eliciting an immune response in that species.

As used herein, the term "multispecific antibody" refers to an antibody that comprises a plurality of immunoglobulin variable domain sequences, wherein a first immunoglobulin variable domain sequence of the plurality has binding specificity for a first epitope and a second immunoglobulin variable domain sequence of the plurality has binding specificity for a second epitope. In an embodiment, the first and second epitopes are on the same antigen, *e.g.*, the same protein (or subunit of a multimeric protein). In an embodiment, the first and second epitopes overlap or substantially overlap. In an embodiment, the first and second epitopes do not overlap or do not substantially overlap. In an embodiment, the first and second epitopes are on different antigens, *e.g.*, the different proteins (or different subunits of a multimeric protein). In an embodiment, a multispecific antibody comprises a third, fourth, or fifth immunoglobulin variable

domain. In an embodiment, a multispecific antibody is a bispecific antibody molecule, a trispecific antibody molecule, or a tetraspecific antibody molecule.

As used herein, the term "bispecific antibody" refers to a multispecific antibody that binds no more than two epitopes or two antigens. A bispecific antibody is characterized by a first immunoglobulin variable domain sequence which has binding specificity for a first epitope and a second immunoglobulin variable domain sequence that has binding specificity for a second epitope. In an embodiment, the first and second epitopes are on the same antigen, *e.g.*, the same protein (or subunit of a multimeric protein). In an embodiment, the first and second epitopes overlap or substantially overlap. In an embodiment, the first and second epitopes are on different antigens, *e.g.*, the different proteins (or different subunits of a multimeric protein). In an embodiment, a bispecific antibody comprises a heavy chain variable domain sequence and a light chain variable domain sequence which have binding specificity for a first epitope and a heavy chain variable domain sequence and a light chain variable domain sequence which have binding specificity for a second epitope. In an embodiment, a bispecific antibody comprises a half antibody, or fragment thereof, having binding specificity for a first epitope and a half antibody, or fragment thereof, having binding specificity for a second epitope. In an embodiment, a bispecific antibody comprises a scFv, or fragment thereof, having binding specificity for a first epitope, and a scFv, or fragment thereof, having binding specificity for a second epitope. In an embodiment, a bispecific antibody comprises a V_HH having binding specificity for a first epitope, and a V_HH having binding specificity for a second epitope. In an embodiment, the term X/Y loop (wherein 'X' and 'Y' are antigens such as CD19 and CD22) refers to an extracellular region in which one scFv (either CD19 or CD22) is nested in between the VL and VH of the other scFv. In some embodiments, X and Y may be the same antigen. In some embodiments, X and Y may be different antigens. In some embodiments, X and Y are tumor antigens.

As used herein, an antigen-binding domain or antigen-binding fragment that "specifically binds to a tumor antigen" refers to an antigen-binding domain or antigen-binding fragment that binds a tumor antigen, with a KD of 1×10^{-7} M or less, preferably 1×10^{-8} M or less, more preferably 5×10^{-9} M or less, 1×10^{-9} M or less, 5×10^{-10} M or less, or 1×10^{-10} M or less. The term "KD" refers to the dissociation constant, which is

obtained from the ratio of Kd to Ka (e.g., Kd/Ka) and is expressed as a molar concentration (M). KD values for antibodies can be determined using methods in the art in view of the present disclosure. For example, the KD of an antigen-binding domain or antigen-binding fragment can be determined by using surface plasmon resonance, such as by using a biosensor system, e.g., a Biacore® system, or by using bio-layer interferometry technology, such as an Octet RED96 system.

The smaller the value of the KD of an antigen-binding domain or antigen-binding fragment, the higher affinity that the antigen-binding domain or antigen-binding fragment binds to a target antigen.

In various embodiments, antibodies or antibody fragments suitable for use in the CAR of the present disclosure include, but are not limited to, monoclonal antibodies, bispecific antibodies, multispecific antibodies, chimeric antibodies, polypeptide-Fc fusions, single-chain Fvs (scFv), single chain antibodies, Fab fragments, F(ab′) fragments, disulfide-linked Fvs (sdFv), masked antibodies (e.g., Probodies®), Small Modular ImmunoPharmaceuticals ("SMIPsTM"), intrabodies, minibodies, single domain antibody variable domains, nanobodies, VHHs, diabodies, tandem diabodies (TandAb®), anti-idiotypic (anti-Id) antibodies (including, e.g., anti-Id antibodies to antigen-specific TCR), and epitope-binding fragments of any of the above. Antibodies and/or antibody fragments may be derived from murine antibodies, rabbit antibodies, human antibodies, fully humanized antibodies, camelid antibody variable domains and humanized versions, shark antibody variable domains and humanized versions, and camelized antibody variable domains.

In some embodiments, the antigen-binding fragment is an Fab fragment, an Fab' fragment, an F(ab')2 fragment, an scFv fragment, an Fv fragment, a dsFv diabody, a VHH, a VNAR, a single-domain antibody (sdAb) or nanobody, a dAb fragment, a Fd' fragment, a Fd fragment, a heavy chain variable region, an isolated complementarity determining region (CDR), a diabody, a triabody, or a decabody. In some embodiments, the antigen-binding fragment is an scFv fragment. In some embodiments, the antigen-binding fragment is a VHH.

In some embodiments, at least one of the extracellular tag-binding domain, the antigen-binding domain, or the tag comprises a single-domain antibody or nanobody.

In some embodiments, at least one of the extracellular tag-binding domain, the antigen-binding domain, or the tag comprises a VHH.

In some embodiments, the extracellular tag-binding domain and the tag each comprise a VHH.

In some embodiments, the extracellular tag-binding domain, the tag, and the antigen-binding domain each comprise a VHH.

In some embodiments, at least one of the extracellular tag-binding domain, the antigen-binding domain, or the tag comprises an scFv.

In some embodiments, the extracellular tag-binding domain and the tag each comprise an scFv.

In some embodiments, the extracellular tag-binding domain, the tag, and the antigen-binding domain each comprise a scFv.

Alternative scaffolds to immunoglobulin domains that exhibit similar functional characteristics, such as high-affinity and specific binding of target biomolecules, may also be used in the CARs of the present disclosure. Such scaffolds have been shown to yield molecules with improved characteristics, such as greater stability or reduced immunogenicity. Non-limiting examples of alternative scaffolds that may be used in the CAR of the present disclosure include engineered, tenascin-derived, tenascin type III domain (e.g., Centyrin™); engineered, gamma-B crystallin-derived scaffold or engineered, ubiquitin-derived scaffold (e.g., Affilins); engineered, fibronectin-derived, 10th fibronectin type III (10Fn3) domain (e.g., monobodies, AdNectins™, or AdNexins™);; engineered, ankyrin repeat motif containing polypeptide (e.g., DARPins™); engineered, low-density-lipoprotein-receptor-derived, A domain (LDLR-A) (e.g., Avimers™); lipocalin (e.g., anticalins); engineered, protease inhibitor-derived, Kunitz domain (e.g., EETI-II/AGRP, BPTI/LACI-D1/ITI-D2); engineered, Protein-A-derived, Z domain (Affibodies™); Sac7d-derived polypeptides (e.g., Nanoffitins® or affitins); engineered, Fyn-derived, SH2 domain (e.g., Fynomers®); $CTLD_3$ (e.g., Tetranectin); thioredoxin (e.g., peptide aptamer); KALBITOR®; the β-sandwich (e.g., iMab); miniproteins; C-type lectin-like domain scaffolds; engineered antibody mimics; and any genetically manipulated counterparts of the foregoing that retains its binding functionality (Wörn A, Pluckthun A, J Mol Biol 305: 989-1010 (2001); Xu L et al., Chem

Biol 9: 933-42 (2002); Wikman M et al., Protein Eng Des Sel 17: 455-62 (2004); Binz H et al., Nat Biolechnol 23: 1257-68 (2005); Hey T et al., Trends Biotechnol 23:514-522 (2005); Holliger P, Hudson P, Nat Biotechnol 23: 1126-36 (2005); Gill D, Damle N, Curr Opin Biotech 17: 653-8 (2006); Koide A, Koide S, Methods Mol Biol 352: 95-109 (2007); Skerra, Current Opin. in Biotech., 2007 18: 295-304; Byla P et al., J Biol Chem 285: 12096 (2010); Zoller F et al., Molecules 16: 2467-85 (2011), each of which is incorporated by reference in its entirety).

In some embodiments, the alternative scaffold is Affilin or Centyrin.

In some embodiments, the first polypeptide of the CARs of the present disclosure comprises a leader sequence. The leader sequence may be positioned at the N-terminus the extracellular tag-binding domain. The leader sequence may be optionally cleaved from the extracellular tag-binding domain during cellular processing and localization of the CAR to the cellular membrane. Any of various leader sequences known to one of skill in the art may be used as the leader sequence. Non-limiting examples of peptides from which the leader sequence may be derived include granulocyte-macrophage colony-stimulating factor receptor (GMCSFR), FcεR, human immunoglobulin (IgG) heavy chain (HC) variable region, CD8α, or any of various other proteins secreted by T cells. In various embodiments, the leader sequence is compatible with the secretory pathway of a T cell. In certain embodiments, the leader sequence is derived from human immunoglobulin heavy chain (HC).

In some embodiments, the leader sequence is derived from GMCSFR. In one embodiment, the GMCSFR leader sequence comprises the amino acid sequence set forth in SEQ ID NO: 1, or a variant thereof having at least 50, at least 55, at least 60, at least 65, at least 70, at least 75, at least 80, at least 85, at least 90, at least 95, at least 96, at least 97, at least 98 or at least 99%, sequence identity with SEQ ID NO: 1.

In some embodiments, the first polypeptide of the CARs of the present disclosure comprise a transmembrane domain, fused in frame between the extracellular tag-binding domain and the cytoplasmic domain.

The transmembrane domain may be derived from the protein contributing to the extracellular tag-binding domain, the protein contributing the signaling or co-signaling domain, or by a totally different protein. In some instances, the transmembrane domain

can be selected or modified by amino acid substitution, deletions, or insertions to minimize interactions with other members of the CAR complex. In some instances, the transmembrane domain can be selected or modified by amino acid substitution, deletions, or insertions to avoid binding of proteins naturally associated with the transmembrane domain. In certain embodiments, the transmembrane domain includes additional amino acids to allow for flexibility and/or optimal distance between the domains connected to the transmembrane domain.

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. Non-limiting examples of transmembrane domains of particular use in this disclosure may be derived from (e.g., comprise at least the transmembrane region(s) of) the α or β chain of the T-cell receptor (TCR), CD28, CD3 epsilon, CD45, CD4, CD5, CD8, CD8α, CD9, CD16, CD22, CD28, CD33, CD37, CD40, CD64, CD80, CD86, CD134, CD137, or CD154. Alternatively, the transmembrane domain may be synthetic, in which case it will comprise predominantly hydrophobic residues such as leucine and valine. For example, a triplet of phenylalanine, tryptophan and/or valine can be found at each end of a synthetic transmembrane domain.

In some embodiments, it will be desirable to utilize the transmembrane domain of the ζ, ɳ or FcεR1γ chains which contain a cysteine residue capable of disulfide bonding, so that the resulting chimeric protein will be able to form disulfide linked dimers with itself, or with unmodified versions of the ζ, ɳ or FcεR1γ chains or related proteins. In some instances, the transmembrane domain will 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. In other cases, it will be desirable to employ the transmembrane domain of ζ, ɳ or FcεR1γ and -β, MB1 (Igα.), B29 or CD3- γ, ζ, or η, in order to retain physical association with other members of the receptor complex.

In some embodiments, the transmembrane domain is derived from CD8 or CD28. In one embodiment, the CD8 transmembrane domain comprises the amino acid sequence set forth in SEQ ID NO: 23, or a variant thereof having at least 50, at least 55, at least 60, at least 65, at least 70, at least 75, at least 80, at least 85, at least 90, at least 95, at least

96, at least 97, at least 98 or at least 99%, sequence identity with SEQ ID NO: 23. In one embodiment, the CD28 transmembrane domain comprises the amino acid sequence set forth in SEQ ID NO: 24, or a variant thereof having at least 50, at least 55, at least 60, at least 65, at least 70, at least 75, at least 80, at least 85, at least 90, at least 95, at least 96, at least 97, at least 98 or at least 99%, sequence identity with SEQ ID NO: 24.

In some embodiments, the first polypeptide of the CAR of the present disclosure comprises a spacer region between the extracellular tag-binding domain and the transmembrane domain, wherein the tag-binding domain, linker, and the transmembrane domain are in frame with each other.

The term "spacer region" as used herein generally means any oligo- or polypeptide that functions to link the tag-binding domain to the transmembrane domain. A spacer region can be used to provide more flexibility and accessibility for the tag-binding domain. A spacer region may comprise up to 300 amino acids, preferably 10 to 100 amino acids and most preferably 25 to 50 amino acids. A spacer region may be derived from all or part of naturally occurring molecules, such as from all or part of the extracellular region of CD8, CD4 or CD28, or from all or part of an antibody constant region. Alternatively, the spacer region may be a synthetic sequence that corresponds to a naturally occurring spacer region sequence, or may be an entirely synthetic spacer region sequence. Non-limiting examples of spacer regions which may be used in accordance to the disclosure include a part of human CD8α chain, partial extracellular domain of CD28, FcγRIIIa receptor, IgG, IgM, IgA, IgD, IgE, an Ig hinge, or functional fragment thereof. In some embodiments, additional linking amino acids are added to the spacer region to ensure that the antigen-binding domain is an optimal distance from the transmembrane domain. In some embodiments, when the spacer is derived from an Ig, the spacer may be mutated to prevent Fc receptor binding.

In some embodiments, the spacer region comprises a hinge domain. The hinge domain may be derived from CD8, CD8α, CD28, or an immunoglobulin (IgG). For example, the IgG hinge may be from IgG1, IgG2, IgG3, IgG4, IgG4 CH3, IgM1, IgM2, IgA1, IgA2, IgD, IgE, or a chimera thereof.

In certain embodiments, the hinge domain comprises an immunoglobulin IgG hinge or functional fragment thereof. In certain embodiments, the IgG hinge is from

IgG1, IgG2, IgG3, IgG4, IgG4 CH3, IgM1, IgM2, IgA1, IgA2, IgD, IgE, or a chimera thereof. In certain embodiments, the hinge domain comprises the CH1, CH2, CH3 and/or hinge region of the immunoglobulin. In certain embodiments, the hinge domain comprises the core hinge region of the immunoglobulin. The term "core hinge" can be used interchangeably with the term "short hinge" (a.k.a "SH"). Non-limiting examples of suitable hinge domains are the core immunoglobulin hinge regions include EPKSCDKTHTCPPCP (SEQ ID NO: 57) from IgG1, ERKCCVECPPCP (SEQ ID NO: 58) from IgG2, ELKTPLGDTTHTCPRCP(EPKSCDTPPPCPRCP)$_3$ (SEQ ID NO: 59) from IgG3, ESKYGPPCPSCP (SEQ ID NO: 60) from IgG4 (*see also* Wypych et al., *JBC* 2008 283(23): 16194-16205, which is incorporated herein by reference in its entirety for all purposes), and ESKYGPPCPPCPGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWES NGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNHY TQKSLSLSLGK (SEQ ID NO: 102), or a variant thereof having at least 50, at least 55, at least 60, at least 65, at least 70, at least 75, at least 80, at least 85, at least 90, at least 95, at least 96, at least 97, at least 98 or at least 99%, sequence identity. In certain embodiments, the hinge domain is a fragment of the immunoglobulin hinge.

In some embodiments, the hinge domain is derived from CD8 or CD28. In one embodiment, the CD8 hinge domain comprises the amino acid sequence set forth in SEQ ID NO: 21, or a variant thereof having at least 50, at least 55, at least 60, at least 65, at least 70, at least 75, at least 80, at least 85, at least 90, at least 95, at least 96, at least 97, at least 98 or at least 99%, sequence identity with SEQ ID NO: 21. In one embodiment, the CD28 hinge domain comprises the amino acid sequence set forth in SEQ ID NO: 22, or a variant thereof having at least 50, at least 55, at least 60, at least 65, at least 70, at least 75, at least 80, at least 85, at least 90, at least 95, at least 96, at least 97, at least 98 or at least 99%, sequence identity with SEQ ID NO: 22.

In some embodiments, the transmembrane domain and/or hinge domain is derived from CD8 or CD28. In some embodiments, both the transmembrane domain and hinge domain are derived from CD8. In some embodiments, both the transmembrane domain and hinge domain are derived from CD28.

In certain aspects, the first polypeptide of CARs of the present disclosure comprise a cytoplasmic domain, which comprises at least one intracellular signaling domain. In some embodiments, cytoplasmic domain also comprises one or more co-stimulatory signaling domains.

The cytoplasmic domain is responsible for activation of at least one of the normal effector functions of the host cell (e.g., T cell) in which the CAR has been placed in. The term "effector function" refers to a specialized function of a cell. Effector function of a T-cell, for example, may be cytolytic activity or helper activity including the secretion of cytokines. Thus, the term "signaling domain" refers to the portion of a protein which transduces the effector function signal and directs the cell to perform a specialized function. While usually the entire signaling domain is present, in many cases it is not necessary to use the entire chain. To the extent that a truncated portion of the intracellular signaling domain is used, such truncated portion may be used in place of the intact chain as long as it transduces the effector function signal. The term intracellular signaling domain is thus meant to include any truncated portion of the signaling domain sufficient to transduce the effector function signal.

Non-limiting examples of signaling domains which can be used in the CARs of the present disclosure include, e.g., signaling domains derived from DAP10, DAP12, Fc epsilon receptor I γ chain (FCER1G), FcR β, CD3δ, CD3ε, CD3γ, CD3ζ, CD5, CD22, CD226, CD66d, CD79A, CD79B, IL18R1, and IL18RAP. In some embodiments, the signaling domain of the CAR can comprise the TIR domain sequence from IL18R1 or IL18RAP.

In some embodiments, the cytoplasmic domain comprises a CD3ζ signaling domain. In one embodiment, the CD3ζ signaling domain comprises the amino acid sequence set forth in SEQ ID NO: 6, or a variant thereof having at least 50, at least 55, at least 60, at least 65, at least 70, at least 75, at least 80, at least 85, at least 90, at least 95, at least 96, at least 97, at least 98 or at least 99%, sequence identity with SEQ ID NO: 6.

In some embodiments, the cytoplasmic domain further comprises one or more co-stimulatory signaling domains. In some embodiments, the one or more co-stimulatory signaling domains are derived from CD28, 41BB, IL2Rb, IL18R1, IL18RAP, CD40, OX40 (CD134), CD80, CD86, CD27, ICOS, NKG2D, DAP10, DAP12, 2B4 (CD244),

BTLA, CD30, GITR, CD226, CD79A, and HVEM. In some embodiments, the co-stimulatory signaling domain of the CAR can comprise the TIR domain sequence from IL18R1 or IL18RAP.

In one embodiment, the co-stimulatory signaling domain is derived from 41BB. In one embodiment, the 41BB co-stimulatory signaling domain comprises the amino acid sequence set forth in SEQ ID NO: 8, or a variant thereof having at least 50, at least 55, at least 60, at least 65, at least 70, at least 75, at least 80, at least 85, at least 90, at least 95, at least 96, at least 97, at least 98 or at least 99%, sequence identity with SEQ ID NO: 8.

In one embodiment, the co-stimulatory signaling domain is derived from IL2Rb. In one embodiment, the IL2Rb co-stimulatory signaling domain comprises the amino acid sequence set forth in SEQ ID NO: 9, or a variant thereof having at least 50, at least 55, at least 60, at least 65, at least 70, at least 75, at least 80, at least 85, at least 90, at least 95, at least 96, at least 97, at least 98 or at least 99%, sequence identity with SEQ ID NO: 9.

In one embodiment, the co-stimulatory signaling domain is derived from CD40. In one embodiment, the CD40 co-stimulatory signaling domain comprises the amino acid sequence set forth in SEQ ID NO: 10, or a variant thereof having at least 50, at least 55, at least 60, at least 65, at least 70, at least 75, at least 80, at least 85, at least 90, at least 95, at least 96, at least 97, at least 98 or at least 99%, sequence identity with SEQ ID NO: 10.

In one embodiment, the co-stimulatory signaling domain is derived from OX40. In one embodiment, the OX40 co-stimulatory signaling domain comprises the amino acid sequence set forth in SEQ ID NO: 11, or a variant thereof having at least 50, at least 55, at least 60, at least 65, at least 70, at least 75, at least 80, at least 85, at least 90, at least 95, at least 96, at least 97, at least 98 or at least 99%, sequence identity with SEQ ID NO: 11.

In one embodiment, the co-stimulatory signaling domain is derived from CD80. In one embodiment, the CD80 co-stimulatory signaling domain comprises the amino acid sequence set forth in SEQ ID NO: 12, or a variant thereof having at least 50, at least 55, at least 60, at least 65, at least 70, at least 75, at least 80, at least 85, at least 90, at least

95, at least 96, at least 97, at least 98 or at least 99%, sequence identity with SEQ ID NO: 12.

In one embodiment, the co-stimulatory signaling domain is derived from CD86. In one embodiment, the CD86 co-stimulatory signaling domain comprises the amino acid sequence set forth in SEQ ID NO: 13, or a variant thereof having at least 50, at least 55, at least 60, at least 65, at least 70, at least 75, at least 80, at least 85, at least 90, at least 95, at least 96, at least 97, at least 98 or at least 99%, sequence identity with SEQ ID NO: 13.

In one embodiment, the co-stimulatory signaling domain is derived from CD27. In one embodiment, the CD27 co-stimulatory signaling domain comprises the amino acid sequence set forth in SEQ ID NO: 14, or a variant thereof having at least 50, at least 55, at least 60, at least 65, at least 70, at least 75, at least 80, at least 85, at least 90, at least 95, at least 96, at least 97, at least 98 or at least 99%, sequence identity with SEQ ID NO: 14.

In one embodiment, the co-stimulatory signaling domain is derived from ICOS. In one embodiment, the ICOS co-stimulatory signaling domain comprises the amino acid sequence set forth in SEQ ID NO: 15, or a variant thereof having at least 50, at least 55, at least 60, at least 65, at least 70, at least 75, at least 80, at least 85, at least 90, at least 95, at least 96, at least 97, at least 98 or at least 99%, sequence identity with SEQ ID NO: 15.

In one embodiment, the co-stimulatory signaling domain is derived from NKG2D. In one embodiment, the NKG2D co-stimulatory signaling domain comprises the amino acid sequence set forth in SEQ ID NO: 16, or a variant thereof having at least 50, at least 55, at least 60, at least 65, at least 70, at least 75, at least 80, at least 85, at least 90, at least 95, at least 96, at least 97, at least 98 or at least 99%, sequence identity with SEQ ID NO: 16.

In one embodiment, the co-stimulatory signaling domain is derived from DAP10. In one embodiment, the DAP10 co-stimulatory signaling domain comprises the amino acid sequence set forth in SEQ ID NO: 17, or a variant thereof having at least 50, at least 55, at least 60, at least 65, at least 70, at least 75, at least 80, at least 85, at least 90, at

least 95, at least 96, at least 97, at least 98 or at least 99%, sequence identity with SEQ ID NO: 17.

In one embodiment, the co-stimulatory signaling domain is derived from DAP12. In one embodiment, the DAP12 co-stimulatory signaling domain comprises the amino acid sequence set forth in SEQ ID NO: 18, or a variant thereof having at least 50, at least 55, at least 60, at least 65, at least 70, at least 75, at least 80, at least 85, at least 90, at least 95, at least 96, at least 97, at least 98 or at least 99%, sequence identity with SEQ ID NO: 18.

In one embodiment, the co-stimulatory signaling domain is derived from IL18R1 or IL18RAP. In one embodiment, the IL18R1 or IL18RAP co-stimulatory signaling domain comprises the amino acid sequence set forth in SEQ ID NO: 198 or 199, or a variant thereof having at least 50, at least 55, at least 60, at least 65, at least 70, at least 75, at least 80, at least 85, at least 90, at least 95, at least 96, at least 97, at least 98 or at least 99%, sequence identity with SEQ ID NO: 198 or 199.

In one embodiment, the co-stimulatory signaling domain is derived from 2B4 (CD244). In one embodiment, the 2B4 (CD244) co-stimulatory signaling domain comprises the amino acid sequence set forth in SEQ ID NO: 19, or a variant thereof having at least 50, at least 55, at least 60, at least 65, at least 70, at least 75, at least 80, at least 85, at least 90, at least 95, at least 96, at least 97, at least 98 or at least 99%, sequence identity with SEQ ID NO: 19.

In some embodiments, the CAR of the present disclosure comprises one co-stimulatory signaling domains. In some embodiments, the CAR of the present disclosure comprises two or more co-stimulatory signaling domains. In certain embodiments, the CAR of the present disclosure comprises two, three, four, five, six or more co-stimulatory signaling domains.

In some embodiments, the signaling domain(s) and co-stimulatory signaling domain(s) can be placed in any order. In some embodiments, the signaling domain is upstream of the co-stimulatory signaling domains. In some embodiments, the signaling domain is downstream from the co-stimulatory signaling domains. In the cases where two or more co-stimulatory domains are included, the order of the co-stimulatory signaling domains could be switched.

Non-limiting exemplary CAR regions and sequences are provided in Table 1, including amino acid and nucleic acid sequences for various CAR constructs shown in Figures 6, 10A, and 11A.

Table 1.

CAR regions	Sequence	UniProt Id	SEQ ID NO
CD19 CAR:			
GMCSFR Signal Peptide	MLLLVTSLLLCELPHPAFLLIP		1
FMC63 VH (amino acid sequence)	EVKLQESGPGLVAPSQSLSVTCTVSGVSLPDY GVSWIRQPPRKGLEWLGVIWGSETTYYNSAL KSRLTIIKDNSKSQVFLKMNSLQTDDTAIYYC AKHYYYGGSYAMDYWGQGTSVTVSS		2
FMC63 VH (nucleotide sequence)	GAAGTGAAACTGCAAGAGTCTGGCCCTGG ACTGGTGGCCCCATCTCAGTCTCTGAGCGT GACCTGTACAGTCAGCGGAGTGTCCCTGCC TGATTACGGCGTGTCCTGGATCAGACAGCC TCCTCGGAAAGGCCTGGAATGGCTGGGAGT GATCTGGGGCAGCGAGACAACCTACTACA ACAGCGCCCTGAAGTCCCGGCTGACCATCA TCAAGGACAACTCCAAGAGCCAGGTGTTCC TGAAGATGAACAGCCTGCAGACCGACGAC ACCGCCATCTACTATTGCGCCAAGCACTAC TACTACGGCGGCAGCTACGCCATGGATTAT TGGGGCCAGGGCACCAGCGTGACCGTGTCT AGC		145
Whitlow Linker (amino acid sequence)	GSTSGSGKPGSGEGSTKG		3
Whitlow Linker (nucleotide sequence)	GGCTCTACAAGCGGCAGCGGCAAACCTGG ATCTGGCGAGGGATCTACCAAGGGC		146
Whitlow Linker (nucleotide sequence)	GGCAGTACTTCTGGTAGCGGAAAACCCGG TAGCGGCGAGGGGTCAACTAAAGGA		150
FMC63 VL (amino acid sequence)	DIQMTQTTSSLSASLGDRVTISCRASQDISKY LNWYQQKPDGTVKLLIYHTSRLHSGVPSRFS GSGSGTDYSLTISNLEQEDIATYFCQQGNTLP YTFGGGTKLEIT		4

FMC63 VL (nucleotide sequence)	GACATCCAGATGACCCAGACCACAAGCAG CCTGTCTGCCAGCCTGGGCGATAGAGTGA CCATCAGCTGTAGAGCCAGCCAGGACATC AGCAAGTACCTGAACTGGTATCAGCAAAA GCCCGACGGCACCGTGAAGCTGCTGATCT ACCACACCAGCAGACTGCACAGCGGCGTG CCAAGCAGATTTTCTGGCAGCGGCTCTGGC ACCGACTACAGCCTGACAATCAGCAACCT GGAACAAGAGGATATCGCTACCTACTTCT GCCAGCAAGGCAACACCCTGCCTTACACC TTTGGCGGAGGCACCAAGCTGGAAATCAC C		147
CD28 (AA 114-220)	IEVMYPPPYLDNEKSNGTIIHVKGKHLCPSPL FPGPSKPFWVLVVVGGVLACYSLLVTVAFIIF WVRSKRSRLLHSDYMNMTPRRPGPTRKHYQ PYAPPRDFAAYRS	P10747-1	5
CD28 (nucleotide sequence)	ATCGAAGTGATGTACCCTCCACCTTACCTG GACAACGAGAAGTCCAACGGCACCATCAT CCACGTGAAGGGCAAGCACCTGTGTCCTTC TCCACTGTTCCCCGGACCTAGCAAGCCTTT CTGGGTGCTCGTTGTTGTTGGCGGCGTGCT GGCCTGTTATAGCCTGCTTGTGACCGTGGC CTTCATCATCTTTTGGGTCCGAAGCAAGCG GAGCCGGCTGCTGCACTCCGACTACATGA ACATGACCCCTAGACGGCCCGGACCAACC AGAAAGCACTACCAGCCTTACGCTCCTCCT AGAGACTTCGCCGCCTACCGGTCC		148
CD3-zeta isoform 3 (AA 52-163)	RVKFSRSADAPAYQQGQNQLYNELNLGRRE EYDVLDKRRGRDPEMGGKPRRKNPQEGLY NELQKDKMAEAYSEIGMKGERRRGKGHDG LYQGLSTATKDTYDALHMQALPPR	P20963-3	6
CD3-zeta isoform 3 (nucleotide sequence)	AGAGTGAAGTTCAGCAGATCCGCCGATGC TCCCGCCTATCAGCAGGGCCAAAACCAGC TGTACAACGAGCTGAACCTGGGGAGAAGA GAAGAGTACGACGTGCTGGACAAGCGGAG AGGCAGAGATCCTGAAATGGGCGGCAAGC CCAGACGGAAGAATCCTCAAGAGGGCCTG TATAATGAGCTGCAGAAAGACAAGATGGC CGAGGCCTACAGCGAGATCGGAATGAAGG GCGAGCGCAGAAGAGGCAAGGGACACGA		149

	TGGACTGTACCAGGGCCTGAGCACCGCCA CCAAGGATACCTATGATGCCCTGCACATGC AGGCCCTGCCTCCAAGA		
FMC63 scFV	EVKLQESGPGLVAPSQSLSVTCTVSGVSLPD YGVSWIRQPPRKGLEWLGVIWGSETTYYNS ALKSRLTIIKDNSKSQVFLKMNSLQTDDTAIY YCAKHYYYGGSYAMDYWGQGTSVTVSSGS TSGSGKPGSGEGSTKGDIQMTQTTSSLSASL GDRVTISCRASQDISKYLNWYQQKPDGTVK LLIYHTSRLHSGVPSRFSGSGSGTDYSLTISNL EQEDIATYFCQQGNTLPYTFGGGTKLEIT		7
CD22 CAR Antigen-binding Domains:			
CD22_D04	QVQLVESGGGLVQAGGSLRLSCAASGSEFT GYPMGWFRQAPGKEREFVAGSVGIGGSTNY ADSVKGRFTISRDNAKNTVYLQMNSLKPED TAVYYCAADKDYYKPYSRYRTVIRYETWG QGTQVTVSS		96
CD22_CNTY_ VHH1_A01 (amino acid sequence)	EVQLLESGGGLVQPGGSLRLSCAASGLTSYS YAMGWYRQAPGKEREFVSAISSGGSAYYAD SVKGRFTISRDNSKNTLYLQMNSLRAEDTAV YYCAVGPYYGFRAVTEADYWGQGTQVTVS S		97
CD22_CNTY_ VHH1_A01 (nucleotide sequence)	GAGGTACAACTTTTGGAGTCAGGCGGTGG ACTGGTACAACCGGGTGGTTCATTGCGTTT GAGCTGCGCTGCCTCTGGTTTGACCTCTTA TTCCTACGCGATGGGCTGGTATCGCCAAGC GCCGGGCAAAGAACGCGAGTTTGTCAGCG CAATCAGCTCGGGTGGTAGCGCGTACTAC GCGGACTCGGTAAAAGGCCGTTTTACGAT CAGTCGTGATAATTCCAAGAATACCTTGTA CCTGCAAATGAATAGCCTTCGCGCAGAAG ACACAGCGGTGTATTATTGTGCCGTTGGAC CGTACTACGGATTTAGAGCGGTTACCGAA GCAGATTATTGGGGCCAGGGTACCCAGGT GACGGTCTCGAGC		100
CD22_CNTY_ VHH1_A01	EVQLLESGGGLVQPGGTLRLSCAASGLTCYS YAMGWYRQAPGKEREFVSAISSGGSAYYAD SVKGRFTICRDNSKNTLYLQMNSLRAEDTA		152

(amino acid sequence)	VYYCAVGPYYGFRAVTEADYWGQGTQVTVSS		
CD22_CNTY_VHH1_A01 (nucleotide sequence)	GAAGTCCAGCTGCTGGAAAGCGGTGGCGGTCTGGTCCAGCCTGGCGGCACCCTGCGCCTGTCCTGTGCCGCTAGCGGCCTGACCTGCTATAGCTATGCCATGGGTTGGTACCGCCAGGCCCCTGGTAAGGAGCGCGAATTCGTGTCCGCTATTTCCAGCGGCGGCTCCGCCTATTATGCTGATAGCGTCAAGGGTCGCTTCACCATTTGCCGCGACAACAGCAAAAACACTCTGTATCTGCAGATGAACTCCCTGCGCGCTGAGGATACCGCCGTCTACTACTGCGCTGTGGGCCCTTATTATGGCTTCCGCGCTGTGACTGAGGCTGACTACTGGGGTCAGGGCACTCAGGTGACCTGTGAGCAGC		153
CD22_CNTY_VHH1_E04 (amino acid sequence)	EVQLLESGGGLVQPGGSLRLSCAASGFTSSSYVMGWYRQAPGKEREFVSSISTGGDAYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAADVWYYHGGAYDYWGQGTQVTVSS		98
CD22_CNTY_VHH1_E04 (nucleotide sequence)	GAGGTACAACTTTTGGAGTCAGGCGGTGGACTGGTACAACCGGGTGGTTCATTGCGTTTGAGCTGCGCTGCCTCTGGTTTTACCAGCTCCTCCTACGTGATGGGCTGGTATCGCCAAGCGCCGGGCAAAGAACGCGAGTTTGTCAGCTCGATCAGCACCGGTGGTGATGCCTACTACGCGGACTCGGTAAAAGGCCGTTTTACGATCAGTCGTGATAATTCCAAGAATACCTTGTACCTGCAAATGAATAGCCTTCGCGCAGAAGACACAGCGGTGTATTATTGTGCCGCTGACGTTTGGTACTACCACGGCGGCGCGTACGATTATTGGGGCCAGGGTACCCAGGTGACGGTCTCGAGC		101
DO4-D11-CD22 VHH (amino acid sequence)	QVQLVESGGGLVQAGGSLRLSCAASGSEFTGYPMGWFRQAPGKEREFVAGSVGIGGSTNYADSVKGRFTISRDNAKNTVYLQMNSLKPEDTAVYYCAADKDYYKPYSRYRTAIRYDTWGQGTQVTVSS		155

DO4-D11-CD22 VHH (nucleotide sequence)	CAGGTGCAGCTGGTTGAGTCTGGGGGAGG CCTTGTCCAGGCTGGGGGGTCCCTGAGACT CTCCTGTGCAGCGTCTGGAAGCGAATTCAC CGGTTATCCCATGGGCTGGTTTCGCCAGGC TCCAGGCAAGGAAAGGGAGTTTGTCGCTG GCTCCGTAGGTATCGGTGGTAGTACAAACT ATGCAGACTCCGTGAAGGGCCGATTCACC ATCTCCAGAGACAATGCGAAGAACACGGT CTATCTGCAAATGAACAGCCTGAAGCCAG AGGACACGGCTGTGTATTACTGTGCGGCC GACAAAGACTACTACAAACCTTATAGTCG ATATAGGACCGCTATCAGGTACGATACCT GGGGCCAAGGGACCCAGGTCACCGTCTCG AGC		156
CD22 CAR w/o Antigen-Binding Domains:			
IgG4(CH3)/ CD28/41BB/ CD3z	IgG4 CH3 Hinge : ESKYGPPCPPCPGQPREPQVYTLPPSQEEMT KNQVSLTCLVKGFYPSDIAVEWESNGQPEN NYKTTPPVLDSDGSFFLYSRLTVDKSRWQEG NVFSCSVMHEALHNHYTQKSLSLSLGK		102
	CD28 Transmembrane Domain: FWVLVVVGGVLACYSLLVTVAFIIFWV		24
	41BB Co-Stimulatory Domain: KRGRKKLLYIFKQPFMRPVQTTQEEDGCSCR FPEEEEGGCEL		8
	CD3z Signaling Domain: RVKFSRSADAPAYQQGQNQLYNELNLGRRE EYDVLDKRRGRDPEMGGKPRRKNPQEGLY NELQKDKMAEAYSEIGMKGERRRGKGHDG LYQGLSTATKDTYDALHMQALPPR		6
CD8/CD28/ 41BB/CD3z	CD8 Hinge : TTTPAPRPPTPAPTIASQPLSLRPEACRPAAG GAVHTRGLDFACD		21

	CD28 Transmembrane Domain: FWVLVVVGGVLACYSLLVTVAFIIFWV		24
	41BB Co-Stimulatory Domain: KRGRKKLLYIFKQPFMRPVQTTQEEDGCSCR FPEEEEGGCEL		8
	CD3z Signaling Domain: RVKFSRSADAPAYQQGQNQLYNELNLGRRE EYDVLDKRRGRDPEMGGKPRRKNPQEGLY NELQKDKMAEAYSEIGMKGERRRGKGHDG LYQGLSTATKDTYDALHMQALPPR		6
CD8/CD8/ DAP10/CD3z	CD8 Hinge : TTTPAPRPPTPAPTIASQPLSLRPEACRPAAG GAVHTRGLDFACD		21
	CD8 Transmembrane Domain: IYIWAPLAGTCGVLLLSLVIT		23
	DAP10 Co-Stimulatory Domain: LCARPRRSPAQEDGKVYINMPGRG		17
	CD3z Signaling Domain: RVKFSRSADAPAYQQGQNQLYNELNLGRRE EYDVLDKRRGRDPEMGGKPRRKNPQEGLY NELQKDKMAEAYSEIGMKGERRRGKGHDG LYQGLSTATKDTYDALHMQALPPR		6
Signaling / Co-stimulatory Domains:			
41BB (AA 214-255)	KRGRKKLLYIFKQPFMRPVQTTQEEDGCSCR FPEEEEGGCEL	Q07011	8
IL2Rb (AA 266-551)	NCRNTGPWLKKVLKCNTPDPSKFFSQLSSEH GGDVQKWLSSPFPSSSFSPGGLAPEISPLEVL ERDKVTQLLPLNTDAYLSLQELQGQDPTHL V	P14784	9
CD40 (AA 216-277)	KKVAKKPTNKAPHPKQEPQEINFPDDLPGSN TAAPVQETLHGCQPVTQEDGKESRISVQERQ	P25942	10
OX40 (AA 236-277)	ALYLLRRDQRLPPDAHKPPGGGSFRTPIQEE QADAHSTLAKI	P43489	11
CD80 (AA 264-288)	TYCFAPRCRERRRNERLRRESVRPV	P33681	12

CD86 (AA269-329)	KWKKKKRPRNSYKCGTNTMEREESEQTKK REKIHIPERSDEAQRVFKSSKTSSCDKSDTCF	P42081	13
CD27 (AA 213-260)	QRRKYRSNKGESPVEPAEPCHYSCPREEEGS TIPIQEDYRKPEPACSP	P26842	14
ICOS (AA 162-199)	CWLTKKKYSSSVHDPNGEYMFMRAVNTAK KSRLTDVTL	Q9Y6W8	15
NKG2D (AA 1-51)	MGWIRGRRSR HSWEMSEFHN YNLDLKKSDF STRWQKQRCP VVKSKCRENAS	P26718	16
DAP10 (AA 70-93)	LCARPRRSPAQEDGKVYINMPGRG	Q9UBK5	17
DAP12 (AA 62-113)	YFLGRLVPRGRGAAEAATRKQRITETESPYQ ELQGQRSDVYSDLNTQRPYYK	O54885	18
2B4/CD244 (AA 251-370)	WRRKRKEKQSETSPKEFLTIYEDVKDLKTRR NHEQEQTFPGGGSTIYSMIQSQSSAPTSQEPA YTLYSLIQPSRKSGSRKRNHSPSFNSTIYEVIG KSQPKAQNPARLSRKELENFDVYS	Q9BZW8	19
CD3-zeta isoform 3 (AA 52-163)	RVKFSRSADAPAYQQGQNQLYNELNLGRRE EYDVLDKRRGRDPEMGGKPRRKNPQEGLY NELQKDKMAEAYSEIGMKGERRRGKGHDG LYQGLSTATKDTYDALHMQALPPR	P20963-3	6
CD28 (AA 180-220)	RSKRSRLLHSDYMNMTPRRPGPTRKHYQPY APPRDFAAYRS	P10747-1	20
IL18R1 TIR domain (373-520)	KTYDAFVSYLKECRPENGEEHTFAVEILPRV LEKHFGYKLCIFERDVVPGGAVVDEIHSLIEK SRRLIIVLSKSYMSNEVRYELESGLHEALVER KIKIILIEFTPVTDFTFLPQSLKLLKSHRVLKW KADKSLSYNSRFWKNLLYLM	Q13478	198
IL18RAP TIR domain (406-559)	KDFDAFVSYAKWSSFPSEATSSLSEEHLALSL FPDVLENKYGYSLCLLERDVAPGGVYAEDIV SIIKRSRRGIFILSPNYVNGPSIFELQAAVNLA LDDQTLKLILIKFCYFQEPESLPHLVKKALRV LPTVTWRGLKSVPPNSRFWAKMRYHM	O95256	199
Spacer/Hinge:			
CD8 (AA 136-182)	TTTPAPRPPTPAPTIASQPLSLRPEACRPAAG GAVHTRGLDFACD	P01732	21
CD28 (AA 114-151)	IEVMYPPPYLDNEKSNGTIIHVKGKHLCPSPL FPGPSKP	P10747-1	22
IgG4 CH3	ESKYGPPCPPCPGQPREPQVYTLPPSQEEMT KNQVSLTCLVKGFYPSDIAVEWESNGQPEN NYKTTPPVLDSDGSFFLYSRLTVDKSRWQEG NVFSCSVMHEALHNHYTQKSLSLSLGK		102

Transmembrane:			
CD8 (AA 183-203)	IYIWAPLAGTCGVLLLSLVIT	P01732	23
CD28 (AA 153-179)	FWVLVVVGGVLACYSLLVTVAFIIFWV	P10747-1	24
Linkers:			
Whitlow Linker	GSTSGSGKPGSGEGSTKG		3
$(G_4S)_3$	GGGGSGGGGSGGGGS		25
Linker 3	GGSEGKSSGSGSESKSTGGS		26
Linker 4	GGGSGGGS		27
Linker 5	GGGSGGGSGGGS		28
Linker 6	GGGSGGGSGGGSGGGS		29
Linker 7	GGGSGGGSGGGSGGGSGGGS		30
Linker 8	GGGGSGGGGSGGGGSGGGGS		31
Linker 9	GGGGSGGGGSGGGGSGGGGSGGGGS		32
Linker 10	IRPRAIGGSKPRVA		33
Linker 11	GKGGSGKGGSGKGGS		34
Linker 12	GGKGSGGKGSGGKGS		35
Linker 13	GGGKSGGGKSGGGKS		36
Linker 14	GKGKSGKGKSGKGKS		37
Linker 15	GGGKSGGKGSGKGGS		38
Linker 16	GKPGSGKPGSGKPGS		39
Linker 17	GKPGSGKPGSGKPGSGKPGS		40
Linker 18	GKGKSGKGKSGKGKSGKGKS		41
Linker 19	STAGDTHLGGEDFD		42
Linker 20	GEGGSGEGGSGEGGS		43
Linker 21	GGEGSGGEGSGGEGS		44
Linker 22	GEGESGEGESGEGES		45
Linker 23	GGGESGGEGSGEGGS		46
Linker 24	GEGESGEGESGEGESGEGES		47
Linker 25	GSTSGSGKPGSGEGSTKG		48
Linker 26	PRGASKSGSASQTGSAPGS		49
Linker 27	GTAAAGAGAAGGAAAGAAG		50
Linker 28	GTSGSSGSGSGGSGSGGGG		51
Linker 29	GKPGSGKPGSGKPGSGKPGS		52
Linker 30	GSGS		53
Linker 31	APAPAPAPAP		54
Linker 32	APAPAPAPAPAPAPAPAPAP		55
Linker 33	AEAAAKEAAAKEAAAAKEAAAAKEAAAAK AAA		56
Linker 34	GGGGS		154
Linker 35	GGGGSGGGGS		151

CAR regions	Sequence	SEQ ID NO
Transmembrane:		
IgK Signal Peptide Variant (amino acid sequence)	MARSPAQLLGLLLLWLSGARC	103
IgK Signal Peptide Variant (nucleotide sequence)	ATGGCCAGATCTCCTGCTCAACTGCTGGGACTGCTGCTGCTGTGGCTTAGCGGAGCCAGATGC	144
Amino Acid Sequences of Mono-specific CARs Targeting CD19 or CD22:		
FMC63_CD28_CD28_CD28_CD3Z (P1209)	MLLLVTSLLLCELPHPAFLLIPDIQMTQTTSSLSASLGDRVTISCRASQDISKYLNWYQQKPDGTVKLLIYHTSRLHSGVPSRFSGSGSGTDYSLTISNLEQEDIATYFCQQGNTLPYTFGGGTKLEITGSTSGSGKPGSGEGSTKGEVKLQESGPGLVAPSQSLSVTCTVSGVSLPDYGVSWIRQPPRKGLEWLGVIWGSETTYYNSALKSRLTIIKDNSKSQVFLKMNSLQTDDTAIYYCAKHYYYGGSYAMDYWGQGTSVTVSSIEVMYPPPYLDNEKSNGTIIHVKGKHLCPSPLFPGPSKPFWVLVVVGGVLACYSLLVTVAFIIFWVRSKRSRLLHSDYMNMTPRRPGPTRKHYQPYAPPRDFAAYRSRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR	104
A01_Ig4CH3_CD28_41BB_CD3Z (P1362)	MARSPAQLLGLLLLWLSGARCEVQLLESGGGLVQPGGSLRLSCAASGLTSYSYAMGWYRQAPGKEREFVSAISSGGSAYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAVGPYYGFRAVTEADYWGQGTQVTVSSESKYGPPCPPCPGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNHYTQKSLSLSLGKFWVLVVVGGVLACYSLLVTVAFIIFWVKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCELRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGL	105

	YNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR	
A01-A01_CD28_CD3Z (P1631)	MARSPAQLLGLLLLWLSGARCEVQLLESGGGLVQPGGSLRLSCAASGLTSYSYAMGWYRQAPGKEREFVSAISSGGSAYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAVGPYYGFRAVTEADYWGQGTQVTVSSGSTSGSGKPGSGEGSTKGEVQLLESGGGLVQPGGSLRLSCAASGLTSYSYAMGWYRQAPGKEREFVSAISSGGSAYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAVGPYYGFRAVTEADYWGQGTQVTVSSIEVMYPPPYLDNEKSNGTIIHVKGKHLCPSPLFPGPSKPFWVLVVVGGVLACYSLLVTVAFIIFWVRSKRSRLLHSDYMNMTPRRPGPTRKHYQPYAPPRDFAAYRSRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR	106
D04_AM_D11_CD8_CD28_41BB_CD3Z (P1729)	MARSPAQLLGLLLLWLSGARCQVQLVESGGGLVQAGGSLRLSCAASGSEFTGYPMGWFRQAPGKEREFVAGSVGIGGSTNYADSVKGRFTISRDNAKNTVYLQMNSLKPEDTAVYYCAADKDYYKPYSRYRTAIRYDTWGQGTQVTVSSTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDFWVLVVVGGVLACYSLLVTVAFIIFWVKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCELRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR	107
E04-E04_CD28_CD28_CD28_CD3Z (P1633)	MARSPAQLLGLLLLWLSGARCEVQLLESGGGLVQPGGSLRLSCAASGFTSSSYVMGWYRQAPGKEREFVSSISTGGDAYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAADVWYYHGGAYDYWGQGTQVTVSSGSTSGSGKPGSGEGSTKGEVQLLESGGGLVQPGGSLRLSCAASGFTSSSYVMGWYRQAPGKEREFVSSISTGGDAYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAADVWYYHGGAYDYWGQGTQVTVSSIEVMYPPPYLDNEKSNGTIIHVKGKHLCPSPLFPGPSKPFWVLVVVGGVLACYSLLVTVAFIIFWVRSKRSRLLHSDYMNMTPRRPGPTRKHYQPYAPPRDFAAYRSRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKR	108

	RGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGM KGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR	
D04AMD11-E04_CD28_CD28_CD28_CD3Z (P1702)	MARSPAQLLGLLLLWLSGARCQVQLVESGGGLVQAGGS LRLSCAASGSEFTGYPMGWFRQAPGKEREFVAGSVGIGG STNYADSVKGRFTISRDNAKNTVYLQMNSLKPEDTAVYY CAADKDYYKPYSRYRTAIRYDTWGQGTQVTVSSGSTSGS GKPGSGEGSTKGEVQLLESGGGLVQPGGSLRLSCAASGF TSSSYVMGWYRQAPGKEREFVSSISTGGDAYYADSVKGR FTISRDNSKNTLYLQMNSLRAEDTAVYYCAADVWYYHG GAYDYWGQGTQVTVSSIEVMYPPPYLDNEKSNGTIIHVK GKHLCPSPLFPGPSKPFWVLVVVGGVLACYSLLVTVAFII FWVRSKRSRLLHSDYMNMTPRRPGPTRKHYQPYAPPRDF AAYRSRVKFSRSADAPAYQQGQNQLYNELNLGRREEYD VLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEA YSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQA LPPR	109
E04_Ig4_CD28_41BB_CD3Z (P1356)	MARSPAQLLGLLLLWLSGARCEVQLLESGGGLVQPGGSL RLSCAASGFTSSSYVMGWYRQAPGKEREFVSSISTGGDA YYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYC AADVWYYHGGAYDYWGQGTQVTVSSESKYGPPCPPCP GKFWVLVVVGGVLACYSLLVTVAFIIFWVKRGRKKLLYI FKQPFMRPVQTTQEEDGCSCRFPEEEEGGCELRVKFSRSA DAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEM GGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRG KGHDGLYQGLSTATKDTYDALHMQALPPR	110
D04AMD11-A01_CD8_CD28_41BB_CD3Z (P1734)	MARSPAQLLGLLLLWLSGARCQVQLVESGGGLVQAGGS LRLSCAASGSEFTGYPMGWFRQAPGKEREFVAGSVGIGG STNYADSVKGRFTISRDNAKNTVYLQMNSLKPEDTAVYY CAADKDYYKPYSRYRTAIRYDTWGQGTQVTVSSGSTSGS GKPGSGEGSTKGEVQLLESGGGLVQPGGSLRLSCAASGL TSYSYAMGWYRQAPGKEREFVSAISSGGSAYYADSVKG RFTISRDNSKNTLYLQMNSLRAEDTAVYYCAVGPYYGFR AVTEADYWGQGTQVTVSSTTTPAPRPPTPAPTIASQPLSL RPEACRPAAGGAVHTRGLDFACDFWVLVVVGGVLACYS LLVTVAFIIFWVKRGRKKLLYIFKQPFMRPVQTTQEEDGC SCRFPEEEEGGCELRVKFSRSADAPAYQQGQNQLYNELN LGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQ	111

	KDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTY DALHMQALPPR	
Polynucleotide Sequences of Mono-specific CARs Targeting CD19 or CD22:		
FMC63_CD28_ CD28_CD28_C D3Z (P1209)	ATGCTGCTGCTGGTTACATCTCTGCTGCTGTGCGAGCT GCCCCATCCTGCCTTTCTGCTGATCCCCGACATCCAGA TGACCCAGACCACAAGCAGCCTGTCTGCCAGCCTGGGC GATAGAGTGACCATCAGCTGTAGAGCCAGCCAGGACA TCAGCAAGTACCTGAACTGGTATCAGCAAAAGCCCGA CGGCACCGTGAAGCTGCTGATCTACCACACCAGCAGA CTGCACAGCGGCGTGCCAAGCAGATTTTCTGGCAGCGG CTCTGGCACCGACTACAGCCTGACAATCAGCAACCTGG AACAAGAGGATATCGCTACCTACTTCTGCCAGCAAGGC AACACCCTGCCTTACACCTTTGGCGGAGGCACCAAGCT GGAAATCACCGGCTCTACAAGCGGCAGCGGCAAACCT GGATCTGGCGAGGGATCTACCAAGGGCGAAGTGAAAC TGCAAGAGTCTGGCCCTGGACTGGTGGCCCCATCTCAG TCTCTGAGCGTGACCTGTACAGTCAGCGGAGTGTCCCT GCCTGATTACGGCGTGTCCTGGATCAGACAGCCTCCTC GGAAAGGCCTGGAATGGCTGGGAGTGATCTGGGGCAG CGAGACAACCTACTACAACAGCGCCCTGAAGTCCCGG CTGACCATCATCAAGGACAACTCCAAGAGCCAGGTGTT CCTGAAGATGAACAGCCTGCAGACCGACGACACCGCC ATCTACTATTGCGCCAAGCACTACTACTACGGCGGCAG CTACGCCATGGATTATTGGGGCCAGGGCACCAGCGTG ACCGTGTCTAGCATCGAAGTGATGTACCCTCCACCTTA CCTGGACAACGAGAAGTCCAACGGCACCATCATCCAC GTGAAGGGCAAGCACCTGTGTCCTTCTCCACTGTTCCC CGGACCTAGCAAGCCTTTCTGGGTGCTCGTTGTTGTTG GCGGCGTGCTGGCCTGTTATAGCCTGCTTGTGACCGTG GCCTTCATCATCTTTTGGGTCCGAAGCAAGCGGAGCCG GCTGCTGCACTCCGACTACATGAACATGACCCCTAGAC GGCCCGGACCAACCAGAAAGCACTACCAGCCTTACGC TCCTCCTAGAGACTTCGCCGCCTACCGGTCCAGAGTGA AGTTCAGCAGATCCGCCGATGCTCCCGCCTATCAGCAG GGCCAAAACCAGCTGTACAACGAGCTGAACCTGGGGA GAAGAGAAGAGTACGACGTGCTGGACAAGCGGAGAG GCAGAGATCCTGAAATGGGCGGCAAGCCCAGACGGAA GAATCCTCAAGAGGGCCTGTATAATGAGCTGCAGAAA GACAAGATGGCCGAGGCCTACAGCGAGATCGGAATGA	112

	AGGGCGAGCGCAGAAGAGGCAAGGGACACGATGGAC TGTACCAGGGCCTGAGCACCGCCACCAAGGATACCTAT GATGCCCTGCACATGCAGGCCCTGCCTCCAAGA	
A01_Ig4 CH3_CD28_41 BB_CD3Z (P1362)	ATGGCCAGATCTCCTGCTCAACTGCTGGGACTGCTGCT GCTGTGGCTTAGCGGAGCCAGATGCGAGGTACAACTTT TGGAGTCAGGCGGTGGACTGGTACAACCGGGTGGTTC ATTGCGTTTGAGCTGCGCTGCCTCTGGTTTGACCTCTTA TTCCTACGCGATGGGCTGGTATCGCCAAGCGCCGGGCA AAGAACGCGAGTTTGTCAGCGCAATCAGCTCGGGTGG TAGCGCGTACTACGCGGACTCGGTAAAAGGCCGTTTTA CGATCAGTCGTGATAATTCCAAGAATACCTTGTACCTG CAAATGAATAGCCTTCGCGCAGAAGACACAGCGGTGT ATTATTGTGCCGTTGGACCGTACTACGGATTTAGAGCG GTTACCGAAGCAGATTATTGGGGCCAGGGTACCCAGG TGACGGTCTCGAGCGAGTCCAAATACGGTCCGCCATGC CCACCATGCCCAGGGCAGCCCCGAGAGCCACAGGTGT ACACCCTGCCCCCATCCCAAGAGGAGATGACCAAGAA CCAAGTCAGCCTGACCTGCCTGGTCAAAGGCTTCTACC CCAGCGACATCGCCGTGGAGTGGGAGAGCAATGGGCA GCCGGAGAACAACTACAAGACCACGCCTCCCGTGCTG GACTCCGACGGCTCCTTCTTCCTCTACTCCCGGCTCACC GTGGACAAGAGCAGGTGGCAGGAGGGGAATGTGTTCT CATGCTCCGTGATGCATGAGGCTCTGCACAACCACTAC ACACAGAAGAGCCTCTCCCTGTCTCTGGGAAAGTTTTG GGTGCTGGTGGTGGTTGGTGGAGTCCTGGCTTGCTATT CCTTGCTAGTAACAGTGGCCTTTATTATTTTCTGGGTGA AACGCGGCCGCAAGAAACTCCTGTATATATTCAAACA ACCATTTATGAGGCCAGTACAAACTACTCAAGAGGAA GATGGCTGTAGCTGCCGATTTCCAGAAGAAGAAGAAG GAGGATGTGAGCTCAGAGTGAAGTTCAGCAGGAGCGC AGACGCCCCCGCGTACCAGCAGGGCCAGAACCAGCTC TATAACGAACTCAATCTAGGACGAAGAGAGGAGTACG ATGTTTTGGACAAGCGGCGTGGCCGGGACCCTGAGAT GGGGGGAAAGCCGAGAAGGAAGAACCCTCAGGAAGG CCTGTACAATGAACTGCAGAAAGATAAGATGGCGGAG GCCTACAGTGAGATTGGGATGAAAGGCGAGCGCCGGA GGGGCAAGGGGCACGATGGCCTTTACCAGGGGCTCAG TACAGCCACCAAGGACACCTACGACGCCCTTCACATGC AGGCCCTGCCCCCTCGC	113

A01-A01_CD28_CD3Z (P1631)	ATGGCCAGATCTCCTGCTCAACTGCTGGGACTGCTGCT GCTGTGGCTTAGCGGAGCCAGATGCGAGGTACAACTTT TGGAGTCAGGCGGTGGACTGGTACAACCGGGTGGTTC ATTGCGTTTGAGCTGCGCTGCCTCTGGTTTGACCTCTTA TTCCTACGCGATGGGCTGGTATCGCCAAGCGCCGGGCA AAGAACGCGAGTTTGTCAGCGCAATCAGCTCGGGTGG TAGCGCGTACTACGCGGACTCGGTAAAAGGCCGTTTTA CGATCAGTCGTGATAATTCCAAGAATACCTTGTACCTG CAAATGAATAGCCTTCGCGCAGAAGACACAGCGGTGT ATTATTGTGCCGTTGGACCGTACTACGGATTTAGAGCG GTTACCGAAGCAGATTATTGGGGCCAGGGTACCCAGG TGACGGTCTCGAGCGGCTCTACAAGCGGCAGCGGCAA ACCTGGATCTGGCGAGGGATCTACCAAGGGCGAGGTA CAACTTTTGGAGTCAGGCGGTGGACTGGTACAACCGG GTGGTTCATTGCGTTTGAGCTGCGCTGCCTCTGGTTTGA CCTCTTATTCCTACGCGATGGGCTGGTATCGCCAAGCG CCGGGCAAAGAACGCGAGTTTGTCAGCGCAATCAGCT CGGGTGGTAGCGCGTACTACGCGGACTCGGTAAAAGG CCGTTTTACGATCAGTCGTGATAATTCCAAGAATACCT TGTACCTGCAAATGAATAGCCTTCGCGCAGAAGACAC AGCGGTGTATTATTGTGCCGTTGGACCGTACTACGGAT TTAGAGCGGTTACCGAAGCAGATTATTGGGGCCAGGG TACCCAGGTGACGGTCTCGAGCATCGAAGTGATGTACC CTCCACCTTACCTGGACAACGAGAAGTCCAACGGCACC ATCATCCACGTGAAGGGCAAGCACCTGTGTCCTTCTCC ACTGTTCCCCGGACCTAGCAAGCCTTTCTGGGTGCTCG TTGTTGTTGGCGGCGTGCTGGCCTGTTATAGCCTGCTTG TGACCGTGGCCTTCATCATCTTTTGGGTCCGAAGCAAG CGGAGCCGGCTGCTGCACTCCGACTACATGAACATGAC CCCTAGACGGCCCGGACCAACCAGAAAGCACTACCAG CCTTACGCTCCTCCTAGAGACTTCGCCGCCTACCGGTC CAGAGTGAAGTTCAGCAGATCCGCCGATGCTCCCGCCT ATCAGCAGGGCCAAAACCAGCTGTACAACGAGCTGAA CCTGGGGAGAAGAGAAGAGTACGACGTGCTGGACAAG CGGAGAGGCAGAGATCCTGAAATGGGCGGCAAGCCCA GACGGAAGAATCCTCAAGAGGGCCTGTATAATGAGCT GCAGAAAGACAAGATGGCCGAGGCCTACAGCGAGATC GGAATGAAGGGCGAGCGCAGAAGAGGCAAGGGACAC GATGGACTGTACCAGGGCCTGAGCACCGCCACCAAGG	114

	ATACCTATGATGCCCTGCACATGCAGGCCCTGCCTCCA AGATAA	
D04_AM_D11_ CD8_CD28_41 BB_CD3Z (P1729)	ATGGCCAGATCTCCTGCTCAACTGCTGGGACTGCTGCT GCTGTGGCTTAGCGGAGCCAGATGCCAGGTGCAGCTG GTTGAGTCTGGGGGAGGCCTTGTCCAGGCTGGGGGGTC CCTGAGACTCTCCTGTGCAGCGTCTGGAAGCGAATTCA CCGGTTATCCCATGGGCTGGTTTCGCCAGGCTCCAGGC AAGGAAAGGGAGTTTGTCGCTGGCTCCGTAGGTATCG GTGGTAGTACAAACTATGCAGACTCCGTGAAGGGCCG ATTCACCATCTCCAGAGACAATGCGAAGAACACGGTCT ATCTGCAAATGAACAGCCTGAAGCCAGAGGACACGGC TGTGTATTACTGTGCGGCCGACAAAGACTACTACAAAC CTTATAGTCGATATAGGACCGCTATCAGGTACGATACC TGGGGCCAAGGGACCCAGGTCACCGTCTCGAGCACAA CAACTCCAGCCCCAAGACCACCTACGCCTGCACCTACT ATCGCATCTCAACCACTGTCCCTGCGCCCTGAGGCATG CCGACCAGCAGCCGGTGGCGCGGTGCATACCCGCGGA CTGGACTTTGCCTGCGATTTTTGGGTGCTGGTGGTGGTT GGTGGAGTCCTGGCTTGCTATAGCTTGCTAGTAACAGT GGCCTTTATTATTTTCTGGGTGAAACGGGGCAGAAAGA AACTCCTGTATATATTCAAACAACCATTTATGCGACCA GTACAAACTACTCAAGAGGAAGATGGCTGTAGCTGCC GATTTCCAGAAGAAGAAGAAGGAGGATGTGAACTGAG AGTGAAGTTCAGCAGGAGCGCAGACGCCCCCGCGTAC CAGCAGGGCCAGAACCAGCTCTATAACGAGCTCAATC TAGGACGAAGAGAGGAGTACGATGTTTTGGACAAGCG ACGTGGCCGGGACCCTGAGATGGGGGGAAAGCCGAGA AGGAAGAACCCTCAGGAAGGCCTGTACAATGAACTGC AGAAAGATAAGATGGCGGAGGCCTACAGTGAGATTGG GATGAAAGGCGAGCGCCGGAGGGGCAAGGGGCACGA TGGCCTTTACCAGGGACTCAGTACAGCCACCAAGGAC ACCTACGACGCCCTTCACATGCAGGCCCTGCCCCCTCG C	115
E04-E04_CD28_CD 28_CD28_CD3 Z (P1633)	ATGGCCAGATCTCCTGCTCAACTGCTGGGACTGCTGCT GCTGTGGCTTAGCGGAGCCAGATGCGAGGTGCAGCTG TTGGAGAGCGGCGGGGGACTTGTTCAACCCGGAGGCT CTCTTAGGTTATCTTGCGCAGCTAGTGGATTTACGAGC TCCAGTTACGTGATGGGATGGTATCGACAGGCTCCTGG AAAAGAAAGAGAGTTCGTGAGCTCTATTAGCACCGGC	116

	GGCGATGCGTATTACGCAGATTCAGTGAAAGGCCGATT CACCATTTCCAGGGATAACTCCAAAAACACTCTCTACC TGCAAATGAACAGCCTGAGAGCCGAAGACACCGCTGT TTATTATTGCGCCGCCGACGTTTGGTATTACCACGGAG GCGCTTATGATTATTGGGGCCAGGGGACTCAGGTGACG GTCTCATCTGGCTCTACAAGCGGCAGCGGCAAACCTGG ATCTGGCGAGGGATCTACCAAGGGCGAGGTACAACTT TTGGAGTCAGGCGGTGGACTGGTACAACCGGGTGGTTC ATTGCGTTTGAGCTGCGCTGCCTCTGGTTTTACCAGCTC CTCCTACGTGATGGGCTGGTATCGCCAAGCGCCGGGCA AAGAACGCGAGTTTGTCAGCTCGATCAGCACCGGTGGT GATGCCTACTACGCGGACTCGGTAAAAGGCCGTTTTAC GATCAGTCGTGATAATTCCAAGAATACCTTGTACCTGC AAATGAATAGCCTTCGCGCAGAAGACACAGCGGTGTA TTATTGTGCCGCTGACGTTTGGTACTACCACGGCGGCG CGTACGATTATTGGGGCCAGGGTACCCAGGTGACGGTC TCGAGCATCGAAGTGATGTACCCTCCACCTTACCTGGA CAACGAGAAGTCCAACGGCACCATCATCCACGTGAAG GGCAAGCACCTGTGTCCTTCTCCACTGTTCCCCGGACC TAGCAAGCCTTTCTGGGTGCTCGTTGTTGTTGGCGGCG TGCTGGCCTGTTATAGCCTGCTTGTGACCGTGGCCTTC ATCATCTTTTGGGTCCGAAGCAAGCGGAGCCGGCTGCT GCACTCCGACTACATGAACATGACCCCTAGACGGCCCG GACCAACCAGAAAGCACTACCAGCCTTACGCTCCTCCT AGAGACTTCGCCGCCTACCGGTCCAGAGTGAAGTTCAG CAGATCCGCCGATGCTCCCGCCTATCAGCAGGGCCAAA ACCAGCTGTACAACGAGCTGAACCTGGGGAGAAGAGA AGAGTACGACGTGCTGGACAAGCGGAGAGGCAGAGAT CCTGAAATGGGCGGCAAGCCCAGACGGAAGAATCCTC AAGAGGGCCTGTATAATGAGCTGCAGAAAGACAAGAT GGCCGAGGCCTACAGCGAGATCGGAATGAAGGGCGAG CGCAGAAGAGGCAAGGGACACGATGGACTGTACCAGG GCCTGAGCACCGCCACCAAGGATACCTATGATGCCCTG CACATGCAGGCCCTGCCTCCAAGA	
D04AMD11-E04_CD28_CD28_CD28_CD3Z (P1702)	ATGGCCAGATCTCCTGCTCAACTGCTGGGACTGCTGCT GCTGTGGCTTAGCGGAGCCAGATGCCAGGTGCAGCTG GTTGAGTCTGGGGGAGGCCTTGTCCAGGCTGGGGGGTC CCTGAGACTCTCCTGTGCAGCGTCTGGAAGCGAATTCA CCGGTTATCCCATGGGCTGGTTTCGCCAGGCTCCAGGC AAGGAAAGGGAGTTTGTCGCTGGCTCCGTAGGTATCG	117

	GTGGTAGTACAAACTATGCAGACTCCGTGAAGGGCCG ATTCACCATCTCCAGAGACAATGCGAAGAACACGGTCT ATCTGCAAATGAACAGCCTGAAGCCAGAGGACACGGC TGTGTATTACTGTGCGGCCGACAAAGACTACTACAAAC CTTATAGTCGATATAGGACCGCTATCAGGTACGATACC TGGGGCCAAGGGACCCAGGTCACCGTCTCGAGCGGGT CTACCTCAGGGTCAGGGAAACCCGGAAGCGGCGAAGG GTCTACAAAAGGTGAGGTACAACTTTTGGAGTCAGGC GGTGGACTGGTACAACCGGGTGGTTCATTGCGTTTGAG CTGCGCTGCCTCTGGTTTTACCAGCTCCTCCTACGTGAT GGGCTGGTATCGCCAAGCGCCGGGCAAAGAACGCGAG TTTGTCAGCTCGATCAGCACCGGTGGTGATGCCTACTA CGCGGACTCGGTAAAAGGCCGTTTTACGATCAGTCGTG ATAATTCCAAGAATACCTTGTACCTGCAAATGAATAGC CTTCGCGCAGAAGACACAGCGGTGTATTATTGTGCCGC TGACGTTTGGTACTACCACGGCGGCGCGTACGATTATT GGGGCCAGGGTACCCAGGTGACGGTCTCGAGCATCGA AGTGATGTACCCTCCACCTTACCTGGACAACGAGAAGT CCAACGGCACCATCATCCACGTGAAGGGCAAGCACCT GTGTCCTTCTCCACTGTTCCCCGGACCTAGCAAGCCTTT CTGGGTGCTCGTTGTTGTTGGCGGCGTGCTGGCCTGTT ATAGCCTGCTTGTGACCGTGGCCTTCATCATCTTTTGGG TCCGAAGCAAGCGGAGCCGGCTGCTGCACTCCGACTA CATGAACATGACCCCTAGACGGCCCGGACCAACCAGA AAGCACTACCAGCCTTACGCTCCTCCTAGAGACTTCGC CGCCTACCGGTCCAGAGTGAAGTTCAGCAGGAGCGCA GACGCCCCCGCGTACCAGCAGGGCCAGAACCAGCTCT ATAACGAGCTCAATCTAGGACGAAGAGAGGAGTACGA TGTTTTGGACAAGCGACGTGGCCGGGACCCTGAGATG GGGGGAAAGCCGAGAAGGAAGAACCCTCAGGAAGGC CTGTACAATGAACTGCAGAAAGATAAGATGGCGGAGG CCTACAGTGAGATTGGGATGAAAGGCGAGCGCCGGAG GGGCAAGGGGCACGATGGCCTTTACCAGGGACTCAGT ACAGCCACCAAGGACACCTACGACGCCCTTCACATGC AGGCCCTGCCCCCTCGC	
E04_Ig4 _CD28_41BB_ CD3Z (P1356)	ATGGCCAGATCTCCTGCTCAACTGCTGGGACTGCTGCT GCTGTGGCTTAGCGGAGCCAGATGCGAGGTACAACTTT TGGAGTCAGGCGGTGGACTGGTACAACCGGGTGGTTC ATTGCGTTTGAGCTGCGCTGCCTCTGGTTTTACCAGCTC CTCCTACGTGATGGGCTGGTATCGCCAAGCGCCGGGCA	118

	AAGAACGCGAGTTTGTCAGCTCGATCAGCACCGGTGGT GATGCCTACTACGCGGACTCGGTAAAAGGCCGTTTTAC GATCAGTCGTGATAATTCCAAGAATACCTTGTACCTGC AAATGAATAGCCTTCGCGCAGAAGACACAGCGGTGTA TTATTGTGCCGCTGACGTTTGGTACTACCACGGCGGCG CGTACGATTATTGGGGCCAGGGTACCCAGGTGACGGTC TCGAGCGAGTCCAAATACGGTCCGCCATGCCCACCATG CCCAGGAAAGTTTTGGGTGCTGGTGGTGGTTGGTGGAG TCCTGGCTTGCTATTCCTTGCTAGTAACAGTGGCCTTTA TTATTTTCTGGGTGAAACGCGGCCGCAAGAAACTCCTG TATATATTCAAACAACCATTTATGAGGCCAGTACAAAC TACTCAAGAGGAAGATGGCTGTAGCTGCCGATTTCCAG AAGAAGAAGAAGGAGGATGTGAGCTCAGAGTGAAGTT CAGCAGGAGCGCAGACGCCCCCGCGTACCAGCAGGGC CAGAACCAGCTCTATAACGAACTCAATCTAGGACGAA GAGAGGAGTACGATGTTTTGGACAAGCGGCGTGGCCG GGACCCTGAGATGGGGGGAAAGCCGAGAAGGAAGAA CCCTCAGGAAGGCCTGTACAATGAACTGCAGAAAGAT AAGATGGCGGAGGCCTACAGTGAGATTGGGATGAAAG GCGAGCGCCGGAGGGGCAAGGGGCACGATGGCCTTTA CCAGGGGCTCAGTACAGCCACCAAGGACACCTACGAC GCCCTTCACATGCAGGCCCTGCCCCCTCGC	
D04AMD11-A01_CD8_CD28_41BB_CD3Z (P1734)	ATGGCCAGATCTCCTGCTCAACTGCTGGGACTGCTGCT GCTGTGGCTTAGCGGAGCCAGATGCCAGGTGCAGCTG GTTGAGTCTGGGGGAGGCCTTGTCCAGGCTGGGGGGTC CCTGAGACTCTCCTGTGCAGCGTCTGGAAGCGAATTCA CCGGTTATCCCATGGGCTGGTTTCGCCAGGCTCCAGGC AAGGAAAGGGAGTTTGTCGCTGGCTCCGTAGGTATCG GTGGTAGTACAAACTATGCAGACTCCGTGAAGGGCCG ATTCACCATCTCCAGAGACAATGCGAAGAACACGGTCT ATCTGCAAATGAACAGCCTGAAGCCAGAGGACACGGC TGTGTATTACTGTGCGGCCGACAAAGACTACTACAAAC CTTATAGTCGATATAGGACCGCTATCAGGTACGATACC TGGGGCCAAGGGACCCAGGTCACCGTCTCGAGCGGGT CTACCTCAGGGTCAGGGAAACCCGGAAGCGGCGAAGG GTCTACAAAAGGTGAGGTACAACTTTTGGAGTCAGGC GGTGGACTGGTACAACCGGGTGGTTCATTGCGTTTGAG CTGCGCTGCCTCTGGTTTGACCTCTTATTCCTACGCGAT GGGCTGGTATCGCCAAGCGCCGGGCAAAGAACGCGAG TTTGTCAGCGCAATCAGCTCGGGTGGTAGCGCGTACTA	119

	CGCGGACTCGGTAAAAGGCCGTTTTACGATCAGTCGTG ATAATTCCAAGAATACCTTGTACCTGCAAATGAATAGC CTTCGCGCAGAAGACACAGCGGTGTATTATTGTGCCGT TGGACCGTACTACGGATTTAGAGCGGTTACCGAAGCA GATTATTGGGGCCAGGGTACCCAGGTGACGGTCTCGA GCACAACAACTCCAGCCCCAAGACCACCTACGCCTGC ACCTACTATCGCATCTCAACCACTGTCCCTGCGCCCTG AGGCATGCCGACCAGCAGCCGGTGGCGCGGTGCATAC CCGCGGACTGGACTTTGCCTGCGATTTTTGGGTGCTGG TGGTGGTTGGTGGAGTCCTGGCTTGCTATAGCTTGCTA GTAACAGTGGCCTTTATTATTTTCTGGGTGAAACGGGG CAGAAAGAAACTCCTGTATATATTCAAACAACCATTTA TGCGACCAGTACAAACTACTCAAGAGGAAGATGGCTG TAGCTGCCGATTTCCAGAAGAAGAAGAAGGAGGATGT GAACTGAGAGTGAAGTTCAGCAGGAGCGCAGACGCCC CCGCGTACCAGCAGGGCCAGAACCAGCTCTATAACGA GCTCAATCTAGGACGAAGAGAGGAGTACGATGTTTTG GACAAGCGACGTGGCCGGGACCCTGAGATGGGGGGAA AGCCGAGAAGGAAGAACCCTCAGGAAGGCCTGTACAA TGAACTGCAGAAAGATAAGATGGCGGAGGCCTACAGT GAGATTGGGATGAAAGGCGAGCGCCGGAGGGGCAAG GGGCACGATGGCCTTTACCAGGGACTCAGTACAGCCA CCAAGGACACCTACGACGCCCTTCACATGCAGGCCCTG CCCCCTCGC	
Amino Acid Sequences of Bispecific CARs Targeting CD19 and CD22:		
FMC63LC_E04_FMC63HC_CD28_CD28_CD28_CD3Z (P1973)	MARSPAQLLGLLLLWLSGARCDIQMTQTTSSLSASLGDR VTISCRASQDISKYLNWYQQKPDGTVKLLIYHTSRLHSGV PSRFSGSGSGTDYSLTISNLEQEDIATYFCQQGNTLPYTFG GGTKLEITGSTSGSGKPGSGEGSTKGEVQLLESGGGLVQP GGSLRLSCAASGFTSSSYVMGWYRQAPGKEREFVSSISTG GDAYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAV YYCAADVWYYHGGAYDYWGQGTQVTVSSGSTSGSGKP GSGEGSTKGEVKLQESGPGLVAPSQSLSVTCTVSGVSLPD YGVSWIRQPPRKGLEWLGVIWGSETTYYNSALKSRLTIIK DNSKSQVFLKMNSLQTDDTAIYYCAKHYYYGGSYAMDY WGQGTSVTVSSIEVMYPPPYLDNEKSNGTIIHVKGKHLCP SPLFPGPSKPFWVLVVVGGVLACYSLLVTVAFIIFWVRSK RSRLLHSDYMNMTPRRPGPTRKHYQPYAPPRDFAAYRSR VKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRR	120

	GRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMK GERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR	
FMC63LC_A01v1_FMC63HC_CD28_CD28_CD28_CD3Z (P1988)	MARSPAQLLGLLLLWLSGARCDIQMTQTTSSLSASLGDR VTISCRASQDISKYLNWYQQKPDGTVKLLIYHTSRLHSGV PSRFSGSGSGTDYSLTISNLEQEDIATYFCQQGNTLPYTFG GGTKLEITGSTSGSGKPGSGEGSTKGEVQLLESGGGLVQP GGSLRLSCAASGLTSYSYAMGWYRQAPGKEREFVSAISS GGSAYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAV YYCAVGPYYGFRAVTEADYWGQGTQVTVSSGSTSGSGK PGSGEGSTKGEVKLQESGPGLVAPSQSLSVTCTVSGVSLP DYGVSWIRQPPRKGLEWLGVIWGSETTYYNSALKSRLTII KDNSKSQVFLKMNSLQTDDTAIYYCAKHYYYGGSYAMD YWGQGTSVTVSSIEVMYPPPYLDNEKSNGTIIHVKGKHL CPSPLFPGPSKPFWVLVVVGGVLACYSLLVTVAFIIFWVR SKRSRLLHSDYMNMTPRRPGPTRKHYQPYAPPRDFAAYR SRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDK RRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIG MKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR	121
FMC63LC_A01v2_FMC63HC_CD28_CD28_CD28_CD3Z (P1988)	MARSPAQLLGLLLLWLSGARCDIQMTQTTSSLSASLGDR VTISCRASQDISKYLNWYQQKPDGTVKLLIYHTSRLHSGV PSRFSGSGSGTDYSLTISNLEQEDIATYFCQQGNTLPYTFG GGTKLEITGSTSGSGKPGSGEGSTKGEVQLLESGGGLVQP GGTLRLSCAASGLTCYSYAMGWYRQAPGKEREFVSAISS GGSAYYADSVKGRFTICRDNSKNTLYLQMNSLRAEDTA VYYCAVGPYYGFRAVTEADYWGQGTQVTVSSGSTSGSG KPGSGEGSTKGEVKLQESGPGLVAPSQSLSVTCTVSGVSL PDYGVSWIRQPPRKGLEWLGVIWGSETTYYNSALKSRLTI IKDNSKSQVFLKMNSLQTDDTAIYYCAKHYYYGGSYAM DYWGQGTSVTVSSIEVMYPPPYLDNEKSNGTIIHVKGKH LCPSPLFPGPSKPFWVLVVVGGVLACYSLLVTVAFIIFWV RSKRSRLLHSDYMNMTPRRPGPTRKHYQPYAPPRDFAAY RSRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLD KRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEI GMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPP R	157
FMC63LC_E04_E04_FMC63HC_CD28_CD28	MARSPAQLLGLLLLWLSGARCDIQMTQTTSSLSASLGDR VTISCRASQDISKYLNWYQQKPDGTVKLLIYHTSRLHSGV PSRFSGSGSGTDYSLTISNLEQEDIATYFCQQGNTLPYTFG	122

_CD28_CD3Z (P1974)	GGTKLEITGSTSGSGKPGSGEGSTKGEVQLLESGGGLVQP GGSLRLSCAASGFTSSSYVMGWYRQAPGKEREFVSSISTG GDAYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAV YYCAADVWYYHGGAYDYWGQGTQVTVSSGGGGSEVQ LLESGGGLVQPGGSLRLSCAASGFTSSCYVMGWYRQAPG KEREFVCTISTGGDAYYADSVKGRFTITRDNSKNTLYLQ MNSLRAEDTAVYYCAADVWYYHGGAYDYWGQGTQVT VSSGSTSGSGKPGSGEGSTKGEVKLQESGPGLVAPSQSLS VTCTVSGVSLPDYGVSWIRQPPRKGLEWLGVIWGSETTY YNSALKSRLTIIKDNSKSQVFLKMNSLQTDDTAIYYCAKH YYYGGSYAMDYWGQGTSVTVSSIEVMYPPPYLDNEKSN GTIIHVKGKHLCPSPLFPGPSKPFWVLVVVGGVLACYSLL VTVAFIIFWVRSKRSRLLHSDYMNMTPRRPGPTRKHYQP YAPPRDFAAYRSRVKFSRSADAPAYQQGQNQLYNELNL GRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQK DKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYD ALHMQALPPR	
FMC63LC_E04 _A01v2_FMC6 3HC_CD28_C D28_CD28_CD 3Z (P2012)	MARSPAQLLGLLLLWLSGARCDIQMTQTTSSLSASLGDR VTISCRASQDISKYLNWYQQKPDGTVKLLIYHTSRLHSGV PSRFSGSGSGTDYSLTISNLEQEDIATYFCQQGNTLPYTFG GGTKLEITGSTSGSGKPGSGEGSTKGEVQLLESGGGLVQP GGSLRLSCAASGFTSSSYVMGWYRQAPGKEREFVSSISTG GDAYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAV YYCAADVWYYHGGAYDYWGQGTQVTVSSGGGGSEVQ LLESGGGLVQPGGTLRLSCAASGLTCYSYAMGWYRQAP GKEREFVSAISSGGGSAYYADSVKGRFTICRDNSKNTLYLQ MNSLRAEDTAVYYCAVGPYYGFRAVTEADYWGQGTQV TVSSGSTSGSGKPGSGEGSTKGEVKLQESGPGLVAPSQSL SVTCTVSGVSLPDYGVSWIRQPPRKGLEWLGVIWGSETT YYNSALKSRLTIIKDNSKSQVFLKMNSLQTDDTAIYYCAK HYYYGGSYAMDYWGQGTSVTVSSIEVMYPPPYLDNEKS NGTIIHVKGKHLCPSPLFPGPSKPFWVLVVVGGVLACYSL LVTVAFIIFWVRSKRSRLLHSDYMNMTPRRPGPTRKHYQ PYAPPRDFAAYRSRVKFSRSADAPAYQQGQNQLYNELNL GRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQK DKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYD ALHMQALPPR	123
FMC63LC_E04 _A01v1_FMC6	MARSPAQLLGLLLLWLSGARCDIQMTQTTSSLSASLGDR VTISCRASQDISKYLNWYQQKPDGTVKLLIYHTSRLHSGV	159

3HC_CD28_CD28_CD28_CD3Z (P2012)	PSRFSGSGSGTDYSLTISNLEQEDIATYFCQQGNTLPYTFGGGTKLEITGSTSGSGKPGSGEGSTKGEVQLLESGGGLVQPGGSLRLSCAASGFTSSSYVMGWYRQAPGKEREFVSSISTGGDAYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAADVWYYHGGAYDYWGQGTQVTVSSGGGGSEVQLLESGGGLVQPGGSLRLSCAASGLTSYSYAMGWYRQAPGKEREFVSAISSGGSAYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAVGPYYGFRAVTEADYWGQGTQVTVSSGSTSGSGKPGSGEGSTKGEVKLQESGPGLVAPSQSLSVTCTVSGVSLPDYGVSWIRQPPRKGLEWLGVIWGSETTYYNSALKSRLTIIKDNSKSQVFLKMNSLQTDDTAIYYCAKHYYYGGSYAMDYWGQGTSVTVSSIEVMYPPPYLDNEKSNGTIIHVKGKHLCPSPLFPGPSKPFWVLVVVGGVLACYSLLVTVAFIIFWVRSKRSRLLHSDYMNMTPRRPGPTRKHYQPYAPPRDFAAYRSRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR	
FMC63LC_E04_D04AMD11_FMC63HC_CD28_CD28_CD28_CD3Z (P2013)	MARSPAQLLGLLLLWLSGARCDIQMTQTTSSLSASLGDRVTISCRASQDISKYLNWYQQKPDGTVKLLIYHTSRLHSGVPSRFSGSGSGTDYSLTISNLEQEDIATYFCQQGNTLPYTFGGGTKLEITGSTSGSGKPGSGEGSTKGEVQLLESGGGLVQPGGSLRLSCAASGFTSSSYVMGWYRQAPGKEREFVSSISTGGDAYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAADVWYYHGGAYDYWGQGTQVTVSSGGGGSQVQLVESGGGLVQAGGSLRLSCAASGSEFTGYPMGWFRQAPGKEREFVAGSVGIGGSTNYADSVKGRFTISRDNAKNTVYLQMNSLKPEDTAVYYCAADKDYYKPYSRYRTAIRYDTWGQGTQVTVSSGSTSGSGKPGSGEGSTKGEVKLQESGPGLVAPSQSLSVTCTVSGVSLPDYGVSWIRQPPRKGLEWLGVIWGSETTYYNSALKSRLTIIKDNSKSQVFLKMNSLQTDDTAIYYCAKHYYYGGSYAMDYWGQGTSVTVSSIEVMYPPPYLDNEKSNGTIIHVKGKHLCPSPLFPGPSKPFWVLVVVGGVLACYSLLVTVAFIIFWVRSKRSRLLHSDYMNMTPRRPGPTRKHYQPYAPPRDFAAYRSRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR	124

E04_FMC63_CD28_CD28_CD28_CD3Z (P1972)	MARSPAQLLGLLLLWLSGARCEVQLLESGGGLVQPGGSLRLSCAASGFTSSSYVMGWYRQAPGKEREFVSSISTGGDAYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAADVWYYHGGAYDYWGQGTQVTVSSGSTSGSGKPGSGEGSTKGDIQMTQTTSSLSASLGDRVTISCRASQDISKYLNWYQQKPDGTVKLLIYHTSRLHSGVPSRFSGSGSGTDYSLTISNLEQEDIATYFCQQGNTLPYTFGGGTKLEITGSTSGSGKPGSGEGSTKGEVKLQESGPGLVAPSQSLSVTCTVSGVSLPDYGVSWIRQPPRKGLEWLGVIWGSETTYYNSALKSRLTIIKDNSKSQVFLKMNSLQTDDTAIYYCAKHYYYGGSYAMDYWGQGTSVTVSSIEVMYPPPYLDNEKSNGTIIHVKGKHLCPSPLFPGPSKPFWVLVVVGGVLACYSLLVTVAFIIFWVRSKRSRLLHSDYMNMTPRRPGPTRKHYQPYAPPRDFAAYRSRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR	125
A01v1_FMC63_CD28_CD28_CD28_CD3Z (P2014)	MARSPAQLLGLLLLWLSGARCEVQLLESGGGLVQPGGSLRLSCAASGLTSYSYAMGWYRQAPGKEREFVSAISSGGSAYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAVGPYYGFRAVTEADYWGQGTQVTVSSGSTSGSGKPGSGEGSTKGDIQMTQTTSSLSASLGDRVTISCRASQDISKYLNWYQQKPDGTVKLLIYHTSRLHSGVPSRFSGSGSGTDYSLTISNLEQEDIATYFCQQGNTLPYTFGGGTKLEITGSTSGSGKPGSGEGSTKGEVKLQESGPGLVAPSQSLSVTCTVSGVSLPDYGVSWIRQPPRKGLEWLGVIWGSETTYYNSALKSRLTIIKDNSKSQVFLKMNSLQTDDTAIYYCAKHYYYGGSYAMDYWGQGTSVTVSSIEVMYPPPYLDNEKSNGTIIHVKGKHLCPSPLFPGPSKPFWVLVVVGGVLACYSLLVTVAFIIFWVRSKRSRLLHSDYMNMTPRRPGPTRKHYQPYAPPRDFAAYRSRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR	126
A01v2_FMC63_CD28_CD28_CD28_CD3Z (P2014)	MARSPAQLLGLLLLWLSGARCEVQLLESGGGLVQPGGTLRLSCAASGLTCYSYAMGWYRQAPGKEREFVSAISSGGSAYYADSVKGRFTICRDNSKNTLYLQMNSLRAEDTAVYYCAVGPYYGFRAVTEADYWGQGTQVTVSSGSTSGSGKPGSGEGSTKGDIQMTQTTSSLSASLGDRVTISCRASQDISKYLNWYQQKPDGTVKLLIYHTSRLHSGVPSRFSGSGSGTDYS	161

	LTISNLEQEDIATYFCQQGNTLPYTFGGGTKLEITGSTSGS GKPGSGEGSTKGEVKLQESGPGLVAPSQSLSVTCTVSGVS LPDYGVSWIRQPPRKGLEWLGVIWGSETTYYNSALKSRL TIIKDNSKSQVFLKMNSLQTDDTAIYYCAKHYYYGGSYA MDYWGQGTSVTVSSIEVMYPPPYLDNEKSNGTIIHVKGK HLCPSPLFPGPSKPFWVLVVVGGVLACYSLLVTVAFIIFW VRSKRSRLLHSDYMNMTPRRPGPTRKHYQPYAPPRDFAA YRSRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVL DKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSE IGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPP R	
D04AMD11_E 04_FMC63_CD 28_CD28_CD2 8_CD3Z (P2015)	MARSPAQLLGLLLLWLSGARCQVQLVESGGGLVQAGGS LRLSCAASGSEFTGYPMGWFRQAPGKEREFVAGSVGIGG STNYADSVKGRFTISRDNAKNTVYLQMNSLKPEDTAVYY CAADKDYYKPYSRYRTAIRYDTWGQGTQVTVSSGGGGS EVQLLESGGGLVQPGGSLRLSCAASGFTSSSYVMGWYRQ APGKEREFVSSISTGGDAYYADSVKGRFTISRDNSKNTLY LQMNSLRAEDTAVYYCAADVWYYHGGAYDYWGQGTQ VTVSSGSTSGSGKPGSGEGSTKGDIQMTQTTSSLSASLGD RVTISCRASQDISKYLNWYQQKPDGTVKLLIYHTSRLHSG VPSRFSGSGSGTDYSLTISNLEQEDIATYFCQQGNTLPYTF GGGTKLEITGSTSGSGKPGSGEGSTKGEVKLQESGPGLVA PSQSLSVTCTVSGVSLPDYGVSWIRQPPRKGLEWLGVIW GSETTYYNSALKSRLTIIKDNSKSQVFLKMNSLQTDDTAI YYCAKHYYYGGSYAMDYWGQGTSVTVSSIEVMYPPPYL DNEKSNGTIIHVKGKHLCPSPLFPGPSKPFWVLVVVGGVL ACYSLLVTVAFIIFWVRSKRSRLLHSDYMNMTPRRPGPTR KHYQPYAPPRDFAAYRSRVKFSRSADAPAYQQGQNQLY NELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLY NELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTAT KDTYDALHMQALPPR	127
D04_E04_A01v 2_D04_FMC63 _CD28_CD28_ CD28_CD3Z (P2016)	MARSPAQLLGLLLLWLSGARCQVQLVESGGGLVQAGGS LRLSCAASGSEFTGYPMGWFRQAPGKEREFVAGSVGIGG STNYADSVKGRFTISRDNAKNTVYLQMNSLKPEDTAVYY CAADKDYYKPYSRYRTAIRYDTWGQGTQVTVSSGGGGS EVQLLESGGGLVQPGGSLRLSCAASGFTSSSYVMGWYRQ APGKEREFVSSISTGGDAYYADSVKGRFTISRDNSKNTLY LQMNSLRAEDTAVYYCAADVWYYHGGAYDYWGQGTQ VTVSSGGGGSEVQLLESGGGLVQPGGTLRLSCAASGLTC	128

	YSYAMGWYRQAPGKEREFVSAISSGGSAYYADSVKGRF TICRDNSKNTLYLQMNSLRAEDTAVYYCAVGPYYGFRA VTEADYWGQGTQVTVSSGGGGSQVQLVESGGGLVQAG GSLRLSCAASGSEFTGYPMGWFRQAPGKEREFVAGSVGI GGSTNYADSVKGRFTISRDNAKNTVYLQMNSLKPEDTAV YYCAADKDYYKPYSRYRTAIRYDTWGQGTQVTVSSGST SGSGKPGSGEGSTKGDIQMTQTTSSLSASLGDRVTISCRA SQDISKYLNWYQQKPDGTVKLLIYHTSRLHSGVPSRFSGS GSGTDYSLTISNLEQEDIATYFCQQGNTLPYTFGGGTKLEI TGSTSGSGKPGSGEGSTKGEVKLQESGPGLVAPSQSLSVT CTVSGVSLPDYGVSWIRQPPRKGLEWLGVIWGSETTYYN SALKSRLTIIKDNSKSQVFLKMNSLQTDDTAIYYCAKHYY YGGSYAMDYWGQGTSVTVSSIEVMYPPPYLDNEKSNGTI IHVKGKHLCPSPLFPGPSKPFWVLVVVGGVLACYSLLVT VAFIIFWVRSKRSRLLHSDYMNMTPRRPGPTRKHYQPYA PPRDFAAYRSRVKFSRSADAPAYQQGQNQLYNELNLGRR EEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDK MAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDAL HMQALPPR	
D04_E04_A01v1_D04_FMC63_CD28_CD28_CD28_CD3Z (P2016)	MARSPAQLLGLLLLWLSGARCQVQLVESGGGLVQAGGS LRLSCAASGSEFTGYPMGWFRQAPGKEREFVAGSVGIGG STNYADSVKGRFTISRDNAKNTVYLQMNSLKPEDTAVYY CAADKDYYKPYSRYRTAIRYDTWGQGTQVTVSSGGGGS EVQLLESGGGLVQPGGSLRLSCAASGFTSSSYVMGWYRQ APGKEREFVSSISTGGDAYYADSVKGRFTISRDNSKNTLY LQMNSLRAEDTAVYYCAADVWYYHGGAYDYWGQGTQ VTVSSGGGGSEVQLLESGGGLVQPGGSLRLSCAASGLTS YSYAMGWYRQAPGKEREFVSAISSGGSAYYADSVKGRF TISRDNSKNTLYLQMNSLRAEDTAVYYCAVGPYYGFRA VTEADYWGQGTQVTVSSGGGGSQVQLVESGGGLVQAG GSLRLSCAASGSEFTGYPMGWFRQAPGKEREFVAGSVGI GGSTNYADSVKGRFTISRDNAKNTVYLQMNSLKPEDTAV YYCAADKDYYKPYSRYRTAIRYDTWGQGTQVTVSSGST SGSGKPGSGEGSTKGDIQMTQTTSSLSASLGDRVTISCRA SQDISKYLNWYQQKPDGTVKLLIYHTSRLHSGVPSRFSGS GSGTDYSLTISNLEQEDIATYFCQQGNTLPYTFGGGTKLEI TGSTSGSGKPGSGEGSTKGEVKLQESGPGLVAPSQSLSVT CTVSGVSLPDYGVSWIRQPPRKGLEWLGVIWGSETTYYN SALKSRLTIIKDNSKSQVFLKMNSLQTDDTAIYYCAKHYY YGGSYAMDYWGQGTSVTVSSIEVMYPPPYLDNEKSNGTI	163

	IHVKGKHLCPSPLFPGPSKPFWVLVVVGGVLACYSLLVT VAFIIFWVRSKRSRLLHSDYMNMTPRRPGPTRKHYQPYA PPRDFAAYRSRVKFSRSADAPAYQQGQNQLYNELNLGRR EEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDK MAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDAL HMQALPPR	
FMC63LC_A01v1_A01v2_FMC63HC_CD28_CD28_CD28_CD3Z (P2193)	MARSPAQLLGLLLLWLSGARCDIQMTQTTSSLSASLGDR VTISCRASQDISKYLNWYQQKPDGTVKLLIYHTSRLHSGV PSRFSGSGSGTDYSLTISNLEQEDIATYFCQQGNTLPYTFG GGTKLEITGSTSGSGKPGSGEGSTKGEVQLLESGGGLVQP GGSLRLSCAASGLTSYSYAMGWYRQAPGKEREFVSAISS GGSAYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAV YYCAVGPYYGFRAVTEADYWGQGTQVTVSSGGGGSEV QLLESGGGLVQPGGTLRLSCAASGLTCYSYAMGWYRQA PGKEREFVSAISSGGSAYYADSVKGRFTICRDNSKNTLYL QMNSLRAEDTAVYYCAVGPYYGFRAVTEADYWGQGTQ VTVSSGSTSGSGKPGSGEGSTKGEVKLQESGPGLVAPSQS LSVTCTVSGVSLPDYGVSWIRQPPRKGLEWLGVIWGSET TYYNSALKSRLTIIKDNSKSQVFLKMNSLQTDDTAIYYCA KHYYYGGSYAMDYWGQGTSVTVSSIEVMYPPPYLDNEK SNGTIIHVKGKHLCPSPLFPGPSKPFWVLVVVGGVLACYS LLVTVAFIIFWVRSKRSRLLHSDYMNMTPRRPGPTRKHY QPYAPPRDFAAYRSRVKFSRSADAPAYQQGQNQLYNEL NLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNEL QKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDT YDALHMQALPPR	129
FMC63LC_A01v2_A01v1_FMC63HC_CD28_CD28_CD28_CD3Z (P2193)	MARSPAQLLGLLLLWLSGARCDIQMTQTTSSLSASLGDR VTISCRASQDISKYLNWYQQKPDGTVKLLIYHTSRLHSGV PSRFSGSGSGTDYSLTISNLEQEDIATYFCQQGNTLPYTFG GGTKLEITGSTSGSGKPGSGEGSTKGEVQLLESGGGLVQP GGTLRLSCAASGLTCYSYAMGWYRQAPGKEREFVSAISS GGSAYYADSVKGRFTICRDNSKNTLYLQMNSLRAEDTA VYYCAVGPYYGFRAVTEADYWGQGTQVTVSSGGGGSE VQLLESGGGLVQPGGSLRLSCAASGLTSYSYAMGWYRQ APGKEREFVSAISSGGSAYYADSVKGRFTISRDNSKNTLY LQMNSLRAEDTAVYYCAVGPYYGFRAVTEADYWGQGT QVTVSSGSTSGSGKPGSGEGSTKGEVKLQESGPGLVAPSQ SLSVTCTVSGVSLPDYGVSWIRQPPRKGLEWLGVIWGSE TTYYNSALKSRLTIIKDNSKSQVFLKMNSLQTDDTAIYYC	173

	AKHYYYGGSYAMDYWGQGTSVTVSSIEVMYPPPYLDNE KSNGTIIHVKGKHLCPSPLFPGPSKPFWVLVVVGGVLACY SLLVTVAFIIFWVRSKRSRLLHSDYMNMTPRRPGPTRKHY QPYAPPRDFAAYRSRVKFSRSADAPAYQQGQNQLYNEL NLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNEL QKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDT YDALHMQALPPR	
FMC63LC_A01v1_A01v1_FMC63HC_CD28_CD28_CD28_CD3Z (P2193)	MARSPAQLLGLLLLWLSGARCDIQMTQTTSSLSASLGDR VTISCRASQDISKYLNWYQQKPDGTVKLLIYHTSRLHSGV PSRFSGSGSGTDYSLTISNLEQEDIATYFCQQGNTLPYTFG GGTKLEITGSTSGSGKPGSGEGSTKGEVQLLESGGGLVQP GGSLRLSCAASGLTSYSYAMGWYRQAPGKEREFVSAISS GGSAYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAV YYCAVGPYYGFRAVTEADYWGQGTQVTVSSGGGGSEV QLLESGGGLVQPGGSLRLSCAASGLTSYSYAMGWYRQA PGKEREFVSAISSGGSAYYADSVKGRFTISRDNSKNTLYL QMNSLRAEDTAVYYCAVGPYYGFRAVTEADYWGQGTQ VTVSSGSTSGSGKPGSGEGSTKGEVKLQESGPGLVAPSQS LSVTCTVSGVSLPDYGVSWIRQPPRKGLEWLGVIWGSET TYYNSALKSRLTIIKDNSKSQVFLKMNSLQTDDTAIYYCA KHYYYGGSYAMDYWGQGTSVTVSSIEVMYPPPYLDNEK SNGTIIHVKGKHLCPSPLFPGPSKPFWVLVVVGGVLACYS LLVTVAFIIFWVRSKRSRLLHSDYMNMTPRRPGPTRKHY QPYAPPRDFAAYRSRVKFSRSADAPAYQQGQNQLYNEL NLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNEL QKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDT YDALHMQALPPR	174
FMC63LC_A01v2_A01v2_FMC63HC_CD28_CD28_CD28_CD3Z (P2193)	MARSPAQLLGLLLLWLSGARCDIQMTQTTSSLSASLGDR VTISCRASQDISKYLNWYQQKPDGTVKLLIYHTSRLHSGV PSRFSGSGSGTDYSLTISNLEQEDIATYFCQQGNTLPYTFG GGTKLEITGSTSGSGKPGSGEGSTKGEVQLLESGGGLVQP GGTLRLSCAASGLTCYSYAMGWYRQAPGKEREFVSAISS GGSAYYADSVKGRFTICRDNSKNTLYLQMNSLRAEDTA VYYCAVGPYYGFRAVTEADYWGQGTQVTVSSGGGGSE VQLLESGGGLVQPGGTLRLSCAASGLTCYSYAMGWYRQ APGKEREFVSAISSGGSAYYADSVKGRFTICRDNSKNTLY LQMNSLRAEDTAVYYCAVGPYYGFRAVTEADYWGQGT QVTVSSGSTSGSGKPGSGEGSTKGEVKLQESGPGLVAPSQ SLSVTCTVSGVSLPDYGVSWIRQPPRKGLEWLGVIWGSE	175

	TTYYNSALKSRLTIIKDNSKSQVFLKMNSLQTDDTAIYYC AKHYYYGGSYAMDYWGQGTSVTVSSIEVMYPPPYLDNE KSNGTIIHVKGKHLCPSPLFPGPSKPFWVLVVVGGVLACY SLLVTVAFIIFWVRSKRSRLLHSDYMNMTPRRPGPTRKHY QPYAPPRDFAAYRSRVKFSRSADAPAYQQGQNQLYNEL NLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNEL QKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDT YDALHMQALPPR	
FMC63LC_D04AMD11_A01v2_FMC63HC_CD28_CD28_CD28_CD3Z (P2191)	MARSPAQLLGLLLLWLSGARCDIQMTQTTSSLSASLGDR VTISCRASQDISKYLNWYQQKPDGTVKLLIYHTSRLHSGV PSRFSGSGSGTDYSLTISNLEQEDIATYFCQQGNTLPYTFG GGTKLEITGSTSGSGKPGSGEGSTKGQVQLVESGGGLVQ AGGSLRLSCAASGSEFTGYPMGWFRQAPGKEREFVAGSV GIGGSTNYADSVKGRFTISRDNAKNTVYLQMNSLKPEDT AVYYCAADKDYYKPYSRYRTAIRYDTWGQGTQVTVSSG GGGSEVQLLESGGGLVQPGGTLRLSCAASGLTCYSYAMG WYRQAPGKEREFVSAISSGGSAYYADSVKGRFTICRDNS KNTLYLQMNSLRAEDTAVYYCAVGPYYGFRAVTEADY WGQGTQVTVSSGSTSGSGKPGSGEGSTKGEVKLQESGPG LVAPSQSLSVTCTVSGVSLPDYGVSWIRQPPRKGLEWLG VIWGSETTYYNSALKSRLTIIKDNSKSQVFLKMNSLQTDD TAIYYCAKHYYYGGSYAMDYWGQGTSVTVSSIEVMYPP PYLDNEKSNGTIIHVKGKHLCPSPLFPGPSKPFWVLVVVG GVLACYSLLVTVAFIIFWVRSKRSRLLHSDYMNMTPRRP GPTRKHYQPYAPPRDFAAYRSRVKFSRSADAPAYQQGQ NQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQ EGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGL STATKDTYDALHMQALPPR	130
FMC63LC_D04AMD11_A01v1_FMC63HC_CD28_CD28_CD28_CD3Z (P2191)	MARSPAQLLGLLLLWLSGARCDIQMTQTTSSLSASLGDR VTISCRASQDISKYLNWYQQKPDGTVKLLIYHTSRLHSGV PSRFSGSGSGTDYSLTISNLEQEDIATYFCQQGNTLPYTFG GGTKLEITGSTSGSGKPGSGEGSTKGQVQLVESGGGLVQ AGGSLRLSCAASGSEFTGYPMGWFRQAPGKEREFVAGSV GIGGSTNYADSVKGRFTISRDNAKNTVYLQMNSLKPEDT AVYYCAADKDYYKPYSRYRTAIRYDTWGQGTQVTVSSG GGGSEVQLLESGGGLVQPGGSLRLSCAASGLTSYSYAMG WYRQAPGKEREFVSAISSGGSAYYADSVKGRFTISRDNS KNTLYLQMNSLRAEDTAVYYCAVGPYYGFRAVTEADY WGQGTQVTVSSGSTSGSGKPGSGEGSTKGEVKLQESGPG	171

	LVAPSQSLSVTCTVSGVSLPDYGVSWIRQPPRKGLEWLG VIWGSETTYYNSALKSRLTIIKDNSKSQVFLKMNSLQTDD TAIYYCAKHYYYGGSYAMDYWGQGTSVTVSSIEVMYPP PYLDNEKSNGTIIHVKGKHLCPSPLFPGPSKPFWVLVVVG GVLACYSLLVTVAFIIFWVRSKRSRLLHSDYMNMTPRRP GPTRKHYQPYAPPRDFAAYRSRVKFSRSADAPAYQQGQ NQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQ EGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGL STATKDTYDALHMQALPPR	
FMC63LC_A01v1_D04AMD11__A01v2_FMC63HC_CD28_CD28_CD28_CD3Z (P2195)	MARSPAQLLGLLLLWLSGARCDIQMTQTTSSLSASLGDR VTISCRASQDISKYLNWYQQKPDGTVKLLIYHTSRLHSGV PSRFSGSGSGTDYSLTISNLEQEDIATYFCQQGNTLPYTFG GGTKLEITGSTSGSGKPGSGEGSTKGEVQLLESGGGLVQP GGSLRLSCAASGLTSYSYAMGWYRQAPGKEREFVSAISS GGSAYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAV YYCAVGPYYGFRAVTEADYWGQGTQVTVSSGGGGSQV QLVESGGGLVQAGGSLRLSCAASGSEFTGYPMGWFRQA PGKEREFVAGSVGIGGSTNYADSVKGRFTISRDNAKNTV YLQMNSLKPEDTAVYYCAADKDYYKPYSRYRTAIRYDT WGQGTQVTVSSGGGGSEVQLLESGGGLVQPGGTLRLSC AASGLTCYSYAMGWYRQAPGKEREFVSAISSGGSAYYA DSVKGRFTICRDNSKNTLYLQMNSLRAEDTAVYYCAVGP YYGFRAVTEADYWGQGTQVTVSSGSTSGSGKPGSGEGST KGEVKLQESGPGLVAPSQSLSVTCTVSGVSLPDYGVSWIR QPPRKGLEWLGVIWGSETTYYNSALKSRLTIIKDNSKSQV FLKMNSLQTDDTAIYYCAKHYYYGGSYAMDYWGQGTS VTVSSIEVMYPPPYLDNEKSNGTIIHVKGKHLCPSPLFPGP SKPFWVLVVVGGVLACYSLLVTVAFIIFWVRSKRSRLLHS DYMNMTPRRPGPTRKHYQPYAPPRDFAAYRSRVKFSRSA DAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEM GGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRG KGHDGLYQGLSTATKDTYDALHMQALPPR	131
FMC63LC_A01v2_D04AMD11__A01v1_FMC63HC_CD28_CD28_CD28_CD3Z (P2195)	MARSPAQLLGLLLLWLSGARCDIQMTQTTSSLSASLGDR VTISCRASQDISKYLNWYQQKPDGTVKLLIYHTSRLHSGV PSRFSGSGSGTDYSLTISNLEQEDIATYFCQQGNTLPYTFG GGTKLEITGSTSGSGKPGSGEGSTKGEVQLLESGGGLVQP GGTLRLSCAASGLTCYSYAMGWYRQAPGKEREFVSAISS GGSAYYADSVKGRFTICRDNSKNTLYLQMNSLRAEDTA VYYCAVGPYYGFRAVTEADYWGQGTQVTVSSGGGGSQ	165

	VQLVESGGGLVQAGGSLRLSCAASGSEFTGYPMGWFRQ APGKEREFVAGSVGIGGSTNYADSVKGRFTISRDNAKNT VYLQMNSLKPEDTAVYYCAADKDYYKPYSRYRTAIRYD TWGQGTQVTVSSGGGGSEVQLLESGGGLVQPGGSLRLSC AASGLTSYSYAMGWYRQAPGKEREFVSAISSGGSAYYA DSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAVGP YYGFRAVTEADYWGQGTQVTVSSGSTSGSGKPGSGEGST KGEVKLQESGPGLVAPSQSLSVTCTVSGVSLPDYGVSWIR QPPRKGLEWLGVIWGSETTYYNSALKSRLTIIKDNSKSQV FLKMNSLQTDDTAIYYCAKHYYYGGSYAMDYWGQGTS VTVSSIEVMYPPPYLDNEKSNGTIIHVKGKHLCPSPLFPGP SKPFWVLVVVGGVLACYSLLVTVAFIIFWVRSKRSRLLHS DYMNMTPRRPGPTRKHYQPYAPPRDFAAYRSRVKFSRSA DAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEM GGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRG KGHDGLYQGLSTATKDTYDALHMQALPPR	
FMC63LC_A01v1_D04AMD11__A01v1_FMC63HC_CD28_CD28_CD28_CD3Z (P2195)	MARSPAQLLGLLLLWLSGARCDIQMTQTTSSLSASLGDR VTISCRASQDISKYLNWYQQKPDGTVKLLIYHTSRLHSGV PSRFSGSGSGTDYSLTISNLEQEDIATYFCQQGNTLPYTFG GGTKLEITGSTSGSGKPGSGEGSTKGEVQLLESGGGLVQP GGSLRLSCAASGLTSYSYAMGWYRQAPGKEREFVSAISS GGSAYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAV YYCAVGPYYGFRAVTEADYWGQGTQVTVSSGGGGSQV QLVESGGGLVQAGGSLRLSCAASGSEFTGYPMGWFRQA PGKEREFVAGSVGIGGSTNYADSVKGRFTISRDNAKNTV YLQMNSLKPEDTAVYYCAADKDYYKPYSRYRTAIRYDT WGQGTQVTVSSGGGGSEVQLLESGGGLVQPGGSLRLSC AASGLTSYSYAMGWYRQAPGKEREFVSAISSGGSAYYA DSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAVGP YYGFRAVTEADYWGQGTQVTVSSGSTSGSGKPGSGEGST KGEVKLQESGPGLVAPSQSLSVTCTVSGVSLPDYGVSWIR QPPRKGLEWLGVIWGSETTYYNSALKSRLTIIKDNSKSQV FLKMNSLQTDDTAIYYCAKHYYYGGSYAMDYWGQGTS VTVSSIEVMYPPPYLDNEKSNGTIIHVKGKHLCPSPLFPGP SKPFWVLVVVGGVLACYSLLVTVAFIIFWVRSKRSRLLHS DYMNMTPRRPGPTRKHYQPYAPPRDFAAYRSRVKFSRSA DAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEM GGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRG KGHDGLYQGLSTATKDTYDALHMQALPPR	166

FMC63LC_A01v2_D04AMD11__A01v2_FMC63HC_CD28_CD28_CD28_CD3Z (P2195)	MARSPAQLLGLLLLWLSGARCDIQMTQTTSSLSASLGDR VTISCRASQDISKYLNWYQQKPDGTVKLLIYHTSRLHSGV PSRFSGSGSGTDYSLTISNLEQEDIATYFCQQGNTLPYTFG GGTKLEITGSTSGSGKPGSGEGSTKGEVQLLESGGGLVQP GGTLRLSCAASGLTCYSYAMGWYRQAPGKEREFVSAISS GGSAYYADSVKGRFTICRDNSKNTLYLQMNSLRAEDTA VYYCAVGPYYGFRAVTEADYWGQGTQVTVSSGGGGSQ VQLVESGGGLVQAGGSLRLSCAASGSEFTGYPMGWFRQ APGKEREFVAGSVGIGGSTNYADSVKGRFTISRDNAKNT VYLQMNSLKPEDTAVYYCAADKDYYKPYSRYRTAIRYD TWGQGTQVTVSSGGGGSEVQLLESGGGLVQPGGTLRLSC AASGLTCYSYAMGWYRQAPGKEREFVSAISSGGSAYYA DSVKGRFTICRDNSKNTLYLQMNSLRAEDTAVYYCAVGP YYGFRAVTEADYWGQGTQVTVSSGSTSGSGKPGSGEGST KGEVKLQESGPGLVAPSQSLSVTCTVSGVSLPDYGVSWIR QPPRKGLEWLGVIWGSETTYYNSALKSRLTIIKDNSKSQV FLKMNSLQTDDTAIYYCAKHYYYGGSYAMDYWGQGTS VTVSSIEVMYPPPYLDNEKSNGTIIHVKGKHLCPSPLFPGP SKPFWVLVVVGGVLACYSLLVTVAFIIFWVRSKRSRLLHS DYMNMTPRRPGPTRKHYQPYAPPRDFAAYRSRVKFSRSA DAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEM GGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRG KGHDGLYQGLSTATKDTYDALHMQALPPR	167
Polynucleotide Sequences of Bispecific CARs Targeting CD19 and CD22:		
FMC63LC_E04_FMC63HC_CD28_CD28_CD28_CD3Z (P1973)	ATGGCCAGATCTCCTGCTCAACTGCTGGGACTGCTGCT GCTGTGGCTTAGCGGAGCCAGATGCGACATCCAGATG ACCCAGACCACAAGCAGCCTGTCTGCCAGCCTGGGCG ATAGAGTGACCATCAGCTGTAGAGCCAGCCAGGACAT CAGCAAGTACCTGAACTGGTATCAGCAAAAGCCCGAC GGCACCGTGAAGCTGCTGATCTACCACACCAGCAGACT GCACAGCGGCGTGCCAAGCAGATTTTCTGGCAGCGGCT CTGGCACCGACTACAGCCTGACAATCAGCAACCTGGA ACAAGAGGATATCGCTACCTACTTCTGCCAGCAAGGCA ACACCCTGCCTTACACCTTTGGCGGAGGCACCAAGCTG GAAATCACCGGCTCTACAAGCGGCAGCGGCAAACCTG GATCTGGCGAGGGATCTACCAAGGGCGAGGTACAACT TTTGGAGTCAGGCGGTGGACTGGTACAACCGGGTGGTT CATTGCGTTTGAGCTGCGCTGCCTCTGGTTTTACCAGCT CCTCCTACGTGATGGGCTGGTATCGCCAAGCGCCGGGC	132

	AAAGAACGCGAGTTTGTCAGCTCGATCAGCACCGGTG GTGATGCCTACTACGCGGACTCGGTAAAAGGCCGTTTT ACGATCAGTCGTGATAATTCCAAGAATACCTTGTACCT GCAAATGAATAGCCTTCGCGCAGAAGACACAGCGGTG TATTATTGTGCCGCTGACGTTTGGTACTACCACGGCGG CGCGTACGATTATTGGGGCCAGGGTACCCAGGTGACG GTCTCGAGCGGCAGTACTTCTGGTAGCGGAAAACCCG GTAGCGGCGAGGGGTCAACTAAAGGAGAAGTGAAACT GCAAGAGTCTGGCCCTGGACTGGTGGCCCCATCTCAGT CTCTGAGCGTGACCTGTACAGTCAGCGGAGTGTCCCTG CCTGATTACGGCGTGTCCTGGATCAGACAGCCTCCTCG GAAAGGCCTGGAATGGCTGGGAGTGATCTGGGGCAGC GAGACAACCTACTACAACAGCGCCCTGAAGTCCCGGC TGACCATCATCAAGGACAACTCCAAGAGCCAGGTGTTC CTGAAGATGAACAGCCTGCAGACCGACGACACCGCCA TCTACTATTGCGCCAAGCACTACTACTACGGCGGCAGC TACGCCATGGATTATTGGGGCCAGGGCACCAGCGTGA CCGTGTCTAGCATCGAAGTGATGTACCCTCCACCTTAC CTGGACAACGAGAAGTCCAACGGCACCATCATCCACG TGAAGGGCAAGCACCTGTGTCCTTCTCCACTGTTCCCC GGACCTAGCAAGCCTTTCTGGGTGCTCGTTGTTGTTGG CGGCGTGCTGGCCTGTTATAGCCTGCTTGTGACCGTGG CCTTCATCATCTTTTGGGTCCGAAGCAAGCGGAGCCGG CTGCTGCACTCCGACTACATGAACATGACCCCTAGACG GCCCGGACCAACCAGAAAGCACTACCAGCCTTACGCT CCTCCTAGAGACTTCGCCGCCTACCGGTCCAGAGTGAA GTTCAGCAGGAGCGCAGACGCCCCCGCGTACCAGCAG GGCCAGAACCAGCTCTATAACGAGCTCAATCTAGGAC GAAGAGAGGAGTACGATGTTTTGGACAAGCGACGTGG CCGGGACCCTGAGATGGGGGGAAAGCCGAGAAGGAA GAACCCTCAGGAAGGCCTGTACAATGAACTGCAGAAA GATAAGATGGCGGAGGCCTACAGTGAGATTGGGATGA AAGGCGAGCGCCGGAGGGGCAAGGGGCACGATGGCCT TTACCAGGGACTCAGTACAGCCACCAAGGACACCTAC GACGCCCTTCACATGCAGGCCCTGCCCCCTCGC	
FMC63LC_A01v1_FMC63HC_CD28_CD28_	ATGGCCAGATCTCCTGCTCAACTGCTGGGACTGCTGCT GCTGTGGCTTAGCGGAGCCAGATGCGACATCCAGATG ACCCAGACCACAAGCAGCCTGTCTGCCAGCCTGGGCG ATAGAGTGACCATCAGCTGTAGAGCCAGCCAGGACAT CAGCAAGTACCTGAACTGGTATCAGCAAAAGCCCGAC	133

CD28_CD3Z (P1988)	GGCACCGTGAAGCTGCTGATCTACCACACCAGCAGACT GCACAGCGGCGTGCCAAGCAGATTTTCTGGCAGCGGCT CTGGCACCGACTACAGCCTGACAATCAGCAACCTGGA ACAAGAGGATATCGCTACCTACTTCTGCCAGCAAGGCA ACACCCTGCCTTACACCTTTGGCGGAGGCACCAAGCTG GAAATCACCGGCTCTACAAGCGGCAGCGGCAAACCTG GATCTGGCGAGGGATCTACCAAGGGCGAGGTACAACT TTTGGAGTCAGGCGGTGGACTGGTACAACCGGGTGGTT CATTGCGTTTGAGCTGCGCTGCCTCTGGTTTGACCTCTT ATTCCTACGCGATGGGCTGGTATCGCCAAGCGCCGGGC AAAGAACGCGAGTTTGTCAGCGCAATCAGCTCGGGTG GTAGCGCGTACTACGCGGACTCGGTAAAAGGCCGTTTT ACGATCAGTCGTGATAATTCCAAGAATACCTTGTACCT GCAAATGAATAGCCTTCGCGCAGAAGACACAGCGGTG TATTATTGTGCCGTTGGACCGTACTACGGATTTAGAGC GGTTACCGAAGCAGATTATTGGGGCCAGGGTACCCAG GTGACGGTCTCGAGCGGCAGTACTTCTGGTAGCGGAA AACCCGGTAGCGGCGAGGGGTCAACTAAAGGAGAAGT GAAACTGCAAGAGTCTGGCCCTGGACTGGTGGCCCCAT CTCAGTCTCTGAGCGTGACCTGTACAGTCAGCGGAGTG TCCCTGCCTGATTACGGCGTGTCCTGGATCAGACAGCC TCCTCGGAAAGGCCTGGAATGGCTGGGAGTGATCTGG GGCAGCGAGACAACCTACTACAACAGCGCCCTGAAGT CCCGGCTGACCATCATCAAGGACAACTCCAAGAGCCA GGTGTTCCTGAAGATGAACAGCCTGCAGACCGACGAC ACCGCCATCTACTATTGCGCCAAGCACTACTACTACGG CGGCAGCTACGCCATGGATTATTGGGGCCAGGGCACC AGCGTGACCGTGTCTAGCATCGAAGTGATGTACCCTCC ACCTTACCTGGACAACGAGAAGTCCAACGGCACCATC ATCCACGTGAAGGGCAAGCACCTGTGTCCTTCTCCACT GTTCCCCGGACCTAGCAAGCCTTTCTGGGTGCTCGTTG TTGTTGGCGGCGTGCTGGCCTGTTATAGCCTGCTTGTG ACCGTGGCCTTCATCATCTTTTGGGTCCGAAGCAAGCG GAGCCGGCTGCTGCACTCCGACTACATGAACATGACCC CTAGACGGCCCGGACCAACCAGAAAGCACTACCAGCC TTACGCTCCTCCTAGAGACTTCGCCGCCTACCGGTCCA GAGTGAAGTTCAGCAGGAGCGCAGACGCCCCCGCGTA CCAGCAGGGCCAGAACCAGCTCTATAACGAGCTCAAT CTAGGACGAAGAGAGGAGTACGATGTTTTGGACAAGC GACGTGGCCGGGACCCTGAGATGGGGGGAAAGCCGAG	

	AAGGAAGAACCCTCAGGAAGGCCTGTACAATGAACTG CAGAAAGATAAGATGGCGGAGGCCTACAGTGAGATTG GGATGAAAGGCGAGCGCCGGAGGGGCAAGGGGCACG ATGGCCTTTACCAGGGACTCAGTACAGCCACCAAGGA CACCTACGACGCCCTTCACATGCAGGCCCTGCCCCCTC GC	
FMC63LC_A01v2_FMC63HC_CD28_CD28_CD28_CD3Z (P1988)	ATGGCCAGATCTCCTGCTCAACTGCTGGGACTGCTGCT GCTGTGGCTTAGCGGAGCCAGATGCGACATCCAGATG ACCCAGACCACAAGCAGCCTGTCTGCCAGCCTGGGCG ATAGAGTGACCATCAGCTGTAGAGCCAGCCAGGACAT CAGCAAGTACCTGAACTGGTATCAGCAAAAGCCCGAC GGCACCGTGAAGCTGCTGATCTACCACACCAGCAGACT GCACAGCGGCGTGCCAAGCAGATTTTCTGGCAGCGGCT CTGGCACCGACTACAGCCTGACAATCAGCAACCTGGA ACAAGAGGATATCGCTACCTACTTCTGCCAGCAAGGCA ACACCCTGCCTTACACCTTTGGCGGAGGCACCAAGCTG GAAATCACCGGCTCTACAAGCGGCAGCGGCAAACCTG GATCTGGCGAGGGATCTACCAAGGGCGAAGTCCAGCT GCTGGAAAGCGGTGGCGGTCTGGTCCAGCCTGGCGGC ACCCTGCGCCTGTCCTGTGCCGCTAGCGGCCTGACCTG CTATAGCTATGCCATGGGTTGGTACCGCCAGGCCCCTG GTAAGGAGCGCGAATTCGTGTCCGCTATTTCCAGCGGC GGCTCCGCCTATTATGCTGATAGCGTCAAGGGTCGCTT CACCATTTGCCGCGACAACAGCAAAAACACTCTGTATC TGCAGATGAACTCCCTGCGCGCTGAGGATACCGCCGTC TACTACTGCGCTGTGGGCCCTTATTATGGCTTCCGCGCT GTGACTGAGGCTGACTACTGGGGTCAGGGCACTCAGG TGACTGTGAGCAGCGGCAGTACTTCTGGTAGCGGAAA ACCCGGTAGCGGCGAGGGGTCAACTAAAGGAGAAGTG AAACTGCAAGAGTCTGGCCCTGGACTGGTGGCCCCATC TCAGTCTCTGAGCGTGACCTGTACAGTCAGCGGAGTGT CCCTGCCTGATTACGGCGTGTCCTGGATCAGACAGCCT CCTCGGAAAGGCCTGGAATGGCTGGGAGTGATCTGGG GCAGCGAGACAACCTACTACAACAGCGCCCTGAAGTC CCGGCTGACCATCATCAAGGACAACTCCAAGAGCCAG GTGTTCCTGAAGATGAACAGCCTGCAGACCGACGACA CCGCCATCTACTATTGCGCCAAGCACTACTACTACGGC GGCAGCTACGCCATGGATTATTGGGGCCAGGGCACCA GCGTGACCGTGTCTAGCATCGAAGTGATGTACCCTCCA CCTTACCTGGACAACGAGAAGTCCAACGGCACCATCAT	158

	CCACGTGAAGGGCAAGCACCTGTGTCCTTCTCCACTGT TCCCCGGACCTAGCAAGCCTTTCTGGGTGCTCGTTGTT GTTGGCGGCGTGCTGGCCTGTTATAGCCTGCTTGTGAC CGTGGCCTTCATCATCTTTTGGGTCCGAAGCAAGCGGA GCCGGCTGCTGCACTCCGACTACATGAACATGACCCCT AGACGGCCCGGACCAACCAGAAAGCACTACCAGCCTT ACGCTCCTCCTAGAGACTTCGCCGCCTACCGGTCCAGA GTGAAGTTCAGCAGGAGCGCAGACGCCCCCGCGTACC AGCAGGGCCAGAACCAGCTCTATAACGAGCTCAATCT AGGACGAAGAGAGGAGTACGATGTTTTGGACAAGCGA CGTGGCCGGGACCCTGAGATGGGGGGAAAGCCGAGAA GGAAGAACCCTCAGGAAGGCCTGTACAATGAACTGCA GAAAGATAAGATGGCGGAGGCCTACAGTGAGATTGGG ATGAAAGGCGAGCGCCGGAGGGGCAAGGGGCACGAT GGCCTTTACCAGGGACTCAGTACAGCCACCAAGGACA CCTACGACGCCCTTCACATGCAGGCCCTGCCCCCTCGC	
FMC63LC_E04_E04_FMC63HC_CD28_CD28_CD28_CD3Z (P1974)	ATGGCCAGATCTCCTGCTCAACTGCTGGGACTGCTGCT GCTGTGGCTTAGCGGAGCCAGATGCGACATCCAGATG ACCCAGACCACAAGCAGCCTGTCTGCCAGCCTGGGCG ATAGAGTGACCATCAGCTGTAGAGCCAGCCAGGACAT CAGCAAGTACCTGAACTGGTATCAGCAAAAGCCCGAC GGCACCGTGAAGCTGCTGATCTACCACACCAGCAGACT GCACAGCGGCGTGCCAAGCAGATTTTCTGGCAGCGGCT CTGGCACCGACTACAGCCTGACAATCAGCAACCTGGA ACAAGAGGATATCGCTACCTACTTCTGCCAGCAAGGCA ACACCCTGCCTTACACCTTTGGCGGAGGCACCAAGCTG GAAATCACCGGCTCTACAAGCGGCAGCGGCAAACCTG GATCTGGCGAGGGATCTACCAAGGGCGAGGTACAACT TTTGGAGTCAGGCGGTGGACTGGTACAACCGGGTGGTT CATTGCGTTTGAGCTGCGCTGCCTCTGGTTTTACCAGCT CCTCCTACGTGATGGGCTGGTATCGCCAAGCGCCGGGC AAAGAACGCGAGTTTGTCAGCTCGATCAGCACCGGTG GTGATGCCTACTACGCGGACTCGGTAAAAGGCCGTTTT ACGATCAGTCGTGATAATTCCAAGAATACCTTGTACCT GCAAATGAATAGCCTTCGCGCAGAAGACACAGCGGTG TATTATTGTGCCGCTGACGTTTGGTACTACCACGGCGG CGCGTACGATTATTGGGGCCAGGGTACCCAGGTGACG GTCTCGAGCGGCGGTGGCGGATCAGAAGTCCAGCTGC TGGAAAGCGGTGGCGGTCTGGTCCAGCCCGGCGGCAG CCTGCGCCTGTCCTGTGCCGCTAGCGGTTTCACTTCCA	134

	GCTGCTATGTCATGGGTTGGTACCGCCAGGCCCCCGGT AAGGAGCGCGAATTCGTGTGCACCATTTCCACTGGCGG CGACGCTTATTATGCTGATAGCGTGAAGGGTCGCTTCA CTATTACCCGCGACAACAGCAAAAACACTCTGTATCTG CAGATGAACTCCCTGCGCGCTGAGGATACCGCCGTCTA CTACTGCGCTGCCGATGTGTGGTATTATCATGGTGGTG CCTATGACTACTGGGGTCAGGGCACTCAGGTCACCGTC AGCTCCGGCAGTACTTCTGGTAGCGGAAAACCCGGTA GCGGCGAGGGGTCAACTAAAGGAGAAGTGAAACTGCA AGAGTCTGGCCCTGGACTGGTGGCCCCATCTCAGTCTC TGAGCGTGACCTGTACAGTCAGCGGAGTGTCCCTGCCT GATTACGGCGTGTCCTGGATCAGACAGCCTCCTCGGAA AGGCCTGGAATGGCTGGGAGTGATCTGGGGCAGCGAG ACAACCTACTACAACAGCGCCCTGAAGTCCCGGCTGAC CATCATCAAGGACAACTCCAAGAGCCAGGTGTTCCTGA AGATGAACAGCCTGCAGACCGACGACACCGCCATCTA CTATTGCGCCAAGCACTACTACTACGGCGGCAGCTACG CCATGGATTATTGGGGCCAGGGCACCAGCGTGACCGT GTCTAGCATCGAAGTGATGTACCCTCCACCTTACCTGG ACAACGAGAAGTCCAACGGCACCATCATCCACGTGAA GGGCAAGCACCTGTGTCCTTCTCCACTGTTCCCCGGAC CTAGCAAGCCTTTCTGGGTGCTCGTTGTTGTTGGCGGC GTGCTGGCCTGTTATAGCCTGCTTGTGACCGTGGCCTT CATCATCTTTTGGGTCCGAAGCAAGCGGAGCCGGCTGC TGCACTCCGACTACATGAACATGACCCCTAGACGGCCC GGACCAACCAGAAAGCACTACCAGCCTTACGCTCCTCC TAGAGACTTCGCCGCCTACCGGTCCAGAGTGAAGTTCA GCAGGAGCGCAGACGCCCCCGCGTACCAGCAGGGCCA GAACCAGCTCTATAACGAGCTCAATCTAGGACGAAGA GAGGAGTACGATGTTTTGGACAAGCGACGTGGCCGGG ACCCTGAGATGGGGGGAAAGCCGAGAAGGAAGAACCC TCAGGAAGGCCTGTACAATGAACTGCAGAAAGATAAG ATGGCGGAGGCCTACAGTGAGATTGGGATGAAAGGCG AGCGCCGGAGGGGCAAGGGGCACGATGGCCTTTACCA GGGACTCAGTACAGCCACCAAGGACACCTACGACGCC CTTCACATGCAGGCCCTGCCCCCTCGC	
FMC63LC_E04 _A01v2_FMC6 3HC_CD28_C	ATGGCCAGATCTCCTGCTCAACTGCTGGGACTGCTGCT GCTGTGGCTTAGCGGAGCCAGATGCGACATCCAGATG ACCCAGACCACAAGCAGCCTGTCTGCCAGCCTGGGCG ATAGAGTGACCATCAGCTGTAGAGCCAGCCAGGACAT	135

D28_CD28_CD3Z (P2012)	CAGCAAGTACCTGAACTGGTATCAGCAAAAGCCCGAC GGCACCGTGAAGCTGCTGATCTACCACACCAGCAGACT GCACAGCGGCGTGCCAAGCAGATTTTCTGGCAGCGGCT CTGGCACCGACTACAGCCTGACAATCAGCAACCTGGA ACAAGAGGATATCGCTACCTACTTCTGCCAGCAAGGCA ACACCCTGCCTTACACCTTTGGCGGAGGCACCAAGCTG GAAATCACCGGCTCTACAAGCGGCAGCGGCAAACCTG GATCTGGCGAGGGATCTACCAAGGGCGAGGTACAACT TTTGGAGTCAGGCGGTGGACTGGTACAACCGGGTGGTT CATTGCGTTTGAGCTGCGCTGCCTCTGGTTTTACCAGCT CCTCCTACGTGATGGGCTGGTATCGCCAAGCGCCGGGC AAAGAACGCGAGTTTGTCAGCTCGATCAGCACCGGTG GTGATGCCTACTACGCGGACTCGGTAAAAGGCCGTTTT ACGATCAGTCGTGATAATTCCAAGAATACCTTGTACCT GCAAATGAATAGCCTTCGCGCAGAAGACACAGCGGTG TATTATTGTGCCGCTGACGTTTGGTACTACCACGGCGG CGCGTACGATTATTGGGGCCAGGGTACCCAGGTGACG GTCTCGAGCGGCGGTGGCGGATCAGAAGTCCAGCTGC TGGAAAGCGGTGGCGGTCTGGTCCAGCCTGGCGGCAC CCTGCGCCTGTCCTGTGCCGCTAGCGGCCTGACCTGCT ATAGCTATGCCATGGGTTGGTACCGCCAGGCCCCTGGT AAGGAGCGCGAATTCGTGTCCGCTATTTCCAGCGGCGG CTCCGCCTATTATGCTGATAGCGTCAAGGGTCGCTTCA CCATTTGCCGCGACAACAGCAAAAACACTCTGTATCTG CAGATGAACTCCCTGCGCGCTGAGGATACCGCCGTCTA CTACTGCGCTGTGGGCCCTTATTATGGCTTCCGCGCTGT GACTGAGGCTGACTACTGGGGTCAGGGCACTCAGGTG ACTGTGAGCAGCGGCAGTACTTCTGGTAGCGGAAAAC CCGGTAGCGGCGAGGGGTCAACTAAAGGAGAAGTGAA ACTGCAAGAGTCTGGCCCTGGACTGGTGGCCCCATCTC AGTCTCTGAGCGTGACCTGTACAGTCAGCGGAGTGTCC CTGCCTGATTACGGCGTGTCCTGGATCAGACAGCCTCC TCGGAAAGGCCTGGAATGGCTGGGAGTGATCTGGGGC AGCGAGACAACCTACTACAACAGCGCCCTGAAGTCCC GGCTGACCATCATCAAGGACAACTCCAAGAGCCAGGT GTTCCTGAAGATGAACAGCCTGCAGACCGACGACACC GCCATCTACTATTGCGCCAAGCACTACTACTACGGCGG CAGCTACGCCATGGATTATTGGGGCCAGGGCACCAGC GTGACCGTGTCTAGCATCGAAGTGATGTACCCTCCACC TTACCTGGACAACGAGAAGTCCAACGGCACCATCATCC	

	ACGTGAAGGGCAAGCACCTGTGTCCTTCTCCACTGTTC CCCGGACCTAGCAAGCCTTTCTGGGTGCTCGTTGTTGT TGGCGGCGTGCTGGCCTGTTATAGCCTGCTTGTGACCG TGGCCTTCATCATCTTTTGGGTCCGAAGCAAGCGGAGC CGGCTGCTGCACTCCGACTACATGAACATGACCCCTAG ACGGCCCGGACCAACCAGAAAGCACTACCAGCCTTAC GCTCCTCCTAGAGACTTCGCCGCCTACCGGTCCAGAGT GAAGTTCAGCAGATCCGCCGATGCTCCCGCCTATCAGC AGGGCCAAAACCAGCTGTACAACGAGCTGAACCTGGG GAGAAGAGAAGAGTACGACGTGCTGGACAAGCGGAG AGGCAGAGATCCTGAAATGGGCGGCAAGCCCAGACGG AAGAATCCTCAAGAGGGCCTGTATAATGAGCTGCAGA AAGACAAGATGGCCGAGGCCTACAGCGAGATCGGAAT GAAGGGCGAGCGCAGAAGAGGCAAGGGACACGATGG ACTGTACCAGGGCCTGAGCACCGCCACCAAGGATACC TATGATGCCCTGCACATGCAGGCCCTGCCTCCAAGA	
FMC63LC_E04_A01v1_FMC63HC_CD28_CD28_CD28_CD3Z (P2012)	ATGGCCAGATCTCCTGCTCAACTGCTGGGACTGCTGCT GCTGTGGCTTAGCGGAGCCAGATGCGACATCCAGATG ACCCAGACCACAAGCAGCCTGTCTGCCAGCCTGGGCG ATAGAGTGACCATCAGCTGTAGAGCCAGCCAGGACAT CAGCAAGTACCTGAACTGGTATCAGCAAAAGCCCGAC GGCACCGTGAAGCTGCTGATCTACCACACCAGCAGACT GCACAGCGGCGTGCCAAGCAGATTTTCTGGCAGCGGCT CTGGCACCGACTACAGCCTGACAATCAGCAACCTGGA ACAAGAGGATATCGCTACCTACTTCTGCCAGCAAGGCA ACACCCTGCCTTACACCTTTGGCGGAGGCACCAAGCTG GAAATCACCGGCTCTACAAGCGGCAGCGGCAAACCTG GATCTGGCGAGGGATCTACCAAGGGCGAGGTACAACT TTTGGAGTCAGGCGGTGGACTGGTACAACCGGGTGGTT CATTGCGTTTGAGCTGCGCTGCCTCTGGTTTTACCAGCT CCTCCTACGTGATGGGCTGGTATCGCCAAGCGCCGGGC AAAGAACGCGAGTTTGTCAGCTCGATCAGCACCGGTG GTGATGCCTACTACGCGGACTCGGTAAAAGGCCGTTTT ACGATCAGTCGTGATAATTCCAAGAATACCTTGTACCT GCAAATGAATAGCCTTCGCGCAGAAGACACAGCGGTG TATTATTGTGCCGCTGACGTTTGGTACTACCACGGCGG CGCGTACGATTATTGGGGCCAGGGTACCCAGGTGACG GTCTCGAGCGGCGGTGGCGGATCAGAGGTACAACTTTT GGAGTCAGGCGGTGGACTGGTACAACCGGGTGGTTCA TTGCGTTTGAGCTGCGCTGCCTCTGGTTTGACCTCTTAT	160

	TCCTACGCGATGGGCTGGTATCGCCAAGCGCCGGGCA AAGAACGCGAGTTTGTCAGCGCAATCAGCTCGGGTGG TAGCGCGTACTACGCGGACTCGGTAAAAGGCCGTTTTA CGATCAGTCGTGATAATTCCAAGAATACCTTGTACCTG CAAATGAATAGCCTTCGCGCAGAAGACACAGCGGTGT ATTATTGTGCCGTTGGACCGTACTACGGATTTAGAGCG GTTACCGAAGCAGATTATTGGGGCCAGGGTACCCAGG TGACGGTCTCGAGCGGCAGTACTTCTGGTAGCGGAAA ACCCGGTAGCGGCGAGGGGTCAACTAAAGGAGAAGTG AAACTGCAAGAGTCTGGCCCTGGACTGGTGGCCCCATC TCAGTCTCTGAGCGTGACCTGTACAGTCAGCGGAGTGT CCCTGCCTGATTACGGCGTGTCCTGGATCAGACAGCCT CCTCGGAAAGGCCTGGAATGGCTGGGAGTGATCTGGG GCAGCGAGACAACCTACTACAACAGCGCCCTGAAGTC CCGGCTGACCATCATCAAGGACAACTCCAAGAGCCAG GTGTTCCTGAAGATGAACAGCCTGCAGACCGACGACA CCGCCATCTACTATTGCGCCAAGCACTACTACTACGGC GGCAGCTACGCCATGGATTATTGGGGCCAGGGCACCA GCGTGACCGTGTCTAGCATCGAAGTGATGTACCCTCCA CCTTACCTGGACAACGAGAAGTCCAACGGCACCATCAT CCACGTGAAGGGCAAGCACCTGTGTCCTTCTCCACTGT TCCCCGGACCTAGCAAGCCTTTCTGGGTGCTCGTTGTT GTTGGCGGCGTGCTGGCCTGTTATAGCCTGCTTGTGAC CGTGGCCTTCATCATCTTTTGGGTCCGAAGCAAGCGGA GCCGGCTGCTGCACTCCGACTACATGAACATGACCCCT AGACGGCCCGGACCAACCAGAAAGCACTACCAGCCTT ACGCTCCTCCTAGAGACTTCGCCGCCTACCGGTCCAGA GTGAAGTTCAGCAGATCCGCCGATGCTCCCGCCTATCA GCAGGGCCAAAACCAGCTGTACAACGAGCTGAACCTG GGGAGAAGAGAAGAGTACGACGTGCTGGACAAGCGG AGAGGCAGAGATCCTGAAATGGGCGGCAAGCCCAGAC GGAAGAATCCTCAAGAGGGCCTGTATAATGAGCTGCA GAAAGACAAGATGGCCGAGGCCTACAGCGAGATCGGA ATGAAGGGCGAGCGCAGAAGAGGCAAGGGACACGAT GGACTGTACCAGGGCCTGAGCACCGCCACCAAGGATA CCTATGATGCCCTGCACATGCAGGCCCTGCCTCCAAGA	
FMC63LC_E04 _D04AMD11_ FMC63HC_CD 28_CD28_CD2	ATGGCCAGATCTCCTGCTCAACTGCTGGGACTGCTGCT GCTGTGGCTTAGCGGAGCCAGATGCGACATCCAGATG ACCCAGACCACAAGCAGCCTGTCTGCCAGCCTGGGCG ATAGAGTGACCATCAGCTGTAGAGCCAGCCAGGACAT	136

8_CD3Z (P2013)	CAGCAAGTACCTGAACTGGTATCAGCAAAAGCCCGAC GGCACCGTGAAGCTGCTGATCTACCACACCAGCAGACT GCACAGCGGCGTGCCAAGCAGATTTTCTGGCAGCGGCT CTGGCACCGACTACAGCCTGACAATCAGCAACCTGGA ACAAGAGGATATCGCTACCTACTTCTGCCAGCAAGGCA ACACCCTGCCTTACACCTTTGGCGGAGGCACCAAGCTG GAAATCACCGGCTCTACAAGCGGCAGCGGCAAACCTG GATCTGGCGAGGGATCTACCAAGGGCGAGGTACAACT TTTGGAGTCAGGCGGTGGACTGGTACAACCGGGTGGTT CATTGCGTTTGAGCTGCGCTGCCTCTGGTTTTACCAGCT CCTCCTACGTGATGGGCTGGTATCGCCAAGCGCCGGGC AAAGAACGCGAGTTTGTCAGCTCGATCAGCACCGGTG GTGATGCCTACTACGCGGACTCGGTAAAAGGCCGTTTT ACGATCAGTCGTGATAATTCCAAGAATACCTTGTACCT GCAAATGAATAGCCTTCGCGCAGAAGACACAGCGGTG TATTATTGTGCCGCTGACGTTTGGTACTACCACGGCGG CGCGTACGATTATTGGGGCCAGGGTACCCAGGTGACG GTCTCGAGCGGCGGTGGCGGATCACAGGTGCAGCTGG TTGAGTCTGGGGGAGGCCTTGTCCAGGCTGGGGGGTCC CTGAGACTCTCCTGTGCAGCGTCTGGAAGCGAATTCAC CGGTTATCCCATGGGCTGGTTTCGCCAGGCTCCAGGCA AGGAAAGGGAGTTTGTCGCTGGCTCCGTAGGTATCGGT GGTAGTACAAACTATGCAGACTCCGTGAAGGGCCGAT TCACCATCTCCAGAGACAATGCGAAGAACACGGTCTAT CTGCAAATGAACAGCCTGAAGCCAGAGGACACGGCTG TGTATTACTGTGCGGCCGACAAAGACTACTACAAACCT TATAGTCGATATAGGACCGCTATCAGGTACGATACCTG GGGCCAAGGGACCCAGGTCACCGTCTCGAGCGGCAGT ACTTCTGGTAGCGGAAAACCCGGTAGCGGCGAGGGGT CAACTAAAGGAGAAGTGAAACTGCAAGAGTCTGGCCC TGGACTGGTGGCCCCATCTCAGTCTCTGAGCGTGACCT GTACAGTCAGCGGAGTGTCCCTGCCTGATTACGGCGTG TCCTGGATCAGACAGCCTCCTCGGAAAGGCCTGGAATG GCTGGGAGTGATCTGGGGCAGCGAGACAACCTACTAC AACAGCGCCCTGAAGTCCCGGCTGACCATCATCAAGG ACAACTCCAAGAGCCAGGTGTTCCTGAAGATGAACAG CCTGCAGACCGACGACACCGCCATCTACTATTGCGCCA AGCACTACTACTACGGCGGCAGCTACGCCATGGATTAT TGGGGCCAGGGCACCAGCGTGACCGTGTCTAGCATCG AAGTGATGTACCCTCCACCTTACCTGGACAACGAGAAG	

	TCCAACGGCACCATCATCCACGTGAAGGGCAAGCACC TGTGTCCTTCTCCACTGTTCCCCGGACCTAGCAAGCCTT TCTGGGTGCTCGTTGTTGTTGGCGGCGTGCTGGCCTGTT ATAGCCTGCTTGTGACCGTGGCCTTCATCATCTTTTGGG TCCGAAGCAAGCGGAGCCGGCTGCTGCACTCCGACTA CATGAACATGACCCCTAGACGGCCCGGACCAACCAGA AAGCACTACCAGCCTTACGCTCCTCCTAGAGACTTCGC CGCCTACCGGTCCAGAGTGAAGTTCAGCAGATCCGCCG ATGCTCCCGCCTATCAGCAGGGCCAAAACCAGCTGTAC AACGAGCTGAACCTGGGGAGAAGAGAAGAGTACGACG TGCTGGACAAGCGGAGAGGCAGAGATCCTGAAATGGG CGGCAAGCCCAGACGGAAGAATCCTCAAGAGGGCCTG TATAATGAGCTGCAGAAAGACAAGATGGCCGAGGCCT ACAGCGAGATCGGAATGAAGGGCGAGCGCAGAAGAG GCAAGGGACACGATGGACTGTACCAGGGCCTGAGCAC CGCCACCAAGGATACCTATGATGCCCTGCACATGCAGG CCCTGCCTCCAAGA	
E04_FMC63_CD28_CD28_CD28_CD3Z (P1972)	ATGGCCAGATCTCCTGCTCAACTGCTGGGACTGCTGCT GCTGTGGCTTAGCGGAGCCAGATGCGAGGTACAACTTT TGGAGTCAGGCGGTGGACTGGTACAACCGGGTGGTTC ATTGCGTTTGAGCTGCGCTGCCTCTGGTTTTACCAGCTC CTCCTACGTGATGGGCTGGTATCGCCAAGCGCCGGGCA AAGAACGCGAGTTTGTCAGCTCGATCAGCACCGGTGGT GATGCCTACTACGCGGACTCGGTAAAAGGCCGTTTTAC GATCAGTCGTGATAATTCCAAGAATACCTTGTACCTGC AAATGAATAGCCTTCGCGCAGAAGACACAGCGGTGTA TTATTGTGCCGCTGACGTTTGGTACTACCACGGCGGCG CGTACGATTATTGGGGCCAGGGTACCCAGGTGACGGTC TCGAGCGGCAGTACTTCTGGTAGCGGAAAACCCGGTA GCGGCGAGGGGTCAACTAAAGGAGACATCCAGATGAC CCAGACCACAAGCAGCCTGTCTGCCAGCCTGGGCGAT AGAGTGACCATCAGCTGTAGAGCCAGCCAGGACATCA GCAAGTACCTGAACTGGTATCAGCAAAAGCCCGACGG CACCGTGAAGCTGCTGATCTACCACACCAGCAGACTGC ACAGCGGCGTGCCAAGCAGATTTTCTGGCAGCGGCTCT GGCACCGACTACAGCCTGACAATCAGCAACCTGGAAC AAGAGGATATCGCTACCTACTTCTGCCAGCAAGGCAAC ACCCTGCCTTACACCTTTGGCGGAGGCACCAAGCTGGA AATCACCGGCTCTACAAGCGGCAGCGGCAAACCTGGA TCTGGCGAGGGATCTACCAAGGGCGAAGTGAAACTGC	137

	AAGAGTCTGGCCCTGGACTGGTGGCCCCATCTCAGTCT CTGAGCGTGACCTGTACAGTCAGCGGAGTGTCCCTGCC TGATTACGGCGTGTCCTGGATCAGACAGCCTCCTCGGA AAGGCCTGGAATGGCTGGGAGTGATCTGGGGCAGCGA GACAACCTACTACAACAGCGCCCTGAAGTCCCGGCTG ACCATCATCAAGGACAACTCCAAGAGCCAGGTGTTCCT GAAGATGAACAGCCTGCAGACCGACGACACCGCCATC TACTATTGCGCCAAGCACTACTACTACGGCGGCAGCTA CGCCATGGATTATTGGGGCCAGGGCACCAGCGTGACC GTGTCTAGCATCGAAGTGATGTACCCTCCACCTTACCT GGACAACGAGAAGTCCAACGGCACCATCATCCACGTG AAGGGCAAGCACCTGTGTCCTTCTCCACTGTTCCCCGG ACCTAGCAAGCCTTTCTGGGTGCTCGTTGTTGTTGGCG GCGTGCTGGCCTGTTATAGCCTGCTTGTGACCGTGGCC TTCATCATCTTTTGGGTCCGAAGCAAGCGGAGCCGGCT GCTGCACTCCGACTACATGAACATGACCCCTAGACGGC CCGGACCAACCAGAAAGCACTACCAGCCTTACGCTCCT CCTAGAGACTTCGCCGCCTACCGGTCCAGAGTGAAGTT CAGCAGGAGCGCAGACGCCCCCGCGTACCAGCAGGGC CAGAACCAGCTCTATAACGAGCTCAATCTAGGACGAA GAGAGGAGTACGATGTTTTGGACAAGCGACGTGGCCG GGACCCTGAGATGGGGGGAAAGCCGAGAAGGAAGAA CCCTCAGGAAGGCCTGTACAATGAACTGCAGAAAGAT AAGATGGCGGAGGCCTACAGTGAGATTGGGATGAAAG GCGAGCGCCGGAGGGGCAAGGGGCACGATGGCCTTTA CCAGGGACTCAGTACAGCCACCAAGGACACCTACGAC GCCCTTCACATGCAGGCCCTGCCCCCTCGC	
A01v1_FMC63_CD28_CD28_CD28_CD3Z (P2014)	ATGGCCAGATCTCCTGCTCAACTGCTGGGACTGCTGCT GCTGTGGCTTAGCGGAGCCAGATGCGAGGTACAACTTT TGGAGTCAGGCGGTGGACTGGTACAACCGGGTGGTTC ATTGCGTTTGAGCTGCGCTGCCTCTGGTTTGACCTCTTA TTCCTACGCGATGGGCTGGTATCGCCAAGCGCCGGGCA AAGAACGCGAGTTTGTCAGCGCAATCAGCTCGGGTGG TAGCGCGTACTACGCGGACTCGGTAAAAGGCCGTTTTA CGATCAGTCGTGATAATTCCAAGAATACCTTGTACCTG CAAATGAATAGCCTTCGCGCAGAAGACACAGCGGTGT ATTATTGTGCCGTTGGACCGTACTACGGATTTAGAGCG GTTACCGAAGCAGATTATTGGGGCCAGGGTACCCAGG TGACGGTCTCGAGCGGCAGTACTTCTGGTAGCGGAAA ACCCGGTAGCGGCGAGGGGTCAACTAAAGGAGACATC	138

	CAGATGACCCAGACCACAAGCAGCCTGTCTGCCAGCCT GGGCGATAGAGTGACCATCAGCTGTAGAGCCAGCCAG GACATCAGCAAGTACCTGAACTGGTATCAGCAAAAGC CCGACGGCACCGTGAAGCTGCTGATCTACCACACCAGC AGACTGCACAGCGGCGTGCCAAGCAGATTTTCTGGCA GCGGCTCTGGCACCGACTACAGCCTGACAATCAGCAA CCTGGAACAAGAGGATATCGCTACCTACTTCTGCCAGC AAGGCAACACCCTGCCTTACACCTTTGGCGGAGGCACC AAGCTGGAAATCACCGGCTCTACAAGCGGCAGCGGCA AACCTGGATCTGGCGAGGGATCTACCAAGGGCGAAGT GAAACTGCAAGAGTCTGGCCCTGGACTGGTGGCCCCAT CTCAGTCTCTGAGCGTGACCTGTACAGTCAGCGGAGTG TCCCTGCCTGATTACGGCGTGTCCTGGATCAGACAGCC TCCTCGGAAAGGCCTGGAATGGCTGGGAGTGATCTGG GGCAGCGAGACAACCTACTACAACAGCGCCCTGAAGT CCCGGCTGACCATCATCAAGGACAACTCCAAGAGCCA GGTGTTCCTGAAGATGAACAGCCTGCAGACCGACGAC ACCGCCATCTACTATTGCGCCAAGCACTACTACTACGG CGGCAGCTACGCCATGGATTATTGGGGCCAGGGCACC AGCGTGACCGTGTCTAGCATCGAAGTGATGTACCCTCC ACCTTACCTGGACAACGAGAAGTCCAACGGCACCATC ATCCACGTGAAGGGCAAGCACCTGTGTCCTTCTCCACT GTTCCCCGGACCTAGCAAGCCTTTCTGGGTGCTCGTTG TTGTTGGCGGCGTGCTGGCCTGTTATAGCCTGCTTGTG ACCGTGGCCTTCATCATCTTTTGGGTCCGAAGCAAGCG GAGCCGGCTGCTGCACTCCGACTACATGAACATGACCC CTAGACGGCCCGGACCAACCAGAAAGCACTACCAGCC TTACGCTCCTCCTAGAGACTTCGCCGCCTACCGGTCCA GAGTGAAGTTCAGCAGATCCGCCGATGCTCCCGCCTAT CAGCAGGGCCAAAACCAGCTGTACAACGAGCTGAACC TGGGGAGAAGAGAAGAGTACGACGTGCTGGACAAGCG GAGAGGCAGAGATCCTGAAATGGGCGGCAAGCCCAGA CGGAAGAATCCTCAAGAGGGCCTGTATAATGAGCTGC AGAAAGACAAGATGGCCGAGGCCTACAGCGAGATCGG AATGAAGGGCGAGCGCAGAAGAGGCAAGGGACACGA TGGACTGTACCAGGGCCTGAGCACCGCCACCAAGGAT ACCTATGATGCCCTGCACATGCAGGCCCTGCCTCCAAG A	
A01v2_FMC63 _CD28_CD28_	ATGGCCAGATCTCCTGCTCAACTGCTGGGACTGCTGCT GCTGTGGCTTAGCGGAGCCAGATGCGAAGTCCAGCTG	162

CD28_CD3Z (P2014)	CTGGAAAGCGGTGGCGGTCTGGTCCAGCCTGGCGGCA CCCTGCGCCTGTCCTGTGCCGCTAGCGGCCTGACCTGC TATAGCTATGCCATGGGTTGGTACCGCCAGGCCCCTGG TAAGGAGCGCGAATTCGTGTCCGCTATTTCCAGCGGCG GCTCCGCCTATTATGCTGATAGCGTCAAGGGTCGCTTC ACCATTTGCCGCGACAACAGCAAAAACACTCTGTATCT GCAGATGAACTCCCTGCGCGCTGAGGATACCGCCGTCT ACTACTGCGCTGTGGGCCCTTATTATGGCTTCCGCGCT GTGACTGAGGCTGACTACTGGGGTCAGGGCACTCAGG TGACTGTGAGCAGCGGCAGTACTTCTGGTAGCGGAAA ACCCGGTAGCGGCGAGGGGTCAACTAAAGGAGACATC CAGATGACCCAGACCACAAGCAGCCTGTCTGCCAGCCT GGGCGATAGAGTGACCATCAGCTGTAGAGCCAGCCAG GACATCAGCAAGTACCTGAACTGGTATCAGCAAAAGC CCGACGGCACCGTGAAGCTGCTGATCTACCACACCAGC AGACTGCACAGCGGCGTGCCAAGCAGATTTTCTGGCA GCGGCTCTGGCACCGACTACAGCCTGACAATCAGCAA CCTGGAACAAGAGGATATCGCTACCTACTTCTGCCAGC AAGGCAACACCCTGCCTTACACCTTTGGCGGAGGCACC AAGCTGGAAATCACCGGCTCTACAAGCGGCAGCGGCA AACCTGGATCTGGCGAGGGATCTACCAAGGGCGAAGT GAAACTGCAAGAGTCTGGCCCTGGACTGGTGGCCCCAT CTCAGTCTCTGAGCGTGACCTGTACAGTCAGCGGAGTG TCCCTGCCTGATTACGGCGTGTCCTGGATCAGACAGCC TCCTCGGAAAGGCCTGGAATGGCTGGGAGTGATCTGG GGCAGCGAGACAACCTACTACAACAGCGCCCTGAAGT CCCGGCTGACCATCATCAAGGACAACTCCAAGAGCCA GGTGTTCCTGAAGATGAACAGCCTGCAGACCGACGAC ACCGCCATCTACTATTGCGCCAAGCACTACTACTACGG CGGCAGCTACGCCATGGATTATTGGGGCCAGGGCACC AGCGTGACCGTGTCTAGCATCGAAGTGATGTACCCTCC ACCTTACCTGGACAACGAGAAGTCCAACGGCACCATC ATCCACGTGAAGGGCAAGCACCTGTGTCCTTCTCCACT GTTCCCCGGACCTAGCAAGCCTTTCTGGGTGCTCGTTG TTGTTGGCGGCGTGCTGGCCTGTTATAGCCTGCTTGTG ACCGTGGCCTTCATCATCTTTTGGGTCCGAAGCAAGCG GAGCCGGCTGCTGCACTCCGACTACATGAACATGACCC CTAGACGGCCCGGACCAACCAGAAAGCACTACCAGCC TTACGCTCCTCCTAGAGACTTCGCCGCCTACCGGTCCA GAGTGAAGTTCAGCAGATCCGCCGATGCTCCCGCCTAT	

	CAGCAGGGCCAAAACCAGCTGTACAACGAGCTGAACC TGGGGAGAAGAGAAGAGTACGACGTGCTGGACAAGCG GAGAGGCAGAGATCCTGAAATGGGCGGCAAGCCCAGA CGGAAGAATCCTCAAGAGGGCCTGTATAATGAGCTGC AGAAAGACAAGATGGCCGAGGCCTACAGCGAGATCGG AATGAAGGGCGAGCGCAGAAGAGGCAAGGGACACGA TGGACTGTACCAGGGCCTGAGCACCGCCACCAAGGAT ACCTATGATGCCCTGCACATGCAGGCCCTGCCTCCAAG A	
D04AMD11_E04_FMC63_CD28_CD28_CD28_CD3Z (P2015)	ATGGCCAGATCTCCTGCTCAACTGCTGGGACTGCTGCT GCTGTGGCTTAGCGGAGCCAGATGCCAGGTGCAGCTG GTTGAGTCTGGGGGAGGCCTTGTCCAGGCTGGGGGGTC CCTGAGACTCTCCTGTGCAGCGTCTGGAAGCGAATTCA CCGGTTATCCCATGGGCTGGTTTCGCCAGGCTCCAGGC AAGGAAAGGGAGTTTGTCGCTGGCTCCGTAGGTATCG GTGGTAGTACAAACTATGCAGACTCCGTGAAGGGCCG ATTCACCATCTCCAGAGACAATGCGAAGAACACGGTCT ATCTGCAAATGAACAGCCTGAAGCCAGAGGACACGGC TGTGTATTACTGTGCGGCCGACAAAGACTACTACAAAC CTTATAGTCGATATAGGACCGCTATCAGGTACGATACC TGGGGCCAAGGGACCCAGGTCACCGTCTCGAGCGGCG GTGGCGGATCAGAGGTACAACTTTTGGAGTCAGGCGG TGGACTGGTACAACCGGGTGGTTCATTGCGTTTGAGCT GCGCTGCCTCTGGTTTTACCAGCTCCTCCTACGTGATG GGCTGGTATCGCCAAGCGCCGGGCAAAGAACGCGAGT TTGTCAGCTCGATCAGCACCGGTGGTGATGCCTACTAC GCGGACTCGGTAAAAGGCCGTTTTACGATCAGTCGTGA TAATTCCAAGAATACCTTGTACCTGCAAATGAATAGCC TTCGCGCAGAAGACACAGCGGTGTATTATTGTGCCGCT GACGTTTGGTACTACCACGGCGGCGCGTACGATTATTG GGGCCAGGGTACCCAGGTGACGGTCTCGAGCGGCAGT ACTTCTGGTAGCGGAAAACCCGGTAGCGGCGAGGGGT CAACTAAAGGAGACATCCAGATGACCCAGACCACAAG CAGCCTGTCTGCCAGCCTGGGCGATAGAGTGACCATCA GCTGTAGAGCCAGCCAGGACATCAGCAAGTACCTGAA CTGGTATCAGCAAAAGCCCGACGGCACCGTGAAGCTG CTGATCTACCACACCAGCAGACTGCACAGCGGCGTGCC AAGCAGATTTTCTGGCAGCGGCTCTGGCACCGACTACA GCCTGACAATCAGCAACCTGGAACAAGAGGATATCGC TACCTACTTCTGCCAGCAAGGCAACACCCTGCCTTACA	139

	CCTTTGGCGGAGGCACCAAGCTGGAAATCACCGGCTCT ACAAGCGGCAGCGGCAAACCTGGATCTGGCGAGGGAT CTACCAAGGGCGAAGTGAAACTGCAAGAGTCTGGCCC TGGACTGGTGGCCCCATCTCAGTCTCTGAGCGTGACCT GTACAGTCAGCGGAGTGTCCCTGCCTGATTACGGCGTG TCCTGGATCAGACAGCCTCCTCGGAAAGGCCTGGAATG GCTGGGAGTGATCTGGGGCAGCGAGACAACCTACTAC AACAGCGCCCTGAAGTCCCGGCTGACCATCATCAAGG ACAACTCCAAGAGCCAGGTGTTCCTGAAGATGAACAG CCTGCAGACCGACGACACCGCCATCTACTATTGCGCCA AGCACTACTACTACGGCGGCAGCTACGCCATGGATTAT TGGGGCCAGGGCACCAGCGTGACCGTGTCTAGCATCG AAGTGATGTACCCTCCACCTTACCTGGACAACGAGAAG TCCAACGGCACCATCATCCACGTGAAGGGCAAGCACC TGTGTCCTTCTCCACTGTTCCCCGGACCTAGCAAGCCTT TCTGGGTGCTCGTTGTTGTTGGCGGCGTGCTGGCCTGTT ATAGCCTGCTTGTGACCGTGGCCTTCATCATCTTTTGGG TCCGAAGCAAGCGGAGCCGGCTGCTGCACTCCGACTA CATGAACATGACCCCTAGACGGCCCGGACCAACCAGA AAGCACTACCAGCCTTACGCTCCTCCTAGAGACTTCGC CGCCTACCGGTCCAGAGTGAAGTTCAGCAGATCCGCCG ATGCTCCCGCCTATCAGCAGGGCCAAAACCAGCTGTAC AACGAGCTGAACCTGGGGAGAAGAGAAGAGTACGACG TGCTGGACAAGCGGAGAGGCAGAGATCCTGAAATGGG CGGCAAGCCCAGACGGAAGAATCCTCAAGAGGGCCTG TATAATGAGCTGCAGAAAGACAAGATGGCCGAGGCCT ACAGCGAGATCGGAATGAAGGGCGAGCGCAGAAGAG GCAAGGGACACGATGGACTGTACCAGGGCCTGAGCAC CGCCACCAAGGATACCTATGATGCCCTGCACATGCAGG CCCTGCCTCCAAGA	
D04_E04_A01v2_D04_FMC63_CD28_CD28_CD28_CD3Z (P2016)	ATGGCCAGATCTCCTGCTCAACTGCTGGGACTGCTGCT GCTGTGGCTTAGCGGAGCCAGATGCCAGGTGCAGCTG GTTGAGTCTGGGGGAGGCCTTGTCCAGGCTGGGGGGTC CCTGAGACTCTCCTGTGCAGCGTCTGGAAGCGAATTCA CCGGTTATCCCATGGGCTGGTTTCGCCAGGCTCCAGGC AAGGAAAGGGAGTTTGTCGCTGGCTCCGTAGGTATCG GTGGTAGTACAAACTATGCAGACTCCGTGAAGGGCCG ATTCACCATCTCCAGAGACAATGCGAAGAACACGGTCT ATCTGCAAATGAACAGCCTGAAGCCAGAGGACACGGC TGTGTATTACTGTGCGGCCGACAAAGACTACTACAAAC	140

	CTTATAGTCGATATAGGACCGCTATCAGGTACGATACC TGGGGCCAAGGGACCCAGGTCACCGTCTCGAGTGGCG GTGGCGGATCAGAGGTACAACTTTTGGAGTCAGGCGG TGGACTGGTACAACCGGGTGGTTCATTGCGTTTGAGCT GCGCTGCCTCTGGTTTTACCAGCTCCTCCTACGTGATG GGCTGGTATCGCCAAGCGCCGGGCAAAGAACGCGAGT TTGTCAGCTCGATCAGCACCGGTGGTGATGCCTACTAC GCGGACTCGGTAAAAGGCCGTTTTACGATCAGTCGTGA TAATTCCAAGAATACCTTGTACCTGCAAATGAATAGCC TTCGCGCAGAAGACACAGCGGTGTATTATTGTGCCGCT GACGTTTGGTACTACCACGGCGGCGCGTACGATTATTG GGGCCAGGGTACCCAGGTGACGGTCTCGAGCGGTGGC GGTGGTTCTGAAGTCCAGCTGCTGGAAAGCGGTGGCG GTCTGGTCCAGCCTGGCGGCACCCTGCGCCTGTCCTGT GCCGCTAGCGGCCTGACCTGCTATAGCTATGCCATGGG TTGGTACCGCCAGGCCCCTGGTAAGGAGCGCGAATTCG TGTCCGCTATTTCCAGCGGCGGCTCCGCCTATTATGCT GATAGCGTCAAGGGTCGCTTCACCATTTGCCGCGACAA CAGCAAAAACACTCTGTATCTGCAGATGAACTCCCTGC GCGCTGAGGATACCGCCGTCTACTACTGCGCTGTGGGC CCTTATTATGGCTTCCGCGCTGTGACTGAGGCTGACTA CTGGGGTCAGGGCACTCAGGTGACTGTGAGCAGCGGT GGTGGCGGATCTCAGGTCCAGCTGGTGGAAAGCGGCG GTGGTCTGGTGCAGGCTGGCGGTAGCCTGCGCCTGAGC TGCGCTGCCAGCGGTTCCGAGTTTACTGGCTACCCTAT GGGTTGGTTCCGCCAGGCCCCCGGTAAAGAGCGCGAA TTCGTGGCCGGTAGCGTCGGCATTGGCGGCTCCACTAA TTACGCTGATAGCGTCAAAGGTCGCTTTACTATTAGCC GCGATAACGCCAAAAATACTGTGTACCTGCAGATGAA TTCCCTGAAACCCGAAGATACCGCCGTCTACTATTGCG CCGCTGATAAGGATTATTATAAGCCCTACTCCCGCTAC CGCACTGCCATTCGCTATGACACTTGGGGTCAGGGCAC TCAGGTGACTGTGAGCTCCGGCAGTACTTCTGGTAGCG GAAAACCCGGTAGCGGCGAGGGGTCAACTAAAGGAGA CATCCAGATGACCCAGACCACAAGCAGCCTGTCTGCCA GCCTGGGCGATAGAGTGACCATCAGCTGTAGAGCCAG CCAGGACATCAGCAAGTACCTGAACTGGTATCAGCAA AAGCCCGACGGCACCGTGAAGCTGCTGATCTACCACA CCAGCAGACTGCACAGCGGCGTGCCAAGCAGATTTTCT GGCAGCGGCTCTGGCACCGACTACAGCCTGACAATCA	

	GCAACCTGGAACAAGAGGATATCGCTACCTACTTCTGC CAGCAAGGCAACACCCTGCCTTACACCTTTGGCGGAGG CACCAAGCTGGAAATCACCGGCTCTACAAGCGGCAGC GGCAAACCTGGATCTGGCGAGGGATCTACCAAGGGCG AAGTGAAACTGCAAGAGTCTGGCCCTGGACTGGTGGC CCCATCTCAGTCTCTGAGCGTGACCTGTACAGTCAGCG GAGTGTCCCTGCCTGATTACGGCGTGTCCTGGATCAGA CAGCCTCCTCGGAAAGGCCTGGAATGGCTGGGAGTGA TCTGGGGCAGCGAGACAACCTACTACAACAGCGCCCT GAAGTCCCGGCTGACCATCATCAAGGACAACTCCAAG AGCCAGGTGTTCCTGAAGATGAACAGCCTGCAGACCG ACGACACCGCCATCTACTATTGCGCCAAGCACTACTAC TACGGCGGCAGCTACGCCATGGATTATTGGGGCCAGG GCACCAGCGTGACCGTGTCTAGCATCGAAGTGATGTAC CCTCCACCTTACCTGGACAACGAGAAGTCCAACGGCAC CATCATCCACGTGAAGGGCAAGCACCTGTGTCCTTCTC CACTGTTCCCCGGACCTAGCAAGCCTTTCTGGGTGCTC GTTGTTGTTGGCGGCGTGCTGGCCTGTTATAGCCTGCTT GTGACCGTGGCCTTCATCATCTTTTGGGTCCGAAGCAA GCGGAGCCGGCTGCTGCACTCCGACTACATGAACATG ACCCCTAGACGGCCCGGACCAACCAGAAAGCACTACC AGCCTTACGCTCCTCCTAGAGACTTCGCCGCCTACCGG TCCAGAGTGAAGTTCAGCAGATCCGCCGATGCTCCCGC CTATCAGCAGGGCCAAAACCAGCTGTACAACGAGCTG AACCTGGGGAGAAGAGAAGAGTACGACGTGCTGGACA AGCGGAGAGGCAGAGATCCTGAAATGGGCGGCAAGCC CAGACGGAAGAATCCTCAAGAGGGCCTGTATAATGAG CTGCAGAAAGACAAGATGGCCGAGGCCTACAGCGAGA TCGGAATGAAGGGCGAGCGCAGAAGAGGCAAGGGAC ACGATGGACTGTACCAGGGCCTGAGCACCGCCACCAA GGATACCTATGATGCCCTGCACATGCAGGCCCTGCCTC CAAGA	
D04_E04_A01v1_D04_FMC63_CD28_CD28_CD28_CD3Z (P2016)	ATGGCCAGATCTCCTGCTCAACTGCTGGGACTGCTGCT GCTGTGGCTTAGCGGAGCCAGATGCCAGGTGCAGCTG GTTGAGTCTGGGGGAGGCCTTGTCCAGGCTGGGGGGTC CCTGAGACTCTCCTGTGCAGCGTCTGGAAGCGAATTCA CCGGTTATCCCATGGGCTGGTTTCGCCAGGCTCCAGGC AAGGAAAGGGAGTTTGTCGCTGGCTCCGTAGGTATCG GTGGTAGTACAAACTATGCAGACTCCGTGAAGGGCCG ATTCACCATCTCCAGAGACAATGCGAAGAACACGGTCT	164

	ATCTGCAAATGAACAGCCTGAAGCCAGAGGACACGGC TGTGTATTACTGTGCGGCCGACAAAGACTACTACAAAC CTTATAGTCGATATAGGACCGCTATCAGGTACGATACC TGGGGCCAAGGGACCCAGGTCACCGTCTCGAGTGGCG GTGGCGGATCAGAGGTACAACTTTTGGAGTCAGGCGG TGGACTGGTACAACCGGGTGGTTCATTGCGTTTGAGCT GCGCTGCCTCTGGTTTTACCAGCTCCTCCTACGTGATG GGCTGGTATCGCCAAGCGCCGGGCAAAGAACGCGAGT TTGTCAGCTCGATCAGCACCGGTGGTGATGCCTACTAC GCGGACTCGGTAAAAGGCCGTTTTACGATCAGTCGTGA TAATTCCAAGAATACCTTGTACCTGCAAATGAATAGCC TTCGCGCAGAAGACACAGCGGTGTATTATTGTGCCGCT GACGTTTGGTACTACCACGGCGGCGCGTACGATTATTG GGGCCAGGGTACCCAGGTGACGGTCTCGAGCGGTGGC GGTGGTTCTGAGGTACAACTTTTGGAGTCAGGCGGTGG ACTGGTACAACCGGGTGGTTCATTGCGTTTGAGCTGCG CTGCCTCTGGTTTGACCTCTTATTCCTACGCGATGGGCT GGTATCGCCAAGCGCCGGGCAAAGAACGCGAGTTTGT CAGCGCAATCAGCTCGGGTGGTAGCGCGTACTACGCG GACTCGGTAAAAGGCCGTTTTACGATCAGTCGTGATAA TTCCAAGAATACCTTGTACCTGCAAATGAATAGCCTTC GCGCAGAAGACACAGCGGTGTATTATTGTGCCGTTGGA CCGTACTACGGATTTAGAGCGGTTACCGAAGCAGATTA TTGGGGCCAGGGTACCCAGGTGACGGTCTCGAGCGGT GGTGGCGGATCTCAGGTCCAGCTGGTGGAAAGCGGCG GTGGTCTGGTGCAGGCTGGCGGTAGCCTGCGCCTGAGC TGCGCTGCCAGCGGTTCCGAGTTTACTGGCTACCCTAT GGGTTGGTTCCGCCAGGCCCCCGGTAAAGAGCGCGAA TTCGTGGCCGGTAGCGTCGGCATTGGCGGCTCCACTAA TTACGCTGATAGCGTCAAAGGTCGCTTTACTATTAGCC GCGATAACGCCAAAAATACTGTGTACCTGCAGATGAA TTCCCTGAAACCCGAAGATACCGCCGTCTACTATTGCG CCGCTGATAAGGATTATTATAAGCCCTACTCCCGCTAC CGCACTGCCATTCGCTATGACACTTGGGGTCAGGGCAC TCAGGTGACTGTGAGCTCCGGCAGTACTTCTGGTAGCG GAAAACCCGGTAGCGGCGAGGGGTCAACTAAAGGAGA CATCCAGATGACCCAGACCACAAGCAGCCTGTCTGCCA GCCTGGGCGATAGAGTGACCATCAGCTGTAGAGCCAG CCAGGACATCAGCAAGTACCTGAACTGGTATCAGCAA AAGCCCGACGGCACCGTGAAGCTGCTGATCTACCACA	

	CCAGCAGACTGCACAGCGGCGTGCCAAGCAGATTTTCT GGCAGCGGCTCTGGCACCGACTACAGCCTGACAATCA GCAACCTGGAACAAGAGGATATCGCTACCTACTTCTGC CAGCAAGGCAACACCCTGCCTTACACCTTTGGCGGAGG CACCAAGCTGGAAATCACCGGCTCTACAAGCGGCAGC GGCAAACCTGGATCTGGCGAGGGATCTACCAAGGGCG AAGTGAAACTGCAAGAGTCTGGCCCTGGACTGGTGGC CCCATCTCAGTCTCTGAGCGTGACCTGTACAGTCAGCG GAGTGTCCCTGCCTGATTACGGCGTGTCCTGGATCAGA CAGCCTCCTCGGAAAGGCCTGGAATGGCTGGGAGTGA TCTGGGGCAGCGAGACAACCTACTACAACAGCGCCCT GAAGTCCCGGCTGACCATCATCAAGGACAACTCCAAG AGCCAGGTGTTCCTGAAGATGAACAGCCTGCAGACCG ACGACACCGCCATCTACTATTGCGCCAAGCACTACTAC TACGGCGGCAGCTACGCCATGGATTATTGGGGCCAGG GCACCAGCGTGACCGTGTCTAGCATCGAAGTGATGTAC CCTCCACCTTACCTGGACAACGAGAAGTCCAACGGCAC CATCATCCACGTGAAGGGCAAGCACCTGTGTCCTTCTC CACTGTTCCCCGGACCTAGCAAGCCTTTCTGGGTGCTC GTTGTTGTTGGCGGCGTGCTGGCCTGTTATAGCCTGCTT GTGACCGTGGCCTTCATCATCTTTTGGGTCCGAAGCAA GCGGAGCCGGCTGCTGCACTCCGACTACATGAACATG ACCCCTAGACGGCCCGGACCAACCAGAAAGCACTACC AGCCTTACGCTCCTCCTAGAGACTTCGCCGCCTACCGG TCCAGAGTGAAGTTCAGCAGATCCGCCGATGCTCCCGC CTATCAGCAGGGCCAAAACCAGCTGTACAACGAGCTG AACCTGGGGAGAAGAGAAGAGTACGACGTGCTGGACA AGCGGAGAGGCAGAGATCCTGAAATGGGCGGCAAGCC CAGACGGAAGAATCCTCAAGAGGGCCTGTATAATGAG CTGCAGAAAGACAAGATGGCCGAGGCCTACAGCGAGA TCGGAATGAAGGGCGAGCGCAGAAGAGGCAAGGGAC ACGATGGACTGTACCAGGGCCTGAGCACCGCCACCAA GGATACCTATGATGCCCTGCACATGCAGGCCCTGCCTC CAAGA	
FMC63LC_A01v1_A01v2_FMC63HC_CD28_CD28_CD3Z (P2193)	ATGGCCAGATCTCCTGCTCAACTGCTGGGACTGCTGCT GCTGTGGCTTAGCGGAGCCAGATGCGACATCCAGATG ACCCAGACCACAAGCAGCCTGTCTGCCAGCCTGGGCG ATAGAGTGACCATCAGCTGTAGAGCCAGCCAGGACAT CAGCAAGTACCTGAACTGGTATCAGCAAAAGCCCGAC GGCACCGTGAAGCTGCTGATCTACCACACCAGCAGACT	141

	GCACAGCGGCGTGCCAAGCAGATTTTCTGGCAGCGGCT CTGGCACCGACTACAGCCTGACAATCAGCAACCTGGA ACAAGAGGATATCGCTACCTACTTCTGCCAGCAAGGCA ACACCCTGCCTTACACCTTTGGCGGAGGCACCAAGCTG GAAATCACCGGCTCTACAAGCGGCAGCGGCAAACCTG GATCTGGCGAGGGATCTACCAAGGGCGAGGTACAACT TTTGGAGTCAGGCGGTGGACTGGTACAACCGGGTGGTT CATTGCGTTTGAGCTGCGCTGCCTCTGGTTTGACCTCTT ATTCCTACGCGATGGGCTGGTATCGCCAAGCGCCGGGC AAAGAACGCGAGTTTGTCAGCGCAATCAGCTCGGGTG GTAGCGCGTACTACGCGGACTCGGTAAAAGGCCGTTTT ACGATCAGTCGTGATAATTCCAAGAATACCTTGTACCT GCAAATGAATAGCCTTCGCGCAGAAGACACAGCGGTG TATTATTGTGCCGTTGGACCGTACTACGGATTTAGAGC GGTTACCGAAGCAGATTATTGGGGCCAGGGTACCCAG GTGACGGTCTCGAGCGGCGGTGGCGGATCAGAAGTCC AGCTGCTGGAAAGCGGTGGCGGTCTGGTCCAGCCTGG CGGCACCCTGCGCCTGTCCTGTGCCGCTAGCGGCCTGA CCTGCTATAGCTATGCCATGGGTTGGTACCGCCAGGCC CCTGGTAAGGAGCGCGAATTCGTGTCCGCTATTTCCAG CGGCGGCTCCGCCTATTATGCTGATAGCGTCAAGGGTC GCTTCACCATTTGCCGCGACAACAGCAAAAACACTCTG TATCTGCAGATGAACTCCCTGCGCGCTGAGGATACCGC CGTCTACTACTGCGCTGTGGGCCCTTATTATGGCTTCCG CGCTGTGACTGAGGCTGACTACTGGGGTCAGGGCACTC AGGTGACTGTGAGCAGCGGCAGTACTTCTGGTAGCGG AAAACCCGGTAGCGGCGAGGGGTCAACTAAAGGAGAA GTGAAACTGCAAGAGTCTGGCCCTGGACTGGTGGCCCC ATCTCAGTCTCTGAGCGTGACCTGTACAGTCAGCGGAG TGTCCCTGCCTGATTACGGCGTGTCCTGGATCAGACAG CCTCCTCGGAAAGGCCTGGAATGGCTGGGAGTGATCTG GGGCAGCGAGACAACCTACTACAACAGCGCCCTGAAG TCCCGGCTGACCATCATCAAGGACAACTCCAAGAGCC AGGTGTTCCTGAAGATGAACAGCCTGCAGACCGACGA CACCGCCATCTACTATTGCGCCAAGCACTACTACTACG GCGGCAGCTACGCCATGGATTATTGGGGCCAGGGCAC CAGCGTGACCGTGTCTAGCATCGAAGTGATGTACCCTC CACCTTACCTGGACAACGAGAAGTCCAACGGCACCAT CATCCACGTGAAGGGCAAGCACCTGTGTCCTTCTCCAC TGTTCCCCGGACCTAGCAAGCCTTTCTGGGTGCTCGTT	

	GTTGTTGGCGGCGTGCTGGCCTGTTATAGCCTGCTTGT GACCGTGGCCTTCATCATCTTTTGGGTCCGAAGCAAGC GGAGCCGGCTGCTGCACTCCGACTACATGAACATGACC CCTAGACGGCCCGGACCAACCAGAAAGCACTACCAGC CTTACGCTCCTCCTAGAGACTTCGCCGCCTACCGGTCC AGAGTGAAGTTCAGCAGATCCGCCGATGCTCCCGCCTA TCAGCAGGGCCAAAACCAGCTGTACAACGAGCTGAAC CTGGGGAGAAGAGAAGAGTACGACGTGCTGGACAAGC GGAGAGGCAGAGATCCTGAAATGGGCGGCAAGCCCAG ACGGAAGAATCCTCAAGAGGGCCTGTATAATGAGCTG CAGAAAGACAAGATGGCCGAGGCCTACAGCGAGATCG GAATGAAGGGCGAGCGCAGAAGAGGCAAGGGACACG ATGGACTGTACCAGGGCCTGAGCACCGCCACCAAGGA TACCTATGATGCCCTGCACATGCAGGCCCTGCCTCCAA GA	
FMC63LC_A01v2_A01v1_FMC63HC_CD28_CD28_CD28_CD3Z (P2193)	ATGGCCAGATCTCCTGCTCAACTGCTGGGACTGCTGCT GCTGTGGCTTAGCGGAGCCAGATGCGACATCCAGATG ACCCAGACCACAAGCAGCCTGTCTGCCAGCCTGGGCG ATAGAGTGACCATCAGCTGTAGAGCCAGCCAGGACAT CAGCAAGTACCTGAACTGGTATCAGCAAAAGCCCGAC GGCACCGTGAAGCTGCTGATCTACCACACCAGCAGACT GCACAGCGGCGTGCCAAGCAGATTTTCTGGCAGCGGCT CTGGCACCGACTACAGCCTGACAATCAGCAACCTGGA ACAAGAGGATATCGCTACCTACTTCTGCCAGCAAGGCA ACACCCTGCCTTACACCTTTGGCGGAGGCACCAAGCTG GAAATCACCGGCTCTACAAGCGGCAGCGGCAAACCTG GATCTGGCGAGGGATCTACCAAGGGCGAAGTCCAGCT GCTGGAAAGCGGTGGCGGTCTGGTCCAGCCTGGCGGC ACCCTGCGCCTGTCCTGTGCCGCTAGCGGCCTGACCTG CTATAGCTATGCCATGGGTTGGTACCGCCAGGCCCCTG GTAAGGAGCGCGAATTCGTGTCCGCTATTTCCAGCGGC GGCTCCGCCTATTATGCTGATAGCGTCAAGGGTCGCTT CACCATTTGCCGCGACAACAGCAAAAACACTCTGTATC TGCAGATGAACTCCCTGCGCGCTGAGGATACCGCCGTC TACTACTGCGCTGTGGGCCCTTATTATGGCTTCCGCGCT GTGACTGAGGCTGACTACTGGGGTCAGGGCACTCAGG TGACTGTGAGCAGCGGCGGTGGCGGATCAGAGGTACA ACTTTTGGAGTCAGGCGGTGGACTGGTACAACCGGGTG GTTCATTGCGTTTGAGCTGCGCTGCCTCTGGTTTGACCT CTTATTCCTACGCGATGGGCTGGTATCGCCAAGCGCCG	176

	GGCAAAGAACGCGAGTTTGTCAGCGCAATCAGCTCGG GTGGTAGCGCGTACTACGCGGACTCGGTAAAAGGCCG TTTTACGATCAGTCGTGATAATTCCAAGAATACCTTGT ACCTGCAAATGAATAGCCTTCGCGCAGAAGACACAGC GGTGTATTATTGTGCCGTTGGACCGTACTACGGATTTA GAGCGGTTACCGAAGCAGATTATTGGGGCCAGGGTAC CCAGGTGACGGTCTCGAGCGGCAGTACTTCTGGTAGCG GAAAACCCGGTAGCGGCGAGGGGTCAACTAAAGGAGA AGTGAAACTGCAAGAGTCTGGCCCTGGACTGGTGGCC CCATCTCAGTCTCTGAGCGTGACCTGTACAGTCAGCGG AGTGTCCCTGCCTGATTACGGCGTGTCCTGGATCAGAC AGCCTCCTCGGAAAGGCCTGGAATGGCTGGGAGTGAT CTGGGGCAGCGAGACAACCTACTACAACAGCGCCCTG AAGTCCCGGCTGACCATCATCAAGGACAACTCCAAGA GCCAGGTGTTCCTGAAGATGAACAGCCTGCAGACCGA CGACACCGCCATCTACTATTGCGCCAAGCACTACTACT ACGGCGGCAGCTACGCCATGGATTATTGGGGCCAGGG CACCAGCGTGACCGTGTCTAGCATCGAAGTGATGTACC CTCCACCTTACCTGGACAACGAGAAGTCCAACGGCACC ATCATCCACGTGAAGGGCAAGCACCTGTGTCCTTCTCC ACTGTTCCCCGGACCTAGCAAGCCTTTCTGGGTGCTCG TTGTTGTTGGCGGCGTGCTGGCCTGTTATAGCCTGCTTG TGACCGTGGCCTTCATCATCTTTTGGGTCCGAAGCAAG CGGAGCCGGCTGCTGCACTCCGACTACATGAACATGAC CCCTAGACGGCCCGGACCAACCAGAAAGCACTACCAG CCTTACGCTCCTCCTAGAGACTTCGCCGCCTACCGGTC CAGAGTGAAGTTCAGCAGATCCGCCGATGCTCCCGCCT ATCAGCAGGGCCAAAACCAGCTGTACAACGAGCTGAA CCTGGGGAGAAGAGAAGAGTACGACGTGCTGGACAAG CGGAGAGGCAGAGATCCTGAAATGGGCGGCAAGCCCA GACGGAAGAATCCTCAAGAGGGCCTGTATAATGAGCT GCAGAAAGACAAGATGGCCGAGGCCTACAGCGAGATC GGAATGAAGGGCGAGCGCAGAAGAGGCAAGGGACAC GATGGACTGTACCAGGGCCTGAGCACCGCCACCAAGG ATACCTATGATGCCCTGCACATGCAGGCCCTGCCTCCA AGA	
FMC63LC_A01v1_A01v1_FMC63HC_CD2	ATGGCCAGATCTCCTGCTCAACTGCTGGGACTGCTGCT GCTGTGGCTTAGCGGAGCCAGATGCGACATCCAGATG ACCCAGACCACAAGCAGCCTGTCTGCCAGCCTGGGCG ATAGAGTGACCATCAGCTGTAGAGCCAGCCAGGACAT	177

8_CD28_CD28 _CD3Z (P2193)	CAGCAAGTACCTGAACTGGTATCAGCAAAAGCCCGAC GGCACCGTGAAGCTGCTGATCTACCACACCAGCAGACT GCACAGCGGCGTGCCAAGCAGATTTTCTGGCAGCGGCT CTGGCACCGACTACAGCCTGACAATCAGCAACCTGGA ACAAGAGGATATCGCTACCTACTTCTGCCAGCAAGGCA ACACCCTGCCTTACACCTTTGGCGGAGGCACCAAGCTG GAAATCACCGGCTCTACAAGCGGCAGCGGCAAACCTG GATCTGGCGAGGGATCTACCAAGGGCGAGGTACAACT TTTGGAGTCAGGCGGTGGACTGGTACAACCGGGTGGTT CATTGCGTTTGAGCTGCGCTGCCTCTGGTTTGACCTCTT ATTCCTACGCGATGGGCTGGTATCGCCAAGCGCCGGGC AAAGAACGCGAGTTTGTCAGCGCAATCAGCTCGGGTG GTAGCGCGTACTACGCGGACTCGGTAAAAGGCCGTTTT ACGATCAGTCGTGATAATTCCAAGAATACCTTGTACCT GCAAATGAATAGCCTTCGCGCAGAAGACACAGCGGTG TATTATTGTGCCGTTGGACCGTACTACGGATTTAGAGC GGTTACCGAAGCAGATTATTGGGGCCAGGGTACCCAG GTGACGGTCTCGAGCGGCGGTGGCGGATCAGAGGTAC AACTTTTGGAGTCAGGCGGTGGACTGGTACAACCGGGT GGTTCATTGCGTTTGAGCTGCGCTGCCTCTGGTTTGACC TCTTATTCCTACGCGATGGGCTGGTATCGCCAAGCGCC GGGCAAAGAACGCGAGTTTGTCAGCGCAATCAGCTCG GGTGGTAGCGCGTACTACGCGGACTCGGTAAAAGGCC GTTTTACGATCAGTCGTGATAATTCCAAGAATACCTTG TACCTGCAAATGAATAGCCTTCGCGCAGAAGACACAG CGGTGTATTATTGTGCCGTTGGACCGTACTACGGATTT AGAGCGGTTACCGAAGCAGATTATTGGGGCCAGGGTA CCCAGGTGACGGTCTCGAGCGGCAGTACTTCTGGTAGC GGAAAACCCGGTAGCGGCGAGGGGTCAACTAAAGGAG AAGTGAAACTGCAAGAGTCTGGCCCTGGACTGGTGGC CCCATCTCAGTCTCTGAGCGTGACCTGTACAGTCAGCG GAGTGTCCCTGCCTGATTACGGCGTGTCCTGGATCAGA CAGCCTCCTCGGAAAGGCCTGGAATGGCTGGGAGTGA TCTGGGGCAGCGAGACAACCTACTACAACAGCGCCCT GAAGTCCCGGCTGACCATCATCAAGGACAACTCCAAG AGCCAGGTGTTCCTGAAGATGAACAGCCTGCAGACCG ACGACACCGCCATCTACTATTGCGCCAAGCACTACTAC TACGGCGGCAGCTACGCCATGGATTATTGGGGCCAGG GCACCAGCGTGACCGTGTCTAGCATCGAAGTGATGTAC CCTCCACCTTACCTGGACAACGAGAAGTCCAACGGCAC	

	CATCATCCACGTGAAGGGCAAGCACCTGTGTCCTTCTC CACTGTTCCCCGGACCTAGCAAGCCTTTCTGGGTGCTC GTTGTTGTTGGCGGCGTGCTGGCCTGTTATAGCCTGCTT GTGACCGTGGCCTTCATCATCTTTTGGGTCCGAAGCAA GCGGAGCCGGCTGCTGCACTCCGACTACATGAACATG ACCCCTAGACGGCCCGGACCAACCAGAAAGCACTACC AGCCTTACGCTCCTCCTAGAGACTTCGCCGCCTACCGG TCCAGAGTGAAGTTCAGCAGATCCGCCGATGCTCCCGC CTATCAGCAGGGCCAAAACCAGCTGTACAACGAGCTG AACCTGGGGAGAAGAGAAGAGTACGACGTGCTGGACA AGCGGAGAGGCAGAGATCCTGAAATGGGCGGCAAGCC CAGACGGAAGAATCCTCAAGAGGGCCTGTATAATGAG CTGCAGAAAGACAAGATGGCCGAGGCCTACAGCGAGA TCGGAATGAAGGGCGAGCGCAGAAGAGGCAAGGGAC ACGATGGACTGTACCAGGGCCTGAGCACCGCCACCAA GGATACCTATGATGCCCTGCACATGCAGGCCCTGCCTC CAAGA	
FMC63LC_A01v2_A01v2_FMC63HC_CD28_CD28_CD28_CD3Z (P2193)	ATGGCCAGATCTCCTGCTCAACTGCTGGGACTGCTGCT GCTGTGGCTTAGCGGAGCCAGATGCGACATCCAGATG ACCCAGACCACAAGCAGCCTGTCTGCCAGCCTGGGCG ATAGAGTGACCATCAGCTGTAGAGCCAGCCAGGACAT CAGCAAGTACCTGAACTGGTATCAGCAAAAGCCCGAC GGCACCGTGAAGCTGCTGATCTACCACACCAGCAGACT GCACAGCGGCGTGCCAAGCAGATTTTCTGGCAGCGGCT CTGGCACCGACTACAGCCTGACAATCAGCAACCTGGA ACAAGAGGATATCGCTACCTACTTCTGCCAGCAAGGCA ACACCCTGCCTTACACCTTTGGCGGAGGCACCAAGCTG GAAATCACCGGCTCTACAAGCGGCAGCGGCAAACCTG GATCTGGCGAGGGATCTACCAAGGGCGAAGTCCAGCT GCTGGAAAGCGGTGGCGGTCTGGTCCAGCCTGGCGGC ACCCTGCGCCTGTCCTGTGCCGCTAGCGGCCTGACCTG CTATAGCTATGCCATGGGTTGGTACCGCCAGGCCCCTG GTAAGGAGCGCGAATTCGTGTCCGCTATTTCCAGCGGC GGCTCCGCCTATTATGCTGATAGCGTCAAGGGTCGCTT CACCATTTGCCGCGACAACAGCAAAAACACTCTGTATC TGCAGATGAACTCCCTGCGCGCTGAGGATACCGCCGTC TACTACTGCGCTGTGGGCCCTTATTATGGCTTCCGCGCT GTGACTGAGGCTGACTACTGGGGTCAGGGCACTCAGG TGACTGTGAGCAGCGGCGGTGGCGGATCAGAAGTCCA GCTGCTGGAAAGCGGTGGCGGTCTGGTCCAGCCTGGC	178

	GGCACCCTGCGCCTGTCCTGTGCCGCTAGCGGCCTGAC CTGCTATAGCTATGCCATGGGTTGGTACCGCCAGGCCC CTGGTAAGGAGCGCGAATTCGTGTCCGCTATTTCCAGC GGCGGCTCCGCCTATTATGCTGATAGCGTCAAGGGTCG CTTCACCATTTGCCGCGACAACAGCAAAAACACTCTGT ATCTGCAGATGAACTCCCTGCGCGCTGAGGATACCGCC GTCTACTACTGCGCTGTGGGCCCTTATTATGGCTTCCGC GCTGTGACTGAGGCTGACTACTGGGGTCAGGGCACTCA GGTGACTGTGAGCAGCGGCAGTACTTCTGGTAGCGGA AAACCCGGTAGCGGCGAGGGGTCAACTAAAGGAGAAG TGAAACTGCAAGAGTCTGGCCCTGGACTGGTGGCCCCA TCTCAGTCTCTGAGCGTGACCTGTACAGTCAGCGGAGT GTCCCTGCCTGATTACGGCGTGTCCTGGATCAGACAGC CTCCTCGGAAAGGCCTGGAATGGCTGGGAGTGATCTG GGGCAGCGAGACAACCTACTACAACAGCGCCCTGAAG TCCCGGCTGACCATCATCAAGGACAACTCCAAGAGCC AGGTGTTCCTGAAGATGAACAGCCTGCAGACCGACGA CACCGCCATCTACTATTGCGCCAAGCACTACTACTACG GCGGCAGCTACGCCATGGATTATTGGGGCCAGGGCAC CAGCGTGACCGTGTCTAGCATCGAAGTGATGTACCCTC CACCTTACCTGGACAACGAGAAGTCCAACGGCACCAT CATCCACGTGAAGGGCAAGCACCTGTGTCCTTCTCCAC TGTTCCCCGGACCTAGCAAGCCTTTCTGGGTGCTCGTT GTTGTTGGCGGCGTGCTGGCCTGTTATAGCCTGCTTGT GACCGTGGCCTTCATCATCTTTTGGGTCCGAAGCAAGC GGAGCCGGCTGCTGCACTCCGACTACATGAACATGACC CCTAGACGGCCCGGACCAACCAGAAAGCACTACCAGC CTTACGCTCCTCCTAGAGACTTCGCCGCCTACCGGTCC AGAGTGAAGTTCAGCAGATCCGCCGATGCTCCCGCCTA TCAGCAGGGCCAAAACCAGCTGTACAACGAGCTGAAC CTGGGGAGAAGAGAAGAGTACGACGTGCTGGACAAGC GGAGAGGCAGAGATCCTGAAATGGGCGGCAAGCCCAG ACGGAAGAATCCTCAAGAGGGCCTGTATAATGAGCTG CAGAAAGACAAGATGGCCGAGGCCTACAGCGAGATCG GAATGAAGGGCGAGCGCAGAAGAGGCAAGGGACACG ATGGACTGTACCAGGGCCTGAGCACCGCCACCAAGGA TACCTATGATGCCCTGCACATGCAGGCCCTGCCTCCAA GA	
FMC63LC_D04AMD11_A01v	ATGGCCAGATCTCCTGCTCAACTGCTGGGACTGCTGCT GCTGTGGCTTAGCGGAGCCAGATGCGACATCCAGATG	142

2_FMC63HC_CD28_CD28_CD28_CD3Z (P2191)	ACCCAGACCACAAGCAGCCTGTCTGCCAGCCTGGGCG ATAGAGTGACCATCAGCTGTAGAGCCAGCCAGGACAT CAGCAAGTACCTGAACTGGTATCAGCAAAAGCCCGAC GGCACCGTGAAGCTGCTGATCTACCACACCAGCAGACT GCACAGCGGCGTGCCAAGCAGATTTTCTGGCAGCGGCT CTGGCACCGACTACAGCCTGACAATCAGCAACCTGGA ACAAGAGGATATCGCTACCTACTTCTGCCAGCAAGGCA ACACCCTGCCTTACACCTTTGGCGGAGGCACCAAGCTG GAAATCACCGGCTCTACAAGCGGCAGCGGCAAACCTG GATCTGGCGAGGGATCTACCAAGGGCCAGGTGCAGCT GGTTGAGTCTGGGGGAGGCCTTGTCCAGGCTGGGGGG TCCCTGAGACTCTCCTGTGCAGCGTCTGGAAGCGAATT CACCGGTTATCCCATGGGCTGGTTTCGCCAGGCTCCAG GCAAGGAAAGGGAGTTTGTCGCTGGCTCCGTAGGTATC GGTGGTAGTACAAACTATGCAGACTCCGTGAAGGGCC GATTCACCATCTCCAGAGACAATGCGAAGAACACGGT CTATCTGCAAATGAACAGCCTGAAGCCAGAGGACACG GCTGTGTATTACTGTGCGGCCGACAAAGACTACTACAA ACCTTATAGTCGATATAGGACCGCTATCAGGTACGATA CCTGGGGCCAAGGGACCCAGGTCACCGTCTCGAGCGG CGGTGGCGGATCAGAAGTCCAGCTGCTGGAAAGCGGT GGCGGTCTGGTCCAGCCTGGCGGCACCCTGCGCCTGTC CTGTGCCGCTAGCGGCCTGACCTGCTATAGCTATGCCA TGGGTTGGTACCGCCAGGCCCCTGGTAAGGAGCGCGA ATTCGTGTCCGCTATTTCCAGCGGCGGCTCCGCCTATT ATGCTGATAGCGTCAAGGGTCGCTTCACCATTTGCCGC GACAACAGCAAAAACACTCTGTATCTGCAGATGAACT CCCTGCGCGCTGAGGATACCGCCGTCTACTACTGCGCT GTGGGCCCTTATTATGGCTTCCGCGCTGTGACTGAGGC TGACTACTGGGGTCAGGGCACTCAGGTGACTGTGAGC AGCGGCAGTACTTCTGGTAGCGGAAAACCCGGTAGCG GCGAGGGGTCAACTAAAGGAGAAGTGAAACTGCAAGA GTCTGGCCCTGGACTGGTGGCCCCATCTCAGTCTCTGA GCGTGACCTGTACAGTCAGCGGAGTGTCCCTGCCTGAT TACGGCGTGTCCTGGATCAGACAGCCTCCTCGGAAAGG CCTGGAATGGCTGGGAGTGATCTGGGGCAGCGAGACA ACCTACTACAACAGCGCCCTGAAGTCCCGGCTGACCAT CATCAAGGACAACTCCAAGAGCCAGGTGTTCCTGAAG ATGAACAGCCTGCAGACCGACGACACCGCCATCTACT ATTGCGCCAAGCACTACTACTACGGCGGCAGCTACGCC	

	ATGGATTATTGGGGCCAGGGCACCAGCGTGACCGTGTC TAGCATCGAAGTGATGTACCCTCCACCTTACCTGGACA ACGAGAAGTCCAACGGCACCATCATCCACGTGAAGGG CAAGCACCTGTGTCCTTCTCCACTGTTCCCCGGACCTA GCAAGCCTTTCTGGGTGCTCGTTGTTGTTGGCGGCGTG CTGGCCTGTTATAGCCTGCTTGTGACCGTGGCCTTCATC ATCTTTTGGGTCCGAAGCAAGCGGAGCCGGCTGCTGCA CTCCGACTACATGAACATGACCCCTAGACGGCCCGGAC CAACCAGAAAGCACTACCAGCCTTACGCTCCTCCTAGA GACTTCGCCGCCTACCGGTCCAGAGTGAAGTTCAGCAG ATCCGCCGATGCTCCCGCCTATCAGCAGGGCCAAAACC AGCTGTACAACGAGCTGAACCTGGGGAGAAGAGAAGA GTACGACGTGCTGGACAAGCGGAGAGGCAGAGATCCT GAAATGGGCGGCAAGCCCAGACGGAAGAATCCTCAAG AGGGCCTGTATAATGAGCTGCAGAAAGACAAGATGGC CGAGGCCTACAGCGAGATCGGAATGAAGGGCGAGCGC AGAAGAGGCAAGGGACACGATGGACTGTACCAGGGCC TGAGCACCGCCACCAAGGATACCTATGATGCCCTGCAC ATGCAGGCCCTGCCTCCAAGA	
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	CCTGGGGCCAAGGGACCCAGGTCACCGTCTCGAGCGG CGGTGGCGGATCAGAGGTACAACTTTTGGAGTCAGGC GGTGGACTGGTACAACCGGGTGGTTCATTGCGTTTGAG CTGCGCTGCCTCTGGTTTGACCTCTTATTCCTACGCGAT GGGCTGGTATCGCCAAGCGCCGGGCAAAGAACGCGAG TTTGTCAGCGCAATCAGCTCGGGTGGTAGCGCGTACTA CGCGGACTCGGTAAAAGGCCGTTTTACGATCAGTCGTG ATAATTCCAAGAATACCTTGTACCTGCAAATGAATAGC CTTCGCGCAGAAGACACAGCGGTGTATTATTGTGCCGT TGGACCGTACTACGGATTTAGAGCGGTTACCGAAGCA GATTATTGGGGCCAGGGTACCCAGGTGACGGTCTCGA GCGGCAGTACTTCTGGTAGCGGAAAACCCGGTAGCGG CGAGGGGTCAACTAAAGGAGAAGTGAAACTGCAAGAG TCTGGCCCTGGACTGGTGGCCCCATCTCAGTCTCTGAG CGTGACCTGTACAGTCAGCGGAGTGTCCCTGCCTGATT ACGGCGTGTCCTGGATCAGACAGCCTCCTCGGAAAGG CCTGGAATGGCTGGGAGTGATCTGGGGCAGCGAGACA ACCTACTACAACAGCGCCCTGAAGTCCCGGCTGACCAT CATCAAGGACAACTCCAAGAGCCAGGTGTTCCTGAAG ATGAACAGCCTGCAGACCGACGACACCGCCATCTACT ATTGCGCCAAGCACTACTACTACGGCGGCAGCTACGCC ATGGATTATTGGGGCCAGGGCACCAGCGTGACCGTGTC TAGCATCGAAGTGATGTACCCTCCACCTTACCTGGACA ACGAGAAGTCCAACGGCACCATCATCCACGTGAAGGG CAAGCACCTGTGTCCTTCTCCACTGTTCCCCGGACCTA GCAAGCCTTTCTGGGTGCTCGTTGTTGTTGGCGGCGTG CTGGCCTGTTATAGCCTGCTTGTGACCGTGGCCTTCATC ATCTTTTGGGTCCGAAGCAAGCGGAGCCGGCTGCTGCA CTCCGACTACATGAACATGACCCCTAGACGGCCCGGAC CAACCAGAAAGCACTACCAGCCTTACGCTCCTCCTAGA GACTTCGCCGCCTACCGGTCCAGAGTGAAGTTCAGCAG ATCCGCCGATGCTCCCGCCTATCAGCAGGGCCAAAACC AGCTGTACAACGAGCTGAACCTGGGGAGAAGAGAAGA GTACGACGTGCTGGACAAGCGGAGAGGCAGAGATCCT GAAATGGGCGGCAAGCCCAGACGGAAGAATCCTCAAG AGGGCCTGTATAATGAGCTGCAGAAAGACAAGATGGC CGAGGCCTACAGCGAGATCGGAATGAAGGGCGAGCGC AGAAGAGGCAAGGGACACGATGGACTGTACCAGGGCC TGAGCACCGCCACCAAGGATACCTATGATGCCCTGCAC ATGCAGGCCCTGCCTCCAAGA	

FMC63LC_A01v1_D04AMD11__A01v2_FMC63HC_CD28_CD28_CD28_CD3Z (P2195)	ATGGCCAGATCTCCTGCTCAACTGCTGGGACTGCTGCT GCTGTGGCTTAGCGGAGCCAGATGCGACATCCAGATG ACCCAGACCACAAGCAGCCTGTCTGCCAGCCTGGGCG ATAGAGTGACCATCAGCTGTAGAGCCAGCCAGGACAT CAGCAAGTACCTGAACTGGTATCAGCAAAAGCCCGAC GGCACCGTGAAGCTGCTGATCTACCACACCAGCAGACT GCACAGCGGCGTGCCAAGCAGATTTTCTGGCAGCGGCT CTGGCACCGACTACAGCCTGACAATCAGCAACCTGGA ACAAGAGGATATCGCTACCTACTTCTGCCAGCAAGGCA ACACCCTGCCTTACACCTTTGGCGGAGGCACCAAGCTG GAAATCACCGGCTCTACAAGCGGCAGCGGCAAACCTG GATCTGGCGAGGGATCTACCAAGGGCGAGGTACAACT TTTGGAGTCAGGCGGTGGACTGGTACAACCGGGTGGTT CATTGCGTTTGAGCTGCGCTGCCTCTGGTTTGACCTCTT ATTCCTACGCGATGGGCTGGTATCGCCAAGCGCCGGGC AAAGAACGCGAGTTTGTCAGCGCAATCAGCTCGGGTG GTAGCGCGTACTACGCGGACTCGGTAAAAGGCCGTTTT ACGATCAGTCGTGATAATTCCAAGAATACCTTGTACCT GCAAATGAATAGCCTTCGCGCAGAAGACACAGCGGTG TATTATTGTGCCGTTGGACCGTACTACGGATTTAGAGC GGTTACCGAAGCAGATTATTGGGGCCAGGGTACCCAG GTGACGGTCTCGAGCGGCGGTGGCGGATCACAGGTGC AGCTGGTTGAGTCTGGGGGAGGCCTTGTCCAGGCTGGG GGGTCCCTGAGACTCTCCTGTGCAGCGTCTGGAAGCGA ATTCACCGGTTATCCCATGGGCTGGTTTCGCCAGGCTC CAGGCAAGGAAAGGGAGTTTGTCGCTGGCTCCGTAGG TATCGGTGGTAGTACAAACTATGCAGACTCCGTGAAGG GCCGATTCACCATCTCCAGAGACAATGCGAAGAACAC GGTCTATCTGCAAATGAACAGCCTGAAGCCAGAGGAC ACGGCTGTGTATTACTGTGCGGCCGACAAAGACTACTA CAAACCTTATAGTCGATATAGGACCGCTATCAGGTACG ATACCTGGGGCCAAGGGACCCAGGTCACCGTCTCGAG CGGTGGCGGTGGTTCTGAAGTCCAGCTGCTGGAAAGC GGTGGCGGTCTGGTCCAGCCTGGCGGCACCCTGCGCCT GTCCTGTGCCGCTAGCGGCCTGACCTGCTATAGCTATG CCATGGGTTGGTACCGCCAGGCCCCTGGTAAGGAGCG CGAATTCGTGTCCGCTATTTCCAGCGGCGGCTCCGCCT ATTATGCTGATAGCGTCAAGGGTCGCTTCACCATTTGC CGCGACAACAGCAAAAACACTCTGTATCTGCAGATGA ACTCCCTGCGCGCTGAGGATACCGCCGTCTACTACTGC	143

	GCTGTGGGCCCTTATTATGGCTTCCGCGCTGTGACTGA GGCTGACTACTGGGGTCAGGGCACTCAGGTGACTGTG AGCAGCGGCAGTACTTCTGGTAGCGGAAAACCCGGTA GCGGCGAGGGGTCAACTAAAGGAGAAGTGAAACTGCA AGAGTCTGGCCCTGGACTGGTGGCCCCATCTCAGTCTC TGAGCGTGACCTGTACAGTCAGCGGAGTGTCCCTGCCT GATTACGGCGTGTCCTGGATCAGACAGCCTCCTCGGAA AGGCCTGGAATGGCTGGGAGTGATCTGGGGCAGCGAG ACAACCTACTACAACAGCGCCCTGAAGTCCCGGCTGAC CATCATCAAGGACAACTCCAAGAGCCAGGTGTTCCTGA AGATGAACAGCCTGCAGACCGACGACACCGCCATCTA CTATTGCGCCAAGCACTACTACTACGGCGGCAGCTACG CCATGGATTATTGGGGCCAGGGCACCAGCGTGACCGT GTCTAGCATCGAAGTGATGTACCCTCCACCTTACCTGG ACAACGAGAAGTCCAACGGCACCATCATCCACGTGAA GGGCAAGCACCTGTGTCCTTCTCCACTGTTCCCCGGAC CTAGCAAGCCTTTCTGGGTGCTCGTTGTTGTTGGCGGC GTGCTGGCCTGTTATAGCCTGCTTGTGACCGTGGCCTT CATCATCTTTTGGGTCCGAAGCAAGCGGAGCCGGCTGC TGCACTCCGACTACATGAACATGACCCCTAGACGGCCC GGACCAACCAGAAAGCACTACCAGCCTTACGCTCCTCC TAGAGACTTCGCCGCCTACCGGTCCAGAGTGAAGTTCA GCAGATCCGCCGATGCTCCCGCCTATCAGCAGGGCCAA AACCAGCTGTACAACGAGCTGAACCTGGGGAGAAGAG AAGAGTACGACGTGCTGGACAAGCGGAGAGGCAGAGA TCCTGAAATGGGCGGCAAGCCCAGACGGAAGAATCCT CAAGAGGGCCTGTATAATGAGCTGCAGAAAGACAAGA TGGCCGAGGCCTACAGCGAGATCGGAATGAAGGGCGA GCGCAGAAGAGGCAAGGGACACGATGGACTGTACCAG GGCCTGAGCACCGCCACCAAGGATACCTATGATGCCCT GCACATGCAGGCCCTGCCTCCAAGA	
FMC63LC_A01v2_D04AMD11__A01v1_FMC63HC_CD28_CD28_CD28_CD3Z (P2195)	ATGGCCAGATCTCCTGCTCAACTGCTGGGACTGCTGCT GCTGTGGCTTAGCGGAGCCAGATGCGACATCCAGATG ACCCAGACCACAAGCAGCCTGTCTGCCAGCCTGGGCG ATAGAGTGACCATCAGCTGTAGAGCCAGCCAGGACAT CAGCAAGTACCTGAACTGGTATCAGCAAAAGCCCGAC GGCACCGTGAAGCTGCTGATCTACCACACCAGCAGACT GCACAGCGGCGTGCCAAGCAGATTTTCTGGCAGCGGCT CTGGCACCGACTACAGCCTGACAATCAGCAACCTGGA ACAAGAGGATATCGCTACCTACTTCTGCCAGCAAGGCA	168

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FMC63LC_A01v1_D04AMD11__A01v1_FMC63HC_CD28_CD28_CD28_CD3Z (P2195)	ATGGCCAGATCTCCTGCTCAACTGCTGGGACTGCTGCT GCTGTGGCTTAGCGGAGCCAGATGCGACATCCAGATG ACCCAGACCACAAGCAGCCTGTCTGCCAGCCTGGGCG ATAGAGTGACCATCAGCTGTAGAGCCAGCCAGGACAT CAGCAAGTACCTGAACTGGTATCAGCAAAAGCCCGAC GGCACCGTGAAGCTGCTGATCTACCACACCAGCAGACT GCACAGCGGCGTGCCAAGCAGATTTTCTGGCAGCGGCT CTGGCACCGACTACAGCCTGACAATCAGCAACCTGGA ACAAGAGGATATCGCTACCTACTTCTGCCAGCAAGGCA ACACCCTGCCTTACACCTTTGGCGGAGGCACCAAGCTG GAAATCACCGGCTCTACAAGCGGCAGCGGCAAACCTG GATCTGGCGAGGGATCTACCAAGGGCGAGGTACAACT TTTGGAGTCAGGCGGTGGACTGGTACAACCGGGTGGTT CATTGCGTTTGAGCTGCGCTGCCTCTGGTTTGACCTCTT ATTCCTACGCGATGGGCTGGTATCGCCAAGCGCCGGGC AAAGAACGCGAGTTTGTCAGCGCAATCAGCTCGGGTG GTAGCGCGTACTACGCGGACTCGGTAAAAGGCCGTTTT ACGATCAGTCGTGATAATTCCAAGAATACCTTGTACCT	169

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CAGGCAAGGAAAGGGAGTTTGTCGCTGGCTCCGTAGG
TATCGGTGGTAGTACAAACTATGCAGACTCCGTGAAGG
GCCGATTCACCATCTCCAGAGACAATGCGAAGAACAC
GGTCTATCTGCAAATGAACAGCCTGAAGCCAGAGGAC
ACGGCTGTGTATTACTGTGCGGCCGACAAAGACTACTA
CAAACCTTATAGTCGATATAGGACCGCTATCAGGTACG
ATACCTGGGGCCAAGGGACCCAGGTCACCGTCTCGAG
CGGTGGCGGTGGTTCTGAGGTACAACTTTTGGAGTCAG
GCGGTGGACTGGTACAACCGGGTGGTTCATTGCGTTTG
AGCTGCGCTGCCTCTGGTTTGACCTCTTATTCCTACGCG
ATGGGCTGGTATCGCCAAGCGCCGGGCAAAGAACGCG
AGTTTGTCAGCGCAATCAGCTCGGGTGGTAGCGCGTAC
TACGCGGACTCGGTAAAAGGCCGTTTTACGATCAGTCG
TGATAATTCCAAGAATACCTTGTACCTGCAAATGAATA
GCCTTCGCGCAGAAGACACAGCGGTGTATTATTGTGCC
GTTGGACCGTACTACGGATTTAGAGCGGTTACCGAAGC
AGATTATTGGGGCCAGGGTACCCAGGTGACGGTCTCG
AGCGGCAGTACTTCTGGTAGCGGAAAACCCGGTAGCG
GCGAGGGGTCAACTAAAGGAGAAGTGAAACTGCAAGA
GTCTGGCCCTGGACTGGTGGCCCCATCTCAGTCTCTGA
GCGTGACCTGTACAGTCAGCGGAGTGTCCCTGCCTGAT
TACGGCGTGTCCTGGATCAGACAGCCTCCTCGGAAAGG
CCTGGAATGGCTGGGAGTGATCTGGGGCAGCGAGACA
ACCTACTACAACAGCGCCCTGAAGTCCCGGCTGACCAT
CATCAAGGACAACTCCAAGAGCCAGGTGTTCCTGAAG
ATGAACAGCCTGCAGACCGACGACACCGCCATCTACT
ATTGCGCCAAGCACTACTACTACGGCGGCAGCTACGCC
ATGGATTATTGGGGCCAGGGCACCAGCGTGACCGTGTC
TAGCATCGAAGTGATGTACCCTCCACCTTACCTGGACA
ACGAGAAGTCCAACGGCACCATCATCCACGTGAAGGG
CAAGCACCTGTGTCCTTCTCCACTGTTCCCCGGACCTA
GCAAGCCTTTCTGGGTGCTCGTTGTTGTTGGCGGCGTG
CTGGCCTGTTATAGCCTGCTTGTGACCGTGGCCTTCATC

	ATCTTTTGGGTCCGAAGCAAGCGGAGCCGGCTGCTGCA CTCCGACTACATGAACATGACCCCTAGACGGCCCGGAC CAACCAGAAAGCACTACCAGCCTTACGCTCCTCCTAGA GACTTCGCCGCCTACCGGTCCAGAGTGAAGTTCAGCAG ATCCGCCGATGCTCCCGCCTATCAGCAGGGCCAAAACC AGCTGTACAACGAGCTGAACCTGGGGAGAAGAGAAGA GTACGACGTGCTGGACAAGCGGAGAGGCAGAGATCCT GAAATGGGCGGCAAGCCCAGACGGAAGAATCCTCAAG AGGGCCTGTATAATGAGCTGCAGAAAGACAAGATGGC CGAGGCCTACAGCGAGATCGGAATGAAGGGCGAGCGC AGAAGAGGCAAGGGACACGATGGACTGTACCAGGGCC TGAGCACCGCCACCAAGGATACCTATGATGCCCTGCAC ATGCAGGCCCTGCCTCCAAGA	
FMC63LC_A01v2_D04AMD11__A01v2_FMC63HC_CD28_CD28_CD28_CD3Z (P2195)	ATGGCCAGATCTCCTGCTCAACTGCTGGGACTGCTGCT GCTGTGGCTTAGCGGAGCCAGATGCGACATCCAGATG ACCCAGACCACAAGCAGCCTGTCTGCCAGCCTGGGCG ATAGAGTGACCATCAGCTGTAGAGCCAGCCAGGACAT CAGCAAGTACCTGAACTGGTATCAGCAAAAGCCCGAC GGCACCGTGAAGCTGCTGATCTACCACACCAGCAGACT GCACAGCGGCGTGCCAAGCAGATTTTCTGGCAGCGGCT CTGGCACCGACTACAGCCTGACAATCAGCAACCTGGA ACAAGAGGATATCGCTACCTACTTCTGCCAGCAAGGCA ACACCCTGCCTTACACCTTTGGCGGAGGCACCAAGCTG GAAATCACCGGCTCTACAAGCGGCAGCGGCAAACCTG GATCTGGCGAGGGATCTACCAAGGGCGAAGTCCAGCT GCTGGAAAGCGGTGGCGGTCTGGTCCAGCCTGGCGGC ACCCTGCGCCTGTCCTGTGCCGCTAGCGGCCTGACCTG CTATAGCTATGCCATGGGTTGGTACCGCCAGGCCCCTG GTAAGGAGCGCGAATTCGTGTCCGCTATTTCCAGCGGC GGCTCCGCCTATTATGCTGATAGCGTCAAGGGTCGCTT CACCATTTGCCGCGACAACAGCAAAAACACTCTGTATC TGCAGATGAACTCCCTGCGCGCTGAGGATACCGCCGTC TACTACTGCGCTGTGGGCCCTTATTATGGCTTCCGCGCT GTGACTGAGGCTGACTACTGGGGTCAGGGCACTCAGG TGACTGTGAGCAGCGGCGGTGGCGGATCACAGGTGCA GCTGGTTGAGTCTGGGGGAGGCCTTGTCCAGGCTGGGG GGTCCCTGAGACTCTCCTGTGCAGCGTCTGGAAGCGAA TTCACCGGTTATCCCATGGGCTGGTTTCGCCAGGCTCC AGGCAAGGAAAGGGAGTTTGTCGCTGGCTCCGTAGGT ATCGGTGGTAGTACAAACTATGCAGACTCCGTGAAGG	170

	GCCGATTCACCATCTCCAGAGACAATGCGAAGAACAC GGTCTATCTGCAAATGAACAGCCTGAAGCCAGAGGAC ACGGCTGTGTATTACTGTGCGGCCGACAAAGACTACTA CAAACCTTATAGTCGATATAGGACCGCTATCAGGTACG ATACCTGGGGCCAAGGGACCCAGGTCACCGTCTCGAG CGGTGGCGGTGGTTCTGAAGTCCAGCTGCTGGAAAGC GGTGGCGGTCTGGTCCAGCCTGGCGGCACCCTGCGCCT GTCCTGTGCCGCTAGCGGCCTGACCTGCTATAGCTATG CCATGGGTTGGTACCGCCAGGCCCCTGGTAAGGAGCG CGAATTCGTGTCCGCTATTTCCAGCGGCGGCTCCGCCT ATTATGCTGATAGCGTCAAGGGTCGCTTCACCATTTGC CGCGACAACAGCAAAAACACTCTGTATCTGCAGATGA ACTCCCTGCGCGCTGAGGATACCGCCGTCTACTACTGC GCTGTGGGCCCTTATTATGGCTTCCGCGCTGTGACTGA GGCTGACTACTGGGGTCAGGGCACTCAGGTGACTGTG AGCAGCGGCAGTACTTCTGGTAGCGGAAAACCCGGTA GCGGCGAGGGGTCAACTAAAGGAGAAGTGAAACTGCA AGAGTCTGGCCCTGGACTGGTGGCCCCATCTCAGTCTC TGAGCGTGACCTGTACAGTCAGCGGAGTGTCCCTGCCT GATTACGGCGTGTCCTGGATCAGACAGCCTCCTCGGAA AGGCCTGGAATGGCTGGGAGTGATCTGGGGCAGCGAG ACAACCTACTACAACAGCGCCCTGAAGTCCCGGCTGAC CATCATCAAGGACAACTCCAAGAGCCAGGTGTTCCTGA AGATGAACAGCCTGCAGACCGACGACACCGCCATCTA CTATTGCGCCAAGCACTACTACTACGGCGGCAGCTACG CCATGGATTATTGGGGCCAGGGCACCAGCGTGACCGT GTCTAGCATCGAAGTGATGTACCCTCCACCTTACCTGG ACAACGAGAAGTCCAACGGCACCATCATCCACGTGAA GGGCAAGCACCTGTGTCCTTCTCCACTGTTCCCCGGAC CTAGCAAGCCTTTCTGGGTGCTCGTTGTTGTTGGCGGC GTGCTGGCCTGTTATAGCCTGCTTGTGACCGTGGCCTT CATCATCTTTTGGGTCCGAAGCAAGCGGAGCCGGCTGC TGCACTCCGACTACATGAACATGACCCCTAGACGGCCC GGACCAACCAGAAAGCACTACCAGCCTTACGCTCCTCC TAGAGACTTCGCCGCCTACCGGTCCAGAGTGAAGTTCA GCAGATCCGCCGATGCTCCCGCCTATCAGCAGGGCCAA AACCAGCTGTACAACGAGCTGAACCTGGGGAGAAGAG AAGAGTACGACGTGCTGGACAAGCGGAGAGGCAGAGA TCCTGAAATGGGCGGCAAGCCCAGACGGAAGAATCCT CAAGAGGGCCTGTATAATGAGCTGCAGAAAGACAAGA	

	TGGCCGAGGCCTACAGCGAGATCGGAATGAAGGGCGA GCGCAGAAGAGGCAAGGGACACGATGGACTGTACCAG GGCCTGAGCACCGCCACCAAGGATACCTATGATGCCCT GCACATGCAGGCCCTGCCTCCAAGA	

In some embodiments, the antigen-binding domain of the second polypeptide binds to an antigen. The antigen-binding domain of the second polypeptide may bind to more than one antigen or more than one epitope in an antigen. For example, the antigen-binding domain of the second polypeptide may bind to two, three, four, five, six, seven, eight or more antigens. As another example, the antigen-binding domain of the second polypeptide may bind to two, three, four, five, six, seven, eight or more epitopes in the same antigen.

The choice of antigen-binding domain may depend upon the type and number of antigens that define the surface of a target cell. For example, the antigen-binding domain may be chosen to recognize an antigen that acts as a cell surface marker on target cells associated with a particular disease state. In certain embodiments, the CARs of the present disclosure can be genetically modified to target a tumor antigen of interest by way of engineering a desired antigen-binding domain that specifically binds to an antigen (e.g., on a tumor cell). Non-limiting examples of cell surface markers that may act as targets for the antigen-binding domain in the CAR of the disclosure include those associated with tumor cells or autoimmune diseases.

In some embodiments, the antigen-binding domain binds to at least one tumor antigen or autoimmune antigen.

In some embodiments, the antigen-binding domain binds to at least one tumor antigen. In some embodiments, the antigen-binding domain binds to two or more tumor antigens. In some embodiments, the two or more tumor antigens are associated with the same tumor. In some embodiments, the two or more tumor antigens are associated with different tumors.

In some embodiments, the antigen-binding domain binds to at least one autoimmune antigen. In some embodiments, the antigen-binding domain binds to two or more autoimmune antigens. In some embodiments, the two or more autoimmune antigens

are associated with the same autoimmune disease. In some embodiments, the two or more autoimmune antigens are associated with different autoimmune diseases.

In some embodiments, the tumor antigen is associated with glioblastoma, ovarian cancer, cervical cancer, head and neck cancer, liver cancer, prostate cancer, pancreatic cancer, renal cell carcinoma, bladder cancer, or hematologic malignancy. Non-limiting examples of tumor antigen associated with glioblastoma include HER2, EGFRvIII, EGFR, CD133, PDGFRA, FGFR1, FGFR3, MET, CD70, ROBO1and IL13Rα2. Non-limiting examples of tumor antigens associated with ovarian cancer include FOLR1, FSHR, MUC16, MUC1, Mesothelin, CA125, EpCAM, EGFR, PDGFRα, Nectin-4, and B7H4. Non-limiting examples of the tumor antigens associated with cervical cancer or head and neck cancer include GD2, MUC1, Mesothelin, HER2, and EGFR. Non-limiting examples of tumor antigen associated with liver cancer include Claudin 18.2, GPC-3, EpCAM, cMET, and AFP. Non-limiting examples of tumor antigens associated with hematological malignancies include CD22, CD79, BCMA, GPRC5D, SLAM F7, CD33, CLL1, CD123, and CD70. Non-limiting examples of tumor antigens associated with bladder cancer include Nectin-4 and SLITRK6.

Additional examples of antigens that may be targeted by the antigen-binding domain include, but are not limited to, alpha-fetoprotein, A3, antigen specific for A33 antibody, Ba 733, BrE3-antigen, carbonic anhydrase EX, CD1, CD1a, CD3, CD5, CD15, CD16, CD19, CD20, CD21, CD22, CD23, CD25, CD30, CD33, CD38, CD45, CD74, CD79a, CD80, CD123, CD138, colon-specific antigen-p (CSAp), CEA (CEACAM5), CEACAM6, CSAp, EGFR, EGP-I, EGP-2, Ep-CAM, EphA1, EphA2, EphA3, EphA4, EphA5, EphA6, EphA7, EphA8, EphA10, EphB1, EphB2, EphB3, EphB4, EphB6, Fit-I, Flt-3, folate receptor, HLA-DR, human chorionic gonadotropin (HCG) and its subunits, hypoxia inducible factor (HIF-I), Ia, IL-2, IL-6, IL-8, insulin growth factor-1 (IGF-I), KC4-antigen, KS-1-antigen, KS1-4, Le-Y, macrophage inhibition factor (MIF), MAGE, MUC2, MUC3, MUC4, NCA66, NCA95, NCA90, antigen specific for PAM-4 antibody, placental growth factor, p53, prostatic acid phosphatase, PSA, PSMA, RS5, S100, TAC, TAG-72, tenascin, TRAIL receptors, Tn antigen, Thomson-Friedenreich antigens, tumor necrosis antigens, VEGF, ED-B fibronectin, 17-1A-antigen, an angiogenesis marker, an oncogene marker or an oncogene product.

In one embodiment, the antigen targeted by the antigen-binding domain is CD19. In one embodiment, the antigen-binding domain comprises an anti-CD19 scFv. In one embodiment, the anti-CD19 scFv comprises a heavy chain variable region (VH) comprising the amino acid sequence set forth in SEQ ID NO: 2, or a variant thereof having at least 50, at least 55, at least 60, at least 65, at least 70, at least 75, at least 80, at least 85, at least 90, at least 95, at least 96, at least 97, at least 98 or at least 99%, sequence identity with SEQ ID NO: 2. In one embodiment, the anti-CD19 scFv comprises a light chain variable region (VL) comprising the amino acid sequence set forth in SEQ ID NO: 4, or a variant thereof having at least 50, at least 55, at least 60, at least 65, at least 70, at least 75, at least 80, at least 85, at least 90, at least 95, at least 96, at least 97, at least 98 or at least 99%, sequence identity with SEQ ID NO: 4. In one embodiment, the anti-CD19 scFv comprises the amino acid sequence set forth in SEQ ID NO: 7, or a variant thereof having at least 50, at least 55, at least 60, at least 65, at least 70, at least 75, at least 80, at least 85, at least 90, at least 95, at least 96, at least 97, at least 98 or at least 99%, sequence identity with SEQ ID NO: 7.

In some embodiments, the antigen is associated with an autoimmune disease or disorder. Such antigens may be derived from cell receptors and cells which produce "self"-directed antibodies. In some embodiments, the antigen is associated with an autoimmune disease or disorder such as Rheumatoid arthritis (RA), multiple sclerosis (MS), Sjögren's syndrome, Systemic lupus erythematosus, sarcoidosis, Type 1 diabetes mellitus, insulin dependent diabetes mellitus (IDDM), autoimmune thyroiditis, reactive arthritis, ankylosing spondylitis, scleroderma, polymyositis, dermatomyositis, psoriasis, vasculitis, Wegener's granulomatosis, Myasthenia gravis, Hashimoto's thyroiditis, Graves' disease, chronic inflammatory demyelinating polyneuropathy, Guillain-Barre syndrome, Crohn's disease or ulcerative colitis.

In some embodiments, autoimmune antigens that may be targeted by the CAR disclosed herein include but are not limited to platelet antigens, myelin protein antigen, Sm antigens in snRNPs, islet cell antigen, Rheumatoid factor, and anticitrullinated protein. Citrullinated proteins and peptides such as CCP-1, CCP-2 (cyclical citrullinated peptides), fibrinogen, fibrin, vimentin, 116longatio, collagen I and II peptides, alpha-enolase, translation initiation factor 4G1, perinuclear factor, keratin, Sa (cytoskeletal protein

vimentin), components of articular cartilage such as collagen II, IX, and XI, circulating serum proteins such as RFs (IgG, IgM), fibrinogen, plasminogen, ferritin, nuclear components such as RA33/hnRNP A2, Sm, eukaryotic 117longation117 117longation factor 1 alpha 1, stress proteins such as HSP-65, -70, -90, BiP, inflammatory/immune factors such as B7-H1, IL-1 alpha, and IL-8, enzymes such as calpastatin, alpha-enolase, aldolase-A, dipeptidyl peptidase, osteopontin, glucose-6-phosphate isomerase, receptors such as lipocortin 1, neutrophil nuclear proteins such as lactoferrin and 25-35 kD nuclear protein, granular proteins such as bactericidal permeability increasing protein (BPI), elastase, cathepsin G, myeloperoxidase, proteinase 3, platelet antigens, myelin protein antigen, islet cell antigen, rheumatoid factor, histones, ribosomal P proteins, cardiolipin, vimentin, nucleic acids such as dsDNA, ssDNA, and RNA, ribonuclear particles and proteins such as Sm antigens (including but not limited to SmD's and SmB′/B), U1RNP, A2/B1 hnRNP, Ro (SSA), and La (SSB) antigens.

In various embodiments, the scFv fragment used in the CAR of the present disclosure may include a linker between the VH and VL domains. The linker can be a peptide linker and may include any naturally occurring amino acid. Exemplary amino acids that may be included into the linker are Gly, Ser Pro, Thr, Glu, Lys, Arg, Ile, Leu, His and The. The linker should have a length that is adequate to link the VH and the VL in such a way that they form the correct conformation relative to one another so that they retain the desired activity, such as binding to an antigen. The linker may be about 5-50 amino acids long. In some embodiments, the linker is about 10-40 amino acids long. In some embodiments, the linker is about 10-35 amino acids long. In some embodiments, the linker is about 10-30 amino acids long. In some embodiments, the linker is about 10-25 amino acids long. In some embodiments, the linker is about 10-20 amino acids long. In some embodiments, the linker is about 15-20 amino acids long. Exemplary linkers that may be used are Gly rich linkers, Gly and Ser containing linkers, Gly and Ala containing linkers, Ala and Ser containing linkers, and other flexible linkers.

In one embodiment, the linker is a Whitlow linker. In one embodiment, the Whitlow linker comprises the amino acid sequence set forth in SEQ ID NO: 3, or a variant thereof having at least 50, at least 55, at least 60, at least 65, at least 70, at least 75, at least 80, at least 85, at least 90, at least 95, at least 96, at least 97, at least 98 or at least 99%, sequence

identity with SEQ ID NO: 3. In another embodiment, the linker is a $(G_4S)_3$ linker. In one embodiment, the $(G_4S)_3$ linker comprises the amino acid sequence set forth in SEQ ID NO: 25, or a variant thereof having at least 50, at least 55, at least 60, at least 65, at least 70, at least 75, at least 80, at least 85, at least 90, at least 95, at least 96, at least 97, at least 98 or at least 99%, sequence identity with SEQ ID NO: 25.

Other linker sequences may include portions of immunoglobulin hinge area, CL or CH1 derived from any immunoglobulin heavy or light chain isotype. Exemplary linkers that may be used include any of SEQ ID NOs: 26-56 in Table 1. Additional linkers are described for example in Int. Pat. Publ. No. WO2019/060695, incorporated by reference herein in its entirety.

II. Artificial Cell Death Polypeptide

According to embodiments of the application, an iPSC cell or a derivative cell thereof comprises an exogenous polynucleotide encoding an artificial cell death polypeptide.

As used herein, the term "artificial cell death polypeptide" refers to an engineered protein designed to prevent potential toxicity or otherwise adverse effects of a cell therapy. The artificial cell death polypeptide could mediate induction of apoptosis, inhibition of protein synthesis, DNA replication, growth arrest, transcriptional and post-transcriptional genetic regulation and/or antibody-mediated depletion. In some instance, the artificial cell death polypeptide is activated by an exogenous molecule, e.g. an antibody, that when activated, triggers apoptosis and/or cell death of a therapeutic cell.

In certain embodiments, an artificial cell death polypeptide comprises an inactivated cell surface receptor that comprises an epitope specifically recognized by an antibody, particularly a monoclonal antibody, which is also referred to herein as a monoclonal antibody-specific epitope. When expressed by iPSCs or derivative cells thereof, the inactivated cell surface receptor is signaling inactive or significantly impaired, but can still be specifically recognized by an antibody. The specific binding of the antibody to the inactivated cell surface receptor enables the elimination of the iPSCs or derivative cells thereof by ADCC and/or ADCP mechanisms, as well as, direct killing with antibody drug conjugates with toxins or radionuclides.

In certain embodiments, the inactivated cell surface receptor comprises an epitope that is selected from epitopes specifically recognized by an antibody, including but not limited to, ibritumomab, tiuxetan, muromonab-CD3, tositumomab, abciximab, basiliximab, brentuximab vedotin, cetuximab, infliximab, rituximab, alemtuzumab, bevacizumab, certolizumab pegol, daclizumab, eculizumab, efalizumab, gemtuzumab, natalizumab, omalizumab, palivizumab, polatuzumab vedotin, ranibizumab, tocilizumab, trastuzumab, vedolizumab, adalimumab, belimumab, canakinumab, denosumab, golimumab, ipilimumab, ofatumumab, panitumumab, or ustekinumab.

Epidermal growth factor receptor, also known as EGFR, ErbB1 and HER1, is a cell-surface receptor for members of the epidermal growth factor family of extracellular ligands. As used herein, "truncated EGFR," "tEGFR," "short EGFR" or "sEGFR" refers to an inactive EGFR variant that lacks the EGF-binding domains and the intracellular signaling domains of the EGFR. An exemplary tEGFR variant contains residues 322-333 of domain 2, all of domains 3 and 4 and the transmembrane domain of the native EGFR sequence containing the cetuximab binding epitope. Expression of the tEGFR variant on the cell surface enables cell elimination by an antibody that specifically binds to the tEGFR, such as cetuximab (Erbitux®), as needed. Due to the absence of the EGF-binding domains and intracellular signaling domains, tEGFR is inactive when expressed by iPSCs or derivative cell thereof.

An exemplary inactivated cell surface receptor of the application comprises a tEGFR variant. In certain embodiments, expression of the inactivated cell surface receptor in an engineered immune cell expressing a chimeric antigen receptor (CAR) induces cell suicide of the engineered immune cell when the cell is contacted with an anti-EGFR antibody. Methods of using inactivated cell surface receptors are described in WO2019/070856, WO2019/023396, WO2018/058002, the disclosure of which is incorporated herein by reference. For example, a subject who has previously received an engineered immune cell of the present disclosure that comprises a heterologous polynucleotide encoding an inactivated cell surface receptor comprising a tEGFR variant can be administered an anti-EGFR antibody in an amount effective to ablate in the subject the previously administered engineered immune cell.

In certain embodiments, the anti-EGFR antibody is cetuximab, matuzumab, necitumumab or panitumumab, preferably the anti-EGFR antibody is cetuximab.

In certain embodiments, the tEGFR variant comprises or consists of an amino acid sequence at least 90%, such as at least 90%, 91%, 82%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100%, identical to SEQ ID NO: 71, preferably the amino acid sequence of SEQ ID NO: 71.

In some embodiments, the inactivated cell surface receptor comprises one or more epitopes of CD79b, such as an epitope specifically recognized by polatuzumab vedotin. In certain embodiments, the CD79b epitope comprises or consists of an amino acid sequence at least 90%, such as at least 90%, 91%, 82%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100%, identical to SEQ ID NO: 78, preferably the amino acid sequence of SEQ ID NO: 78.

In some embodiments, the inactivated cell surface receptor comprises one or more epitopes of CD20, such as an epitope specifically recognized by rituximab. In certain embodiments, the CD20 epitope comprises or consists of an amino acid sequence at least 90%, such as at least 90%, 91%, 82%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100%, identical to SEQ ID NO: 80, preferably the amino acid sequence of SEQ ID NO: 80.

In some embodiments, the inactivated cell surface receptor comprises one or more epitopes of Her 2 receptor or ErbB, such as an epitope specifically recognized by trastuzumab. In certain embodiments, the monoclonal antibody-specific epitope comprises or consists of an amino acid sequence at least 90%, such as at least 90%, 91%, 82%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100%, identical to SEQ ID NO: 82, preferably the amino acid sequence of SEQ ID NO: 82.

In some embodiments the inactivated cell surface receptor further comprises a cytokine, such as interleukin-15 or interleukin-2.

As used herein “Interleukin-15” or “IL-15” refers to a cytokine that regulates T and NK cell activation and proliferation, or a functional portion thereof. A “functional portion” (“biologically active portion”) of a cytokine refers to a portion of the cytokine that retains one or more functions of full length or mature cytokine. Such functions for IL-15 include the promotion of NK cell survival, regulation of NK cell and T cell activation and proliferation as well as the support of NK cell development from

hematopoietic stem cells. As will be appreciated by those of skill in the art, the sequence of a variety of IL-15 molecules are known in the art. In certain embodiments, the IL-15 is a wild-type IL-15. In certain embodiments, the IL-15 is a human IL-15. In certain embodiments, the IL-15 comprises an amino acid sequence at least 90%, such as at least 90%, 91%, 82%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100%, identical to SEQ ID NO: 72, preferably the amino acid sequence of SEQ ID NO: 72.

As used herein "Interleukin-2" refers to a cytokine that regulates T and NK cell activation and proliferation, or a functional portion thereof. In certain embodiments, the IL-2 is a wild-type IL-2. In certain embodiments, the IL-2 is a human IL-2. In certain embodiments, the IL-2 comprises an amino acid sequence at least 90%, such as at least 90%, 91%, 82%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100%, identical to SEQ ID NO: 76, preferably the amino acid sequence of SEQ ID NO: 76.

In certain embodiments, an inactivated cell surface receptor comprises a monoclonal antibody-specific epitope operably linked to a cytokine, preferably by an autoprotease peptide sequence. Examples of the autoprotease peptide include, but are not limited to, a peptide sequence selected from the group consisting of porcine teschovirus-1 2A (P2A), a foot-and-mouth disease virus (FMDV) 2A (F2A), an Equine Rhinitis A Virus (ERAV) 2A (E2A), a Thosea asigna virus 2A (T2A), a cytoplasmic polyhedrosis virus 2A (BmCPV2A), a Flacherie Virus 2A (BmIFV2A), and a combination thereof. In one embodiment, the autoprotease peptide is an autoprotease peptide of porcine tesehovirus-1 2A (P2A). In certain embodiments, the autoprotease peptide comprises an amino acid sequence at least 90%, such as at least 90%, 91%, 82%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100%, identical to SEQ ID NO: 73, preferably the amino acid sequence of SEQ ID NO: 73.

In certain embodiments, an inactivated cell surface receptor comprises a truncated epithelial growth factor (tEGFR) variant operably linked to an interleukin-15 (IL-15) or IL-2 by an autoprotease peptide sequence. In a particular embodiment, the inactivated cell surface receptor comprises an amino acid sequence at least 90%, such as at least 90%, 91%, 82%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100%, identical to SEQ ID NO: 74, preferably the amino acid sequence of SEQ ID NO: 74.

In some embodiments, an inactivated cell surface receptor further comprises a signal sequence. In certain embodiments, the signal sequence comprises an amino acid sequence at least 90%, such as at least 90%, 91%, 82%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100%, identical to SEQ ID NO: 77, preferably the amino acid sequence of SEQ ID NO: 77.

In some embodiments, an inactivated cell surface receptor further comprises a hinge domain. In some embodiments, the hinge domain is derived from CD8. In one embodiment, the CD8 hinge domain comprises the amino acid sequence set forth in SEQ ID NO: 21, or a variant thereof having at least 50, at least 55, at least 60, at least 65, at least 70, at least 75, at least 80, at least 85, at least 90, at least 95, at least 96, at least 97, at least 98 or at least 99%, sequence identity with SEQ ID NO: 21.

In certain embodiments, an inactivated cell surface receptor further comprises a transmembrane domain. In some embodiments, the transmembrane domain is derived from CD8. In one embodiment, the CD8 transmembrane domain comprises the amino acid sequence set forth in SEQ ID NO: 23, or a variant thereof having at least 50, at least 55, at least 60, at least 65, at least 70, at least 75, at least 80, at least 85, at least 90, at least 95, at least 96, at least 97, at least 98 or at least 99%, sequence identity with SEQ ID NO: 23.

In certain embodiment, an inactivated cell surface receptor comprises one or more epitopes specifically recognized by an antibody in its extracellular domain, a transmembrane region and a cytoplasmic domain. In some embodiments, the inactivated cell surface receptor further comprises a hinge region between the epitope(s) and the transmembrane region. In some embodiments, the inactivated cell surface receptor comprises more than one epitopes specifically recognized by an antibody, the epitopes can have the same or different amino acid sequences, and the epitopes can be linked together via a peptide linker, such as a flexible peptide linker have the sequence of (GGGGS)n, wherein n is an integer of 1-8 (SEQ ID NO: 25). In some embodiments, the inactivated cell surface receptor further comprises a cytokine, such as an IL-15 or IL-2. In certain embodiments, the cytokine is in the cytoplasmic domain of the inactivated cell surface receptor. Preferably, the cytokine is operably linked to the epitope(s) specifically recognized by an antibody, directly or indirectly, via an autoprotease peptide sequence,

such as those described herein. In some embodiments, the cytokine is indirectly linked to the epitope(s) by connecting to the transmembrane region via the autoprotease peptide sequence.

In some embodiments, the artificial cell death polypeptide can comprise an inducible Caspase 9 sequence (iCasp9). Caspase 9 homodimerizes to become activated. The homodimer undergoes a conformational change and the proteolytic domain of one of a pair of dimers becomes active. Physiologically, this occurs by binding of the CARD domain of Caspase 9 to APAF-1. In iCasp9, the APAF-1 domain is replaced with a modified FKBP12 which has been mutated to selectively bind a chemical inducer of dimerization (CID). Presence of the CID results in homodimerization and activation. iCasp9 is based on a modified human caspase 9 fused to a human FK506 binding protein (FKBP) (Straathof et al (2005) Blood 105:4247-4254). It enables conditional dimerization in the presence of a small molecule CID, known as AP1903. AP1903 is an experimental drug and is considered biologically inert since it does not interact with wild-type FKBP12. However clinical experience with this agent is limited to a very small number of patients (Di Stasi, A. et al. (2011) N. Engl. J. Med. 365, 1673-1683; and Iuliucci, J. D. et al. (2001) J. Clin. Pharmacol. 41, 870-879). AP1903 is also a relatively large and polar molecule and unlikely to cross the blood-brain barrier.

Non-limiting exemplary artificial cell death polypeptide / inactivated cell surface receptor regions and sequences are provided in Table 2.

Table 2.

Regions	Sequence	SEQ ID NO
tEGFR-IL-15:		
tEGFR	MRPSGTAGAALLALLAALCPASRAGVRKCKKCEGPCRK VCNGIGIGEFKDSLSINATNIKHFKNCTSISGDLHILPVAF RGDSFTHTPPLDPQELDILKTVKEITGFLLIQAWPENRTD LHAFENLEIIRGRTKQHGQFSLAVVSLNITSLGLRSLKEIS DGDVIISGNKNLCYANTINWKKLFGTSGQKTKIISNRGE NSCKATGQVCHALCSPEGCWGPEPRDCVSCRNVSRGRE CVDKCNLLEGEPREFVENSECIQCHPECLPQAMNITCTG RGPDNCIQCAHYIDGPHCVKTCPAGVMGENNTLVWKY ADAGHVCHLCHPNCTYGCTGPGLEGCPTNGPKIPSIATG MVGALLLLLVVALGIGLFM	71
P2A	ATNFSLLKQAGDVEENPGP	73

IL-15	MRISKPHLRSISIQCYLCLLLNSHFLTEAGIHVFILGCFSA GLPKTEANWVNVISDLKKIEDLIQSMHIDATLYTESDVH PSCKVTAMKCFLLELQVISLESGDASIHDTVENLIILANN SLSSNGNVTESGCKECEELEEKNIKEFLQSFVHIVQMFIN TS	72
CD79b-IL-15:		
Signal Sequence	MEFGLSWVFLVALFRGVQC	77
CD79b epitope	ARSEDRYRNPKGSACSRIWQS	78
CD8 (AA 136-182)	TTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGL DFACD	21
CD8 (AA 183-203)	IYIWAPLAGTCGVLLLSLVIT	23
P2A	ATNFSLLKQAGDVEENPGP	73
IL-15	MRISKPHLRSISIQCYLCLLLNSHFLTEAGIHVFILGCFSA GLPKTEANWVNVISDLKKIEDLIQSMHIDATLYTESDVH PSCKVTAMKCFLLELQVISLESGDASIHDTVENLIILANN SLSSNGNVTESGCKECEELEEKNIKEFLQSFVHIVQMFIN TS	72
CD20 mimitope-IL-15:		
Signal Sequence	MEFGLSWVFLVALFRGVQC	77
CD20 mimitope	ACPYANPSLC	80
Linker	GGGSGGGS	27
CD8 (AA 136-182)	TTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGL DFACD	21
CD8 (AA 183-203)	IYIWAPLAGTCGVLLLSLVIT	23
P2A	ATNFSLLKQAGDVEENPGP	73
IL-15	MRISKPHLRSISIQCYLCLLLNSHFLTEAGIHVFILGCFSA GLPKTEANWVNVISDLKKIEDLIQSMHIDATLYTESDVH PSCKVTAMKCFLLELQVISLESGDASIHDTVENLIILANN SLSSNGNVTESGCKECEELEEKNIKEFLQSFVHIVQMFIN TS	72
ErbB epitope-IL-15:		
Signal Sequence	MEFGLSWVFLVALFRGVQC	77
ErbB epitope	EGLACHQLCARGHCWGPGPTQCVNCSQFLRGQECVEE CRVLQGLPREYVNARHCLPCHPECQPQNGSVTCFGPEA DQCVACAHYKDPPFCVARCPSGVKPDLSYMPIWKFPDE EGACQPCPINCTHSCVDLDDKGCPAEQRASPLTSIISAVV GILLVVVLGVVFGILIGGGGSGG	82
P2A	ATNFSLLKQAGDVEENPGP	73

IL-15	MRISKPHLRSISIQCYLCLLLNSHFLTEAGIHVFILGCFSA GLPKTEANWVNVISDLKKIEDLIQSMHIDATLYTESDVH PSCKVTAMKCFLLELQVISLESGDASIHDTVENLIILANN SLSSNGNVTESGCKECEELEEKNIKEFLQSFVHIVQMFIN TS	72
Inducible Caspase:		
FKBP12-dCasp9	MLEGVQVETISPGDGRTFPKRGQTCVVHYTGMLEDGKK VDSSRDRNKPFKFMLGKQEVIRGWEEGVAQMSVGQRA KLTISPDYAYGATGHPGIIPPHATLVFDVELLKLESGGGS GVDGFGDVGALESLRGNADLAYILSMEPCGHCLIINNVN FCRESGLRTRTGSNIDCEKLRRRFSSLHFMVEVKGDLTA KKMVLALLELARQDHGALDCCVVVILSHGCQASHLQFP GAVYGTDGCPVSVEKIVNIFNGTSCPSLGGKPKLFFIQAC GGEQKDHGFEVASTSPEDESPGSNPEPDATPFQEGLRTF DQLDAISSLPTPSDIFVSYSTFPGFVSWRDPKSGSWYVET LDDIFEQWAHSEDLQSLLLRVANAVSVKGIYKQMPGCF NFLRKKLFFKTS	200
FRB-FKBP12-dCasp9	MASRILWHEMWHEGLEEASRLYFGERNVKGMFEVLEP LHAMMERGPQTLKETSFNQAYGRDLMEAQEWCRKYM KSGNVKDLLQAWDLYYHVFRRISKLEYSGGGSLEGVQV ETISPGDGRTFPKRGQTCVVHYTGMLEDGKKFDSSRDR NKPFKFMLGKQEVIRGWEEGVAQMSVGQRAKLTISPDY AYGATGHPGIIPPHATLVFDVELLKLESGGGGSGGGGSG GGGSGVDGFGDVGALESLRGNADLAYILSMEPCGHCLII NNVNFCRESGLRTRTGSNIDCEKLRRRFSSLHFMVEVKG DLTAKKMVLALLELAQQDHGALDCCVVVILSHGCQAS HLQFPGAVYGTDGCPVSVEKIVNIFNGTSCPSLGGKPKL FFIQACGGEQKDHGFEVASTSPEDESPGSNPEPDATPFQE GLRTFDQLDAISSLPTPSDIFVSYSTFPGFVSWRDPKSGS WYVETLDDIFEQWAHSEDLQSLLLRVANAVSVKGIYKQ MPGCFNFLRKKLFFKTSAS	201
IL-15-IL-15RA:		
IL-15-IL-15RA	MAPRRARGCRTLGLPALLLLLLLRPPATRGNWVNVISD LKKIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLLEL QVISLESGDASIHDTVENLIILANNSLSSNGNVTESGCKE CEELEEKNIKEFLQSFVHIVQMFINTSGGGGSGGGGSGG GGSGGGGSGGGGSITCPPPMSVEHADIWVKSYSLYSRER YICNSGFKRKAGTSSLTECVLNKATNVAHWTTPSLKCIR	202

	DPALVHQRPAPPSTVTTAGVTPQPESLSPSGKEPAASSPS SNNTAATTAAIVPGSQLMPSKSPSTGTTEISSHESSHGTPS QTTAKNWELTASASHQPPGVYPQGHSDTTVAISTSTVLL CGLSAVSLLACYLKSRQTPPLASVEMEAMEALPVTWGT SSRDEDLENCSHHL	

In a particular embodiment, the inactivated cell surface receptor comprises an amino acid sequence at least 90%, such as at least 90%, 91%, 82%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100%, identical to SEQ ID NO: 79, preferably the amino acid sequence of SEQ ID NO: 79.

In a particular embodiment, the inactivated cell surface receptor comprises an amino acid sequence at least 90%, such as at least 90%, 91%, 82%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100%, identical to SEQ ID NO: 81, preferably the amino acid sequence of SEQ ID NO: 81.

In a particular embodiment, the inactivated cell surface receptor comprises an amino acid sequence at least 90%, such as at least 90%, 91%, 82%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100%, identical to SEQ ID NO: 83, preferably the amino acid sequence of SEQ ID NO: 83.

III. HLA Expression

In certain embodiments, an iPSC or derivative cell thereof of the application can be further modified by introducing an exogenous polynucleotide encoding one or more proteins related to immune evasion, such as non-classical HLA class I proteins (e.g., HLA-E and HLA-G). In particular, disruption of the B2M gene eliminates surface expression of all MHC class I molecules, leaving cells vulnerable to lysis by NK cells through the “missing self” response. Exogenous HLA-E expression can lead to resistance to NK-mediated lysis (Gornalusse et al., Nat Biotechnol. 2017 Aug; 35(8): 765–772).

In certain embodiments, the iPSC or derivative cell thereof comprises an exogenous polypeptide encoding at least one of a human leukocyte antigen E (HLA-E) and human leukocyte antigen G (HLA-G). In a particular embodiment, the HLA-E comprises an amino acid sequence at least 90%, such as at least 90%, 91%, 82%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100%, identical to SEQ ID NO: 65, preferably the

amino acid sequence of SEQ ID NO: 65. In a particular embodiment, the HLA-G comprises an amino acid sequence at least 90%, such as at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100%, identical to SEQ ID NO: 68, preferably SEQ ID NO: 68.

In certain embodiments, the third exogenous polynucleotide encodes a polypeptide comprising a signal peptide operably linked to a mature B2M protein that is fused to an HLA-E via a linker. In a particular embodiment, the third exogenous polypeptide comprises an amino acid sequence at least sequence at least 90%, such as at least 90%, 91%, 82%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100%, identical to SEQ ID NO: 66.

In other embodiments, the third exogenous polynucleotide encodes a polypeptide comprising a signal peptide operably linked to a mature B2M protein that is fused to an HLA-G via a linker. In a particular embodiment, the third exogenous polypeptide comprises an amino acid sequence at least sequence at least 90%, such as at least 90%, 91%, 82%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100%, identical to SEQ ID NO: 69.

IV. Other Optional Genome Edits

In one embodiment of the above described cell, the genomic editing at one or more selected sites may comprise insertions of one or more exogenous polynucleotides encoding other additional artificial cell death polypeptides, targeting modalities, receptors, signaling molecules, transcription factors, pharmaceutically active proteins and peptides, drug target candidates, or proteins promoting engraftment, trafficking, homing, viability, self-renewal, persistence, and/or survival of the genome-engineered iPSCs or derivative cells thereof.

In some embodiments, the exogenous polynucleotides for insertion are operatively linked to (1) one or more exogenous promoters comprising CMV, Efla, PGK, CAG, UBC, or other constitutive, inducible, temporal-, tissue-, or cell type-specific promoters; or (2) one or more endogenous promoters comprised in the selected sites comprising AAVS1, CCR5, ROSA26, collagen, HTRP, Hll, beta-2 microglobulin, GAPDH, TCR or RUNX1, or other locus meeting the criteria of a genome safe harbor. In

some embodiments, the genome-engineered iPSCs generated using the above method comprise one or more different exogenous polynucleotides encoding proteins comprising caspase, thymidine kinase, cytosine deaminase, B-cell CD20, ErbB2 or CD79b wherein when the genome-engineered iPSCs comprise two or more suicide genes, the suicide genes are integrated in different safe harbor locus comprising AAVSl, CCR5, ROSA26, collagen, HTRP, Hll, Hll, beta-2 microglobulin, GAPDH, TCR or RUNX1. Other exogenous polynucleotides encoding proteins may include those encoding PET reporters, homeostatic cytokines, and inhibitory checkpoint inhibitory proteins such as PD1, PD-L1, and CTLA4 as well as proteins that target the CD47/signal regulatory protein alpha (SIRPα) axis. In some other embodiments, the genome-engineered iPSCs generated using the method provided herein comprise in/del at one or more endogenous genes associated with targeting modality, receptors, signaling molecules, transcription factors, drug target candidates, immune response regulation and modulation, or proteins suppressing engraftment, trafficking, homing, viability, self-renewal, persistence, and/or survival of the iPSCs or derivative cells thereof.

V. Targeted Genome Editing at Selected Locus in iPSCs

According to embodiments of the application, one or more of the exogenous polynucleotides are integrated at one or more loci on the chromosome of an iPSC.

Genome editing, or genomic editing, or genetic editing, as used interchangeably herein, is a type of genetic engineering in which DNA is inserted, deleted, and/or replaced in the genome of a targeted cell. Targeted genome editing (interchangeable with "targeted genomic editing" or "targeted genetic editing") enables insertion, deletion, and/or substitution at pre-selected sites in the genome. When an endogenous sequence is deleted or disrupted at the insertion site during targeted editing, an endogenous gene comprising the affected sequence can be knocked-out or knocked-down due to the sequence deletion or disruption. Therefore, targeted editing can also be used to disrupt endogenous gene expression with precision. Similarly used herein is the term "targeted integration," referring to a process involving insertion of one or more exogenous sequences at pre-selected sites in the genome, with or without deletion of an endogenous sequence at the insertion site.

Targeted editing can be achieved either through a nuclease-independent approach, or through a nuclease-dependent approach. In the nuclease-independent targeted editing approach, homologous recombination is guided by homologous sequences flanking an exogenous polynucleotide to be inserted, through the enzymatic machinery of the host cell.

Alternatively, targeted editing could be achieved with higher frequency through specific introduction of double strand breaks (DSBs) by specific rare-cutting endonucleases. Such nuclease-dependent targeted editing utilizes DNA repair mechanisms including non-homologous end joining (NHEJ), which occurs in response to DSBs. Without a donor vector containing exogenous genetic material, the NHEJ often leads to random insertions or deletions (in/dels) of a small number of endogenous nucleotides. In comparison, when a donor vector containing exogenous genetic material flanked by a pair of homology arms is present, the exogenous genetic material can be introduced into the genome during homology directed repair (HDR) by homologous recombination, resulting in a "targeted integration."

Available endonucleases capable of introducing specific and targeted DSBs include, but not limited to, zinc-finger nucleases (ZFN), transcription activator-like effector nucleases (TALEN), RNA-guided CRISPR (Clustered Regular Interspaced Short Palindromic Repeats) systems. Additionally, DICE (dual integrase cassette exchange) system utilizing phiC31 and Bxbl integrases is also a promising tool for targeted integration.

ZFNs are targeted nucleases comprising a nuclease fused to a zinc finger DNA binding domain. By a "zinc finger DNA binding domain" or "ZFBD" it is meant a polypeptide domain that binds DNA in a sequence-specific manner through one or more zinc fingers. A zinc finger is a domain of about 30 amino acids within the zinc finger binding domain whose structure is stabilized through coordination of a zinc ion. Examples of zinc fingers include, but not limited to, C2H2 zinc fingers, C3H zinc fingers, and C4 zinc fingers. A "designed" zinc finger domain is a domain not occurring in nature whose design/composition results principally from rational criteria, e.g., application of substitution rules and computerized algorithms for processing information in a database storing information of existing ZFP designs and binding data. See, for example, U.S. Pat.

Nos. 6,140,081; 6,453,242; and 6,534,261; see also WO 98/53058; WO 98/53059; WO 98/53060; WO 02/016536 and WO 03/016496. A "selected" zinc finger domain is a domain not found in nature whose production results primarily from an empirical process such as phage display, interaction trap or hybrid selection. ZFNs are described in greater detail in U.S. Pat. No. 7,888,121 and U.S. Pat. No. 7,972,854, the complete disclosures of which are incorporated herein by reference. The most recognized example of a ZFN in the art is a fusion of the Fokl nuclease with a zinc finger DNA binding domain.

A TALEN is a targeted nuclease comprising a nuclease fused to a TAL effector DNA binding domain. By "transcription activator-like effector DNA binding domain", "TAL effector DNA binding domain", or "TALE DNA binding domain" it is meant the polypeptide domain of TAL effector proteins that is responsible for binding of the TAL effector protein to DNA. TAL effector proteins are secreted by plant pathogens of the genus Xanthomonas during infection. These proteins enter the nucleus of the plant cell, bind effector-specific DNA sequences via their DNA binding domain, and activate gene transcription at these sequences via their transactivation domains. TAL effector DNA binding domain specificity depends on an effector-variable number of imperfect 34 amino acid repeats, which comprise polymorphisms at select repeat positions called repeat variable-diresidues (RVD). TALENs are described in greater detail in U.S. Patent Application No. 2011/0145940, which is herein incorporated by reference. The most recognized example of a TALEN in the art is a fusion polypeptide of the Fokl nuclease to a TAL effector DNA binding domain.

Another example of a targeted nuclease that finds use in the subject methods is a targeted Spoll nuclease, a polypeptide comprising a Spol l polypeptide having nuclease activity fused to a DNA binding domain, e.g. a zinc finger DNA binding domain, a TAL effector DNA binding domain, etc. that has specificity for a DNA sequence of interest. See, for example, U.S. Application No. 61/555,857, the disclosure of which is incorporated herein by reference.

Additional examples of targeted nucleases suitable for the present application include, but not limited to Bxbl, phiC3 l, R4, PhiBTl, and Wp/SPBc/TP90l-l, whether used individually or in combination.

Other non-limiting examples of targeted nucleases include naturally occurring and recombinant nucleases; CRISPR related nucleases from families including cas, cpf, cse, csy, csn, csd, cst, csh, csa, csm, and cmr; restriction endonucleases; meganucleases; homing endonucleases, and the like. As an example, CRISPR/Cas9 requires two major components: (1) a Cas9 endonuclease and (2) the crRNA-tracrRNA complex. When co-expressed, the two components form a complex that is recruited to a target DNA sequence comprising PAM and a seeding region near PAM. The crRNA and tracrRNA can be combined to form a chimeric guide RNA (gRNA) to guide Cas9 to target selected sequences. These two components can then be delivered to mammalian cells via transfection or transduction. As another example, CRISPR/Cpf1 comprises two major components: (1) a CPf1 endonuclease and (2) a crRNA. When co-expressed, the two components form a ribobnucleoprotein (RNP) complex that is recruited to a target DNA sequence comprising PAM and a seeding region near PAM. The crRNA can be combined to form a chimeric guide RNA (gRNA) to guide Cpf1 to target selected sequences. These two components can then be delivered to mammalian cells via transfection or transduction.

MAD7 is an engineered Cas12a variant originating from the bacterium *Eubacterium rectale* that has a preference for 5′-TTTN-3′ and 5′-CTTN-3′ PAM sites and does not require a tracrRNA. See, for example, PCT Publication No. 2018/236548, the disclosure of which is incorporated herein by reference.

DICE mediated insertion uses a pair of recombinases, for example, phiC31 and Bxbl, to provide unidirectional integration of an exogenous DNA that is tightly restricted to each enzymes' own small attB and attP recognition sites. Because these target att sites are not naturally present in mammalian genomes, they must be first introduced into the genome, at the desired integration site. See, for example, U.S. Application Publication No. 2015/0140665, the disclosure of which is incorporated herein by reference.

One aspect of the present application provides a construct comprising one or more exogenous polynucleotides for targeted genome integration. In one embodiment, the construct further comprises a pair of homologous arm specific to a desired integration site, and the method of targeted integration comprises introducing the construct to cells to enable site specific homologous recombination by the cell host enzymatic machinery. In

another embodiment, the method of targeted integration in a cell comprises introducing a construct comprising one or more exogenous polynucleotides to the cell, and introducing a ZFN expression cassette comprising a DNA-binding domain specific to a desired integration site to the cell to enable a ZFN-mediated insertion. In yet another embodiment, the method of targeted integration in a cell comprises introducing a construct comprising one or more exogenous polynucleotides to the cell, and introducing a TALEN expression cassette comprising a DNA-binding domain specific to a desired integration site to the cell to enable a TALEN-mediated insertion. In another embodiment, the method of targeted integration in a cell comprises introducing a construct comprising one or more exogenous polynucleotides to the cell, introducing a Cpf1 expression cassette, and a gRNA comprising a guide sequence specific to a desired integration site to the cell to enable a Cpf1-mediated insertion. In another embodiment, the method of targeted integration in a cell comprises introducing a construct comprising one or more exogenous polynucleotides to the cell, introducing a Cas9 expression cassette, and a gRNA comprising a guide sequence specific to a desired integration site to the cell to enable a Cas9-mediated insertion. In still another embodiment, the method of targeted integration in a cell comprises introducing a construct comprising one or more att sites of a pair of DICE recombinases to a desired integration site in the cell, introducing a construct comprising one or more exogenous polynucleotides to the cell, and introducing an expression cassette for DICE recombinases, to enable DICE-mediated targeted integration.

Sites for targeted integration include, but are not limited to, genomic safe harbors, which are intragenic or extragenic regions of the human genome that, theoretically, are able to accommodate predictable expression of newly integrated DNA without adverse effects on the host cell or organism. In certain embodiments, the genome safe harbor for the targeted integration is one or more loci of genes selected from the group consisting of AAVS1, CCR5, ROSA26, collagen, HTRP, Hll, GAPDH, TCR and RUNX1 genes.

In other embodiments, the site for targeted integration is selected for deletion or reduced expression of an endogenous gene at the insertion site. As used herein, the term "deletion" with respect to expression of a gene refers to any genetic modification that abolishes the expression of the gene. Examples of "deletion" of expression of a gene

include, e.g., a removal or deletion of a DNA sequence of the gene, an insertion of an exogenous polynucleotide sequence at a locus of the gene, and one or more substitutions within the gene, which abolishes the expression of the gene.

Genes for target deletion include, but are not limited to, genes of major histocompatibility complex (MHC) class I and MHC class II proteins. Multiple MHC class I and class II proteins must be matched for histocompatibility in allogeneic recipients to avoid allogeneic rejection problems. "MHC deficient", including MHC-class I deficient, or MHC-class II deficient, or both, refers to cells that either lack, or no longer maintain, or have reduced level of surface expression of a complete MHC complex comprising a MHC class I protein heterodimer and/or a MHC class II heterodimer, such that the diminished or reduced level is less than the level naturally detectable by other cells or by synthetic methods. MHC class I deficiency can be achieved by functional deletion of any region of the MHC class I locus (chromosome 6p2l), or deletion or reducing the expression level of one or more MHC class-I associated genes including, not being limited to, beta-2 microglobulin (B2M) gene, TAP 1 gene, TAP 2 gene and Tapasin genes. For example, the B2M gene encodes a common subunit essential for cell surface expression of all MHC class I heterodimers. B2M null cells are MHC-I deficient. MHC class II deficiency can be achieved by functional deletion or reduction of MHC-II associated genes including, not being limited to, RFXANK, CIITA, RFX5 and RFXAP. CIITA is a transcriptional coactivator, functioning through activation of the transcription factor RFX5 required for class II protein expression. CIITA null cells are MHC-II deficient. In certain embodiments, one or more of the exogenous polynucleotides are integrated at one or more loci of genes selected from the group consisting of B2M, TAP 1, TAP 2, Tapasin, RFXANK, CIITA, RFX5 and RFXAP genes to thereby delete or reduce the expression of the gene(s) with the integration.

In certain embodiments, the exogenous polynucleotides are integrated at one or more loci on the chromosome of the cell, preferably the one or more loci are of genes selected from the group consisting of AAVS1, CCR5, ROSA26, collagen, HTRP, Hl l, GAPDH, RUNX1, B2M, TAPI, TAP2, Tapasin, NLRC5, CIITA, RFXANK, CIITA, RFX5, RFXAP, TCR a or b constant region, NKG2A, NKG2D, CD38, CIS, CBL-B, SOCS2, PD1, CTLA4, LAG3, TIM3, or TIGIT genes, provided at least one of the one or

more loci is of a MHC gene, such as a gene selected from the group consisting of B2M, TAP 1, TAP 2, Tapasin, RFXANK, CIITA, RFX5 and RFXAP genes. Preferably, the one or more exogenous polynucleotides are integrated at a locus of an MHC class-I associated gene, such as a beta-2 microglobulin (B2M) gene, TAP 1 gene, TAP 2 gene or Tapasin gene; and at a locus of an MHC-II associated gene, such as a RFXANK, CIITA, RFX5, RFXAP, or CIITA gene; and optionally further at a locus of a safe harbor gene selected from the group consisting of AAVS1, CCR5, ROSA26, collagen, HTRP, Hll, GAPDH, TCR and RUNX1 genes. More preferably, the one or more of the exogenous polynucleotides are integrated at the loci of CIITA, AAVS1 and B2M genes.

In certain embodiments, (i) the first exogenous polynucleotide is integrated at a locus of AAVS1 gene; (ii) the second exogenous polypeptide is integrated at a locus of CIITA gene; and (iii) the third exogenous polypeptide is integrated at a locus of B2M gene; wherein integrations of the exogenous polynucleotides delete or reduce expression of CIITA and B2M genes.

In certain embodiments, (i) the first exogenous polynucleotide comprises the polynucleotide sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to SEQ ID NO: 62; (ii) the second exogenous polynucleotide comprises the polynucleotide sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to SEQ ID NO: 75; and (iii) the third exogenous polynucleotide comprises the polynucleotide sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to SEQ ID NO: 67.

In certain embodiments, (i) the first exogenous polynucleotide comprises the polynucleotide sequence of SEQ ID NO: 62; (ii) the second exogenous polynucleotide comprises the polynucleotide sequence of SEQ ID NO: 75; and (iii) the third exogenous polynucleotide comprises the polynucleotide sequence of SEQ ID NO: 67.

In some aspects, the present disclosure describes genetically engineered iPSCs and cells derived therefrom that exogenously express recombinant CD16 and recombinant NKG2D. In some aspects, such cells also express one or more CARs (e.g., one or more CARs comprising a CD22 antigen binding domain and/or a CD19 antigen binding domain). For example, in some embodiments, such cells may express a single

CAR comprising one or more antigen binding domains that bind CD22 and/or CD19. In another example, such cells may express two or more CARs, wherein each CAR comprises an antigen binding domain that binds a tumor antigen, wherein the tumor antigens are independently selected from the group consisting of CD22 and CD19.

Described herein is a method for exogenously expressing or overexpressing CD16 and NKG2D proteins and transgenes in cells, as well as such cells and therapeutic uses thereof. The surface receptor CD16 (FcγRIIIA) affects human natural killer (NK) cells during maturation. NK cells bind the Fc portion of IgG via CD16, and execute antibody-dependent cellular cytotoxicity, which is critical for the effectiveness of several anti-tumor monoclonal antibody therapies. NKG2D is an stimulatory/activating receptor that is mostly expressed on cells of the cytotoxic arm of the immune system including NK cells and subsets of T cells. NKG2D is crucial in diverse aspects of innate and adaptive immune functions. In some embodiments, CD16 and NKG2D are expressed from in a single polynucleotide construct as it is advantageous to reduce the number of gene edits of a cell.

In certain aspects, provided is an iPSC or derivative cell thereof containing an exogenous or isolated polynucleotide construct encoding a CD16 protein and an NKG2D protein. In some embodiments, described herein is an iPSC or derivative cell thereof expressing recombinant CD16 proteins and recombinant NKG2D proteins. In some embodiments, the recombinant proteins are encoded by an exogenous or isolated polynucleotide construct. In some embodiments, the polynucleotide construct encoding the CD16 protein and the NKG2D protein also includes a polynucleotide sequence encoding an autoprotease peptide or self-cleaving peptide. In some embodiments, an exogenous polynucleotide construct encoding the CD16 protein, the NKG2D protein and the self-cleaving peptide is introduced into the iPSC or derivative cell thereof. The exogenous or isolated polynucleotide construct can be introduced into a gene locus of the iPSC or derivative cell thereof.

In some embodiments, the iPSC or derivative cell thereof expressing recombinant CD16 proteins and recombinant NKG2D proteins also expresses chimeric antigen receptors (CARs). In some embodiments, the cell expressing recombinant CD16 proteins and recombinant NKG2D proteins also expresses either recombinant HLA-E, HLA-G, or

both. In several embodiments, the iPSC or derivative cell thereof expressing recombinant CD16 proteins and recombinant NKG2D proteins also expresses CARs and either recombinant HLA-E, HLA-G, or both. In many embodiments, the cell expressing recombinant CD16 proteins, recombinant NKG2D proteins and CARs also expresses recombinant IL-15 proteins. In many embodiments, the cell expresses recombinant CD16 proteins, recombinant NKG2D proteins, CARs, recombinant IL-15 proteins, and either recombinant HLA-E, HLA-G, or both.

In many embodiments, the cell expressing recombinant CD16 proteins, recombinant NKG2D proteins and CARs also expresses recombinant fusion proteins containing IL-15 and IL-15Ra. In many embodiments, the cell expresses recombinant CD16 proteins, recombinant NKG2D proteins, CARs, recombinant fusion proteins containing IL-15 and IL-15Ra, and either recombinant HLA-E, HLA-G, or both. In some embodiments, the cell expressing recombinant CD16 proteins and recombinant NKG2D proteins also expresses recombinant IL-15 proteins. In some embodiments, the cell expressing recombinant CD16 proteins and recombinant NKG2D proteins also expresses recombinant fusion proteins containing IL-15 and IL-15Ra. In some embodiments, the cell expressing recombinant CD16 proteins, recombinant NKG2D proteins, and recombinant IL-15 proteins also expresses CARs. In some embodiments, the cell expressing recombinant CD16 proteins, recombinant NKG2D proteins, and recombinant fusion proteins containing IL-15 and IL-15Ra also expresses CARs.

In one aspect, provided is an exogenous or isolated polynucleotide construct encoding a CD16 protein and an NKG2D protein. In some embodiments of the exogenous polynucleotide construct, the polynucleotide sequence encoding a CD16 protein and the polynucleotide sequence encoding an NKG2D protein are operably linked by a polynucleotide sequence encoding an autoprotease peptide or self-cleaving peptide. In some embodiments, the polynucleotide construct includes from 5' to 3' end: a polynucleotide sequence encoding a CD16 protein, a polynucleotide sequence encoding an autoprotease peptide or self-cleaving peptide and a polynucleotide sequence encoding a NKG2D protein. In some embodiments, the polynucleotide construct includes from 5' to 3' end: a polynucleotide sequence encoding an NKG2D protein, a polynucleotide sequence encoding an autoprotease peptide or self-cleaving peptide and a polynucleotide

sequence encoding a CD16 protein. In some embodiments, the exogenous polynucleotide construct comprises the nucleic acid sequence of SEQ ID NO: 179. In some embodiments, the exogenous polynucleotide construct encodes for the amino acid sequence of SEQ ID NO: 180.

In some embodiments, the CD16 protein (which is also referred to as "low affinity immunoglobulin gamma Fc region receptor III-A" or "Fc gamma receptor IIIa") is a wildtype CD16 protein. In some embodiments, the human wildtype CD16 protein has the amino acid sequence set forth in NCBI Ref. Seq. No. NP_000560.7 or UniProt No. P08637. In some instance, the coding sequence of human wildtype CD16 is set forth in NCBI Ref. No. NM_000569.8.

In some embodiments, the CD16 protein is a CD16 variant protein. In some instances, the CD16 variant protein has an amino acid sequence having at least 90%, e.g., at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity to wildtype CD16 such as that of SEQ ID NO: 181. In some instances, the CD16 variant is a high affinity CD16 variant. In other instances, the CD16 variant is a non-cleavable CD16 variant. In some instances, the CD16 variant is a high affinity and non-cleavable CD16 variant.

In some embodiments, the CD16 variant comprises one or more amino acid substitutions selected from the group consisting of F158V, F176V, S197P, D205A, S219A, T220A, and any combination thereof. In some embodiments, the CD16 variant has an F158V substitution and one or more substitutions selected from F176V, S197P, D205A, S219A, T220A, and any combination thereof. In one embodiment, the CD16 variant has an F176V substitution and one or more substitutions selected from F158V, S197P, D205A, S219A, T220A, and any combination thereof. In many embodiments, the CD16 variant has an S197P, substitution and one or more substitutions selected from F158V, F176V, D205A, S219A, T220A, and any combination thereof. In various embodiments, the CD16 variant has a D205A substitution and one or more substitutions selected from F158V, F176V, S197P, S219A, T220A, and any combination thereof. In some embodiments, the CD16 variant has a substitution and one or more substitutions selected from F158V, F176V, S197P, D205A, S219A, T220A, and any combination thereof. In some embodiments, the CD16 variant has an S219A substitution and one or

more substitutions selected from F158V, F176V, S197P, D205A, T220A, and any combination thereof. In some embodiments, the CD16 variant has a T220A substitution and one or more substitutions selected from F158V, F176V, S197P, D205A, S219A, T220A, and any combination thereof. In some embodiments, the variant CD16 protein has the sequence of SEQ ID NO: 182. In some embodiments, the nucleic acid sequence encoding the variant CD16 protein has the sequence of SEQ ID NO: 183. In some embodiments, the wildtype CD16 protein has the sequence of SEQ ID NO: 181.

In some embodiments, the NKG2D protein (which is also referred to as NKG2-D type II integral membrane protein, CD314, killer cell lectin-like receptor subfamily K1 member 1 or KLRK1) is a wildtype NKG2D protein. In some embodiments, the human wildtype NKG2D protein has the amino acid sequence set forth in NCBI Ref. Seq. Nos. NP_001186734.1 or NP_031386.2 or UniProt No. P26718. In some instance, the coding sequence of human wildtype NKG2D is set forth in NCBI Ref. Nos. NM_001199805.1 or NM_007360.3. In some embodiments, the NKG2D protein is a NKG2D variant protein. In some instances, the NKG2D variant protein has an amino acid sequence having at least 90%, e.g., at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity to wildtype NKG2D such as that of SEQ ID NO: 184. In some embodiments, the NKG2D protein has the amino acid sequence of SEQ ID NO: 184. In some embodiments, the nucleic acid sequence encoding the NKG2D protein has sequence of SEQ ID NO: 185.

As discussed above, provided herein are constructs containing autoprotease peptide sequences including 2A peptides that can induce ribosomal skipping during translation of an polypeptide. 2A peptides function to “cleave” an mRNA transcript by making the ribosome skip the synthesis of a peptide bond at the C-terminus, between the glycine (G) and proline (P) residues, thereby leading to separation between the end of the 2A sequence and the next peptide downstream. 2A peptides include, but are not limited to, a porcine tesehovirus-1 2A (P2A) peptide, a foot-and-mouth disease virus (FMDV) 2A (F2A) peptide, an Equine Rhinitis A Virus (ERAV) 2A (E2A) peptide, a Thosea asigna virus 2A (T2A) peptide, a cytoplasmic polyhedrosis virus 2A (BmCPV2A) peptide, and a Flacherie Virus 2A (BmIFV2A) peptide.

An exemplary P2a peptide can include an amino acid sequence having at least 90%, such as 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to SEQ ID NO: 186. In some embodiment, the P2A peptide has the amino acid sequence of SEQ ID NO: 186.

Derivative Cells

In another aspect, the invention relates to a cell derived from differentiation of an iPSC, a derivative cell. As described above, the genomic edits introduced into the iPSC cell are retained in the derivative cell. In certain embodiments of the derivative cell obtained from iPSC differentiation, the derivative cell is a hematopoietic cell, including, but not limited to, HSCs (hematopoietic stem and progenitor cells), hematopoietic multipotent progenitor cells, T cell progenitors, NK cell progenitors, T cells, NKT cells, NK cells, B cells, antigen presenting cells (APC), monocytes and macrophages. In certain embodiments, the derivative cell is an immune effector cell, such as a NK cell or a T cell.

In certain embodiments, the application provides a natural killer (NK) cell or a T cell comprising: (i) an exogenous polynucleotide encoding a chimeric antigen receptor (CAR); (ii) a deletion or reduced expression of an MHC class I associated gene and an MHC class II associated gene, such as an MHC class-I associated gene selected from the group consisting of a B2M gene, TAP 1 gene, TAP 2 gene and Tapasin gene, and an MHC-II associated gene selected from the group consisting of a RFXANK gene, CIITA gene, RFX5 gene, RFXAP gene, and CIITA gene, preferably the B2M gene and CIITA gene; and optionally (iii) an exogenous polynucleotide encoding a chimeric IL-15RA and an interleukin 15 (IL-15), wherein the IL-15RA and IL-15 are operably linked.

In certain embodiments, the NK cell or T cell further comprises an exogenous polynucleotide encoding at least one of a human leukocyte antigen E (HLA-E) and a human leukocyte antigen G (HLA-G).

Also provided is a NK cell or a T cell comprising: (i) an exogenous polynucleotide encoding a chimeric antigen receptor (CAR) having the amino acid sequence of SEQ ID NO: 61; (ii) an exogenous polynucleotide encoding a human leukocyte antigen E (HLA-E) having the amino acid sequence of SEQ ID NO: 66; and optionally (iii) an exogenous polynucleotide encoding a chimeric IL-15RA and an

interleukin 15 (IL-15), wherein the IL-15RA and IL-15 are operably linked, having the amino acid sequence of SEQ ID NO: 202.

wherein the exogenous polynucleotides are integrated at loci of AAVS1, B2M, and CIITA genes, respectively, to thereby delete or reduce expression of CIITA and B2M.

In certain embodiments, the first exogenous polynucleotide comprises the polynucleotide sequence of SEQ ID NO: 62; the second exogenous polynucleotide comprises the polynucleotide sequence of SEQ ID NO: 75; and the third exogenous polynucleotide comprises the polynucleotide sequence of SEQ ID NO: 67.

Also provided is a CD34+ hematopoietic progenitor cell (HPC) derived from an induced pluripotent stem cell (iPSC) comprising: (i) an exogenous polynucleotide encoding a chimeric antigen receptor (CAR); (ii) an exogenous polynucleotide encoding an inactivated cell surface receptor that comprises a monoclonal antibody-specific epitope and an interleukin 15 (IL-15), wherein the inactivated cell surface receptor and IL-15 are operably linked by an autoprotease peptide sequence; and (iii) a deletion or reduced expression of one or more of B2M, TAP 1, TAP 2, Tapasin, RFXANK, CIITA, RFX5 and RFXAP genes.

In certain embodiments, the CD34+ HPC further comprises an exogenous polynucleotide encoding a human leukocyte antigen E (HLA-E) and/or human leukocyte antigen G (HLA-G).

In certain embodiments, the CAR comprises (i) a signal peptide comprising a signal peptide; (ii) an extracellular domain comprising a binding domain that specifically binds the CD19 antigen; (iii) a hinge region; (iv) a transmembrane domain; (v) an intracellular signaling domain; and (vi) a co-stimulatory domain, such as a co-stimulatory domain comprising a CD28 signaling domain.

Also provided is a method of manufacturing the derivative cell. The method comprises differentiating the iPSC under conditions for cell differentiation to thereby obtain the derivative cell.

An iPSC of the application can be differentiated by any method known in the art. Exemplary methods are described in US8846395, US8945922, US8318491, WO2010/099539, WO2012/109208, WO2017/070333, WO2017/179720, WO2016/010148, WO2018/048828 and WO2019/157597, each of which are herein

incorporated by reference in its entirety. The differentiation protocol may use feeder cells or may be feeder-free. As used herein, "feeder cells" or "feeders" are terms describing cells of one type that are co-cultured with cells of a second type to provide an environment in which the cells of the second type can grow, expand, or differentiate, as the feeder cells provide stimulation, growth factors and nutrients for the support of the second cell type.

In another embodiment of the invention, the iPSC derivative cells of the invention are NK cells which are prepared by a method of differentiating an iPSC cell into an NK cell by subjecting the cells to a differentiation protocol including the addition of recombinant human IL-12p70 for the final 24 hours of culture. By including the IL-12 in the differentiation protocol, cells that are primed with IL-12 demonstrate more rapid cell killing compared to those that are differentiated in the absence of IL-12. In addition, the cells differentiated using the IL-12 conditions demonstrate improved cancer cell growth inhibition.

Polynucleotides, vectors, and host cells

(1) Nucleic acids encoding a CAR

In another general aspect, the invention relates to an isolated nucleic acid encoding a chimeric antigen receptor (CAR) useful for an invention according to embodiments of the application. It will be appreciated by those skilled in the art that the coding sequence of a CAR can be changed (e.g., replaced, deleted, inserted, etc.) without changing the amino acid sequence of the protein. Accordingly, it will be understood by those skilled in the art that nucleic acid sequences encoding CARs of the application can be altered without changing the amino acid sequences of the proteins.

In certain embodiments, the isolated nucleic acid encodes a CAR targeting CD19. In a particular embodiment, the isolated nucleic acid encoding the CAR comprises a polynucleotide sequence at least 90%, such as at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 100%, identical to SEQ ID NO: 62, preferably the polynucleotide sequence of SEQ ID NO: 62.

In another general aspect, the application provides a vector comprising a polynucleotide sequence encoding a CAR useful for an invention according to

embodiments of the application. Any vector known to those skilled in the art in view of the present disclosure can be used, such as a plasmid, a cosmid, a phage vector or a viral vector. In some embodiments, the vector is a recombinant expression vector such as a plasmid. The vector can include any element to establish a conventional function of an expression vector, for example, a promoter, ribosome binding element, terminator, enhancer, selection marker, and origin of replication. The promoter can be a constitutive, inducible, or repressible promoter. A number of expression vectors capable of delivering nucleic acids to a cell are known in the art and can be used herein for production of a CAR in the cell. Conventional cloning techniques or artificial gene synthesis can be used to generate a recombinant expression vector according to embodiments of the application.

In a particular aspect, the application provides vectors for targeted integration of a CAR useful for an invention according to embodiments of the application. In certain embodiments, the vector comprises an exogenous polynucleotide having, in the 5' to 3' order, (a) a promoter; (b) a polynucleotide sequence encoding a CAR according to an embodiment of the application; and (c) a terminator/polyadenylation signal.

In certain embodiments, the promoter is a CAG promoter. In certain embodiments, the CAG promoter comprises the polynucleotide sequence at least 90%, such as at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 100%, identical to SEQ ID NO: 63. Other promoters can also be used, examples of which include, but are not limited to, EF1a, UBC, CMV, SV40, PGK1, and human beta actin.

In certain embodiments, the terminator/ polyadenylation signal is a SV40 signal. In certain embodiments, the SV40 signal comprises the polynucleotide sequence at least 90%, such as at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 100%, identical to SEQ ID NO: 64. Other terminator sequences can also be used, examples of which include, but are not limited to, BGH, hGH, and PGK.

In certain embodiments, the polynucleotide sequence encoding a CAR comprises the polynucleotide sequence at least 90%, such as at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 100%, identical to SEQ ID NO: 62.

In some embodiment, the vector further comprises a left homology arm and a right homology arm flanking the exogenous polynucleotide. As used herein, "left homology arm" and "right homology arm" refers to a pair of nucleic acid sequences that

flank an exogenous polynucleotide and facilitate the integration of the exogenous polynucleotide into a specified chromosomal locus. Sequences of the left and right arm homology arms can be designed based on the integration site of interest. In some embodiment, the left or right arm homology arm is homologous to the left or right side sequence of the integration site.

In certain embodiments, the left homology arm comprises the polynucleotide sequence at least 90%, such as at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 100%, identical to SEQ ID NO: 90. In certain embodiments, the right homology arm comprises the polynucleotide sequence at least 90%, such as at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 100%, identical to SEQ ID NO: 91.

In a particular embodiment, the vector comprises a polynucleotide sequence at least 85%, such as at least 85%, 86%, 87%, 88%, 89%,90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 100%, identical to SEQ ID NO: 92, preferably the polynucleotide sequence of SEQ ID NO: 92.

(2) Nucleic acids encoding an inactivated cell surface receptor

In another general aspect, the invention relates to an isolated nucleic acid encoding an inactivated cell surface receptor useful for an invention according to embodiments of the application. It will be appreciated by those skilled in the art that the coding sequence of an inactivated cell surface receptor can be changed (e.g., replaced, deleted, inserted, etc.) without changing the amino acid sequence of the protein. Accordingly, it will be understood by those skilled in the art that nucleic acid sequences encoding an inactivated cell surface receptor of the application can be altered without changing the amino acid sequences of the proteins.

In certain embodiments, an isolated nucleic acid encodes any inactivated cell surface receptor described herein, such as that comprises a monoclonal antibody-specific epitope, and a cytokine, such as an IL-15 or IL-2, wherein the monoclonal antibody-specific epitope and the cytokine are operably linked by an autoprotease peptide sequence.

In some embodiments, the isolated nucleic acid encodes an inactivated cell surface receptor comprising an epitope specifically recognized by an antibody, such as ibritumomab, tiuxetan, muromonab-CD3, tositumomab, abciximab, basiliximab,

brentuximab vedotin, cetuximab, infliximab, rituximab, alemtuzumab, bevacizumab, certolizumab pegol, daclizumab, eculizumab, efalizumab, gemtuzumab, natalizumab, omalizumab, palivizumab, ranibizumab, tocilizumab, trastuzumab, vedolizumab, adalimumab, belimumab, canakinumab, denosumab, golimumab, ipilimumab, ofatumumab, panitumumab, or ustekinumab.

In certain embodiments, the isolated nucleic acid encodes an inactivated cell surface receptor having a truncated epithelial growth factor (tEGFR) variant. Preferably, the inactivated cell surface receptor comprises an epitope specifically recognized by cetuximab, matuzumab, necitumumab or panitumumab, preferably cetuximab.

In certain embodiments, the isolated nucleic acid encodes an inactivated cell surface receptor having one or more epitopes of CD79b, such as an epitope specifically recognized by polatuzumab vedotin.

In certain embodiments, the isolated nucleic acid encodes an inactivated cell surface receptor having one or more epitopes of CD20, such as an epitope specifically recognized by rituximab.

In certain embodiments, the isolated nucleic acid encodes an inactivated cell surface receptor having one or more epitopes of Her 2 receptor, such as an epitope specifically recognized by trastuzumab

In certain embodiments, the autoprotease peptide sequence is porcine tesehovirus-1 2A (P2A).

In certain embodiments, the truncated epithelial growth factor (tEGFR) variant consists of an amino acid sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to the amino acid sequence of SEQ ID NO: 71.

In certain embodiments, the monoclonal antibody-specific epitope specifically recognized by polatuzumab vedotin consists of an amino acid sequence at least 90%, such as at least 90%, 91%, 82%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100%, identical to SEQ ID NO: 78.

In certain embodiments, the monoclonal antibody-specific epitope specifically recognized by rituximab consists of an amino acid sequence at least 90%, such as at least

90%, 91%, 82%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100%, identical to SEQ ID NO: 80.

In certain embodiments, the monoclonal antibody-specific epitope specifically recognized by trastuzumab consists of an amino acid sequence at least 90%, such as at least 90%, 91%, 82%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100%, identical to SEQ ID NO: 82.

In certain embodiments, the IL-15 comprises an amino acid sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to the amino acid sequence of SEQ ID NO: 72.

In certain embodiments, the autoprotease peptide has an amino acid sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to the amino acid sequence of SEQ ID NO: 73.

In certain embodiments, the polynucleotide sequence encodes a polypeptide comprising an amino acid sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to the amino acid sequence of SEQ ID NO: 74.

In a particular embodiment, the isolated nucleic acid encoding the inactivated cell surface receptor comprises a polynucleotide sequence at least 90%, such as at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 100%, identical to SEQ ID NO: 75, preferably the polynucleotide sequence of SEQ ID NO: 75.

In certain embodiments, the polynucleotide sequence encodes a polypeptide comprising an amino acid sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to the amino acid sequence of SEQ ID NO: 79.

In another general aspect, the application provides a vector comprising a polynucleotide sequence encoding an inactivated cell surface receptor useful for an invention according to embodiments of the application. Any vector known to those skilled in the art in view of the present disclosure can be used, such as a plasmid, a cosmid, a phage vector or a viral vector. In some embodiments, the vector is a recombinant expression vector such as a plasmid. The vector can include any element to establish a conventional function of an expression vector, for example, a promoter,

ribosome binding element, terminator, enhancer, selection marker, and origin of replication. The promoter can be a constitutive, inducible, or repressible promoter. A number of expression vectors capable of delivering nucleic acids to a cell are known in the art and can be used herein for production of a inactivated cell surface receptor in the cell. Conventional cloning techniques or artificial gene synthesis can be used to generate a recombinant expression vector according to embodiments of the application.

In a particular aspect, the application provides a vector for targeted integration of an inactivated cell surface receptor useful for an invention according to embodiments of the application. In certain embodiments, the vector comprises an exogenous polynucleotide having, in the 5' to 3' order, (a) a promoter; (b) a polynucleotide sequence encoding an inactivated cell surface receptor, such as an inactivated cell surface receptor comprising a truncated epithelial growth factor (tEGFR) variant and an interleukin 15 (IL-15), wherein the tEGFR variant and IL-15 are operably linked by an autoprotease peptide sequence, such as porcine tesehovirus-1 2A (P2A), and (c) a terminator/polyadenylation signal.

In certain embodiments, the promoter is a CAG promoter. In certain embodiments, the CAG promoter comprises the polynucleotide sequence at least 90%, such as at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 100%, identical to SEQ ID NO: 63. Other promoters can also be used, examples of which include, but are not limited to, EF1a, UBC, CMV, SV40, PGK1, and human beta actin.

In certain embodiments, the terminator/polyadenylation signal is a SV40 signal. In certain embodiments, the SV40 signal comprises the polynucleotide sequence at least 90%, such as at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 100%, identical to SEQ ID NO: 64. Other terminator sequences can also be used, examples of which include, but are not limited to BGH, hGH, and PGK.

In certain embodiments, the polynucleotide sequence encoding an inactivated cell surface receptor comprises the polynucleotide sequence at least 90%, such as at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 100%, identical to SEQ ID NO: 75.

In some embodiment, the vector further comprises a left homology arm and a right homology arm flanking the exogenous polynucleotide.

In certain embodiments, the left homology arm comprises the polynucleotide sequence at least 90%, such as at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 100%, identical to SEQ ID NO: 84. In certain embodiments, the right homology arm comprises the polynucleotide sequence at least 90%, such as at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 100%, identical to SEQ ID NO: 85

In a particular embodiment, the vector comprises a polynucleotide sequence at least 85%, such as at least 85%, 86%, 87%, 88%, 89%,90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 100%, identical to SEQ ID NO: 86, preferably the polynucleotide sequence of SEQ ID NO: 86.

(3) Nucleic acids encoding an HLA construct

In another general aspect, the invention relates to an isolated nucleic acid encoding an HLA construct useful for an invention according to embodiments of the application. It will be appreciated by those skilled in the art that the coding sequence of an HLA construct can be changed (e.g., replaced, deleted, inserted, etc.) without changing the amino acid sequence of the protein. Accordingly, it will be understood by those skilled in the art that nucleic acid sequences encoding an HLA construct of the application can be altered without changing the amino acid sequences of the proteins.

In certain embodiments, the isolated nucleic acid encodes an HLA construct comprising a signal peptide, such as an HLA-G signal peptide, operably linked to an HLA coding sequence, such as a coding sequence of a mature B2M, and/or a mature HLA-E. In some embodiments, the HLA coding sequence encodes the HLA-G and B2M, which are operably linked by a 4X GGGGS linker, and/or the B2M and HLA-E, which are operably linked by a 3X GGGGS linker. In a particular embodiment, the isolated nucleic acid encoding the HLA construct comprises a polynucleotide sequence at least 90%, such as at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 100%, identical to SEQ ID NO: 67, preferably the polynucleotide sequence of SEQ ID NO: 67. In another embodiment, the isolated nucleic acid encoding the HLA construct comprises a polynucleotide sequence at least 90%, such as at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 100%, identical to SEQ ID NO: 70, preferably the polynucleotide sequence of SEQ ID NO: 70.

In another general aspect, the application provides a vector comprising a polynucleotide sequence encoding a HLA construct useful for an invention according to embodiments of the application. Any vector known to those skilled in the art in view of the present disclosure can be used, such as a plasmid, a cosmid, a phage vector or a viral vector. In some embodiments, the vector is a recombinant expression vector such as a plasmid. The vector can include any element to establish a conventional function of an expression vector, for example, a promoter, ribosome binding element, terminator, enhancer, selection marker, and origin of replication. The promoter can be a constitutive, inducible, or repressible promoter. A number of expression vectors capable of delivering nucleic acids to a cell are known in the art and can be used herein for production of a HLA construct in the cell. Conventional cloning techniques or artificial gene synthesis can be used to generate a recombinant expression vector according to embodiments of the application.

In a particular aspect, the application provides vectors for targeted integration of a HLA construct useful for an invention according to embodiments of the application. In certain embodiments, the vector comprises an exogenous polynucleotide having, in the 5' to 3' order, (a) a promoter; (b) a polynucleotide sequence encoding an HLA construct; and (c) a terminator/polyadenylation signal.

In certain embodiments, the promoter is a CAG promoter. In certain embodiments, the CAG promoter comprises the polynucleotide sequence at least 90%, such as at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 100%, identical to SEQ ID NO: 63. Other promoters can also be used, examples of which include, but are not limited to, EF1a, UBC, CMV, SV40, PGK1, and human beta actin.

In certain embodiments, the terminator/ polyadenylation signal is a SV40 signal. In certain embodiments, the SV40 signal comprises the polynucleotide sequence at least 90%, such as at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 100%, identical to SEQ ID NO: 64. Other terminator sequences can also be used, examples of which include, but are not limited to BGH, hGH, and PGK.

In certain embodiments, a polynucleotide sequence encoding a HLA construct comprises a signal peptide, such as a HLA-G signal peptide, a mature B2M, and a mature HLA-E, wherein the HLA-G and B2M are operably linked by a 4X GGGGS linker (SEQ

ID NO: 31) and the B2M transgene and HLA-E are operably linked by a 3X GGGGS linker (SEQ ID NO: 25). In particular embodiments, the HLA construct comprises the polynucleotide sequence at least 90%, such as at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 100%, identical to SEQ ID NO: 67, preferably the polynucleotide sequence of SEQ ID NO: 67. In another embodiment, the HLA construct comprises the polynucleotide sequence at least 90%, such as at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 100%, identical to SEQ ID NO: 70, preferably the polynucleotide sequence of SEQ ID NO: 70.

In some embodiment, the vector further comprises a left homology arm and a right homology arm flanking the exogenous polynucleotide.

In certain embodiments, the left homology arm comprises the polynucleotide sequence at least 90%, such as at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 100%, identical to SEQ ID NO: 87. In certain embodiments, the right homology arm comprises the polynucleotide sequence at least 90%, such as at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 100%, identical to SEQ ID NO: 88.

In a particular embodiment, the vector comprises a polynucleotide sequence at least 85%, such as at least 85%, 86%, 87%, 88%, 89%,90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 100%, identical to SEQ ID NO: 89, preferably the polynucleotide sequence of SEQ ID NO: 89.

(4) Host cells

In another general aspect, the application provides a host cell comprising a vector of the application and/or an isolated nucleic acid encoding a construct of the application. Any host cell known to those skilled in the art in view of the present disclosure can be used for recombinant expression of exogenous polynucleotides of the application. According to particular embodiments, the recombinant expression vector is transformed into host cells by conventional methods such as chemical transfection, heat shock, or electroporation, where it is stably integrated into the host cell genome such that the recombinant nucleic acid is effectively expressed.

Examples of host cells include, for example, recombinant cells containing a vector or isolated nucleic acid of the application useful for the production of a vector or construct of interest; or an engineered iPSC or derivative cell thereof containing one or

more isolated nucleic acids of the application, preferably integrated at one or more chromosomal loci. A host cell of an isolated nucleic acid of the application can also be an immune effector cell, such as a T cell or NK cell, comprising the one or more isolated nucleic acids of the application. The immune effector cell can be obtained by differentiation of an engineered iPSC of the application. Any suitable method in the art can be used for the differentiation in view of the present disclosure. The immune effector cell can also be obtained transfecting an immune effector cell with one or more isolated nucleic acids of the application.

Compositions

In another general aspect, the application provides a composition comprising an isolated polynucleotide of the application, a host cell and/or an iPSC or derivative cell thereof of the application.

In certain embodiments, the composition further comprises one or more therapeutic agents selected from the group consisting of a peptide, a cytokine, a checkpoint inhibitor, a mitogen, a growth factor, a small RNA, a dsRNA (double stranded RNA), siRNA, oligonucleotide, mononuclear blood cells, , a vector comprising one or more polynucleic acids of interest, an antibody, a chemotherapeutic agent or a radioactive moiety, or an immunomodulatory drug (iMiD).

In certain embodiments, the composition is a pharmaceutical composition comprising an isolated polynucleotide of the application, a host cell and/or an iPSC or derivative cell thereof of the application and a pharmaceutically acceptable carrier. The term "pharmaceutical composition" as used herein means a product comprising an isolated polynucleotide of the application, an isolated polypeptide of the application, a host cell of the application, and/or an iPSC or derivative cell thereof of the application together with a pharmaceutically acceptable carrier. Polynucleotides, polypeptides, host cells, and/or iPSCs or derivative cells thereof of the application and compositions comprising them are also useful in the manufacture of a medicament for therapeutic applications mentioned herein.

As used herein, the term "carrier" refers to any excipient, diluent, filler, salt, buffer, stabilizer, solubilizer, oil, lipid, lipid containing vesicle, microsphere, liposomal

encapsulation, or other material well known in the art for use in pharmaceutical formulations. It will be understood that the characteristics of the carrier, excipient or diluent will depend on the route of administration for a particular application. As used herein, the term "pharmaceutically acceptable carrier" refers to a non-toxic material that does not interfere with the effectiveness of a composition described herein or the biological activity of a composition described herein. According to particular embodiments, in view of the present disclosure, any pharmaceutically acceptable carrier suitable for use in a polynucleotide, polypeptide, host cell, and/or iPSC or derivative cell thereof can be used.

The formulation of pharmaceutically active ingredients with pharmaceutically acceptable carriers is known in the art, e.g., Remington: The Science and Practice of Pharmacy (e.g. 21st edition (2005), and any later editions). Non-limiting examples of additional ingredients include: buffers, diluents, solvents, tonicity regulating agents, preservatives, stabilizers, and chelating agents. One or more pharmaceutically acceptable carrier may be used in formulating the pharmaceutical compositions of the application.

Methods of use

Primary cancer cells can be readily distinguished from non-cancerous cells by well-established techniques, particularly histological examination. The definition of a cancer cell, as used herein, includes not only a primary cancer cell, but any cell derived from a cancer cell ancestor. This includes metastasized cancer cells, and in vitro cultures and cell lines derived from cancer cells. When referring to a type of cancer that normally manifests as a solid tumor, a "clinically detectable" tumor is one that is detectable on the basis of tumor mass; e.g., by procedures such as computed tomography (CT) scan, magnetic resonance imaging (MRI), X-ray, ultrasound or palpation on physical examination, and/or which is detectable because of the expression of one or more cancer-specific antigens in a sample obtainable from a patient.

Cancer conditions may be characterized by the abnormal proliferation of malignant cancer cells and may include leukemias, such as AML, CML, ALL and CLL, lymphomas, such as Hodgkin lymphoma, non-Hodgkin lymphoma and multiple

myeloma, and solid cancers such as sarcomas, skin cancer, melanoma, bladder cancer, brain cancer, breast cancer, uterus cancer, ovary cancer, prostate cancer, lung cancer, colorectal cancer, cervical cancer, liver cancer, head and neck cancer, esophageal cancer, pancreatic cancer, renal cancer, adrenal cancer, stomach cancer, testicular cancer, cancer of the gall bladder and biliary tracts, thyroid cancer, thymus cancer, cancer of bone, and cerebral cancer, as well as cancer of unknown primary (CUP).

Cancer cells within an individual may be immunologically distinct from normal somatic cells in the individual (e.g., the cancerous tumor may be immunogenic). For example, the cancer cells may be capable of eliciting a systemic immune response in the individual against one or more antigens expressed by the cancer cells. The tumor antigens that elicit the immune response may be specific to cancer cells or may be shared by one or more normal cells in the individual.

The cancer cells of an individual suitable for treatment as described herein may express the antigen and/or may be of correct HLA type to bind the antigen receptor expressed by the T cells.

An individual suitable for treatment as described above may be a mammal. In preferred embodiments, the individual is a human. In other preferred embodiments, non-human mammals, especially mammals that are conventionally used as models for demonstrating therapeutic efficacy in humans (e.g. murine, primate, porcine, canine, or rabbit animals) may be employed.

In some embodiments, the individual may have minimal residual disease (MRD) after an initial cancer treatment. In some embodiments, the individual may have no minimal residual disease after one or more cancer treatments or repeated dosing.

An individual with cancer may display at least one identifiable sign, symptom, or laboratory finding that is sufficient to make a diagnosis of cancer in accordance with clinical standards known in the art. Examples of such clinical standards can be found in textbooks of medicine such as Harrison's Principles of Internal Medicine, 15th Ed., Fauci AS et al., eds., McGraw-Hill, New York, 2001. In some instances, a diagnosis of a cancer in an individual may include identification of a particular cell type (e.g. a cancer cell) in a sample of a body fluid or tissue obtained from the individual.

An anti-tumor effect is a biological effect which can be manifested by a reduction in the rate of tumor growth, decrease in tumor volume, a decrease in the number of tumor cells, a decrease in the number of metastases, an increase in life expectancy, or amelioration of various physiological symptoms associated with the cancerous condition. An "anti-tumor effect" can also be manifested by the ability of the peptides, polynucleotides, cells and antibodies, also T cells which may be obtained according to the methods of the present invention, as described herein in prevention of the occurrence of tumors in the first place.

Treatment may be any treatment and/or therapy, whether of a human or an animal (e.g. in veterinary applications), in which some desired therapeutic effect is achieved, for example, the inhibition or delay of the progress of the condition, and includes a reduction in the rate of progress, a halt in the rate of progress, amelioration of the condition, cure or remission (whether partial or total) of the condition, preventing, delaying, abating or arresting one or more symptoms and/or signs of the condition or prolonging survival of a subject or patient beyond that expected in the absence of treatment.

Treatment may also be prophylactic (e.g., prophylaxis). For example, an individual susceptible to or at risk of the occurrence or re-occurrence of cancer may be treated as described herein. Such treatment may prevent or delay the occurrence or re-occurrence of cancer in the individual.

In particular, treatment may include inhibiting cancer growth, including complete cancer remission, and/or inhibiting cancer metastasis. Cancer growth generally refers to any one of a number of indices that indicate change within the cancer to a more developed form. Thus, indices for measuring an inhibition of cancer growth include a decrease in cancer cell survival, a decrease in tumor volume or morphology (for example, as determined using computed tomographic (CT), sonography, or other imaging method), a delayed tumor growth, a destruction of tumor vasculature, improved performance in delayed hypersensitivity skin test, an increase in the activity of T cells, and a decrease in levels of tumor-specific antigens. Administration of T cells modified as described herein may improve the capacity of the individual to resist cancer growth, in particular growth of a cancer already present the subject and/or decrease the propensity for cancer growth in the individual.

This application provides a method of treating a disease or a condition in a subject in need thereof. The methods comprise administering to the subject in need thereof a therapeutically effective amount of cells of the application and/or a composition of the application. In certain embodiments, the disease or condition is cancer. The cancer can, for example, be a solid or a liquid cancer. The cancer, can, for example, be selected from the group consisting of a lung cancer, a gastric cancer, a colon cancer, a liver cancer, a renal cell carcinoma, a bladder urothelial carcinoma, a metastatic melanoma, a breast cancer, an ovarian cancer, a cervical cancer, a head and neck cancer, a pancreatic cancer, an endometrial cancer, a prostate cancer, a thyroid cancer, a glioma, a glioblastoma, and other solid tumors, and a non-Hodgkin's lymphoma (NHL), Hodgkin's lymphoma/disease (HD), an acute lymphocytic leukemia (ALL), a chronic lymphocytic leukemia (CLL), a chronic myelogenous leukemia (CML), a multiple myeloma (MM), an acute myeloid leukemia (AML), and other liquid tumors. In a preferred embodiment, the cancer is a non-Hodgkin's lymphoma (NHL).

According to embodiments of the application, the composition comprises a therapeutically effective amount of an isolated polynucleotide, an isolated polypeptide, a host cell, and/or an iPSC or derivative cell thereof. As used herein, the term "therapeutically effective amount" refers to an amount of an active ingredient or component that elicits the desired biological or medicinal response in a subject. A therapeutically effective amount can be determined empirically and in a routine manner, in relation to the stated purpose.

As used herein with reference to a cell of the application and/or a pharmaceutical composition of the application a therapeutically effective amount means an amount of the cells and/or the pharmaceutical composition that modulates an immune response in a subject in need thereof.

According to particular embodiments, a therapeutically effective amount refers to the amount of therapy which is sufficient to achieve one, two, three, four, or more of the following effects: (i) reduce or ameliorate the severity of the disease, disorder or condition to be treated or a symptom associated therewith; (ii) reduce the duration of the disease, disorder or condition to be treated, or a symptom associated therewith; (iii) prevent the progression of the disease, disorder or condition to be treated, or a symptom

associated therewith; (iv) cause regression of the disease, disorder or condition to be treated, or a symptom associated therewith; (v) prevent the development or onset of the disease, disorder or condition to be treated, or a symptom associated therewith; (vi) prevent the recurrence of the disease, disorder or condition to be treated, or a symptom associated therewith; (vii) reduce hospitalization of a subject having the disease, disorder or condition to be treated, or a symptom associated therewith; (viii) reduce hospitalization length of a subject having the disease, disorder or condition to be treated, or a symptom associated therewith; (ix) increase the survival of a subject with the disease, disorder or condition to be treated, or a symptom associated therewith; (xi) inhibit or reduce the disease, disorder or condition to be treated, or a symptom associated therewith in a subject; and/or (xii) enhance or improve the prophylactic or therapeutic effect(s) of another therapy.

The therapeutically effective amount or dosage can vary according to various factors, such as the disease, disorder or condition to be treated, the means of administration, the target site, the physiological state of the subject (including, e.g., age, body weight, health), whether the subject is a human or an animal, other medications administered, and whether the treatment is prophylactic or therapeutic. Treatment dosages are optimally titrated to optimize safety and efficacy.

According to particular embodiments, the compositions described herein are formulated to be suitable for the intended route of administration to a subject. For example, the compositions described herein can be formulated to be suitable for intravenous, subcutaneous, or intramuscular administration.

The cells of the application and/or the pharmaceutical compositions of the application can be administered in any convenient manner known to those skilled in the art. For example, the cells of the application can be administered to the subject by aerosol inhalation, injection, ingestion, transfusion, implantation, and/or transplantation. The compositions comprising the cells of the application can be administered transarterially, subcutaneously, intradermaly, intratumorally, intranodally, intramedullary, intramuscularly, inrapleurally, by intravenous (i.v.) injection, or intraperitoneally. In certain embodiments, the cells of the application can be administered with or without lymphodepletion of the subject.

The pharmaceutical compositions comprising cells of the application can be provided in sterile liquid preparations, typically isotonic aqueous solutions with cell suspensions, or optionally as emulsions, dispersions, or the like, which are typically buffered to a selected pH. The compositions can comprise carriers, for example, water, saline, phosphate buffered saline, and the like, suitable for the integrity and viability of the cells, and for administration of a cell composition.

Sterile injectable solutions can be prepared by incorporating cells of the application in a suitable amount of the appropriate solvent with various other ingredients, as desired. Such compositions can include a pharmaceutically acceptable carrier, diluent, or excipient such as sterile water, physiological saline, glucose, dextrose, or the like, that are suitable for use with a cell composition and for administration to a subject, such as a human. Suitable buffers for providing a cell composition are well known in the art. Any vehicle, diluent, or additive used is compatible with preserving the integrity and viability of the cells of the application.

The cells of the application and/or the pharmaceutical compositions of the application can be administered in any physiologically acceptable vehicle. A cell population comprising cells of the application can comprise a purified population of cells. Those skilled in the art can readily determine the cells in a cell population using various well known methods. The ranges in purity in cell populations comprising genetically modified cells of the application can be from about 50% to about 55%, from about 55% to about 60%, from about 60% to about 65%, from about 65% to about 70%, from about 70% to about 75%, from about 75% to about 80%, from about 80% to about 85%, from about 85% to about 90%, from about 90% to about 95%, or from about 95% to about 100%. Dosages can be readily adjusted by those skilled in the art, for example, a decrease in purity could require an increase in dosage.

The cells of the application are generally administered as a dose based on cells per kilogram (cells/kg) of body weight of the subject to which the cells and/or pharmaceutical compositions comprising the cells are administered. Generally, the cell doses are in the range of about 10^4 to about 10^{10} cells/kg of body weight, for example, about 10^5 to about 10^9, about 10^5 to about 10^8, about 10^5 to about 10^7, or about 10^5 to about 10^6, depending on the mode and location of administration. In general, in the case

of systemic administration, a higher dose is used than in regional administration, where the immune cells of the application are administered in the region of a tumor and/or cancer. Exemplary dose ranges include, but are not limited to, 1×10^4 to 1×10^8, 2×10^4 to 1×10^8, 3×10^4 to 1×10^8, 4×10^4 to 1×10^8, 5×10^4 to 6×10^8, 7×10^4 to 1×10^8, 8×10^4 to 1×10^8, 9×10^4 to 1×10^8, 1×10^5 to 1×10^8, 1×10^5 to 9×10^7, 1×10^5 to 8×10^7, 1×10^5 to 7×10^7, 1×10^5 to 6×10^7, 1×10^5 to 5×10^7, 1×10^5 to 4×10^7, 1×10^5 to 4×10^7, 1×10^5 to 3×10^7, 1×10^5 to 2×10^7, 1×10^5 to 1×10^7, 1×10^5 to 9×10^6, 1×10^5 to 8×10^6, 1×10^5 to 7×10^6, 1×10^5 to 6×10^6, 1×10^5 to 5×10^6, 1×10^5 to 4×10^6, 1×10^5 to 4×10^6, 1×10^5 to 3×10^6, 1×10^5 to 2×10^6, 1×10^5 to 1×10^6, 2×10^5 to 9×10^7, 2×10^5 to 8×10^7, 2×10^5 to 7×10^7, 2×10^5 to 6×10^7, 2×10^5 to 5×10^7, 2×10^5 to 4×10^7, 2×10^5 to 4×10^7, 2×10^5 to 3×10^7, 2×10^5 to 2×10^7, 2×10^5 to 1×10^7, 2×10^5 to 9×10^6, 2×10^5 to 8×10^6, 2×10^5 to 7×10^6, 2×10^5 to 6×10^6, 2×10^5 to 5×10^6, 2×10^5 to 4×10^6, 2×10^5 to 4×10^6, 2×10^5 to 3×10^6, 2×10^5 to 2×10^6, 2×10^5 to 1×10^6, 3×10^5 to 3×10^6 cells/kg, and the like. Additionally, the dose can be adjusted to account for whether a single dose is being administered or whether multiple doses are being administered. The precise determination of what would be considered an effective dose can be based on factors individual to each subject.

As used herein, the terms "treat," "treating," and "treatment" are all intended to refer to an amelioration or reversal of at least one measurable physical parameter related to a cancer, which is not necessarily discernible in the subject, but can be discernible in the subject. The terms "treat," "treating," and "treatment," can also refer to causing regression, preventing the progression, or at least slowing down the progression of the disease, disorder, or condition. In a particular embodiment, "treat," "treating," and "treatment" refer to an alleviation, prevention of the development or onset, or reduction in the duration of one or more symptoms associated with the disease, disorder, or condition, such as a tumor or more preferably a cancer. In a particular embodiment, "treat," "treating," and "treatment" refer to prevention of the recurrence of the disease, disorder, or condition. In a particular embodiment, "treat," "treating," and "treatment" refer to an increase in the survival of a subject having the disease, disorder, or condition. In a particular embodiment, "treat," "treating," and "treatment" refer to elimination of the disease, disorder, or condition in the subject.

The cells of the application and/or the pharmaceutical compositions of the application can be administered in combination with one or more additional therapeutic agents. In certain embodiments the one or more therapeutic agents are selected from the group consisting of a peptide, a cytokine, a checkpoint inhibitor, a mitogen, a growth factor, a small RNA, a dsRNA (double stranded RNA), siRNA, oligonucleotide, mononuclear blood cells, , a vector comprising one or more polynucleic acids of interest, an antibody, a chemotherapeutic agent or a radioactive moiety, or an immunomodulatory drug (IMiD).

EXAMPLES

Abbreviations

BSA	Bovine Serum Albumin	nM	Nanomolar
CAR	Chimeric Antigen Receptor	UTD	untransduced
CD	Cluster Of Differentiation	VHH	Single variable domain on a heavy chain
E:T	Effector To Target Ratio	KO	Knockout
Fc	Fragment Crystallizable		
Ig	Immunoglobulin		
mM	Micromolar		

Example 1. Development of cells expressing CD22-specific VHH CARs

CARs were constructed with VHH binding domains on the extracellular side of the protein construct, singly or in multiples. The VHH antibody binding fragments were isolated through phage display of VHH libraries and selection on the soluble CD22 protein. VHH proteins were tested as VHH-IgG1 Fc fusions for protein affinity and specific binding to CD22+ cells. Epitope mapping was done by binding competition and using CD22 protein variants with particular domains deleted from the full-length protein. Two clones demonstrated binding to the N-terminal domains, IgV and IgC1-2, of CD22 and another clone demonstrated binding to the C-terminal IgC domains 4-6. The clone binding to the C-terminal IgC domains was affinity matured by scanning mutagenesis of CDR3. This improved affinity from 30nM to 5nM.

VHH fragments were cloned into lentiviral vectors in frame with a CAR consisting of a hinge domain (IgG4 Fc or CD8 hinge), a transmembrane domain (CD28tm), a co-stimulatory domain (41BB, CD28, DAP10) and a primary signaling

domain from CD3z. As monomeric VHH-CARs, low levels of T-cell mediated cytotoxicity was observed. When put in a tandem format, VHH1-VHH1 or VHH1-VHH2, greater levels of cytotoxcity were observed, suggesting that improved affinity/avidity for CD22 on cells was critical for full target cell engagement.

Anti-CD22 VHH antibodies were isolated from VHH phage display libraries through panning against the CD22 full length ECD. Three clones of interest demonstrated were labeled as D04, A01 and E04 (Table 3). VHH sequences were fused to the human IgG1 Fc domain in a mammalian expression construct and expressed as VHH-Fc fusion proteins in Expi293 cells and purified with ProteinA (MabSelect SuRe) resin. VHH-Fc proteins were eluted with 0.1M sodium acetate (pH=3.5) and neutralized with 2.5M sodium acetate (pH=7.5).

VHH-Fc fusion proteins were assessed for affinity against monomeric CD22 (Acro Biosystems) using the ForteBio Octet Red BLI instrument. VHH-Fc proteins were captured onto an anti-human IgG Fc sensor at 50nM. Sensor tips were quenched with human IgG Fc protein at 100nM, then CD22 antigen was added at 250nM to assess binding kinetics. Association was examined for 600 seconds and dissociation was examined for 600 seconds, allowing for calculation of Kon, Kdis and KD. The A01 clone (PROT739) has an affinity of 96nM, the E04 (PROT740) clone has an affinity of 23nM and the D04 clone (PROT265) has an affinity of 32nM (Table 4).

Table 3: VHH clone sequences

Clone name	VHH sequence
CD22_D04	QVQLVESGGGLVQAGGSLRLSCAASGSEFTGYPMGWFRQAPG KEREFVAGSVGIGGSTNYADSVKGRFTISRDNAKNTVYLQMNS LKPEDTAVYYCAADKDYYKPYSRYRTVIRYETWGQGTQVTVS S (SEQ ID NO.: 96) CAGGTGCAGCTGGTTGAGTCTGGGGGAGGCCTTGTCCAGGC TGGGGGGTCCCTGAGACTCTCCTGTGCAGCGTCTGGAAGCG AATTCACCGGTTATCCCATGGGCTGGTTTCGCCAGGCTCCAG GCAAGGAAAGGGAGTTTGTCGCTGGCTCCGTAGGTATCGGT GGTAGTACAAACTATGCAGACTCCGTGAAGGGCCGATTCAC CATCTCCAGAGACAATGCGAAGAACACGGTCTATCTGCAAA TGAACAGCCTGAAGCCAGAGGACACGGCTGTGTATTACTGT GCGGCCGACAAAGACTACTACAAACCTTATAGTCGATATAG

	GACCGCTATCAGGTACGATACCTGGGGCCAAGGGACCCAGG TCACCGTCTCGAGC (SEQ ID NO.: 99)
CD22_CNTY_VHH1_A01	EVQLLESGGGLVQPGGSLRLSCAASGLTSYSYAMGWYRQAPG KEREFVSAISSGGSAYYADSVKGRFTISRDNSKNTLYLQMNSLR AEDTAVYYCAVGPYYGFRAVTEADYWGQGTQVTVSS (SEQ ID NO.: 97) GAGGTACAACTTTTGGAGTCAGGCGGTGGACTGGTACAACC GGGTGGTTCATTGCGTTTGAGCTGCGCTGCCTCTGGTTTGAC CTCTTATTCCTACGCGATGGGCTGGTATCGCCAAGCGCCGGG CAAAGAACGCGAGTTTGTCAGCGCAATCAGCTCGGGTGGTA GCGCGTACTACGCGGACTCGGTAAAAGGCCGTTTTACGATC AGTCGTGATAATTCCAAGAATACCTTGTACCTGCAAATGAAT AGCCTTCGCGCAGAAGACACAGCGGTGTATTATTGTGCCGTT GGACCGTACTACGGATTTAGAGCGGTTACCGAAGCAGATTA TTGGGGCCAGGGTACCCAGGTGACGGTCTCGAGC (SEQ ID NO.: 100)
CD22_CNTY_VHH1_E04	EVQLLESGGGLVQPGGSLRLSCAASGFTSSSYVMGWYRQAPG KEREFVSSISTGGDAYYADSVKGRFTISRDNSKNTLYLQMNSLR AEDTAVYYCAADVWYYHGGAYDYWGQGTQVTVSS (SEQ ID NO.: 98) GAGGTACAACTTTTGGAGTCAGGCGGTGGACTGGTACAACC GGGTGGTTCATTGCGTTTGAGCTGCGCTGCCTCTGGTTTTAC CAGCTCCTCCTACGTGATGGGCTGGTATCGCCAAGCGCCGGG CAAAGAACGCGAGTTTGTCAGCTCGATCAGCACCGGTGGTG ATGCCTACTACGCGGACTCGGTAAAAGGCCGTTTTACGATCA GTCGTGATAATTCCAAGAATACCTTGTACCTGCAAATGAATA GCCTTCGCGCAGAAGACACAGCGGTGTATTATTGTGCCGCTG ACGTTTGGTACTACCACGGCGGCGCGTACGATTATTGGGGCC AGGGTACCCAGGTGACGGTCTCGAGC (SEQ ID NO.: 101)

Table 4: Binding affinity of anti-CD22 VHH proteins

Sample ID	KD (M)	K_{on} (1/Ms)	K_{dis} (1/s)
PROT265 (D04)	3.19E-08	8.08E+04	2.57E-03
PROT739 (A01)	9.61E-08	1.06E+05	1.02E-02
PROT740 (E04)	2.33E-08	2.30E+04	5.36E-04
PROT810 (D04-AM-D11)	6.97E-09	9.43E+04	6.57E-04

VHH-Fc proteins were tested for binding to CD22 expressed on the cell surface. VHH-Fc proteins were serially diluted and added to Raji cells and incubated at 4C for 30 minutes. Cells were washed once in BD staining buffer, BSA (BD Pharmingen), then PE-labeled anti-human IgG Fc (Jackson Immnoresearch) was added to detect VHH-Fc cell binding and incubated at 4C for 30 minutes. Cells were washed once, then resuspended in staining buffer with 0.1% pluronic acid and run through a flow cytometer to detect the fluorescent labeling of the VHH-Fc cells. Binding curves are displayed in Figure 1, showing that the three clones all bind to CD22 positive Raji cells.

To determine the domains to which the VHH-Fc proteins were binding, two deletion variant proteins were constructed. One variant, CD22Δ2-3, deletes IgC domains 2 & 3 and the other, CD22ΔV-1, deletes the N-terminal IgV and IgC1 domains. VHH-Fc protein binding to the full length CD22 was compared to binding to these variants using the ForteBio Octet. VHH-Fc proteins were captured in kinetics buffer (ForteBio) on anti-human IgG Fc biosensor tips. Tips were quenched with human IgG Fc fragment (Jackson Immunoresearch), then CD22 protein was allowed to associate with the VHH-Fc for 10 minutes. Biosensor tips were then transferred to kinetics buffer for dissociation of the CD22 proteins. PROT265 (D04) bound to all proteins tested, suggesting that it binds to the IgC domains 4-6. PROT739 (A01) bound to the full length CD22 and had weak binding to the CD22Δ2-3 protein with no binding to the CD22ΔV-1 protein, suggesting that it binds to the IgC1-2 domains. PROT740 (E04) bound to the full length CD22 and the CD22Δ2-3 protein, but not the CD22ΔV-1 protein, suggesting that it binds to the IgV-IgC1 domains (Figure 2).

The D04 clone was affinity matured by scanning mutagenesis of the CDRs and phage display selections for higher affinity clones. Variants were isolated and expressed as VHH-Fc fusions for affinity assessment. Several clones showed improvement, with the best clone, D04-AM-D11 (PROT810), showing 5-fold improvement to 7nM (Table 4). This clone also showed improved binding to CD22+ Raji cells (Figure 1B)

VHH sequences were cloned into lentiviral CAR constructs with the human IgG4 (CH3) hinge, CD28 transmembrane domain, 41BB co-stimulatory domain and CD3z signaling domain (Table 5). Lentivirus was produced for the various constructs and transduced into Jurkat cells to examine antigen-dependent activation of this T-cell line.

VHH-CAR transduced cells were cultured for one week, then co-cultured with other tumor cell lines with or without CD22 surface expression. Jurkat cells were then stained for expression of activation marker CD69 and examined by flow cytometry. Activated CAR-Jurkat cells were compared to untransduced control (UTD) cells, lacking a CAR. Figure 3a shows activation of cells with the A01 (P952) and E04 (P953) VHH-CARs when exposed to CD22+ cells, Daudi and Raji, but no when exposed to Raji CD22 knock-out or K-562 cells. The D04 VHH-CAR (D04.P262) was tested in a separate experiment (Figure 3b) and also showed activation with exposure to Daudi and Raji cells.

Table 5. CAR sequences

CAR	Hinge	Transmembrane	Co-stimulatory domain	Signaling domain (CD3z)
IgG4(CH3)/CD28/41BB/CD3z	IgG4 CH3: ESKYGPPCPP CPGQPREPQ VYTLPPSQEE MTKNQVSLT CLVKGFYPS DIAVEWESN GQPENNYKT TPPVLDSDG SFFLYSRLTV DKSRWQEG NVFSCSVMH EALHNHYTQ KSLSLSLGK (SEQ ID NO.: 102)	CD28: FWVLVVVG GVLACYSLL VTVAFIIFWV (SEQ ID NO.: 24)	41BB: KRGRKKLLY IFKQPFMRPV QTTQEEDGC SCRFPEEEEG GCEL (SEQ ID NO.: 8)	RVKFSRSAD APAYQQGQ NQLYNELNL GRREEYDVL DKRRGRDPE MGGKPRRK NPQEGLYNE LQKDKMAE AYSEIGMKG ERRRGKGHD GLYQGLSTA TKDTYDALH MQALPPR (SEQ ID NO.: 6)
CD8/CD28/41BB/CD3z	CD8: TTTPAPRPPT PAPTIASQPL SLRPEACRP AAGGAVHT RGLDFACD (SEQ ID NO.: 21)	CD28: FWVLVVVG GVLACYSLL VTVAFIIFWV (SEQ ID NO.: 24)	41BB: KRGRKKLLY IFKQPFMRPV QTTQEEDGC SCRFPEEEEG GCEL (SEQ ID NO.: 8)	RVKFSRSAD APAYQQGQ NQLYNELNL GRREEYDVL DKRRGRDPE MGGKPRRK NPQEGLYNE LQKDKMAE AYSEIGMKG ERRRGKGHD GLYQGLSTA TKDTYDALH MQALPPR

				(SEQ ID NO.: 6)
CD8/CD8/ DAP10/ CD3z	CD8: TTTPAPRPPT PAPTIASQPL SLRPEACRP AAGGAVHT RGLDFACD (SEQ ID NO.: 21)	CD8: IYIWAPLAGT CGVLLLSLVI T (SEQ ID NO.: 23)	DAP10: LCARPRRSP AQEDGKVYI NMPGRG (SEQ ID NO.: 17)	RVKFSRSAD APAYQQGQ NQLYNELNL GRREEYDVL DKRRGRDPE MGGKPRRK NPQEGLYNE LQKDKMAE AYSEIGMKG ERRRGKGHD GLYQGLSTA TKDTYDALH MQALPPR (SEQ ID NO.: 6)

Example 2. Cytotoxicity Assays

VHH-CARs were tested in primary T-cells for killing CD22+ tumor target cells. Lentivirus for each of the CARs described was transduced into primary donor T-cells and grown for 14 days. Expression of the CARs was assessed by flow cytometry. CD22+ Raji cells were labeled with CellTrace Violet (ThermoFisher) and co-cultured with the CAR-T cells at various E:T ratios for 48 hours. Raji cell viability was then assessed by flow cytometry (Figure 4). The P403 construct (CD22_D04) showed modest Raji cell killing over background (UTD). The affinity improved variant P1278 (CD22_D04_AM_D11) had significantly better cytotoxicity, up to 80% at the 5:1 E:T ratio. The P952 (CD22_CNTY_VHH1_A01) and P953 (CD22_CNTY_VHH1_E04) constructs showed modest cytotoxicity above background.

To improve the cytotoxicity profile, VHHs were assembled in tandem on the CAR protein. As the A01 and E04 clones bound to N-terminal epitopes and the D04 clone bound to a C-terminal epitope, combinations of these VHHs were made such that the VHH-CAR could bind the two different epitopes. VHHs were assembled in tandem, separated by a linker having the sequence GSTSGSGKPGSGEGSTKG (SEQ ID NO: 3). and paired with different CAR domains: 1) CD8 hinge/CD28 transmembrane/41BB/CD3z and 2) CD8 hinge/transmembrane/DAP10/CD3z (Table 3).

Lentivirus was prepared from these constructs and transduced into primary T-cells. Cytotoxicity was assessed as described. Results show improved cell killing of Raji cells with tandem biparatopic VHH-CARs with the "CD22_D04_AM_D11-linker-CD22_A01" format having the most robust cell killing (Figure 5).

Example 3. Cytotoxicity Assays Using Therapeutic Cells Expressing Mono- and Bispecific CARs Against Various Target Cells

Bispecific CAR ectodomains were engineered with select CD22 binders in combination with FMC63 (Figure 6). Selected CARs (e.g., identified by P####) were expressed and tested for cytotoxicity activity in T-cells along with mono-specific CAR-T cells.

The selected bispecific CARs were tested in primary T-cells for killing CD19+, CD22+ or CD19+/CD22+ tumor target cells (Table 6).

Table 6. Tumor target cells

Cell Line	CD22 Expression	CD19 Expression
CHO (Parent)	Negative	Negative
CHO-CD19	Negative	Positive
CHO-CD22	Positive	Negative
Raji (Parent)	Positive	Positive
Raji CD22 KO	Negative	Positive
Raji CD19 KO	Positive	Negative
NALM6	Positive	Positive
NALM6 CD19 KO	Positive	Negative
Reh	Positive	Positive
Jeko	Positive	Positive

Lentivirus for each of the CARs described was transduced into primary donor T-cells and grown for 14 days. Expression of the CARs was assessed by flow cytometry.

The target expressing cells were co-cultured with the CAR-T cells at various E:T ratios for 48 hours. Cell viability was assessed by flow cytometry (Figures 9 & 10B). CD19-, CD22-, and CD19/CD22-dependent cytotoxicity was assessed at a 1.25:1 E:T ratio (Figures 7 and 8). The data represent the cumulative cytotoxicity (e.g., percent target killing) for the cell lines tested. All of the bispecific CAR-T cells tested exhibited CD19-targeted cytotoxicity activity equivalent to the FMC63 CAR P1209 (Figure 7A). The bispecific CARS showed comparable CD22-dependent cytotoxicity as the mono-specific tandem CARs P1631, P1633, P1702 and P1734 (Figure 7B). This study demonstrated the successful engineering of several bispecific CD19-CD22 CAR-T cells.

It will be appreciated by those skilled in the art that changes could be made to the embodiments described above without departing from the broad inventive concept thereof. It is understood, therefore, that this invention is not limited to the particular embodiments disclosed, but it is intended to cover modifications within the spirit and scope of the present invention as defined by the present description.

CLAIMS

It is claimed:

1. An induced pluripotent stem cell (iPSC) or a derivative cell thereof comprising:

one or more exogenous polynucleotides encoding a chimeric antigen receptor (CAR) comprising an antigen binding domain targeting a CD22 antigen; and

optionally, at least one of:

(i) a CD19 antigen-binding domain encoded by the one or more exogenous polynucleotides;

(ii) a deletion or reduced expression of one or more of B2M, TAP 1, TAP 2, Tapasin, RFXANK, CIITA, RFX5, RFXAP genes;

(iii) an exogenous polynucleotide encoding a human leukocyte antigen E (HLA-E) and/or human leukocyte antigen G (HLA-G);

(iv) an exogenous polynucleotide encoding an NK cell receptor immunoglobulin gamma Fc region receptor III (FcyRIII, cluster of differentiation 16 (CD16) and/or an NKG2D protein;

(v) a deletion or reduced expression of one or more of NKG2A or CD70 genes;

(vi) an exogeneous polynucleotide encoding a cytokine;

(vii) an exogenous polynucleotide encoding a safety switch; and

(viii) an exogeneous polynucleotide encoding a PSMA cell tracer.

2. The iPSC or the derivative cell according to claim 1 comprising the CD19 antigen-binding domain, wherein:

(i) the CAR is a bispecific CAR comprising the CD19 antigen-binding domain, or

(ii) the one or more exogenous polynucleotides encode a additional CAR comprising the CD19 antigen-binding domain.

3. The iPSC or the derivative cell according to claim 1 or 2, wherein the CAR comprises an anti-CD22 VHH domain, and/or wherein the CD19 antigen-binding domain comprises an anti-CD19 VHH domain.

4. The iPSC or the derivative cell thereof according to any one of claims 1-3, wherein the cytokine comprises an IL-15 protein.

5. The iPSC or derivative cell according to claim 4, wherein the IL-15 protein comprises an inactivated cell surface receptor that comprises a monoclonal antibody-specific epitope and an interleukin 15 (IL-15), and wherein the inactivated cell surface receptor and the IL-15 are operably linked by an autoprotease peptide.

6. The iPSC or the derivative cell thereof according to claim 4, wherein the IL-15 protein comprises (i) a fusion polypeptide comprising an IL-15 and an IL-15 receptor alpha (IL-15Rα), or (ii) a polypeptide sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to SEQ ID NO: 202.

7. The iPSC or the derivative cell thereof according to any one of claims 4-6, wherein the IL-15 comprises an amino acid sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to SEQ ID NO: 72.

8. The iPSC or the derivative cell according to any one of claims 1-7, comprising the deletion or reduced expression of one or more of B2M, TAP 1, TAP 2, Tapasin, RFXANK, CIITA, RFX5 and RFXAP genes.

9. The iPSC or the derivative cell according to any one of claims 1-8, further comprising an exogenous polynucleotide encoding a human leukocyte antigen E (HLA-E) and/or human leukocyte antigen G (HLA-G).

10. The iPSC or the derivative cell thereof according to any one of claims 1-9, wherein the CD16 is a CD16 variant protein.

11. The iPSC or the derivative cell thereof according to claim 10, wherein the CD16 variant protein is a high affinity CD16 variant.

12. The iPSC or the derivative cell thereof according to claim 10 or 11, wherein the CD16 variant protein is a non-cleavable CD16 variant.

13. The iPSC or the derivative cell thereof according to any one of claims 10-12, wherein the CD16 variant protein comprises one or more amino acid substitutions selected from the group consisting of F158V, F176V, S197P, D205A, S219A, T220A.

14. The iPSC or the derivative cell thereof according to any one of claims 10-13, wherein the CD16 variant protein comprises an amino acid sequence having at least 90% sequence identity to any one of SEQ ID NOS:181 and 182.

15. The iPSC or the derivative cell thereof according to any one of claims 1-14 comprising an exogenous polynucleotide encoding the CD16 protein and the NKG2D protein, wherein the CD16 protein and the NKG2D protein are operably linked by an autoprotease peptide.

16. The iPSC or the derivative cell thereof according to claim 15, wherein the NKG2D protein is a wildtype NKG2D protein.

17. The iPSC or the derivative cell thereof according to claim 15 or 16, wherein the NKG2D protein comprises an amino acid sequence having at least 90% sequence identity to SEQ ID NO: 184.

18. The iPSC or the derivative cell thereof according to any one of claims 15-17, wherein the autoprotease peptide is selected from the group consisting of a

porcine tesehovirus-1 2A (P2A) peptide, a foot-and-mouth disease virus 2A (F2A) peptide, an Equine Rhinitis A Virus (ERAV) 2A (E2A) peptide, a Thosea asigna virus 2A (T2A) peptide, a cytoplasmic polyhedrosis virus 2A (BmCPV2A) peptide, and a Flacherie Virus 2A (BmIFV2A) peptide.

19. The iPSC or the derivative cell thereof according to claim 18, wherein the autoprotease peptide is a P2A peptide comprising an amino acid sequence having at least 90% sequence identity to SEQ ID NO: 186.

20. The iPSC or the derivative cell thereof according to any one of claims 15-19, wherein the exogenous polynucleotide encoding the CD16 protein and the NKG2D protein comprises a nucleic acid sequence having at least 90% sequence identity to SEQ ID NO: 186.

21. The iPSC or the derivative cell according to any one of claims 1-20, wherein one or more of the exogenous polynucleotides are integrated at one or more loci on the chromosome of the cell selected from the group consisting of AAVS1, CCR5, ROSA26, collagen, HTRP, Hl l, GAPDH, RUNX1, B2M, TAPI, TAP2, Tapasin, NLRC5, RFXANK, CIITA, RFX5, RFXAP, TCR a or b constant region, NKG2A, NKG2D, CD38, CIS, CBL-B, SOCS2, PD1, CTLA4, LAG3, TIM3, and TIGIT genes, provided at least one of the exogenous polynucleotides is integrated at a locus of a gene selected from the group consisting of AAVS1, B2M, TAP 1, TAP 2, Tapasin, RFXANK, CIITA, RFX5 and RFXAP genes to thereby result in a deletion or reduced expression of the gene.

22. The iPSC or the derivative cell according to any one of claims 1-20, wherein one or more of the exogenous polynucleotides are integrated at the loci of the AAVS1 and B2M genes.

23. The iPSC or the derivative cell according to any one of claims 1-22 having a deletion or reduced expression of one or more of B2M or CIITA genes.

24. The iPSC or the derivative cell thereof according to claim 23, comprising the deletion or reduced expression of B2M and CIITA genes.

25. The iPSC of any one of claims 1-24, where the iPSC is reprogrammed from whole peripheral blood mononuclear cells (PBMCs).

26. The iPSC of any one of claim 1-24, which is derived from a re-programmed T-cell.

27. The iPSC or the derivative cell according to any one of claims 1-26, wherein the CAR comprises:
(i) a signal peptide;
(ii) a extracellular domain comprising the antigen binding domain targeting the CD22 antigen;
(iii) a hinge region;
(iv) a transmembrane domain;
(v) an intracellular signaling domain; and
(vi) a co-stimulatory domain.

28. The iPSC or the derivative cell according to claim 27, wherein the extracellular domain comprises a VHH single domain antibody that specifically binds the CD22 antigen.

29. The iPSC or the derivative cell according to any one of claims 27 or 28, wherein the extracellular domain comprises an amino acid sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to one of SEQ ID NOs: 96-98, 152, and 155.

30. The iPSC or the derivative cell according to any one of claims 1-29 wherein the extracellular domain comprises a polynucleotide sequence having at least 90%,

91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to one of SEQ ID NOs: 99-101, 153, and 156.

31. The iPSC or the derivative cell according to any one of claims 2-30, wherein the additional CAR comprises:
    (i) a signal peptide;
    (ii) an additional extracellular domain comprising a binding domain that specifically binds the CD19 antigen;
    (iii) a hinge region;
    (iv) a transmembrane domain;
    (v) an intracellular signaling domain; and
    (vi) a co-stimulatory domain.

32. The iPSC or the derivative cell according to claim 31, wherein the additional extracellular domain comprises an scFv derived from an antibody that specifically binds the CD19 antigen.

33. The iPSC or the derivative cell according to claim 31 or 32, wherein the additional extracellular domain comprises (i) an amino acid sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to one or more of SEQ ID NOs: 2, 4, and 7, or (ii) is encoded by a polynucleotide sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to one or more of SEQ ID NOs: 145 and 147.

34. The iPSC or the derivative cell according to any one of claims 27-33, wherein the signal peptide comprises a GMCSFR signal peptide.

35. The iPSC or the derivative cell according to any one of claims 27-34, wherein the hinge region for each of the CAR and the additional CAR are independently selected from the group consisting of a CD28 hinge region, an IgG4 hinge region, and a CD8 hinge region.

36. The iPSC or the derivative cell according to any one of claims 27-35, wherein the transmembrane domain for each of the CAR and the additional CAR are independently selected from the group consisting of a CD28 transmembrane domain and a Cd8 transmembrane domain.

37. The iPSC or the derivative cell according to any one of claims 31-36, wherein the intracellular signaling domain comprises a CD3ζ intracellular domain.

38. The iPSC or the derivative cell according to any one of claims 31-37, wherein the co-stimulatory domain for each of the CAR and the additional CAR are independently selected from the group consisting of a CD28 signaling domain, a 41BB signaling domain, a DAP10 signaling domain, an IL18R1 signaling domain, and an IL18RAP signaling domain.

39. The iPSC or the derivative cell according to any one of claims 31-38, wherein the additional CAR comprises:

(i) the signal peptide comprising an amino acid sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to SEQ ID NOs: 1, 103, or 144;

(ii) the additional extracellular domain comprising an amino acid sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to SEQ ID NOs: 2, 4, or 7, or the additional extracellular domain encoded by a polynucleotide sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to SEQ ID NOs: 145 or 147;

(iii) the hinge region comprising an amino acid sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to SEQ ID NO: 22;

(iv) the transmembrane domain comprising an amino acid sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to SEQ ID NO: 24;

(v) the intracellular signaling domain comprising an amino acid sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to SEQ ID NO: 6, or the intracellular signaling domain encoded by a polynucleotide sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to SEQ ID NO: 149; and

(vi) the co-stimulatory domain comprising an amino acid sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to SEQ ID NO: 20.

40. The iPSC or the derivative cell according to any one of claims 31-39, wherein the additional CAR comprises:

(i) the signal peptide comprising the amino acid sequence of SEQ ID NOs: 1, 103, or 144;

(ii) the additional extracellular domain (i) comprising the amino acid sequence of SEQ ID NO: 2, 4, and 7, or (ii) encoded by the polynucleotide sequence of SEQ ID NOs: 145 and 147;

(iii) the hinge region comprising the amino acid sequence of SEQ ID NO: 22;

(iv) the transmembrane domain comprising the amino acid sequence of SEQ ID NO: 24;

(v) the intracellular signaling domain comprising the amino acid sequence of SEQ ID NO: 6, or the intracellular signaling domain encoded by the polynucleotide sequence of SEQ ID NO: 149; and

(vi) the co-stimulatory domain comprising the amino acid sequence of SEQ ID NO: 20.

41. The iPSC or the derivative cell according to any one of claims 31-40, wherein the CAR comprises:

(i) the signal peptide comprising an amino acid sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to SEQ ID NOs: 1, 103, or 144;

(ii) the extracellular domain comprising an amino acid sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to one of SEQ ID NOs: 96-98, 152, and 155;

(iii) the hinge region comprising an amino acid sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to SEQ ID NO: 21 or 102;

(iv) the transmembrane domain comprising an amino acid sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to SEQ ID NO: 23 or 24;

(v) the intracellular signaling domain comprising an amino acid sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to SEQ ID NO: 6, 198, or 199, or the intracellular signaling domain encoded by a polynucleotide sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to SEQ ID NO: 149; and

(vi) the co-stimulatory domain comprising an amino acid sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to SEQ ID NO: 8, 17, 198, or 199.

42. The iPSC or the derivative cell according to any one of claims 31-39, wherein the CAR comprises:

(i) the signal peptide comprising the amino acid sequence of SEQ ID NO: 1, 103, or 144;

(ii) the extracellular domain comprising the amino acid sequence of one of SEQ ID NOs: 96-98, 152, and 155;

(iii) the hinge region comprising the amino acid sequence of SEQ ID NO: 21 or 102;

(iv) the transmembrane domain comprising the amino acid sequence of SEQ ID NO: 23 or 24;

(v) the intracellular signaling domain comprising the amino acid sequence of SEQ ID NO: 6, 198, or 199, or the intracellular signaling domain encoded by the polynucleotide sequence of SEQ ID NO: 149; and

(vi) the co-stimulatory domain comprising the amino acid sequence of SEQ ID NO: 8, 17, 198, or 199.

43. The iPSC or the derivative cell thereof of any one of claims 1-42, further comprising an exogenous polynucleotide encoding a safety switch.

44. The iPSC or the derivative cell thereof of claim 43, wherein the safety switch comprises an exogenous polynucleotide encoding an inactivated cell surface receptor that comprises a monoclonal antibody-specific epitope.

45. The iPSC or the derivative cell according to claim 44, wherein the inactivated cell surface protein is selected from the group of monoclonal antibody specific epitopes selected from epitopes specifically recognized by ibritumomab, tiuxetan, muromonab-CD3, tositumomab, abciximab, basiliximab, brentuximab vedotin, cetuximab, infliximab, rituximab, alemtuzumab, bevacizumab, certolizumab pegol, daclizumab, eculizumab, efalizumab, gemtuzumab, natalizumab, omalizumab, palivizumab, polatuzumab vedotin, ranibizumab, tocilizumab, trastuzumab, vedolizumab, adalimumab, belimumab, canakinumab, denosumab, golimumab, ipilimumab, ofatumumab, panitumumab, and ustekinumab.

46. The iPSC or the derivative cell according to claim 44, wherein the inactivated cell surface protein is a truncated epithelial growth factor (tEGFR) variant.

47. The iPSC or the derivative cell according to claim 46, wherein the tEGFR variant consists of an amino acid sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to SEQ ID NO: 71.

48. The iPSC or the derivative cell thereof according to any one of claims 1-48, wherein the safety switch comprises (i) an intracellular domain having a herpes simplex virus thymidine kinase (HSV-TK) or (ii) an inducible Caspase 9 (iCasp9).

49. The iPSC or the derivative cell thereof according to any one of claims 1-48 comprising the exogeneous polynucleotide encoding the PSMA cell tracer, wherein the PSMA cell tracer comprises an extracellular domain comprising a PSMA extracellular domain or fragment thereof.

50. The iPSC or the derivative cell thereof according to claim 49, comprising a combined artificial cell death/reporter system polypeptide comprising an intracellular domain having a herpes simplex virus thymidine kinase (HSV-TK) and a linker, a transmembrane region, and an extracellular domain comprising the PSMA extracellular domain or fragment thereof.

51. The iPSC or the derivative cell thereof according to any one of claims 48-50, wherein (i) the HSV-TK comprises an amino acid sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to SEQ ID NO: 187 or 188, or (ii) the iCasp9 comprises an amino acid sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to SEQ ID NO: 200 or 201.

52. The iPSC or the derivative cell thereof according to claim 50, wherein the combined artificial cell death/reporter system polypeptide comprises the HSV-TK fused to a truncated variant PSMA polypeptide via the linker.

53. The iPSC or the derivative cell thereof according to claim 52, wherein the truncated variant PSMA polypeptide comprises an amino acid sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to SEQ ID NO: 189.

54. The iPSC or the derivative cell thereof according to any one of claims 50-53, wherein the linker comprises an autoprotease peptide sequence selected from the group consisting of P2A peptide sequence, T2A peptide sequence, E2A peptide sequence, and F2A peptide sequence.

55. The iPSC or the derivative cell thereof according to any one of claims 50-54, wherein the artificial cell death/reporter system polypeptide comprises an amino acid sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to a sequence selected from the group consisting of SEQ ID NO: 190.

56. The iPSC or the derivative cell thereof according to claim 55, wherein the artificial cell death/reporter system polypeptide comprises an amino acid sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to a sequence selected from the group consisting of SEQ ID NOS: 191-193.

57. The iPSC or the derivative cell thereof according to any one of claims 50-56, wherein the artificial cell death/reporter system polypeptide comprises nucleic acid sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to a sequence selected from the group consisting of SEQ ID NOS: 194-196.

58. The iPSC or the derivative cell according to any one of claims 9-57, wherein the HLA-E comprises an amino acid sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to SEQ ID NO: 66 or the HLA-G comprises an amino acid sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to SEQ ID NO: 69.

59. The iPSC or the derivative cell according to any one of claims 1-58, wherein:

(i) the one or more exogenous polynucleotides encoding a chimeric antigen receptor (CAR) comprising an antigen binding domain targeting a CD22 and/or CD19 antigen comprises a sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to one or more polynucleotide sequences selected from the group consisting of SEQ ID NOs: 62, 99-101, 112-119, 132-143, 153, 156, 158, 160, 162, 164, 168-170, 172, and 176-178;

(ii) the exogenous polynucleotide encoding a human leukocyte antigen E (HLA-E) and/or human leukocyte antigen G (HLA-G) comprises the polynucleotide sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to one or more of SEQ ID NOs: 67 and 70;

(iii) the exogenous polynucleotide encoding an NK cell receptor immunoglobulin gamma Fc region receptor III (FcyRIII, cluster of differentiation 16 (CD16)) and/or an NKG2D protein comprises the polynucleotide sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to one or more of SEQ ID NOs: 179, 183, and 185;

(iv) the exogeneous polynucleotide encoding a cytokine comprises the polynucleotide sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to SEQ ID NO: 197;

(v) the exogenous polynucleotide encoding a safety switch comprises the polynucleotide sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to one or more of SEQ ID NO: 194-196; and/or

(vi) the exogeneous polynucleotide encoding a PSMA cell tracer comprising the amino acid sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to SEQ ID NO: 189.

60. The iPSC or the derivative cell thereof according to any one of claims 1-59, wherein:

(i) the one or more exogenous polynucleotides encoding a chimeric antigen receptor (CAR) comprising an antigen binding domain targeting a CD22 and/or CD19 antigen comprises one or more sequences selected from the group consisting of SEQ ID NOs: 62, 99-101, 112-119, 132-143, 153, 156, 158, 160, 162, 164, 168-170, 172, and 176-178;

(ii) the exogenous polynucleotide encoding a human leukocyte antigen E (HLA-E) and/or human leukocyte antigen G (HLA-G) comprises the polynucleotide sequence having the sequence SEQ ID NO: 67 or 70;

(iii) the exogenous polynucleotide encoding an NK cell receptor immunoglobulin gamma Fc region receptor III (FcyRIII, cluster of differentiation 16 (CD16)) and/or an NKG2D protein comprises the polynucleotide sequence of SEQ ID NO: 179, 183, or 185;

(iv) the exogeneous polynucleotide encoding the cytokine comprises the polynucleotide sequence of SEQ ID NO: 197; and/or

(v) the exogenous polynucleotide encoding the safety switch comprises the polynucleotide sequence having the sequence of one of SEQ ID NOs: 194-196.

61. The iPSC or the derivative cell thereof according to claim 59 or 60, wherein the exogenous polynucleotides are integrated into a gene locus independently selected from the group consisting of an AAVS1 locus, a B2M locus, a CIITA locus, a CCR5 locus, a CD70 locus, a CLYBL locus, an NKG2A locus, an NKG2D locus, a CD33 locus, a CD38 locus, a TRAC locus, a TRBC1 locus, a ROSA26 locus, an HTRP locus, a GAPDH locus, a RUNX1 locus, a TAP1 locus, a TAP2 locus, a TAPBP locus, an NLRC5 locus, a RFXANK locus, a RFX5 locus, a RFXAP locus, a CISH locus, a CBLB locus, a SOCS2 locus, a PD1 locus, a CTLA4 locus, a LAG3 locus, a TIM3 locus, and a TIGIT locus.

62. The iPSC or the derivative cell thereof according to claim 61, wherein:

(i) the one or more exogenous polynucleotides encoding a chimeric antigen receptor (CAR) comprising one or more antigen binding domains targeting CD22 and/or CD19 antigens is integrated at a locus of the AAVS1 gene;

(ii) the exogenous polynucleotide encoding a human leukocyte antigen E (HLA-E) and/or human leukocyte antigen G (HLA-G) is integrated at a locus of the B2M gene;

(iii) the exogenous polynucleotide encoding an NK cell receptor immunoglobulin gamma Fc region receptor III (FcyRIII, cluster of differentiation 16 (CD16)) and/or an NKG2D is integrated at a locus of the CD70 gene;

(iv) the exogeneous polynucleotide encoding the cytokine is integrated at the locus of the NKG2A gene;

(v) there is a deletion or reduced expression of the CIITA gene; and

(vi) optionally, there a safety switch or PSMA is integrated at the locus of the CIITA gene.

63. The iPSC or the derivative cell according to any one of claims 2-62, wherein the CAR is a bispecific CAR comprising a CD22/CD19 loop.

64. The iPSC or the derivative cell according to any one of claims 2-62, comprising the bispecific CAR, wherein the bispecific CAR comprises one or more amino acid sequences having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to a sequence selected from the group consisting of SEQ ID NOs: 61, 96-98, 104-111, 120-131, 152, 155, 157, 159, 161, 163, 165-167, 171, and 173-175.

65. The iPSC or the derivative cell according to any one of claims 2-62 comprising the bispecific CAR, wherein the bispecific CAR comprises one or more polynucleotide sequences having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to a sequence selected from the group consisting of SEQ

ID NOs: 62, 99-101, 112-119, 132-143, 153, 156, 158, 160, 162, 164, 168-170, 172, and 176-178.

66. The derivative cell of any one of claims 1-65, wherein the derivative cell is a natural killer (NK) cell or a T cell.

67. The derivative cell of claim 66, wherein the derivative cell is a natural killer (NK) cell.

68. The derivative cell of claim 66, wherein the derivative cell is a T cell.

69. The derivative cell of claim 68, wherein the T cell is a gamma delta T cell.

70. The derivative cell of claim 68, wherein the T cell is a gamma delta Vγ9/Vδ1 T cell.

71. A composition comprising the cell according to any one of the claims 1-70.

72. The composition according to claim 71, further comprising or being used in combination with, one or more therapeutic agents selected from the group consisting of a peptide, a cytokine, a checkpoint inhibitor, a mitogen, a growth factor, a small RNA, a dsRNA (double stranded RNA), siRNA, oligonucleotide, mononuclear blood cells, a vector comprising one or more polynucleic acids of interest, an antibody, a chemotherapeutic agent or a radioactive moiety, or an immunomodulatory drug (IMiD).

73. An induced pluripotent stem cell (iPSC) or a derivative cell thereof comprising:

one or more exogenous polynucleotides encoding a chimeric antigen receptor (CAR) targeting a CD22 antigen and a CD19 antigen; and

at least one of:

(i) a deletion or reduced expression of one or more of B2M, TAP 1, TAP 2, Tapasin, RFXANK, CIITA, RFX5, RFXAP genes;

(ii) an exogenous polynucleotide encoding a human leukocyte antigen E (HLA-E) and/or human leukocyte antigen G (HLA-G);

(iii) an exogenous polynucleotide encoding an NK cell receptor immunoglobulin gamma Fc region receptor III (FcγRIII, cluster of differentiation 16 (CD16) and/or an NKG2D protein;

(iv) a deletion or reduced expression of one or more of NKG2A or CD70 genes;

(v) an exogeneous polynucleotide encoding a cytokine;

(vi) an exogenous polynucleotide encoding a safety switch; and

(vii) an exogeneous polynucleotide encoding a PSMA cell tracer.

74. The iPSC or the derivative cell according to claim 73, wherein the CAR is a bispecific CAR comprising a CD22/CD19 loop.

75. The iPSC or the derivative cell according to claim 73 or 74, wherein the CAR comprises an anti-CD22 VHH domain.

76. The iPSC or the derivative cell according to any one of claims 73-75, wherein the one or more exogenous polynucleotides each comprise a sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to one or more sequences independently selected from the group consisting of SEQ ID NOs: 62, 99-101, 112-119, 132-143, 153, 156, 158, 160, 162, 164, 168-170, 172, and 176-178.

77. A CD34+ hematopoietic progenitor cell (HPC) derived from an induced pluripotent stem cell (iPSC) comprising:

one or more exogenous polynucleotides encoding a chimeric antigen receptor (CAR) comprising an antigen binding domain targeting a CD22 antigen; and

optionally, at least one of:

(i) a CD19 antigen-binding domain encoded by the one or more exogenous polynucleotides;

(ii) a deletion or reduced expression of one or more of B2M, TAP 1, TAP 2, Tapasin, RFXANK, CIITA, RFX5, and RFXAP genes;

(iii) an exogenous polynucleotide encoding a human leukocyte antigen E (HLA-E) and/or human leukocyte antigen G (HLA-G);

(iv) an exogenous polynucleotide encoding an NK cell receptor immunoglobulin gamma Fc region receptor III (FcγRIII, cluster of differentiation 16 (CD16) and/or an NKG2D protein;

(v) a deletion or reduced expression of one or more of NKG2A or CD70 genes

(vi) an exogeneous polynucleotide encoding a cytokine;

(vii) an exogenous polynucleotide encoding a safety switch; and

(viii) an exogeneous polynucleotide encoding a PSMA cell tracer.

78. The CD34+ HPC according to claim 77 comprising the CD19 antigen-binding domain, wherein:

(i) the CAR is a bispecific CAR comprising the CD19 antigen-binding domain, or

(ii) the one or more exogenous polynucleotides encode an additional CAR comprising the CD19 antigen-binding domain.

79. The CD34+ HPC according to claim 77 or 78, wherein the CAR comprises an anti-CD22 VHH domain, and/or wherein the CD19 antigen-binding domain comprises an anti-CD19 VHH domain.

80. The CD34+ HPC according to any one of claims 77-79, wherein the cytokine comprises an IL-15 protein.

81. The CD34+ HPC according to claim 80, wherein the IL-15 protein comprises an inactivated cell surface receptor that comprises a monoclonal antibody-specific

epitope and an interleukin 15 (IL-15), and wherein the inactivated cell surface receptor and the IL-15 are operably linked by an autoprotease peptide.

82. The CD34+ HPC according to claim 80, wherein the IL-15 protein comprises a fusion polypeptide comprising an IL-15 and an IL-15 receptor alpha (IL-15Rα).

83. The CD34+ HPC according to any one of claims 80-82, wherein the IL-15 comprises an amino acid sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to SEQ ID NO: 72.

84. The CD34+ HPC according to any one of claims 77-83, comprising the deletion or reduced expression of one or more of B2M, TAP 1, TAP 2, Tapasin, RFXANK, CIITA, RFX5 and RFXAP genes.

85. The CD34+ HPC according to any one of claims 77-84, comprising the exogenous polynucleotide encoding a human leukocyte antigen E (HLA-E) and/or human leukocyte antigen G (HLA-G).

86. The CD34+ HPC according to any one of claims 77-85, wherein one or more of the exogenous polynucleotides are integrated at one or more loci on the chromosome of the cell independently selected from the group consisting of AAVS1, CCR5, ROSA26, collagen, HTRP, Hl l, GAPDH, RUNX1, B2M, TAPI, TAP2, Tapasin, NLRC5, RFXANK, CIITA, RFX5, RFXAP, TCR a or b constant region, NKG2A, NKG2D, CD38, CIS, CBL-B, SOCS2, PD1, CTLA4, LAG3, TIM3, and TIGIT genes, provided at least one of the exogenous polynucleotides is integrated at a locus of a gene selected from the group consisting of AAVS1, B2M, TAP 1, TAP 2, Tapasin, RFXANK, CIITA, RFX5 and RFXAP genes to thereby result in a deletion or reduced expression of the gene.

87. The CD34+ HPC according to claim 86, wherein one or more of the exogenous polynucleotides are integrated at the loci of the CIITA, AAVS1 and B2M genes.

88. The CD34+ HPC according to any one of claims 77-87 having a deletion or reduced expression of one or more of B2M or CIITA genes.

89. The CD34+ HPC according to any one of claims 77-88, wherein the CAR comprises:
    (i) a signal peptide;
    (ii) a extracellular domain comprising a binding domain that specifically binds the CD22 antigen and, optionally, a binding domain that specifically binds the CD19 antigen;
    (iii) a hinge region;
    (iv) a transmembrane domain;
    (v) an intracellular signaling domain; and
    (vi) a co-stimulatory domain.

90. The CD34+ HPC according to claim 89, wherein the extracellular domain comprises a VHH single domain antibody that specifically binds the CD22 antigen.

91. The CD34+ HPC according to any one of claims 89 or 90, wherein the extracellular domain comprises an amino acid sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to one of SEQ ID NOs: 96-98, 152, and 155.

92. The CD34+ HPC according to any one of claims 89-91, wherein the extracellular domain comprises a polynucleotide sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to one of SEQ ID NOs: 99-101, 153, and 156.

93. The CD34+ HPC according to any one of claims 78-92 comprising the bispecific CAR, wherein the bispecific CAR comprises one or more amino acid sequences having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100%

sequence identity to a sequence selected from the group consisting of SEQ ID NOs: 61, 96-98, 104-111, 120-131, 152, 155, 157, 159, 161, 163, 165-167, 171, and 173-175.

94. The CD34+ HPC according to any one of claims 78-92 comprising the bispecific CAR, wherein the bispecific CAR comprises an amino acid sequence encoded by one or more polynucleotide sequences having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to a sequence selected from the group consisting of SEQ ID NOs: 62, 99-101, 112-119, 132-143, 153, 156, 158, 160, 162, 164, 168-170, 172, and 176-178.

95. The CD34+ HPC according to any one of claims 78-92, wherein the additional CAR comprises:
(i) a signal peptide;
(ii) an additional extracellular domain comprising a binding domain that specifically binds the CD19 antigen;
(iii) a hinge region;
(iv) a transmembrane domain;
(v) an intracellular signaling domain; and
(vi) a co-stimulatory domain.

96. The CD34+ HPC according to claim 95, wherein the additional extracellular domain comprises an scFv derived from an antibody that specifically binds the CD19 antigen.

97. The CD34+ HPC according to claim 95 or 96, wherein the additional extracellular domain comprises (i) an amino acid sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to one or more of SEQ ID NOs: 2, 4, and 7, or (ii) is encoded by a polynucleotide sequence having at least

90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to one or more of SEQ ID NOs: 145 and 147.

98. A chimeric antigen receptor (CAR) polypeptide comprising an extracellular domain that specifically binds to CD22 having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to SEQ ID NOs: 62, 99-101, 112-119, 132-143, 153, 156, 158, 160, 162, 164, 168-170, 172, and 176-178.

99. A method of treating cancer in a subject in need thereof, comprising administering the derivative cell according to any one of claims 66-70 or the composition according claim 71 or 72 to a subject in need thereof.

100. The method of treatment according to any claim 99, wherein the cancer is selected from the group consisting of leukemia, such as AML, CML, acute lymphocytic leukemia (ALL), B-cell acute lymphocytic leukemia (B-ALL), and chronic lymphocytic leukemia (CLL), lymphomas, such as Hodgkin lymphoma, non-Hodgkin lymphoma, multiple myeloma, and follicular lymphoma, and solid cancers such as sarcomas, skin cancer, melanoma, bladder cancer, brain cancer, breast cancer, uterus cancer, ovary cancer, prostate cancer, lung cancer, colorectal cancer, cervical cancer, liver cancer, head and neck cancer, esophageal cancer, pancreatic cancer, renal cancer, adrenal cancer, stomach cancer, testicular cancer, cancer of the gall bladder and biliary tracts, thyroid cancer, thymus cancer, cancer of bone, and cerebral cancer, as well as cancer of unknown primary (CUP).

101. The method of treatment according to claim 100, wherein the cancer is a B-cell malignancy, acute lymphocytic leukemia (ALL), B-cell acute lymphocytic leukemia (B-ALL), chronic lymphocytic leukemia (CLL), or non-Hodgkin lymphoma, follicular lymphoma.

102. The method of treatment according to any one of claims 99-101, wherein the subject has minimal residual disease (MRD) after an initial cancer treatment.

103. The method of treatment according to any one of claims 99-101, wherein the subject has no minimal residual disease (MRD) after one or more cancer treatments or repeated dosing.

104. A method of manufacturing the derivative cell according to any one of claims 1-70, and 73-76 comprising differentiating the iPSC cell under conditions for cell differentiation to thereby obtain the derivative cell.

105. The method according to claim 104, wherein the iPSC is obtained by genomic engineering an unmodified iPSC, wherein the genomic engineering comprises targeted editing.

106. The method according to claim 104, wherein the targeted editing comprises deletion, insertion, or in/del carried out by CRISPR, ZFN, TALEN, homing nuclease, homology recombination, or any other functional variation of these methods.

107. A method of differentiating an induced pluripotent stem cell (iPSC) into an NK cell, comprising subjecting the iPSCs to a differentiation protocol including culturing the cells in a medium containing a recombinant human IL-12 for the final 24 hours of culturing under the differentiation protocol.

108. The method according to claim 107, wherein the recombinant IL-12 comprises IL12p70.

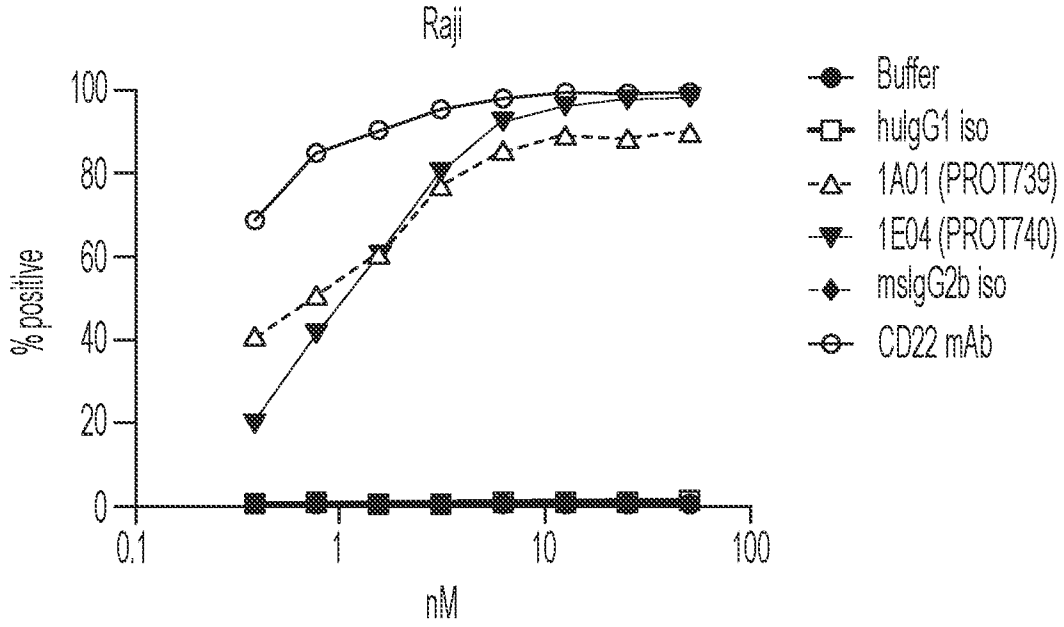

Raji
% positive
100
80
60
40
20
0
0.1
1
10
100
nM
Buffer
hulgG1 iso
1A01 (PROT739)
1E04 (PROT740)
mslgG2b iso
CD22 mAb

FIG. 1A

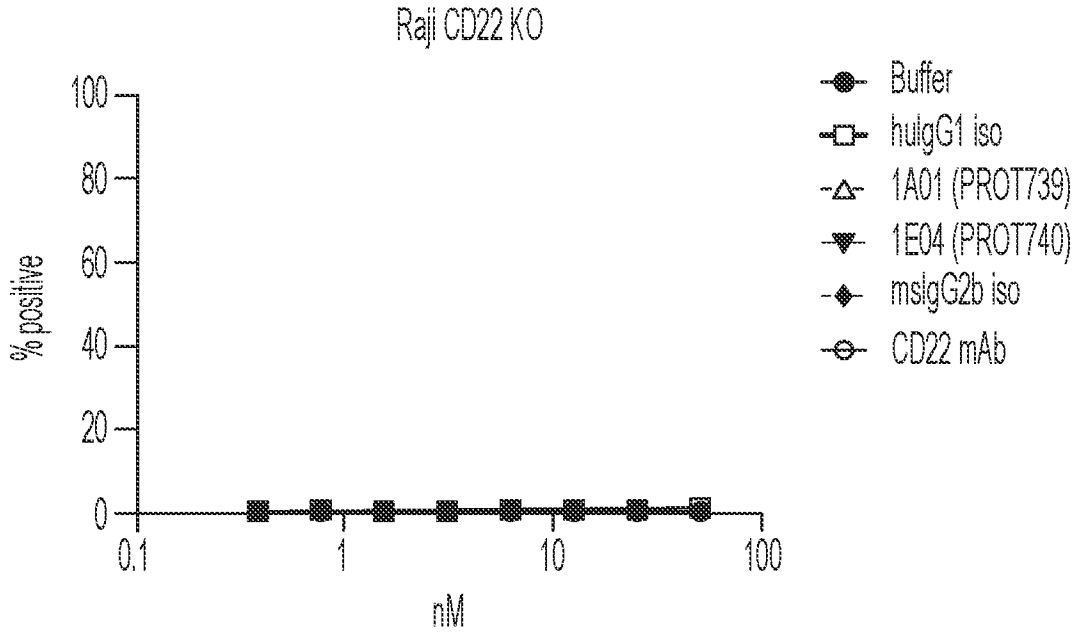

Raji CD22 KO
% positive
100
80
60
40
20
0
0.1
1
10
100
nM
Buffer
hulgG1 iso
1A01 (PROT739)
1E04 (PROT740)
mslgG2b iso
CD22 mAb

FIG. 1B

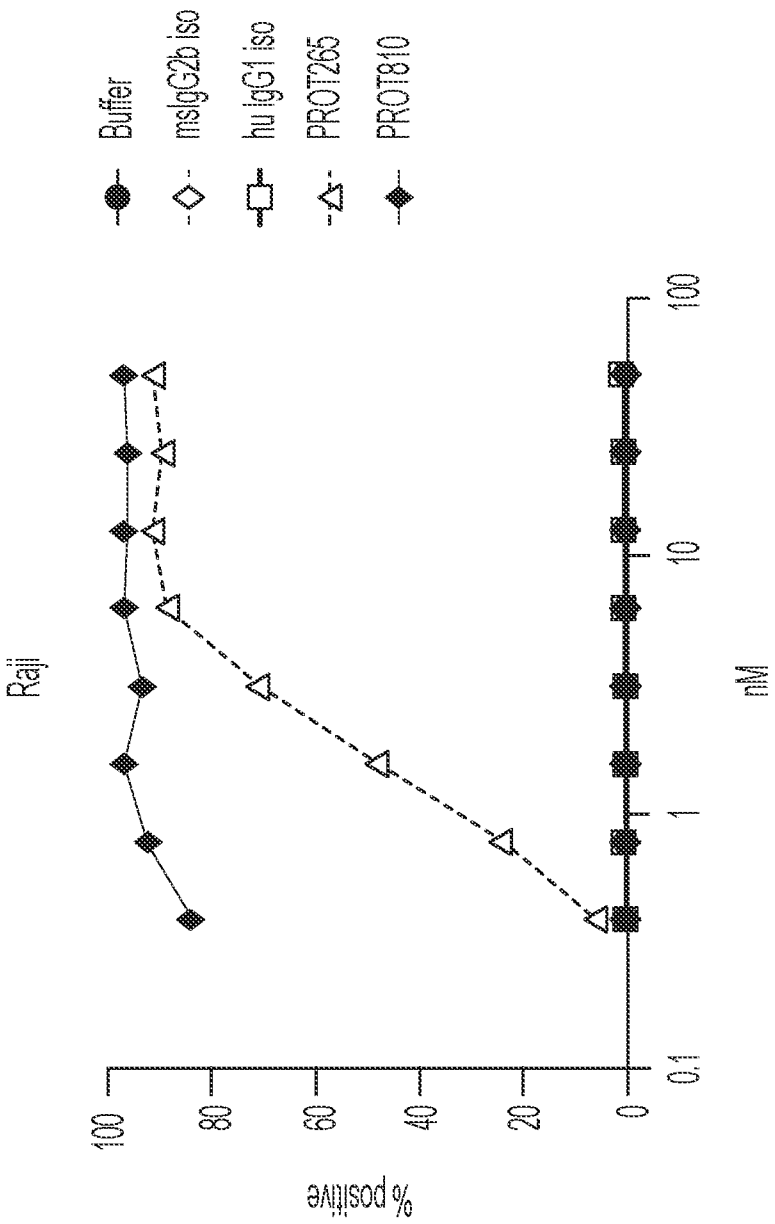

Buffer
msIgG2b iso
hu IgG1 iso
PROT265
PROT810
Raji
% positive
100
80
60
40
20
0
0.1
1
10
100
nM

FIG. 1C

	PROT265	PROT739	PROT740
CD22	++++	++++	++++
CD22Δ2-3	++++	+	++++
CD22ΔV-1	++++	-	-

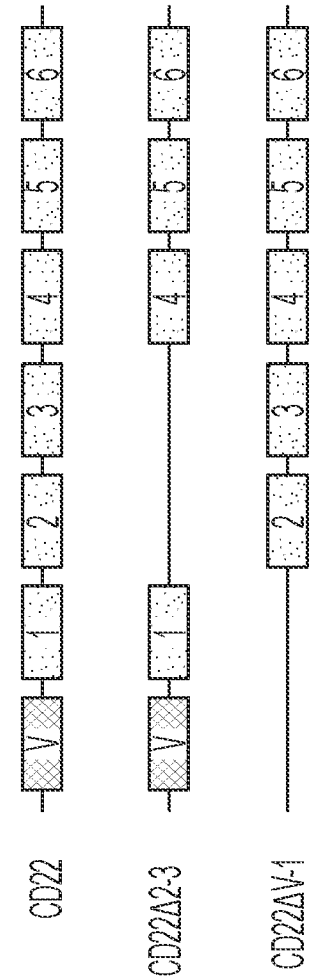


FIG. 2

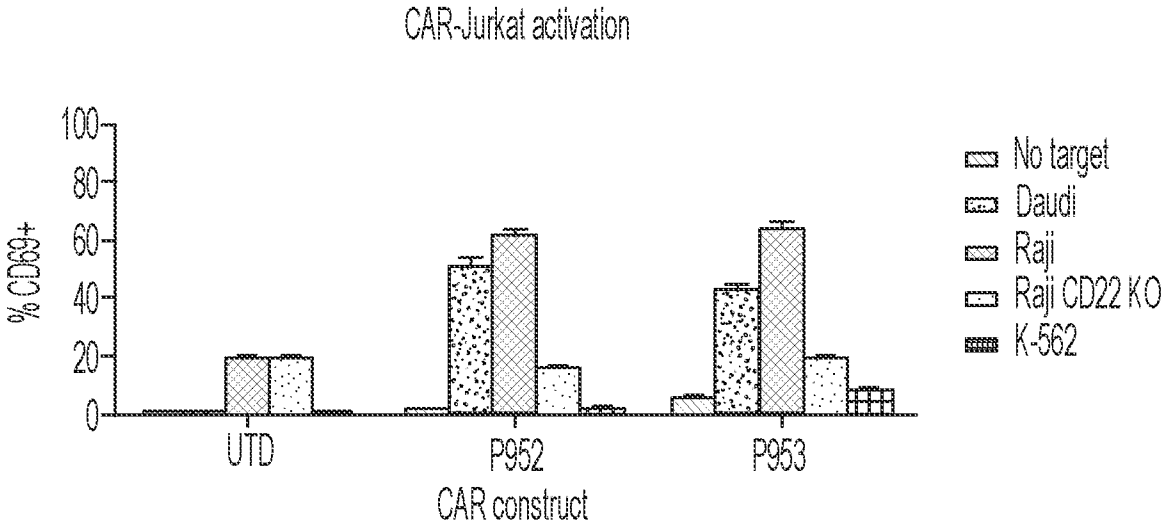

CAR-Jurkat activation
% CD69+
100
80
60
40
20
0
UTD
P952
P953
CAR construct
No target
Daudi
Raji
Raji CD22 KO
K-562

FIG. 3A

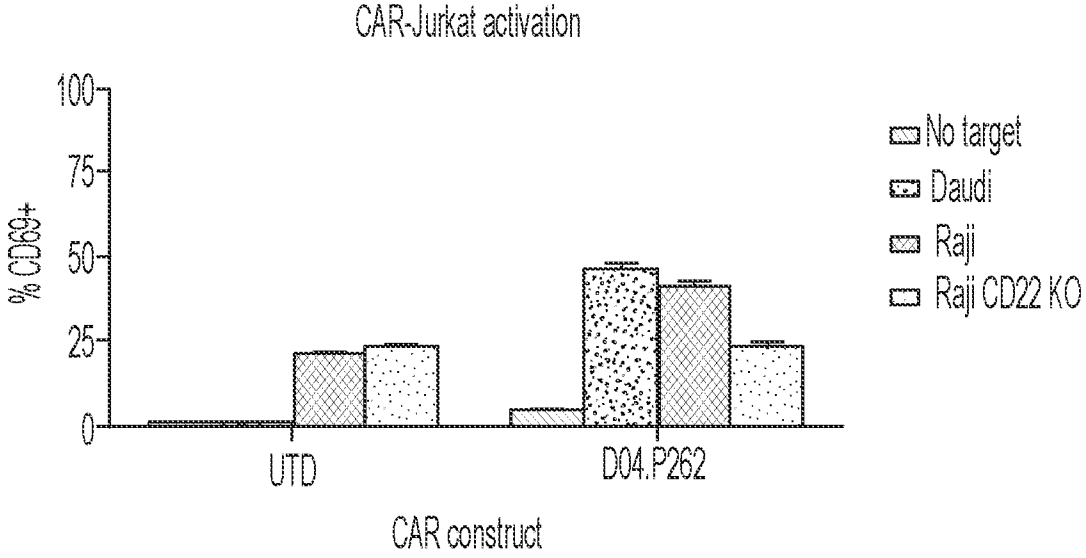

CAR-Jurkat activation
% CD69+
100
75
50
25
0
UTD
D04.P262
CAR construct
No target
Daudi
Raji
Raji CD22 KO

FIG. 3B

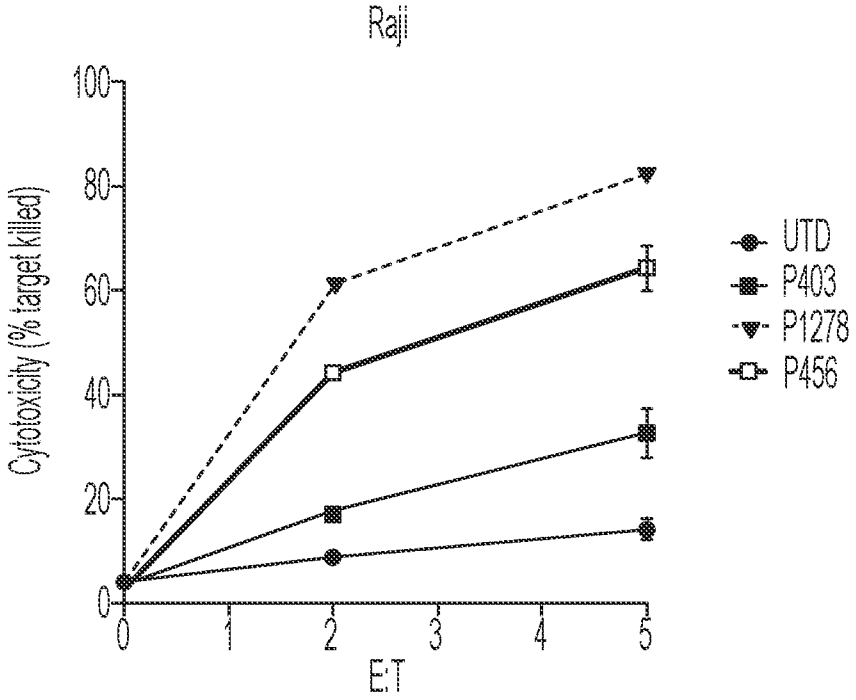

Raji
Cytotoxicity (% target killed)
100
80
60
40
20
0
0
1
2
3
4
5
E:T
UTD
P403
P1278
P456

FIG. 4A

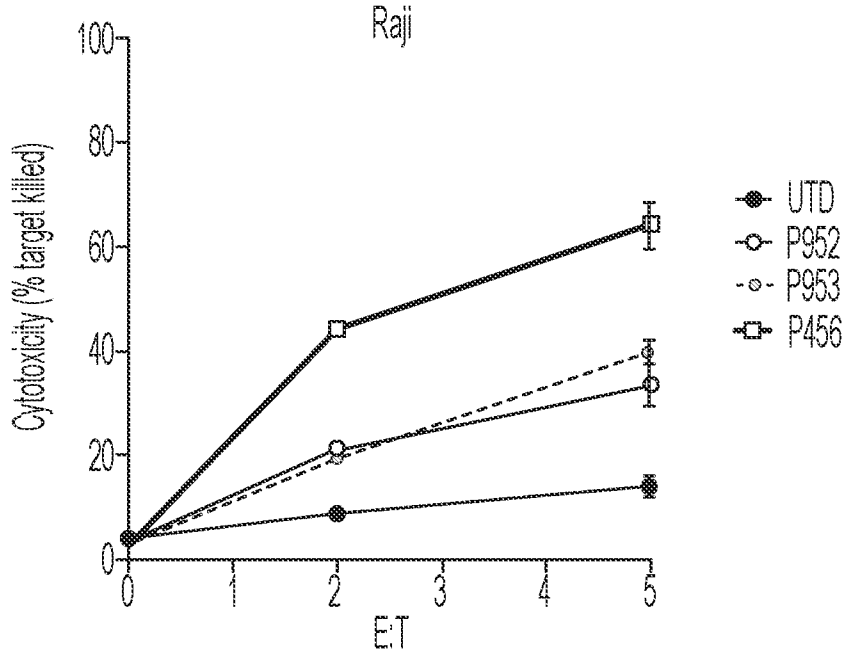

Raji
Cytotoxicity (% target killed)
100
80
60
40
20
0
0
1
2
3
4
5
E:T
UTD
P952
P953
P456

FIG. 4B

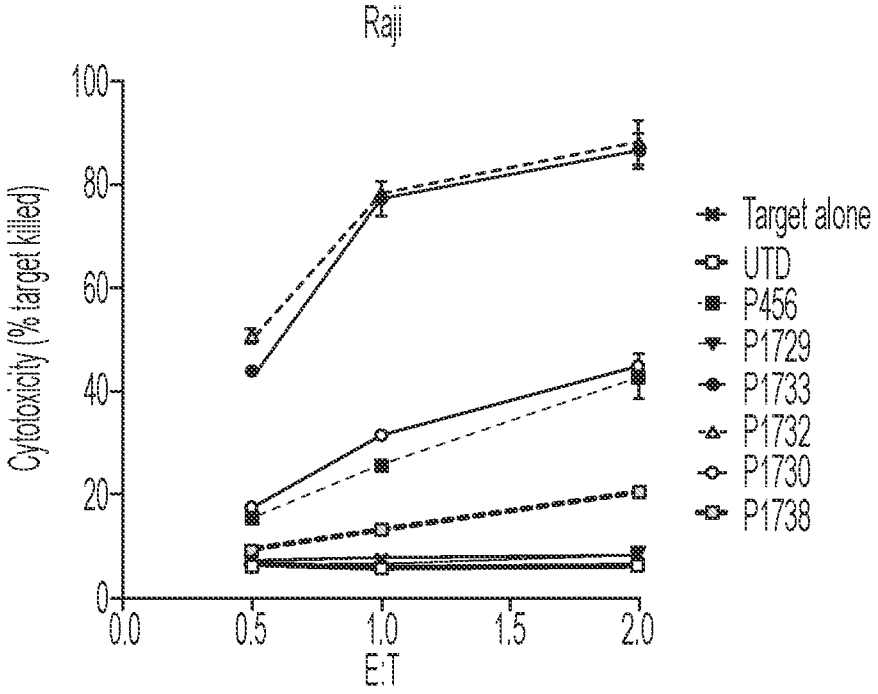

Raji
Cytotoxicity (% target killed)
100
80
60
40
20
0
0.0
0.5
1.0
1.5
2.0
E:T
Target alone
UTD
P456
P1729
P1733
P1732
P1730
P1738

FIG. 5A

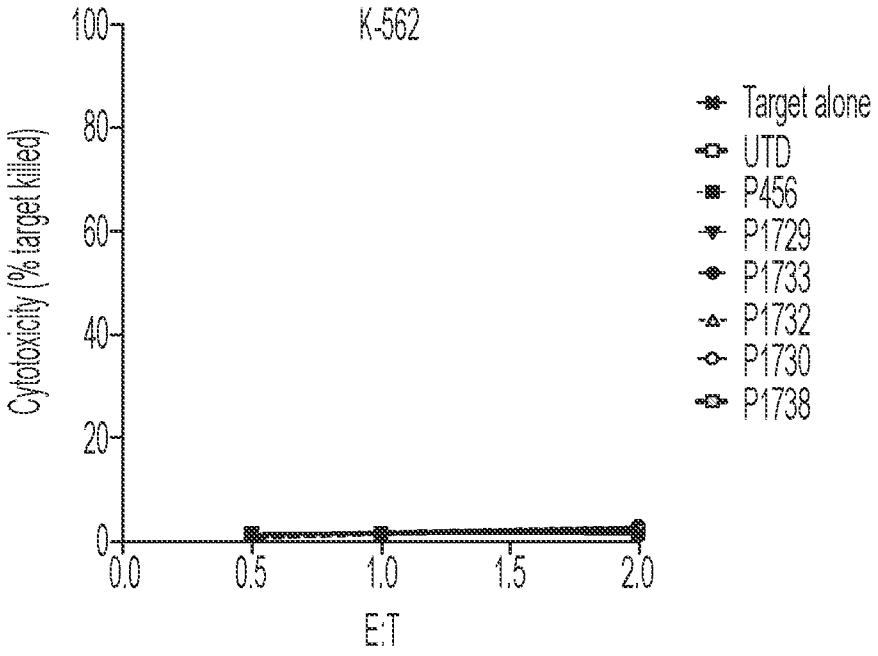

K-562
Cytotoxicity (% target killed)
100
80
60
40
20
0
0.0
0.5
1.0
1.5
2.0
E:T
Target alone
UTD
P456
P1729
P1733
P1732
P1730
P1738

FIG. 5B

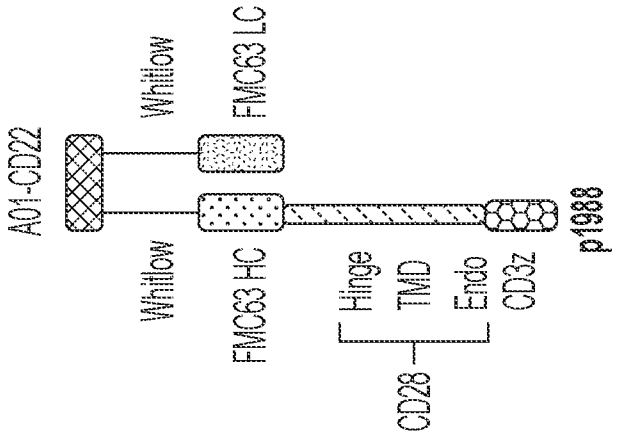

A01-CD22
Whitlow
FMC63 LC
Whitlow
FMC63 HC
Hinge
TMD
Endo
CD3z
CD28
p1988

FIG. 6B

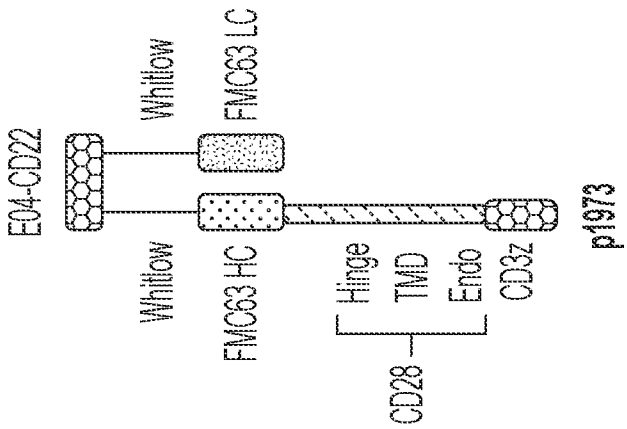

E04-CD22
Whitlow
FMC63 LC
Whitlow
FMC63 HC
Hinge
TMD
Endo
CD3z
CD28
p1973

FIG. 6A

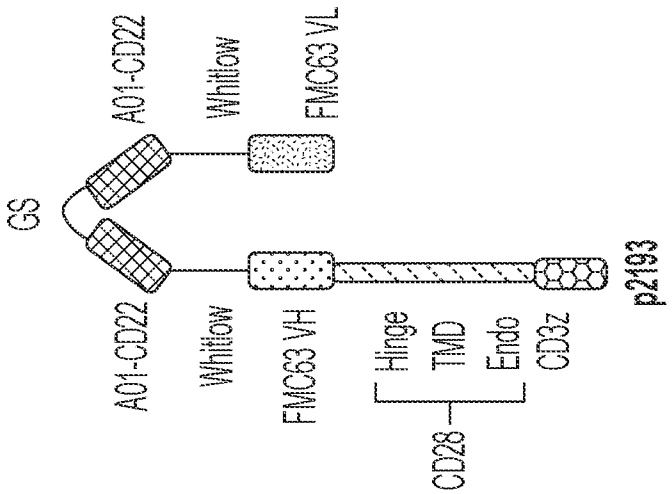

A01-CD22
Whitlow
FMC63 VL
GS
A01-CD22
Whitlow
FMC63 VH
Hinge
TMD
Endo
CD3z
CD28
p2193

FIG. 6D

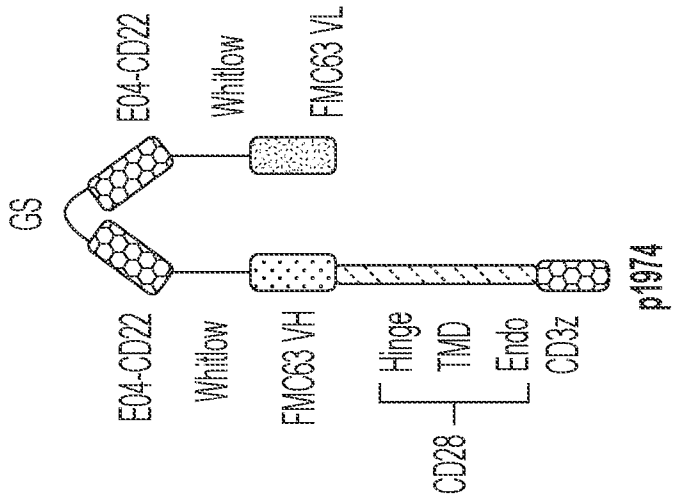

E04-CD22
Whitlow
FMC63 VL
GS
E04-CD22
Whitlow
FMC63 VH
Hinge
TMD
Endo
CD3z
CD28
p1974

FIG. 6C

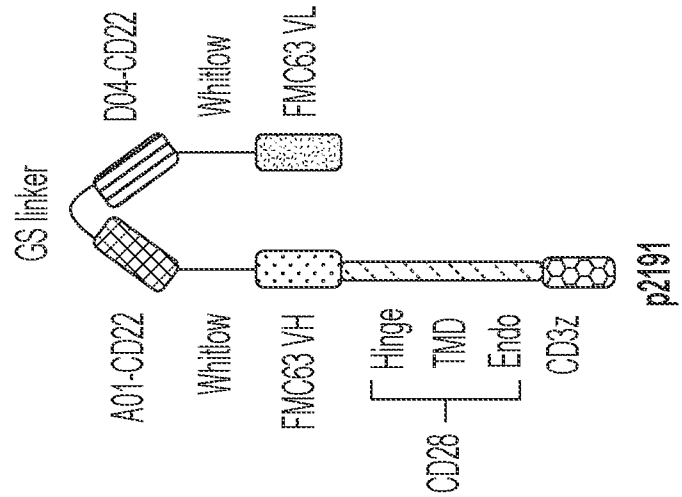

GS linker
D04-CD22
Whitlow
FMC63 VL
A01-CD22
Whitlow
FMC63 VH
Hinge
TMD
Endo
CD3z
CD28
p2191

FIG. 6F

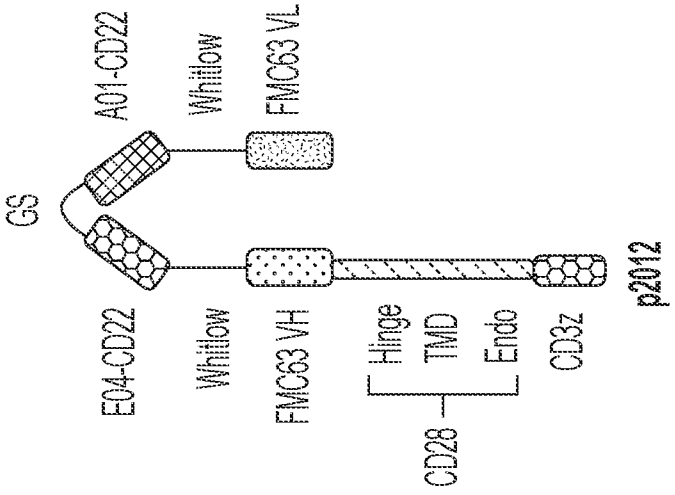

GS
A01-CD22
Whitlow
FMC63 VL
E04-CD22
Whitlow
FMC63 VH
Hinge
TMD
Endo
CD3z
CD28
p2012

FIG. 6E

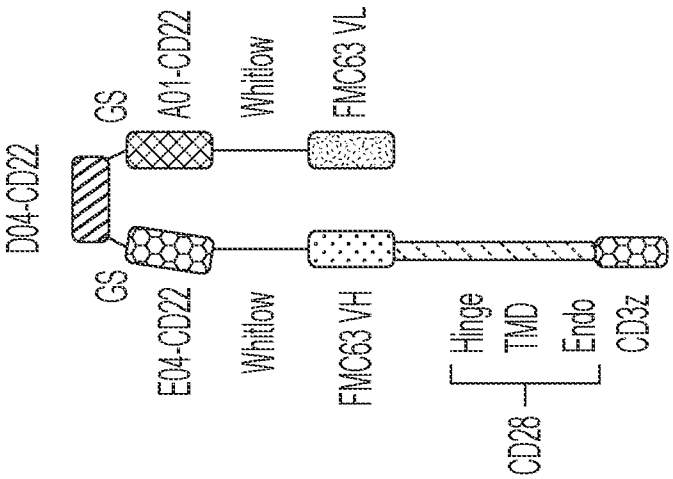

D04-CD22
GS
A01-CD22
Whitlow
FMC63 VL
GS
E04-CD22
Whitlow
FMC63 VH
Hinge
TMD
Endo
CD3z
CD28

FIG. 6H

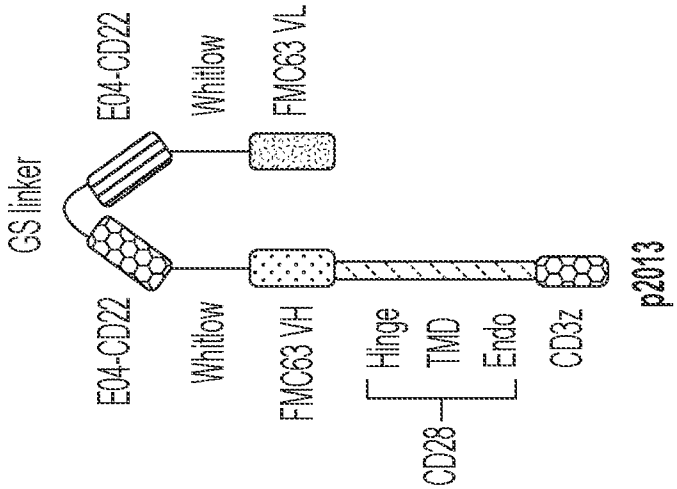

GS linker
E04-CD22
Whitlow
FMC63 VL
E04-CD22
Whitlow
FMC63 VH
Hinge
TMD
Endo
CD3z
CD28
p2013

FIG. 6G

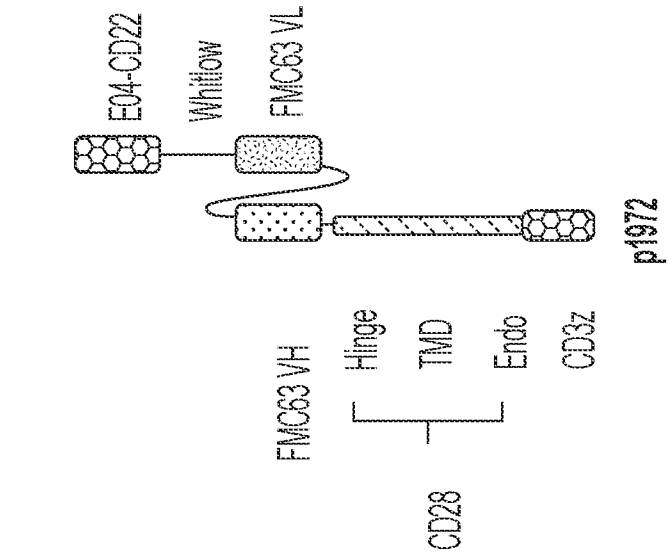

E04-CD22
Whitlow
FMC63 VL
p1972
FMC63 VH
Hinge
TMD
Endo
CD3z
CD28

FIG. 6J

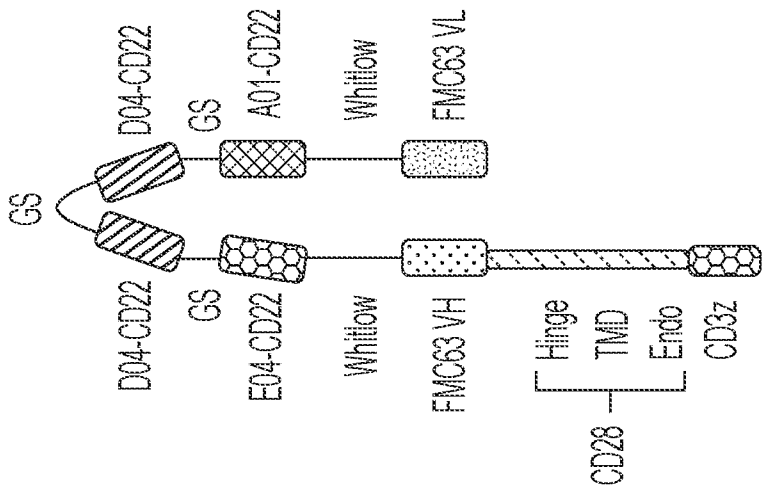

D04-CD22
GS
A01-CD22
Whitlow
FMC63 VL
GS
D04-CD22
GS
E04-CD22
Whitlow
FMC63 VH
Hinge
TMD
Endo
CD3z
CD28

FIG. 6I

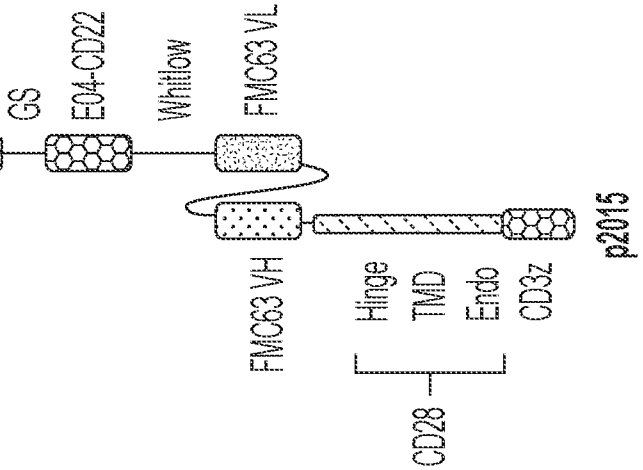

D04-CD22
GS
E04-CD22
Whitlow
FMC63 VL
FMC63 VH
Hinge
TMD
Endo
CD3z
CD28
p2015

FIG. 6L

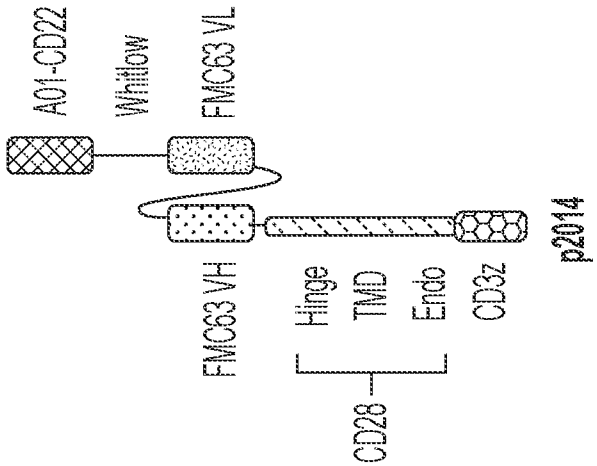

A01-CD22
Whitlow
FMC63 VL
FMC63 VH
Hinge
TMD
Endo
CD3z
CD28
p2014

FIG. 6K

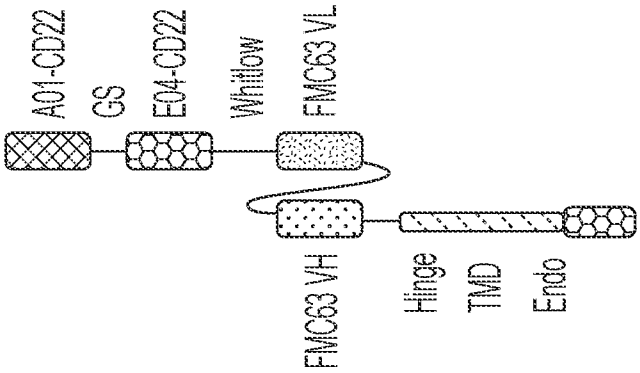

A01-CD22
GS
E04-CD22
Whitlow
FMC63 VL
FMC63 VH
Hinge
TMD
Endo

FIG. 6N

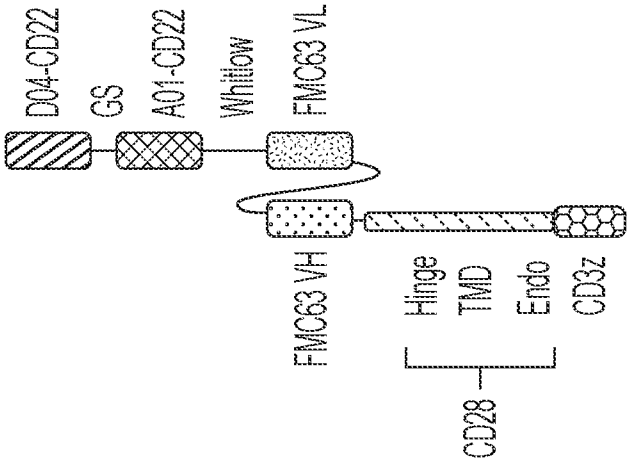

D04-CD22
GS
A01-CD22
Whitlow
FMC63 VL
FMC63 VH
Hinge
TMD
Endo
CD3z
CD28

FIG. 6M

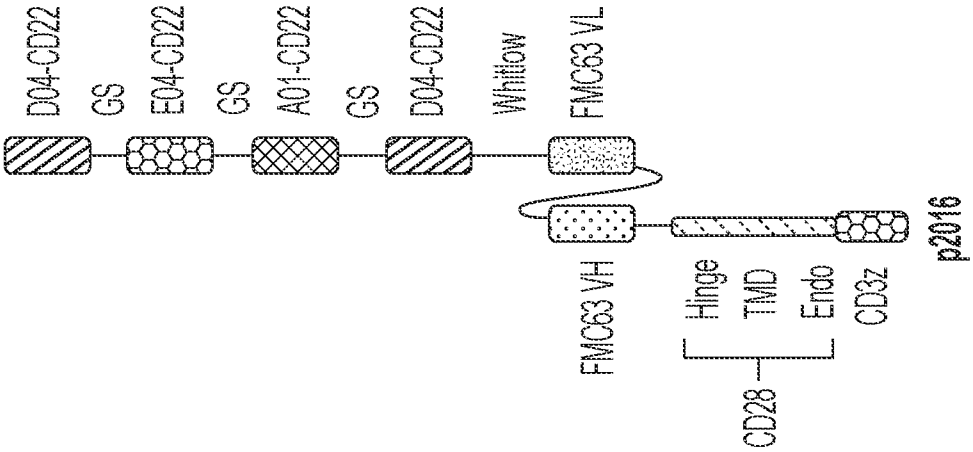

D04-CD22
GS
E04-CD22
GS
A01-CD22
GS
D04-CD22
Whitlow
FMC63 VL
FMC63 VH
Hinge
TMD
Endo
CD3z
CD28
p2016

FIG. 6P

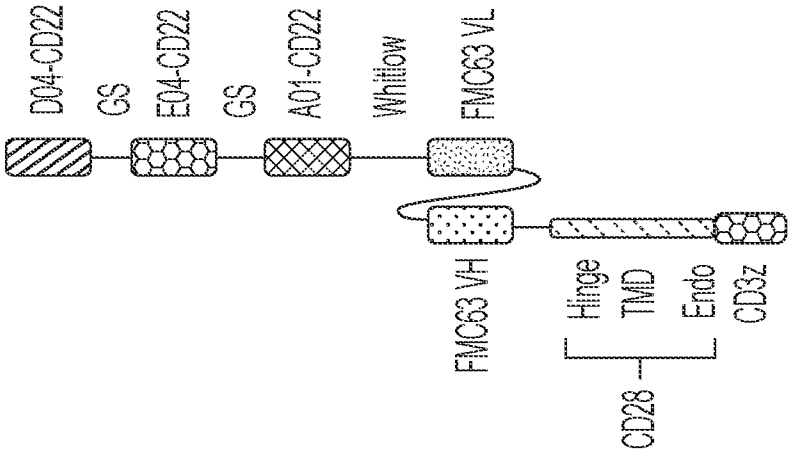

D04-CD22
GS
E04-CD22
GS
A01-CD22
Whitlow
FMC63 VL
FMC63 VH
Hinge
TMD
Endo
CD3z
CD28

FIG. 6O

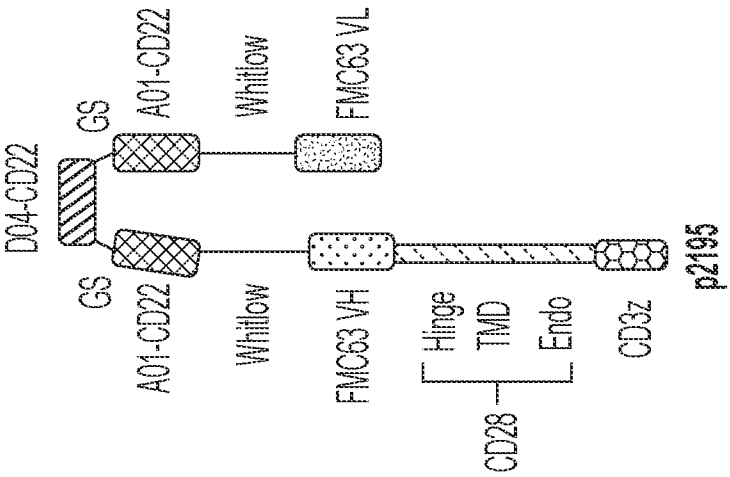

D04-CD22
GS
A01-CD22
Whitlow
FMC63 VL
GS
A01-CD22
Whitlow
FMC63 VH
Hinge
TMD
Endo
CD3z
CD28
p2195

FIG. 6R

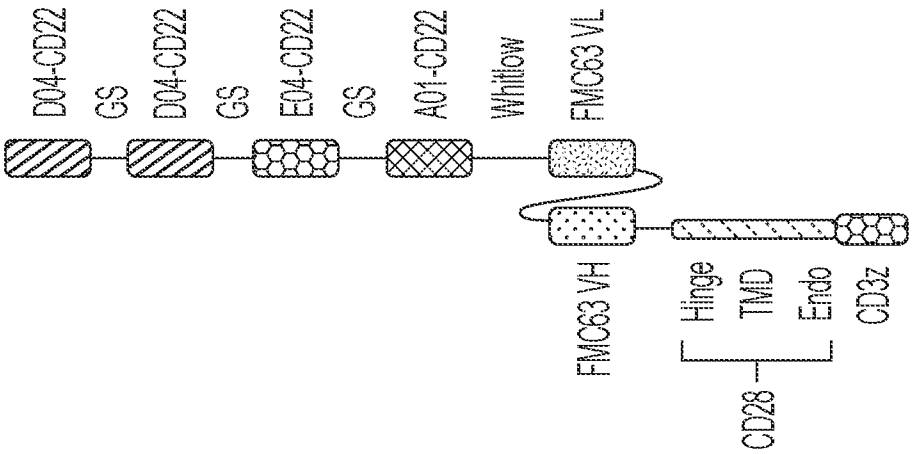

D04-CD22
GS
D04-CD22
GS
E04-CD22
GS
A01-CD22
Whitlow
FMC63 VL
FMC63 VH
Hinge
TMD
Endo
CD3z
CD28

FIG. 6Q

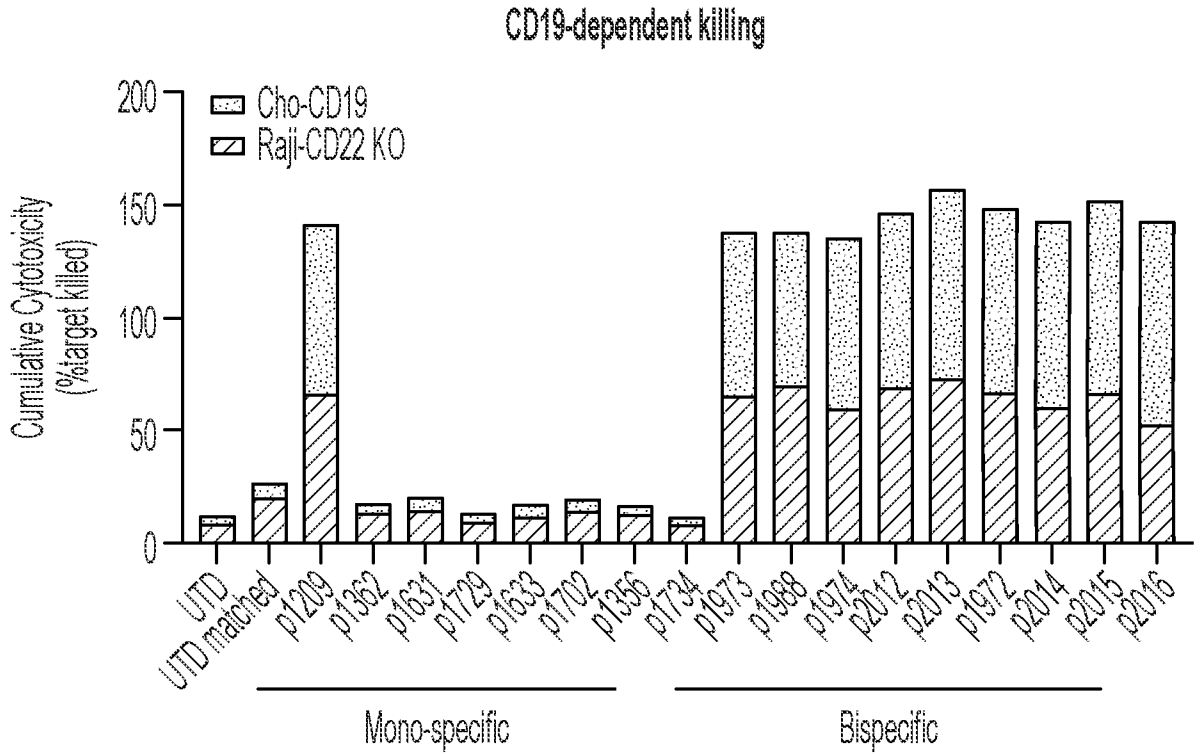

CD19-dependent killing
Cho-CD19
Raji-CD22 KO
Cumulative Cytotoxicity (%target killed)
200
150
100
50
0
UTD
UTD matched
p1209
p1362
p1631
p1729
p1633
p1702
p1356
p1734
p1973
p1988
p1974
p2012
p2013
p1972
p2014
p2015
p2016
Mono-specific
Bispecific

FIG. 7A

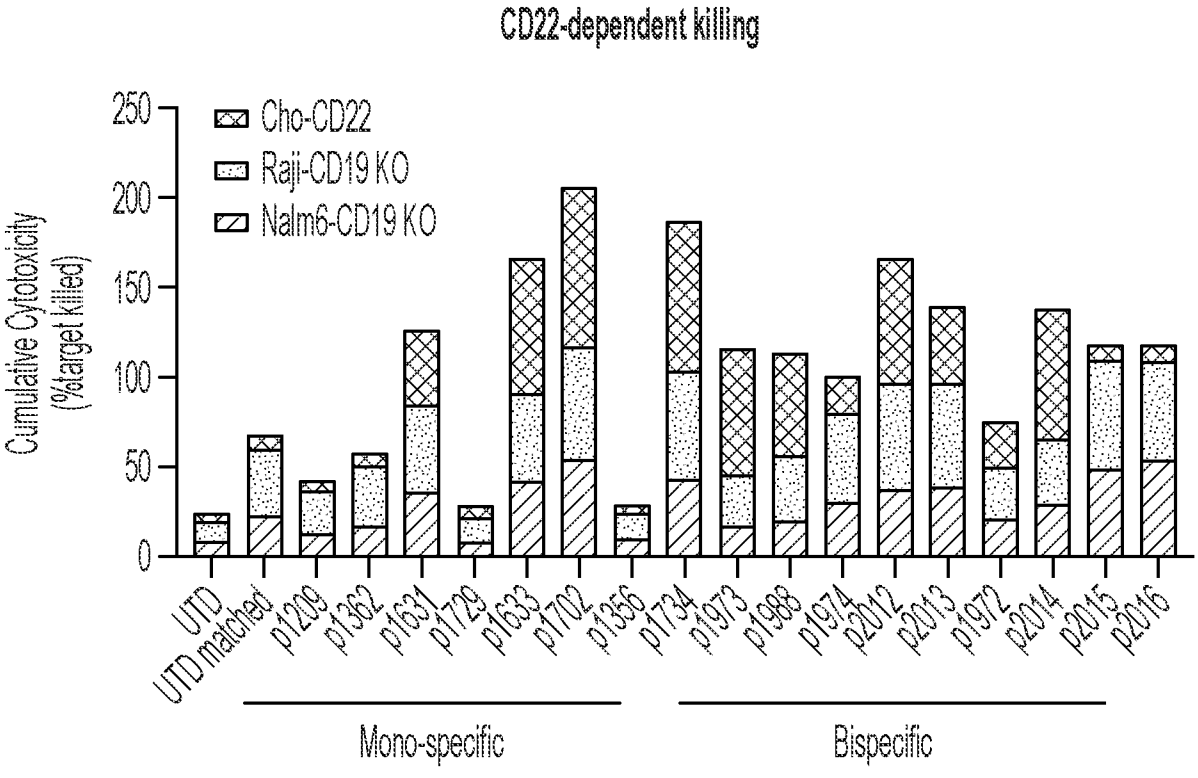

CD22-dependent killing
Cho-CD22
Raji-CD19 KO
Nalm6-CD19 KO
Cumulative Cytotoxicity (%target killed)
250
200
150
100
50
0
UTD
UTD matched
p1209
p1362
p1631
p1729
p1633
p1702
p1356
p1734
p1973
p1988
p1974
p2012
p2013
p1972
p2014
p2015
p2016
Mono-specific
Bispecific

FIG. 7B

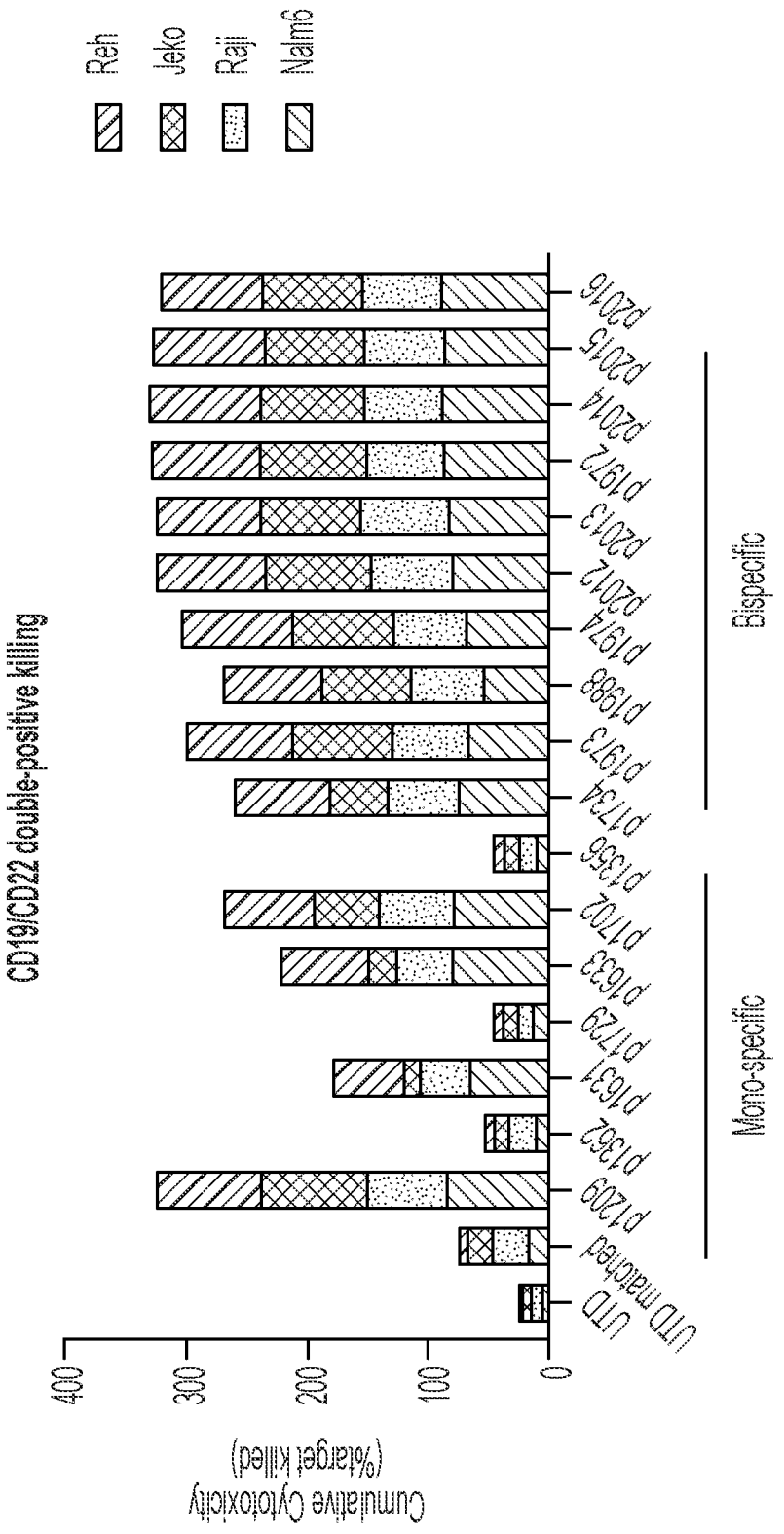

Reh
Jeko
Raji
Nalm6
CD19/CD22 double-positive killing
Cumulative Cytotoxicity
(%target killed)
400
300
200
100
0
UTD
UTD matched
p1209
p1362
p1631
p1729
p1633
p1702
p1356
p1724
p1973
p1988
p1974
p2012
p2013
p1972
p2014
p2015
p2016
Mono-specific
Bispecific

FIG. 8

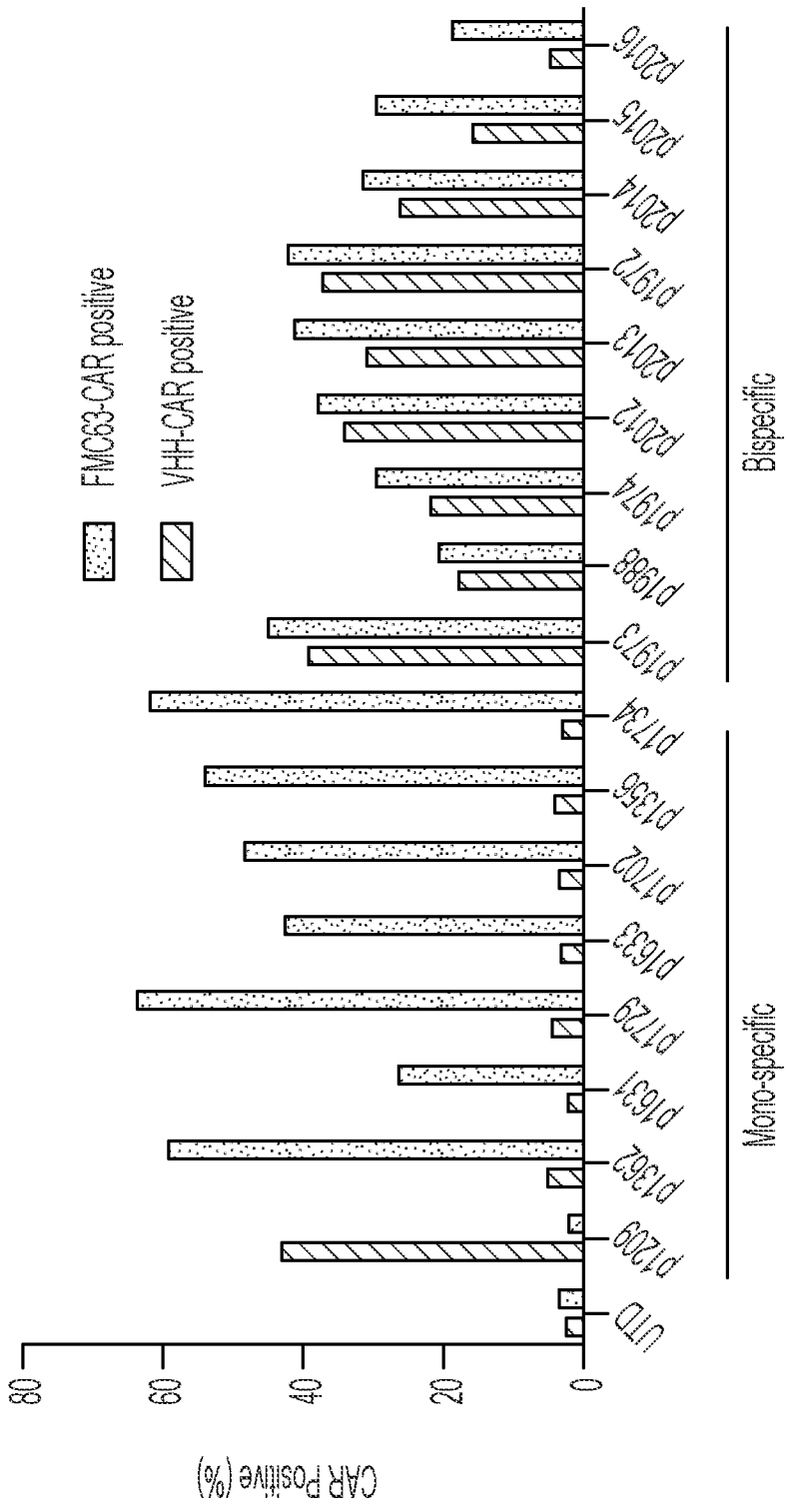

FMC63-CAR positive
VHH-CAR positive
CAR Positive (%)
0
20
40
60
80
UTD
p1209
p1362
p1631
p1729
p1633
p1702
p1356
p1734
p1973
p1988
p1974
p2012
p2013
p1972
p2014
p2015
p2016
Mono-specific
Bispecific

FIG. 9

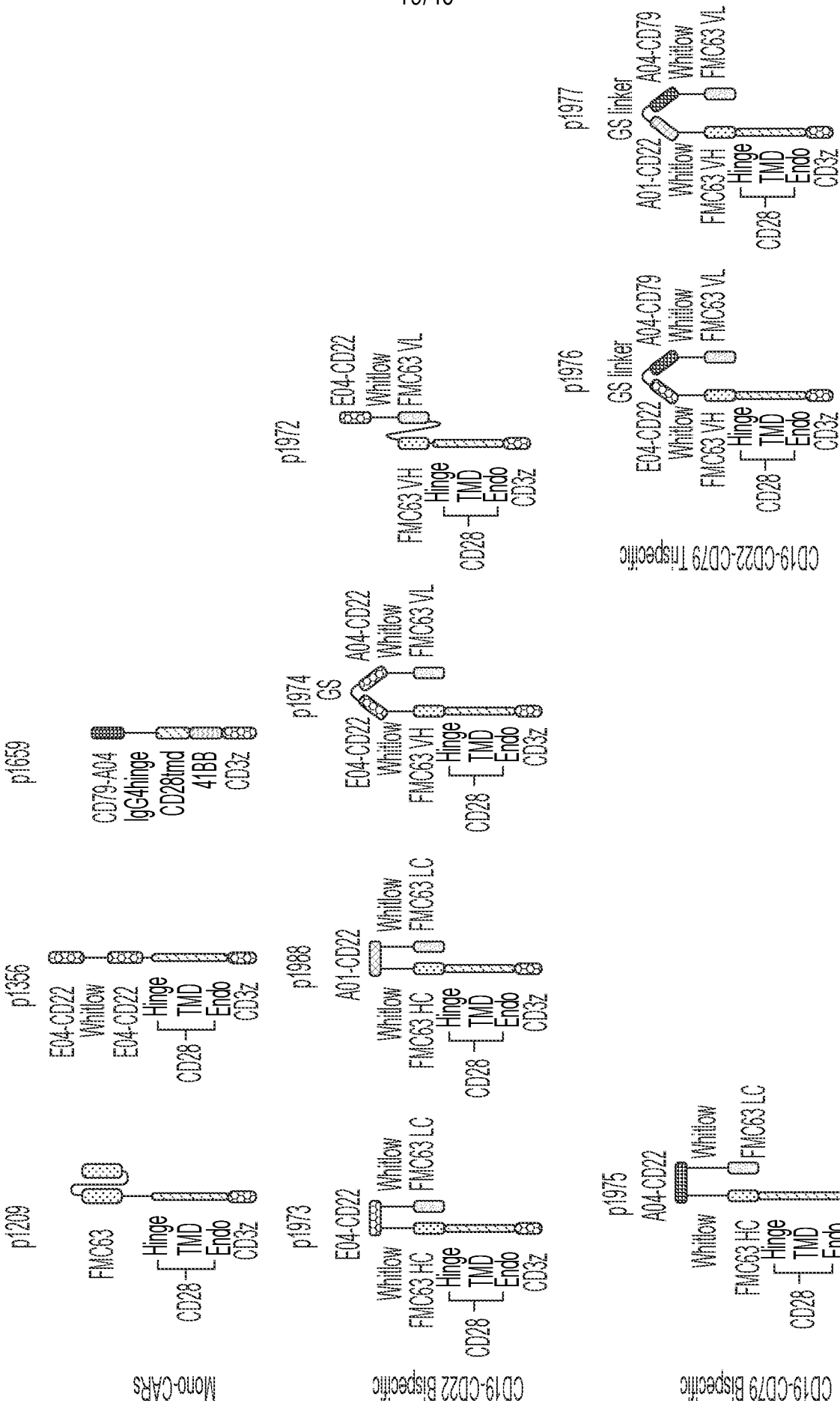

Mono-CARs
p1209
FMC63
Hinge
CD28
TMD
Endo
CD3z
p1356
E04-CD22
Whitlow
E04-CD22
Hinge
CD28
TMD
Endo
CD3z
p1659
CD79-A04
IgG4hinge
CD28tmd
41BB
CD3z
CD19-CD22 Bispecific
p1973
E04-CD22
Whitlow
FMC63 HC
Hinge
CD28
TMD
Endo
CD3z
FMC63 LC
p1988
A01-CD22
Whitlow
FMC63 HC
Hinge
CD28
TMD
Endo
CD3z
FMC63 LC
p1974
GS
E04-CD22
Whitlow
FMC63 VH
Hinge
CD28
TMD
Endo
CD3z
A04-CD22
Whitlow
FMC63 VL
p1972
E04-CD22
Whitlow
FMC63 VL
FMC63 VH
Hinge
CD28
TMD
Endo
CD3z
CD19-CD22-CD79 Trispecific
p1976
GS linker
E04-CD22
Whitlow
FMC63 VH
Hinge
CD28
TMD
Endo
CD3z
A04-CD79
Whitlow
FMC63 VL
p1977
GS linker
A01-CD22
Whitlow
FMC63 VH
Hinge
CD28
TMD
Endo
CD3z
A04-CD79
Whitlow
FMC63 VL
CD19-CD79 Bispecific
p1975
A04-CD22
Whitlow
FMC63 HC
Hinge
CD28
TMD
Endo
CD3z
Whitlow
FMC63 LC

FIG. 10A

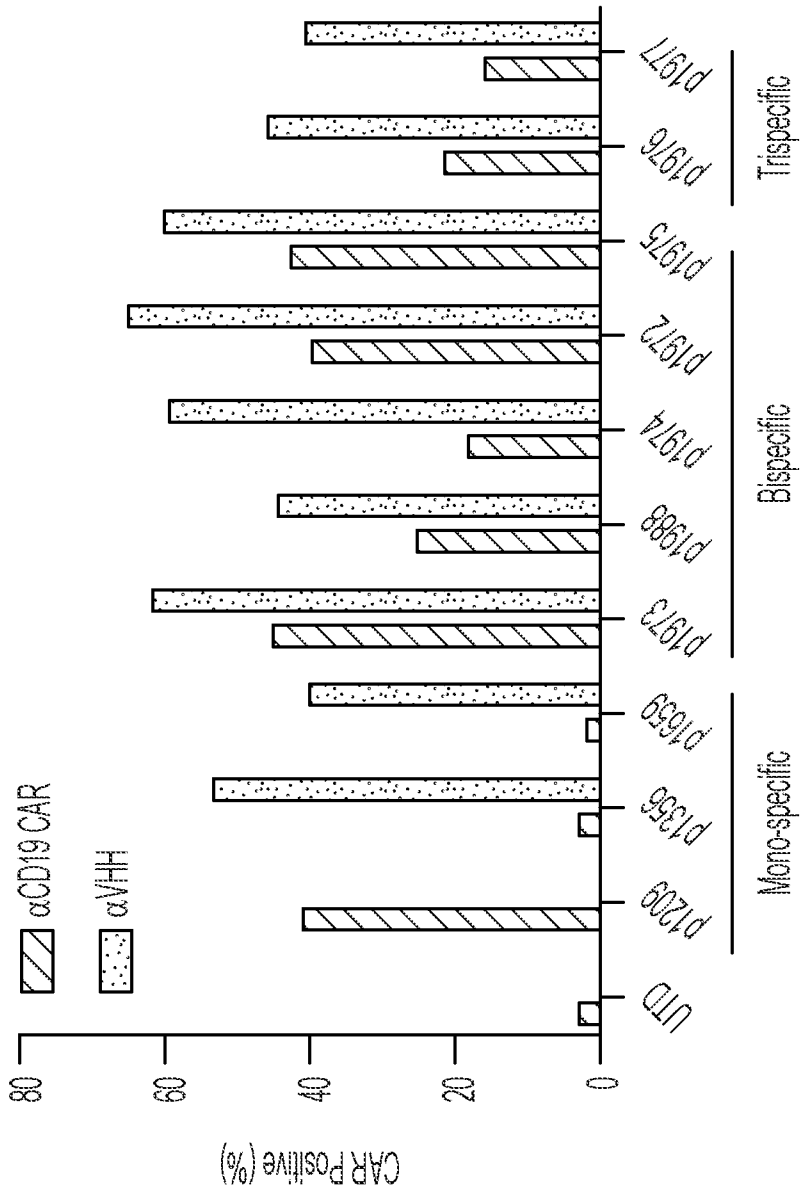

CAR Positive (%)
0
20
40
60
80
αCD19 CAR
αVHH
UTD
p1209
p1366
p1659
p1973
p1988
p1974
p1972
p1975
p1976
p1977
Mono-specific
Bispecific
Trispecific

FIG. 10B

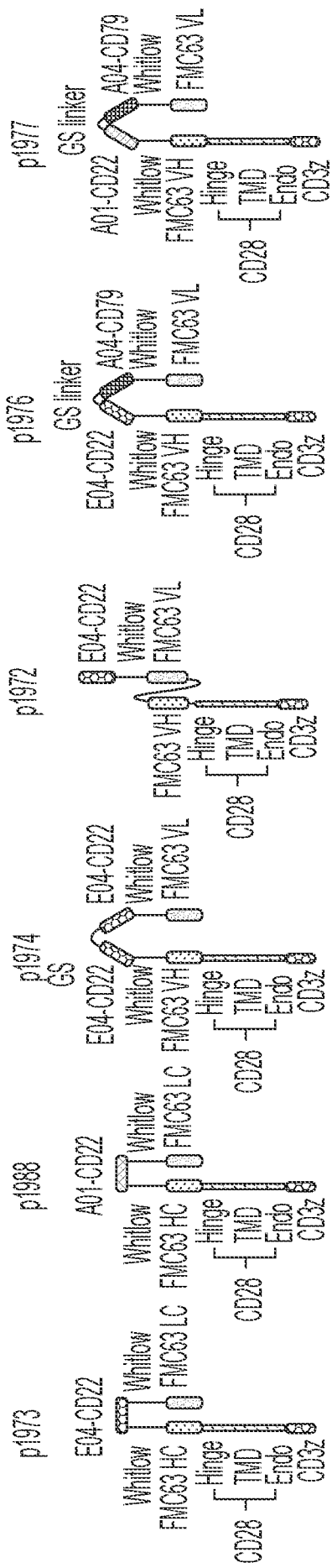


FIG. 11A

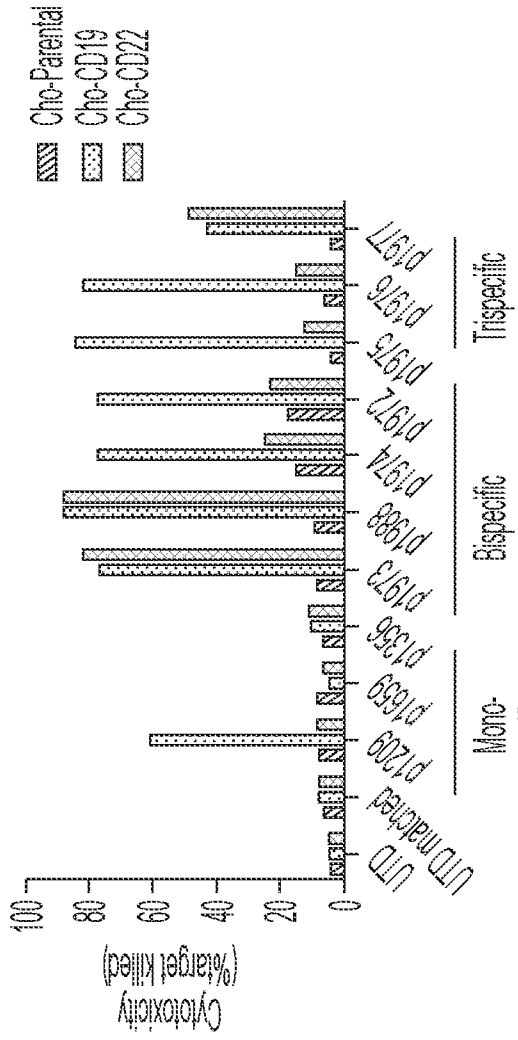


FIG. 11B

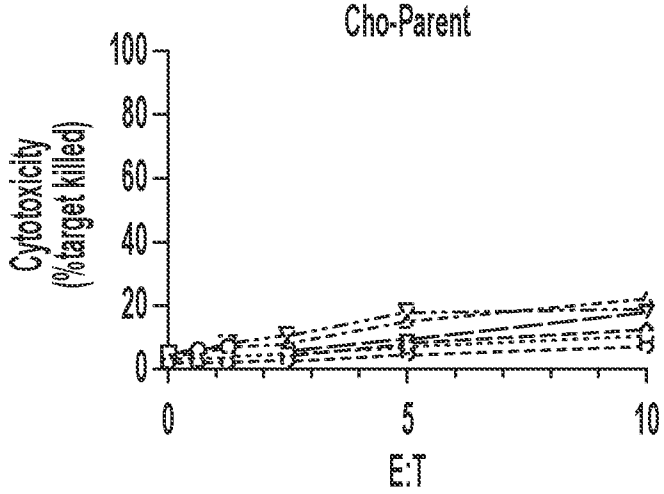


FIG. 12A

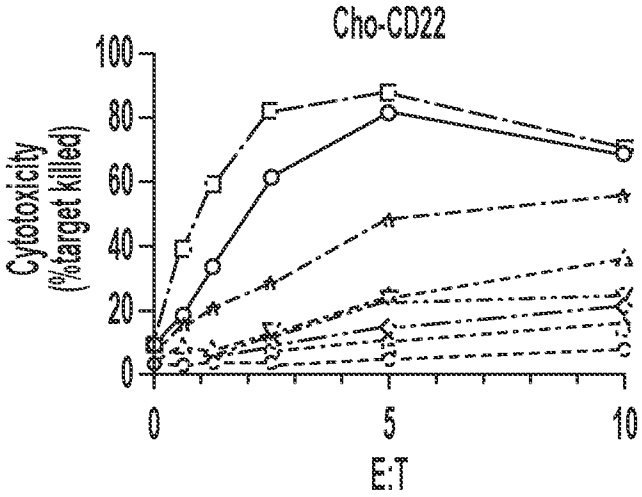


FIG. 12B

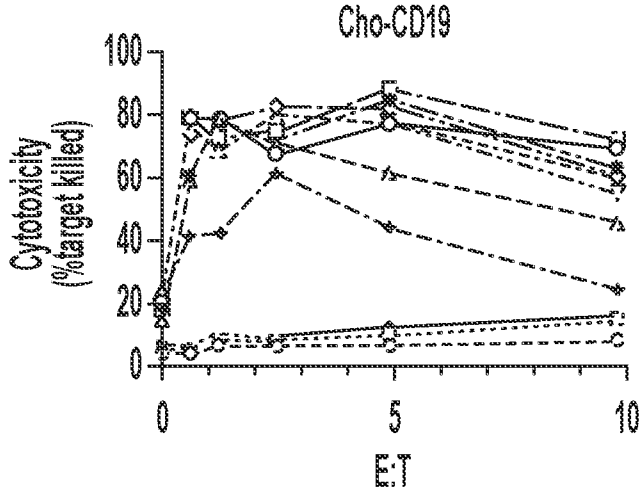


FIG. 12C

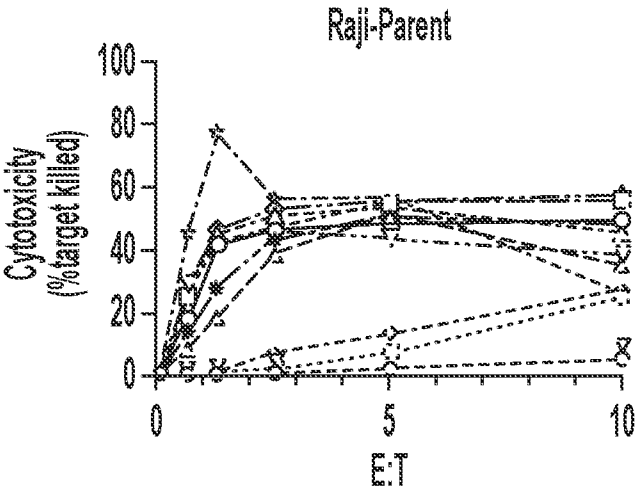


FIG. 12D

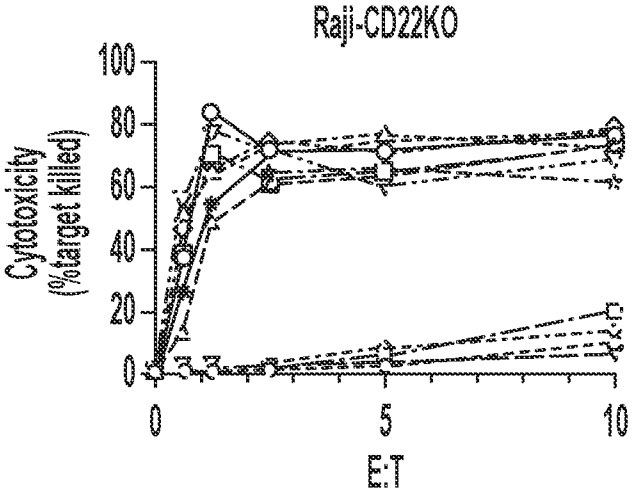


FIG. 12E

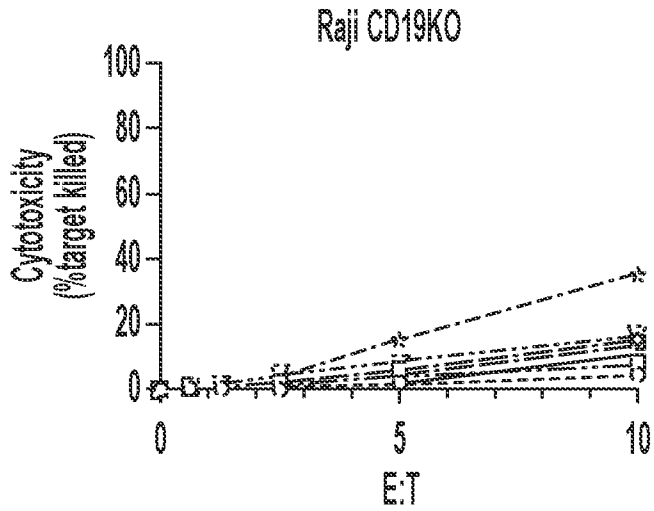


FIG. 12F

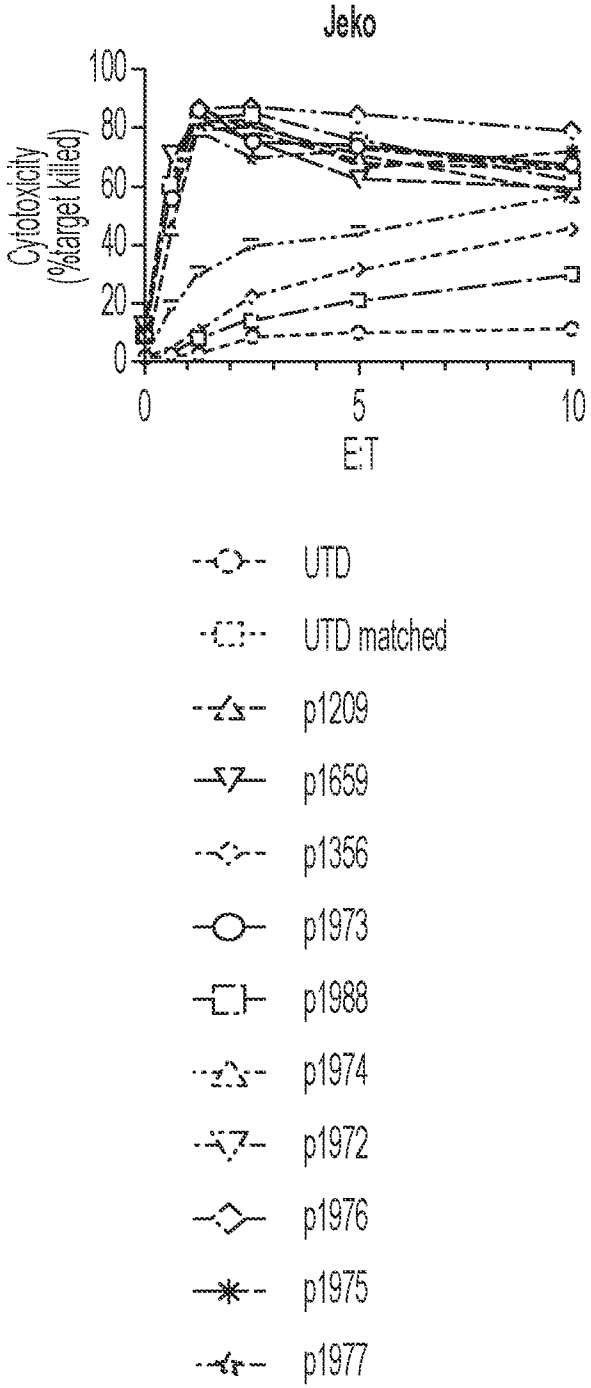

Jeko
Cytotoxicity (%target killed)
100
80
60
40
20
0
0
5
10
E:T
UTD
UTD matched
p1209
p1659
p1356
p1973
p1988
p1974
p1972
p1976
p1975
p1977

FIG. 12G

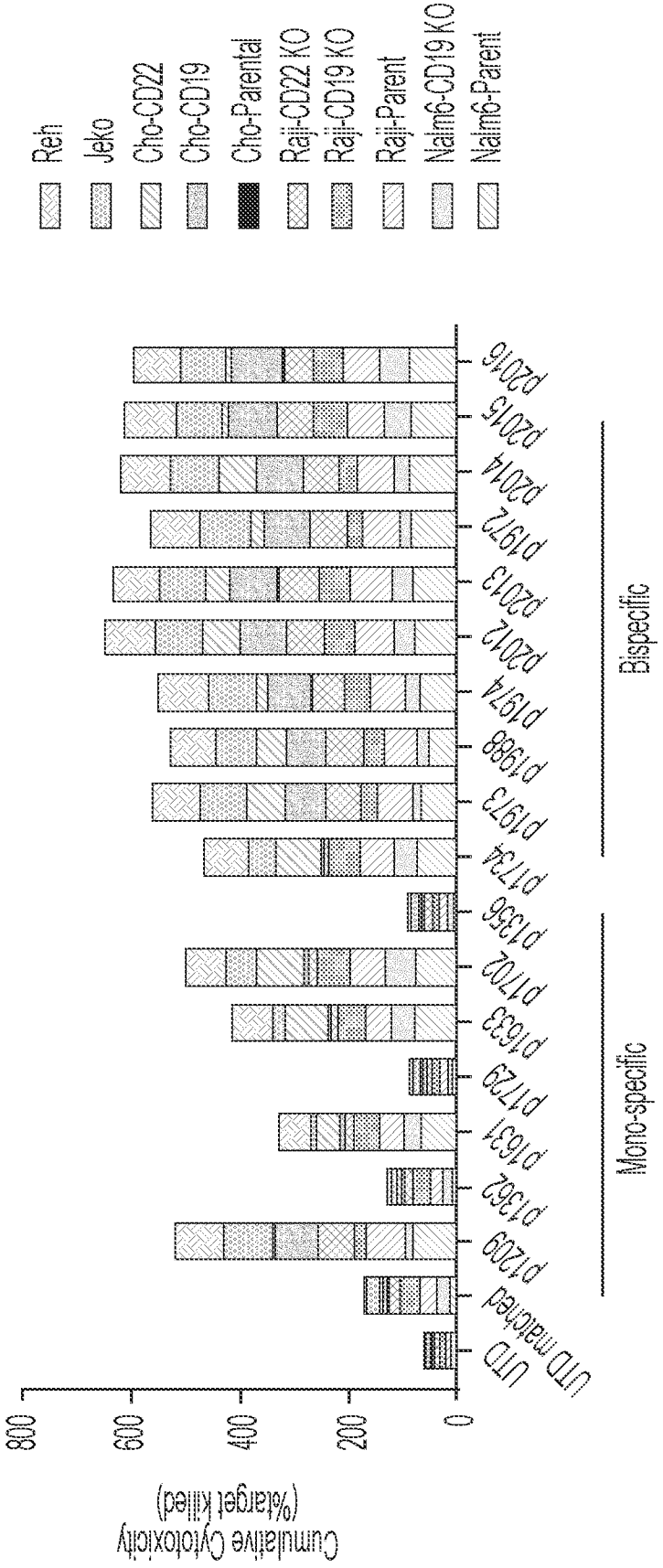

Reh
Jeko
Cho-CD22
Cho-CD19
Cho-Parental
Raji-CD22 KO
Raji-CD19 KO
Raji-Parent
Nalm6-CD19 KO
Nalm6-Parent
Cumulative Cytotoxicity
(%target killed)
800
600
400
200
0
UTD
UTD matched
P1209
P1362
P1631
P1729
P1633
P1702
P1356
P1734
P1973
P1988
P1974
P2012
P2013
P1972
P2014
P2015
P2016
Mono-specific
Bispecific

FIG. 13

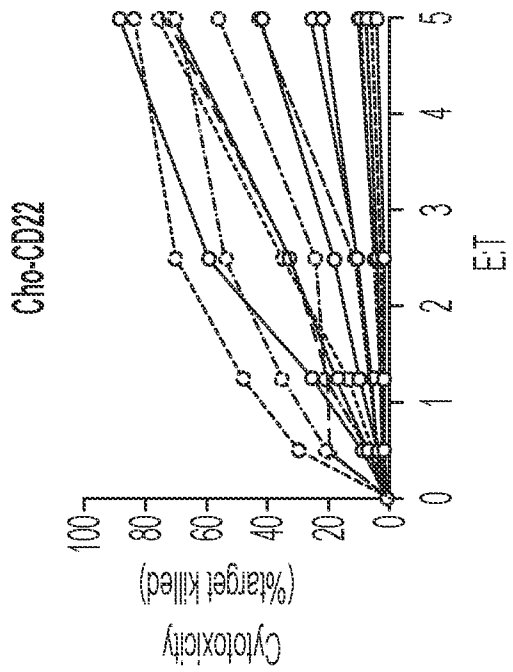


FIG. 14B

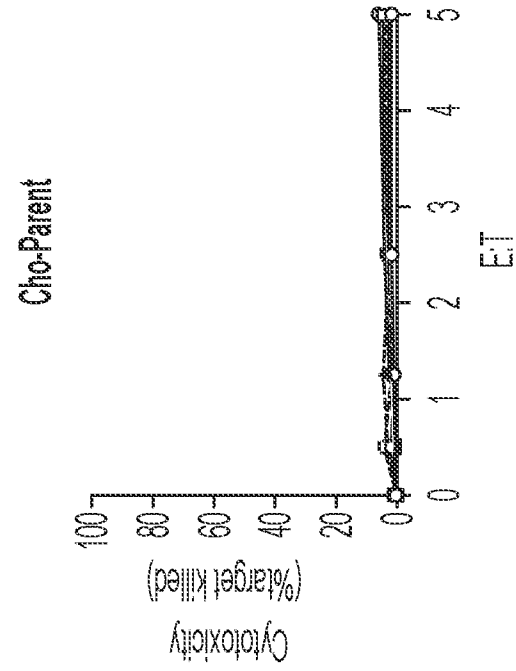


FIG. 14A

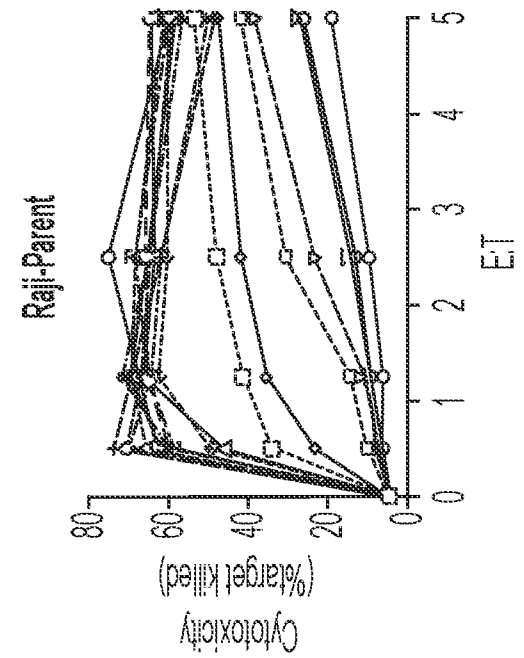


FIG. 14D

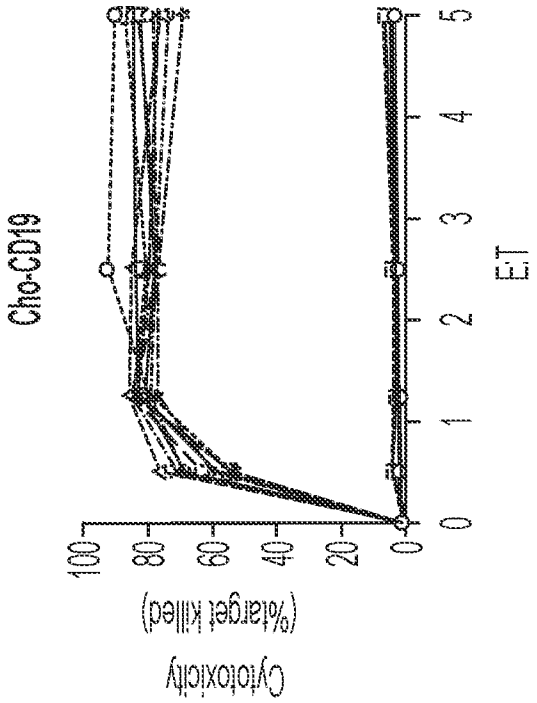


FIG. 14C

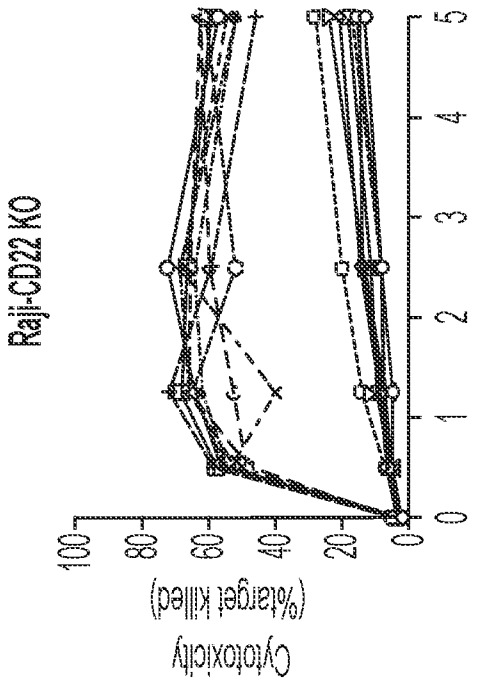


FIG. 14F

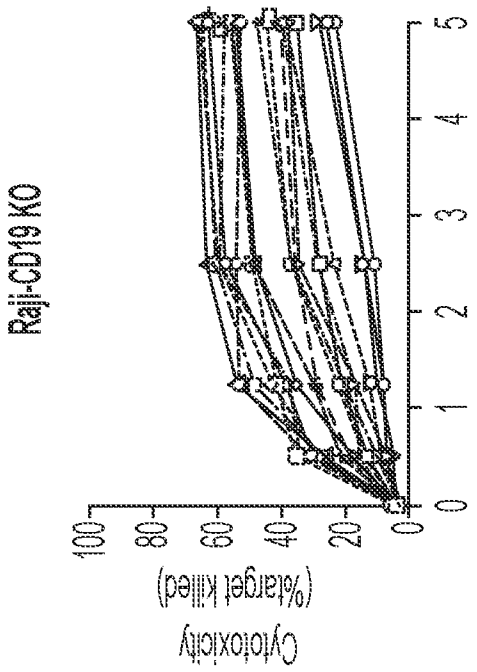


FIG. 14E

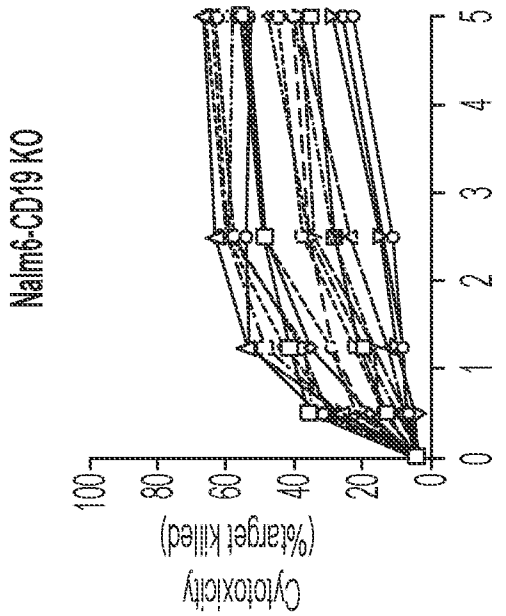


FIG. 14H

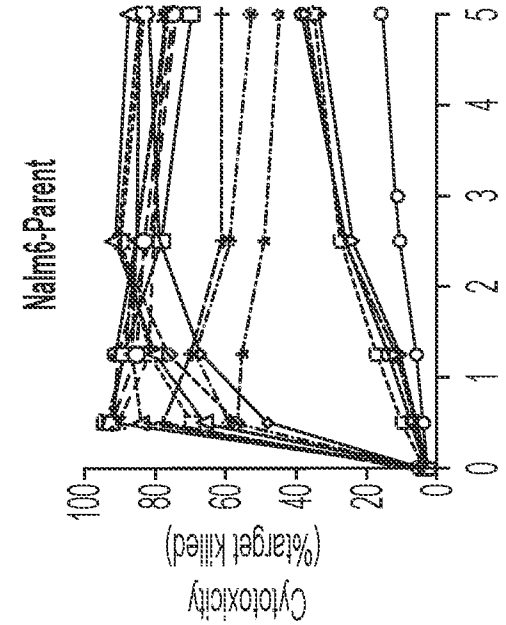


FIG. 14G

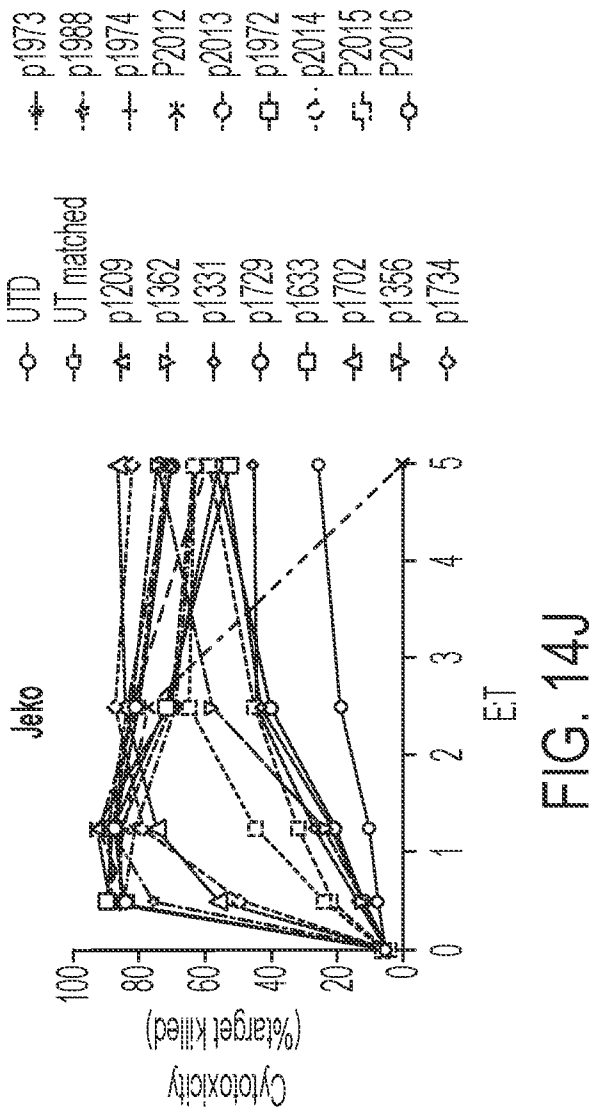


FIG. 14J

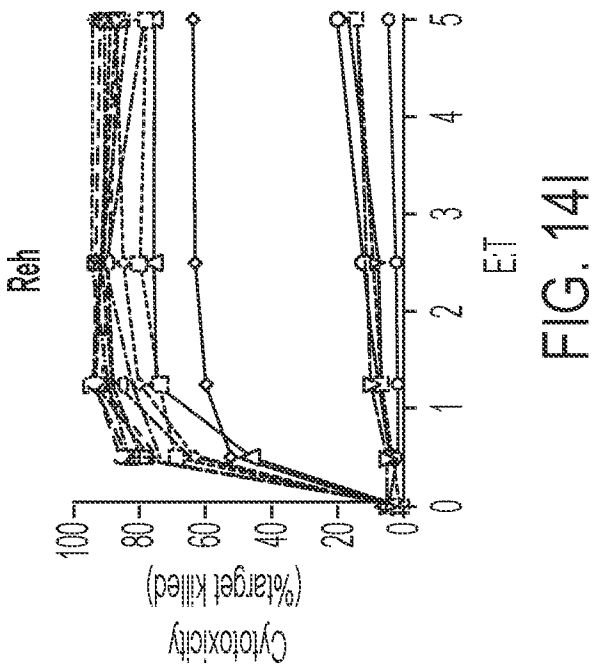


FIG. 14I

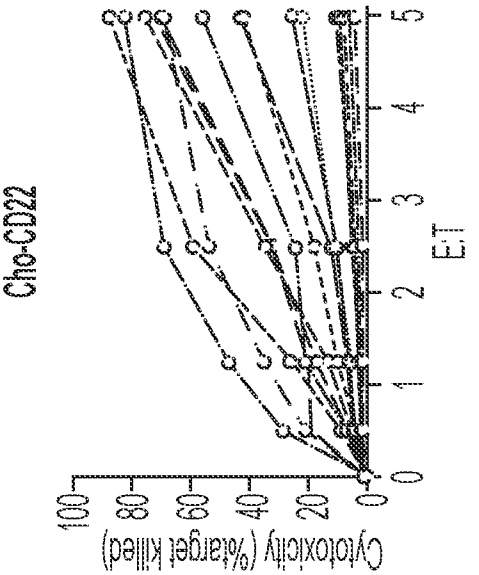
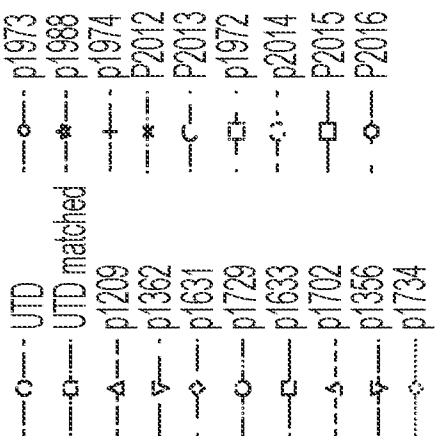


FIG. 15B



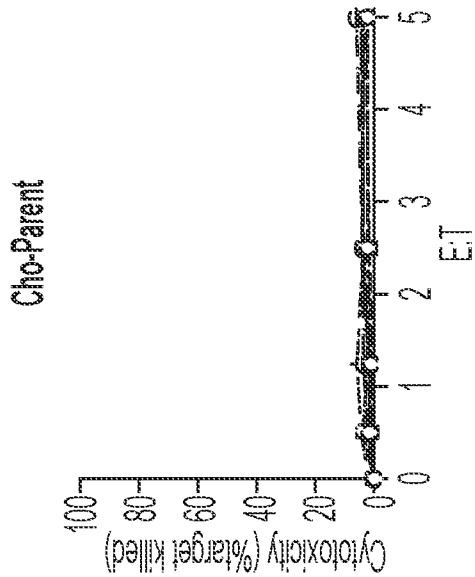


FIG. 15A

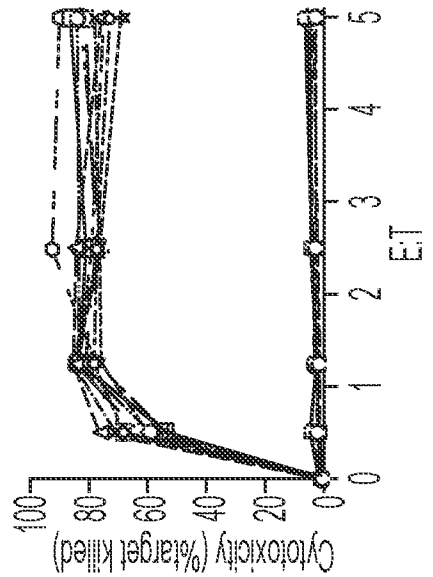


FIG. 15C

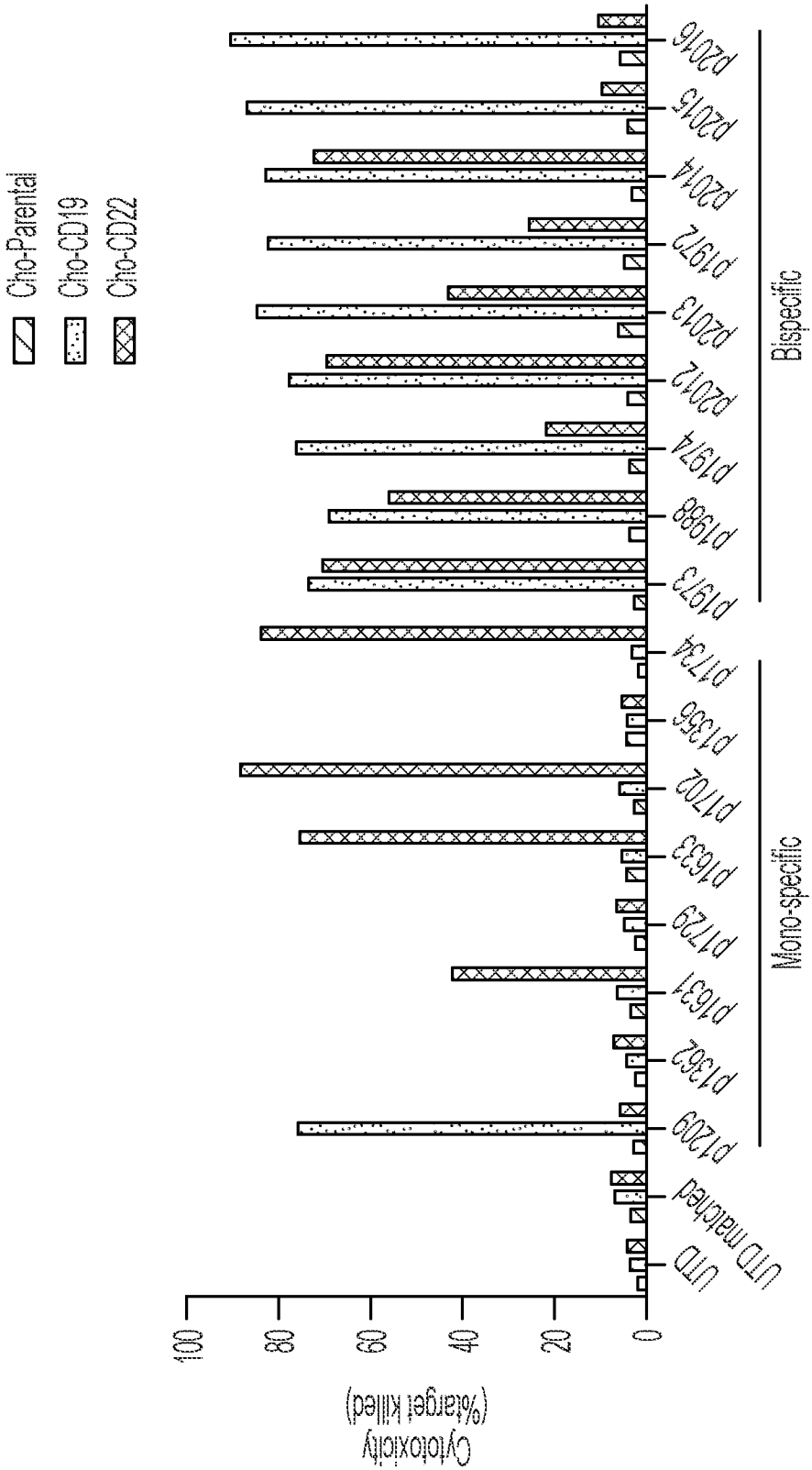

Cho-Parental
Cho-CD19
Cho-CD22
Cytotoxicity
(%target killed)
100
80
60
40
20
0
UTD
UTD matched
p1209
p1362
p1634
p1729
p1633
p1702
p1356
p1734
p1973
p1988
p1974
p2012
p2013
p1972
p2014
p2015
p2016
Mono-specific
Bispecific

FIG. 16

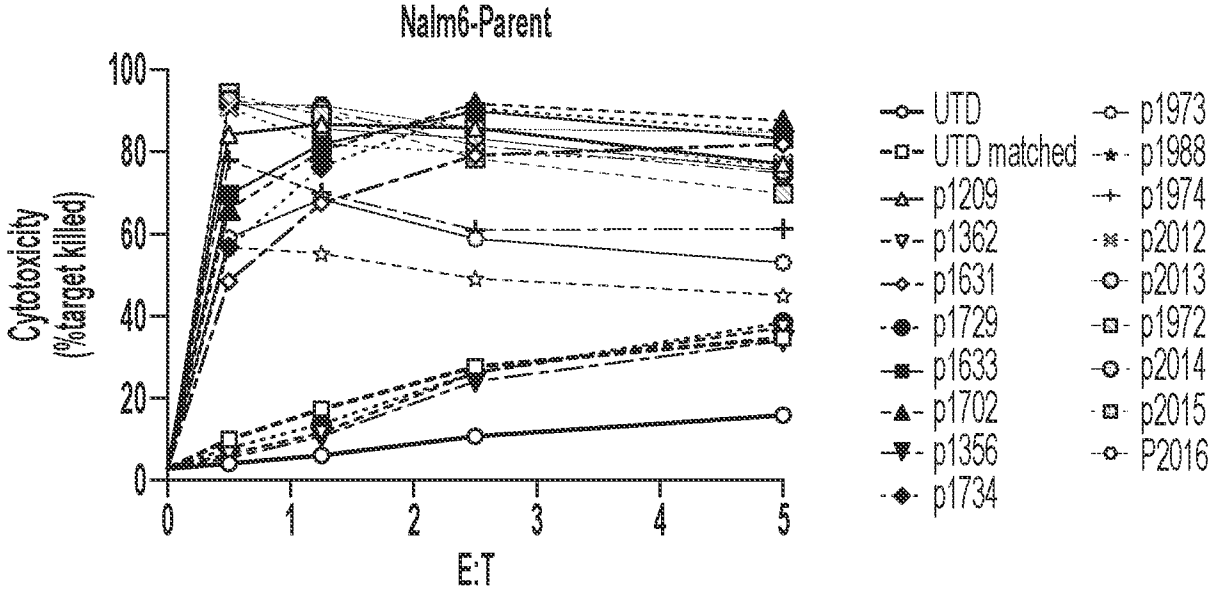


FIG. 17A

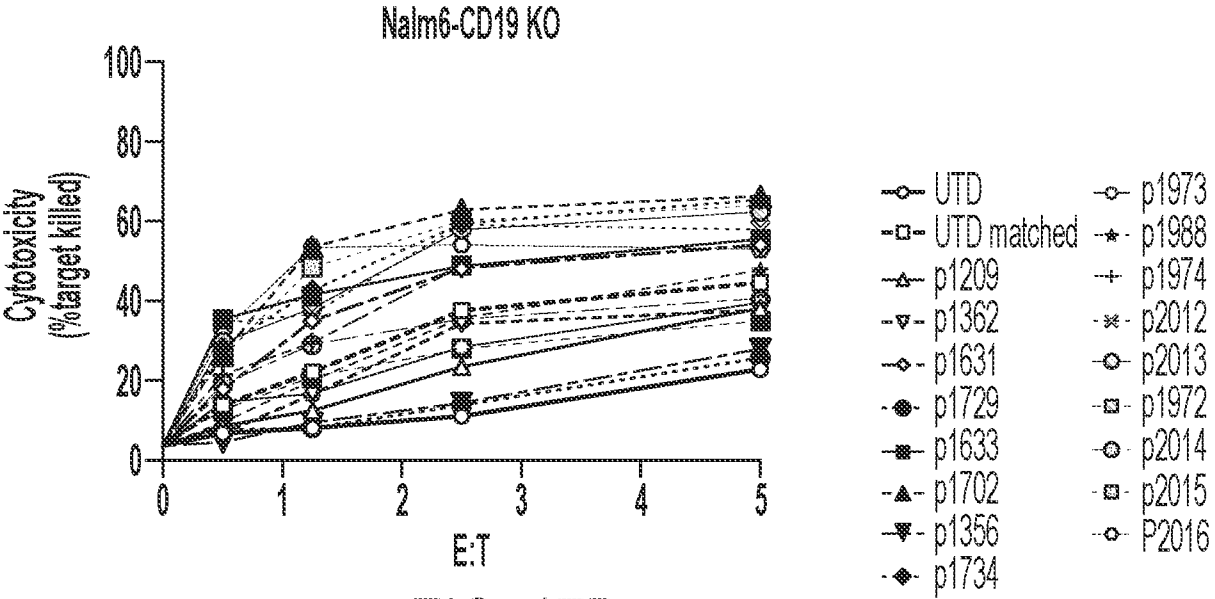


FIG. 17B

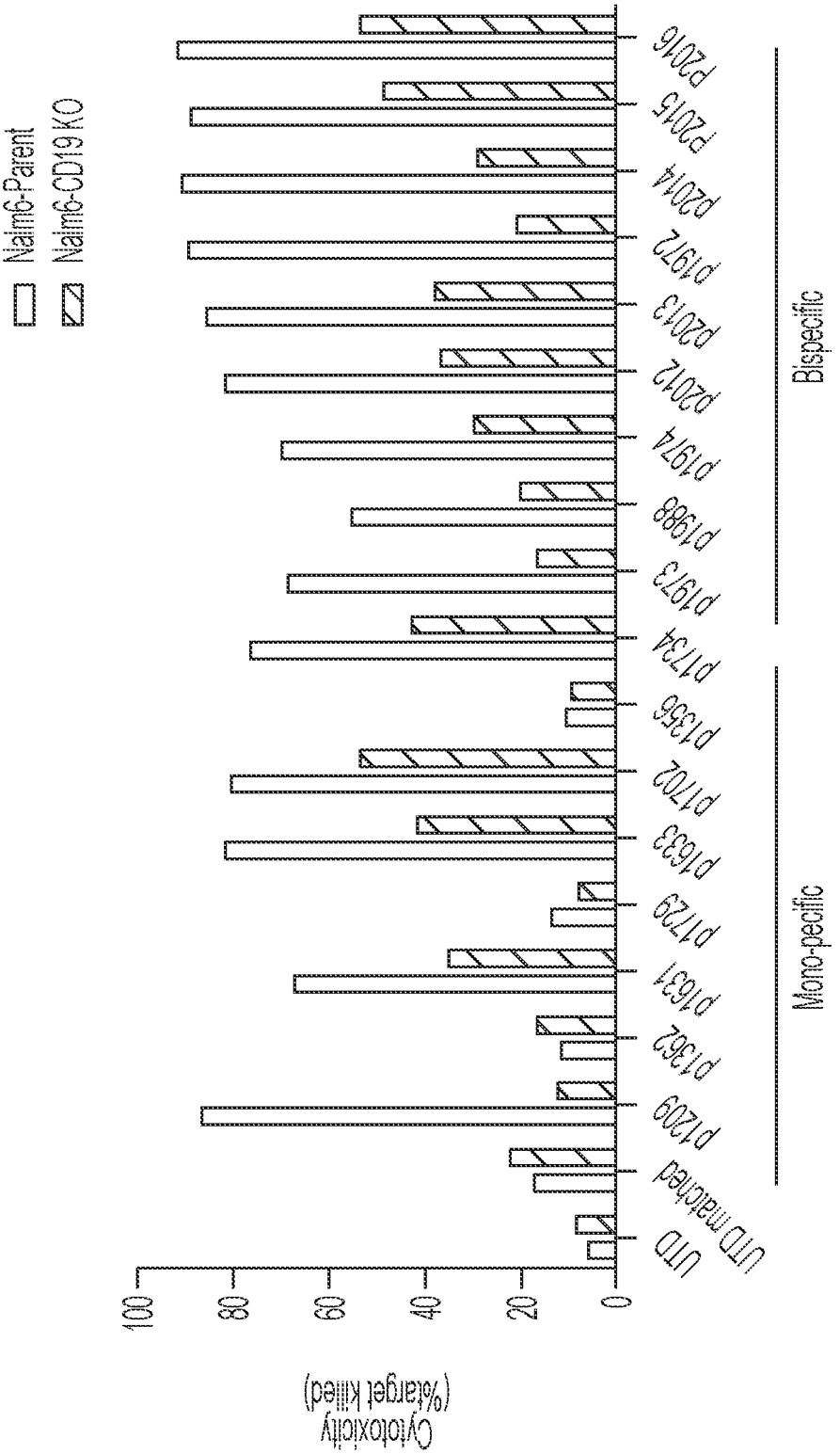

Nalm6-Parent
Nalm6-CD19 KO
Cytotoxicity (%target killed)
0
20
40
60
80
100
UTD
UTD matched
p1209
p1362
p1631
p1729
p1633
p1702
p1656
p1734
p1973
p1988
p1974
p2012
p2013
p1972
p2014
p2015
p2016
Mono-pecific
Bispecific

FIG. 18

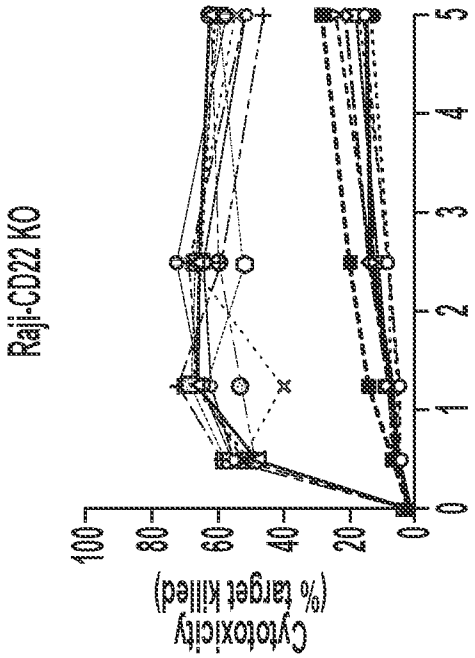
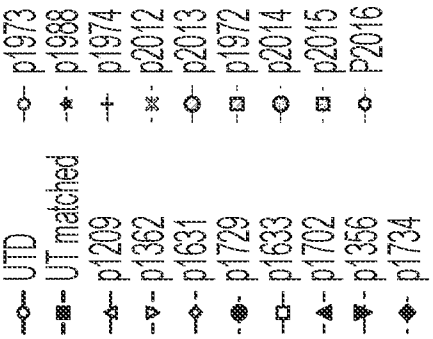


FIG. 19B



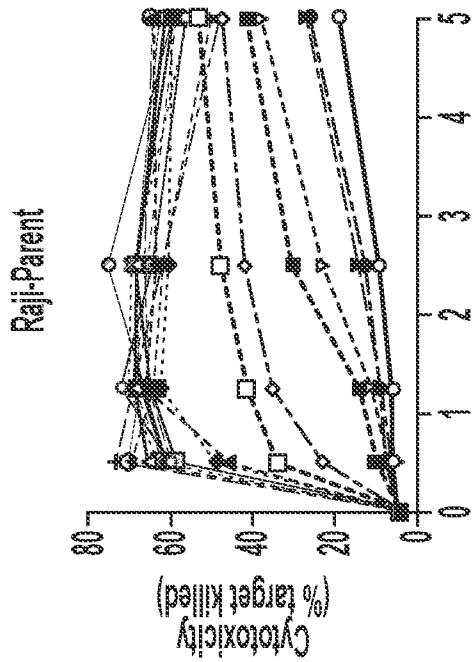


FIG. 19A

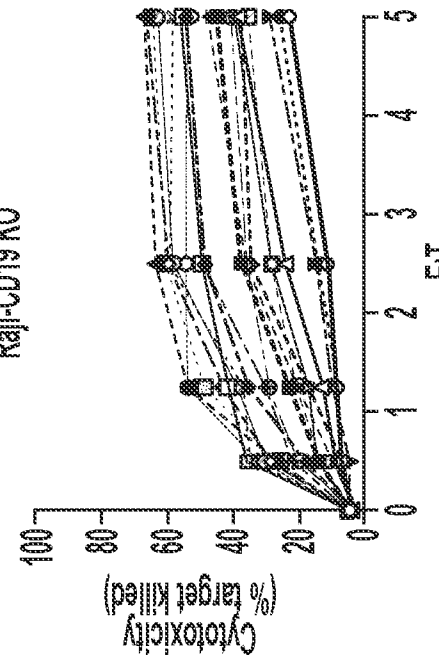


FIG. 19C

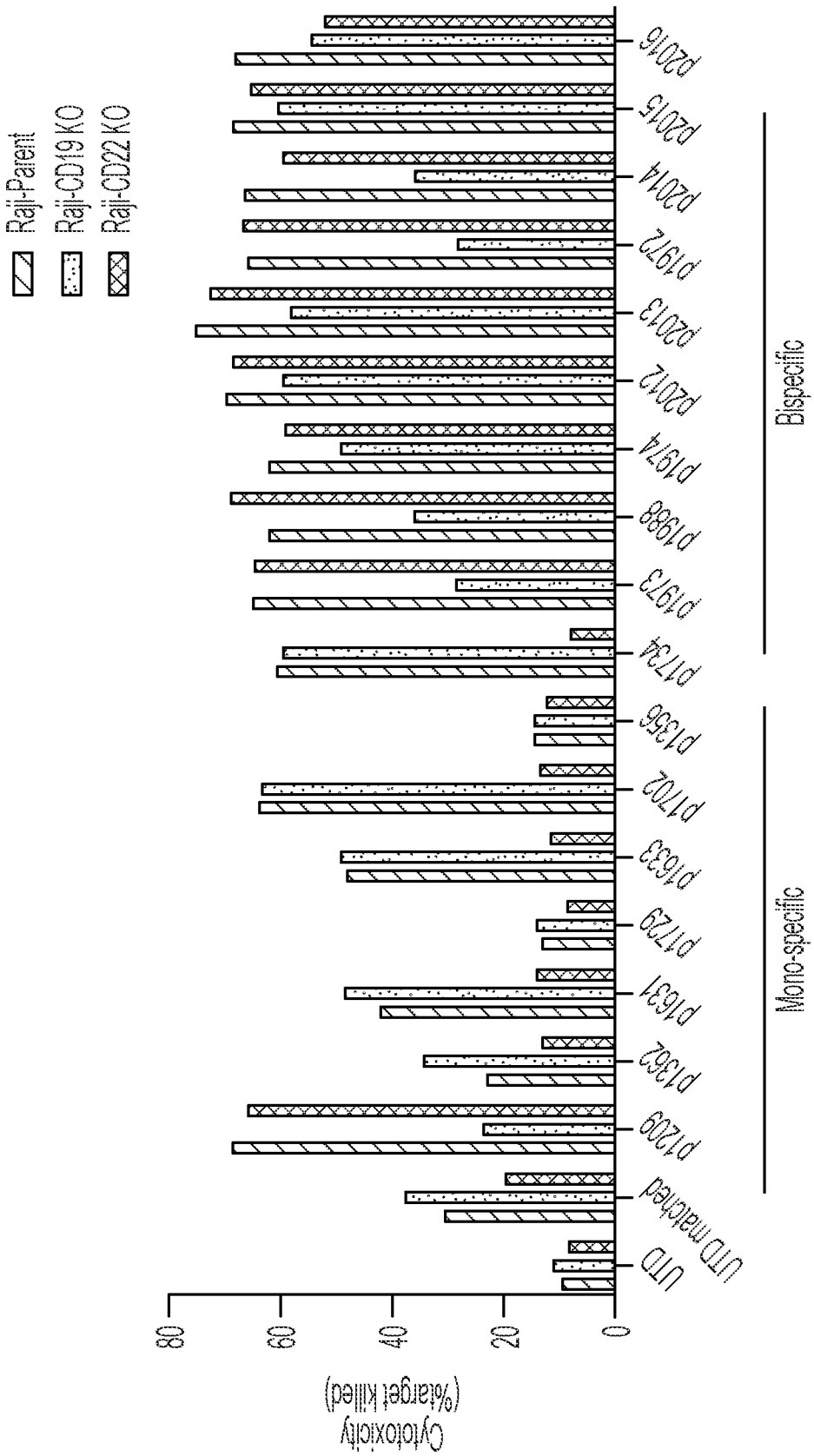

Raji-Parent
Raji-CD19 KO
Raji-CD22 KO
Cytotoxicity
(%target killed)
0
20
40
60
80
UTD
UTD matched
p1209
p1862
p1631
p1729
p1633
p1702
p1956
p1734
p1973
p1988
p1974
p2012
p2013
p1972
p2014
p2015
p2016
Mono-specific
Bispecific

FIG. 20

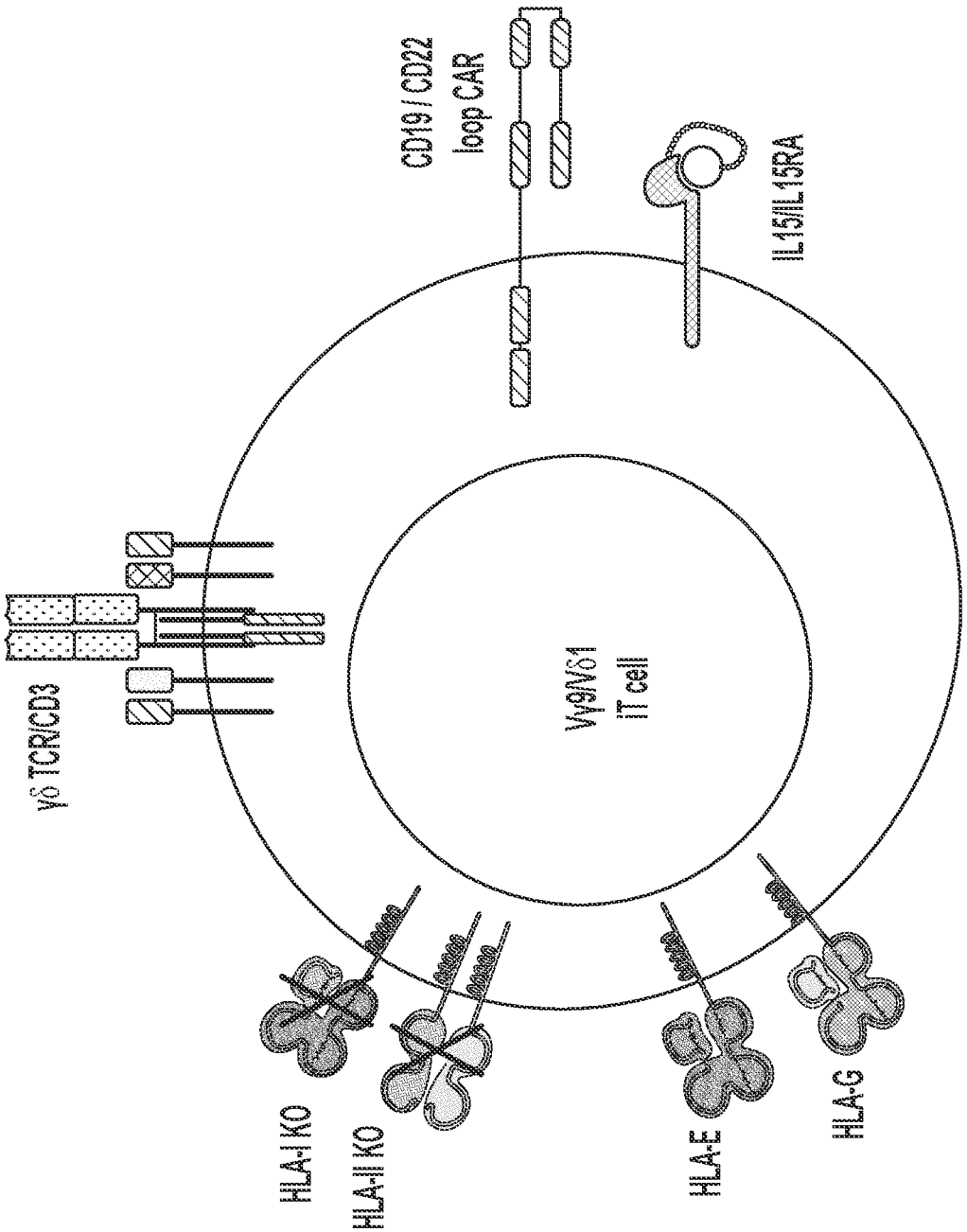

CD19 / CD22
loop CAR
IL15/IL15RA
Vγ9Vδ1
iT cell
γδ TCR/CD3
HLA-I KO
HLA-II KO
HLA-E
HLA-G

FIG. 21

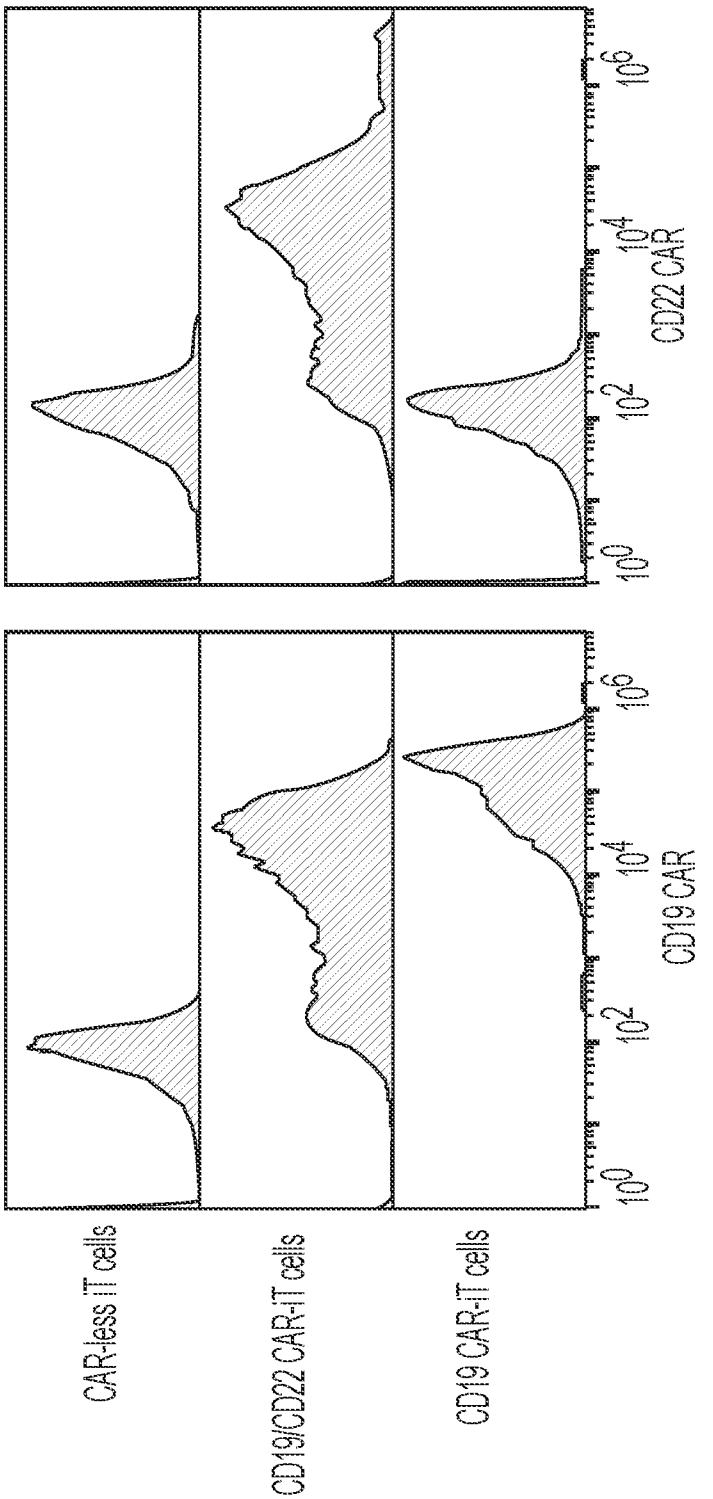

CAR-less iT cells
CD19/CD22 CAR-iT cells
CD19 CAR-iT cells
CD19 CAR
CD22 CAR
10^0
10^2
10^4
10^6

FIG. 22

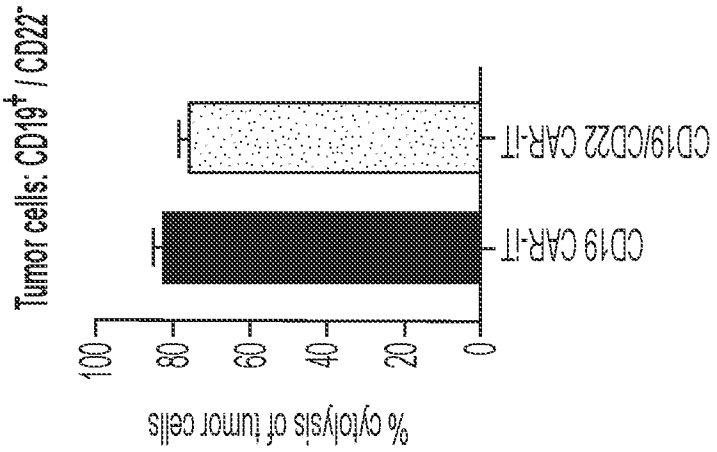


FIG. 23C

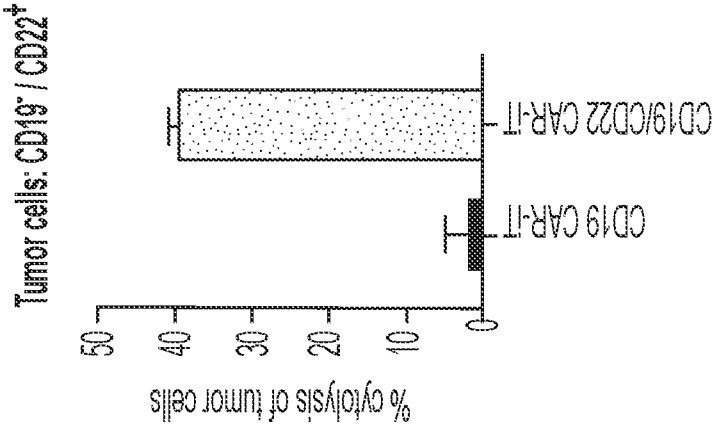


FIG. 23B

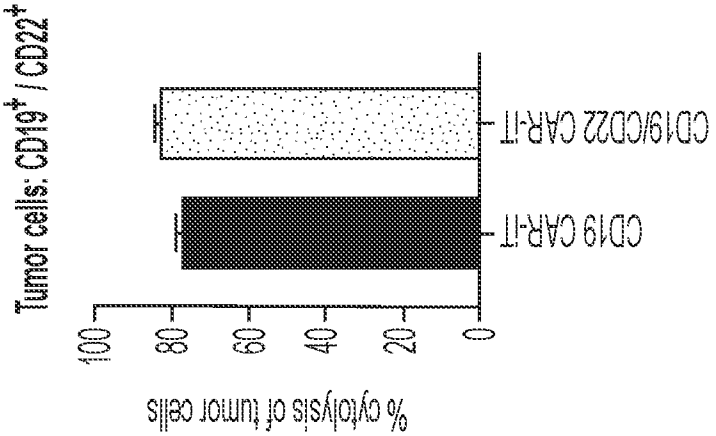


FIG. 23A

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2022/082441

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61K39/00 C12N5/074 C12N5/0783 C12N5/0789 C07K14/725
C07K16/30
ADD.
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)
A61K C12N C07K

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

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal, WPI Data, BIOSIS, CHEM ABS Data, Sequence Search, EMBASE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2021/258016 A1 (FATE THERAPEUTICS INC [US]) 23 December 2021 (2021-12-23) paragraphs [0008], [0013], [0018], [0027], [0032]	1-108
X	WO 2021/071962 A1 (FATE THERAPEUTICS INC [US]) 15 April 2021 (2021-04-15) paragraphs [0006], [0013], [0016], [0021], [0024], [0026], [0146], [0196], [0197], [0280]; table 2	1-108
X	WO 2021/092252 A1 (FORMAN STEPHEN J [US]; BROWN CHRISTINE E [US]; WANG ZHIQIANG [US]) 14 May 2021 (2021-05-14) paragraphs [0027] - [0028], [0030]; claims 17-18	1-108

-/--

[X] Further documents are listed in the continuation of Box C.

[X] 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
24 May 2023	13/06/2023

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Petri, Bernhard

2

Form PCT/ISA/210 (second sheet) (April 2005)

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2021/188599 A1 (UNIV SOUTHERN CALIFORNIA [US]) 23 September 2021 (2021-09-23) paragraphs [0032], [0033], [0321], [0646]	1-108
X	WO 2021/108613 A1 (NOVARTIS AG [CH]; ENGELS BORIS [US] ET AL.) 3 June 2021 (2021-06-03) abstract figure 2; examples 1, 2; tables 1A, 3A	1-108
X	ZHOU Z J ET AL: "Tri-Specific CD19xCD20xCD22 VHH CAR-T Cells (LCAR-AIO) Eradicate Antigen-Heterogeneous B Cell Tumors, Enhance Expansion, and Prolong Persistence in Preclinical In Vivo Models", BLOOD; 63RD ASH ANNUAL MEETING; 20211211 TO 20211214; ATLANTA GA, USA, AMERICAN SOCIETY OF HEMATOLOGY, US, vol. 138, no. Supplement 1, 23 November 2021 (2021-11-23), page 1700, XP009544563, ISSN: 0006-4971, DOI: 10.1182/BLOOD-2021-150650 the whole document	1-108
X	WO 2020/069184 A2 (LENTIGEN TECH INC [US]) 2 April 2020 (2020-04-02) figures 1A, 1B; examples 1B, 2	1-108
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Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:

   a. [X] forming part of the international application as filed.

   b. [ ] furnished subsequent to the international filing date for the purposes of international search (Rule 13*ter*.1(a)).

      [ ] accompanied by a statement to the effect that the sequence listing does not go beyond the disclosure in the international application as filed.

2. [ ] With regard to any nucleotide and/or amino acid sequence disclosed in the international application, this report has been established to the extent that a meaningful search could be carried out without a WIPO Standard ST.26 compliant sequence listing.

3. Additional comments:

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