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

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
14 December 2023 (14.12.2023)

WIPO | PCT

(10) International Publication Number
WO 2023/240147 A1

(51) International Patent Classification:
C07K 14/735 (2006.01) *A61K 35/545* (2015.01)
C07K 14/705 (2006.01) *A61K 38/00* (2006.01)
C12N 5/0783 (2010.01) *A61P 35/00* (2006.01)
A61K 35/17 (2015.01) *C12N 5/074* (2010.01)

(21) International Application Number:
PCT/US2023/068079

(22) International Filing Date:
07 June 2023 (07.06.2023)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
63/350,298 08 June 2022 (08.06.2022) US

(71) Applicant: **CENTURY THERAPEUTICS, INC.** [US/US]; 3675 Market Street, Philadelphia, Pennsylvania 19104 (US).

(72) Inventors: **NASO, Michael**; 3675 Market Street, Philadelphia, Pennsylvania 19104 (US). **GURUNG, Buddha**; 3675 Market Street, Philadelphia, Pennsylvania 19104 (US). **MORSE, Barry**; 3675 Market Street, Philadelphia, Pennsylvania 19104 (US). **BORGES, Luis**; 3675 Market Street, Philadelphia, Pennsylvania 19104 (US).

(74) Agent: **NG, Jennifer** et al.; Orrick, Herrington & Sutcliffe LLP, 2050 Main Street, Suite 1100, Irvine, California 92614-8255 (US).

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

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

Declarations under Rule 4.17:
— *as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(ii))*
— *as to the applicant's entitlement to claim the priority of the earlier application (Rule 4.17(iii))*

Published:
— *with international search report (Art. 21(3))*
— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(h))*
— *with sequence listing part of description (Rule 5.2(a))*

(54) Title: GENETICALLY ENGINEERED CELLS EXPRESSING CD16 VARIANTS AND NKG2D AND USES THEREOF

(57) Abstract: Disclosed herein are engineered iPSCs and cells derived therefrom that express CD16 variants and NKG2D. Also, the engineered iPSCs and cells derived therefrom can express a chimeric antigen receptor. Additionally, provided herein are polynucleotide constructs and methods of using thereof that are useful for expressing CD16 variants and NKG2D in such cells.

WO 2023/240147 A1

GENETICALLY ENGINEERED CELLS EXPRESSING CD16 VARIANTS AND NKG2D AND USES THEREOF

CROSS-REFERENCE

[0001] This application claims the benefit of U.S. Provisional Application No. 63/350,298 filed June 8, 2022, which is incorporated herein by reference in its entirety.

SEQUENCE LISTING INCORPORATION

[0002] This instant application contains a Sequence Listing, which has been submitted electronically in XML format in accordance to the WIPO Standard ST.26 and is hereby incorporated by reference in its entirety. The XML copy, created on June 7, 2023 is named "SL.xml" and is 150,741 bytes in size.

BACKGROUND

[0003] Cancer continues to be a significant global health issue despite substantial research efforts and scientific advances towards treating the disease. Cancer immunotherapies are desirable because they are highly specific and can facilitate removal of the cancer cells by using the patient's immune system. Research is ongoing to develop effective immune cell-based therapies for treating cancer.

[0004] Activated NK cells can kill target cells such as cancer cells by means similar to cytotoxic T cells (i.e., via cytolytic granules that contain perforin and granzymes as well as via death receptor pathways). Activated NK cells also secrete inflammatory cytokines such as IFN-γ and chemokines that promote the recruitment of other leukocytes to target tissues such as cancer tissues.

[0005] Sources of immune cells include those that have been differentiated from induced pluripotent stem cells (iPSCs). These cells can be modified to be allogeneic. There remains an unmet need for therapeutically sufficient and functional engineered allogeneic iPSC-derived therapies for treating cancer. Further, in engineering cell therapies, it is desirable to minimize the number of genetic edits that need to be made to the cells. Thus, there is a need for engineered cell therapies with multiple functionalities that can be engineered with a minimum number of edits.

BRIEF SUMMARY

[0006] In one aspect, described is an induced pluripotent stem cell (iPSC) or a derivative cell thereof comprising an exogenous polynucleotide encoding a CD16 protein and an NKG2D protein, wherein the CD16 protein and the NKG2D protein are operably linked by an autoprotease peptide.

[0007] In some embodiments, the CD16 protein is a CD16 variant protein. In some embodiments, the CD16 variant is a high affinity CD16 variant. In many embodiments, the CD16 variant is a non-cleavable CD16 variant. In various 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 comprises an amino acid sequence having at least 90% sequence identity to any one of SEQ ID NOS: 2 and 5.

[0008] In some embodiments, the NKG2D protein is a wildtype NKG2D protein. In many embodiments, the NKG2D protein comprises an amino acid sequence having at least 90% sequence identity to SEQ ID NO:4.

[0009] 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 many embodiments, the autoprotease peptide is a P2A peptide comprising an amino acid sequence having at least 90% sequence identity to SEQ ID NO:3.

[0010] 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:6.

[0011] In some embodiments, the exogenous polynucleotide is integrated into a gene locus 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 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.

[0012] In some embodiments, the exogenous polynucleotide is integrated into a gene locus selected from the group consisting of an AAVS1 locus, a B2M locus, a CIITA locus, a CD70 locus, a CLYBL locus, an NKG2A locus, an NKG2D locus, a TAP1 locus, a TAP2 locus, a TAPBP locus, a TRAC locus, a TRBC1 locus, a RFXANK locus, a RFX5 locus, and a RFXAP locus, thereby disrupting expression of the gene.

[0013] In some embodiments, the exogenous polynucleotide is integrated into a gene locus selected from the group consisting of an AAVS1 locus, a B2M locus, a CIITA locus, a CD70 locus, a CLYBL locus, an NKG2A locus and a TRAC locus, thereby disrupting expression of the gene.

[0014] In some embodiments, the disruption of the gene comprises an elimination of or reduced expression of the gene. In some embodiments, the integration into the gene locus is generated by targeted genome editing.

[0015] In some embodiments, the targeted genome editing comprises using a method selected from the group consisting of a CRISPR method, a zinc finger nuclease method, a TALEN method, a homing nuclease method, a homology recombination method, and any functional variation thereof.

[0016] In some embodiments, the method further comprises a disruption of one or more genes selected from the group consisting of an AAVS1 gene, a B2M gene, a CIITA gene, a CD70 gene, a CLYBL gene, an NKG2A gene, an NKG2D gene, a TAP1 gene, a TAP2 gene, a TAPBP gene, a TRAC gene, a TRBC1 gene, a RFXANK gene, a RFX5 gene, a RFXAP gene, and any combination thereof.

[0017] In some embodiments, the disruption is of the B2M gene and the CIITA gene.

[0018] T In some embodiments, the iPSC or the derivative cell thereof of claim 18 or 19, wherein the disruption of the one or more genes comprises an elimination of or reduced expression of the one or more genes. In many embodiments, the disruption of the one or more genes is generated by targeted genome editing.

[0019] In some embodiments, the targeted genome editing comprises using a method selected from the group consisting of a CRISPR method, a zinc finger nuclease method, a TALEN method, a homing nuclease method, a homology recombination method, and any functional variation thereof.

[0020] In some embodiments, the iPSC or the derivative cell further comprises a second exogenous polynucleotide encoding an IL-15 protein. In some embodiments, the IL-15 protein comprise an amino acid sequence having at least 90% sequence identity to SEQ ID NO:16.

[0021] In some embodiments, the iPSC or the derivative cell further comprises a second exogenous polynucleotide encoding a fusion polypeptide comprising an IL-15 and an IL-15 receptor alpha (IL-15Rα). In some embodiments, the fusion polypeptide comprises an amino acid sequence having at least 90% sequence identity to SEQ ID NO:17. In some embodiments, the fusion polypeptide comprises the amino acid sequence of SEQ ID NO:17.

[0022] In some embodiments, the iPSC or the derivative cell further comprises a third exogenous polynucleotide encoding a human leukocyte antigen E (HLA-E) protein. In some embodiments, the HLA-E comprises an amino acid sequence having at least 90% sequence identity to SEQ ID NO:14.

[0023] In some embodiments, the iPSC or the derivative cell further comprises a fourth exogenous polynucleotide encoding a human leukocyte antigen G (HLA-G) protein. In some embodiments, the HLA-G comprises an amino acid sequence having at least 90% sequence identity to SEQ ID NO:15.

[0024] In some embodiments, the HLA-E protein and the HLA-G protein are operably linked by a second autoprotease peptide. In some embodiments, the second autoprotease peptide is selected from the group consisting of a P2A peptide, an F2A peptide, an E2A peptide, a T2A peptide, a BmCPV2A peptide and a BmIFV2A peptide.

[0025] In some embodiments, the second, third, and/or fourth exogenous polynucleotides are integrated into one or more gene loci 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 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, and any combination thereof.

[0026] In some embodiments, the second, third, and/or fourth exogenous polynucleotides are integrated into one or more gene loci selected from the group consisting of an AAVS1 locus, a B2M locus, a CIITA locus, a CD70 locus, a CLYBL locus, an NKG2A locus, an NKG2D locus, a TAP1 locus, a TAP2 locus, a TAPBP locus, a TRAC locus, a TRBC1 locus, a RFXANK locus, a RFX5 locus, a RFXAP locus, and any combination thereof.

[0027] In some embodiments, the second, third, and/or fourth exogenous polynucleotides are integrated into one or more gene loci selected from the group consisting of an AAVS1 locus, a B2M locus, a CIITA locus, a CD70 locus, a CLYBL locus, an NKG2A locus, an NKG2D locus, a TAP1 locus, a TAP2 locus, a TAPBP locus, a TRAC locus, a TRBC1 locus, a RFXANK locus, a RFX5 locus, a RFXAP locus, and any combination thereof, thereby disrupting the one or more genes. In some embodiments, the disruption in the one or more genes comprises an elimination or reduced expression of the one or more genes.

[0028] In some embodiments, the second, third, and/or fourth exogenous polynucleotides are integrated into one or more gene loci selected from the group consisting of an AAVS1 locus, a B2M locus, a CIITA locus, a CD70 locus, a CLYBL locus, an NKG2A locus, a TRAC locus, and any combination thereof, thereby disrupting the one or more genes. In some embodiments, the disruption in the one or more genes comprises an elimination or reduced expression of the one or more genes.

[0029] In some embodiments, the iPSC is reprogrammed from whole peripheral blood mononuclear cells (PBMCs). In some embodiments, the iPSC is derived from a reprogrammed NK or T cell.

[0030] In some embodiments, the iPSC or the derivative cell thereof further comprises a fifth exogenous polynucleotide encoding a chimeric antigen receptor (CAR) that binds a target antigen.

[0031] In some embodiments, the target antigen is selected from the group consisting of 17-1A antigen, A3, A33 antigen, AFP, B7H4, Ba 733, BCMA, BrE3 antigen, CA125, CA9 (CAIX),

CD1, CD1a, CD3, CD5, CD15, CD16, CD19, CD20, CD21, CD22, CD22, CD23, CD25, CD30, CD33, CD33, CD38, CD45, CD70, CD74, CD79, CD79a, CD80, CD123, CD133, CD138, CEACAM5, CEACAM6, CLDN18.2, CLL1, cMET, colon-specific antigen-p (CSAp), ED-B fibronectin, EGFR, EGFRvIII, EGP-1, EGP-2, EpCAM, EphA1, EphA2, EphA3, EphA4, EphA5, EphA6, EphA7, EphA8, EphA10, EphB1, EphB2, EphB3, EphB4, EphB6, FGFR1, FGFR3, Flt-1, Flt-3, FOLR1, FOLR2, FOLR3, FSHR, GD2, GPC-3, GPRC5D, HCG, a HCG subunit, HER2, HIF-I, HLA-DR, Ia, IGF-I, IL13Rα2, IL-2, IL-6, IL-8, KC4 antigen, KS-1 antigen, KS1-4 antigen, Le-Y, MAGE, MET, MIF, MSLN, MUC1, MUC2, MUC3, MUC4, MUC16, NCA66, NCA90, NCA95, Nectin-4, p53, PAP, PDGFRA, PLGF, PSA, PSMA, ROBO1, RS5, S100, SLAM F7, SLITRK6, TAC, TAG-72, tenascin-C, tenascin-R, tenascin-W, tenascin-X, Thomson-Friedenreich antigen, Tn antigen, TRAILR1, TRAILR2, TRAILR3, TRAILR4, VEGF, a tumor necrosis antigen, an angiogenesis antigen, and an oncogene antigen. In some embodiments, the CAR comprises an antigen-binding domain selected from the group consisting of any provided in Tables 1, 2 and 3.

[0032] In some embodiments, the CAR comprises: (i) a signal peptide; (ii) an extracellular domain comprising a binding domain that specifically binds the target antigen; (iii) a hinge region; (iv) a transmembrane domain, (v) an intracellular signaling domain; and (vi) one or more co-stimulatory domains. In some embodiments, the signal peptide of a CAR comprises a GMCSFR signal peptide. In some embodiments, the extracellular domain of a CAR comprises an single chain Fv (scFv) or a VHH domain that specifically binds the target antigen. In some embodiments, the hinge region of a CAR comprises a CD28 hinge region. In some embodiments, the transmembrane domain of a CAR comprises a CD28 transmembrane domain. In some embodiments, the intracellular signaling domain of a CAR comprises a CD3ζ intracellular domain. In some embodiments, the one or more co-stimulatory domains of a CAR com prise a CD28 signaling domain.

[0033] In some embodiments, the derivative cell is an NK cell or a T cell. In other embodiments, the derivative cell is an NK cell. In many embodiments, the derivative cell is a T cell. In some embodiments, the derivative cell is a CD34+ hematopoietic progenitor cell.

[0034] In some embodiments, provided is a composition comprising a population of any one of the iPSCs or the derivative cells thereof described herein.

[0035] In another aspect, provided is an engineered cell comprising: (i) a first exogenous polynucleotide encoding a CD16 protein and an NKG2D protein, wherein the CD16 protein and the NKG2D protein are operably linked by an autoprotease peptide; (ii) a second exogenous polynucleotide encoding an exogenous polypeptide comprising an IL-15 protein; and (iii) optionally, a third exogenous polynucleotide encoding a human leukocyte antigen E (HLA-E) and/or a fourth exogenous polynucleotide encoding a human leukocyte antigen G (HLA-G).

[0036] In some embodiments, the engineered cell further comprises a fifth polynucleotide encoding 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 a prostate-specific membrane antigen (PSMA) extracellular domain or fragment thereof.

[0037] In some embodiments, 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:23 or 29.

[0038] 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.

[0039] 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:24.

[0040] 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 certain embodiments, the linker is selected from any one of the group consisting of those set forth in Table 4.

[0041] 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:25.

[0042] 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:27, 30 and 31.

[0043] 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:26, 28 and 32.

[0044] In another aspect, provided is an engineered cell comprising: (i) a first exogenous polynucleotide encoding a CD16 protein and an NKG2D protein, wherein the CD16 protein and the NKG2D protein are operably linked by an autoprotease peptide; (ii) a second exogenous polynucleotide encoding a fusion polypeptide comprising an IL-15 protein and an IL-15 receptor alpha (IL-15Rα) protein; and (iii) optionally, a third exogenous polynucleotide encoding a human leukocyte antigen E (HLA-E) protein and/or a fourth exogenous polynucleotide encoding a human leukocyte antigen G (HLA-G) protein.

[0045] is an engineered induced pluripotent stem cell (iPSC), an engineered natural killer (NK) cell or an engineered T cell.

[0046] In some embodiments, the first exogenous polynucleotide comprises the nucleic acid sequence of SEQ ID NO:6.

[0047] In some embodiments, the second exogenous polynucleotide comprises the nucleic acid sequence encoding an IL-15/IL-15Rα fusion protein of SEQ ID NO:17. In some embodiments, the second exogenous polynucleotide comprises the nucleic acid sequence of SEQ ID NO:38. In some embodiments, the second exogenous polynucleotide comprises the nucleic acid sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to SEQ ID NO:38. In some embodiments, the IL-15 protein comprises an amino acid sequence of SEQ ID NO:16.

[0048] In some embodiments, the third exogenous polynucleotide comprises the nucleic acid sequence of SEQ ID NO:21 and the fourth exogenous polynucleotide comprises the nucleic acid sequence of SEQ ID NO:22.

[0049] In some embodiments, the HLA-E protein and HLA-G protein are linked by an autoprotease peptide. In some embodiments, the HLA-E protein of the engineered cell comprises

the amino acid sequence of SEQ ID NO:19. In some embodiments, the HLA-G protein of the engineered cell comprises the amino acid sequence of SEQ ID NO:20. Described herein is a nucleic acid sequence of SEQ ID NO:21 which encodes the amino acid sequence of SEQ ID NO:19. Described herein is a nucleic acid sequence of SEQ ID NO:22 which encodes the amino acid sequence of SEQ ID NO:20.

[0050] In some embodiments, the engineered cell further comprises disruption of the B2M and CIITA genes. In some embodiments, the disruption of the B2M and CIITA genes is generated by targeted genome editing. In some embodiments, the targeted genome editing comprises using a method selected from the group consisting of a CRISPR method, a zinc finger nuclease method, a TALEN method, a homing nuclease method, a homology recombination method, and any functional variation thereof.

[0051] In some embodiments, the first exogenous polynucleotide is integrated into a gene locus 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 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.

[0052] In some embodiments, the second exogenous polynucleotide is integrated into a gene locus 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 CD38 locus, a TRAC locus, a TRBC1 locus, a ROSA26 locus, a collagen 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.

[0053] In some embodiments, the third exogenous polynucleotide is integrated into a gene locus 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 CD38 locus, a TRAC locus, a TRBC1 locus, a ROSA26 locus, a collagen 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.

[0054] In some embodiments, the fourth exogenous polynucleotide is integrated into a gene locus 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 CD38 locus, a TRAC locus, a TRBC1 locus, a ROSA26 locus, a collagen 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.

[0055] In some embodiments, the first exogenous polynucleotide and either the second, third or fourth exogenous polynucleotides are integrated into the B2M gene locus and the CIITA gene locus, thereby disrupting the B2M and CIITA genes. In certain embodiments, the first exogenous polynucleotide is integrated into the CD70 locus and the second exogenous polynucleotide is integrated into the B2M gene locus, thereby disrupting the CD70 and B2M genes. In various embodiments, the first exogenous polynucleotide is integrated into the CD70 locus and the second exogenous polynucleotide is integrated into the CIITA gene locus, thereby disrupting the CD70 and CIITA genes.

[0056] In some embodiments, the first exogenous polynucleotide is integrated into the CD70 locus and the third or fourth exogenous polynucleotide is integrated into the B2M gene locus, thereby disrupting the CD70 and B2M genes.

[0057] In many embodiments, the first exogenous polynucleotide is integrated into the CD70 locus and the third or fourth exogenous polynucleotide is integrated into the CIITA gene locus, thereby disrupting the CD70 and CIITA genes. In various embodiments, the integration into the CD70 locus is into exon 1 of the CD70 gene.

[0058] In some embodiments, the integration into the gene locus is generated by targeted genome editing. In many embodiments, the targeted genome editing comprises using a method selected from the group consisting of a CRISPR method, a zinc finger nuclease method, a TALEN method, a homing nuclease method, a homology recombination method, and any functional variation thereof.

[0059] In some embodiments, the engineered cell further comprises a fifth exogenous polynucleotide encoding a chimeric antigen receptor (CAR) that binds a target antigen. In many embodiments, the target antigen is selected from the group consisting of 17-1A antigen, A3, A33 antigen, AFP, B7H4, Ba 733, BCMA, BrE3 antigen, CA125, CA9 (CAIX), CD1, CD1a, CD3, CD5, CD15, CD16, CD19, CD20, CD21, CD22, CD22, CD23, CD25, CD30, CD33, CD33, CD38, CD45, CD70, CD74, CD79, CD79a, CD80, CD123, CD133, CD138, CEACAM5, CEACAM6, CLDN18.2, CLL1, cMET, colon-specific antigen-p (CSAp), ED-B fibronectin, EGFR, EGFRvIII, EGP-1, EGP-2, EpCAM, EphA1, EphA2, EphA3, EphA4, EphA5, EphA6, EphA7, EphA8, EphA10, EphB1, EphB2, EphB3, EphB4, EphB6, FGFR1, FGFR3, Flt-1, Flt-3, FOLR1, FOLR2, FOLR3, FSHR, GD2, GPC-3, GPRC5D, HCG, a HCG subunit, HER2, HIF-I, HLA-DR, Ia, IGF-I, IL13Rα2, IL-2, IL-6, IL-8, KC4 antigen, KS-1 antigen, KS1-4 antigen, Le-Y, MAGE, MET, MIF, MSLN, MUC1, MUC2, MUC3, MUC4, MUC16, NCA66, NCA90, NCA95, Nectin-4, p53, PAP, PDGFRA, PLGF, PSA, PSMA, ROBO1, RS5, S100, SLAM F7, SLITRK6, TAC, TAG-72, tenascin-C, tenascin-R, tenascin-W, tenascin-X, Thomson-Friedenreich antigen, Tn antigen, TRAILR1, TRAILR2, TRAILR3, TRAILR4, VEGF, a tumor necrosis antigen, an angiogenesis antigen, and an oncogene antigen. In some embodiments, the CAR comprises an antigen-binding domain selected from the group consisting of any provided in Tables 1, 2 and 3.

[0060] In some embodiments, the CAR comprises: (i) a signal peptide; (ii) an extracellular domain comprising a binding domain that specifically binds the target antigen; (iii) a hinge region; (iv) a transmembrane domain, (v) an intracellular signaling domain; and (vi) one or more co-stimulatory domains.

[0061] In certain embodiments, the signal peptide of a CAR comprises a GMCSFR signal peptide. In many embodiments, the extracellular domain comprises an single chain Fv (scFv) or a VHH domain that specifically binds the target antigen. In various embodiments, the hinge region comprises a CD28 hinge region. In some embodiments, the transmembrane domain comprises a CD28 transmembrane domain. In certain embodiments, the intracellular signaling domain comprises a CD3ζ intracellular domain. In some embodiments, the one or more co-stimulatory domains comprise a CD28 signaling domain. In some embodiments, the engineered iPSC is differentiated into an engineered differentiated cell.

[0062] In some embodiments, the engineered iPSC is differentiated into an engineered NK cell. In some embodiments, the engineered iPSC is differentiated into an engineered T cell. In some embodiments, the engineered iPSC is differentiated into an engineered CD34+ hematopoietic progenitor cell.

[0063] Provided is a composition comprising a population of any one of the engineered iPSCs described herein. Provided is a composition comprising a population of any one of the engineered differentiated cells described herein. Also provided is a composition comprising a population of any one of the engineered NK cells described herein. And also provided is a composition comprising a population of any one of the engineered T cells described herein. Also provided is a composition comprising a population of any one of the engineered CD34+ hematopoietic progenitor cells described herein.

[0064] In one aspect, disclosed is a method of treating cancer in a subject in need thereof, comprising administering any of the derivative cells described, any of the engineered NK cells described, any of the engineered T cells described, any of the engineered CD34+ hematopoietic progenitor cells described, and any of the compositions described to the subject in need thereof.

[0065] In some embodiments, the cancer is selected from the group consisting of acute lymphocytic leukemia (ALL), acute myeloid leukemia (AML), adenomas, benign lesions, bladder cancers, bone cancers, breast cancers, cancers of the thyroid gland, carcinomas of the larynx, carcinomas of the lung, carcinomas of the mouth, carcinomas of the throat, cervical cancers, chronic lymphocytic leukemia (CLL), chronic myeloid leukemias (CML), cutaneous melanomas, endocrine cancers, endometrial cancers, gastrointestinal cancers, genitourinary cancers, glioblastomas, head and neck cancers, hematologic malignancy, hematopoietic cancers, Hodgkin's lymphoma, intraocular melanomas, leukemias, liver cancers, lymphomas, melanomas, myelomas, myeloproliferative disorders, nervous system cancers, non-Hodgkin's lymphoma, ovarian cancers, pancreatic cancers, papillomas, parathyroid gland cancers, prostate cancers, renal cell carcinomas, sarcomas, skin cancers, solid tissue carcinomas, squamous cell carcinomas, and uterine cancers.

[0066] In some aspects, provided is a method of differentiating the iPSC cell into an NK cell, comprising subjecting any one of the iPSC cells described to a differentiation protocol comprising culturing the cell in a medium comprising a recombinant human IL-12 protein for the

final 24 hours of culturing under the differentiation protocol, thereby generating the NK cell. In some embodiments, the recombinant human IL-12 protein 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:33.

[0067] In some aspects, provided is a method of differentiating the iPSC cell into a T cell, comprising subjecting any one of the iPSC cells described to a differentiation protocol comprising culturing the cell in a medium comprising a recombinant DLL4 variant polypeptide having an amino acid sequence selected from the group consisting of SEQ ID NOS:35-37, thereby generating the T cell. Provided is a recombinant DLL4 variant polypeptide having an amino acid sequence selected from the group consisting of SEQ ID NOS:35-37.

[0068] A method of differentiating the iPSC cell into a CD34+ hematopoietic progenitor cell, comprising subjecting any one of the iPSC cells described to a differentiation protocol comprising culturing the cell in a pre-selected medium, thereby generating the CD34+ hematopoietic progenitor cell.

[0069] In some aspects, provided is a polynucleotide encoding a CD16 protein and an NKG2D protein, wherein the CD16 protein and the NKG2D protein are operably linked by an autoprotease peptide. In many embodiments, the CD16 protein comprises an amino acid sequence having at least 90% sequence identity to SEQ ID NO:5. In some embodiments, the CD16 protein is encoded by a nucleic acid sequence having at least 90% sequence identity to SEQ ID NO:7. In various embodiments, the CD16 protein is a CD16 variant protein. 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, and any combination thereof.

[0070] In some embodiments, the CD16 variant comprises an amino acid sequence having at least 90% sequence identity to SEQ ID NO:2 or SEQ ID NO:5. In various embodiments, the CD16 variant is encoded by a nucleic acid sequence having at least 90% sequence identity, e.g., at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity, to SEQ ID NO:7. In many embodiments, the NKG2D protein comprises an amino acid sequence having at least 90% sequence identity to SEQ ID NO:4. In some embodiments, the NKG2D protein is encoded by a nucleic acid sequence having at least 90% sequence identity, e.g., at least

90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity, to SEQ ID NO:9. In certain embodiments, the NKG2D protein is an NKG2D variant protein. In some embodiments, the NKG2D variant comprises an amino acid sequence having at least 90% sequence identity, e.g., at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity, to SEQ ID NO: 4.

[0071] 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 (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. In some embodiments, the autoprotease peptide is a P2A peptide comprising an amino acid sequence having at least 90% sequence identity, e.g., at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity, to SEQ ID NO:3. In some embodiments, the autoprotease peptide is a P2A peptide encoded by an nucleic acid sequence having at least 90% sequence identity, e.g., at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity, to SEQ ID NO:8.

[0072] In some embodiments, the exogenous polynucleotide encoding the CD16 protein and the NKG2D protein comprises the nucleic acid sequence having at least 90% sequence identity, e.g., at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity, to SEQ ID NO:6. In some embodiments, the exogenous polynucleotide encoding the CD16 protein and the NKG2D protein has the nucleic acid sequence of SEQ ID NO:6.

[0073] Provided is a vector comprising any of the polynucleotides described. In some embodiments, the vector comprises from 5' to 3': (i) a left homology sequence; (ii) a promoter; (iii) any of polynucleotides described; (iv) a terminator and/or a polyadenylation signal sequence; and (iv) a right homology sequence.

[0074] In some embodiments, the left homology sequence comprises a nucleic acid sequence having at least 90% sequence identity to SEQ ID NO:11. In some embodiments, the right homology sequence comprises a nucleic acid sequence having at least 90% sequence identity to SEQ ID NO:12.

[0075] In some embodiments, the vector comprises a nucleic acid sequence having at least 90% sequence identity, e.g., at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100%

sequence identity to SEQ ID NO:13. In some embodiments, the vector comprises the nucleic acid sequence of SEQ ID NO:13.

[0076] In some embodiments, the vector comprises a nucleic acid sequence having at least 90% sequence identity, e.g., at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity, to SEQ ID NO:39. In some embodiments, the vector comprises the nucleic acid sequence of SEQ ID NO:39.

BRIEF DESCRIPTION OF THE DRAWINGS

[0077] FIGS. 1A-1C depict an amino acid sequence and a nucleic acid sequence of the CD16 (F176V)-P2A-NKG2D transgene (SEQ ID NOS:1-9).

[0078] FIGS. 2A-2C depict sequences for CD70 targeted knock-in or knock-out in cells such as iPSC-derived natural killer cells (iNK cells). FIG. 2A provides a CD70 gRNA target sequence (SEQ ID NO:10). FIG. 2B provides a schematic diagram of exon 1 of the CD70 gene (SEQ ID NOS: 78-80). FIG. 2C depicts the nucleic acid sequence of an exemplary left homology arm (LHA) of the CD70 exon 1 targeting construct. FIG. 2D depicts the nucleic acid sequence of an exemplary right homology arm (RHA) of the CD70 exon 1 targeting construct (SEQ ID NOS:11-12).

[0079] FIG. 3 provides a schematic diagram of a targeting construct design including human CD16 transgene. The targeting construct includes a LHA targeting exon 1 of CD70, a constitutive promoter (CAG promoter), a KOZAK sequence, a human CD16 transgene, a SV40 terminator-poly adenylation signal, a RHA targeting exon 1 of CD70, and an selection marker (kanamycin-resistance marker).

[0080] FIGS. 4A-4G depict a nucleic acid sequence of the exemplary targeting construct sequence depicted in FIG. 3 and others. The CD70 exon homology arms are shown in bold, double underlined. The CAG promoter is shown in underlined. The human CD16 transgene is shown in bold. The SV40 terminator is shown in bold, underlined and the kanamycin resistance sequence is shown in double underlined. FIGS. 4D-4G present the nucleic acid sequence of an exemplary embodiment of a target sequence containing CD16-2A-NKG2D (SEQ ID NO:39).

[0081] FIGS. 5A-5N; FIGS. 5A-5D provide additional amino acid and nucleic acid sequences of IL-15-IL-15Rα, HLA-E and HLA-G fusions and components thereof (SEQ ID NOS:14-22 and

38). FIGS. 5E-5L provide additional amino acid and nucleic acid sequences of HSV-TK-PSMA fusions and components thereof (SEQ ID NOS:23-32). FIGS. 5L and 5M provide amino acid and nucleic acid sequences of IL-12 (SEQ ID NOS:33-34). FIGS. 5M and 5N provide amino acid sequences of various DLL4-Fc fusion proteins (SEQ ID NOS: 35-37).

[0082] FIG. 6 provides flow cytometry data detecting CD16 engineered into the CD70 locus using homology directed repair and CRISPR nuclease into iPSCs and differentiation into gamma/delta iT cells. iPSC1283 and iPSC1303 cells are iPSC cell lines expressing a CD16 transgene and a CAR. CD16 expression was measured on D0, D7, D14 and D21 of the cells undergoing differentiation into gamma/delta iT cells.

[0083] FIGS. 7A-7C show enhanced anti-tumor activity of iNK cells overexpressing NKG2D protein and enhanced antibody-dependent cellular cytotoxicity (ADCC) of iNK cells overexpressing high-affinity CD16. Engineered overexpression of NKG2D on iNK cells enhances anti-tumor activity is shown in FIGS. 7A-7B. iPSCs were engineered to constitutively express NKG2D. When iPSCs were differentiated into iNK cells, expression of NKG2D was quantified by flow cytometry and it is demonstrated that NKG2D expression was increased to 95.5% of iNK cells compared to 72.1% of non-engineered iNK cells (FIG. 7A). Non-engineered or NKG2D-engineered iNK cells were used in a killing assay with U87 glioblastoma cells that express stress ligands that trigger NKG2D activity. The NKG2D-engineered iNK cells more potently killed U87 cells. To confirm that enhanced killing was due to NKG2D expression, a neutralizing antibody against NKG2D was used in some conditions (to block the interaction of NKG2D with stress ligands on U87 cells). There was a marked reduction in U87 killing when the NKG2D neutralizing (blocking) antibody was included with the NKG2D-engineered iNK cells (FIG. 7B). Engineered overexpression of high-affinity CD16 on iNK cells enhances antibody-dependent cellular cytotoxicity (ADCC) (FIG. 7C). iPSCs were engineered to constitutively express one of two different naturally occurring variants of CD16. The iPSCs were then differentiated into iNK cells and used in a tumor killing assay where the targets were CD20+ lymphoblastic B cells. To trigger ADCC, anti-CD20 therapeutic antibody rituximab (black bars) was included at various concentrations. As a negative control, non-binding isotype control antibody was used in some conditions (grey bars) (top panel) When iPSCs were differentiated into iNK cells expressing the low affinity variant of CD16, ADCC was evident (increased dead tumor cells) only when rituximab was included. (bottom panel) When iPSCs

were differentiated into iNK cells expressing the high affinity variant of CD16, greater ADCC was observed compared to the low affinity version of CD16.

[0084] FIG. 8 shows a gating strategy for ADCC assay. Lymphocytes were gated based on forward scatter area (FSC-A) and side scatter area (SSC-A), followed by gating on CellTrace Violet (CTV)+ target cells (Raji cells in FIG. 9 and RajiΔCD19 cells in FIG. 10), and finally gating on 7-AAD-positive cells to determine % of dead therapeutic iNK target cells. FSC-A = forward scatter area, SSC-A = side scatter area, CTV = CellTrace Violet, 7-AAD = 7-amino-actinomycin D.

[0085] FIGS. 9A and 9B show rituximab-mediated ADCC using therapeutic iNK cells and Raji target cells. Rituximab at different concentrations (10, 1, 0.1, 0.01 and 0 μg/mL rituximab; right bars with circles) was tested and compared to corresponding concentrations of a host-matched isotype control (left bars with squares). The percentage of 7-AAD+ cells on CTV-labeled targets were graphed by antibody concentration. The test effector cells included (i) therapeutic iNK cells expressing a low-affinity CD16 variant (iPSC16), (ii) therapeutic iNK cells expressing a high-affinity CD16 variant (iPSC17 or iPSC18), (iii) therapeutic iNK cells expressing both a low-affinity CD16 variant (iPSC16) and a CD19-specific CAR (a p1209 transgene encoding a CD19-specific CAR), and (iv) therapeutic iNK cells expressing both a high-affinity CD16 variant (iPSC17 or iPSC18) and a CD19-specific CAR (a p1209 transgene encoding a CD19-specific CAR). FIG. 9A shows results of an ADCC assay of control iNK effector cells that do not express a CD16 variant (left graphs) and iNK effector cells expressing a low affinity CD16 variant (iPSC16; right graphs). FIG. 9B shows results of an ADCC assay of iNK effector cells expressing a high affinity CD16 variant (iPSC17 or iPSC18; left and middle graphs). The results of a control ADCC assay with Raji target cells alone are shown in FIG. 9B (right bar graphs).

[0086] FIGS. 10A and 10B show rituximab-mediated ADCC using therapeutic iNK cells and RajiΔCD19 target cells. Rituximab at different concentrations (10, 1, 0.1, 0.01 and 0 μg/mL rituximab; right bars with circles) was tested and compared to corresponding concentrations of a host-matched isotype control (left bars with squares). The percentage of 7-AAD+ cells on CTV-labeled targets were graphed by antibody concentration. The test effector cells included (i) therapeutic iNK cells expressing a low-affinity CD16 variant (iPSC16), (ii) therapeutic iNK cells expressing a high-affinity CD16 variant (iPSC17 or iPSC18), (iii) therapeutic iNK cells

expressing both a low-affinity CD16 variant (iPSC16) and a CD19-specific CAR (a p1209 transgene encoding a CD19-specific CAR), and (iv) therapeutic iNK cells expressing both a high-affinity CD16 variant (iPSC17 or iPSC18) and a CD19-specific CAR (a p1209 transgene encoding a CD19-specific CAR). FIG. 10A shows results of an ADCC assay of control iNK effector cells that do not express a CD16 variant (left graphs) and iNK effector cells expressing a low affinity CD16 variant (iPSC16; right graphs). FIG. 10B shows results of an ADCC assay of iNK effector cells expressing a high affinity CD16 variant (iPSC17 or iPSC18; left and middle graphs). The results of a control ADCC assay with RajiΔCD19 target cells alone are shown in FIG. 10B (right graphs).

DETAILED DESCRIPTION

I. Introduction

[0087] Provided herein are induced pluripotent stem cell (iPSC)-derived cell therapies such as iPSC-derived natural killer (iNK) cells and iPSC-derived T (iT) cells for immuno-oncology. In some aspects, the genetically engineered iPSC-derived immune cells express CARs and other molecules that can mediate the persistence, functionality, and/or activation of these engineered immune cells. Also, provided herein are methods of generating and using such iPSC-derived immune cells.

[0088] Additionally described are genetically engineered iPSCs and cells derived therefrom that exogenously express recombinant CD16 and recombinant NKG2D. In some aspects, such cells also express a CAR. Also provided are related constructs (e.g., vectors), polynucleotides, and pharmaceutical compositions.

II. Definitions

[0089] Unless defined otherwise, all terms of art, notations and other technical and scientific terms or terminology used herein have the same meaning as is commonly understood by one of skill in the art to which the claimed subject matter belongs. In some cases, terms with commonly understood meanings are defined herein for clarity and/or for ready reference, and the inclusion of such definitions herein should not necessarily be construed to represent a substantial difference over what is generally understood in the art. All patents and publications referred to herein are incorporated by reference in their entireties.

[0090] Unless defined otherwise, all technical and scientific terms, acronyms, and abbreviations used herein have the same meaning as commonly understood by one of ordinary skill in the art to which the invention pertains. Unless indicated otherwise, abbreviations and symbols for chemical and biochemical names is per IUPAC-IUB nomenclature. Unless indicated otherwise, all numerical ranges are inclusive of the values defining the range as well as all integer values in-between.

[0091] As used herein, the articles "a" and "an" refer to one or to more than one (i.e. to at least one) of the grammatical object of the article. By way of example, "an element" means one element or more than one element.

[0092] Furthermore, "and/or" where used herein is to be taken as specific disclosure of each of the two specified features or components with or without the other. Thus, the term "and/or" as used in a phrase such as "A and/or B" herein is intended to include "A and B," "A or B," "A (alone)", and "B (alone)".

[0093] As used herein, the term "about" will be understood by persons of ordinary skill in the art and will vary to some extent on the context in which it is used. As used herein, "about" when referring to a measurable value such as an amount, a temporal duration, and the like, is meant to encompass variations of ±20% or ±10%, more preferably ±5%, even more preferably ±1%, and still more preferably ±0.1% from the specified value, as such variations are appropriate to perform the disclosed methods.

[0094] 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.

[0095] 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.

[0096] 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.

[0097] 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.

[0098] 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.

[0099] 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.

[0100] 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.

[0101] 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)).

[0102] 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.

[0103] 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)).

[0104] 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. Natl. 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.

[0105] 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.

[0106] 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, i.e., 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.

[0107] As used herein, the term "polynucleotide," synonymously referred to as "nucleic acid molecule," "nucleotides," "nucleic acids," or "polynucleic 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.

[0108] 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.

[0109] By "integration" it is meant that one or more nucleotides of a construct is stably inserted into the cellular genome, i.e., 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.

[0110] 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.

[0111] 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 (i.e. a polypeptide found in nature) or fragment thereof; a variant polypeptide (i.e. 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.

[0112] "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 (i.e., 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.

[0113] 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.

[0114] 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.

[0115] 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.

[0116] 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.

[0117] As used herein, a “porcine tesehovirus-1 2A peptide” or “P2A peptide” or “P2A”, refers to a “self-cleaving peptide” of a picornavirus. The average length of P2A peptides is 18–22 amino acids. A P2A peptide was first identified in a foot-and-mouth disease virus (FMDV), a member of the picornavirus (Ryan et al., *J Gen Virol,* 1991, 72(Pt 11): 2727–2732). It was reported that ribosomes skip the synthesis of the glycyl-prolyl peptide bond at the C-terminus of a 2A peptide, leading to the cleavage between a 2A peptide and its immediate downstream peptide (see, e.g., Donnelly et al., *J Gen Virol.,* 2001, 82: 1013–1025.

[0118] 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).

[0119] 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 (i.e., 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.

[0120] 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.

[0121] 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.

[0122] 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.

[0123] 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 or γδ 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.

[0124] "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.

[0125] As used herein, the term "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.

[0126] 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 an NK cell modified to express a chimeric antigen receptor (CAR). The NK cell can also be differentiated from a stem cell or progenitor cell.

[0127] 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.

[0128] 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.

[0129] 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.

[0130] 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.

[0131] 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, i.e., the extracellular domain and the transmembrane domain of the CAR protein.

[0132] 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.

[0133] The term "hinge region" or "spacer region" as used herein generally means any oligo- or polypeptide that functions to link the extracellular domain to the transmembrane domain. A hinge region can be used to provide more flexibility and accessibility for the extracellular domain.

[0134] 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.

[0135] 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").

[0136] 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.

[0137] 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 (i.e., 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 (i.e., 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.

[0138] 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.

[0139] As used herein, the term "monoclonal antibody" refers to an antibody obtained from a population of substantially homogeneous antibodies, i.e., 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.

[0140] 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.

[0141] 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).

[0142] 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.

[0143] 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.

[0144] 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.

[0145] 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.

[0146] 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.

[0147] 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.

[0148] 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" or "Kd" refers to the dissociation constant, which is obtained from the ratio of Kd to Ka (i.e., 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.

[0149] 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.

[0150] As used herein, the terms "cancer", "malignancy", "neoplasm", "tumor", and "carcinoma", are used interchangeably herein to refer to cells that exhibit relatively abnormal, uncontrolled, and/or autonomous growth, so that they exhibit an aberrant growth phenotype characterized by a significant loss of control of cell proliferation. In general, cells of interest for treatment in the present application include precancerous (e.g., benign), malignant, pre-metastatic, metastatic, and non-metastatic cells. The teachings of the present disclosure may be relevant to any and all cancers. Non-limiting examples of one or more cancers include, for example, hematopoietic cancers including leukemias, lymphomas (Hodgkin's and non-Hodgkin's), myelomas and myeloproliferative disorders; sarcomas, melanomas, adenomas, carcinomas of solid tissue, squamous cell carcinomas of the mouth, throat, larynx, and lung, liver cancer, genitourinary cancers such as prostate, cervical, bladder, uterine, and endometrial cancer and renal cell carcinomas, bone cancer, pancreatic cancer, skin cancer, cutaneous or intraocular melanoma, cancer of the endocrine system, cancer of the thyroid gland, cancer of the parathyroid gland, head and neck cancers, breast cancer, gastro-intestinal cancers and nervous system cancers, benign lesions such as papillomas, and the like.

III. NKG2D and CD16 transgene

[0151] 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.

[0152] In some aspects, provided is an iPSC cell 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 cell 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 cell or derivative cell thereof. The exogenous or isolated polynucleotide construct can be introduced into a gene locus of the iPSC cell or derivative cell thereof.

[0153] In some embodiments, the iPSC cell 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 cell 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.

[0154] In many embodiments, the cell expressing recombinant CD16 proteins, recombinant NKG2D proteins and CARs also expresses recombinant fusion proteins containing IL-15 and IL-15Rα. In many embodiments, the cell expresses recombinant CD16 proteins, recombinant NKG2D proteins, CARs, recombinant fusion proteins containing IL-15 and IL-15Rα, 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-15Rα. 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-15Rα also expresses CARs.

[0155] 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 an 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:6. In some embodiments, the exogenous polynucleotide construct encodes for the amino acid sequence of SEQ ID NO:1.

[0156] 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.

[0157] 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:5. 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.

[0158] 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 CD16 variant protein has a F176V substitution. In some embodiments, the variant CD16 protein has the sequence of SEQ ID NO:2. In some embodiments, the nucleic acid sequence encoding the variant CD16 protein has the sequence of SEQ ID NO:7. In some embodiments, the wildtype CD16 protein has the sequence of SEQ ID NO:5.

[0159] 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 embodiment, the NKG2D protein is an 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:4. In some embodiment, the NKG2D protein has the amino acid sequence of SEQ ID NO:4. In some embodiment, the nucleic acid sequence encoding the NKG2D protein has sequence of SEQ ID NO:9.

A. Autoprotease peptides

[0160] 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.

[0161] 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:3. In some embodiment, the P2A peptide has the amino acid sequence of SEQ ID NO:3.

IV. Chimeric Antigen Receptors (CARs)

[0162] In some embodiments, an iPSC cell or derivative cell thereof contains an exogenous polynucleotide encoding a chimeric antigen receptor (CAR), such as a CAR targeting a tumor antigen. In some instances, the recombinant CAR polypeptide includes at least an extracellular domain that binds specifically to an antigen (or more than one antigen), a transmembrane domain and an intracellular signaling domain. In some instances, the recombinant CAR polypeptide includes a signal peptide, an extracellular domain that binds specifically to an antigen (or more than one antigen), a transmembrane domain, an intracellular signaling domain and one or more co-stimulatory domains. In other instances, the recombinant CAR polypeptide includes a signal peptide, an extracellular domain that binds specifically to an antigen (or more than one antigen), a hinge region, a transmembrane domain, an intracellular signaling domain and one or more co-stimulatory domains.

A. Extracellular domains

[0163] In certain embodiments, an extracellular domain of a CAR includes an antibody, an antibody fragment, 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. In some embodiments, the antigen-binding domains or fragments 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.

[0164] In some embodiments, antibodies or antibody fragments suitable for use in the CAR 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.

[0165] 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.

[0166] In some embodiments, the extracellular domain of the CAR is a single-domain antibody or nanobody. In some embodiments, the extracellular domain is a VHH. In some embodiments, the extracellular domain is an scFv.

[0167] Optionally, 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 described. Such scaffolds have been shown to yield molecules with improved characteristics, such as greater stability or reduced immunogenicity. Non-limiting examples of alternative scaffolds 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 Biotechnol 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, Curr 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.

i. Antigen-binding domains

[0168] In some embodiments, an antigen-binding domain of a CAR binds to a target antigen. The antigen-binding domain may bind to more than one antigen or more than one epitope in an

antigen. For example, the antigen-binding domain may bind to 2, 3, 4, 5, 6, 7, 8 or more antigens. As another example, the antigen-binding domain may bind 2, 3, 4, 5, 6, 7, 8 or more epitopes in the same antigen.

[0169] 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 some embodiments, CAR 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 include those associated with tumor cells or autoimmune diseases.

[0170] In some embodiments, the antigen-binding domain binds to at least one tumor antigen or autoimmune antigen.

[0171] 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.

[0172] 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.

[0173] 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, ROBO1 and 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 CD19, 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. Non-limiting examples of tumor antigens associated with renal cancer include CD70 and FOLR1.

[0174] 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.

[0175] 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 as set forth in PCT/US2021/072646. 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 as set forth in

PCT/US2021/072646. 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 as set forth in PCT/US2021/072646.

[0176] 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.

[0177] In some embodiments, autoimmune antigens that may be targeted by the CAR 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, filaggrin, 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 translation elongation 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.

[0178] Non-limiting exemplary antigen targets are provided in Tables 1-3. Table 1 provides antigen binding domains that bind to exemplary antigen targets. The antigen-binding domain may comprise a VH sequence, a VL sequence, and/or CDRs thereof, such as those described in the cited publications, the contents of each publication are incorporated herein by reference in their entirety for all purposes.

TABLE 1

Antigen target	Type	Examples of Source	Type	Examples of Source
5T4	VH	Identifier 2, 4 in WO2016022939	VL	Identifier 1, 3 in WO2016022939
AGR2	VH	Identifier 10, 18 in WO2016040321	VL	Identifier 11, 19 in WO2016040321
ALK	VH	Identifier 1, 11, 13, 15, 3, 5, 7, 9 in US20160280798Al; Identifier 9, 1, 3, 5, 11, 13, 15, 7, 9 in WO2015069922	VL	Identifier 10, 12, 14, 16, 2, 4, 6, 8 in US20160280798Al; Identifier 10, 12, 14, 16, 8 in WO2015069922; Identifier 2, 4, 6 in WO2015069922
AMC	VH	Identifier 17, 18, 19, 20, 21, 22, 23, 24, 25, 26 in WO2016161390	VL	Identifier 27, 28, 29, 31, 32, 33, 34, 35, 36 in WO2016161390
ANG2	VH	Identifier 1, 3 in WO2015091655	VL	Identifier 2 , 4 in WO2015091655
APCDD1	VH	Identifier 10, 102, 106, 110, 114, 118, 122, 126, 130, 134, 14, 6, 98 in WO2012019061	VL	Identifier 136, 100, 104, 108, 112, 116, 12, 120, 124, 128, 132, 16, 8 in WO2012019061
APRIL	VH	Identifier 12, 14, 16. 18. 3. 32, 34, 36, 38, 40, 42, 44, 46, 48, 52 in US20160264674	VL	Identifier 20, 22, 24, 26, 28, 30, 4, 50 in US2016026467
AXL	VH	Identifier 21, 3, 45 in WO2016097370	VL	Identifier 22, 4 in WO2016097370
B2MG	VH	Identifier 28 in WO2016126213A1	VL	Identifier 29 in WO2016126213A1
B7H1	VH	Identifier 12, 32, 42, 52, 72, 2, 62 in US20130034559	VL	Identifier 17, 37, 47, 57, 7, 77, 27, 67 in US20130034559
B7H3	VH	Identifier 10, 11, 12, 13, 14, 15, 16, 9 in WO2016033225	VL	Identifier 1, 2, 3, 4, 5, 6, 7, 8 in WO2016033225
B7H3 (CD276)	VH	Identifier 17, 26, 7 in WO2016044383	VL	Identifier 18, 27, 8 in WO2016044383

B7H4	VH	Identifier 100, 101, 102, 103, 107, 108, 109, 110, 111, 112, 113, 114, 12, 127, 130, 131, 132, 133, 137, 2, 20, 28, 36, 37, 38, 4, 56, 99, 144 in US20160159910; Identifier 13, 15, 17 in WO2016160620	VL	Identifier 104, 11, 126, 134, 138, 19, 27, 3, 35, 55, 93, 95, 97, 98, 145, 146, 147, 148 in US20160159910; Identifier 29, 31, 33 in WO2016160620
BAT1	VH	Identifier 5, 6, 7, 8, 9 in WO2013014668	VL	Identifier 1, 2, 3, 4 in WO2013014668
BCMA	VH	Identifier 26 in WO2016168773 A3; Identifier 142, 148, 154, 160, 166, 172, 178, 184, 190, 196, 202, 208, 214, 220, 226, 232, 238, 244, 250, 256, 262, 268, 274, 280, 286, 292, 298, 304, 310, 316, 322, 328, 334, 340, 346, 352 in WO2016168595A1; Identifier 8 in WO2016094304A3; Identifier 171, 172, 173, 174, 175, 176, 177, 178, 179, 180, 181, 182, 183, 184, 185, 186, 187, 190, 255, 257, 258, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81 WO2016014565; Identifier 38 in EP3057994A1; Identifier 55 in WO2016187349A1; Identifier 1, 13, 17, 21, 25, 29, 33, 37, 41, 45, 49, 5, 53, 57, 61, 65, 9 in WO2016090320; Identifier 101, 743, 174, 758, 95, 759, 97, 760, 99 in WO2016120216; Identifier 11, 741, 17 in WO2015158671A1; Identifier 10, 11, 12, 13, 14 in WO2016014789; Identifier 15 in WO2016168766A1	VL	Identifier 25 in WO2016168773 A3; Identifier 42 in WO2016097231; Identifier 143, 149, 155, 161, 167, 173, 179, 185, 191, 197, 203, 209, 215, 221, 227, 233, 239, 245, 251, 257, 263, 269, 275, 281, 287, 293, 299, 305, 311, 317, 323, 329, 335, 341, 347, 353 in WO2016168595 A1; Identifier 192, 193, 194, 195, 196, 197, 198, 199, 200, 201, 204, 205, 207, 208, 211, 259, 260, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98 in WO2016014565; Identifier 53 in WO2016187349A1; Identifier 7 in WO2016094304 A3; Identifier 10, 14, 18, 2, 22, 26, 30, 34, 38, 42, 46, 50, 54, 58, 6, 62, 66 in WO2016090320; Identifier 100, 102, 175, 96, 98 in WO2016120216; Identifier 12, 14, 16, 18 in WO2015158671A1; Identifier 7, 8, 9 in WO2016014789; Identifier 14 in WO2016168766A1

BMPR1A	VH	Identifier 12 in WO2011116212	-	-
CA19.9	VH	Identifier 117 in US20160333114A1	VL	Identifier 118 in US20160333114A1
Campath 1	VH	Identifier 34 in US20160333114A1	VL	Identifier 31, 33 in US20160333114A1
CD105	VH	Identifier 13, 14, 16 in WO2014039682	VL	Identifier 1, 17, 20, 22, 23 in WO2014039682
CD123	VH	Identifier 11, 13, 14, 21 in WO2015140268A1; Identifier 113, 115, 57, 59, 63 in WO2016120216; Identifier 12, 123, 24, 25, 26, 27, 28, 29, 30, 9 in WO2016120220; Identifier 216, 217, 218, 219, 274 in WO2016028896	VL	Identifier 9, 11, 18, 19, 20, 21, 22, 23 in WO2016120220; Identifier 12, 16, 18, 19, 22 in WO2015140268A1; Identifier 275, 276, 277, 278, 307, 308, 309, 310 in WO2016028896; Identifier 5 in US20160333108A1; Identifier 114, 116, 58, 60, 64 in WO2016120216
CD148	VH	Identifier 10, 14, 18, 2, 22, 26, 30, 6 in WO2005118643	VL	Identifier 12, 16, 20, 24, 28, 32, 4, 8 in WO2005118643
CD16	VH	Identifier 25 in WO2015158868	VL	Identifier 26 in WO2015158868
CD19	VH	Identifier 53, 55 in WO2016120216	VL	Identifier 27, 31 in WO2016168773 A3; Identifier 49 in WO2016187349A1; Identifier 11 in WO2016134284; Identifier 194 in US20140134142A1; Identifier 54, 56 in WO2016120216; Identifier 13, 14, 15, 16, 17, 186, 187, 188, 189, 192, 196, 197, 198, 199, 200, 201, 202, 203, 204, 205, 64, 66, 67, 68, 69, 70, 71, 91 US20160152723; Identifier 22 in US20160039942; 3361 Identifier 63 in WO2016097231; Identifier 3 in US20160145337A1; Identifier 112 in US20160333114A1; Identifier 114 in

				US20160333114A1; Identifier 13, 6 US20160319020
CD19H 803	VH	Identifier 28, 29, 32, 33, 34, 35 in WO2016168773 A3; Identifier 51 in WO2016187349A1; Identifier 20 in US20160039942; Identifier 1 in WO2014184143; Identifier 5 in US20160145337A1; Identifier 166, 167, 168, 172, 176, 177, 181, 183, 184, 185, 62 in US20160152723; Identifier 15 US20160319020; Identifier 17, 33, 34, 35 in EP3057994A1; Identifier 62 in WO2016097231; Identifier 12 in WO2016134284; Identifier 111, 113 in US20160333114A1	-	-
CD2	VH	Identifier 103, 117, 119 in WO2016122701	VL	Identifier 102, 116 in WO2016122701
CD20	VH	Identifier 45 in WO2016097231; Identifier 11, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 7, 9 in WO2017004091; Identifier 26 in US20170000900; Identifier 54 in US20160333114A1; Identifier 25 in US20170000900; Identifier 24 in US20170000900; Identifier 23 in US20170000900	VL	Identifier 46 in WO2016097231; Identifier 10, 12, 8 in WO2017004091; Identifier 51 in US20160333114A1
CD22	VH	Identifier 3 in WO2013059593; Identifier 10, 11, 12, 7, 9, 8 in US20150299317; Identifier 201 in WO2016164731; CD22 VH 869 Identifier	VL	Identifier 17, 8, 14, 15 in US20150239974; Identifier 7 in US20150299317; Identifier 681 in WO2016164731; Identifier 682 in WO2016164731;

		671 in WO2016164731; Identifier 672 in WO2016164731; Identifier 673 in WO2016164731; Identifier 676 in WO2016164731; Identifier 678 in WO2016164731; Identifier 679 in WO2016164731; Identifier 680 in WO2016164731; Identifier 700 in WO2016164731; Identifier 701 in WO2016164731; Identifier 702 in WO2016164731; Identifier 703 in WO2016164731; Identifier 704 in WO2016164731; Identifier 705 in WO2016164731; Identifier 706 in WO2016164731; Identifier 707 in WO2016164731; Identifier 708 in WO2016164731, Identifier 709 in WO2016164731; Identifier 711 in WO2016164731; Identifier 712 in WO2016164731; Identifier 713 in WO2016164731; Identifier 714 in WO2016164731; Identifier 715 in WO2016164731; Identifier 716 in WO2016164731; Identifier 717 in WO2016164731; Identifier 718 in WO2016164731; Identifier 719 in WO2016164731; Identifier 720 in WO2016164731; Identifier 721 in WO2016164731; Identifier 722 in WO2016164731; Identifier 723 in WO2016164731; Identifier 724 in WO2016164731;		Identifier 683, 2020 in WO2016164731; Identifier 684 in WO2016164731; Identifier 685 in WO2016164731; Identifier 686 in WO2016164731; Identifier 687 in WO2016164731; Identifier 688 in WO2016164731; Identifier 690 in WO2016164731; Identifier 740 in WO2016164731; Identifier 741 in WO2016164731; Identifier 742 in WO2016164731; Identifier 743 in WO2016164731; Identifier 744 in WO2016164731; Identifier 745 in WO2016164731; Identifier 746 in WO2016164731; Identifier 747 in WO2016164731; Identifier 748 in WO2016164731; Identifier 749 in WO2016164731; Identifier 750 in WO2016164731; Identifier 752 in WO2016164731; Identifier 753 in WO2016164731; Identifier 754 in WO2016164731; Identifier 755 in WO2016164731; Identifier 756 in WO2016164731; Identifier 757 in WO2016164731; Identifier 758 in WO2016164731; Identifier 759 in WO2016164731; Identifier 760 in WO2016164731; Identifier 761 in WO2016164731; Identifier 762 in WO2016164731; Identifier 763 in WO2016164731; Identifier 764 in

		Identifier 725 in WO2016164731; Identifier 726 in WO2016164731; Identifier 727 in WO2016164731; Identifier 728 in WO2016164731; Identifier 729 in WO2016164731; Identifier 730 in WO2016164731; Identifier 731 in WO2016164731; Identifier 732 in WO2016164731; Identifier 733 in WO2016164731; Identifier 734 in WO2016164731; Identifier 735 in WO2016164731; Identifier 736 in WO2016164731; Identifier 737 in WO2016164731; Identifier 738 in WO2016164731		WO2016164731; Identifier 765 in WO2016164731; Identifier 766 in WO2016164731; Identifier 767 in WO2016164731; Identifier 768 in WO2016164731; Identifier 769 in WO2016164731; Identifier 770 in WO2016164731; Identifier 771 in WO2016164731; Identifier 772 in WO2016164731; Identifier 773 in WO2016164731; Identifier 774 in WO2016164731; Identifier 775 in WO2016164731; Identifier 776 in WO2016164731; Identifier 777 in WO2016164731; Identifier 124 in WO2016122701
CD276	VH	Identifier 17, 26, 7 in US20160053017	VL	Identifier 18, 27 in US20160053017
CD28	VH	Identifier 19 in WO2015158868	VL	Identifier 20 in WO2015158868
CD3	VH	Identifier 108, 112, 115 in WO2016122701; Identifier 29 in WO2014144722 A2; Identifier 12 in WO2016126213A1	VL	Identifier 104 in WO2016122701; Identifier 13 in WO2016126213A1
CD30	VH	Identifier 14, 16 in WO2016134284	VL	Identifier 13, 15 in WO2016134284
CD324	VH	Identifier 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49, 51, 53, 55, 57, 59, 61, 63, 65, 67, 69, 71 in US9534058	VL	Identifier 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, 64, 66, 68, 70 in US9534058
CD32B	VH	Identifier 127 in WO2016122701	VL	Identifier 126 in WO2016122701
CD33	VH	Identifier 65, 67, 69, 71, 77, 79, 81, 83, 84 in WO2016120216; Identifier 11, 13, 15; 17 in WO2015150526A2; Identifier 57, 58, 59, 60, 61,	VL	Identifier 12, 14, 16, 18 in WO2015150526A2; Identifier 66, 68, 70, 72, 78, 80, 82 in WO2016120216; Identifier

		62, 63, 64, 65 in WO2016014576		66, 67, 68, 69, 70, 71, 72, 73, 74 in WO2016014576
CD37	VH	Identifier 11, 12, 18 in US20170000900	VL	Identifier 14, 15 in US20170000900
CD38	VH	Identifier 2 in WO2009080830; Identifier 10 in WO2015121454	VL	Identifier 1, 11 in WO2009080830
CD3s	VH	Identifier 7 in WO2014144722A2	VL	Identifier 8 in WO2014144722A2
CD40	VH	Identifier 1 in WO2016069919; Identifier 5, 7, 8 in WO2015091655	VL	Identifier 2 in WO2016069919; Identifier 6 in WO2015091655
CD45	VH	Identifier 24 in WO2016126213A1	VL	Identifier 25 in WO2016126213A1
CD46	VH	Identifier 39, 47, 59, 15, 19, 23, 27, 31, 35, 43, 51, 55, 63, 67, 71, 75, 79, 83, 69, 71, 83 in WO2012031273; Identifier 1, 10, 11, 12, 13, 14, 15, 16, 17, 3, 5, 6, 7, 9, 18, 19, 20, 21 in WO2016040683	VL	Identifier 41, 61, 21, 25, 29, 33, 37, 45, 49, 53, 57, 65, 69, 73, 77, 81, 85, 17, 73, 77 in WO2012031273; Identifier 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42 in WO2016040683
CD4BS	VH	Identifier 15, 3 in US20160194375A1	VL	Identifier 14, 2 in US20160194375A1
CD4i	VH	Identifier 5 in US20160194375A1	VL	Identifier 4 in US20160194375A1
CD52	VH	Identifier 103, 136, 137 in WO2010132659	VL	Identifier 102, 138 in WO2010132659
CD64	VH	Identifier 129 in WO2016122701	VL	Identifier 128 in WO2016122701
CD7	VH	Identifier 16, 20 in WO2016126213A1	VL	Identifier 17, 21 in WO2016126213A1
CD70	VH	Identifier 81, 85, 89 in WO2015121454	VL	Identifier 83, 87, 91 in WO2015121454; Identifier 83 in WO2015121454; Identifier 87 in WO2015121454; Identifier 91 in WO2015121454
CD71	VH	Identifier 1, 3, 325, 4, 5, 699 in US20160355599	VL	Identifier 2,327, 329, 331, 333, 335, 337, 6, 650, 652, 654, 656, 658, 660, 670, 671, 672, 673, 7, 701, 702, 703, 704, 705, 706, 707, 708, 709, 710, 711, 712, 721, 722, 723, 724, 725, 726, 727, 728, 729, 730,

				731, 732, 733, 734, 735, 736, 737, 738, 739, 740, 741, 742, 743, 744, 745, 746, 747, 748, 749, 750, 751, 752, 753, 754, 755, 756, 757, 758, 759, 760, 761, 762, 763, 764, 765, 766, 767, 768, 769, 770, 771, 772, 773, 774, 775, 776, 777, 778, 779, 780, 781, 782, 783, 784 785, 786, 787, 788, 8, 810, 811, 812, 813, 814, 815, 816, 817, 818, 819, 820, 821, 822, 823, 824, 825, 826, 827, 828, 829, 830, 831, 832, 833, 834, 835, 836, 810, 842, 843, 844, 845, 846, 847, 848, 849, 850, 851, 852, 853, 854, 855, 856, 857, 858, 859, 860, 861, 862, 863, 864, 865, 866, 867, 868, 869, 870, 871, 872, 873, 874, 875, 876, 877, 879, 879, 880, 881, 882, 883, 884, 885, 886, 887, 888, 889, 890, 891, 892, 893, 894, 895, 896, 897, 898, 899, 900, 901, 902, 903, 904, 905, 906, 907, 908 in US20160355599
CD73	VH	Identifier 100, 103, 107, 109, 112, 114, 116, 119, 121, 16, 32, 4, 52, 60, 68, 80, 88 in US20160145350; Identifier 135, 40, 21, 3, 28, 36 in WO2016055609A1	VL	Identifier 12. 20, 44, 72, 76, 8, 84, 92 in US20160145350; Identifier 22, 29, 37, 4 in WO2016055609A1; Identifier 101, 102, 104, 106, 110, 117, 118, 120, 122 in US20160145350
CD74	VH	Identifier 6 in US20100284906A1; Identifier 10, 11, 9 in US20040115193A1; Identifier 23, 27, 30, 33 in US20130171064	VL	Identifier 25, 29, 31, 35 in US20130171064; Identifier 12, 13, 14, 11, 4 in US20040115193A1

CD76b	VH	Identifier 15, 17, 19, 23, 27, 29, 37, 57, 59, 61 in US20160159906	VL	Identifier 16, 18, 22, 38, 58, 60, 62 in US20160159906
CD79	VH	Identifier 131 in WO2016122701	VL	Identifier 130 in WO2016122701
CDIM	VH	Identifier 1, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 2, 20, 21, 22, 3, 4, 5, 6, 7, 8, 9 in WO2013120012	VL	Identifier 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49 in WO2013120012
CEA	VH	Identifier 8 in US8287865	VL	Identifier 10, 38, 39, 7, 9 in US8287865
Claudin	VH	Identifier 101, 103, 105, 107, 109, 111, 113, 115, 117, 119, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, 150, 23, 27, 31, 35, 39, 43, 47, 51, 55, 59, 63, 67, 71, 75, 79, 81, 83, 85, 87, 89, 91, 93, 95, 97, 99 in WO2016073649A1	VL	Identifier 114, 116, 118, 120, 22, 25, 29, 33, 37, 41, 45, 49, 53, 57, 61, 65, 69, 73, 77 in WO2016073649A1
CLDN18.2	VH	Identifier 12, 2 in US20160347815A1	VL	Identifier 13, 3 in US20160347815A1
CLL1	VH	Identifier 13, 14, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35 in WO2016120219; Identifier 195, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77 in WO2016014535; Identifier 31, 33, 34, 36, 38, 40, 42, 46 in US20160075787; Identifier 150, 103, 105, 107, 109, 111, 113, 115, 117 in WO2016179319A1	VL	Identifier 16, 18, 20, 22, 24, 26, 28, 30, 32, 24, 36 in WO2016120219; Identifier 196, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90 in WO2016014535; Identifier 30, 32, 35, 37, 39, 41 in US20160075787; Identifier 152, 104, 106, 108, 110, 112, 114, 116, 118 in WO2016179319A1
CLL3	VH	Identifier 101, 103, 105, 107, 109, 111, 113, 115, 117, 119, 121, 123, 125, 127, 129, 131, 133, 135, 137, 139, 141, 145, 147, 149, 151, 153, 155, 157, 159, 161, 163, 165, 167,	VL	Identifier 100, 102, 104, 106, 108, 110, 112, 114, 116, 118, 120, 122, 124, 126, 128, 130, 132, 134, 136, 138, 140, 144, 146, 148, 150, 152, 154, 156, 158, 160, 162, 164, 166,

		169, 171, 173, 175, 177, 179, 181, 183, 185, 187, 191, 193, 195, 197, 199, 201, 203, 205, 207, 209, 21, 211, 213, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49, 51, 53, 55, 57, 59, 61, 63, 65, 67, 69, 71, 73, 75, 77, 79, 81, 83, 85, 87, 89, 91, 93, 95, 97, 99 in US20170000901		170, 172, 174, 176, 178, 180, 182, 184, 186, 190, 192, 194, 196, 198, 20, 200, 202, 204, 206, 208, 210, 212, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 54, 56, 58, 60, 62, 64, 66, 68, 70, 72, 74, 76, 78, 80, 82, 84, 86, 88, 90, 92, 94, 96, 98 in US20170000901
collagen	VH	Identifier 21, 4, 15, 17, 18, 19, 20, 5, 6, 7, 1, 2, 3 in WO2007024921	VL	Identifier 11, 12, 14, 23, 25, 26, 27, 8, 9 in WO2007024921
CS1	VH	Identifier 22 in WO2016168773 A3; 103, 105, 107, 109 in WO2016120216; Identifier 13, 15, 17, 19 in WO2015166056A1; Identifier 38, 40, 42, 44, 46 in WO2015121454; Identifier 26 in US20160075784A1	VL	Identifier 104, 106, 108 in WO2016120216; Identifier 14, 16, 18, 20, 22 in WO2015166056A1; Identifier 39, 41, 43, 45, 47 in WO2015121454; Identifier 110, 112 in WO2016120216
CSF	VH	Identifier 10, 102, 14, 18, 2, 22, 26, 30, 34, 38, 46, 50, 54, 58, 6, 62, 66, 70, 74, 78, 82, 86, 90, 94, 98 in US20050059113A1	VL	Identifier 12, 32, 44, 48, 60 in US20050059113A1
CSPG4	VH	Identifier 10, 16, 18, 4, 6, 8 in WO2016077638; Identifier 8 in WO2016164429	VL	Identifier 7 in WO2016164429; Identifier 12, 14 in WO2016077638
CTLA4	VH	Identifier 3, 31, 32, 33, 34, 35, 41, 42, 43, 44, 45, 7 in US20140105914; Identifier 4 in US8697845; Identifier 19 in US20150283234; Identifier 17 in WO2014066532	VL	Identifier 36, 37, 38, 39, 40, 46, 47, 48, 49, 50, 8, 4 in US20140105914; Identifier 2 in US8697845; Identifier 20 in US20150283234; Identifier 18 in WO2014066532
CXCR4	VH	Identifier 72, 73, 74, 75, 84 in US20110020218	VL	Identifier 76, 77, 78, 79, 80, 81, 82, 87, 88, 90, 91, 92, 93 in US20110020218
Daclizumab	VH	Identifier 44, 46 in US20160333114A1	VL	Identifier 43, 45 in US20160333114A1

DR5	VH	Identifier 18, 82, 90, 98, 8 in WO2016122701	VL	Identifier 13, 23, 25, 27, 3, 78, 86, 94, 29 in WO2016122701; Identifier 62 in WO2016122701; Identifier 54 in WO2016122701; Identifier 70 in WO2016122701
E7MC	VH	Identifier 15, 16, 17, 18, 19, 20, 21, 22, 23, 233, 234, 235, 236, 237, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35 in WO2016182957A1	VL	Identifier 238, 239, 240, 241, 242, 243, 36, 37, 38, 39, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56 in WO2016182957A1
EFNA	VH	Identifier 149, 153, 157, 161 in US20150125472	VL	Identifier 151, 155, 159, 163 in WO2012118547; Identifier 27, 53 in US20150125472
EFNA4	VH	Identifier 13, 39 in US20150125472	-	-
EGFR	VH	Identifier 14, 50, 9 in WO2015143382; Identifier 12, 14, 15, 21 in US20100008978A1; Identifier 2123 in WO2018231759	VL	Identifier 15 in WO2015143382; Identifier 14 in WO2014143765; Identifier 4051, 4052, 4053, 4054, 4055, 4056, 4057, 4058, 4059, 4060, 4061, 4062, 4063, 4064, 4065, 4066, 4067, 4068, 4069, 4070, 4071, 4072, 4073, 4074, 4075, 4076, 4077, 4078, 4079, 4080, 4081, 4082, 4083, 4084, 4085, 4086, 4087, 4088, 4089. 4090, 4091, 4092, 4093, 4094, 4095, 4096, 4097, 4098, 4099, 4100, 4101,4102, 4103, 4104, 4105, 4106, 4107, 4108, 4109, 4110, 4111, 4112, 4113, 4114, 4115, 4116, 4117, 4118, 4119, 4120, 4121, 4122, 4123, 4124, 4125, 4126, 4127, 4128, 4129, 4130, 4131, 4132, 4133, 4134, 4135, 4136, 4137, 4138, 4139, 4140, 4141, 4142, 4143, 4144,

				4145, 4146, 4147, 4148, 4149, 4150, 4151, 4152, 4153, 4154, 4155, 4156, 4157, 4158, 4159, 4160, 4161, 4162, 4163, 4164, 4165, 4166, 4167, 4168, 4169, 4170, 4171, 4172, 4173, 4174, 4175, 4176, 4177, 4178, 4179, 4180, 4181, 4182, 4183, 4184, 4185, 4186, 4187, 4188, 4189, 4190, 4191, 4192, 4193, 4194, 4195, 4196, 4197, 4198, 4199, 4200, 4201, 4202, 4203, 4204, 4205, , 4206, 4207, 4208, 4209, 4210, 4211, 4212, 4213, 4214, 4215, 4216, 4217, 4218, 4219, 4220, 4221, 4222, 4223, 4224, 4225, 4226, 4227, 4228, 4229, 4230, 4231, 4232, 4233, 4234, 4235, 4236, 4237, 4238, 4239, 4240, 4241, 4242, 4243, 4244, 4245, 4246, 4247, 4248, 4249, 4250, 4251, 4252, 4253, 4254, 4255, 4256, 4257, 4258, 4259, 4260, 4261, 4262, 4263, 4264, 4265, 4266, 4267, 4268, 4269, 4270, 4271, 4272, 4273, 4274, 4275, 4276, 4277, 4278, 4279, 42870, 4281, 4282, 42834284, 4285, 4286, 4287, 4288, 4289, 4290, 4291, 4292, 4293, 4294, 4295, 4296, 4297, 4298, 4299, 4300, 4301, 4302, 4303, 4304, 4305, 4306, 4307, 4308, 4309, 4310, 4311, 4312, 4313, 4314, 4315, 4316, 4317, 4318, 4319, 4320, 4321, 4322, 4323, 4324, 4325, 4326, 4327, 4328,

				4329, 4330, 4331, 4332, 4333, 4334, 4335, 4336, 4337, 4338, 4339, 4340, 4341, 4342, 4343, 4344, 4345, 4346, 4347, 4348, 4349, 4350, 4351, 4352, 4353, 4354, 4355, 4356, 4357, 4358, 4359, 4360, 4361, 4362, 4363, 4364, 4365, 4366, 4367, 4368, 4369, 4370, 4371, 4372, 4373, 4374, 4375, 4376, 4377, 4378, 4379, 4380, 4381, 4382, 4383, 4384, 4385, 4386, 4387, 4388, 4389, 4390, 4391, 4392, 4393, 4394, 4395, 4396, 4397, 4398, 4399, 4400, 4401, 4402, 4403, 4404, 4405, 4406, 4407, 4408, 4409, 4410, 4411, 4412, 4413, 4414, 4415, 4416, 4417, 4418, 4419, 4420, 4421, 4422, 4423, 4424, 4425, 4426, 4427, 4428, 4429, 4430, 4431, 4432, 4433, 4434, 4435, 4436, 4437, 4438, 4439, 4440, 4441, 4442, 4443, 4444, 4445, 4446, 4447, 4448, 4449, 4450, 4451, 4452, 4453, 4454, 4455, 4456, 4457, 4458, 4459, 4460, 4461, 4462, 4463, 4464, 4465, 4466, 4467, 4468, 4469, 4470, 4471, 4472, 4473, 4474, 4475, 4476, 4477, 4478, 4479, 4480, 4481, 4482, 4483, 4484, 4485, 4486, 4487, 4488, 4489, 4490, 4491, 4492, 4493, 4494, 4495, 4496, 4497, 4498, 4499, 4500, 4501, 4502, 4503, 4504, 4505, 4506, 4507, 4508, 4509, 4510, 4511, 4512,

				4513, 4514, 4515, 4516, 4517, 4518, 4519, 4520, 4521, 4522, 4523, 4524, 4525, 4526, 4527, 4528, 4529, 4530, 4531, 4532, 4533, 4534, 4535, 4536, 4537, 4538, 4539, 4540, 4541, 4542, 4543, 4544, 4545, 4546, 4547, 4548, 4549, 4550, 4551, 4552, 4553, 4554, 4555, 4556, 4557, 4558, 4559, 4560, 4561, 4562, 4563, 4564, 4565, 4566, 4567, 4568, 4569, 4570, 4571, 4572, 4573, 4574, 4575, 4576, 4577, 4578, 4579, 4580, 4581, 4582, 4583, 4584, 4585, 4586, 4587, 4588, 4589, 4590, 4591, 4592, 4593, 4594, 4595, 4596, 4597, 4598, 4599, 4600, 4601, 4602, 4603, 4604, 4605, 4606, 4607, 4608, 4609, 4610, 4611, 4612, 4613, 4614, 4615, 4616, 4617, 4618, 4619, 4620, 4621, 4622, 4623, 4624, 4625, 4626, 4627, 4629, 4629, 4630, 4631, 4632, 4633, 4634, 4635, 4636, 4637, 4638, 4639 in WO2018231759
EGFR (EGFRvIII)	VH	Identifier 2124 in WO2018231759; Identifier 13 in WO2016016341; Identifier 24 in WO2016168773 A3; Identifier 34 in US20160304615; Identifier 2 in US20160200819A1; Identifier 91, 93 in WO2016120216	VL	4640, 4641, 4642, 4643, 4644, 4645, 4646, 4647, 4648, 4649, 4650, 4651, 4652, 4653, 4654, 4655, 4656, 4657, 4658 in WO2018231759; Identifier 14 in WO2016016341; Identifier 23 in WO2016168773 A3; Identifier 42 in US20160304615; Identifier 1 in US20160200819A1;

				Identifier 92, 94 in WO2016120216
Endoglin	VH	Identifier 41, 42, 43, 71, 73, 75, 88, 89, 90, 91, 92 in US20160009811	VL	Identifier 103, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 102, 100 in WO2011041441; Identifier 100, 102, 103, 3, 4, 5, 70, 72, 74, 93, 94, 95, 96, 97 in US20160009811
EphA2 receptor	VH	Identifier 20, 22, 24, 32, 34, 36, 37, 38, 40, 42, 43, 45, 74, 76 in US20150274824	VL	Identifier 26, 28, 30, 47, 48, 49, 50, 52, 78, 80 in US20150274824
ERBB2	VH	Identifier 2, 4 in US20110129464; Identifier 10, 2, 26, 30, 38, 4, 40, 42, 52, 54, 56, 57, 58, 6 in US20130089544; Identifier 8 in US20130266564; Identifier 1 in US20150104443	VL	Identifier 1 in US20110129464; Identifier 12, 16, 20, 24, 32, 36, 44, 50, 51, 53, 8 in US20130089544; Identifier 7 in US20130266564; Identifier 3 in US20110129464
Factor D	VH	Identifier 17, 20, 27, 29, 30, 31, 32, 33, 4 in US20160017052	VL	Identifier 16, 18, 19, 26, 3 in US20160017052
Factor XII	VH	Identifier 15 in WO2014089493	VL	Identifier 17 in WO2014089493
FAP	VH	Identifier 1, 5 in WO2015118030; Identifier 170, 172 in WO2016120216; Identifier 8 in US20160326265 A1	VL	Identifier 2, 6 in WO2015118030; Identifier 171, 173 in WO2016120216; Identifier 9 in US20160326265A1
FcRL5 (FcReceptorLike 5)	VH	Identifier 12, 16, 20, 24, 28, 32, 36, 4, 40, 44, 48, 8, 915, 919 in WO2016090337	VL	Identifier 11, 15, 19, 23, 27, 3, 31, 35, 39, 43, 47, 7, 917, 921 WO2016090337
FGFR3	VH	Identifier 132, 134, 136 in US9499623	VL	Identifier 133, 135, 137, 139 in US9499623
Frizzled Receptor	VH	Identifier 10 in WO2010037041	VL	Identifier 12, 14 in WO2010037041
GAH	VH	Identifier 7 in US20060057147A1	VL	Identifier 8 in US20060057147A1
GCC1	VH	Identifier 1 in US20160030595A1	VL	Identifier 4, 2 in US20160030595A1
GD2	VH	Identifier 17 in WO2016134284; Identifier 1 in US20130216528; Identifier 10, 9 in WO2015132604; Identifier	VL	Identifier 10, 2, 5, 7, 9 in US20130216528; Identifier 11, 12 in WO2015132604; Identifier 18 in WO2016134284

		3, 4, 6, 8 in US20130216528		
GD3	VH	Identifier 11, 13, 15, 17 in WO2016185035A1	VL	Identifier 12, 14, 16, 18 in WO2016185035A1
Glyco epitope and ErbBB I Specific	VH	Identifier 7 in WO2012007167A1	VL	Identifier 10 in WO2012007167A1
GM2	VH	Identifier 20, 22, 23, 26, 27, 28, 29, 30 in US20090028877	VL	Identifier 21, 24, 25, 31, 32, 33, 34, 35 in US20090028877
GPC3	VH	Identifier 10, 14, 2, 3, 4, 5, 6, 7, 8, 9 in US20160208015 A1; Identifier 22 in WO2016049459; Identifier 12, 16, 20, 37, 8 in US9409994B2	VL	Identifier 10, 14, 18, 22, 24, 26 in US9409994B2; Identifier 16, 31 in US20160208015A1; Identifier 23 in WO2016049459
GPDL1	VH	Identifier 20 in US20160108123	-	-
GPRC5D	VH	Identifier 13, 17, 21, 25, 29, 314, 326, 33, 338, 350, 362, 37, 374, 386, 41, 45, 49, 5, 53, 57, 61, 65, 69, 73, 77, 81, 85, 89, 93, 1, 9 in WO2016090312	VL	Identifier 10, 14, 18, 2, 22, 26, 30, 303, 315, 327, 339, 34, 351, 363, 375, 38, 387, 42, 46, 50, 54, 58, 6, 62, 66, 70, 74, 78, 82, 86, 94 in WO2016090312
Her2	VH	Identifier 141, 262, 264, 266, 268, 270 in WO2016168773A3; Identifier 11 in US9518118; Identifier 62 in US20160333114A1; Identifier 19, 24 in US9518118	VL	Identifier 140 in WO2016054555A2; Identifier 261, 263, 265, 267, 269 in WO2016168773A3; Identifier 10, 18, 23 in US9518118; Identifier 3 in WO2016168769A1; Identifier 59, 61 in US20160333114A1
Her1/her3	VH	Identifier 8 of WO2016073629	VL	Identifier 4 of WO2016073629
HLAG	VH	Identifier 10, 8 in WO2016160622A2	VL	Identifier 18, 20 in WO2016160622A2
HSP70	VH	Identifier 11, 12 in WO2016120217	VL	Identifier 16, 17 in WO2016120217
Human collagen VII	VH	Identifier 31 in WO2016112870	VL	Identifier 32 in WO2016112870
Human CD79b	VH	Identifier 27 in WO2016112870 humanCD79b VH 2304	VL	Identifier 28, 30 in WO2016112870

		Identifier 29 in WO2016112870		
Human ERBB3	VH	Identifier 19, 29, 38, 45, 55, 61, 9 in WO2013052745	VL	Identifier 10, 20, 30, 39, 46, 56, 62 in WO2013052745
ICOS	VH	Identifier 15, 16, 19, 23, 7 in US20160215059	VL	Identifier 17, 18, 20, 24, 8 in US20160215059
IGFI	VH	Identifier 1, 3, 7 in WO2007118214; Identifier 7 in WO2015073575	VL	Identifier 2, 4, 6, 8 in WO2007118214
IGFR1	VH	Identifier 7 in WO2015073575 A2	VL	Identifier 8 in WO2015073575 A2
IL13	VH	Identifier 302 in US20160168242	VL	Identifier 303 in US20160168242
IL13Ra2	VH	Identifier 7, 8 in WO2016123143	-	-
IL21	VH	Identifier 2, 3 in US20160145332	-	-
IL33	VH	Identifier 134, 136, 138, 185, 187, 189, 216, 218, 220, 221,236, 246, 282, 284, 286, 36, 38, 40, 84, 86, 88 in US20160168242	VL	Identifier 135, 137, 139, 184, 188, 217, 219, 237, 247, 283, 285, 287, 37, 39, 41, 87 in US20160168242
IL3alpha	VH	Identifier 22 in WO2008127735	VL	Identifier 27, 37 in WO2008127735
IL4R	VH	Identifier 10, 11, 14, 15, 9 in WO2009121847	VL	Identifier 13, 7, 8 in WO2009121847
ILIRAP	VH	Identifier 1, 10, 19, 8, 9 in WO2016020502; Identifier 120, 122, 124 in WO2016179319A1	VL	Identifier 14, 15, 17, 18, 2, 20 in WO2016020502; Identifier 121, 123, 125 in WO2016179319A1
Integrin	VH	Identifier 3, 4, 5 in US 20140161794	VL	Identifier 10, 11, 8, 9 in US 20140161794
KDR	VH	Identifier 20, 24, 26, 29, 31, 33 in WO2003075840	VL	Identifier 22 in WO2003075840
KIR (Lirilumab)	VH	Identifier 3 in US20150290316; 2371 Identifier 1 in WO2014055648	VL	Identifier 5 in US20150290316; Identifier 2 in WO2014055648
KIR2DL 1	VH	Identifier 36 in WO2016126213A1	VL	Identifier 37 in WO2016126213A1
KIR2DL 2/3	VH	Identifier 36 in WO2016126213A1	VL	Identifier 37 in WO2016126213A1
Klon43	VH	Identifier 47 in WO2016097231	VL	Identifier 48 in WO2016097231

KMA	VH	Identifier 22 in WO2016172703 A2	VL	Identifier 2, 21 in WO2016172703A2
LAG3	VH	Identifier 102, 106, 110, 113, 122, 18, 30, 66, 70, 74, 78 in US20150259420; Identifier 100, 104, 108, 28, 64, 68, 72, 76, 8, 80 in US20150259420; Identifier 1 in WO2015042246	VL	Identifier 32, 36, 40, 44, 48, 52, 56, 60, 84, 88, 92, 96, 134, 34, 38, 42, 46, 50, 54, 58, 60, 86, 90, 94, 98 in US20150259420; Identifier 2 in WO2015042246
Leukocytegen A2	VH	Identifier 25 in WO2010065962 A2	-	-
Leukocytegen A	VH	Identifier 9 in WO2010065962 A2	VL	Identifier 24 in WO2010065962 A2
LGR4	VH	Identifier 12, 13, 5, 9 in US20160046723	VL	Identifier 10, 11, 6 in US20160046723
LGR5	VH	Identifier 10, 12, 16, 18, 20, 22, 24, 26, 4 in US20160102146	VL	Identifier 15, 19, 21, 23, 25, 3 in US20160102146
LHR	VH	Identifier 1, 2, 3, 4, 5, 6, 7, 8 in WO2016160618A3	-	-
Lymphotoxin beta receptor	VH	Identifier 10, 12, 14, 16, 2 in WO2004002431	VL	Identifier 1, 15, 4, 6, 8 in WO2004002431
Lysyloxidase-like 2	VH	Identifier 42, 44 in WO2011097513	VL	Identifier 43, 45 in WO2011097513
Malignant Variable Receptor	VH	Identifier 1 in WO2015133817A1	VL	Identifier 5 in WO2015133817A1
MCAM	VH	Identifier 115, 116, 117, 118, 119, 157, 158, 159, 160, 161, 178, 179 in US20150259419; Identifier 35, 45, 55, 65, 77, 89 in US20150239980; Identifier 101, 102, 103, 104, 105, 106, 107 in US20150259419	VL	Identifier 109, 110, 111, 112, 121, 122, 123 in US20150259419; Identifier 30, 40, 50, 60, 70, 71, 72 in US20150239980
MCSF	VH	Identifier 102, 10, 14, 18, 2, 22, 26, 30, 34, 38, 46, 50, 54, 58, 6, 66, 70, 74, 78, 82, 86, 90, 94, 98 in WO2005030124	VL	Identifier 8, 32, 52, 60, 28, 36, 4, 44, 48, 56, 62, 12, 16, 20, 24 in WO2005030124
Mesothelin	VH	Identifier 1, 6 in WO2015188141; Identifier 119, 50 in US20160333114A1; Identifier 5, 6 in	VL	Identifier 3, 5 WO2015188141; Identifier 1, 2, 3 in WO2013142034; Identifier 11, 15, 19, 23, 27 in US20160229919A1;

		WO2013142034; Identifier 15, 2 in US9416190B2; Identifier 13, 17, 21, 25, 29, 9 in US20160229919A1		Identifier 120, 47, 49 in US20160333114A1
MN	VH	Identifier 133, 135, 137, 139, 141, 143, 145, 147, 149, 151 in WO2007070538	VL	Identifier 134, 136, 138, 140, 142, 144, 146, 148, 150, 152 in WO2007070538
MPER	VH	Identifier 13 in US20160194375A1	VL	Identifier 12 in US20160194375A1
MUC1	VH	Identifier 5 in US20160130357; Identifier 2, 14 in WO2013023162; Identifier 15, 19, 23, 60, 64, 68 in WO2015116753	VL	Identifier 7 in US20160130357; Identifier 16, 7 in WO2013023162; Identifier 17, 21, 25, 62, 66, 70 in WO2015116753
MUC16	VH	Identifier 1, 21, 41, 81, 61 in WO2016149368; Identifier 11, 4, 6, 8 in US20130171152	VL	Identifier 2, 22, 42, 62, 82 in WO2016149368
MUC1C ECD	VH	Identifier 15, 19, 23, 60, 64, 68, 72 in US20160340442A1	VL	Identifier 17, 21, 25, 62, 66, 70, 75 in US20160340442A1
MUCIN1	VH	Identifier 101, 106, 109, 115, 119, 123, 127, 141, 15, 23, 28, 33, 39, 42, 47, 5, 57, 66, 70, 75, 80, 83, 87, 92 in EP3049812A2	VL	Identifier 148, 158, 162, 167, 170, 174, 184, 190, 193, 203, 208, 211, 220, 225, 229, 234, 242, 246, 250, 255, 261, 270, 275, 279, 283, 291, 297, 303, 308, 315, 319, 323, 333, 340 in EP3049812A2
MVR	VH	Identifier 1 in US20160257762A1	VL	Identifier 5 in US20160257762A1
N Glycan	VH	Identifier 7, 9 in US20160194375A1	VL	Identifier 6, 8 in US20160194375A1 N
NKG2A	VH	Identifier 32 in WO2016126213A1; Identifier 2, 3, 4, 5, 6 in WO2016041947	VL	Identifier 33 in WO2016126213A1; Identifier 7 in WO2016041947
NKG2D	VH	Identifier 135, 137 in WO2016122701	VL	Identifier 134, 136 in WO2016122701
NOTCH 1	VH	Identifier 58 in US20160333114A1; Identifier 12 in WO2013074596	VL	Identifier 16, 20 in WO2013074596; Identifier 55, 57 in US20160333114A1
NOTCH 2/3	VH	Identifier 29 in WO2013074596	VL	Identifier 31 in WO2013074596

Notum	VH	Identifier 56, 331 in WO2012027723	VL	Identifier 332, 58 in WO2012027723
NYBR1	VH	Identifier 19 in US20160333422A1	VL	Identifier 18 in US20160333422A1
OlfmB	VH	Identifier 1 in WO2015054441A1	VL	Identifier 2 in WO2015054441A1
Olfml3	VH	Identifier 19, 3 in WO2015054441A1	VL	Identifier 20, 4 in WO2015054441A1
Oncofetal fibronectin	-	-	VL	Identifier 1, 2, 7 in US20070202103A1
Osteonectin	VH	Identifier 58 in WO2016112870	VL	Identifier 59 in WO2016112870
OTK3	VH	Identifier 17 in WO2015158868	VL	Identifier 18 in WO2015158868
OX40	VH	Identifier 101, 103, 105, 107, 109, 111, 113, 115, 117, 119, 121, 123, 124, 125, 17, 28, 318, 37, 48, 50, 58, 66,, 74, 85, 93, 95, 97, 99 in WO2016196228; Identifier 31, 34, 36, 38, 40, 42, 44, 46, 48, 50, 53, 54, 55, 58, 59, 61 in US20150190506; Identifier 33, 35, 37, 39, 41, 43, 45, 47, 49, 51, 53, 55, 57, 59, 61, 63, 65, 67, 71 in US20160137740; Identifier 44, 46, 48, 7, 9 in US8283450; Identifier 9, 15 in US9428570; Identifier 19, 21, 22, 23, 29, 58, 59, 7, 77, 78, 79, 80 in US8748585	VL	Identifier 10, 45, 47, 49, 8 in US8283450; Identifier 11, 7 in US9428570; Identifier 116, 120, 122, 30, 38, 49, 57, 65, 73, 84, 86, 94, 98 in WO2016196228; Identifier 24, 26, 27, 28, 30, 60, 8, 81, 82, 83, 84, 85, 86, 87, 88, 89 in US8748585; Identifier 30, 32 in US20160137740; Identifier 32, 35, 39, 41, 43, 45, 47, 49, 51, 52, 56, 57, 62 in US20150190506; Identifier 29, 37 in US20160137740
PD1	VH	Identifier 19 in US20150290316; Identifier 25, 26, 27, 28, 29 in US20130291136; Identifier 29, 3, 38 in US 20160159905; Identifier 38, 50 in WO2015112900; Identifier 4, 4, 6 in US 20160159905; Identifier 82, 86 in WO2015112900; Identifier 17 in WO2014055648; Identifier	VL	Identifier 2, 39, 7, 8, 9 in US 20160159905; Identifier 21 in US20150290316; Identifier 30, 31, 32, 33 in US20130291136; Identifier 42, 46, 54 in WO2015112900; Identifier 58, 62, 66, 70, 74, 78 in WO2015112900; Identifier 18 in WO2014055648; Identifier 5 in

		4 in WO2016040892; Identifier 12 in US20150190506		WO2016040892; Identifier 13 in US20150190506
PDK1	VH	Identifier 2 in WO2016090365	VL	Identifier 9 in WO2016090365
PDl (Nivolumab)	VH	Identifier 2 in WO2016040892; Identifier 10 in US20150190506	VL	Identifier 11 in US20150190506
PDL1	VH	Identifier 10, 32, 8 in US20160319022; Identifier 18, 30, 38, 46, 50, 54, 62, 70, 78 in WO2016061142; Identifier 29, 7 in US20150190506; Identifier 16, 18, 197, 247, 248, 250, 251, 252, 253, 254, 255, 256, 257, 258, 259, 260, 30, 308, 310, 312, 319, 32, 324, 339, 356, 38, 40, 46, 48, 50, 52, 54, 6, 62, 70, 72, 78, 80, 91, 96 in US20160108123; Identifier 358, 56, 64 in US20160108123	VL	Identifier 22, 26, 34, 42, 58, 66, 74, 82, 86 in WO2016061142; Identifier 30, 8, 9 in US20150190506; Identifier 7, 9 in US20160319022; Identifier 17, 22, 24, 249, 26, 28, 309, 311, 313, 320, 325, 34, 340, 357, 359, 36, 42, 44, 58, 60, 66, 68, 74, 76, 8, 82, 84, 86, 88 in US20160108123
PDL2	VH	Identifier 43, 44, 56, 46 in US20130291136	VL	Identifier 47, 48, 49, 50, 51 in US20130291136
PG16	VH	Identifier 13 in EP3074419A2	VL	Identifier 12 in EP3074419A2
PG9	VH	Identifier 11 in EP3074419A2	VL	Identifier 10 in EP3074419A2
PGT1	VH	Identifier 15 in EP307441	-	-
PGT2	VH	Identifier 17 in EP3074419A2	VL	Identifier 16 in EP3074419A2
PGT3	VH	Identifier 19 in EP3074419A2	VL	Identifier 18 in EP3074419A2
PGT4	VH	Identifier 21 in EP3074419A2	VL	Identifier 20 in EP3074419A2
PGT5	VH	Identifier 23 in EP3074419A2	VL	Identifier 22 in EP3074419A2
PRAME	VH	Identifier 50, 52, 54, 56, 58, 60, 62 in WO2016191246A2	VL	Identifier 49, 51, 53, 55, 57, 59, 61 in WO2016191246A2
PRP	VH	Identifier 42 in US20160333114A1	VL	Identifier 39, 41 in US20160333114A1
PSMA	VH	Identifier 43 in WO2016097231	VL	Identifier 44 in WO2016097231; Identifier 44 in WO2016097231

PTK7	VH	Identifier 1, 25, 49 in US20150315293; Identifier 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49, 51, 53, 55, 57, 59, 61, 63, 65, 67, 69 in WO2012112943A1	VL	Identifier 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, 64, 66, 68 in WO2012112943A1; Identifier 15, 39, 63 in US20150315293
RAS	VH	Identifier 17, 47, 57, 67, 7, 77 in WO2016154047	VL	Identifier 19, 49, 59, 69, 79, 9 in WO2016154047
RHAMM	VH	Identifier 4 in US20020127227A1	-	-
RHAMM antagonist body	VH	Identifier 2 in WO2000029447	VL	Identifier 4 in WO2000029447
Rituximab	VH	Identifier 66 in US20160333114A1	VL	Identifier 63, 65 in US20160333114A1
ROR1	VH	Identifier 12, 20, 28, 36, 44, 60, 68 WO2016016343A1; Identifier 57, 19, 31, 45, 53, 71 in WO2016016344A1; Identifier 85, 87, 89 in WO2016120216; Identifier 122, 125, 175, 176, 179, 180, 181, 182, 183, 184, 185, 186, 187, 188, 189, 190, 191, 192, 193, 194, 195, 196, 197, 197, 199, 200, 201, 202, 203, 204, 205, 206, 207, 208, 209 in US20160208018A1; Identifier 55 in EP3083671A1; Identifier 104, 112, 120, 128, 152, 16, 160, 168, 176, 184, 192, 200, 208, 216, 224, 232, 24, 240, 248, 256, 264, 272, 280, 288, 296, 304, 312, 32, 320, 336, 344, 352, 360, 40, 48, 56, 64, 72, 8, 80, 88 in WO2016187216A1	VL	Identifier 16, 24, 32, 40, 56, 64, 72, 36, 62, 23, 49, 58 WO2016016343A1; Identifier 86, 88, 90 in WO2016120216; Identifier 126, 127, 234, 235, 236, 237, 238, 240, 241, 242, 243, 244, 245, 246, 247, 248 in US20160208018A1; Identifier 56 in EP3083671A1; Identifier 103, 111, 127, 135, 143, 15, 151, 159, 167, 175, 183, 191, 199, 207, 215, 223, 23, 231, 239, 247, 255, 263, 271, 279, 287, 295, 303, 31, 311, 319, 327, 335, 343, 351, 359, 39, 47, 55, 63, 7, 71, 79, 87, 95 in WO2016187216A1
SEMAPHORIN 4D	VH	Identifier 10, 25, 9 in US20160115240A1	VL	Identifier 17, 18, 29 in US20160115240A1
TAG72	VH	Identifier 115 in US20160333114A1	VL	Identifier 116 in US20160333114A1
TCR	VH	Identifier 133 in WO2016122701	VL	Identifier 132 in WO2016122701

TEM8	VH	Identifier 1, 3, 5, 7 in US20160264662A1	VL	Identifier 4, 6, 8 in US20160264662A1
Tie	VH	Identifier 723 in US20060057138A1	VL	Identifier 724 in US20060057138A1
TIGIT	VH	Identifier 10, 11, 12, 124, 125, 126, 127, 128, 129, 13, 136, 138, 14, 143, 144, 149, 15, 150, 16, 17, 18, 19, 20, 21, 22, 23, 24, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 63, 94, 7, 9 in US20160355589	VL	Identifier 130, 131, 132, 133, 137, 139, 145, 146, 151, 152, 25, 26, 27, 28, 29, 30, 50, 51, 52, 64, 95, 8 in US20160355589
TIM3	VH	Identifier 102, 112, 12, 2, 22, 32, 42, 52, 62, 72, 82, 91 in US20150086574; Identifier 82 in WO2013006490; Identifier 13, 21, 29, 37, 45, 5, 53, 61, 69, 77, 85, 93 in WO2016179319A1; Identifier 7 in WO2013006490; Identifier 107, 117, 17, 27, 37, 47, 57, 67, 7, 77, 87, 97 in US20150086574; Identifier 17, 25, 33, 41, 49, 57, 65, 73, 81, 89, 9, 97 in WO2016179319A1	-	-
Tissue factor	VH	Identifier 10, 19, 23, 27, 29, 6 in WO2004094475; Identifier 38 in US20160333114A1	VL	Identifier 25. 31 in US20040229301A1; Identifier 12, 21, 25, 31, 8 in WO2004094475; Identifier 35, 37 in US20160333114A1
Tn Glycopeptide	VH	Identifier 19, 20 in WO2015120180	-	-
TRBC1	VH	Identifier 1 in WO2015132598	VL	Identifier 2 in WO2015132598
Trophoblast Glycoprotein 5T4	VH	Identifier 17, 13, 15, 11 in WO2016034666A1	VL	Identifier 18, 12, 14, 16 in WO2016034666A1
Upar	VH	Identifier 72 in US20160333114A1	VL	Identifier 71, 73 in US20160333114A1
V2	VH	Identifier 11 in US20160194375A1	-	-

VEGF	VH	Identifier 4, 8, in WO2000034337; Identifier 12, 20, 4, 44 in WO2006012688A1; Identifier 7 in US20030175276A1; Identifier 152, 153, 154, 155, 156, 157, 158, 159 in US20160090427	VL	Identifier 2, 6 in WO2000034337; Identifier 9 in US20030175276A1; Identifier 11, 19, 27, 28, 3, 43 in WO2006012688A1; Identifier 160, 161, 162, 163, 164, 165, 166, 167 in US20160090427
VEGFR2	VH	Identifier 100, 101, 102, 103, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 95, 96, 97, 98, 99 in WO2017004254	VL	Identifier 107, 108, 109, 110, 111, 112, 113, 86, 87, 88, 89, 90, 91, 92, 93, 94 in WO2017004254
VISTA	VH	Identifier 37, 38, 39, 40 in WO2015097536	VL	Identifier 41, 42, 43, 44, 45 in WO2015097536
VMS2	VH	FIG. 1 in WO2000058363	-	-
WT1/HLA Bispecific	VH	Identifier 104, 111, 128, 14, 32, 50, 68, 86 in WO2015070061	VL	Identifier 106, 112, 130, 34, 52, 70, 88 in WO2015070061

[001] Table 2 provides exemplary antigen targets. The antigen-binding domain may comprise an scFv derived from an antibody or antibody fragment that binds to an antigen target such as those described in the cited publications, the contents of each publication are incorporated herein by reference in their entirety for all purposes.

TABLE 2

Antigen Target	**Examples of Source**
Activated alpha-v-beta-3 integrin receptor	Identifier 8, 2, 4 in US20090117096A1
Adalimumab	Identifier 41 in US20160208021; Identifier 41 in WO2016112870
ALK	Identifier 17, 18, 19, 20, 21, 22, 23 in WO2015069922; Identifier 17, 18, 19, 20, 21, 22, 23, 24 in US20160280798A1; Identifier 24 in WO23015069922
B7H3	Identifier 99, 100, 101, 102, 103, 104, 102, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 87, 88, 89, 90, 91, 92, 94, 95, 96, 97, 98 in WO2016033225
B7H4	Identifier 1, 2, 3, 4 in WO2013067492; Identifier 1 in US9422351B2
BCMA	Identifier 152, 158, 176, 185, 188, 200, 212, 218, 224, 284, 290, 296, 302, 314, 326, 344, 129, 130, 131, 132, 133, 134, 135, 136, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, 263, 264, 265, 266, 271, 273, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 64, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146,

	147, 148, 149, 263, 264, 265, 266, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53 in WO2016014565; Identifier 214, 215, 216, 217, 218, 219, 220, 221, 222, 223, 224, 225, 226, 227, 228, 229, 230, 231, 232, 233, 234, 235, 236, 237, 238, 239, 240, 241, 242, 243, 244, 245, 246, 247, 248, 249, 251 in US20160311907A1
CCR4	Identifier 7, 9 in WO2015191997
CD123	Identifier 157, 158, 159, 160, 184, 185, 186, 187, 188, 189, 190, 191, 192, 193, 194, 195, 196, 197, 198, 199, 200, 201, 202, 203, 204, 205, 206, 207, 208, 209, 210, 211, 212, 213, 214, 215, 478, 480, 483, 485 in WO2016028896; Identifier 36 in WO2015092024A2; Identifier 57 in WO2016115482A1; Identifier 36 in EP3083691A2; Identifier 157 in US20160311907A1
CD124	Identifier 158 in US20160311907A1
CD125	Identifier 159 in US20160311907A1
CD126	Identifier 160 in US20160311907A1
CD127	Identifier 161 in US20160311907A1
CD128	Identifier 162 in US20160311907A1
CD129	Identifier 163 in US20160311907A1
CD130	Identifier 164 in US20160311907A1
CD131	Identifier 165 in US20160311907A1
CD138	Identifier 36 in WO2016130598A1
CD19	Identifier 53, 54, 37 in EP3083671A1; Identifier 1, 10, 11, 12, 2 in WO2015157252; Identifier 10, 2, 206, 207, 208, 209, 210, 211, 213, 214, 215, 216, 217, 218, 219, 220, 221, 222, 223, 224, 225, 4, 45, 47, 49, 51, 53, 55, 57, 51, 53, 55, 57, 59, 6, 8, 87 in WO2016033570; Identifier 3, 4, 5, 59, 6, 7, 8, 9 in WO2015157252; 5754 Identifier 5 in WO2015155341A1; Identifier 7 in WO2014184143; Identifier 9 in WO2016139487; Identifier 10, 2, 206, 207, 208, 209, 210, 211, 212, 213, 214, 215, 216, 217, 218, 219, 220, 221, 222, 223, 224, 225, 4, 45, 47, 49, 51, 53, 55, 57, 59, 6, 8, 87, 89 in US20160152723; Identifier 32, 35, 38 in EP3083691A2; Identifier 174 in WO2016115482A1; Identifier 20 in WO2012079000; Identifier 32, 33, 35, 38 in WO2015092024A2; Identifier 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51 in WO2016109410; Identifier 5, 6 in WO2015155341A1; Identifier 7, 9 in US20160145337A1; Identifier 20 in US9499629B2; Identifier 73 in WO2016164580; Identifier 10, 2, 206, 207, 209, 210, 212, 216, 218, 219, 220, 221, 222, 223, 224, 225, 4, 45, 47, 49, 51, 53, 55, 57, 59, 6, 8, 87, 89 in US20160152723; Identifier 5 in WO2016055551
CD19/CD22 Bispecific	Identifier 1303, 1307 in WO2016164731A2
CD20	Identifier 691 in WO2016164731A100; Identifier 692 in WO2016164731A101; Identifier 693 in WO2016164731A102; Identifier 694 in WO2016164731A103; Identifier 695 in WO2016164731A104; Identifier 696 in WO2016164731A105; Identifier 175 in WO2016115482A1

CD22	Identifier 5, 6 in WO2013059593; Identifier 9 in US20150299317; Identifier 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 203, 209, 215, 221, 227, 232, 238, 244, 250, 256, 262, 268, 274, 280, 286, 292, 298, 304, 310, 316, 322, 328, 334, 340, 346, 353, 358, 364, 370, 376, 383, 388, 394, 400, 406, 412, 418, 423 in WO2016164731A2
CD276	Identifier 10, 19, 28 in US20160053017
CD3	Identifier 46, 47 in WO2015153912A1
CD30	Identifier 20 in WO2016116035A1; Identifier 2 in US20160200824A1
CD33	Identifier 262, 263, 264, 265, 266, 267, 268, 39, 40, 41, 42, 43, 44, 45, 46, 47 in WO2016014576; Identifier 37 in WO2015092024 A2; Identifier 37 in EP3083691A2; Identifier 153, 154, 155, 156, 157, 158, 159, 160, 161, 162, 163 in WO2016115482A1
CD33/CD3s bispecific	Identifier 33, 34, 84 in WO2014144722A2
CD37	Identifier 21, 22 in US20170000900
CD44	Identifier 17 in WO2016042461A1
CD46	Identifier 1-42 in WO2016040683
CD5	Identifier 16 in WO2016138491
CD79b	Identifier 33 in US20160208021
CEA	Identifier 1 in US20160303166A1; Identifier 22 in US20140242701A1
Cetuximab	Identifier 37 in WO2016112870; Identifier 37 in US20160208021
Claudin	Identifier 11, 5, 7, 9 in WO2016073649A1; Identifier 17 in WO2014179759A1
Claudin6	Identifier 164 in WO2016115482A1
Claudin7	Identifier 165 in WO2016115482A1
Claudin8	Identifier 166 in WO2016115482A1
CLDN6	Identifier 2 in WO2016150400
CLDN7	Identifier 4 in WO2016150400
CLDN8	Identifier 6 in WO2016150400
CLL1	Identifier 39, 40, 41, 42, 43, 44, 45, 46, 48, 49, 50, 51 in WO2016014535; Identifier 200, 201, 202, 203, 204, 205, 206, 207, 208, 209, 210, 211, 212, 213 in US20160311907A1
CMet	Identifier 11, 12, 13, 14, 15, 16, 17, 18, 19, 2, 21, 22, 23, 25, 26, 27, 28, 3, 30, 31, 33, 34, 35, 36, 37, 38, 39, 4; 40, 41, 42, 43, 44, 48, 49, 5, 50, 51, 52, 53, 54, 55, 56, 57, 58, 6, 60, 7, 9, 29 in US20040166544; Identifier 26, 27, 28, 29, 30, 32 in US20150299326; Identifier 32 in US20130034559
CS1	Identifier 1 of WO2016090369; Identifier 17 in WO2014179759A1
CSPG4	Identifier 2 in WO2015080981; Identifier 2 in EP3074025A1
CXCR4	Identifier 83, 85, 86, 89 in US20110020218
E7MC	Identifier 223, 224, 225, 226, 227, 228, 229, 230, 231, 232 in WO2016182957A1
EGFR	Identifier 11, 38, 41, 44, 47, 50, 53, 56, 59, 62, 65, 68, 71, 74, 77, 80, 83, 88, 91, 94 in WO2014130657
EGFR VIII	Identifier 5 in US20140037628; Identifier 174 in US20160311907A1; Identifier 38 in US9394368B2; Identifier 5 in US20160200819A1

END 0180	Identifier 6 in WO2013098813
ERBB2	Identifier 26, 27 in US20110059076A1; Identifier 1, 2 in US7244826
ESKAVT	Identifier 173 in WO2016115482A1
FcRL	Identifier 11, 15, 19, 23, 27, 31, 35, 39, 3, 43, 7, 594, 596, 598, 600, 602, 604, 606, 608, 610, 612, 614, 616, 618, 620, 622, 624, 626, 628, 630, 632, 634, 636, 638, 640, 642, 644, 646, 648, 652, 654, 656; 658, 660, 662, 664, 666, 668, 670, 672, 674, 676, 680, 682, 684, 686, 688, 690, 692, 694, 696, 700, 702, 704, 706, 708, 710, 712, 714, 716, 718, 720, 722, 724, 726, 728, 730, 732, 734, 736, 738, 740, 742, 744, 746, 748, 750, 752, 754, 756, 758, 760, 762, 764, 766, 768, 770, 772, 774, 776, 778, 780, 782, 784, 786, 788, 790, 792, 794, 796, 798, 800, 802, 804, 806, 808, 810, 812, 814, 816, 818, 820, 822, 824, 826, 828, 830, 832, 834, 836, 838, 840, 842, 844; 846, 848, 850, 852, 854, 856, 858, 860, 862, 864, 866, 868, 870, 874, 876, 878, 880, 882, 884, 886, 888, 890, 892, 894, 896, 650, 678 in WO2016090337
Folate receptor	Identifier 15 in US20170002072A1
Folate receptor alpha	Identifier 15, 23 in WO2012099973
FOLRl/CD3s bispecific	Identifier 90 in WO2014144722A2
GCN4	Identifier 165, 166, 167, 168, 169, 170 in WO2016168773 A3
GD2	Identifier 19, 20, 21, in WO2016134284; Identifier 8 in WO2015132604
GPC3	Identifier 1 in WO2016049459; Identifier 12 in US20160208015A1
GPC4	Identifier 24 in WO2016049459
GPRC5D	Identifier 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123,301, 313, 325, 337, 349, 361, 373, 385 in WO2016090312
HER2/CD3	Identifier 9 in WO2014144722 A2
Human CD79b	Identifier 33 in WO2016112870
Human collagen VII	Identifier 34 in WO2016112870
IL4	Identifier 17, 16 in WO2009121847
Integrin bivalent	Identifier 2, 1 in WO2009070753
Ipilimumab	Identifier 39 in US20160208021; Identifier 39 in WO2016112870
Mec/CD3s bispecific	Identifier 78 in WO2014144722A2
Mesothelin	Identifier 7 WO2015188141; Identifier NO 47, 46, 57, 48, 49, 50, 51, 53, 54, 55, 56, 58, 59, 62, 64, 65, 66, 67, 68, 69, 70, 52, 60, 61, 63 in WO2016090034; Identifier 10, 11, 12 in WO2013142034; Identifier 11 in WO2013063419
MUC1	Identifier 15 in US20160130357
MUC2	Identifier 17 in US20160130357
MUC3	Identifier 15 in US20160130357
MUC4	Identifier 17 in US20160130357
Nivolumab	Identifier 38 in US20160208021; Identifier 38 in WO2016112870

NYBR1	Identifier 21 in US20160333422A1; Identifier 21, 18, 19 in WO2015112830
O-acetylated GD2 ganglioside	Identifier 29, 31 in US20150140023
OX40	Identifier 33 in US20150190506
PD1	Identifier 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61 in US20160311917A1
PDK1	Identifier 15 in WO2016090365
PDL1 nanobody	Identifier 22, 23, 24, 25, 26, 27 in US20110129458
PDL2 nanobody 6462	Identifier 28, 29, 30, 31, 32, 33 in US20110129458
PRAME	Identifier 63, 64, 65, 66, 67, 68, 69 in WO2016191246A2
PSMA	Identifier 19, 21, 30, 31, 34, 35 in WO2012145714
PSMA diabody	Identifier 12, 13, 14, 15 in WO2011069019
Radiation inducible neoantigen	Identifier 22, 24 in WO2005042780A1
Ranibizumab	Identifier 40 in US20160208021; Identifier 40 in WO2016112870
RAS	Identifier 81 in WO2016154047
Rituximab	Identifier 36 in US20160208021; Identifier 36 in WO2016112870
RORI	Identifier 34 in EP3083691A2; Identifier 249, 250 251, 252, 253, 254, 255, 256, 257, 258, 259, 260, 261, 262, 263, 264, 265, 266, 267, 268 in US20160208018A1; Identifier 57 in EP3083671A1; Identifier 1, 2 in US20160304619A1; Identifier 34 in WO2015092024A2
Teplizumab	Identifier 42 in WO2016112870
Teplizumab (mutated)	Identifier 42 in US20160208021
TOSO	Identifier 2 in EP3098237A1
Trastuzumab	Identifier 35 in US20160208021; Identifier 35 in WO2016112870
TRBC1	Identifier 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 3 in WO2015132598
TRBC2	Identifier 23, 24, 25, 26, 27, 28, 29, 30, 31, 32 in WO2015132598
TSLPR	Identifier 1, 2 in US20160311910A1; Identifier 1, 2 in WO2015084513
VEGF	Identifier 168, 169, 170, 171, 172, 173, 174, 175 in US20160090427; Identifier 498, 500, 502, 504, 506, 508 in US20110177074A1
VEGFR2	Identifier 1, 2 in US20120213783
WT1/HLA bispecific	Identifier 108, 113, 18, 36, 54, 72, 90 in WO2015070061

[002] Table 3 provides exemplary antigen targets. The antigen-binding domain may comprise an antigen-binding domain derived from a CAR that binds to an antigen target, such as those described in the cited publications, the contents of each publication are incorporated herein by reference in their entirety for all purposes.

TABLE 3

Antigen Target	Examples of Source
Acid/base leucine zipper domains	Identifier 34, 35 in WO2016124930
ALK	Identifier 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 84, 85, 86, 87, 88, 89, 90 in WO2015069922
APRIL-based CAR	Identifier 53 in US20160296562A1; Identifier 52 in US20160296562A1
BCMA	Identifier 180, 162, 168, 174, 144, 150, 186, 192, 198, 204, 210, 156, 216, 222, 228, 234, 240, 246, 252, 258, 264, 270, 276 330, 282, 300, 306, 336, 354, 288, 312, 294, 342, 324, 318, 348 in WO2016168595A1; Identifier 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42 in WO2015158671A1; Identifier 124, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 125, 126, 127, 128, 234, 235, 236, 237, 238, 239, 240, 241, 242, 243, 244, 245, 246, 247, 248, 249, 250, 251, 252, 253, 254, 267, 268, 269, 270 in WO2016014565; Identifier 1, 2, 3, 4, 5, 20 in WO2015052538; Identifier 1, 2, 3, 4, 5, 6 in US20160237139A1; Identifier 9 in WO2016094304 A3; Identifier 4, 5, 6, 8, 9, 10, 11, 12 in WO2013154760; Identifier 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 71, 73 in WO2016014789; Identifier 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 145, 146, 147, 148, 149, 150 in WO2016120216; Identifier 102, 106, 107, 108, 109, 110, 111, 112, 129, 130, 131, 132, 133, 134, 135, 136, 113, 114, 115, 116, 117, 118, 101, 100, 137, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 103, 104, 105, 213 in WO2016097231
CAR and gate (CD19 and CD33) CD148 phosphatase	Identifier 2 in US20160296562
CAR and gate (CD19 and CD5)	Identifier 43 in US20160296562
CAR and gate (CD19 and EGFR VIII)	Identifier 45 in US20160296562
CAR and gate (CD19 and GD2)	Identifier 41 in US20160296562
CAR and gate (CD19 or CD33) CD45 phosphatase	Identifier 3 in US20160296562
CAR and not gate (CD19 and not CD33)	Identifier 4, 5 in US20160296562
CAR and not gate (CD19 and not CD33)	Identifier 6 in US20160296562A1
CAR and not gate 1	Identifier 48 in US20160296562

CAR and not gate 2	Identifier 49 in US20160296562
CAR and not gate 3	Identifier 50 in US20160296562
CAR or gate (DC19 or DC33)	Identifier 1 in US20160296562
CAT19 CAR with CD28 zeta endodomain	Identifier 12 in WO2016139487
CAT19 CAR with OX40 zeta endodomain	Identifier 11 in WO2016139487
CAT19, campana architecture	Identifier 10 in WO2016139487
CD123	Identifier 69 in WO2016142532; Identifier 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 44, 45, 46, 47, 48 in WO2015140268A1; Identifier 9, 10, 11, 12 in US20140271582; Identifier 56, 57, 58, 59, 60, 61 in WO2016097231; Identifier 98, 99, 100, 101, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, 150, 151, 152, 153, 154, 155, 156 in WO2016028896; Identifier 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, 150, 151, 152, 153, 154, 155, 156, 157, 158, 159, 160, 172, 173, 174, 175, 176, 177, 178, 179, 180, 181, 182, 183, 184, 185, 186, 187, 188, 189, 190, 191, 193, 194, 195, 196, 197 in WO2016120220; Identifier 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 142 in WO2016120216
CD19	Identifier 12 in US9499629B2; Identifier 24 in US20160333108A1; Identifier 25, 29 in US20160333108A1; Identifier 27 in US20160333108A1; Identifier 1 in EP2997134A4; Identifier 19, 20 in EP3071687A1; Identifier 181 in WO2016168773A3; Identifier 2 in WO2015157399A9; Identifier 56, 62 in WO2016174409A1; Identifier 145, 293, 294, 295, 296, 297, 298 in WO2016179319A1; Identifier 73 in WO2013176915A1; Identifier 73 in WO2013176916A1; Identifier 73 in US20130315884A1; Identifier 73 in US20140134142A1; Identifier 73 in US20150017136A1; Identifier 73 in US20150203817A1; Identifier 73 in US20160120905A1; Identifier 73 in US20160120906A1; Identifier 8, 5 in WO2015124715; Identifier 73 in WO2014184744; Identifier 73 in WO2014184741; Identifier 14, 15 in US20160145337A1; Identifier 14, 15 in WO2014184143; Identifier 15, 16 in WO2015075175; Identifier 16 in US20160145337A1; Identifier 16 in

	WO2014184143; Identifier 12 in WO2012079000; Identifier 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 58 in WO2016164580; Identifier 14, 15 in US20160296563A1; Identifier 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42 in WO2015157252; Identifier 14, 15 in WO2016139487; Identifier 53, 54, 55, 56, 57, 58 in US20160250258A1; Identifier 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13 in WO2015187528; Identifier 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 58 in WO2015157252; Identifier 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42 in WO2014153270; WO2016134284 (no Identifier); Identifier 13 in WO2016139487
CD19 or CD33	Identifier 1 in WO2015075468 which recognizes CD19 OR CD33
CD19/CD20 bispecific	Identifier 1308 in WO2016164731A2; Identifier 2, 8, 11 in US9447194B2
CD19/IL13 bispecific	Identifier 10 in US20160340649A1
CD2	Identifier 10, 11 in WO2016138491
CD20	Identifier 25 in WO2015157399A9; Identifier 177, 181, 182, 183, 184, 185, 186, 187, 205, 206, 207, 208, 209, 210, 211, 188, 189, 190, 191, 192, 193, 176, 212, 194, 195, 196, 197, 198, 199, 200, 201, 202, 203, 178, 179, 180 in WO2016097231
CD22	Identifier 380, 204, 260, 266, 272, 278, 284, 290, 296, 302, 308, 341, 213, 320, 326, 332, 338, 347, 350, 356, 362, 368, 374, 219, 386, 392, 398, 404, 410, 416, 421, 427, 225, 230, 1109, 236, 242, 248, 254 in WO2016164731A2; Identifier 15, 16, 17, 18, 19, 20, 32 in WO2013059593; Identifier 22, 23, 24 in US20150299317
CD22/CD19 bispecific	Identifier 29, 30 in WO2016149578; Identifier 1304 in WO2016164731A2
CD276	Identifier 39, 40, 41, 42, 43, 44, 45, 46, 47, 122, 123, 124, 125, 126, 127, 128, 129, 130 in US20160053017
CD3	Identifier 12 in WO2016138491
CD30	Identifier 20 in WO2016008973A1; Identifier 1 in WO2016116035A1; WO2016134284 (no Identifier); Identifier 2 in WO2016008973
CD33	Identifier 48, 49, 50, 51, 52, 53, 54, 55, 83 in WO2016014576; Identifier 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71 in WO2015150526A2
CD38	Identifier 70, 71, 72, 64, 65, 66, 67, 68, 69 in WO2016097231; Identifier 35, 36, 37 in WO2015121454
CD4	Identifier 13, 14 in WO2016138491
CD410	Identifier 7 in EP3074419A2
CD435	Identifier 5 in EP3074419A2
CD44	Identifier 21, 22, 23, 24, 25, 26, 27, 28, 31, 32, 33, 34, 35 in WO2016042461A1
CD4-DDY3	Identifier 9 in EP3074419A2
CD5	Identifier 15, 13 in WO2016138491

CD52	Identifier 18 in WO2016138491
CD7	Identifier 17 in WO2016138491
CD70	Identifier 99 in WO2015121454
CD70	Identifier 100, 93, 94, 96, 101, 95, 97, 98 in WO2015121454
CD8 stalk APRIL	Identifier 51 in US20160296562A1
CEA	Identifier 4 in WO2016008973A1; Identifier 29, 30 in US20140242701A
CLDN6	Identifier 22, 23, 24 in WO2016150400
CLL1	Identifier 148 in WO2016179319A1; Identifier 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112 in WO2016120218; Identifier 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 197 in WO2016014535
COM22	Identifier 358, 359, 360 in US20160297884A1
CS1	Identifier 55, 57, 60, 54, 56, 48, 49, 50, 51, 52, 53, 58, 59, 61, 62 in WO2015121454; Identifier 28 in WO2014179759A1
DDD1/AD1 zip	Identifier 37 in WO2016124930
DDD1/AD1-based zip	Identifier 36 in WO2016124930
EGFR	Identifier 3, 2in WO2014130657; Identifier 36, 37, 38, 39, 35 in US20140242701 A; Identifier 43, 96, 49, 55, 61, 67, 73, 79, 85, 90, 1 in WO2014130657
EGFR vIII	Identifier 15, 16, 17, 18, 24, 25, 26, 27 in WO2016016341; Identifier 5, 10, 12, 8, 31, 30, 3 in US20160311907A1; Identifier 10 in US20160200819A1; Identifier 43, 49, 55, 61, 67, 73, 79, 85, 90, 96 in US9394368B2; Identifier 49, 55, 61, 67, 73, 79, 85, 90, 2, 1 in US20170008963A1; Identifier 10, 11 in US20140037628
FcRL5	Identifier 11 in US20170008963A1
Folate receptor	Identifier 12 in US20170008963A1
FR	Identifier 22 in US20170002072A1
FR beta	Identifier 13, 22 in US9402865B2; Identifier 2, 4, 6 in US9446105B2
FRa	Identifier 13, 14 in US20120213783
Fra	Identifier 959 in WO2016090337; Identifier 13 in US20170002072A1
GCN4	Identifier 8, 10 in US9446105B2
GD2	Identifier 12 in US9446105B2; Identifier 273, 274 in WO2016168773A3; Identifier 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37 in WO2015132604; WO2016134284 (no Identifier)
GD3	Identifier 19 in WO2016185035A1; Identifier 20, 21, 22, 23, 24, 25, 26 in WO2016185035A1; WO2016134284 (no Identifier)
GFR alpha	Identifier 27 in WO2016185035A1
GPC3	Identifier 28, 29 in WO2016185035A1; Identifier 3, 27, 10, 29, 14, 30, 31, 18, 33 in WO2016049459; Identifier 22 in US20160215261A1

HER2	Identifier 25 in US20160215261A1; Identifier 9, 10 of WO2016073629; Identifier 17, 28, 98, 110 in US20160333114A1; Identifier 271, 272 in WO2016168773A3; Identifier 5 in WO2016168769A1
Herl/Her3 bispecific	Identifier 23, 24 in US20160215261A1
HERVK	Identifier 6 in WO2016168769A1
HIV Env	Identifier 48, 49 in WO2016168766A1; Identifier 4 in EP2997134A4; Identifier 7, 9, 47, 49 in WO2015077789
HSP70	Identifier 51, 53, 5 in WO2015077789; Identifier 21, 22, 23, 24, 25, 26, 27, 28, 29 in WO2016120217
IL13	Identifier 30, 31, 32 in WO2016120217
IL13Ra2specific	Identifier 4, 5, 6 in WO2016089916A1; Identifier 47, 49 in WO2016123143; Identifier 51, 53, 55 in WO2016123143; Identifier 1, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45 in US20160340649A1
KMA	Identifier 46 in US20160340649A1
Mesothelin	Identifier 47, 48 in US20160340649A1; Identifier 48 in US20160340649A1; Identifier 27 in WO2016172703A2; Identifier 18, 19, 20, 21, 22, 23 in WO2013142034; Identifier 3 in WO2013067492
MUC1	Identifier 5, 7 in WO2013063419; Identifier 51 in US20160340406A1; Identifier 30, 32, 34 in US20160130357; Identifier 295, 298, 301, 304, 307, 607, 609, 611, 613 in WO2016130726
NCAR with RQR82 ACD 19	Identifier 615 in WO2016130726
NYBR1	Identifier 617, 619 in WO2016130726; Identifier 218 in WO2016097231; Identifier 26, 29, 60 in WO2015112830; Identifier 1 in US20160333422A1
P5A	Identifier 26, 29, 60 in US20160333422A1
P5AC1	Identifier 343, 344, 345, 346 in US20160297884A1
P5AC16	Identifier 347, 396, 348 in US20160297884A1
P6AP	Identifier 349, 350, 351 in US20160297884A1
P6DY	Identifier 364, 365, 366 in US20160297884A1
PC1C12	Identifier 352, 353, 354 in US20160297884A1
PCI	Identifier 361, 362, 363 in US20160297884A1
PD1	Identifier 355, 356, 357 in US20160297884A1; Identifier 119 in WO2014153270; Identifier 121 in WO2014153270; Identifier 22, 24, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86 in US20160311917A1; Identifier 26, 39 in WO2016172537A1; Identifier 40 in US20160311907A1; Identifier 121, 119 in WO2015157252; Identifier 24 in WO2016014565; Identifier 22 in WO2016014565
PD1	Identifier 23 in WO2016014565; Identifier 26 in WO2015142675
PSMA	Identifier 39 in WO2015142675; Identifier 28, 29 in US20160311907A1; Identifier 140, 144, 145, 146, 147, 148, 149,

	150, 167, 168, 169, 170, 171, 172, 173, 174, 151, 152, 153, 154, 155, 156, 139, 138, 175, 157, 158, 159, 160, 161, 162, 163, 164, 165, 166, 141, 142, 143, 214 in WO2016097231
ROR1	Identifier 216, 217, 215 in WO2016097231; Identifier 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 127, 128, 129, 130, 131,132, 133,134, 1335, 136, 137, 138, 97, 98, 99, 100, 101, 102, 121, 122, 123, 124, 125, 126 in WO2016016344A1; Identifier 386, 387, 388, 389, 390, 391, 392, 393, 394 in WO2016187216A1
SNAP	Identifier 395 in WO2016187216A1
SSEA4	Identifier 396, 397 in WO2016187216A1
Tan	Identifier 19 in US20160311907A1 recognizes CD19 AND CD33 using a CD45 phosphatase; Identifier 5 in WO2016026742A1 recognizes CD19 AND CD33 using a CD148 phosphatase; Identifier 6 in WO2016026742A1 which recognizes CD19 AND NOT CD33 and is based on an ITIM containing endodomain from LAIR1; Identifier 3 in WO2015075468 which recognizes CD19 AND NOT CD33 based on PTPN6 phosphatase; Identifier 2 in WO2015075468 which recognizes CD19 AND NOT CD33 and recruits a PTPN6/CD148 fusion protein to an ITIM containing endodomain
TOSO	Identifier 5, 4 in WO2015075468
Trophoblast Glycoprotein 5T4	Identifier 6 in WO2015075468; Identifier 4 in US20160347854A1; Identifier 4 in EP3098237A1; Identifier 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39 in WO2016034666A1
TSLPR	Identifier 40, 41, 42 in WO2016034666A1; Identifier 39, 40, 41, 42, 43, 44, 45, 46 in WO2015084513; Identifier 39, 40, 41, 42, 43 in US20160311910A1
VEGFR2	Identifier 44, 45, 46 in US20160311910A1; Identifier 10, 11, 12 in US20120213783
VNAR	Identifier 105, 106, 107, 108, 109, 110 in US20160333094A1

ii. Linkers

[0179] In some embodiments, an scFv fragment of an extracellular domain of a CAR includes 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 Phe. The linker should have a length that is adequate to connect 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.

[0180] In some embodiments, the linker is a Whitlow linker. In one embodiment, the Whitlow linker includes 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 to SEQ ID NO:3 of PCT/US2021/072646.

[0181] In another embodiment, the linker is a $(G4S)_3$ linker. In one embodiment, the $(G4S)_3$ linker includes 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 to SEQ ID NO:25 of PCT/US2021/072646.

[0182] 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 of PCT/US2021/072646, the disclosure including the sequence listing is incorporated herein by reference. Additional linkers are described for example in WO2019/060695, incorporated by reference herein in its entirety.

[0183] The linkers described herein including SEQ ID NOS: 40-73 of Table 4 can be used in any of the polypeptides provided including those containing CD16, NKG2D, IL-15, IL-15Rα, HLA-E, HLA-G, HSV-TK, PSMA, and the like.

[003] Table 4 provides exemplary linkers (SEQ ID NOS:40-73), which correspond to SEQ ID NOS: 3 and 25-56 of US Application No. 17/657,803 filed April 4, 2022, the contents of which are incorporated herein by reference in its entirety.

TABLE 4

[0184]

Name	Linker Sequence	SEQ ID NO:	SEQ ID NO: of US17/657,803
Whitlow Linker	GSTSGSGKPGSGEGSTKG	40	3
GGGGS or multimer thereof	GGGGS	41	
$(G_4S)_3$	GGGGSGGGGSGGGGS	42	25
Linker 3	GGSEGKSSGSGSESKSTGGS	43	26
Linker 4	GGGSGGGS	44	27
Linker 5	GGGSGGGSGGGS	45	28
Linker 6	GGGSGGGSGGGSGGGS	46	29
Linker 7	GGGSGGGSGGGSGGGSGGGS	47	30
Linker 8	GGGGSGGGGSGGGGSGGGGS	48	31
Linker 9	GGGGSGGGGSGGGGSGGGGSGGGGS	49	32
Linker 10	IRPRAIGGSKPRVA	50	33
Linker 11	GKGGSGKGGSGKGGS	51	34
Linker 12	GGKGSGGKGSGGKGS	52	35
Linker 13	GGGKSGGGKSGGGKS	53	36
Linker 14	GKGKSGKGKSGKGKS	54	37
Linker 15	GGGKSGGKGSGKGGS	55	38
Linker 16	GKPGSGKPGSGKPGS	56	39
Linker 17	GKPGSGKPGSGKPGSGKPGS	57	40
Linker 18	GKGKSGKGKSGKGKSGKGKS	58	41
Linker 19	STAGDTHLGGEDFD	59	42
Linker 20	GEGGSGEGGSGEGGS	60	43
Linker 21	GGEGSGGEGSGGEGS	61	44
Linker 22	GEGESGEGESGEGES	62	45
Linker 23	GGGESGGEGSGEGGS	63	46
Linker 24	GEGESGEGESGEGESGEGES	64	47
Linker 25	GSTSGSGKPGSGEGSTKG	65	48
Linker 26	PRGASKSGSASQTGSAPGS	66	49
Linker 27	GTAAAGAGAAGGAAAGAAG	67	50
Linker 28	GTSGSSGSGSGGSGSGGGG	68	51
Linker 29	GKPGSGKPGSGKPGSGKPGS	69	52
Linker 30	GSGS	70	53
Linker 31	APAPAPAPAP	71	54
Linker 32	APAPAPAPAPAPAPAPAPAP	72	55
Linker 33	AEAAAKEAAAKEAAAAKEAAAAKEAAAAK AAA	73	56

B. Signal peptides

[0185] In some embodiments, a CAR polypeptide includes a signal peptide (e.g., a leader peptide or localization peptide). The signal peptide may be positioned at the N-terminus of the extracellular domain. The signal peptide may be optionally cleaved from the extracellular

domain during cellular processing and localization of the CAR to the cellular membrane. Any of various signal peptide sequences known to one of skill in the art may be used. Non-limiting examples of signal peptides from which the 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 some embodiments, the signal sequence is compatible with the secretory pathway of a T cell. In certain embodiments, the signal sequence is derived from a human immunoglobulin heavy chain.

[0186] In some embodiments, the signal sequence is derived from GMCSFR. In one embodiment, the GMCSFR signal sequence includes 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 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 SEQ ID NO:1 as set forth in PCT/US2021/072646, the disclosure of which is incorporated herein by reference.

C. Transmembrane domains

[0187] In some embodiments, a CAR polypeptide includes a transmembrane domain, fused in frame between an extracellular domain and a cytoplasmic domain.

[0188] The transmembrane domain may be derived from the protein contributing to the extracellular domain, the protein contributing the signaling or co-signaling domain, or by a completely different protein. In some embodiments, the transmembrane domain is selected or modified by amino acid substitution, deletions, or insertions to minimize interactions with other members of the CAR polypeptide. In some instances, the transmembrane domain is selected or modified by amino acid substitution, deletions, or insertions to avoid binding of proteins naturally associated with the transmembrane domain. In some embodiments, the transmembrane domain includes additional amino acids to allow for flexibility and/or optimal distance between the domains connected to the transmembrane domain.

[0189] 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 may be derived from (i.e. comprise at least the transmembrane region(s) of) the α, β or ζ chain of the T-

cell receptor (TCR), CD28, CD3 epsilon, CD45, CD4, CD5, CD8, CD8α, CD9, CD16, CD22, CD33, CD37, CD40, CD64, CD80, CD86, CD134, CD137, or CD154. In some instances, the transmembrane domain may be synthetic, in which case it may include 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.

[0190] In some embodiments, it is desirable to utilize the transmembrane domain of the ζ, η 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, η or FcεR1γ chains or related proteins. In some embodiments, the transmembrane domain is selected or modified by amino acid substitution to avoid binding of such domains to the transmembrane domains of the same or different surface membrane proteins to minimize interactions with other members of the receptor complex. In some embodiments, it is desirable to employ the transmembrane domain of ζ, η 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.

[0191] In some embodiments, the transmembrane domain of a CAR is derived from CD8 or CD28. In some embodiments, 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 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 SEQ ID NO:23 as set forth in PCT/US2021/072646, the disclosure of which is incorporated herein by reference. In an 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 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 SEQ ID NO:24 as set forth in PCT/US2021/072646, the disclosure of which is incorporated herein by reference.

D. Hinge regions

[0192] In some embodiments, a CAR polypeptide includes a hinge region (e.g., a spacer region) between an extracellular domain and a transmembrane domain, such that the extracellular domain, hinge region, and transmembrane domain are in frame with each other.

[0193] A hinge region may contain up to 300 amino acids, preferably 10 to 100 amino acids and most preferably 25 to 50 amino acids. A hinge 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 hinge 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 hinge regions 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 hinge region to ensure that the antigen-binding domain is an optimal distance from the transmembrane domain. In some embodiments, when the hinge region is derived from an immunoglobulin, the region may be mutated to prevent Fc receptor binding.

[0194] In some embodiments, the hinge region includes a hinge domain of a recognized protein. The hinge domain may be derived from CD8α, CD28, or an immunoglobulin (IgG). For example, the IgG hinge may be from IgG1, IgG2, IgG3, IgG4, IgM1, IgM2, IgA1, IgA2, IgD, IgE, or a chimera thereof.

[0195] In some 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, IgM1, IgM2, IgA1, IgA2, IgD, IgE, or a chimera thereof. In various embodiments, the hinge domain comprises the CH1, CH2, CH3 and/or hinge region of the immunoglobulin. In many 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" ("SH"). Non-limiting examples of suitable hinge domains are the core immunoglobulin hinge regions include EPKSCDKTHTCPPCP (SEQ ID NO:74; SEQ ID NO:57 of PCT/US2021/072646) from IgG1, ERKCCVECPPCP (SEQ ID NO:75; SEQ ID NO: 58 of PCT/US2021/072646) from IgG2, ELKTPLGDTTHTCPRCP(EPKSCDTPPPCPRCP)$_3$ (SEQ ID NO:76; SEQ ID NO: 59 of PCT/US2021/072646) from IgG3, and ESKYGPPCPSCP (SEQ ID NO:77; SEQ ID NO: 60 of PCT/US2021/072646) 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). In many embodiments, the hinge domain is a fragment of the immunoglobulin hinge.

[0196] 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 to SEQ ID NO:21 of PCT/US2021/072646, the disclosure of which is incorporated herein by reference.

[0197] 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 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 SEQ ID NO:22 of PCT/US2021/072646, the disclosure of which is incorporated herein by reference.

[0198] 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.

E. Cytoplasmic domains including co-stimulatory domains

[0199] In some aspects, a CAR polypeptide includes a cytoplasmic domain, which contains at least one intracellular signaling domain. In some embodiments, a cytoplasmic domain also comprises one or more co-stimulatory signaling domains.

[0200] The cytoplasmic domain is responsible for activation of at least one of the normal effector functions (e.g., specialized function) 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. A signaling domain can include a 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 domain.

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. An intracellular signaling domain includes any truncated portion of the signaling domain sufficient to transduce the effector function signal. Non-limiting examples of signaling domains which can be used include, e.g., signaling domains derived from DAP10, DAP12, Fc epsilon receptor I γ chain (FCER1G), FcRβ, CD3δ, CD3ε, CD3γ, CD3ζ, CD5, CD22, CD226, CD66d, CD79A, and CD79B.

[0201] In some embodiments, the cytoplasmic domain comprises a CD3ζ signaling domain. In some embodiments, the CD3ζ signaling domain includes 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 to SEQ ID NO:6 of PCT/US2021/072646, the disclosure of which is incorporated herein by reference in its entirety.

[0202] In some embodiments, the cytoplasmic domain contains one or more co-stimulatory signaling domains. In some embodiments, the one or more co-stimulatory signaling domains are derived from CD28, 41BB, IL2Rb, CD40, OX40 (CD134), CD80, CD86, CD27, ICOS, NKG2D, DAP10, DAP12, 2B4 (CD244), BTLA, CD30, GITR, CD226, CD79A, and HVEM in its entirety.

[0203] In some embodiments, the co-stimulatory signaling domain is derived from 4-1BB. In one embodiment, the 4-1BB 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 to SEQ ID NO:8 of PCT/US2021/072646, the disclosure of which is incorporated herein by reference in its entirety.

[0204] In some embodiments, 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 to SEQ ID NO:9 of PCT/US2021/072646, the disclosure of which is incorporated herein by reference in its entirety.

[0205] In some embodiments, the co-stimulatory signaling domain is derived from CD40. In one embodiment, the CD40 co-stimulatory signaling domain includes 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 to SEQ ID NO:10 of PCT/US2021/072646, the disclosure of which is incorporated herein by reference in its entirety.

[0206] In some embodiments, the co-stimulatory signaling domain is derived from OX40. In one embodiment, the OX40 co-stimulatory signaling domain includes 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 to SEQ ID NO:11 of PCT/US2021/072646, the disclosure of which is incorporated herein by reference in its entirety.

[0207] In some embodiments, the co-stimulatory signaling domain is derived from CD80. In one embodiment, the CD80 co-stimulatory signaling domain includes 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 to SEQ ID NO:12 of PCT/US2021/072646, the disclosure of which is incorporated herein by reference in its entirety.

[0208] In some embodiments, the co-stimulatory signaling domain is derived from CD86. In one embodiment, the CD86 co-stimulatory signaling domain includes 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 to SEQ ID NO:13 of PCT/US2021/072646, the disclosure of which is incorporated herein by reference in its entirety.

[0209] In some embodiments, the co-stimulatory signaling domain is derived from CD27. In one embodiment, the CD27 co-stimulatory signaling domain includes 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 to SEQ ID NO:14 of PCT/US2021/072646, the disclosure of which is incorporated herein by reference in its entirety.

[0210] In some embodiments, the co-stimulatory signaling domain is derived from ICOS. In one embodiment, the ICOS co-stimulatory signaling domain includes 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 to SEQ ID NO:15 of PCT/US2021/072646, the disclosure of which is incorporated herein by reference in its entirety.

[0211] In some embodiments, the co-stimulatory signaling domain is derived from NKG2D. In one embodiment, the NKG2D co-stimulatory signaling domain includes 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 to SEQ ID NO:16 of PCT/US2021/072646, the disclosure of which is incorporated herein by reference in its entirety.

[0212] In some embodiments, the co-stimulatory signaling domain is derived from DAP10. In one embodiment, the DAP10 co-stimulatory signaling domain includes 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 to SEQ ID NO:17 of PCT/US2021/072646, the disclosure of which is incorporated herein by reference in its entirety.

[0213] In some embodiments, the co-stimulatory signaling domain is derived from DAP12. In one embodiment, the DAP12 co-stimulatory signaling domain includes 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 to SEQ ID NO:18 of PCT/US2021/072646, the disclosure of which is incorporated herein by reference in its entirety.

[0214] In some embodiments, the co-stimulatory signaling domain is derived from 2B4 (CD244). In one embodiment, the 2B4 (CD244) co-stimulatory signaling domain includes 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 to

SEQ ID NO:19 of PCT/US2021/072646, the disclosure of which is incorporated herein by reference in its entirety.

[0215] In some embodiments, the CAR polypeptide includes one costimulatory signaling domains. In many embodiments, the CAR includes 2 or more costimulatory signaling domains. In various embodiments, the CAR includes 2, 3, 4, 5, 6, or more costimulatory signaling domains.

V. Non-classical HLA class I

[0216] In some embodiments, an iPSC or derivative cell thereof 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 some cases, disruption of the beta-2-microglobulin (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 some embodiments, an iPSC or derivative cell thereof is engineered to exogenously express HLA-E and/or HLA-G. In certain embodiments, an iPSC or derivative cell thereof with disruption or elimination of B2M expression is engineered to exogenously express HLA-E and/or HLA-G.

[0217] In some 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 some embodiments, the iPSC or derivative cell thereof comprises an exogenous polynucleotide encoding HLA-E, HLA-G or both HLA-E and HLA-G. In many embodiments, the exogenous polynucleotide encodes HLA-E and HLA-G such that they are operably linked by an autoprotease peptide. For example, the polynucleotide can include from 5' to 3' order: an HLA-E sequence, a P2A sequence, and an HLA-G sequence. In some cases, the polynucleotide can include from 5' to 3' order: an HLA-G sequence, a P2A sequence, and an HLA-E sequence.

[0218] In some embodiments, the full-length HLA-E protein has the amino acid sequence set forth in NCBI Ref. Seq. No. NP_005507.3 or UniProt No. P13747. In some instance, the coding sequence of full-length HLA-E is set forth in NCBI Ref. No. NM_005516.5. In some

embodiments, the mature HLA-E protein has the sequence from amino acid positions 22-358 of the sequence set forth in NCBI Ref. Seq. No. NP_005507.3 or UniProt No. P13747.

[0219] In some embodiments, the HLA-E protein comprises an amino acid sequence having at least 90%, such as at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100%, sequence identity to SEQ ID NO:65, preferably the amino acid sequence of SEQ ID NO:65 as set forth in PCT/US2021/072646, the disclosure of which is incorporated herein by reference. In some embodiments, the HLA-E protein comprises an amino acid sequence having at least 90%, such as at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100%, sequence identity to SEQ ID NO:14, preferably the amino acid sequence of SEQ ID NO:14 as set forth in FIG. 5.

[0220] In some embodiments, the full-length HLA-G protein has the amino acid sequence set forth in NCBI Ref. Seq. No. NP_002118.1 or UniProt No. P17693. In some instance, the coding sequence of full-length HLA-G is set forth in NCBI Ref. No. NM_002127.5. In some embodiments, the mature HLA-G protein has the sequence from amino acid positions 25-358 of the sequence set forth in NCBI Ref. Seq. No. NP_002118.1 or UniProt No. P17693.

[0221] In some embodiments, the HLA-G protein comprises an amino acid sequence having at least 90%, such as at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100%, sequence identity to SEQ ID NO:68, preferably SEQ ID NO:68 as set forth in PCT/US2021/072646, the disclosure of which is incorporated herein by reference. In some embodiments, the HLA-G protein comprises an amino acid sequence having at least 90%, such as at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100%, sequence identity to SEQ ID NO:15, preferably SEQ ID NO:15 as set forth FIG. 5.

[0222] In various embodiments, the 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 (including those described herein). In some embodiments, the exogenous polypeptide comprises an amino acid sequence having at least 90%, such as at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100%, sequence identity to SEQ ID NO:66, preferably SEQ ID NO:66 as set forth in PCT/US2021/072646, the disclosure of which is incorporated herein by reference. In various embodiments, the 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

(including those described herein). In some embodiments, the exogenous polypeptide comprises an amino acid sequence having at least 90%, such as at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100%, sequence identity to SEQ ID NO:69, preferably SEQ ID NO:69 as set forth in PCT/US2021/072646, the disclosure of which is incorporated herein by reference.

[0223] In some instances, the exogenous polynucleotide encodes an HLA polypeptide that includes a signal peptide, such as an HLA-E signal peptide, which is linked to an HLA sequence, such as a mature B2M protein, and/or a mature HLA-E protein. In some embodiments, the B2M and HLA-E are linked by a flexible linker such as, but not limited to, a glycine-serine linker and other described herein. In some instances, the HLA polypeptide that includes a signal peptide, such as an HLA-G signal peptide, which is linked to the HLA sequence, such as a mature B2M protein, and/or a mature HLA-G protein. In some embodiments, the B2M and HLA-G are linked by a flexible linker such as, but not limited to, a glycine-serine linker and other described herein.

VI. IL-15 and IL-15Ra polypeptides

[0224] In some embodiments, an iPSC or derivative cell thereof is modified to express exogenous polynucleotide encoding an IL-15 protein. In some embodiments, an iPSC or derivative cell thereof is modified to express an exogenous polynucleotide encoding a fusion protein comprising an IL-15 protein and an IL-15 receptor alpha (IL-15Rα, IL-15Ra, and IL-15RA). Such an exogenous polynucleotide construct can be introduced into a specific genomic site or gene locus of the iPSC or derivative cell.

[0225] In some embodiments, a full-length IL-15 protein has the amino acid sequence set forth in NCBI Ref. Seq. Nos. NP_000576.1 or NP_751915.1 or UniProt No. P40933. In some instance, the coding sequence of full-length IL-15 is set forth in NCBI Ref. Nos. NM_000585.4 or NM_172175.2. In some embodiments, the mature IL-15 protein has the sequence from amino acid positions 49-162 of the sequence set forth in NCBI Ref. Seq. Nos. NP_000576.1 or NP_751915.1 or UniProt No. P40933. In some embodiments, the IL-15 protein includes an amino acid sequence having at least 90%, such as at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100%, sequence identity to SEQ ID NO:16, preferably the amino acid sequence of SEQ ID NO:16 as set forth in FIG. 5.

[0226] In some embodiments, a full-length IL-15Ra protein has the amino acid sequence set forth in NCBI Ref. Seq. Nos. NP_001230468.1, NP_001243694.1. NP_002180.1 or

NP_751950.2 or UniProt No. Q13261. In some instance, the coding sequence of full-length IL-15Ra is set forth in NCBI Ref. Nos. NM_001243539.1, NM_001256765.1, NM_002189.3 or NM_172200.2. In some embodiments, the IL-15Ra protein includes an amino acid sequence having at least 90%, such as at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100%, sequence identity to SEQ ID NO:18, preferably the amino acid sequence of SEQ ID NO:18 as set forth in FIG. 5.

[0227] In some embodiments, the fusion protein comprising an IL-15 protein and an IL-15 receptor alpha (IL-15Rα) protein include an amino acid sequence having at least 90%, such as at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100%, sequence identity to SEQ ID NO:17, preferably the amino acid sequence of SEQ ID NO:17 as set forth in FIG. 5.

[0228] The exogenous polynucleotide encoding an IL-15 protein or a fusion protein comprising an IL-15 protein and an IL-15 receptor alpha (IL-15Rα) can be integrated into a genomic site by way of genomic editing.

VII. Combined artificial cell death/reporter system polypeptides

[0229] In some embodiments, an iPSC or derivative cell thereof described herein is modified to express exogenous combined artificial cell death/reporter system polypeptides. In some embodiments, described herein is an iPSC or derivative cell thereof that expresses exogenous combined artificial cell death/reporter system polynucleotides. In some embodiments, provided herein is a polynucleotide encoding a combined artificial cell death/reporter polypeptide and an iPSC or derivative thereof engineered to express. In some embodiments, provided herein is a combined artificial cell death/reporter polypeptide and an iPSC or derivative thereof engineered to express.

[0230] A combined artificial cell death/reporter polypeptide acts as a safety switch so the cells can be killed if the patient has an adverse reaction. In some embodiments, the polypeptide or components thereof are useful for imaging such as, but not limited to, molecular imaging and PET imaging. It is advantageous to engineer cells to include a safety switch to eliminate the cells that have been infused into a patient in case of adverse events.

[0231] In some embodiments, provided is a combined artificial cell death/reporter polypeptide that can function as an artificial cell death polypeptide, a reporter polypeptide, or both an artificial cell death polypeptide and a reporter polypeptide. Having the combined artificial cell

death and reporter polypeptide in a single polynucleotide that can be expressed as a single polypeptide has the advantage of reducing the number of gene edits of the cell. Descriptions of combined artificial cell death/reporter polypeptides can be found, for example, in US2022/0332782, the contents of which are incorporated herein by reference in its entirety.

[0232] In some embodiments, an artificial cell death/reporter 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:25. Also provided is a polynucleotide encoding the artificial cell death/reporter polypeptide comprising 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:25.

[0233] In some embodiments, an artificial cell death/reporter 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:27, 30 and 31. Also provided is a polynucleotide encoding the artificial cell death/reporter polypeptide comprising 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:31.

[0234] In some embodiments, an artificial cell death/reporter 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:26, 28 and 32. Also provided is a polynucleotide encoding the artificial cell death/reporter polypeptide comprising 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:32.

[0235] In some embodiments, a combined artificial cell death/reporter polypeptide includes an HSV-TK fused to a truncated variant PSMA polypeptide via a linker. In some embodiments, a combined artificial cell death/reporter polypeptide comprises (1) an intracellular domain having a herpes simplex virus thymidine kinase (HSV-TK) and a linker, (2) a transmembrane region, and (3) an extracellular domain comprising a prostate-specific membrane antigen (PSMA) extracellular domain or fragment thereof. In some embodiments, the linker includes a Whitlow

linker, an autoprotease peptide sequence, such as an autoprotease peptide sequence selected from the group consisting of porcine teschovirus-1 2A (P2A), thosea asigna virus 2A (T2A), equine rhinitis A virus 2A (E2A), foot-and-mouth disease virus 18 2A (F2A), and any linker described. In some embodiments, the artificial cell death/reporter polypeptide includes an intracellular domain of HSV-TK fused to a truncated variant PSMA polypeptide via a linker. As such, the PSMA portion is extracellular and the HSV-TK is located intracellular. In some embodiments, provided is a polynucleotide encoding a combined artificial cell death/reporter polypeptide and an iPSC or derivative thereof engineered to express.

[0236] In some embodiments, an artificial cell death polypeptide comprises a viral enzyme that is recognized by an antiviral drug. In some embodiments, the viral enzyme is a herpes simplex virus thymidine kinase (HSV-TK) (see, e.g., Bonini et al., Science, 1997 Jun 13;276(5319):1719-24). In some embodiments, an HSV-TK polypeptide includes 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 29. Also provided is a polynucleotide encoding an HSV-TK polypeptide comprising 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:23 or 29.

[0237] In some embodiments, the artificial cell death/reporter system polypeptide is encoded by an HSV-TK-T2A-PSMA transgene. In some embodiments, it is encoded by an HSV-TK (H168A)-T2A-PSMA transgene. In some embodiments, it is encoded by an HSV-TK (A168H)-T2A-PSMA transgene.

[0238] In some embodiments, such cells have been engineered to include a gene for an artificial cell death polypeptide (a "suicide gene") which is a genetically encoded molecule that allows selective destruction of the cells (e.g., allowing selective ablation of the gene modified cells), thereby preventing collateral damage to contiguous cells and/or tissues. An artificial cell death polypeptide includes an engineered protein designed to prevent potential toxicity or otherwise adverse effects of a cell therapy. The artificial cell death/reporter 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 embodiments, provided herein is an artificial cell death polypeptide. In some embodiments,

provided herein is a polynucleotide encoding an artificial cell death and an iPSC or derivative thereof engineered to express. In some instance, the artificial cell death polypeptide is activated by an exogenous molecule, e.g., an antibody, anti-viral drug, or radioisotopic conjugate drug, that when activated, triggers apoptosis and/or cell death of a therapeutic cell.

[0239] A reporter polypeptide refers to and includes an engineered protein that, in combination with an imaging probe, can be used to mark cells. In some embodiments, a reporter polypeptide comprises an antigen targeted by an entity, such as a small molecule compound, a radioisotopic conjugate, or an antibody or an antigen binding fragment thereof. In certain embodiments, the antigen is a prostate-specific membrane antigen (PSMA) polypeptide, also referred to as glutamate carboxypeptidase 2. PSMA is a type II membrane protein that is targeted to the secretary pathway by its transmembrane domain, which biochemically resembles a signal sequence without being cleaved. In various embodiments, the reporter polypeptide comprises a prostate-specific membrane antigen (PSMA) extracellular domain or fragment thereof.

[0240] In some embodiments, the PSMA polypeptide is a truncated variant as described WO2015143029A1 and WO2018187791A1, the disclosures of which are incorporated herein by reference in their entirety. In many embodiments, the prostate-specific membrane antigen (PSMA) polypeptide comprises or consists of 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:24, preferably the amino acid sequence of SEQ ID NO:24. In many embodiments, the PSMA antigen may also function as an artificial cell death polypeptide since expression of truncated PSMA in a cell induces cell death of the engineered cell when the cell is contacted with a radioisotopic conjugate drug that binds to PSMA via a peptide. PSMA-targeting compounds are described in WO2010/108125, the disclosure of which is incorporated herein by reference. In some embodiments, a 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:24. In some embodiments, described is a polynucleotide encoding a 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:24.

[0241] In some embodiments, the artificial cell death/reporter polypeptide comprises a viral enzyme that is recognized by an antiviral drug. In certain embodiments, the viral enzyme is a herpes simplex virus thymidine kinase (HSV-TK). In certain embodiments, the HSV-TK 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:23, preferably the amino acid sequence of SEQ ID NO:23. This enzyme phosphorylates the nontoxic prodrugs acyclovir or ganciclovir, which then become phosphorylated by endogenous kinases to GCV-triphosphate, causing chain termination and single-strand breaks upon incorporation into DNA, thereby killing dividing cells. In some embodiments, described is a polynucleotide encoding an HSV-TK polynucleotide encoding 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:23.

[0242] In some embodiments, expression of the viral enzyme in an engineered immune cell expressing a chimeric antigen receptor (CAR) induces cell death of the engineered immune cell when the cell is contacted with one or more antiviral drugs. In certain embodiments, the one or more antiviral drugs comprise acyclovir or a derivative thereof, or ganciclovir or a derivative thereof.

[0243] In some embodiments, the cell expressing the artificial cell death/reporter system also expresses one or more of the other exogenous polypeptides described. In some instances, the cell expresses a CAR. In some instances, the cell expresses a CD16 polypeptide such as a CD16 variant. In some instances, the cell expresses an NKG2D polypeptide.

VIII. Other exogenous polypeptides

[0244] In some embodiments, the genomic editing at one or more selected genomic sites described may include insertions of one or more exogenous polynucleotides encoding any of polypeptides including, but not limited to, 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 genome-engineered iPSCs or derivative cells thereof.

[0245] Other exogenous polynucleotides encoding polypeptides 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 embodiments, the genome-engineered iPSCs generated using the methods provided herein contain an insertion or deletion (in/del) modification 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.

[0246] In some embodiments, the genome-engineered iPSCs generated using the methods provided contain one or more different exogenous polynucleotides encoding proteins comprising caspase, thymidine kinase, cytosine deaminase, CD20, ErbB2 or CD79b such that when the genome-engineered iPSCs contain two or more suicide genes, the suicide genes are integrated in different safe harbor loci such as, but not limited to, an AAVS1 locus, a CCR5 locus, a ROSA26 locus, a collagen locus, an HTRP locus, a beta-2 microglobulin locus, a GAPDH locus, a TCR locus and a RUNX1 locus.

IX. Gene locus

[0247] In one aspect, any of the exogenous polynucleotides described can be integrated into a specific gene locus 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 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, any of the exogenous polynucleotide constructs described can be integrated into a specific gene locus selected from the group consisting of: an AAVS1 locus, a B2M locus, a CIITA locus, a CD70 locus, a CLYBL locus, an NKG2A locus, an NKG2D locus, a TAP1 locus, a TAP2 locus, a TAPBP locus, a TRAC locus, a TRBC1 locus, a RFXANK locus, a RFX5 locus, and a RFXAP locus, and optionally the integration into the gene locus disrupts (such as, reduces or eliminates) expression of the gene. In some embodiments, any of the exogenous polynucleotide is integrated into a gene locus selected from the group consisting of an AAVS1 locus, a B2M locus, a CIITA locus, a CD70 locus, a CLYBL locus, an NKG2A locus and a

TRAC locus, and optionally the integration into the gene locus disrupts (such as, reduces or eliminates) expression of the gene.

[0248] In other words, integration of an exogenous polynucleotide described can produce a disruption of one or more genes selected from the group consisting of an AAVS1 gene, a B2M gene, a CIITA gene, a CD70 gene, a CLYBL gene, an NKG2A gene, an NKG2D gene, a TAP1 gene, a TAP2 gene, a TAPBP gene, a TRAC (TRA) gene, a TRBC1 (TRB) gene, a RFXANK gene, a RFX5 gene, a RFXAP gene, and any combination thereof. In some instances, the disruption is to the B2M gene. In some instances, the disruption is to the CIITA gene. In some instances, the disruption is to the B2M gene and the CIITA gene.

[0249] In some embodiments, the exogenous polynucleotide encoding the CD16 protein or variant thereof and the NKG2D protein or variant thereof is integrated into any of the gene locus described. In some embodiments, the exogenous polynucleotide encoding HLA-E is integrated into any of the gene locus described. In some embodiments, the exogenous polynucleotide encoding HLA-G is integrated into any of the gene locus described. In some embodiments, the exogenous polynucleotide encoding IL-15 is integrated into any of the gene locus described. In some embodiments, the exogenous polynucleotide encoding IL-15Ra is integrated into any of the gene locus described. In some embodiments, the exogenous polynucleotide encoding an fusion protein containing IL-15 and IL-15Ra is integrated into any of the gene locus described.

X. Targeted genome editing at a selected gene locus

[0250] In some aspect, one or more of the exogenous polynucleotides described are integrated at one or more loci on the chromosome of a cell such as an iPSC. In some instances, the integration of the exogenous polynucleotide into the gene locus is produced by way of targeted genome editing. Non-limiting examples of targeted genome editing include any method selected from the group consisting of a CRISPR method, a zinc finger nuclease method, a TALEN method, a homing nuclease method, a homology recombination method, and any functional variation thereof.

[0251] 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.

[0252] 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."

[0253] 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.

[0254] 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, US6,140,081; US6,453,242; and US6,534,261; 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 US7,888,121 and US7,972,854, 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 FokI nuclease with a zinc finger DNA binding domain.

[0255] 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 US2011/0145940, which is herein incorporated by reference. The most recognized example of a TALEN in the art is a fusion polypeptide of the FokI nuclease to a TAL effector DNA binding domain.

[0256] Another example of a targeted nuclease that finds use in the subject methods is a targeted SpoII nuclease, a polypeptide comprising a SpoII 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, US61/555,857, the disclosure of which is incorporated herein by reference.

[0257] Additional examples of targeted nucleases suitable for the present application include, but not limited to Bxbl, phiC3 1, R4, PhiBTl, and Wp/SPBc/TP90l-l, whether used individually or in combination.

[0258] 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 ribonucleoprotein (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.

[0259] 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, WO2018/236548, the disclosure of which is incorporated herein by reference. Additional descriptions of CRISPR-MAD7 methods can be found in, e.g., CRISPR J., April 2020, 3(2):97-108.

[0260] 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, US2015/0140665 and Farriggo et al., Methods Mol Biol, 2017, 1642:69-85, the disclosures of which are incorporated herein by reference.

[0261] In one aspect, provided herein a construct comprising one or more exogenous polynucleotides for targeted genome integration. In some embodiments, 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.

[0262] 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, HTRP, GAPDH, TCR and RUNX1 genes. In some embodiments, a TCR gene is selected from the group consisting of a TRA gene, a TRB gene, a TRD gene, and a TRG gene.

[0263] In other embodiments, the site for targeted integration is selected for deletion or reduced expression of an endogenous gene at the insertion site. A deletion respect to expression of a gene includes any genetic modification that abolishes the expression of the gene. Examples of a 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.

[0264] 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, TAP1 gene, TAP2 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 some embodiments, one or more of the exogenous polynucleotides are integrated at one or more loci of genes selected from the group consisting of B2M, TAP1, TAP2, Tapasin, RFXANK, CIITA, RFX5 and RFXAP genes to thereby delete or reduce the expression of the gene(s) with the integration.

[0265] In some 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, HTRP, GAPDH, RUNX1, B2M, TAP1, TAP2, Tapasin, NLRC5, CIITA, RFXANK, CIITA, RFX5, RFXAP, TCRa constant region, TCRb 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, TAP1, TAP2, Tapasin, RFXANK, CIITA, RFX5 and RFXAP genes. In many embodiments, the one or more exogenous polynucleotides are integrated at a locus of an MHC class-I associated gene, such as a B2M gene, TAP1 gene, TAP2 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, HTRP, GAPDH, TCR and RUNX1 genes. In some embodiments, a TCR gene is selected from the group consisting of a TRA gene, a TRB gene, a TRD gene, and a TRG gene. In various embodiments, the one or more of the exogenous polynucleotides are integrated at the loci of CIITA, AAVS1 and B2M genes.

[0266] In some embodiments, (i) an exogenous polynucleotide is integrated at a gene locus; (ii) a different exogenous polynucleotide is integrated at a locus of CIITA gene; and (iii) another

different exogenous polynucleotide is integrated at a locus of B2M gene; wherein integrations of the exogenous polynucleotides delete or reduce expression of CIITA and B2M genes. In some embodiments, (i) an exogenous polynucleotide is integrated at a gene locus; (ii) another exogenous polynucleotide is integrated at a locus of CIITA gene; and (iii) yet another exogenous polynucleotide is integrated at a locus of B2M gene; wherein integrations of the exogenous polynucleotides eliminate or reduce expression of CIITA and B2M genes. In certain embodiments, (i) a first exogenous polynucleotide is integrated at a safe harbor locus; (ii) a second exogenous polynucleotide is integrated at a locus of CIITA gene; and (iii) a third exogenous polynucleotide is integrated at a locus of B2M gene; wherein integrations of the exogenous polynucleotides eliminate or reduce expression of CIITA and B2M genes. In some embodiments, (i) a first exogenous polynucleotide is integrated at a AAVS1, CCR5, ROSA26, HTRP, GAPDH, TRA, TRB, TRD, TRG or RUNX1 gene locus; (ii) a second exogenous polynucleotide is integrated at a CIITA gene locus; and (iii) a third exogenous polynucleotide is integrated at a B2M gene locus; wherein integrations of the exogenous polynucleotides eliminate or reduce expression of CIITA and B2M genes.

[0267] In some embodiments, an exogenous polynucleotide is integrated at a CD70 gene locus. In some embodiments, (i) an exogenous polynucleotide is integrated at a gene locus; (ii) a different exogenous polynucleotide is integrated at a CIITA gene locus; (iii) another different exogenous polynucleotide is integrated at a B2M gene locus; and (iv) yet another different exogenous polynucleotide is integrated at a CD70 gene locus; wherein integrations of the exogenous polynucleotides eliminate or reduce expression of the CD70, CIITA and B2M genes.

[0268] In some embodiments, (i) a first exogenous polynucleotide is integrated at a gene locus; (ii) a second exogenous polynucleotide is integrated at a CIITA gene locus; (iii) a third exogenous polynucleotide is integrated at a B2M gene locus; and (iv) a fourth exogenous polynucleotide is integrated at a CD70 gene locus; wherein integrations of the exogenous polynucleotides eliminate or reduce expression of the CD70, CIITA and B2M genes.

[0269] In certain embodiments, (i) a first exogenous polynucleotide is integrated at a safe harbor gene locus; (ii) a second exogenous polynucleotide is integrated at a CIITA gene locus; (iii) a third exogenous polynucleotide is integrated at a B2M gene locus; and (iv) a fourth exogenous

polynucleotide is integrated at a CD70 gene locus; wherein integrations of the exogenous polynucleotides eliminate or reduce expression of the CD70, CIITA and B2M genes.

[0270] In some embodiments, (i) a first exogenous polynucleotide is integrated at a AAVS1, CCR5, ROSA26, HTRP, GAPDH, TRA, TRB, TRD, TRG or RUNX1 gene locus; (ii) a second exogenous polynucleotide is integrated at a CIITA gene locus; (iii) a third exogenous polynucleotide is integrated at a B2M gene locus; and (iv) a fourth exogenous polynucleotide is integrated at a CD70 gene locus; wherein integrations of the exogenous polynucleotides eliminate or reduce expression of the CD70, CIITA and B2M genes.

XI. Derivative cells from iPSC cells

[0271] In some aspects, provided is a cell differentiated from an iPSC cell or a derivative thereof. In some embodiments, iPSCs are differentiated into a cell type which is then cultured and differentiated into another cell type. For instance, an iPSC can be differentiated into a progenitor cell such as an NK progenitor cell, which is then cultured under conditions to become a mature cell such as an NK cell. As described above, the genomic edits introduced into the iPSC are retained in the derivative cell. In some embodiments of the derivative cell obtained from iPSC differentiation, the derivative cell is a hematopoietic cell, including, but not limited to, hematopoietic stem and progenitor cells (HSCs), hematopoietic multipotent progenitor cells, T cell progenitors, natural killer (NK) cell progenitors, B cell progenitors, CD34+ hematopoietic progenitor cells, T cells, NKT cells, NK cells, B cells, antigen presenting cells (APC), monocytes and macrophages. In some embodiments, the derivative cell is an immune effector cell, such as an NK cell or a T cell.

[0272] In some embodiments, the iPSC is produced from whole peripheral blood mononuclear cells. In some embodiments, the iPSC is produced from an NK cell. In some embodiments, the iPSC is produced from a T cell. In some embodiments, the iPSC is produced from a reprogrammed NK cell. In some embodiments, the iPSC is produced from a reprogrammed T cell. In some embodiments, the iPSC is produced from a reprogrammed whole peripheral blood mononuclear cell.

[0273] Also provided is a method of manufacturing the differentiated cell or a derivative thereof. In some embodiments, the method includes differentiating the iPSC under conditions to promote, facilitate or generate a specific differentiated cell. In some instances, the differentiated

cells is further cultured to produce a cell derived from the differentiated cell, e.g., a derivative cell.

[0274] An iPSC can be differentiated by any method known in the art. Exemplary methods are described in US10,947,502; US8,846,395; US8,945,922; US8,318,491; WO2010/099539; WO2010/141801; WO2012/109208; WO2016/010148; WO2017/070333; WO2017/070337; WO2017/179720; WO2018/048828; and WO2019/157597 and WO2020/252477; the contents of which are herein incorporated by reference in their entireties. The differentiation protocol may use feeder cells or may be feeder-free. Feeder cells or feeders include 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.

[0275] In one embodiment, the differentiated iPSCs are NK cells which are prepared by a method of differentiating an iPSC into an NK cell. In some embodiments, the iPSCs are subjected to a differentiation protocol including the addition of recombinant human IL-12p70 (e.g., IL-12) to the culture media 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. Descriptions of methods for generating iPSC-derived NK cells can be found, for example, in WO2010/099539; Euchner et al., Front Immunol, 2021 May 04, 12:640672; Li et al., Cell Stem Cell, 2018 Aug 2, 23:181-192; and Karagiannis and Kim, Mol Cells, 2021 Aug 31, 44(8):541-548. In many embodiments, recombinant human IL-12p70 (human IL-12) includes a IL-12 p40 subunit and/or a IL-12 p35 subunit. In many embodiments, a IL-12 p40 subunit is connected to a IL-12 p35 subunit by way of a linker which can be any of those described herein. In some embodiments, recombinant human IL-12p70 includes a IL-12 p40 subunit, a Whitlow linker, and a IL-12 p35 subunit. In some embodiments, the recombinant human IL-12p70 protein has at least 90%, such as at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100%, sequence identity to SEQ ID NO:33. In some embodiments, the recombinant human IL-12p70 protein is 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:34.

[0276] In many embodiments, the differentiated iPSCs are T cells which are prepared by a method of differentiating an iPSC into a T cell. Protocols for generating a T cell from an iPSC include those disclosed, for example, in WO2010/099539, WO2010/141801, WO2017/070333, WO2017/070337, WO2017/179720, WO2018/048828, WO2019/157597 and WO2020/252477, the contents of which are herein incorporated by reference in their entireties.

[0277] In one embodiment, the differentiated iPSCs are T cells which are prepared by a method of differentiating an iPSC into an T cell. In some embodiments, the iPSCs are subjected to a differentiation protocol including the addition of recombinant human DLL-4 protein to the culture media. In some cases, the cells are cultured in medium comprising human DLL-4 protein for the final hours (e.g., 12, 18, 20, or 24 hours) of culture. In some embodiments, recombinant human DLL-4 protein comprises an amino acid sequence having at least 90%, such as at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100%, sequence identity to SEQ ID NO:35. In some embodiments, recombinant human DLL-4 comprises an amino acid sequence having at least 90%, such as at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100%, sequence identity to SEQ ID NO:36. In some embodiments, recombinant human DLL-4 comprises an amino acid sequence having at least 90%, such as at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100%, sequence identity to SEQ ID NO:37.

[0278] Provided herein is a recombinant DLL4 variant protein having an amino acid sequence selected from the group consisting of SEQ ID NOS:35-37. In some embodiments, the recombinant DLL4 variant protein has an amino acid sequence provided in SEQ ID NOS:90-95 of US2022/0333074, the contents including Table 2 and the sequence listing of which are herein incorporated by reference.

[0279] In many embodiments, the differentiated iPSCs are CD34-positive (CD34+) cells which are prepared by a method of differentiating an iPSC into a CD34+ cell. Protocols for generating a CD34+ cell from an iPSC include those disclosed, for example, in WO2010/099539, WO2010/141801, WO2017/070333, WO2017/070337, WO2018/048828, WO2019/157597 and WO2020/252477, the contents of which are herein incorporated by reference in their entireties.

[0280] In some embodiments, iPSC cells are differentiated into hematopoietic progenitor cells (HPCs). In an exemplary embodiment of a differentiation protocol, iPSC cells are in HDM-I media plus H1152. In some instances, HDM media contains IMDM medium, Ham's F12

medium, CTS B27 minus vitamin A supplement, non-essential amino acids, ascorbic acid, Mg 2-phosphate, monothioglycerol, and heparin. HDM-I media can contain HDM + CHIR99021 GSK3 inhibitor, FGF2, and VEGF. In some instances, the cells are further cultured in HDM-II medium comprising HDM media in addition to BMP4, FGF2, and VEGF. In some instances, the cells are further cultured in HDM-III medium comprising HDM in addition to BMP4, SCF, TPO, FLT3L, and IL3. The resulting HPCs can be collected.

[0281] In some embodiments, HPCs are differentiated to produce NK or T cells. In some instances, the HPCs are cultured in retronectin/DLL4-coated bioreactors, e.g., G-Rex bioreactors. Notch signaling factors, cytokines, and growth factors can be added to culture medium to facilitate differentiation into lymphoid lineage and subsequent NK or T cell maturation and activation.

[0282] In some embodiments, maturation and/or activation of NK or T cells from HPCs includes culturing the HPCs in a culture medium comprising a recombinant IL-12 protein. IL-12 is a cytokine that stimulates the production of interferon-gamma (IFN-γ) and tumor necrosis factor-alpha (TNF-α) from T cells and natural killer (NK) cells. In some embodiments, a recombinant IL-12 protein comprises human IL-12p70. In various embodiments, recombinant IL-12 comprises a human IL-12p70 p40 subunit and a human IL-12p70 p35 subunit. In various embodiments, recombinant IL-12 protein comprises a human IL-12p70 p40 subunit, a human IL-12p70 p35 subunit and a linker. In various embodiments, recombinant IL-12 protein comprises from N- to C-terminus: a human IL-12p70 p40 subunit, a linker, and a human IL-12p70 p35 subunit. In certain embodiments, recombinant IL-12 protein comprises from N- to C-terminus: a human IL-12p70 p35 subunit, a linker, and a human IL-12p70 p40 subunit. In some embodiments, recombinant IL-12 protein comprises from N- to C-terminus: a human IL-12p70 p40 subunit, a Whitlow linker, and a human IL-12p70 p35 subunit. In various embodiments, recombinant IL-12 protein comprises from N- to C-terminus: a human IL-12p70 p35 subunit, a Whitlow linker, and a human IL-12p70 p40 subunit. In some embodiments, a recombinant IL-12 protein comprises an amino acid sequence having at least 90%, at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100%, sequence identity to SEQ ID NO:33. In various embodiments, a recombinant IL-12 protein is encoded by a polynucleotide having at least 90%, at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100%, sequence identity to SEQ ID NO:34.

[0283] Detailed descriptions of generating iPSC-derived γδ T cells are known in the art and can be found in WO2022/216514. Briefly, peripheral blood mononuclear cells (PBMCs) from healthy donors can be reprogrammed into iPSCs using methods known in the art and described in WO2022/120334; WO2022/216514; WO2022/216624;WO2023/049918; US8,183,038; US8,268,620; US8,440,461; US8,546,140; US8,765,470; US8,952,801; US9,328,332; US9,499,786; US9,644,184; and US10,865,381; the contents of each are incorporated herein by reference in their entirety. The iPSCs can be differentiated into hematopoietic progenitor cells (HSCs) such as CD34+ HSCs using methods known in the art and described in WO2022/216514. The CD34+ HSCs can be further differentiated to generate T cells in particular, γδ T cells using methods known in the art and described in Examples 1 and 3 of WO2022/216514.

[0284] Detailed descriptions of generating iPSC-derived αβ T cells are known in the art and can be found in Example 2 of WO2023049918, the contents are incorporated herein by reference in its entirety.

[0285] In some embodiments, the iPSCs and derivatives thereof express a polynucleotide encoding the amino acid sequence of SEQ ID NO:1. In some embodiments, the iPSCs and derivatives thereof express a polynucleotide encoding the amino acid sequence of SEQ ID NO:2. In some embodiments, the iPSCs and derivatives thereof express a polynucleotide encoding the amino acid sequence of SEQ ID NO:4. In some embodiments, the iPSCs and derivatives thereof express a polynucleotide comprising the nucleic acid sequence of SEQ ID NO:6. In some embodiments, the iPSCs and derivatives thereof express a polynucleotide comprising the nucleic acid sequence of SEQ ID NO:7. In some embodiments, the iPSCs and derivatives thereof express a polynucleotide comprising the nucleic acid sequence of SEQ ID NO:9. In some embodiments, the iPSCs and derivatives thereof express a polynucleotide comprising the nucleic acid sequence of SEQ ID NO:13. In some embodiments, the iPSCs and derivatives thereof express a polynucleotide comprising the nucleic acid sequence of SEQ ID NO:39. In some embodiments, the iPSCs and derivatives thereof also express an IL-15 protein of SEQ ID NO:16. In particular embodiments, the iPSCs and derivatives thereof also express an IL-15 and IL-15Rα fusion protein of SEQ ID NO:17. In some instances, the iPSCs and derivatives thereof express an exogenous polynucleotide encoding an IL-15 protein of SEQ ID NO:16. In some instances, the iPSCs and derivatives thereof express an exogenous polynucleotide encoding an IL-15 and IL-

15Rα fusion protein of SEQ ID NO:17. In some instances, the iPSCs and derivatives thereof express an exogenous polynucleotide comprising the nucleic acid sequence of SEQ ID NO:38. In some embodiments, the iPSCs and derivatives thereof also express an HLA-E protein of SEQ ID NO:14. In some embodiments, the iPSCs and derivatives thereof also express an HLA-G protein of SEQ ID NO:15. In some embodiments, the iPSCs and derivatives thereof also express an exogenous polynucleotide encoding an HLA-E protein of SEQ ID NO:14. In some embodiments, the iPSCs and derivatives thereof also express an exogenous polynucleotide encoding an HLA-G protein of SEQ ID NO:15. In some cases, the iPSCs and derivatives thereof express the polypeptide of SEQ ID NO:19. In some cases, the iPSCs and derivatives thereof express the polypeptide of SEQ ID NO:20. In some cases, the iPSCs and derivatives thereof express the polynucleotide of SEQ ID NO:21 and/or SEQ ID NO:22. In some embodiments, the iPSCs and derivatives thereof express a chimeric antigen receptor described herein and in WO2022/120334, the contents are herein incorporated by reference in its entirety. In some embodiments, iPSCs and derivatives thereof express an artificial cell death polypeptide described herein and in US2022/0332782, the contents are herein incorporated by reference in its entirety. In certain embodiments, the iPSCs and derivatives thereof express a polypeptide comprising an amino acid sequence of SEQ ID NO:23, 24, 25, 27, 29, 30, or 31. In other embodiments, the iPSCs and derivatives thereof express a polynucleotide comprising a nucleic acid sequence of SEQ ID NO:26, 28 or 32. The iPSCs and derivatives thereof include, but are not limited to, human iPSCs generated from reprogrammed whole blood mononuclear cells, human iPSCs generated by reprogrammed NK cells, human iPSCs generated by reprogrammed T cells, CD34+ hematopoietic progenitor cells derived from human iPSCs, NK cells derived from human iPSCs, T cells derived from human iPSCs, NK cells differentiated from CD34+ hematopoietic progenitor cells derived from human iPSCs, and T cells differentiated from CD34+ hematopoietic progenitor cells derived from human iPSCs. The T cells can be αβ T cells or γδ T cells.

XII. Vectors

[0286] In some aspects, provided is an isolated vector (construct) comprising a polynucleotide sequence encoding a useful polypeptide according to embodiments of the disclosure. Any vector known to those skilled in the art 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 recombinant protein in the cell. Conventional cloning techniques or artificial gene synthesis can be used to generate a recombinant expression vector according to embodiments described.

[0287] In some embodiments, any of the exogenous polynucleotides are operatively linked to one or more exogenous promoters such as, but not limited to, CAG, CMV, EF1a, PGK1, SV40, UBC and human beta actin, as well as other constitutive, inducible, temporal-specific, tissue-specific, and cell type-specific promoters. In some embodiments, any of the exogenous polynucleotides are operatively linked to one or more endogenous promoters found in a selected genomic site (e.g., gene locus) such as, but not limited to, AAVS1, CCR5, ROSA26, collagen, HTRP, beta-2 microglobulin (B2M), GAPDH, TCR (e.g., TRA, TRB, TRD and TRG) and RUNX1, as well as other locus that meet the criteria of a genome safe harbor.

[0288] . In some embodiments, the vector comprises an exogenous polynucleotide having, in the 5' to 3' order, (a) a promoter; (b) a polynucleotide sequence; and (c) a terminator/polyadenylation signal. Non-limiting examples of constitutive promoters include CAG, EF1a, UBC, CMV, SV40, PGK1, and human beta actin. Non-limiting examples of terminator/polyadenylation signal include a SV40 signal, BGH signal, hGH signal, and PGK signal.

[0289] In some embodiment, the vector includes 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 some embodiments, the left homology arm and a right homology arm target the CD70 gene.

XIII. Compositions

[0290] In some aspects, provided are compositions or populations of iPSCs or derivative cells thereof that express one or more recombinant proteins described herein. In some embodiments, the cells are CD34+ cells, NK cells, T cells, iNK cells or iT cells. In certain embodiments, the cells are NK cells derived from iPSCs. In some embodiments, the cells are T cells derived from iPSCs. In some instances, the T cells derived from iPSCs are αβ T cells. In certain instances, the T cells derived from iPSCs are γδ T cells.

[0291] In some aspects, provided are compositions or populations of iPSCs or derivative cells thereof that can express one or more recombinant proteins including, but not limited to, a recombinant CD16 protein such as a CD16 variant protein, a recombinant NKG2D protein, a CAR protein, a fusion protein containing IL-15 and IL-15Ra, HLA-E, and HLA-G. In some embodiments, the compositions or populations of iPSCs or derivative cells thereof express a recombinant CD16 protein such as a CD16 variant protein, a recombinant NKG2D protein, and a CAR protein. In some embodiments, the compositions or populations of iPSCs or derivative cells thereof express a recombinant CD16 protein such as a CD16 variant protein, a recombinant NKG2D protein, a CAR protein, and a fusion protein containing IL-15 and IL-15Ra. In some embodiments, the compositions or populations of iPSCs or derivative cells thereof express recombinant CD16 protein such as a CD16 variant protein, a recombinant NKG2D protein, a CAR protein, and an IL-15 protein. In some embodiments, the compositions or populations of iPSCs or derivative cells thereof express a recombinant CD16 protein such as a CD16 variant protein, a recombinant NKG2D protein, a CAR protein, and either HLA-E, HLA-G or both HLA-E and HLA-G. In some embodiments, the compositions or populations of iPSCs or derivative cells thereof express a recombinant HSV-TK-PSMA fusion.

[0292] In some embodiments, the compositions include a population of either NK cells, T cells or CD34+ cells that have been differentiated from iPSCs and contain an exogenous polynucleotide construct encoding a CD16 protein (such as a high affinity CD16 variant), an NKG2D protein and a self-cleavage peptide. In some instances, the population of NK cells that have been differentiated from iPSCs contains an exogenous polynucleotide construct encoding a CD16 protein (such as a high affinity CD16 variant), an NKG2D protein and a self-cleavage peptide. In some embodiments, the population of either NK cells, T cells or CD34+ cells that have been differentiated from iPSCs contains an exogenous polynucleotide construct encoding a CD16 protein (such as a high affinity CD16 variant), an NKG2D protein and a self-cleavage

peptide; and an exogenous polynucleotide construct encoding a CAR. In some embodiments, the population of either NK cells, T cells or CD34+ cells that have been differentiated from iPSCs contains an exogenous polynucleotide construct encoding a CD16 protein (such as a high affinity CD16 variant), an NKG2D protein and a self-cleavage peptide; and an exogenous polynucleotide construct encoding HLA-E, HLA-G or both. In some embodiments, the population of either NK cells, T cells or CD34+ cells that have been differentiated from iPSCs contains an exogenous polynucleotide construct encoding a CD16 protein (such as a high affinity CD16 variant), an NKG2D protein and a self-cleavage peptide and an exogenous polynucleotide construct encoding a fusion protein containing IL-15 and IL-15Ra. In some embodiments, the population of either NK cells, T cells or CD34+ cells that have been differentiated from iPSCs contains an exogenous polynucleotide construct encoding a CD16 protein (such as a high affinity CD16 variant), an NKG2D protein and a self-cleavage peptide and an exogenous polynucleotide construct encoding an IL-15 protein. In some embodiments, the population of either NK cells, T cells or CD34+ cells that have been differentiated from iPSCs contains an exogenous polynucleotide construct encoding a CD16 protein (such as a high affinity CD16 variant), an NKG2D protein and a self-cleavage peptide; an exogenous polynucleotide construct encoding a CAR; and an exogenous polynucleotide construct encoding HLA-E, HLA-G or both. In some embodiments, the population of either NK cells, T cells or CD34+ cells that have been differentiated from iPSCs contains an exogenous polynucleotide construct encoding a CD16 protein (such as a high affinity CD16 variant), an NKG2D protein and a self-cleavage peptide; an exogenous polynucleotide construct encoding a CAR; and an exogenous polynucleotide construct encoding a fusion protein containing IL-15 and IL-15Ra. In some embodiments, the population of either NK cells, T cells or CD34+ cells that have been differentiated from iPSCs contains an exogenous polynucleotide construct encoding a CD16 protein(such as a high affinity CD16 variant), an NKG2D protein and a self-cleavage peptide; an exogenous polynucleotide construct encoding a CAR; an exogenous polynucleotide construct encoding HLA-E, HLA-G or both; and an exogenous polynucleotide construct encoding a fusion protein containing IL-15 and IL-15Ra. In some embodiments, the population of either NK cells, T cells or CD34+ cells that have been differentiated from iPSCs contains an exogenous polynucleotide construct encoding a CD16 protein(such as a high affinity CD16 variant), an NKG2D protein and a self-cleavage peptide; an exogenous polynucleotide construct encoding a CAR; an exogenous polynucleotide

construct encoding HLA-E, HLA-G or both; and an exogenous polynucleotide construct encoding an IL-15 protein. In some embodiments, any of the cell populations can also express an exogenous polynucleotide construct comprising HSV-TK-PMSA as described herein.

[0293] In some instances, one or more of the exogenous polynucleotide constructs have been introduced into a specific genomic site (e.g., gene locus). In many instances, one or more of the exogenous polynucleotide constructs have been introduced into a safe harbor genomic site (e.g., gene locus). In some instances, one or more of the exogenous polynucleotide constructs have been introduced into a gene locus such that the expression of the gene is reduced or eliminated.

[0294] In some aspects, provided is a composition comprising an isolated polynucleotide, a host cell and/or an iPSC or derivative cell thereof described herein. In certain embodiments, the composition also includes 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 polynucleotides of interest, an antibody, a chemotherapeutic agent, a radioactive moiety or agent, or an immunomodulatory drug.

[0295] In some embodiments, the composition comprises an isolated polynucleotide, a host cell and/or an iPSC or derivative cell thereof described herein and a pharmaceutically acceptable carrier. Non-limiting examples of a pharmaceutically acceptable carrier include 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. A pharmaceutically acceptable carrier includes 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. 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 described.

XIV. Methods of treating cancer

[0296] In some aspects, provided are methods of treating a disease or disorder such as a cancer and/or an autoimmune disease by administering any of the cells described herein. The teachings of the present disclosure may be relevant to any and all cancers. In some embodiments, the cancer treated by methods of the present disclosure is a solid tumor. In some embodiments, the cancer treated by methods of the present disclosure is a hematologic malignancy.

[0297] In some embodiments, NK cells described herein that have been differentiated from iPSCs and engineered (e.g., modified) to express one or more of the exogenous polynucleotide constructs provided can be administered to a patient for treating cancer and/or an autoimmune disease. In some embodiments, T cells (e.g., gamma-delta T cells or γδ T cells) described herein that have been differentiated from iPSCs and engineered to express one or more of the exogenous polynucleotide constructs provided can be administered to a patient for treating cancer and/or an autoimmune disease. In some embodiments, CD34+ cells described herein that have been differentiated from iPSCs and engineered to express one or more of the exogenous polynucleotide constructs provided can be administered to a patient for treating cancer and/or an autoimmune disease. In many embodiments, the one or more of the exogenous polynucleotide constructs include, but are not limited to, a exogenous polynucleotide construct encoding a CD16 protein (such as a high affinity CD16 variant), an NKG2D protein and a self-cleavage peptide; an exogenous polynucleotide construct encoding a CAR; an exogenous polynucleotide construct encoding an HLA-E protein; an exogenous polynucleotide construct encoding an HLA-G protein; an exogenous polynucleotide construct encoding an HLA-E protein and an HLA-G protein; an exogenous polynucleotide construct encoding an HLA-E protein, an HLA-G protein, and a self-cleavage peptide; an exogenous polynucleotide construct encoding a fusion protein containing IL-15 and IL-15Ra; and an exogenous polynucleotide construct encoding an IL-15 protein. In various embodiments such cells can be administered to treat a patient with cancer, such as any type of cancer. In some embodiments, the cancer treated by methods of the present disclosure include a glioblastoma, ovarian cancer, cervical cancer, head and neck cancer, liver cancer, prostate cancer, pancreatic cancer, renal cell carcinoma, bladder cancer, other solid tumor cancer, or hematologic malignancy. In some embodiment, the hematologic malignancy is a leukemia (e.g., acute lymphocytic (ALL), chronic lymphocytic (CLL), acute myeloid (AML), chronic myeloid (CML)), myeloma, or lymphoma (e.g., Hodgkin's or non-Hodgkin's (NHL)).

[0298] In various embodiments of the treatment methods described herein, the disease is an autoimmune disease or disorder. In some embodiments, the autoimmune disease or disorder is 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.

[0299] In some embodiments, when more than one polypeptide with unique antigen-binding specificities are administered via cells described, the methods can be used to target multiple antigens (or multiple epitopes in the same antigen) in the same disease (e.g., tumor or autoimmune disease), or multiple antigens in different diseases (e.g., tumor or autoimmune disease). In various embodiments, when engineered cells expressing more than one polypeptide with unique antigen-binding specificities are administered, the methods as described can be used to target multiple antigens (or multiple epitopes in the same antigen) in the same disease (e.g., tumor or autoimmune disease), or multiple antigens in different diseases (e.g., tumor or autoimmune disease).

XV. Examples

Example 1: Exogenous expression of CD16 in iPSC cells expressing a chimeric antigen receptor (CAR) and gamma-delta iT cells derived therefrom

[0300] The CD16 transgene described herein was introduced into the CD70 locus of an iPSC cell carrying a CD19-specific CAR using homology directed repair and a CRISPR nuclease. An exemplary targeting construct is provided in FIG. 3 "p1630 CD16 at CD70" and SEQ ID NO:13. The resulting engineered iPSC cells were differentiated into gamma/delta iT -CAR cells (iPSC-derived γδ CAR T cells) as described below.

[0301] Detailed descriptions of useful methods for producing hematopoietic progenitor cells from iPSCs can be found in WO2022/120334, the contents including the examples are herein incorporated by reference in its entirety. iPSC derived hematopoietic progenitor cells (CD34-expressing HSCs) were differentiated into iPSC-derived γδ T cells on plates coated with retronectin and DLL4-Fc proteins and cultured in complete medium containing basal TCDM

medium supplemented with SCF (50 ng/ml), FLT3L (50 ng/ml), IL-7 (50 ng/ml), and TPO (50 ng/ml). At day 7, the cells were cultured in complete medium supplemented with CHIR09921 (2μM final concentration) on plates coated with RetroNectin® and DLL4-Fc proteins. At day 14, day 17, and day 21, the cells were re-seeded and cultured in complete medium supplemented with IL-2 (5 ng/ml) on plates coated with RetroNectin® and DLL4-Fc proteins. Culture medium was refreshed as needed. CD16 expression in the cells (see iPSC1283 and iPSC1302 of FIG. 6) was evaluated from day 0 to day 21.

Example 2: Enhanced anti-tumor activity of iNK cells overexpressing NKG2D protein

[0302] iPSCs were engineered to constitutively express NKG2D. When iPSCs were differentiated into iNK cells, expression of NKG2D was quantified by flow cytometry. The data demonstrated that NKG2D expression was increased to 95.5% of the engineered iNK cells compared to only 72.1% of the non-engineered iNK cells (FIG. 7A, left).

[0303] Non-engineered or NKG2D-engineered iNK cells were used in a killing assay with NLR-labeled U87 glioblastoma cells that express stress ligands (MIC-A and MIC-B) and can trigger NKG2D activity. The NKG2D-engineered iNK cells more potently killed U87 cells (FIG. 7A, right). To confirm that enhanced killing was due to NKG2D expression, a neutralizing (blocking) antibody against NKG2D was used in some conditions to block the interaction of NKG2D with stress the ligands on U87 cells. There was a marked reduction in U87 killing when the NKG2D neutralizing antibody was included with the NKG2D-engineered iNK cells (FIG. 7B). An isotype IgG1 control was used as an antibody control.

Example 3: Enhanced antibody-dependent cellular cytotoxicity (ADCC) of iNK cells overexpressing high-affinity CD16 variant protein

[0304] iPSCs were engineered to constitutively express one of two different naturally occurring variants of CD16 (a low affinity CD16 and a high affinity CD16). The iPSCs were then differentiated into iNK cells and used in a tumor killing assay where the targets were CD20+ lymphoblastic B cells. To trigger ADCC, an anti-CD20 therapeutic antibody rituximab (black bars) was included at various concentrations. As a negative control, non-binding isotype control antibody was used in some conditions (grey bars). When iPSCs were differentiated into iNK cells expressing the low affinity variant of CD16, ADCC was evident (increased dead tumor

cells) only when rituximab was included (FIG. 7C; top panel). When iPSCs were differentiated into iNK cells expressing the high affinity variant of CD16, greater ADCC was observed compared to the low affinity version of CD16 (FIG. 7C; bottom panel).

Example 4: Enhanced ADCC of CD16 overexpressing iNK cells using a CAR-mediated tumor killing assay of fluorescently-labeled Raji lymphoblastic B cells

[0305] iNK cells expressing a CD19-specific CAR (FMC63-CAR) or iNK cells without the CAR were engineered to express a CD16 variant construct – a low affinity CD16 variant (iPSC16) or a high affinity CD16 variant (iPSC17 or iPSC18). In an ADCC assay, the resulting iNK cells were tested for killing of either parental Raji or modified RajiΔCD19 target cells. The parental Raji cells are a lymphoblastic B cell line, which expresses B cell antigens CD19 and CD20. The modified RajiΔCD19 cells have been modified via CRISPR gene editing to knockout the gene encoding CD19. To trigger ADCC, an anti-CD20 therapeutic antibody rituximab (right bars with circle at top) was included at various concentrations. An IgG1 isotype control was included as a control (left bars with square at top).

[0306] The engineered iNK cells were co-cultured with either CellTrace Violet (CTV) labeled Raji cells or CTV labeled RajiΔCD19 target cells at an E:T of 3:1 in the presence of different concentrations of rituximab or a host-isotype (human IgG1) control (10, 1, 0.1, 0.01 and 0 μg/mL Rituximab or host-matched isotype) for 3 hours. The percentage of CTV-positive and non-viable or dead (7-AAD positive) cells were measured using a BD Symphony cytometer. The percentages of CTV+/7-AAD+ cells were graphed by rituximab or isotype control concentration.

[0307] ADCC was seen in iNK cells expressing the low affinity CD16 variant only when rituximab was included. Rituximab enabled iNK cells expressing high affinity CD16 variants were able to kill Raji cells and RajiΔCD19 cells to a greater extent (FIGS. 9-10).

[0308] While various embodiments of the invention(s) of the present disclosure have been shown and described herein, it will be obvious to those skilled in the art that such embodiments are provided by way of example only. Numerous variations, changes, and substitutions may occur to those skilled in the art without departing from the invention(s). It should be understood that various alternatives to the embodiments of the invention(s) described herein may be employed in practicing any one of the inventions(s) set forth herein.

[0309] The detailed descriptions above are presented in order to more fully illustrate some embodiments of the invention. They should, in no way be construed, however, as limiting the broad scope of the invention. One skilled in the art can readily devise many variations and modifications of the principles disclosed herein without departing from the scope of the invention.

WHAT IS CLAIMED IS:

1. An induced pluripotent stem cell (iPSC) or a derivative cell thereof comprising an exogenous polynucleotide encoding a CD16 protein and an NKG2D protein, wherein the CD16 protein and the NKG2D protein are operably linked by an autoprotease peptide.

2. The iPSC or the derivative cell thereof of claim 1, wherein the CD16 protein is a CD16 variant protein.

3. The iPSC or the derivative cell thereof of claim 1 or 2, wherein the CD16 variant protein is a high affinity CD16 variant.

4. The iPSC or the derivative cell thereof of any one of claims 1-3, wherein the CD16 variant protein is a non-cleavable CD16 variant.

5. The iPSC or the derivative cell thereof any one of claims 1-4, wherein the CD16 variant protein comprises one or more amino acid substitutions selected from the group consisting of F158V, F176V, S197P, D205A, S219A, T220A, and any combination thereof.

6. The iPSC or the derivative cell thereof of any one of claims 1-5, wherein the CD16 variant protein comprises an amino acid sequence having at least 90% sequence identity to any one of SEQ ID NOS:2 and 5.

7. The iPSC or the derivative cell thereof of any one of claims 1-6, wherein the NKG2D protein is a wildtype NKG2D protein.

8. The iPSC or the derivative cell thereof of any one of claims 1-6, wherein the NKG2D protein comprises an amino acid sequence having at least 90% sequence identity to SEQ ID NO:4.

9. The iPSC or the derivative cell thereof of any one of claims 1-8, 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.

10. The iPSC or the derivative cell thereof of any one of claims 1-9, wherein the autoprotease peptide is a P2A peptide comprising an amino acid sequence having at least 90% sequence identity to SEQ ID NO:3.

11. The iPSC or the derivative cell thereof of any one of claims 1-10, 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:6.

12. The iPSC or the derivative cell thereof of any one of claims 1-11, wherein the exogenous polynucleotide is integrated into a gene locus 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 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.

13. The iPSC or the derivative cell thereof of any one of claims 1-12, wherein the exogenous polynucleotide is integrated into a gene locus selected from the group consisting of an AAVS1 locus, a B2M locus, a CIITA locus, a CD70 locus, a CLYBL locus, an NKG2A locus, an NKG2D locus, a TAP1 locus, a TAP2 locus, a TAPBP locus, a TRAC locus, a TRBC1 locus, a RFXANK locus, a RFX5 locus, and a RFXAP locus, thereby disrupting expression of the gene.

14. The iPSC or the derivative cell thereof of any one of claims 1-13, wherein the exogenous polynucleotide is integrated into a gene locus selected from the group consisting of an AAVS1 locus, a B2M locus, a CIITA locus, a CD70 locus, a CLYBL locus, an NKG2A locus and a TRAC locus, thereby disrupting expression of the gene.

15. The iPSC or the derivative cell thereof of claim 13 or 14, wherein the disruption of the gene comprises an elimination of or reduced expression of the gene.

16. The iPSC or the derivative cell thereof of any one of claims 12-14, wherein the integration into the gene locus is generated by targeted genome editing.

17. The iPSC or the derivative cell thereof of claim 16, wherein the targeted genome editing comprises using a method selected from the group consisting of a CRISPR method, a zinc finger nuclease method, a TALEN method, a homing nuclease method, a homology recombination method, and any functional variation thereof.

18. The iPSC or the derivative cell thereof of any one of claims 1-17, further comprising a disruption of one or more genes selected from the group consisting of an AAVS1 gene, a B2M gene, a CIITA gene, a CD70 gene, a CLYBL gene, an NKG2A gene, an NKG2D gene, a TAP1 gene, a TAP2 gene, a TAPBP gene, a TRAC gene, a TRBC1 gene, a RFXANK gene, a RFX5 gene, a RFXAP gene, and any combination thereof.

19. The iPSC or the derivative cell thereof of claim 18, wherein the disruption is of the B2M gene and the CIITA gene.

20. The iPSC or the derivative cell thereof of claim 18 or 19, wherein the disruption of the one or more genes comprises an elimination of or reduced expression of the one or more genes.

21. The iPSC or the derivative cell thereof of any one of claims 18-20, wherein the disruption of the one or more genes is generated by targeted genome editing.

22. The iPSC or the derivative cell thereof of claim 21, wherein the targeted genome editing comprises using a method selected from the group consisting of a CRISPR method, a zinc finger nuclease method, a TALEN method, a homing nuclease method, a homology recombination method, and any functional variation thereof.

23. The iPSC or the derivative cell thereof of any one of claims 1-22, further comprising a second exogenous polynucleotide encoding an IL-15 protein.

24. The iPSC or the derivative cell thereof of claim 23, wherein the IL-15 protein comprises an amino acid sequence having at least 90% sequence identity to SEQ ID NO:16.

25. The iPSC or the derivative cell thereof of any one of claims 1-22, further comprising a second exogenous polynucleotide encoding a fusion polypeptide comprising an IL-15 and an IL-15 receptor alpha (IL-15Rα).

26. The iPSC or the derivative cell thereof of claim 25, wherein the fusion polypeptide comprises an amino acid sequence having at least 90% sequence identity to SEQ ID NO:17.

27. The iPSC or the derivative cell thereof of claim 25 or 26, wherein the fusion polypeptide comprises the amino acid sequence of SEQ ID NO:17.

28. The iPSC or the derivative cell thereof of any one of claims 1-27, further comprising a third exogenous polynucleotide encoding a human leukocyte antigen E (HLA-E) protein.

29. The iPSC or the derivative cell thereof of claim 27, wherein the HLA-E comprises an amino acid sequence having at least 90% sequence identity to SEQ ID NO:14.

30. The iPSC or the derivative cell thereof of any one of claims 1-29, further comprising a fourth exogenous polynucleotide encoding a human leukocyte antigen G (HLA-G) protein.

31. The iPSC or the derivative cell thereof of claim 30, wherein the HLA-G comprises an amino acid sequence having at least 90% sequence identity to SEQ ID NO:15.

32. The iPSC or the derivative cell thereof of claim 30 or 31, wherein the HLA-E protein and the HLA-G protein are operably linked by a second autoprotease peptide.

33. The iPSC or the derivative cell thereof of claim 32, wherein the second autoprotease peptide is selected from the group consisting of a P2A peptide, an F2A peptide, an E2A peptide, a T2A peptide, a BmCPV2A peptide and a BmIFV2A peptide.

34. The iPSC or the derivative cell thereof of any one of claims 23-33, wherein the second, third, and/or fourth exogenous polynucleotides are integrated into one or more gene loci 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 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, and any combination thereof.

35. The iPSC or the derivative cell thereof of any one of claims 23-34, wherein the second, third, and/or fourth exogenous polynucleotides are integrated into one or more gene loci selected from the group consisting of an AAVS1 locus, a B2M locus, a CIITA locus, a CD70 locus, a CLYBL locus, an NKG2A locus, an NKG2D locus, a TAP1 locus, a TAP2 locus, a TAPBP locus, a TRAC locus, a TRBC1 locus, a RFXANK locus, a RFX5 locus, a RFXAP locus, and any combination thereof.

36. The iPSC or the derivative cell thereof of any one of claims 23-35, wherein the second, third, and/or fourth exogenous polynucleotides are integrated into one or more gene loci selected from the group consisting of an AAVS1 locus, a B2M locus, a CIITA locus, a CD70 locus, a CLYBL locus, an NKG2A locus, an NKG2D locus, a TAP1 locus, a TAP2 locus, a TAPBP locus, a TRAC locus, a TRBC1 locus, a RFXANK locus, a RFX5 locus, a RFXAP locus, and any combination thereof, thereby disrupting the one or more genes.

37. The iPSC or the derivative cell thereof of any one of claims 23-36, wherein the second, third, and/or fourth exogenous polynucleotides are integrated into one or more gene loci selected from the group consisting of an AAVS1 locus, a B2M locus, a CIITA locus, a CD70 locus, a CLYBL locus, an NKG2A locus, a TRAC locus, and any combination thereof, thereby disrupting the one or more genes.

38. The iPSC or the derivative cell thereof of claim 36 or 37, wherein the disruption in the one or more genes comprises an elimination or reduced expression of the one or more genes.

39. The iPSC or the derivative cell thereof of any one of claims 1-38, wherein the iPSC is reprogrammed from whole peripheral blood mononuclear cells (PBMCs).

40. The iPSC or the derivative cell thereof of any one of claims 1-39, wherein the iPSC is derived from a reprogrammed NK or T cell.

41. The iPSC or the derivative cell thereof of any one of claims 1-40, further comprising a fifth exogenous polynucleotide encoding a chimeric antigen receptor (CAR) that binds a target antigen.

42. The iPSC or the derivative cell thereof of claim 41, wherein the target antigen is selected from the group consisting of 17-1A antigen, A3, A33 antigen, AFP, B7H4, Ba 733, BCMA, BrE3 antigen, CA125, CA9 (CAIX), CD1, CD1a, CD3, CD5, CD15, CD16, CD19, CD20, CD21, CD22, CD22, CD23, CD25, CD30, CD33, CD33, CD38, CD45, CD70, CD74, CD79, CD79a, CD80, CD123, CD133, CD138, CEACAM5, CEACAM6, CLDN18.2, CLL1, cMET, colon-specific antigen-p (CSAp), ED-B fibronectin, EGFR, EGFRvIII, EGP-1, EGP-2, EpCAM, EphA1, EphA2, EphA3, EphA4, EphA5, EphA6, EphA7, EphA8, EphA10, EphB1, EphB2, EphB3, EphB4, EphB6, FGFR1, FGFR3, Flt-1, Flt-3, FOLR1, FOLR2, FOLR3, FSHR, GD2, GPC-3, GPRC5D, HCG, a HCG subunit, HER2, HIF-I, HLA-DR, Ia, IGF-I, IL13Rα2, IL-2, IL-6, IL-8, KC4 antigen, KS-1 antigen, KS1-4 antigen, Le-Y, MAGE, MET, MIF, MSLN, MUC1, MUC2, MUC3, MUC4, MUC16, NCA66, NCA90, NCA95, Nectin-4, p53, PAP, PDGFRA, PLGF, PSA, PSMA, ROBO1, RS5, S100, SLAM F7, SLITRK6, TAC, TAG-72, tenascin-C, tenascin-R, tenascin-W, tenascin-X, Thomson-Friedenreich antigen, Tn antigen, TRAILR1, TRAILR2, TRAILR3, TRAILR4, VEGF, a tumor necrosis antigen, an angiogenesis antigen, and an oncogene antigen.

43. The iPSC or the derivative cell thereof of claim 41 or 42, wherein the CAR comprises an antigen-binding domain selected from the group consisting of any provided in Tables 1, 2 and 3.

44. The iPSC or the derivative cell thereof of any one of claims 41-43, wherein the CAR comprises:

(i) a signal peptide;

(ii) an extracellular domain comprising a binding domain that specifically binds the target antigen;

(iii) a hinge region;

(iv) a transmembrane domain,

(v) an intracellular signaling domain; and

(vi) one or more co-stimulatory domains.

45. The iPSC or the derivative cell thereof of claim 44, wherein the signal peptide comprises a GMCSFR signal peptide.

46. The iPSC or the derivative cell thereof of claim 44 or 45, wherein the extracellular domain comprises a single chain Fv (scFv) or a VHH domain that specifically binds the target antigen.

47. The iPSC or the derivative cell thereof of any one of claims 44-46, wherein the hinge region comprises a CD28 hinge region.

48. The iPSC or the derivative cell thereof of any one of claims 44-47, wherein the transmembrane domain comprises a CD28 transmembrane domain.

49. The iPSC or the derivative cell thereof of any one of claims 44-48, wherein the intracellular signaling domain comprises a CD3ζ intracellular domain.

50. The iPSC or the derivative cell thereof of any one of claims 44-49, wherein the one or more co-stimulatory domains comprise a CD28 signaling domain.

51. The derivative cell thereof of any one of claims 1-50, wherein the derivative cell is an NK cell or a T cell.

52. The derivative cell thereof of claim 51, wherein the derivative cell is an NK cell.

53. The derivative cell thereof of claim 51, wherein the derivative cell is a T cell.

54. The derivative cell thereof of any one of claims 1-53, wherein the derivative cell is a CD34+ hematopoietic progenitor cell.

55. A composition comprising a population of the iPSCs or the derivative cells thereof of any one of claims 1-54.

56. An engineered cell comprising:

(i) a first exogenous polynucleotide encoding a CD16 protein and an NKG2D protein, wherein the CD16 protein and the NKG2D protein are operably linked by an autoprotease peptide;

(ii) a second exogenous polynucleotide encoding an exogenous polypeptide comprising an IL-15 protein; and

(iii) optionally, a third exogenous polynucleotide encoding a human leukocyte antigen E (HLA-E) and/or a fourth exogenous polynucleotide encoding a human leukocyte antigen G (HLA-G).

57. The engineered cell thereof of claim 56, further comprising a fifth polynucleotide encoding 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 a prostate-specific membrane antigen (PSMA) extracellular domain or fragment thereof.

58. The engineered cell thereof of claim 56 or 57, wherein 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:23 or 29.

59. The engineered cell thereof of any one of claims 56-58, wherein the combined artificial cell death/reporter system polypeptide comprises the HSV-TK fused to a truncated variant PSMA polypeptide via the linker.

60. The engineered cell thereof of any one of claims 56-59, 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:24.

61. The engineered cell thereof of any one of claims 56-59, 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.

62. The engineered cell thereof of any one of claims 56-60, 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:25.

63. The engineered cell thereof of any one of claims 56-62, 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:27, 30 and 31.

64. The engineered cell thereof of any one of claims 56-63, 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:26, 28 and 32.

65. An engineered cell comprising:

(i) a first exogenous polynucleotide encoding a CD16 protein and an NKG2D protein, wherein the CD16 protein and the NKG2D protein are operably linked by an autoprotease peptide;

(ii) a second exogenous polynucleotide encoding a fusion polypeptide comprising an IL-15 protein and an IL-15 receptor alpha (IL-15Rα) protein; and

(iii) optionally, a third exogenous polynucleotide encoding a human leukocyte antigen E (HLA-E) protein and/or a fourth exogenous polynucleotide encoding a human leukocyte antigen G (HLA-G) protein.

66. The engineered cell of any one of claims 56-65, wherein the engineered cell is an engineered induced pluripotent stem cell (iPSC), an engineered natural killer (NK) cell or an engineered T cell.

67. The engineered cell of any one of claims 56-66, wherein the first exogenous polynucleotide comprises the nucleic acid sequence of SEQ ID NO:6.

68. The engineered cell of any one of claims 56-67, wherein the IL-15 protein comprises an amino acid sequence of SEQ ID NO:16.

69. The engineered cell of any one of claims 56-67, wherein the second exogenous polynucleotide comprises the nucleic acid sequence encoding an IL-15/IL-15Rα fusion protein of SEQ ID NO:17.

70. The engineered cell of any one of claims 56-69, wherein the third exogenous polynucleotide comprises the nucleic acid sequence of SEQ ID NO:21 and the fourth exogenous polynucleotide comprises the nucleic acid sequence of SEQ ID NO:22.

71. The engineered cell of any one of claims 56-69, wherein the HLA-E protein and HLA-G protein are linked by an autoprotease peptide.

72. The engineered cell of any one of claims 56-70, further comprising a disruption of the B2M and CIITA genes.

73. The engineered cell of claim 72, wherein the disruption of the B2M and CIITA genes is generated by targeted genome editing.

74. The engineered cell of 73, wherein the targeted genome editing comprises using a method selected from the group consisting of a CRISPR method, a zinc finger nuclease method, a TALEN method, a homing nuclease method, a homology recombination method, and any functional variation thereof.

75. The engineered cell of any one of claims 56-74, wherein the first exogenous polynucleotide is integrated into a gene locus 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 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.

76. The engineered cell of any one of claims 56-75, wherein the second exogenous polynucleotide is integrated into a gene locus 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 CD38 locus, a TRAC locus, a TRBC1 locus, a ROSA26 locus, a

collagen 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.

77. The engineered cell of any one of claims 56-76, wherein the third exogenous polynucleotide is integrated into a gene locus 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 CD38 locus, a TRAC locus, a TRBC1 locus, a ROSA26 locus, a collagen 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.

78. The engineered cell of any one of claims 56-77, wherein the fourth exogenous polynucleotide is integrated into a gene locus 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 CD38 locus, a TRAC locus, a TRBC1 locus, a ROSA26 locus, a collagen 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.

79. The engineered cell of any one of claims 56-78, wherein the first exogenous polynucleotide and either the second, third or fourth exogenous polynucleotides are integrated into the B2M gene locus and the CIITA gene locus, thereby disrupting the B2M and CIITA genes.

80. The engineered cell of any one of claims 56-78, wherein the first exogenous polynucleotide is integrated into the CD70 locus and the second exogenous polynucleotide is integrated into the B2M gene locus, thereby disrupting the CD70 and B2M genes.

81. The engineered cell of any one of claims 56-78, wherein the first exogenous polynucleotide is integrated into the CD70 locus and the second exogenous polynucleotide is integrated into the CIITA gene locus, thereby disrupting the CD70 and CIITA genes.

82. The engineered cell of any one of claims 56-78, wherein the first exogenous polynucleotide is integrated into the CD70 locus and the third or fourth exogenous polynucleotide is integrated into the B2M gene locus, thereby disrupting the CD70 and B2M genes.

83. The engineered cell of any one of claims 56-78, wherein the first exogenous polynucleotide is integrated into the CD70 locus and the third or fourth exogenous polynucleotide is integrated into the CIITA gene locus, thereby disrupting the CD70 and CIITA genes.

84. The engineered cell of any one of claims 80-83, wherein the integration into the CD70 locus is into exon 1 of the CD70 gene.

85. The engineered cell of any one of claims 75-84, wherein the integration into the gene locus is generated by targeted genome editing.

86. The engineered cell of claim 85, wherein the targeted genome editing comprises using a method selected from the group consisting of a CRISPR method, a zinc finger nuclease method, a TALEN method, a homing nuclease method, a homology recombination method, and any functional variation thereof.

87. The engineered cell of any one of claims 78-86, further comprising a fifth exogenous polynucleotide encoding a chimeric antigen receptor (CAR) that binds a target antigen.

88. The engineered cell of claim 87, wherein the target antigen is selected from the group consisting of 17-1A antigen, A3, A33 antigen, AFP, B7H4, Ba 733, BCMA, BrE3 antigen, CA125, CA9 (CAIX), CD1, CD1a, CD3, CD5, CD15, CD16, CD19, CD20, CD21, CD22, CD22, CD23, CD25, CD30, CD33, CD33, CD38, CD45, CD70, CD74, CD79, CD79a, CD80, CD123, CD133, CD138, CEACAM5, CEACAM6, CLDN18.2, CLL1, cMET, colon-specific

antigen-p (CSAp), ED-B fibronectin, EGFR, EGFRvIII, EGP-1, EGP-2, EpCAM, EphA1, EphA2, EphA3, EphA4, EphA5, EphA6, EphA7, EphA8, EphA10, EphB1, EphB2, EphB3, EphB4, EphB6, FGFR1, FGFR3, Flt-1, Flt-3, FOLR1, FOLR2, FOLR3, FSHR, GD2, GPC-3, GPRC5D, HCG, a HCG subunit, HER2, HIF-I, HLA-DR, Ia, IGF-I, IL13Rα2, IL-2, IL-6, IL-8, KC4 antigen, KS-1 antigen, KS1-4 antigen, Le-Y, MAGE, MET, MIF, MSLN, MUC1, MUC2, MUC3, MUC4, MUC16, NCA66, NCA90, NCA95, Nectin-4, p53, PAP, PDGFRA, PLGF, PSA, PSMA, ROBO1, RS5, S100, SLAM F7, SLITRK6, TAC, TAG-72, tenascin-C, tenascin-R, tenascin-W, tenascin-X, Thomson-Friedenreich antigen, Tn antigen, TRAILR1, TRAILR2, TRAILR3, TRAILR4, VEGF, a tumor necrosis antigen, an angiogenesis antigen, and an oncogene antigen.

89. The engineered cell of claim 88, wherein the CAR comprises an antigen-binding domain selected from the group consisting of any provided in Tables 1, 2 and 3.

90. The engineered cell of any one of claims 87-89, wherein the CAR comprises:

(i) a signal peptide;

(ii) an extracellular domain comprising a binding domain that specifically binds the target antigen;

(iii) a hinge region;

(iv) a transmembrane domain;

(v) an intracellular signaling domain; and

(vi) one or more co-stimulatory domains.

91. The engineered cell of claim 90, wherein the signal peptide comprises a GMCSFR signal peptide.

92. The engineered cell of claim 90 or 91, wherein the extracellular domain comprises a single chain Fv (scFv) or a VHH domain that specifically binds the target antigen.

93. The engineered cell of any one of claims 90-92, wherein the hinge region comprises a CD28 hinge region.

94. The engineered cell of any one of claims 90-93, wherein the transmembrane domain comprises a CD28 transmembrane domain.

95. The engineered cell of any one of claims 90-94, wherein the intracellular signaling domain comprises a CD3ζ intracellular domain.

96. The engineered cell of any one of claims 90-95, wherein the one or more co-stimulatory domains comprise a CD28 signaling domain.

97. The engineered cell of any one of claims 56-96, wherein the engineered iPSC is differentiated into an engineered differentiated cell.

98. The engineered cell of any one of claims 56-97, wherein the engineered iPSC is differentiated into an engineered NK cell.

99. The engineered cell of any one of claims 56-97, wherein the engineered iPSC is differentiated into an engineered T cell.

100. The engineered cell of any one of claims 56-97, wherein the engineered iPSC is differentiated into an engineered CD34+ hematopoietic progenitor cell.

101. A composition comprising a population of the engineered iPSCs of any one of claims 56-96.

102. A composition comprising a population of the engineered differentiated cells of any one of claims 56-101.

103. A composition comprising a population of the engineered NK cells of any one of claims 56-98.

104. A composition comprising a population of the engineered T cells of any one of claims 56-97 and 99.

105. A composition comprising a population of the engineered CD34+ hematopoietic progenitor cells of any one of claims 56-97 and 100.

106. A method of treating cancer in a subject in need thereof, comprising administering the derivative cells thereof of any one of claims 1-54, the engineered NK cells of any one of claims 56-98, the engineered T cells of any one of claims 56-97 and 99, the

engineered CD34+ hematopoietic progenitor cells of any one of claims 56-97 and 100, or the composition of any one of claims 55 and 101-105 to the subject in need thereof.

107. The method of claim 106, wherein the cancer is selected from the group consisting of acute lymphocytic leukemia (ALL), acute myeloid leukemia (AML), adenomas, benign lesions, bladder cancers, bone cancers, breast cancers, cancers of the thyroid gland, carcinomas of the larynx, carcinomas of the lung, carcinomas of the mouth, carcinomas of the throat, cervical cancers, chronic lymphocytic leukemia (CLL), chronic myeloid leukemias (CML), cutaneous melanomas, endocrine cancers, endometrial cancers, gastrointestinal cancers, genitourinary cancers, glioblastomas, head and neck cancers, hematologic malignancy, hematopoietic cancers, Hodgkin's lymphoma, intraocular melanomas, leukemias, liver cancers, lymphomas, melanomas, myelomas, myeloproliferative disorders, nervous system cancers, non-Hodgkin's lymphoma, ovarian cancers, pancreatic cancers, papillomas, parathyroid gland cancers, prostate cancers, renal cell carcinomas, sarcomas, skin cancers, solid tissue carcinomas, squamous cell carcinomas, and uterine cancers.

108. A method of differentiating the iPSC cell into an NK cell, comprising subjecting the iPSC cell of any one of claims 1-50 and 56-96 to a differentiation protocol comprising culturing the cell in a medium comprising a recombinant human IL-12 protein for the final 24 hours of culturing under the differentiation protocol, thereby generating the NK cell.

109. The method of claim 108, wherein the recombinant human IL-12 protein 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:33.

110. A method of differentiating the iPSC cell into a T cell, comprising subjecting the iPSC cell of any one of claims 1-50 and 56-96 to a differentiation protocol comprising culturing the cell in a medium comprising a recombinant DLL4 variant polypeptide having an amino acid sequence selected from the group consisting of SEQ ID NOS:35-37, thereby generating the T cell.

111. A recombinant DLL4 variant polypeptide having an amino acid sequence selected from the group consisting of SEQ ID NOS:35-37.

112. A method of differentiating the iPSC cell into a CD34+ hematopoietic progenitor cell, comprising subjecting the iPSC cell of any one of claims 1-50 and 56-96 to a differentiation protocol comprising culturing the cell in a pre-selected medium, thereby generating the CD34+ hematopoietic progenitor cell.

113. A polynucleotide encoding a CD16 protein and an NKG2D protein, wherein the CD16 protein and the NKG2D protein are operably linked by an autoprotease peptide.

114. The polynucleotide of claim 113, wherein the CD16 protein comprises an amino acid sequence having at least 90% sequence identity to SEQ ID NO:5.

115. The polynucleotide of claim 113 or 114, wherein the CD16 protein is encoded by a nucleic acid sequence having at least 90% sequence identity to SEQ ID NO:7.

116. The polynucleotide of claim 113, wherein the CD16 protein is a CD16 variant protein.

117. The polynucleotide of claim 115, wherein the CD16 variant protein comprises one or more amino acid substitutions selected from the group consisting of F158V, F176V, S197P, D205A, S219A, T220A, and any combination thereof.

118. The polynucleotide of claim 115 or 117, wherein the CD16 variant comprises an amino acid sequence having at least 90% sequence identity to SEQ ID NO:2 or SEQ ID NO:5.

119. The polynucleotide of any one of claims 115-118, wherein the CD16 variant is encoded by a nucleic acid sequence having at least 90% sequence identity to SEQ ID NO:7.

120. The polynucleotide of any one of claims 115-119, wherein the NKG2D protein comprises an amino acid sequence having at least 90% sequence identity to SEQ ID NO:4.

121. The polynucleotide of any one of claims 115-120, wherein the NKG2D protein is encoded by a nucleic acid sequence having at least 90% sequence identity to SEQ ID NO:9.

122. The polynucleotide of any one of claims 115-120, wherein the NKG2D protein is an NKG2D variant protein.

123. The polynucleotide of claim 121, wherein the NKG2D variant comprises an amino acid sequence having at least 90% sequence identity to SEQ ID NO:4.

124. The polynucleotide of any one of claims 113-123, wherein the autoprotease peptide is selected from the group consisting of 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.

125. The polynucleotide of any one of claims 113-124, wherein the autoprotease peptide is a P2A peptide comprising an amino acid sequence having at least 90% sequence identity to SEQ ID NO:3.

126. The polynucleotide of any one of claims 113-125, wherein the autoprotease peptide is a P2A peptide encoded by a nucleic acid sequence having at least 90% sequence identity to SEQ ID NO:8.

127. The polynucleotide of any one of claims 113-125, wherein the exogenous polynucleotide encoding the CD16 protein and the NKG2D protein comprises the nucleic acid sequence having at least 90% sequence identity to SEQ ID NO:6.

128. The polynucleotide of any one of claims 113-126, wherein the exogenous polynucleotide encoding the CD16 protein and the NKG2D protein has the nucleic acid sequence of SEQ ID NO:6.

129. A vector comprising the polynucleotide of any one of claims 113-127.

130. The vector of claim 128, comprising from 5' to 3':

(i) a left homology sequence;

(ii) a promoter;

(iii) the polynucleotide of any one of claims 113-127;

(iv) a terminator and/or a polyadenylation signal sequence; and

(iv) a right homology sequence.

131. The vector of claim 130, wherein the left homology sequence comprises a nucleic acid sequence having at least 90% sequence identity to SEQ ID NO:11.

132. The vector of claim 130 or 131, wherein the right homology sequence comprises a nucleic acid sequence having at least 90% sequence identity to SEQ ID NO:12.

133. The vector of any one of claims 128-132, wherein the vector comprises a nucleic acid sequence having at least 90% sequence identity to SEQ ID NO:13.

134. The vector of any one of claims 128-132, wherein the vector comprises the nucleic acid sequence of SEQ ID NO:13.

135. The vector of any one of claims 128-132, wherein the vector comprises a nucleic acid sequence having at least 90% sequence identity to SEQ ID NO:39.

136. The vector of any one of claims 128-132, wherein the vector comprises the nucleic acid sequence of SEQ ID NO:39.

FIG. 1A

CD16 (F176V)-P2A-NKG2D transgene – amino acid sequence (SEQ ID NO:1)

MWQLLLPTALLLLVSAGMRTEDLPKAVVFLEPQWYRVLEKDSVTLKCQGAYSPEDNSTQWFHNESLISSQ
ASSYFIDAATVDDSGEYRCQTNLSTLSDPVQLEVHIGWLLLQAPRWVFKEEDPIHLRCHSWKNTALHKVT
YLQNGKGRKYFHHNSDFYIPKATLKDSGSYFCRGLVGSKNVSSETVNITITQGLAVSTISSFFPPGYQVS
FCLVMVLLFAVDTGLYFSVKTNIRSSTRDWKDHKFKWRKDPQDKGSGATNFSLLKQAGDVEENPGP**MGWI
RGRRSRHSWEMSEFHNYNLDLKKSDFSTRWQKQRCPVVKSKCRENASPFFFCCFIAVAMGIRFIIMVTIW
SAVFLNSLFNQEVQIPLTESYCGPCPKNWICYKNNCYQFFDESKNWYESQASCMSQNASLLKVYSKEDQD
LLKLVKSYHWMGLVHIPTNGSWQWEDGSILSPNLLTIIEMQKGDCALYASSFKGYIENCSTPNTYICMQR
TV**

CD16 (F176V) – SEQ ID NO:2
MWQLLLPTALLLLVSAGMRTEDLPKAVVFLEPQWYRVLEKDSVTLKCQGAYSPEDNSTQWFHNESLISSQ
ASSYFIDAATVDDSGEYRCQTNLSTLSDPVQLEVHIGWLLLQAPRWVFKEEDPIHLRCHSWKNTALHKVT
YLQNGKGRKYFHHNSDFYIPKATLKDSGSYFCRGLVGSKNVSSETVNITITQGLAVSTISSFFPPGYQVS
FCLVMVLLFAVDTGLYFSVKTNIRSSTRDWKDHKFKWRKDPQDK

P2A – SEQ ID NO:3
GSGATNFSLLKQAGDVEENPGP

NKG2D – SEQ ID NO:4
MGWIRGRRSRHSWEMSEFHNYNLDLKKSDFSTRWQKQRCPVVKSKCRENASPFFFCCFIAVAMGIRFIIM
VTIWSAVFLNSLFNQEVQIPLTESYCGPCPKNWICYKNNCYQFFDESKNWYESQASCMSQNASLLKVYSK
EDQDLLKLVKSYHWMGLVHIPTNGSWQWEDGSILSPNLLTIIEMQKGDCALYASSFKGYIENCSTPNTYI
CMQRTV

wildtype CD16 – SEQ ID NO:5
MWQLLLPTALLLLVSAGMRTEDLPKAVVFLEPQWYRVLEKDSVTLKCQGAYSPEDNSTQWFHNESLISSQ
ASSYFIDAATVDDSGEYRCQTNLSTLSDPVQLEVHIGWLLLQAPRWVFKEEDPIHLRCHSWKNTALHKVT
YLQNGKGRKYFHHNSDFYIPKATLKDSGSYFCRGLFGSKNVSSETVNITITQGLAVSTISSFFPPGYQVS
FCLVMVLLFAVDTGLYFSVKTNIRSSTRDWKDHKFKWRKDPQDK

FIG. 1B

CD16 (F176V)-P2A-NKG2D transgene – nucleic acid sequence (SEQ ID NO:6)

ATGTGGCAACTGTTGCTGCCTACAGCTCTGCTGCTGCTGGTGTCTGCCGGCATGAGAACAGAGGATCTGC
CTAAGGCCGTGGTGTTCCTGGAACCTCAGTGGTACAGAGTGCTGGAAAAGGACAGCGTGACCCTGAAGTG
CCAGGGCGCCTATTCTCCCGAGGACAATAGCACCCAGTGGTTCCACAACGAGAGCCTGATCAGCAGCCAG
GCCAGCAGCTACTTTATCGATGCCGCCACCGTGGACGACAGCGGCGAGTACAGATGCCAGACCAATCTGA
GCACCCTGAGCGACCCTGTGCAGCTGGAAGTGCACATTGGATGGCTGCTGCTTCAGGCCCCTAGATGGGT
GTTCAAAGAAGAGGACCCCATCCACCTGAGATGCCACTCTTGGAAGAACACAGCCCTGCACAAAGTGACC
TACCTGCAGAACGGCAAGGGCAGAAAGTACTTCCACCACAACAGCGACTTCTACATCCCCAAGGCCACAC
TGAAGGACTCCGGCTCCTACTTCTGTAGAGGCCTCGTGGGCAGCAAGAACGTGTCCAGCGAGACAGTGAA
CATCACCATCACACAGGGCCTCGCCGTGTCTACCATCAGCAGCTTTTTCCCACCTGGCTATCAGGTGTCC
TTCTGCCTGGTCATGGTGCTGCTGTTCGCCGTGGATACCGGCCTGTACTTCAGCGTCAAGACCAACATCC
GGTCCAGCACCAGAGACTGGAAGGACCACAAGTTCAAGTGGCGGAAGGACCCTCAGGACAAAGGCAGCGG
CGCCACCAATTTCAGCCTGCTGAAACAGGCTGGCGACGTGGAAGAGAACCCTGGACCT**ATGGGCTGGATC**
CGGGGCAGAAGAAGCAGACACAGCTGGGAGATGAGCGAGTTCCACAATTACAACCTGGACCTGAAGAAGT
CCGACTTCAGCACCCGGTGGCAGAAACAGAGATGCCCCGTGGTCAAGAGCAAGTGCAGAGAGAACGCTAG
CCCCTTCTTCTTCTGTTGCTTTATCGCCGTGGCCATGGGCATCCGCTTCATCATCATGGTCACAATTTGG
AGCGCCGTGTTCCTGAACTCCCTGTTCAATCAAGAGGTGCAGATCCCTCTGACCGAGAGCTACTGTGGCC
CCTGTCCTAAGAACTGGATCTGCTACAAGAACAACTGCTACCAGTTCTTCGACGAGAGCAAGAATTGGTA
CGAGAGCCAGGCCTCCTGCATGAGCCAGAATGCTTCCCTGCTGAAGGTGTACAGCAAAGAGGACCAGGAT
CTGCTGAAGCTGGTCAAGTCCTACCACTGGATGGGCCTCGTGCACATCCCTACCAATGGCTCTTGGCAGT
GGGAGGACGGCAGCATTCTGAGCCCTAACCTGCTGACCATCATCGAGATGCAGAAGGGCGACTGCGCCCT
GTACGCCAGCTCTTTCAAGGGCTACATCGAGAACTGCAGCACCCCTAACACCTACATCTGTATGCAGCGG
ACCGTGTGA

FIG. 1C

CD16 (F176V) – SEQ ID NO:7

ATGTGGCAACTGTTGCTGCCTACAGCTCTGCTGCTGCTGGTGTCTGCCGGCATGAGAACAGAGGATCTGC
CTAAGGCCGTGGTGTTCCTGGAACCTCAGTGGTACAGAGTGCTGGAAAAGGACAGCGTGACCCTGAAGTG
CCAGGGCGCCTATTCTCCCGAGGACAATAGCACCCAGTGGTTCCACAACGAGAGCCTGATCAGCAGCCAG
GCCAGCAGCTACTTTATCGATGCCGCCACCGTGGACGACAGCGGCGAGTACAGATGCCAGACCAATCTGA
GCACCCTGAGCGACCCTGTGCAGCTGGAAGTGCACATTGGATGGCTGCTGCTTCAGGCCCCTAGATGGGT
GTTCAAAGAAGAGGACCCCATCCACCTGAGATGCCACTCTTGGAAGAACACAGCCCTGCACAAAGTGACC
TACCTGCAGAACGGCAAGGGCAGAAAGTACTTCCACCACAACAGCGACTTCTACATCCCCAAGGCCACAC
TGAAGGACTCCGGCTCCTACTTCTGTAGAGGCCTCGTGGGCAGCAAGAACGTGTCCAGCGAGACAGTGAA
CATCACCATCACACAGGGCCTCGCCGTGTCTACCATCAGCAGCTTTTTCCCACCTGGCTATCAGGTGTCC
TTCTGCCTGGTCATGGTGCTGCTGTTCGCCGTGGATACCGGCCTGTACTTCAGCGTCAAGACCAACATCC
GGTCCAGCACCAGAGACTGGAAGGACCACAAGTTCAAGTGGCGGAAGGACCCTCAGGACAAA

P2A – SEQ ID NO:8

GGCAGCGGCGCCACCAATTTCAGCCTGCTGAAACAGGCTGGCGACGTGGAAGAGAACCCTGGACCT

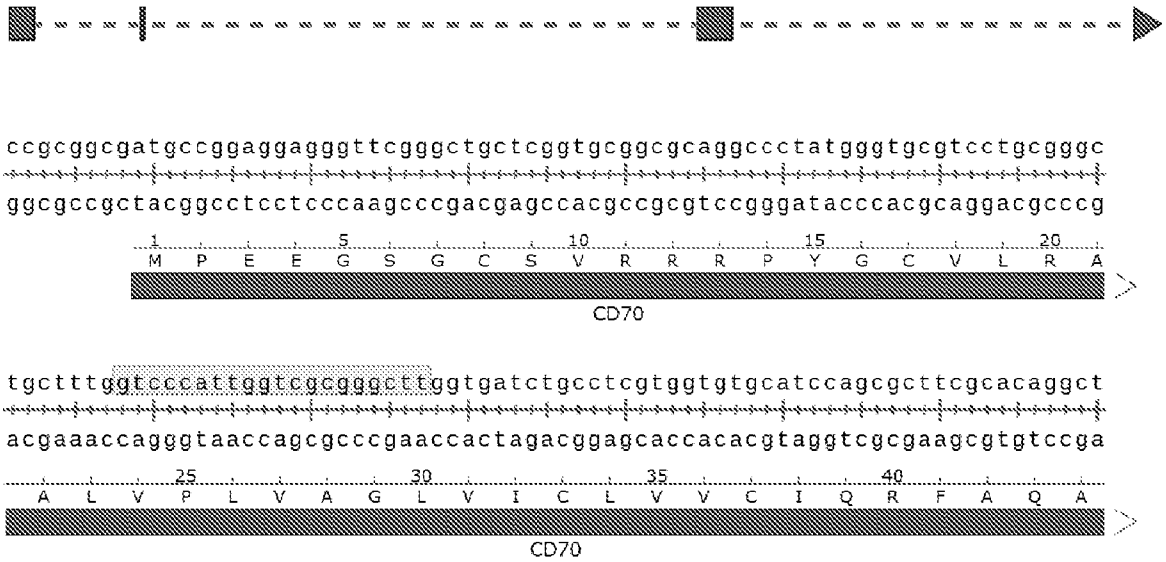
NKG2D – SEQ ID NO:9

ATGGGCTGGATCCGGGGCAGAAGAAGCAGACACAGCTGGGAGATGAGCGAGTTCCACAATTACAACCTGG
ACCTGAAGAAGTCCGACTTCAGCACCCGGTGGCAGAAACAGAGATGCCCCGTGGTCAAGAGCAAGTGCAG
AGAGAACGCTAGCCCCTTCTTCTTCTGTTGCTTTATCGCCGTGGCCATGGGCATCCGCTTCATCATCATG
GTCACAATTTGGAGCGCCGTGTTCCTGAACTCCCTGTTCAATCAAGAGGTGCAGATCCCTCTGACCGAGA
GCTACTGTGGCCCCTGTCCTAAGAACTGGATCTGCTACAAGAACAACTGCTACCAGTTCTTCGACGAGAG
CAAGAATTGGTACGAGAGCCAGGCCTCCTGCATGAGCCAGAATGCTTCCCTGCTGAAGGTGTACAGCAAA
GAGGACCAGGATCTGCTGAAGCTGGTCAAGTCCTACCACTGGATGGGCCTCGTGCACATCCCTACCAATG
GCTCTTGGCAGTGGGAGGACGGCAGCATTCTGAGCCCTAACCTGCTGACCATCATCGAGATGCAGAAGGG
CGACTGCGCCCTGTACGCCAGCTCTTTCAAGGGCTACATCGAGAACTGCAGCACCCCTAACACCTACATC
TGTATGCAGCGGACCGTGTGA

FIG. 2A

CD70 gRNA target sequence (SEQ ID NO:10): GTCCCATTGGTCGCGGGCTT

FIG. 2B



Top strand of exon 1 of the CD70 gene (SEQ ID NO: 78)
CCGCGGCGATGCCGGAGGAGGGTTCGGGCTGCTCGGTGCGGCGCAGGCCCTATGGGTGCGTCCTGCGGGCTGCTTTG
GTCCCATTGGTCGCGGGCTTGGTGATCTGCCTCGTGGTGTGCATCCAGCGCTTCGCACAGGCT

Bottom strand of exon 1 of the CD70 gene (SEQ ID NO: 79)
GGCGCCGCTACGGCCTCCTCCCAAGCCCGACGAGCCACGCCGCGTCCGGGATACCCACGCAGGACGCCCGACGAAAC
CAGGGTAACCAGCGCCCGAACCACTAGACGGAGCACCACACGTAGGTCGCGAAGCGTGTCCGA

CD70 portion encoded by exon 1 (SEQ ID NO: 80)
MPEEGSGCSVRRRPYGCVLRAALVPLVAGLVICLVVCIQRFAQA

FIG. 2C

CD70 exon 1 LHA sequence (SEQ ID NO:11)

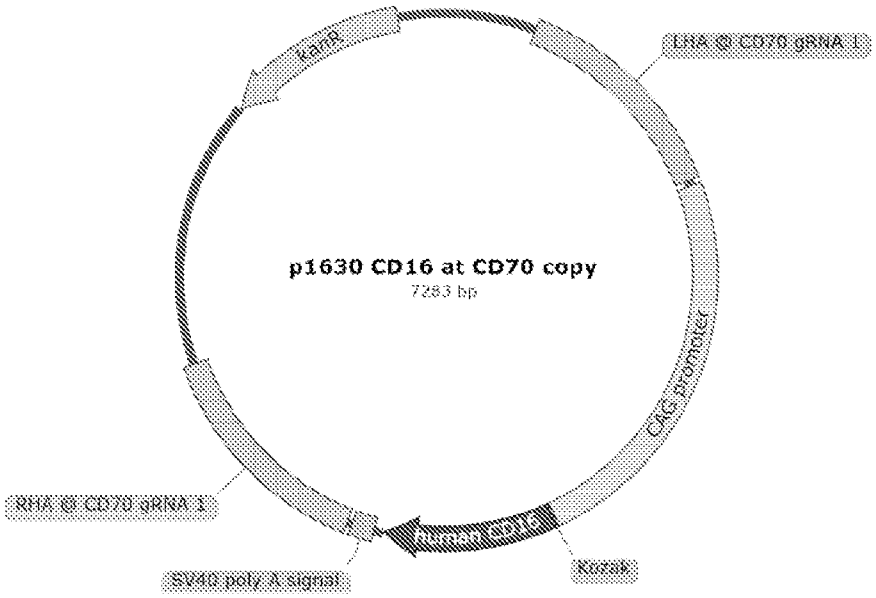
AAAAATAAAAAATAAAAATATTAACTTAATTTAACTTTAAACAAAAAAGCAGGTGGTCTCCAAGAATGCA
GGAGATAACTGACCGGGTGCAGTGTCTCATGCCTTTAATCCCAGCACTTTGGAAGGCCAAGGCGGGTGGA
TCACCCAAGGTCAGGAGTTCAAGTCCAGCCTGGCCAACATGGTGAAACCCCATCTCTACTAAAAATACAA
AAAATTAGCCAGGCATGGTGGCGCGCGCATGTTACTCCCAGCTACTCGCGAGGCTCAGACAGGAGAATCG
CTTGAACCCAGGAGATCGAGGTTGCGGCGAGCTGAGATGGCGCCACTGCACTCCAGCCTGGGTGACAGAG
GGAGACCTCCGTCTCAAAAACAAAACAAATCAAAAAAATGCAGGAGAGGGGTACACGAATATTTGGGGAG
CACCCCCAATTCTTGGATGTCTGCTGTATCCCCAGTGCACAGCACAATCTAATCCCTAATAAATGTGCAG
TGGAGGTTTGTTGAATAAATGAATGGGCCCCAGAAGAATGAGGTGGAGAGGGGAATAGGAAGATTGAATG
TCTCCTGCCTGAAGGTCGGGCGGGGAGGGGTTGGGGGCAGGCAACTCTGAGGCTCACCCGGGGCCACTGC
CTGCATCCTGGCAACTGCCTCCACCCACTTTAGGATCTTCAGACTGGCAGCGGTTGGAGGGAATTTCCCC
TCGCCAATTGCTCAAGTCCCTCCCCTCGACCGGCCGGACATCCCCAGAGAGGGGCAGGCTGGTCCCCTGA
CAGGTTGAAGCAAGTAGACGCCCAGGAGCCCCGGGAGGGGGCTGCAGTTTCCTTCCTTCCTTCTCGGCAG
CGCTCCGCGCCCCCATCGCCCCTCCTGCGCTAGCGGAGGTGATCGCCGCGGCGATGCCGGAGGAGGGTTC
GGGCTGCTCGGTGCGGCGCAGGCCCTATGGGTGCGTCCTGCGGGCTGCTTTGGTCCCATTGGT

CD70 exon 1 RHA (SEQ ID NO:12)

CGCGGGCTTGGTGATCTGCCTCGTGGTGTGCATCCAGCGCTTCGCACAGGCTCAGCAGCAGCTGCCGCTC
GAGTCACTTGGGGTGAGTTGAGATGGAAAAGTTGGGAAGAAAACATAGAGAGGCGCGTGACCGAAAAGAC
AGAATGAGATGGGTACAAAGAGGCCAGAGAGGAAGATCTGGTAGGGCAGAGACAGAGACCAGAACAGGGA
GGCGAGGCGGGGACCAGGCTGCCCGGTGTAGGGGCTACGAGACAGGCAGCCCTGCCAGGAGGTACAGGGA
GATCCCGGGATGGGAAAGGTAGGCACACATGGAAATGGAAGATGACTCGGCTCTGGTGTTCCCCCGGCAG
GCTGACTCAGAGGCTGCTGGGGGCTTCACAAGGCTGGGCGTGGGGGCTTCCTGGGGCCTCCTAGGACGGG
ATGGCCCCAGCCACTCGCTCCGGGTGGGGGAGGGGTCCCTTTGGGGACCGCGCCGGGCGCCTTTGCAGCG
TAGAGAGTCCGCTGCGCGCGGTGCTCTCGCGCCCAGTGACATCCAGGAAAACGATTCGGGAAACGAAGAA
GTTCTTTTGAAGGTCTCGACTTCACGTTCCCCGCTGGTTCAGACCTGCTTCCTCTTTAAGAAGTCTTAAG
AGTAAAAAAAAATAAAATGAAATAAAATCACCAGTGCGCGCCGTGGGATGAGAGGTGGAAAGGAGGATGG
ACAGAGAAAAGAGAGCTCCTGGCACAGGGGACACATAGAACCTCTCTGCTTACGTCCGTGCCCTGTTTTC
TGGTCTTTTCTTCCAGTGGGACGTAGCTGAGCTGCAGCTGAATCACACAGGTAACACGGGGGACGTGGAG
GGACGGGGAGAAGAAGAGGCACAGAGAGAGAAGGAAGGAGAGGTAGAAAGACAAGTGGGGAGAGACAGAG
AGAAAGAGACACAGACAGAGACGGAGGGAGAGAGGGAGGGAGAGATAGGGAGGGAAACGGAGAGGGGGAG
ACAGAGAGAAGACAGAGAGG

FIG. 3

Targeting construct design (CD16 transgene)



Targeting construct design (CD16-2A-NKG2D)

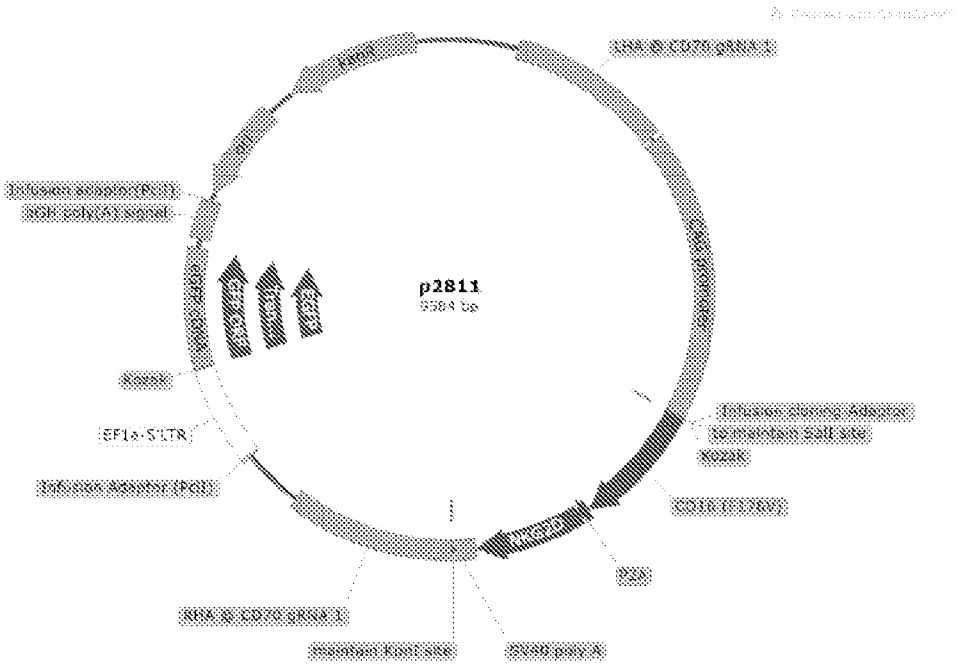


FIG. 4A

Targeting construct sequence (CD16 transgene; SEQ ID NO:13)

TCGCGCGTTTCGGTGATGACGGTGAAAACCTCTGACACATGCAGCTCCCGGAGACTGTCACAGCTTGTCT
GTAAGCGGATGCCGGGAGCAGACAAGCCCGTCAGGGCGCGTCAGCGGGTGTTGGCGGGTGTCGGGGCTGG
CTTAACTATGCGGCATCAGAGCAGATTGTACTGAGAGTGCACCATATGCGGTGTGAAATACCGCACAGAT
GCGTAAGGAGAAAATACCGCATCAGGCGCCATTCGCCATTCAGGCTGCGCAACTGTTGGGAAGGGCGATC
GGTGCGGGCCTCTTCGCTATTACGCCAGCTGGCGAAAGGGGGATGTGCTGCAAGGCGATTAAGTTGGGTA
ACGCCAGGGTTTTCCCAGTCACGACGTTGTAAAACGACGGCCAGTGAATTGACGCGTATTGGGAT**AAAAA**
TAAAAAATAAAAATATTAACTTAATTTAACTTTAAACAAAAAAGCAGGTGGTCTTGCTTTGGTCCCATTG
GT**CCAAGAATGCAGGAGATAACTGACCGGGTGCAGTGTCTCATGCCTTTAATCCCAGCACTTTGGAAGGC**
CAAGGCGGGTGGATCACCCAAGGTCAGGAGTTCAAGTCCAGCCTGGCCAACATGGTGAAACCCCATCTCT
ACTAAAAATACAAAAAATTAGCCAGGCATGGTGGCGCGCGCATGTTACTCCCAGCTACTCGCGAGGCTCA
GACAGGAGAATCGCTTGAACCCAGGAGATCGAGGTTGCGGCGAGCTGAGATGGCGCCACTGCACTCCAGC
CTGGGTGACAGAGGGAGACCTCCGTCTCAAAAACAAAACAAATCAAAAAAATGCAGGAGAGGGGTACACG
AATATTTGGGGAGCACCCCCAATTCTTGGATGTCTGCTGTATCCCCAGTGCACAGCACAATCTAATCCCT
AATAAATGTGCAGTGGAGGTTTGTTGAATAAATGAATGGGCCCCAGAAGAATGAGGTGGAGAGGGGAATA
GGAAGATTGAATGTCTCCTGCCTGAAGGTCGGGCGGGGAGGGGTTGGGGGCAGGCAACTCTGAGGCTCAC
CCGGGGCCACTGCCTGCATCCTGGCAACTGCCTCCACCCACTTTAGGATCTTCAGACTGGCAGCGGTTGG
AGGGAATTTCCCCTCGCCAATTGCTCAAGTCCCTCCCCTCGACCGGCCGGACATCCCCAGAGAGGGGCAG
GCTGGTCCCCTGACAGGTTGAAGCAAGTAGACGCCCAGGAGCCCCGGGAGGGGGCTGCAGTTTCCTTCCT
TCCTTCTCGGCAGCGCTCCGCGCCCCCATCGCCCCTCCTGCGCTAGCGGAGGTGATCGCCGCGGCGATGC
CGGAGGAGGGTTCGGGCTGCTCGGTGCGGCGCAGGCCCTATGGGTGCGTCCTGCGGGCTGCTTTGGTCCC
ATTGGTGGCCTCCAACGCGTTAATTCGAAATTGATTATTGACTAGTTATTAATAGTAATCAATTACGGGG
TCATTAGTTCATAGCCCATATATGGAGTTCCGCGTTACATAACTTACGGTAAATGGCCCGCCTGGCTGAC
CGCCCAACGACCCCCGCCCATTGACGTCAATAATGACGTATGTTCCCATAGTAACGCCAATAGGGACTTT
CCATTGACGTCAATGGGTGGACTATTTACGGTAAACTGCCCACTTGGCAGTACATCAAGTGTATCATATG
CCAAGTACGCCCCCTATTGACGTCAATGACGGTAAATGGCCCGCCTGGCATTATGCCCAGTACATGACCT
TATGGGACTTTCCTACTTGGCAGTACATCTACGTATTAGTCATCGCTATTACCATGGGTCGAGGTGAGCC
CCACGTTCTGCTTCACTCTCCCCATCTCCCCCCCCTCCCCACCCCCAATTTTGTATTTATTTATTTTTTA
ATTATTTTGTGCAGCGATGGGGGCGGGGGGGGGGGGGCGCGCCAGGCGGGGCGGGGCGGGGCGAGGG
GCGGGGCGGGGCGAGGCGGAGAGGTGCGGCGGCAGCCAATCAGAGCGGCGCGCTCCGAAAGTTTCCTTTT
ATGGCGAGGCGGCGGCGGCGGCGGCCCTATAAAAAGCGAAGCGCGCGGCGGGCGGGAGTCGCTGCGTTGC
CTTCGCCCCGTGCCCCGCTCCGCGCCGCCTCGCGCCGCCCGCCCCGGCTCTGACTGACCGCGTTACTCCC
ACAGGTGAGCGGGCGGGACGGCCCTTCTCCTCCGGGCTGTAATTAGCGCTTGGTTTAATGACGGCTCGTT
TCTTTTCTGTGGCTGCGTGAAAGCCTTAAAGGGCTCCGGGAGGGCCCTTTGTGCGGGGGGGAGCGGCTCG
GGGGGTGCGTGCGTGTGTGTGTGCGTGGGGAGCGCCGCGTGCGGCCCGCGCTGCCCGGCGGCTGTGAGCG
CTGCGGGCGCGGCGCGGGGCTTTGTGCGCTCCGCGTGTGCGCGAGGGGAGCGCGGCCGGGGGCGGTGCCC
CGCGGTGCGGGGGGGCTGCGAGGGGAACAAAGGCTGCGTGCGGGGTGTGTGCGTGGGGGGGTGAGCAGGG
GGTGTGGGCGCGGCGGTCGGGCTGTAACCCCCCCCTGCACCCCCCTCCCCGAGTTGCTGAGCACGGCCCG
GCTTCGGGTGCGGGGCTCCGTGCGGGGCGTGGCGCGGGGCTCGCCGTGCCGGGCGGGGGGTGGCGGCAGG
TGGGGGTGCCGGGCGGGGCGGGGCCGCCTCGGGCCGGGGAGGGCTCGGGGGAGGGGCGCGGCGGCCCCGG
AGCGCCGGCGGCTGTCGAGGCGCGGCGAGCCGCAGCCATTGCCTTTTATGGTAATCGTGCGAGAGGGCGC
AGGGACTTCCTTTGTCCCAAATCTGGCGGAGCCGAAATCTGGGAGGCGCCGCCGCACCCCCTCTAGCGGG
CGCGGGCGAAGCGGTGCGGCGCCGGCAGGAAGGAAATGGGCGGGGAGGGCCTTCGTGCGTCGCCGCGCCG
CCGTCCCCTTCTCCATCTCCAGCCTCGGGGCTGCCGCAGGGGGACGGCTGCCTTCGGGGGGGACGGGGCA
GGGCGGGGTTCGGCTTCTGGCGTGTGACCGGCGGGATATCTACGAAGCGGCCGCCCTCTGCTAACCATGT
TCATGCCTTCTTCTTTTTCCTACAGCTCCTGGGCAACGTGCTGGTTATTGTGCTGTCTCATCATTTTGGC

FIG. 4B

SEQ ID NO:13 - continued

AAAGTCGACCGCCACC**ATGTGGCAGCTGCTGCTGCCCACCGCCCTGCTGCTGCTGGTGAGCGCCGGCATG**
AGAACCGAGGACCTGCCCAAGGCCGTGGTGTTCCTGGAGCCCCAGTGGTACAGAGTGCTGGAGAAGGACA
GCGTGACCCTGAAGTGCCAGGGCGCCTACAGCCCCGAGGACAACAGCACCCAGTGGTTCCACAACGAGAG
CCTGATCAGCAGCCAGGCCAGCAGCTACTTCATCGACGCCGCCACCGTGGACGACAGCGGCGAGTACAGA
TGCCAGACCAACCTGAGCACCCTGAGCGACCCCGTGCAGCTGGAGGTGCACATCGGCTGGCTGCTGCTGC
AGGCCCCCAGATGGGTGTTCAAGGAGGAGGACCCCATCCACCTGAGATGCCACAGCTGGAAGAACACCGC
CCTGCACAAGGTGACCTACCTGCAGAACGGCAAGGGCAGAAAGTACTTCCACCACAACAGCGACTTCTAC
ATCCCCAAGGCCACCCTGAAGGACAGCGGCAGCTACTTCTGCAGAGGCCTGGTGGGCAGCAAGAACGTGA
GCAGCGAGACCGTGAACATCACCATCACCCAGGGCCTGGCCGTGAGCACCATCAGCAGCTTCTTCCCCCC
CGGCTACCAGGTGAGCTTCTGCCTGGTGATGGTGCTGCTGTTCGCCGTGGACACCGGCCTGTACTTCAGC
GTGAAGACCAACATCAGAAGCAGCACCAGAGACTGGAAGGACCACAAGTTCAAGTGGAGAAAGGACCCCC
AGGACAAGTGATAAGGCATGCAAGCTT**AACTTGTTTATTGCAGCTTATAATGGTTACAAATAAAGCAATA**
GCATCACAAATTTCACAAATAAAGCATTTTTTTCACTGCATTCTAGTTGTGGTTTGTCCAAACTCATCAA
TGTATCTTATCATGTCTGGGTACCGAATTC**CGCGGGCTTGGTGATCTGCCTCGTGGTGTGCATCCAGCGC**
TTCGCACAGGCTCAGCAGCAGCTGCCGCTCGAGTCACTTGGGGTGAGTTGAGATGGAAAAGTTGGGAAGA
AAACATAGAGAGGCGCGTGACCGAAAAGACAGAATGAGATGGGTACAAAGAGGCCAGAGAGGAAGATCTG
GTAGGGCAGAGACAGAGACCAGAACAGGGAGGCGAGGCGGGGACCAGGCTGCCCGGTGTAGGGGCTACGA
GACAGGCAGCCCTGCCAGGAGGTACAGGGAGATCCCGGGATGGGAAAGGTAGGCACACATGGAAATGGAA
GATGACTCGGCTCTGGTGTTCCCCCGGCAGGCTGACTCAGAGGCTGCTGGGGGCTTCACAAGGCTGGGCG
TGGGGGCTTCCTGGGGCCTCCTAGGACGGGATGGCCCCAGCCACTCGCTCCGGGTGGGGGAGGGGTCCCT
TTGGGGACCGCGCCGGGCGCCTTTGCAGCGTAGAGAGTCCGCTGCGCGCGGTGCTCTCGCGCCCAGTGAC
ATCCAGGAAAACGATTCGGGAAACGAAGAAGTTCTTTTGAAGGTCTCGACTTCACGTTCCCCGCTGGTTC
AGACCTGCTTCCTCTTTAAGAAGTCTTAAGAGTAAAAAAAAAATAAAATGAAATAAAATCACCAGTGCGCG
CCGTGGGATGAGAGGTGGAAAGGAGGATGGACAGAGAAAAGAGAGCTCCTGGCACAGGGGACACATAGAA
CCTCTCTGCTTACGTCCGTGCCCTGTTTTCTGGTCTTTTCTTCCAGTGGGACGTAGCTGAGCTGCAGCTG
AATCACACAGGTAACACGGGGGACGTGGAGGGACGGGGAGAAGAAGAGGCACAGAGAGAGAAGGAAGGAG
AGGTAGAAAGACAAGTGGGGAGAGACAGAGAGAAAGAGACACAGACAGAGACGGAGGGAGAGAGGGAGGG
AGAGATAGGGAGGGAAACGGAGAGGGGGAGACAGAGAGAAGACAGAGAGGATCCCAATGGCGCGCCGAGC
TTGGCTCGAGCATGGTCATAGCTGTTTCCTGTGTGAAATTGTTATCCGCTCACAATTCCACACAACATAC
GAGCCGGAAGCATAAAGTGTAAAGCCTGGGGTGCCTAATGAGTGAGCTAACTCACATTAATTGCGTTGCG
CTCACTGCCCGCTTTCCAGTCGGGAAACCTGTCGTGCCAGCTGCATTAATGAATCGGCCAACGCGCGGGG
AGAGGCGGTTTGCGTATTGGGCGCTGTTCCGCTTCCTCGCTCACTGACTCGCTGCGCTCGGTCGTTCGGC
TGCGGCGAGCGGTATCAGCTCACTCAAAGGCGGTAATACGGTTATCCACAGAATCAGGGGATAACGCAGG
AAAGAACATGTGAGCAAAAGGCCAGCAAAAGGCCAGGAACCGTAAAAAGGCCGCGTTGCTGGCGTTTTTC
CATAGGCTCCGCCCCCCTGACGAGCATCACAAAAATCGACGCTCAAGTCAGAGGTGGCGAAACCCGACAG
GACTATAAAGATACCAGGCGTTTCCCCCTGGAAGCTCCCTCGTGCGCTCTCCTGTTCCGACCCTGCCGCT
TACCGGATACCTGTCCGCCTTTCTCCCTTCGGGAAGCGTGGCGCTTTCTCATAGCTCACGCTGTAGGTAT
CTCAGTTCGGTGTAGGTCGTTCGCTCCAAGCTGGGCTGTGTGCACGAACCCCCCGTTCAGCCCGACCGCT
GCGCCTTATCCGGTAACTATCGTCTTGAGTCCAACCCGGTAAGACACGACTTATCGCCACTGGCAGCAGC
CACTGGTAACAGGATTAGCAGAGCGAGGTATGTAGGCGGTGCTACAGAGTTCTTGAAGTGGTGGCCTAAC
TACGGCTACACTAGAAGAACAGTATTTGGTATCTGCGCTCTGCTGAAGCCAGTTACCTTCGGAAAAAGAG
TTGGTAGCTCTTGATCCGGCAAACAAACCACCGCTGGTAGCGGTGGTTTTTTTGTTTGCAAGCAGCAGAT
TACGCGCAGAAAAAAAGGATCTCAAGAAGATCCTTTGATCTTTTCTACGGGGTCTGACGCTCAGTGGAAC
GAAAACTCACGTTAAGGGATTTTGGTCATGAGATTATCAAAAAGGATCTTCACCTAGATCCTTTTAAATT

FIG. 4C

SEQ ID NO:13 continued

AAAAATGAAGTTTTAAATCAATCTAAAGTATATATGAGTAAACTTGGTCTGACAGTTAGAAAAACTCATC
GAGCATCAAATGAAACTGCAATTTATTCATATCAGGATTATCAATACCATATTTTTGAAAAAGCCGTTTC
TGTAATGAAGGAGAAAACTCACCGAGGCAGTTCCATAGGATGGCAAGATCCTGGTATCGGTCTGCGATTC
CGACTCGTCCAACATCAATACAACCTATTAATTTCCCCTCGTCAAAAATAAGGTTATCAAGTGAGAAATC
ACCATGAGTGACGACTGAATCCGGTGAGAATGGCAAAAGTTTATGCATTTCTTTCCAGACTTGTTCAACA
GGCCAGCCATTACGCTCGTCATCAAAATCACTCGCATCAACCAAACCGTTATTCATTCGTGATTGCGCCT
GAGCGAAACGAAATACGCGATCGCTGTTAAAAGGACAATTACAAACAGGAATCGAATGCAACCGGCGCAG
GAACACTGCCAGCGCATCAACAATATTTTCACCTGAATCAGGATATTCTTCTAATACCTGGAATGCTGTT
TTCCCAGGGATCGCAGTGGTGAGTAACCATGCATCATCAGGAGTACGGATAAAATGCTTGATGGTCGGAA
GAGGCATAAATTCCGTCAGCCAGTTTAGTCTGACCATCTCATCTGTAACATCATTGGCAACGCTACCTTT
GCCATGTTTCAGAAACAACTCTGGCGCATCGGGCTTCCCATACAATCGATAGATTGTCGCACCTGATTGC
CCGACATTATCGCGAGCCCATTTATACCCATATAAATCAGCATCCATGTTGGAATTTAATCGCGGCCTAG
AGCAAGACGTTTCCCGTTGAATATGGCTCATACTCTTCCTTTTTCAATATTATTGAAGCATTTATCAGGG
TTATTGTCTCATGAGCGGATACATATTTGAATGTATTTAGAAAAATAAACAAATAGGGGTTCCGCGCACA
TTTCCCCGAAAAGTGCCACCTGACGTCTAAGAAACCATTATTATCATGACATTAACCTATAAAAATAGGC
GTATCACGAGGCCCTTTTGTC

CD70 exon 1 homology arms

CAG promoter

CD16 Transgene

SV40 terminator

Kanamycin resistance sequence

FIG. 4D

Targeting construct sequence (CD16-2A-NKG2D; SEQ ID NO:39)

tcgcgcgtttcggtgatgacggtgaaaacctctgacacatgcagctcccggagactgtcacagcttgtct
gtaagcggatgccgggagcagacaagcccgtcagggcgcgtcagcgggtgttggcgggtgtcggggctgg
cttaactatgcggcatcagagcagattgtactgagagtgcaccatatgcggtgtgaaataccgcacagat
gcgtaaggagaaaataccgcatcaggcgccattcgccattcaggctgcgcaactgttgggaagggcgatc
ggtgcgggcctcttcgctattacgccagctggcgaaaggggatgtgctgcaaggcgattaagttgggta
acgccagggttttcccagtcacgacgttgtaaaacgacggccagtgaattgacgcgtattgggat**AAAAA**
TAAAAAATAAAAATATTAACTTAATTTAACTTTAAACAAAAAAGCAGGTGGTCTCCAAGAATGCAGGAGA
TAACTGACCGGGTGCAGTGTCTCATGCCTTTAATCCCAGCACTTTGGAAGGCCAAGGCGGGTGGATCACC
CAAGGTCAGGAGTTCAAGTCCAGCCTGGCCAACATGGTGAAACCCCATCTCTACTAAAAATACAAAAAAT
TAGCCAGGCATGGTGGCGCGCGCATGTTACTCCCAGCTACTCGCGAGGCTCAGACAGGAGAATCGCTTGA
ACCCAGGAGATCGAGGTTGCGGCGAGCTGAGATGGCGCCACTGCACTCCAGCCTGGGTGACAGAGGGAGA
CCTCCGTCTCAAAAACAAAACAAATCAAAAAAATGCAGGAGAGGGGTACACGAATATTTGGGGAGCACCC
CCAATTCTTGGATGTCTGCTGTATCCCCAGTGCACAGCACAATCTAATCCCTAATAAATGTGCAGTGGAG
GTTTGTTGAATAAATGAATGGGCCCCAGAAGAATGAGGTGGAGAGGGGAATAGGAAGATTGAATGTCTCC
TGCCTGAAGGTCGGGCGGGGAGGGGTTGGGGGCAGGCAACTCTGAGGCTCACCCGGGGCCACTGCCTGCA
TCCTGGCAACTGCCTCCACCCACTTTAGGATCTTCAGACTGGCAGCGGTTGGAGGGAATTTCCCCTCGCC
AATTGCTCAAGTCCCTCCCCTCGACCGGCCGGACATCCCCAGAGAGGGGCAGGCTGGTCCCCTGACAGGT
TGAAGCAAGTAGACGCCCAGGAGCCCCGGGAGGGGGCTGCAGTTTCCTTCCTTCCTTCTCGGCAGCGCTC
CGCGCCCCCATCGCCCCTCCTGCGCTAGCGGAGGTGATCGCCGCGGCGATGCCGGAGGAGGGTTCGGGCT
GCTCGGTGCGGCGCAGGCCCTATGGGTGCGTCCTGCGGGCTGCTTTGGTCCCATTGGTGGCCTCCAACGC
GTTAATTCGAAATTGATTATTGACTAGTTATTAATAGTAATCAATTACGGGGTCATTAGTTCATAGCCCA
TATATGGAGTTCCGCGTTACATAACTTACGGTAAATGGCCCGCCTGGCTGACCGCCCAACGACCCCCGCC
CATTGACGTCAATAATGACGTATGTTCCCATAGTAACGCCAATAGGGACTTTCCATTGACGTCAATGGGT
GGACTATTTACGGTAAACTGCCCACTTGGCAGTACATCAAGTGTATCATATGCCAAGTACGCCCCCTATT
GACGTCAATGACGGTAAATGGCCCGCCTGGCATTATGCCCAGTACATGACCTTATGGGACTTTCCTACTT
GGCAGTACATCTACGTATTAGTCATCGCTATTACCATGGGTCGAGGTGAGCCCCACGTTCTGCTTCACTC
TCCCCATCTCCCCCCCCTCCCCACCCCCAATTTTGTATTTATTTATTTTTTAATTATTTTGTGCAGCGAT
GGGGGCGGGGGGGGGGGGGCGCGCGCCAGGCGGGGCGGGGCGGGGCGAGGGGCGGGGCGGGGCGAGGCG
GAGAGGTGCGGCGGCAGCCAATCAGAGCGGCGCGCTCCGAAAGTTTCCTTTTATGGCGAGGCGGCGGCGG
CGGCGGCCCTATAAAAAGCGAAGCGCGCGGCGGGCGGGAGTCGCTGCGTTGCCTTCGCCCCGTGCCCCGC
TCCGCGCCGCCTCGCGCCGCCCGCCCCGGCTCTGACTGACCGCGTTACTCCCACAGGTGAGCGGGCGGGA
CGGCCCTTCTCCTCCGGGCTGTAATTAGCGCTTGGTTTAATGACGGCTCGTTTCTTTTCTGTGGCTGCGT
GAAAGCCTTAAAGGGCTCCGGGAGGGCCCTTTGTGCGGGGGGGAGCGGCTCGGGGGGTGCGTGCGTGTGT
GTGTGCGTGGGGAGCGCCGCGTGCGGCCCGCGCTGCCCGGCGGCTGTGAGCGCTGCGGGCGCGGCGCGGG
GCTTTGTGCGCTCCGCGTGTGCGCGAGGGGAGCGCGGCCGGGGGCGGTGCCCCGCGGTGCGGGGGGGCTG
CGAGGGGAACAAAGGCTGCGTGCGGGGTGTGTGCGTGGGGGGGTGAGCAGGGGGTGTGGGCGCGGCGGTC
GGGCTGTAACCCCCCCCTGCACCCCCCTCCCCGAGTTGCTGAGCACGGCCCGGCTTCGGGTGCGGGGCTC
CGTGCGGGGCGTGGCGCGGGGCTCGCCGTGCCGGGCGGGGGGTGGCGGCAGGTGGGGGTGCCGGGCGGGG
CGGGGCCGCCTCGGGCCGGGGAGGGCTCGGGGGAGGGGCGCGGCGGCCCCGGAGCGCCGGCGGCTGTCGA
GGCGCGGCGAGCCGCAGCCATTGCCTTTTATGGTAATCGTGCGAGAGGGCGCAGGGACTTCCTTTGTCCC
AAATCTGGCGGAGCCGAAATCTGGGAGGCGCCGCCGCACCCCCTCTAGCGGGCGCGGGCGAAGCGGTGCG
GCGCCGGCAGGAAGGAAATGGGCGGGGAGGGCCTTCGTGCGTCGCCGCGCCGCCGTCCCCTTCTCCATCT
CCAGCCTCGGGGCTGCCGCAGGGGGACGGCTGCCTTCGGGGGGGACGGGGCAGGGCGGGGTTCGGCTTCT
GGCGTGTGACCGGCGGGATATCTACGAAGCGGCCGCCCTCTGCTAACCATGTTCATGCCTTCTTCTTTTT
CCTACAGCTCCTGGGCAACGTGCTGGTTATTGTGCTGTCTCATCATTTTGGCAAAGTCGACGCCACC**ATG**

FIG. 4E

SEQ ID NO:39 - continued

TGGCAACTGTTGCTGCCTACAGCTCTGCTGCTGCTGGTGTCTGCCGGCATGAGAACAGAGGATCTGCCTA
AGGCCGTGGTGTTCCTGGAACCTCAGTGGTACAGAGTGCTGGAAAAGGACAGCGTGACCCTGAAGTGCCA
GGGCGCCTATTCTCCCGAGGACAATAGCACCCAGTGGTTCCACAACGAGAGCCTGATCAGCAGCCAGGCC
AGCAGCTACTTTATCGATGCCGCCACCGTGGACGACAGCGGCGAGTACAGATGCCAGACCAATCTGAGCA
CCCTGAGCGACCCTGTGCAGCTGGAAGTGCACATTGGATGGCTGCTGCTTCAGGCCCCTAGATGGGTGTT
CAAAGAAGAGGACCCCATCCACCTGAGATGCCACTCTTGGAAGAACACAGCCCTGCACAAAGTGACCTAC
CTGCAGAACGGCAAGGGCAGAAAGTACTTCCACCACAACAGCGACTTCTACATCCCCAAGGCCACACTGA
AGGACTCCGGCTCCTACTTCTGTAGAGGCCTCGTGGGCAGCAAGAACGTGTCCAGCGAGACAGTGAACAT
CACCATCACACAGGGCCTCGCCGTGTCTACCATCAGCAGCTTTTTCCCACCTGGCTATCAGGTGTCCTTC
TGCCTGGTCATGGTGCTGCTGTTCGCCGTGGATACCGGCCTGTACTTCAGCGTCAAGACCAACATCCGGT
CCAGCACCAGAGACTGGAAGGACCACAAGTTCAAGTGGCGGAAGGACCCTCAGGACAAAGGCAGCGGCGC
CACCAATTTCAGCCTGCTGAAACAGGCTGGCGACGTGGAAGAGAACCCTGGACCTATGGGCTGGATCCGG
GGCAGAAGAAGCAGACACAGCTGGGAGATGAGCGAGTTCCACAATTACAACCTGGACCTGAAGAAGTCCG
ACTTCAGCACCCGGTGGCAGAAACAGAGATGCCCCGTGGTCAAGAGCAAGTGCAGAGAGAACGCTAGCCC
CTTCTTCTTCTGTTGCTTTATCGCCGTGGCCATGGGCATCCGCTTCATCATCATGGTCACAATTTGGAGC
GCCGTGTTCCTGAACTCCCTGTTCAATCAAGAGGTGCAGATCCCTCTGACCGAGAGCTACTGTGGCCCCT
GTCCTAAGAACTGGATCTGCTACAAGAACAACTGCTACCAGTTCTTCGACGAGAGCAAGAATTGGTACGA
GAGCCAGGCCTCCTGCATGAGCCAGAATGCTTCCCTGCTGAAGGTGTACAGCAAAGAGGACCAGGATCTG
CTGAAGCTGGTCAAGTCCTACCACTGGATGGGCCTCGTGCACATCCCTACCAATGGCTCTTGGCAGTGGG
AGGACGGCAGCATTCTGAGCCCTAACCTGCTGACCATCATCGAGATGCAGAAGGGCGACTGCGCCCTGTA
CGCCAGCTCTTTCAAGGGCTACATCGAGAACTGCAGCACCCCTAACACCTACATCTGTATGCAGCGGACC
GTGTGATAAtaatgaaacttgtttattgcagcttataatggttacaaataaagcaatagcatcacaaatt
tcacaaataaagcatttttttcactgcattctagttgtggtttgtccaaactcatcaatgtatcttaTCA
TGTCTGggtacCGAATTCCGCGGGCTTGGTGATCTGCCTCGTGGTGTGCATCCAGCGCTTCGCACAGGCT
CAGCAGCAGCTGCCGCTCGAGTCACTTGGGGTGAGTTGAGATGGAAAAGTTGGGAAGAAAACATAGAGAG
GCGCGTGACCGAAAAGACAGAATGAGATGGGTACAAAGAGGCCAGAGAGGAAGATCTGGTAGGGCAGAGA
CAGAGACCAGAACAGGGAGGCGAGGCGGGGACCAGGCTGCCCGGTGTAGGGGCTACGAGACAGGCAGCCC
TGCCAGGAGGTACAGGGAGATCCCGGGATGGGAAAGGTAGGCACACATGGAAATGGAAGATGACTCGGCT
CTGGTGTTCCCCCGGCAGGCTGACTCAGAGGCTGCTGGGGGCTTCACAAGGCTGGGCGTGGGGGCTTCCT
GGGGCCTCCTAGGACGGGATGGCCCCAGCCACTCGCTCCGGGTGGGGGAGGGGTCCCTTTGGGGACCGCG
CCGGGCGCCTTTGCAGCGTAGAGAGTCCGCTGCGCGCGGTGCTCTCGCGCCCAGTGACATCCAGGAAAAC
GATTCGGGAAACGAAGAAGTTCTTTTGAAGGTCTCGACTTCACGTTCCCCGCTGGTTCAGACCTGCTTCC
TCTTTAAGAAGTCTTAAGAGTAAAAAAAAATAAAATGAAATAAAATCACCAGTGCGCGCCGTGGGATGAG
AGGTGGAAAGGAGGATGGACAGAGAAAAGAGAGCTCCTGGCACAGGGGACACATAGAACCTCTCTGCTTA
CGTCCGTGCCCTGTTTTCTGGTCTTTTCTTCCAGTGGGACGTAGCTGAGCTGCAGCTGAATCACACAGGT
AACACGGGGGACGTGGAGGGACGGGGAGAAGAAGAGGCACAGAGAGAGAAGGAAGGAGAGGTAGAAAGAC
AAGTGGGGAGAGACAGAGAGAAAGAGACACAGACAGAGACGGAGGGAGAGAGGGAGGGAGAGATAGGGAG
GGAAACGGAGAGGGGGAGACAGAGAGAAGACAGAGAGGatcccaatggcgcgccgagcttggctcgagca
tggtcatagctgtttcctgtgtgaaattgttatccgctcacaattccacacaacatacgagccggaagca
taaagtgtaaagcctggggtgcctaatgagtgagctaactcacattaattgcgttgcgctcactgcccgc
tttccagtcgggaaacctgtcgtgccagctgcattaatgaatcggccaacgcgcggggagaggcggtttg
cgtattgggcgctgttccgcttcctcgctcactgactcgctgcgctcggtcgttcggctgcggcgagcgg
tatcagctcactcaaaggcggtaatacggttatccacagaatcaggggataacgcaggaaagaacatgGG
CCTCCAAGGCCTTAATTCGAAACTAGTAAGGATCTGCGATCGCTCCGGTGCCCGTCAGTGGGCAGAGCGC

FIG. 4F

SEQ ID NO:39 - continued

ACATCGCCCACAGTCCCCGAGAAGTTGGGGGGAGGGGTCGGCAATTGAACGGGTGCCTAGAGAAGGTGGC
GCGGGGTAAACTGGGAAAGTGATGTCGTGTACTGGCTCCGCCTTTTTCCCGAGGGTGGGGGAGAACCGTA
TATAAGTGCAGTAGTCGCCGTGAACGTTCTTTTTCGCAACGGGTTTGCCGCCAGAACACAGCTGAAGCTT
CGAGGGGCTCGCATCTCTCCTTCACGCGCCCGCCGCCCTACCTGAGGCCGCCATCCACGCCGGTTGAGTC
GCGTTCTGCCGCCTCCCGCCTGTGGTGCCTCCTGAACTGCGTCCGCCGTCTAGGTAAGTTTAAAGCTCAG
GTCGAGTCCGGGCCTTTGTCCGGCGCTCCCTTGGAGCCTACCTAGACTCAGCCGGCTCTCCACGCTTTGC
CTGACCCTGCTTGCTCAACTCTACGTCTTTGTTTCGTTTTCTGTTCTGCGCCGTTACAGATCCAAGCTGT
GACCGGCGCCTACTCTAGATGTACGCCACC*atggtgagcaagggcgaggagctgttcaccggggtggtgc*
ccatcctggtcgagctggacggcgacgtaaacggccacaagttcagcgtgtccggcgagggcgagggcga
tgccacctacggcaagctgaccctgaagttcatctgcaccaccggcaagctgcccgtgccctggcccacc
ctcgtgaccaccctgacctacggcgtgcagtgcttcagccgctaccccgaccacatgaagcagcacgact
tcttcaagtccgccatgcccgaaggctacgtccaggagcgcaccatcttcttcaaggacgacggcaacta
caagacccgcgccgaggtgaagttcgagggcgacaccctggtgaaccgcatcgagctgaagggcatcgac
ttcaaggaggacggcaacatcctggggcacaagctggagtacaactacaacagccacaacgtctatatca
tggccgacaagcagaagaacggcatcaaggtgaacttcaagatccgccacaacatcgaggacggcagcgt
gcagctcgccgaccactaccagcagaacaccccatcggcgacggccccgtgctgctgcccgacaaccac
tacctgagcacccagtccgccctgagcaaagaccccaacgagaagcgcgatcacatggtcctgctggagt
*tcgtgaccgccgccgggatcactctcggcatggacgagctgtacaagtaa*TGAacgctagcg***ctgccggt***
tagggcccgtttaaacccgctgatcagcctcgactgtgccttctagttgccagccatctgttgtttgcc
cctcccccgtgccttccttgaccctggaaggtgccactcccactgtcctttcctaataaaatgaggaaat
tgcatcgcattgtctgagtaggtgtcattctattctgggggggtggggtggggcaggacagcaaggggggag
gattgggaagacaatagcaggcatgctggggatgcggtgggctctatggcatgtgagcaaaaggccagca
aaaggccaggaaccgtaaaaaggccgcgttgctggcgtttttccataggctccgccccctgacgagcat
cacaaaaatcgacgctcaagtcagaggtggcgaaacccgacaggactataaagataccaggcgtttcccc
ctggaagctccctcgtgcgctctcctgttccgaccctgccgcttaccggatacctgtccgcctttctccc
ttcgggaagcgtggcgctttctcatagctcacgctgtaggtatctcagttcggtgtaggtcgttcgctcc
aagctgggctgtgtgcacgaaccccccgttcagcccgaccgctgcgccttatccggtaactatcgtcttg
agtccaacccggtaagacacgacttatcgccactggcagcagccactggtaacaggattagcagagcgag
gtatgtaggcggtgctacagagttcttgaagtggtggcctaactacggctacactagaagaacagtattt
ggtatctgcgctctgctgaagccagttaccttcggaaaaagagttggtagctcttgatccggcaaacaaa
ccaccgctggtagcggtggtttttttgtttgcaagcagcagattacgcgcagaaaaaaggatctcaaga
agatcctttgatcttttctacggggtctgacgctcagtggaacgaaaactcacgttaagggattttggtc
atgagattatcaaaaaggatcttcacctagatccttttaaattaaaaatgaagttttaaatcaatctaaa
gtatatatgagtaaacttggtctgacagttagaaaaactcatcgagcatcaaatgaaactgcaatttatt
catatcaggattatcaataccatatttttgaaaaagccgtttctgtaatgaaggagaaaactcaccgagg
cagttccataggatggcaagatcctggtatcggtctgcgattccgactcgtccaacatcaatacaaccta
ttaatttcccctcgtcaaaaataaggttatcaagtgagaaatcaccatgagtgacgactgaatccggtga
gaatggcaaaagtttatgcatttctttccagacttgttcaacaggccagccattacgctcgtcatcaaaa
tcactcgcatcaaccaaaccgttattcattcgtgattgcgcctgagcgaaacgaaatacgcgatcgctgt
taaaaggacaattacaaacaggaatcgaatgcaaccggcgcaggaacactgccagcgcatcaacaatatt
ttcacctgaatcaggatattcttctaatacctggaatgctgttttcccagggatcgcagtggtgagtaac
catgcatcatcaggagtacggataaaatgcttgatggtcggaagaggcataaattccgtcagccagtttta
gtctgaccatctcatctgtaacatcattggcaacgctacctttgccatgtttcagaaacaactctggcgc
atcgggcttcccatacaatcgatagattgtcgcacctgattgcccgacattatcgcgagcccatttatac
ccatataaatcagcatccatgttggaatttaatcgcggcctagagcaagacgtttcccgttgaatatggc

FIG. 4G

SEQ ID NO:39 - continued

tcatactcttccttttttcaatattattgaagcatttatcagggttattgtctcatgagcggatacatatt
tgaatgtatttagaaaaataaacaaatagggttccgcgcacatttccccgaaaagtgccacctgacgtc
taagaaaccattattatcatgacattaacctataaaaataggcgtatcacgaggccctttttgtc

CD70 exon 1 homology arms

CAG promoter

CD16 Transgene

SV40 terminator

EF1A promoter

GFP transgene

BGH terminator

Kanamycin resistance sequence

FIG. 5A

IL-15 protein (SEQ ID NO:16)

MRISKPHLRSISIQCYLCLLLNSHFLTEAGIHVFILGCFSAGLPKTEANWVNVISDLKKIEDLIQSMHID
ATLYTESDVHPSCKVTAMKCFLLELQVISLESGDASIHDTVENLIILANNSLSSNGNVTESGCKECEELE
EKNIKEFLQSFVHIVQMFINTS

Fusion protein including IL-15 and IL-15Ra (SEQ ID NO:17)

MRISKPHLRSISIQCYLCLLLNSHFLTEAGIHVFILGCFSAGLPKTEANWVNVISDLKKIEDLIQSMHID
ATLYTESDVHPSCKVTAMKCFLLELQVISLESGDASIHDTVENLIILANNSLSSNGNVTESGCKECEELE
EKNIKEFLQSFVHIVQMFINTSGGGGSGGGGSGGGGSGGGGSGGGGITCPPPMSVEHADIWVKSYSLYSR
ERYICNSGFKRKAGTSSLTECVLNKATNVAHWTTPSLKCIRDPALVHQRPAPPSTVTTAGVTPQPESLSP
SGKEPAASSPSSNNTAATTAAIVPGSQLMPSKSPSTGTTEISSHESSHGTPSQTTAKNWELTASASHQPP
GVYPQGHSDTTVAISTSTVLLCGLSAVSLLACYLKSRQTPPLASVEMEAMEALPVTWGTSSRDEDLENCS
HHL

IL-15RA protein (SEQ ID NO:18)

ITCPPPMSVEHADIWVKSYSLYSRERYICNSGFKRKAGTSSLTECVLNKATNVAHWTTPSLKCIRDPALV
HQRPAPPSTVTTAGVTPQPESLSPSGKEPAASSPSSNNTAATTAAIVPGSQLMPSKSPSTGTTEISSHES
SHGTPSQTTAKNWELTASASHQPPGVYPQGHSDTTVAISTSTVLLCGLSAVSLLACYLKSRQTPPLASVE
MEAMEALPVTWGTSSRDEDLENCSHHL

IL15-IL15RA nucleic acid coding sequence (SEQ ID NO:38)

atgcgcatctctaagcctcatctgcgatccatcagtatccaatgttacctgtgtctgctgcttaatagtc
attttctgactgaagccggaatccatgtctttatcctgggctgcttcagtgcgggattgcccaaaactga
agccaactgggttaacgtgatctctgatttgaagaaaatagaagaccttatacaaagtatgcatattgat
gccacgctctataccgaatcagacgttcatccctcatgcaaagtcacagctatgaagtgttttctgctgg
agctgcaggttattagtctggagagtggagacgccagcatccacgatacggtcgagaatctcattatctt
ggcaaataattcactctcttcaaacggaaacgtgaccgaatcagggtgcaaggagtgcgaggaactcgaa
gaaaagaatatcaaagagttcctgcagagcttcgtgcacatcgtgcaaatgttcatcaatacctctggggg
gaggtggttcaggggtggaggttctggtgggggggctcaggaggaggggtagcggcggtggtggaag
catcacgtgtcccccaccgatgtctgtagaacacgctgacatctgggtcaaatcttactctctgtactct
cgcgaacgctacatttgcaactcagggtttaaacggaaagccggaacctctagtctgacggagtgtgtgc
tgaataaggcgacaaacgtagcgcactggaccacccctagtctcaagtgcataagggacccagctttggt
acaccagaggcctgcaccgccctccaccgtcactacggctggcgtaacaccgcaaccggagtctctgagt
cctagcggaaaagagcccgccgcaagtagcccttcaagtaacaacaccgccgcgacgacggcggctattg
tgcccggtagtcaattgatgccttccaagtctccgtccacaggcacgacagagataagttcacacgaaag
tagccatgggactccttcacagacgaccgcaaagaattgggagttgacggcttcagcctcacaccagccc
ccgggcgtgtatccgcagggacatagcgataccacggtagccataagtactagtacagtattgctgtgcg
gactctctgccgtcagtcttcttgcttgttatttgaaatccagacagacgccaccgctggcctccgtgga
aatggaagcgatggaagccctccctgtaacgtggggaacaagcagtcgagacgaagatctggagaactgc
tcacatcacttg

IL15
GS linker
IL15RA

FIG. 5B

Mature HLA-E protein (SEQ ID NO:14)

HSLKYFHTSVSRPGRGEPRFISVGYVDDTQFVRFDNDAASPRMVPRAPWMEQEGSEYWDRETRSARDTAQ
IFRVNLRTLRGYYNQSEAGSHTLQWMHGCELGPDGRFLRGYEQFAYDGKDYLTLNEDLRSWTAVDTAAQI
SEQKSNDASEAEHQRAYLEDTCVEWLHKYLEKGKETLLHLEPPKTHVTHHPISDHEATLRCWALGFYPAE
ITLTWQQDGEGHTQDTELVETRPAGDGTFQKWAAVVVPSGEEQRYTCHVQHEGLPEPVTLRWKPASQPTI
PIVGIIAGLVLLGSVVSGAVVAAVIWRKKSSGGKGGSYSKAEWSDSAQGSESHSL

Mature HLA-G protein (SEQ ID NO:15)

HSMRYFSAAVSRPGRGEPRFIAMGYVDDTQFVRFDSDSACPRMEPRAPWVEQEGPEYWEEETRNTKAHAQ
TDRMNLQTLRGYYNQSEASSHTLQWMIGCDLGSDGRLLRGYEQYAYDGKDYLALNEDLRSWTAADTAAQI
SKRKCEAANVAEQRRAYLEGTCVEWLHRYLENGKEMLQRADPPKTHVTHHPVFDYEATLRCWALGFYPAE
IILTWQRDGEDQTQDVELVETRPAGDGTFQKWAAVVVPSGEEQRYTCHVQHEGLPEPLMLRWKQSSLPTI
PIMGIVAGLVVLAAVVTGAAVAAVLWRKKSSD

signal peptide-B2M-linker-HLA-E (SEQ ID NO:19; in PCT/US2021/072646 as SEQ ID NO:66)

MVVMAPRTLFLLLSGALTLTETWAVMAPRTLILGGGGSGGGGSGGGGSGGGGSIQRTPKIQVYSRHPAEN
GKSNFLNCYVSGFHPSDIEVDLLKNGERIEKVEHSDLSFSKDWSFYLLYYTEFTPTEKDEYACRVNHVTL
SQPKIVKWDRDMGGGGSGGGGSGGGGSGSHSLKYFHTSVSRPGRGEPRFISVGYVDDTQFVRFDNDAASP
RMVPRAPWMEQEGSEYWDRETRSARDTAQIFRVNLRTLRGYYNQSEAGSHTLQWMHGCELGPDGRFLRGY
EQFAYDGKDYLTLNEDLRSWTAVDTAAQISEQKSNDASEAEHQRAYLEDTCVEWLHKYLEKGKETLLHLE
PPKTHVTHHPISDHEATLRCWALGFYPAEITLTWQQDGEGHTQDTELVETRPAGDGTFQKWAAVVVPSGE
EQRYTCHVQHEGLPEPVTLRWKPASQPTIPIVGIIAGLVLLGSVVSGAVVAAVIWRKKSSGGKGGSYSKA
EWSDSAQGSESHSL

signal peptide-B2M-linker-HLA-G (SEQ ID NO:20; in PCT/US2021/072646 as SEQ ID NO:69)

MVVMAPRTLFLLLSGALTLTETWARIIPRHLQLGGGGSGGGGSIQRTPKIQVYSRHPAENGKSNFLNCYV
SGFHPSDIEVDLLKNGERIEKVEHSDLSFSKDWSFYLLYYTEFTPTEKDEYACRVNHVTLSQPKIVKWDR
DMGGGGSGGGGSGGGGSGSHSMRYFSAAVSRPGRGEPRFIAMGYVDDTQFVRFDSDSACPRMEPRAPWVE
QEGPEYWEEETRNTKAHAQTDRMNLQTLRGYYNQSEASSHTLQWMIGCDLGSDGRLLRGYEQYAYDGKDY
LALNEDLRSWTAADTAAQISKRKCEAANVAEQRRAYLEGTCVEWLHRYLENGKEMLQRADPPKTHVTHHP
VFDYEATLRCWALGFYPAEIILTWQRDGEDQTQDVELVETRPAGDGTFQKWAAVVVPSGEEQRYTCHVQH
EGLPEPLMLRWKQSSLPTIPIMGIVAGLVVLAAVVTGAAVAAVLWRKKSSD

FIG. 5C

signal peptide-B2M-linker-HLA-E (SEQ ID NO:21; in PCT/US2021/072646 as SEQ ID NO:67)

atggtggtca tggcccctag aacactgttc ctgctgctgt ctggcgccct gacactgaca
gagacatggg ccgtgatggc ccccagaacc ctgatcctgg gcggcggtgg ttcaggcgga
ggaggttcag gaggaggggg tagtggaggt ggtggttcta tccagcggac ccctaagatc
caggtgtaca gcagacaccc cgccgagaac ggcaagagca acttcctgaa ctgctacgtg
tccggctttc accccagcga cattgaggtg gacctgctga agaacggcga gcggatcgag
aaggtggaac acagcgatct gagcttcagc aaggactggt ccttctacct gctgtactac
accgagttca cccctaccga gaaggacgag tacgcctgca gagtgaacca cgtgacactg
agccagccta agatcgtgaa gtgggatcgc gatatgggcg gaggcggatc tggtggcgga
ggaagtggcg gcggaggatc tggctcccac tccttgaagt atttccacac ttccgtgtcc
cggcccggcc gcggggagcc ccgcttcatc tctgtgggct acgtggacga cacccagttc
gtgcgcttcg acaacgacgc cgcgagtccg aggatggtgc cgcgggcgcc gtggatggag
caggaggggt cagagtattg ggaccgggag acacggagcg ccagggacac cgcacagatt
ttccgagtga atctgcggac gctgcgcggc tactacaatc agagcgaggc cgggtctcac
accctgcagt ggatgcatgg ctgcgagctg gggcccgacg ggcgcttcct ccgcgggtat
gaacagttcg cctacgacgg caaggattat ctcaccctga atgaggacct gcgctcctgg
accgcggtgg acacggcggc tcagatctcc gagcaaaagt caaatgatgc ctctgaggcg
gagcaccaga gagcctacct ggaagacaca tgcgtggagt ggctccacaa atacctggag
aaggggaagg agacgctgct tcacctggag cccccaaaga cacacgtgac tcaccacccc
atctctgacc atgaggccac cctgaggtgc tgggccctgg gcttctaccc tgcggagatc
acactgacct ggcagcagga tggggagggc catacccagg acacggagct cgtggagacc
aggcctgcag gggatggaac cttccagaag tgggcagctg tggtggtgcc ttctggagag
gagcagagat acacgtgcca tgtgcagcat gaggggctac ccgagcccgt caccctgaga
tggaagccgg cttcccagcc caccatcccc atcgtgggca tcattgctgg cctggttctc
cttggatctg tggtctctgg agctgtggtt gctgctgtga tatggaggaa gaagagctca
ggtggaaaag gagggagcta ctctaaggct gagtggagcg acagtgccca ggggtctgag
tctcacagct tgtaa

FIG. 5D

signal peptide-B2M-linker-HLA-G (SEQ ID NO:22; in PCT/US2021/072646 as SEQ ID NO:70)

gccaccatgg tggtcatggc gccccgaacc ctcttcctgc tgctctcggg ggccctgacc
ctgaccgaga cctgggcgcg gatcattccc cgacatctgc aactgggagg cggcggttca
ggaggggcg gatcgatcca acgcaccccc aagatccagg tctactccag acacccggcc
gaaaacggaa agtcgaactt cctgaactgc tatgtgtcag gattccaccc gtccgacatc
gaggtggacc tcctgaagaa cggcgaacgc attgagaagg tcgagcactc cgatctgtcg
ttctccaagg actggtcctt ctaccttctc tactataccg aattcacccc gaccgagaag
gacgaatacg cctgccgggt caaccacgtg accctgagcc agccaaagat cgtgaaatgg
gaccgcgata tgggaggagg aggttccggc ggaggaggaa gcggaggcgg aggttccggc
tcccactcca tgaggtattt cagcgccgcc gtgtcccggc ctggccgcgg agagcctcgc
ttcatcgcca tgggatacgt ggacgacacc cagttcgtca gattcgacag cgacagcgcc
tgtcctcgga tggaacctag agcaccttgg gtcgagcaag agggccctga gtactgggaa
gaagagacac ggaacaccaa ggctcacgcc cagaccgaca gaatgaacct gcagaccctg
cggggctact acaatcagtc tgaggccagc agccatactc tgcagtggat gatcggctgc
gatctgggct ctgatggcag actgctgaga ggctacgagc agtacgccta cgacggcaag
gattatctgg ccctgaacga ggacctgcgg tcttggacag ctgccgatac agccgctcag
atcagcaaga gaaagtgcga ggccgccaat gtggccgaac agagaagggc ttacctggaa
ggcacctgtg tggaatggct gcacagatac ctggaaaacg gcaaagagat gctgcagcgg
gccgatcctc ctaagacaca tgtgacccac catcctgtgt tcgactacga ggccacactg
agatgttggg ccctgggctt ttaccctgcc gagatcatcc tgacctggca gcgagatggc
gaggatcaga cccaggatgt ggaactggtg gaaaccagac ctgccggcga cggcaccttt
cagaaatggg ctgctgtggt ggtgcccagc ggagaggaac agagatacac ctgtcacgtg
cagcacgagg gactgcctga acctctgatg ctgagatgga agcagagcag cctgcctaca
atccccatca tgggaatcgt ggccggactg gtggttctgg ccgctgttgt tacaggtgct
gcagtggctg ccgtgctgtg gcggaagaaa agcagcgact ga

FIG. 5E

HSV thymidine kinase polypeptide (SEQ ID NO:23; in US17/657,803 as SEQ ID NO:71)

MASYPCHQHASAFDQAARSRGHSNRRTALRPRRQQEATEVRLEQKMPTLLRVYIDGPHGMGKTTTTQLLV
ALGSRDDIVYVPEPMTYWQVLGASETIANIYTTQHRLDQGEISAGDAAVVMTSAQITMGMPYAVTDAVLA
PHIGGEAGSSHAPPPALTLIFDRHPIAALLCYPAARYLMGSMTPQAVLAFVALIPPTLPGTNIVLGALPE
DRHIDRLAKRQRPGERLDLAMLAAIRRVYGLLANTVRYLQGGGSWREDWGQLSGTAVPPQGAEPQSNAGP
RPHIGDTLFTLFRAPELLAPNGDLYNVFAWALDVLAKRLRPMHVFILDYDQSPAGCRDALLQLTSGMVQT
HVTTPGSIPTICDLARTFAREMGEAN

PSMA variant polypeptide (SEQ ID NO:24; in US17/657,803 as SEQ ID NO:72)

MWNLLARRPRWLCAGALVLAGGFFLLGFLFGWFIKSSNEATNITPKHNMKAFLDELKAENIKKFLYNFTQ
IPHLAGTEQNFQLAKQIQSQWKEFGLDSVELAHYDVLLSYPNKTHPNYISIINEDGNEIFNTSLFEPPPP
GYENVSDIVPPFSAFSPQGMPEGDLVYVNYARTEDFFKLERDMKINCSGKIVIARYGKVFRGNKVKNAQL
AGAKGVILYSDPADYFAPGVKSYPDGWNLPGGGVQRGNILNLNGAGDPLTPGYPANEYAYRRGIAEAVGL
PSIPVHPIGYYDAQKLLEKMGGSAPPDSSWRGSLKVPYNVGPGFTGNFSTQKVKMHIHSTNEVTRIYNVI
GTLRGAVEPDRYVILGGHRDSWVFGGIDPQSGAAVVHEIVRSFGTLKKEGWRPRRTILFASWDAEEFGLL
GSTEWAEENSRLLQERGVAYINADSSIEGNYTLRVDCTPLMYSLVHNLTKELKSPDEGFEGKSLYESWTK
KSPSPEFSGMPRISKLGSGNDFEVFFQRLGIASGRARYTKNWETNKFSGYPLYHSVYETYELVEKFYDPM
FKYHLTVAQVRGGMVFELANSIVLPFDCRDYAVVLRKYADKIYSISMKHPQEMKTYSVSFDSLFSAVKNF
TEIASKFSERLQDFDKSNPIVLRMMNDQLMFLERAFIDPLGLPDRPFYRHVIYAPSSHNKYAGESFPGIY
DALFDIESKVDPSKAWGEVKRQIYVAAFTVQAAAETLSEVA

HSV-TK-PSMA fusion amino acid (SEQ ID NO:25; in US17/657,803 as SEQ ID NO:73)

MASYPCHQHASAFDQAARSRGHSNRRTALRPRRQQEATEVRLEQKMPTLLRVYIDGPHGMGKTTTTQLLV
ALGSRDDIVYVPEPMTYWQVLGASETIANIYTTQHRLDQGEISAGDAAVVMTSAQITMGMPYAVTDAVLA
PHIGGEAGSSHAPPPALTLIFDRHPIAALLCYPAARYLMGSMTPQAVLAFVALIPPTLPGTNIVLGALPE
DRHIDRLAKRQRPGERLDLAMLAAIRRVYGLLANTVRYLQGGGSWREDWGQLSGTAVPPQGAEPQSNAGP
RPHIGDTLFTLFRAPELLAPNGDLYNVFAWALDVLAKRLRPMHVFILDYDQSPAGCRDALLQLTSGMVQT
HVTTPGSIPTICDLARTFAREMGEANGSTSGSGKPGSGEGSTKGMWNLLARRPRWLCAGALVLAGGFFLL
GFLFGWFIKSSNEATNITPKHNMKAFLDELKAENIKKFLYNFTQIPHLAGTEQNFQLAKQIQSQWKEFGL
DSVELAHYDVLLSYPNKTHPNYISIINEDGNEIFNTSLFEPPPPGYENVSDIVPPFSAFSPQGMPEGDLV
YVNYARTEDFFKLERDMKINCSGKIVIARYGKVFRGNKVKNAQLAGAKGVILYSDPADYFAPGVKSYPDG
WNLPGGGVQRGNILNLNGAGDPLTPGYPANEYAYRRGIAEAVGLPSIPVHPIGYYDAQKLLEKMGGSAPP
DSSWRGSLKVPYNVGPGFTGNFSTQKVKMHIHSTNEVTRIYNVIGTLRGAVEPDRYVILGGHRDSWVFGG
IDPQSGAAVVHEIVRSFGTLKKEGWRPRRTILFASWDAEEFGLLGSTEWAEENSRLLQERGVAYINADSS
IEGNYTLRVDCTPLMYSLVHNLTKELKSPDEGFEGKSLYESWTKKSPSPEFSGMPRISKLGSGNDFEVFF
QRLGIASGRARYTKNWETNKFSGYPLYHSVYETYELVEKFYDPMFKYHLTVAQVRGGMVFELANSIVLPF
DCRDYAVVLRKYADKIYSISMKHPQEMKTYSVSFDSLFSAVKNFTEIASKFSERLQDFDKSNPIVLRMMN
DQLMFLERAFIDPLGLPDRPFYRHVIYAPSSHNKYAGESFPGIYDALFDIESKVDPSKAWGEVKRQIYVA
AFTVQAAAETLSEVA

FIG. 5F

HSV-TK-PSMA fusion polynucleotide (SEQ ID NO:26; in US17/657,803 as SEQ ID NO:74)

atggcctcat atccctgcca ccagcatgcg agtgcgtttg atcaagctgc acggtcaaga
ggacattcca ataggcgaac tgcactgaga cctaggcgac aacaagaagc aacggaggtc
cgccttgagc agaagatgcc tactcttctt agggtttaca tcgatggtcc gcacggaatg
ggtaagacca cgactactca actgcttgtc gcattgggct ccagggatga catagtttac
gttccggaac ctatgactta ttggcaggtg ttgggggcat ccgaaaccat agctaatatt
tataccacgc aacaccgctt ggaccaagga gagatctctg cgggagatgc agcggtggta
atgaccagcg cacaaatcac catggggatg ccgtatgccg taacagacgc ggtgctggcc
ccccatatcg gtggtgaagc cggttcaagt catgcgcctc cgccggcgct gacattgatc
tttgatcggc atcctattgc ggcactcctg tgttatccag ccgcacgcta tctgatggga
agcatgaccc cacaagcggt tctggccttt gtggcattga tcccaccaac attgcctgga
actaatattg tactgggcgc tctccccgag gatcgacata ttgataggct cgcgaaacgc
caacgccctg gtgaaaggct tgatttggcc atgctcgcgg ctatacgccg cgtctatggg
ttgctcgcca atactgtgag atacctgcag ggcgggggct catggcgcga ggattggggt
cagttgtcag gcacggcagt gcccccacag ggggcggagc ctcagtcaaa tgcaggccca
agaccgcata ttggtgatac tctcttcacg ttgtttcggg cgccagaatt gcttgccccc
aacggtgatc tttataacgt cttcgcatgg gccctcgatg tcttggccaa gaggctgcga
ccgatgcacg tttttattct cgactatgac cagtcacctg cgggatgcag ggatgcgttg
ctccagttga caagtggcat ggtgcagaca catgtgacga cacccggatc aatacctacg
atatgtgatc tcgcaaggac ctttgcgagg gaaatggggg aagctaatgg ctcaacttct
ggatccggaa agcccggtag cggcgaaggt tctacaaagg gtatgtggaa cctgttggca
agacgccccc gctggctctg tgccggtgct ctggtactgg cggggggatt ttttctcttg
ggatttcttt ttgggtggtt cataaaaagt tctaatgagg ccacaaatat cactccgaaa
cacaatatga aggcatttct ggacgagctc aaagcggaaa atattaagaa attcctgtat
aactttactc agatacctca tctggctggc accgagcaaa acttccagtt ggctaagcag
attcaatcac agtggaaaga gttcggtctc gatagcgttg aattggcaca ctacgatgtc
cttcttagct atcctaataa aacacatccg aactacataa gtatcattaa tgaggacggc
aacgaaattt tcaacacctc tctttttgaa cctcctccgc cgggatatga aaacgtgagc
gatatcgttc ccccctttc cgcgttctca ccacagggaa tgccggaggg tgaccttgtt
tatgtaaatt atgccagaac ggaagatttc tttaaacttg aacgcgatat gaaaatcaac
tgctctggta agattgttat tgcccgatac gggaaggtat tcaggggtaa caaagtgaaa
aacgctcagc tggcgggtgc caaaggagtg atcctttatt ctgaccctgc ggattatttc
gcaccaggcg taaaatcata tcctgacggt tggaaccttc ctggaggtgg agtacagcgc
ggaaatatat tgaatcttaa cggagccggt gacccactga ctcctggata ccccgcaaac
gagtatgcct atcgacgcgg cattgccgaa gcggtgggac tgccctcaat acctgtacat
cctattggat actatgatgc tcagaaactt ttggagaaga tgggtggaag tgccccgcct
gatagttcct ggagaggctc ccttaaggtt ccatataacg taggtccagg gtttacgggc
aacttttcaa cacaaaaggt aaagatgcat atacattcaa ctaacgaggt gacgaggata
tacaatgtaa tcggaactct gaggggagcc gtagagcctg atcgatatgt catcttgggg
ggccacaggg atagttgggt ctttggtgga attgatcctc agtcaggtgc ggctgtagtc
cacgagattg tccgctcttt cggcacgctg aagaaggagg ggtggagacc ccgaaggact
attttgtttg cctcttggga tgctgaagaa tttggtctgc tcggatcaac ggagtgggct
gaagagaatt ctaggttgtt gcaagaacgc ggtgtggcct acataaatgc ggacagtagt
atagaaggca attacacact tcgagtggat tgcaccccgc ttatgtacag tctggtacat
aacctgacga aggagcttaa atcacctgat gaaggattcg aggggaaatc cctttacgaa
tcatggacta aaaagtcacc ttcccctgaa tttagtggga tgccgcgcat aagtaaactc
gggtccggaa acgacttcga agttttcttc caacgattgg gtatcgcctc tggacgagca
cggtacacca aaaattggga aacgaacaaa ttttccggat atcctctcta ccactctgtc
tatgaaacct acgagctggt ggaaaagttt tacgatccga tgtttaagta ccatttgacc

FIG. 5G

HSV-TK-PSMA fusion polynucleotide (SEQ ID NO:26 – continued)

gtcgcccagg tgcggggagg aatggtcttt gaattggcaa atagtatagt ccttcctttt
gattgtcgag attatgccgt cgtccttagg aagtatgctg acaagattta ttcaatatct
atgaagcacc cccaagaaat gaagacctac tcagtgtcct ttgactccct gttctccgct
gtgaagaact tcactgagat cgcctctaag ttctcagagc gactgcaaga ttttgacaag
agtaacccca ttgttcttag gatgatgaat gaccagctca tgtttttgga gagagcattt
attgatccgc tgggccttcc cgaccggcca ttttacagac acgttatcta tgctccttca
agtcacaata aatatgcagg agaatccttt cctgggatct acgatgccct cttcgacata
gaaagtaagg ttgatccctc caaggcgtgg ggggaagtta aacgacagat atatgtcgct
gcattcaccg tacaggccgc cgcagagaca cttagtgagg ttgcgtga

HSV-TK-T2A-PSMA polypeptide (SEQ ID NO:27; in US17/657,803 as SEQ ID NO:76)

MASYPCHQHASAFDQAARSRGHSNRRTALRPRRQQEATEVRLEQKMPTLLRVYIDGPHGMGKTTTTQLLV
ALGSRDDIVYVPEPMTYWQVLGASETIANIYTTQHRLDQGEISAGDAAVVMTSAQITMGMPYAVTDAVLA
PHIGGEAGSSHAPPPALTLIFDRHPIAALLCYPAARYLMGSMTPQAVLAFVALIPPTLPGTNIVLGALPE
DRHIDRLAKRQRPGERLDLAMLAAIRRVYGLLANTVRYLQGGGSWREDWGQLSGTAVPPQGAEPQSNAGP
RPHIGDTLFTLFRAPELLAPNGDLYNVFAWALDVLAKRLRPMHVFILDYDQSPAGCRDALLQLTSGMVQT
HVTTPGSIPTICDLARTFAREMGEANSGSGEGRGSLLTCGDVEENPGPMWNLLARRPRWLCAGALVLAGG
FFLLGFLFGWFIKSSNEATNITPKHNMKAFLDELKAENIKKFLYNFTQIPHLAGTEQNFQLAKQIQSQWK
EFGLDSVELAHYDVLLSYPNKTHPNYISIINEDGNEIFNTSLFEPPPPGYENVSDIVPPFSAFSPQGMPE
GDLVYVNYARTEDFFKLERDMKINCSGKIVIARYGKVFRGNKVKNAQLAGAKGVILYSDPADYFAPGVKS
YPDGWNLPGGGVQRGNILNLNGAGDPLTPGYPANEYAYRRGIAEAVGLPSIPVHPIGYYDAQKLLEKMGG
SAPPDSSWRGSLKVPYNVGPGFTGNFSTQKVKMHIHSTNEVTRIYNVIGTLRGAVEPDRYVILGGHRDSW
VFGGIDPQSGAAVVHEIVRSFGTLKKEGWRPRRTILFASWDAEEFGLLGSTEWAEENSRLLQERGVAYIN
ADSSIEGNYTLRVDCTPLMYSLVHNLTKELKSPDEGFEGKSLYESWTKKSPSPEFSGMPRISKLGSGNDF
EVFFQRLGIASGRARYTKNWETNKFSGYPLYHSVYETYELVEKFYDPMFKYHLTVAQVRGGMVFELANSI
VLPFDCRDYAVVLRKYADKIYSISMKHPQEMKTYSVSFDSLFSAVKNFTEIASKFSERLQDFDKSNPIVL
RMMNDQLMFLERAFIDPLGLPDRPFYRHVIYAPSSHNKYAGESFPGIYDALFDIESKVDPSKAWGEVKRQ
IYVAAFTVQAAAETLSEVA

FIG. 5H

HSV-TK-T2A-PSMA polynucleotide (SEQ ID NO:28; in US17/657,803 as SEQ ID NO:76)

atggcaagtt acccttgcca ccagcatgct agcgctttcg atcaagcggc ccgcagtcgg
ggccatagca atagaaggac cgcattgcgc ccgcgccgac agcaggaagc taccgaagta
cgactggaac aaaaaatgcc cactcttctg agagtataca tcgatgggcc acacggcatg
ggcaaaacga cgactaccca actcttggta gctcttggta gccgggatga tatcgtatat
gtgccagaac ctatgaccta ttggcaggtc ctcggcgcca gtgaaaccat cgccaacata
tatacgacgc aacataggct tgaccagggt gaaatctccg caggtgacgc ggcagtggtc
atgactagcg cccaaatcac gatgggaatg ccttatgcgg tgactgacgc agtactcgct
cctcacattg gaggtgaagc ggggagctcc cacgcaccgc cgcccgctct tacgctcatt
ttcgatcgcc accctatagc tgccctgctc tgttatcccg cggccaggta cttgatgggg
tccatgaccc cccaggcggt gctggccttc gttgcgttga taccgccaac tctccccggc
actaatattg ttctcggagc acttccagaa gacaggcata ttgacaggtt ggctaagcgc
caaaggcccg gtgaacgact cgacctggct atgcttgctg ccatccgccg cgtctatggg
ctgctggcta atacggtcag gtatcttcaa ggcggcggat cttggaggga agattgggga
cagctcagtg gaacggctgt acctccacaa ggggccgaac ctcagtcaaa tgcaggtcct
cgccctcaca ttggagatac acttttact ctttccggg ctccagaact gctggcacca
aatggcgacc tgtacaatgt ttttgcctgg gctctggatg ttttggcaaa aaggcttcgg
ccgatgcacg tatttatttt ggactatgat cagtcaccgg ctggttgtag agacgcattg
cttcaactta catccgggat ggtacaaacg catgtaacaa ccccagggtc aattccaact
atctgcgatc tcgcccgcac attcgcaaga gaaatgggtg aggctaactc tggcagtggt
gaaggccgcg gatctctcct gacttgtggg gatgtcgaag aaaacccggg acccatgtgg
aacctgttgg caagacgccc ccgctggctc tgtgccggtg ctctggtact ggcgggggga
ttttttctct tgggatttct ttttgggtgg ttcataaaaa gttctaatga ggccacaaat
atcactccga aacacaatat gaaggcattt ctggacgagc tcaaagcgga aaatattaag
aaattcctgt ataactttac tcagatacct catctggctg gcaccgagca aaacttccag
ttggctaagc agattcaatc acagtggaaa gagttcggtc tcgatagcgt tgaattggca
cactacgatg tccttcttag ctatcctaat aaaacacatc cgaactacat aagtatcatt
aatgaggacg gcaacgaaat tttcaacacc tctcttttg aacctcctcc gccgggatat
gaaaacgtga gcgatatcgt tccccccttt tccgcgttct caccacaggg aatgccggag
ggtgaccttg tttatgtaaa ttatgccaga acggaagatt tctttaaact tgaacgcgat
atgaaaatca actgctctgg taagattgtt attgcccgat acgggaaggt attcaggggt
aacaaagtga aaaacgctca gctggcgggt gccaaaggag tgatccttta ttctgaccct
gcggattatt tcgcaccagg cgtaaaatca tatcctgacg gttggaacct tcctggaggt
ggagtacagc gcggaaatat attgaatctt aacggagccg gtgacccact gactcctgga
taccccgcaa acgagtatgc ctatcgacgc ggcattgccg aagcggtggg actgccctca
atacctgtac atcctattgg atactatgat gctcagaaac ttttggagaa gatgggtgga
agtgccccgc ctgatagttc ctggagaggc tcccttaagg ttccatataa cgtaggtcca
gggtttacgg gcaacttttc aacacaaaag gtaaagatgc atatacattc aactaacgag
gtgacgagga tatacaatgt aatcggaact ctgaggggag ccgtagagcc tgatcgatat
gtcatcttgg ggggccacag ggatagttgg gtctttggtg gaattgatcc tcagtcaggt
gcggctgtag tccacgagat tgtccgctct ttcggcacgc tgaagaagga ggggtggaga
ccccgaagga ctattttgtt tgcctcttgg gatgctgaag aatttggtct gctcggatca
acggagtggg ctgaagagaa ttctaggttg ttgcaagaac gcggtgtggc ctacataaat
gcggacagta gtatagaagg caattacaca cttcgagtgg attgcacccc gcttatgtac
agtctggtac ataacctgac gaaggagctt aaatcacctg atgaaggatt cgaggggaaa
tccctttacg aatcatggac taaaaagtca ccttcccctg aatttagtgg gatgccgcgc
ataagtaaac tcgggtccgg aaacgacttc gaagttttct tccaacgatt gggtatcgcc
tctggacgag cacggtacac caaaaattgg gaaacgaaca aattttccgg atatcctctc
taccactctg tctatgaaac ctacgagctg gtggaaaagt tttacgatcc gatgtttaag

FIG. 5I

HSV-TK-T2A-PSMA polypeptide (SEQ ID NO:28 – continued)

taccatttga ccgtcgccca ggtgcgggga ggaatggtct ttgaattggc aaatagtata
gtccttcctt ttgattgtcg agattatgcc gtcgtcctta ggaagtatgc tgacaagatt
tattcaatat ctatgaagca cccccaagaa atgaagacct actcagtgtc ctttgactcc
ctgttctccg ctgtgaagaa cttcactgag atcgcctcta agttctcaga gcgactgcaa
gattttgaca agagtaaccc cattgttctt aggatgatga atgaccagct catgtttttg
gagagagcat ttattgatcc gctgggcctt cccgaccggc cattttacag acacgttatc
tatgctcctt caagtcacaa taaatatgca ggagaatcct ttcctgggat ctacgatgcc
ctcttcgaca tagaaagtaa ggttgatccc tccaaggcgt ggggggaagt taaacgacag
atatatgtcg ctgcattcac cgtacaggcc gccgcagaga cacttagtga ggttgcgtga

HSV thymidine kinase A168H variant polypeptide (SEQ ID NO:29; in US17/657,803 as SEQ ID NO:89)

MASYPCHQHASAFDQAARSRGHSNRRTALRPRRQQEATEVRLEQKMPTLLRVYIDGPHGMGKTTTTQLLV
ALGSRDDIVYVPEPMTYWQVLGASETIANIYTTQHRLDQGEISAGDAAVVMTSAQITMGMPYAVTDAVLA
PHIGGEAGSSHAPPPALTLIFDRHPIAHLLCYPAARYLMGSMTPQAVLAFVALIPPTLPGTNIVLGALPE
DRHIDRLAKRQRPGERLDLAMLAAIRRVYGLLANTVRYLQGGGSWREDWGQLSGTAVPPQGAEPQSNAGP
RPHIGDTLFTLFRAPELLAPNGDLYNVFAWALDVLAKRLRPMHVFILDYDQSPAGCRDALLQLTSGMVQT
HVTTPGSIPTICDLARTFAREMGEAN

HSV-TK-T2A-PSMA (N9del) polypeptide (SEQ ID NO:30; in US17/657,803 as SEQ ID NO:93)

MASYPCHQHASAFDQAARSRGHSNRRTALRPRRQQEATEVRLEQKMPTLLRVYIDGPHGMGKTTTTQLLV
ALGSRDDIVYVPEPMTYWQVLGASETIANIYTTQHRLDQGEISAGDAAVVMTSAQITMGMPYAVTDAVLA
PHIGGEAGSSHAPPPALTLIFDRHPIAALLCYPAARYLMGSMTPQAVLAFVALIPPTLPGTNIVLGALPE
DRHIDRLAKRQRPGERLDLAMLAAIRRVYGLLANTVRYLQGGGSWREDWGQLSGTAVPPQGAEPQSNAGP
RPHIGDTLFTLFRAPELLAPNGDLYNVFAWALDVLAKRLRPMHVFILDYDQSPAGCRDALLQLTSGMVQT
HVTTPGSIPTICDLARTFAREMGEANGSGEGRGSLLTCGDVEENPGPMWNLLARRPRWLCAGALVLAGGF
FLLGFLFGWFIKSSNEATNITPKHNMKAFLDELKAENIKKFLYNFTQIPHLAGTEQNFQLAKQIQSQWKE
FGLDSVELAHYDVLLSYPNKTHPNYISIINEDGNEIFNTSLFEPPPPGYENVSDIVPPFSAFSPQGMPEG
DLVYVNYARTEDFFKLERDMKINCSGKIVIARYGKVFRGNKVKNAQLAGAKGVILYSDPADYFAPGVKSY
PDGWNLPGGGVQRGNILNLNGAGDPLTPGYPANEYAYRRGIAEAVGLPSIPVHPIGYYDAQKLLEKMGGS
APPDSSWRGSLKVPYNVGPGFTGNFSTQKVKMHIHSTNEVTRIYNVIGTLRGAVEPDRYVILGGHRDSWV
FGGIDPQSGAAVVHEIVRSFGTLKKEGWRPRRTILFASWDAEEFGLLGSTEWAEENSRLLQERGVAYINA
DSSIEGNYTLRVDCTPLMYSLVHNLTKELKSPDEGFEGKSLYESWTKKSPSPEFSGMPRISKLGSGNDFE
VFFQRLGIASGRARYTKNWETNKFSGYPLYHSVYETYELVEKFYDPMFKYHLTVAQVRGGMVFELANSIV
LPFDCRDYAVVLRKYADKIYSISMKHPQEMKTYSVSFDSLFSAVKNFTEIASKFSERLQDFDKSNPIVLR
MMNDQLMFLERAFIDPLGLPDRPFYRHVIYAPSSHNKYAGESFPGIYDALFDIESKVDPSKAWGEVKRQI
YVAAFTVQAAAETLSEVA

FIG. 5J

(A168H) HSV-TK-T2A-PSMA (N9del) polypeptide (SEQ ID NO:31; in US17/657,803 as SEQ ID NO:94)

MASYPCHQHASAFDQAARSRGHSNRRTALRPRRQQEATEVRLEQKMPTLLRVYIDGPHGMGKTTTTQLLV
ALGSRDDIVYVPEPMTYWQVLGASETIANIYTTQHRLDQGEISAGDAAVVMTSAQITMGMPYAVTDAVLA
PHIGGEAGSSHAPPPALTLIFDRHPIAHLLCYPAARYLMGSMTPQAVLAFVALIPPTLPGTNIVLGALPE
DRHIDRLAKRQRPGERLDLAMLAAIRRVYGLLANTVRYLQGGGSWREDWGQLSGTAVPPQGAEPQSNAGP
RPHIGDTLFTLFRAPELLAPNGDLYNVFAWALDVLAKRLRPMHVFILDYDQSPAGCRDALLQLTSGMVQT
HVTTPGSIPTICDLARTFAREMGEANGSGEGRGSLLTCGDVEENPGPMWNLLARRPRWLCAGALVLAGGF
FLLGFLFGWFIKSSNEATNITPKHNMKAFLDELKAENIKKFLYNFTQIPHLAGTEQNFQLAKQIQSQWKE
FGLDSVELAHYDVLLSYPNKTHPNYISIINEDGNEIFNTSLFEPPPPGYENVSDIVPPFSAFSPQGMPEG
DLVYVNYARTEDFFKLERDMKINCSGKIVIARYGKVFRGNKVKNAQLAGAKGVILYSDPADYFAPGVKSY
PDGWNLPGGGVQRGNILNLNGAGDPLTPGYPANEYAYRRGIAEAVGLPSIPVHPIGYYDAQKLLEKMGGS
APPDSSWRGSLKVPYNVGPGFTGNFSTQKVKMHIHSTNEVTRIYNVIGTLRGAVEPDRYVILGGHRDSWV
FGGIDPQSGAAVVHEIVRSFGTLKKEGWRPRRTILFASWDAEEFGLLGSTEWAEENSRLLQERGVAYINA
DSSIEGNYTLRVDCTPLMYSLVHNLTKELKSPDEGFEGKSLYESWTKKSPSPEFSGMPRISKLGSGNDFE
VFFQRLGIASGRARYTKNWETNKFSGYPLYHSVYETYELVEKFYDPMFKYHLTVAQVRGGMVFELANSIV
LPFDCRDYAVVLRKYADKIYSISMKHPQEMKTYSVSFDSLFSAVKNFTEIASKFSERLQDFDKSNPIVLR
MMNDQLMFLERAFIDPLGLPDRPFYRHVIYAPSSHNKYAGESFPGIYDALFDIESKVDPSKAWGEVKRQI
YVAAFTVQAAAETLSEVA

FIG. 5K

(A168H) HSV-TK-T2A-PSMA (N9del) polynucleotide (SEQ ID NO:32; in US17/657,803 as SEQ ID NO:95)

atggccagct atccttgtca ccagcacgcc agcgcctttg atcaggccgc aagatctaga
ggccacagca acagaagaac agccctgcgg cctcggagac agcaagaggc tacagaagtt
cggctggaac agaagatgcc cacactgctg cgggtgtaca tcgatggccc tcacggcatg
ggcaagacca ccacaacaca gctgctggtg gccctgggca gcagagatga tatcgtgtac
gtgcccgagc ctatgaccta ctggcaggtt ctgggagcca gcgagacaat cgccaacatc
tacaccacac agcaccggct ggatcagggc gaaatttctg ctggcgacgc cgccgtggtt
atgacatctg cccagatcac catgggcatg ccttacgccg tgacagatgc tgtgctggcc
cctcacattg gcggagaagc cggatcttct catgcccctc caccagctct gaccctgatc
ttcgacagac accctatcgc ccatctgctg tgttatcctg ccgccagata cctgatgggc
agcatgacac ctcaggccgt gctggctttc gtggccctga ttcctcctac actgcccggc
accaatatcg tgctgggagc cctgcctgag gaccggcaca ttgatagact ggccaagaga
cagcggcctg gcgagagact ggatctggct atgctggccg ccatcagaag agtgtacggc
ctgctggcca acaccgtgcg gtatcttcaa ggcggcggat cttggagaga ggactgggga
caactgagcg gcacagcagt tcctccacaa ggcgctgagc ctcagtctaa cgctggaccc
agacctcaca tcggcgacac cctgtttacc ctgttcagag cccctgagct gctggctcct
aacggcgacc tgtacaacgt gttcgcctgg gctcttgacg tgctggcaaa aagactgcgg
cccatgcacg tgttcatcct ggactacgat cagtcccctg ccggctgtag agatgctctg
ctgcagctga caagcggcat ggtgcagacc cacgttacaa cccctggcag catccccacc
atctgtgacc tggccagaac cttcgccaga gagatgggag aagccaacgg cagcggcgaa
ggcagaggat ctctgctgac atgtggcgac gtggaagaga accccggacc tatgtggaac
ctgctggcta gacggcccag atggctttgt gctggtgctc tggttctggc tggcggcttt
ttcctgctgg gcttcctgtt tggctggttc atcaagagca gcaacgaggc caccaacatc
acccctaagc acaacatgaa ggcctttctg gacgagctga aggccgagaa tatcaagaag
ttcctctaca acttcacgca gatccctcac ctggccggca ccgagcagaa ttttcagctg
gccaagcaga tccagagcca gtggaaagag ttcggcctgg actctgtgga actggcccac
tacgatgtgc tgctgagcta ccccaacaag acacacccca actacatcag catcatcaac
gaggacggca acgagatctt caacaccagc ctgttcgagc ctccacctcc tggctacgag
aacgtgtccg atatcgtgcc tccattcagc gctttcagcc ctcaagggat gcctgagggc
gatctggtgt acgtgaacta cgccagaacc gaggacttct tcaagctgga acgggacatg
aagatcaact gctccggcaa gatcgtgatc gcccgctacg gcaaagtgtt ccggggcaac
aaagtgaaga acgcccagct ggcaggcgcc aaaggcgtga tcctgtatag cgaccccgcc
gactattttg cccctggcgt gaagtcttac cccgacggct ggaatcttcc tggtggcgga
gtgcagagag gcaacatcct gaaccttaac ggcgcaggcg accctctgac acctggctat
cctgccaatg agtacgccta cagacgggga attgccgagg ctgtgggact gccttctatc
cctgtgcacc ccatcggcta ctacgacgcc cagaaactgc tggaaaagat gggcggaagc
gcccctcctg actctagttg gagaggctct ctgaaggtgc cctacaatgt cggcccaggc
ttcaccggca acttcagcac ccagaaagtg aaaatgcaca tccacagcac caacgaagtg
acccggatct acaacgtgat cggcacactg agaggcgccg tggaacccga cagatatgtg
atcctcggcg gccacagaga tagctgggtg ttcggaggaa tcgaccctca gtctggtgcc
gctgtggtgc acgaaatcgt gcggtctttt ggcaccctga agaaagaagg ctggcgcccc
agacggacca tcctgtttgc ctcttgggac gccgaggaat tcggactgct gggatctaca
gagtgggccg aagagaacag cagactgctg caagaaagag gcgtggccta catcaacgcc
gacagcagca tcgagggcaa ctacaccctg agagtggact gcacccctct gatgtacagc
ctggtgcaca acctgaccaa agagctgaag tcccctgacg agggctttga gggcaagagc
ctgtacgaga gctggaccaa gaagtcccca tctcctgagt tcagcggcat gccccggatc
tctaagctcg gctctggcaa cgacttcgag gtgttcttcc agcggctggg aatcgcttct
ggcagagcca gatacaccaa gaactgggag acaaacaagt tctccggcta tcccctgtac
cacagcgtgt acgagacata cgagctggtc gagaagttct acgaccccat gttcaagtac

FIG. 5L

(A168H) HSV-TK-T2A-PSMA (N9del) polynucleotide (SEQ ID NO:32 – continued)

```
cacctgacag tggcccaagt gcgcggaggc atggtgttcg aactggccaa tagcatcgtg
ctgcccttcg actgcagaga ctatgccgtg gtgctgcgga agtacgccga taagatctac
agcatcagca tgaagcaccc gcaagagatg aagacctaca gcgtgtcctt cgatagcctg
ttcagcgccg tgaagaactt caccgagatc gccagcaagt tcagcgagcg gctgcaggac
ttcgacaaga gcaacccaat cgtgctgagg atgatgaacg accagctgat gttcctggaa
cgcgccttca tcgacccact gggcttgccc gatagaccct tctaccggca cgtgatctat
gcccctagca gccacaacaa atacgccggc gagagcttcc ccggcatcta cgatgcctg
ttcgacatcg agagcaaggt ggacccatct aaggcctggg gcgaagtgaa gcggcagatc
tacgtggccg cattcacagt gc
```

IL-12 protein (SEQ ID NO:33)

MCHQQLVISWFSLVFLASPLVAIWELKKDVYVVELDWYPDAPGEMVVLTCDTPEEDGITWTLDQSSEVLG
SGKTLTIQVKEFGDAGQYTCHKGGEVLSHSLLLLHKKEDGIWSTDILKDQKEPKNKTFLRCEAKNYSGRF
TCWWLTTISTDLTFSVKSSRGSSDPQGVTCGAATLSAERVRGDNKEYEYSVECQEDSACPAAEESLPIEV
MVDAVHKLKYENYTSSFFIRDIIKPDPPKNLQLKPLKNSRQVEVSWEYPDTWSTPHSYFSLTFCVQVQGK
SKREKKDRVFTDKTSATVICRKNASISVRAQDRYYSSSWSEWASVPCSGSTSGSGKPGSGEGSTKG**RNLP**
VATPDPGMFPCLHHSQNLLRAVSNMLQKARQTLEFYPCTSEEIDHEDITKDKTSTVEACLPLELTKNESC
LNSRETSFITNGSCLASRKTSFMMALCLSSIYEDLKMYQVEFKTMNAKLLMDPKRQIFLDQNMLAVIDEL
MQALNFNSETVPQKSSLEEPDFYKTKIKLCILLHAFRIRAVTIDRVMSYLNAS

IL12 Signal Sequence
IL12p70 p40 subunit
Whitlow linker
IL12p70 p35 subunit

FIG. 5M

Polynucleotide sequence encoding IL-12 protein (SEQ ID NO:34)

ATGTGCCATCAACAATTGGTTATATCATGGTTCTCTCTCGTGTTTCTGGCATCACCACTTGTTGCAATCT
GGGAACTGAAAAAAGATGTATATGTAGTCGAATTGGACTGGTATCCAGATGCGCCCGGCGAGATGGTTGT
TCTGACTTGTGACACACCTGAGGAAGACGGCATTACGTGGACGCTGGATCAATCTTCCGAGGTCCTCGGA
TCTGGTAAAACCCTTACAATTCAAGTTAAGGAATTTGGTGATGCAGGACAATACACCTGCCACAAGGGTG
GTGAAGTACTGTCCCATTCCCTCCTGTTGCTGCACAAGAAGGAGGACGGAATATGGAGTACAGACATCCT
GAAGGACCAGAAAGAGCCCAAAAACAAGACGTTTTTGAGATGCGAGGCAAAGAACTACAGTGGTCGGTTC
ACGTGCTGGTGGTTGACTACCATTTCAACAGATCTGACATTTTCAGTCAAGTCAAGTAGAGGGTCTTCAG
ACCCGCAAGGTGTTACATGTGGCGCTGCAACGCTCTCCGCAGAGAGGGTTAGGGGAGACAACAAGGAGTA
TGAGTATAGTGTCGAGTGTCAAGAAGACAGTGCCTGTCCCGCGGCAGAGGAATCCCTCCCAATTGAGGTC
ATGGTAGATGCTGTTCACAAGCTCAAGTATGAAAATTATACTTCAAGTTTTTTTATCAGAGACATTATCA
AGCCGGATCCCCCTAAAAATCTCCAGCTTAAGCCCCTCAAAAATAGTCGGCAGGTCGAAGTGAGCTGGGA
ATATCCCGACACGTGGTCTACCCCGCACTCATACTTCAGTCTGACTTTTTGCGTCCAAGTACAAGGAAAG
TCCAAGAGAGAAAAAAAGGATAGAGTGTTTACCGACAAGACTAGCGCGACGGTTATTTGTCGGAAGAACG
CGAGCATTTCAGTTCGAGCACAGGACAGGTATTATTCATCTTCATGGTCAGAATGGGCTTCAGTTCCGTG
CAGCGGCTCTACTTCCGGCTCAGGTAAGCCGGGCTCTGGAGAGGGTAGCACTAAGGGCAGGAACTTGCCT
GTCGCCACCCCGGACCCAGGCATGTTCCCTTGTTTGCATCACAGTCAGAATTTGCTGCGAGCGGTCTCCA
ACATGCTTCAAAAAGCTCGGCAAACCCTCGAATTCTATCCGTGCACTAGCGAGGAAATAGACCACGAAGA
CATAACTAAGGACAAAACAAGTACTGTGGAAGCCTGCCTGCCCCTCGAACTTACGAAAAACGAGAGCTGC
CTGAATAGCCGAGAAACCTCTTTCATTACTAATGGGAGCTGTCTGGCGAGTCGGAAGACCTCATTTATGA
TGGCGCTTTGTCTTTCCTCAATTTACGAAGACCTCAAGATGTACCAGGTTGAATTTAAAACGATGAACGC
CAAGTTGCTTATGGATCCGAAGCGGCAGATATTTCTTGATCAGAATATGCTTGCTGTTATAGATGAGTTG
ATGCAGGCTCTCAACTTCAACAGCGAGACTGTTCCACAGAAGTCATCTCTGGAAGAACCCGATTTCTACA
AGACCAAGATAAAACTCTGTATTCTCTTGCATGCTTTTCGGATTCGGGCAGTGACGATCGACAGGGTTAT
GTCTTACCTTAATGCCAGT

DLL4-Fc fusion 4 (SEQ ID NO:35)

SSVFQLQLQEFINERGVLASGRPCEPGCRTFFRVCLKHFQAVVSPGPCTFGTVSTPVLGTNSFAVRDDSS
GGGRNPLQLPLNFTWPGTFSLIIEAWHAPGDDLRPEALPPDALISKFAIQGSLAVGQNWLLDEQTSTLTR
LRYSYRVICSDNYYGDNCSRLCKKRNDYFGHYVCQPDGNPSCLPGWTGEYCQQPICLSGCHEQNGYCSKP
AECLCRPGWQGRLCNECIPHNGCRHGTCSTPWQCTCDEGWGGLFCDQDKTHTCPPCPAPELLGGPSVFLF
PPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDW
LNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWES
NGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

DLL4-Fc fusion 5 (SEQ ID NO:36)

SSVFQLQLQEFINERGVLASGRPCEPGCRTFFRVCLKHFQAVVSPGPCTFGTVSTPVLGTNSFAVRDDSS
GGGRNPLQLPLNFTWPGTFSLIIEAWHAPGDDLRPEALPPDALISKFAIQGSLAVGQNWLLDEQTSTLTR
LRYSYRVICSDNYYGDNCSRLCKKRNDYFGHYVCQPDGNPSCLPGWTGEYCQQPICLSGCHEQNGYCSKP
AECLCRPGWQGRLCNECIPHNGCRHGTCSTPWQCTCDEGWGGLFCDQDKTHTCPPCPAPELLGGPSVFLF
PPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDW
LNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWES
NGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

FIG. 5N

DLL4-Fc fusion 6 (SEQ ID NO:37)

SSVFQLQLQEFINERGVLASGRPCEPGCRTFFRVCLKHFQAVVSPGPCTFGTVSTPVLGTNSFAVRDDSS
GGGRNPLQLPLNFTWPGTFSLIIEAWHAPGDDLRPEALPPDALISKFAIQGSLAVGQNWLLDEQTSTLTR
LRYSYRVICSDNYYGDNCSRLCKKRNDYFGHYVCQPDGNPSCLPGWTGEYCQQPICLSGCHEQNGYCSKP
AECLCRPGWQGRLCNECIPHNGCRHGTCSTPWQCTCDEGWGGLFCDQDLNYCTHHSPCKNGATCSNSGQR
SYTCTCRPGYTGVDCELELSECDSNPCRNGGSCKDQEDGYHCLCPPGYYGLHCEHSTLSCADSPCFNGGS
CRERNQGANYACECPPNFTGSNCEKKVDRCTSNPCANGGQCLNRGPSRMCRCRPGFTGTYCELHVSDCAR
NPCAHGGTCHDLENGLMCTCPAGFSGRRCEVRTSIDACASSPCFNRATCYTDLSTDTFVCNCPYGFVGSR
CEFPVGLPDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGV
EVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLP
PSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNV
FSCSVMHEALHNHYTQKSLSLSPGK

FIG. 6

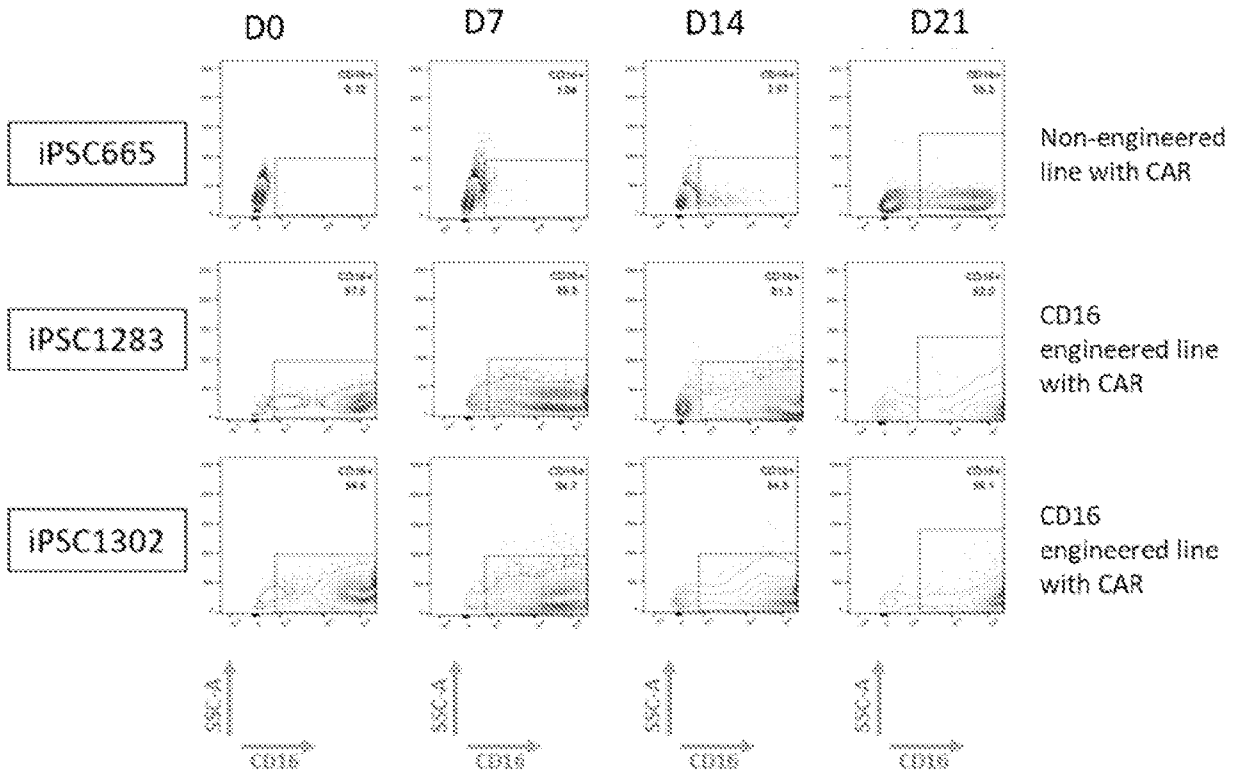

D0
D7
D14
D21
iPSC665
iPSC1283
iPSC1302
Non-engineered line with CAR
CD16 engineered line with CAR
CD16 engineered line with CAR
SSC-A
CD16

FIG. 7A

NKG2D engineered iNK cells

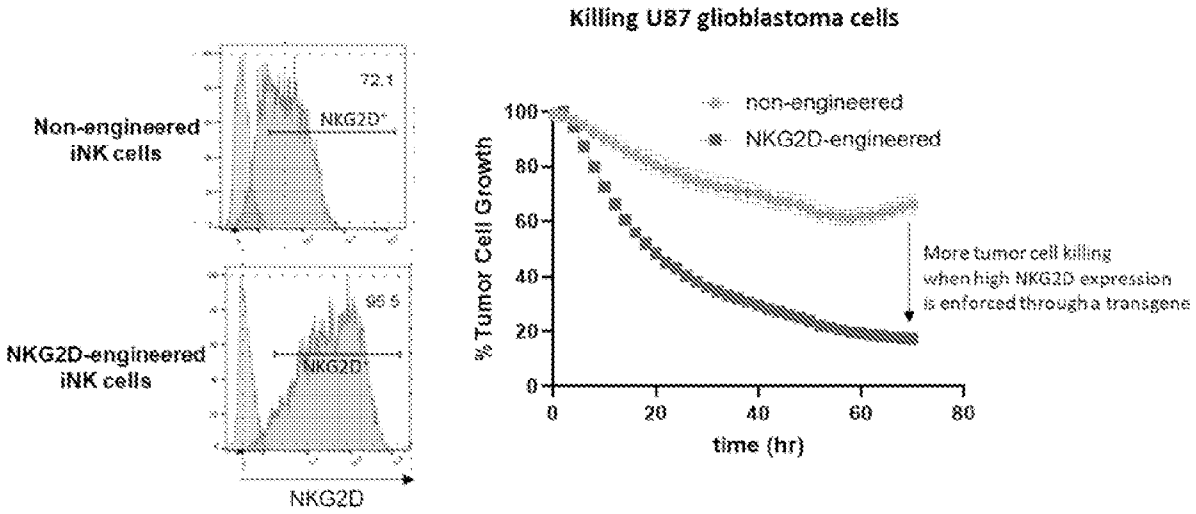


FIG. 7B

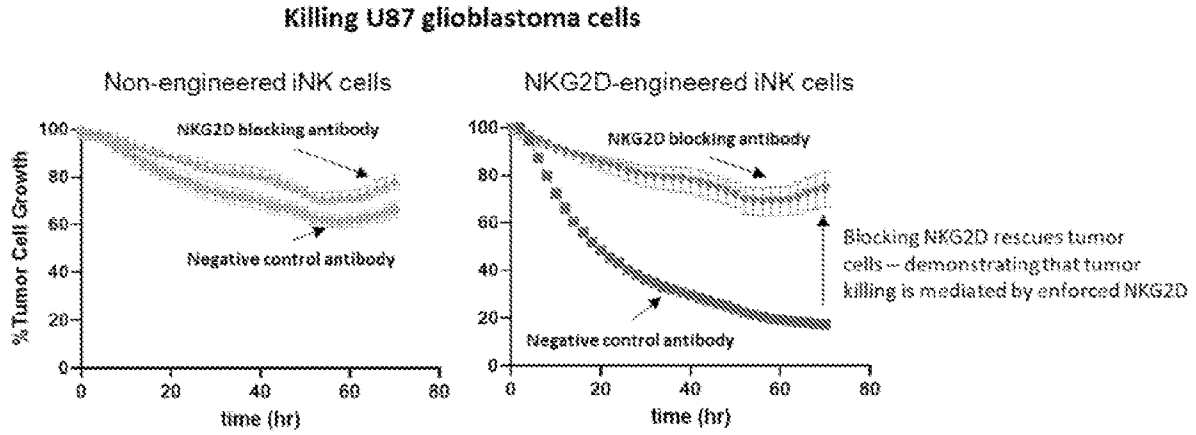


FIG. 7C

CD16 engineered iNK cells

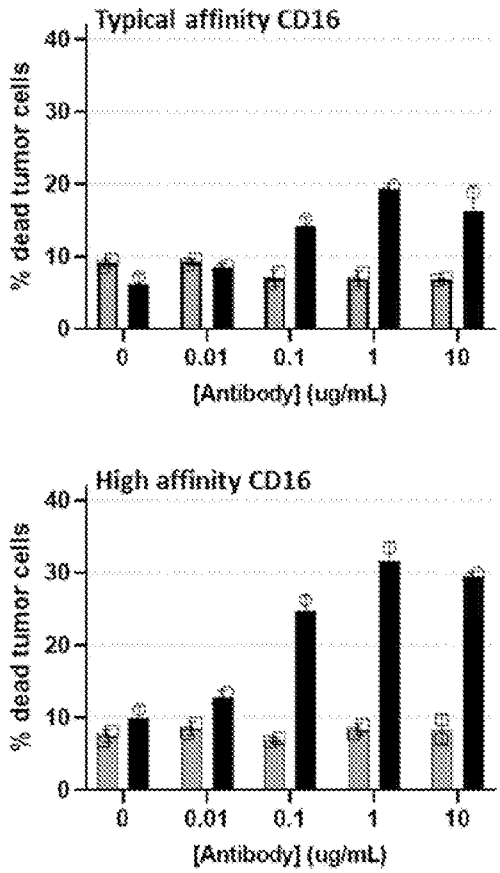


FIG. 8

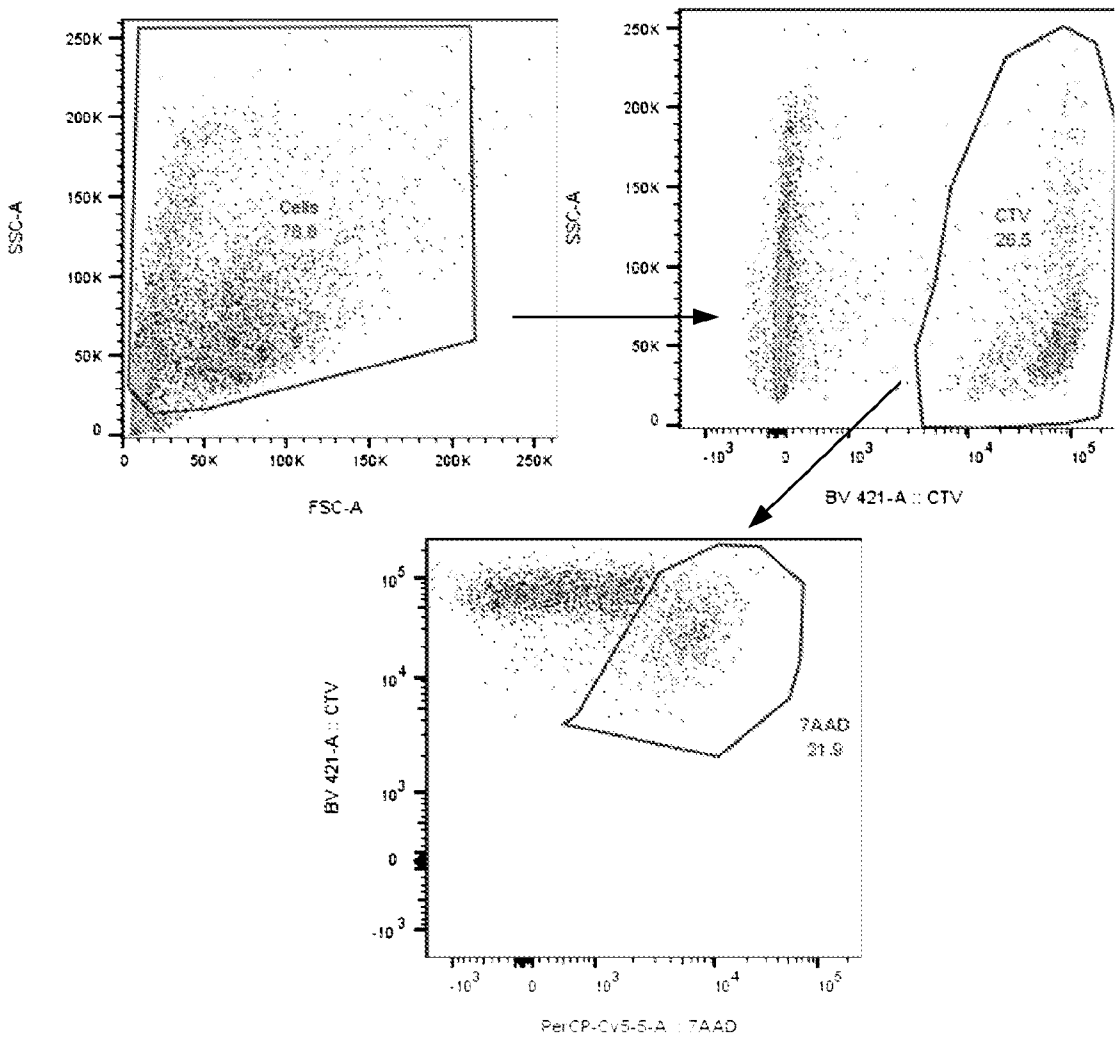

SSC-A
FSC-A
Cells
78.8
CTV
28.5
BV 421-A :: CTV
7AAD
21.9
PerCP-Cy5-5-A :: 7AAD

FIG. 9A

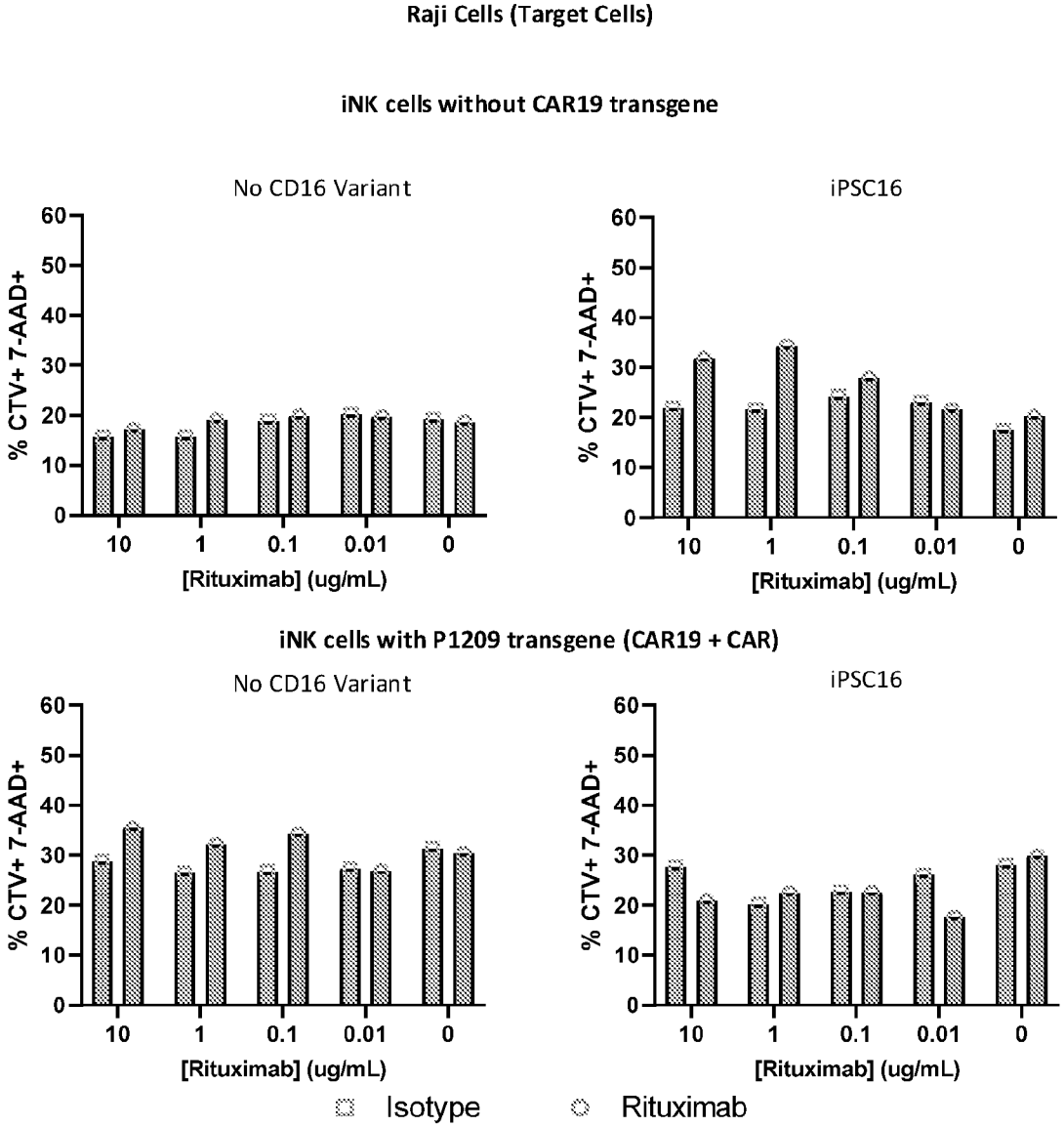

Raji Cells (Target Cells)
iNK cells without CAR19 transgene
No CD16 Variant
iPSC16
% CTV+ 7-AAD+
60
50
40
30
20
10
0
10
1
0.1
0.01
0
[Rituximab] (ug/mL)
iNK cells with P1209 transgene (CAR19 + CAR)
No CD16 Variant
iPSC16
% CTV+ 7-AAD+
[Rituximab] (ug/mL)
Isotype
Rituximab

FIG. 9B

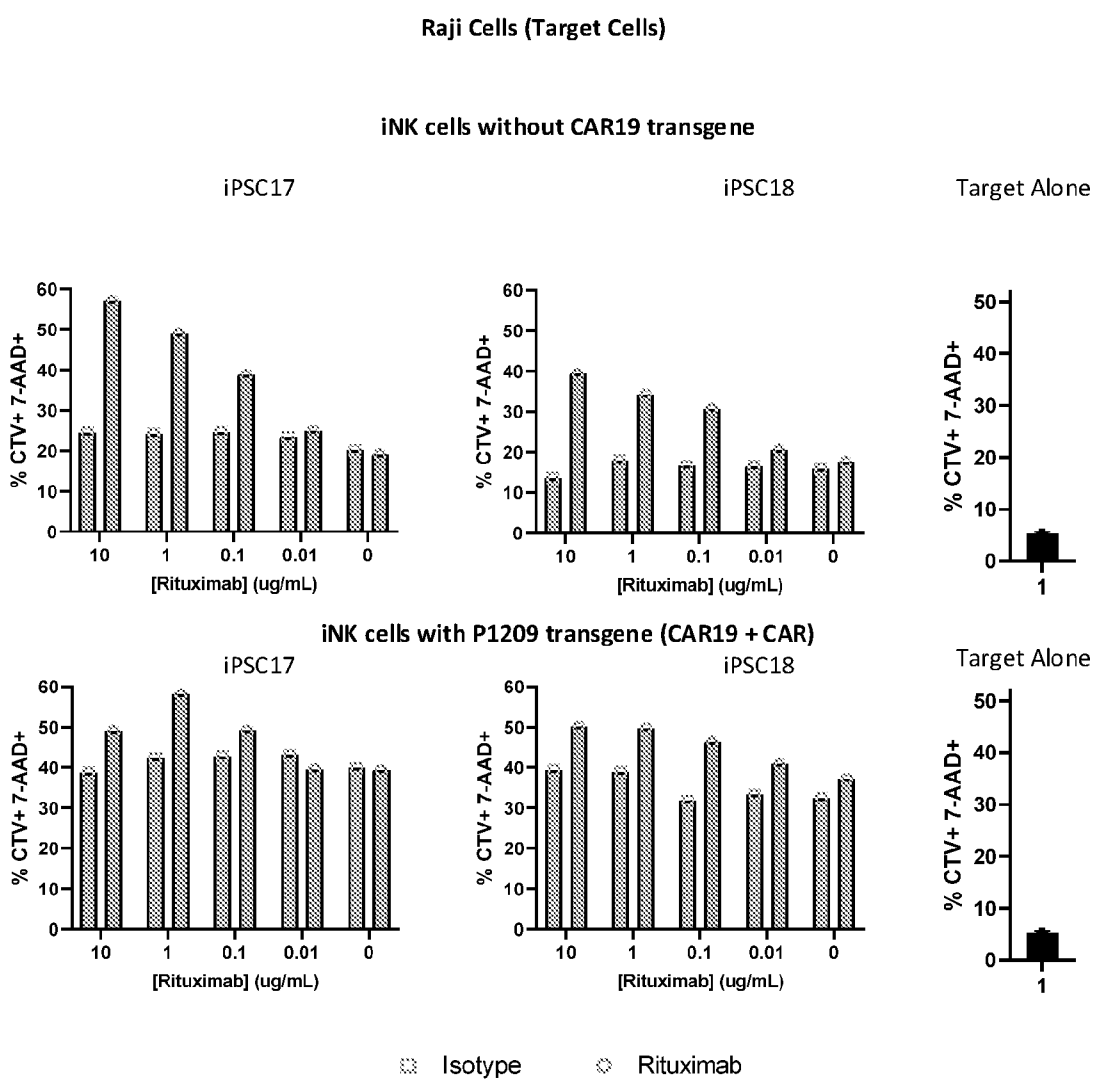

Raji Cells (Target Cells)
iNK cells without CAR19 transgene
iPSC17
iPSC18
Target Alone
% CTV+ 7-AAD+
[Rituximab] (ug/mL)
iNK cells with P1209 transgene (CAR19 + CAR)
Isotype
Rituximab

FIG. 10A

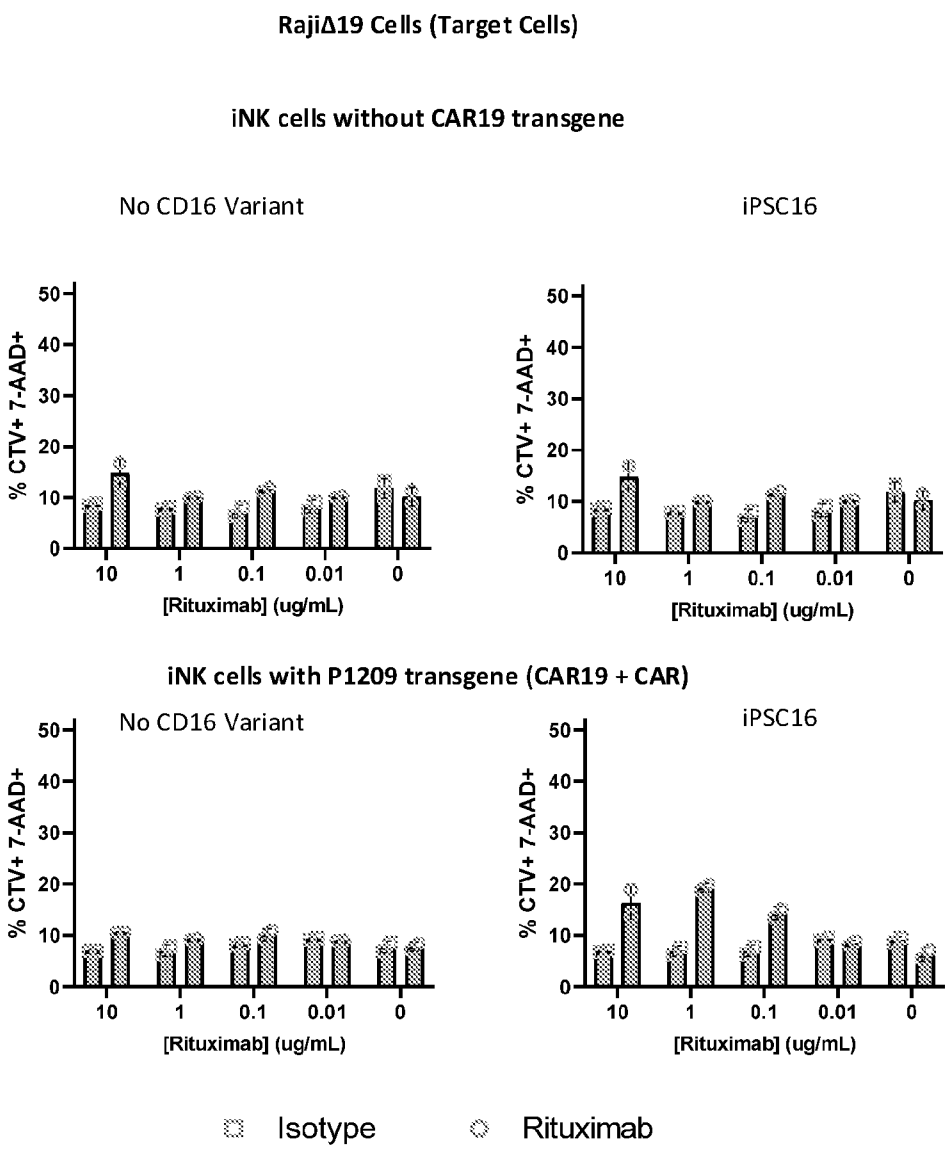
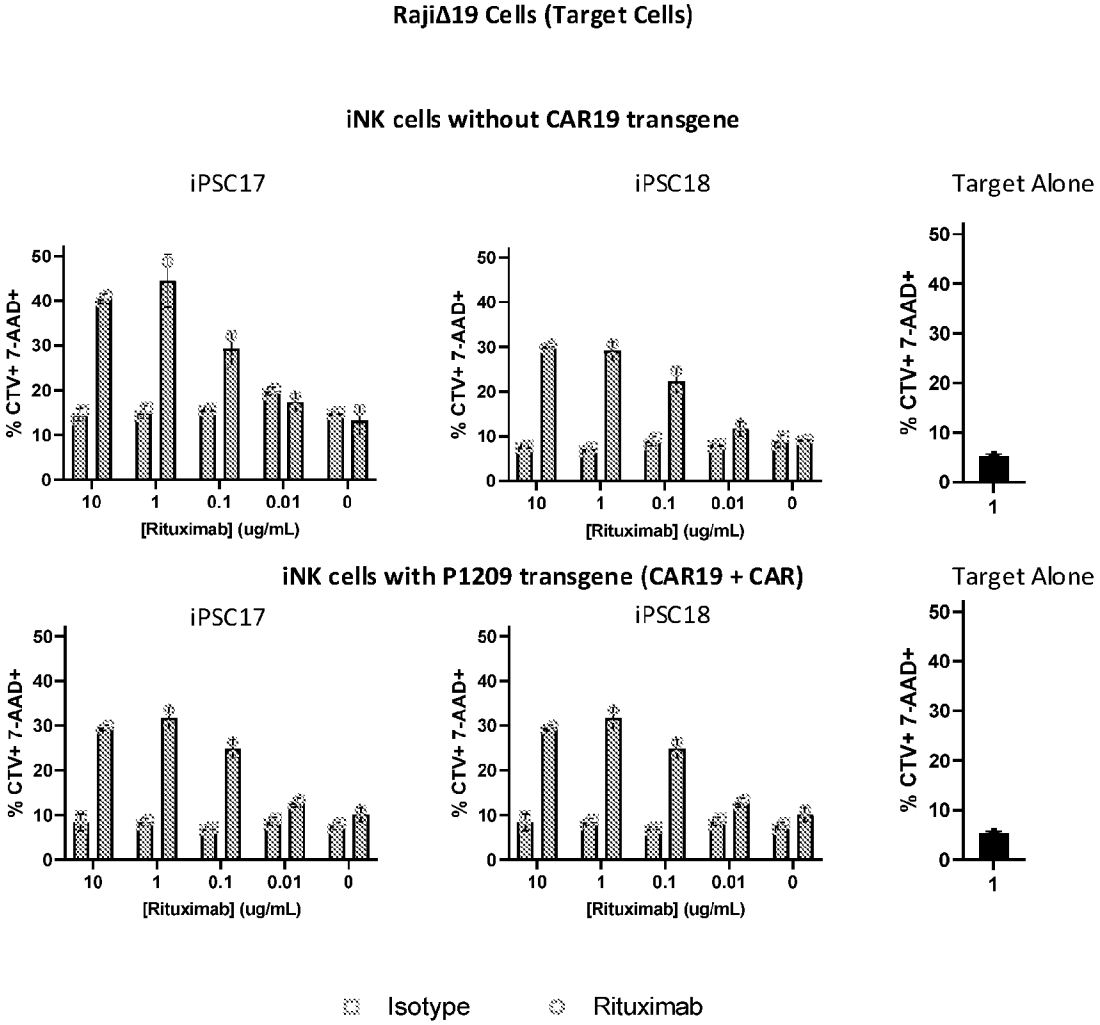

RajiΔ19 Cells (Target Cells)
iNK cells without CAR19 transgene
No CD16 Variant
iPSC16
% CTV+ 7-AAD+
50
40
30
20
10
0
10
1
0.1
0.01
0
[Rituximab] (ug/mL)
iNK cells with P1209 transgene (CAR19 + CAR)
No CD16 Variant
iPSC16
Isotype
Rituximab

FIG. 10B


RajiΔ19 Cells (Target Cells)
iNK cells without CAR19 transgene
iPSC17
iPSC18
Target Alone
% CTV+ 7-AAD+
[Rituximab] (ug/mL)
iNK cells with P1209 transgene (CAR19 + CAR)
Isotype
Rituximab

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2023/068079

A. CLASSIFICATION OF SUBJECT MATTER
INV. C07K14/735 C07K14/705 C12N5/0783 A61K35/17 A61K35/545
A61K38/00 A61P35/00 C12N5/074
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)
C07K C12N A61K A61P

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, Sequence Search, BIOSIS, EMBASE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	BACHANOVA VERONIKA ET AL: "Safety and Efficacy of FT596, a First-in-Class, Multi-Antigen Targeted, Off-the-Shelf, iPSC-Derived CD19 CAR NK Cell Therapy in Relapsed/Refractory B-Cell Lymphoma", BLOOD, vol. 138, 5 November 2021 (2021-11-05), pages 823-825, XP093074018, the whole document ----- -/--	1-110, 112-136

[x] Further documents are listed in the continuation of Box C.
[ ] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance;; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance;; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
18 August 2023	23/10/2023

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	Mabit, Hélène

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

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2023/068079

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	Y.-H. CHANG ET AL: "A Chimeric Receptor with NKG2D Specificity Enhances Natural Killer Cell Activation and Killing of Tumor Cells", CANCER RESEARCH, vol. 73, no. 6, 15 March 2013 (2013-03-15) , pages 1777-1786, XP055262100, US ISSN: 0008-5472, DOI: 10.1158/0008-5472.CAN-12-3558 abstract -----	1-110, 112-136
Y	YE LI ET AL: "Human iPSC-Derived Natural Killer Cells Engineered with Chimeric Antigen Receptors Enhance Anti-tumor Activity", CELL STEM CELL, vol. 23, no. 2, 28 June 2018 (2018-06-28), pages 181-192, XP055700643, AMSTERDAM, NL ISSN: 1934-5909, DOI: 10.1016/j.stem.2018.06.002 paragraph [discussion] -----	1-110, 112-136
Y	SCHMIDT DAYANE ET AL: "Engineering CAR-NK cells: how to tune innate killer cells for cancer immunotherapy", IMMUNOTHERAPY ADVANCES, vol. 2, no. 1, 3 February 2022 (2022-02-03), pages 1-14, XP093074650, DOI: 10.1093/immadv/ltac003 page 2 table 2 page 6 - page 7; figure 2 page 6, column 2 -----	1-110, 112-136
Y	GOLDENSON BENJAMIN H. ET AL: "iPSC-Derived Natural Killer Cell Therapies - Expansion and Targeting", FRONTIERS IN IMMUNOLOGY, vol. 13, 3 February 2022 (2022-02-03), XP093073962, DOI: 10.3389/fimmu.2022.841107 the whole document -----	1-110, 112-136

-/--

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	ZHU HUANG ET AL: "Pluripotent stem cell-derived NK cells with high-affinity noncleavable CD16a mediate improved antitumor activity", BLOOD, vol. 135, no. 6, 6 February 2020 (2020-02-06), pages 399-410, XP055967123, US ISSN: 0006-4971, DOI: 10.1182/blood.2019000621 Retrieved from the Internet: URL:http://ashpublications.org/blood/article-pdf/135/6/399/1633322/bloodbld2019000621.pdf> abstract ----- ZHANG CAI ET AL: "Chimeric antigen receptor- and natural killer cell receptor-engineered innate killer cells in cancer immunotherapy", CELLULAR & MOLECULAR IMMUNOLOGY, NATURE PUBLISHING GROUP UK, LONDON, vol. 18, no. 9, 15 July 2021 (2021-07-15), pages 2083-2100, XP037559982, ISSN: 1672-7681, DOI: 10.1038/S41423-021-00732-6 [retrieved on 2021-07-15] figure 1 page 2086 - page 2087 table 1 ----- GURUNG BUDDHA ET AL: "265?Empowering iPSC-derived iNK cells with multiple gene edits to improve persistence and anti-tumor efficacy", J. IMMUNOTHER. CANCER, vol. 10, no. 2, 7 November 2022 (2022-11-07), pages A280-A280, XP093074333, DOI: 10.1136/jitc-2022-SITC2022.0265 the whole document -----	1-110, 112-136 1-110, 112-136 1-110, 112-136

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2023/068079

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:

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2023/068079

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

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

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

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

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

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

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

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

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

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

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims;; it is covered by claims Nos.:

1-109, 112-136(completely); 110(partially)

Remark on Protest

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

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

☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. claims: 1-109, 112-136(completely); 110(partially)

Subject matter of claims 1-110 when related to a CD16 protein linked by an autoprotease peptide to a NKG2D protein

---

2. claims: 111(completely); 110(partially)

Subject matter related to DDL4 variants of SEQ ID N°35-37
