

SOLID TUMOR TARGETING BACKBONE PROMOTING EFFECTOR CELL DIFFERENTIATION AND FUNCTION

RELATED APPLICATION

[0001] The present application claims priority to U.S. Provisional Application Serial No. 63/517,343, filed August 2, 2023, the disclosure of which is hereby incorporated by reference in its entirety.

INCORPORATION BY REFERENCE OF SEQUENCE LISTING

[0002] The Sequence Listing titled 184143-653101_SL.xml, which was created on August 2, 2023 and is 184,114 bytes in size, is hereby incorporated by reference in its entirety.

FIELD OF THE INVENTION

[0003] The present disclosure is broadly concerned with the field of off-the-shelf immunocellular products. More particularly, the present disclosure is concerned with strategies for developing multifunctional effector cells capable of delivering therapeutically relevant properties *in vivo*. Cell products developed under the present disclosure address critical limitations of patient-sourced cell therapies.

BACKGROUND OF THE INVENTION

[0004] The field of adoptive cell therapy is currently focused on using patient- and donor-sourced cells, which makes it particularly difficult to achieve consistent manufacturing of cancer immunotherapies and to deliver therapies to all patients who may benefit therefrom. There is also a need to improve the efficacy and persistence of adoptively transferred lymphocytes to promote favorable patient outcomes. Lymphocytes such as T cells and natural killer (NK) cells are potent anti-tumor effectors that play an important role in innate and adaptive immunity. However, the use of these immune cells for adoptive cell therapies remains challenging and has unmet needs for improvement. Therefore, there remain significant opportunities to harness the full potential of T and NK cells, or other immune effector cells in adoptive immunotherapy.

SUMMARY OF THE INVENTION

[0005] There is a need for functionally improved effector cells that address issues ranging from response rate, cell exhaustion, loss of transfused cells (survival and/or persistence), tumor

escape through target loss or lineage switch, tumor targeting precision, off-target toxicity, off-tumor effect, to efficacy against solid tumors, i.e., tumor microenvironment and related immune suppression, recruiting, trafficking and infiltration.

[0006] It is an object of embodiments of the present invention to provide methods and compositions to generate derivative non-pluripotent cells differentiated from a single cell derived iPSC (induced pluripotent stem cell) clonal line, which iPSC line comprises one or several genetic modifications in its genome. In some embodiments, the one or several genetic modifications include one or more of DNA insertion, deletion, and substitution, and which modifications are retained and remain functional in subsequently derived cells after differentiation, expansion, passaging and/or transplantation.

[0007] The iPSC derived non-pluripotent cells of the present application include, but are not limited to, CD34⁺ cells, hemogenic endothelium cells, HSCs (hematopoietic stem and progenitor cells), hematopoietic multipotent progenitor cells, T cell progenitors, NK cell progenitors, T cells, NKT cells, NK cells, and B cells. The iPSC-derived non-pluripotent cells of the present application comprise one or several genetic modifications in their genome through differentiation from an iPSC comprising the same genetic modifications. In some embodiments, the engineered clonal iPSC differentiation strategy for obtaining genetically engineered derivative cells benefits from the developmental potential of the iPSC in a directed differentiation that is not significantly adversely impacted by the engineered modality in the iPSC, and also that the engineered modality functions as intended in the derivative cell. Further, this strategy overcomes the present barrier in engineering primary lymphocytes, such as T cells or NK cells obtained from peripheral blood, as such cells are difficult to engineer, with engineering of such cells often lacking reproducibility and uniformity, resulting in cells exhibiting poor cell persistence with high cell death and low cell expansion. Moreover, this strategy avoids production of a heterogenous effector cell population otherwise obtained using primary cell sources which are heterogenous to start with.

[0008] In general, this application provides compositions and methods for generating a Master Cell Bank (MCB) having at least three targeted integration sites hosting multiplex configured edits via multiple MCB intermediaries (pre-MCBs). One aspect of the present application provides compositions and methods to generate iPSC and derivative effector cells comprising an TCR promoter-driven ADR and/or a cytokine, and a constitutively expressed TCE, in addition to edits comprising a CAR, a TGFβ-SRR, a C-X-C-motif chemokine receptor or a variant thereof, a CD16 variant, and a CD38 knockout, among other modifications as provided herein.

[0009] In one aspect, the present disclosure provides a cell or population thereof, wherein (i) the cell is (a) an immune cell; (b) an induced pluripotent cell (iPSC); or (c) a derivative effector cell obtained from differentiating the iPSC; (ii) the cell comprises a construct comprising at least one of: (a) a polynucleotide encoding an allo-immune defense receptor (ADR); and (b) a polynucleotide encoding IL2, IL18, or a IL7RF; (iii) the construct is inserted into a T cell receptor (TCR) locus, thereby knocking out the TCR; and (iv) the construct is expressed under control of an endogenous TCR promoter.

[00010] In some embodiments, the cell or population thereof further comprises a construct at a first integration site (site 1), wherein: (i) the site 1 construct comprises two or more of: (a) a polynucleotide encoding a TGF β signaling redirector receptor (TGF β -SRR) comprising a partial or full peptide of the extracellular domain (ECD) of transforming growth factor beta receptor (TGF β R); (b) a polynucleotide encoding a C-X-C motif chemokine receptor or a variant thereof; and (c) a polynucleotide encoding an exogenous CD16 or a variant thereof; and (ii) the site 1 construct comprises an exogenous promoter that regulates expression of the polynucleotides in the site 1 construct. In some embodiments, the cell or population thereof further comprises a construct at a second integration site (site 2), wherein: (i) the site 2 construct comprises a polynucleotide encoding a first chimeric antigen receptor (CAR1); (ii) the site 2 construct optionally further comprises one or more of: (a) a polynucleotide encoding a second chimeric antigen receptor (CAR2) that is different from CAR1 in antigen specificity; (b) a polynucleotide encoding a T cell enhancer (TCE); and (c) a polynucleotide encoding a cytokine; (iii) the site 2 differs from the site 1; and (iv) the site 2 construct comprises an exogenous promoter that regulates expression of the polynucleotides in the site 2 construct. In some embodiments, the site 1 comprises one of AAVS1, CCR5, ROSA26, collagen, HTRP, H11, PH12, GAPDH, TCR constant region, RUNX1, B2M, TAP1, TAP2, tapasin, NLRC5, CIITA, RFXANK, RFX5, RFXAP, TCR α or β constant region (TRAC or TRBC), NKG2A, NKG2D, CD38, CD25, CD69, CD71, CD44, CD54, CD56, CD58, CIS, CBL-B, SOCS2, PD1, CTLA4, LAG3, TIM3, ASB2, or TIGIT. In some embodiments, (i) the site 1 comprises one of CD38, CD54, CD56, CD58, TIM3, ASB2, TIGIT, H11 or PH12; and (ii) integration of the site 1 construct at any of CD38, CD54, CD56, CD58, TIM3, or TIGIT knocks out an endogenous gene at the site 1. In some embodiments, the site 2 comprises one of AAVS1, CCR5, ROSA26, collagen, HTRP, H11, PH12, GAPDH, TCR constant region, RUNX1, B2M, TAP1, TAP2, tapasin, NLRC5, CIITA, RFXANK, RFX5, RFXAP, TCR α or β constant region (TRAC or TRBC), NKG2A, NKG2D, CD38, CD25, CD69, CD71, CD44, CD54, CD56, CD58, CIS, CBL-B, SOCS2, PD1, CTLA4, LAG3, TIM3, ASB2, or TIGIT. In some embodiments, (i) the site 2 comprises one of CD38, CD54, CD56, CD58, TIM3, ASB2, TIGIT, H11 or PH12; and (ii) integration of the site 2

construct at any of CD38, CD54, CD56, CD58, TIM3, ASB2, or TIGIT knocks out an endogenous gene at the site 2.

[00011] In some embodiments, the TCR locus is a constant region of TCR alpha (TRAC) or TCR beta (TRBC), wherein the gene at the TCR locus is TCR α or TCR β , and wherein the endogenous TCR promoter is a TRAC promoter or a TRBC promoter. In some embodiments, the ADR is specific to 41BB. In some embodiments, the ADR comprises a 41BB-specific ligand operably linked to a signaling domain promoting effector cell activation, and wherein ADR comprises an amino acid sequence of at least 75%, 80%, 85%, 90%, 95% or 99% identity to SEQ ID NOs: 66 - 69. In some embodiments, the ADR comprises a signaling domain, and wherein the signaling domain comprises an amino acid sequence of at least 75%, 80%, 85%, 90%, 95% or 99% identity to SEQ ID NO: 60.

[00012] In some embodiments, (i) the TGF β -SRR further comprises a partial or full peptide of the intracellular domain (ICD) of a cytokine receptor comprising an IL2R, IL12R, IL18R, IL21R, or any combination thereof; (ii) the C-X-C motif chemokine receptor comprises CXCR2 or CXCR3; (iii) the exogenous CD16 or variant thereof comprises at least one of: (a) a high affinity non-cleavable CD16 (hnCD16); (b) F176V and S197P in ectodomain domain of CD16; (c) a full or partial ectodomain originated from CD64; (d) a non-native (or non-CD16) transmembrane domain; (e) a non-native (or non-CD16) intracellular domain; (f) a non-native (or non-CD16) signaling domain; (g) a non-native stimulatory domain; and (h) transmembrane, signaling, and stimulatory domains that are not originated from CD16, and are originated from a same or different polypeptide; or (iv) the integration site PH12 is a safe harbor locus having a coordinate of chr17:44210001-44271500 in human reference genome assembly hg38. In some embodiments, the cytokine receptor is IL2R β , thereby forming a TGF β R2-IL2R β redirector receptor, and the intracellular domain (ICD) of IL2R β comprises an amino acid sequence represented by SEQ ID NO: 4. In some embodiments, the cytokine receptor is IL12R β , thereby forming a TGF β R2-IL12R β redirector receptor, and the intracellular domain (ICD) of IL12R β comprises an amino acid sequence represented by SEQ ID NO: 5 or SEQ ID NO: 6. In some embodiments, the cytokine receptor is IL18R β , thereby forming a TGF β R2-IL18R β redirector receptor, and the intracellular domain (ICD) of IL18R β comprises an amino acid sequence represented by SEQ ID NO: 7. In some embodiments, the cytokine receptor is IL21R β , thereby forming a TGF β R2-IL21R β redirector receptor, and the intracellular domain (ICD) of IL21R β comprises an amino acid sequence represented by SEQ ID NO: 8. In some embodiments, the extracellular domain (ECD) of TGF β R comprises an amino acid sequence represented by SEQ ID NO: 3. In some embodiments, the cytokine receptor is a fragment of IL2R β , forming a TGF β R2-trIL12R β redirector receptor which comprises an amino acid sequence having sequence

identity of at least 80%, 85%, 90%, 95%, or 97%, 98%, or 99% to SEQ ID NO: 9, wherein an amino acid sequence represented by SEQ ID NO: 10 comprised in SEQ ID NO: 9 is variable.

[00013] In some embodiments, the first CAR (and optionally the second CAR, if present) are specific to different antigens, wherein: (i) the antigen comprises ADGRE2, B7H3, carbonic anhydrase IX (CAIX), CCR1, CCR4, carcinoembryonic antigen (CEA), CD3, CD5, CD7, CD8, CD10, CD20, CD22, CD30, CD33, CD34, CD38, CD41, CD44, CD44V6, CD49f, CD56, CD70, CD74, CD79b, CD99, CD123, CD133, CD138, CDS, CLEC12A, an antigen of a cytomegalovirus (CMV) infected cell, epithelial glycoprotein-2 (EGP-2), epithelial glycoprotein-40 (EGP-40), epithelial cell adhesion molecule (EpCAM), EGFRvIII, receptor tyrosine-protein kinases erb- B2,3,4, EGFR, EGFR-VIII, ERBB folate-binding protein (FBP), fetal acetylcholine receptor (AChR), folate receptor- α , Ganglioside G2 (GD2), Ganglioside G3 (GD3), GPRC5D, human Epidermal Growth Factor Receptor 2 (HER2), human telomerase reverse transcriptase (hTERT), ICAM-1, Integrin B7, Interleukin-13 receptor subunit alpha-2 (IL-13R α 2), κ -light chain, kinase insert domain receptor (KDR), KLK2, Lewis A (CA19.9), Lewis Y (LeY), L1 cell adhesion molecule (L1-CAM), LILRB2, melanoma antigen family A 1 (MAGE-A1), MICA/B, Mucin 1 (Muc-1), Mucin 16 (Muc-16), Mesothelin (MSLN), NKCSI, NKG2D ligands, c-Met, cancer-testis antigen NY-ESO-1, oncofetal antigen (h5T4), PRAME, prostate stem cell antigen (PSCA), PRAME prostate-specific membrane antigen (PSMA), tumor-associated glycoprotein 72 (TAG-72), TIM-3, TRBC1, TRBC2, vascular endothelial growth factor R2 (VEGF-R2), Wilms tumor protein (WT-1), and a pathogen antigen; or (ii) the antigen comprises B7H3, BCMA, CD19, CD20, CD22, CD38, CD52, CD79b, CD123, EGFR, EGP2/EpCAM, GD2, GPRC5D, HER2, KLK2, MICA/B, MSLN, VEGF-R2, PSMA and PDL1.

[00014] In some embodiments, the first CAR (and optionally the second CAR, if present) comprise (i) an ectodomain comprising an antigen binding domain specific to a tumor associated antigen; (ii) a transmembrane domain; and (iii) an endodomain comprising at least one signaling domain; wherein the at least one signaling domain responds specifically to binding of the CAR to the tumor associated antigen, thereby generating a cancer antigen specific response. In some embodiments, the at least one signaling domain comprises: (i) any one of: 2B4 (Natural killer Cell Receptor 2B4), 4-1BB (Tumor necrosis factor receptor superfamily member 9), CD28 (T-cell-specific surface glycoprotein CD28), CD3 ζ (T-cell surface glycoprotein CD3 zeta chain), DAP10 (Hematopoietic cell signal transducer), DAP12 (TYRO protein tyrosine kinase-binding protein), DNAM1 (CD226 antigen), FcERI γ (High affinity immunoglobulin epsilon receptor subunit gamma), IL21R (Interleukin-21 receptor), IL2R β /IL15R β (Interleukin-2 receptor subunit beta), IL2R γ (Cytokine receptor common subunit gamma), IL-7R (Interleukin-7 receptor subunit alpha), KIR2DS2 (Killer cell immunoglobulin-like receptor 2DS2), NKG2D (NKG2-D type II

integral membrane protein), NKp30 (Natural cytotoxicity triggering receptor 3), NKp44 (Natural cytotoxicity triggering receptor 2), NKp46 (Natural cytotoxicity triggering receptor 1), CS1 (SLAM family member 7), and CD8 (T-cell surface glycoprotein CD8 alpha chain); (ii) an amino acid sequence that has at least about 85%, about 90%, about 95%, about 96%, about 97%, about 98%, or about 99% identity to the cytoplasmic domain, or a portion thereof, of 2B4, 41BB, CD16, CD2, CD28, CD28H, CD3 ζ , DAP10, DAP12, DNAM1, FcERI γ , IL21R, IL2R β (IL15R β), IL2R γ , IL7R, KIR2DS2, NKG2D, NKp30, NKp44, NKp46, CD3 ζ 1XX, CS1, or CD8, represented by SEQ ID NOs: 98-120, respectively; and/or (iii) an amino acid sequence that has at least about 85%, about 90%, about 95%, about 96%, about 97%, about 98%, or about 99% identity to the cytoplasmic domain, or a portion thereof, of 2B4, CD28, CD3 ζ , DAP10, NKG2D, CD3 ζ , CD3 ζ 1XX, DNAM1, CS1, or combinations thereof. In some embodiments, the endodomain comprises two different signaling domains, and wherein said endodomain domain comprises fused cytoplasmic domains, or portions thereof, in any one of the forms: CD28-CD3 ζ , CD28-CD3 ζ 1XX, 41BB-CD3 ζ , 41BB-CD3 ζ 1XX, 2B4-CD3 ζ and 2B4-CD3 ζ 1XX. In some embodiments, the transmembrane domain comprises an amino acid sequence that has at least about 85%, about 90%, about 95%, about 96%, about 97%, about 98%, or about 99% identity to a transmembrane region, or a portion thereof, of CD2, CD3 δ , CD3 ϵ , CD3 γ , CD3 ζ , CD4, CD8, CD8a, CD8b, CD16, CD27, CD28, CD28H, CD40, CD84, CD166, 4-1BB, OX40, ICOS, ICAM-1, CTLA4, PD1, LAG3, 2B4, BTLA, DNAM1, DAP10, DAP12, FcERI γ , IL7, IL12, IL15, KIR2DL4, KIR2DS1, KIR2DS2, NKp30, NKp44, NKp46, NKG2C, NKG2D, CS1, or T cell receptor polypeptide. In some embodiments, the transmembrane domain comprises an amino acid sequence that has at least about 85%, about 90%, about 95%, about 96%, about 97%, about 98%, or about 99% identity to a transmembrane region, or a portion thereof, of 2B4, CD16, CD2, CD28, CD28H, CD3 ζ , DAP10, DAP12, DNAM1, FcERI γ , KIR2DS2, NKG2D, NKp30, NKp44, NKp46, CS1, or CD8, represented by SEQ ID NOs: 76, 78-86, 91-97, respectively. In some embodiments, the transmembrane domain and its immediately linked signaling domain are from a same protein or from different proteins. In some embodiments, the antigen binding domain comprises an amino acid sequence that has at least about 85%, about 90%, about 95%, about 96%, about 97%, about 98%, or about 99% identity to any of SEQ ID NOs: 158, 159, 166-177, and 184-186.

[00015] In some embodiments, the TCE is TCF1 or CD27. In some embodiments, the site 1 is CD38, and wherein the site 2 is one of CD58, TIM3, ASB2, TIGIT, or PH12. In some embodiments, the iPSC is a clonal iPSC, a single cell dissociated iPSC, an iPSC cell line cell, or an iPSC master cell bank (MCB) cell. In some embodiments, the derivative cell comprises a derivative CD34⁺ cell, a derivative hematopoietic stem and progenitor cell, a derivative

hematopoietic multipotent progenitor cell, a derivative T cell progenitor, a derivative NK cell progenitor, a derivative T lineage cell, a derivative NKT lineage cell, a derivative NK lineage cell, or a derivative B lineage cell. In some embodiments, the derivative cell comprises a derivative effector cell having one or more functional features that are not present in a counterpart primary T, NK, NKT, and/or B cell. In some embodiments, the derivative cell has therapeutic properties comprising one or more of: (i) increased cytotoxicity; (ii) improved persistency and/or survival; (iii) enhanced ability in migrating, and/or activating or recruiting bystander immune cells, to tumor sites; (iv) improved tumor infiltration; (v) enhanced ability to reduce tumor immunosuppression; (vi) improved ability in rescuing tumor antigen escape; (vii) controlled apoptosis; (viii) enhanced or acquired ADCC; and (ix) ability to avoid fratricide, in comparison to its counterpart primary cell obtained from peripheral blood, umbilical cord blood, or any other donor tissues without the same genetic edit(s).

[00016] In one aspect, the present disclosure provides a cell or a population thereof, wherein (i) the cell is (a) an immune cell; (b) an induced pluripotent cell (iPSC); or (c) a derivative effector cell obtained from differentiating the iPSC; and (ii) comprising a construct at an integration site, wherein the construct comprises a polynucleotide encoding a first chimeric antigen receptor (CAR1), and wherein the construct further comprises one or more of: (a) a polynucleotide encoding a second chimeric antigen receptor (CAR2) that is different from CAR1 in antigen specificity; (b) a polynucleotide encoding a T cell enhancer (TCE); (c) a polynucleotide encoding a cytokine; (iii) the integration site comprises one of CD38, CD54, CD56, CD58, TIM3, ASB2, TIGIT, H11 and PH12; (iv) the construct comprises an exogenous promoter that regulates expression of the polynucleotides in the construct; and (v) integration of the construct at any of CD38, CD54, CD56, CD58, TIM3, ASB2, or TIGIT knocks out an endogenous gene at the integration site. In some embodiments, the integration site is not TRAC. In some embodiments, the first CAR (and optionally the second CAR, if present) are specific to different antigens, wherein: (i) the antigen comprises ADGRE2, B7H3, carbonic anhydrase IX (CAIX), CCR1, CCR4, carcinoembryonic antigen (CEA), CD3, CD5, CD7, CD8, CD10, CD20, CD22, CD30, CD33, CD34, CD38, CD41, CD44, CD44V6, CD49f, CD56, CD70, CD74, CD79b, CD99, CD123, CD133, CD138, CDS, CLEC12A, an antigen of a cytomegalovirus (CMV) infected cell, epithelial glycoprotein-2 (EGP-2), epithelial glycoprotein-40 (EGP-40), epithelial cell adhesion molecule (EpCAM), EGFRvIII, receptor tyrosine-protein kinases erb-B2,3,4, EGFR, EGFR-VIII, ERBB folate-binding protein (FBP), fetal acetylcholine receptor (AChR), folate receptor- α , Ganglioside G2 (GD2), Ganglioside G3 (GD3), GPRC5D, human Epidermal Growth Factor Receptor 2 (HER2), human telomerase reverse transcriptase (hTERT), ICAM-1, Integrin B7, Interleukin-13 receptor subunit alpha-2 (IL-13R α 2), κ -light chain, kinase

insert domain receptor (KDR), KLK2, Lewis A (CA19.9), Lewis Y (LeY), L1 cell adhesion molecule (L1-CAM), LILRB2, melanoma antigen family A 1 (MAGE-A1), MICA/B, Mucin 1 (Muc-1), Mucin 16 (Muc-16), Mesothelin (MSLN), NKCSI, NKG2D ligands, c-Met, cancer-testis antigen NY-ESO-1, oncofetal antigen (h5T4), PRAME, prostate stem cell antigen (PSCA), PRAME prostate-specific membrane antigen (PSMA), tumor-associated glycoprotein 72 (TAG-72), TIM-3, TRBC1, TRBC2, vascular endothelial growth factor R2 (VEGF-R2), Wilms tumor protein (WT-1), and a pathogen antigen; or (ii) the antigen comprises B7H3, BCMA, CD19, CD20, CD22, CD38, CD52, CD79b, CD123, EGFR, EGP2/EpCAM, GD2, GPRC5D, HER2, KLK2, MICA/B, MSLN, VEGF-R2, PSMA and PDL1. In some embodiments, the TCE is TCF1 or CD27.

[00017] In one aspect, the present disclosure provides a cell or population thereof, wherein: (i) the cell is (a) an immune cell; (b) an induced pluripotent cell (iPSC); or (c) a derivative effector cell obtained from differentiating the iPSC; and (ii) comprising an insertion at an integration site PH12, wherein the integration site PH12 is a safe harbor locus having a coordinate of chr17:44210001-44271500 in human reference genome assembly hg38. In some embodiments, the insertion comprises a construct comprising at least one exogenous polynucleotide encoding a peptide of interest, and a regulatory region. In some embodiments, the regulatory region comprises a promoter (e.g., a constitutive promoter). In some embodiments, the construct comprises polynucleotides encoding one or more CARs, a cytokine, a T cell enhancer, a TGF β -SRR, a C-X-C motif chemokine receptor or a variant thereof, an exogenous CD16 or a variant thereof, or any combination thereof.

[00018] In one aspect, the present disclosure provides a cell or population thereof, wherein: (i) the cell is (a) an immune cell; (b) an induced pluripotent cell (iPSC); or (c) a derivative effector cell obtained from differentiating the iPSC; and (ii) the cell comprises a polynucleotide encoding an allo-immune defense receptor (ADR); wherein the polynucleotide is inserted into a TCR locus, thereby knocking out a TCR gene; and wherein the construct is expressed under an endogenous TCR promoter.

[00019] In one aspect, the present disclosure provides a cell or population thereof, wherein: (i) the cell is (a) an immune cell; (b) an induced pluripotent cell (iPSC); or (c) a derivative effector cell obtained from differentiating the iPSC; and (ii) the cell comprises a polynucleotide encoding a IL2, a IL18, or a IL7/IL7R fusion protein; wherein the polynucleotide is inserted into a TCR locus, thereby knocking out a TCR gene; and wherein the construct is expressed under an endogenous TCR promoter.

[00020] In one aspect, the present disclosure provides, a cell or population thereof, wherein: (i) the cell is (a) an immune cell; (b) an induced pluripotent cell (iPSC); or (c) a derivative

effector cell obtained from differentiating the iPSC; and (ii) the cell comprises at least three targeted loci for transgene integration, wherein the at least three targeted loci comprise TRAC and two of CD38, CD54, CD56, CD58, TIM3, ASB2, TIGIT, H11 and PH12. In some embodiments, the at least three targeted loci comprise TRAC, CD38, and one or more of CD54, CD56, CD58, TIM3, ASB2, TIGIT, H11 and PH12. In some embodiments, the cell is TCR^{-/-}, and optionally, CD38^{-/-}, CD54^{-/-}, CD56^{-/-}, CD58^{-/-}, TIM3^{-/-}, ASB2^{-/-}, and/or TIGIT^{-/-}. In some embodiments, the cell comprises CD38 targeted insertion of TGFβ-SRR-hnCD16-CXCR2, TRAC targeted insertion of ADR-sIL2, CD58 target insertion of CD58_CAR-TCF1, and knock-out of CD38, TRAC and CD58.

[00021] In one aspect, the present disclosure provides a composition comprising the cell or population thereof according to any of the various aspects and embodiments herein. In some embodiments, the composition further comprises one or more therapeutic agents. In some embodiments, the one or more therapeutic agents comprise a peptide, a cytokine, a checkpoint inhibitor, a mitogen, a growth factor, a small RNA, a dsRNA (double stranded RNA), mononuclear blood cells, feeder cells, feeder cell components or replacement factors thereof, a vector comprising one or more polynucleic acids of interest, an antibody, an engager, a chemotherapeutic agent or a radioactive moiety, or an immunomodulatory drug (IMiD). In some embodiments, the checkpoint inhibitor comprises: (a) one or more antagonists to checkpoint molecules comprising PD-1, PDL-1, TIM-3, TIGIT, LAG-3, CTLA-4, 2B4, 4-1BB, 4-1BBL, A_{2A}R, BATE, BTLA, CD39, CD47, CD73, CD94, CD96, CD160, CD200, CD200R, CD274, CEACAM1, CSF-1R, Foxp1, GARP, HVEM, IDO, EDO, TDO, LAIR-1, MICA/B, NR4A2, MAFB, OCT-2, Rara (retinoic acid receptor alpha), TLR3, VISTA, NKG2A/HLA-E, or inhibitory KIR; (b) one or more of atezolizumab, avelumab, durvalumab, ipilimumab, IPH4102, IPH43, IPH33, lirimumab, monalizumab, nivolumab, pembrolizumab, and their derivatives or functional equivalents; or (c) at least one of atezolizumab, nivolumab, and pembrolizumab. In some embodiments, the antibody comprises: (a) an anti-CD20 antibody, an anti-HER2 antibody, an anti-CD52 antibody, an anti-EGFR antibody, an anti-CD123 antibody, an anti-GD2 antibody, an anti-PDL1 antibody, or an anti-CD38 antibody; or (b) one or more of rituximab, velutuzumab, ofatumumab, ublituximab, ocaratuzumab, obinutuzumab, trastuzumab, pertuzumab, alemtuzumab, cetuximab, dinutuximab, avelumab, daclizumab, basiliximab, M-A251, 2A3, BC69, 24204, 22722, 24212, MAB23591, FN50, 298614, AF2359, CY1G4, DF1513, bivatumumab, RG7356, G44-26, 7G3, CSL362, elotuzumab, daratumumab, isatuximab, MOR202, and their humanized or Fc modified variants or fragments and their functional equivalents and biosimilars thereof. In some embodiments, the engager comprises: (i) a bispecific T cell engager (BiTE); (ii) a bispecific killer cell engager (BiKE); or (iii) a tri-specific

killer cell engager (TriKE); or wherein the engage comprises (a) a first binding domain recognizing an extracellular portion of CD3, CD28, CD5, CD16, CD64, CD32, CD33, CD89, NKG2C, NKG2D, or any functional variants thereof of the cell or a by-stander immune effector cell; and (b) a second binding domain specific to an antigen comprising any one of: B7H3, CD10, CD19, CD20, CD22, CD24, CD30, CD33, CD34, CD38, CD44, CD52, CD79a, CD79b, CD123, CD138, CD179b, CEA, CLEC12A, CS-1, DLL3, EGFR, EGFRvIII, EpCAM, FLT-3, FOLR1, FOLR3, GD2, gpA33, HER2, HM1.24, LGR5, MSLN, MCSP, MICA/B, Muc1, Muc16, PDL1, PSMA, PAMA, P-cadherin, ROR1, or VEGF-R2.

[00022] In one aspect, the present disclosure provides a master cell bank (MCB) comprising the iPSC according to any of the various aspects or embodiments herein.

[00023] In one aspect, the present disclosure provides a therapeutic use of a composition disclosure herein. In some embodiments, the therapeutic use comprises introducing the composition to a subject in need of an adoptive cell therapy, wherein the subject has an autoimmune disorder, a hematological malignancy, a solid tumor, cancer, or a virus infection.

[00024] In one aspect, the present disclosure provides a method of improving T cell differentiation. In some embodiments, the method comprises regulating ADR expression under an endogenous promoter that is temporally regulated during T cell differentiation. In some embodiments, the endogenous promoter is activated upon commitment to lymphoid lineage, and wherein the endogenous promoter activity is further heightened upon commitment to T cell lineage. In some embodiments, the endogenous promoter comprises a promoter of at least one of TRAC, TIM-3, ASB2, and TIGIT. In some embodiments, the ADR is co-expressed with a cytokine comprising IL2, IL18, or IL7RF.

[00025] In one aspect, the present disclosure provides a method of manufacturing a derivative effector cell according to any of the various aspects or embodiments herein. In some embodiments, the derivative effector cell is an immune effector cell, and the method comprises: (a) obtaining a genetically engineered iPSC, wherein the iPSC comprises edits comprising: (i) a polynucleotide encoding a TCR promoter-driven ADR and/or a cytokine, (ii) a polynucleotide encoding a TCE, (iii) a polynucleotide encoding a CAR, (iv) a polynucleotide encoding a TGFβ-SRR, (v) a polynucleotide encoding a C-X-C-motif chemokine receptor or a variant thereof, (vi) a polynucleotide encoding an exogenous CD16 or variant thereof, and (vii) a CD38 knockout; (b) differentiating the genetically engineered iPSC to a derivative CD34⁺ cell; and (c) differentiating the derivative CD34⁺ cell to an immune effector cell, wherein the immune effector cell retains the edits. In some embodiments, obtaining the genetically engineered iPSC comprises: (i) generating a first pre-MCB (master cell bank) iPSC by introducing to an iPSC a construct comprising polynucleotides encoding a TGFβ-SRR, a C-X-C-motif chemokine

receptor or a variant thereof, and a CD16 variant to a first integration site (site 1); (ii) generating a second pre-MCB iPSC by introducing to the first pre-MCB iPSC a construct comprising a polynucleotide encoding an allo-immune defense receptor (ADR) and a polynucleotide encoding one of IL2, IL18 and a IL7RF at a TCR locus; and (iii) generating a MCB iPSC by introducing to the second pre-MCB iPSC a construct comprising one or more CAR and a TCE, at a second integration site (site 2). In some embodiments, the site 2 differs from the site 1, and wherein the site 1 and site 2 comprise any two of AAVS1, CCR5, ROSA26, collagen, HTRP, H11, PH12, GAPDH, TCR constant region, RUNX1, B2M, TAP1, TAP2, tapasin, NLRC5, CIITA, RFXANK, RFX5, RFXAP, NKG2A, NKG2D, CD38, CD25, CD69, CD71, CD44, CD54, CD56, CD58, CIS, CBL-B, SOCS2, PD1, CTLA4, LAG3, TIM3, ASB2, and TIGIT loci. In some embodiments, the site 2 comprise CD38, CD54, CD56, CD58, TIM3, TIGIT, H11 and PH12; wherein integration at any of CD38, CD54, CD56, CD58, TIM3, ASB2, or TIGIT knocks out an endogenous gene. In some embodiments, the TCR constant region is a constant region of TCR alpha (TRAC) or TCR beta (TRBC); wherein endogenous gene at TRAC or TRBC is knocked out; and wherein the construct is under control of TRAC or TRBC endogenous promoter. In some embodiments, (i) the C-X-C motif chemokine receptor comprises CXCR2 or CXCR3; (ii) the TGF β -SRR comprises a TGF β R2-IL2R β , a TGF β R2-IL12R β , a TGF β R2-IL18R β , or a TGF β R2-trIL12R β redirector receptor; (iii) the CD16 variant is a high affinity non-cleavable CD16 (hnCD16); (iv) the ADR is specific to 4-1BB; or (v) the TCE is TCF1 or CD27. In some embodiments, the method further comprises genetically engineering the first pre-MCB iPSC, the second pre-MCB iPSC, or the MCB iPSC by one or more of: (a) introducing HLA-I deficiency, and/or HLA-II deficiency; (b) deleting or disrupting one or more of B2M, CIITA, TAP1, TAP2, Tapasin, NLRC5, RFXANK, RFX5, RFXAP, TCR, NKG2A, NKG2D, CD25, CD44, CD54, CD56, CD58, CD69, CIS, CBL-B, SOCS2, PD1, CTLA4, LAG3, TIM3, ASB2, and TIGIT; or (c) introducing at least one of HLA-G, HLA-E, 4-1BBL, CD3, CD4, CD8, CD16, CD47, CD113, CD131, CD137, CD80, PDL1, A_{2A}R, antigen-specific TCR, chimeric fusion receptor (CFR), Fc receptor, an antibody or functional variant or fragment thereof, a checkpoint inhibitor, an engager, and surface triggering receptor for coupling with an agonist. In some embodiments, the genetic engineering comprises targeted editing. In some embodiments, the targeted editing is carried out by CRISPR, ZFN, TALEN, homing nuclease, homology recombination, or any other functional variation of these methods.

[00026] In one aspect, the present disclosure provides a method of treating a subject in need of an adoptive cell therapy, wherein the method comprises infusing the subject with effector cells, wherein the effector cells comprise the derivative cell or population thereof according to any of the various aspect or embodiments herein. In some embodiments, the effector cells

comprise a CAR specific to an antigen expressed on a cancer cell, wherein the antigen comprises at least one of B7H3, BCMA, CD19, CD20, CD22, CD38, CD52, CD79b, CD123, EGFR, EGP2/EpCAM, GD2, GPRC5D, HER2, KLK2, MICA/B, MSLN, VEGF-R2, PSMA and PDL1. In some embodiments, the method further comprises administering one or more therapeutic agents to the subject, wherein the one or more therapeutic agents comprise: (i) a cytokine, an antibody, an engager, a checkpoint inhibitor, a chemotherapeutic agent or a radioactive moiety, or an immunomodulatory drug (IMiD); (ii) an anti-CD38 antibody comprising daratumumab, isatuximab, or MOR202; (iii) an engager comprising a BiTE (bi-specific T cell engager) or a TriKE (tri-specific Killer cell engager); (iv) a checkpoint inhibitor comprising atezolizumab, avelumab, durvalumab, ipilimumab, IPH4102, IPH43, IPH33, lirimumab, monalizumab, nivolumab, or pembrolizumab; and/or (v) a chemotherapeutic agent comprising cyclophosphamide and fludarabine (Cy/Flu). In some embodiments, the effector cells comprise a CD38 knockout, a TCR knockout, and an ADR; wherein the method comprises administering to the subject an anti-CD38 antibody; and wherein the method does not require, or requires minimal, lymphodepletion comprising administering Cy/Flu to the subject. In some embodiments, the effector cells are allogeneic, and wherein infusing the subject with effector cells is in an out-patient setting.

[00027] In one aspect, the present disclosure provides a method of improving an adoptive cell therapy in treating a subject having a solid tumor, the method comprising administering a population of derivative cells according to any of the various aspect or embodiments herein. In some embodiments, the derivative cells are T lineage cells.

[00028] Various objects and advantages of the compositions and methods as provided herein will become apparent from the following description taken in conjunction with the accompanying drawings wherein are set forth, by way of illustration and example, certain embodiments of this invention.

BRIEF DESCRIPTION OF THE DRAWINGS

[00029] FIG. 1 shows an illustrative schematic for evaluating T cell enhancer (TCE) candidates, in which the TCE is introduced to T cells differentiated from iPSC (iT) using lentiviral transduction, which are then expanded and subjected to various tests.

[00030] FIG. 2 provides illustrative results showing that TCF1 or CD27 enhances CAR-iT cell in tumor growth inhibition (TGI) using a serial restimulation assay.

[00031] FIG. 3 provides illustrative results showing that effector cells comprising a selected TCE and/or cytokine signaling in a configuration as indicated have T cell signatures marked by TRAC-CAR and intracellular CD3 (icCD3) expression.

[00032] FIGS. 4A-4B show illustrative results for tumor volume for individual mice plotted against days post tumor implant for each effector cell group as indicated, and show that certain edits and/or configurations resulted in enhanced TGI compared to the CAR-iT cell control group.

[00033] FIGS. 5A-5B show illustrative total effector counts in tumor mass, spleen, and bone marrow graphed for both D8 (circle) and D15 (rectangle) for each mouse group having the indicated effector cells. The legend of effector cells in FIG. 5A is applicable to the graphs in both FIG. 5A and 5B.

[00034] FIGS. 6A-6B show effector cell counts and tumor cell counts in each mouse group having the indicated effector cells on D8 (circle) and D15 (square).

[00035] FIG. 7 provides illustrative results showing that the constitutively expressed ADR is detrimental to iT differentiation.

[00036] FIG. 8 shows illustrative results for progressive and titrated expression of ADR under endogenous TRAC promoter regulation during the iT cell differentiation process.

[00037] FIG. 9 shows illustrative results for appropriate lymphoid commitment in the process of T cell differentiation from iPSC comprising TRAC regulated ADR.

[00038] FIGS. 10 A-10B provide illustrative results showing that the TRAC regulated ADR expression in CAR iT cells enables resistance to allogenic immune cells *in vivo*.

[00039] FIGS. 11A-11B provide illustrative results showing that CD58 knockout does not impact ADR function, and synergizes with ADR to resist allogeneic rejection.

[00040] FIG. 12 provides illustrative results showing that TRAC regulated IL2 expression improves the anti-tumor efficacy and intratumoral persistence of CAR iT cells *in vivo*.

[00041] FIGS. 13A-13B provide illustrative results showing that IL2 production regulated temporally by TRAC following CAR activation is much lower than that of primary CAR T cells.

[00042] FIGS. 14A-14D provide illustrative results showing that engineering of IL2 into alternative T cell loci enables higher IL2 expression after CAR activation, but only TRAC regulated IL2 expression enhances the persistence and sustained *in vivo* efficacy of CAR iT cells.

[00043] FIGS. 15A-15C provide schematics and illustrative results showing that TRAC regulated IL2 supports hnCD16 and ADR mediated efficacy in effector cells.

[00044] FIGS. 16A-16B provide illustrative results showing that iT cells with constitutively regulated CAR maintain cytolytic activity over multiple rounds of tumor challenge *in vitro* without expected exhaustion.

[00045] FIG. 17 provides illustrative results showing that effector cells having a constitutive CAR outperform those having TRAC regulated CAR in a subcutaneous *in vivo* model of solid tumor.

[00046] FIGS. 18A-18B provide illustrative results showing that constitutive expression of TCF1 reinforces T cell lineage commitment in engineered iPSC differentiation.

[00047] FIGS. 19A-19B provide schematics and illustrative results showing that iT cells comprising an ADR_2 backbone (TRAC-ADR-IL2, CD38KO_ TGF β -SRR-hnCD16-CXCR2), and constitutively expressed CAR-TCF1 from the CD58 locus demonstrated improved *in vivo* antitumor activity in comparison control cells without TCF1.

[00048] FIGS. 20A-20C provide illustrative results showing that constitutively expressed TCF1 improves ADR_2 iT cells in both allogenic defense and antitumor function.

[00049] FIG. 21 provides illustrative results showing the workflow and strategic design for generating a Master Cell Bank (MCB) having at least three targeted integration sites hosting multiplex configured edits via multiple MCB intermediaries (pre-MCBs).

[00050] FIGS. 22A-22C provide illustrative flow cytometry analyses confirming CD58 targeted CAR and TCF1 insertion in various configurations.

DETAILED DESCRIPTION OF THE INVENTION

[00051] Genomic modification of iPSCs (induced pluripotent stem cells) can include one or more of polynucleotide insertion, deletion, and substitution. Exogenous gene expression in genome-engineered iPSCs often encounters problems such as gene silencing or reduced gene expression after prolonged clonal expansion of the original genome-engineered iPSCs, after cell differentiation, and in dedifferentiated cell types from the cells derived from the genome-engineered iPSCs. On the other hand, direct engineering of primary immune cells such as T or NK cells is challenging and presents a hurdle to the preparation and delivery of engineered immune cells for adoptive cell therapy. In various embodiments, the present invention provides an efficient, reliable, and targeted approach for stably integrating one or more exogenous genes, including suicide genes and other functional modalities, which provide improved therapeutic properties relating to engraftment, trafficking, homing, migration, cytotoxicity, viability, maintenance, expansion, longevity, self-renewal, persistence, and/or survival, into iPSC derivative cells, including but not limited to HSCs (hematopoietic stem and progenitor cells), T cell progenitor cells, NK cell progenitor cells, T lineage cells, NKT lineage cells, and NK lineage cells.

[00052] Definitions

[00053] Unless otherwise defined herein, scientific and technical terms used in connection with the present application shall have the meanings that are commonly understood by those of ordinary skill in the art. Further, unless otherwise required by context, singular terms shall include pluralities and plural terms shall include the singular.

[00054] It should be understood that this invention is not limited to the particular methodology, protocols, and reagents, etc., described herein and as such may vary. The terminology used herein is for the purpose of describing particular embodiments only, and is not intended to limit the scope of the present invention, which is defined solely by the claims.

[00055] As used herein, the articles “a,” “an,” and “the” are used herein to 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.

[00056] The use of the alternative (e.g., “or”) should be understood to mean either one, both, or any combination thereof of the alternatives.

[00057] The term “and/or” should be understood to mean either one, or both of the alternatives.

[00058] As used herein, the term “about” or “approximately” refers to a quantity, level, value, number, frequency, percentage, dimension, size, amount, weight or length that varies by as much as 15%, 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2% or 1% compared to a reference quantity, level, value, number, frequency, percentage, dimension, size, amount, weight or length. In one embodiment, the term “about” or “approximately” refers a range of quantity, level, value, number, frequency, percentage, dimension, size, amount, weight or length $\pm 15\%$, $\pm 10\%$, $\pm 9\%$, $\pm 8\%$, $\pm 7\%$, $\pm 6\%$, $\pm 5\%$, $\pm 4\%$, $\pm 3\%$, $\pm 2\%$, or $\pm 1\%$ of a reference quantity, level, value, number, frequency, percentage, dimension, size, amount, weight or length.

[00059] As used herein, the term “substantially” or “essentially” refers to a quantity, level, value, number, frequency, percentage, dimension, size, amount, weight or length that is about 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% or higher compared to a reference quantity, level, value, number, frequency, percentage, dimension, size, amount, weight or length. In one embodiment, the terms “essentially the same” or “substantially the same” refer a range of quantity, level, value, number, frequency, percentage, dimension, size, amount, weight or length that is about the same as a reference quantity, level, value, number, frequency, percentage, dimension, size, amount, weight or length.

[00060] As used herein, the terms “substantially free of” and “essentially free of” are used interchangeably, and when used to describe a composition, such as a cell population or culture media, refer to a composition that is free of a specified substance or its source thereof, such as, 95% free, 96% free, 97% free, 98% free, 99% free of the specified substance or its source thereof, or is undetectable as measured by conventional means. The term “free of” or “essentially free of” a certain ingredient or substance in a composition also means that no such ingredient or substance is (1) included in the composition at any concentration, or (2) included in the composition at a functionally inert, low concentration. Similar meaning can be applied to the

term “absence of,” where referring to the absence of a particular substance or its source thereof of a composition.

[00061] Throughout this specification, unless the context requires otherwise, the words “comprise,” “comprises” and “comprising” will be understood to imply the inclusion of a stated step or element or group of steps or elements but not the exclusion of any other step or element or group of steps or elements. In particular embodiments, the terms “include,” “has,” “contains,” and “comprise” are used synonymously.

[00062] By “consisting of” is meant including, and limited to, whatever follows the phrase “consisting of.” Thus, the phrase “consisting of” indicates that the listed elements are required or mandatory, and that no other elements may be present.

[00063] By “consisting essentially of” is meant including any elements listed after the phrase, and limited to other elements that do not interfere with or contribute to the activity or action specified in the disclosure for the listed elements. Thus, the phrase “consisting essentially of” indicates that the listed elements are required or mandatory, but that no other elements are optional and may or may not be present depending upon whether or not they affect the activity or action of the listed elements.

[00064] Reference throughout this specification to “one embodiment,” “an embodiment,” “a particular embodiment,” “a related embodiment,” “a certain embodiment,” “an additional embodiment,” or “a further embodiment” or combinations thereof means that a particular feature, structure or characteristic described in connection with the embodiment is included in at least one embodiment of the present invention. Thus, the appearances of the foregoing phrases in various places throughout this specification are not necessarily all referring to the same embodiment. Furthermore, the particular features, structures, or characteristics may be combined in any suitable manner in one or more embodiments.

[00065] The term “*ex vivo*” refers generally to activities that take place outside an organism, such as experimentation or measurements done in or on living tissue in an artificial environment outside the organism, preferably with minimum alteration of the natural conditions. In particular embodiments, “*ex vivo*” procedures involve living cells or tissues taken from an organism and cultured in a laboratory apparatus, usually under sterile conditions, and typically for a few hours or up to about 24 hours, but including up to 48 or 72 hours or longer, depending on the circumstances. In certain embodiments, such tissues or cells can be collected and frozen, and later thawed for *ex vivo* treatment. Tissue culture experiments or procedures lasting longer than a few days using living cells or tissue are typically considered to be “*in vitro*,” though in certain embodiments, this term can be used interchangeably with *ex vivo*.

[00066] The term “*in vivo*” refers generally to activities that take place inside an organism.

[00067] As used herein, the terms “reprogramming” or “dedifferentiation” or “increasing cell potency” or “increasing developmental potency” refer 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.

[00068] 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 such as, 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 (i.e., 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 germ 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).

[00069] As used herein, the term “induced pluripotent stem cells” or “iPSCs”, refers to stem cells that are produced *in vitro* from differentiated adult, neonatal or fetal cells that have been induced or changed, i.e., reprogrammed into cells capable of differentiating into tissues of all three germ or dermal layers: mesoderm, endoderm, and ectoderm. In some embodiments, the reprogramming process uses reprogramming factors and/or small molecule chemical driven methods. The iPSCs produced do not refer to cells as they are found in nature.

[00070] As used herein, the term “embryonic stem cell” refers to naturally occurring pluripotent stem cells of the inner cell mass of the embryonic blastocyst. Embryonic stem cells are pluripotent and give rise during development to all derivatives of the three primary germ layers: ectoderm, endoderm and mesoderm. They do not contribute to the extra-embryonic membranes or the placenta (i.e., are not totipotent).

[00071] As used herein, the term “multipotent stem cell” refers to a cell that has the developmental potential to differentiate into cells of one or more germ layers (i.e., ectoderm,

mesoderm and endoderm), but not all three. Thus, a multipotent cell can also be termed a “partially differentiated cell.” Multipotent cells are known in the art, and examples of multipotent cells include adult stem cells, such as for example, hematopoietic stem cells and neural stem cells. “Multipotent” indicates that a cell may form many types of cells in a given lineage, but not cells of other lineages. For example, a multipotent hematopoietic cell can form the many different types of blood cells (red, white, platelets, etc.), but it cannot form neurons. Accordingly, the term “multipotency” refers to the state of a cell with a degree of developmental potential that is less than totipotent and pluripotent.

[00072] Pluripotency can be determined, in part, by assessing pluripotency characteristics of the cells. Pluripotency characteristics include, but are not limited to: (i) pluripotent stem cell morphology; (ii) the potential for unlimited self-renewal; (iii) expression of pluripotent stem cell markers including, but not limited to SSEA1 (mouse only), SSEA3/4, SSEA5, TRA1-60/81, TRA1-85, TRA2-54, GCTM-2, TG343, TG30, CD9, CD29, CD133/prominin, CD140a, CD56, CD73, CD90, CD105, OCT4, NANOG, SOX2, CD30 and/or CD50; (iv) the ability to differentiate to all three somatic lineages (ectoderm, mesoderm and endoderm); (v) teratoma formation consisting of the three somatic lineages; and (vi) formation of embryoid bodies consisting of cells from the three somatic lineages.

[00073] Two types of pluripotency have previously been described: the “primed” or “metastable” state of pluripotency akin to the epiblast stem cells (EpiSC) of the late blastocyst, and the “naïve” or “ground” state of pluripotency akin to the inner cell mass of the early/preimplantation blastocyst. While both pluripotent states exhibit the characteristics as described above, the naïve or ground state further exhibits: (i) pre-inactivation or reactivation of the X-chromosome in female cells; (ii) improved clonality and survival during single-cell culturing; (iii) global reduction in DNA methylation; (iv) reduction of H3K27me3 repressive chromatin mark deposition on developmental regulatory gene promoters; and (v) reduced expression of differentiation markers relative to primed state pluripotent cells. Standard methodologies of cellular reprogramming in which exogenous pluripotency genes are introduced to a somatic cell, expressed, and then either silenced or removed from the resulting pluripotent cells are generally seen to have characteristics of the primed state of pluripotency. Under standard pluripotent cell culture conditions such cells remain in the primed state unless the exogenous transgene expression is maintained, wherein characteristics of the ground state are observed.

[00074] As used herein, the term “pluripotent stem cell morphology” refers to the classical morphological features of an embryonic stem cell. Normal embryonic stem cell morphology is

characterized by being round and small in shape, with a high nucleus-to-cytoplasm ratio, the notable presence of nucleoli, and typical inter-cell spacing.

[00075] As used herein, the term “subject” refers to any animal, preferably a human patient, livestock, or other domesticated animal.

[00076] A “pluripotency factor,” or “reprogramming factor,” refers to an agent capable of increasing the developmental potency of a cell, either alone or in combination with other agents. Pluripotency factors include, without limitation, polynucleotides, polypeptides, and small molecules capable of increasing the developmental potency of a cell. Exemplary pluripotency factors include, for example, transcription factors and small molecule reprogramming agents.

[00077] “Culture” or “cell culture” refers to the maintenance, growth and/or differentiation of cells in an *in vitro* environment. “Cell culture media,” “culture media” (singular “medium” in each case), “supplement” and “media supplement” refer to nutritive compositions that cultivate cell cultures.

[00078] “Cultivate” or “maintain” refers to the sustaining, propagating (growing) and/or differentiating of cells outside of tissue or the body, for example in a sterile plastic (or coated plastic) cell culture dish or flask. “Cultivation” or “maintaining” may utilize a culture medium as a source of nutrients, hormones and/or other factors helpful to propagate and/or sustain the cells.

[00079] As used herein, the term “mesoderm” refers to one of the three germinal layers that appears during early embryogenesis and which gives rise to various specialized cell types including blood cells of the circulatory system, muscles, the heart, the dermis, skeleton, and other supportive and connective tissues.

[00080] As used herein, the term “definitive hemogenic endothelium” (HE) or “pluripotent stem cell-derived definitive hemogenic endothelium” (iHE) refers to a subset of endothelial cells that give rise to hematopoietic stem and progenitor cells in a process called endothelial-to-hematopoietic transition. The development of hematopoietic cells in the embryo proceeds sequentially from lateral plate mesoderm through the hemangioblast to the definitive hemogenic endothelium and hematopoietic progenitors.

[00081] The term “hematopoietic stem and progenitor cells,” “hematopoietic stem cells,” “hematopoietic progenitor cells,” or “hematopoietic precursor cells” refers to cells which are committed to a hematopoietic lineage but are capable of further hematopoietic differentiation and include, 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). The term “definitive hematopoietic stem cell” as used herein, refers to CD34⁺ hematopoietic cells capable of giving rise to both mature myeloid and lymphoid cell types including T lineage cells, NK lineage cells and B lineage cells. Hematopoietic cells also include various subsets of primitive hematopoietic cells that give rise to primitive erythrocytes, megakaryocytes and macrophages.

[00082] As used herein, the terms “T lymphocyte” and “T cell” are used interchangeably and refer to a principal type of white blood cell that completes maturation in the thymus and that has various roles in the immune system, including the identification of specific foreign antigens in the body and the activation and deactivation of other immune cells in an MHC class I-restricted manner. 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, SupT1, etc., or a T cell obtained from a mammal. The T cell can be a CD3⁺ cell. 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., Th1 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, naïve T cells, regulator T cells, gamma delta T cells ($\gamma\delta$ T cells), and the like. Additional types of helper T cells include cells such as Th3 (Treg), Th17, Th9, or Tfh cells. Additional types of memory T cells include cells such as central memory T cells (T_{cm} cells), effector memory T cells (T_{em} cells and TEMRA cells). The term “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). A T cell or T cell like effector cell can also be differentiated from a stem cell or progenitor cell (“a derived T cell” or “a derived T cell like effector cell”, or collectively, “a derivative T lineage cell”). A derived T cell like effector cell may have a T cell lineage in some respects, but at the same time has one or more functional features that are not present in a primary T cell. In this application, a T cell, a T cell like effector cell, a derived T cell, a derived T cell like effector cell, or a derivative T lineage cell, are collectively termed as “a T lineage cell”.

[00083] “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 secretion profiles following stimulation, which may include secretion of cytokines such as IFN-gamma, TNF-alpha, IL2, IL4 and IL10. “CD4” molecules 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.

[00084] “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.

[00085] As used herein, the term “NK cell” or “Natural Killer cell” refer to a subset of peripheral blood lymphocytes defined by the expression of CD56 or CD16 and the absence of the T cell receptor (CD3). An NK cell can be any NK cell, such as a cultured NK cell, e.g., a primary NK cell, or an NK cell from a cultured or expanded NK cell or a cell-line NK cell, e.g., NK-92, or an NK cell obtained from a mammal that is healthy or with a disease condition. As used herein, the terms “adaptive NK cell” and “memory NK cell” are interchangeable and refer to a subset of NK cells that are phenotypically CD3⁻ and CD56⁺, expressing at least one of NKG2C and CD57, and optionally, CD16, but lack expression of one or more of the following: PLZF, SYK, FcεRγ, and EAT-2. In some embodiments, isolated subpopulations of CD56⁺ NK cells comprise expression of CD16, NKG2C, CD57, NKG2D, NCR ligands, NKp30, NKp40, NKp46, activating and inhibitory KIRs, NKG2A and/or DNAM-1. CD56⁺ can be dim or bright expression. An NK cell, or an NK cell like effector cell may be differentiated from a stem cell or progenitor cell (“a derived NK cell” or “a derived NK cell like effector cell”, or collectively, “a derivative NK lineage cell”). A derivative NK cell like effector cell may have an NK cell lineage in some respects, but at the same time has one or more functional features that are not present in a primary NK cell. In this application, an NK cell, an NK cell like effector cell, a derived NK cell, a derived NK cell like effector cell, or a derivative NK lineage cell, are collectively termed as “an NK lineage cell”.

[00086] As used herein, the term “NKT cells” or “natural killer T cells” or “NKT lineage cells” refers to CD1d-restricted T cells, which express a T cell receptor (TCR). Unlike conventional T cells that detect peptide antigens presented by conventional major histocompatibility (MHC) molecules, NKT cells recognize lipid antigens presented by CD1d, a non-classical MHC molecule. Two types of NKT cells are recognized. Invariant or type I NKT cells express a very limited TCR repertoire - a canonical α-chain (Vα24-Jα18 in humans) associated with a limited spectrum of β chains (Vβ11 in humans). The second population of NKT cells, called non-classical or non-invariant type II NKT cells, display a more heterogeneous TCR αβ usage. Type I NKT cells are considered suitable for immunotherapy. Adaptive or invariant

(type I) NKT cells can be identified by the expression of one or more of the following markers: TCR Va24-Ja18, Vb11, CD1d, CD3, CD4, CD8, aGalCer, CD161 and CD56.

[00087] The term “effector cell” generally is applied to certain cells in the immune system that carry out a specific activity in response to stimulation and/or activation, or to cells that effect a specific function upon activation. As used herein, the term “effector cell” includes, and in some contexts is interchangeable with, immune cells, “differentiated immune cells,” and primary or differentiated cells that are edited and/or modulated to carry out a specific activity in response to stimulation and/or activation. Non-limiting examples of effector cells include primary-sourced or iPSC-derived T cells, NK cells, NKT cells, B cells, macrophages, and neutrophils.

[00088] As used herein, the term “isolated” or the like refers to a cell, or a population of cells, which has been separated from its original environment, i.e., the environment of the isolated cells is substantially free of at least one component as found in the environment in which the “un-isolated” reference cells exist. The term includes a cell that is removed from some or all components as it is found in its natural environment, for example, isolated from a tissue or biopsy sample. The term also includes a cell that is removed from at least one, some or all components as the cell is found in non-naturally occurring environments, for example, isolated from a cell culture or cell suspension. Therefore, an “isolated cell” is partly or completely separated from at least one component, including other substances, cells or cell populations, as it is found in nature or as it is grown, stored or subsisted in non-naturally occurring environments. Specific examples of isolated cells include partially pure cell compositions, substantially pure cell compositions and cells cultured in a medium that is non-naturally occurring. Isolated cells may be obtained by separating the desired cells, or populations thereof, from other substances or cells in the environment, or by removing one or more other cell populations or subpopulations from the environment.

[00089] As used herein, the term “purify” or the like refers to increasing purity. For example, the purity can be increased to at least 50%, 60%, 70%, 80%, 90%, 95%, 99%, or 100%.

[00090] As used herein, the term “encoding” refers to the inherent property of specific sequences of nucleotides in a polynucleotide, such as a gene, a cDNA, or a mRNA, to serve as templates for synthesis of other polymers and macromolecules in biological processes having either a defined sequence of nucleotides (i.e., rRNA, tRNA and mRNA) or a defined sequence of amino acids and the biological properties resulting therefrom. Thus, a gene encodes a protein if transcription and translation of mRNA corresponding to that gene produces the protein in a cell or other biological system. Both the coding strand, the nucleotide sequence of which is identical to the mRNA sequence and is usually provided in sequence listings, and the non-coding strand,

used as the template for transcription of a gene or cDNA, can be referred to as “encoding” the protein or other product of that gene or cDNA.

[00091] 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. Thus, the term “vector” 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 vectors, viral vectors, cosmids, and artificial chromosomes. Viral vectors include, but are not limited to, adenovirus vectors, adeno-associated virus vectors, retrovirus vectors, lentivirus vectors, Sendai virus vectors, and the like.

[00092] As used from time to time throughout the application, the expression of “TRAC_[construct]”, with “[construct]” being a variable expression construct having components and arrangement thereof specified in a given context, means that the expression construct is inserted at the TRAC locus to knock out TCR and with the component(s) of the expression construct expressed or co-expressed under the control of the endogenous TCR promoter.

[00093] As used from time to time throughout the application, the expression of “CD38_[construct]”, with “[construct]” being a variable expression construct having components and arrangement thereof specified in a given context, means that the expression construct is inserted at the CD38 locus to knock out CD38 and with the component(s) of the expression construct expressed or co-expressed, whether under control of the endogenous CD38 promoter or under an exogenous promoter in the construct.

[00094] 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” may further comprise replacement of the endogenous sequence or a nucleotide that is deleted with the one or more inserted nucleotides.

[00095] As used herein, the term “exogenous” is intended to mean that the referenced molecule or the referenced activity is introduced into, or is 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. Similarly, the term when used in reference to expression of an encoding nucleic acid refers to expression of an encoding nucleic acid contained within the cell and not exogenously introduced.

[00096] 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.

[00097] As used herein, the term “polynucleotide” refers to a polymeric form of nucleotides of any length, either deoxyribonucleotides or ribonucleotides or analogs thereof. The sequence of a polynucleotide is composed of four nucleotide bases: adenine (A); cytosine (C); guanine (G); thymine (T); and uracil (U) for thymine when the polynucleotide is RNA. A polynucleotide can include a gene or gene fragment (for example, a probe, primer, EST or SAGE tag), exons, introns, messenger RNA (mRNA), transfer RNA, ribosomal RNA, ribozymes, cDNA, recombinant polynucleotides, branched polynucleotides, plasmids, vectors, isolated DNA of any sequence, isolated RNA of any sequence, nucleic acid probes and primers. “Polynucleotide” also refers to both double- and single-stranded molecules.

[00098] As used herein, the terms “peptide,” “polypeptide,” and “protein” are used interchangeably and refer to a molecule having amino acid residues covalently linked by peptide bonds. A polypeptide must contain at least two amino acids, and no limitation is placed on the maximum number of amino acids of a polypeptide. As used herein, the terms refer to both short chains, which are also commonly referred to in the art as peptides, oligopeptides and oligomers, for example, and to longer chains, which generally are referred to in the art as polypeptides or proteins. “Polypeptides” include, for example, biologically active fragments, substantially homologous polypeptides, oligopeptides, homodimers, heterodimers, variants of polypeptides, modified polypeptides, derivatives, analogs, fusion proteins, among others. The polypeptides

include natural polypeptides, recombinant polypeptides, synthetic polypeptides, or a combination thereof.

[00099] As used herein, the term “subunit” refers to each separate polypeptide chain of a protein complex, where each separate polypeptide chain can form a stable folded structure by itself. Many protein molecules are composed of more than one subunit, where the amino acid sequences can either be identical for each subunit, or similar, or completely different. For example, CD3 complex is composed of CD3 α , CD3 ϵ , CD3 δ , CD3 γ , and CD3 ζ subunits, which form the CD3 ϵ /CD3 γ , CD3 ϵ /CD3 δ , and CD3 ζ /CD3 ζ dimers. Within a single subunit, contiguous portions of the polypeptide chain frequently fold into compact, local, semi-independent units that are called “domains”. Many protein domains may further comprise independent “structural subunits”, also called subdomains, contributing to a common function of the domain. As such, the term “subdomain” as used herein refers to a protein domain inside of a larger domain, for example, a binding domain within an ectodomain of a cell surface receptor; or a stimulatory domain or a signaling domain of an endodomain of a cell surface receptor.

[000100] “Operably-linked” or “operatively linked,” interchangeable with “operably connected” or “operatively connected,” refers to the association of nucleic acid sequences on a single nucleic acid fragment (or amino acids in a polypeptide with multiple domains) 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. As a further example, a receptor-binding domain can be operatively connected to an intracellular signaling domain, such that binding of the receptor to a ligand transduces a signal responsive to said binding.

[000101] “Fusion proteins” or “chimeric proteins”, as used herein, are proteins created through genetic engineering to join two or more partial or whole polynucleotide coding sequences encoding separate proteins, and the expression of these joined polynucleotides results in a single peptide or multiple polypeptides with functional properties derived from each of the original proteins or fragments thereof. Between two neighboring polypeptides of different sources in the fusion protein, a linker (or spacer) peptide can be added.

[000102] 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 cells or derivative T 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 iPSCs using genomic editing. In the aspect of a source cell obtained from a specifically selected donor, disease or treatment context, the genetic imprint contributing to preferential therapeutic attributes may include any context-specific genetic or epigenetic modifications which manifest a retainable phenotype, i.e., a preferential therapeutic attribute, that is passed on to derivative cells of the selected source cell, irrespective of the underlying molecular events being identified or not. Donor-, disease-, or treatment response- specific source cells may comprise genetic imprints that are retainable in iPSCs and derived hematopoietic lineage cells, which genetic imprints include but are not limited to, prearranged monospecific TCR, for example, from a viral specific T cell or invariant natural killer T (iNKT) cell; trackable and desirable genetic polymorphisms, for example, homozygous for a point mutation that encodes for the high-affinity CD16 receptor in selected donors; and predetermined HLA requirements, i.e., selected HLA-matched donor cells exhibiting a haplotype with increased population. As used herein, preferential therapeutic attributes include improved engraftment, trafficking, homing, viability, self-renewal, persistence, immune response regulation and modulation, survival, and cytotoxicity of a derived cell. A preferential therapeutic attribute may also relate to antigen targeting receptor expression; HLA presentation or lack thereof; resistance to tumor microenvironment; induction of bystander immune cells and immune modulations; improved on-target specificity with reduced off-tumor effect; and/or resistance to treatment such as chemotherapy. When derivative cells having one or more therapeutic attributes are obtained from differentiating an iPSC that has genetic imprint(s) conferring a preferential therapeutic attribute incorporated thereto, such derivative cells are also called “synthetic cells”. In general, a synthetic cell possesses one or more non-native cell functions when compared to its closest counterpart primary cell, whether the synthetic cell is differentiated from engineered pluripotent cells or obtained by engineering a primary cell from natural/native sources, such as peripheral blood, umbilical cord blood, or other donor tissues. For example, synthetic effector cells, or synthetic NK cells or synthetic T cells, as used throughout this application are cells differentiated from a genomically modified iPSC, as compared to their primary counterpart

obtained from natural/native sources such as peripheral blood, umbilical cord blood, or other donor tissues. In some embodiments, the synthetic cell possesses one or more non-native cell functions when compared to its closest counterpart primary cell.

[000103] The term “enhanced therapeutic property” as used herein, refers to a therapeutic property of a cell that is enhanced as compared to a typical immune cell of the same general cell type. For example, an NK cell with an “enhanced therapeutic property” will possess an enhanced, improved, and/or augmented therapeutic property as compared to a typical, unmodified, and/or naturally occurring NK cell. Therapeutic properties of an immune cell may include, but are not limited to, cell engraftment, trafficking, homing, viability, self-renewal, persistence, immune response regulation and modulation, survival, and cytotoxicity. Therapeutic properties of an immune cell are also manifested by antigen targeting receptor expression; HLA presentation or lack thereof; resistance to tumor microenvironment; induction of bystander immune cells and immune modulations; improved on-target specificity with reduced off-tumor effect; and/or resistance to treatment such as chemotherapy.

[000104] As used herein, the term “engager” refers to a molecule, e.g., a fusion polypeptide, which is capable of forming a link between an immune cell (e.g., a T cell, a NK cell, a NKT cell, a B cell, a macrophage, a neutrophil), and a tumor cell; and activating the immune cell. Examples of engagers include, but are not limited to, bi-specific T cell engagers (BiTEs), bi-specific killer cell engagers (BiKEs), tri-specific killer cell engagers (TriKEs), or multi-specific killer cell engagers, or universal engagers compatible with multiple immune cell types.

[000105] As used herein, the term “surface triggering receptor” refers to a receptor capable of triggering or initiating an immune response, e.g., a cytotoxic response. Surface triggering receptors may be engineered, and may be expressed on effector cells, e.g., a T cell, a NK cell, a NKT cell, a B cell, a macrophage, or a neutrophil. In some embodiments, the surface triggering receptor facilitates bi- or multi-specific antibody engagement between the effector cells and a specific target cell (e.g., a tumor cell) independent of the effector cells’ natural receptors and cell types. Using this approach, one may generate iPSCs comprising a universal surface triggering receptor, and then differentiate such iPSCs into populations of various effector cell types that express the universal surface triggering receptor. By “universal”, it is meant that the surface triggering receptor can be expressed in, and activate, any effector cells irrespective of the cell type, and all effector cells expressing the universal receptor can be coupled or linked to the engagers recognizable by the surface triggering receptor, regardless of the engager’s tumor binding specificities. In some embodiments, engagers having the same tumor targeting specificity are used to couple with the universal surface triggering receptor. In some embodiments, engagers having different tumor targeting specificity are used to couple with the

universal surface triggering receptor. As such, one or multiple effector cell types can be engaged to kill one specific type of tumor cells in some cases, and to kill two or more types of tumors in other cases. A surface triggering receptor generally comprises a co-stimulatory domain for effector cell activation and an anti-epitope that is specific to the epitope of an engager. A bi-specific engager is specific to the anti-epitope of a surface triggering receptor on one end, and is specific to a tumor antigen on the other end.

[000106] As used herein, the term “safety switch protein” refers to an engineered protein designed to prevent potential toxicity or otherwise adverse effects of a cell therapy. In some instances, the safety switch protein expression is conditionally controlled to address safety concerns for transplanted engineered cells that have permanently incorporated the gene encoding the safety switch protein into its genome. This conditional regulation could be variable and might include control through a small molecule-mediated post-translational activation and tissue-specific and/or temporal transcriptional regulation. The safety switch protein could mediate induction of apoptosis, inhibition of protein synthesis, DNA replication, growth arrest, transcriptional and post-transcriptional genetic regulation and/or antibody-mediated depletion. In some instance, the safety switch protein is activated by an exogenous molecule, e.g., a prodrug, that when activated, triggers apoptosis and/or cell death of a therapeutic cell. Examples of safety switch proteins include, but are not limited to, suicide genes such as caspase 9 (or caspase 3 or 7), thymidine kinase, cytosine deaminase, B cell CD20, modified EGFR, and any combination thereof. In this strategy, a prodrug that is administered in the event of an adverse event is activated by the suicide-gene product and kills the transduced cell.

[000107] As used herein, the term “pharmaceutically active proteins or peptides” refers to proteins or peptides that are capable of achieving a biological and/or pharmaceutical effect on an organism. A pharmaceutically active protein has healing, curative or palliative properties against a disease and may be administered to ameliorate, relieve, alleviate, reverse or lessen the severity of a disease. A pharmaceutically active protein also has prophylactic properties and is used to prevent the onset of a disease or to lessen the severity of such disease or pathological condition when it does emerge. “Pharmaceutically active proteins” include an entire protein or peptide or pharmaceutically active fragments thereof. The term also includes pharmaceutically active analogs of the protein or peptide or analogs of fragments of the protein or peptide. The term pharmaceutically active protein also refers to a plurality of proteins or peptides that act cooperatively or synergistically to provide a therapeutic benefit. Examples of pharmaceutically active proteins or peptides include, but are not limited to, receptors, binding proteins, transcription and translation factors, tumor growth suppressing proteins, antibodies or fragments thereof, growth factors, and/or cytokines.

[000108] As used herein, the term “signaling molecule” refers to any molecule that modulates, participates in, inhibits, activates, reduces, or increases, cellular signal transduction. “Signal transduction” refers to the transmission of a molecular signal in the form of chemical modification by recruitment of protein complexes along a pathway that ultimately triggers a biochemical event in the cell. Examples of signal transduction pathways are known in the art, and include, but are not limited to, G protein coupled receptor signaling, tyrosine kinase receptor signaling, integrin signaling, toll gate signaling, ligand-gated ion channel signaling, ERK/MAPK signaling pathway, Wnt signaling pathway, cAMP-dependent pathway, and IP3/DAG signaling pathway.

[000109] As used herein, the term “targeting modality” refers to a molecule, e.g., a polypeptide, that is genetically incorporated into a cell to promote antigen and/or epitope specificity that includes, but is not limited to, i) antigen specificity as it relates to a unique chimeric antigen receptor (CAR) or T cell receptor (TCR), ii) engager specificity as it relates to monoclonal antibodies or bispecific engagers, iii) targeting of transformed cells, iv) targeting of cancer stem cells, and v) other targeting strategies in the absence of a specific antigen or surface molecule.

[000110] As used herein, the term “specific” or “specificity” can be used to refer to the ability of a molecule, e.g., a receptor or an engager, to selectively bind to a target molecule, in contrast to non-specific or non-selective binding.

[000111] The term “adoptive cell therapy” as used herein refers to a cell-based immunotherapy that relates to the transfusion of autologous or allogeneic lymphocytes, whether the immune cells are isolated from a human donor, or effector cells obtained from *in vitro* differentiation of a pluripotent cell; whether they are genetically modified or not; or whether they are primary donor cells or cells that have been passaged, expanded, or immortalized, *ex vivo*, after isolation from a donor.

[000112] As used herein, “radiation” refers to the emission or transmission of energy in the form of waves or particles. Exemplary forms of radiation include, but are not limited to, electromagnetic radiation (e.g., radio waves, microwaves, infrared, visible light, ultraviolet, x-rays, and gamma radiation), particle radiation (e.g., alpha radiation, beta radiation, proton radiation and neutron radiation), and acoustic radiation (e.g., ultrasound, sound and seismic waves). In various embodiments, the amount of radiation is measured as a Gray (Gy), which is defined as the absorption of one joule of radiation energy per kilogram of matter. In radiation therapy, the amount of radiation applied varies depending on the type and stage of cancer being treated. For curative cases, the typical dose for a solid epithelial tumor ranges from 60 to 80 Gy, while lymphomas are typically treated with 20 to 40 Gy. Preventive (adjuvant) doses are

typically around 45–60 Gy in 1.8–2 Gy fractions (for, e.g., breast, head, and neck cancers). In various embodiments, radiation may be used as a sensitizing agent as disclosed herein.

[000113] As used herein, “radiation therapy” or “radiotherapy” are used interchangeably to refer to a type of cancer treatment that involves use of radiation to damage cells by destroying the genetic material that controls how cells grow and divide. While both healthy and cancerous cells are damaged by radiation therapy, the goal of radiation therapy is to destroy as few normal, healthy cells as possible. The term “radiation therapy” often refers to external beam radiation therapy, wherein high-energy beams (e.g., x-rays, gamma rays, photons, protons, neutrons, ions, and any other forms of energy applicable to such treatments) are produced by a machine outside of the subject being treated, and are aimed at a precise point on the subject’s body. However, the term “radiation therapy” also includes brachytherapy, wherein seeds, ribbons, or capsules that contain or are otherwise linked to a radiation source are placed inside the subject’s body in or near a tumor or cancer cell. Included in brachytherapy are low-dose rate implants, high-dose rate implants, and permanent implants. Also included in the term “radiation therapy” is systemic radiation therapy, wherein radioactive drugs (e.g., radiopharmaceuticals or radionuclides, including radiopeptides) are given to the subject orally or intravenously and collect within the subject’s body at the tumor or area where cancers cells are located. Similar to antibody-drug candidates, where an antibody that binds to a tumor antigen is linked to a toxic drug, radiopharmaceuticals incorporate a radioactive compound linked to a targeting molecule (such as an antibody) that specifically binds to a tumor antigen. Examples of radioactive compounds useful in radiopharmaceuticals include, but are not limited to calcium-47, carbon-11, carbon-14, chromium-51, cobalt-57, cobalt-58, erbium-169, fluorine-18, gallium-67, gallium-68, hydrogen-3, indium-111, iodine-123, iodine-125, iodine-131, iron-59, krypton-81m, lutetium-177, nitrogen-13, oxygen-15, phosphorus-32, radium-223, rubidium-82, samarium-153, selenium-75, sodium-22, sodium-24, strontium-89, technetium-99m, thallium-201, xenon-133, and yttrium-90. In various embodiments, radiation therapy may be used as a sensitizing agent as disclosed herein.

[000114] As used herein, “lymphodepletion” and “lympho-conditioning” are used interchangeably to refer to the destruction of lymphocytes and T cells, typically prior to immunotherapy. The purpose of lympho-conditioning prior to the administration of an adoptive cell therapy is to promote homeostatic proliferation of effector cells as well as to eliminate regulatory immune cells and other competing elements of the immune system that compete for homeostatic cytokines. Thus, lympho-conditioning is typically accomplished by administering one or more chemotherapeutic agents to the subject prior to a first dose of the adoptive cell therapy. In various embodiments, lympho-conditioning precedes the first dose of the adoptive

cell therapy by a few hours to a few days. Exemplary chemotherapeutic agents useful for lympho-conditioning include, but are not limited to, cyclophosphamide (CY), fludarabine (FLU), and those described below. However, a sufficient lymphodepletion through anti-CD38 mAb could provide an alternative conditioning process for the present iNK cell therapy, without or with minimal need of a CY/FLU-based lympho-conditioning procedure, as further described herein.

[000115] As used herein, “homing” or “trafficking” refers to active navigation (migration) of a cell to a target site (e.g., a cell, tissue (e.g., tumor), or organ). A “homing molecule” refers to a molecule that directs cells to a target site. In some embodiments, a homing molecule functions to recognize and/or initiate interaction of a cell to a target site. In some embodiments, a homing molecule is a chemokine receptor. As used herein, “chemokine receptor” refers to a cell surface molecule that binds to a chemokine. A chemokine receptor can comprise a naturally occurring or recombinant chemokine receptor or a variant thereof. Exemplary chemokine receptors include, but are not limited to, a CXC chemokine receptor (for example, CXCR1, CXCR2, CXCR3, CXCR4, CXCR5, CXCR6, or CXCR7), a CC chemokine receptor (for example, CCR1, CCR2, CCR3, CCR4, CCR5, CCR6, CCR7, CCR8, CCR9, CCR10, or CCR11), a CX3C chemokine receptor (for example, CX3CR1), an XC chemokine receptor (for example, XCR1), or a variant thereof.

[000116] A “therapeutically sufficient amount”, as used herein, includes within its meaning a non-toxic, but sufficient and/or effective amount of a particular therapeutic agent and/or pharmaceutical composition to which it is referring to provide a desired therapeutic effect. The exact amount required will vary from subject to subject, depending on factors such as the patient’s general health, the patient’s age and the stage and severity of the condition being treated. In particular embodiments, a “therapeutically sufficient amount” is sufficient and/or effective to ameliorate, reduce, and/or improve at least one symptom associated with a disease or condition of the subject being treated.

[000117] Differentiation of pluripotent stem cells requires a change in the culture system, such as changing the stimuli agents in the culture medium or the physical state of the cells. The most conventional strategy utilizes the formation of embryoid bodies (EBs) as a common and critical intermediate to initiate lineage-specific differentiation. “Embryoid bodies” are three-dimensional clusters that have been shown to mimic embryo development as they give rise to numerous lineages within their three-dimensional area. Through the differentiation process, typically a few hours to days, simple EBs (for example, aggregated pluripotent stem cells elicited to differentiate) continue maturation and develop into a cystic EB at which time, typically days to a few weeks, they are further processed to continue differentiation. EB formation is initiated

by bringing pluripotent stem cells into close proximity with one another in three-dimensional multilayered clusters of cells. Typically, this is achieved by one of several methods including allowing pluripotent cells to sediment in liquid droplets, sedimenting cells into “U” bottomed well-plates or by mechanical agitation. To promote EB development, the pluripotent stem cell aggregates require further differentiation cues, as aggregates maintained in pluripotent culture maintenance medium do not form proper EBs. As such, the pluripotent stem cell aggregates need to be transferred to differentiation medium that provides eliciting cues towards the lineage of choice. EB-based culture of pluripotent stem cells typically results in generation of differentiated cell populations (i.e., ectoderm, mesoderm and endoderm germ layers) with modest proliferation within the EB cell cluster. Although proven to facilitate cell differentiation, EBs, however, give rise to heterogeneous cells in variable differentiation states because of the inconsistent exposure of the cells in the three-dimensional structure to the differentiation cues within the environment. In addition, EBs are laborious to create and maintain. Moreover, cell differentiation through EB formation is accompanied with modest cell expansion, which also contributes to low differentiation efficiency.

[000118] In comparison, “aggregate formation,” as distinct from “EB formation,” can be used to expand the populations of pluripotent stem cell derived cells. For example, during aggregate-based pluripotent stem cell expansion, culture media are selected to maintain proliferation and pluripotency. Cell proliferation generally increases the size of the aggregates, forming larger aggregates, which can be mechanically or enzymatically dissociated into smaller aggregates to maintain cell proliferation within the culture and increase numbers of cells. As distinct from EB culture, cells cultured within aggregates in maintenance culture media maintain markers of pluripotency. The pluripotent stem cell aggregates require further differentiation cues to induce differentiation.

[000119] As used herein, “monolayer differentiation” is a term referring to a differentiation method distinct from differentiation through three-dimensional multilayered clusters of cells, i.e., “EB formation.” Monolayer differentiation, among other advantages disclosed herein, avoids the need for EB formation to initiate differentiation. Because monolayer culturing does not mimic embryo development such as is the case with EB formation, differentiation towards specific lineages is deemed to be minimal as compared to all three germ layer differentiation in EB formation.

[000120] As used herein, a “dissociated cell” or “single dissociated cell” refers to a cell that has been substantially separated or purified away from other cells or from a surface (e.g., a culture plate surface). For example, cells can be dissociated from an animal or tissue by mechanical or enzymatic methods. Alternatively, cells that aggregate *in vitro* can be

enzymatically or mechanically dissociated from each other, such as by dissociation into a suspension of clusters, single cells or a mixture of single cells and clusters. In yet another alternative embodiment, adherent cells can be dissociated from a culture plate or other surface. Dissociation thus can involve breaking cell interactions with extracellular matrix (ECM) and substrates (e.g., culture surfaces), or breaking the ECM between cells.

[000121] As used herein, a “master cell bank” or “MCB” refers to a clonal master engineered iPSC line, which is a clonal population of iPSCs that have been engineered to comprise one or more therapeutic attributes, have been characterized, tested, qualified, and expanded, and have been shown to reliably serve as the starting cellular material for the production of cell-based therapeutics through directed differentiation in manufacturing settings. In various embodiments, an MCB is maintained, stored, and/or cryopreserved in multiple vessels to prevent genetic variation and/or potential contamination by reducing and/or eliminating the total number of times the iPS cell line is passaged, thawed or handled during the manufacturing processes.

[000122] As used herein, “feeder cells” or “feeders” are terms describing cells of one type that are co-cultured with cells of a second type to provide an environment in which the cells of the second type can grow, expand, or differentiate, as the feeder cells provide stimulation, growth factors and nutrients for the support of the second cell type. The feeder cells are optionally from a different species as the cells they are supporting. For example, certain types of human cells, including stem cells, can be supported by primary cultures of mouse embryonic fibroblasts, or immortalized mouse embryonic fibroblasts. In another example, peripheral blood derived cells or transformed leukemia cells support the expansion and maturation of natural killer cells. The feeder cells may typically be inactivated when being co-cultured with other cells by irradiation or treatment with an anti-mitotic agent such as mitomycin to prevent them from outgrowing the cells they are supporting. Feeder cells may include endothelial cells, stromal cells (for example, epithelial cells or fibroblasts), and leukemic cells. Without limiting the foregoing, one specific feeder cell type may be a human feeder, such as a human skin fibroblast. Another feeder cell type may be mouse embryonic fibroblasts (MEF). In general, various feeder cells can be used in part to maintain pluripotency, direct differentiation towards a certain lineage, enhance proliferation capacity and promote maturation to a specialized cell type, such as an effector cell.

[000123] As used herein, a “feeder-free” (FF) environment refers to an environment such as a culture condition, cell culture or culture media which is essentially free of feeder or stromal cells, and/or which has not been pre-conditioned by the cultivation of feeder cells. “Pre-conditioned” medium refers to a medium harvested after feeder cells have been cultivated within the medium for a period of time, such as for at least one day. Pre-conditioned medium contains many mediator substances, including growth factors and cytokines secreted by the feeder cells

cultivated in the medium. In some embodiments, a feeder-free environment is free of both feeder or stromal cells and is also not pre-conditioned by the cultivation of feeder cells.

[000124] “Functional” as used in the context of genomic editing or modification of iPSC, and derived non-pluripotent cells differentiated therefrom, or genomic editing or modification of non-pluripotent cells and derived iPSCs reprogrammed therefrom, refers to (1) at the gene level—successful knocked-in, knocked-out, knocked-down gene expression, transgenic or controlled gene expression such as inducible or temporal expression at a desired cell development stage, which is achieved through direct genomic editing or modification, or through “passing-on” via differentiation from or reprogramming of a starting cell that is initially genomically engineered; or (2) at the cell level—successful removal, addition, or alteration of a cell function/characteristic via (i) gene expression modification obtained in said cell through direct genomic editing, (ii) gene expression modification maintained in said cell through “passing-on” via differentiation from or reprogramming of a starting cell that is initially genomically engineered; (iii) down-stream gene regulation in said cell as a result of gene expression modification that only appears in an earlier development stage of said cell, or only appears in the starting cell that gives rise to said cell via differentiation or reprogramming; or (iv) enhanced or newly attained cellular function or attribute displayed within the mature cellular product, initially derived from the genomic editing or modification conducted at the iPSC, progenitor or dedifferentiated cellular origin.

[000125] “HLA deficient”, including HLA class I deficient, HLA class II deficient, or both, refers to cells that either lack, or no longer maintain, or have a reduced level of surface expression of a complete MHC complex comprising an HLA class I protein heterodimer and/or an HLA class II heterodimer, such that the diminished or reduced level is less than the level naturally detectable by other cells or by synthetic methods.

[000126] “Modified HLA deficient iPSC,” as used herein, refers to an HLA deficient iPSC that is further modified by introducing genes expressing proteins related, but not limited to improved differentiation potential, antigen targeting, antigen presentation, antibody recognition, persistence, immune evasion, resistance to suppression, proliferation, costimulation, cytokine stimulation, cytokine production (autocrine or paracrine), chemotaxis, and cellular cytotoxicity, such as non-classical HLA class I proteins (e.g., HLA-E and HLA-G), chimeric antigen receptor (CAR), T cell receptor (TCR), CD16 Fc Receptor, BCL11b, NOTCH, RUNX1, IL15, 4-1BB, DAP10, DAP12, CD24, CD3 ζ , 4-1BBL, CD47, CD113, and PDL1. The cells that are “modified HLA deficient” also include cells other than iPSCs.

[000127] The term “ligand” refers to a substance that forms a complex with a target molecule to produce a signal by binding to a site on the target. The ligand may be a natural or artificial

substance capable of specific binding to the target. The ligand may be in the form of a protein, a peptide, an antibody, an antibody complex, a conjugate, a nucleic acid, a lipid, a polysaccharide, a monosaccharide, a small molecule, a nanoparticle, an ion, a neurotransmitter, or any other molecular entity capable of specific binding to a target. The target to which the ligand binds, may be a protein, a nucleic acid, an antigen, a receptor, a protein complex, or a cell. A ligand that binds to and alters the function of the target and triggers a signaling response is called “agonistic” or “an agonist”. A ligand that binds to a target and blocks or reduces a signaling response is “antagonistic” or “an antagonist.”

[000128] The term “antibody” is used herein in the broadest sense and refers generally to an immune-response generating molecule that contains at least one binding site that specifically binds to a target, wherein the target may be an antigen, or a receptor that is capable of interacting with certain antibodies. For example, an NK cell can be activated by the binding of an antibody or the Fc region of an antibody to its Fc-gamma receptors (FcγR), thereby triggering the ADCC (antibody-dependent cellular cytotoxicity) mediated effector cell activation. A specific piece or portion of an antigen or receptor, or a target in general, to which an antibody binds is known as an epitope or an antigenic determinant. The term “antibody” includes, but is not limited to, native antibodies and variants thereof, fragments of native antibodies and variants thereof, peptibodies and variants thereof, and antibody mimetics that mimic the structure and/or function of an antibody or a specified fragment or portion thereof, including single chain antibodies and fragments thereof. An antibody may be a murine antibody, a human antibody, a humanized antibody, a camel IgG, a single variable new antigen receptor (VNAR), a shark heavy-chain antibody (Ig-NAR), a chimeric antibody, a recombinant antibody, a single-domain antibody (dAb), an anti-idiotypic antibody, a bi-specific-, multi-specific- or multimeric- antibody, or antibody fragment thereof. Anti-idiotypic antibodies are specific for binding to an idiotope of another antibody, wherein the idiotope is an antigenic determinant of an antibody. A bi-specific antibody may be a BiTE (bi-specific T cell engager) or a BiKE (bi-specific killer cell engager), and a multi-specific antibody may be a TriKE (tri-specific Killer cell engager). Non-limiting examples of antibody fragments include Fab, Fab', F(ab')₂, F(ab')₃, Fv, Fabc, pFc, Fd, single chain fragment variable (scFv), tandem scFv (scFv)₂, single chain Fab (scFab), disulfide stabilized Fv (dsFv), minibody, diabody, triabody, tetrabody, single-domain antigen binding fragments (sdAb), camelid heavy-chain IgG and Nanobody® fragments, recombinant heavy-chain-only antibody (VHH), and other antibody fragments that maintain the binding specificity of the antibody.

[000129] “Fc receptors,” abbreviated FcR, are classified based on the type of antibody that they recognize. For example, those that bind the most common class of antibody, IgG, are called

Fc-gamma receptors (Fc γ R), those that bind IgA are called Fc-alpha receptors (Fc α R) and those that bind IgE are called Fc-epsilon receptors (Fc ϵ R). The classes of FcRs are also distinguished by the cells that express them (macrophages, granulocytes, natural killer cells, T and B cells) and the signaling properties of each receptor. Fc-gamma receptors (Fc γ R) include several members, Fc γ RI (CD64), Fc γ RIIA (CD32), Fc γ RIIB (CD32), Fc γ RIIIA (CD16a), and Fc γ RIIIB (CD16b), which differ in their antibody affinities due to their different molecular structures.

[000130] “Chimeric Receptor” is a general term used to describe an engineered, artificial, or a hybrid receptor protein molecule that is made to comprise two or more portions of amino acid sequences that are originated from at least two different proteins. The chimeric receptor proteins have been engineered to give a cell the ability to initiate signal transduction and carry out downstream function upon binding of an agonistic ligand to the receptor. Exemplary “chimeric receptors” include, but are not limited to, chimeric antigen receptors (CARs), chimeric fusion receptors (CFRs), chimeric Fc receptors (CFcRs), as well as fusions of two or more receptors.

[000131] “Chimeric Fc Receptor,” abbreviated as CFcR, is a term used to describe engineered Fc receptors having their native transmembrane and/or intracellular signaling domains modified or replaced with non-native transmembrane and/or intracellular signaling domains. In some embodiments of the chimeric Fc receptor, in addition to having one of, or both of, the transmembrane and signaling domains being non-native, one or more stimulatory domains can be introduced to the intracellular portion of the engineered Fc receptor to enhance cell activation, expansion and function upon triggering of the receptor. Unlike a chimeric antigen receptor (CAR), which contains an antigen binding domain to a target antigen, the chimeric Fc receptor binds to an Fc fragment, or the Fc region of an antibody, or the Fc region comprised in an engager or a binding molecule and activates the cell function with or without bringing the targeted cell close in vicinity. For example, a Fc γ receptor can be engineered to comprise selected transmembrane, stimulatory, and/or signaling domains in the intracellular region that respond to the binding of IgG at the extracellular domain, thereby generating a CFcR. In one example, a CFcR is produced by engineering CD16, a Fc γ receptor, by replacing its transmembrane domain and/or intracellular domain. To further improve the binding affinity of the CD16-based CFcR, the extracellular domain of CD64 or the high-affinity variants of CD16 (F176V, for example) can be incorporated. In some embodiments of the CFcR where a high affinity CD16 extracellular domain is involved, the proteolytic cleavage site comprising a serine at position 197 is eliminated or is replaced such that the extracellular domain of the receptor is non-cleavable, i.e., not subject to shedding, thereby obtaining a hnCD16-based CFcR.

[000132] CD16, a Fc γ R receptor, has been identified to have two isoforms, Fc receptors Fc γ RIIIa (CD16a) and Fc γ RIIIB (CD16b). CD16a is a transmembrane protein expressed by NK

cells, which binds monomeric IgG attached to target cells to activate NK cells and facilitate antibody-dependent cell-mediated cytotoxicity (ADCC). “High affinity CD16,” “non-cleavable CD16,” or “high affinity non-cleavable CD16” (abbreviated as hnCD16), as used herein, refers to a natural or non-natural variant of CD16. The wildtype CD16 has low affinity and is subject to ectodomain shedding, a proteolytic cleavage process that regulates the cells surface density of various cell surface molecules on leukocytes upon NK cell activation. F176V and F158V are exemplary CD16 polymorphic variants having high affinity. A CD16 variant having the cleavage site (position 195-198) in the membrane-proximal region (position 189-212) altered or eliminated is not subject to shedding. The cleavage site and the membrane-proximal region are described in detail in WO2015/148926, the complete disclosure of which is incorporated herein by reference. The CD16 S197P variant is an engineered non-cleavable version of CD16. A CD16 variant comprising both F158V and S197P has high affinity and is non-cleavable. Another exemplary high affinity and non-cleavable CD16 (hnCD16) variant is an engineered CD16 comprising an ectodomain originated from one or more of the 3 exons of the CD64 ectodomain.

[000133] In some embodiments, provided herein are cells comprising a set of engineered components that collectively complement (and in some cases synergize with) one another to enhance the activity of an effector cell, in the context of treating a tumor in general, and for a solid tumor microenvironment in particular. The selected set of engineered components are referred to herein as a “backbone,” for its compatibility with any tumor antigen binding molecule to be expressed in the effector cell, including but not limited to, a CAR, an antibody, a bispecific antibody, and a TCR. However, the term “backbone” does not require any particular physical relationship between the individual components of the set, or their location within the cell; although certain association and/or arrangements (e.g., order in a co-expression construct of two or more of the individual components) may be optimized for higher expression level or ease of processing, among other considerations in a manufacturing setting. For example, a backbone may comprise integration of two expression cassettes, each at a different location in the genome of the cell. In some embodiments, the backbone comprises a plurality of genomic modifications, such as the insertion of one or more polynucleotides and/or modification to knockout one or more genes. Modifications may be made simultaneously or sequentially. Non-limiting examples of effector cell function that may be increased by the modifications of the backbone include one or more of improving cell growth, proliferation, expansion, and/or effector function autonomously without contacting additionally supplied soluble cytokines *in vitro* or *in vivo*, as well as enhanced homing, trafficking, depletion or reduction of alloreactive host immune cells, and retention at tumor sites, in which the tumor cells could be sensitized to synergize with the functional features provided to the effector cells. A solid tumor targeting backbone of the present

disclosure can be particularly beneficial in the context of an iPSC comprising the backbone, such as by providing a master cell bank providing a source of starting cells that can be modified by the simple addition of a tumor antigen binding molecule for an indication intended to be treated, and then being used as a source for differentiating enhanced effector cells with therapeutic properties for one or more intended tumor indications.

I. Cells and Compositions Useful for Adoptive Cell Therapies with Enhanced Properties

[000134] Provided herein is a strategy to systematically engineer the regulatory circuitry of a clonal iPSC without impacting the differentiation potency and cell development biology of the iPSC and its derivative cells, while enhancing the therapeutic properties of the derivative cells differentiated from the iPSC. The iPSC-derived cells are functionally improved and suitable for adoptive cell therapies following a combination of selective modalities being introduced to the cells at the level of iPSC through genomic engineering. It was previously unclear whether altered iPSCs comprising one or more provided genetic edits still have the capacity to enter cell development, and/or to mature and generate functional differentiated cells while retaining modified activities and/or properties. Unanticipated failures during directed cell differentiation from iPSCs have been attributed to aspects including, but not limited to, development stage specific gene expression or lack thereof, requirements for HLA complex presentation, protein shedding of introduced surface expressing modalities, and the need for reconfiguration of differentiation protocols enabling phenotypic and/or functional change in the cell. The present application has shown that the one or more selected genomic modifications as provided herein does not negatively impact iPSC differentiation potency, and the functional effector cells derived from the engineered iPSC have enhanced and/or acquired therapeutic properties attributable to the individual or combined genomic modifications retained in the effector cells following the iPSC differentiation. Further, all genomic modifications and combinations thereof as may be described in the context of iPSC and iPSC-derived effector cells are applicable to primary sourced cells, including primary immune cells such as T, NK, or immunoregulatory cells, whether cultured or expanded, the modification of which results in engineered immune cells useful for adoptive cell therapy.

[000135] Further, while CAR-T cells have been shown to be effective and potent in treating several hematologic malignancies, engineered T cell therapies have had limited success in addressing solid tumors. Unlike liquid tumors where uniformly-expressed antigens are accessible and can be effectively targeted, tumor access, lack of tumor-exclusive antigen targets, and antigen heterogeneity are significant barriers to the successful development of CAR-T cells

in solid tumors. In addition, inherent genetic engineering variability seen with patient- and donor- derived immune cells limits the wide application of CAR-T cell therapy. The present application provides genomic engineering aspects in the form of a solid tumor targeting backbone, as well as other genetic modalities, to improve on-target specificity with reduced off-tumor effect in the off-the-shelf adoptive cell therapy setting using effector cells derived from engineered iPSCs, to evade alloreactivity, as well as to overcome suppressive tumor microenvironment, a heightened challenge especially with solid tumors.

1. C-X-C Motif Chemokine Receptor Overexpression

[000136] Chemokines are a family of homogeneous serum proteins of about 7 to about 16 kDa originally characterized by their ability to induce leukocyte migration. Most of chemokines have four characteristic cysteines (Cys) and are classified into C-X-C (or alpha, CXC), C-C (or beta), C (or gamma), and CX3C (or delta) chemokine classes, according to motifs displayed by the first two cysteines. Subfamilies of C-X-C (or alpha, CXC) are further classified, according to the presence of an ELR motif (Glu-Leu-Arg) preceding the first cysteine, into two groups: ELR-CXC chemokines and non-ELR-CXC chemokines.

[000137] CXC chemokine receptor 2 (CXCR2), also known as CD128, interleukin 8 receptor beta (IL8R β), or L8 receptor type B, is a chemokine receptor mostly expressed by neutrophils, mast cells, monocytes, and macrophages. It is known that CD56 dim NK cells express CXCR2, however its expression can be downregulated upon NK cell activation. T cells typically do not express CXCR2. iPSCs and iPSC-derived T cells do not express CXCR2 without transducing exogenous polynucleotides encoding CXCR2 as disclosed in this application. The chemokine IL8 (also known as CXCL8) is secreted by mononuclear macrophages, neutrophils, eosinophils, T lymphocytes, epithelial cells, and fibroblasts, and functions as a chemotactic factor by guiding the neutrophils to the site of infection. CXCL8 is also secreted by tumor cells and promotes tumor migration, invasion, angiogenesis and metastasis. CXCL8 is one of the ligands to multiple CXC chemokine receptors including CXCR1 and CXCR2. Additional chemokines known to bind to CXCR2 include, but are not limited to, CXCL1, GRO β (CXCL2), CXCL3, CXCL5, CXCL6, and CXCL7.

[000138] CXC chemokine receptor 3 (CXCR3), also known as G Protein-coupled Receptor 9 (GPR9) and CD183, is a G Protein-coupled receptor that binds to the chemokines CXCL9, CXCL10, and CXCL11. CXCR3 is expressed primarily in activated T-helper type 1 (Th1) lymphocytes, but is also present in natural killer cells, macrophages, dendritic cells, and B lymphocyte subsets. The interaction of CXCR3 and its ligands is involved in guiding receptor-

bearing cells to specific parts of the body, especially sites of inflammation, immune impairment, and immune dysfunction.

[000139] In various embodiments, the present application provides effector cells or iPSCs genetically engineered to comprise, among other editing as contemplated and described herein, a solid tumor targeting backbone comprising, among other genetic modalities, a C-X-C motif chemokine receptor. In various embodiments, the C-X-C motif chemokine receptor comprises CXCR2 or CXCR3, or variants thereof. A non-limiting example of the amino acid sequence of human CXCR2 is one registered as UniProtKB No: P25025. In one embodiment, the CXCR2 comprises an amino acid sequence of at least 75%, 80%, 85%, 90%, 95% or 99% identity to SEQ ID NO: 1. In some embodiments, the CXCR2 comprises an amino acid sequence of at least 90% identity to SEQ ID NO: 1. In some embodiments, the CXCR2 comprises an amino acid sequence of at least 95% identity to SEQ ID NO: 1. In some embodiments, the CXCR2 comprises the amino acid sequence of SEQ ID NO: 1. As used herein and throughout the application, the percent identity between two sequences is a function of the number of identical positions shared by the sequences (i.e., % identity = # of identical positions/total # of positions x 100), taking into account the number of gaps, and the length of each gap, which need to be introduced for optimal alignment of the two sequences. The comparison of sequences and determination of percent identity between two sequences can be accomplished using a mathematical algorithm recognized in the art.

SEQ ID NO: 1

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MEDFNMESDSFEDFWKGEDLSNYSYSTLPPFLLDAAPCEPESLEINKYFVVIYALVFLSLLGNSLVM
LVILYSRVGRSVTDVYLLNLALADLLFALTLP IWAASKVNGWIFGTFLCKVVSLLKEVNFYSGILLACI
SVDRYLAIVHATRTLTQKRYLVKFICLSIWGLSLLLALPVLLFRRTVYSSNVSPACYEDMGNNNTANWRML
LRILPQSFGFIVPLLIIMLFCYGFTLRTL FKAHMGQKHRAMRVI FAVVLI FLLCWLPYNLVLLADTL MRTQ
VIQETCERRNHIDRALDATEILGILHSCLNPLIYAFIGQKFRHGLLKILAIHGLISKDSL PKDSRPSFVG
SSSGHTSTTL
```

(360 a.a. CXCR2; UniProtKB No: P25025)

[000140] A non-limiting example of the amino acid sequence of human CXCR3 is one registered as UniProtKB No: P49682. In one embodiment, the CXCR3 comprises an amino acid sequence of at least 75%, 80%, 85%, 90%, 95% or 99% identity to SEQ ID NO: 2. In some embodiments, the CXCR3 comprises an amino acid sequence of at least 90% identity to SEQ ID NO: 2. In some embodiments, the CXCR3 comprises an amino acid sequence of at least 95% identity to SEQ ID NO: 2. In some embodiments, the CXCR3 comprises the amino acid sequence of SEQ ID NO: 2.

SEQ ID NO: 2

MVLEVSDHQVLNDAEVAALLENFSSSYDYGENESDSCCTSPPCPQDFSLNFDRAFLPALYSLLFLLGLLG
 NGAVAAVLLSRRTALSSTDTFLLHLAVADTLLVLTPLWAVDAAVQWVFGSGLCKVAGALFNINFYAGAL
 LLACISFDRLNIVHATQLYRRGPPARVTLTCLAVWGLCLLFALPDFIFLSAHHDERLNATHCQYNFPQV
 GRTALRVLQLVAGFLLPLLVMAYCYAHILAVLLVSRGQRRRLRAMRLVVVVVAFALCWTPYHLVVLVDIL
 MDLGALARNCGRESRVDVAKSVTSGLGYMHCCLNPLLYAFVGVKFRERMWMLLLRLGCPNQRGLQRQPSS
 SRRDSSWSETSEASYSGL

(368 a.a. CXCR3; UniProtKB No: P49682)

[000141] In various embodiments, the polynucleotide encoding the C-X-C motif chemokine receptor or variant thereof is inserted in a selected locus of a primary-sourced effector cell or an iPSC for deriving functional effector cells comprising the same genetic editing through directed differentiation. In some embodiments, the selected locus for insertion of the C-X-C motif chemokine receptor comprises a safe harbor locus, a gene locus intended to be disrupted or knocked out, a gene locus that provides an endogenous promoter that provides spacial and/or temporal control of the exogenous gene expression. In some embodiments, the selected locus for C-X-C motif chemokine receptor insertion comprises AAVS1, CCR5, ROSA26, collagen, HTRP, H11, PH12, GAPDH, RUNX1, B2M, TAP1, TAP2, Tapasin, NLRC5, CIITA, RFXANK, RFX5, RFXAP, TCR, NKG2A, NKG2D, CD38, CD25, CD69, CD44, CD58, CD54, CD56, CD71, CIS, CBL-B, SOCS2, PD1, CTLA4, LAG3, TIM3, or TIGIT. In one embodiment, the selected locus for C-X-C motif chemokine receptor insertion is the TCR locus. In one embodiment, the selected locus for C-X-C motif chemokine receptor insertion is the CD38 locus.

[000142] In some embodiments, the C-X-C motif chemokine receptor is co-expressed with one or more exogenous polynucleotides encoding a polypeptide of interest through separate expression constructs, or a single bi- or tri- cistronic expression cassette. In some embodiments, the single bi- or tri- cistronic expression cassette comprising the C-X-C motif chemokine receptor and one or more exogenous polynucleotides encoding a polypeptide of interest comprises a 2A sequence, such that the C-X-C motif chemokine receptor and the additional polynucleotide(s) are in a single open reading frame (ORF). The bi-cistronic design allows coordinated expression of multiple polynucleotides both in timing and quantity, and under the same control mechanism that may be chosen to incorporate, for example, an inducible promoter for the expression of the single ORF. Self-cleaving peptides are found in members of the Picornaviridae virus family, including aphthoviruses such as foot-and-mouth disease virus (FMDV), equine rhinitis A virus (ERAV), Thosia asigna virus (TaV) and porcine tescho virus- 1 (PTV-I) (Donnelly, ML, et al, J. Gen. Virol, 82, 1027-101 (2001); Ryan, MD, et al., J. Gen. Virol., 72, 2727-2732 (2001)), and cardioviruses such as Theilovirus (e.g., Theiler's murine encephalomyelitis) and encephalomyocarditis viruses. The 2A peptides derived from FMDV, ERAV, PTV-I, and TaV are

sometimes also referred to as “F2A”, “E2A”, “P2A”, and “T2A”, respectively. In some embodiments, the exogenous polynucleotides that could be co-expressed with the C-X-C motif chemokine receptor encode one or more polypeptides comprising a CAR, a CD16 or a variant thereof, a cytokine, a cytokine receptor, a cytokine signaling complex, a chimeric fusion receptor, a chimeric Fc receptor, an engager, a checkpoint inhibitor, an Fc receptor, or an antibody or functional variant or fragment thereof. In one embodiment, the exogenous polynucleotides that are co-expressed with the C-X-C motif chemokine receptor in a bi-cistronic cassette do not encode a CAR. In one embodiment, at least one exogenous polynucleotide that is co-expressed in a bi-cistronic cassette with the C-X-C motif chemokine receptor encodes an exogenous CD16. In some embodiments, the primary-sourced or derived effector cells comprising the C-X-C motif chemokine receptor or variant thereof are T lineage cells. In some embodiments, the primary-sourced or derived effector cells comprising the C-X-C motif chemokine receptor or a variant thereof are NK lineage cells.

[000143] Additionally provided in this application is a master cell bank comprising single cell sorted and expanded clonal engineered iPSCs having at least one modification or phenotype as provided herein, including but not limited to, a C-X-C motif chemokine receptor or a variant thereof, wherein the cell bank provides clonal engineered iPSCs for additional engineering and a renewable source for manufacturing off-the-shelf, engineered, homogeneous cell therapy products, including but not limited to derivative NK and T cells, which are well-defined and uniform in composition, and can be mass produced at significant scale in a cost-effective manner.

2. Exogenously introduced TGFβ Redirector Receptor

[000144] Transforming growth factor beta (TGFβ) is a multipotent immunosuppressive cytokine with complex roles in tumorigenesis including epithelial to mesenchymal transition, angiogenesis, tumor cell motility and metastasis, cancer associated fibroblast (CAF) proliferation, and immunosuppression. TGFβ exists in its latent form in the tumor microenvironment, and is known to suppress T cell effector function, in part, through Smad-mediated downregulation of the target genes granzyme, perforin, and interferon. Furthermore, the detection of a TGFβ gene expression signature correlates with T cell exclusion from tumors and resistance to immunotherapy. One aspect of the present application provides a multi-element solid tumor targeting backbone design that incorporates a synthetic transforming growth factor beta receptor (TGFβR) signaling redirector receptor, among other editing as contemplated and described herein, to equip allogeneic effector cells, including those derived from genetically engineered iPSCs for better efficacy in tumors in general, and in solid tumors in particular. In general, a “signaling (or signal) redirector receptor” or “SRR” redirects the signaling of an

extracellular domain from a first receptor (e.g., a TGF β receptor) through an intracellular domain from a different receptor (e.g., a cytokine receptor) by joining the extracellular domain of the first receptor and intracellular domains of the different receptor. In the context of TGF β R, the signaling redirector receptor may be referred to as a “TGF β R redirector” or “TGF β R redirector receptor” or “TGF β signal redirector receptor” or “TGF β -SRR” throughout this application.

[000145] In some embodiments, iPSCs and derivative cells therefrom comprise a polynucleotide encoding a TGF β redirector receptor (TGF β -SRR), which comprises a partial or full peptide of an extracellular domain (ECD) of TGF β R. In some embodiments, the TGF β redirector receptor comprises: (i) an extracellular domain, or a fragment thereof, of transforming growth factor beta receptor (TGF β R); and (ii) an intracellular domain (ICD), or a fragment thereof, of a cytokine receptor comprising IL2R, IL12R, IL18R, IL21R, or any combination thereof.

[000146] In some embodiments, the TGF β redirector receptor comprising the ECD and ICD as described above further comprises a transmembrane domain (TM). In various embodiments, the transmembrane (TM) domain of the TGF β redirector receptor can: (i) originate from the same molecule providing the intracellular domain, (ii) originate from the same molecule providing the extracellular domain, or (iii) may be modified or replaced with a transmembrane domain of any other membrane bound proteins. In some embodiments, the cytokine receptor providing an intracellular domain or a fragment thereof of the TGF β redirector receptor comprises at least one of an IL2R (e.g., IL2R β), IL4R, IL6R, IL7R, IL9R, IL10R, IL11R, IL12R (e.g., IL12R β), IL15R, IL18R (e.g., IL18R β), and IL21R (e.g., IL21R β).

[000147] In some embodiments, the extracellular domain (ECD) of TGF β R comprises an amino acid sequence having at least about 85%, about 90%, about 95%, about 96%, about 97%, about 98%, or about 99% identity to SEQ ID NO: 3. In some embodiments, the extracellular domain (ECD) of TGF β R comprises an amino acid sequence having at least about 90% identity to SEQ ID NO: 3. In some embodiments, the extracellular domain (ECD) of TGF β R comprises an amino acid sequence having at least about 95% identity to SEQ ID NO: 3. In some embodiments, the extracellular domain (ECD) of TGF β R comprises the amino acid sequence of SEQ ID NO: 3. In some embodiments, the intracellular domain (ICD) of IL2R β comprises an amino acid sequence having at least about 85%, about 90%, about 95%, about 96%, about 97%, about 98%, or about 99% identity to SEQ ID NO: 4. In some embodiments, the intracellular domain (ICD) of IL2R β comprises an amino acid sequence having at least about 90% identity to SEQ ID NO: 4. In some embodiments, the intracellular domain (ICD) of IL2R β comprises an amino acid sequence having at least about 95% identity to SEQ ID NO: 4. In some embodiments, the intracellular domain (ICD) of IL2R β comprises the amino acid sequence of

SEQ ID NO: 4. In some embodiments, the intracellular domain (ICD) of IL12R β comprises an amino acid sequence having at least about 85%, about 90%, about 95%, about 96%, about 97%, about 98%, or about 99% identity to SEQ ID NO: 5. In some embodiments, the intracellular domain (ICD) of IL12R β comprises an amino acid sequence having at least about 90% identity to SEQ ID NO: 5. In some embodiments, the intracellular domain (ICD) of IL12R β comprises an amino acid sequence having at least about 95% identity to SEQ ID NO: 5. In some embodiments, the intracellular domain (ICD) of IL12R β comprises the amino acid sequence of SEQ ID NO: 5. In some embodiments, a fragment of the intracellular domain of IL12R β comprises an amino acid sequence having at least about 85%, about 90%, about 95%, about 96%, about 97%, about 98%, or about 99% identity to SEQ ID NO: 6. In some embodiments, a fragment of the intracellular domain of IL12R β comprises an amino acid sequence having at least about 90% identity to SEQ ID NO: 6. In some embodiments, a fragment of the intracellular domain of IL12R β comprises an amino acid sequence having at least about 95% identity to SEQ ID NO: 6. In some embodiments, a fragment of the intracellular domain of IL12R β comprises the amino acid sequence of SEQ ID NO: 6. In some embodiments, the intracellular domain (ICD) of IL18R β comprises an amino acid sequence having at least about 85%, about 90%, about 95%, about 96%, about 97%, about 98%, or about 99% identity to SEQ ID NO: 7. In some embodiments, the intracellular domain (ICD) of IL18R β comprises an amino acid sequence having at least about 90% identity to SEQ ID NO: 7. In some embodiments, the intracellular domain (ICD) of IL18R β comprises an amino acid sequence having at least about 95% identity to SEQ ID NO: 7. In some embodiments, the intracellular domain (ICD) of IL18R β comprises the amino acid sequence of SEQ ID NO: 7. In some embodiments, the intracellular domain (ICD) of IL21R β comprises an amino acid sequence having at least about 85%, about 90%, about 95%, about 96%, about 97%, about 98%, or about 99% identity to SEQ ID NO: 8. In some embodiments, the intracellular domain (ICD) of IL21R β comprises an amino acid sequence having at least about 90% identity to SEQ ID NO: 8. In some embodiments, the intracellular domain (ICD) of IL21R β comprises an amino acid sequence having at least about 95% identity to SEQ ID NO: 8. In some embodiments, the intracellular domain (ICD) of IL21R β comprises the amino acid sequence of SEQ ID NO: 8.

SEQ ID NO: 3

TIPPHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSITSICEKPQEVCAVWRKND
ENITLETVCHDKPLPYHDFILEDAAAPKICIMKEKKKPGETFFMCSCSSDECNDNIIIFSEEYNTSNPDLLL
VIFQ
(ECD of TGF β R)

SEQ ID NO: 4

NCRNTGFWLKKVLKCNTPDPSKFFSQLSSEHGGDVQKWLSSPFPSSSFSPGGLAPEISPLEVLERDKVTQ
 LLLQQDKVPEPASLSSNHSLSCTFTNQGYFFFHLPDALEIEACQVYFTYDPYSEEDPDDEGVAGAPTGSSP
 QPLQPLSGEDDAYCTFPSRDDLLLFSPSLLGGPSPPSTAPGGSGAGEERMPPSLQERVPRDWDQPPLGPP
 TPGVPDLVDFQPPPELVREAGEEVPDAGPREGVSFPWSRPPGQGEFRALNARLPLNTDAYLSLQELQGQ
 DPTHLV
 (ICD of IL2R β)

SEQ ID NO: 5

HYFQQKVFLAALRPQWCSREIPDPANSTCAKKYPIAEKTKQLPLDRLLIDWPTPEDPEPLVISEVLHQ
 VTPVFRHPPCSNWPQREKGIQGHQASEKMMHSASSPPPPRALQAESRQLVDLYKVLESRGSDPKPENPA
 CPWTVLPAGDLPTHGDLPSNIDDLPSHEAPLADSLEELEPQHISLSVFPSSSLHPLTFSCGDKLTLDQL
 KMRCDLSML
 (ICD of IL12R β)

SEQ ID NO: 6

SDPKPENPACPWTVLPAGDLPTHGDLPSNIDDLPSHEAPLADSLEELEPQ
 (an ICD fragment of IL12R β)

SEQ ID NO: 7

YRVDLVLFYRHLTRRDETLDGKTYDAFVSYLKECRPENGEHTFAVEILPRVLEKHFYKLCIFERDVV
 PGGAUVDEIHSLEKSRRLIIVLSKSYMSNEVRYELESGLHEALVERKIKIILIEFTPVTDFTFLPQSLK
 LLKSHRVLKWKADKSLSYNSRFWKNLLYLMPAKTVKPRDEPEVLPVLSSES
 (ICD of IL8R β)

SEQ ID NO: 8

SLKTHPLWRLWKKIWAVPSPERFFMPPLYKGCSDGFKKWVGAPFTGSSLELGPWSPEVPSTLEVYSCHPPR
 SPAKRLQLTELQEPALVESDGVKPSFWPTAQNSGGSAYSEERDRPYGLVSIIDTVTVLDAEGPCTWPCS
 CEDDGYPALDLDAGLEPSPGLEDPDLLDAGTTVLSCGCVSAGSPGLGGPLGSLLDRLKPPLADGEDWAGGL
 PWGGRSPGGVSESEAGSPLAGLMDTFDGFGVSDCSSPVECDFTSPGDEGPPRSILRQWVVIPLPLSSP
 GPQAS
 (ICD of IL21R β)

[000148] In some embodiments, the signaling receptor comprises an extracellular domain or a fragment thereof of TGF β R and an intracellular domain or a fragment thereof of the cytokine receptor IL2R β , thereby forming a TGF β R2-IL2R β signaling redirector receptor. In some embodiments, the signaling receptor comprises an extracellular domain or a fragment thereof of

TGF β R and an intracellular domain or a fragment thereof of the cytokine receptor IL12R β , thereby forming a TGF β R2-IL12R β signaling redirector receptor. In some embodiments, the signaling receptor comprises an extracellular domain or a fragment thereof of TGF β R and an intracellular domain or a fragment thereof of the cytokine receptor IL18R β , thereby forming a TGF β R2-IL18R β signaling redirector receptor. In some embodiments, the signaling receptor comprises an extracellular domain or a fragment thereof of TGF β R and an intracellular domain or a fragment thereof of the cytokine receptor IL21R, thereby forming a TGF β R2-IL21R signaling redirector receptor.

[000149] In some embodiments, TGF β R2-IL12R β signaling redirector receptor comprises an amino acid sequence having sequence identity of at least 80%, 85%, 90%, 95%, or 97%, 98%, or 99% to a sequence represented by SEQ ID NO: 9 (termed specifically as TGF β R2-trIL12R β throughout the application). In some embodiments, TGF β R2-IL12R β signaling redirector receptor comprises an amino acid sequence having sequence identity of at least 90% to SEQ ID NO: 9. In some embodiments, TGF β R2-IL12R β signaling redirector receptor comprises an amino acid sequence having sequence identity of at least 95% to SEQ ID NO: 9. In some embodiments, TGF β R2-IL12R β signaling redirector receptor comprises the amino acid sequence of SEQ ID NO: 9. In some embodiments, the transmembrane domain (TM) sequence represented by SEQ ID NO: 10 that is comprised within SEQ ID NO: 9 may vary in sequence or in length, or may even be replaced with a transmembrane domain of another transmembrane protein.

SEQ ID NO: 9

TIPPHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSITSICEKPQEVCAVWRKND
ENITLETVCHDPKLPYHDFILEDAAAPKCIMKEKKKPGETFFMCSCSSDECNDNIIIFSEYNTSNPDL
LLLVIFQ**VTGISLLPPLGVAISVIIIFYCYRVN**SDPKPENPACPWTVLPAAGDLPTHGGLPSNIDDLPSHEAP
LADSLLEELEPQ
(TGF β R2-~~TM~~-trIL12R β)

SEQ ID NO: 10

VTGISLLPPLGVAISVIIIFYCYRVN
(exemplary and variable portion of TGF β R2-trIL12R β)

[000150] As such, in various embodiments, any of the TGF β -SRRs provided herein may be introduced to iPSCs using one or more of the construct designs described above, and to their derivative cells upon iPSC differentiation. In addition to an induced pluripotent cell (iPSC), a

clonal iPSC, a clonal iPS cell line, or iPSC-derived cells comprising at least one engineered modality as disclosed herein are provided. Also provided is a master cell bank comprising single cell sorted and expanded clonal engineered iPSCs having at least a TGF β -SRR as described in this section, wherein the cell bank provides a platform for additional iPSC engineering and a renewable source for manufacturing off-the-shelf, engineered, homogeneous cell therapy products, which are well-defined and uniform in composition, and can be mass produced at a significant scale in a cost-effective manner.

[000151] Accordingly, in some embodiments, the present invention provides immune cells, iPSCs, and iPSC derived cells comprising a solid tumor targeting backbone comprising a polynucleotide encoding a TGF β redirector receptor (“TGF β -SRR” in Table 4), among other genetic modalities, wherein the cells, such as derivative T and NK cells, are useful for overcoming or reducing tumor microenvironment suppression associated with a tumor, and particularly, a solid tumor. In some embodiments, the iPSC and derivative cells thereof comprise a solid tumor targeting backbone comprising two or more of: a polynucleotide encoding a C-X-C motif chemokine receptor or a variant thereof, a polynucleotide encoding a TGF β redirector receptor, and and/or one or more additional genomic edits as described herein, without adversely impacting the differentiation potential of the iPSC and function of the derived effector cells, such as derivative T and NK cells.

[000152] Also provided is a master cell bank comprising single cell sorted and expanded clonal engineered iPSCs having at least an exogenously introduced polynucleotide encoding a TGF β redirector receptor, and optionally a polynucleotide encoding a C-X-C motif chemokine receptor or a variant thereof, wherein the cell bank provides a platform for additional iPSC engineering and a renewable source for manufacturing off-the-shelf, engineered, homogeneous cell therapy products, which are well-defined and uniform in composition, and can be mass produced at a significant scale in a cost-effective manner.

3. *CD16 knock-in*

[000153] CD16 has been identified as two isoforms, Fc receptors Fc γ RIIIa (CD16a; NM_000569.6) and Fc γ RIIIb (CD16b; NM_000570.4). CD16a is a transmembrane protein expressed by NK cells, which binds monomeric IgG attached to target cells to activate NK cells and facilitate antibody-dependent cell-mediated cytotoxicity (ADCC). CD16b is exclusively expressed by human neutrophils. “High affinity CD16,” “non-cleavable CD16,” or “high affinity non-cleavable CD16” (abbreviated as hnCD16), as used herein, refers to various CD16 variants. The wildtype CD16 has low affinity and is subject to ectodomain shedding, a proteolytic cleavage process that regulates cell surface density of various cell surface molecules on

leukocytes upon NK cell activation. F176V (also called F158V in some publications) is an exemplary CD16 polymorphic variant having high affinity; whereas S197P variant is an example of genetically engineered non-cleavable version of CD16. An engineered CD16 variant comprising both F176V and S197P has high affinity and is non-cleavable, which was described in greater detail in WO2015/148926, the complete disclosure of which is incorporated herein by reference. In addition, a chimeric CD16 receptor with the ectodomain of CD16 essentially replaced with at least a portion of CD64 ectodomain can also achieve the desired high affinity and non-cleavable features of a CD16 receptor capable of carrying out ADCC. In some embodiments, the replacement ectodomain of a chimeric CD16 comprises one or more of EC1, EC2, and EC3 exons of CD64 (UniPRotKB_P12314 or its isoform or polymorphic variant).

[000154] As such, various embodiments of an exogenous CD16 introduced to a cell include functional CD16 variants and chimeric receptors thereof. In some embodiments, the functional CD16 variant is a high-affinity non-cleavable CD16 receptor (hnCD16). An hnCD16, in some embodiments, comprises both F176V and S197P; and in some embodiments, comprises F176V and with the cleavage region eliminated. In some embodiments, an hnCD16 comprises a sequence having identity of at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 99%, 100%, or any percentage in-between, when compared to any of the exemplary sequences, SEQ ID NOs. 11, 12 and 13, and each comprises at least a portion of CD64 ectodomain. In some embodiments, the hnCD16 comprises an amino acid sequence of at least 90% identity to any of SEQ ID NOs. 11-13, and optionally one or more of F176V, S197P, and at least a portion of CD64 ectodomain. In some embodiments, the hnCD16 comprises an amino acid sequence of at least 95% identity to any of SEQ ID NOs. 11-13, and optionally one or more of F176V, S197P, and at least a portion of CD64 ectodomain. In some embodiments, the hnCD16 comprises the amino acid sequence of SEQ ID NO 11. In some embodiments, the hnCD16 comprises the amino acid sequence of SEQ ID NO 12. In some embodiments, the hnCD16 comprises the amino acid sequence of SEQ ID NO 13.

SEQ ID NO: 11

MWFLTTLLLLWVPVDGQVDTTKAVITLQPPWVSFQEETVTLHCEVLHLPGSSSTQWFLNGTATQTSTPSY
RITSASVNDSGEYRCQRGLSGRSDPIQLEIHRGWLLQVSSRVFTEGEPLALRCHAWKDKLVYNVLYYRN
GKAFKFFHWNNSNLTILKTNISHNGTYHCSGMGKHRYTSAGISVTVKELFPAPVLNASVTSPLLEGNLVTL
SCETKLLQRPGLQLYFSFYMGSKTLRGRNTSSEYQILTARREDSGLYWCEAATEDGNVLKRSPELELQV
LGLQLPTPVWFHYQVSFCLVMVLLFAVDTGLYFSVKTNIRSSSTRDWKDHKFKWRKDPQDK
 (340 a.a. CD64 domain-based construction; CD16TM; CD16^{ICD})

SEQ ID NO: 12

MWFLTTLLWVPVDGQVDTTKAVITLQPPWVSVFQEETVTLHCEVLHLPGSSSTQWFLNGTATQTSTPSY
RITSASVNDSGEYRCQRGLSGRSDPIQLEIHRGWLLLQVSSRVFTEGEPLALRCHAWKDKLVYNVLYYRN
GKAFKFFHWSNLTILKTNISHNGTYHCSGMGKHRYTSAGISVTVKELFPAPVLNASVTSPLLEGNLVTL
SCETKLLLQRPGLQLYFSFYMGSKTLRGRNTSSEYQILTARREDSGLYWCEAATEDGNVLKRSPELELQV
LGLFFPPGYQVSFCLVMVLLFAVDTGLYFSVKTNIRSSSTRDWKDHKFKWRKDPQDK
 (336 a.a. CD64 exon-based construction; CD16TM; CD16ICD)

SEQ ID NO: 13

MWFLTTLLWVPVDGQVDTTKAVITLQPPWVSVFQEETVTLHCEVLHLPGSSSTQWFLNGTATQTSTPSY
RITSASVNDSGEYRCQRGLSGRSDPIQLEIHRGWLLLQVSSRVFTEGEPLALRCHAWKDKLVYNVLYYRN
GKAFKFFHWSNLTILKTNISHNGTYHCSGMGKHRYTSAGISVTVKELFPAPVLNASVTSPLLEGNLVTL
SCETKLLLQRPGLQLYFSFYMGSKTLRGRNTSSEYQILTARREDSGLYWCEAATEDGNVLKRSPELELQV
LGLFFPPGYQVSFCLVMVLLFAVDTGLYFSVKTNIRSSSTRDWKDHKFKWRKDPQDK
 (335 a.a. CD64 exon-based construction; CD16TM; CD16ICD)

[000155] Accordingly, provided herein are effector cells or iPSCs genetically engineered to comprise a solid tumor targeting backbone that comprises, among other editing as contemplated and described herein, an exogenous CD16 or a variant thereof, wherein the effector cells are cells from primary sources or derived from iPSC differentiation, or wherein the genetically engineered iPSCs are capable of differentiating into derived effector cells comprising the exogenous CD16 or a variant thereof introduced to the iPSCs. In some embodiments, the exogenous CD16 is a high-affinity non-cleavable CD16 receptor (hnCD16). In some embodiments, the exogenous CD16 comprises at least a portion of the CD64 ectodomain. In some embodiments, the exogenous CD16 is in a form of a CD16-based chimeric Fc receptor (CFcR) that comprises a transmembrane domain, a stimulatory domain and/or a signaling domain that is not derived from CD16.

[000156] In some embodiments, the primary-sourced or derived effector cells comprising the exogenous CD16 or variant thereof are NK lineage cells. In some embodiments, the primary-sourced or derived effector cells comprising the exogenous CD16 or variant thereof are T lineage cells. In some embodiments, the exogenous CD16 or functional variants thereof comprised in iPSC or effector cells has high affinity in binding to a ligand that triggers downstream signaling upon such binding. Non-limiting examples of ligands binding to the exogenous CD16 or functional variants thereof include not only ADCC antibodies or fragments thereof, but also to bi-, tri-, or multi- specific engagers or binders that recognize the CD16 or CD64 extracellular binding domains of the exogenous CD16. Examples of bi-, tri-, or multi- specific engagers or binders are further described below in this application. As such, at least one of the aspects of the present application provides a derivative effector cell comprising a solid tumor targeting

backbone, or a cell population thereof, preloaded with one or more pre-selected ADCC antibodies through an exogenous CD16 expressed on the derivative effector cell, in an amount sufficient for therapeutic use in a treatment of a condition, a disease, or an infection as further detailed in this application, wherein the exogenous CD16 comprises an extracellular binding domain of CD64, or of a CD16 having F176V and S197P.

[000157] In some other embodiments, an exogenous CD16 comprises a CD16-, or variants thereof, based CFcR. A chimeric Fc receptor (CFcR) is produced to comprise a non-native transmembrane domain, a non-native stimulatory domain and/or a non-native signaling domain by modifying or replacing the native CD16 transmembrane- and/or the intracellular-domain. The term “non-native” used herein means that the transmembrane, stimulatory or signaling domain are derived from a different receptor other than the receptor which provides the extracellular domain.

[000158] The various embodiments of the CD16-based chimeric Fc receptor as described above are capable of binding, with high affinity, to the Fc region of an antibody or fragment thereof; or to a bi-, tri-, or multi- specific engager or binder. Upon binding, the stimulatory and/or signaling domains of the chimeric receptor enable the activation and cytokine secretion of the effector cells, and the killing of the tumor cells targeted by the antibody, or the bi-, tri-, or multi- specific engager or binder having a tumor antigen binding component as well as the Fc region. Without being limited by theory, through the non-native transmembrane, stimulatory and/or signaling domains, or through an engager binding to the ectodomain, of the CD16-based chimeric Fc receptor, the CFcR could contribute to effector cells' killing ability while increasing the effector cells' proliferation and/or expansion potential. The antibody and the engager can bring tumor cells expressing the antigen and the effector cells expressing the CFcR into close proximity, which also contributes to the enhanced killing of the tumor cells. .

[000159] In some embodiments, the present disclosure also provides a derivative NK or T cell comprising a solid tumor targeting backbone, or a cell population thereof, preloaded with one or more pre-selected ADCC antibodies in an amount sufficient for therapeutic use in a treatment of a condition, a disease, or an infection as further detailed in this application. In some embodiments, the preloaded CD38 antibody is daratumumab. In some embodiments, the derived NK or T cells comprise a solid tumor targeting backbone comprising a TCR promoter-driven ADR and/or a cytokine, and a constitutively expressed TCE, in addition to edits comprising a CAR, a TGF β -SRR, a C-X-C-motif chemokine receptor or a variant thereof, a CD16 variant, and a CD38 knockout, among other edits as provided herein. In some embodiments, said derived NK or T cells are preloaded with one or more of an anti-HER2 antibody (e.g., trastuzumab,

pertuzumab), an anti-EGFR antibody (e.g., cetuximab), or an anti-PDL1 antibody (e.g., avelumab).

[000160] As provided further, the cell or population thereof, comprising the solid tumor targeting backbone, and optionally a CAR, and an exogenous CD16 or a variant thereof (“CD16^{exo}” in Table 4), may further comprise one or more additional engineered modalities described herein, and/or as shown in Table 4. Additionally provided in this application is a master cell bank comprising single cell sorted and expanded clonal engineered iPSCs having at least one phenotype as provided herein, including but not limited to, a solid tumor targeting backbone comprising, among other genetic modalities, an exogenous CD16 or a variant thereof, wherein the cell bank provides a platform for additional iPSC engineering and a renewable source for manufacturing off-the-shelf, engineered, homogeneous cell therapy products, including but not limited to derivative NK and T cells, which are well-defined and uniform in composition, and can be mass produced at significant scale in a cost-effective manner.

4. Allo-Immune Defense Receptor (ADR) expression

[000161] Unwanted activation of T- and NK- cells often promotes allo-immune reactions leading to development of graft-versus-host disease (GvHD). Although some steps may be taken to reduce the reactivity of allogeneic cells in the recipient individual, such cells would still be targeted by the immune system of the recipient (primarily T- and NK-cells), which would recognize them as foreign leading to rejection and limiting therapeutic benefit. On the other hand, modulating a host immune system to reduce allo-immune reactions, for example, by lympho-conditioning using chemotherapy such as Cy/Flu (cyclophosphamide/fludarabine) often leads to associated hematologic toxicities, including increased susceptibility to severe infections, due to indiscriminate lymphodepletion and a severely compromised immune system as a result. To control pathogenic conditions due to unwanted activation of the immune system, in various embodiments, the present application provides a solid tumor targeting backbone comprising an allo-immune defense receptor (ADR), among other components. Another aspect of the application provides immune cells, iPSCs, and iPSC-derived effector cells that are genetically engineered to comprise, among other editing as contemplated and described herein, a 4-1BB or CD38 specific allo-immune defense receptor (ADR) for effector cell potentiation as well as selective depletion of alloreactive host NK cells and T cells with upregulated 4-1BB and/or CD38 expression, the latter of which include pathogenic T cells, and regulatory T cells, while sparing resting cells in the recipient.

[000162] In some embodiments of the ADR that is specific to 4-1BB (CD137, also referred to as “41BB”), the ADR comprises an extracellular domain that targets 4-1BB upregulated on

host T or NK cells when they are activated, and a signaling domain promoting effector cell activation. For example, the 41BB-ADR extracellular domain may comprise any suitable ligand for 4-1BB, including 4-1BBL, an antibody (or functional fragment thereof) that targets 4-1BB, a fusion of Fc with 4-1BBL, or functional derivatives or fragments thereof. In some embodiments, the 41BB-ADR extracellular domain comprises 4-1BBL, or a fragment thereof effective to bind 4-1BB. In some embodiments, the 41BB-ADR extracellular domain comprises an amino acid sequence with at least about 85%, about 90%, about 95%, about 96%, about 97%, about 98%, or about 99% sequence identity to SEQ ID NO: 14. In some embodiments, the 41BB-ADR extracellular domain comprises an amino acid sequence with at least about 90% sequence identity to SEQ ID NO: 14. In some embodiments, the 41BB-ADR extracellular domain comprises an amino acid sequence with at least about 95% sequence identity to SEQ ID NO: 14. In some embodiments, the 41BB-ADR extracellular domain comprises the amino acid sequence of SEQ ID NO: 14.

SEQ ID NO: 14

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GLLDLRQGMFAQLVAQNVLIDGPLSWYSDPGLAGVSLTGGLSYKEDTKELVVAKAGVYYVFFQLELRRV
VAGEGSGSVSLALHLQPLRSAAGAAALALTVDLPPASSEARNSAFGFQGRLLHLSAGQRLGVHLHTEARA
RHAWQLTQGATVLGLFRVTPEIPAGLPSPRSE
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[000163] In one embodiment of the CD38 specific ADR, the CD38-ADR comprises an extracellular domain comprising a CD38 binding domain or fragments thereof. In some embodiments, the CD38 binding domain or fragment thereof is from an anti-CD38 antibody. In some embodiments, the anti-CD38 antibody comprises a murine antibody, a human antibody, a humanized antibody, a camel Ig, a single variable new antigen receptor (VNAR), a shark heavy-chain-only antibody (Ig NAR), a chimeric antibody, a recombinant antibody, or an antibody fragment thereof. Non-limiting examples of antibody binding domain or fragments thereof include Fab, Fab', F(ab')₂, F(ab')₃, Fv, single chain antigen binding fragment (scFv), (scFv)₂, disulfide stabilized Fv (dsFv), minibody, diabody, triabody, tetrabody, single-domain antigen binding fragments (sdAb, Nanobody), recombinant heavy-chain-only antibody (VHH), and other antibody fragments that maintain the binding specificity of the whole antibody.

[000164] In some embodiments, the CD38 binding domain or fragments thereof comprised in the CD38-ADR comprises a variable region of the heavy chain and/or a variable region of the light chain represented by an amino acid sequence that is of at least about 99%, about 98%, about 96%, about 95%, about 90%, about 85%, and/or at least about 80% identity to SEQ ID NOs: 15 and 16 respectively, SEQ ID NOs: 17 and 18 respectively, SEQ ID NOs: 19 and 20

respectively, SEQ ID NOs: 21 and 22 respectively, SEQ ID NOs: 23 and 24 respectively, SEQ ID NOs: 25 and 26 respectively, SEQ ID NOs: 27 and 28 respectively, SEQ ID NOs: 29 and 30 respectively, SEQ ID NOs: 31 and 32 respectively, SEQ ID NOs: 33 and 34 respectively, SEQ ID NOs: 35 and 36 respectively, SEQ ID NOs: 37 and 38 respectively, SEQ ID NOs: 39 and 40 respectively, SEQ ID NOs: 41 and 42 respectively, SEQ ID NOs: 43 and 44 respectively, SEQ ID NOs: 45 and 46 respectively, SEQ ID NOs: 47 and 48 respectively, SEQ ID NOs: 49 and 50 respectively, SEQ ID NOs: 51 and 52 respectively, SEQ ID NOs: 53 and 54 respectively, SEQ ID NOs: 55 and 56 respectively, or SEQ ID NOs: 57 and 58 respectively,. Selected VH and VL sequences of exemplary CD38 binding domains are provided in Table 1A as numbered pairs 1-23. In some embodiments, the CD38-ADR extracellular domain comprises an amino acid sequence with at least about 90% sequence identity to the VH and/or VL sequence of any of pairs 1-23 in Table 1A. In some embodiments, the CD38-ADR extracellular domain comprises an amino acid sequence with at least about 95% sequence identity to the VH and/or VL sequence of any of pairs 1-23 in Table 1A. In some embodiments, the CD38-ADR extracellular domain comprises the amino acid sequence of the VH and/or VL sequence of any of pairs 1-23 in Table 1A.

Table 1A

	VH	VL
1	QVQLKQSGPSLVQPSQRLSITCTVSGFSLISY GVHWVRQSPGKGLEWLGVIWRGGSTDYNA AFMSRLSITKDNSKSQVFFKMNSLQADDTAI YFCAKTLITTGYAMDYWGQGTSTVTVSS (SEQ ID NO: 15)	DIQLTQSSSFVSVSLGDRVTTITCKASEDI YNRLAWYQQKPGNAPRLISGATSLET GVPSRFGSGSGSKDYTLSTLSLQTEDVA TYYCQQYWSTPTFGGGTKLEIK (SEQ ID NO: 16)
2	QVELVESGGSLKLSCAASGDFSRWMNW VRQAPGKGLEWIGEINPDSTINYTTSLKDK FIISRDNAKNTLYLQMTKVRSEDALYYCA RYGNWFPYWGQGTSLTVTVSS (SEQ ID NO: 17)	DILMTQSQKIMPTSVGDRVSVTCKASQ NVDTNVAWYQQKPGQSPKALIYSASY RYSQVPDRFTGSGSGTDFTLTITNVQSE DLAEYFCQQYDSYPLTFGAGTKLCLK (SEQ ID NO: 18)
3	QVQLKQSGPGLVHPSQSLSITCTVSGFSLTSY GVHWVRQSPGKGLEWLGVMWRGGSTDYN AAFMSRLNITKDNSKRQVFFKMNSLQADDT AIYYCAKSMITTGFVMDSWGQGTSTVTVSS (SEQ ID NO: 19)	DIQLTQSPSSFVSVSLGDRVTTITCKASEDI YNRLTWYQQKPGNAPRLISGATSLET GVPSRFGSGSGSKDYTLSTLSLQTEDVA TYYCQQYWSNPYTFGGGKLEIR (SEQ ID NO: 20)
4	QVQLVESGGGLVQPGGSLRLSCAASGFTFSS YYMNWVRQAPGKGLEWVSGISGDPSTYY ADSVKGRFTISRDNKNTLYLQMNSLRAED TAVYYCARDLPLVYTGFAIWGQGTSLTVS S (SEQ ID NO: 21)	DIELTQPPSVSVAPGQTARISCSGDNLR HYVYVYVYQQKPGQAPVLVIYGDSKRP SGIPERFSGSNSGNTATLTISGTQAEDE ADYYCQTYTGASLVFGGGTKLTVL (SEQ ID NO: 22)
5	QVQLVQSGAEVKKPGASVKVSKASGYTFT SYSINWVRQAPGQGLEWMGYIDPNRGNTN YAQKFQGRVTMTRDTSISTAYMELSSLRSE	DIVMTQSPLSLPVTGPGEPAISCRSSQSL LFIDGNLYLNWYLQKPGQSPQLLIYLG SNRASGVDPDRFSGSGSGTDFTLKISRVE

	DTAVYYCAREYIYFIHGMLDFWGQGTLVTVSS (SEQ ID NO: 23)	AEDVGVYYCQQYSSKSATFGQGTKVEIK (SEQ ID NO: 24)
6	QVQLVESGGGLVQPGGSLRLSCAASGFTFSNYGMHWVRQAPGKGLEWVSNIRSDGSWTYYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCARRYWSKSHASVTDYWGQGTLVTVSS (SEQ ID NO: 25)	DIQMTQSPSSLSASVGDRTITCRASQDISAFLNWWYQQKPGKAPKLLIYKVSNLQSGVPSRFSGSGSGTDFTLTISLQPEDFATYYCQQAYSGSITFGQGTKVEIK (SEQ ID NO: 26)
7	EVQLLES GGGLVQP GGSLRLSCAASGFTFDDYGM SWVRQAPGKGLEWVSDISWNGGKTHYVDSVKGQFTISRDN SKNTLYLQMNSLRAEDTAVYYCARGSLFHDSSGFYFGHWGQGTLVTVSS (SEQ ID NO: 27)	QSVLTQPPSASGTPGQRVTISCSGSSSNI GDNYVSWYQQLPGTAPKLLIYRDSQRP SGVPDRFSGSGSGTSASLAISGLRSEDE ADYYCQSYDSSLGSGSVFGGGTKLTVL (SEQ ID NO: 28)
8	EVQLLES GGGLVQP GGSLRLSCAASGFTFNNYDMTWVRQAPGKGLEWVAVISYDGSDDKYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCARVYYGFGSGPSMDVWGQGTLVTVSS (SEQ ID NO: 29)	QSVLTQPPSASGTPGQRVTISCSGSSSNI GSNTVNWYQQLPGTAPKLLIYSDSNRP SGVPDRFSGSGSGTSASLAISGLRSEDE ADYYCQSYDSSLGSGSRVFGGGTKLTVL (SEQ ID NO: 30)
10	EVQLLES GGGLVQP GGSLRLSCAASGFTFSSYGMNWVRQAPGKGLEWVSGISGSGGSTYYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCAKDSNYDFWSGYYYGMDVWGQGTLVTVSS (SEQ ID NO: 31)	QSVLTQPPSASGTPGQRVTISCSGSSSNI GSKTVSWYQQLPGTAPKLLIYDNNKRP SGVPDRFSGSGSGTSASLAISGLRSEDE ADYYCSSY AARSTNIIFGGGTKLTVL (SEQ ID NO: 32)
11	EVQLLES GGGLVQP GGSLRLSCAASGFTFSSYGMHWVRQAPGKGLEWVSRINSDGSSTSYADSMKGQFTISRDN SKNTLYLQMNSLRAEDTAVYYCARGGYYYAMDVWGQGTLVTVSS (SEQ ID NO: 33)	QSVLTQPPSASGTPGQRVTISCSGSSSNI GYKTVNWYQQLPGTAPKLLIYDNNKR PSGVPDRFSGSGSGTSASLAISGLRSEDEADYYCAAWDDSLNGLVFGGGTKLTVL (SEQ ID NO: 34)
12	QIQLVQSGPELKKPGETVKISCKASGYTLTSYGMNWVKQAPGKGLKWMGWINTYTGEPTYADDFKGRFAFSLETSASTAFLQINN LKNE DTATYFCVRRGFAYWGQGTLVTVSA (SEQ ID NO: 35)	NIVLTQSPASLAVSLGQRATISCRASESVEIYGNGFMNWFQQKPGQPPKLLIYRASNLESGIPARFSGSGSRTEFTLTIDPVEADDVATYYCQQINEDPFTFGSGTKLEIK (SEQ ID NO: 36)
13	QIQLVQSGPELKKPGETVKISCKASGYTFTN SGMNWVKQAPGKGLKWMGWINTYTGEPTYADDFKGRFAFSLETSASSAYLQISNLKNE DTATYFCARRGFVYWQGT LVTVSA (SEQ ID NO: 37)	DIVLTQSPASLAVSLGQRATISCRASESVAIYGNSFLKWFQQKPGQPPKLLIYRASNLESGIPARFSGSGSGTDFTLTINPVEADDVATYYCQQINEDPYTFGGGTKLEIK (SEQ ID NO: 38)
14	QVQLQQSGAELARPGTSVKLSCKASGYTFTDYWMQWVKQRPGQGLEWIGTIYPGDGDTGYAQKFQKATLTADKSSKTVYMHLSSLASEDSAVYYCARGDYYGSNSLDYWGQGTSTVTVSS (SEQ ID NO: 39)	DIVMAQSHKFMSTSVGDRVSITCKASQDVSTVVAWYQQKPGQSPKRLIYSASYRYIGVPDRFTGSGSGTDFTFTISSVQAE DLAVYYCQQHYSPPYTFGGGTKLEIK (SEQ ID NO: 40)
15	NVQLVES GGGLVQP GGSRKLSAASGFTFSNFGMHWRQAPGKGLEWVAYIRSGSGTIYSDTVKGRFTISRDNPKNTLFLQMTSLRSEDTAMYYCARSYYDFGAWFAYWGQGT LVTVSA (SEQ ID NO: 41)	DIVMTQSQKFMSTSVGDRVSVTCKASQNVGTNVAWYQHKPGQSPKIMIYSASSRYSGVPDRFTGSGSGTLFTLTINNVS EDLAEYFCQQYNSYPLTFGSGTKLEIK (SEQ ID NO: 42)
16	QVQLVQSGAEVAKPGTSVKLSCKASGYTFTDYWMQWVKQRPGQGLEWIGTIYPGDGDTGYAQKFQKATLTADKSSKTVYMHLSSLASE	DIVMTQSHLSMSTSLGDPVSITCKASQDVSTVVAWYQQKPGQSPRRLIYSASYRYIGVPDRFTGSGAGTDFTFTISSVQAE

	DSAVYYCARGDYYGSNSLDYWGQGTSTVTVSS (SEQ ID NO: 43)	LAVYYCQQHYSPPYTFGGGKLEIK (SEQ ID NO: 44)
17	EVQLLESQGGGLVQPGGSLRLSCAVSGFTFNSFAMSWVRQAPGKGLEWVSAISGSGGGTYADSVKGRFTISRDNKNTLYLQMNSLRSED TAVYFCAKDILWFGEVFDYWGQGTSLVTVSS (SEQ ID NO: 45)	EIVLTQSPATLSLSPGERATLSCRASQSVSSYLAWYQQKPGQAPRLLIYDASNRATGIPARFSGSGSGTDFTLTISSELPEDF AVYYCQQRSNWPPTFGQGTKEIK (SEQ ID NO: 46)
18	QVQLVQSGAEVKKPGSSVKVSCKAFGGTFSYAIWVRQAPGQGLEWMGRIIRFLGIANYAQKFQGRVTLIADKSTNTAYMELSSLRSEDTAVYYCAGEPGERDPDAVDIWGQGTMTVTVSS (SEQ ID NO: 47)	DIQMTQSPSSLSASVGDRTITCRASQGIKRWLAWYQQKPEKAPKSLIYAASSLQSGVPSRFSGSGSGTDFTLTISSELPEDFA TYYCQQYNSYPLTFGGGKLEIK (SEQ ID NO: 48)
19	EVQLVQSGAEVKKPGESLKISCKGSGYSFSNYWIGWVRQMPGKGLEWMGIHYPHDSARYSPSFQGVTFSDAKSISTAYLQWSSLKASDTAMYYCARHVGWGSRYWYFDLWGRGTLVTVSS (SEQ ID NO: 49)	EIVLTQSPATLSLSPGERATLSCRASQSVSSYLAWYQQKPGQAPRLLIYDASNRATGIPARFSGSGSGTDFTLTISSELPEDF AVYYCQQRSNWPPTFGQGTKEIK (SEQ ID NO: 50)
20	QVQLVQSGAEVKKPGSSVKVSCKASGGTFSYAFSWVRQAPGQGLEWMGRVIPFLGIANS AQKFQGRVTITADKSTSTAYMDLSSLRSEDTAVYYCARDIAALGPFDYWGQGTSLVTVSSAS (SEQ ID NO: 51)	DIQMTQSPSSLSASVGDRTITCRASQGIKRWLAWYQQKPEKAPKSLIYAASSLQSGVPSRFSGSGSGTDFTLTISSELPEDFA TYYCQQYNSYPRTFGQGTKEIK (SEQ ID NO: 52)
21	QVQLVQSGAEVKKPGSSVKVSCKAFGGTFSYAIWVRQAPGQGLEWMGRIIRFLGKTNH AQKFQGRVTLTADKSTNTAYMELSSLRSEDTAVYYCAGEPGRDPDAVDIWGQGTMTVTVSS (SEQ ID NO: 53)	DIQMTQSPSSLSASVGDRTITCRASQGIKRWLAWYQQKPEKAPKSLIYAASSLQSGVPSRFSGSGSGTDFTLTISSELPEDFA TYYCQQYNSYPLTFGGGKLEIK (SEQ ID NO: 54)
22	QVQLVQSGAEVKKPGSSVKVSCKPSGGTFRSYAIWVRQAPGQGLEWMGRIIVFLGKVN YAQRQGRVTLTADKSTTTAYMELSSLRSEDTAVYYCTGEPGARDPDAFDIWGQGTMTVTVSS (SEQ ID NO: 55)	DIQMTQSPSSLSASVGDRTITCRASQGIKRWLAWYQQKPEKAPKSLIYAASSLQSGVPSRFSGSGSGTDFTLTISSELPEDFA TYYCQQYNNYPLTFGGGKLEIK (SEQ ID NO: 56)
23	QVQLVQSGAEVKKPGSSVKVSCKAFGGTFSYAIWVRQAPGQGLEWMGRIIRFLGIANYAQKFQGRVTLIADKSTNTAYMELSSLRSEDTAVYYCAGEPGERDPDAVDIWGQGTMTVTVSS (SEQ ID NO: 57)	DIQMTQSPSSLSASVGDRTITCRASQGISNYLAWFQQKPGKAPKSLIYAASSLQSGVPSRFSGSGSGTDFTLTISSELPEDFA TYYCQQYNSYPYTFGQGTKEIK (SEQ ID NO: 58)

[000165] In some embodiments, the extracellular domain of the 41BB-ADR or CD38-ADR may be operably linked to one or more signaling domains that mediate downstream signaling upon effector cell activation upon the binding to the 4-1BB or CD38, respectively, of alloreactive host immune cells. In particular embodiments, the ADR comprises CD3 ζ , represented by an amino acid sequence of at least about 85%, about 90%, about 95%, about 96%, about 97%, about 98%, or about 99% sequence identity to SEQ ID NO: 59 or a functional fragment thereof, or comprises a CD3 ζ derivative (for example, CD3 ζ 1XX, represented by an amino acid sequence of at least about 85%, about 90%, about 95%, about 96%, about 97%, about 98%, or about 99% sequence identity to SEQ ID NO: 60 or a functional fragment thereof). In some embodiments,

the CD3 ζ comprises an amino acid sequence of at least about 90% sequence identity to SEQ ID NO: 59. In some embodiments, the CD3 ζ comprises an amino acid sequence of at least about 95% sequence identity to SEQ ID NO: 59. In some embodiments, the CD3 ζ comprises the amino acid sequence of SEQ ID NO: 59. In some embodiments, the CD3 ζ derivative comprises an amino acid sequence of at least about 90% sequence identity to SEQ ID NO: 60. In some embodiments, the CD3 ζ derivative comprises an amino acid sequence of at least about 95% sequence identity to SEQ ID NO: 60. In some embodiments, the CD3 ζ derivative comprises the amino acid sequence of SEQ ID NO: 60. CD3 ζ mediates downstream ITAM-derived signaling during effector T or NK cell activation. Other ITAM-containing signaling domains may include those derived from DAP12, Fc receptors, and other CD3 subunits.

SEQ ID NO: 59

RVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPQRRKNPQEGLYNELQKDKMAE
AYSEIGMKGERRRGKGGHDGLYQGLSTATKDTYDALHMQALPPR
(CD3 ζ)

SEQ ID NO: 60

RVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLFNELQKDKMAEA
FSEIGMKGERRRGKGGHDGLFQGLSTATKDTFDALHMQALPPR
(CD3 ζ 1XX - containing 2 mutations in ITAM1)

[000166] In some embodiments, the intracellular domain of ADR comprising a signaling domain further comprises one, two, three, or more costimulatory domains that enhance cytokine production from the effector cells that express the ADR. The costimulatory domains may be derived from the intracellular signaling domains of costimulatory proteins including, but not limited to, CD28, CD27, 4-1BB, OX40, ICOS, CD30, HVEM, CD40, and so forth. In some embodiments the ADR comprising CD3 ζ further comprises a costimulatory domain derived from 4-1BB endodomain. In some embodiments, the endodomain is represented by an amino acid sequence of at least about 85%, about 90%, about 95%, about 96%, about 97%, about 98%, or about 99% sequence identity to SEQ ID NO: 63 or a functional fragment thereof. In some embodiments, the endodomain comprises an amino acid sequence of at least about 90% sequence identity to SEQ ID NO: 63. In some embodiments, the endodomain comprises an amino acid sequence of at least about 95% sequence identity to SEQ ID NO: 63. In some embodiments, the endo-domain comprises the amino acid sequence of SEQ ID NO: 63. In one embodiment, when the ADR comprises 4-1BBL in its extracellular domain, the costimulatory domain of the ADR is not derived from 4-1BB.

SEQ ID NO: 63

KRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCELRVKFSRSADAPAYQQGQNQLYNELNLGR
REEYDVLDKRRGRDPFEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDLGLYQGLSTATK
 DTYDALHMQALPPR
 (41BB endo-CD3ζ)

[000167] The intracellular domain of an ADR may be non-covalently linked to the extracellular domain of the ADR via a transmembrane domain. In some embodiments, the ADR comprises a transmembrane domain that may be of any kind so long as it allows the CD3ζ component of the ADR to be located intracellularly and the extracellular domain that targets 4-1BB or CD38 to be located extracellularly. In other instances, ADRs are soluble proteins that can bind to the respective ligand on activated T cells and promote cytotoxicity by crosslinking TCR (e.g., ADR-CD3 T-cell engager protein). In a case wherein the extracellular domain is from a surface protein having a transmembrane domain, (CD40, for example), the ADR may comprise the transmembrane domain from that corresponding endogenous molecule. In some embodiments in which the ADR molecule comprises one or more costimulatory domains, the transmembrane domain (TM) may be from the same endogenous molecule that has the costimulatory domain. Non limiting examples of TMs include those from CD3, CD8a, CD27, CD28, 4-1BB, OX40, and CD4.

[000168] In some embodiments, the ADR comprises a spacer between the extracellular protein and the transmembrane domain. In some embodiments, the spacer may comprise a sequence that is inert or contributes substantially little or nothing with respect to any function the ADR may have; whereas in other cases the spacer comprises a sequence that enhances a function of the ADR and/or allows it to be detectable and/or able to be targeted for inhibition. In specific embodiments, the spacer comprises an encoded protein sequence that facilitates detection of cells that express the ADR. For example, the spacer may encode an Fc region or fragments thereof that would allow for surface detection of the cells expressing the ADR, such as by using anti-Fc antibodies. In particular embodiments, the spacer provides separation between the ligand binding extracellular domain and the membrane to avoid potential steric hindrances. As understood by one skilled in the art, the spacer can vary in sequence and/or in length, whether a function other than being a physical separation is intended or not. Exemplary spacers that may be included in the ADR are commonly known in the art, including, but not limited to, IgG4 spacers, CD28 spacers, CD8 spacers, or combinations of more than one spacer. The length of the spacers may also vary, from about 15 amino acids (a.a.) to about 300 a.a. or more. Non-limiting exemplary spacer peptides include those represented by an amino acid sequence of at least about 80%, about

85%, about 90%, about 95%, about 96%, about 97%, about 98%, or about 99% identity to SEQ ID NO: 64 or 65. In some embodiments, the spacer peptide comprises an amino acid sequence of at least about 90% identity to SEQ ID NO: 64 or 65. In some embodiments, the spacer peptide comprises an amino acid sequence of at least about 95% identity to SEQ ID NO: 64 or 65. In some embodiments, the spacer peptide comprises the amino acid sequence of SEQ ID NO: 64. In some embodiments, the spacer peptide comprises the amino acid sequence of SEQ ID NO: 65.

SEQ ID NO: 64

ESKYGPPCPPCPGGGSSGGSGGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPE
NNYKTTPPVLDSDGSFFL
(88 a.a.)

SEQ ID NO: 65

ESKYGPPCPPCPGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPVL
DSDGSFFLYSKLTVDKSRWQQGNVFSQVMHEALHNAYTQKSLSLSPGKKDPK
(123 a.a. *IgG4 hinge-IgG1 CH3*)

[000169] In one embodiment of the 4-1BB specific ADR, the 41BB-ADR is represented by an amino acid sequence of at least about 85%, about 90%, about 95%, about 96%, about 97%, about 98%, or about 99% sequence identity to any of SEQ ID NOs: 66-69. In some embodiments, the 41BB-ADR comprises an amino acid sequence of at least about 90% identity to any one of SEQ ID NOs: 66-69. In some embodiments, the the 41BB-ADR comprises an amino acid sequence of at least about 95% identity to any one of SEQ ID NOs: 66-69. In some embodiments, the the 41BB-ADR comprises the amino acid sequence of SEQ ID NO: 66. In some embodiments, the the 41BB-ADR comprises the amino acid sequence of SEQ ID NO: 67. In some embodiments, the the 41BB-ADR comprises the amino acid sequence of SEQ ID NO: 68. In some embodiments, the the 41BB-ADR comprises the amino acid sequence of SEQ ID NO: 69.

SEQ ID NO: 66

MEFGLSWLFLVAILKGVQCGLLDLRQGMFAQLVAQNVLIDGPLSWYSDPGLAGVSLTGGLSYKEDTKEL
VVAKAGVYVFFQLELRRVAGEGSGSVSLALHLQPLRSAAGAAALALTVDLPPASSEARNSAFGFQGR
LHLASAGQRLGVHLHTEARARHAWQLTQGATVLGLFRVTPEIPAGLPSRSEESKYGPPCPPCPGQPREPQ
VYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRW
QQGNVFSQVMHEALHNAYTQKSLSLSPGKKDPKFWVLVVVGVLACYSLVTVAFIIFWVRSRVKFSRS

ADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMK
GERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR

Signal peptide-41BBL-spacer-CD28 (TM)-CD3z (the signal peptide, spacer and TM/transmembrane domain may vary)

SEQ ID NO: 67

MEFGLSWLFLVAILKGVQCGLLDLRQGMFAQLVAQNVLIDGPLSWYSDPGLAGVSLTGGLSYKEDTKEL
VVAKAGVYYVFFQLELRRVVAGEGSGSVSLALHLQPLRSAAGAAALALTVDLPPASSEARNSAFGFQGR
LHLSAGQRLGVHLHTEARARHAWQLTQGATVLGLFRVTPEIPAGLPSRSEESKYGPPCPPCPGQPREPO
VYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPVLDSGDSFFLYSKLTVDKSRW
QQGNVFSCSVMHEALHNAYTQKSLSLSPGKKDPFWVLVVVGGVLACYSLLVTVAFIIFWVRSKRSRL
SDYMNMTPRRPGPTRKHYPYAPPRDFAAYRSRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDK
RGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQA
LPPR

Signal peptide-41BBL-spacer-CD28 (TM)-CD28(ICD)-CD3z (the signal peptide, spacer and TM/transmembrane domain may vary)

SEQ ID NO: 68

MEFGLSWLFLVAILKGVQCGLLDLRQGMFAQLVAQNVLIDGPLSWYSDPGLAGVSLTGGLSYKEDTKEL
VVAKAGVYYVFFQLELRRVVAGEGSGSVSLALHLQPLRSAAGAAALALTVDLPPASSEARNSAFGFQGR
LHLSAGQRLGVHLHTEARARHAWQLTQGATVLGLFRVTPEIPAGLPSRSEESKYGPPCPPCPGQPREPO
VYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPVLDSGDSFFLYSKLTVDKSRW
QQGNVFSCSVMHEALHNAYTQKSLSLSPGKKDPFWVLVVVGGVLACYSLLVTVAFIIFWVRSRVKFSRS
ADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLFNELQKDKMAEAFSEIGMK
GERRRGKGHDGLFQGLSTATKDTFDALHMQALPPR

Signal peptide-41BBL-spacer-CD28 (TM)-CD3z1xx (the signal peptide, spacer and TM/transmembrane domain may vary)

SEQ ID NO: 69

MEFGLSWLFLVAILKGVQCGLLDLRQGMFAQLVAQNVLIDGPLSWYSDPGLAGVSLTGGLSYKEDTKEL
VVAKAGVYYVFFQLELRRVVAGEGSGSVSLALHLQPLRSAAGAAALALTVDLPPASSEARNSAFGFQGR
LHLSAGQRLGVHLHTEARARHAWQLTQGATVLGLFRVTPEIPAGLPSRSEESKYGPPCPPCPGQPREPO
VYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPVLDSGDSFFLYSKLTVDKSRW
QQGNVFSCSVMHEALHNAYTQKSLSLSPGKKDPFWVLVVVGGVLACYSLLVTVAFIIFWVRSKRSRL
SDYMNMTPRRPGPTRKHYPYAPPRDFAAYRSRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDK
RGRDPEMGGKPRRKNPQEGLFNELQKDKMAEAFSEIGMKGERRRGKGHDGLFQGLSTATKDTFDALHMQA
LPPR

Signal peptide-41BBL-spacer-CD28 (TM)-CD28(ICD)-CD3z1xx (the signal peptide, spacer and TM/transmembrane domain may vary)

[000170] In one embodiment of the CD38 specific ADR, the CD38-ADR is represented by an amino acid sequence of at least about 85%, about 90%, about 95%, about 96%, about 97%, about 98%, or about 99% sequence identity to SEQ ID NO: 70. In some embodiments, the CD38-ADR comprises an amino acid sequence of at least about 90% sequence identity to SEQ

ID NO: 70. In some embodiments, the CD38-ADR comprises an amino acid sequence of at least about 95% sequence identity to SEQ ID NO: 70. In some embodiments, the CD38-ADR comprises the amino acid sequence of SEQ ID NO: 70.

SEQ ID NO: 70

MDFQVQIFSFLLISASVIMSRDIQMTQSPSSLSASVGRVTITCRASQGIRSWLAWYQQKPEKAPKSLIYAASSLQS
*GVPSRFSGSGSGTDFTLTITSSLPEDFATYYCQQYNNYPLTFGGGTKVEIK**GGGGSGGGSGGGSGVQLVQSGAEV*
KKPGSSVKVSKKPSGGRFTRSYAISWVRQAPGQGLEWMGRIIVFLGKVNYAQRFGGRVTLTADKSTTTAYMELSSLRS
*EDTAVYYCTGEPGARDPDAFDIWGQGTMTVSS**STTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFAC*
DIYIWAPLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEEGGCELRVKFSRSADA
PAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQDKMAEAYSEIGMKGERRRGKGHD
GLYQGLSTATKDTYDALHMQALPPR

Signal peptide-antiCD38VH-linker-antiCD38VL-CD8 (TM) -41BB (endo) -
CD3z (endo) (the signal peptide, linker, and TM/transmembrane domain may vary)

[000171] Accordingly, in some embodiments, the present application provides a solid tumor targeting backbone comprising a polynucleotide encoding an ADR specific to 4-1BB or CD38, among other components of the backbone, to equip an allogeneic effector cell with the ability to selectively deplete activated host immune cells while potentiating the effector cell through increased expansion in a tumor environment. Also provided in this application are immune cells, iPSCs, and iPSC-derived effector cells comprising a solid tumor targeting backbone comprising a polynucleotide encoding a 4-1BB specific ADR or a CD38 specific ADR, among other selected components, wherein the effector cells, including the genetically engineered T and NK cells, possess alloreactive resistance to the host immune system associated with the allogeneic use of the effector cells for treatment of tumors and infectious diseases in a patient.

5. Exogenously introduced cytokine signaling complex

[000172] By avoiding systemic high-dose administration of clinically relevant cytokines, the risk of dose-limiting toxicities due to such a practice is reduced while cytokine-mediated cell autonomy is being established. To achieve lymphocyte autonomy without the need to additionally administer soluble cytokines, a cytokine signaling complex comprising a partial or full length peptide of one or more of IL2, IL4, IL6, IL7, IL9, IL10, IL11, IL12, IL15, IL18, IL21, and/or their respective receptors may be introduced to the cell as part of the solid tumor targeting backbone to enable cytokine signaling with or without the expression of the cytokine itself, thereby maintaining or improving cell growth, proliferation, expansion, and/or effector function with reduced risk of cytokine toxicities. In some embodiments, the introduced cytokine and/or its respective native or modified receptor for cytokine signaling (signaling complex) are

expressed on the cell surface. In some embodiments, the cytokine signaling is constitutively activated. In some embodiments, the activation of the cytokine signaling is inducible. In some embodiments, the activation of the cytokine signaling is transient and/or temporal. In some embodiments, the transient/temporal expression of a cell surface cytokine/cytokine receptor is through an expression construct carried by a retrovirus, Sendai virus, an adenovirus, an episome, mini-circle, or RNAs including mRNA.

[000173] Various construct designs for introducing a protein complex for signaling of one, two, or more cytokines including, but not limited to, IL2, IL4, IL6, IL7, IL9, IL10, IL11, IL12, IL15, IL18 and IL21, into the cell are provided herein. For example, in embodiments where the signaling complex is for IL15, the transmembrane (TM) domain can be native to the IL15 receptor or may be modified or replaced with the transmembrane domain of any other membrane bound proteins. In various embodiments, the cytokine signaling complex comprises an IL15 receptor fusion (IL15RF) comprising a full or partial length of IL15 and a full or partial length of IL15 receptor (IL15R). In some embodiments, IL15 and IL15R α are co-expressed by using a self-cleaving peptide, mimicking trans-presentation of IL15, without eliminating cis-presentation of IL15. In other embodiments, IL15R α is fused to IL15 at the C-terminus through a linker, mimicking trans-presentation without eliminating cis-presentation of IL15 as well as ensuring that IL15 is membrane-bound. In other embodiments, IL15R α with truncated intracellular domain is fused to IL15 at the C-terminus through a linker, mimicking trans-presentation of IL15, maintaining IL15 membrane-bound, and additionally eliminating cis-presentation and/or any other potential signal transduction pathways mediated by a normal IL15R through its intracellular domain. In other embodiments, IL15R α is fused to IL15 without an intracellular domain (IL15 Δ), as described in International Pub. Nos. WO 2019/191495 and WO 2019/126748, the entire disclosure of each of which is incorporated herein by reference.

[000174] In other various embodiments, the cytokine signaling complex comprises an IL7 receptor fusion (IL7RF) comprising a full or partial length of IL7 and a full or partial length of IL7 receptor. The transmembrane (TM) domain can be native to the IL7 receptor or may be modified or replaced with a transmembrane domain of any other membrane bound proteins. In one embodiment, a native (or wildtype) or modified IL7R may be fused to IL7 at the C-terminus through a linker, enabling constitutive signaling and maintaining membrane-bound IL7. In some embodiments, such a construct comprises an amino acid sequence of at least 75%, 80%, 85%, 90%, 95% or 99% identity to SEQ ID NO: 71, with transmembrane domain, signal peptide and linker being flexible and varying in length and/or sequences. In some embodiments, the IL7 construct comprises an amino acid sequence of at least 90% identity to SEQ ID NO: 71, with

transmembrane domain, signal peptide and linker being flexible and varying in length and/or sequences. In some embodiments, the IL7 construct comprises an amino acid sequence of at least 95% identity to SEQ ID NO: 71, with transmembrane domain, signal peptide and linker being flexible and varying in length and/or sequences. In some embodiments, the IL7 construct comprises the amino acid sequence of SEQ ID NO: 71.

SEQ ID NO: 71

MDWTWILFLVAAATRVHSDCDIEGKDGKQYESVLMVSIQLLDSMKEIGSNCLNNEFNFFKRHI
CDANKEGMFLFRAARKLRQFLKMNSTGDFDLHLLKVSEGTITLLNCTGQVKGRKPAALGEAQPT
KSLEENKSLKEQKKLNDLCFLKRLLEIKTCWNKILMGTKEHSGGGSGGGSGGGSGGGSGG
GSLQESGYAQNGDLEDAELDDYSFSCYSQLEVNGSQHSLTCAFEDPDVNITNLEFEICGALVEV
KCLNFRKLQEIYFIETKKFLLIGKSNICVKVGEKSLTCKKIDLTITVKPEAPFDLSVVYREGAN
DFVVTFNTSHLQKKYVKVLMHDVAYRQEKDENKWTHTVNLSSSTKLTLQKRLQPAAMYEIKVRSI
PDHYFKGFWEWSPSYFRTPEINNSSGEMDPIILLTISILSFFSVALLVILACVLWKKRIKPIV
WPSLPDHKKTLEHLCKKPRKNLNVSNPESFLDCQIHRVDDIQARDEVEGFLQDTFPQQLEESE
KQRLGGDVQSPNCPSEDVVITPESFGRDSSLTCLAGNVSACDAPILSSSRSLDCRESGKNPHV
YQDLLLSLGTNTSTLPPPFSLQSGILTLNPVAQGQPIILTSLSNQEAYVTMSSFYQNG

(Signal peptide-IL7-linker-IL7R; transmembrane domain (TM), signal peptide and linker can vary in length and sequences)

[000175] In another embodiment, a native or modified common receptor γ C is fused to IL7 at the C-terminus through a linker for constitutive and membrane-bound cytokine signaling complex. In addition, engineered IL7R that forms a homodimer in the absence of IL7 is useful for producing constitutive signaling of the cytokine as well.

[000176] In yet another embodiment, cytokine signaling is provided by exogenous and soluble IL2 or IL18. IL-2 is a cytokine that promotes proliferation and differentiation of T cells, while IL-18 enhances T cell effector function. In the context of iPSC derived T cells, temporal and dose control of the exogenous cytokine can have important implications for the lymphoid lineage and T cell lineage commitment during the iPSC differentiation process. In some embodiments, the soluble cytokine expression is under the control of an endogenous TRAC promoter. In some embodiments, the soluble cytokine expression is under the control of an endogenous Tim-3 promoter. In some embodiments, the cell comprises a polynucleotide encoding a soluble IL2 (sIL2), such as a polypeptide having the sequence of SEQ ID NO: 72. In some embodiments, the cell comprises a polynucleotide encoding a soluble IL18 (sIL18), such as a polypeptide having the sequence of SEQ ID NO: 73.

SEQ ID NO: 72

MYRMQLLSICIALSLALVTNSAPTSSSTKKTQLQLEHLLLDLQMI L N G I N N Y K N P K L T R M L T F K F Y M P K K A
 TELKHLQCLEEELKPLEEVLNLAQSKNFHLRPRDLISNINVIVLELKGSETTFMCEYADETATIVEFLNR
 WITFCQSIISTLT

(sIL2)

SEQ ID NO: 73

MAAEPVEDNCINFVAMKFIDNTLYFIAEDDENLESDFGKLESKLSVIRNLNDQVLFIDQGNRPLFEDMT
 DSDCRDNAPRTIFIISMYKDSQPRGMAVTISVKCEKISTLSCENKIISFKEMNPPDNIKDTKSDIIFQQR
 SVPGHDNKMQFESSYEGYFLACEKERDLFKLILKKEDELGDRSIMFTVQNE

(sIL18)

[000177] One having ordinary skill in the art would appreciate that the signal peptide and the linker sequences above are illustrative and in no way limit their variations suitable for use as a signal peptide or linker. There are many suitable signal peptide or linker sequences known and available to those in the art. The ordinary skilled in the art understands that the signal peptide and/or linker sequences may be substituted for another sequence without altering the activity of the functional peptide led by the signal peptide or linked by the linker.

[000178] As such, in various embodiments, the cytokines and/or receptors thereof, may be introduced to iPSCs using one or more of the construct designs described herein, and to their derivative cells upon iPSC differentiation. Also provided is a master cell bank comprising single cell sorted and expanded clonal engineered iPSCs having a solid tumor targeting backbone as disclosed herein, wherein the cell bank provides a platform for additional iPSC engineering and a renewable source for manufacturing off-the-shelf, engineered, homogeneous cell therapy products, which are well-defined and uniform in composition, and can be mass produced at a significant scale in a cost-effective manner.

6. Chimeric Antigen Receptor (CAR) expression

[000179] Applicable to the genetically engineered immune cells, iPSCs and derivative effector cells thereof may be any CAR design known in the art. CAR is a fusion protein generally including an ectodomain that comprises a target binding region (for example, an antigen recognition domain), a transmembrane domain, and an endodomain. In some embodiments, the ectodomain can further include a signal peptide or leader sequence and/or a spacer. In some embodiments, the endodomain can further comprise a signaling peptide that activates the effector cell expressing the CAR. In some embodiments, the signaling peptide of the endodomain (or intracellular domain) comprises a full length or at least a portion of a polypeptide of 2B4, CD2, CD3 ζ , CD3 ζ 1XX, CD8, CD28, CD28H, CD137 (4-1BB), CS1,

DAP10, DAP12, DNAM1, FcERI γ , IL2R γ , IL7R, IL21R, IL2R β (IL15R β), IL21, IL7, IL12, IL15, IL21, KIR2DS2, NKp30, NKp44, NKp46, NKG2C, or NKG2D. In one embodiment, the signaling peptide of a CAR comprises an amino acid sequence that has at least about 85%, about 90%, about 95%, about 96%, about 97%, about 98%, or about 99% identity to at least one ITAM (immunoreceptor tyrosine-based activation motif) of CD3 ζ . Exemplary N-terminal signal peptides include MALPVTALLLPLALLHA (SEQ ID NO: 74; CD8asp) or MDFQVQIFSLLISASVIMSR (SEQ ID NO: 75; IgKsp), or any signal peptide sequence or functional variants thereof known in the art.

[000180] In some embodiments, the antigen recognition domain can specifically bind an antigen. In some embodiments, the CAR is suitable to activate T, NK or NKT cells expressing said CAR. In some embodiments, the CAR is NK cell specific for comprising NK-specific signaling components. In some embodiments, the CAR is NKT cell specific for comprising NKT-specific signaling components. In certain embodiments, said T cells are derived from a CAR expressing iPSCs comprising a solid tumor targeting backbone as described herein, and the derivative T cells may comprise T helper cells, cytotoxic T cells, memory T cells, regulatory T cells, natural killer T cells, $\alpha\beta$ T cells, $\gamma\delta$ T cells, or a combination thereof. In certain embodiments, said NK cells are derived from a CAR expressing iPSCs comprising a solid tumor targeting backbone as described herein. In certain embodiments, said NKT cells are derived from a CAR expressing iPSCs comprising a solid tumor targeting backbone as described herein.

[000181] In various embodiments, the antigen recognition region comprises a murine antibody, a human antibody, a humanized antibody, a camel Ig, a single variable new antigen receptor (VNAR), a shark heavy-chain antibody (Ig-NAR), a chimeric antibody, a recombinant antibody, a single-domain antibody (dAb), an anti-idiotypic antibody, a bi-specific-, multi-specific- or multimeric- antibody, or antibody fragment thereof. Anti-idiotypic antibodies are specific for binding to an idiotope of another antibody, wherein the idiotope is an antigenic determinant of an antibody. A bi-specific antibody may be a BiTE (bi-specific T cell engager) or a BiKE (bi-specific killer cell engager), and a multi-specific antibody may be a TriKE (tri-specific Killer cell engager). Non-limiting examples of antibody fragments include Fab, Fab', F(ab')₂, F(ab')₃, Fv, Fabc, pFc, Fd, single chain fragment variable (scFv), tandem scFv (scFv)₂, single chain Fab (scFab), disulfide stabilized Fv (dsFv), minibody, diabody, triabody, tetrabody, single-domain antigen binding fragments (sdAb), camelid heavy-chain IgG and Nanobody® fragments, recombinant heavy-chain-only antibody (VHH), and other antibody fragments that maintain the binding specificity of the antibody. In some embodiments an antigen binding domain of a CAR comprises CDR1, CDR2, and CDR3 of a heavy chain (H-CDRs) of an antibody or fragments thereof. In some embodiments, the antigen binding domain of a CAR

comprising the H-CDRs of an antibody further comprises the CDRs of a light chain (L-CDRs) of the antibody.

[000182] In some embodiments, the antigen recognition domain of a CAR specifically binds an antigen associated with a disease or pathogen. In some embodiments, the disease-associated antigen is a tumor antigen, wherein the tumor may be a liquid or a solid tumor. In some embodiments of a CAR, the CAR targets antigens of hematological malignancies, which include, but are not limited to, acute and chronic leukemias (acute myelogenous leukemia (AML), acute lymphoblastic leukemia (ALL), chronic myelogenous leukemia (CML), lymphomas, non-Hodgkin lymphoma (NHL), Hodgkin's disease, multiple myeloma, and myelodysplastic syndromes.

[000183] In some embodiments of CARs targeting solid cancer antigens, the antigens are associated with sarcomas and carcinomas. In some embodiments, the solid cancers suitable for CAR targeting include, but are not limited to, bladder cancer, bone cancer, brain/CNS cancer, breast cancer, breast lung cancer, cervical cancer, colorectal cancer, esophageal cancer, gastric/stomach cancer, head and neck cancer, kidney cancer, larynx cancer, liver cancer, lung cancer, metastatic cancer, ovarian cancer, pancreatic cancer, prostate cancer, rectal cancer, renal cancer, salivary gland cancer, skin cancer, testicular tumor, thyroid tumor, urothelial cancer, and uterine/endometrial cancer. More specifically, in some embodiments, the CAR targets an antigen associated to adenocarcinoma, basal cell carcinoma, bile duct carcinoma, bladder carcinoma, bronchogenic carcinoma, cholangiocarcinoma, chondrosarcoma, choriocarcinoma, colon carcinoma, Ewing's tumor, fibrosarcoma, gallbladder carcinoma, hepatocellular carcinoma, hepatoma, leiomyosarcoma, liposarcoma, lymphoid malignancy, medullary carcinoma, medullary thyroid carcinoma, melanoma, mesothelioma, myxosarcoma, non-small cell lung cancer, osteosarcoma, papillary adenocarcinoma, papillary carcinoma, papillary thyroid carcinoma, pheochromocytomas sebaceous gland carcinoma, peritoneal carcinoma, renal cell carcinoma, rhabdomyosarcoma, sarcoma, seminoma, squamous cell carcinoma, sweat gland carcinoma, synovial sarcoma, synovioma, and Wilms' tumor. In some embodiments, the CAR targets antigens of CNS tumors including, but not limited to, acoustic neuroma, astrocytoma, CNS lymphoma, ependymoma, hemangioblastoma, germinoma, glioma (including brainstem glioma and mixed gliomas), glioblastoma (also known as glioblastoma multiforme), medulloblastoma, meningioma, neuroblastoma, oligodendroglioma, pinealoma, retinoblastoma, Schwannoma craniopharyngioma, and brain metastases.

[000184] Non-limiting examples of antigens that may be targeted by a CAR include oncofetal antigen (h5T4), 8H9, 9D7, ACPP, α actinin-4 (ACTN4), ADAM12, ADRB3, ADGRE2/EMR2, AFP, AKAP-4, ALK, ALPP, ALPPL2, Androgen receptor, ASGR1

(asialoglycoprotein receptor 1), ASGR2 (asialoglycoprotein receptor 2), AXL, B7H3, B7H6, BAGE, β -catenin, BCR, BCR-ABL, Bigh3, BING-4, BORIS, BRCA1/2, BST2, carbonic anhydrase IX (CAIX/CA9), CA125, C-C motif chemokine receptor 1 (CCR1), CCR4, carcinoembryonic antigen (CEA/CECAM5), Calcium-activated chloride channel 2 (CLCA4), Carbohydrates (Le), CD3, CD4, CD5, CD7, CD8, CD10, CD19, CD20, CD22, CD24, CD30, CD33, CD34, CD37, CD38, CD41, CD44, CD44v6, CD44v7/8, CD47, CD49f, CD52, CD56, CD70, CD72, CD74, CD79a, CD79b, CD97, CD99, CD123, CD133, CD138, CD171, CD179a, CD207, CD269 (BCMA), CD300LF, CDC1 27, CDH3 (p-cadherin), CDH6, cadherin 19 (CDH19), CDK4, CFC1, CLCA1, CLDN6, CLDN18.2, CLEC12A, CLL-1, c-MET, CML66, an antigen of a cytomegalovirus (CMV) infected cell (e.g., a cell surface antigen), CR1L, CS-1, CSPG4, CXCR2, CXCR5, CXORF61, Cyclin B1 (CCNB1), CYP1B1, DLL3, EFNA4, EGFR (or erbB-1), EGFRvIII, EGF1R, epithelial cell adhesion molecule/epithelial glycoprotein-2 (EpCAM/EGP2), epithelial glycoprotein-40 (EGP40), ELF2M, ENPP3, EphA2, EphA3, EphB2, ERBB2 (or HER2/neu), ERBB3, ERBB4, ERG (TMPRSS2 ETS fusion gene), ETA, ETV6-AML, FAP, folate-binding protein (FBP), FCAR, FCRL5, fetal acetylcholine receptor (AChR), Fibronectin, FLT3, folate receptor- α , (FR- α /FOLR1), Folate receptor beta (FR- β /FOLR2), FOLR3, Fos-related antigen 1 (FOSL1), FRcc, FZD10, GAGE, gangliosides (GM1, FucGM1, GM2, GM3, GD2, o-acetyl-GD2, GD3), GloboH, GpA33, Gp75, Gp100, Glypican-1 (GPC1), Glypican-2 (GPC2), Glypican-3 (GPC3), GPNMB, GPR20, GPR27, GPR35, GPR119, GPRC5D, guanylate cyclase C (GC-C), GUCY2C, HAVCR1, HERV-envelope protein, HLA-A1, HM1.24, HMWMAA, HPV E6, HPV E7, human telomerase reverse transcriptase (hTERT), IGF1R/IGF1R, IGLL1 (CD179b), IL11R α , Interleukin-13 receptor subunit alpha-2 (IL13R α 2), IL13Rcc2, Immature laminin receptor (iLRP), Integrin α V β 3, Integrin α 5 β , Integrin B7, intercellular adhesion molecule 1 (ICAM1), intestinal carboxyl esterase (iCE), κ -light chain, kinase insert domain receptor (KDR), KIT, KISS1R, LAIR1, LAGE-1a, LAMP-1, LCK, legumain, Lewis A (CA19.9), Lewis Y (LeY), L1 cell adhesion molecule (L1-CAM), LILRA2, LILRB2, LIV-1, LMP2, LRRC15, LY6K, LY75, LYPD3, MAD-CT-1, MAD-CT-2, melanoma antigen family A1 (MAGE-A1), MC1R, MelanA/MART1, MART2, melanoma-associated chondroitin sulfate proteoglycan (MCSP), c-Met, MICA/B, Mesothelin (MSLN), ML-IAP, MR1, multidrug resistance-associated protein 3 (MRP3), MS4A12, Mucin 1 (MUC1, tMUC1), MUC2, MUC5A, MUC12, MUC16, MUC17, MUC21, Mud, MUM1, MUM2, MUM3, mut hsp70-2, MYCN, NA17, NA88-1, NCAM, Nectin4, NKCSI, NKG2D ligands, NPM, NY-BR-1, cancer-testis antigen NY-ESO-1, OA1, OGT, OR51E2, OY-TES1, p53, p53 mutant, PANX3, PAP, PAX3, PAX5, PCTA-1/Galectin 8, PDGFR-beta, PDL1, periostin, PLAC1, PRAME, PRLR, Prostase (KLK2, KLK4), prostein (P501S), PRSS21, Polysialic acid (PSA), prostate stem cell

antigen (PSCA), PSC1, PRAME prostate-specific membrane antigen (PSMA/FOLH1), PTK7, QRFPR, RAGE-1, RANKL, Ras, Ras mutant, RCC, RhoC, Ron Kinase, ROR1, RU1, RU2, SAGE, SAP1, sarcoma translocation breakpoints, SART3, SIGLEC-15, Sialo-epitope CA6, SLC6A3, SLC12A3, SLC13A5, SLC22A1, SLC22A7, SLC30A4, SLC30A8, SLC34A2, SLC45A3, sLe, SLITRK6, SPARC, Sperm protein 17 (SP17), SSEA-4, SSTR1, SSX2, STEAP, sTN, Survivin, tumor-associated glycoprotein 72 (TAG72), TARP, TEM1/CD248, TEM7R, TEMs, Telomerase, TGF-B receptor, TGS5, Tie 2, Tissue Factor (TF), TIM-3, TMEFF2 (TENB2), TMEM238, TMPRSS11B, TMPRSS11E, Tn Ag, TNC, TP-3, TRAILR1, TRAILR2, TRBC1, TRBC2, TRF2, TRG, TROP2, TRP1, TRP2, TSHR, TSTA, Tyrosinase, UGT1A1, UPK1B, UPK2, VEGF, VEGFR, vascular endothelial growth factor R2 (VEGF-R2), VTCN1 (B7H4), Wilms tumor protein (WT1), XAGE1, and various pathogen antigen known in the art. Non-limiting examples of pathogens include viruses, bacteria, fungi, parasites and protozoa capable of causing diseases.

[000185] Non-limiting examples of solid cancers with corresponding tumor antigens are provided in Table 1B.

Table 1B – Exemplary Solid Tumors and Solid Tumor Associated Antigens

Cancer Type	Antigen	Antibody
Bladder Cancer	HER2, MICA/B, CD207, EFNA4, LY6K, LYPD3, Nectin4, PTK7, SLITRK6, TIM-3, TNC, UPK1B, UPK2	enfortumab, trastuzumab, pertuzumab, SLITRK6
Bone Cancer	MICA/B, ADAM12, CCR1, CD99, CD248, EPHA2, GPNMB, LRRC15, TP-3	huM25, DS-8895a variant 1, DS-8895a variant 2, glembatumab
Brain Cancer	MICA/B, CD133, DLL3, EGFRvIII, TNC	AMG595, ABT806, rovalpituzumab, depatuxizumab
Breast Cancer	HER2, MICA/B, ADAM12, ADGRE2/EMR2, CCR4, CD49f, CD133, CDH3 (p-cadherin), CLDN6, c-MET, CXCR2, EFNA4, EGFR, EPCAM/EGP2, EPHA2, GPNMB, ICAM1, LAMP-1, LIV-1, LILRB2, LRRC15, LYPD3, MUC1, tMUC1, PRLR, PTK7, Sialo-epitope CA6, TNC, TROP2	trastuzumab, pertuzumab, sacituzumab, ladiratuzumab, huLiv1-14, Liv1-1.7A4, huLiv1-22, huDS6, glembatumumab, PF-0664720, MEDI-547, DS-8895a variant 1, DS-08895a variant 2
Breast Lung Cancer	HER2, MICA/B, ADGRE2/EMR2, EPCAM/EGP2, ROR1	
Cervical Cancer	MICA/B, EFNA4, LY6K, MUC1, MUC16, LYPD3, PTK7, SLC12A3, SSTR1	PF-0664720, anetumumab, 4H11, 4H5, huDS6, sofituzumab
Cholangiocarcinoma	MICA/B, tMUC1	
Colorectal Cancer	HER2, MICA/B, ADAM12, CA19.9, CD3, CD49f, CD133, CEA/CECAM5, CLCA1, c-MET, EFNA4, EPHB2,	huM25, PR1A3, humanized PR1A3, pantumumab,

	GPA33, GPR35, GUCY2C, ICAM1, LGR5/GPR49, LRRC15, MS4A12, MUC12, MUC17, TIM-3, TMEM238	cetuximab, nimotuzumab, zalutumumab
Esophageal Cancer	HER2, MICA/B, CA19.9, CD10, CEA/CECAM5, EFNA4, EPHB2, MUC21, TMEM238, TMPRSS11B, TMPRSS11E	
Gallbladder Carcinoma	EPCAM/EGP2	
Gastric (Stomach) Cancer	HER2, MICA/B, CEA/CECAM5, CLDN18.2, c-MET, CRIL, EFNA4, EPHB2, LGR5/GPR49, MUC17, PSCA, TIM-3, TMEM238	sofituzumab, anetumab, pertuzumab, trastuzumab, humanized PR1A3
Glioma	MICA/B, ADGRE2/EMR2, CD49f, CD133, EGFR, EGFRvIII, EPHA2, HM1.24, IL13-R α 2	
Head and Neck Cancer	HER2, MICA/B, ADAM12, CD3, c-MET, EFNA4, LRRC15, LY6K, LYPD3, PTK7, TNC	cetuximab, panitumumab, nintuzumab, PF-0664720, pantumumab, cetuximab, nimotuzumab, zalutumumab
Kidney Cancer	MICA/B, CD70, CDH6, c-MET, ENPP3, HAVCR1	AGS-16M8F, AGS-16C3, the antibody of CDX-014, onartuzumab
Liver Cancer	MICA/B, ASGR1, ASGR2, C9 (CAIX), CA19.9, CEA/CECAM5, CCR1, CD3, CD133, EPCAM/EGP2, GPC3, ICAM1, LGR5/GPR49, SLC13A5, SLC22A1, SLC22A7, TIM-3, TRF2, UGT1A1	codrituzumab, oportuzumab, humanized PR1A3
Lung Cancer	HER2, MICA/B, ADAM12, ADGRE2/EMR2, CCR1, CCR4, CD56, CD133, CEA/CECAM5, CXCR2, DLL3, EFNA4, EGFR, EGFRvIII, FOLR1, GPC3, HM1.24, ICAM1, LILRB2, LRRC15, LY6K, LYPD3, MSLN, MUC1, MUC16, PDL1, PTK7, SLC34A2, TIM-3	panitumumab, cetuximab, pembrolizumab, nivolumab, atezolizumab, and nimotuzumab, lifastuzumab, anetumab, PF-0664720, farletuzumab, rovalpituzumab, lifastuzumab, sofituzumab, huDS6, ABT806, AMG595, huM25
Mesothelioma	MICA/B, FAP, MSLN	
Metastatic Cancer	MICA/B, MSLN, VEGFR-II	
Neuroblastoma	MICA/B, GD2	
Non-Small Cell Lung Cancer (NSCLC)	MICA/B, c-MET, EGFR	
Ovarian Cancer	HER2, MICA/B, CCR1, CD3, CD133, CLDN6, c-MET, EFNA4, EPCAM/EGP2, FAP, FOLR1, FOLR3, FR- α , FZD10, GPR27, GPR119, LRRC15, MSLN, MUC1, MUC16, PTK7, SLC34A2, sTN, TMEM238, VTCN1	sofituzumab, 4H11, 4H5, huDS6, farletuzumab, anetumab, trastuzumab, pertuzumab, PF-0664720, sibrotuzumab, huM25, lifastuzumab
Pancreatic Cancer	MICA/B, ADAM12, CA19.9, CFC1, EFNA4, EPCAM/EGP2, ICAM1, LILRB2, LRRC15, MSLN, MUC1, tMUC1, MUC5A, MUC16, MUC17, PSCA, PTK7, SLC30A8	PF-0664720, clivatuzumab, 4H11, 4H5, anetumumab, huDS6, sofituzumab, huM25, RG7841

Peritoneal Carcinoma	FOLR3	
Prostate Cancer	MICA/B, ACPP, CD10, CD49f, CD133, EFNA4, OR51E2, PSCA, PSMA/FOLH1, PTK7, SLC30A4, SLC45A3, STEAP, TIM-3, TMEFF2 (TENB2)	mirvetuximab, J591 variant 1, J591 variant 2
Renal Cancer	MICA/B, CD3, CD70, ICAM1, KISS1R, LILRB2, QRFPR, SLC6A3, TIM-3	
Sarcoma	MICA/B, LRRC15	
Salivary Gland Cancer	HER2, MICA/B	
Skin Cancer	CCR4, CD3, CD10, ICAM1	
Synovial Sarcoma (Soft Tissue Cancer)	CD99	
Thyroid Tumor	MICA/B, CD10, c-MET, PTK7, TSHR	
Urothelial Cancer	MICA/B, CLDN6, EPCAM/EGP2, SIGLEC-15, TIM-3, UPK2	
Uterine/Endometrial Cancer	HER2, MICA/B, ALPP, ALPPL2, CCR1, CLDN6, EFNA4, EPHB2, FOLR1, LILRB2, LY6K, LYPD3, MUC1, MUC16, PTK7	PF-0664720, anetumumab, 4H11, 4H5, huDS6, sofituzumab, farletuzumab

[000186] In some embodiments, the antigen recognition domain of a CAR comprises CDRs of the heavy chain (H-CDRs), CDRs of both the heavy and the light chains (H- and L- CDRs), the variable region of the heavy chain (VH), or a single chain of the variable regions of both the heavy and light chains (VH and VL) of the binding domains of an antibody that is specific to a tumor antigen, including those exemplified in this application. In some embodiments, the CAR is designed based on the binding domains of an antibody comprising trastuzumab, cetuximab, panitumumab, ofatumumab, belimumab, ipilimumab, pertuzumab, tremelimumab, nivolumab, pembrolizumab, atezolizumab, MDX-1105, dacetuzumab, urelumab, MPDL3280A, lambrolizumab, blinatumomab, nimotuzumab, zalutumumab, onartuzumab, patritumab, clivatuzumab, sofituzumab, edrecolomab, adecatumumab, anetumab, huDS6, lifastuzumab, sacituzumab, PR1A3, humanized PR1A3, humanized Ab2-3, claudiximab, AMG595, ABT806, sibrotuzumab, DS-8895a variant 1, DS-8895a variant 2, MEDI-547, narnatumab, RG7841, farletuzumab, mirvetuximab, J591 variant 1, J591 variant 2, rovalpituzumab, PF-06647020, ladiratuzumab, cirmtuzumab, ladiratuzumab, huLiv1-14, Liv1-1.7A4, huLiv1-22, 4H11, 4H5, glembatumumab, oportuzumab, enfortumab, depatuxizumab, or codrituzumab.

[000187] Accordingly, in some embodiments, the antigen recognition domain of the CAR specifically binds to an antigen present on bladder cancer. In some embodiments, the CAR targeting a bladder cancer associated antigen specifically binds to HER2, MICA/B, CD207, EFNA4, LY6K, LYPD3, Nectin4, PTK7, SLITRK6, TIM-3, TNC, UPK1B, or UPK2. In some embodiments, the antigen recognition domain of said CAR comprises the H-CDRs, H- and L-

CDRs, the VH, or a single chain of VH and VL of an antibody comprising enfortumab, trastuzumab, pertuzumab or SLITRK6.

[000188] In some embodiments, the antigen recognition domain of the CAR specifically binds to an antigen present on bone cancer. In some embodiments, the CAR targeting a bone cancer associated antigen specifically binds to MICA/B, ADAM12, CCR1, CD99, CD248, EPHA2, GPNMB, LRRC15, or TP-3. In some embodiments, the antigen recognition domain of said CAR comprises the H-CDRs, H- and L- CDRs, the VH, or a single chain of VH and VL of an antibody comprising huM25, DS-8895a variant 1, DS-8895a variant 2, or glembatumab.

[000189] In some embodiments, the antigen recognition domain of the CAR specifically binds to an antigen present on brain cancer. In some embodiments, the CAR targeting a brain cancer associated antigen specifically binds to MICA/B, CD133, DLL3, EGFRvIII, or TNC. In some embodiments, the antigen recognition domain of said CAR comprises the H-CDRs, H- and L- CDRs, the VH, or a single chain of VH and VL of an antibody comprising AMG595, ABT806, rovalpituzumab or depatuxizumab.

[000190] In some embodiments, the antigen recognition domain of the CAR specifically binds to an antigen present on a breast cancer cell. In some embodiments, the CAR targeting a breast cancer associated antigen specifically binds to HER2, MICA/B, ADAM12, ADGRE2/EMR2, CCR4, CD49f, CD133, CDH3 (p-cadherin), CLDN6, c-MET, CXCR2, EFNA4, EGFR, EPCAM/EGP2, EPHA2, GPNMB, ICAM1, LAMP-1, LIV-1, LILRB2, LRRC15, LYPD3, MUC1, tMUC1, PRLR, PTK7, Sialo-epitope CA6, TNC, or TROP2. In some embodiments, the antigen recognition domain of said CAR comprises the H-CDRs, H- and L- CDRs, the VH, or a single chain of VH and VL of an antibody comprising trastuzumab, pertuzumab, sacituzumab, ladiratuzumab, huLiv1-14, Liv1-1.7A4, huLiv1-22, huDS6, glembatumumab, PF-0664720, MEDI-547, DS-8895a variant 1, or DS-08895a variant 2.

[000191] In some embodiments, the antigen recognition domain of the CAR specifically binds to an antigen present on a breast lung cancer cell. In some embodiments, the CAR targeting a breast lung cancer associated antigen specifically binds to HER2, MICA/B, ADGRE2/EMR2, EPCAM/EGP2, or ROR1.

[000192] In some embodiments, the antigen recognition domain of the CAR specifically binds to an antigen present on cervical/uterine/endometrial cancer. In some embodiments, the CAR targeting a cervical/uterine/endometrial cancer associated antigen specifically binds to MICA/B, EFNA4, LY6K, MUC1, MUC16, LYPD3, PTK7, SLC12A3, or SSTR1. In some embodiments, the antigen recognition domain of said CAR comprises the H-CDRs, H- and L- CDRs, the VH, or a single chain of VH and VL of an antibody comprising PF-0664720, anetumumab, 4H11, 4H5, huDS6, or sofituzumab.

[000193] In some embodiments, the antigen recognition domain of the CAR specifically binds to an antigen present on a cholangiocarcinoma cell. In some embodiments, the CAR targeting a cholangiocarcinoma associated antigen specifically binds to MICA/B or tMUC1.

[000194] In some embodiments, the antigen recognition domain of the CAR specifically binds to an antigen present on colorectal cancer. In some embodiments, the CAR targeting a colorectal cancer associated antigen specifically binds to HER2, MICA/B, ADAM12, CA19.9, CD3, CD49f, CD133, CEA/CECAM5, CLCA1, c-MET, EFNA4, EPHB2, GPA33, GPR35, GUCY2C, ICAM1, LGR5/GPR49, LRRC15, MS4A12, MUC12, MUC17, TIM-3, or TMEM238. In some embodiments, the antigen recognition domain of said CAR comprises the H-CDRs, H- and L- CDRs, the VH, or a single chain of VH and VL of an antibody comprising huM25, PR1A3, humanized PR1A3, pantumumab, cetuximab, nimotuzumab, or zalutumumab.

[000195] In some embodiments, the antigen recognition domain of the CAR specifically binds to an antigen present on an esophageal cancer cell. In some embodiments, the CAR targeting an esophageal cancer associated antigen specifically binds to HER2, MICA/B, CA19.9, CD10, CEA/CECAM5, EFNA4, EPHB2, MUC21, TMEM238, TMPRSS11B, or TMPRSS11E.

[000196] In some embodiments, the antigen recognition domain of the CAR specifically binds to an antigen present on a gall bladder carcinoma cell. In some embodiments, the CAR targeting a gall bladder carcinoma associated antigen specifically binds to EPCAM/EGP2.

[000197] In some embodiments, the antigen recognition domain of the CAR specifically binds to an antigen present on gastric/stomach cancer. In some embodiments, the CAR targeting a gastric/stomach cancer associated antigen specifically binds to HER2, MICA/B, CEA/CECAM5, CLDN18.2, c-MET, CR1L, EFNA4, EPHB2, LGR5/GPR49, MUC17, PSCA, TIM-3, or TMEM238. In some embodiments, the antigen recognition domain of said CAR comprises the H-CDRs, H- and L- CDRs, the VH, or a single chain of VH and VL of an antibody comprising sofituzumab, anetumab, pertuzumab, trastuzumab, or humanized PR1A3.

[000198] In some embodiments, the antigen recognition domain of the CAR specifically binds to an antigen present on a glioma cancer cell. In some embodiments, the CAR targeting a glioma cancer associated antigen specifically binds to MICA/B, ADGRE2/EMR2, CD49f, CD133, EGFR, EGFRvIII, EPHA2, HM1.24, or IL13-R α 2.

[000199] In some embodiments, the antigen recognition domain of the CAR specifically binds to an antigen present on head and neck cancer. In some embodiments, the CAR targeting a head and neck cancer associated antigen specifically binds to HER2, MICA/B, ADAM12, CD3, c-MET, EFNA4, LRRC15, LY6K, LYPD3, PTK7, or TNC. In some embodiments, the antigen recognition domain of said CAR comprises the H-CDRs, H- and L- CDRs, the VH, or a single

chain of VH and VL of an antibody comprising cetuximab, panitumumab, nimtuzumab, PF-0664720, pantumumab, cetuximab, nimotuzumab, or zalutumumab.

[000200] In some embodiments, the antigen recognition domain of the CAR specifically binds to an antigen present on kidney cancer. In some embodiments, the CAR targeting a kidney cancer associated antigen specifically binds to MICA/B, CD70, CDH6, c-MET, ENPP3, or HAVCR1. In some embodiments, the antigen recognition domain of said CAR comprises the H-CDRs, H- and L- CDRs, the VH, or a single chain of VH and VL of an antibody comprising AGS-16M8F, AGS-16C3, the antibody of CDX-014, or onartuzumab.

[000201] In some embodiments, the antigen recognition domain of the CAR specifically binds to an antigen present on liver cancer. In some embodiments, the CAR targeting a liver cancer associated antigen specifically binds to MICA/B, ASGR1, ASGR2, C9 (CAIX), CA19.9, CEA/CECAM5, CCR1, CD3, CD133, EPCAM/EGP2, GPC3, ICAM1, LGR5/GPR49, SLC13A5, SLC22A1, SLC22A7, TIM-3, TRF2, or UGT1A1. In some embodiments, the antigen recognition domain of said CAR comprises the H-CDRs, H- and L- CDRs, the VH, or a single chain of VH and VL of an antibody comprising codrituzumab, oportuzumab, or humanized PR1A3.

[000202] In some embodiments, the antigen recognition domain of the CAR specifically binds to an antigen present on lung cancer. In some embodiments, the CAR targeting a lung cancer associated antigen specifically binds to HER2, MICA/B, ADAM12, ADGRE2/EMR2, CCR1, CCR4, CD56, CD133, CEA/CECAM5, CXCR2, DLL3, EFNA4, EGFR, EGFRvIII, FOLR1, GPC3, HM1.24, ICAM1, LILRB2, LRRC15, LY6K, LYPD3, MSLN, MUC1, MUC16, PDL1, PTK7, SLC34A2, or TIM-3. In some such embodiments, the antigen recognition domain of said CAR comprises the H-CDRs, H- and L- CDRs, the VH, or a single chain of VH and VL of an antibody comprising panitumumab, cetuximab, pembrolizumab, nivolumab, atezolizumab, and nimotuzumab, lifastuzumab, anetumab, PF-0664720, farletuzumab, rovalpituzumab, lifastuzumab, sofituzumab, huDS6, ABT806, AMG595, or huM25.

[000203] In some embodiments, the antigen recognition domain of the CAR specifically binds to an antigen present on a mesothelioma cell. In some embodiments, the CAR targeting a mesothelioma associated antigen specifically binds to MICA/B, FAP, or MSLN.

[000204] In some embodiments, the antigen recognition domain of the CAR specifically binds to an antigen present on a metastatic cancer cell. In some embodiments, the CAR targeting a metastatic cancer cell associated antigen specifically binds to MICA/B, MSLN, or VEGFR-II.

[000205] In some embodiments, the antigen recognition domain of the CAR specifically binds to an antigen present on a neuroblastoma cell. In some embodiments, the CAR targeting a neuroblastoma associated antigen specifically binds to MICA/B or GD2.

[000206] In some embodiments, the antigen recognition domain of the CAR specifically binds to an antigen present on a non-small cell lung cancer (NSCLC) cell. In some embodiments, the CAR targeting a non-small cell lung cancer associated antigen specifically binds to MICA/B, c-MET, or EGFR.

[000207] In some embodiments, the antigen recognition domain of the CAR specifically binds to an antigen present on ovarian cancer. In some embodiments, the CAR targeting an ovarian cancer associated antigen specifically binds to HER2, MICA/B, CCR1, CD3, CD133, CLDN6, c-MET, EFNA4, EPCAM/EGP2, FAP, FOLR1, FOLR3, FR- α , FZD10, GPR27, GPR119, LRRC15, MSLN, MUC1, MUC16, PTK7, SLC34A2, sTN, TMEM238, or VTCN1. In some embodiments, the antigen recognition domain of said CAR comprises the H-CDRs, H- and L- CDRs, the VH, or a single chain of VH and VL of an antibody comprising sofutuzumab, 4H11, 4H5, huDS6, farletuzumab, anetumab, trastuzumab, pertuzumab, PF-0664720, sibrotuzumab, huM25, or lifastuzumab.

[000208] In some embodiments, the antigen recognition domain of the CAR specifically binds to an antigen present on pancreatic cancer. In some embodiments, the CAR targeting a pancreatic cancer associated antigen specifically binds to MICA/B, ADAM12, CA19.9, CFC1, EFNA4, EPCAM/EGP2, ICAM1, LILRB2, LRRC15, MSLN, MUC1, tMUC1, MUC5A, MUC16, MUC17, PSCA, PTK7, or SLC30A8. In some embodiments, the antigen recognition domain of said CAR comprises the H-CDRs, H- and L- CDRs, the VH, or a single chain of VH and VL of an antibody comprising PF-0664720, clivatuzumab, 4H11, 4H5, anetumumab, huDS6, sofutuzumab, huM25, or RG7841.

[000209] In some embodiments, the antigen recognition domain of the CAR specifically binds to an antigen present on a peritoneal carcinoma cell. In some embodiments, the CAR targeting a peritoneal carcinoma associated antigen specifically binds to FOLR3.

[000210] In some embodiments, the antigen recognition domain of the CAR specifically binds to an antigen present on prostate cancer. In some embodiments, the CAR targeting a prostate cancer associated antigen specifically binds to MICA/B, ACPP, CD10, CD49f, CD133, EFNA4, OR51E2, PSCA, PSMA/FOLH1, PTK7, SLC30A4, SLC45A3, STEAP, TIM-3, or TMEFF2/TENB2. In some embodiments, the antigen recognition domain of said CAR comprises the H-CDRs, H- and L- CDRs, the VH, or a single chain of VH and VL of an antibody comprising mirvetuximab, or J591 variant 1 or 2.

[000211] In some embodiments, the antigen recognition domain of the CAR specifically binds to an antigen present on a renal cancer cell. In some embodiments, the CAR targeting a renal cancer associated antigen specifically binds to MICA/B, CD3, CD70, ICAM1, KISS1R, LILRB2, QRFPR, SLC6A3, or TIM-3.

[000212] In some embodiments, the antigen recognition domain of the CAR specifically binds to an antigen present on a sarcoma. In some embodiments, the CAR targeting a sarcoma associated antigen specifically binds to MICA/B or LRRC15.

[000213] In some embodiments, the antigen recognition domain of the CAR specifically binds to an antigen present on a salivary gland cancer cell. In some embodiments, the CAR targeting a salivary gland cancer associated antigen specifically binds to HER2 or MICA/B.

[000214] In some embodiments, the antigen recognition domain of the CAR specifically binds to an antigen present on skin cancer. In some embodiments, the CAR targeting a skin cancer associated antigen specifically binds to CCR4, CD3, CD10, or ICAM1.

[000215] In some embodiments, the antigen recognition domain of the CAR specifically binds to an antigen present on a synovial sarcoma. In some embodiments, the CAR targeting a synovial sarcoma associated antigen specifically binds to CD99.

[000216] In some embodiments, an antibody specifically binds to an antigen present on a thyroid cancer/tumor cell. In some embodiments, the CAR targeting a thyroid cancer/tumor associated antigen specifically binds to MICA/B, CD10, c-MET, PTK7, or TSHR.

[000217] In some embodiments, an antibody specifically binds to an antigen present on a urothelial cancer cell. In some embodiments, the CAR targeting a urothelial cancer associated antigen specifically binds to MICA/B, CLDN6, EPCAM/EGP2, SIGLEC-15, TIM-3, or UPK2.

[000218] In some embodiments, the antigen recognition domain of the CAR specifically binds to an antigen present on uterine/endometrial cancer cell. In some embodiments, the CAR targeting a uterine/endometrial cancer associated antigen specifically binds to HER2, MICA/B, ALPP, ALPPL2, CCR1, CLDN6, EFNA4, EPHB2, FOLR1, LILRB2, LY6K, LYPD3, MUC1, MUC16, or PTK7. In some embodiments, the antigen recognition domain of said CAR comprises the H-CDRs, H- and L- CDRs, the VH, or a single chain of VH and VL of an antibody comprising PF-0664720, farletuzumab, sofituzumab, 4H11, or 4H5.

[000219] In various embodiments, the antigen recognition domain of the CAR specifically binds to a tumor antigen known to be associated with three or more cancer types (sometimes referred to as “pan-tumor antigen”). A non-limiting set of such pan-tumor antigens comprises at least ADAM12, ADGRE2/EMR2, CA19.9, CCR1, CCR4, CD3, CD10, CD49f, CD133, CEA/CECAM5, CLDN6, c-MET, EFNA4, EGFR, EGFRvIII, EPHA2, EPHB2, FOLR1, HER2, ICAM1, LILRB2, LRRC15, LY6K, LYPD3, MICA/B, MSLN, MUC1, tMUC1, MUC16, MUC17, PSCA, PTK7, TIM-3, TMEM238, and TNC as exemplified in Table 2.

Table 2 – Exemplary Pan-Tumor Antigens and Associated Cancers

Antigen	Associated Cancers
ADAM12	Bone cancer, Breast cancer, Colorectal cancer, Head and Neck cancer, Lung cancer, Pancreatic cancer
ADGRE2/EMR2	Breast cancer, Breast Lung cancer, Glioma, Lung cancer
CA19.9	Colorectal cancer, Esophageal cancer, Liver cancer, Pancreatic cancer
CCR1	Bone cancer, Liver cancer, Lung cancer, Ovarian cancer, Uterine/Endometrial cancer
CCR4	Breast cancer, Lung cancer, Skin cancer
CD3	Colorectal cancer, Head and Neck cancer, Liver cancer, Ovarian cancer, Renal cancer, Skin cancer
CD10	Esophageal cancer, Prostate cancer, Skin cancer, Thyroid tumor
CD49f	Breast cancer, Colorectal cancer, Glioma, Prostate cancer
CD133	Brain cancer, Breast cancer, Colorectal cancer, Glioma, Liver cancer, Lung cancer, Ovarian cancer, Prostate cancer
CEA/CECAM5	Colorectal cancer, Esophageal cancer, Gastric/Stomach cancer, Liver cancer, Lung cancer
CLDN6	Breast cancer, Ovarian cancer, Urothelial cancer, Uterine/Endometrial cancer
c-MET	Breast cancer, Colorectal cancer, Gastric/Stomach cancer, Head and Neck cancer, Kidney cancer, Non-Small Cell Lung cancer, Ovarian cancer, Thyroid tumor
EFNA4	Bladder cancer, Breast cancer, Cervical cancer, Colorectal cancer, Esophageal cancer, Gastric/Stomach cancer, Head and Neck cancer, Lung cancer, Ovarian cancer, Pancreatic cancer, Prostate cancer, Uterine/Endometrial cancer
EGFR	Breast cancer, Glioma, Lung cancer, Non-Small Cell Lung cancer, Neuroblastoma
EGFRvIII	Brain cancer, Glioma, Lung cancer
EPCAM/EGP2	Breast cancer, Breast Lung cancer, Gallbladder carcinoma, Liver cancer, Ovarian cancer, Urothelial cancer
EPHA2	Bone cancer, Breast cancer, Glioma
EPHB2	Colorectal cancer, Esophageal cancer, Gastric/Stomach cancer, Uterine/Endometrial cancer
FOLR1	Lung cancer, Ovarian cancer, Uterine/Endometrial cancer
HER2	Bladder cancer, Breast cancer, Breast Lung cancer, Colorectal cancer, Esophageal cancer, Gastric/Stomach cancer, Head and Neck cancer, Lung cancer, Ovarian cancer, Salivary Gland cancer
ICAM1	Breast cancer, Colorectal cancer, Liver cancer, Lung cancer, Pancreatic cancer, Renal cancer, Skin cancer
LILRB2	Breast cancer, Lung cancer, Pancreatic cancer, Renal cancer, Uterine/Endometrial cancer
LRRC15	Bone cancer, Breast cancer, Colorectal cancer, Head and Neck cancer, Lung cancer, Ovarian cancer, Pancreatic cancer, Renal cancer
LY6K	Bladder cancer, Cervical cancer, Head and Neck cancer, Lung cancer, Uterine/Endometrial cancer
LYPD3	Bladder cancer, Breast cancer, Cervical cancer, Head and Neck cancer, Lung cancer, Uterine/Endometrial cancer
MICA/B	Bladder cancer, Bone cancer, Brain cancer, Breast cancer, Breast Lung cancer, Cervical cancer, Cholangiocarcinoma, Colorectal cancer,

	Esophageal cancer, Gastric (Stomach) cancer, Glioma, Head and Neck cancer, Kidney cancer, Liver cancer, Lung cancer, Mesothelioma, Metastatic cancer, Neuroblastoma, Non-Small Cell Lung cancer, Ovarian cancer, Pancreatic cancer, Prostate cancer, Renal cancer, Sarcoma, Salivary Gland cancer, Thyroid cancer, Urothelial cancer, Uterine/Endometrial cancer
MSLN	Lung cancer, Metastatic cancer, Mesothelioma, Ovarian cancer, Pancreatic cancer
MUC1	Breast cancer, Cervical cancer, Lung cancer, Ovarian cancer, Pancreatic cancer, Uterine/Endometrial cancer
tMUC1	Breast cancer, Cholangiocarcinoma, Pancreatic cancer
MUC16	Cervical cancer, Lung cancer, Ovarian cancer, Pancreatic cancer, Uterine/Endometrial cancer
MUC17	Colorectal cancer, Gastric (Stomach) cancer, Pancreatic cancer
PSCA	Gastric (Stomach) cancer, Pancreatic cancer, Prostate cancer
PTK7	Bladder cancer, Breast cancer, Cervical cancer, Head and Neck cancer, Lung cancer, Ovarian cancer, Pancreatic cancer, Prostate cancer, Thyroid tumor, Uterine/Endometrial cancer
TIM-3	Bladder cancer, Colorectal cancer, Gastric (Stomach) cancer, Liver cancer, Lung cancer, Prostate cancer, Renal cancer, Urothelial cancer
TMEM238	Colorectal cancer, Esophageal cancer, Gastric (Stomach) cancer, Ovarian cancer
TNC	Bladder cancer, Brain cancer, Breast cancer, Head and Neck cancer

[000220] In some embodiments, the antigen recognition domain of the CAR specifically binds to tumor associated HER2, wherein an effector cell comprising said CAR and a solid tumor targeting backbone as disclosed is useful for treating one or more cancers comprising at least bladder cancer, breast cancer, breast lung cancer, colorectal cancer, esophageal cancer, gastric/stomach cancer, head and neck cancer, lung cancer, ovarian cancer, or salivary gland cancer.

[000221] In some embodiments, the antigen recognition domain of the CAR specifically binds to tumor associated MICA/B, wherein an effector cell comprising said CAR and a solid tumor targeting backbone as disclosed is useful for treating one or more cancers comprising at least bladder cancer, bone cancer, brain cancer, breast cancer, breast lung cancer, cervical cancer, cholangiocarcinoma, colorectal cancer, esophageal cancer, gastric/stomach cancer, glioma, head and neck cancer, kidney cancer, liver cancer, lung cancer, mesothelioma, metastatic cancer, neuroblastoma, non-small cell lung cancer, ovarian cancer, pancreatic cancer, prostate cancer, renal cancer, sarcoma, salivary gland cancer, thyroid cancer, urothelial cancer, or uterine/endometrial cancer.

[000222] In some embodiments, the antigen recognition domain of the CAR specifically binds to tumor associated MSLN, wherein an effector cell comprising said CAR and a solid

tumor targeting backbone as disclosed is useful for treating one or more cancers comprising at least lung cancer, metastatic cancer, mesothelioma, ovarian cancer, or pancreatic cancer.

[000223] In some embodiments, the antigen recognition domain of the CAR specifically binds to tumor associated MUC1, wherein an effector cell comprising said CAR and a solid tumor targeting backbone as disclosed is useful for treating one or more cancers comprising at least breast cancer, cervical cancer, lung cancer, ovarian cancer, pancreatic cancer, or uterine/endometrial cancer.

[000224] In some embodiments, the antigen recognition domain of the CAR specifically binds to tumor associated PSCA, wherein an effector cell comprising said CAR and a solid tumor targeting backbone as disclosed is useful for treating one or more cancers comprising at least gastric/stomach cancer, pancreatic cancer, or prostate cancer.

[000225] In various embodiments, the CARs applicable to the cells described herein include at least an ectodomain, a transmembrane domain, and an endodomain. In some embodiments, the endodomain of the CAR comprises at least one signaling domain that is activated upon antigen binding. In some embodiments of the CAR endodomain, one or more co-stimulation domains (oftentimes referred to as “additional signaling domain(s)”) is further included for optimized functionality. Exemplary signal transducing proteins suitable for a CAR design include, but are not limited to, 2B4, 4-1BB, CD16, CD2, CD28, CD28H, CD3 ζ /1XX (i.e., CD3 ζ or CD3 ζ 1XX), DAP10, DAP12, DNAM1, FcERI γ , IL21R, IL-2R β (IL-15R β), IL-2R γ , IL-7R, KIR2DS2, NKG2D, NKp30, NKp44, NKp46, CS1 and CD8. The description of the exemplary signal transducing proteins, including transmembrane and cytoplasmic sequences of the proteins are provided below, and further in Table 3A.

Table 3A:

	Protein name	UniProtKB Accession No.	Transmembrane Sequence	Cytoplasmic Sequence
2B4	Natural killer cell receptor 2B4	Q9BZW8	FLVIVILSALFLGT LACFCV (SEQ ID NO: 76)	WRRKRKEKQSETSPKEFLTIYED VKDLKTRRNHEQEQTTPGGGSTI YSMIQSQSSAPTSQEPAYTLYSLI QPSRKSGSRKRNHSPSFNSTIYE VIGKSQPKAQNPALSRKELENF DVYS (SEQ ID NO: 98)
4-1BB	Tumor necrosis factor receptor superfamily member 9	Q07011	IISFFLALTSTALLF LLFFLTLRFSVV (SEQ ID NO: 77)	KRGRKKLLYIFKQPFMRPVQTT QEEDGCSCRFPEEEEGGCEL (SEQ ID NO: 99)

CD16	IgG Fc region Receptor III-A	P08637	VSFCLVMVLLFAV DTGLYFSVKTNIR SSTRD (SEQ ID NO: 78)	WKDHKFKWRKDPQDK (SEQ ID NO: 100)
CD2	T-cell surface antigen CD2	P06729	IYLIIGICGGGSL MVFVALLVFYITK RKKQRSRRNDEEL ETRAHRVATEERG RKPHQIPASTPQN PATSQHPPPPPGH RSQAPSHRPPPPG HRVQ (SEQ ID NO: 79)	HQPQKRPPAPSGTQVHQKGGP LPRPRVQPKPPHGAAENSLSPSS N (SEQ ID NO: 101)
CD28	T-cell-specific surface glycoprotein CD28	P10747	FWVLVVVGGLA CYSLLVTVAFIIFW V (SEQ ID NO: 80)	RSKRSRLHSDYMNMTPRRPGP TRKHYPYAPPRDFAAYRS (SEQ ID NO: 102)
CD28H	Transmembrane and immunoglobulin domain-containing protein 2	Q96BF3	FLFVLLGVGSMG VAAIVWGAW (SEQ ID NO: 81)	FWGRRSCQQRDSGNPNAFY NVLYRPRGAPKKSEDCSGEGKD QRGQSIYSTSFPQPAPRPHLAS RPCSPRPCSPRPGHPVSMVRV SPRPSPTQQPRPKGFPKVGEE (SEQ ID NO: 103)
CD3ζ/1XX	T-cell surface glycoprotein CD3 zeta chain	P20963	LCYLLDGILFIYGV ILTALFL (SEQ ID NO: 82)	RVKFSRSADAPAYQQGQNQLY NELNLGRREEYDVLDKRRGRDP EMGGKQRRKNPQEGLYNELQ KDKMAEAYSEIGMKGERRRGK GHDGLYQGLSTATKDTYDALH MQALPPR (SEQ ID NO: 104; CD3ζ) Or RVKFSRSADAPAYQQGQNQLY NELNLGRREEYDVLDKRRGRDP EMGGKQRRKNPQEGLFNELQKD KMAEAFSEIGMKGERRRGKGH DGLFQGLSTATKDTFDALHMQA LPPR (SEQ ID NO: 105; containing 2 mutations in ITAM1; CD3ζ1XX)
DAP10	Hematopoietic cell signal transducer	Q9UBK5	LLAGLVAADAVA SLLIVGAVF (SEQ ID NO: 83)	LCARPRSPAQEDGKVYINMPG RG (SEQ ID NO: 106)
DAP12	TYRO protein tyrosine kinase-binding protein	O43914	GVLGIVMGDLV LTVLIALAV (SEQ ID NO: 84)	YFLGRLVPRGRGAAEAATRKQR ITETESPYQELQGQRSDVYSDLN TQRPPYK (SEQ ID NO: 107)

DNAM1	CD226 antigen	Q15762	GGTVLLLLFVISIT TIIVIFL (SEQ ID NO: 85)	NRRRRRERRDLFTESWDTQKAP NNYRSPISTSQPTNQSMDDTRED IYVNYPTFSRRPKTRV (SEQ ID NO: 108)
FcERI γ	High affinity immunoglobulin epsilon receptor subunit gamma	P30273	CYILDAILFLYGIV LTLLYC (SEQ ID NO: 86)	RLKIQVRKAAITSYEKSDGVYTG LSTRNQETYETLKHEKPPQ (SEQ ID NO: 109)
IL-21R	Interleukin-21 receptor	Q9HBE5	GWNPHLLLLLLL IVFIPAFW (SEQ ID NO: 87)	SLKTHPLWRLWKKIWAVSPER FFMPLYKGCSDGDFKKWVGAPFT GSSLELGPWSPEVPSTLEVYSCH PPRSPAKRLQLTELQEPALVES DGVPKPSFWPTAQNSGGSAYSE ERDRPYGLVSIDTVTVLDAEGPC TWPCSCEDDGYPALDLDAGLEP SPGLEDPLLDAGTTVLSCGCVSA GSPGLGGPLGSLLDRLKPPLADG EDWAGGLPWGGRSPGGVSESE AGSPLAGLMDMTFDSGFVGSDC SSPVECDFTSPGDEGPSPSYLRQ WVVIPPLSSPGPQAS (SEQ ID NO: 110)
IL-2R β (IL-15R β)	Interleukin-2 receptor subunit beta	P14784	IPWLGHLLVGLSG AFGFILVYLLI (SEQ ID NO: 88)	NCRNTGPWLKKVLKCNTPDPSPK FFSQLSSEHGGDVQKWLSSPFPS SSFSPGGLAPEISPVEVLERDKVT QLLLQQDKVPEPASLSSNHSLS CFTNQGYFFFHLPDALEIEACQV YFTYDPYSEEDPDEGVAGAPTG SSPQLQPLSGEDDAYCTFPSRD DLLLLFSPSLLGGSPSTAPGGSG AGEERMPPSLQERVPRDWDQP LGPPTPGVPDLVDFQPPPELVLR EAGEEVPDAGPREGVSFPWSRPP GQGEFRALNARLPLNTDAYLSL QELQGQDPHTLV (SEQ ID NO: 111)
IL-2R γ	Cytokine receptor common subunit gamma	P31785	VVISVGSMGLIISL LCVYFWL (SEQ ID NO: 89)	ERTMPRIPTLKNLEDLVTEYHGN FSAWSGVSKGLAESLQPDYSER LCLVSEIPKGGALGEGPGASPC NQHSPYWAPPCYTLKPET (SEQ ID NO: 112)
IL-7R	Interleukin-7 receptor subunit alpha	P16871	PILLTISILSFFSVA LLVILACVLW (SEQ ID NO: 90)	KKRIKPIVWPSLPDHKKTLEHLC KKPRKNLNVSNPESFLDCQIHR VDDIQARDEVEGFLQDTFPQQL EESEKQRLGGDVQSPNCPSADV VITPESFGRDSSLTCLAGNVSA DAPILSSSRSLDCRESGKNGPHV YQDLLLSLGTNSTLPPPSLQS

				GILTLNPVAQGQPILTSLSGNQE EAYVTMSSFYQNNQ (SEQ ID NO: 113)
KIR2DS2	Killer cell immunoglobulin-like receptor 2DS2	P43631	VLIGTSVVKIPFTI LLFFLL (SEQ ID NO: 91)	HRWCSNKKNAAVMDQEPAGNR TVNSEDSDQDHQEVSYA (SEQ ID NO: 114)
NKG2D	NKG2-D type II integral membrane protein	P26718	PFFFCFIAVAMGI RFIMVA (SEQ ID NO: 92)	IWSAVFLNSLFNQEVQIPLTESY CGPCPKNWICYKNNCYQFFDES KNWYESQASCMSQNASLLKVY SKEDQDLLKLVKSYHWMGLVH IPTNGSWQWEDGSILSPNLLTIE MQKGDICALYASSFKGYIENCST PNTYICMQRTV (SEQ ID NO: 115)
NKp30	Natural cytotoxicity triggering receptor 3	O14931	AGTVLLLRAGFY AVSFLSVAV (SEQ ID NO: 93)	GSTVYYQGKCLTWKGPRRQLP AVVPAPLPPPCGSSAHLPPVPG G (SEQ ID NO: 116)
NKp44	Natural cytotoxicity triggering receptor 2	O95944	LVPVFCGLLVAKS LVLSALLV (SEQ ID NO: 94)	WWGDIWWKTMMELRSLDTQK ATCHLQQVTDLPWTSVSSPVER EILYHTVARTKISDDDDDEHTL (SEQ ID NO: 117)
NKp46	Natural cytotoxicity triggering receptor 1	O76036	GLAFLVLVALVW FLVEDWLS (SEQ ID NO: 95)	RKRTRERASRASTWEGRRRLNT QTL (SEQ ID NO: 118)
CS1	SLAM family member 7	Q9NQ25	VLLCLLLVPLLLS LFVLGLFL (SEQ ID NO: 96)	WFLKRERQEEYIEKKRVDICRE TPNICPHSGENTYDTPHTNRTI LKEDPANTVYSTVEIPKKMENP HSLLTMPDTPRLFAYENVI (SEQ ID NO: 119)
CD8	T-cell surface glycoprotein CD8 alpha chain	P01732	IYIWAPLAGTC (SEQ ID NO: 97)	GVLLLSLVITLYCNHRNRRRVC KCPRPVVKSGDKPSLSARYV (SEQ ID NO: 120)

[000226] In some embodiments of the CAR applicable to the cells provided herein, the endodomain of the CAR comprises at least a first signaling domain having an amino acid sequence that has at least about 85%, about 90%, about 95%, about 96%, about 97%, about 98%, or about 99% identity to the cytoplasmic domain, or a portion thereof, of 2B4, 4-1BB, CD16, CD2, CD28, CD28H, CD3 ζ , CD3 ζ 1XX, DAP10, DAP12, DNAM1, FcERI γ , IL21R, IL-2R β (IL-15R β), IL-2R γ , IL-7R, KIR2DS2, NKG2D, NKp30, NKp44, NKp46, CS1, or CD8, represented by SEQ ID NOs: 98-120, respectively. In some embodiments, the first signaling domain comprises an amino acid sequence of at least 90% identity to any of SEQ ID NOs: 98-120. In

some embodiments, the first signaling domain comprises an amino acid sequence of at least 95% identity to any of SEQ ID NOs: 98-120. In some embodiments, the first signaling domain comprises the amino acid sequence of any of SEQ ID NOs: 98-120. In some embodiments, the signaling domain of the CAR comprises only a portion of the cytoplasmic domain of 2B4, 4-1BB, CD16, CD2, CD28, CD28H, CD3 ζ , CD3 ζ 1XX, DAP10, DAP12, DNAM1, FcERI γ IL21R, IL-2R β (IL-15R β), IL-2R γ , IL-7R, KIR2DS2, NKG2D, NKp30, NKp44, NKp46, CS1, or CD8, represented by SEQ ID NOs: 98-120. In some embodiments, the portion of the cytoplasmic domain selected for the CAR signaling domain comprises an amino acid sequence that has at least about 85%, about 90%, about 95%, about 96%, about 97%, about 98%, or about 99% identity to an ITAM (immunoreceptor tyrosine-based activation motif), a YxxM motif, a TxYxxV/I motif, FcR γ , hemi-ITAM, and/or an ITT-like motif.

[000227] In some embodiments of the CAR as provided, the endodomain of the CAR comprising a first signaling domain further comprises a second signaling domain comprising an amino acid sequence that has at least about 85%, about 90%, about 95%, about 96%, about 97%, about 98%, or about 99% identity to the cytoplasmic domain, or a portion thereof, of 2B4, 4-1BB, CD16, CD2, CD28, CD28H, CD3 ζ , CD3 ζ 1XX, DAP10, DAP12, DNAM1, FcERI γ IL21R, IL-2R β (IL-15R β), IL-2R γ , IL-7R, KIR2DS2, NKG2D, NKp30, NKp44, NKp46, CS1 or CD8, represented by SEQ ID NOs: 98-120, respectively, wherein the second signaling domain is different from the first signaling domain. In some embodiments, the second signaling domain comprises an amino acid sequence of at least 90% identity to any of SEQ ID NOs: 98-120. In some embodiments, the second signaling domain comprises an amino acid sequence of at least 95% identity to any of SEQ ID NOs: 98-120. In some embodiments, the second signaling domain comprises the amino acid sequence of any of SEQ ID NOs: 98-120.

[000228] In some embodiments of the CAR as provided, the endodomain of the CAR comprising a first and a second signaling domain further comprises a third signaling domain comprising an amino acid sequence that has at least about 85%, about 90%, about 95%, about 96%, about 97%, about 98%, or about 99% identity to the cytoplasmic domain, or a portion thereof, of 2B4, 4-1BB, CD16, CD2, CD28, CD28H, CD3 ζ , CD3 ζ 1XX, DAP10, DAP12, DNAM1, FcERI γ , IL21R, IL-2R β (IL-15R β), IL-2R γ , IL-7R, KIR2DS2, NKG2D, NKp30, NKp44, NKp46, CS1, or CD8, represented by SEQ ID NOs: 98-120, respectively, wherein the third signaling domain is different from the first and the second signaling domains. In some embodiments, the third signaling domain comprises an amino acid sequence of at least 90% identity to any of SEQ ID NOs: 98-120. In some embodiments, the third signaling domain comprises an amino acid sequence of at least 95% identity to any of SEQ ID NOs: 98-120. In some embodiments, the third signaling domain comprises the amino acid sequence of any of

SEQ ID NOs: 98-120. In some embodiments, signal transducing proteins suitable for designing a signaling domain of a CAR endodomain further comprise CD27, OX40, ICOS, PD-1, LAG-3, BTLA, or CTLA-4.

[000229] In some exemplary embodiments of a CAR having an endodomain comprised of only one signaling domain, said endodomain comprises an amino acid sequence that has at least about 85%, about 90%, about 95%, about 96%, about 97%, about 98%, or about 99% identity to the cytoplasmic domain or a portion thereof, of a protein including, but not limited to, DNAM1, CD28H, KIR2DS2, DAP12 or DAP10.

[000230] In some exemplary embodiments of a CAR having an endodomain comprised of two different signaling domains, said endodomain comprises fused cytoplasmic domains, or portions thereof, in a form including, but not limited to, 2B4-CD3 ζ /1XX (i.e., 2B4-CD3 ζ or 2B4-CD3 ζ 1XX; same below), 2B4-DNAM1, 2B4-FcERI γ , 2B4-DAP10, CD16-DNAM1, CD16-DAP10, CD16-DAP12, CD2-CD3 ζ /1XX, CD2-DNAM1, CD2-FcERI γ , CD2-DAP10, CD28-DNAM1, CD28-FcERI γ , CD28-DAP10, CD28-DAP12, CD28-CD3 ζ /1XX, CD28H-CD3 ζ /1XX, DAP10-CD3 ζ /1XX, DAP10-DAP12, DAP12-CD3 ζ /1XX, DAP12-DAP10, DNAM1-CD3 ζ /1XX, KIR2DS2-CD3 ζ /1XX, KIR2DS2-DAP10, KIR2DS2-2B4, or NKp46-2B4.

[000231] In some exemplary embodiments of a CAR having an endodomain comprised of three different signaling domains, said endodomain comprises fused cytoplasmic domains, or portions thereof, in a form including, but not limited to, 2B4-DAP10-CD3 ζ /1XX, 2B4-IL21R-DAP10, 2B4-IL2RB-DAP10, 2B4-IL2RB-CD3 ζ /1XX, 2B4-41BB-DAP10, CD16-2B4-DAP10, or KIR2DS2-2B4-CD3 ζ /1XX.

[000232] In some embodiments, the transmembrane domain of the CAR comprises an amino acid sequence that has at least about 85%, about 90%, about 95%, about 96%, about 97%, about 98%, or about 99% identity to a full length or a portion of the transmembrane region of CD2, CD3 δ , CD3 ϵ , CD3 γ , CD3 ζ , CD4, CD8, CD8a, CD8b, CD16, CD27, CD28, CD28H, CD40, CD84, CD166, 4-1BB, OX40, ICOS, ICAM-1, CTLA4, PD1, LAG3, 2B4, BTLA, DNAM1, DAP10, DAP12, FcERI γ , IL7, IL12, IL15, KIR2DL4, KIR2DS1, KIR2DS2, NKp30, NKp44, NKp46, NKG2C, NKG2D, CS1, or T cell receptor polypeptide. In some other embodiments, the transmembrane domain of a CAR comprises an amino acid sequence that has at least about 85%, about 90%, about 95%, about 96%, about 97%, about 98%, or about 99% identity to a full length or a portion of the transmembrane region of (a) 2B4, CD16, CD2, CD28, CD28H, CD3 ζ , DAP10, DAP12, DNAM1, FcERI γ , KIR2DS2, NKG2D, NKp30, NKp44, NKp46, CS1, or CD8, represented by SEQ ID NOs: 76, 78-86, 91-97, respectively; or of (b) 2B4, CD28, CD28H, DAP10, DNAM1, KIR2DS2, and NKG2D. In some embodiments, the transmembrane domain comprises an amino acid sequence of at least about 90% identity to any of SEQ ID NOs: 76, 78-

86, 91-97. In some embodiments, the transmembrane domain comprises an amino acid sequence of at least about 95% identity to any of SEQ ID NOs: 76, 78-86, 91-97. In some embodiments, the transmembrane domain comprises the amino acid sequence of any of SEQ ID NOs: 76, 78-86, 91-97. In some embodiments of the CAR, the transmembrane domain and its immediately linked signaling domain are from the same protein. In some other embodiments of the CAR, the transmembrane domain and the signaling domain that is immediately linked are from different proteins.

[000233] Non-limiting examples of CAR constructs comprising a transmembrane domain (TM) and an endodomain (labelled as: TM-(endodomain)) are shown in Table 3B. In general, the illustrated CAR constructs each comprise a transmembrane domain, and an endodomain comprising one or more signaling domains derived from the cytoplasmic region of one or more signal transducing proteins. In general, a transmembrane domain is a three-dimensional protein structure which is thermodynamically stable in a membrane such as the phospholipid bilayer of a biological membrane (e.g., a membrane of a cell or cell vesicle). Thus, in some embodiments, the transmembrane domain of the CAR applicable to the cells provided herein comprises a single alpha helix, a stable complex of several transmembrane alpha helices, a transmembrane beta barrel, a beta-helix of gramicidin A, or any combination thereof. In various embodiments, the transmembrane domain of the CAR comprises all or a portion of a “transmembrane protein” or “membrane protein” that is within the membrane. As used herein, a “transmembrane protein” or “membrane protein” is a protein located at and/or within a membrane. Examples of transmembrane proteins that are suitable for providing a transmembrane domain comprised in a CAR according to some embodiments of the invention include, but are not limited to, a receptor, a ligand, an immunoglobulin, a glycoprotein, or any combination thereof. In some embodiments, the transmembrane domain comprised in the CAR comprises all or a portion of a transmembrane domain of 2B4, 4-1BB, BTLA, CD2, CD3 δ , CD3 ϵ , CD3 γ , CD3 ζ , CD4, CD8, CD8a, CD8b, CD16, CD27, CD28, CD28H, CD40, CD84, CD166, CS1, CTLA-4, DNAM1, DAP10, DAP12, FcERI γ , ICOS, ICAM-1, IL7, IL12, IL15, KIR2DL4, KIR2DS1, KIR2DS2, LAG3, PD1, NKp30, NKp44, NKp46, NKG2C, NKG2D, OX40, T cell receptor polypeptide (such as TCR α and/or TCR β), a nicotinic acetylcholine receptor, a GABA receptor, or any combination thereof.

[000234] In some embodiments, one or more signaling domains comprised in the CAR endodomain are derived from the same or a different protein from which the TM is derived. As shown in Table 3B, the portion representing the transmembrane domain of the CAR is underlined, the domains comprised in the endodomain appear in parenthesis, “()”, with each of the TM and signaling domains designated by the name of the signal transducing protein from which the domain sequence is derived. In embodiments, the amino acid sequence of each TM or

signaling domains may be of about 85%, about 90%, about 95%, about 96%, about 97%, about 98%, or about 99% identity to a full length or a portion of the corresponding transmembrane or cytoplasmic regions of the designated signal transducing protein. Exemplary CAR constructs comprising a transmembrane domain and an endodomain as provided herein include, but are not limited to: NKG2D-(2B4-IL2RB-CD3ζ), CD8-(41BB-CD3ζ1XX), CD28-(CD28-2B4-CD3ζ), CD28-(CD28-CD3 ζ 1XX), CD28H-(CD28H-CD3ζ), DNAM1-(DNAM1-CD3ζ), DAP10-(DAP10-CD3ζ), KIR2DS2-(KIR2DS2-CD3ζ), KIR2DS2-(KIR2DS2-DAP10), KIR2DS2-(KIR2DS2-2B4), CD16-(CD16-2B4-DAP10), CD16-(CD16-DNAM1), NKp46-(NKp46-2B4), NKp46-(NKp46-2B4-CD3ζ), NKp46-(NKp46-CD2-DAP10), CD2-(CD2-CD3ζ), 2B4-(2B4-CD3ζ), 2B4-(2B4-FcERIγ), and CS1-(CS1-CD3ζ). In some embodiments, each of the above exemplary CAR constructs comprising a transmembrane domain and an endodomain comprises an amino acid sequence of at least about 85%, about 90%, about 95%, about 96%, about 97%, about 98%, about 99%, or 100% identity to a sequence represented by each of SEQ ID NOs: 121-139 in Table 3B. In some embodiments, the CAR comprises an amino acid sequence of at least about 90% identity to any of SEQ ID NOs: 121-139. In some embodiments, the CAR comprises an amino acid sequence of at least about 95% identity to any of SEQ ID NOs: 121-139. In some embodiments, the CAR comprises the amino acid sequence of any of SEQ ID NOs: 121-139. The illustrative sequence for each construct provided in Table 3B has text formatted to match the formatting of the corresponding region in the illustration at left of the sequence (i.e., underlined, normal, or bolded text). For most of the illustrative constructs of Table 3B, the TM is the first sequence region; however, constructs may include an extracellular domain preceeding the TM (see, e.g., Construct 7 in Table 3B), and may be from the same or a different protein as the TM. In some embodiments, two or more signaling domains comprised in the CAR endodomain may be separated by one or more additional sequences, such as a spacer or a linker.

Table 3B:

Construct	Sequence Domains <u>TM</u> -(endodomain)	Illustrative Sequence	SEQ ID NO:
1	<u>NKG2D</u> -(<u>2B4</u> - <u>IL2Rβ</u> - <u>CD3ζ</u>)	<u>SNLFVASWIAVMIIFRIGMAVAIFCCFFPSWRRKRKE</u> <u>KQSETSPKEFLTIYEDVKDLKTRRNHEQEQTTPGGG</u> <u>STIYSMIQSQSSAPTSQEPAYTLYSLIQPSRKSGSRKR</u> <u>NHSPSFNSTIYEVIGKSQPKAQNPARLSRKELENFDV</u> <u>YSNCRNTGPWLKKVLKCNTPDPSKFSSQLSSEHGGDV</u> <u>QKWLSSPFPSSSFSPGGLAPEISPLEVLERDKVTQLLLQ</u> <u>QDKVPEPASLSSNHSLTSCFTNQGYFFFHLPDALEIEAC</u> <u>QVYFTYDPYSEEDPDEGVAGAPTGSSPQPLQPLSGEDD</u> <u>AYCTFPSRDDLLLFSPSLLGGPSPPSTAPGGSGAGEERM</u> <u>PPSLQERVPRDWPQPLGPPTPGVVDLVDLQPPPELVLR</u>	121

		EAGEEVPDAGPREGVSFPWSRPPGQGEFRALNARLPN TDAYLSLQELQGQDPHTLVVRVKFSRSADAPAYQQGQ NQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRR KNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKG HDGLYQGLSTATKDTYDALHMQALPPR	
2	<u>CD8-(41BB- CD3ζ1XX)</u>	<u>IYIWAPLAGTCGVLLLSLVITLYCKRGRKLLYIFKQP</u> FMRPVQTTQEEDGCSCRFPEEEEGGCELRVKFSRSA DAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPE MGGKPRRKNPQEGLFNELQKDKMAEAFSEIGMKGERR RGKGHDGLFQGLSTATKDTFDALHMQALPPR	122
3	<u>CD28-(CD28- 2B4-CD3ζ)</u>	<u>FWVLVVVGVLACYSLLVTVAFIIFWVRSKRSRLHSD</u> YMNMTPRRPGPTRKHYQPYAPPRDFAAYRSWRRKRK EKQSETSPKEFLTIEDVKDLKTRRNHEQEQTFFGG GSTIYSMIQSQSSAPTSQEPAYTLYSLIQPSRKSGSRK RNHSPSFNSTIYEVIGKSQPKAQNPAPLSRKELENFD VYSRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDV LDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEA YSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHM QALPPR	123
4	<u>CD28-(CD28- CD3ζ1XX)</u>	<u>FWVLVVVGVLACYSLLVTVAFIIFWVRSKRSRLHSD</u> YMNMTPRRPGPTRKHYQPYAPPRDFAAYRSRVKFSRS ADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGR DPEMGGKPRRKNPQEGLFNELQKDKMAEAFSEIGM KGERRRGKGHDGLFQGLSTATKDTFDALHMQALP PR	124
5	<u>CD28H- (CD28H-CD3ζ)</u>	<u>FLFVLLGVGSMGVAAIVWGAFWGRRSCQQRDSGNS</u> PGNAFYSNVLYRPRGAPKKSEDCSGEGKDQRGQSIYST SFPQPAPRQPHLASRPCSPRCPSPRPGHPVSMVRVSP RPSPTQQPRPKGFVKVGEERVKFSRSADAPAYQQGQN QLYNELNLGRREEYDVLDKRRGRDPEMGGKPRR KNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGH DGLYQGLSTATKDTYDALHMQALPPR	125
6	<u>DNAM1- (DNAM1- CD3ζ)</u>	<u>GGTVLLLLFVISITTIIVIFLNRRRRRERRDLFTESWDTQ</u> KAPNNYRSPISTSQPTNQSMDDTREDIYVNYPTFSRRPK TRVRVKFSRSADAPAYQQGQNQLYNELNLGRREEY DVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDK MAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDT YDALHMQALPPR	126
7	<u>DAP10- (DAP10-CD3ζ)</u>	<u>TTPGERSSLPAYFPGTSGSCSGCSLSLPLLAGLVAADA</u> VASLLIVGAVFLCARPRRSPAQEDGKVYINMPGRGRV KFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDK RRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAY SEIGMKGERRRGKGHDGLYQGLSTATKDTYDALH MQALPPR	127
8	<u>KIR2DS2- (KIR2DS2- CD3ζ)</u>	<u>VLIGTSVVKIPFTILLFFLLHRWCSNKKNAAVMDQEP</u> GNRTVNSEDSDEQDHQEVSYARVKFSRSADAPAYQQ GQNQLYNELNLGRREEYDVLDKRRGRDPEMGGK PRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRG KGHDGLYQGLSTATKDTYDALHMQALPPR	128
9	<u>KIR2DS2- (KIR2DS2- DAP10)</u>	<u>VLIGTSVVKIPFTILLFFLLHRWCSNKKNAAVMDQEP</u> GNRTVNSEDSDEQDHQEVSYALCARPRRSPAQEDGK VYINMPGRG	129
10	<u>KIR2DS2- (KIR2DS2- 2B4)</u>	<u>VLIGTSVVKIPFTILLFFLLHRWCSNKKNAAVMDQEP</u> GNRTVNSEDSDEQDHQEVSYAWRRKRKEKQSETSPK EFLTIEDVKDLKTRRNHEQEQTFFGGGSTIYSMIQS	130

		QSSAPTSQEPAYTLYSLIQPSRKSGSRKRNHSPSFNST IYEVIGKSQPKAQNPARLSRKELENFDVYS	
11	CD16-(CD16- 2B4-DAP10)	VSFCLVMVLLFAVDTGLYFSVKTNIRSSSTRDWKDHKF KWRKDPQDKWRRKRKEKQSETSPKEFLTIYEDVKD LKTRRNHEQEQTTPGGGSTIYSMIQSQSSAPTSQEP YTLYSLIQPSRKSGSRKRNHSPSFNSTIYEVIGKSQPK AQNPARLSRKELENFDVYSLCARPRRSPAQEDGKVYI NMPGRG	131
12	CD16-(CD16- DNAM1)	VSFCLVMVLLFAVDTGLYFSVKTNIRSSSTRDWKDHKF KWRKDPQDKNRRRRRERRDLFTESWDTQKAPNNYR SPISTSQPTNQSMDDTREDIYVNYPTFSRRPKTRV	132
13	NKp46- (NKp46-2B4)	MGLAFLVLVALVWFLVEDWLSRKTRERASRASTWE GRRRLNTQTLWRRKRKEKQSETSPKEFLTIYEDVKD LKTRRNHEQEQTTPGGGSTIYSMIQSQSSAPTSQEP YTLYSLIQPSRKSGSRKRNHSPSFNSTIYEVIGKSQPK AQNPARLSRKELENFDVYS	133
14	NKp46- (NKp46-2B4- CD3ζ)	MGLAFLVLVALVWFLVEDWLSRKTRERASRASTWE GRRRLNTQTLWRRKRKEKQSETSPKEFLTIYEDVKD LKTRRNHEQEQTTPGGGSTIYSMIQSQSSAPTSQEP YTLYSLIQPSRKSGSRKRNHSPSFNSTIYEVIGKSQPK AQNPARLSRKELENFDVYSRVKFSRSADAPAYQQGQ NQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKN PQEGLYNELQKDKMAEAYSEIGMKGERRRGKHDGL YQGLSTATKDTYDALHMQALPPR	134
15	NKp46- (NKp46-CD2- DAP10)	MGLAFLVLVALVWFLVEDWLSRKTRERASRASTWE GRRRLNTQTLKRKKQSRNRNDEELETRAHRVATEER GRKPHQIPASTPQNPA TSQHPPPPGHRSAQPSHRPP PPGHRVQHQPQKRPPAPSGTQVHQKGPPLPRPRV QPKPPHGA AENSLSPSSNLCARPRRSPAQEDGKVYIN MPGRG	135
16	CD2-(CD2- CD3ζ)	IYLIIGICGGGSLLMVFVALLVFYITKRKKQSRNRNDEE LETRAHRVATEERGRKPHQIPASTPQNPA TSQHPPPPG HRSQAPSHRPPPPGHRVQHQPQKRPPAPSGTQVHQK GPPLPRPRVQPKPPHGA AENSLSPSSNRVKFSRSADAP AYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEM GGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGE RRRGKHDGLYQGLSTATKDTYDALHMQALPPR	136
17	2B4-(2B4- CD3ζ)	FLVIIIVLSALFLGTLACFCVWRRKRKEKQSETSPKEFLT IYEDVKDLKTRRNHEQEQTTPGGGSTIYSMIQSQSSAPT SQEPAYTLYSLIQPSRKSGSRKRNHSPSFNSTIYEVIGKS QPKAQNPARLSRKELENFDVYSRVKFSRSADAPAYQQ GQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKP RRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRG KHDGLYQGLSTATKDTYDALHMQALPPR	137
18	2B4-(2B4- FcER1γ)	FLVIIIVLSALFLGTLACFCVWRRKRKEKQSETSPKEFLT IYEDVKDLKTRRNHEQEQTTPGGGSTIYSMIQSQSSAPT SQEPAYTLYSLIQPSRKSGSRKRNHSPSFNSTIYEVIGKS QPKAQNPARLSRKELENFDVYSRLKIQVRKAAITSYE KSDGVYTGLSTRNQETYETLKHEKPPQ	138
19	CS1-(CS1- CD3ζ)	VLLCLLLVPLLLSLFVLGLFLWFLKRERQEEYIEEKRV DICRETPNICPHSGENTYDTIPTNRTILKEDPANTVYS TVEIPKKMENPHSLTTPDTPLRFAYENVIRVKFSRSA DAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRD PEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGM KGERRRGKHDGLYQGLSTATKDTYDALHMQALP PR	139

[000235] In some embodiments, the ectodomain can further include a signal peptide or leader sequence and/or a spacer/hinge. In some embodiments, there is a spacer/hinge between the antigen recognition region/domain and the transmembrane domain of the CAR, although in some other embodiments such spacer/hinge is not required. Exemplary spacers that may be included in a CAR or an ADR are commonly known in the art, including, but not limited to, IgG4 spacers, CD28 spacers, CD8 spacers, or combinations of more than one spacer. The length of the spacers may also vary, from about 15 amino acids (a.a.) to about 300 a.a. or more. In this application, for ease of description, a spacer of less than around 80 a.a., for example 10-80 a.a., is considered short; a spacer of about 80-180 a.a. is considered medium; and a spacer of more than 180 a.a. is considered long. Non-limiting exemplary spacer peptides include those represented by an amino acid sequence of at least about 80%, about 85%, about 90%, about 95%, about 96%, about 97%, about 98%, or about 99% identity to any of SEQ ID NOs: 140-144. In some embodiments, the spacer peptide comprises an amino acid sequence of at least about 90% identity to any of SEQ ID NOs: 140-144. In some embodiments, the spacer peptide comprises an amino acid sequence of at least about 95% identity to any of SEQ ID NOs: 140-144. In some embodiments, the spacer peptide comprises the amino acid sequence of any of SEQ ID NOs: 140-144.

SEQ ID NO: 140

IEVMYPPPYLDNEKSNGTIIHVKGKHLCPSPFLFPGPSKP

(39 a.a.)

SEQ ID NO: 141ESKYGPPCPPCPGGSSGGSGGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPE
NNYKTTTPVLDSGDSFFL

(88 a.a.)

SEQ ID NO: 142ESKYGPPCPPCPAPEFEGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAK
TKPREEQFQSTYRVVSVLT

(89 a.a.)

SEQ ID NO: 143ESKYGPPCPPCPGGSSGGSGGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPE
NNYKTTTPVLDSGDSFFLYSRLTVDKSRWQEGNVFSCSVMEALHNHYTQKSLSLSLGK

(129 a.a.)

SEQ ID NO: 144

ESKYGPPCPPCPAPEFEGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAK
TKPREEQFQSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTIKAKGQPREPQVYTLPPSQEEM
TKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVM
HEALHNHYTQKSLSLGLGK
(229 a.a.)

[000236] In one embodiment, the CAR provided herein comprises a co-stimulatory domain derived from CD28, and a signaling domain comprising the native or modified ITAM1 of CD3 ζ . In a further embodiment, the CAR comprising a co-stimulatory domain derived from CD28, and a native or modified ITAM1 of CD3 ζ also comprises a hinge domain (or “spacer”) and transmembrane domain derived from CD28, wherein an scFv may be connected to the transmembrane domain through the hinge, and the CAR comprises an amino acid sequence of at least about 85%, about 90%, about 95%, about 96%, about 97%, about 98%, or about 99% identity to SEQ ID NO: 145, wherein the spacer may vary in length and sequence. In some embodiments, the CAR comprises an amino acid sequence of at least 80% to SEQ ID NO: 145, wherein the spacer may vary in length and sequence. In some embodiments, the CAR comprises an amino acid sequence of at least 90% to SEQ ID NO: 145, wherein the spacer may vary in length and sequence. In some embodiments, the CAR comprises an amino acid sequence of at least 95% to SEQ ID NO: 145, wherein the spacer may vary in length and sequence. In some embodiments, the CAR comprises the amino acid sequence of SEQ ID NO: 145.

SEQ ID NO: 145

ESKYGPPCPPCPGGSSGGGSGGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPE
NNYKTTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNHYTQKSLSLGLGKMFVVLVVGGV
LACYSLLVTVAFIIFWVRSKRSRLHSDYMNMTPRRPGPTRKHYQPYAPPRDFAAYRSRVKFSRSADAPA
YQQGQNQLYNEINLGRREYDVLDKRRGRDPEMGGKPRRKNPQEGFLNELQKDKMAEAFSEIGMKGERRR
GKGHDGLFQGLSTATKDTFDALHMQALPPR
(spacer-CD28 TM-CD28 Costim-CD3 ζ IXX activation)

[000237] In another embodiment, the CAR applicable to the cells provided herein comprises a transmembrane domain derived from NKG2D, a co-stimulatory domain derived from 2B4, and a signaling domain comprising the native or modified CD3 ζ , represented by an amino acid sequence of at least about 85%, about 90%, about 95%, about 96%, about 97%, about 98%, or about 99% identity to SEQ ID NO: 146. In some embodiments, the CAR comprises an amino acid sequence of at least about 90% identity to SEQ ID NO: 146. In some embodiments, the CAR comprises an amino acid sequence of at least about 95% identity to SEQ ID NO: 146. In some embodiments, the CAR comprises the amino acid sequence of SEQ ID NO: 146. In some

embodiments, said CAR comprising a transmembrane domain derived from NKG2D, a co-stimulatory domain derived from 2B4, and a signaling domain comprising the native or modified CD3 ζ may further comprise a hinge.

SEQ ID NO: 146

SNLFVASWIAVMII FRIGMAVAIFCCFFPSWRRKRKEKQSETSPKEFLTIIYEDVKDLKTRRNHEQEQT
PGGGSTIYSMIQSQSSAPTSQEPAYTLYSLIQPSRKSGSRKRNHSPSFNSTIYEVIGKSQPKAQNP
RKELNFDVYSRVKFSRSADAPAYKQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLY
NELQKDKMAEAYSEIGMKGERRRGKGDGLYQGLSTATKDTYDALHMQALPPR

(263 a.a NKG2D TM-2B4-CD3 ζ)

[000238] In one example, the genetically engineered immune cells, iPSCs and derivative effector cells comprise a solid tumor targeting backbone as disclosed herein and a CAR comprising an antigen recognition region specific to a tumor cell surface HER2 antigen. Unless otherwise specified, the antigen binding domain of the HER2-CAR in this application is based on the CDRs of CasMab250, a HER2 cancer-specific monoclonal antibody (CasMab), and this CasMab250 based HER2-CAR is also referred to “CasMab250-CAR” from time to time in this application.

[000239] In some embodiments the antigen binding domain of the HER2-CAR comprises a single chain variable fragment (scFV) having a sequence identity of at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 99%, 100%, or any percentage in-between, when compared to the exemplary sequences represented by SEQ ID NO: 153 or SEQ ID NO: 154, wherein each of SEQ ID NOs: 153 and 154 comprise a linker that can vary in length and/or sequence. In some embodiments, the scFV comprises an amino acid sequence of at least 90% identity to SEQ ID NO: 153 or 154. In some embodiments, the scFV comprises an amino acid sequence of at least 95% identity to SEQ ID NO: 153 or 154. In some embodiments, the scFV comprises the amino acid sequence of SEQ ID NO: 153. In some embodiments, the scFV comprises the amino acid sequence of SEQ ID NO: 154.

SEQ ID NO: 153

EVQLVESGGGLVQPGGSLKLSCAASGFTFSNYGMSWVRQTPDRRLQLVATINNNGGGTYYPDSVKGRFTI
SRDNAKNTLYLQMSLKSSEDAMYYCTSPGLLWDAGAGTTVTVSSGSTSGGGSGGGSSDVMTQ
TPLTSLSVSIGQPASISCKSSQSLDSDGRTYLNWLLQRPQGQSPKRLIYLVSKLDSGAPDRFTGSGSGTDF
TLKISRVEAEDLGVIYCWQGTGTHFPQTFGGGTKLEIK

SEQ ID NO: 154

DVVTQTPLTSLSVSIGQPASISCKSSQSLDSDGRTYLNWLLQRPQGQSPKRLIYLVSKLDSGAPDRFTGS
SGTDFTLKISRVEAEDLGVIYCWQGTGTHFPQTFGGGTKLEIKGSTSGGGSGGGSSSEVQLVESGGG

LVQPGGSLKLSAASGFTFSNYGMSWVRQTPDRRLELVATINNNGGGTTYYPDSVKGRFTISRDNANTLY
LQMSLKSSEDAMYCTSPGLLWDAGAGTTTVTVSS

[000240] In one embodiment, the CAR provided herein comprises an amino acid sequence of at least about 85%, about 90%, about 95%, about 96%, about 97%, about 98%, or about 99% identity to SEQ ID NO: 155, wherein the linker in the ectodomain and the spacer between the ectodomain and transmembrane domain may vary in length and sequence. In some embodiments, the CAR comprises an amino acid sequence of at least about 90% identity to SEQ ID NO: 155, wherein the linker in the ectodomain and the spacer between the ectodomain and transmembrane domain may vary in length and sequence. In some embodiments, the CAR comprises an amino acid sequence of at least about 95% identity to SEQ ID NO: 155, wherein the linker in the ectodomain and the spacer between the ectodomain and transmembrane domain may vary in length and sequence. In some embodiments, the CAR comprises the amino acid sequence of SEQ ID NO: 155. In some embodiments, the CAR provided herein recognizes a HER2 antigen specific to cells of solid tumors. In some embodiments, the CAR provided herein recognizes a HER2 antigen of a tumor comprising breast cancer, ovary cancer, endometrium cancer, lung cancer, esophageal cancer, salivary gland cancer, bladder cancer, gastric cancer, colorectal cancer, or head and neck cancer. In yet some other embodiments, the CAR provided herein recognizes a HER2 antigen of a tumor and does not respond, or has a low level of response, to HER2 expressed on non-cancer or normal cells.

SEQ ID NO: 155

EVQLVESGGGLVQPGGSLKLSAASGFTFSNYGMSWVRQTPDRRLELVATINNNGGGTTYYPDSVKGRFTISRDNANTLYLQMSLKSSEDAMYCTSPGLLWDAGAGTTTVTVSSSGSTSGGGSGGGSGGGSSDVMTQTPLTSLVSIGQPASISCKSSQSLDSDGRTYLNWLLQRPQSPKRLIYLVSKLDSGAPDRFTGSGSGTDFTLKISRVEAEDLGVIYCWQGTHTFPQTFGGGTKLEIKESKYGPPCPPCGGGSSGGGSGGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMEALHNHYTQKSLSLSLGKMFVVLVVGGLVACYSLLVTVAFTIIFWVRSKRSRLHSDYMNMTPRRPGPTRKHYPYAPPRDFAAYRSRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPENMGKPRRKNPQEGFLNELQKDKMAEAFSEIGMKGERRRGKGHDGLFQGLSTATKDTFDALHMQALPPR

(anti-HER2 scFV[linker]- *spacer*- CD28 TM- CD28 Costim-CD3ζ1XX activation)

[000241] In another example, the genetically engineered immune cells, iPSCs and derivative effector cells comprise a solid tumor targeting backbone as disclosed herein and a CAR comprising an antigen recognition region that targets tumor antigen MICA and MICB (MICA/B). In some embodiments of the MICA/B targeting CAR, the antigen recognition region is a scFV that specifically binds to the conserved α3 domain of MICA and MICB. In one

embodiment, the scFV comprises a variable region of the heavy chain and a variable region of the light chain, respectively represented by an amino acid sequence that is of at least about 99%, about 98%, about 96%, about 95%, about 90%, about 85%, or at least about 80% identity to SEQ ID NO: 156 and 157. In one embodiment of the MICA/B scFV, the scFV is represented by an amino acid sequence that is of at least about 99%, about 98%, about 96%, about 95%, about 90%, about 85%, or at least about 80% identity to any of SEQ ID NOs: 158 and 159. In some embodiments, the scFV comprises an amino acid sequence of at least 90% identity to SEQ ID NO: 158 or 159. In some embodiments, the scFV comprises an amino acid sequence of at least 95% identity to SEQ ID NO: 158 or 159. In some embodiments, the scFV comprises the amino acid sequence of SEQ ID NO: 158 or 159.

SEQ ID NO: 156

QIQLVQSGPELKKPGETVKVSCASGYMFTNYAMNWVKQAPEKGLKWMGWINTHTGDPTYADDFKGR IAF
SLETSASTAYLQINNKNEDTATYFCVRTYGNYAMDYWGQGTSTVTVSS
(118AA. MICA/B scFV heavy chain (HC))

SEQ ID NO: 157

DIQMTQTSSLSASLGDRVTISCSASQDISNYLNWYQQKPDGTVKLLIYDTSILHLGVPSRFSGSGSGTD
YSLTISNLEPEDIATYYCQQYSKFPRTFGGGTLEIK
(107AA. MICA/B scFV light chain (LC))

SEQ ID NO: 158

MDFQVQIFSFL~~LL~~ISASVIMSRDIQLVQSGPELKKPGETVKVSCASGYMFTNYAMNWVKQAPEKGLKWMG
WINTHTGDPTYADDFKGR IAFSLETSASTAYLQINNKNEDTATYFCVRTYGNYAMDYWGQGTSTVTVSSG
GGGSGGGSGGGGSDIQMTQTSSLSASLGDRVTISCSASQDISNYLNWYQQKPDGTVKLLIYDTSILHL
GVPSRFSGSGSGTDYSLTISNLEPEDIATYYCQQYSKFPRTFGGGTLEIK

(MICA/B scFV; HC-Linker-LC; Signal peptide/Leader – other signal peptides are also possible;
Linker – other linkers are also possible)

SEQ ID NO: 159

MDFQVQIFSFL~~LL~~ISASVIMSRDIQMTQTSSLSASLGDRVTISCSASQDISNYLNWYQQKPDGTVKLLIY
DTSILHLGVPSRFSGSGSGTDYSLTISNLEPEDIATYYCQQYSKFPRTFGGGTLEIKGGGSGGGSGGG
GGSQIQLVQSGPELKKPGETVKVSCASGYMFTNYAMNWVKQAPEKGLKWMGWINTHTGDPTYADDFKGR
IAFSLETSASTAYLQINNKNEDTATYFCVRTYGNYAMDYWGQGTSTVTVSS

(MICA/B scFV; LC-Linker-HC; Signal peptide/Leader – other signal peptides are also possible;
Linker – other linkers are also possible)

[000242] In another example, the genetically engineered iPSC and its derivative cell comprise a solid tumor targeting backbone as disclosed herein and a CAR that targets tumor antigen BCMA (B cell maturation antigen). In some embodiments of the BCMA targeting CAR, the antigen recognition region is a scFV that specifically binds to the extracellular domain of CD269. In one embodiment of the BCMA scFV for CAR construction, the scFV comprises a V_H and a V_L, represented by an amino acid sequence that is of at least about 99%, about 98%, about 96%, about 95%, about 90%, about 85%, or at least about 80% identity to SEQ ID NO: 160 and SEQ ID NO: 161, respectively; or SEQ ID NO: 162 and SEQ ID NO: 163, or SEQ ID NO: 164 and SEQ ID NO: 165, respectively.

[000243] In one embodiment of the BCMA scFV, the scFV is represented by an amino acid sequence that is of at least about 99%, about 98%, about 96%, about 95%, about 90%, about 85%, or at least about 80% identity to any of SEQ ID NOs: 166-171. In some embodiments, the scFV comprises an amino acid sequence of at least 90% identity to any of SEQ ID NOs: 166-171. In some embodiments, the scFV comprises an amino acid sequence of at least 95% identity to any of SEQ ID NOs: 166-171. In some embodiments, the scFV comprises an amino acid sequence of any of SEQ ID NOs: 166-171.

SEQ ID NO: 160

EVQLVESGGGLVQPGGSLRLSCAASGFTFSRYWFSWVRQAPGKGLVWVGEINPSSSTINYAPSLKDKFTI
SRDNAKNTLYLQMNLSRAEDTAVYYCASLYDYGDYDYGQGTTLVTVSS
(BCMA scFV heavy chain-1 (VH))

SEQ ID NO: 161

EIVMTQSPATLSVSPGERATLSCASQSVESNVAWYQQKPGQAPRALIYSASLRFSGIPARFSGSGSGTE
FTLTISSLQSEDFAVYYCQQYNNYPLTFGAGTKLELK
(BCMA scFV light chain-1 (VL))

SEQ ID NO: 162

QVQLVQSGAEVKKPGASVKVSCKASGYSFPDYINWVRQAPGQGLEWMGWIIYFASGNSEYNQKFTGRVTM
TRDTSINTAYMELSSLTSEDYAVYFCASLYDYDWYFDVWGQGTMTVTSS
(BCMA scFV heavy chain-2 (VH))

SEQ ID NO: 163

DIVMTQTPLSLSVTPGQPASISCKSSQSLVHSNGNTYLHWYLQKPGQSPQLLIYKVSNRFSQVDFRSGS
GSGTDFTLKISRVEAEDVGIYYCSQSSIYPWTFGQGTKLEIK
(BCMA scFV light chain-2 (VL))

SEQ ID NO: 164

QVQLVQSGAEVKKPGASVKVSCKASGYSPFDYYINWVRQAPGQGLEWMGWIYFASGNSEYNQKFTGRVTM
TRDTSSSTAYMELSSLRSEDTAVYFCASLYDYDWYFDVWGQGTMTVTVSS

(BCMA scFV heavy chain-3 (VH))

SEQ ID NO: 165

DIVMTQTPLSLSVTPGEPASISCKSSQSLVHSNGNTYLHWYLQKPGQSPQLLIYKVSNRFSQVPDRFSGS
GSGADFTLKISRVEAEDVGVYYCAETSHVPWTFGQGTKLEIK

(BCMA scFV light chain-3 (VL))

SEQ ID NO: 166

MDFQVQIFSFLLISASVIMSREVQLVESGGGLVQPGGSLRLSCAASGFTFSRYWFSWVRQAPGKGLVWVG
EINPSSSTINYAPSLKDKFTISRDNKNTLYLQMNSLRAEDTAVYYCASLYDYDAYDYWGQGTTLTVTS
SGSTSGSGKPGSGEGSTKGEIVMTQSPATLSVSPGERATLSCKASQSVESNVAWYQQKPGQAPRALIYSA
SLRFSGIPARFSGSGSGTEFTLTISLQSEDAVYYCQQYNNYPLTFGAGTKLELK

(BCMA scFV-1; VH-Linker-VL; Signal peptide/Leader – other signal peptides are also possible;

Linker – other linkers are also possible)

SEQ ID NO: 167

MDFQVQIFSFLLISASVIMSREIVMTQSPATLSVSPGERATLSCKASQSVESNVAWYQQKPGQAPRALIY
SASLRFSGIPARFSGSGSGTEFTLTISLQSEDAVYYCQQYNNYPLTFGAGTKLELKGGGSGGGSGG
GGSEVQLVESGGGLVQPGGSLRLSCAASGFTFSRYWFSWVRQAPGKGLVWVGEINPSSSTINYAPSLKDK
FTISRDNKNTLYLQMNSLRAEDTAVYYCASLYDYDAYDYWGQGTTLTVTVSS

(BCMA scFV-2; VL-Linker-VH; Signal peptide/Leader – other signal peptides are also possible;

Linker – other linkers are also possible)

SEQ ID NO: 168

MDFQVQIFSFLLISASVIMSRQVQLVQSGAEVKKPGASVKVSCKASGYSPFDYYINWVRQAPGQGLEWMG
WIYFASGNSEYNQKFTGRVTMTRDTSINTAYMELSSLTSEDVAVYFCASLYDYDWYFDVWGQGTMTVTVSS
GSTSGSGKPGSGEGSTKGDIVMTQTPLSLSVTPGQPASISCKSSQSLVHSNGNTYLHWYLQKPGQSPQLL
IYKVSNRFSQVPDRFSGSGSGTDFTLKISRVEAEDVGIYYCSQSSIYPWTFGQGTKLEIK

(BCMA scFV-3; VH-Linker-VL; Signal peptide/Leader – other signal peptides are also possible;

Linker – other linkers are also possible)

SEQ ID NO: 169

MDFQVQIFSFLLISASVIMSRDIVMTQTPLSLSVTPGQPASISCKSSQSLVHSNGNTYLHWYLQKPGQSP
QLLIYKVSNRFSQVPDRFSGSGSGTDFTLKISRVEAEDVGIYYCSQSSIYPWTFGQGTKLEIKGSTSGSG
KPGSGEGSTKQVQLVQSGAEVKKPGASVKVSCKASGYSPFDYYINWVRQAPGQGLEWMGWIYFASGNSE
YNQKFTGRVTMTRDTSINTAYMELSSLTSEDVAVYFCASLYDYDWYFDVWGQGTMTVTVSS

(BCMA scFV-4; VL-Linker-VH; Signal peptide/Leader – other signal peptides are also possible;

Linker – other linkers are also possible)

SEQ ID NO: 170

MDFQVQIFSFLLISASVIMSRQVQLVQSGAEVKKPGASVKVSCASGYSFPDYYINWVRQAPGQGLEWMG
WIYFASGNSEYNQKFTGRVTMTTRDTSSSTAYMELSSLRSEDVAVYFCASLYDYDWYFDVWGQGTMTVTSS
GSTSGSGKPGSGEGSTKGDIVMTQTPLSLSVTPGEPASISCKSSQSLVHSNGNTYLHWYLQKPGQSPQLL
IYKVSNRFSGVDPDRFSGSGSGADFTLKISRVEAEDVGVYYCAETSHVPWTFGQGTKLEIK

(BCMA scFV-5; VH-Linker-VL; Signal peptide/Leader – other signal peptides are also possible;
Linker – other linkers are also possible)

SEQ ID NO: 171

MDFQVQIFSFLLISASVIMSRDIVMTQTPLSLSVTPGEPASISCKSSQSLVHSNGNTYLHWYLQKPGQSP
QLLIYKVSNRFSGVDPDRFSGSGSGADFTLKISRVEAEDVGVYYCAETSHVPWTFGQGTKLEIKGSTSGSG
KPGSGEGSTKQVQLVQSGAEVKKPGASVKVSCASGYSFPDYYINWVRQAPGQGLEWMGWIYFASGNSE
YNQKFTGRVTMTTRDTSSSTAYMELSSLRSEDVAVYFCASLYDYDWYFDVWGQGTMTVTSS

(BCMA scFV-6; VL-Linker-VH; Signal peptide/Leader – other signal peptides are also possible;
Linker – other linkers are also possible)

[000244] In yet another example, the genetically engineered iPSC and its derivative cell comprise a solid tumor targeting backbone as disclosed herein and a CAR that targets tumor antigen B7H3 (CD276). In various embodiments of the CAR targeting a B7H3 tumor antigen, the CAR comprises a recombinant heavy-chain-only antibody (VHH) that specifically binds to B7H3. In one embodiment, the CAR comprises a binding domain comprising an amino acid sequence that is of at least about 99%, about 98%, about 96%, about 95%, about 90%, about 85%, or at least about 80% identity to any of SEQ ID NOs: 172-177. In some embodiments, the binding domain comprises an amino acid sequence of at least about 90% identity to any of SEQ ID NOs: 172-177. In some embodiments, the binding domain comprises an amino acid sequence of at least about 95% identity to any of SEQ ID NOs: 172-177. In some embodiments, the binding domain comprises the sequence of any of SEQ ID Nos: 172-177.

[000245] In certain embodiments, the CAR comprises a binding domain comprising a variant of SEQ ID NO: 172, and wherein the variant has one or more mutations at positions comprising 1, 40, 46, 79, 87, 88, 89, 97, 98, and 117 of SEQ ID NO: 172. In other embodiments, the CAR comprises an amino acid sequence represented by a variant of SEQ ID NO: 172, wherein the variant has one or more substitutions comprising Q1E, T40A, E46V, G79L, K87R, P88A, D89E, V97A, S98R, and Q117L according to SEQ ID NO: 172.

SEQ ID NO: 172

QVQLVESGGGLVQPGGSLRLSCAASGFTFSSYWMYVWRQTPGKGLEWVSTINRDGSATWYADSVKGRFTI
SRDNAKNTGYLQMNSLKPDDTAVYYCVSDPDNYSSDEMVPYWGQGTQVTVSS

(122 a.a. VHH camelid B7H3)

SEQ ID NO: 173

EVQLVESGGGLVQPGGSLRLSCAASGFTFSSYWMYWVRQAPGKGLVWVSTINRDGSATWYADSVKGRFTI
SRDNAKNTLYLQMNSLRAEDTAVYYCARDPDNYSSDEMVPYWGQGLVTVSS

(122 a.a. VHH1)

SEQ ID NO: 174

EVQLVESGGGLVQPGGSLRLSCAASGFTFSSYWMYWVRQAPGKGLVWVSTINRDGSATWYADSVKGRFTI
SRDNAKNTLYLQMNSLRAEDTAVYYCVSDPDNYSSDEMVPYWGQGLVTVSS

(122 a.a. VHH2)

SEQ ID NO: 175

EVQLVESGGGLVQPGGSLRLSCAASGFTFSSYWMYWVRQTPGKGLVWVSTINRDGSATWYADSVKGRFTI
SRDNAKNTLYLQMNSLRAEDTAVYYCVSDPDNYSSDEMVPYWGQGLVTVSS

(122 a.a. VHH3)

SEQ ID NO: 176

EVQLVESGGGLVQPGGSLRLSCAASGFTFSSYWMYWVRQAPGKGLEWVSTINRDGSATWYADSVKGRFTI
SRDNAKNTLYLQMNSLRAEDTAVYYCVSDPDNYSSDEMVPYWGQGLVTVSS

(122 a.a. VHH4)

SEQ ID NO: 177

EVQLVESGGGLVQPGGSLRLSCAASGFTFSSYWMYWVRQTPGKGLEWVSTINRDGSATWYADSVKGRFTI
SRDNAKNTGYLQMNSLRPEDTAVYYCVSDPDNYSSDEMVPYWGQGLVTVSS

(122AA. VHH5)

[000246] In some embodiments, the antigen binding domain of the CAR comprises a VH and a VL domain having a sequence identity of at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 99%, 100%, or any percentage in-between, when compared to the exemplary sequences represented by SEQ ID NOs: 178 and 179, or SEQ ID NOs: 180 and 181, or SEQ ID NOs: 182 and 183. These VH and VL sequences are described in detail in WO2020148677 (GPRC5D binding specificity), WO2021099944 (CD79b binding specificity), and WO2021019386 (KLK2 binding specificity) respectively. In some embodiments the antigen binding domain of the CAR comprises a single chain variable fragment (scFV) having a sequence identity of at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 99%, 100%, or any percentage in-between, when compared to the exemplary sequence represented by SEQ ID NOs: 184, 185, or 186, wherein SEQ ID NOs: 184, 185, or 186 comprises a linker that can vary in length and/or sequence. In some embodiments, the scFV comprises an amino acid sequence of at least 90% identity to any of SEQ ID NOs: 184-186. In some embodiments, the scFV comprises an amino acid sequence of at least 95% identity to any of SEQ ID NOs: 184-186. In some embodiments, the scFV comprises the amino acid sequence of any of SEQ ID NOs: 184-186.

SEQ ID NO: 178

QLQLQESGPGLVKPSETLSLTCTVSGGSLSSSSYWWGWTRQPPGRGLEWIGTMYYSGNIYYNPS
LQSRATISVDTSKNQFSLKLSSVTAADTAVYYCARHVGYSYGRRFWYFDLWGRGTLVTVSS

SEQ ID NO: 179

EIVLTQSPATLSLSPGERATLSCRASQSVSSYLAWYQQKPGQAPRLLIYDASN RATGIPARFSGSG
SGTDFTLTISLLEPEDFAVYYCQQRSNWPPTFGQGTKVEIK

SEQ ID NO: 180

QVQLQQSGPGLVKPSQTLSTCAISGDSVSSKSGAWN WIRQSPSRGLEWLGR TYYRSKWYNEYA
VSVKSRITINPDTSKNQFSLQLNSVTPEDTAVYYCTRVD TDFDYWGQGT LTVTVSS

SEQ ID NO: 181

QSALTQPPSVSEAPRQRTISCSGSASNIGNNGVNWYQQLP GKTPKLLIYNDDLLPSGVSDRFSGS
KSGTSASLAISGLQSEDEADYFCAAWDDSLNGLVFGGGTKLTVL

SEQ ID NO: 182

EVQLVESGGGLVQPGGSLRLSCAASGFTFSYWM TWVRQAPGKGLEWVANIKQDGSERY YVDS
VKGRFTISRDN AKNSLYLQMNSLRAEDTAVYYCARDQNYDILTGHYGM DVWGQGT TTVTVSS

SEQ ID NO: 183

EIVLTQSPSFLSASVGDRV TITCRASQGISSYLSWYQQKPGKAPKLLIYATSTLQSGVPSRFSGSGS
GTEFTLTISLQPEDFATYYCQQLNSYPRTFGQGTKVEIK

SEQ ID NO: 184

QLQLQESGPGLVKPSETLSLTCTVSGGSLSSSSYWWGWTRQPPGRGLEWIGTMYYSGNIYYNPS
LQSRATISVDTSKNQFSLKLSSVTAADTAVYYCARHVGYSYGRRFWYFDLWGRGTLVTVSSGGG
EGKSSGSGSESKSTGGSEIVLTQSPATLSLSPGERATLSCRASQSVSSYLAWYQQKPGQAPRLLIYD
ASN RATGIPARFSGSGSGTDFTLTISLLEPEDFAVYYCQQRSNWPPTFGQGTKVEIK

SEQ ID NO: 185

QVQLQQSGPGLVKPSQTLSTCAISGDSVSSKSGAWN WIRQSPSRGLEWLGR TYYRSKWYNEYA
VSVKSRITINPDTSKNQFSLQLNSVTPEDTAVYYCTRVD TDFDYWGQGT LTVTVSSGGSEKSSGS
GSESKSTGGQSALTQPPSVSEAPRQRTISCSGSASNIGNNGVNWYQQLP GKTPKLLIYNDDLLP
SGVSDRFSGSKSGTSASLAISGLQSEDEADYFCAAWDDSLNGLVFGGGTKLTVL

SEQ ID NO: 186

EIVLTQSPSFLSASVGDRV TITCRASQGISSYLSWYQQKPGKAPKLLIYATSTLQSGVPSRFSGSGS
GTEFTLTISLQPEDFATYYCQQLNSYPRTFGQGTKVEIKGGSEKSSGSGSESKSTGGSEVQLVES
GGGLVQPGGSLRLSCAASGFTFSYWM TWVRQAPGKGLEWVANIKQDGSERY YVDSVKGRFTI
SRDN AKNSLYLQMNSLRAEDTAVYYCARDQNYDILTGHYGM DVWGQGT TTVTVSS

[000247] Non-limiting CAR strategies further include heterodimeric, conditionally activated CAR through dimerization of a pair of intracellular domains (see for example, U.S. Pat. No. 9,587,020); a split CAR, where homologous recombination of antigen binding, hinge, and endo-domains to generate a CAR (see for example, U.S. Pub. No. 2017/0183407); a multi-chain CAR that allows non-covalent link between two transmembrane domains connected to an antigen binding domain and a signaling domain, respectively (see for example, U.S. Pub. No. 2014/0134142); CARs having bispecific antigen binding domain (see for example, U.S. Pat. No. 9,447,194), or having a pair of antigen binding domains recognizing same or different antigens or epitopes (see for example, U.S. Pat. No. 8,409,577), or a tandem CAR (see for example, Hegde et al., J Clin Invest. 2016;126(8):3036-3052); an inducible CAR (see for example, U.S. Pub. Nos. 2016/0046700, 2016/0058857, and 2017/0166877); a switchable CAR (see for example, U.S. Pub. No. 2014/0219975); and any other designs known in the art.

[000248] In some embodiments, the polynucleotide encoding a CAR as disclosed is operatively linked to an endogenous promoter. In some embodiments, the polynucleotide encoding a CAR as disclosed is operatively linked to an exogenous promoter. The promoters may be inducible, or constitutive, and may be temporal-, tissue- or cell type- specific. Suitable constitutive promoters for methods disclosed herein include, but are not limited to, cytomegalovirus (CMV), elongation factor 1 α (EF1 α), phosphoglycerate kinase (PGK), hybrid CMV enhancer/chicken β -actin (CAG) and ubiquitin C (UBC) promoters. In one embodiment, the exogenous promoter is CAG.

[000249] As described herein, in some embodiments, the cells comprising a solid tumor targeting backbone comprise a polynucleotide encoding a CAR and/or one or more additional modified modalities provided herein. Additionally provided in this application is a master cell bank comprising single cell sorted and expanded clonal engineered iPSCs having at least one phenotype as provided herein, including but not limited to, a solid tumor targeting backbone as described herein and a CAR, wherein the cell bank provides a platform for additional iPSC engineering and a renewable source for manufacturing off-the-shelf, engineered, homogeneous cell therapy products, including but not limited to derivative NK and T cells, which are well-defined and uniform in composition, and can be mass produced at significant scale in a cost-effective manner.

7. TCE

[000250] According to some embodiments, the T Cell Enhancer (TCE) as a T cell potentiator as disclosed herein is aimed at enhancing the antitumor activity and persistence of iPSC-derived

CAR-T cells. TCE edits according to some embodiments have the ability to activate one or more of cytokine signaling (e.g., soluble cytokines, or cytokine-receptor fusion constructs), costimulatory signals (e.g., over-expression of costimulatory receptors or other important signal transduction proteins), or gene expression profiles (e.g., over-expression of transcription factors). In the context of iPSC derived T cells according to some embodiments, the TCE edits are compatible with T cell differentiation process. As disclosed herein, TCF1 and CD27, among other candidates are suitable TCEs for potentiating iPSC derived T cells. In some embodiments, the TCF1 has a sequence of SEQ ID NO: 187. In some embodiments, the CD27 has a sequence of SEQ ID NO: 188.

SEQ ID NO: 187

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MPQLDSGGGGAGGGDDLGAPEDELLAFQDEGEEQDDKSRDSAAGPERDLAELKSSLVNESEGAAGGAGIPG
VPGAGAGARGEAEALGREHAAQRLFPDKLPEPLEDGLKAPECTSGMYKETVYSAFNLLMHYPPPSGAGQH
PQPQPPLHKANQPPHGVPLSLYEHFNSPHPTPAPADISQKQVHRPLQTPDLSGFYSLTSGSMGQLPHTV
SWFTHPSLMLGSGVPGHPAAIPHPAIVPPSGKQELQPFDRNLKTQAESKAEKEAKKPTIKKPLNAFMLYM
KEMRAKVIAECTLKESAAINQILGRRWHALSREEQAKY YELARKERQLHMQLYPGWSARDNYGKKRRSR
EKHQESTTGGKRNAFGTYPEKAAAPAPFLPMTVL
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(TCF1)

SEQ ID NO: 188

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MARPHPWWLCVLGTLVGLSATPAPKSCPERHYWAQGKLCCQMCEPGTFLVKDCDQHRKAAQCDPCIPGV
FSPDHHTRPHCESCRHCNSGLLVNRCTITANAECACRNGWQCRDKECTECDPLPNPSLTARSSQALSPHP
QPHTLPYVSEMLEARTAGHMQLTADFRQLPARTLSTHWPPQRSILCSSDFIRILVIFSGMFLVFTLAGALF
LHQRKRYRSNKGESPEPAEPCHYSCPREEEGSTIPIQEDYRKPEPACSP
```

(CD27)

[000251] In some embodiments, the TCE is provided by a polynucleotide encoding the TCE. In some embodiments, the polynucleotide encoding a TCE as disclosed is operatively linked to an exogenous promoter. The promoters may be inducible, or constitutive, and may be temporal-, tissue- or cell type- specific. Suitable constitutive promoters for methods disclosed herein include, but are not limited to, cytomegalovirus (CMV), elongation factor 1 α (EF1 α), phosphoglycerate kinase (PGK), hybrid CMV enhancer/chicken β -actin (CAG) and ubiquitin C (UBC) promoters. In one embodiment, the exogenous promoter is CAG.

8. CD38 knockout

[000252] The cell surface molecule CD38 is highly upregulated in multiple hematologic malignancies derived from both lymphoid and myeloid lineages, including multiple myeloma and a CD20 negative B-cell malignancy, which makes it an attractive target for antibody

therapeutics to deplete cancer cells. Antibody mediated cancer cell depletion is usually attributable to a combination of direct cell apoptosis induction and activation of immune effector mechanisms such as ADCC (antibody-dependent cell-mediated cytotoxicity). In addition to ADCC, the immune effector mechanisms in concert with the therapeutic antibody may also include antibody-dependent cell-mediated phagocytosis (ADCP) and/or complement-dependent cytotoxicity (CDC).

[000253] Other than being highly expressed on malignant cells, CD38 is also expressed on plasma cells, as well as on NK cells and activated T and B cells. During hematopoiesis, CD38 is expressed on CD34⁺ stem cells and lineage-committed progenitors of lymphoid, erythroid, and myeloid, and during the final stages of maturation which continues through the plasma cell stage. As a type II transmembrane glycoprotein, CD38 carries out cell functions as both a receptor and a multifunctional enzyme involved in the production of nucleotide-metabolites. As an enzyme, CD38 catalyzes the synthesis and hydrolysis of the reaction from NAD⁺ to ADP-ribose, thereby producing secondary messengers CADPR and NAADP which stimulate release of calcium from the endoplasmic reticulum and lysosomes, critical for the calcium dependent process of cell adhesion. As a receptor, CD38 recognizes CD31 and regulates cytokine release and cytotoxicity in activated NK cells. CD38 is also reported to associate with cell surface proteins in lipid rafts, to regulate cytoplasmic Ca²⁺ flux, and to mediate signal transduction in lymphoid and myeloid cells.

[000254] In malignancy treatment, systemic use of CD38 antigen binding receptor transduced T cells has been shown to lyse the CD38⁺ fractions of CD34⁺ hematopoietic progenitor cells, monocytes, NK cells, T cells and B cells, leading to incomplete treatment responses and reduced or eliminated efficacy because of the impaired recipient immune effector cell function. In addition, in multiple myeloma patients treated with daratumumab, a CD38-specific antibody, NK cell reduction in both bone marrow and peripheral blood was observed, although other immune cell types, such as T cells and B cells, were unaffected despite their CD38 expression (Casneuf et al., Blood Advances. 2017; 1(23):2105-2114).

[000255] Without being limited by theories, the present application includes a strategy to leverage the full potential of CD38 targeted cancer treatment by knocking out CD38 in the effector cell, thereby overcoming CD38-specific antibody and/or CD38 antigen binding domain-induced effector cell depletion or reduction through fratricide. In addition, since CD38 is upregulated on activated lymphocytes such as T or B cells, by suppressing activation of these recipient lymphocytes using a CD38-specific antibody, such as daratumumab, in the recipient of allogeneic effector cells, host allojection against these effector cells would be reduced and/or prevented, thereby increasing effector cell survival and persistency. As such, a CD38-specific

antibody, a secreted CD38-specific engager or a CD38-CAR (chimeric antigen receptor) against activation of recipient T, Treg, NK, and/or B cells can be used as a replacement for lymphodepletion using chemotherapy such as Cy/Flu (cyclophosphamide/fludarabine) prior to adoptive cell transferring.

[000256] In addition, when targeting CD38⁺ T and pbNK cells using CD38⁻ effector cells in the presence of anti-CD38 antibodies or CD38 inhibitors, the depletion of CD38⁺ alloreactive cells increases the NAD⁺ (nicotinamide adenine dinucleotide, a substrate of CD38) availability and decreases NAD⁺ consumption related cell death, which, among other advantages, boosts effector cell responses in an immunosuppressive tumor microenvironment and supports cell rejuvenation in aging, degenerative or inflammatory diseases.

[000257] Moreover, the strategy as provided herein, i.e., CD38 knockout, is compatible with other components and processes contemplated for establishing a solid tumor targeting backbone as disclosed in this application, thereby providing an immune cell, an iPSC and differentiated effector cell therefrom comprising a CD38 knockout with additional backbone edits. As disclosed herein, in various embodiments, the solid tumor targeting backbone comprised in the iPSC line or a derivative thereof, comprises an TCR promoter-driven ADR and/or a cytokine, and a constitutively expressed TCE, in addition to edits comprising a CAR, a TGFβ-SRR, a C-X-C-motif chemokine receptor or a variant thereof, a CD16 variant, and a CD38 knockout, among other edits as provided herein. In some embodiments, the provided CD38^{neg} iPSC line optionally comprises one or more additional engineered modalities described herein, and as shown in Table 4. As such, these CD38^{neg} derivative effector cells comprising a solid tumor targeting backbone are protected against fratricide and allorejection when CD38 targeted therapeutic moieties are employed with the effector cells, among other advantages including improved metabolic fitness, increased resistance to oxidative stress and inducing a protein expression program in the effector cell that enhances cell activation and effector function. In addition, anti-CD38 monoclonal antibody therapy significantly depletes a patient's activated immune system without adversely affecting the patient's hematopoietic stem cell compartment. A CD38^{neg} derivative cell has the ability to resist CD38 antibody mediated depletion, and may be effectively administered in combination with an anti-CD38 antibody or CD38-CAR without the use of toxic conditioning agents, thereby reducing and/or replacing chemotherapy-based lymphodepletion.

[000258] In one embodiment as provided herein, the CD38 knockout in an iPSC line is a bi-allelic knockout. In another embodiment, knocking out CD38 at the same time as inserting one or more transgenes, including a C-X-C-motif chemokine receptor or a variant thereof, a TGFβ-SRR, and/or a CD16 variant as provided herein, at a selected position in CD38 can be achieved, for example, by a CD38-targeted knock-in/knockout (CD38-KI/KO) construct. In some

embodiments of the construct, the construct comprises a pair of CD38 targeting homology arms for position-selective insertion within the CD38 locus. In some embodiments, the preselected targeting site is within an exon of CD38. The CD38-KI/KO constructs provided herein allow the transgene(s) to express either under the CD38 endogenous promoter or under an exogenous promoter comprised in the construct. When two or more transgenes are to be inserted at a selected location in the CD38 locus, a linker sequence, for example, a 2A linker or IRES, is placed between any two transgenes. The 2A linker encodes a self-cleaving peptide derived from FMDV, ERAV, PTV-I, and TaV (referred to as “F2A”, “E2A”, “P2A”, and “T2A”, respectively), allowing for separate proteins to be produced from a single translation. In some embodiments, insulators are included in the construct to reduce the risk of transgene and/or exogenous promoter silencing. The exogenous promoter comprised in a CD38-KI/KO construct may be CAG, or other constitutive, inducible, temporal-, tissue-, or cell type- specific promoters including, but not limited to CMV, EF1 α , PGK, and UBC.

[000259] In various embodiments, said iPSC is capable of directed differentiation to produce functional derivative hematopoietic cells including, but not limited to, mesodermal cells with definitive hemogenic endothelium (HE) potential, definitive HE, CD34⁺ hematopoietic cells, hematopoietic stem and progenitor cells, hematopoietic multipotent progenitors (MPP), T cell progenitors, NK cell progenitors, myeloid cells, neutrophil progenitors, T cells, NKT cells, NK cells, B cells, neutrophils, dendritic cells, and macrophages. In some embodiments, the CD38 negative effector cells are NK lineage cells derived from iPSCs. In some embodiments, the CD38 negative effector cells are T lineage cells derived from iPSCs. In some embodiments, the iPSC and derivative cells thereof comprise a solid tumor targeting backbone comprising CD38^{neg} and at least two of: a polynucleotide encoding a C-X-C motif chemokine receptor or a variant thereof, a polynucleotide encoding a TGF β -SRR, and a polynucleotide encoding a CD16 variant, and optionally include one or more additional genomic edits as described herein.

9. Stealth related edits

[000260] Multiple HLA class I and class II proteins must be matched for histocompatibility in allogeneic recipients to avoid allogeneic rejection problems. Provided herein is an iPSC cell line and its derivative cells differentiated therefrom with eliminated or substantially reduced expression of HLA class I and/or HLA class II proteins. HLA class I deficiency can be achieved by functional deletion of any region of the HLA class I locus (chromosome 6p21), or deletion or disruption of HLA class-I associated genes including, but not limited to, beta-2 microglobulin (B2M) gene, TAP1 gene, TAP2 gene and Tapasin. For example, the B2M gene encodes a common subunit essential for cell surface expression of all HLA class I heterodimers. B2M

negative cells are HLA-I deficient. HLA class II deficiency can be achieved by functional deletion or disruption of HLA class II associated genes including, but not 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 negative cells are HLA-II deficient. As such, this application provides an iPSC and derivative cells therefrom comprising HLA-I and/or HLA-II deficiency, for example by lacking B2M and/or CIITA expression, wherein the obtained derivative effector cells enable allogeneic cell therapies by eliminating the need for MHC (major histocompatibility complex) matching, and avoiding recognition and killing by host (allogeneic) T cells.

[000261] Furthermore, a lack of HLA class I expression leads to lysis by host NK cells. Therefore, in addition to the above-discussed approach of CD38 conditioning to remove activated CD38-expressing host NK cells, to overcome this “missing self” response, HLA-E, HLA-G or other non-classical HLA-I proteins may be optionally knocked in to avoid NK cell recognition and killing of the HLA-I deficient effector cells derived from an engineered iPSC. In one embodiment, the provided HLA-I deficient iPSC and its derivative cells further comprise HLA-G knock-in.

[000262] Alternatively, in one embodiment, the provided HLA-I deficient iPSC and its derivative cells further comprise one or both of CD58 knockout and CD54 knockout. CD58 (or LFA-3) and CD54 (or ICAM-1) are adhesion proteins initiating signal-dependent cell interactions, and facilitating cell, including immune cell, migration. It was previously shown that CD58 and/or CD54 disruption effectively reduces the susceptibility of HLA-I deficient iPSC-derived effector cells to allogeneic NK cell killing. While it was shown that CD58 knockout has a higher efficiency in reducing allogeneic NK cell activation than CD54 knockout, double knockout of both CD58 and CD54 was shown to provide the most enhanced reduction of NK cell activation. In some observations, the CD58 and CD54 double knockout is even more effective than HLA-G overexpression for HLA-I deficient cells in overcoming “missing-self” effect.

[000263] As provided herein, in some embodiments, the iPSC and its derivative cells comprising a solid tumor targeting backbone comprising two or more of: a C-X-C-motif chemokine receptor or a variant thereof, a TGF β -SRR, and a CD16 variant. In some embodiments, said iPSC and its derivative cells are CD58 negative. In some embodiments, said iPSC and its derivative cells are CD54 negative. In yet some other embodiments, said iPSC and its derivative cells are CD54 negative and CD58 negative.

[000264] In some embodiments, the engineering for HLA-I and/or HLA-II deficiency may be bypassed, or kept intact, by expressing an inactivation CAR targeting an upregulated surface

protein in activated recipient immune cells to avoid allojection. In some embodiments, the upregulated surface protein in the activated recipient immune cells includes, but is not limited to, CD38, CD25, CD69, CD44, 4-1BB, OX40, or CD40L. When the cell expresses such an inactivation CAR, it is preferable that the cell does not express, or has knockout of, the same surface protein targeted by CAR. In some embodiments, the inactivation CAR comprises at least one of a CD38-CAR, a CD25-CAR, a CD69-CAR, a CD44-CAR, a 4-1BB-CAR, an OX40-CAR, and a CD40L-CAR.

[000265] Additionally provided in this application is a master cell bank comprising single cell sorted and expanded clonal engineered iPSCs having at least one phenotype as provided herein, including but not limited to, a solid tumor targeting backbone as described herein and HLA modification (“HLA” in Table 4: HLA-I and/or HLA-II deficiency with or without HLA-E or HLA-G knock in, or with knockout of one or both of CD58 and CD54), wherein the cell bank provides a platform for additional iPSC engineering and a renewable source for manufacturing off-the-shelf, engineered, homogeneous cell therapy products, including but not limited to derivative NK and T cells, which are well-defined and uniform in composition, and can be mass produced at significant scale in a cost-effective manner.

10. Genetically engineered iPSC line and derivative cells provided herein

[000266] In light of the above, the present application provides an immune cell, an iPSC, an iPS cell line cell, or a population thereof, and a derivative functional cell obtained from differentiating the iPSC, wherein each cell comprises a solid tumor targeting backbone comprising an TCR promoter-driven ADR and/or a cytokine, and a constitutively expressed TCE, in addition to edits comprising a CAR, a TGFβ-SRR, a C-X-C-motif chemokine receptor or a variant thereof, a CD16 variant, and a CD38 knockout, among other modifications as described in the application, wherein the cell is an eukaryotic cell, an animal cell, a human cell, an induced pluripotent cell (iPSC), an iPSC-derived effector cell, an immune cell, or a feeder cell. In some embodiments, said cells are suitable for homing or migration of the effectors to tumor sites for CAR targeted tumor killing. In some embodiments, the tumor cells at the tumor sites secrete or overexpress a chemokine that binds to a C-X-C-motif chemokine receptor or a variant thereof. In some embodiments, the C-X-C motif chemokine receptor comprises CXCR2 or CXCR3. In some embodiments, the secreted or overexpressed chemokine by the tumor cells at the tumor sites comprises IL8 (CXCL8). In some embodiments, the functional derivative cells are hematopoietic cells including, but not limited to, mesodermal cells with definitive hemogenic endothelium (HE) potential, definitive HE, CD34⁺ hematopoietic cells, hematopoietic stem and progenitor cells, hematopoietic multipotent progenitors (MPP), T cell progenitors, NK cell

progenitors, myeloid cells, neutrophil progenitors, T lineage cells, NKT lineage cells, NK lineage cells, B lineage cells, neutrophils, dendritic cells, and macrophages. In some embodiments, the functional derivative hematopoietic cells comprise effector cells having one or more functional features that are not present in a counterpart primary T, NK, NKT, and/or B cell.

[000267] Said effector cells have improved ability to home or migrate to, and remain in, tumor sites which include solid tumors, and provide a tumor antigen dual targeting mechanism to tackle tumor antigen heterogeneity and tumor antigen escape. The dual targeting through CAR binding and CD16-mediated ADCC further increases tumor targeting precision, enhancing tumor killing and minimizing the impact of tumor antigen escape.

[000268] In some further embodiments, the iPSC, iPS cell line cell, or clonal population thereof, and/or derivative effector cells therefrom comprising a solid tumor targeting backbone as described herein, wherein the solid tumor targeting backbone further comprises CD38 knockout, and said cells are suitable for a subject undergoing an adoptive cell therapy. In certain embodiments, the subject may additionally receive a tumor sensitizing procedure (e.g., administration of a sensitizing agent, such as a chemotherapeutic agent, radiation, or radiotherapeutic) to upregulate tumor cell chemokine expression including, but not limited to, CXCL8 overexpression, to further enhance C-X-C motif chemokine receptor overexpressing effector cell homing, trafficking and retention, and cytotoxicity at the tumor sites. In some embodiments, said effector cells comprise T lineage cells. In some other embodiments, said effector cells comprise NK lineage cells.

[000269] In some embodiments of the derivative effector cells, the iPSCs and their derivative cells that comprise a solid tumor targeting backbone comprising an TCR promoter-driven ADR and/or a cytokine, and a constitutively expressed TCE, in addition to edits comprising a CAR, a TGFβ-SRR, a C-X-C-motif chemokine receptor or a variant thereof, a CD16 variant, and a CD38 knockout, among other modifications as described herein. In some embodiments, said cells have the ADR and/or a cytokine inserted in a TCR constant region (TRAC or TRBC), leading to TCR knockout, and optionally placing ADR and/or cytokine expression under the control of the endogenous TCR promoter to promote T cell differentiation, T cell identity and lineage commitment, and to avoid T cell exhaustion. The disruption of the constant region of TCRα or TCRβ (TRAC or TRBC) produces a TCR^{neg} cell. TCR^{neg} cells do not require HLA matching, have reduced alloreactivity, and are able to prevent GvHD (Graft versus Host Disease) when used in allogeneic adoptive cell therapies.

[000270] Additional insertion sites of polynucleotides of interest include, but are not limited to, AAVS1, CCR5, ROSA26, collagen, HTRP, H11, PH12, GAPDH, RUNX1, B2M, TAP1, TAP2, tapasin, NLRC5, CIITA, RFXANK, RFX5, RFXAP, NKG2A, NKG2D, CD25, CD38,

CD44, CD58, CD54, CD56, CD69, CD71, CIS, CBL-B, SOCS2, PD1, CTLA4, LAG3, TIM3, ASB2, and TIGIT. In some embodiments, the polynucleotides of interest comprise those encoding a CAR, a TGF β -SRR, a C-X-C-motif chemokine receptor or a variant thereof, a CD16 variant, a TCE. In one embodiment, the effector cell, the iPSC and its derivative NK or T cell described herein comprises one or more CARs, where the CAR is inserted in the CD58, TIM3, TIGIT, NKG2A or NKG2D locus, leading to CD58, TIM3, TIGIT, NKG2A or NKG2D knockout. In one embodiment, the effector cell, the iPSC and its derivative NK or T cell described herein comprises co-expressed TGF β -SRR, C-X-C-motif chemokine receptor or a variant thereof, and a CD16 variant at a CD38 locus, wherein the CD38 is knocked out upon the insertion of the transgenes.

[000271] Additionally provided is an iPSC comprising a solid tumor targeting backbone comprising an TCR promoter-driven ADR and/or a cytokine, and a constitutively expressed TCE, wherein the polynucleotide encoding an interleukin cytokine or a cytokine signaling complex (IL) comprising a full or partial length of cytokine and/or a full or partial length of a cytokine receptor to enable cytokine signaling contributing to cell survival, persistence and/or expansion, wherein the iPSC line is capable of directed differentiation to produce functional derivative hematopoietic cells having improved survival, persistency, expansion, and effector cell function, as well as homing, trafficking, tumor site retention and cytotoxicity. In various embodiments, the exogenously introduced IL cytokine signaling(s) comprise the signaling of any one, two, or more of IL2, IL4, IL6, IL7, IL9, IL10, IL11, IL12, IL15, IL18, and IL21. In some embodiments, the introduced IL cytokine signaling complex is for IL15 signaling in the cell, and the cell is optionally an NK lineage cell. In some other specific embodiments, the introduced IL cytokine signaling complex is for IL2, IL7 or IL18 signaling in the cell, and the cell is optionally a T lineage cell. In some embodiments, the introduced IL cytokine signaling complex is expressed on the cell surface. In some embodiments, the introduced IL cytokine is soluble. In some embodiments, the IL cytokine signaling is constitutively activated. In some embodiments, the IL cytokine signaling is under the control of temporal and dose regulation of an endogenous or exogenous promoter. In some embodiments, activation of the IL cytokine signaling is inducible. In some embodiments, activation of the IL cytokine signaling is transient and/or temporal. In some embodiments, the transient/temporal expression of a cell surface or soluble cytokine/cytokine receptor is through a retrovirus, Sendai virus, an adenovirus, an episome, mini-circle, or RNAs including mRNA. In some embodiments, the transient/temporal expression of a cell surface or soluble cytokine/cytokine receptor is through endogenous promoter including TRAC, TIM3, TIGIT, and ASB2.

[000272] In some embodiments, effector cells comprising a solid tumor targeting backbone comprising polynucleotides encoding an TCR promoter-driven ADR and/or a cytokine, and a constitutively expressed TCE, in addition to edits comprising a CAR, a TGF β -SRR, a C-X-C-motif chemokine receptor or a variant thereof, a CD16 variant, and a CD38 knockout, and optionally one or more additional genetic modifications as provided in Table 4 and throughout the application are capable of maintaining or improving cell growth, proliferation, expansion, and/or effector function autonomously without contacting additionally supplied soluble cytokines *in vitro* or *in vivo*, as well as enhanced homing, trafficking, and retention at tumor sites, in which the tumor cells could be sensitized to synergize with the functional features provided to the effector cells through rational design and precision engineering of a primary-sourced immune cell or a clonal iPSC.

[000273] As such, the present application provides iPSCs and their functional derivative hematopoietic cells, which comprise any one of the following genotypes in Table 4. “**TRAC**” or “**CD38**” in the genotype indicates the insertion locus. When insertion locus is not specified, the insertion takes place in any locus as provided in this application. “\” is used to separate transgenes at different loci.

Table 4: Applicable Exemplary Genotypes of the Cells Provided:

TGFβ-SRR/ C-X-C/ CD16 ^{exo}	CD38 ^{-/-}	ADR and TCR ^{-/-}	IL2 and TCR ^{-/-}	IL7RF and TCR ^{-/-}	CAR1	CAR2	TCE	IL18	Genotype
		✓							1. TRAC_ADR TCR ^{-/-}
			✓						2. TRAC_IL2 TCR ^{-/-}
		✓	✓						3. TRAC_ADR IL2 TCR ^{-/-}
		✓		✓					4. TRAC_ADR IL7RF TCR ^{-/-}
✓									5. TGFβ-SRR/C-X-C/ CD16 ^{exo}
✓	✓								6. CD38_TGFβ-SRR C-X-C CD16 ^{exo} CD38 ^{-/-}
✓		✓							7. TGFβ-SRR C-X-C CD16 ^{exo} \\TRAC_ADR TCR ^{-/-}
✓		✓	✓						8. TGFβ-SRR C-X-C CD16 ^{exo} \\TRAC_ADR IL2 TCR ^{-/-}
✓		✓		✓					9. TGFβ-SRR C-X-C CD16 ^{exo} \\TRAC_ADR IL7RF TCR ^{-/-}
✓	✓	✓							10. CD38_TGFβ-SRR C-X-C CD16 ^{exo} CD38 ^{-/-} \\TRAC_ADR TCR ^{-/-}
✓	✓	✓	✓						11. CD38_TGFβ-SRR C-X-C CD16 ^{exo} CD38 ^{-/-} \\TRAC_ADR IL2 TCR ^{-/-}
✓	✓	✓		✓					12. CD38_TGFβ-SRR C-X-C CD16 ^{exo} CD38 ^{-/-} \\TRAC_ADR IL7RF TCR ^{-/-}
✓		✓			✓				13. TGFβ-SRR C-X-C CD16 ^{exo} CD38 ^{-/-} \\TRAC_ADR TCR ^{-/-} \\CAR1
✓		✓			✓	✓			14. TGFβ-SRR C-X-C CD16 ^{exo} CD38 ^{-/-} \\TRAC_ADR TCR ^{-/-} \\CAR1 CAR2
✓		✓			✓		✓		15. TGFβ-SRR C-X-C CD16 ^{exo} CD38 ^{-/-} \\TRAC_ADR TCR ^{-/-} \\CAR1 TCE
✓		✓			✓			✓	16. TGFβ-SRR C-X-C CD16 ^{exo} CD38 ^{-/-} \\TRAC_ADR TCR ^{-/-} \\CAR1 IL18
✓		✓			✓	✓	✓		17. TGFβ-SRR C-X-C CD16 ^{exo} CD38 ^{-/-} \\TRAC_ADR TCR ^{-/-} \\CAR1 CAR2 TCE
✓			✓		✓				18. TGFβ-SRR C-X-C CD16 ^{exo} CD38 ^{-/-} \\TRAC_IL2 TCR ^{-/-} \\CAR1
✓			✓		✓	✓			19. TGFβ-SRR C-X-C CD16 ^{exo} CD38 ^{-/-} \\TRAC_IL2 TCR ^{-/-} \\CAR1 CAR2
✓			✓		✓		✓		20. TGFβ-SRR C-X-C CD16 ^{exo} CD38 ^{-/-} \\TRAC_IL2 TCR ^{-/-} \\CAR1 TCE
✓			✓		✓			✓	21. TGFβ-SRR C-X-C CD16 ^{exo} CD38 ^{-/-} \\TRAC_IL2 TCR ^{-/-} \\CAR1 IL18
✓			✓		✓	✓	✓		22. TGFβ-SRR C-X-C CD16 ^{exo} CD38 ^{-/-} \\TRAC_IL2 TCR ^{-/-} \\CAR1 CAR2 TCE
✓		✓	✓		✓				23. TGFβ-SRR C-X-C CD16 ^{exo} CD38 ^{-/-} \\TRAC_ADR IL2 TCR ^{-/-} \\CAR1
✓		✓	✓		✓	✓			24. TGFβ-SRR C-X-C CD16 ^{exo} CD38 ^{-/-} \\TRAC_ADR IL2 TCR ^{-/-} \\CAR1 CAR2
✓		✓	✓		✓		✓		25. TGFβ-SRR C-X-C CD16 ^{exo} CD38 ^{-/-} \\TRAC_ADR IL2 TCR ^{-/-} \\CAR1 TCE
✓		✓	✓		✓			✓	26. TGFβ-SRR C-X-C CD16 ^{exo} CD38 ^{-/-} \\TRAC_ADR IL2 TCR ^{-/-} \\CAR1 IL18
✓		✓	✓		✓	✓	✓		27. TGFβ-SRR C-X-C CD16 ^{exo} CD38 ^{-/-} \\TRAC_ADR IL2 TCR ^{-/-} \\CAR1 CAR2 TCE
✓				✓	✓				28. TGFβ-SRR C-X-C CD16 ^{exo} CD38 ^{-/-} \\TRAC_IL7RF TCR ^{-/-} \\CAR1

✓					✓	✓	✓	✓	✓	29. TGFβ-SRR C-X-C CD16 ^{exo} CD38 ^{-/-} \\TRAC_IL7RF TCR ^{-/-} \\CAR1 CAR2
✓					✓	✓	✓	✓	✓	30. TGFβ-SRR C-X-C CD16 ^{exo} CD38 ^{-/-} \\TRAC_IL7RF TCR ^{-/-} \\CAR1 TCE
✓					✓	✓			✓	31. TGFβ-SRR C-X-C CD16 ^{exo} CD38 ^{-/-} \\TRAC_IL7RF TCR ^{-/-} \\CAR1 IL18
✓					✓	✓	✓	✓	✓	32. TGFβ-SRR C-X-C CD16 ^{exo} CD38 ^{-/-} \\TRAC_IL7RF TCR ^{-/-} \\CAR1 CAR2 TCE
✓					✓	✓	✓	✓	✓	33. TGFβ-SRR C-X-C CD16 ^{exo} CD38 ^{-/-} \\TRAC_ADR IL7RF TCR ^{-/-} \\CAR1
✓	✓				✓	✓	✓	✓	✓	34. TGFβ-SRR C-X-C CD16 ^{exo} CD38 ^{-/-} \\TRAC_ADR IL7RF TCR ^{-/-} \\CAR1 CAR2
✓	✓	✓			✓	✓	✓	✓	✓	35. TGFβ-SRR C-X-C CD16 ^{exo} CD38 ^{-/-} \\TRAC_ADR IL7RF TCR ^{-/-} \\CAR1 TCE
✓	✓	✓	✓		✓	✓	✓	✓	✓	36. TGFβ-SRR C-X-C CD16 ^{exo} CD38 ^{-/-} \\TRAC_ADR IL7RF TCR ^{-/-} \\CAR1 IL18
✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	37. TGFβ-SRR C-X-C CD16 ^{exo} CD38 ^{-/-} \\TRAC_ADR IL7RF TCR ^{-/-} \\CAR1 CAR2 TCE
✓					✓	✓	✓	✓	✓	38. CD38_TGFβ-SRR C-X-C CD16 ^{exo} CD38 ^{-/-} \\TRAC_ADR TCR ^{-/-} \\CAR1
✓	✓				✓	✓	✓	✓	✓	39. CD38_TGFβ-SRR C-X-C CD16 ^{exo} CD38 ^{-/-} \\TRAC_ADR TCR ^{-/-} \\CAR1 CAR2
✓	✓	✓			✓	✓	✓	✓	✓	40. CD38_TGFβ-SRR C-X-C CD16 ^{exo} CD38 ^{-/-} \\TRAC_ADR TCR ^{-/-} \\CAR1 TCE
✓	✓	✓	✓		✓	✓	✓	✓	✓	41. CD38_TGFβ-SRR C-X-C CD16 ^{exo} CD38 ^{-/-} \\TRAC_ADR TCR ^{-/-} \\CAR1 IL18
✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	42. CD38_TGFβ-SRR C-X-C CD16 ^{exo} CD38 ^{-/-} \\TRAC_ADR TCR ^{-/-} \\CAR1 CAR2 TCE
✓					✓	✓	✓	✓	✓	43. CD38_TGFβ-SRR C-X-C CD16 ^{exo} CD38 ^{-/-} \\TRAC_IL2 TCR ^{-/-} \\CAR1
✓	✓				✓	✓	✓	✓	✓	44. CD38_TGFβ-SRR C-X-C CD16 ^{exo} CD38 ^{-/-} \\TRAC_IL2 TCR ^{-/-} \\CAR1 CAR2
✓	✓				✓	✓	✓	✓	✓	45. CD38_TGFβ-SRR C-X-C CD16 ^{exo} CD38 ^{-/-} \\TRAC_IL2 TCR ^{-/-} \\CAR1 TCE
✓	✓				✓	✓	✓	✓	✓	46. CD38_TGFβ-SRR C-X-C CD16 ^{exo} CD38 ^{-/-} \\TRAC_IL2 TCR ^{-/-} \\CAR1 IL18
✓	✓	✓			✓	✓	✓	✓	✓	47. CD38_TGFβ-SRR C-X-C CD16 ^{exo} CD38 ^{-/-} \\TRAC_IL2 TCR ^{-/-} \\CAR1 CAR2 TCE
✓	✓	✓	✓		✓	✓	✓	✓	✓	48. CD38_TGFβ-SRR C-X-C CD16 ^{exo} CD38 ^{-/-} \\TRAC_ADR IL2 TCR ^{-/-} \\CAR1
✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	49. CD38_TGFβ-SRR C-X-C CD16 ^{exo} CD38 ^{-/-} \\TRAC_ADR IL2 TCR ^{-/-} \\CAR1 CAR2 TCE
✓					✓	✓	✓	✓	✓	50. CD38_TGFβ-SRR C-X-C CD16 ^{exo} CD38 ^{-/-} \\TRAC_ADR IL2 TCR ^{-/-} \\CAR1 TCE
✓	✓	✓			✓	✓	✓	✓	✓	51. CD38_TGFβ-SRR C-X-C CD16 ^{exo} CD38 ^{-/-} \\TRAC_ADR IL2 TCR ^{-/-} \\CAR1 IL18
✓	✓	✓	✓		✓	✓	✓	✓	✓	52. CD38_TGFβ-SRR C-X-C CD16 ^{exo} CD38 ^{-/-} \\TRAC_ADR IL2 TCR ^{-/-} \\CAR1 CAR2 TCE
✓	✓				✓	✓	✓	✓	✓	53. CD38_TGFβ-SRR C-X-C CD16 ^{exo} CD38 ^{-/-} \\TRAC_IL7RF TCR ^{-/-} \\CAR1
✓	✓				✓	✓	✓	✓	✓	54. CD38_TGFβ-SRR C-X-C CD16 ^{exo} CD38 ^{-/-} \\TRAC_IL7RF TCR ^{-/-} \\CAR1 CAR2
✓	✓	✓			✓	✓	✓	✓	✓	55. CD38_TGFβ-SRR C-X-C CD16 ^{exo} CD38 ^{-/-} \\TRAC_IL7RF TCR ^{-/-} \\CAR1 TCE
✓	✓	✓			✓	✓	✓	✓	✓	56. CD38_TGFβ-SRR C-X-C CD16 ^{exo} CD38 ^{-/-} \\TRAC_IL7RF TCR ^{-/-} \\CAR1 IL18
✓	✓	✓			✓	✓	✓	✓	✓	57. CD38_TGFβ-SRR C-X-C CD16 ^{exo} CD38 ^{-/-} \\TRAC_IL7RF TCR ^{-/-} \\CAR1 CAR2 TCE

✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	58. CD38_TGFB-SRR C-X-C CD16 ^{exo} CD38 ⁺ \\TRAC_ADR IL7RF TCR ⁺ \\CAR1
✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	59. CD38_TGFB-SRR C-X-C CD16 ^{exo} CD38 ⁺ \\TRAC_ADR IL7RF TCR ⁺ \\CAR1 CAR2
✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	60. CD38_TGFB-SRR C-X-C CD16 ^{exo} CD38 ⁺ \\TRAC_ADR IL7RF TCR ⁺ \\CAR1 TCE
✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	61. CD38_TGFB-SRR C-X-C CD16 ^{exo} CD38 ⁺ \\TRAC_ADR IL7RF TCR ⁺ \\CAR1 IL18
✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	62. CD38_TGFB-SRR C-X-C CD16 ^{exo} CD38 ⁺ \\TRAC_ADR IL7RF TCR ⁺ \\CAR1 CAR2 TCE

11. Engagers

[000274] In some embodiments, the genetically modified modalities further comprise one or more of: safety switch proteins, 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, immune response regulation and modulation, and/or survival of the iPSCs or derivative cells thereof. In some embodiments, the genetically modified iPSC and the derivative cells thereof comprise a genotype listed in Table 4. In some embodiments, the iPSC and its derivative effector cells comprising any one of the genotypes in Table 4 may additionally comprise disruption of at least one of TAP1, TAP2, Tapasin, NLRC5, PD1, LAG3, TIM3, RFXANK, RFX5, RFXAP, and any gene in the chromosome 6p21 region; or introduction of at least one of HLA-E, 4-1BBL, CD3, CD4, CD8, CD47, CD113, CD131, CD137, CD80, PDL1, A_{2A}R, antigen-specific TCR, an Fc receptor, an engager, and a surface triggering receptor for coupling with bi-, multi- specific or universal engagers.

[000275] Engagers are fusion proteins consisting of two or more single-chain variable fragments (scFvs) of different antibodies, with at least one scFv that binds to an effector cell surface molecule or surface triggering receptor, and at least another to a target cell via a target cell specific surface molecule. Examples of engagers include, but are not limited to, bi-specific T cell engagers (BiTEs), bi-specific killer cell engagers (BiKEs), tri-specific killer cell engagers (TriKEs), multi-specific killer cell engagers, or universal engagers compatible with multiple immune cell types. Thus, engagers can be bi-specific or multi-specific. Such bi-specific or multi-specific engagers are capable of directing an effector cell (e.g., a T cell, a NK cell, an NKT cell, a B cell, a macrophage, and/or a neutrophil) to a tumor cell and activating the immune effector cell, and have shown great potential to maximize the benefits of CAR-T cell therapy.

[000276] In some embodiments, the engager is used in combination with a population of the effector cells comprising a solid tumor targeting backbone as described herein by concurrent or consecutive administration, wherein the effector cells comprise a surface molecule, or surface triggering receptor, that is recognized by the engager. In some other embodiments, the engager is a bi-specific antibody expressed by a derivative effector cell through genetically engineering an iPSC comprising a solid tumor targeting backbone as described herein, and directed differentiation of the engineered iPSC. Exemplary effector cell surface molecules, or surface triggering receptors, that can be used for bi- or multi- specific engager recognition, or coupling, include, but are not limited to, CD3, CD28, CD5, CD16, NKG2D, CD64, CD32, CD89, NKG2C, and a chimeric Fc receptor as disclosed herein. As described herein, in some embodiments, the exogenous CD16 expressed on the surface of the derivative effector cells for

engager recognition is a hnCD16, comprising a CD16 (containing F176V and optionally S197P) or CD64 extracellular domain, and native or non-native transmembrane, stimulatory and/or signaling domains as described herein. In some embodiments, the exogenous CD16 expressed on the surface of effector cells for engager recognition is a CD16-based chimeric Fc receptor (CFcR). In some embodiments, the CD16-based CFcR comprises a transmembrane domain of NKG2D, a stimulatory domain of 2B4, and a signaling domain of CD3 ζ ; wherein the extracellular domain of the exogenous CD16 is derived from a full length or partial sequence of the extracellular domain of CD64 or CD16; and wherein the extracellular domain of CD16 comprises F176V and optionally S197P.

[000277] In some embodiments, the target cell for an engager is a tumor cell. Exemplary tumor cell surface molecules for bi- or multi- specific engager recognition include, but are not limited to, B7H3, BCMA, CD10, CD19, CD20, CD22, CD24, CD30, CD33, CD34, CD38, CD44, CD79a, CD79b, CD123, CD138, CD179b, CEA, CLEC12A, CS-1, DLL3, EGFR, EGFRvIII, EPCAM, FLT-3, FOLR1, FOLR3, GD2, gpA33, HER2, HM1.24, LGR5, MSLN, MCSP, MICA/B, PSMA, PAMA, P-cadherin, and ROR1. In one embodiment, the bi-specific engager is a bi-specific antibody specific to CD3 and CD19 (CD3-CD19). In another embodiment, the bi-specific antibody is CD16-CD30 or CD64-CD30. In another embodiment, the bi-specific antibody is CD16-BCMA or CD64-BCMA. In still another embodiment, the bi-specific antibody is CD3-CD33.

[000278] In yet another embodiment, the bi-specific antibody further comprises a linker between the effector cell and tumor cell antigen binding domains. For example, a modified IL15 may be used as a linker for effector NK cells to facilitate effector cell expansion (called TriKE, or Tri-specific Killer Engager, in some publications). In one embodiment, the TriKE is CD16-IL15-EPCAM or CD64-IL15-EPCAM. In another embodiment, the TriKE is CD16-IL15-CD33 or CD64-IL15-CD33. In yet another embodiment, the TriKE is NKG2C-IL15-CD33 ("2C1533"). In addition to IL15, cytokines suitable for inclusion in the TriKE include, but are not limited to, IL2, IL4, IL6, IL7, IL9, IL10, IL11, IL12, IL18, and IL21.

[000279] In some embodiments, the surface triggering receptor for bi- or multi- specific engagers could be endogenous to the effector cells, sometimes depending on the cell types. In some other embodiments, one or more exogenous surface triggering receptors could be introduced to the effector cells using the methods and compositions provided herein, i.e., through additional engineering of an iPSC comprising a genotype listed in Table 4, then directing the differentiation of the iPSC to T, NK or any other effector cells comprising the same genotype and the surface triggering receptor as the source iPSC.

12. *Antibodies for immunotherapy*

[000280] In some embodiments, in addition to the genomically engineered effector cells comprising a solid tumor targeting backbone as provided herein, additional therapeutic agents comprising an antibody, or an antibody fragment that targets an antigen associated with a condition, a disease, or an indication may be used with these effector cells in a combinational therapy. In some embodiments, the antibody is used in combination with a population of the effector cells comprising a solid tumor targeting backbone as described herein by concurrent or consecutive administration to a subject. In other embodiments, such antibody or a fragment thereof may be expressed by the effector cells by genetically engineering an iPSC using an exogenous polynucleotide sequence encoding said antibody or fragment thereof, and directing differentiation of the engineered iPSC. In some embodiments, the effector cell expresses an exogenous CD16 variant, wherein the cytotoxicity of the effector cell is enhanced by the antibody via ADCC.

[000281] In some embodiments, the therapeutic antibody is a monoclonal antibody. In some embodiments, the therapeutic antibody is a humanized antibody, a humanized monoclonal antibody, or a chimeric antibody. In some embodiments, the therapeutic antibody, or antibody fragment, specifically binds to a viral antigen. In other embodiments, the antibody, or antibody fragment, specifically binds to a tumor antigen. In some embodiments, the tumor- or viral-specific antigen activates the administered iPSC-derived effector cells to enhance their killing ability. In some embodiments, the therapeutic antibodies suitable for combinational treatment as an additional therapeutic agent to the administered iPSC-derived effector cells include, but are not limited to, anti-CD20 antibodies (rituximab, veltuzumab, ofatumumab, ublituximab, ocaratuzumab, obinutuzumab), anti-HER2 antibodies (trastuzumab, pertuzumab), anti-CD52 antibodies (alemtuzumab), anti-EGFR antibodies (cetuximab), anti-GD2 antibodies (dinutuximab), anti-PDL1 antibodies (avelumab), anti-CD38 antibodies (daratumumab, isatuximab, MOR202), anti-CD123 antibodies (7G3, CSL362), anti-SLAMF7 antibodies (elotuzumab), anti-MICA/B antibodies (7C6, 6F11, 1C2) and their humanized or Fc modified variants or fragments or their functional equivalents and biosimilars. In some embodiments, the antibodies suitable for combinational treatment as an additional therapeutic agent to the administered iPSC-derived effector cells further include bi-specific or multi-specific antibodies that target more than one antigen or epitope on a target cell or recruit effector cells (e.g., T cells, NK cells, or macrophage cells) toward target cells while targeting the target cells. Such bi-specific or multi-specific antibodies function as engagers capable of directing an effector cell (e.g., a T cell, a NK cell, an NKT cell, a B cell, a macrophage, and/or a neutrophil) to a tumor

cell and activating the immune effector cell, and have shown great potential to maximize the benefits of antibody therapy.

[000282] In some embodiments, the iPSC-derived effector cells comprise hematopoietic lineage cells comprising a genotype listed in Table 4. In some embodiments, the iPSC-derived effector cells comprise NK cells comprising a genotype listed in Table 4. In some embodiments, the iPSC-derived effector cells comprise T cells comprising a genotype listed in Table 4.

[000283] In some embodiments of a combination useful for treating liquid or solid tumors, the combination comprises iPSC-derived NK or T cells comprising a solid tumor targeting backbone comprising an TCR promoter-driven ADR and/or a cytokine, and a constitutively expressed TCE, in addition to edits comprising a CAR, a TGF β -SRR, a C-X-C-motif chemokine receptor or a variant thereof, a CD16 variant, and a CD38 knockout, and optionally one or more additional genetic modifications as provided herein; and a therapeutic antibody as described above. In some embodiments of a combination useful for treating liquid or solid tumors, the combination comprises iPSC-derived NK or T cells comprising a solid tumor targeting backbone as described herein, and optionally TCR knockout, a CAR, a cytokine signaling complex, exogenous CD16 or a variant thereof, and CD38 knockout; and a therapeutic antibody as described above. In some embodiments of a combination useful for treating liquid or solid tumors, the combination comprises iPSC-derived NK or T cells comprising a solid tumor targeting backbone as described herein, and optionally TCR knockout, a CAR, IL cytokine signaling complex, exogenous CD16 or a variant thereof, CD38 knockout, and HLA-I and/or HLA-II deficiency; and a therapeutic antibody as described above. In various embodiments of said combination, the CAR targets a solid tumor antigen as set forth herein. In various embodiments of said combination, the exogenous CD16 is hnCD16. Without being limited by the theory, hnCD16 provides enhanced ADCC of the monoclonal antibody, whereas the CAR not only targets a specific tumor antigen but also prevents tumor antigen escape using a dual targeting strategy in combination with a monoclonal antibody targeting a different tumor antigen.

13. Checkpoint inhibitors

[000284] Checkpoints are cell molecules, often cell surface molecules, capable of suppressing or downregulating immune responses when not inhibited. It is now clear that tumors co-opt certain immune-checkpoint pathways as a major mechanism of immune resistance, particularly against T cells that are specific for tumor antigens. Checkpoint inhibitors (CIs) are antagonists capable of reducing checkpoint gene expression or gene products, or decreasing activity of checkpoint molecules, thereby blocking inhibitory checkpoints, and restoring immune system function. The development of checkpoint inhibitors targeting PD1/PDL1 or CTLA4 has

transformed the oncology landscape, with these agents providing long term remissions in multiple indications. However, many tumor subtypes are resistant to checkpoint blockade therapy, and relapse remains a significant concern. Thus, one aspect of the present application provides a therapeutic approach to overcome CI resistance by including genomically-engineered functional iPSC-derived cells as provided herein in a combination therapy with CI. In one embodiment of the combination therapy described herein, the iPSC-derived cells are NK cells. In another embodiment of the combination therapy described herein, the iPSC-derived cells are T cells. In addition to exhibiting direct antitumor capacity, the derivative NK cells provided herein have been shown to resist PDL1-PD1 mediated inhibition, and to have the ability to enhance T cell migration, to recruit T cells to the tumor microenvironment, and to augment T cell activation at the tumor site. Therefore, the tumor infiltration of T cells facilitated by the functionally potent genomically engineered derivative NK cells indicate that said NK cells are capable of synergizing with T cell targeted immunotherapies, including the checkpoint inhibitors, to relieve local immunosuppression and to reduce tumor burden.

[000285] In some embodiments of the combination therapy, the checkpoint inhibitor is used in combination with a population of the effector cells comprising a solid tumor targeting backbone as described herein by concurrent or consecutive administration thereof to a subject. In some other embodiments, the checkpoint inhibitor is expressed by the effector cells by genetically engineering an iPSC using an exogenous polynucleotide sequence encoding said checkpoint inhibitor, or a fragment or variant thereof, and directing differentiation of the engineered iPSC. Some embodiments of the combination therapy with the effector cells comprising a solid tumor targeting backbone as described herein, comprise at least one checkpoint inhibitor to target at least one checkpoint molecule; wherein the effector cells have a genotype listed in Table 4.

[000286] In one embodiment, the iPSC-derived effector cell for checkpoint inhibitor combination therapy comprises a solid tumor targeting backbone comprising a TCR promoter-driven ADR and/or a cytokine, and a constitutively expressed TCE, in addition to edits comprising a CAR, a TGF β -SRR, a C-X-C-motif chemokine receptor or a variant thereof, a CD16 variant, and a CD38 knockout, among other modifications as provided herein. In some embodiments, the above derivative effector cell comprising a solid tumor targeting backbone as provided herein comprises knock-out of CD54, CD58, TIM3, TIGIT. In some embodiment, said derivative effector cell may additionally comprise deletion, disruption, or reduced expression of at least one of B2M, TAP1, TAP2, Tapasin, NLRC5, PD1, LAG3, TIM3, RFXANK, RFX5, RFXAP, RAG1, and any gene in the chromosome 6p21 region; or introduction of at least one of HLA-E, 4-1BBL, CD3, CD4, CD8, CD47, CD113, CD131, CD137, CD80, PDL1, A_{2A}R, CAR,

Fc receptor, and surface triggering receptor for coupling with bi-, multi- specific or universal engagers.

[000287] Suitable checkpoint inhibitors for combination therapy with the derivative NK or T cells as provided herein include, but are not limited to, antagonists of PD1 (Pdccl, CD279), PDL-1 (CD274), TIM3 (Havcr2), TIGIT (WUCAM and Vstm3), LAG3 (CD223), CTLA4 (CD152), 2B4 (CD244), 4-1BB (CD137), 4-1BBL (CD137L), A_{2A}R, BATE, BTLA, CD39 (Entpd1), CD47, CD73 (NT5E), CD94, CD96, CD160, CD200, CD200R, CD274, CEACAM1, CSF-1R, Foxp1, GARP, HVEM, IDO, EDO, TDO, LAIR-1, MICA/B, NR4A2, MAFB, OCT-2 (Pou2f2), retinoic acid receptor alpha (Rara), TLR3, VISTA, NKG2A/HLA-E, and inhibitory KIR (for example, 2DL1, 2DL2, 2DL3, 3DL1, and 3DL2).

[000288] In some embodiments, the antagonist inhibiting any of the above checkpoint molecules is an antibody. In some embodiments, the checkpoint inhibitory antibodies may be murine antibodies, human antibodies, humanized antibodies, a camel Ig, a single variable new antigen receptor (VNAR), a shark heavy-chain-only antibody (Ig NAR), chimeric antibodies, recombinant antibodies, or antibody fragments thereof. Non-limiting examples of antibody fragments include Fab, Fab', F(ab')₂, F(ab')₃, Fv, single chain antigen binding fragments (scFv), (scFv)₂, disulfide stabilized Fv (dsFv), minibody, diabody, triabody, tetrabody, single-domain antigen binding fragments (sdAb, Nanobody), recombinant heavy-chain-only antibody (VHH), and other antibody fragments that maintain the binding specificity of the whole antibody, which may be more cost-effective to produce, more easily used, or more sensitive than the whole antibody. In some embodiments, the one, or two, or three, or more checkpoint inhibitors comprise at least one of atezolizumab (anti-PDL1 mAb), avelumab (anti-PDL1 mAb), durvalumab (anti-PDL1 mAb), tremelimumab (anti-CTLA4 mAb), ipilimumab (anti-CTLA4 mAb), IPH4102 (anti-KIR antibody), IPH43 (anti-MICA antibody), IPH33 (anti-TLR3 antibody), lirimumab (anti-KIR antibody), monalizumab (anti-NKG2A antibody), nivolumab (anti-PD1 mAb), pembrolizumab (anti-PD1 mAb), and any derivatives, functional equivalents, or biosimilars thereof.

[000289] In some embodiments, the antagonist inhibiting any of the above checkpoint molecules is microRNA-based, as many miRNAs are found as regulators that control the expression of immune checkpoints (Dragomir et al., Cancer Biol Med. 2018, 15(2):103-115). In some embodiments, the checkpoint antagonistic miRNAs include, but are not limited to, miR-28, miR-15/16, miR-138, miR-342, miR-20b, miR-21, miR-130b, miR-34a, miR-197, miR-200c, miR-200, miR-17-5p, miR-570, miR-424, miR-155, miR-574-3p, miR-513, and miR-29c.

[000290] Some embodiments of the combination therapy with the provided iPSC-derived effector cells comprise at least one checkpoint inhibitor to target at least one checkpoint

molecule; wherein the iPSC-derived cells have a genotype listed in Table 4. Some other embodiments of the combination therapy with the provided derivative effector cells comprise two, three or more checkpoint inhibitors such that two, three, or more checkpoint molecules are targeted. In some embodiments of the combination therapy comprising at least one checkpoint inhibitor and the iPSC-derived cells having a genotype listed in Table 4, said checkpoint inhibitor is an antibody, or a humanized or Fc modified variant or fragment, or a functional equivalent or biosimilar thereof, and said checkpoint inhibitor is produced by the iPSC-derived cells by expressing an exogenous polynucleotide sequence encoding said antibody, or a fragment or variant thereof. In some embodiments, the exogenous polynucleotide sequence encoding the antibody, or a fragment or a variant thereof that inhibits a checkpoint is co-expressed with a CAR, either in separate constructs or in a bi- or tri- cistronic construct comprising both the CAR and the sequence encoding the antibody, or the fragment thereof. In some further embodiments, the sequence encoding the antibody or the fragment thereof can be linked to either the 5' or the 3' end of a CAR expression construct through a self-cleaving 2A coding sequence, illustrated as, for example, CAR-2A-CI or CI-2A-CAR. As such, the coding sequences of the checkpoint inhibitor and the CAR may be in a single open reading frame (ORF). When the checkpoint inhibitor is delivered, expressed and secreted as a payload by the derivative effector cells capable of infiltrating the tumor microenvironment (TME), it counteracts the inhibitory checkpoint molecule upon engaging the TME, allowing activation of the effector cells by activating modalities such as CAR or activating receptors. In some embodiments, the checkpoint inhibitor co-expressed with CAR inhibits at least one of the checkpoint molecules: PD-1, PDL-1, TIM-3, TIGIT, LAG-3, CTLA-4, 2B4, 4-1BB, 4-1BBL, A_{2A}R, BATE, BTLA, CD39 (Entpd1), CD47, CD73 (NT5E), CD94, CD96, CD160, CD200, CD200R, CD274, CEACAM1, CSF-1R, Foxp1, GARP, HVEM, IDO, EDO, TDO, LAIR-1, MICA/B, NR4A2, MAFB, OCT-2 (Pou2f2), retinoic acid receptor alpha (Rara), TLR3, VISTA, NKG2A/HLA-E, and inhibitory KIR. In some embodiments, the checkpoint inhibitor co-expressed with CAR in a derivative cell having a genotype listed in Table 4 comprises atezolizumab, avelumab, durvalumab, tremelimumab, ipilimumab, IPH4102, IPH43, IPH33, lirimumab, monalizumab, nivolumab, pembrolizumab, or their humanized, or Fc modified variants, fragments and their functional equivalents or biosimilars. In some embodiments, the checkpoint inhibitor co-expressed with CAR is atezolizumab, or its humanized, or Fc modified variants, fragments or their functional equivalents or biosimilars. In some other embodiments, the checkpoint inhibitor co-expressed with CAR is nivolumab, or its humanized, or Fc modified variants, fragments or their functional equivalents or biosimilars. In some other embodiments, the checkpoint inhibitor co-expressed

with CAR is pembrolizumab, or its humanized, or Fc modified variants, fragments or their functional equivalents or biosimilars.

[000291] In some other embodiments of the combination therapy comprising the iPSC-derived cells comprising a solid tumor targeting backbone as provided herein and at least one antibody inhibiting a checkpoint molecule, said antibody is not produced by, or in, the iPSC-derived cells and is additionally administered before, with, or after the administering of the derivative cells having a genotype listed in Table 4. In some embodiments, the administering of one, two, three or more checkpoint inhibitors in a combination therapy with the provided derivative effector cells are simultaneous or sequential. In one embodiment of the combination treatment comprising derived NK cells or T cells having a genotype listed in Table 4, the checkpoint inhibitor included in the treatment is one or more of atezolizumab, avelumab, durvalumab, tremelimumab, ipilimumab, IPH4102, IPH43, IPH33, lirimumab, monalizumab, nivolumab, pembrolizumab, and their humanized or Fc modified variants, fragments and their functional equivalents or biosimilars. In some embodiments of the combination treatment comprising derived NK cells or T cells having a genotype listed in Table 4, the checkpoint inhibitor included in the treatment is atezolizumab, or its humanized or Fc modified variant, fragment and its functional equivalent or biosimilar. In some embodiments of the combination treatment comprising derived NK cells or T cells having a genotype listed in Table 4, the checkpoint inhibitor included in the treatment is nivolumab, or its humanized or Fc modified variant, fragment or its functional equivalent or biosimilar. In some embodiments of the combination treatment comprising derived NK cells or T cells having a genotype listed in Table 4, the checkpoint inhibitor included in the treatment is pembrolizumab, or its humanized or Fc modified variant, fragment or its functional equivalent or biosimilar.

14. Chimeric Fusion Receptor (CFR)

[000292] Also provided herein, according to some embodiments, are Chimeric Fusion Receptors (CFRs). In general, a CFR is a fusion protein with a receptor functionality. In some embodiments, a CFR enables an effector cell to initiate an appropriate signal transduction cascade through CFR binding with a selected agonist for enhanced therapeutic properties of the effector cell expressing the CFR. Such enhanced effector cell therapeutic properties may include, but are not limited to, increased activation and cytotoxicity, acquired dual targeting capability, prolonged persistency, improved trafficking and tumor penetration, enhanced ability in priming, activating or recruiting bystander immune cells to tumor sites, enhanced ability to resist immunosuppression, improved ability in rescuing tumor antigen escape, and/or controlled cell signaling feedback, metabolism and apoptosis.

[000293] In some embodiments, a CFR comprises an ectodomain, a transmembrane domain, and an endodomain, wherein the ectodomain, the transmembrane domain and the endodomain do not comprise any endoplasmic reticulum (ER) retention signals or endocytosis signals. The ectodomain of the CFR is for initiating signal transduction upon binding to an engager; the transmembrane domain is for membrane anchoring of the CFR; and the endodomain comprises at least one signaling domain that regulates (e.g., activates or deactivates) a signaling pathway of choice for enhancing cell therapeutic properties including, but not limited to, tumor killing, persistence, mobility, differentiation, TME counteracting, and/or controlled apoptosis. The elimination of ER retention signals from the CFR permits CFR cell surface presentation by itself when expressed, and the elimination of endocytosis signals from the CFR reduces CFR internalization and surface downregulation. In some embodiments, it is important to either select domain components that have neither ER retention nor endocytosis signals, or remove ER retention or endocytosis signals from selected components of the CFR using molecular engineering tools. In addition, the domains of the CFRs as provided by some embodiments herein are modular, meaning for a given endodomain of a CFR, the ectodomain of the CFR is switchable depending on the binding specificity of a selected agonist, such as an antibody, a BiTE, a TriKE, or any other type of engager, to be used with said CFR; and for a given ectodomain and a specificity matching agonist, the endodomain is switchable depending on the desired signaling pathway to be activated. Additionally, the transmembrane domain in accordance with some embodiments is switchable for a given ectodomain and/or a given endodomain, so long as the transmembrane domain does not comprise any endoplasmic reticulum (ER) retention signals or endocytosis signals. Non-limiting examples of CFRs are described in US20240033355A1, which are incorporated herein by reference.

II. Methods for Targeted Genome Editing at Selected Locus in iPSCs

[000294] 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 at the insertion site during targeted editing, an endogenous gene comprising the affected sequence may be knocked-out or knocked-down due to the sequence deletion. Therefore, targeted editing may 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, with or without deletion of an endogenous sequence at the insertion site. In comparison, randomly

integrated genes are subject to position effects and silencing, making their expression unreliable and unpredictable. For example, centromeres and sub-telomeric regions are particularly prone to transgene silencing. Reciprocally, newly integrated genes may affect the surrounding endogenous genes and chromatin, potentially altering cell behavior or favoring cellular transformation. Therefore, inserting exogenous DNA in a pre-selected locus such as a safe harbor locus, or genomic safe harbor (GSH) is important for safety, efficiency, copy number control, and for reliable gene response control.

[000295] 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.

[000296] 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.” In some situations, the targeted integration site is intended to be within a coding region of a selected gene, and thus the targeted integration could disrupt the gene expression, resulting in simultaneous knock-in and knock-out (KI/KO) in one single editing step.

[000297] Inserting one or more transgenes can be achieved at a selected position such as a genomic safe harbor (GSH) locus or at a gene locus of interest (GOI), and may also thereby knock out the gene at the locus of insertion. In some embodiments, the knocking out is desirable at the same time of knocking-in the transgene.

[000298] Genomic safe harbor (GSH), 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. A useful safe harbor locus must permit sufficient transgene expression to yield desired levels of the vector-encoded protein or non-coding RNA. A safe harbor also must not predispose cells to malignant transformation nor alter cellular functions. For an genomic site to be a potential safe harbor locus, it ideally needs to meet criteria including, but not limited to: absence of disruption of regulatory elements or genes, as judged by sequence annotation; is an intergenic region in a gene dense area, or a

location at the convergence between two genes transcribed in opposite directions; keep distance to minimize the possibility of long-range interactions between vector-encoded transcriptional activators and the promoters of adjacent genes, particularly cancer-related and microRNA genes; and has apparently ubiquitous transcriptional activity, as reflected by broad spatial and temporal expressed sequence tag (EST) expression patterns, indicating ubiquitous transcriptional activity. This latter feature is especially important in stem cells, where during differentiation, chromatin remodeling typically leads to silencing of some loci and potential activation of others. Within the region suitable for exogenous insertion, a locus chosen for insertion should be devoid of repetitive elements and conserved sequences and to which primers for amplification of homology arms could easily be designed.

[000299] As disclosed herein, a newly discovered PH12 site in a non-coding region on human chromosome 17, having a coordinate of chr17:44210001-44271500 in human reference genome assembly hg38, is a suitable genomic safe harbor locus for transgene integration. Additional suitable sites for human genome editing, or specifically, targeted integration, include, but are not limited to, the adeno-associated virus site 1 (AAVS1), the chemokine (CC motif) receptor 5 (*CCR5*) gene locus and the human orthologue of the mouse ROSA26 locus. Further, the human orthologue of the mouse H11 locus may also be a suitable site for insertion using the composition and method of targeted integration disclosed herein. Furthermore, collagen and HTRP gene loci may also be used as safe harbor for targeted integration. However, validation of each selected site is typically needed, especially in stem cells for specific integration events, and optimization of insertion strategy including promoter election, exogenous gene sequence and arrangement, and construct design is often needed as well.

[000300] For targeted simultaneous knock-in and knock-out (targeted in/del, or KI/KO), the editing site is often comprised in an endogenous gene whose expression and/or function is intended to be disrupted. In some embodiments, the endogenous gene comprising a targeted in/del is associated with a specific development stage, a 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 cells, including stem cells and/or progenitor cells, and the derived cells therefrom.

[000301] As such, in one 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 construct comprising a pair of homologous arms specific to a desired integration site and one or more exogenous sequence, to enable site specific homologous recombination by the cell host enzymatic machinery, wherein the desired integration site comprises AAVS1,

CCR5, ROSA26, collagen, HTRP, H11, PH12, GAPDH, TCR or RUNX1, or other loci meeting the criteria of a genome safe harbor. Additional integration sites include an endogenous gene locus intended for disruption, such as reduction or knockout, which comprises B2M, TAP1, TAP2, tapasin, NLRC5, CIITA, RFXANK, RFX5, RFXAP, TCR α or β constant region (TRAC or TRBC), NKG2A, NKG2D, CD38, CD25, CD69, CD71, CD44, CD54, CD56, CD58, CIS, CBL-B, SOCS2, PD1, CTLA4, LAG3, TIM3, or TIGIT. In some embodiments, the method of targeted integration in a cell comprises introducing multiple constructs to two or more distinctive desired integration sites comprising TRAC, CD38, CD54, CD56, CD58, TIM3, TIGIT, H11, PH12 or combinations thereof. In some embodiments of the cell comprising three integration sites, the sites are TRAC, CD38, and one of CD54, CD56, CD58, TIM3, TIGIT, H11, or PH12. In some embodiments, the three integration sites are TRAC, CD38, and CD58, with all three endogenous genes knocked out upon transgene insertion. In some embodiments, the three integration sites are TRAC, CD38, and TIM3, with all three endogenous genes knocked out upon transgene insertion. In some embodiments, the three integration sites are TRAC, CD38, and TIGIT, with all three endogenous genes knocked out upon transgene insertion. In some embodiments, the three integration sites are TRAC, CD38, and PH12, with endogenous genes at TRAC and CD38 knocked out upon transgene insertion. In some embodiments, the three integration sites are TRAC, CD38, and H11, with endogenous genes at TRAC and CD38 knocked out upon transgene insertion.

[000302] With respective site-specific targeting homology arms for position-selective insertion, it allows the transgene(s) to express either under an endogenous promoter at the site or under an exogenous promoter comprised in the construct. When two or more transgenes are to be inserted at a selected location, a linker sequence, for example, a 2A linker or IRES, may be placed between any two transgenes. The 2A linker encodes a self-cleaving peptide derived from, e.g., FMDV, ERAV, PTV-I, or TaV (referred to as “F2A”, “E2A”, “P2A”, and “T2A”, respectively), allowing for separate proteins to be produced from a single translation. In some embodiments, insulators are included in the construct to reduce the risk of transgene and/or exogenous promoter silencing. In various embodiments, the exogenous promoter may be CAG, or other constitutive, inducible, temporal-, tissue-, or cell type- specific promoters including, but not limited to CMV, EF1 α , PGK, and UBC.

[000303] In some embodiments, one or more exogenous polynucleotides integrated by the method described herein are driven by operatively-linked exogenous promoters comprised in the construct for targeted integration. The promoters may be inducible, or constitutive, and may be temporal-, tissue- or cell type- specific. Suitable constitutive promoters for methods of the invention include, but not limited to, cytomegalovirus (CMV), elongation factor 1 α (EF1 α),

phosphoglycerate kinase (PGK), hybrid CMV enhancer/chicken β -actin (CAG) and ubiquitin C (UBC) promoters. In one embodiment, the exogenous promoter is CAG.

[000304] The exogenous polynucleotides integrated by the method described herein may be driven by endogenous promoters in the host genome, at the integration site. In one embodiment, the method described herein is used for targeted integration of one or more exogenous polynucleotides at TRAC or TRBC locus in the genome of a cell. In one embodiment, at least one integrated polynucleotide is driven by the endogenous TRAC or TRBC promoter. In one embodiment, at least one integrated polynucleotide is driven by the endogenous Tim-3 promoter. In one embodiment, at least one integrated polynucleotide is driven by the endogenous TIGIT promoter. In one embodiment, at least one integrated polynucleotide is driven by the endogenous ASB2 promoter.

[000305] In some embodiments, the one or more exogenous polynucleotides comprised in the construct for the methods of targeted integration are driven by one promoter. In some embodiments, the construct comprises one or more linker sequences between two adjacent polynucleotides driven by the same promoter to provide greater physical separation between the moieties and maximize the accessibility to enzymatic machinery. The linker peptide of the linker sequences may consist of amino acids selected to make the physical separation between the moieties (exogenous polynucleotides, and/or the protein or peptide encoded therefrom) more flexible or more rigid depending on the relevant function. The linker sequence may be cleavable by a protease or cleavable chemically to yield separate moieties. Examples of enzymatic cleavage sites in the linker include sites for cleavage by a proteolytic enzyme, such as enterokinase, Factor Xa, trypsin, collagenase, and thrombin. In some embodiments, the protease is one which is produced naturally by the host or it is exogenously introduced. Alternatively, the cleavage site in the linker may be a site capable of being cleaved upon exposure to a selected chemical, e.g., cyanogen bromide, hydroxylamine, or low pH. The optional linker sequence may serve a purpose other than the provision of a cleavage site. The linker sequence should allow effective positioning of the moiety with respect to another adjacent moiety for the moieties to function properly. The linker may also be a simple amino acid sequence of a sufficient length to prevent any steric hindrance between the moieties. In addition, the linker sequence may provide for post-translational modification including, but not limited to, e.g., phosphorylation sites, biotinylation sites, sulfation sites, γ -carboxylation sites, and the like. In some embodiments, the linker sequence is flexible so as not to hold the biologically active peptide in a single undesired conformation. The linker may be predominantly comprised of amino acids with small side chains, such as glycine, alanine, and serine, to provide for flexibility. In some embodiments about 80 to 90 percent or greater of the linker sequence comprises glycine, alanine, or serine

residues, particularly glycine and serine residues. In several embodiments, a G4S linker peptide separates the end-processing and endonuclease domains of the fusion protein. In other embodiments, a 2A linker sequence allows for two separate proteins to be produced from a single translation. Suitable linker sequences can be readily identified empirically. Additionally, suitable size and sequences of linker sequences also can be determined by conventional computer modeling techniques. In one embodiment, the linker sequence encodes a self-cleaving peptide. In one embodiment, the self-cleaving peptide is 2A. In some other embodiments, the linker sequence provides an Internal Ribosome Entry Sequence (IRES). In some embodiments, any two consecutive linker sequences are different.

[000306] Further, as provided herein, the above method for targeted integration in a preselected locus is used to insert any polynucleotide of interest, for example, polynucleotides encoding safety switch proteins, targeting modality, receptors, signaling molecules, transcription factors, pharmaceutically active proteins and peptides, drug target candidates, and proteins promoting engraftment, trafficking, homing, viability, self-renewal, persistence, and/or survival of stem cells and/or progenitor cells. In some other embodiments, the construct comprising one or more exogenous polynucleotides further comprises one or more marker genes. In one embodiment, the exogenous polynucleotide in a construct of the invention is a suicide gene encoding a safety switch protein.

[000307] The method of introducing into cells a construct comprising exogenous polynucleotides for targeted integration can be achieved using a method of gene transfer to cells known per se. In one embodiment, the construct comprises backbones of viral vectors such as adenovirus vectors, adeno-associated virus vectors, retrovirus vectors, lentivirus vectors, or Sendai virus vectors. In some embodiments, the plasmid vectors are used for delivering and/or expressing the exogenous polynucleotides to target cells (e.g., pAI-11, pXTI, pRc/CMV, pRc/RSV, pcDNAI/Neo) and the like. In some other embodiments, the episomal vector is used to deliver the exogenous polynucleotide to target cells. In some embodiments, recombinant adeno-associated viruses (rAAV) can be used for genetic engineering to introduce insertions, deletions or substitutions through homologous recombination. Unlike lentiviruses, rAAVs do not integrate into the host genome. In addition, episomal rAAV vectors mediate homology-directed gene targeting at much higher rates compared to transfection of conventional targeting plasmids. In some embodiments, an AAV6 or AAV2 vector is used to introduce insertions, deletions or substitutions in a target site in the genome of iPSCs.

[000308] Available endonucleases capable of introducing specific and targeted DSBs include, but are not limited to, zinc-finger nucleases (ZFN), transcription activator-like effector nucleases (TALEN), RNA-guided CRISPR (Clustered Regular Interspaced Short Palindromic

Repeats) systems. Additionally, the DICE (dual integrase cassette exchange) system utilizing phiC31 and Bxb1 integrases is also a promising tool for targeted integration.

[000309] 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 are not limited to, C₂H₂ zinc fingers, C₃H zinc fingers, and C₄ zinc fingers. A “designed” zinc finger domain is a domain not occurring in nature whose design/composition results principally from rational criteria, e.g., application of substitution rules and computerized algorithms for processing information in a database storing information of existing ZFP designs and binding data. See, for example, U.S. Pat. Nos. 6,140,081; 6,453,242; and 6,534,261; see also WO 98/53058; WO 98/53059; WO 98/53060; WO 02/016536 and WO 03/016496. A “selected” zinc finger domain is a domain not found in nature whose production results primarily from an empirical process such as phage display, interaction trap or hybrid selection. ZFNs are described in greater detail in U.S. Pat. No. 7,888,121 and U.S. Pat. No. 7,972,854, the complete disclosures of which are incorporated herein by reference. The most recognized example of a ZFN in the art is a fusion of the FokI nuclease with a zinc finger DNA binding domain.

[000310] 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 US Pub. No. 2011/0145940, which is herein incorporated by reference. The most recognized example of a TALEN in the art is a fusion polypeptide of the FokI nuclease to a TAL effector DNA binding domain.

[000311] Another example of a targeted nuclease that finds use in the subject methods is a targeted Spo11 nuclease, a polypeptide comprising a Spo11 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.

[000312] Additional examples of targeted nucleases suitable for embodiments of the present invention include, but not limited to Bxb1, phiC31, R4, PhiBT1, and Wβ/SPBc/TP901-1, whether used individually or in combination.

[000313] 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.

[000314] Using Cas9 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.

[000315] DICE-mediated insertion uses a pair of recombinases, for example, phiC31 and Bxb1, 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 that are not naturally present in mammalian genomes, they must be first introduced into the genome, at the desired integration site. See, for example, U.S. Pub. No. 2015/0140665, the disclosure of which is incorporated herein by reference.

[000316] One aspect of the present invention provides a construct comprising one or more exogenous polynucleotides for targeted genome integration. In one embodiment, the construct further comprises a pair of homologous arms 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 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.

[000317] 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, wherein the desired integration site comprises AAVS1, CCR5, ROSA26, collagen, HTRP, H11, PH12, GAPDH, RUNX1, B2M, TAP1, TAP2, tapasin, NLRC5, CIITA, RFXANK, RFX5, RFXAP, TCR α or β constant region, NKG2A, NKG2D, CD25, CD38, CD44, CD54, CD56, CD58, CD69, CD71, OX40, 4-1BB, CIS, CBL-B, SOCS2, PD1, CTLA4, LAG3, TIM3, or TIGIT. 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, wherein the desired integration site comprises AAVS1, CCR5, ROSA26, collagen, HTRP, H11, PH12, GAPDH, RUNX1, B2M, TAP1, TAP2, tapasin, NLRC5, CIITA, RFXANK, RFX5, RFXAP, TCR α or β constant region, NKG2A, NKG2D, CD25, CD38, CD44, CD54, CD56, CD58, CD69, CD71, OX40, 4-1BB, CIS, CBL-B, SOCS2, PD1, CTLA4, LAG3, TIM3, or TIGIT. 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, wherein the desired integration site comprises AAVS1, CCR5, ROSA26, collagen, HTRP, H11, PH12, GAPDH, RUNX1, B2M, TAP1, TAP2, tapasin, NLRC5, CIITA, RFXANK, RFX5, RFXAP, TCR α or β constant region, NKG2A, NKG2D, CD25, CD38, CD44, CD54, CD56, CD58, CD69, CD71, OX40, 4-1BB, CIS, CBL-B, SOCS2, PD1, CTLA4, LAG3, TIM3, or TIGIT. 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, wherein the desired integration site comprises AAVS1, CCR5, ROSA26, collagen, HTRP, H11, PH12, GAPDH, RUNX1, B2M, TAP1, TAP2, tapasin, NLRC5, CIITA, RFXANK, RFX5, RFXAP, TCR α or β constant region, NKG2A, NKG2D, CD25, CD38, CD44,

CD54, CD56, CD58, CD69, CD71, OX40, 4-1BB, CIS, CBL-B, SOCS2, PD1, CTLA4, LAG3, TIM3, or TIGIT.

[000318] In some embodiments, the genomically modified iPSCs and their derivative cells obtained using the methods and compositions described herein comprise at least one genotype listed in Table 4.

III. Method of Obtaining and Maintaining Genome-engineered iPSCs

[000319] In various embodiments, the present invention also provides a method of obtaining and maintaining genome-engineered iPSCs comprising one or more targeted edits (e.g., multiplex engineering) at one or more desired sites, wherein the one or more targeted edits remain intact and functional in expanded genome-engineered iPSCs or the iPSC-derived non-pluripotent cells at the respective selected editing sites. The targeted editing introduces into the genome of the iPSC, and derivative cells thereof, insertions, deletions, and/or substitutions (i.e., targeted integration and/or in/dels at selected sites). In comparison to direct engineering of patient-sourced, peripheral blood originated primary effector cells, the many benefits of obtaining genomically-engineered derivative cells through editing and differentiating iPSC as provided herein include, but are not limited to: unlimited source for engineered effector cells; no need for repeated manipulation of the effector cells, especially when multiple engineered modalities are involved; the obtained effector cells are rejuvenated for having elongated telomere and experiencing less exhaustion; the effector cell population is homogeneous in terms of editing site, copy number, and void of allelic variation, random mutations and expression variegation, largely due to the enabled clonal selection in engineered iPSCs as provided herein.

[000320] In some embodiments, the genome-engineered iPSCs comprising one or more targeted edits at one or more selected sites are maintained, passaged and expanded as single cells for an extended period in cell maintenance culture medium (FMM), wherein the iPSCs retain the targeted editing and functional modification at the selected site(s). The iPSCs cultured in FMM have been shown to continue to maintain their undifferentiated, and ground or naïve, profile; provide genomic stability without the need for culture cleaning or selection; and readily to give rise to all three somatic lineages, *in vitro* differentiation via embryoid bodies or monolayer (without formation of embryoid bodies); and by *in vivo* differentiation via teratoma formation. See, for example, International Pub. No. WO2015/134652, the disclosure of which is incorporated herein by reference.

[000321] In some embodiments, the genome-engineered iPSCs comprising one or more targeted integrations and/or in/dels are maintained, passaged and expanded in a medium (FMM) comprising a MEK inhibitor, a GSK3 inhibitor, and a ROCK inhibitor, and free of, or essentially

free of, TGF β receptor/ALK5 inhibitors, wherein the iPSCs retain the intact and functional targeted edits at the selected sites.

[000322] Another aspect of the invention provides a method of generating genome-engineered iPSCs through targeted editing of iPSCs; or through first generating genome-engineered non-pluripotent cells by targeted editing, and then reprogramming the selected/isolated genome-engineered non-pluripotent cells to obtain iPSCs comprising the same targeted editing as the non-pluripotent cells. A further aspect of the invention provides genome-engineering non-pluripotent cells which are concurrently undergoing reprogramming by introducing targeted integration and/or targeted in/dels to the cells, wherein the contacted non-pluripotent cells are under sufficient conditions for reprogramming, and wherein the conditions for reprogramming comprise contacting non-pluripotent cells with one or more reprogramming factors and small molecules. In various embodiments of the method for concurrent genome-engineering and reprogramming, the targeted integrations and/or targeted in/dels may be introduced to the non-pluripotent cells prior to, or essentially concomitantly with, initiating reprogramming by contacting the non-pluripotent cells with one or more reprogramming factors and optionally one or more small molecules.

[000323] In some embodiments, to concurrently genome-engineer and reprogram non-pluripotent cells, the targeted integrations and/or in/dels may also be introduced to the non-pluripotent cells after the multi-day process of reprogramming is initiated by contacting the non-pluripotent cells with one or more reprogramming factors and small molecules, and wherein the vectors carrying the constructs are introduced before the reprogramming cells present stable expression of one or more endogenous pluripotent genes including but not limited to, SSEA4, Tra181 and CD30.

[000324] In some embodiments, the reprogramming is initiated by contacting the non-pluripotent cells with at least one reprogramming factor, and optionally a combination of a TGF β receptor/ALK inhibitor, a MEK inhibitor, a GSK3 inhibitor and a ROCK inhibitor. In some embodiments, the genome-engineered iPSCs produced through any methods above are further maintained and expanded using a mixture comprising a combination of a MEK inhibitor, a GSK3 inhibitor and a ROCK inhibitor.

[000325] In some embodiments of the method of generating genome-engineered iPSCs, the method comprises: genomically engineering an iPSC by introducing one or more targeted integrations and/or in/dels into iPSCs to obtain genome-engineered iPSCs having a genotype provided herein. Alternatively, the method of generating genome-engineered iPSCs comprises: (a) introducing one or more targeted edits into non-pluripotent cells to obtain genome-engineered non-pluripotent cells comprising targeted integrations and/or in/dels at selected sites, and (b)

contacting the genome-engineered non-pluripotent cells with one or more reprogramming factors, and optionally a small molecule composition comprising a TGF β receptor/ALK inhibitor, a MEK inhibitor, a GSK3 inhibitor and/or a ROCK inhibitor, to obtain genome-engineered iPSCs comprising targeted integrations and/or in/dels at selected sites. Alternatively, the method of generating genome-engineered iPSCs comprises: (a) contacting non-pluripotent cells with one or more reprogramming factors, and optionally a small molecule composition comprising a TGF β receptor/ALK inhibitor, a MEK inhibitor, a GSK3 inhibitor and/or a ROCK inhibitor to initiate the reprogramming of the non-pluripotent cells; (b) introducing one or more targeted integrations and/or in/dels into the reprogramming non-pluripotent cells for genome-engineering; and (c) obtaining clonal genome-engineered iPSCs comprising the targeted integrations and/or in/dels at selected sites. Any of the above methods may further comprise single cell sorting of the genome-engineered iPSCs to obtain a clonal iPSC, and/or screening for off-target editing and abnormal karyotypes in the genome-engineered iPSCs. Through clonal expansion of the genome-engineered iPSCs, a master cell bank is generated to comprise single cell sorted and expanded clonal engineered iPSCs having at least one phenotype as provided herein. The master cell bank is subsequently cryopreserved, providing a platform for additional iPSC engineering and a renewable source for manufacturing off-the-shelf, engineered, homogeneous cell therapy products, which are well-defined and uniform in composition, and can be mass produced at significant scale in a cost-effective manner.

[000326] The reprogramming factors are selected from the group consisting of OCT4, SOX2, NANOG, KLF4, LIN28, C-MYC, ECAT1, UTF1, ESRRB, SV40LT, HESRG, CDH1, TDGF1, DPPA4, DNMT3B, ZIC3, L1TD1, and any combinations thereof as disclosed in International Pub. Nos. WO2015/134652 and WO 2017/066634, the disclosures of which are incorporated herein by reference. The one or more reprogramming factors may be in the form of polypeptides. The reprogramming factors may also be in the form of polynucleotides encoding the reprogramming factors, and thus may be introduced to the non-pluripotent cells by vectors such as, a retrovirus, a Sendai virus, an adenovirus, an episome, a plasmid, and a mini-circle. In some embodiments, the one or more polynucleotides encoding at least one reprogramming factor are introduced by a lentiviral vector. In some embodiments, the one or more polynucleotides are introduced by an episomal vector. In various other embodiments, the one or more polynucleotides are introduced by a Sendai viral vector. In some embodiments, the one or more polynucleotides introduced by a combination of plasmids. See, for example, International Pub. No. WO2019/075057A1, the disclosure of which is incorporated herein by reference.

[000327] In some embodiments, the non-pluripotent cells are transfected with multiple constructs comprising different exogenous polynucleotides and/or different promoters by

multiple vectors for targeted integration at the same or different selected sites. These exogenous polynucleotides may comprise a suicide gene, or a gene encoding targeting modalities, receptors, signaling molecules, transcription factors, pharmaceutically active proteins and peptides, drug target candidates, or a gene encoding a protein promoting engraftment, trafficking, homing, viability, self-renewal, persistence, and/or survival of the iPSCs or derivative cells thereof. In some embodiments, the exogenous polynucleotides encode RNA, including but not limited to siRNA, shRNA, miRNA and antisense nucleic acids. These exogenous polynucleotides may be driven by one or more promoters selected from the group consisting of constitutive promoters, inducible promoters, temporal-specific promoters, and tissue or cell type specific promoters. Accordingly, the polynucleotides are expressible when under conditions that activate the promoter, for example, in the presence of an inducing agent or in a particular differentiated cell type. In some embodiments, the polynucleotides are expressed in iPSCs and/or in cells differentiated from the iPSCs. In one embodiment, one or more suicide gene is driven by a constitutive promoter, for example Caspase-9 driven by CAG. These constructs comprising different exogenous polynucleotides and/or different promoters can be transfected to non-pluripotent cells either simultaneously or consecutively. The non-pluripotent cells subjected to targeted integration of multiple constructs can simultaneously contact the one or more reprogramming factors to initiate the reprogramming concurrently with the genomic engineering, thereby obtaining genome-engineered iPSCs comprising multiple targeted integrations in the same pool of cells. As such, this robust method enables a concurrent reprogramming and engineering strategy to derive a clonal genomically-engineered iPSC with multiple modalities integrated into one or more selected target sites.

IV. A method of Obtaining Genetically-Engineered Effector Cells by Differentiating Genome-engineered iPSC

[000328] In some embodiments, the genome-engineered iPSCs comprising one or more genetic modifications as provided herein are used to derive hematopoietic cell lineages or any other specific cell types *in vitro*, wherein the derived non-pluripotent cells retain the functional genetic modifications including targeted editing at the selected site(s). In some embodiments, the genome-engineered iPSCs used to derive hematopoietic cell lineages or any other specific cell types *in vitro* are master cell bank cells that are cryopreserved and thawed right before their usage. In one embodiment, the genome-engineered iPSC-derived cells include, but are not limited to, mesodermal cells with definitive hemogenic endothelium (HE) potential, definitive HE, CD34⁺ hematopoietic cells, hematopoietic stem and progenitor cells, hematopoietic multipotent progenitors (MPP), T cell progenitors, NK cell progenitors, myeloid cells, neutrophil

progenitors, T cells, NKT cells, NK cells, B cells, neutrophils, dendritic cells, and macrophages, wherein the cells derived from the genome-engineered iPSCs retain the functional genetic modifications including targeted editing at the desired site(s).

[000329] Applicable differentiation methods and compositions for obtaining iPSC-derived hematopoietic cell lineages include those depicted in, for example, International Pub. No. WO2017/078807, the disclosure of which is incorporated herein by reference. As provided, the methods and compositions for generating hematopoietic cell lineages are through definitive hemogenic endothelium (HE) derived from pluripotent stem cells, including iPSCs under serum-free, feeder-free, and/or stromal-free conditions and in a scalable and monolayer culturing platform without the need of EB formation. Cells that may be differentiated according to the provided methods range from pluripotent stem cells, to progenitor cells that are committed to particular terminally differentiated cells and transdifferentiated cells, and to cells of various lineages directly transitioned to hematopoietic fate without going through a pluripotent intermediate. Similarly, the cells that are produced by differentiating stem cells range from multipotent stem or progenitor cells, to terminally differentiated cells, and to all intervening hematopoietic cell lineages.

[000330] The methods for differentiating and expanding cells of the hematopoietic lineage from pluripotent stem cells in monolayer culturing comprise contacting the pluripotent stem cells with a BMP pathway activator, and optionally, bFGF. As provided, the pluripotent stem cell-derived mesodermal cells are obtained and expanded without forming embryoid bodies from pluripotent stem cells. The mesodermal cells are then subjected to contact with a BMP pathway activator, bFGF, and a WNT pathway activator to obtain expanded mesodermal cells having definitive hemogenic endothelium (HE) potential without forming embryoid bodies from the pluripotent stem cells. By subsequent contact with bFGF, and optionally, a ROCK inhibitor, and/or a WNT pathway activator, the mesodermal cells having definitive HE potential are differentiated to definitive HE cells, which are also expanded during differentiation.

[000331] The methods provided herein for obtaining cells of the hematopoietic lineage are superior to EB-mediated pluripotent stem cell differentiation, because EB formation leads to modest to minimal cell expansion, does not allow monolayer culturing which is important for many applications requiring homogeneous expansion and homogeneous differentiation of the cells in a population, and is laborious and of low efficiency.

[000332] The provided monolayer differentiation platform facilitates differentiation towards definitive hemogenic endothelium resulting in the derivation of hematopoietic stem cells and differentiated progeny such as T, B, NKT and NK cells. The monolayer differentiation strategy combines enhanced differentiation efficiency with large-scale expansion, and enables the

delivery of a therapeutically relevant number of pluripotent stem cell-derived hematopoietic cells for various therapeutic applications. Further, monolayer culturing using the methods provided herein leads to functional hematopoietic lineage cells that enable a full range of *in vitro* differentiation, *ex vivo* modulation, and *in vivo* long term hematopoietic self-renewal, reconstitution and engraftment. As provided, the iPSC-derived hematopoietic lineage cells include, but are not limited to, definitive hemogenic endothelium, hematopoietic multipotent progenitor cells, hematopoietic stem and progenitor cells, T cell progenitors, NK cell progenitors, T cells, NK cells, NKT cells, B cells, macrophages, and neutrophils.

[000333] Thus, in various embodiments, the method for directing differentiation of pluripotent stem cells into cells of a definitive hematopoietic lineage, comprises: (i) contacting pluripotent stem cells with a composition comprising a BMP activator, and optionally bFGF, to initiate differentiation and expansion of mesodermal cells from the pluripotent stem cells; (ii) contacting the mesodermal cells with a composition comprising a BMP activator, bFGF, and a GSK3 inhibitor, wherein the composition is optionally free of TGF β receptor/ALK inhibitor, to initiate differentiation and expansion of mesodermal cells having definitive HE potential from the mesodermal cells; (iii) contacting the mesodermal cells having definitive HE potential with a composition comprising a ROCK inhibitor; one or more growth factors and cytokines selected from the group consisting of bFGF, VEGF, SCF, IGF, EPO, IL6, and IL11; and optionally, a Wnt pathway activator, wherein the composition is optionally free of TGF β receptor/ALK inhibitor, to initiate differentiation and expansion of definitive hemogenic endothelium from pluripotent stem cell-derived mesodermal cells having definitive hemogenic endothelium potential.

[000334] In some embodiments, the method further comprises contacting pluripotent stem cells with a composition comprising a MEK inhibitor, a GSK3 inhibitor, and a ROCK inhibitor, wherein the composition is free of TGF β receptor/ALK inhibitors, to seed and expand the pluripotent stem cells. In some embodiments, the pluripotent stem cells are iPSCs, or naïve iPSCs, or iPSCs comprising one or more genetic imprints; and the one or more genetic imprints comprised in the iPSCs are retained in the hematopoietic cells differentiated therefrom. In some embodiments of the method for directing differentiation of pluripotent stem cells into cells of a hematopoietic lineage, the differentiation of the pluripotent stem cells into cells of hematopoietic lineage is void of generation of embryoid bodies and is in a monolayer culturing form.

[000335] In some embodiments of the above method, the obtained pluripotent stem cell-derived definitive hemogenic endothelium cells are CD34⁺. In some embodiments, the obtained definitive hemogenic endothelium cells are CD34⁺CD43⁻. In some embodiments, the definitive hemogenic endothelium cells are CD34⁺CD43⁻CXCR4⁻CD73⁻. In some embodiments, the definitive hemogenic endothelium cells are CD34⁺CXCR4⁻CD73⁻. In some embodiments, the

definitive hemogenic endothelium cells are $CD34^{+}CD43^{-}CD93^{-}$. In some embodiments, the definitive hemogenic endothelium cells are $CD34^{+}CD93^{-}$.

[000336] In some embodiments of the above method, the method further comprises (i) contacting pluripotent stem cell-derived definitive hemogenic endothelium with a composition comprising a ROCK inhibitor; one or more growth factors and cytokines selected from the group consisting of VEGF, bFGF, SCF, Flt3L, TPO, and IL7; and optionally a BMP activator; to initiate the differentiation of the definitive hemogenic endothelium to pre-T cell progenitors; and optionally, (ii) contacting the pre-T cell progenitors with a composition comprising one or more growth factors and cytokines selected from the group consisting of SCF, Flt3L, and IL7, but free of one or more of VEGF, bFGF, TPO, BMP activators and ROCK inhibitors, to initiate the differentiation of the pre-T cell progenitors to T cell progenitors or T cells. In some embodiments of the method, the pluripotent stem cell-derived T cell progenitors are $CD34^{+}CD45^{+}CD7^{+}$. In some embodiments of the method, the pluripotent stem cell-derived T cell progenitors are $CD45^{+}CD7^{+}$.

[000337] In yet some embodiments of the above method for directing differentiation of pluripotent stem cells into cells of a hematopoietic lineage, the method further comprises: (i) contacting pluripotent stem cell-derived definitive hemogenic endothelium with a composition comprising a ROCK inhibitor; one or more growth factors and cytokines selected from the group consisting of VEGF, bFGF, SCF, Flt3L, TPO, IL3, IL7, and IL15; and optionally, a BMP activator, to initiate differentiation of the definitive hemogenic endothelium to pre-NK cell progenitor; and optionally, (ii) contacting pluripotent stem cells-derived pre-NK cell progenitors with a composition comprising one or more growth factors and cytokines selected from the group consisting of SCF, Flt3L, IL3, IL7, and IL15, wherein the medium is free of one or more of VEGF, bFGF, TPO, BMP activators and ROCK inhibitors, to initiate differentiation of the pre-NK cell progenitors to NK cell progenitors or NK cells. In some embodiments, the pluripotent stem cell-derived NK progenitors are $CD3^{-}CD45^{+}CD56^{+}CD7^{+}$. In some embodiments, the pluripotent stem cell-derived NK cells are $CD3^{-}CD45^{+}CD56^{+}$, and optionally further defined by being $NKp46^{+}$, $CD57^{+}$ and $CD16^{+}$.

[000338] In some embodiments, the genome-engineered iPSC-derived cells obtained from the above methods comprise one or more inducible suicide genes integrated at one or more desired integration sites comprising AAVS1, CCR5, ROSA26, collagen, HTRP, H11, PH12, GAPDH, RUNX1, B2M, TAP1, TAP2, tapasin, NLRC5, CIITA, RFXANK, RFX5, RFXAP, TCR α or β constant region, NKG2A, NKG2D, CD25, CD38, CD44, CD54, CD56, CD58, CD69, CD71, OX40, 4-1BB, CIS, CBL-B, SOCS2, PD1, CTLA4, LAG3, TIM3, ASB2, and TIGIT, or other loci meeting the criteria of a genome safe harbor. In some other embodiments,

the genome-engineered iPSC-derived cells comprise polynucleotides encoding safety switch proteins, targeting modality, receptors, signaling molecules, transcription factors, pharmaceutically active proteins and peptides, drug target candidates, or proteins promoting trafficking, homing, viability, self-renewal, persistence, and/or survival of stem cells and/or progenitor cells. In some embodiments, the genome-engineered iPSC-derived cells comprising one or more suicide genes further comprise one or more in/dels comprised in one or more endogenous genes associated with immune response regulation and mediation, including, but not limited to, checkpoint genes, endogenous T cell receptor genes, and MHC class I suppressor genes. In one embodiment, the genome-engineered iPSC-derived cells comprising one or more suicide genes further comprise an in/del in B2M gene, wherein the B2M is knocked-out.

[000339] Additionally, applicable dedifferentiation methods and compositions for obtaining genomic-engineered hematopoietic cells of a first fate to genomic-engineered hematopoietic cells of a second fate include those depicted in, for example, International Pub. No. WO2011/159726, the disclosure of which is incorporated herein by reference. The method and composition provided therein allows partially reprogramming a starting non-pluripotent cell to a non-pluripotent intermediate cell by limiting the expression of endogenous Nanog gene during reprogramming; and subjecting the non-pluripotent intermediate cell to conditions for differentiating the intermediate cell into a desired cell type.

V. Therapeutic Use of Derivative Immune Cells with Functional Modalities Differentiated from Genetically Engineered iPSCs

[000340] The present invention provides, in some embodiments, a composition comprising an isolated population or subpopulation of functionally enhanced derivative immune cells that have been differentiated from genomically engineered iPSCs using the methods and compositions as disclosed. In some embodiments, the iPSCs of the composition comprise one or more targeted genetic edits as disclosed herein, which are retainable in the iPSC-derived effector cells, wherein the genetically engineered iPSCs and derivative cells thereof are suitable for cell-based adoptive therapies. In one embodiment, the isolated population or subpopulation of genetically engineered effector cells of the composition comprises iPSC-derived CD34⁺ cells. In one embodiment, the isolated population or subpopulation of genetically engineered effector cells of the composition comprises iPSC-derived HSC cells. In one embodiment, the isolated population or subpopulation of genetically engineered effector cells of the composition comprises iPSC-derived proT or T cells. In one embodiment, the isolated population or subpopulation of genetically engineered effector cells of the composition comprises iPSC-derived proNK or NK cells. In one embodiment, the isolated population or subpopulation of

genetically engineered effector cells of the composition comprises iPSC-derived immune regulatory cells or myeloid derived suppressor cells (MDSCs).

[000341] In some embodiments of the composition, the iPSC-derived genetically engineered effector cells are further modulated *ex vivo* for improved therapeutic potential. In one embodiment of the composition, an isolated population or subpopulation of genetically engineered effector cells that have been derived from iPSCs comprises an increased number or ratio of naïve T cells, stem cell memory T cells, and/or central memory T cells. In one embodiment of the composition, the isolated population or subpopulation of genetically engineered effector cells that have been derived from iPSCs comprises an increased number or ratio of type I NKT cells. In another embodiment of the composition, the isolated population or subpopulation of genetically engineered effector cells that have been derived from iPSCs comprises an increased number or ratio of adaptive NK cells. In some embodiments of the composition, the isolated population or subpopulation of genetically engineered CD34⁺ cells, HSC cells, T cells, NK cells, or myeloid derived suppressor cells derived from iPSCs are allogeneic. In some other embodiments of the composition, the isolated population or subpopulation of genetically engineered CD34⁺ cells, HSC cells, T cells, NK cells, or MDSCs derived from iPSC are autologous.

[000342] In some embodiments of the composition, the iPSC for differentiation comprises genetic imprints selected to convey desirable therapeutic attributes in derived effector cells, provided that cell development biology during differentiation is not disrupted, and provided that the genetic imprints are retained and functional in the differentiated hematopoietic cells derived from said iPSC.

[000343] In some embodiments of the composition, the genetic imprints of the pluripotent stem cells comprise (i) one or more genetically modified modalities obtained through genomic insertion, deletion or substitution in the genome of the pluripotent cells during or after reprogramming a non-pluripotent cell to iPSC; or (ii) one or more retainable therapeutic attributes of a source specific immune cell that is donor-, disease-, or treatment response-specific, and wherein the pluripotent cells are reprogrammed from the source specific immune cell, wherein the iPSC retain the source therapeutic attributes, which are also comprised in the iPSC-derived hematopoietic lineage cells.

[000344] In some embodiments of the composition, the genetically modified modalities comprise one or more of: safety switch proteins, 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, immune response regulation and modulation, and/or survival of the iPSCs or

derivative cells thereof. In some embodiments of the composition, the genetically modified iPSC and the derivative cells thereof comprise a genotype listed in Table 4. In some other embodiments of the composition, the genetically modified iPSC and the derivative cells thereof comprising a genotype listed in Table 4 further comprise additional genetically modified modalities comprising (1) deletion or disruption of B2M, TAP1, TAP2, tapasin, NLRC5, CIITA, RFXANK, RFX5, RFXAP, TCR α or TCR β constant region (TRAC or TRBC), NKG2A, NKG2D, CD38, CD25, CD69, CD71, CD44, CD54, CD56, CD58, OX40, 4-1BB, CIS, CBL-B, SOCS2, PD1, CTLA4, LAG3, TIM3, or TIGIT; and/or (2) introduction of HLA-E, HLA-G, 4-1BBL, CD3, CD4, CD8, CD47, CD113, CD131, CD137, CD80, PDL1, A_{2A}R, CAR, TCR, Fc receptor, or surface triggering receptors for coupling with bi- or multi- specific or universal engagers.

[000345] In still some other embodiments of the composition, the iPSC-derived hematopoietic lineage cells comprise the therapeutic attributes of the source specific immune cell relating to one or more of: (i) increased cytotoxicity; (ii) improved persistency and/or survival; (iii) enhanced ability in migrating, and/or activating or recruiting bystander immune cells, to tumor sites; (iv) improved tumor infiltration; (v) enhanced ability to reduce tumor immunosuppression; (vi) improved ability in rescuing tumor antigen escape; (vii) controlled apoptosis; (viii) enhanced or acquired ADCC; and (ix) ability to avoid fratricide, in comparison to its counterpart primary cell obtained from peripheral blood, umbilical cord blood, or any other donor tissues without the same genetic edit(s). In some embodiments of the composition, the iPSC-derived hematopoietic lineage cells additionally comprise the therapeutic attributes of promoting homing or trafficking and retention of the effector cells at a tumor site.

[000346] In some embodiments of the composition, the iPSC-derived hematopoietic cells comprising a genotype listed in Table 4. In some embodiments of the composition, the engineered expression of the cytokine(s) and the CAR(s) is NK cell specific. In some other embodiments of the composition, the engineered expression of the cytokine(s) and the CAR(s) is T cell specific. In some embodiments of the composition, the iPSC-derived hematopoietic effector cells are antigen specific. In some embodiments of the composition, the antigen specific derivative effector cells target a liquid tumor. In some embodiments of the composition, the antigen specific derivative effector cells target a solid tumor. In some embodiments of the composition, the antigen specific iPSC-derived hematopoietic effector cells are capable of rescuing tumor antigen escape. Additionally, the present application makes possible a combined therapeutic approach by providing rationally designed effector cells capable of synergize with a tumor sensitizing procedure that upregulates tumor cell expression of a matching chemokine to

augment effector cell tumor site homing, trafficking and retention, which contributes to increased effector cell cytotoxicity and persistency.

[000347] As provided herein, exposing a tumor cell to a sensitizing agent (e.g., radiation) elevates secretion and/or surface expression of stress ligands including, but limited to, the chemokine IL8, by the tumor cells. Tumor preconditioning by sensitization, as described herein therefore provides an additional strategy to further enhance the therapeutic efficacy of the effector cells overexpressing a C-X-C motif chemokine receptor or a variant thereof. Without being limited by theory, tumor sensitization may be utilized to overcome tumor resistance by modulating potential tumorigenic mechanisms (including, but not limited to cell cycle progression, inflammation, proliferation, apoptosis, invasion, perfusion, metastasis, and angiogenesis) to make the tumor cells more susceptible to activities of another selective drugs, such as the allogeneic effector cells with desired engineered therapeutic attributes as described herein, thereby enhancing the efficacy of the therapeutic effector cells targeting the tumor.

[000348] Without being bound by theory, exemplary sensitizing agents useful in compositions and methods disclosed herein include, but are not limited to, radiation therapy, radiopharmaceuticals, or chemotherapeutic agents. Thus, the above-discussed compositions may further comprise a sensitizing agent, as described above. In various embodiments, the sensitizing agent increases secretion and/or surface expression of a chemokine, including CXCL8, by a tumor cell upon contact therewith.

[000349] Embodiments of radiation therapy include, but are not limited to, external beam radiation therapy, wherein high-energy beams (e.g., x-rays, gamma rays, photons, protons, neutrons, ions, and any other forms of energy applicable to such treatments) are produced by a machine and aimed at the tumor; brachytherapy, wherein seeds, ribbons, or capsules that contain or are otherwise linked to a radiation source/particle are placed in or near a tumor or cancer cell. Embodiments of radioactive drugs (e.g., radiopharmaceuticals or radionuclides, including radiopeptides) comprise a radioactive compound linked to a targeting molecule (e.g., an antibody conjugate).

[000350] In various embodiments, the amount of radiation agent being exposed to, or contacted with, a cancer or tumor cell ranges from about 0.0001 Gy to about 80Gy. Thus, in some embodiments, the amount of sensitizing agent provided to a subject and/or included in the compositions provided herein is at least about 0.0001 Gy, at least about 0.0005 Gy, at least about 0.001 Gy, at least about 0.0015 Gy, at least about 0.01 Gy, at least about 0.015 Gy, at least about 0.1 Gy, at least about 0.15 Gy, at least about 1.0 Gy, at least about 1.5 Gy, at least about 10.0 Gy, at least about 15 Gy, at least about 20.0 Gy, at least about 25.0 Gy, at least about 30.0 Gy, at least about 35.0 Gy, at least about 40.0 Gy, at least about 45.0 Gy, at least about 50.0 Gy, at least about

55.0 Gy, at least about 60.0 Gy, at least about 65.0 Gy, at least about 70.0 Gy, at least about 75.0 Gy, at least about 80.0 Gy or any range in-between. In some embodiments, the amount of sensitizing agent is about 25.0 Gy.

[000351] Examples of radioactive compounds useful as radiopharmaceuticals include, but are not limited to calcium-47, carbon-11, carbon-14, chromium-51, cobalt-57, cobalt-58, erbium-169, fluorine-18, gallium-67, gallium-68, hydrogen-3, indium-111, iodine-123, iodine-125, iodine-131, iorn-59, krypton-81m, lutetium-177, nitrogen-13, oxygen-15, phosphorus-32, radium-223, rubidium-82, samarium-153, selenium-75, sodium-22, sodium-24, strontium-89, technetium-99m, thallium-201, xenon-133, and yttrium-90.

[000352] Exemplary chemotherapeutic agents that can be potentially used for tumor cell sensitization include, but are not limited to, alkylating agents (cyclophosphamide, mechlorethamine, mephalin, chlorambucil, heamethylmelamine, thiotepa, busulfan, carmustine, lomustine, semustine), animetabolites (methotrexate, fluorouracil, floxuridine, cytarabine, 6-mercaptopurine, thioguanine, pentostatin), vinca alkaloids (vincristine, vinblastine, vindesine), epipodophyllotoxins (etoposide, etoposide orthoquinone, and teniposide), antibiotics (daunorubicin, doxorubicin, mitoxantrone, bisanthrene, actinomycin D, plicamycin, puromycin, and gramicidine D), colchicine, cytochalasin B, emetine, maytansine, and amsacrine. Additional agents include aminglutethimide, cisplatin, carboplatin, mitomycin, altretamine, cyclophosphamide, lomustine (CCNU), carmustine (BCNU), irinotecan (CPT-11), alemtuzumab, altretamine, anastrozole, L-asparaginase, azacitidine, bevacizumab, bexarotene, bleomycin, bortezomib, busulfan, calusterone, capecitabine, celecoxib, cetuximab, cladribine, clofurabine, cytarabine, dacarbazine, denileukin diftitox, diethylstilbestrol, docetaxel, dromostanolone, epirubicin, erlotinib, estramustine, etoposide, ethinyl estradiol, exemestane, floxuridine, 5-flourouracil, fludarabine, flutamide, fulvestrant, gefitinib, gemcitabine, goserelin, hydroxyurea, ibritumomab, idarubicin, ifosfamide, imatinib, interferon alpha (2a, 2b), irinotecan, letrozole, leucovorin, leuprolide, levamisole, mecllorethamine, megestrol, melphalin, mercaptopurine, methotrexate, methoxsalen, mitomycin C, mitotane, mitoxantrone, nandrolone, nofetumomab, oxaliplatin, paclitaxel, pamidronate, pemetrexed, pegademase, pegasparagase, pentostatin, pipobroman, plicamycin, polifeprosan, porfimer, procarbazine, quinacrine, rituximab, sargramostim, streptozocin, tamoxifen, temozolomide, teniposide, testolactone, thioguanine, thiotepa, topotecan, toremifene, tositumomab, trastuzumab, tretinoin, uracil mustard, valrubicin, vinorelbine, and zoledronate. Other suitable chemotherapeutic agents are those that are approved for human use, including those that will be approved, as chemotherapeutics or radiotherapeutics, and known in the art. Such agents can be referenced through any of a number of standard physicians' and oncologists' references (e.g., Goodman & Gilman's The Pharmacological Basis

of Therapeutics, Ninth Edition, McGraw-Hill, N.Y., 1995) or through the National Cancer Institute website ([fda.gov/cder/cancer/druglistframe.htm](https://www.fda.gov/cder/cancer/druglistframe.htm)), both as updated from time to time.

[000353] A variety of diseases may be ameliorated by introducing the derivative effector cells and/or the compositions disclosed herein to a subject suitable for adoptive cell therapy. In some embodiments, the iPSC-derived hematopoietic cells or the compositions as provided herein are for allogeneic adoptive cell therapies. Additionally, the present invention provides, in some embodiments, therapeutic use of the above immune cells and/or therapeutic compositions and/or combination therapies by introducing the cells or composition to a subject suitable for adoptive cell therapy, wherein the subject has an autoimmune disorder; a hematological malignancy; a solid tumor; or an infection associated with HIV, RSV, EBV, CMV, adenovirus, or BK polyomavirus. Examples of hematological malignancies include, but are not limited to, acute and chronic leukemias (acute myelogenous leukemia (AML), acute lymphoblastic leukemia (ALL), chronic myelogenous leukemia (CML), lymphomas, non-Hodgkin lymphoma (NHL), Hodgkin's disease, multiple myeloma, and myelodysplastic syndromes. Examples of solid cancers include, but are not limited to, bladder cancer, bone cancer, brain/CNS cancer, breast cancer, breast lung cancer, cervical cancer, colorectal cancer, esophageal cancer, gastric/stomach cancer, head and neck cancer, kidney cancer, larynx cancer, liver cancer, lung cancer, metastatic cancer, ovarian cancer, pancreatic cancer, prostate cancer, rectal cancer, renal cancer, salivary gland cancer, skin cancer, testicular tumor, thyroid tumor, urothelial cancer, and uterine/endometrial cancer. Examples of various autoimmune disorders include, but are not limited to, alopecia areata, autoimmune hemolytic anemia, autoimmune hepatitis, dermatomyositis, diabetes (type 1), some forms of juvenile idiopathic arthritis, glomerulonephritis, Graves' disease, Guillain-Barré syndrome, idiopathic thrombocytopenic purpura, myasthenia gravis, some forms of myocarditis, multiple sclerosis, pemphigus/pemphigoid, pernicious anemia, polyarteritis nodosa, polymyositis, primary biliary cirrhosis, psoriasis, rheumatoid arthritis, scleroderma/systemic sclerosis, Sjögren's syndrome, systemic lupus, erythematosus, some forms of thyroiditis, some forms of uveitis, vitiligo, granulomatosis with polyangiitis (Wegener's). Examples of viral infections include, but are not limited to, HIV- (human immunodeficiency virus), HSV- (herpes simplex virus), KSHV- (Kaposi's sarcoma-associated herpesvirus), RSV- (Respiratory Syncytial Virus), EBV- (Epstein-Barr virus), CMV- (cytomegalovirus), VZV (Varicella zoster virus), adenovirus-, a lentivirus-, a BK polyomavirus- associated disorders.

[000354] The treatment using the derived hematopoietic lineage cells of embodiments disclosed herein, or the compositions provided herein, could be carried out upon symptom presentation, or for relapse prevention. The terms "treating," "treatment," and the like are used

herein to generally mean obtaining a desired pharmacologic and/or physiologic effect. The effect may be prophylactic in terms of completely or partially preventing a disease and/or may be therapeutic in terms of a partial or complete cure for a disease and/or adverse effect attributable to the disease. "Treatment" as used herein covers any intervention of a disease in a subject and includes: preventing the disease from occurring in a subject which may be predisposed to the disease but has not yet been diagnosed as having it; and inhibiting the disease, i.e., arresting its development; or relieving the disease, i.e., causing regression of the disease. The therapeutic agent(s) and/or compositions may be administered before, during or after the onset of a disease or an injury. Treatment of ongoing disease, where the treatment stabilizes or reduces the undesirable clinical symptoms of the patient, is also of particular interest. In some embodiments, the subject in need of a treatment has a disease, a condition, and/or an injury that can be contained, ameliorated, and/or improved in at least one associated symptom by a cell therapy. Certain embodiments contemplate that a subject in need of cell therapy, includes, but is not limited to, a candidate for bone marrow or stem cell transplantation, a subject who has received chemotherapy or irradiation therapy, a subject who has or is at risk of having a hyperproliferative disorder or a cancer, e.g., a hyperproliferative disorder or a cancer of hematopoietic system, a subject having or at risk of developing a tumor, e.g., a solid tumor, a subject who has or is at risk of having a viral infection or a disease associated with a viral infection.

[000355] When evaluating responsiveness to the treatment comprising the derived hematopoietic lineage cells of embodiments disclosed herein, the response can be measured by criteria comprising at least one of: clinical benefit rate, survival until mortality, pathological complete response, semi-quantitative measures of pathologic response, clinical complete remission, clinical partial remission, clinical stable disease, recurrence-free survival, metastasis free survival, disease free survival, circulating tumor cell decrease, circulating marker response, and RECIST (Response Evaluation Criteria In Solid Tumors) criteria.

[000356] The therapeutic composition comprising iPSC-derived effector cells as disclosed herein can be administered to a subject before, during, and/or after other treatments, including sensitization of cancer or tumor cells, as described above. As such a method of combinational therapy can involve the administration or preparation of iPSC-derived effector cells before, during, and/or after the use of one or more additional therapeutic agents. As provided above, the one or more additional therapeutic agents comprise a peptide, a cytokine, a checkpoint inhibitor, an engager, a mitogen, a growth factor, a small RNA, a dsRNA (double stranded RNA), mononuclear blood cells, feeder cells, feeder cell components or replacement factors thereof, a vector comprising one or more polynucleic acids of interest, an antibody, a chemotherapeutic agent or a radioactive moiety, or an immunomodulatory drug (IMiD). The administration of the

iPSC-derived immune cells can be separated in time from the administration of an additional therapeutic agent by hours, days, or even weeks. Additionally, or alternatively, the administration can be combined with other biologically active agents or modalities such as, but not limited to, an antineoplastic agent, a non-drug therapy, such as, surgery.

[000357] In some embodiments of a combinational cell therapy, the therapeutic combination comprises the iPSC-derived effector cells provided herein and an additional therapeutic agent that is an antibody, or an antibody fragment. In some embodiments, the antibody is a monoclonal antibody. In some embodiments, the antibody may be a humanized antibody, a humanized monoclonal antibody, or a chimeric antibody. In some embodiments, the antibody, or antibody fragment, specifically binds to a viral antigen. In other embodiments, the antibody, or antibody fragment, specifically binds to a tumor antigen. In some embodiments, the tumor or viral specific antigen activates the administered iPSC-derived hematopoietic lineage cells to enhance their killing ability. In some embodiments, the antibodies suitable for combinational treatment as an additional therapeutic agent to the administered iPSC-derived hematopoietic lineage cells include, but are not limited to, anti-CD20 antibodies (e.g., rituximab, velutuzumab, ofatumumab, ublituximab, ocaratuzumab, obinutuzumab), anti-HER2 antibodies (e.g., trastuzumab, pertuzumab), anti-CD52 antibodies (e.g., alemtuzumab), anti-EGFR antibodies (e.g., cetuximab), anti-GD2 antibodies (e.g., dinutuximab), anti-PDL1 antibodies (e.g., avelumab), anti-CD38 antibodies (e.g., daratumumab, isatuximab, MOR202), anti-CD123 antibodies (e.g., 7G3, CSL362), anti-SLAMF7 antibodies (elotuzumab), MICA/B antibodies (7C6, 6F11, 1C2), and their humanized or Fc modified variants or fragments or their functional equivalents or biosimilars. In some embodiments, the present invention provides therapeutic compositions comprising effector cells, including the iPSC-derived hematopoietic lineage cells, having a genotype listed in Table 4 and an additional therapeutic agent that is an antibody, or an antibody fragment, as described above.

[000358] In some embodiments, the additional therapeutic agent comprises one or more checkpoint inhibitors. Checkpoints are referred to cell molecules, often cell surface molecules, capable of suppressing or downregulating immune responses when not inhibited. Checkpoint inhibitors are antagonists capable of reducing checkpoint gene expression or gene products, or decreasing activity of checkpoint molecules. Suitable checkpoint inhibitors for combination therapy with the derivative effector cells include, but are not limited to, antagonists of PD1 (Pdccl, CD279), PDL-1 (CD274), TIM3 (Havcr2), TIGIT (WUCAM and Vstm3), LAG3 (CD223), CTLA4 (CD152), 2B4 (CD244), 4-1BB (CD137), 4-1BBL (CD137L), A2AR, BATE, BTLA, CD39 (Entpd1), CD47, CD73 (NT5E), CD94, CD96, CD160, CD200, CD200R, CD274, CEACAM1, CSF-1R, Foxp1, GARP, HVEM, IDO, EDO, TDO, LAIR-1, MICA/B, NR4A2,

MAFB, OCT-2 (Pou2f2), retinoic acid receptor alpha (Rara), TLR3, VISTA, NKG2A/HLA-E, and inhibitory KIR (for example, 2DL1, 2DL2, 2DL3, 3DL1, and 3DL2).

[000359] Some embodiments of the combination therapy comprising the provided derivative effector cells further comprise at least one inhibitor targeting a checkpoint molecule. Some other embodiments of the combination therapy with the provided derivative effector cells comprise two, three or more inhibitors such that two, three, or more checkpoint molecules are targeted. In some embodiments, the effector cells for combination therapy as described herein are derivative NK cells as provided. In some embodiments, the effector cells for combination therapy as described herein are derivative T cells. In some embodiments, the derivative NK or T cells for combination therapies are functionally enhanced as provided herein. In some embodiments, the two, three or more checkpoint inhibitors may be administered in a combination therapy with, before, or after the administering of the derivative effector cells. In some embodiments, the two or more checkpoint inhibitors are administered at the same time, or one at a time (sequential). In some embodiments, the present invention provides therapeutic compositions comprising effector cells, including the iPSC-derived effector cells, having a genotype listed in Table 4 and one or more checkpoint inhibitors, as described above.

[000360] In some embodiments, the antagonist inhibiting any of the above checkpoint molecules is an antibody. In some embodiments, the checkpoint inhibitory antibodies may be murine antibodies, human antibodies, humanized antibodies, a camel Ig, a single variable new antigen receptor (VNAR), a shark heavy-chain-only antibody (Ig NAR), chimeric antibodies, recombinant antibodies, or antibody fragments thereof. Non-limiting examples of antibody fragments include Fab, Fab', F(ab')₂, F(ab')₃, Fv, single chain antigen binding fragments (scFv), (scFv)₂, disulfide stabilized Fv (dsFv), minibody, diabody, triabody, tetrabody, single-domain antigen binding fragments (sdAb, Nanobody), recombinant heavy-chain-only antibody (VHH), and other antibody fragments that maintain the binding specificity of the whole antibody, which may be more cost-effective to produce, more easily used, or more sensitive than the whole antibody. In some embodiments, the one, or two, or three, or more checkpoint inhibitors comprise at least one of atezolizumab, avelumab, durvalumab, ipilimumab, IPH4102, IPH43, IPH33, lirimumab, monalizumab, nivolumab, pembrolizumab, and their derivatives or functional equivalents.

[000361] The combination therapies comprising the derivative effector cells described herein, and one or more check inhibitors are applicable to treatment of liquid and solid cancers, including but not limited to cutaneous T-cell lymphoma, non-Hodgkin lymphoma (NHL), Mycosis fungoides, Pagetoid reticulosis, Sezary syndrome, Granulomatous slack skin, Lymphomatoid papulosis, Pityriasis lichenoides chronica, Pityriasis lichenoides et varioliformis

acuta, CD30⁺ cutaneous T-cell lymphoma, Secondary cutaneous CD30⁺ large cell lymphoma, non- mycosis fungoides CD30 cutaneous large T-cell lymphoma, Pleomorphic T-cell lymphoma, Lennert lymphoma, subcutaneous T-cell lymphoma, angiocentric lymphoma, blastic NK-cell lymphoma, B-cell Lymphomas, hodgkins lymphoma (HL), Head and neck tumor; Squamous cell carcinoma, rhabdomyosarcoma, Lewis lung carcinoma (LLC), non-small cell lung cancer, esophageal squamous cell carcinoma, esophageal adenocarcinoma, renal cell carcinoma (RCC), colorectal cancer (CRC), acute myeloid leukemia (AML), breast cancer, gastric cancer, prostatic small cell neuroendocrine carcinoma (SCNC), liver cancer, glioblastoma, liver cancer, oral squamous cell carcinoma, pancreatic cancer, thyroid papillary cancer, intrahepatic cholangiocellular carcinoma, hepatocellular carcinoma, bone cancer, metastasis, and nasopharyngeal carcinoma.

[000362] In some embodiments, other than the derivative effector cells as provided herein, a combination for therapeutic use comprises one or more additional therapeutic agents comprising a chemotherapeutic agent or a radioactive moiety. “Chemotherapeutic agent” refers to cytotoxic antineoplastic agents, that is, chemical agents which preferentially kill neoplastic cells or disrupt the cell cycle of rapidly proliferating cells, or which are found to eradicate stem cancer cells, and which are used therapeutically to prevent or reduce the growth of neoplastic cells. Chemotherapeutic agents are also sometimes referred to as antineoplastic or cytotoxic drugs or agents, examples of which are known in the art.

[000363] In some embodiments, the chemotherapeutic agent comprises an anthracycline, an alkylating agent, an alkyl sulfonate, an aziridine, an ethylenimine, a methylmelamine, a nitrogen mustard, a nitrosourea, an antibiotic, an antimetabolite, a folic acid analog, a purine analog, a pyrimidine analog, an enzyme, a podophyllotoxin, a platinum-containing agent, an interferon, and an interleukin. Exemplary chemotherapeutic agents include, but are not limited to, alkylating agents (cyclophosphamide, mechlorethamine, mephalin, chlorambucil, heamethylmelamine, thiotepa, busulfan, carmustine, lomustine, semustine), animetabolites (methotrexate, fluorouracil, floxuridine, cytarabine, 6-mercaptopurine, thioguanine, pentostatin), vinca alkaloids (vincristine, vinblastine, vindesine), epipodophyllotoxins (etoposide, etoposide orthoquinone, and teniposide), antibiotics (daunorubicin, doxorubicin, mitoxantrone, bisanthrene, actinomycin D, plicamycin, puromycin, and gramicidine D), paclitaxel, colchicine, cytochalasin B, emetine, maytansine, and amsacrine. Additional agents include aminglutethimide, cisplatin, carboplatin, mitomycin, altretamine, cyclophosphamide, lomustine (CCNU), carmustine (BCNU), irinotecan (CPT-11), alemtuzamab, altretamine, anastrozole, L-asparaginase, azacitidine, bevacizumab, bexarotene, bleomycin, bortezomib, busulfan, calusterone, capecitabine, celecoxib, cetuximab, cladribine, clofurabine, cytarabine, dacarbazine, denileukin diftitox, diethylstilbestrol, docetaxel,

dromostanolone, epirubicin, erlotinib, estramustine, etoposide, ethinyl estradiol, exemestane, floxuridine, 5-fluorouracil, fludarabine, flutamide, fulvestrant, gefitinib, gemcitabine, goserelin, hydroxyurea, ibritumomab, idarubicin, ifosfamide, imatinib, interferon alpha (2a, 2b), irinotecan, letrozole, leucovorin, leuprolide, levamisole, meclorothamine, megestrol, melphalin, mercaptopurine, methotrexate, methoxsalen, mitomycin C, mitotane, mitoxantrone, nandrolone, nofetumomab, oxaliplatin, paclitaxel, pamidronate, pemetrexed, pegademase, pegasparagase, pentostatin, pipobroman, plicamycin, polifeprosan, porfimer, procarbazine, quinacrine, rituximab, sargramostim, streptozocin, tamoxifen, temozolomide, teniposide, testolactone, thioguanine, thiotepa, topotecan, toremifene, tositumomab, trastuzumab, tretinoin, uracil mustard, valrubicin, vinorelbine, and zoledronate. Other suitable agents are those that are approved for human use, including those that will be approved, as chemotherapeutics or radiotherapeutics, and known in the art. Such agents can be referenced through any of a number of standard physicians' and oncologists' references (e.g., Goodman & Gilman's The Pharmacological Basis of Therapeutics, Ninth Edition, McGraw-Hill, N.Y., 1995) or through the National Cancer Institute website (fda.gov/cder/cancer/druglistframe.htm), both as updated from time to time.

[000364] Immunomodulatory drugs (IMiDs) such as thalidomide, lenalidomide, and pomalidomide stimulate both NK cells and T cells. As provided herein, IMiDs may be used with the iPSC-derived therapeutic immune cells for cancer treatments.

[000365] Other than an isolated population of iPSC-derived hematopoietic lineage cells included in the therapeutic compositions, the compositions suitable for administration to a subject/patient can further include one or more pharmaceutically acceptable carriers (additives) and/or diluents (e.g., pharmaceutically acceptable medium, for example, cell culture medium), or other pharmaceutically acceptable components. Pharmaceutically acceptable carriers and/or diluents are determined in part by the particular composition being administered, as well as by the particular method used to administer the therapeutic composition. Accordingly, there is a wide variety of suitable formulations of therapeutic compositions of embodiments of the present invention (see, e.g., Remington's Pharmaceutical Sciences, 17th ed. 1985, the disclosure of which is hereby incorporated by reference in its entirety).

[000366] In one embodiment, the therapeutic composition comprises the iPSC-derived T cells made by the methods and composition disclosed herein. In one embodiment, the therapeutic composition comprises the pluripotent cell derived NK cells made by the methods and composition disclosed herein. In one embodiment, the therapeutic composition comprises the iPSC-derived CD34⁺ HE cells made by the methods and composition disclosed herein. In one embodiment, the therapeutic composition comprises the pluripotent cell derived HSCs made by

the methods and composition disclosed herein. In one embodiment, the therapeutic composition comprises the pluripotent cell derived MDSC made by the methods and composition disclosed herein. A therapeutic composition comprising a population of iPSC-derived hematopoietic lineage cells as disclosed herein can be administered separately by intravenous, intraperitoneal, enteral, or tracheal administration methods or in combination with other suitable compounds to affect the desired treatment goals.

[000367] These pharmaceutically acceptable carriers and/or diluents can be present in amounts sufficient to maintain a pH of the therapeutic composition of between about 3 and about 10. As such, a buffering agent can be as much as about 5% on a weight to weight basis of the total composition. Electrolytes such as, but not limited to, sodium chloride and potassium chloride can also be included in the therapeutic composition. In one aspect, the pH of the therapeutic composition is in the range from about 4 to about 10. Alternatively, the pH of the therapeutic composition is in the range from about 5 to about 9, from about 6 to about 9, or from about 6.5 to about 8. In another embodiment, the therapeutic composition includes a buffer having a pH in one of said pH ranges. In another embodiment, the therapeutic composition has a pH of about 7. Alternatively, the therapeutic composition has a pH in a range from about 6.8 to about 7.4. In still another embodiment, the therapeutic composition has a pH of about 7.4.

[000368] The invention also provides, in some embodiments, the use of a pharmaceutically acceptable cell culture medium in particular compositions and/or cultures disclosed herein. Such compositions are suitable for administration to human subjects. Generally speaking, any medium that supports the maintenance, growth, and/or health of the iPSC-derived effector cells in accordance with embodiments of the invention are suitable for use as a pharmaceutical cell culture medium. In some embodiments, the pharmaceutically acceptable cell culture medium is a serum free, and/or feeder-free medium. In various embodiments, the serum-free medium is animal-free, and can optionally be protein-free. Optionally, the medium can contain biopharmaceutically acceptable recombinant proteins. Animal-free medium refers to medium wherein the components are derived from non-animal sources. Recombinant proteins replace native animal proteins in animal-free medium and the nutrients are obtained from synthetic, plant or microbial sources. Protein-free medium, in contrast, is defined as substantially free of protein. One having ordinary skill in the art would appreciate that the above examples of media are illustrative and in no way limit the formulation of media suitable for use in the present invention and that there are many suitable media known and available to those in the art.

[000369] In various embodiments, the iPSC-derived hematopoietic lineage cells can have at least 50%, 60%, 70%, 80%, 90%, 95%, 98%, or 99% T cells, NK cells, NKT cells, proT cells, proNK cells, CD34⁺ HE cells, HSCs, B cells, myeloid-derived suppressor cells (MDSCs),

regulatory macrophages, regulatory dendritic cells, or mesenchymal stromal cells. In some embodiments, the iPSC-derived hematopoietic lineage cells have about 95% to about 100% T cells, NK cells, proT cells, proNK cells, CD34⁺ HE cells, or myeloid-derived suppressor cells (MDSCs). In some embodiments, the present invention provides therapeutic compositions having purified T cells or NK cells, such as a composition having an isolated population of about 95% T cells, NK cells, proT cells, proNK cells, CD34⁺ HE cells, or myeloid-derived suppressor cells (MDSCs) to treat a subject in need of the cell therapy.

[000370] One aspect of the present application provides a method of treating a subject in need by administering one or more therapeutic doses of effector cells comprising an TCR promoter-driven ADR and/or a cytokine, and a constitutively expressed TCE, in addition to edits comprising a CAR, a TGF β -SRR, a C-X-C-motif chemokine receptor or a variant thereof, a CD16 variant, and a CD38 knockout, among other modifications as provided herein, or a genotype listed in Table 4. In some embodiments, the present application provides a method of treating a subject having cancer or a tumor by first sensitizing the cancer or tumor cell in the subject to increase or enhance secretion and/or surface expression of one or more chemokines that are ligands to a C-X-C-motif chemokine receptor, as compared to chemokine secretion and/or surface expression prior to such contacting/exposure. Following sensitization of the cancer or tumor cell, an effector cell or population thereof, as described above, is given/administered to the subject, wherein the effector cell comprises a solid tumor targeting backbone as provided herein, and optionally one or more additional edits described herein, or the effector cell comprises a genotype listed in Table 4. In various embodiments, the effector cell or population thereof may be provided prior to or concurrently with one or more additional therapeutic agents, as described above.

[000371] Another aspect of the present application provides a method of treating a subject in need using a combinational cell therapy. In some embodiments of the combinational cell therapy, the method of treating a subject in need comprises administering one or more therapeutic doses of effector cells comprising a solid tumor targeting backbone as provided herein, and optionally one or more other edits described herein, or a genotype listed in Table 4; and one or more therapeutic agents comprising a peptide, a cytokine, a checkpoint inhibitor, an engager, a mitogen, a growth factor, a small RNA, a dsRNA (double stranded RNA), mononuclear blood cells, feeder cells, feeder cell components or replacement factors thereof, a vector comprising one or more polynucleic acids of interest, an antibody, a chemotherapeutic agent or a radioactive moiety, or an immunomodulatory drug (IMiD), and optionally preconditioning tumor cells in the subject by administering a sensitizing agent. In some embodiments, the combinational cell therapy, or composition used therefor, comprises a

population of effector cells derived from genomically engineered iPSCs and one or more therapeutic agents, wherein the engineered iPSCs and the derived effector cells comprise a solid tumor targeting backbone as provided herein, and optionally one or more other edits described herein, or a genotype listed in Table 4. In some embodiments of the method of combinational cell therapy, the method comprises preconditioning tumor cells in the subject by administering a sensitizing agent, wherein the sensitizing agent comprises radiation therapy, radiopharmaceuticals, or chemotherapeutic agents as provided herein. In various embodiments, preconditioning of the tumor cells in the subject occurs prior to, or concurrently with administering the one or more therapeutic doses of effector cells described herein.

[000372] As a person of ordinary skill in the art would understand, both autologous and allogeneic hematopoietic lineage cells derived from iPSC based on the methods and compositions provided herein can be used in cell therapies as described above. For autologous transplantation, the isolated population of derived hematopoietic lineage cells are either complete or partial HLA-match with the patient. In another embodiment, the derived hematopoietic lineage cells are not HLA-matched to the subject, wherein the derived hematopoietic lineage cells are NK cells or T cell with HLA-I and/or HLA-II deficiency.

[000373] In some embodiments, the number of derived hematopoietic lineage cells in the therapeutic composition is at least 0.1×10^5 cells, at least 1×10^5 cells, at least 5×10^5 cells, at least 1×10^6 cells, at least 5×10^6 cells, at least 1×10^7 cells, at least 5×10^7 cells, at least 1×10^8 cells, at least 5×10^8 cells, at least 1×10^9 cells, or at least 5×10^9 cells, per dose. In some embodiments, the number of derived hematopoietic lineage cells in the therapeutic composition is about 0.1×10^5 cells to about 1×10^6 cells, per dose; about 0.5×10^6 cells to about 1×10^7 cells, per dose; about 0.5×10^7 cells to about 1×10^8 cells, per dose; about 0.5×10^8 cells to about 1×10^9 cells, per dose; about 1×10^9 cells to about 5×10^9 cells, per dose; about 0.5×10^9 cells to about 8×10^9 cells, per dose; about 3×10^9 cells to about 3×10^{10} cells, per dose, or any range in-between. Generally, 1×10^8 cells/dose translates to 1.67×10^6 cells/kg for a 60 kg patient/subject.

[000374] In one embodiment, the number of derived hematopoietic lineage cells in the therapeutic composition is the number of immune cells in a partial or single cord of blood, or is at least 0.1×10^5 cells/kg of bodyweight, at least 0.5×10^5 cells/kg of bodyweight, at least 1×10^5 cells/kg of bodyweight, at least 5×10^5 cells/kg of bodyweight, at least 10×10^5 cells/kg of bodyweight, at least 0.75×10^6 cells/kg of bodyweight, at least 1.25×10^6 cells/kg of bodyweight, at least 1.5×10^6 cells/kg of bodyweight, at least 1.75×10^6 cells/kg of bodyweight, at least 2×10^6 cells/kg of bodyweight, at least 2.5×10^6 cells/kg of bodyweight, at least 3×10^6 cells/kg of bodyweight, at least 4×10^6 cells/kg of bodyweight, at least 5×10^6 cells/kg of bodyweight, at least 10×10^6 cells/kg of bodyweight, at least 15×10^6 cells/kg of bodyweight, at

least 20×10^6 cells/kg of bodyweight, at least 25×10^6 cells/kg of bodyweight, at least 30×10^6 cells/kg of bodyweight, 1×10^8 cells/kg of bodyweight, 5×10^8 cells/kg of bodyweight, or 1×10^9 cells/kg of bodyweight.

[000375] In one embodiment, a dose of derived hematopoietic lineage cells is delivered to a subject. In one illustrative embodiment, the effective amount of cells provided to a subject is at least 2×10^6 cells/kg, at least 3×10^6 cells/kg, at least 4×10^6 cells/kg, at least 5×10^6 cells/kg, at least 6×10^6 cells/kg, at least 7×10^6 cells/kg, at least 8×10^6 cells/kg, at least 9×10^6 cells/kg, or at least 10×10^6 cells/kg, or more cells/kg, including all intervening doses of cells.

[000376] In another illustrative embodiment, the effective amount of cells provided to a subject is about 2×10^6 cells/kg, about 3×10^6 cells/kg, about 4×10^6 cells/kg, about 5×10^6 cells/kg, about 6×10^6 cells/kg, about 7×10^6 cells/kg, about 8×10^6 cells/kg, about 9×10^6 cells/kg, or about 10×10^6 cells/kg, or more cells/kg, including all intervening doses of cells.

[000377] In another illustrative embodiment, the effective amount of cells provided to a subject is from about 2×10^6 cells/kg to about 10×10^6 cells/kg, about 3×10^6 cells/kg to about 10×10^6 cells/kg, about 4×10^6 cells/kg to about 10×10^6 cells/kg, about 5×10^6 cells/kg to about 10×10^6 cells/kg, 2×10^6 cells/kg to about 6×10^6 cells/kg, 2×10^6 cells/kg to about 7×10^6 cells/kg, 2×10^6 cells/kg to about 8×10^6 cells/kg, 3×10^6 cells/kg to about 6×10^6 cells/kg, 3×10^6 cells/kg to about 7×10^6 cells/kg, 3×10^6 cells/kg to about 8×10^6 cells/kg, 4×10^6 cells/kg to about 6×10^6 cells/kg, 4×10^6 cells/kg to about 7×10^6 cells/kg, 4×10^6 cells/kg to about 8×10^6 cells/kg, 5×10^6 cells/kg to about 6×10^6 cells/kg, 5×10^6 cells/kg to about 7×10^6 cells/kg, 5×10^6 cells/kg to about 8×10^6 cells/kg, or 6×10^6 cells/kg to about 8×10^6 cells/kg, including all intervening doses of cells.

[000378] In some embodiments, the therapeutic use of derived hematopoietic lineage cells is a single-dose treatment. In some embodiments, the therapeutic use of derived hematopoietic lineage cells is a multi-dose treatment. In some embodiments, the multi-dose treatment is one dose every day, every 3 days, every 7 days, every 10 days, every 15 days, every 20 days, every 25 days, every 30 days, every 35 days, every 40 days, every 45 days, or every 50 days, or any number of days in-between. In some embodiments, the multi-dose treatment comprises three, four, or five, once-weekly doses. In some embodiments of the multi-dose treatment comprising three, four, or five, once-weekly doses further comprise an observation period for determining whether additional single or multi doses are needed.

[000379] The compositions comprising a population of derived hematopoietic lineage cells of embodiments of the invention can be sterile, and can be suitable and ready for administration (i.e., can be administered without any further processing) to human patients/subjects. A cell-based composition that is ready for administration means that the composition does not require

any further processing or manipulation prior to transplant or administration to a subject. In other embodiments, the invention provides an isolated population of derived hematopoietic lineage cells that are expanded and/or modulated prior to administration with one or more agents including small chemical molecules. The compositions and methods for modulating immune cells including iPSC-derived effector cells are described in greater detail, for example, in International Pub. No. WO2017/127755, the relevant disclosure of which is incorporated herein by reference. For derived hematopoietic lineage cells that are genetically engineered to express recombinant TCR or CAR, the cells can be activated and expanded using methods as described, for example, in U.S. Patents 6,352,694.

[000380] In certain embodiments, the primary stimulatory signal and the co-stimulatory signal for the derived hematopoietic lineage cells can be provided by different protocols. For example, the agents providing each signal can be in solution or coupled to a surface. When coupled to a surface, the agents can be coupled to the same surface (i.e., in "cis" formation) or to separate surfaces (i.e., in "trans" formation). Alternatively, one agent can be coupled to a surface and the other agent in solution. In one embodiment, the agent providing the co-stimulatory signal can be bound to a cell surface and the agent providing the primary activation signal is in solution or coupled to a surface. In certain embodiments, both agents can be in solution. In another embodiment, the agents can be in soluble form, and then cross-linked to a surface, such as a cell expressing Fc receptors or an antibody or other binding agent which will bind to the agents such as disclosed in U.S. Pub. Nos. 2004/0101519 and 2006/0034810, the disclosures of which are incorporated by reference, for artificial antigen presenting cells (aAPCs) that are contemplated for use in activating and expanding T lymphocytes in embodiments of the present invention.

[000381] Some variation in dosage, frequency, and protocol will necessarily occur depending on the condition of the subject being treated. The person responsible for administration will, in any event, determine the appropriate dose, frequency and protocol for the individual subject.

EXAMPLES

[000382] The following examples are offered by way of illustration and not by way of limitation.

EXAMPLE 1 – Materials and Methods

[000383] To effectively select and test exogenous gene control and regulation systems under the control of various promoters in combination with different safe harbor loci integration strategies in the context of pluripotent stem cells, a hiPSC platform was used for single cell

passaging and high-throughput, 96-well plate-based flow cytometry sorting, to allow for the engineering and derivation of clonal hiPSCs with single or multiple genetic modulations.

[000384] *hiPSC Maintenance in Small Molecule Culture:* hiPSCs were routinely passaged as single cells once confluency of the culture reached 75%–90%. For single-cell dissociation, hiPSCs were washed with PBS (Mediatech) and treated with Accutase (Millipore). The single-cell suspension was then mixed with conventional medium, centrifuged, resuspended in FMM, and plated on Matrigel-coated surface. Passages were typically 1:6–1:8, and fed every 2–3 days with FMM. Cell cultures were maintained in a humidified incubator set at 37°C and 5–10% CO₂.

[000385] *Human iPSC engineering with ZFN, CRISPR for targeted editing of modalities of interest:* Using ROSA26 targeted insertion as an example, for ZFN mediated genome editing, 2 million iPSCs were transfected with a mixture of 2.5 µg ZFN-L, 2.5 µg ZFN-R and 5 µg donor construct, for AAVS1 targeted insertion. For CRISPR mediated genome editing, 2 million iPSCs were transfected with a mixture of 5 µg ROSA26-gRNA/Cas9 and 5 µg donor construct, for ROSA26 targeted insertion. Transfection was done using a Neon transfection system (Life Technologies). On day 2 or 3 after transfection, transfection efficiency was measured using flow cytometry if the plasmids contain artificial promoter-driven GFP and/or RFP expression cassette.

[000386] *Bulk sort and clonal sort of genome-edited iPSCs:* iPSCs with genomic targeted editing using ZFN or CRISPR-Cas9 were bulk sorted and clonal sorted of GFP⁺SSEA4⁺TRA181⁺ iPSCs. Single cell dissociated targeted iPSC pools were resuspended in staining buffer made fresh for optimal performance. Conjugated primary antibodies, including SSEA4-PE, TRA181-Alexa Fluor-647 (BD Biosciences), were added to the cell solution. The solution was washed in staining buffer, spun and resuspended in staining buffer containing 10 µM Thiazovivn for flow cytometry sorting. Upon completion of the sort, the 96-well plates were incubated. Colony formation was detected as early as day 2 and most colonies were expanded between days 7–10 post sort. In the first passage, wells were washed with PBS and dissociated with 30 µL Accutase. The dissociated colony is transferred to another well of a 96-well plate previously coated with Matrigel. Subsequent passages were done routinely. Each clonal cell line was analyzed for GFP fluorescence level and TRA1-81 expression level. Clonal lines with near 100% GFP⁺ and TRA1-81⁺ were selected for further screening and analysis including but not limited to off-target editing, and/or karyotype of the engineered iPSCs, before the clonal population is cryopreserved to serve as a master cell bank.

EXAMPLE 2 – T Cell Enhancer (TCE) Screening and Identification

[000387] Lentiviral transduction to D35 iT cells and *in vitro* IncuCyte-based restimulation assay were used to screen for gene edits that can function as a T cell potentiator (a.k.a. T cell

enhancer; TCE) with cytokine autonomy (FIG. 1). A TCE has the capability to promote T cell identity, function and performance of iPSC derived T cells (iT cell).

[000388] To evaluate the contribution of an exogenous gene to iT persistence, lentiviral-transduced iT cells (having an exemplary CAR specific to a tumor target GPRC5D for illustration purposes) were magnetically enriched with thy1.1 and proceeded with IncuCyte based restimulation assay *in vitro* at a 1:1 ratio of effector to H929-NLR, a target cell line endogenously expressing the tumor target of interest. The study was performed for 3 rounds with partial media change every 48 hr followed by cell transferring to a new plate with target cells. No cytokine support was given throughout the study. The readout was the target cell count by IncuCyte.

[000389] In this example, the H929-NLR cell count was normalized to time point 0 and the relative H929 count was plotted on the y axis across different time points for individual rounds. The lower the relative tumor count the better the tumor growth inhibition (TGI). As shown in FIG. 2, CD27 contributed to increased TGI for two rounds and the effect of TCF1 (the Transcription Factor 1 protein, encoded by the human gene *TCF7*) lasted further beyond the first two rounds to round 3. TCF1 and CD27 therefore were advanced for further analyses as potential TCEs for CAR-iT functionalities.

[000390] Candidate edits were then placed in different engineering configurations and in combination with an exemplary KLK2 CAR. Candidate edits tested included IL7RF, sIL2 (soluble IL2), TCF1, CD27, IL15RF, and IL2RaF. The engineering configurations included (a) TRAC knockout and insertion (KO/I) with CAR alone (1 of FIG. 3), (b) TRAC KO/I with a bicistron (2-4 of FIG. 3), (c) TRAC KO/I with a tricistron (6-9 of FIG. 3), or (d) TRAC KO/I with a bicistron in combination with a constitutively expressed TCE candidate at a different insertion site (10-11 of FIG. 3; and CD38 KO/I in this example). The primary CAR T cells comprising the same CAR but without TRAC knockout were used as control.

[000391] All editing constructs were introduced to iPSCs and followed by T cell differentiation. As shown in FIG. 3, the iT identity marked by CAR expression and intracellular CD3 expression in each differentiated cell group confirmed the proper T cell differentiation from engineered iPSCs having the edits as indicated (FIG. 3). The various groups of iT cells were then used as effector cells for subsequent functional tests, including *in vivo* studies.

EXAMPLE 3 – T Cell Enhancer (TCE) Function Analysis

[000392] Two million of antigen-expressing tumor cells PC3 were implanted subcutaneously to NSG mice on D0. Tumor volume measurement was performed to assess tumor cell engraftment in each group of mice that was randomly assigned to a treatment arm. Effector were

dosed at 1×10^7 for iT or 2×10^6 for primary T cells on D6. Tumor growth was monitored twice per week. No cytokine support was provided through the entire course.

[000393] During this *in vivo* study, tumor volume for each individual mice was plotted against days post tumor implant for each arm. As shown in FIGs. 4A-4B, with each graph categorized according to engineering designs, certain edits and configurations resulted in enhanced TGI as compared to the standard TRAC CAR-iT cell control group. As shown, the bi-cistronic configurations that co-express sIL2 or IL15RF with a CAR had better control of tumor growth than that co-expresses IL7RF. It is also observed that the TRAC regulated sIL2 expression enhances tumor cell growth control as compared to CAR-T cell without the sIL2 driven by the TRAC endogenous promoter. Notably, when in combination with constitutively expressed TCF7 or CD27, the bi-cistronic construct co-expressing IL7RF has improved tumor volume reduction when compared to without the constitutively expressed TCE (FIGS. 4A-4B). However, the TCEs in a form of tri-cistronic configuration did not improve tumor control of TRAC CAR co-expressing IL7RF (FIGS. 4A-4B).

[000394] The tumor mass, spleen, bone marrow of NSG mice of indicated groups from the above study were harvested for *ex vivo* function evaluation on D8 (3 days post effector dosing) or D15 (10 days post effector dosing). By measuring the total effector count in tumor mass, spleen and bone marrow on both D8 and D15, it was observed that the effector cells infiltrated into tumor mass as early as day 3 post effector cell treatment. Modest tumor antigen mediated effector cell proliferation was observed in tumor mass. Whereas in spleen and bone marrow, cell persistence independent of tumor antigen was observed with iT cells expressing sIL2 in comparison to effector cells having other indicated constructs but without sIL2 (FIGS. 5A-5B).

[000395] In FIGS. 6A-6B, for D8 and D15, the total effector cell count in tumor mass is plotted on the x axis to show the effector cell expansion, with the total tumor cell count plotted on y axis for each individual mouse of the indicated group to show the effectiveness of the tumor cell killing. It was observed that sIL2 iT cells not only have as good iT expansion as effector cells having, for example, engineering edits 3, 4 or 5, as indicated in FIGS. 6A-6B, the sIL2 iT cells further demonstrated better tumor clearance in comparison. It is also notable that iT cells having engineering edits 6 (TCF7 iT) led to the greatest tumor cell count reduction, thus a highly effective tumor cell killing, despite that these cells showed less cell expansion, suggesting better potency of these iT cells.

EXAMPLE 4 – PH12 as an alternative integration site for TCE insertion

[000396] PH12 is a newly discovered and confirmed genomic safe harbor (GSH) locus in a non-coding region on human chromosome 17, having a coordinate of chr17:44210001-44271500 in human reference genome assembly hg38. The sequence of the human reference genome hg38 is available from Genome Reference Consortium, with a reference number GRCh38/hg38, and also available in the Genome Browser provided by The UC Santa Cruz Genomics Institute.

[000397] A previously known GSH locus H11 was used as a control for confirmatory study of PH12 for engineering various cell types, including primary cells, iPSC obtained from reprogramming fibroblast (FiPSC) or T cells (TiPSC). Polynucleotides encoding CD19-CAR, BCMA-CAR, or EGFR were engineered into PH12 for the purpose of verifying the site's suitability as a genomic safe harbor locus. The insertion of various polynucleotides at PH12 in various cells using CRISPR showed a cutting efficiency more than 80%, with the knock-in efficiency (both bi-allelic and mono-allelic) ranging from about 40% to about 80%. Random integration appeared to be less than 50%, lower than expected, in PH12 site specific engineering.

[000398] The EGFR or CAR expression after insertion at PH12 is stable during prolonged culturing and at a level comparable to that of insertion at H11 locus. Both the mono-allelic and bi-allelic PH12-targeted iPSC clones maintained pluripotency and had iPSC marker expression patterns well preserved. The iPSCs with genomic editing at PH12 were also subjected to cryopreservation. The cell survival and pluripotency profile were evaluated after thaw and appeared to be normal. No genetic abnormality, including abnormal karyotype, was discovered in engineered iPSC clones. The engineered iPSCs were further differentiated to NK cells and expanded for phenotype and function evaluation. The differentiated cells maintained expected phenotype, function, and genome stability.

[000399] The suitability of PH12 as a genomic safe harbor locus was thereby confirmed for inserting exogenous polynucleotides encoding one or more proteins or peptides driven by constitutive or inducible promoter as desired. The exogenous polynucleotide can be any one of TGFbR redirector, CXCR2, CD16, CAR, TCE (CD27 or TCF1), or any other gene of interest as disclosed herein.

EXAMPLE 5 – iT cell Differentiation Compatible Configuration of Alloimmune Defense Receptor (ADR)

[000400] It is noted that 41BB-ADR was previously configured for constitutive and strong expression, for example driven by the synthetic promoter CAG engineered into the CD38 locus. It was later discovered that, in some cases, such a configuration is not optimally compatible with

iPSC-derived T cell differentiation processes. It was observed that CAG-driven expression of 41BB-ADR can negatively impact iT cell differentiation for having limited lymphoid commitment shown by low expression of CD7 and a suppressed T cell lineage commitment shown by few CD4⁺ single positive, CD8⁺ single positive and CD4⁺CD8⁺ double positive cells. In one example as shown in FIG. 7, T cell derived iPSCs engineered to express either constitutive ADR engineered into CD38 loci (+ADR) or control engineered cells that lacked ADR (No ADR) were differentiated into CAR-T cells. Lymphoid and T cell commitment was evaluated by flow cytometry, assessing CD45, CD7, CD8 α , and CD4 expression. Unlike control (No ADR) CAR-iT cells that demonstrated complete lymphoid commitment (98.9% CD45⁺CD7⁺) and appropriate populations of DN (CD8 α -CD4⁻), DP (CD8 α +CD4⁺), and SP (CD8 α +CD4⁻ or CD8 α -CD4⁺), CAR-iT cells with constitutively expressed ADR demonstrated aberrant lymphoid commitment with 63.7% of cells lacking CD7 expression and 88.4% lacking either CD4 and/or CD8 α expression.

[000401] To investigate whether temporal and dose regulation of 41BB-ADR expression could promote its compatibility with iT cell differentiation, 41BB-ADR was inserted at TRAC locus to be driven by TRAC endogenous promoter.

[000402] The TRAC targeted 41BB-ADR (TRAC_41BB-ADR) iPSCs are differentiated alongside control TRAC_CAR iPSC and CAG_41BB-ADR iPSC towards CD34-expressing hemogenic endothelium (iHE) followed by differentiation towards hematopoietic cells, lymphoid cells and subsequently T cells using the compositions and methods described herein. The temporal control of 41BB-ADR expression is demonstrated by similar kinetics of ADR expression to the kinetics of CAR expression observed in the control TRAC_CAR-derived iT cells (FIG.8). The improved compatibility of TRAC inserted 41BB-ADR iPSC with iT cell differentiation is demonstrated by the similar expression level of 41BB-ADR to the CAR expression in the control TRAC_CAR iPSC-derived iT cells, which iT cells of both groups have the hematopoietic marker CD45, the lymphoid marker CD7 and the T cell lineage marker intracellular CD3. As shown in FIG. 9, flow cytometry was performed confirming (top row) appropriate lymphoid commitment (99% CD45⁺CD7⁺) for both TRAC control (left) and TRAC ADR (right) engineered CAR iT cells. Flow cytometry also confirmed (i) equivalent CAR expression (97.5% CAR⁺, control vs 96.5% CAR⁺, TRAC ADR) in both engineered CAR iT cells and (ii) specific ADR expression by TRAC ADR engineered cells (26.6% 4-1BBL⁺ control vs 94.6% 4-1BBL⁺ TRAC ADR).

[000403] The functional integrity of the TRAC_41BB-ADR-derived iT cells is assessed in a co-culture assay with primed alloreactive immune cells, including T cells. The co-cultures are monitored for (1) a reduction in ADR-negative iT cell counts compared to the ADR-positive iT

cell counts, and (2) the expansion of alloreactive T cells in co-cultures with ADR-negative iT cells compared to a decrease in alloreactive T cell counts in co-cultures with ADR-positive iT cells. These observations demonstrate the protection of 41BB-ADR-expressing iT cells from alloreactive T cell-mediated cytotoxicity, and thus confirm that the temporal and dose controlled 41BB-ADR expression is essential for iT differentiation using engineered iPSC comprising a 41BB-ADR. In this case, an endogenous promoter enables later T cell developmental stage expression of 41BB-ADR at a lower expression level and is beneficial for retaining 41BB-ADR functionality without impeding lymphoid and T cell lineage commitment during the iPSC-derived T cell differentiation process.

[000404] ADR⁺ or ADR⁻ iPSC-derived CAR-T cells (CAR iT cells) were administered to NSG mice previously administered primed allogeneic T cells (pAllo T, or pAT) in the presence or absence of subcutaneous SKOV3 tumors. 14 days after effector administration, bone marrows were removed and CAR iT cells and pAllo T were enumerated via flow cytometry. As shown in FIG. 10A, approximately 5× ($p < 0.01$) more ADR⁺ iT cells compared to ADR⁻ iT cells were observed in the bone marrows of effector and pAllo T co-administered mice. Similarly, ~2.5× ($p < 0.01$) more pAllo T were observed in the bone marrows of mice co-administered ADR⁻ iT cells, compared to pAllo T alone and pAllo T + ADR(+) iT cells. Tumor burden was evaluated in SKOV3 bearing NSG mice administered pAlloT and/or ADR⁺ iT cells, and FIG. 10B demonstrates that the anti-tumor activity of ADR⁺ iT cells was unaffected in the presence of pAllo T cells.

[000405] CD58 was intermediately knocked out during the differentiation of ADR⁺ and ADR⁻ CAR iT cells and the allogeneic resistance of differentiated CAR iT cells was evaluated in co-culture with primed allogeneic T cells. FIG. 11A shows CD58 expression of fully differentiated ADR⁺ and ADR⁻ CAR iT cells following intermediate knockout, generating 86.2% CD58KO ADR⁻ and 55.7% CD58KO ADR⁺ iT cells. After 72 hours of co-culture with primed allo T cells from two independent donors, total remaining CAR iT (FIG. 11B left) and primed allo T cells (FIG. 11B right) were evaluated and normalized to their respective CAR iT alone or primed allo T alone control conditions. As shown in FIG. 11B left panel, only 50% of CD58⁺ ADR⁻ CAR iT cells (first bar from left) remained after primed allo T co-culture, whereas the deletion of either CD58 (second bar from left) or the expression of ADR (third bar from left) alone led to the preservation of about 75% of CAR iT cells. The combination of both CD58 knockout and ADR expression (fourth bar from left) led to the greatest resistance of CAR iT cells to primed allogeneic T cells with co-cultured wells indistinguishable from CAR iT alone wells (~100%). Primed allo-T cell numbers were also quantified from two independent donors following CAR iT cell co-culture and normalized to primed allo T cell alone. As shown in FIG.

11B right panel, the combination of CD58 knockout and ADR (fourth bar from left) led to the least recovery (~67% of primed allo T alone) of primed allo T, further underscoring the synergy of CD58 knockout and ADR to limit immune synapse formation and/or selectively target activated allo T cells, and thus synergistically resist allogeneic rejection.

EXAMPLE 6 – TRAC Regulated Cytokine Expression in Cell Configuration for Enhanced Property

[000406] Soluble IL2 expression at the TRAC locus was demonstrated to have improved CAR-iT cell tumor growth inhibition (see for example FIG. 4A). Without being limited by theory, a proper temporal and dose regulation of exogenous cytokine signaling may have contributed to iPSC-derived T cell development and to generating iT cells that are less exhausted, without exerting negative impact to the differentiation process before the lymphoid lineage and T cell lineage commitment. Using a bi-cistronic vector sIL2 and 41BB-ADR were inserted at TRAC locus, and the co-expression of these two transgenes are driven by the endogenous TRAC promoter. For comparison, iPSCs comprising TRAC targeted 41BB-ADR and IL7RF co-expression were also made, differentiated, and analyzed alongside with TRAC_ADR-sIL2. As shown in FIG. 12, iPSC derived CAR-T cells engineered to express either IL7RF or soluble IL2 (sIL2) were evaluated in two separate prostate and ovarian cancer xenograft models. Expression of sIL2 controlled by TRAC led to the greatest anti-tumor activity in both *in vivo* models and similarly led to an increase in intratumoral CAR iT cell persistence, compared to IL7RF expressing CAR iT cells. It was shown that CAR-iT cells secreting IL-2 show enhanced CAR and ADCC activity *in vivo*, and that ADR in combination with sIL2 leads to improved performance in a tri-culture assay with tumor, primed allo T cells, and iT cells.

[000407] IL-2 production by iPSC derived CAR-T cells (CAR iT) engineered to express soluble IL2 from the TRAC locus were evaluated under steady state and under CAR activation conditions and compared to CAR iT cells that lacked sIL2 or primary, healthy donor derived CAR-T cells. Under steady state conditions (e.g. without activation, FIG. 13A), limited but enhanced IL2 production (1.5-2.0 pg/ml) was observed by TRAC sIL2 engineered CAR iT cells compared to primary CAR-T cells and control CAR iT cells after 48 and 72 hours of culture. Following CAR activation on tumor targets at the indicated effector:target [E:T] ratios, sIL2 production was quantified at 72 hours. More than 100pg/ml of sIL2 was observed following activation of primary CAR-T cells at each indicated setting, while sIL2 production by TRAC sIL2 engineered CAR iT cells was less than 8.5 pg/ml across activation conditions (FIG. 13B). Therefore it appears that IL2 production is regulated temporally by TRAC following CAR activation to promote CAR iT efficacy at a low expression level.

[000408] It was also observed that the TRAC regulation of IL2 is unique in enhancing the persistence and sustained *in vivo* efficacy of CAR iT cells, in that other selected insertion sites (“T cell loci” or TCL) produced different results. For example, FIG. 14A shows the differential IL2 mRNA expression after insertion at TRAC, TCL1, and TCL2 loci respectively, and during CAR iT cell differentiation and CAR activation. The *in vivo* performance of CAR iT cells engineered to express IL-2 from TRAC (FIG. 14B), TCL1 (FIG. 14C), and TCL2 (FIG. 14D) was determined in a subcutaneous xenograft model. As shown, CAR iT cells engineered to produce a lower level of IL2 under TRAC regulation demonstrate sustained anti-tumor activity compared to similar IL-2 engineering into TCL1 or TCL2. Further, engineering of IL2 into alternative “T cell loci” that produce higher level of IL2 after CAR activation leads to poorer anti-tumor activity of the effector cells *in vivo*. Without being bound by theory, it seems that when IL2 expression level is too low it leads to poor effector cell persistence, however if the IL2 expression level is too high, it leads to effector cell exhaustion. TRAC regulated IL2 expression is at a level uniquely beneficial to both persistence and efficacy of CAR iT cells.

[000409] It was further demonstrated that TRAC regulated IL2 supports hnCD16 and ADR mediated efficacy. iPSC derived T (iT) cells were engineered to constitutively express TGFβ-SRR, hnCD16, and CXCR2 with either TRAC regulated ADR and IL7RF (“ADR 7”) or TRAC regulated ADR and soluble IL-2 (“ADR 2”) (FIG. 15A). The anti-tumor activity mediated by hnCD16 in either scenario was evaluated in an *in vivo* ovarian xenograft model (FIG. 15B) and in an *in vitro* co-culture with tumor targets and primed allogeneic T cells (pAT) (FIG. 15C).

[000410] In FIG. 15B, NSG mice previously challenged subcutaneously with SKOV3 target cells were administered Herceptin alone, Herceptin and ADR 2 iT cells, or Herceptin and ADR 7 iT cells, and tumor burden was evaluated up to 50 days post tumor challenge. Although both ADR 2 and ADR 7 iT cells demonstrated specific *in vivo* antibody dependent cytotoxicity when combined with Herceptin, ADR 2 (0 mm³ Avg tumor burden; 5 of 5 tumor clear) outperformed ADR 7 (64.8 mm³ Avg tumor burden; 0 of 5 tumor clear) iT cells.

[000411] An extended *in vitro* triculture assay was performed with ADR 7 and ADR 2 iT cells co-cultured with SKOV3 tumor cells and pAT in the presence of Herceptin; and the tumor burden was evaluated where indicated via luminescence assay. As shown in FIG. 15C, unlike ADR 7 iT cells which lost anti-tumor control after three days of tri-culture, reaching $\sim 7 \times 10^4$ RLU (relative luminescence unit) on Day 8, ADR 2 iT cells demonstrated sustained anti-tumor activity in the presence of primed allogeneic T cells out to Day 8 of tri-culture (3×10^2 RLU). Therefore the ADR 2 iT having TRAC regulated ADR and IL2 demonstrated enhanced ADCC activity and sustained anti-tumor efficacy and resistance to allo-rejection.

EXAMPLE 7 – TRAC-Driven vs. Constitutive CAR in Cell Configuration for Enhanced Property

[000412] T cell derived iPSCs, engineered to express a constitutive CAR engineered into CD58 along with ADR and soluble IL2 in the TRAC locus and constitutive TGF β -SRR, hnCD16, and CXCR2 from CD38, were differentiated into constitutive CAR iT cells (ADR/IL2 + Const. CAR). The CAR expression by the constitutive CAR iT cell population was evaluated prior to tumor challenge and at the end of each round of tumor challenge. Geometric mean fluorescent intensities are shown as a heat map indicative of CAR expression level by each effector cell population (FIG. 16A). As expected, the constitutive CAR expression level is much higher than the TRAC controlled CAR expression. Previously, a TRAC controlled CAR was preferred with the belief that lower CAR expression level would decrease the level of cell exhaustion, and that the temporal controlled CAR expression also steers progenitor cells to commit to T cell lineage during the iPSC differentiation. However, when the CAR mediated anti-tumor activity of the constitutive CAR iT cells (line “3”) was evaluated in a repeat tumor challenge model at a 4:1 effector to target ratio with E:T resets at each round and compared to primary CAR-T cells (line “1”) or CAR iT cells with TRAC engineered CAR and soluble IL2 (TRAC: CAR/IL2, line “2”), with normalized tumor counts shown in FIG. 16B, it was shown that the constitutive CAR expression by CAR iT cells with TRAC regulated ADR and soluble IL2 enabled sustained anti-tumor activity over three separate rounds of tumor challenge, similar to healthy donor-derived primary CAR-T cells and CAR iT cells expressing TRAC regulated CAR and soluble IL2, surprisingly without the cell exhaustion effect to the constitutive CAR iT cells despite their consistent high CAR expression level.

[000413] Next, the *in vivo* anti-tumor activity of CAR iT cells engineered to express (i) TRAC regulated CAR and soluble IL2 (TRAC CAR/IL2), or (ii) constitutive CAR along with TRAC regulated ADR and soluble IL2 (Const. CAR + ADR/IL2) in a prostate xenograft model was evaluated. The NSG mice previously challenged subcutaneously with PC3 tumor cells were administered (A) 1×10^7 or (B) 5×10^6 TRAC CAR/IL2 or (C) 5×10^6 Constitutive CAR + ADR/IL2, with the tumor burden measured out to 45 days post tumor challenge. As shown in FIG. 17, at stress effector dosing (5×10^6) Constitutive CAR + TRAC_ADR/IL2 (Avg tumor burden = 408 mm^3) significantly outperformed TRAC_CAR/IL2 (Avg tumor burden = 1033 mm^3) engineered iT cells ($p < 0.01$) in controlling solid tumor burden in the subcutaneous *in vivo* model.

EXAMPLE 8 – Constitutive Expression of TCF1 Reinforces T cell Lineage Commitment

[000414] CAR iPSCs comprising a TRAC regulated cytokine were CRISPR engineered to insert a constitutive promoter driven TCF1 cassette in a selected genomic locus. In this illustrative example, the insertion is at CD38. After iPSC differentiation, flow cytometric staining for TCF1 in control CAR-iT cells (trace “1”) compared to over-expression of TCF1 (trace “2”) is shown in FIG. 18A. Further, the progenitor iT cells (around D24 iPSC differentiation) were profiled by flow cytometry for expression of the GATA3 and Bcl11b, two representative transcription factors that are regulated by TCF1 and are responsible for preventing differentiation toward B, myeloid, and innate cell lineages. As shown in FIG. 18B, over-expression of TCF1 results in a significantly increased proportion of GATA3+ Bcl11b+ cells (43% vs 13%). Next, the end-of-process iT cells (around D42 differentiation) were profiled by flow cytometry for expression of the T cell markers CD7 and CD5. While both the control and TCF1-overexpressing iT cells were >99% CD7 positive, overexpression of TCF1 increased the proportion of iT cells that were double positive for CD7 and CD5 (59% vs 13%), demonstrating the reinforced T cell lineage commitment with TCF1 overexpression in the CAR iT cells.

[000415] FIG. 19A is a schematic showing an ADR_2 backbone (TRAC_ADR-IL2 and CD38KO_TGFβR2-hnCD16-CXCR2) engineered to also express a CAG-promoter driven bicistronic cassette (CAR-2a-TCF1) that was engineered into the CD58 locus (CD58KO_CAR-TCF1). FIG. 19B represents BLI data from mice that were given 1e5 Nalm6 tumor on day 0 and treated with the indicated effector cells on day 1. It is shown that CAR-iT cells expressing TCF1 had complete tumor growth inhibition on day 21, whereas CAR-iT cells that did not overexpress TCF1 were starting to show tumor rebound past day 15.

[000416] FIG. 20A shows the flow cytometric measurement of the indicated iT cells co-cultured with primed allo T cells. It was shown that over-expression of TCF1 leads to enhanced persistence of the ADR_2 iT cells when co-cultured with primed allo T cells (pAT) that target the iT cells. In a tri-culture setting where iT cells need to eliminate tumor while also protecting themselves from primed allo T cell attack, the TCF1 positive ADR_2 iT cells showed increased persistence over the TCF1 negative ADR_2 iT cells, and the flow cytometric measurement of the indicated iT cells are shown in FIG. 20B. The increased persistence of the TCF1 positive ADR_2 iT cells also showed improved control of tumor growth in a tri-culture setting, as shown by the luciferase-based readout of tumor cells in the tri-culture assay over time (FIG. 20C).

EXAMPLE 9 – Pre-Master Cell Bank iPSC Construction for Deriving Effector Cells with Enhanced Property And Function

[000417] The general design of the master cell bank (MCB) for deriving effector cells through iPSC differentiation includes generation of two intermediary engineered iPS cell lines (pre-MCBs) having multiplexed genome editing at various genome engineering sites for gene knock-ins and knockouts. A series of bi-cistronic and tri-cistronic constructs were designed and prepared for engineering cells at multiple loci for endogenous gene knockout and at the same time to insert (KO/KI) multiple functional modalities in desired combinations and desired placement in order to explore a functional backbone arrangement that better equips effector cells for solid tumor immuno-therapies.

[000418] A first pre-MCB (pre-MCB1) comprises knock-in of one or more of TGF β R redirector (TGF β -SRR), CXCR2 and hnCD16 at a preselected site (site 1). Suitable genome engineering sites for the knock-in include, but are not limited to, a TCR locus (e.g., TRAC or TRBC), CD38, CD54, CD56, CD58, TIM3, TIGIT, H11 and PH12. In this example, CD38 was selected as site 1 for pre-MCB1 generation. While inserting at CD38 two or more of CD16, CXCR2 and a TGF β -SRR using a bi- or tri- cistronic construct to address challenges of the solid tumor environment, the endogenous CD38 gene is knocked out as a result such that an anti-CD38 monoclonal antibody or a CD38-CAR may be applied to selectively deplete CD38-expressing activated host immune cells. The TGF β R redirector, CXCR2 and/or hnCD16 are driven by an exogenous promoter for constitutive expression. The TGF β R2 redirector is a fusion protein of TGF β R2 with one of IL12R β , IL18R, IL21R, or fragments thereof, referred to as TGF β R2-IL12R β , TGF β R2-IL18R, TGF β R2-IL21R specifically or as TGF β -SRR in general. For illustration, the TGF β -SRR comprises IL18R. The pre-MCB1 iPSCs were differentiated into CAR iT or CAR iNK cells to verify the pluripotency and was used for further genomic editing to generate pre-MCB2.

[000419] A second pre-MCB (pre-MCB2) comprises additional edits introduced into pre-MCB1 cells at TRAC locus. PreMCB2 comprises an insertion of IL7RF or IL2 at TRAC, optionally, co-expressed with 41BB-ADR. The TRAC gene is knocked out by the insertion, and the expression of the inserted gene(s) is subjected to control of TRAC endogenous promoter. The pre-MCB2 iPSCs having both CD38 and TRAC sites editing were used for further genomic editing to generate MCB. It is noted here that an antigen-specific CAR was not included at the TRAC locus as compared to the TRAC_CAR configuration, which was shown to improve CAR-T cell efficacy and delay CAR-T cell exhaustion upon activation by tuning down the CAR expression level through TRAC endogenous promoter (Eyquem et al. Nature. 2017 Mar 2; 543(7643): 113–117).

[000420] The pre-MCB2 clone was engineered at a preselected site (site 2) to knock in a first CAR (CAR1) and TCE, optionally with an additional CAR (CAR2) to generate MCB for effector cell differentiation. (1) A vector containing a CAR1 only, (2) a bi-cistronic vector containing a CAR1 and a cytokine IL18, (3) a bi-cistronic vector containing a CAR1 and a TCE, (4) a bi-cistronic vector containing a CAR1 and a CAR2, or (5) a tri-cistronic vector containing a CAR1, a CAR2, and a TCE were used to determine whether any of the designed configuration supports effector cell fitness and/or persistency among other advantages, especially with one or more CARs being expressed. All transgenes at site 2 are driven by an exogenous promoter. Site 2 is a preselected genome engineering site that is different from site 1. In this example, with site 1 being CD38, sites CD58, TIM3 and PH12 are each being used as site 2 in parallel experimentations. In the case of using CD38, CD58 or TIM3 locus, the endogenous genes are knocked out by targeted insertion. CAR1 and CAR2 have different targeting specificity and are driven by CAG promoter as an example. For illustration purposes, BCMA-CAR and CD38-CAR were used where two CARs are in the design. TCF1 is the selected TCE for illustration purposes. It was observed that the combination of IL7RF and TCF1 enhanced antitumor activity of CAR-iT cells in a subcutaneous xenograft tumor model.

[000421] FIG. 21 illustrates the engineering designs and process to generate MCB for effector cell differentiation through pre-MCB1 and pre-MCB2. FIGS. 22A-22C show the flow cytometry confirmation of CAR1 expression after CD58 targeted editing of pre-MCB2.

[000422] Following differentiation, tri-site edited MCB iPSCs were evaluated for their lymphoid commitment by assessing their CD45 and CD7 expression via flow cytometry on individual clones from each cell group having indicated backbone configurations. All evaluated clones co-expressed CD45 and CD7, demonstrating (i) successful differentiation into immune effector cells and (ii) compatibility of the iPSC engineering strategy with regard to the individual edits of the solid tumor targeting backbone with the effector cell differentiation capacity of the iPSC having the modifications. Differentiated CAR iT cell groups were further evaluated for CAR1, CAR2, ADR, hnCD16, TGF β -SRR, TCF1 and cytokine transgene expression via flow cytometry, and *in vitro* and *in vivo* effector functions.

[000423] According to previous design, the ADR and sIL2 are constitutively expressed at a high level whilst with CAR expression controlled by TRAC endogenous promoter. The tumor targeting backbone of this application, however, was consciously designed to have ADR and sIL2 driven by TRAC endogenous promoter, while constitutively expressing a TCE, optionally co-expressed with a CAR, at a non-TRAC locus. This arrangement of the elements is unconventional and was with unknown advantages until the disclosure herein. By coupling cytokine secretion with temporal promoter activity, we can ensure that the differentiation of iT

cells is not impacted. Other candidate promoters include those which are mostly inactive or exhibit low activity during the entire T cell differentiation process but the activity is upregulated upon T cell activation, for example, via engagement of the CAR and cognate antigen. In addition to TRAC endogenous promoter, suitable temporally regulated endogenous promoters include, but are not limited to Tim-3, TIGIT, and ASB2.

[000424] Autonomous cytokine signaling delivered by sIL2, IL7RF and/or IL18 improves *in vivo* persistence and expansion of the engineered iT cell independent of cytokine support. The ability to expand independently of cytokine support is also useful for *in vitro* cell selection during stepwise cell engineering because the cytokine autonomy allows modified cells to expand while unmodified cells remain inactive. Such outgrowth of modified cells during culture alleviates the need for sorting, which increases the screening throughput and scale for a large number of cell positive for intended edits.

[000425] After the master cell bank (MCB) of engineered iPSCs, for example, comprising CD38_TGFβ-SRR-hnCD16-CXCR2, TRAC_ADR-sIL2, CD58_CAR-TCF1, and knock-out of CD38, TRAC and CD58 is established, validated, and cryopreserved, the MCB can be used repeatedly as a resource for effector cells.

[000426] One skilled in the art would readily appreciate that the methods, compositions, and products described herein are representative of exemplary embodiments, and not intended as limitations on the scope of the invention. It will be readily apparent to one skilled in the art that varying substitutions and modifications may be made to the present disclosure disclosed herein without departing from the scope and spirit of the invention.

[000427] All patents and publications mentioned in the specification are indicative of the levels of those skilled in the art to which the present disclosure pertains. All patents and publications are herein incorporated by reference to the same extent as if each individual publication was specifically and individually indicated as incorporated by reference.

[000428] The present disclosure illustratively described herein suitably may be practiced in the absence of any element or elements, limitation or limitations that are not specifically disclosed herein. Thus, for example, in each instance herein any of the terms “comprising,” “consisting essentially of,” and “consisting of” may be replaced with either of the other two terms. The terms and expressions which have been employed are used as terms of description and not of limitation, and there is no intention that in the use of such terms and expressions of excluding any equivalents of the features shown and described or portions thereof, but it is recognized that various modifications are possible within the scope of the present disclosure claimed. Thus, it should be understood that although the present disclosure has been specifically

disclosed by preferred embodiments and optional features, modification and variation of the concepts herein disclosed may be resorted to by those skilled in the art, and that such modifications and variations are considered to be within the scope of this invention as defined by the appended claims.

CLAIMS

What is claimed is:

1. A cell or a population thereof, wherein
 - (i) the cell is (a) an immune cell; (b) an induced pluripotent cell (iPSC); or (c) a derivative effector cell obtained from differentiating the iPSC; and
 - (ii) the cell comprises a construct comprising at least one of:
 - (a) a polynucleotide encoding an allo-immune defense receptor (ADR); and
 - (b) a polynucleotide encoding IL2, IL18, or a IL7RF;
 - (iii) the construct is inserted into a T cell receptor (TCR) locus, thereby knocking out the TCR; and
 - (iv) the construct is expressed under control of an endogenous TCR promoter.
2. The cell or population thereof of claim 1, further comprising a construct at a first integration site (site 1), wherein:
 - (i) the site 1 construct comprises two or more of:
 - (a) a polynucleotide encoding a TGF β signaling redirector receptor (TGF β -SRR) comprising a partial or full peptide of the extracellular domain (ECD) of transforming growth factor beta receptor (TGF β R);
 - (b) a polynucleotide encoding a C-X-C motif chemokine receptor or a variant thereof; and
 - (c) a polynucleotide encoding an exogenous CD16 or a variant thereof; and
 - (ii) the site 1 construct comprises an exogenous promoter that regulates expression of the polynucleotides in the site 1 construct.
3. The cell or population thereof of claim 2, further comprising a construct at a second integration site (site 2), wherein:
 - (i) the site 2 construct comprises a polynucleotide encoding a first chimeric antigen receptor (CAR1);
 - (ii) the site 2 construct optionally further comprises one or more of:
 - (a) a polynucleotide encoding a second chimeric antigen receptor (CAR2) that is different from CAR1 in antigen specificity;
 - (b) a polynucleotide encoding a T cell enhancer (TCE); and
 - (c) a polynucleotide encoding a cytokine;

- (iii) the site 2 differs from the site 1; and
- (iv) the site 2 construct comprises an exogenous promoter that regulates expression of the polynucleotides in the site 2 construct.

4. The cell or population thereof of claim 2, wherein the site 1 comprises one of AAVS1, CCR5, ROSA26, collagen, HTRP, H11, PH12, GAPDH, TCR constant region, RUNX1, B2M, TAP1, TAP2, tapasin, NLRC5, CIITA, RFXANK, RFX5, RFXAP, TCR α or β constant region (TRAC or TRBC), NKG2A, NKG2D, CD38, CD25, CD69, CD71, CD44, CD54, CD56, CD58, CIS, CBL-B, SOCS2, PD1, CTLA4, LAG3, TIM3, ASB2, or TIGIT.

5. The cell or population thereof of claim 4, wherein (i) the site 1 comprises one of CD38, CD54, CD56, CD58, TIM3, ASB2, TIGIT, H11 or PH12; and (ii) integration of the site 1 construct at any of CD38, CD54, CD56, CD58, TIM3, ASB2, or TIGIT knocks out an endogenous gene at the site 1.

6. The cell or population thereof of claim 3, wherein the site 2 comprises one of AAVS1, CCR5, ROSA26, collagen, HTRP, H11, PH12, GAPDH, TCR constant region, RUNX1, B2M, TAP1, TAP2, tapasin, NLRC5, CIITA, RFXANK, RFX5, RFXAP, TCR α or β constant region (TRAC or TRBC), NKG2A, NKG2D, CD38, CD25, CD69, CD71, CD44, CD54, CD56, CD58, CIS, CBL-B, SOCS2, PD1, CTLA4, LAG3, TIM3, ASB2, or TIGIT.

7. The cell or population thereof of claim 6, wherein (i) the site 2 comprises one of CD38, CD54, CD56, CD58, TIM3, ASB2, TIGIT, H11 or PH12; and (ii) integration of the site 2 construct at any of CD38, CD54, CD56, CD58, TIM3, ASB2, or TIGIT knocks out an endogenous gene at the site 2.

8. The cell or population thereof of claim 1, wherein the TCR locus is a constant region of TCR alpha (TRAC) or TCR beta (TRBC), wherein the gene at the TCR locus is TCR α or TCR β , and wherein the endogenous TCR promoter is a TRAC promoter or a TRBC promoter.

9. The cell or population thereof of claim 1, wherein the ADR is specific to 41BB.

10. The cell or population thereof of claim 1, wherein the ADR comprises a 41BB-specific ligand operably linked to a signaling domain promoting effector cell activation, and wherein

ADR comprises an amino acid sequence of at least 75%, 80%, 85%, 90%, 95% or 99% identity to SEQ ID NOs: 66 - 69.

11. The cell or population thereof of claim 1, wherein the ADR comprises a signaling domain, and wherein the signaling domain comprises an amino acid sequence of at least 75%, 80%, 85%, 90%, 95% or 99% identity to SEQ ID NO: 60.

12. The cell or population thereof of claim 2, wherein

(i) the TGF β -SRR further comprises a partial or full peptide of the intracellular domain (ICD) of a cytokine receptor comprising an IL2R, IL12R, IL18R, IL21R, or any combination thereof;

(ii) the C-X-C motif chemokine receptor comprises CXCR2 or CXCR3;

(iii) the exogenous CD16 or variant thereof comprises at least one of:

(a) a high affinity non-cleavable CD16 (hnCD16);

(b) F176V and S197P in ectodomain domain of CD16;

(c) a full or partial ectodomain originated from CD64;

(d) a non-native (or non-CD16) transmembrane domain;

(e) a non-native (or non-CD16) intracellular domain;

(f) a non-native (or non-CD16) signaling domain;

(g) a non-native stimulatory domain; and

(h) transmembrane, signaling, and stimulatory domains that are not originated from CD16, and are originated from a same or different polypeptide;

or

(iv) the integration site PH12 is a safe harbor locus having a coordinate of chr17:44210001-44271500 in human reference genome assembly hg38.

13. The cell or population thereof of claim 12, wherein

(i) the cytokine receptor is IL2R β , thereby forming a TGF β R2-IL2R β redirector receptor, and the intracellular domain (ICD) of IL2R β comprises an amino acid sequence represented by SEQ ID NO: 4; or

(ii) the cytokine receptor is IL12R β , thereby forming a TGF β R2-IL12R β redirector receptor, and the intracellular domain (ICD) of IL12R β comprises an amino acid sequence represented by SEQ ID NO: 5 or SEQ ID NO: 6; or

(iii) the cytokine receptor is IL18R β , thereby forming a TGF β R2-IL18R β redirector receptor, and the intracellular domain (ICD) of IL18R β comprises an amino acid sequence represented by SEQ ID NO: 7; or

(iv) the cytokine receptor is IL21R β , thereby forming a TGF β R2-IL21R β redirector receptor, and the intracellular domain (ICD) of IL21R β comprises an amino acid sequence represented by SEQ ID NO: 8; or

(v) the extracellular domain (ECD) of TGF β R comprises an amino acid sequence represented by SEQ ID NO: 3.

14. The cell or population thereof of claim 13, wherein the cytokine receptor is a fragment of IL2R β , forming a TGF β R2-trIL12R β redirector receptor which comprises an amino acid sequence having sequence identity of at least 80%, 85%, 90%, 95%, or 97%, 98%, or 99% to SEQ ID NO: 9, wherein an amino acid sequence represented by SEQ ID NO: 10 comprised in SEQ ID NO: 9 is variable.

15. The cell or population thereof of claim 3, wherein the first CAR and the second CAR are specific to different antigens, and wherein:

(i) the antigen comprises ADGRE2, B7H3, carbonic anhydrase IX (CAIX), CCR1, CCR4, carcinoembryonic antigen (CEA), CD3, CD5, CD7, CD8, CD10, CD20, CD22, CD30, CD33, CD34, CD38, CD41, CD44, CD44V6, CD49f, CD56, CD70, CD74, CD79b, CD99, CD123, CD133, CD138, CDS, CLEC12A, an antigen of a cytomegalovirus (CMV) infected cell, epithelial glycoprotein-2 (EGP-2), epithelial glycoprotein-40 (EGP-40), epithelial cell adhesion molecule (EpCAM), EGFRvIII, receptor tyrosine-protein kinases erb- B2,3,4, EGFR, EGFR-VIII, ERBB folate-binding protein (FBP), fetal acetylcholine receptor (AChR), folate receptor- α , Ganglioside G2 (GD2), Ganglioside G3 (GD3), GPRC5D, human Epidermal Growth Factor Receptor 2 (HER2), human telomerase reverse transcriptase (hTERT), ICAM-1, Integrin B7, Interleukin-13 receptor subunit alpha-2 (IL-13R α 2), κ -light chain, kinase insert domain receptor (KDR), KLK2, Lewis A (CA19.9), Lewis Y (LeY), L1 cell adhesion molecule (L1-CAM), LILRB2, melanoma antigen family A 1 (MAGE-A1), MICA/B, Mucin 1 (Muc-1), Mucin 16 (Muc-16), Mesothelin (MSLN), NKCSI, NKG2D ligands, c-Met, cancer-testis antigen NY-ESO-1, oncofetal antigen (h5T4), PRAME, prostate stem cell antigen (PSCA), PRAME prostate-specific membrane antigen (PSMA), tumor-associated glycoprotein 72 (TAG-72), TIM-3, TRBC1, TRBC2, vascular endothelial growth factor R2 (VEGF-R2), Wilms tumor protein (WT-1), and a pathogen antigen; or

(ii) the antigen comprises B7H3, BCMA, CD19, CD20, CD22, CD38, CD52, CD79b, CD123, EGFR, EGP2/EpCAM, GD2, GPRC5D, HER2, KLK2, MICA/B, MSLN, VEGF-R2, PSMA and PDL1.

16. The cell or population thereof of claim 3 or 15, wherein the first CAR and the second CAR each comprises:

(i) an ectodomain comprising an antigen binding domain specific to a tumor associated antigen;

(ii) a transmembrane domain; and

(iii) an endodomain comprising at least one signaling domain;

wherein the at least one signaling domain responds specifically to binding of the CAR to the tumor associated antigen, thereby generating a cancer antigen specific response.

17. The cell or population thereof of claim 16, wherein the at least one signaling domain comprises:

(i) any one of: 2B4 (Natural killer Cell Receptor 2B4), 4-1BB (Tumor necrosis factor receptor superfamily member 9), CD28 (T-cell-specific surface glycoprotein CD28), CD3 ζ (T-cell surface glycoprotein CD3 zeta chain), DAP10 (Hematopoietic cell signal transducer), DAP12 (TYRO protein tyrosine kinase-binding protein), DNAM1 (CD226 antigen), FcERI γ (High affinity immunoglobulin epsilon receptor subunit gamma), IL21R (Interleukin-21 receptor), IL2R β /IL15R β (Interleukin-2 receptor subunit beta), IL2R γ (Cytokine receptor common subunit gamma), IL-7R (Interleukin-7 receptor subunit alpha), KIR2DS2 (Killer cell immunoglobulin-like receptor 2DS2), NKG2D (NKG2-D type II integral membrane protein), NKp30 (Natural cytotoxicity triggering receptor 3), NKp44 (Natural cytotoxicity triggering receptor 2), NKp46 (Natural cytotoxicity triggering receptor 1), CS1 (SLAM family member 7), and CD8 (T-cell surface glycoprotein CD8 alpha chain);

(ii) an amino acid sequence that has at least about 85%, about 90%, about 95%, about 96%, about 97%, about 98%, or about 99% identity to the cytoplasmic domain, or a portion thereof, of 2B4, 41BB, CD16, CD2, CD28, CD28H, CD3 ζ , DAP10, DAP12, DNAM1, FcERI γ , IL21R, IL2R β (IL15R β), IL2R γ , IL7R, KIR2DS2, NKG2D, NKp30, NKp44, NKp46, CD3 ζ 1XX, CS1, or CD8, represented by SEQ ID NOs: 98-120, respectively; and/or

(iii) an amino acid sequence that has at least about 85%, about 90%, about 95%, about 96%, about 97%, about 98%, or about 99% identity to the cytoplasmic domain, or a portion thereof, of 2B4, CD28, CD3 ζ , DAP10, NKG2D, CD3 ζ , CD3 ζ 1XX, DNAM1, CS1, or combinations thereof.

18. The cell or population thereof of claim 16, wherein the endodomain comprises two different signaling domains, and wherein said endodomain domain comprises fused cytoplasmic domains, or portions thereof, in any one of the forms: CD28-CD3 ζ , CD28-CD3 ζ 1XX, 41BB-CD3 ζ , 41BB-CD3 ζ 1XX, 2B4-CD3 ζ and 2B4-CD3 ζ 1XX.
19. The cell or population thereof of claim 16, wherein the transmembrane domain comprises an amino acid sequence that has at least about 85%, about 90%, about 95%, about 96%, about 97%, about 98%, or about 99% identity to a transmembrane region, or a portion thereof, of CD2, CD3 δ , CD3 ϵ , CD3 γ , CD3 ζ , CD4, CD8, CD8a, CD8b, CD16, CD27, CD28, CD28H, CD40, CD84, CD166, 4-1BB, OX40, ICOS, ICAM-1, CTLA4, PD1, LAG3, 2B4, BTLA, DNAM1, DAP10, DAP12, FcERI γ , IL7, IL12, IL15, KIR2DL4, KIR2DS1, KIR2DS2, NKp30, NKp44, NKp46, NKG2C, NKG2D, CS1, or T cell receptor polypeptide.
20. The cell or population thereof of claim 16, wherein the transmembrane domain comprises an amino acid sequence that has at least about 85%, about 90%, about 95%, about 96%, about 97%, about 98%, or about 99% identity to a transmembrane region, or a portion thereof, of 2B4, CD16, CD2, CD28, CD28H, CD3 ζ , DAP10, DAP12, DNAM1, FcERI γ , KIR2DS2, NKG2D, NKp30, NKp44, NKp46, CS1, or CD8, represented by SEQ ID NOs: 76, 78-86, 91-97, respectively.
21. The cell or population thereof of claim 16, wherein the transmembrane domain and its immediately linked signaling domain are from a same protein or from different proteins.
22. The cell or population thereof of claim 16, wherein the antigen binding domain comprises an amino acid sequence that has at least about 85%, about 90%, about 95%, about 96%, about 97%, about 98%, or about 99% identity to any of SEQ ID NOs: 158, 159, 166-177, and 184-186.
23. The cell or population thereof of claim 3, wherein the TCE is TCF1 or CD27.
24. The cell or population thereof of claim 3, wherein the site 1 is CD38, and wherein the site 2 is one of CD58, TIM3, ASB2, TIGIT, or PH12.

25. The cell or population thereof of claim 1, wherein (i) the iPSC is a clonal iPSC, a single cell dissociated iPSC, an iPSC cell line cell, or an iPSC master cell bank (MCB) cell; or (ii) the derivative cell comprises a derivative CD34⁺ cell, a derivative hematopoietic stem and progenitor cell, a derivative hematopoietic multipotent progenitor cell, a derivative T cell progenitor, a derivative NK cell progenitor, a derivative T lineage cell, a derivative NKT lineage cell, a derivative NK lineage cell, or a derivative B lineage cell; or (iii) the derivative cell comprises a derivative effector cell having one or more functional features that are not present in a counterpart primary T, NK, NKT, and/or B cell.

26. The cell or population thereof of claim 25, wherein the derivative cell has therapeutic properties comprising one or more of:

- (i) increased cytotoxicity;
- (ii) improved persistency and/or survival;
- (iii) enhanced ability in migrating, and/or activating or recruiting bystander immune cells, to tumor sites;
- (iv) improved tumor infiltration;
- (v) enhanced ability to reduce tumor immunosuppression;
- (vi) improved ability in rescuing tumor antigen escape;
- (vii) controlled apoptosis;
- (viii) enhanced or acquired ADCC; and
- (ix) ability to avoid fratricide,

in comparison to its counterpart primary cell obtained from peripheral blood, umbilical cord blood, or any other donor tissues without the same genetic edit(s).

27. A cell or a population thereof, wherein:

- (i) the cell is (a) an immune cell; (b) an induced pluripotent cell (iPSC); or (c) a derivative effector cell obtained from differentiating the iPSC; and
- (ii) comprising a construct at an integration site, wherein the construct comprises a polynucleotide encoding a first chimeric antigen receptor (CAR1), and wherein the construct further comprises one or more of:

- (a) a polynucleotide encoding a second chimeric antigen receptor (CAR2) that is different from CAR1 in antigen specificity;
- (b) a polynucleotide encoding a T cell enhancer (TCE);
- (c) a polynucleotide encoding a cytokine;

- (iii) the integration site comprises one of CD38, CD54, CD56, CD58, TIM3, ASB2, TIGIT, H11 and PH12;
- (iv) the construct comprises an exogenous promoter that regulates expression of the polynucleotides in the construct; and
- (v) integration of the construct at any of CD38, CD54, CD56, CD58, TIM3, ASB2, or TIGIT knocks out an endogenous gene at the integration site.

28. The cell or population thereof of claim 27, wherein the first CAR and the second CAR are specific to different antigens, wherein

(i) the antigen comprises ADGRE2, B7H3, carbonic anhydrase IX (CAIX), CCR1, CCR4, carcinoembryonic antigen (CEA), CD3, CD5, CD7, CD8, CD10, CD20, CD22, CD30, CD33, CD34, CD38, CD41, CD44, CD44V6, CD49f, CD56, CD70, CD74, CD79b, CD99, CD123, CD133, CD138, CDS, CLEC12A, an antigen of a cytomegalovirus (CMV) infected cell, epithelial glycoprotein-2 (EGP-2), epithelial glycoprotein-40 (EGP-40), epithelial cell adhesion molecule (EpCAM), EGFRvIII, receptor tyrosine-protein kinases erb- B2,3,4, EGFR, EGFR-VIII, ERBB folate-binding protein (FBP), fetal acetylcholine receptor (AChR), folate receptor- α , Ganglioside G2 (GD2), Ganglioside G3 (GD3), GPRC5D, human Epidermal Growth Factor Receptor 2 (HER2), human telomerase reverse transcriptase (hTERT), ICAM-1, Integrin B7, Interleukin-13 receptor subunit alpha-2 (IL-13R α 2), κ -light chain, kinase insert domain receptor (KDR), KLK2, Lewis A (CA19.9), Lewis Y (LeY), L1 cell adhesion molecule (L1-CAM), LILRB2, melanoma antigen family A 1 (MAGE-A1), MICA/B, Mucin 1 (Muc-1), Mucin 16 (Muc-16), Mesothelin (MSLN), NKCSI, NKG2D ligands, c-Met, cancer-testis antigen NY-ESO-1, oncofetal antigen (h5T4), PRAME, prostate stem cell antigen (PSCA), PRAME prostate-specific membrane antigen (PSMA), tumor-associated glycoprotein 72 (TAG-72), TIM-3, TRBC1, TRBC2, vascular endothelial growth factor R2 (VEGF-R2), Wilms tumor protein (WT-1), and a pathogen antigen; or

(ii) the antigen comprises B7H3, BCMA, CD19, CD20, CD22, CD38, CD52, CD79b, CD123, EGFR, EGP2/EpCAM, GD2, GPRC5D, HER2, KLK2, MICA/B, MSLN, VEGF-R2, PSMA and PDL1.

29. The cell or population thereof of claim 27, wherein the TCE is TCF1 or CD27.

30. A cell or population thereof, wherein:

(i) the cell is (a) an immune cell; (b) an induced pluripotent cell (iPSC); or (c) a derivative effector cell obtained from differentiating the iPSC; and

(ii) comprising an insertion at an integration site PH12, wherein the integration site PH12 is a safe harbor locus having a coordinate of chr17:44210001-44271500 in human reference genome assembly hg38.

31. The cell or population thereof of claim 30, wherein the insertion comprises a construct comprising at least one exogenous polynucleotide encoding a peptide of interest, and a regulatory region.

32. The cell or population thereof of claim 31, wherein the regulatory region comprises a promoter.

33. The cell or population thereof of claim 32, wherein the promoter is a constitutive promoter.

34. The cell or population thereof of claim 31, wherein the construct comprises polynucleotides encoding one or more CARs, a cytokine, a T cell enhancer, a TGF β -SRR, a C-X-C motif chemokine receptor or a variant thereof, an exogenous CD16 or a variant thereof, or any combination thereof.

35. A cell or population thereof, wherein:

(i) the cell is (a) an immune cell; (b) an induced pluripotent cell (iPSC); or (c) a derivative effector cell obtained from differentiating the iPSC; and

(ii) the cell comprises a polynucleotide encoding an allo-immune defense receptor (ADR);

wherein the polynucleotide is inserted into a TCR locus, thereby knocking out a TCR gene; and wherein the construct is expressed under an endogenous TCR promoter.

36. A cell or population thereof, wherein:

(i) the cell is (a) an immune cell; (b) an induced pluripotent cell (iPSC); or (c) a derivative effector cell obtained from differentiating the iPSC; and

(ii) the cell comprises a polynucleotide encoding a IL2, a IL18, or a IL7/IL7R fusion protein;

wherein the polynucleotide is inserted into a TCR locus, thereby knocking out a TCR gene; and wherein the construct is expressed under an endogenous TCR promoter.

37. A cell or population thereof, wherein:
- (i) the cell is (a) an immune cell; (b) an induced pluripotent cell (iPSC); or (c) a derivative effector cell obtained from differentiating the iPSC; and
 - (ii) the cell comprises at least three targeted loci for transgene integration, wherein the at least three targeted loci comprise TRAC and two of CD38, CD54, CD56, CD58, TIM3, ASB2, TIGIT, H11 and PH12.
38. The cell or population thereof of claim 37, wherein the at least three targeted loci comprise TRAC, CD38, and one or more of CD54, CD56, CD58, TIM3, ASB2, TIGIT, H11 and PH12.
39. The cell or population thereof of claim 37, wherein the cell is TCR^{-/-}, and optionally, CD38^{-/-}, CD54^{-/-}, CD56^{-/-}, CD58^{-/-}, TIM3^{-/-}, ASB2^{-/-}, and/or TIGIT^{-/-}.
40. The cell or population thereof of claim 37, wherein the cell comprises CD38 targeted insertion of TGFβ-SRR-hnCD16-CXCR2, TRAC targeted insertion of ADR-sIL2, CD58 targeted insertion of CD58_CAR-TCF1, and knock-out of CD38, TRAC and CD58.
41. A composition comprising the cell or population thereof of any one of claims 1-40.
42. The composition of claim 41, further comprising one or more therapeutic agents.
43. The composition of claim 42, wherein the one or more therapeutic agents comprise a peptide, a cytokine, a checkpoint inhibitor, a mitogen, a growth factor, a small RNA, a dsRNA (double stranded RNA), mononuclear blood cells, feeder cells, feeder cell components or replacement factors thereof, a vector comprising one or more polynucleic acids of interest, an antibody, an engager, a chemotherapeutic agent or a radioactive moiety, or an immunomodulatory drug (IMiD).
44. The composition of claim 43, wherein the checkpoint inhibitor comprises:
- (a) one or more antagonists to checkpoint molecules comprising PD-1, PDL-1, TIM-3, TIGIT, LAG-3, CTLA-4, 2B4, 4-1BB, 4-1BBL, A_{2A}R, BATE, BTLA, CD39, CD47, CD73, CD94, CD96, CD160, CD200, CD200R, CD274, CEACAM1, CSF-1R, Foxp1, GARP, HVEM, IDO, EDO, TDO, LAIR-1, MICA/B, NR4A2, MAFB, OCT-2, Rara (retinoic acid receptor alpha), TLR3, VISTA, NKG2A/HLA-E, or inhibitory KIR;

(b) one or more of atezolizumab, avelumab, durvalumab, ipilimumab, IPH4102, IPH43, IPH33, lirimumab, monalizumab, nivolumab, pembrolizumab, and their derivatives or functional equivalents; or

(c) at least one of atezolizumab, nivolumab, and pembrolizumab.

45. The composition of claim 43, wherein the antibody comprises:

(a) an anti-CD20 antibody, an anti-HER2 antibody, an anti-CD52 antibody, an anti-EGFR antibody, an anti-CD123 antibody, an anti-GD2 antibody, an anti-PDL1 antibody, or an anti-CD38 antibody; or

(b) one or more of rituximab, veltuzumab, ofatumumab, ublituximab, ocaratuzumab, obinutuzumab, trastuzumab, pertuzumab, alemtuzumab, cetuximab, dinutuximab, avelumab, daclizumab, basiliximab, M-A251, 2A3, BC69, 24204, 22722, 24212, MAB23591, FN50, 298614, AF2359, CY1G4, DF1513, bivatuzumab, RG7356, G44-26, 7G3, CSL362, elotuzumab, daratumumab, isatuximab, MOR202, and their humanized or Fc modified variants or fragments and their functional equivalents and biosimilars thereof.

46. The composition of claim 43, wherein the engager comprises:

(i) a bispecific T cell engager (BiTE);

(ii) a bispecific killer cell engager (BiKE); or

(iii) a tri-specific killer cell engager (TriKE); or

wherein the engager comprises:

(a) a first binding domain recognizing an extracellular portion of CD3, CD28, CD5, CD16, CD64, CD32, CD33, CD89, NKG2C, NKG2D, or any functional variants thereof of the cell or a by-stander immune effector cell; and

(b) a second binding domain specific to an antigen comprising any one of: B7H3, CD10, CD19, CD20, CD22, CD24, CD30, CD33, CD34, CD38, CD44, CD52, CD79a, CD79b, CD123, CD138, CD179b, CEA, CLEC12A, CS-1, DLL3, EGFR, EGFRvIII, EpCAM, FLT-3, FOLR1, FOLR3, GD2, gpA33, HER2, HM1.24, LGR5, MSLN, MCSP, MICA/B, Muc1, Muc16, PDL1, PSMA, PAMA, P-cadherin, ROR1, or VEGF-R2.

47. A master cell bank (MCB) comprising the iPSC of any one of claims 1-40.

48. Therapeutic use of the composition of any one of the claims 41-46 by introducing the composition to a subject in need of an adoptive cell therapy, wherein the subject has an autoimmune disorder, a hematological malignancy, a solid tumor, cancer, or a virus infection.
49. A method of improving T cell differentiation of a cell comprising an ADR, the method comprising regulating ADR expression under an endogenous promoter that is temporally regulated during T cell differentiation.
50. The method of claim 49, wherein the endogenous promoter is activated upon commitment to lymphoid lineage, and wherein the endogenous promoter activity is further heightened upon commitment to T cell lineage.
51. The method of claim 49, wherein the endogenous promoter comprises a promoter of at least one of TRAC, TIM-3, ASB2, and TIGIT.
52. The method of claim 49, wherein the ADR is co-expressed with a cytokine comprising IL2, IL18, or IL7RF.
53. A method of manufacturing the derivative effector cell of any one of claims 1-40, wherein the derivative effector cell is an immune effector cell, and the method comprises:
- (a) obtaining a genetically engineered iPSC, wherein the iPSC comprises edits comprising: (i) a polynucleotide encoding a TCR promoter-driven ADR and/or a cytokine, (ii) a polynucleotide encoding a TCE, (iii) a polynucleotide encoding a CAR, (iv) a polynucleotide encoding a TGF β -SRR, (v) a polynucleotide encoding a C-X-C-motif chemokine receptor or a variant thereof, (vi) a polynucleotide encoding an exogenous CD16 or variant thereof, and (vii) a CD38 knockout;
 - (b) differentiating the genetically engineered iPSC to a derivative CD34⁺ cell; and
 - (c) differentiating the derivative CD34⁺ cell to an immune effector cell, wherein the immune effector cell retains the edits.
54. The method of claim 53, wherein obtaining the genetically engineered iPSC comprises:
- (i) generating a first pre-MCB (master cell bank) iPSC by introducing to an iPSC a construct comprising polynucleotides encoding a TGF β -SRR, a C-X-C-motif chemokine receptor or a variant thereof, and a CD16 variant to a first integration site (site 1);

- (ii) generating a second pre-MCB iPSC by introducing to the first pre-MCB iPSC a construct comprising a polynucleotide encoding an allo-immune defense receptor (ADR) and a polynucleotide encoding one of IL2, IL18 and a IL7RF at a TCR locus; and
- (iii) generating a MCB iPSC by introducing to the second pre-MCB iPSC a construct comprising one or more CAR and a TCE, at a second integration site (site 2).

55. The method of claim 54, wherein the site 2 differs from the site 1, and wherein the site 1 and site 2 comprise any two of AAVS1, CCR5, ROSA26, collagen, HTRP, H11, PH12, GAPDH, TCR constant region, RUNX1, B2M, TAP1, TAP2, tapasin, NLRC5, CIITA, RFXANK, RFX5, RFXAP, NKG2A, NKG2D, CD38, CD25, CD69, CD71, CD44, CD54, CD56, CD58, CIS, CBL-B, SOCS2, PD1, CTLA4, LAG3, TIM3, ASB2, and TIGIT loci.

56. The method of claim 55, wherein the site 2 comprise CD38, CD54, CD56, CD58, TIM3, TIGIT, H11 and PH12; and wherein integration at any of CD38, CD54, CD56, CD58, TIM3, ASB2, or TIGIT knocks out an endogenous gene.

57. The method of claim 54, wherein the TCR constant region is a constant region of TCR alpha (TRAC) or TCR beta (TRBC); wherein endogenous gene at TRAC or TRBC is knocked out; and wherein the construct is under control of TRAC or TRBC endogenous promoter.

58. The method of claim 54, wherein

- (i) the C-X-C motif chemokine receptor comprises CXCR2 or CXCR3;
- (ii) the TGF β -SRR comprises a TGF β R2-IL2R β , a TGF β R2-IL12R β , a TGF β R2-IL18R β , or a TGF β R2-trIL12R β redirector receptor;
- (iii) the CD16 variant is a high affinity non-cleavable CD16 (hnCD16);
- (iv) the ADR is specific to 4-1BB; or
- (v) the TCE is TCF1 or CD27.

59. The method of claim 54, further comprising genetically engineering the first pre-MCB iPSC, the second pre-MCB iPSC, or the MCB iPSC by one or more of:

- (a) introducing HLA-I deficiency, and/or HLA-II deficiency;
- (b) deleting or disrupting one or more of B2M, CIITA, TAP1, TAP2, Tapasin, NLRC5, RFXANK, RFX5, RFXAP, TCR, NKG2A, NKG2D, CD25, CD44, CD54, CD56, CD58, CD69, CIS, CBL-B, SOCS2, PD1, CTLA4, LAG3, TIM3, ASB2, and TIGIT; or

(c) introducing at least one of HLA-G, HLA-E, 4-1BBL, CD3, CD4, CD8, CD16, CD47, CD113, CD131, CD137, CD80, PDL1, A_{2A}R, antigen-specific TCR, chimeric fusion receptor (CFR), Fc receptor, an antibody or functional variant or fragment thereof, a checkpoint inhibitor, an engager, and surface triggering receptor for coupling with an agonist.

60. The method of any one of claims 53-59, wherein the genetic engineering comprises targeted editing.

61. The method of claim 60, wherein the targeted editing is carried out by CRISPR, ZFN, TALEN, homing nuclease, homology recombination, or any other functional variation of these methods.

62. A method of treating a subject in need of an adoptive cell therapy, wherein the method comprises infusing the subject with effector cells, wherein the effector cells comprise the derivative cell or population thereof according to any one of claims 1-40.

63. The method of claim 62, wherein the effector cells comprise a CAR specific to an antigen expressed on a cancer cell, wherein the antigen comprises at least one of B7H3, BCMA, CD19, CD20, CD22, CD38, CD52, CD79b, CD123, EGFR, EGP2/EpCAM, GD2, GPRC5D, HER2, KLK2, MICA/B, MSLN, VEGF-R2, PSMA and PDL1.

64. The method of claim 62, further comprising administering one or more therapeutic agents to the subject, wherein the one or more therapeutic agents comprise:

- (i) a cytokine, an antibody, an engager, a checkpoint inhibitor, a chemotherapeutic agent or a radioactive moiety, or an immunomodulatory drug (IMiD);
- (ii) an anti-CD38 antibody comprising daratumumab, isatuximab, or MOR202;
- (iii) an engager comprising a BiTE (bi-specific T cell engager) or a TriKE (tri-specific Killer cell engager);
- (iv) a checkpoint inhibitor comprising atezolizumab, avelumab, durvalumab, ipilimumab, IPH4102, IPH43, IPH33, lirimumab, monalizumab, nivolumab, or pembrolizumab; and/or
- (v) a chemotherapeutic agent comprising cyclophosphamide and fludarabine (Cy/Flu).

65. The method of claim 62, wherein the effector cells comprise a CD38 knockout, a TCR knockout, and an ADR; wherein the method comprises administering to the subject an anti-CD38 antibody; and wherein the method does not require, or requires minimal, lymphodepletion comprising administering Cy/Flu to the subject.

66. The method of claim 62, wherein the effector cells are allogeneic, and wherein infusing the subject with effector cells is in an out-patient setting.

67. A method of improving an adoptive cell therapy in treating a subject having a solid tumor, the method comprising administering a population of derivative cells of any one of claims 1-40.

68. The method of claim 67, wherein the derivative cells are T lineage cells.