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(54) **Titre : CELLULES EFFECTRICES MODIFIEES ET PROCEDES D'AMELIORATION DU CIBLAGE OMNIPRESENT DE TUMEURS SOLIDES**

(54) **Title: ENGINEERED EFFECTOR CELLS AND METHODS OF ENHANCING UBIQUITOUS TARGETING OF SOLID TUMORS**

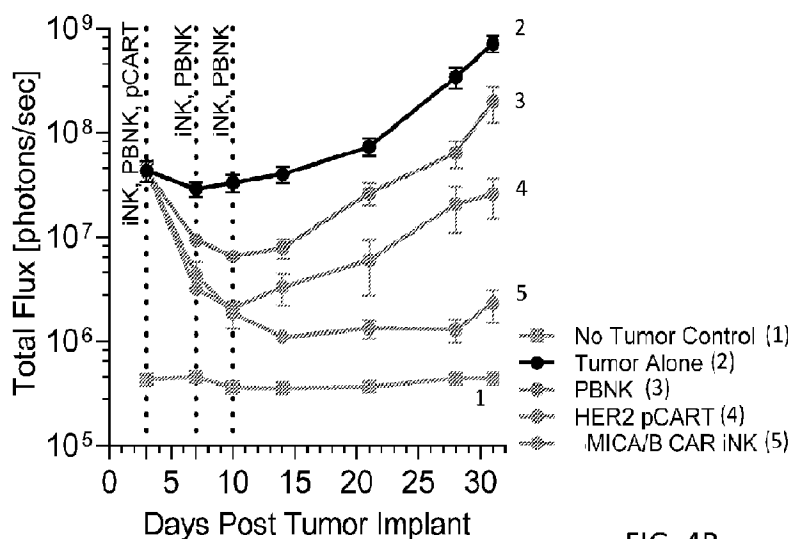


FIG. 4B

(57) **Abrégé/Abstract:**

Provided are methods and compositions for obtaining functionally enhanced derivative effector cells obtained from directed differentiation of genomically engineered iPSCs. Also provided are derivative cells having stable and functional genome editing that delivers improved or enhanced therapeutic effects. Also provided are therapeutic compositions and the use thereof comprising the functionally enhanced derivative effector cells alone, or with antibodies or checkpoint inhibitors in combination therapies.

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Abstract:

Provided are methods and compositions for obtaining functionally enhanced derivative effector cells obtained from directed differentiation of genomically engineered iPSCs. Also provided are derivative cells having stable and functional genome editing that delivers improved or enhanced therapeutic effects. Also provided are therapeutic compositions and the use thereof comprising the functionally enhanced derivative effector cells alone, or with antibodies or checkpoint inhibitors in combination therapies.

ENGINEERED EFFECTOR CELLS AND METHODS OF ENHANCING UBIQUITOUS TARGETING OF SOLID TUMORS

RELATED APPLICATIONS

[0001] This application claims priority to U.S. Provisional Application Serial No. 63/263,758, filed November 8, 2021, and to U.S. Provisional Application Serial No. 63/265,192, filed December 9, 2021, the disclosure of each of which is hereby incorporated by reference in their entireties.

INCORPORATION BY REFERENCE OF SEQUENCE LISTING

[0002] The Sequence Listing titled 184143-639601_SL.xml, which was created on November 7, 2022 and is 32,367 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 the 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 the 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 lymphocytes 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. Said one or several genetic modifications include, in some embodiments, 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 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 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 heterogeneous effector cell population otherwise obtained using primary cell sources which are heterogeneous to start with.

[0008] Some aspects of the present invention provide genome-engineered iPSCs obtained using a method comprising (I), (II) or (III), reflecting a strategy of genomic engineering subsequently to, simultaneously with, and prior to the reprogramming process, respectively:

(I): genetically engineering iPSCs by one or both of (i) and (ii), in any order: (i) introducing into iPSCs one or more construct(s) to allow targeted integration at selected site(s); (ii) (a) introducing into iPSCs one or more double stranded break(s) at selected site(s) using one or more endonuclease capable of selected site recognition; and (b) culturing the iPSCs of step

(I)(ii)(a) to allow endogenous DNA repair to generate targeted in/dels at the selected site(s); thereby obtaining genome-engineered iPSCs capable of differentiation into partially or fully differentiated cells.

(II): genetically engineering reprogramming non-pluripotent cells to obtain the genome-engineered iPSCs comprising: (i) 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 reprogramming of the non-pluripotent cells; and (ii) introducing into the reprogramming non-pluripotent cells of step (II)(i) one or both of (a) and (b), in any order: (a) one or more construct(s) to allow targeted integration at selected site(s); (b) one or more double stranded break(s) at a selected site using at least one endonuclease capable of selected site recognition, then the cells of step (II)(ii)(b) are cultured to allow endogenous DNA repair to generate targeted in/dels at the selected site(s); as such the obtained genome-engineered iPSCs comprise at least one functional targeted genomic editing, and said genome-engineered iPSCs are capable of differentiation into partially or fully differentiated cells.

(III): genetically engineering non-pluripotent cells for reprogramming to obtain genome-engineered iPSCs comprising (i) and (ii): (i) introducing into non-pluripotent cells one or both of (a) and (b), in any order: (a) one or more construct(s) to allow targeted integration at selected site(s); (b) one or more double stranded break(s) at a selected site using at least one endonuclease capable of selected site recognition, wherein the cells of step (III)(i)(b) are cultured to allow endogenous DNA repair to generate targeted in/dels at the selected sites; and (ii) contacting the cells of step (III)(i) 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 editing at selected sites; thereby obtaining genome-engineered iPSCs comprising at least one functional targeted genomic editing, and said genome-engineered iPSCs are capable of being differentiated into partially differentiated cells or fully-differentiated cells.

[0009] In one embodiment of the above method, the at least one targeted genomic editing at one or more selected sites comprises insertion of one or more exogenous polynucleotides encoding 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, and/or survival of the genome-engineered iPSCs or derivative cells thereof. In some embodiments, the exogenous polynucleotides for insertion are operatively linked to (1) one or more exogenous promoters comprising CMV, EF1 α , PGK, CAG, UBC, or other constitutive, inducible, temporal-,

tissue-, or cell type- specific promoters; or (2) one or more endogenous promoters comprised in the selected sites comprising AAVS1, CCR5, ROSA26, collagen, HTRP, H11, beta-2 microglobulin, GAPDH, TCR or RUNX1, or other locus meeting the criteria of a genome safe harbor. In some embodiments, the genome-engineered iPSCs generated using the above method comprise one or more different exogenous polynucleotides encoding protein comprising caspase, thymidine kinase, cytosine deaminase, modified EGFR, or B-cell CD20, wherein when the genome-engineered iPSCs comprise two or more suicide genes, the suicide genes are integrated in different safe harbor locus comprising AAVS1, CCR5, ROSA26, collagen, HTRP, H11, H11, beta-2 microglobulin, GAPDH, TCR or RUNX1. In one embodiment, the exogenous polynucleotide encodes a partial or full length peptide of IL2, IL4, IL6, IL7, IL9, IL10, IL11, IL12, IL15, IL18, IL21, and/or of respective receptors thereof. In some embodiments, the partial or full length peptide of IL2, IL4, IL6, IL7, IL9, IL10, IL11, IL12, IL15, IL18, IL21, and/or of respective receptors thereof encoded by the exogenous polynucleotide is in a form of fusion protein.

[00010] In some other embodiments, the genome-engineered iPSCs generated using the method provided herein comprise in/del at one or more endogenous genes associated with targeting modality, receptors, signaling molecules, transcription factors, drug target candidates, immune response regulation and modulation, or proteins suppressing engraftment, trafficking, homing, viability, self-renewal, persistence, and/or survival of the iPSCs or derivative cells thereof. In some embodiments, the endogenous gene for disruption comprises at least one of B2M, TAP1, TAP2, Tapasin, NLRC5, PD1, LAG3, TIM3, RFXANK, CIITA, RFX5, RFXAP, and any gene in the chromosome 6p21 region.

[00011] In yet some other embodiments, the genome-engineered iPSCs generated using the method provided herein comprise a caspase encoding exogenous polynucleotide at AAVS1 locus, and a thymidine kinase encoding exogenous polynucleotide at H11 locus.

[00012] In still some other embodiments, approach (I), (II) and/or (III) further comprises: contacting the genome-engineered iPSCs with a small molecule composition comprising a MEK inhibitor, a GSK3 inhibitor and a ROCK inhibitor, to maintain the pluripotency of the genomic-engineered iPSCs. In one embodiment, the obtained genome engineered iPSCs comprising at least one targeted genomic editing are functional, are differentiation potent, and are capable of differentiating into non-pluripotent cells comprising the same functional genomic editing.

[00013] Accordingly, in one aspect, the present invention also provides a composition comprising a cell or population thereof, wherein the cell is an immune cell, an induced pluripotent cell (iPSC), or a derivative cell obtained from differentiating the iPSC; and wherein the cell comprises a MICA/B tumor antigen targeting specificity; wherein the cell

optionally comprises one or more exogenous polynucleotides encoding a MICA/B-CAR (chimeric antigen receptor), a CD16 or a variant thereof that mediates ADCC when in combination with an anti-MICA/B antibody, an exogenous TCR targeting a MICA/B tumor antigen, or any combination thereof; and wherein: (i) the cell or population thereof further comprises a CAR having a tumor antigen targeting specificity other than MICA/B, or (ii) the composition further comprises a sensitizing agent, wherein the sensitizing agent increases tumor cell surface levels of MICA/B.

[00014] In various embodiments of the composition, 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 the derivative cell is a hematopoietic cell, and comprises longer telomeres in comparison to its native counterpart cell obtained from peripheral blood, umbilical cord blood, or any other donor tissues; or wherein the MICA/B-CAR has at least one of the following characteristics: (i) being T cell specific; (ii) being NK cell specific; (iii) binding to surface MICA/B; (iv) comprising a scFV (single chain variable fragment) binding to the conserved $\alpha 3$ domain of MICA/B; (v) comprising a heavy chain variable region represented by an amino acid sequence that is of at least about 99%, 98%, 96%, 95%, 90%, 85%, or 80% identity to SEQ ID NO: 1; (vi) comprising a light chain variable region represented by an amino acid sequence that is of at least about 99%, 98%, 96%, 95%, 90%, 85%, or 80% identity to SEQ ID NO: 2; (vii) comprising a scFV represented by an amino acid sequence that is of at least about 99%, 98%, 96%, 95%, 90%, 85%, or 80% identity to SEQ ID NOs: 3 or 4; (viii) comprising a heavy chain variable region of a MICA/B binding scFV functionally linked to a first constant region of a T cell receptor (TCR), and a light chain variable region of a MICA/B binding scFV functionally linked to a second constant region of a T cell receptor (TCR); and (ix) being inserted at one of the gene loci: B2M, TAP1, TAP2, Tapasin, NLRC5, CIITA, RFXANK, RFX5, RFXAP, TCR α or β constant region, NKG2A, NKG2D, CD38, CD25, CD44, CD58, CD54, CD69, CD71, CD56, CIS, CBL-B, SOCS2, PD1, CTLA4, LAG3, TIM3, or TIGIT; and, optionally, wherein the insertion knocks out expression of the gene in the locus. In some embodiments of the composition, the cell further comprises one or more of: (i) CD38 knockout; (ii) HLA-I deficiency and/or HLA-II deficiency; (iii) introduction of HLA-G or non-cleavable HLA-G, or knockout in one or both of CD58 and CD54; (iv) an exogenous CD16 or a variant thereof; (v) a chimeric antigen receptor (CAR) with targeting specificity other than MICA/B; (vi) a cytokine signaling complex comprising a partial or full peptide of a cell surface expressed exogenous cytokine or a receptor thereof; (vii) at least one of the genotypes listed in Table 1; (viii) disruption of at least one of TAP1, TAP2, Tapasin, NLRC5, CIITA, RFXANK, RFX5, RFXAP, TCR α or β constant region, NKG2A, NKG2D, CD56, CIS, CBL-B, SOCS2, PD1, CTLA4, LAG3, TIM3, and TIGIT, in comparison to its native counterpart

cell; and (ix) introduction of at least one of HLA-E, 4-1BBL, CD3, CD4, CD8, CD16, CD47, CD113, CD131, CD137, CD80, PDL1, A_{2A}R, antigen-specific TCR, Fc receptor, an engager, and surface triggering receptor for coupling with bi- or multi- specific or universal engagers, in comparison to its native counterpart cell.

[00015] In some embodiments of the composition, the exogenous CD16 comprises a high affinity non-cleavable CD16 (hnCD16) or a variant thereof. In particular embodiments, the the high affinity non-cleavable CD16 (hnCD16) or a variant thereof comprises at least one of: (a) F176V and S197P in ectodomain domain of CD16; (b) a full or partial ectodomain originated from CD64; (c) a non-native (or non-CD16) transmembrane domain; (d) a non-native (or non-CD16) intracellular domain; (e) a non-native (or non-CD16) signaling domain; (f) a stimulatory domain; and (g) transmembrane, signaling, and stimulatory domains that are not originated from CD16, and are originated from a same or different polypeptide. In particular embodiments, (a) the transmembrane domain is derived from CD3 δ , CD3 ϵ , CD3 γ , CD3 ζ , CD4, CD8, CD8a, CD8b, CD27, CD28, CD40, CD84, CD166, 4-1BB, OX40, ICOS, ICAM-1, CTLA-4, PD-1, LAG-3, 2B4, BTLA, CD16, IL7, IL12, IL15, KIR2DL4, KIR2DS1, NKp30, NKp44, NKp46, NKG2C, NKG2D, or T cell receptor (TCR) polypeptide; (b) the stimulatory domain is derived from CD27, CD28, 4-1BB, OX40, ICOS, PD-1, LAG-3, 2B4, BTLA, DAP10, DAP12, CTLA-4, or NKG2D polypeptide; (c) the signaling domain is derived from CD3 ζ , 2B4, DAP10, DAP12, DNAM1, CD137 (4-1BB), IL21, IL7, IL12, IL15, NKp30, NKp44, NKp46, NKG2C, or NKG2D polypeptide; or (d) the transmembrane domain is derived from NKG2D, the non-native stimulatory domain is derived from 2B4, and the non-native signaling domain is derived from CD3 ζ .

[00016] In some embodiments, the sensitizing agent comprises at least one of external beam radiation, brachytherapy, a radiopharmaceutical, and a chemotherapeutic agent. In particular embodiments, the sensitizing agent increases MICA/B surface expression by a tumor cell upon contact therewith. In some embodiments, the sensitizing agent comprises at least one of (i) 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; or (ii) paclitaxel.

[00017] In various embodiments of the composition, the composition further comprises one or more therapeutic agents. In some embodiments, the therapeutic agents comprise a peptide, a cytokine, a checkpoint inhibitor, an engager, an antibody, an antibody conjugate, 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). In those embodiments where the therapeutic agent is a checkpoint inhibitor, the checkpoint inhibitor may comprise: (i) one or more antagonists 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; (ii) one or more of atezolizumab, avelumab, cetuximab, durvalumab, ipilimumab, IPH4102, IPH43, IPH33, lirimumab, monalizumab, nivolumab, pembrolizumab, trastuzumab, pertuzumab, and their derivatives or functional equivalents; (iii) at least one of atezolizumab, nivolumab, and pembrolizumab; or the therapeutic agents comprise one or more of venetoclax, azacitidine, and pomalidomide. In those embodiments where the therapeutic agent is an antibody, the antibody may comprise: (a) an anti-CD20, an anti-CD22, an anti-HER2, an anti-CD52, an anti-EGFR, an anti-CD123, an anti-GD2, an anti-PDL1, and/or an anti-CD38 antibody; or (b) one or more of rituximab, veltuzumab, ofatumumab, ublituximab, ocaratuzumab, obinutuzumab, ibritumomab, ocrelizumab, inotuzumab, moxetumomab, epratuzumab, trastuzumab, pertuzumab, alemtuzumab, cetuximab, amivantamab, dinutuximab, avelumab, daratumumab, isatuximab, MOR202, 7G3, CSL362, elotuzumab, and their humanized or Fc modified variants or fragments and their functional equivalents and biosimilars.

[00018] In various embodiments of the composition, the composition further comprises a multi-specific engager. In some embodiments, the multi-specific engager is at least one of: (i) a T cell engager; (ii) an NK cell engager; (iii) a bi-specific T cell engager (BiTE); (iv) a bi-specific killer cell engager (BiKE); (v) a tri-specific killer cell engager (TriKE); (vi) a MICA/B engager; or (vii) a CD16 engager.

[00019] In various embodiments of the composition, the cell is a derivative NK or a derivative T cell, and has at least one of the following characteristics comprising: (i) improved persistency and/or survival; (ii) increased resistance to native immune cells; (iii) increased cytotoxicity; (iv) improved tumor penetration; (v) enhanced or acquired ADCC; (vi) enhanced ability in migrating, and/or activating or recruiting bystander immune cells, to tumor sites; (vii) enhanced ability to reduce tumor immunosuppression; (viii) improved ability in rescuing tumor antigen escape; (ix) ability to stabilize tumor antigen; and (x) ability to avoid fratricide, in comparison to its native counterpart cell obtained from peripheral blood, umbilical cord blood, or any other donor tissues.

[00020] In various embodiments of the composition, the CAR having a tumor antigen targeting specificity other than MICA/B is: (i) T cell specific or NK cell specific; (ii) a bi-specific antigen binding CAR; (iii) a switchable CAR; (iv) a dimerized CAR; (v) a split CAR; (vi) a multi-chain CAR; (vii) an inducible CAR; (viii) a recombinant TCR; (ix) co-expressed with another CAR; (x) co-expressed with a cytokine signaling complex comprising a partial or full peptide of a cell surface expressed exogenous cytokine or a receptor thereof, optionally in separate constructs or in a bi-cistronic construct; (xi) co-expressed with a checkpoint inhibitor, optionally in separate constructs or in a bi-cistronic construct; (xii) specific to at least one of CD19, BCMA, CD20, CD22, CD38, CD123, HER2, CD52, EGFR, GD2, MSLN, VEGF-R2, PSMA and PDL1; and/or (xiii) specific to any one of ADGRE2, 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, CD99, CD123, CD133, CD138, CDS, CLEC12A, an antigen of a cytomegalovirus (CMV) infected cell, epithelial glycoprotein2 (EGP-2), epithelial glycoprotein-40 (EGP-40), epithelial cell adhesion molecule (EpCAM), EGFRvIII, receptor tyrosine-protein kinases erbB2,3,4, EGFR, EGFR-VIII, ERBB folate-binding protein (FBP), fetal acetylcholine receptor (AChR), folate receptor- α , Ganglioside G2 (GD2), Ganglioside G3 (GD3), 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), 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; wherein the CAR of any one of (i) to (xiii) is optionally inserted at TRAC locus, and/or is driven by an endogenous promoter of TCR, and/or the TCR is knocked out by the CAR insertion. In one embodiment, the CAR having a tumor antigen targeting specificity other than MICA/B is specific to BCMA. In various embodiments of the composition, the cytokine signaling complex (a) comprises a partial or full peptide of at least one of IL2, IL4, IL6, IL7, IL9, IL10, IL11, IL12, IL15, IL18, IL21, and its respective receptor; or (b) comprises at least one of: (i) co-expression of IL15 and IL15R α by using a self-cleaving peptide; (ii) a fusion protein of IL15 and IL15R α ; (iii) an IL15/IL15R α fusion protein with intracellular domain of IL15R α truncated; (iv) a fusion protein of IL15 and membrane bound Sushi domain of IL15R α ; (v) a fusion protein of IL15 and IL15R β ; (vi) a fusion protein of IL15 and common receptor γ C, wherein the common receptor γ C

is native or modified; and (vii) a homodimer of IL15R β ; wherein any one of (b)(i)-(vii) can be co-expressed with a CAR in separate constructs or in a bi-cistronic construct; (c) comprises at least one of: (i) a fusion protein of IL7 and IL7R α ; (ii) a fusion protein of IL7 and common receptor γ C, wherein the common receptor γ C is native or modified; and (iii) a homodimer of IL7R β , wherein any one of (c)(i)-(iii) is optionally co-expressed with a CAR in separate constructs or in a bi-cistronic expression cassette; and optionally, (c) is transiently expressed. In various embodiments of the composition, the cell is a derivative NK or a derivative T cell, wherein the derivative NK cell is capable of recruiting, and/or migrating T cells to tumor sites, and wherein the derivative NK or the derivative T cell is capable of reducing tumor immunosuppression in the presence of one or more checkpoint inhibitors.

[00021] In those embodiments of the composition that include one or more checkpoint inhibitors, the checkpoint inhibitors may be antagonists to one or more 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. In some embodiments, the checkpoint inhibitors comprise: (a) one or more of atezolizumab, avelumab, durvalumab, ipilimumab, IPH4102, IPH43, IPH33, lirimumab, monalizumab, nivolumab, pembrolizumab, and their derivatives or functional equivalents; or (b) at least one of atezolizumab, nivolumab, and pembrolizumab.

[00022] In various embodiments of the composition, 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 cell, a derivative NKT cell, a derivative NK cell, or a derivative B cell. In some embodiments, the cell comprises: (i) one or more exogenous polynucleotides integrated in one safe harbor locus or a selected gene locus; or (ii) more than two exogenous polynucleotides integrated in different safe harbor loci or two or more selected gene locus. In particular embodiments, the safe harbor locus comprises at least one of AAVS1, CCR5, ROSA26, collagen, HTRP, H11, GAPDH, or RUNX1; and wherein the selected gene locus is one of B2M, TAP1, TAP2, Tapasin, NLRC5, CIITA, RFXANK, RFX5, RFXAP, TCR, NKG2A, NKG2D, CD38, CD25, CD44, CD58, CD54, CD56, CD69, CD71, CIS, CBL-B, SOCS2, PD1, CTLA4, LAG3, TIM3, or TIGIT; and wherein the integration of the exogenous polynucleotide optionally knocks out expression of the gene in the locus, and optionally the exogenous polynucleotide expresses under an endogenous promoter at the gene locus. In some embodiments, the TCR locus is a constant region of TCR alpha or TCR beta.

[00023] In another aspect, the invention provides therapeutic use of the compositions described herein by introducing the composition into a subject suitable for adoptive cell therapy, wherein the subject has an autoimmune disorder; a hematological malignancy; a solid tumor; cancer, or a virus infection. In another aspect, the invention provides a method of treating a subject comprising administering to the subject the compositions described herein. In various embodiments, the method further comprises administering one or more therapeutic agents to the subject.

[00024] In yet another aspect, the invention provides a method of treating a subject comprising: (a) administering a sensitizing agent to the subject to precondition tumor cells in the subject; and (b) administering a cell or population thereof to the subject following preconditioning the tumor cells, wherein: the cell is an immune cell or a derivative cell obtained from differentiating an iPSC; wherein the cell comprises a MICA/B tumor antigen targeting specificity; and wherein the cell optionally comprises one or more exogenous polynucleotides encoding a MICA/B-CAR (chimeric antigen receptor), a CD16 or a variant thereof that mediates ADCC when in combination with an anti-MICA/B antibody, an exogenous TCR targeting a MICA/B tumor antigen, or any combination thereof, and wherein the subject has an autoimmune disorder; a hematological malignancy; a solid tumor; cancer, or a virus infection. In various embodiments of the method of treating, the sensitizing agent comprises at least one of a chemotherapeutic agent, external beam radiation, brachytherapy, and a radiopharmaceutical. In various embodiments, the sensitizing agent increases MICA/B surface expression and/or density on the tumor cell upon contact therewith. In particular embodiments, the sensitizing agent comprises: (i) at least one of x-ray radiation, gamma radiation, photon radiation, proton radiation, and neutron radiation; or (ii) at least one of 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; or (iii) paclitaxel. In various embodiments, the method further comprises administering an antibody that binds to a MIC alpha 3-domain of MICA/B tumor cell surface antigen prior to or during administering the cell or population thereof.

[00025] In some embodiments, the derivative cell is a hematopoietic cell, and comprises longer telomeres in comparison to its native counterpart cell obtained from peripheral blood, umbilical cord blood, or any other donor tissues; or wherein the MICA/B-CAR has at least one of the following characteristics: (i) being T cell specific; (ii) being NK cell specific; (iii) binding to surface MICA/B; (iv) comprising a scFV (single chain variable fragment) binding to the

conserved $\alpha 3$ domain of MICA/B; (v) comprising a heavy chain variable region represented by an amino acid sequence that is of at least about 99%, 98%, 96%, 95%, 90%, 85%, or 80% identity to SEQ ID NO: 1; (vi) comprising a light chain variable region represented by an amino acid sequence that is of at least about 99%, 98%, 96%, 95%, 90%, 85%, or 80% identity to SEQ ID NO: 2; (vii) comprising a scFV represented by an amino acid sequence that is of at least about 99%, 98%, 96%, 95%, 90%, 85%, or 80% identity to SEQ ID NOs: 3 or 4; (viii) comprising a heavy chain variable region of a MICA/B binding scFV functionally linked to a first constant region of a T cell receptor (TCR), and a light chain variable region of a MICA/B binding scFV functionally linked to a second constant region of a T cell receptor (TCR); and (ix) being inserted at one of the gene loci: B2M, TAP1, TAP2, Tapasin, NLRC5, CIITA, RFXANK, RFX5, RFXAP, TCR α or β constant region, NKG2A, NKG2D, CD38, CD25, CD44, CD58, CD54, CD56, CD69, CD71, CIS, CBL-B, SOCS2, PD1, CTLA4, LAG3, TIM3, or TIGIT; and, optionally, wherein the insertion knocks out expression of the gene in the locus. In some embodiments of the method, the cell further comprises one or more of: (i) CD38 knockout; (ii) HLA-I deficiency and/or HLA-II deficiency; (iii) introduction of HLA-G or non-cleavable HLA-G, or knockout in one or both of CD58 and CD54; (iv) an exogenous CD16 or a variant thereof; (v) a chimeric antigen receptor (CAR) with targeting specificity other than MICA/B; (vi) a cytokine signaling complex comprising a partial or full peptide of a cell surface expressed exogenous cytokine or a receptor thereof; (vii) at least one of the genotypes listed in Table 1; (viii) disruption of at least one of TAP1, TAP2, Tapasin, NLRC5, CIITA, RFXANK, RFX5, RFXAP, TCR α or β constant region, NKG2A, NKG2D, CD56, CIS, CBL-B, SOCS2, PD1, CTLA4, LAG3, TIM3, and TIGIT, in comparison to its native counterpart cell; and (ix) introduced or increased expression in at least one of HLA-E, 4-1BBL, CD3, CD4, CD8, CD16, CD47, CD113, CD131, CD137, CD80, PDL1, A2AR, antigen-specific TCR, Fc receptor, an engager, and surface triggering receptor for coupling with bi- or multi- specific or universal engagers, in comparison to its native counterpart cell. In some embodiments of the method, the exogenous CD16 comprises a high affinity non-cleavable CD16 (hnCD16) or a variant thereof. In particular embodiments, the high affinity non-cleavable CD16 (hnCD16) or a variant thereof comprises at least one of: (a) F176V and S197P in ectodomain domain of CD16; (b) a full or partial ectodomain originated from CD64; (c) a non-native (or non-CD16) transmembrane domain; (d) a non-native (or non-CD16) intracellular domain; (e) a non-native (or non-CD16) signaling domain; (f) a stimulatory domain; and (g) transmembrane, signaling, and stimulatory domains that are not originated from CD16, and are originated from a same or different polypeptide.

[00026] In various embodiments of the method of treating, the chimeric antigen receptor (CAR) of (v) with targeting specificity other than MICA/B is: (i) T cell specific or NK cell

specific; (ii) a bi-specific antigen binding CAR; (iii) a switchable CAR; (iv) a dimerized CAR; (v) a split CAR; (vi) a multi-chain CAR; (vii) an inducible CAR; (viii) a recombinant TCR; (ix) co-expressed with another CAR; (x) co-expressed with a cytokine signaling complex comprising a partial or full peptide of a cell surface expressed exogenous cytokine or a receptor thereof, optionally in separate constructs or in a bi-cistronic construct; (xi) co-expressed with a checkpoint inhibitor, optionally in separate constructs or in a bi-cistronic construct; (xii) specific to at least one of CD19, BCMA, CD20, CD22, CD38, CD123, HER2, CD52, EGFR, GD2, MSLN, VEGF-R2, PSMA and PDL1; and/or (xiii) specific to any one of ADGRE2, 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, 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 erbB2,3,4, EGFR, EGFR-VIII, ERBB folate-binding protein (FBP), fetal acetylcholine receptor (AChR), folate receptor- α , Ganglioside G2 (GD2), Ganglioside G3 (GD3), 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), 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; wherein the CAR of any one of (i) to (xiii) is optionally inserted at TRAC locus, and/or is driven by an endogenous promoter of TCR, and/or the TCR is knocked out by the CAR insertion. In one embodiment, the CAR is specific to BCMA.

[00027] In various embodiments of the method of treating, the method further comprises administering one or more therapeutic agents. In some embodiments, the one or more therapeutic agents comprise a peptide, a cytokine, an antibody, an antibody conjugate, 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, a chemotherapeutic agent or a radioactive moiety, or an immunomodulatory drug (IMiD). In those embodiments where the therapeutic agent is a checkpoint inhibitor, the checkpoint inhibitor may comprise: (i) one or more antagonists 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; (ii) one or more of atezolizumab, avelumab, cetuximab, durvalumab, ipilimumab, IPH4102, IPH43, IPH33, lirimumab, monalizumab, nivolumab, pembrolizumab, trastuzumab, pertuzumab, and their derivatives or functional equivalents; (iii) at least one of atezolizumab, nivolumab, and pembrolizumab; or (b) the therapeutic agents comprise one or more of venetoclax, azacitidine, and pomalidomide. In those embodiments where the therapeutic agent is an antibody, the antibody may comprise: (a) an MICA/B antibody that binds to a MIC alpha 3-domain of a cell surface MICA/B antigen; (b) an antibody triggering antibody-dependent cellular cytotoxicity (ADCC); (c) an anti-CD20, an anti-CD22, an anti-HER2, an anti-CD52, an anti-EGFR, an anti-CD123, an anti-GD2, an anti-PDL1, and/or an anti-CD38 antibody; or (d) one or more of rituximab, velutuzumab, ofatumumab, ublituximab, ocaratuzumab, obinutuzumab, ibritumomab, ocrelizumab, inotuzumab, moxetumomab, epratuzumab, trastuzumab, pertuzumab, alemtuzumab, cetuximab, amivantamab, dinutuximab, avelumab, daratumumab, isatuximab, MOR202, 7G3, CSL362, elotuzumab, and their humanized or Fc modified variants or fragments and their functional equivalents and biosimilars. In various embodiments of the method of treating, the method further comprises administering a multi-specific engager. In some embodiments, the multi-specific engager is at least one of: (i) a T cell engager; (ii) an NK cell engager; (iii) a bi-specific T cell engager (BiTE); (iv) a bi-specific killer cell engager (BiKE); (v) a tri-specific killer cell engager (TriKE); (vi) a MICA/B engager; or (vii) a CD16 engager.

[00028] In yet another aspect, the invention provides a method of treating a subject comprising: administering a cell or population thereof to the subject, wherein the cell is an immune cell or a derivative cell obtained from differentiating an iPSC, wherein the cell: (a) comprises a MICA/B tumor antigen targeting specificity; and (b) comprises an exogenous polynucleotide encoding a CAR having specificity to a tumor antigen other than MICA/B; and optionally wherein the MICA/B tumor antigen targeting specificity comprises one or more exogenous polynucleotides encoding: (i) a MICA/B-CAR (chimeric antigen receptor); (ii) a CD16 or a variant thereof that mediates ADCC when in combination with an anti-MICA/B antibody; (iii) an exogenous TCR targeting a MICA/B tumor antigen; or (iv) any combination thereof; and wherein the subject has an autoimmune disorder; a hematological malignancy; a solid tumor; cancer, or a virus infection. In various embodiments of the method of treating, the CAR of (b) is: (i) T cell specific or NK cell specific; (ii) a bi-specific antigen binding CAR; (iii) a switchable CAR; (iv) a dimerized CAR; (v) a split CAR; (vi) a multi-chain CAR; (vii) an

inducible CAR; (viii) a recombinant TCR; (ix) co-expressed with another CAR; (x) co-expressed with a cytokine signaling complex comprising a partial or full peptide of a cell surface expressed exogenous cytokine or a receptor thereof, optionally in separate constructs or in a bi-cistronic construct; (xi) co-expressed with a checkpoint inhibitor, optionally in separate constructs or in a bi-cistronic construct; (xii) specific to at least one of CD19, BCMA, CD20, CD22, CD38, CD123, HER2, CD52, EGFR, GD2, MSLN, VEGF-R2, PSMA and PDL1; and/or (xiii) specific to any one of ADGRE2, 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, 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 erbB2,3,4, EGFR, EGFR-VIII, ERBB folate-binding protein (FBP), fetal acetylcholine receptor (AChR), folate receptor- α , Ganglioside G2 (GD2), Ganglioside G3 (GD3), 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), 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; wherein the CAR of any one of (i) to (xiii) is optionally inserted at TRAC locus, and/or is driven by an endogenous promoter of TCR, and/or the TCR is knocked out by the CAR insertion. In one embodiment, the CAR of (b) is specific to BCMA.

[00029] In various embodiments of the method of treating, the method further comprises administering a sensitizing agent to the subject to precondition tumor cells in the subject. In some embodiments, the sensitizing agent increases MICA/B surface expression and/or MICA/B density on the tumor cell upon contact therewith. In various embodiments of the method of treating, the sensitizing agent comprises at least one of a chemotherapeutic agent, external beam radiation, brachytherapy, and a radiopharmaceutical. In particular embodiments, the sensitizing agent comprises: (i) at least one of x-ray radiation, gamma radiation, photon radiation, proton radiation, and neutron radiation; or (ii) at least one of 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; or (iii) paclitaxel. In various embodiments of the method of treating, the method further comprises administering an antibody that binds to a MIC alpha 3-domain of MICA/B tumor cell surface antigen prior to or during administering the cell or population thereof. In some embodiments of the method of treating, the derivative cell is a hematopoietic cell, and comprises longer telomeres in comparison to its native counterpart cell obtained from peripheral blood, umbilical cord blood, or any other donor tissues; or wherein the MICA/B-CAR has at least one of the following characteristics: (i) being T cell specific; (ii) being NK cell specific; (iii) binding to surface MICA/B; (iv) comprising a scFV (single chain variable fragment) binding to the conserved $\alpha 3$ domain of MICA/B; (v) comprising a heavy chain variable region represented by an amino acid sequence that is of at least about 99%, 98%, 96%, 95%, 90%, 85%, or 80% identity to SEQ ID NO: 1; (vi) comprising a light chain variable region represented by an amino acid sequence that is of at least about 99%, 98%, 96%, 95%, 90%, 85%, or 80% identity to SEQ ID NO: 2; (vii) comprising a scFV represented by an amino acid sequence that is of at least about 99%, 98%, 96%, 95%, 90%, 85%, or 80% identity to SEQ ID NOs: 3 or 4; (viii) comprising a heavy chain variable region of a MICA/B binding scFV functionally linked to a first constant region of a T cell receptor (TCR), and a light chain variable region of a MICA/B binding scFV functionally linked to a second constant region of a T cell receptor (TCR); and (ix) being inserted at one of the gene loci: B2M, TAP1, TAP2, Tapasin, NLRC5, CIITA, RFXANK, RFX5, RFXAP, TCR α or β constant region, NKG2A, NKG2D, CD38, CD25, CD44, CD58, CD54, CD56, CD69, CD71, CIS, CBL-B, SOCS2, PD1, CTLA4, LAG3, TIM3, or TIGIT; and, optionally, wherein the insertion knocks out expression of the gene in the locus.

[00030] In various embodiments of the method of treating, the cell further comprises one or more of: (i) CD38 knockout; (ii) HLA-I deficiency and/or HLA-II deficiency; (iii) introduction of HLA-G or non-cleavable HLA-G, or knockout in one or both of CD58 and CD54; (iv) an exogenous CD16 or a variant thereof; (v) a cytokine signaling complex comprising a partial or full peptide of a cell surface expressed exogenous cytokine or a receptor thereof; (vi) at least one of the genotypes listed in Table 1; (vii) disruption in at least one of TAP1, TAP2, Tapasin, NLRC5, CIITA, RFXANK, RFX5, RFXAP, TCR α or β constant region, NKG2A, NKG2D, CD56, CIS, CBL-B, SOCS2, PD1, CTLA4, LAG3, TIM3, and TIGIT, in comparison to its native counterpart cell; and (viii) introduction of at least one of HLA-E, 4-1BBL, CD3, CD4, CD8, CD16, CD47, CD113, CD131, CD137, CD80, PDL1, A2AR, antigen-specific TCR, Fc receptor, an engager, and surface triggering receptor for coupling with bi- or multi- specific or universal engagers, in comparison to its native counterpart cell. In some embodiments, the exogenous

CD16 comprises a high affinity non-cleavable CD16 (hnCD16) or a variant thereof. In particular embodiments, the high affinity non-cleavable CD16 (hnCD16) or a variant thereof comprises at least one of: (a) F176V and S197P in ectodomain domain of CD16; (b) a full or partial ectodomain originated from CD64; (c) a non-native (or non-CD16) transmembrane domain; (d) a non-native (or non-CD16) intracellular domain; (e) a non-native (or non-CD16) signaling domain; (f) a stimulatory domain; and (g) transmembrane, signaling, and stimulatory domains that are not originated from CD16, and are originated from a same or different polypeptide.

[00031] In various embodiments of the method of treating, the method further comprises administering one or more therapeutic agents. In some embodiments, the one or more therapeutic agents comprise a peptide, a cytokine, an antibody, an antibody conjugate, 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, a chemotherapeutic agent or a radioactive moiety, or an immunomodulatory drug (IMiD). In those embodiments where the therapeutic agent is a checkpoint inhibitor, the checkpoint inhibitor may comprise: (i) one or more antagonists checkpoint molecules comprising PD-1, PDL-1, TIM-3, TIGIT, LAG-3, CTLA-4, 2B4, 4-1BB, 4-1BBL, A₂AR, 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; (ii) one or more of atezolizumab, avelumab, cetuximab, durvalumab, ipilimumab, IPH4102, IPH43, IPH33, lirimumab, monalizumab, nivolumab, pembrolizumab, trastuzumab, pertuzumab, and their derivatives or functional equivalents; (iii) at least one of atezolizumab, nivolumab, and pembrolizumab; or (b) the therapeutic agents comprise one or more of venetoclax, azacitidine, and pomalidomide. In those embodiments where the therapeutic agent is an antibody, the antibody may comprise: (a) an MICA/B antibody that binds to a MIC alpha 3-domain of a cell surface MICA/B antigen; (b) an antibody triggering antibody-dependent cellular cytotoxicity (ADCC); (c) an anti-CD20, an anti-CD22, an anti-HER2, an anti-CD52, an anti-EGFR, an anti-CD123, an anti-GD2, an anti-PDL1, and/or an anti-CD38 antibody; or (d) one or more of rituximab, velutuzumab, ofatumumab, ublituximab, ocaratuzumab, obinutuzumab, ibritumomab, ocrelizumab, inotuzumab, moxetumomab, epratuzumab, trastuzumab, pertuzumab, alemtuzumab, cetuximab, amivantamab, dinutuximab, avelumab, daratumumab, isatuximab, MOR202, 7G3, CSL362, elotuzumab, and their humanized or Fc modified variants or fragments and their functional equivalents and biosimilars. In various embodiments of the method of treating, the method further comprises administering a multi-specific engager. In some embodiments, the multi-specific engager is at

least one of: (i) a T cell engager; (ii) an NK cell engager; (iii) a bi-specific T cell engager (BiTE); (iv) a bi-specific killer cell engager (BiKE); (v) a tri-specific killer cell engager (TriKE); (vi) a MICA/B engager; or (vii) a CD16 engager.

[00032] In yet another aspect, the invention provides a method of enhancing tumor surface MICA/B expression comprising contacting a tumor cell with a sensitizing agent, thereby increasing surface MICA/B expression as compared to MICA/B expression prior to contact with the sensitizing agent. In various embodiments, the sensitizing agent comprises at least one of external beam radiation, brachytherapy, a radiopharmaceutical, and a chemotherapeutic agent. In particular embodiments, the sensitizing agent comprises: (i) at least one of x-ray radiation, gamma radiation, photon radiation, proton radiation, and neutron radiation; or (ii) at least one of 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; or (iii) paclitaxel. In various embodiments, the the method further comprises contacting the tumor cell with an antibody that binds to a MIC alpha 3-domain of tumor cell surface MICA/B antigen.

[00033] In one aspect, the invention provides kits comprising one or more compositions described herein, or one or more components thereof. In some embodiments, the kit comprises (i) a cell or population thereof, such as a cell or population thereof in accordance with any of the various aspects described herein; and (ii) a sensitizing agent, such as a sensitizing agent in accordance with any of the various aspects described herein. In some embodiments, the cell or population thereof is an immune cell, an induced pluripotent cell (iPSC) or a derivative cell obtained from differentiating the iPSC; the cell comprises a MICA/B tumor antigen targeting specificity; and the cell optionally comprises one or more exogenous polynucleotides encoding a MICA/B-CAR (chimeric antigen receptor), a CD16 or a variant thereof that mediates ADCC when in combination with an anti-MICA/B antibody, an exogenous TCR targeting a MICA/B tumor antigen, or any combination thereof. In some embodiments, the sensitizing agent increases tumor cell surface levels of MICA/B.

[00034] 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

[00035] FIGs. 1A-1C show that MICA and MICB are broadly expressed across a range of tumor types and indications, and that multiple antigens were observed within the same tumor indication.

[00036] FIG. 2 shows that iPSC-derived NK effector cells expressing a MICA/B-CAR demonstrate pan tumor reactivity using a wide range of tumor cell models.

[00037] FIG. 3A shows a transgene profile of exemplary NK effector cells expressing a MICA/B-CAR. FIG. 3B shows MICA/B-specific cytokine release in response to indicated high, medium, and low natively expressing MICA/B tumor cell lines. In FIG. 3B, each entry along the x-axes presents results as three groups of dots, each group from left to right representing cells indicated in the legend from top to bottom, respectively.

[00038] FIGs. 4A-4C show that that iPSC-derived NK effector cells expressing a MICA/B-CAR demonstrated *in vivo* ADCC efficacy against a human lung adenocarcinoma xenograft model.

[00039] FIG. 5 shows that iPSC-derived NK effector cells expressing a MICA/B-CAR demonstrate compatibility with ADCC-competent antibodies.

[00040] FIGs. 6A and 6B show that iPSC-derived effector cells expressing a MICA/B-CAR demonstrate both direct monotherapy reactivity and enhanced reactivity in combination with ADCC in a wide range of solid tumor cell models.

[00041] FIG. 7 shows that iPSC-derived effector cells expressing a MICA/B-CAR demonstrate enhanced reactivity in combination with ADCC in the triple negative breast cancer (TNBC) cell line cell MDA-MB-231 wildtype or with MICA/B knockout.

[00042] FIGs. 8A-8B show that iPSC-derived effector cells expressing a MICA/B-CAR demonstrate both direct monotherapy reactivity and enhanced reactivity in combination with monoclonal antibodies targeting different antigens expressed by the NSCLC adenocarcinoma cell line cell NCI-H1975 having heterogeneous tumor associated antigens.

[00043] FIGs. 9A-9B show that iPSC-derived effector cells expressing a MICA/B-CAR demonstrated *in vivo* efficacy in single-dose treatment regimens against a human lung adenocarcinoma xenograft model.

[00044] FIGs. 10A-10B show that iPSC-derived effector cells expressing a MICA/B-CAR demonstrated *in vivo* efficacy with anti-HER2 mAb mediated ADCC in multi-dose treatment regimens against a human lung adenocarcinoma xenograft model.

[00045] FIG. 11 shows generation of BCMA/MICA dual CAR-T cells.

[00046] FIGs. 12A and 12B show that dual expression of BCMA- and MICA- CAR increases avidity and functional responses of dual CAR-T cells compared to single CAR-T cells.

[00047] FIG. 13 shows that dual CAR-T cells exhibit elevated cytokine responses compared to single CAR-T cells.

[00048] FIG. 14 shows that multiple myeloma tumor lines express BCMA and/or MICA/B.

[00049] FIG. 15 shows that BCMA and MICA/B dual CAR-T cells exhibit greater cytotoxicity to multiple myeloma tumor lines compared to each individual single CAR-T cells.

[00050] FIG. 16 shows that BCMA and MICA/B dual CAR-T cells recognize and effectively kill several multiple myeloma tumor lines.

[00051] FIG. 17 shows that BCMA and MICA/B dual CAR-T cells control tumor cell growth of multiple myeloma cell line, MM1S, *in vivo*.

[00052] FIGs. 18A-18B show that sensitization of tumor cells augments MICA/B surface expression and enhances MICA/B-CAR effector cell antigen-specific cytotoxicity.

[00053] FIG. 19 show that sensitization of tumor cells augments MICA/B surface expression and enhances MICA/B-CAR effector cell antigen-specific cytotoxicity.

DETAILED DESCRIPTION OF THE INVENTION

[00054] 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 cells, NKT cells, NK cells.

[00055] Definitions

[00056] 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.

[00057] 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.

[00058] 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.

[00059] The use of the alternative (e.g., “or”) should be understood to mean either one, both, or any combination thereof of the alternatives.

[00060] The term “and/or” should be understood to mean either one, or both of the alternatives.

[00061] 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.

[00062] 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.

[00063] 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.

[00064] 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.

[00065] 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.

[00066] 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.

[00067] 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.

[00068] 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*.

[00069] The term “*in vivo*” refers generally to activities that take place inside an organism.

[00070] 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.

[00071] 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).

[00072] 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.

[00073] 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).

[00074] 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.

[00075] 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.

[00076] 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.

[00077] 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.

[00078] As used herein, the term “subject” refers to any animal, preferably a human patient, livestock, or other domesticated animal.

[00079] 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.

[00080] “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.

[00081] “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.

[00082] 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.

[00083] 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.

[00084] 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.

[00085] 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”. In some embodiments, the derivative T lineage cell is an iPSC-derived T cell obtained by differentiating an iPSC, which cells are also referred to herein as “iT” cells.

[00086] “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- γ , TNF- α , 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.

[00087] “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.

[00088] 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). 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”. In some embodiments, the derivative NK lineage cell is an iPSC-derived NK cell obtained by differentiating an iPSC, which cells are also referred to herein as “iNK” cells.

[00089] As used herein, the term “NKT cells” or “natural killer T 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 Vα24-Jα18, Vβ11, CD1d, CD3, CD4, CD8, aGalCer, CD161 and CD56.

[00090] 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.

[00091] 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.

[00092] 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%.

[00093] 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.

[00094] 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.

[00095] 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.

[00096] 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.

[00097] 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.

[00098] 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.

[00099] 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.

[000100] 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.

[000101] “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.

[000102] “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.

[000103] 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 lineage 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”. 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, a synthetic cell possesses one or more non-native cell functions when compared to its closest counterpart primary cell.

[000104] 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.

[000105] 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.

[000106] 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.

[000107] 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.

[000108] 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.

[000109] 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.

[000110] 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.

[000111] 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.

[000112] 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.

[000113] 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.

[000114] 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.

[000115] 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 (e.g., for use in an iNK cell therapy in accordance with various embodiments herein), without or with minimal need of a CY/FLU-based lympho-conditioning procedure, as further described herein.

[000116] 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.

[000117] 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.

[000118] 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.

[000119] 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.

[000120] 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.

[000121] 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.

[000122] 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 iPSC line is passaged, thawed or handled during the manufacturing processes.

[000123] 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.

[000124] 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.

[000125] “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) downstream 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.

[000126] “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.

[000127] “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.

[000128] 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.”

[000129] 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 (Fc γ R), 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 bispecific-, 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.

[000130] “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 (FcγR), those that bind IgA are called Fc-alpha receptors (FcαR) and those that bind IgE are called Fc-epsilon receptors (FcεR). 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 (FcγR) include several members, FcγRI (CD64), FcγRIIA (CD32), FcγRIIB (CD32), FcγRIIIA (CD16a), and FcγRIIIB (CD16b), which differ in their antibody affinities due to their different molecular structures.

[000131] “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.

[000132] “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.

[000133] 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.

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 shows that one or more selected genomic modifications of various embodiments provided herein do 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] Also provided here is a solution for increasing MICA/B expression on a tumor cell surface through contact with one or more sensitizing agents, such as chemotherapeutic agents, radiation and/or radioactive molecules, to improve on-target specificity with reduced off-tumor effect and/or improve homing, persistence, and cytotoxicity in the off-the-shelf adoptive cell therapy setting using effector cells derived from engineered iPSCs. In addition, the present application provides further genomic engineering aspects to achieve enhanced functionality of the effector cells, as detailed herein.

1. MICA/B-CAR

[000136] MICA and MICB are expressed family members of human major histocompatibility complex class I chain-related gene (MIC). The members of the MIC family are highly polymorphic (more than 100 human alleles) but with structurally conserved motifs. Applicable to the genetically engineered iPSC and derivative effector cells thereof may be one or more CAR designs. CAR, a chimeric antigen receptor, is a fusion protein generally including an ectodomain that comprises an antigen recognition region, a transmembrane domain, and an

endodomain. In some embodiments, the ectodomain can further include a signal peptide or leader sequence and/or a spacer (also called a hinge). In some embodiments, the endodomain can further comprise a signaling domain, where the signaling domain originates from a cytoplasmic domain of a signal transducing protein specific to T and/or NK cell activation or functioning. In some embodiments, the antigen recognition domain can specifically bind an antigen. In some embodiments, the antigen recognition domain can specifically bind 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, the CAR is suitable to activate T, NK or NKT cells expressing the CAR. In some embodiments, the CAR is NK cell specific for comprising NK-specific signaling components. In certain embodiments, the NK cells are derived from iPSCs comprising a polynucleotide encoding the CAR. In some embodiments, the CAR is T cell specific for comprising T cell specific signaling components. In certain embodiments, the T cells are derived from an iPSC comprising a polynucleotide encoding the CAR, 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.

[000137] In certain embodiments, the antigen recognition domain comprises a murine antibody, a human antibody, a humanized antibody, a camel Ig, a shark heavy-chain-only antibody (VNAR), Ig NAR, a chimeric antibody, a recombinant antibody, or antibody fragment thereof. Non-limiting examples of antibody fragments include Fab, Fab', F(ab')₂, F(ab')₃, Fv, antigen binding single chain variable 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. In some embodiments, the antigen recognition region of a CAR originates from the binding domain of a T cell receptor (TCR) that targets a tumor associated antigen (TAA).

[000138] In one example, the present specification provides a CAR comprising an antigen recognition region that targets tumor antigen MICA and MICB. In some embodiments of the MICA/B-targeting CAR, the antigen recognition region is a scFV that specifically binds to the conserved $\alpha 3$ domain of MICA and MICB (sometimes referred to as "MICA/B-CAR"). In one embodiment, the scFV comprises a variable region of the heavy chain 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: 1, and 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%, or at least about 80% identity to SEQ ID NO: 2. 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 SEQ ID NO: 3, in which the linker and/or signal peptide are exemplary and are replaceable. In another 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 SEQ ID NO: 4, in which the linker and/or signal peptide are exemplary and their length and sequence can vary. 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

QIQLVQSGPELKKPGETVKVSCASGYMFTNYAMNWVKQAPEKGLKWMGWINTHTGDPTYADDFKGRIAFS
LETSASTAYLQINNLKNEDTATYFCVRTYGNYAMDYWGQGTSTVTVSS

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

SEQ ID NO: 2

DIQMTQTTSSLSASLGDRVTISCSASQDISNYLNWYQQKPDGTVKLLIYDTSILHLGVPSRFSGSGSGTDY
SLTISNLEPEDIATYYCQQYSKFPRTFGGGTTLEIK

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

SEQ ID NO: 3 (HC-Linker-LC)

MDFQVQIFSFLLISASVIMSRDIQIQLVQSGPELKKPGETVKVSCASGYMFTNYAMNWVKQAPEKGLKWMGW
INTHTGDPTYADDFKGRIAFSLETSASTAYLQINNLKNEDTATYFCVRTYGNYAMDYWGQGTSTVTVSSGGG
GSGGGGSGGGGSDIQMTQTTSSLSASLGDRVTISCSASQDISNYLNWYQQKPDGTVKLLIYDTSILHLGVP
SRFSGSGSGTDYSLTISNLEPEDIATYYCQQYSKFPRTFGGGTTLEIK

(Signal peptide – other signal peptides are also possible; Linker – other linkers are also possible)

SEQ ID NO: 4 (LC-Linker-HC)

MDFQVQIFSFLLISASVIMSRDIQMTQTTSSLSASLGDRVTISCSASQDISNYLNWYQQKPDGTVKLLIYD
TSILHLGVPSRFSGSGSGTDYSLTISNLEPEDIATYYCQQYSKFPRTFGGGTTLEIKGGGGSGGGGSGGGG
SQIQLVQSGPELKKPGETVKVSCASGYMFTNYAMNWVKQAPEKGLKWMGWINTHTGDPTYADDFKGRIAF
SLETSASTAYLQINNLKNEDTATYFCVRTYGNYAMDYWGQGTSTVTVSS

(Signal peptide – other signal peptides are also possible; Linker – other linkers are also possible)

[000139] Another aspect of the present specification provides a genetically engineered iPSC and its derivative cell, wherein the cell comprises MICA/B tumor antigen targeting specificity and comprises an exogenous polynucleotide encoding at least a MICA/B-CAR. In some embodiments, the iPSC-derived effector cell comprising MICA/B tumor antigen targeting specificity and optionally an exogenous polynucleotide encoding at least a MICA/B-CAR is a T cell. In some embodiments, the iPSC-derived effector cell comprising MICA/B tumor antigen targeting specificity and optionally an exogenous polynucleotide encoding at least a MICA/B-CAR is an NK cell. In some other embodiments, the iPSC-derived effector cell comprising MICA/B tumor antigen targeting specificity and optionally an exogenous polynucleotide encoding at least a MICA/B-CAR is an NKT cell.

[000140] MICA/B as a tumor associated antigen is predominantly expressed in GI epithelium, endothelial cells and fibroblasts, and its expression is induced by cellular/genotoxic stress, and is highly expressed on epithelial and melanoma cancers. The shedding of MICA/B on tumor cells, on the other hand, results in increased soluble MICA/B which is not recognized by NKG2D expressed on NK and T cell subsets, possibly enables tumor evasion/escape and inhibits immunosurveillance. As shown in the present specification, the MICA/B tumor antigen targeted by the MICA/B-CAR as provided herein inhibits surface MICA/B shedding observed in many human and murine tumor cell lines, resulting in an increase in MICA/B cell surface density, reduced soluble shedding of MICA/B, and enhanced NK and/or T cell mediated tumor killing. Capable of targeting and stabilizing tumor cell surface MICA/B, the MICA/B-CAR as provided herein does not interfere with NKG2D binding to the tumor MICA and MICB, and is capable of enhancing immunosurveillance and preventing or reducing tumor evasion through tumor antigen shedding, while activating the immune cells expressing the MICA/B-CAR, including, but not limited to, primary T cells, NK cells, iPSC-derived T lineage cells, and iPSC-derived NK lineage cells to carry out MICA/B specific targeted tumor cell killing. Further, the immune cells carrying the provided MICA/B-CAR are capable of pan MICA/B (tumor) targeting and killing as shown by a wide range of tumor cell types expressing various MICA/B alleles.

[000141] An immune cell, including a genetically engineered iPSC and its derivative effector cell, comprising a MICA/B tumor antigen targeting specificity and optionally a MICA/B-CAR, as provided herein, may further comprise one or more additional CARs targeting one or more tumor antigens that are different from MICA/B. Non-limiting examples of antigens that may be targeted by additional CAR(s) comprised in a genetically engineered iPSC and derivative effector cells therefrom include ADGRE2, carbonic anhydrase IX (CAIX), CCR1, CCR4, carcinoembryonic antigen (CEA), CD3, CD5, CD7, CD8, CD10, CD19, CD20, CD22, CD30, CD33, CD34, CD38, CD41, CD44, CD44V6, CD49f, CD56, CD70, CD74, CD99, CD123,

CD133, CD138, CD269 (BCMA), CDS, CLEC12A, an antigen of a cytomegalovirus (CMV) infected cell (e.g., a cell surface antigen), epithelial glycoprotein-2 (EGP-2), epithelial glycoprotein-40 (EGP-40), epithelial cell adhesion molecule (EpCAM), EGFRvIII, receptor tyrosine-protein kinases erbB2,3,4, EGFR, EGFR-VIII, ERBB folate-binding protein (FBP), fetal acetylcholine receptor (AChR), folate receptor- α , Ganglioside G2 (GD2), Ganglioside G3 (GD3), 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), Lewis A (CA19.9), Lewis Y (LeY), L1 cell adhesion molecule (L1-CAM), LILRB2, melanoma antigen family A 1 (MAGE-A1), 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 various pathogen antigens known in the art. Non-limiting examples of pathogens include viruses, bacteria, fungi, parasites and protozoa capable of causing diseases.

[000142] In one embodiment of iPSCs and derivative effector cells therefrom comprising MICA/B tumor antigen targeting specificity and optionally a MICA/B-CAR, the cell further comprises a polynucleotide encoding a CD19-CAR. Non-limiting examples of CD19-targeting CARs are described in US2020/0317777A1, which is incorporated herein by reference. In another embodiment of iPSCs and derivative effector cells therefrom comprising MICA/B tumor antigen targeting specificity and optionally a MICA/B-CAR, the cell further comprises a polynucleotide encoding a BCMA-CAR. Non-limiting examples of BCMA-targeting CARs are described in US2019/0307797A1, which is incorporated herein by reference. In yet another embodiment of iPSCs and derivative effector cells therefrom comprising MICA/B tumor antigen targeting specificity and optionally a MICA/B-CAR, the cell further comprises a polynucleotide encoding a HER2-CAR. In still another embodiment of iPSCs and derivative effector cells therefrom comprising MICA/B tumor antigen targeting specificity and optionally a MICA/B-CAR, the cell further comprises a polynucleotide encoding a MSLN-CAR. In a further embodiment of iPSCs and derivative effector cells therefrom comprising MICA/B tumor antigen targeting specificity and optionally a MICA/B-CAR, the cell further comprises a polynucleotide encoding a PSMA-CAR. In still another embodiment of iPSCs and derivative effector cells therefrom comprising MICA/B tumor antigen targeting specificity and optionally a MICA/B-CAR, the cell further comprises a polynucleotide encoding a VEGF-R2 CAR.

[000143] In some embodiments of the MICA/B-CAR, there is a spacer/hinge between the MICA/B binding domain and the transmembrane domain of the CAR. Exemplary spacers that may be included in a CAR 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 could also vary, from about 25 amino acids up to about 300 amino acids or more. In this application, a spacer of less than 100 amino acids, or less than 50 amino acids, is considered short; whereas a spacer of more than 100 amino acids, or more than 200 amino acids is considered long.

[000144] In some embodiments, the transmembrane domain of a CAR comprises a full length or at least a portion of the native (i.e., wildtype) or modified transmembrane region of a transmembrane protein, including, but not limited to, CD3 δ , CD3 ϵ , CD3 γ , CD3 ζ , CD4, CD8, CD8a, CD8b, CD27, CD28, CD40, CD84, CD166, 4-1BB, OX40, ICOS, ICAM-1, CTLA4, PD1, LAG3, 2B4, BTLA, CD16, IL7, IL12, IL15, KIR2DL4, KIR2DS1, NKp30, NKp44, NKp46, NKG2C, NKG2D, and T cell receptor polypeptide. In one embodiment, the MICA/B-CAR and/or an additional CAR (targeting an antigen other than MICA/B) comprises a transmembrane domain derived from CD28. In one embodiment, the MICA/B-CAR and/or an additional CAR comprises a transmembrane domain derived from NKG2D.

[000145] In some embodiments, the signaling domain of the endodomain (or intracellular domain) comprises a full length or at least a portion of a signaling molecule, including, but not limited to, CD3 ζ , 2B4, DAP10, DAP12, DNAM1, CD137 (4-1BB), IL21, IL7, IL12, IL15, NKp30, NKp44, NKp46, NKG2C, NKG2D, or T cell receptor (TCR) polypeptide. In one embodiment, the signaling peptide of a CAR disclosed herein 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 .

[000146] In certain embodiments, the endodomain of a CAR further comprises at least one co-stimulatory signaling region. The co-stimulatory signaling region can comprise a full length or at least a portion of a signaling molecule, including, but not limited to, CD27, CD28, 4-1BB, OX40, ICOS, PD1, LAG3, 2B4, BTLA, DAP10, DAP12, CTLA4, or NKG2D, or any combination thereof.

[000147] In one embodiment, the CAR applicable to the cells provided herein comprises a co-stimulatory domain derived from CD28, and a signaling domain comprising the native or modified ITAM1 of 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: 5. 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 and trans-membrane domain

derived from CD28, wherein an scFv may be connected to the trans-membrane 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: 6, wherein the length and/or the sequence of the hinge/spacer can vary.

SEQ ID NO: 5

RSKRSRLHSDYMNMTPRRPGPTRKHYPYAPPRDFAAYRSRVKFSRSADAPAYQQGQNQ
LYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLFNELQKDKMAEAFSEIGMKGE
RRRGKGDGLFQGLSTATKDTFDALHMQALPPR

(153 a.a. CD28 co-stim + CD3ζITAM)

SEQ ID NO: 6

IEVMYPPPPYLDNEKSNGTIIHVKGKHLCPSPLEFPGPSKPFWVLVVVGGVLACYSLLVTVA
FIIFWVRSKRSRLHSDYMNMTPRRPGPTRKHYPYAPPRDFAAYRSRVKFSRSADAPAY
QQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLFNELQKDKMAEAFSE
IGMKGERRRRGKGDGLFQGLSTATKDTFDALHMQALPPR

(219 a.a. CD28 *hinge* + CD28 TM + CD28 co-stim + CD3ζITAM)

[000148] 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 CD3ζ, 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: 7. The CAR comprising a transmembrane domain derived from NKG2D, a co-stimulatory domain derived from 2B4, and a signaling domain comprising the native or modified CD3ζ may further comprise a CD8 hinge, wherein the amino acid sequence of such a structure is of at least about 85%, about 90%, about 95%, about 96%, about 97%, about 98%, or about 99% identity to SEQ ID NO: 8.

SEQ ID NO: 7

SNLFVASWIAVMIIFRIGMAVAIFCCFFFPSWRRKRKEKQSETSPKEFLTIIYEDVKDLKT
RRNHEQEQTFFPGGGSTIYSMIQSQSSAPTSQEPAYTLYSLIQPSRKSGSRKRNHSPSFNS
TIYEVIGKSQPKAQNPALRSRKELENFDVYSRVKFSRSADAPAYKQGQNQLYNELNLGR
EEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRRGKGDGL
YQGLSTATKDTYDALHMQALPPR

(263 a.a NKG2D TM + 2B4 + CD3ζ)

SEQ ID NO: 8

TTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDSNLFVASWIAVMIIF
RIGMAVAIFCCFFFPSWRRKRKEKQSETSPKEFLTIIYEDVKDLKTRRNHEQEQTFFPGGS
TIYSMIQSQSSAPTSQEPAYTLYSLIQPSRKSGSRKRNHSPSFNSTIYEVIGKSQPKAQNP
ALRSRKELENFDVYSRVKFSRSADAPAYKQGQNQLYNELNLGRREEYDVLDKRRGRDPE

MGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDAL
 HMQALPPR

(308 a.a CD8 *hinge* + NKG2D TM + 2B4 + CD3ζ)

[000149] Non-limiting CAR strategies further include heterodimeric, conditionally activated CAR through dimerization of a pair of intracellular domain (see for example, U.S. Pat. No. 9,587,020); 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); multi-chain CAR that allows non-covalent linking between two transmembrane domains connected to an antigen binding domain and a signaling domains, 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 the same or different antigens or epitopes (see for example, U.S. Pat. No. 8,409,577), a tandem CAR (see for example, Hegde et al., *J Clin Invest.* 2016;126(8):3036-3052); inducible CAR (see for example, U.S. Pub. Nos. 2016/0046700, 2016/0058857, 2017/0166877); switchable CAR (see for example, U.S. Pub. No. 2014/0219975); and any other designs known in the art.

[000150] Further examples of a CAR utilize a recombinant TCR (T cell receptor) for signaling transduction, resulting in a recombinant TCRα and/or a recombinant TCRβ, each comprising a respective constant region (i.e., TRAC and TRBC) linked to a scFV heavy chain or a scFV light chain, respectively, optionally through a flexible linker. In some embodiments of the MICA/B-CAR utilizing a recombinant TCR, the recombinant TCRα comprising TRAC comprises a light chain (LC; SEQ ID NO: 2), whereas the recombinant TCRβ comprising TRBC comprises a heavy chain (HC; SEQ ID NO: 1), of the MICA/B scFV as provided herein. In some other embodiments, the recombinant TCRα comprising TRAC comprises a heavy chain (HC; SEQ ID NO: 1), whereas the recombinant TCRβ comprising TRBC comprises a light chain (LC; SEQ ID NO: 2), of the MICA/B scFV as provided herein. In some embodiments of the recombinant TCR comprising the binding element of MICA/B scFV is suitable for TCR locus insertion and can integrate with endogenous CD3 for CD3 surface expression. In some embodiments, the MICA/B-CAR utilizing a recombinant TCR complex is more sensitive and/or specific to the tumor MICA/B antigen. In some embodiments, the amino acid sequence of TRAC is of at least about 85%, about 90%, about 95%, about 96%, about 97%, about 98%, or about 99% identity to SEQ ID NO: 9. In some embodiments, the amino acid sequence of TRBC is of at least about 85%, about 90%, about 95%, about 96%, about 97%, about 98%, or about 99% identity to SEQ ID NO: 10 or 11.

SEQ ID NO: 9

IQNPDPVYQLRDSKSSDKSVCLFTDFDSQTNVSQSKSDVYITDKTVLDMRSMDFKSNSAVAWSNKSDFA
CANAFNNSIIPEDTFFPSPRESSCDVKLVEKSFETDTNLFQNLVIGFRILLKLVAGFNLLMTLRLWSS

SEQ ID NO: 10

DLKNVFPPEVAVFEPSEAEISHTQKATLVCLATGFYPDHVELSWVNGKEVHSGVSTDPQPLKEQPALNDS
RYCLSSRLRVSATFWQNPРНHFRСQVQFYGLSENDEWTQDRAKPVТQIVSAEAWGRADCGFTSESYQQGVЛ
SATILYEILLGKATLYAVLVСALVLMAMVKRKDSRG

SEQ ID NO: 11

DLKNVFPPEVAVFEPSEAEISHTQKATLVCLATGFYPDHVELSWVNGKEVHSGVSTDPQPLKEQPALNDS
RYCLSSRLRVSATFWQNPРНHFRСQVQFYGLSENDEWTQDRAKPVТQIVSAEAWGRADCGFTSVSYQQGVЛ
SATILYEILLGKATLYAVLVСALVLMAMVKRKDF

[000151] In alternative embodiments of the above, the TRAC may be replaced with the constant region of TCR delta (TRDC), and wherein TRBC is replaced with a constant region of TCR gamma (TRGC). In some embodiments, the amino acid sequence of TRDC is of at least about 85%, about 90%, about 95%, about 96%, about 97%, about 98%, or about 99% identity to SEQ ID NO: 12. In some embodiments, the amino acid sequence of TRBC is of at least about 85%, about 90%, about 95%, about 96%, about 97%, about 98%, or about 99% identity to SEQ ID NO: 13 or 14.

SEQ ID NO: 12

SQPHTKPSVFVMKNGTNVACLVEFYPKDIRINLVSSKKITEFDPAIVISPSGKYNAVKLGKYEDSNSVTC
SVQHDNKTVHSTDFEVKTDSTDHVKPKETENTKQPSKSKCHKPKAIVHTEKVNMMSLTVLGLRMLFAKTAVV
NFLLTAKLFFL

SEQ ID NO: 13

DKQLDADVSPKPTIFLPSIAETKLQKAGTYLCLLEKFFPDVIKIHWQEKKSNTILGSQEGNTMKTNDTYMK
FSWLTVPKESLDKEHRCIVRHENNKNGVDQEIIFFPIKTDVITMDPKDNC SKDANDTLLLQLTNTSAYYMY
LLLLLKS VVYFAIITCCLLRRTAFCCNGEKS

SEQ ID NO: 14

DKQLDADVSPKPTIFLPSIAETKLQKAGTYLCLLEKFFPDIIKIHWQEKKSNTILGSQEGNTMKTNDTYMK
FSWLTVPKESLDKEHRCIVRHENNKNGIDQEIIFFPIKTDVITMDPKDNC SKDANDVITMDPKDNWSKDAN
DTLLLQLTNTSAYYTYLLLLLKS VVYFAIITCCLLRRTAFCCNGEKS

[000152] The genomic loci suitable for MICA/B-CAR and/or additional CAR (targeting an antigen other than MICA/B) insertion include loci meeting the criteria of a genome safe harbor as provided herein and gene loci where the knock-down or knockout of the gene in the selected locus as a result of the integration is desired. In some embodiments, the genomic loci suitable for MICA/B CAR insertion include, not are not limited to, AAVS1, CCR5, ROSA26, collagen,

HTRP, H11, GAPDH, RUNX1, B2M, TAP1, TAP2, Tapasin, NLRC5, CIITA, RFXANK, RFX5, RFXAP, TCR α or β constant region, NKG2A, NKG2D, CD38, CD25, CD44, CD58, CD54, CD56, CD69, CD71, CIS, CBL-B, SOCS2, PD1, CTLA4, LAG3, TIM3, and TIGIT.

[000153] In one embodiment, the iPSC and its derivative cells comprising MICA/B tumor antigen targeting specificity via a MICA/B-CAR, the cells have the CAR inserted in a TCR constant region (TRAC or TRBC), leading to endogenous TCR knockout (TCR^{neg}), and optionally placing CAR expression under the control of the endogenous TCR promoter. In one embodiment, the iPSC and its derivative cells comprising MICA/B tumor antigen targeting specificity, the MICA/B tumor antigen targeting specificity may be via a MICA/B-CAR, an exogenous TCR targeting a MICA/B tumor antigen or a combination thereof. In one particular embodiment of the iPSC derivative cell comprising MICA/B tumor antigen targeting specificity described herein, the cells comprise endogenous TCR knockout (TCR^{neg}), wherein the derivative cell is a T lineage cell. In another embodiment, the iPSC and its derivative cells comprising MICA/B tumor antigen targeting specificity described herein, the cells are TCR^{neg} and the CAR is inserted in the NKG2A locus or NKG2D locus, leading to NKG2A or NKG2D knockout, and optionally placing CAR expression under the control of the endogenous NKG2A or NKG2D promoter. In one particular embodiment of the iPSC derivative cell comprising MICA/B tumor antigen targeting specificity described herein, wherein the cell is NKG2A or NKG2D null and, the derivative cell is an NK lineage cell. In yet another embodiment, the iPSC and its derivative cells comprising MICA/B tumor antigen targeting specificity described herein have the CAR inserted in a CD38 coding region, leading to CD38 knockout, and optionally placing CAR expression under the control of the endogenous CD38 promoter. In one embodiment, the iPSC and its derivative cells comprising a MICA/B tumor antigen targeting specificity described herein have the CAR inserted in a CD58 coding region, leading to CD58 knockout. In one embodiment, the iPSC and its derivative cells comprising MICA/B tumor antigen targeting specificity described herein have the CAR inserted in a CD54 coding region, leading to CD54 knockout. In one embodiment, the iPSC and its derivative cells comprising MICA/B tumor antigen targeting specificity described herein have the CAR inserted in a CIS (Cytokine-Inducible SH2-containing protein) coding region, leading to CIS knockout. In one embodiment, the iPSC and its derivative cells comprising MICA/B tumor antigen targeting specificity described herein have the CAR inserted in a CBL-B (E3 ubiquitin-protein ligase CBL-B) coding region, leading to CBL-B knockout. In one embodiment, the iPSC and its derivative cells comprising MICA/B tumor antigen targeting specificity described herein have the CAR inserted in a SOCS2 (E3 ubiquitin-protein ligase CBL-B) coding region, leading to SOCS2 knockout. In one embodiment, the iPSC and its derivative cells comprising MICA/B tumor antigen targeting specificity described herein

have the CAR inserted in a CD56 (NCAM1) coding region, leading to NCAM1 knockout. In another embodiment, the iPSC and its derivative cells comprising MICA/B tumor antigen targeting specificity described herein have the CAR inserted in a coding region of any one of PD1, CTLA4, LAG3 and TIM3, leading to the gene knockout at the insertion site. In a further embodiment, the iPSC and its derivative cells comprising MICA/B tumor antigen targeting specificity herein have the CAR inserted in a coding region of TIGIT, leading to TIGIT knockout.

[000154] Provided herein therefore are effector cells obtained from differentiating genomically engineered iPSCs, wherein both the iPSCs and the derivative cells comprise a polynucleotide encoding a MICA/B tumor antigen targeting specificity via: an exogenous TCR targeting a MICA/B tumor antigen, a MICA/B-CAR, or a combination 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 MICA/B tumor antigen targeting specificity described herein. In some embodiments, the primary-sourced or derived effector cells comprising MICA/B tumor antigen targeting specificity described herein are T lineage cells. In some embodiments, the primary-sourced or derived effector cells comprising MICA/B tumor antigen targeting specificity described herein are NK lineage cells.

[000155] 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, MICA/B tumor antigen targeting specificity via: MICA/B-CAR, an exogenous TCR targeting a MICA/B tumor antigen, or a combination 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.

2. CD38 knockout

[000156] 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).

[000157] 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.

[000158] In malignancy treatment, systemic use of CD38 antigen binding receptor transduced T cells have 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).

[000159] Without being limited by theories, the present application provides a strategy to leverage the full potential of CD38 targeted cancer treatment by overcoming CD38 specific antibody and/or CD38 antigen binding domain induced effector cell depletion or reduction through fratricide. In various embodiments, the present application provides CD38 negative effector cells comprising a MICA/B-CAR, which can overcome CD38 mediated fratricide, and avoid 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 and/or eliminating these activated lymphocytes using a CD38-specific antibody such as daratumumab in the recipient of allogeneic effector cells, host alloreactivity 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.

[000160] 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.

[000161] Moreover, in various embodiments, strategies provided herein for CD38 knockout are compatible with other components and processes contemplated herein. , thereby generating an iPSC line having MICA/B tumor antigen targeting specificity described herein and a CD38 knockout, a master cell bank comprising single cell sorted and expanded clonal iPSCs, and obtaining CD38 negative (CD38^{neg} or CD38^{-/-}) derivative effector cells comprising the MICA/B tumor antigen targeting specificity described herein through directed differentiation of the engineered iPSC line, wherein the derivative effector cells 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 anti-CD38 antibody or CD38-CAR without the use of toxic conditioning agents and thus reduce and/or replace chemotherapy-based lymphodepletion.

[000162] 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 MICA/B-CAR and/or an exogenous TCR targeting a MICA/B tumor antigen, 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. In one embodiment, the MICA/B-CAR is inserted in the CD38 locus to simultaneously knockout CD38 in iPSC.

[000163] As such, this application provides an iPSC and derivative cells therefrom comprising MICA/B tumor antigen targeting specificity via a MICA/B-CAR, an exogenous TCR targeting a tumor MICA/B peptide, or a combination thereof, where the cells further comprise a CD38 knockout.

[000164] 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 CD38 negative iPSC and its derivative cells comprise one or more additional genomic edits as described herein, including but not limited to, MICA/B tumor antigen targeting specificity via a MICA/B-CAR, an exogenous TCR targeting a tumor MICA/B peptide, or a combination thereof, and may further comprise one or more additional engineered modalities described herein, and as shown in Table 1.

[000165] 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, 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.

3. CD16 knock-in

[000166] 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 an S197P variant is an example of a 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 International Pub. No. 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).

[000167] 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 other embodiments, a 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. 15, 16 and 17, each comprises at least a portion of CD64 ectodomain.

SEQ ID NO: 15

MWFLTTLLWVPVDGQVDTTKAVITLQPPWVSVEEETVTLHCEVLHLPGSSSTQWFLNGTATQTSTPSYR
ITSASVNDSGEYRCQRGLSGRSDPIQLEIHRGWLLLQVSSRVFTEGEPLALRCHAWKDKLVYNVLYYRNGK
AFKFFHWSNLTILKTNI SHNGTYHCSGMGKHRYTSAGISVTVKELFPAPVLNASVTSPLLEGNLVTLSC
E
TKLLLRPGLQLYFSFYMGSKTLRGRNTSSEYQILTARREDSGLYWCEAATEDGNVLKRSPELELQVLGLQ
LPTPVWFHYQVSFCLVMVLLFAVDTGLYFSVKTNI RSSTRDWKDHKFKWRKDPQDK
 (340 a.a. CD64 domain-based construction; CD16TM; CD16^{ICD})

SEQ ID NO: 16

MWFLTTLLWVPVDGQVDTTKAVITLQPPWVSVEEETVTLHCEVLHLPGSSSTQWFLNGTATQTSTPSYR
ITSASVNDSGEYRCQRGLSGRSDPIQLEIHRGWLLLQVSSRVFTEGEPLALRCHAWKDKLVYNVLYYRNGK
AFKFFHWSNLTILKTNI SHNGTYHCSGMGKHRYTSAGISVTVKELFPAPVLNASVTSPLLEGNLVTLSC
E

TKLLLQRPGLQLYFSFYMGSKTLRGRNTSSEYQILTARREDSGLYWCEAATEDGNVLKRSPELELQVLGLFF
FPPGYQVSFCLVMVLLFAVDTGLYFSVKTNIIRSSTRDWKDHKFKWRKDPQDK
 (336 a.a. CD64 exon-based construction; CD16TM; CD16ICD)

SEQ ID NO: 17

MWFLTTLTLLLVFPVDGQVDITTKAVITLQPPWVSVEFQEEITVTLHCEVLHLPGSSSTQWFLNGTATQTSTPSYR
ITSASVNDSGEYRCQRGLSGRSDPIQLEIHRGWLLQLVSSRVFTEGEPLALRCHAWKDKLVYNVLYYRNGK
AFKFFHWNNSLTIKTNISHNGTYHCSGMGKHRYTSAGISVTVKELFPAPVLNASVTSPLLEGNLVTLSC
TKLLLQRPGLQLYFSFYMGSKTLRGRNTSSEYQILTARREDSGLYWCEAATEDGNVLKRSPELELQVLGLFF
PPGYQVSFCLVMVLLFAVDTGLYFSVKTNIIRSSTRDWKDHKFKWRKDPQDK
 (335 a.a. CD64 exon-based construction; CD16TM; CD16ICD)

[000168] Accordingly, provided herein are effector cells or iPSCs genetically engineered to comprise, among other editing as contemplated and described herein, an exogenous CD16 or 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 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.

[000169] 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 comprises hnCD16. In some embodiments, the hnCD16 comprises a full or a partial length extracellular domain of CD64. 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 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 this application, wherein the exogenous CD16 comprises an extracellular binding domain of CD64, or of a CD16 having F176V and S197P.

[000170] 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. In the illustration here, the CFcR based on CD16 or variants thereof does not have a transmembrane, stimulatory or signaling domain that is derived from CD16. In some embodiments, the exogenous CD16-based CFcR comprises a non-native transmembrane domain derived from CD3δ, CD3ε, CD3γ, CD3ζ, CD4, CD8, CD8a, CD8b, CD27, CD28, CD40, CD84, CD166, 4-1BB, OX40, ICOS, ICAM-1, CTLA4, PD1, LAG3, 2B4, BTLA, CD16, IL7, IL12, IL15, KIR2DL4, KIR2DS1, NKp30, NKp44, NKp46, NKG2C, NKG2D, or T cell receptor polypeptide. In some embodiments, the exogenous CD16-based CFcR comprises a non-native stimulatory/inhibitory domain derived from CD27, CD28, 4-1BB, OX40, ICOS, PD1, LAG3, 2B4, BTLA, DAP10, DAP12, CTLA4, or NKG2D polypeptide. In some embodiments, the exogenous CD16-based CFcR comprises a non-native signaling domain derived from CD3ζ, 2B4, DAP10, DAP12, DNAM1, CD137 (4-1BB), IL21, IL7, IL12, IL15, NKp30, NKp44, NKp46, NKG2C, or NKG2D polypeptide. In one embodiment of CD16-based CFcR, the provided chimeric Fc receptor comprises a transmembrane domain and a signaling domain both derived from one of IL7, IL12, IL15, NKp30, NKp44, NKp46, NKG2C, and NKG2D polypeptide. One particular embodiment of the CD16-based chimeric Fc receptor comprises a transmembrane domain of NKG2D, a stimulatory domain of 2B4, and a signaling domain of CD3ζ; wherein the extracellular domain of the CFcR 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 S197P. Another exemplary embodiment of the CD16-based chimeric Fc receptor comprises a transmembrane domain and a signaling domain of CD3ζ; wherein the extracellular domain of the CFcR is derived from a full length or partial sequence of the extracellular domain of CD64 or CD16, wherein the extracellular domain of CD16 comprises F176V and S197P.

[000171] The various embodiments of 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. Exemplary tumor antigens for bi-, tri-, multi- specific engager or binders 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. Some non-limiting exemplary bi-, tri-, multi- specific engagers or binders suitable for engaging effector cells expressing the CD16-based CFcR in attacking tumor cells include CD16 (or CD64)-CD30, CD16 (or CD64)-BCMA, CD16 (or CD64)-IL15-EPCAM, and CD16 (or CD64)-IL15-CD33.

[000172] Unlike the endogenous CD16 expressed by primary NK cells which gets cleaved from the cellular surface following NK cell activation, the various non-cleavable versions of CD16 in derivative NK cells avoid CD16 shedding and maintain constant expression. In derivative NK cells, non-cleavable CD16 increases expression of TNF α and CD107a, indicative of improved cell functionality. Non-cleavable CD16 also enhances the antibody-dependent cell-mediated cytotoxicity (ADCC), and the engagement of bi-, tri-, or multi- specific engagers. ADCC is a mechanism of NK cell-mediated lysis through the binding of CD16 to antibody-coated target cells. The additional high affinity characteristics of the introduced hnCD16 in a derived NK cell also enables *in vitro* loading of an ADCC antibody to the NK cell through hnCD16 before administering the cell to a subject in need of a cell therapy. As provided herein, the hnCD16 may comprise F176V and S197P in some embodiments, or may comprise a full or partial ectodomain originated from CD64 as exemplified by SEQ ID NO: 15, 16 or 17, or may further comprise at least one of non-native transmembrane domain, stimulatory domain and signaling domain. As disclosed, the present application also provides a derivative NK cell 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 preloaded antibody is an anti-MICA/B antibody.

[000173] In some embodiments, the derived NK cells comprising MICA/B tumor antigen targeting specificity as described herein, MICA/B tumor antigen targeting specificity is via an exogenous CD16 or a variant thereof that mediates ADCC when in combination with an anti-MICA/B antibody, or additionally via a MICA/B-CAR, and/or an exogenous TCR targeting a tumor MICA/B peptide. In some embodiments, the derived NK cells comprising MICA/B tumor antigen targeting specificity described herein further comprise CD38 knockout. In some embodiments, the derived NK cells comprising MICA/B tumor antigen targeting specificity described herein and CD38 knockout, are preloaded with a CD38 antibody. In some embodiments, the preloaded CD38 antibody is daratumumab. In some embodiments, the derived NK cells comprising MICA/B tumor antigen targeting specificity described herein and CD38 knockout, are preloaded with one or more of an anti-HER2 antibody (e.g., trastuzumab, pertuzumab), an anti-EGFR antibody (e.g., cetuximab, amivantamab), or an anti-PDL1 antibody (e.g., avelumab). In some embodiments, the derived NK cells comprising MICA/B tumor antigen targeting specificity described herein and CD38 knockout, are preloaded with one or more anti-MICA/B antibodies.

[000174] Unlike primary NK cells, mature T cells from a primary source (i.e., natural/native sources such as peripheral blood, umbilical cord blood, or other donor tissues) do not express CD16. It was previously unexpected that iPSC comprising an expressed exogenous non-cleavable CD16 did not impair the T cell developmental biology and was able to differentiate into functional derivative T lineage cells that not only express the exogenous CD16, but also are capable of carrying out function through an acquired ADCC mechanism. This acquired ADCC in the derivative T lineage cell can additionally be used as an approach for dual targeting and/or to rescue antigen escape often occurred with CAR-T cell therapy, where the tumor relapses with reduced or lost CAR-T targeted antigen expression or expression of a mutated antigen to avoid recognition by the CAR (chimerical antigen receptor). When the derivative T lineage cell comprises acquired ADCC through exogenous CD16, including functional variants and CD16-based CFcR, expression, and when an antibody targets a different tumor antigen from the one targeted by the CAR, the antibody can be used to rescue CAR-T antigen escape and reduce or prevent relapse or recurrence of the targeted tumor often seen in CAR-T treatment. Such a strategy to reduce and/or prevent antigen escape while achieving dual targeting is equally applicable to NK cells expressing one or more CARs.

[000175] As such, the application provides a derivative T lineage cell comprising MICA/B tumor antigen targeting specificity via an exogenous CD16 or a variant thereof that mediates ADCC when in combination with an anti-MICA/B antibody, or additionally via a MICA/B-CAR, and/or an exogenous TCR targeting a tumor MICA/B peptide. In a further provided

embodiment, the derivative T lineage cell obtained herein comprises CD38 knockout in addition to the MICA/B tumor antigen targeting specificity described herein, such that the cells can avoid being eliminated when in the presence of a therapeutic targeting the tumor antigen CD38. In one embodiment, the therapeutic targeting the tumor antigen CD38 is an anti-CD38 antibody. In another embodiment, the therapeutic targeting the tumor antigen CD38 is a CAR comprising a CD38 binding region, for example, an anti-CD38 scFV. In some embodiments, the exogenous CD16 comprised in the derivative T lineage cell is an hnCD16 comprising F176V and S197P. In some other embodiments, the hnCD16 comprised in the derivative T cell comprises a full or partial ectodomain originated from CD64 as exemplified by SEQ ID NO: 15, 16 or 17; or may further comprise at least one of non-native transmembrane domain, stimulatory domain and signaling domain. As explained herein, such derivative T lineage cells have an acquired mechanism to target tumors with a monoclonal antibody mediated by ADCC to enhance the therapeutic effect of the antibody. As disclosed, the present application also provides a derivative T lineage cell, 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 below.

[000176] As provided further, the cell or population thereof, comprising MICA/B tumor antigen targeting specificity described herein may further comprise a second CAR and/or an exogenous CD16 or a variant thereof (“CD16^{exo}” in Table 1) and/or one or more additional engineered modalities described herein, and/or as shown in Table 1. 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, MICA/B tumor antigen targeting specificity described herein and at least one phenotype as provided herein, including but not limited to, 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. Exogenously introduced cytokine signaling complex

[000177] 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 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 to enable cytokine signaling with or without the expression of the cytokine itself to achieve lymphocyte autonomy without administered soluble cytokines, 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.

[000178] 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 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.

[000179] In various embodiments, such a truncated construct comprises an amino acid sequence of at least 75%, 80%, 85%, 90%, 95% or 99% identity to SEQ ID NO: 18. In one embodiment of the truncated IL15/IL15R α , the construct does not comprise the last 4 amino acid (KSRQ) of SEQ ID NO:18, and comprises an amino acid sequence of at least 75%, 80%, 85%, 90%, 95% or 99% identity to SEQ ID NO: 19. In some embodiments, the construct comprises

the amino acid sequence of SEQ ID NO: 19. In some embodiments, the construct comprises the amino acid sequence of SEQ ID NO: 18.

SEQ ID NO: 18

MDWTWILFLVAAATRVHSGIHVFILGCFSAGLPKTEANWVNVISDLKKIEDLIQSMHIDATLYTESDVHPS
CKVTAMKCFLELQVISLESGDASIHDTVENLIILANNSLSSNGNVTESGCKECEELEEKNIKEFLQSFVH
IVQMFINTSSGGGSGGGGSGGGGSGGGGSLQITCPPPMSVEHADIWVKSYSLSRERYICNSGFKRK
ACTSSLTECVLNKATNVAHWTTPSLKCIRDPALVHQRPAPPSTVTTAGVTPQPESLSPSCKEPAASSPSSN
NTAATTAAIVPGSQLMPSPSTGTTEISSHESHGTPSQTTAKNWELTASASHQPPGVYPQGHSDTTVAI
STSTVLLCGLSAVSLACYLKSRQ

(379 a.a.; signal and linker peptides are underlined)

SEQ ID NO: 19

MDWTWILFLVAAATRVHSGIHVFILGCFSAGLPKTEANWVNVISDLKKIEDLIQSMHIDATLYTESDVHPS
CKVTAMKCFLELQVISLESGDASIHDTVENLIILANNSLSSNGNVTESGCKECEELEEKNIKEFLQSFVH
IVQMFINTSSGGGSGGGGSGGGGSGGGGSLQITCPPPMSVEHADIWVKSYSLSRERYICNSGFKRK
AGTSSLTECVLNKATNVAHWTTPSLKCIRDPALVHQRPAPPSTVTTAGVTPQPESLSPSGKEPAASSPSSN
NTAATTAAIVPGSQLMPSPSTGTTEISSHESHGTPSQTTAKNWELTASASHQPPGVYPQGHSDTTVAI
STSTVLLCGLSAVSLACYL

(375 a.a.; signal and linker peptides are underlined)

[000180] In yet other embodiments, the cytoplasmic domain of IL15R α can be omitted without negatively impacting the autonomous feature of the effector cell equipped with IL15. In other embodiments, essentially the entire IL15R α is removed except for the Sushi domain fused with IL15 at one end and a transmembrane domain on the other (mb-Sushi), optionally with a linker between the Sushi domain and the trans-membrane domain. The fused IL15/mb-Sushi is expressed at the cell surface through the transmembrane domain of any membrane bound protein. Thus, unnecessary signaling through IL15R α , including cis-presentation, is eliminated when only the desirable trans-presentation of IL15 is retained. In some embodiments, the component comprising IL15 fused with Sushi domain comprises an amino acid sequence of at least 75%, 80%, 85%, 90%, 95% or 99% identity to SEQ ID NO: 20.

SEQ ID NO: 20

MDWTWILFLVAAATRVHSGIHVFILGCFSAGLPKTEANWVNVISDLKKIEDLIQSMHIDATLYTESDVHPS
CKVTAMKCFLELQVISLESGDASIHDTVENLIILANNSLSSNGNVTESGCKECEELEEKNIKEFLQSFVH
IVQMFINTSSGGGSGGGGSGGGGSGGGGSLQITCPPPMSVEHADIWVKSYSLSRERYICNSGFKRK
AGTSSLTECVLNKATNVAHWTTPSLKCIR

(242 a.a.; signal and linker peptides are underlined)

[000181] In other embodiments, a native or modified IL15R β is fused to IL15 at the C-terminus through a linker, enabling constitutive signaling and maintaining IL15 membrane-bound

and trans-representation. In other embodiments, a native or modified common receptor γ C is fused to IL15 at the C-terminus through a linker for constitutive signaling and membrane bound trans-presentation of the cytokine. The common receptor γ C is also called the common gamma chain or CD132, also known as IL2 receptor subunit gamma or IL2RG. γ C is a cytokine receptor sub-unit that is common to the receptor complexes for many interleukin receptors, including, but not limited to, IL2, IL4, IL7, IL9, IL15 and IL21 receptor. In other embodiments, engineered IL15R β that forms homodimer in absence of IL15 is useful for producing constitutive signaling of the cytokine.

[000182] 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 some embodiments, 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: 21, with transmembrane domain, signal peptide and linker being flexible and varying in length and/or sequences. In some embodiments, the construct comprises the amino acid sequence of SEQ ID NO: 21.

SEQ ID NO: 21

MDWTWILFLVAAATRVHSDCDIEGKDGKQYESVLMVSIQLLDSMKEIGSNCLNNEFNFFKRHIC
 DANKEGMFLFRAARKLRQFLKMNSTGDFDLHLLKVSEGTITLLNCTGQVKGRKPAALGEAQPTKS
 LEENKSLKEQKKLNDLCFLKRLLEQIKTCWNKILMGTKEHSGGGSGGGSGGGSGGGSGGGSL
 QESGYAQNGDLEDAELDDYSFSCYSQLEVNGSQHSLTCAFEDPDVNI TNLEFEICGALVEVKCLN
 FRKLQEIIYFIETKKFLLIGKSNICVKVGEKSLTCKKIDLTITVKPEAPFDLSVVYREGANDFVVT
 FNTSHLQKKYVKVLMHDVAYRQEKDENKWTHTVNLSSSTKLTLQKRLQPAAMYEEKVRSIPDHYFK
 GFWSEWSPSYFRTPEINNSSGEMDPILLTISILSFFSVALLVILACVLWKKRIKPIVWPSLPDH
 KKTLEHLCKKPRKNLNVSNPESFLDCQIHRVDDIQARDEVEGFLQDTFPQQLEESEKQRLGGDV
 QSPNCPSEDVVITPESFGRDSSLTCLAGNVSACDAPILSSSRSLDCRESGKNPHVYQDLLLSLG
 TTNSTLPPPFSLQSGILTLPVAQGQPILTSLSGNSQEEAYVTMSSFYQNO

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

[000183] 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.

[000184] 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.

[000185] In iPSCs and derivative cells therefrom comprising both CAR and exogenous cytokine and/or cytokine receptor signaling (signaling complex or “IL” in Table 1), the CAR and IL may be expressed in separate constructs, or may be co-expressed in a bi-cistronic construct comprising both CAR and IL. In some further embodiments, the signaling complex can be linked to either the 5' or the 3' end of a CAR expression construct through a self-cleaving 2A coding sequence. As such, an IL signaling complex (e.g., IL7 signaling complex) and CAR may be in a single open reading frame (ORF). In one embodiment, the signaling complex is comprised in CAR-2A-IL or IL-2A-CAR construct. When CAR-2A-IL or IL-2A-CAR is expressed, the self-cleaving 2A peptide allows the expressed CAR and IL to dissociate, and the dissociated IL can then be presented at the cell surface, with the transmembrane domain anchored in the cell membrane. The CAR-2A-IL or IL-2A-CAR bi-cistronic design allows for coordinated CAR and IL signaling complex expression both in timing and quantity, and under the same control mechanism that may be chosen to incorporate, for example, an inducible promoter or promoter with temporal or spatial specificity 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), Thosa 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.

[000186] The bi-cistronic CAR-2A-IL or IL-2A-CAR embodiment as disclosed herein is also contemplated for expression of any other cytokine or cytokine signaling complex provided herein, for example, any one or more of IL2, IL4, IL6, IL9, IL10, IL11, IL12, IL18, and IL21. In some embodiments, the bi-cistronic CAR-2A-IL or IL-2A-CAR is for expression of one or more of IL2, IL4, IL7, IL9, IL15 and IL21.

[000187] In some embodiments of the iPSC and its derivative cells comprising MICA/B tumor antigen targeting specificity via: a MICA/B-CAR, an exogenous TCR targeting a tumor MICA/B peptide, an exogenous and CD16 or a variant thereof that mediates ADCC when in

combination with an anti-MICA/B antibody, or a combination thereof, the cells optionally comprise one or more of a second CAR, CD38 knockout, and exogenous cytokine and/or cytokine receptor signaling, where the cells may further comprise any one of the additional attributes listed in Table 1. In particular embodiments of the iPSC and its derivative cells comprising MICA/B tumor antigen targeting specificity and optionally a MICA/B-CAR, the second CAR comprises a BCMA-CAR. In some embodiments, the iPSC, and its derivative effector cells comprising any one of the genotypes in Table 1 may additionally comprise disruption of at least one of 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, CD4, CD8, CD47, CD113, CD131, CD137, CD80, PDL1, A_{2A}R, TCR, Fc receptor, an antibody, and surface triggering receptor for coupling with bi-, multi- specific or universal engagers.

[000188] As such, in various embodiments, the cytokines IL15 or IL7 and/or receptors thereof, 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, provided herein is an induced pluripotent cell (iPSC), a clonal iPSC, a clonal iPS cell line, or iPSC-derived cells comprising a polynucleotide encoding a MICA/B tumor antigen targeting specificity described herein and at least one phenotype as provided herein, including but not limited to, a second CAR, exogenous CD16 or a variant thereof, CD38 knockout, cytokine signaling complex (IL) and/or one or more engineered modalities as disclosed herein, wherein the cytokine signaling complex comprises a partial or full peptide of a cell surface expressed exogenous cytokine and/or a receptor thereof, as described in this section, and 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.

5. *HLA-I- and HLA-II- deficiency*

[000189] 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 both HLA class I and 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 being 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-II associated genes including, not being limited to, RFXANK, CIITA, RFX5 and RFXAP. CIITA is a transcriptional coactivator, functioning through activation of the transcription factor RFX5 required for class II protein expression. CIITA negative cells are HLA-II deficient. Provided herein is an iPSC line and its derivative cells with both HLA-I and HLA-II deficiency, for example for lacking both B2M and 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.

[000190] 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.

[000191] 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.

[000192] As provided herein, in some embodiments, the iPSC and its derivative cells comprising at least MICA/B tumor antigen targeting specificity via one or more of: a MICA/B-CAR, an exogenous TCR targeting a tumor MICA/B peptide, and an exogenous CD16 or a variant thereof that mediates ADCC when in combination with an anti-MICA/B antibody as described, said cells are HLA-I and/or HLA-II deficient. In some embodiments, said HLA-I and/or HLA-II deficient iPSC and its derivative cells are CD58 negative. In some other embodiments, the HLA-I and/or HLA-II deficient iPSC and its derivative cells are CD54 negative. In yet some other embodiments, the HLA-I and/or HLA-II deficient iPSC and its derivative cells are CD54 negative and CD58 negative. Further, in some embodiments of the

iPSC and its derivative cells comprising MICA/B tumor antigen targeting specificity and optionally a MICA/B-CAR and/or an exogenous TCR targeting a tumor MICA/B peptide and/or an exogenous CD16 or a variant thereof that mediates ADCC when in combination with an anti-MICA/B antibody, said cells are HLA-I and/or HLA-II deficient and have an exogenous polynucleotide encoding HLA-G. In some embodiments of the iPSC and its derivative cells comprising MICA/B tumor antigen targeting specificity as described, the cells are HLA-I and/or HLA-II deficient and are CD58 negative. In some embodiments of the iPSC and its derivative cells comprising MICA/B tumor antigen targeting specificity as described, the cells are HLA-I and/or HLA-II deficient and are CD54 negative. In yet some other embodiments of the iPSC and its derivative cells comprising MICA/B tumor antigen targeting specificity as described, the cells are HLA-I and/or HLA-II deficient, and are both CD58 negative and CD54 negative.

[000193] 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.

[000194] 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, HLA modification (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.

6. Genetically engineered iPSC line and derivative cells provided herein

[000195] 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 polynucleotide encoding a MICA/B tumor antigen targeting specificity, and wherein the cell comprises one or more polynucleotides encoding a MICA/B-CAR (chimeric antigen receptor), a CD16 or a variant thereof that mediates

ADCC when in combination with an anti-MICA/B antibody, an exogenous TCR targeting a MICA/B tumor antigen, or any combination thereof, wherein the cell may further comprise one or more additional genetic modifications as described in the application, and 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.

[000196] In some embodiments of the derivative effector cells, the iPSCs and their derivative cells comprise a polynucleotide encoding a CAR (e.g., a MICA/B-CAR), wherein the CAR is inserted in a TCR constant region (TRAC or TRBC), leading to TCR knockout, and optionally placing CAR expression under the control of the endogenous TCR promoter. The disruption of the constant region of TCR α or TCR β (TRAC or TRBC) produces a TCRneg cell. In addition, the expression of TCR is also negative in a NK lineage effector cell that is differentiated from an iPSC. TCRneg 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. Additional insertion sites of a CAR include, but are not limited to, AAVS1, CCR5, ROSA26, collagen, HTRP, H11, 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, and TIGIT. In another embodiment, the effector cell, the iPSC and its derivative cell (e.g., a derivative NK cell) described herein comprises a polynucleotide encoding a CAR, where the CAR is inserted in the NKG2A locus or NKG2D locus, leading to NKG2A or NKG2D knockout, thereby placing CAR expression under the control of the endogenous NKG2A or NKG2D promoter. As such, in another embodiment, the cells comprising a MICA/B tumor antigen targeting specificity as described above, further comprise TCR^{neg} and/or one or more additional genetic modifications as described in the application.

[000197] 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.

[000198] Also provided herein is an immune cell, iPSC, iPS cell line cell, or a population thereof, and derived functional derivative cells comprising a polynucleotide encoding a MICA/B tumor antigen targeting specificity; wherein each cell comprises one or more polynucleotides

encoding a MICA/B-CAR (chimeric antigen receptor), a CD16 or a variant thereof that mediates ADCC when in combination with an anti-MICA/B antibody, an exogenous TCR targeting a MICA/B tumor antigen, or any combination thereof, and wherein the cells are also TCR^{neg} and CD38 negative, such that when an CD38 antibody is used to induce the CD16-mediated enhanced ADCC, the iPSC and/or its derivative effector cells comprising CD38 knockout, MICA/B-CAR and exogenous CD16 or a variant thereof can target the CD38 expressing (tumor) cells without causing effector cell elimination, i.e., reduction or depletion of CD38 expressing effector cells, thereby increasing the iPSC and its effector cell persistence and/or survival. In some embodiments, the effector cells comprise T lineage cells. In some other embodiments, said effector cells comprise NK lineage cells. The herein described immune cells, iPSCs, iPS cell line cells, or a population thereof, and derivative functional cells obtained from differentiating the iPSCs, wherein each cell comprises a polynucleotide encoding a MICA/B tumor antigen targeting specificity as described herein, optionally comprises one or more of TCR^{neg}, CD38 negative, an engager, a checkpoint inhibitor, an antibody and/or other attributes provided in this application.

[000199] Also provided is an iPSC comprising a polynucleotide encoding a MICA/B tumor antigen targeting specificity described herein, wherein the cell additionally comprises one or more of a polynucleotide encoding a second chimeric antigen receptor (CAR) with a target specificity other than MICA/B, TCR^{neg}, CD38 negative, an engager, a checkpoint inhibitor, an antibody and/or other attributes provided in this application, and wherein the iPSC is capable of directed differentiation to produce functional derivative effector cells having two CARs targeting two different tumor antigens. In various embodiments, the second CAR comprised in the iPSC and its derivative effector cells targets tumor cell surface protein CD19, BCMA, CD20, CD22, CD38, CD123, HER2, CD52, EGFR, GD2, MSLN, VEGF-R2, PSMA and/or PDL1. In one embodiment, the second CAR comprised in the iPSC and its derivative effector cells is a BCMA-CAR. In one embodiment, the iPSCs and/or their derivative effector cells have a second CAR targeting CD38, and said cells are also CD38 negative. As such, the CD38-CAR does not lead to elimination of iPSCs and/or their derivative effector cells due to CD38-mediated fratricide. In some embodiments, the CAR comprised in the iPSC and its derivative effector cells comprising CD38 knockout does not target CD38.

[000200] Additionally provided is an iPSC comprising a polynucleotide encoding a MICA/B tumor antigen targeting specificity described herein, wherein the cell additionally comprises one or more of a polynucleotide encoding at least one exogenous cytokine signaling complex (IL), TCR^{neg}, CD38 negative, a second CAR, an engager, a checkpoint inhibitor, an antibody and/or other attributes provided in this application, and wherein the IL enables 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. In various embodiments, the exogenously introduced 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 cytokine signaling complex is for IL15 signaling in the cell, and the cell is optionally an NK lineage cell. In some other embodiments, the introduced cytokine signaling complex is for IL7 signaling in the cell, and the cell is optionally a T lineage cell. In some embodiments, the introduced cytokine signaling complex comprising a partial or full peptide of cytokine and/or its respective receptor 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 a retrovirus, Sendai virus, an adenovirus, an episome, mini-circle, or RNAs including mRNA. In some embodiments, the cytokine signaling complex comprised in the iPSC comprising a MICA/B tumor antigen targeting specificity describe herein, or derivative cells thereof, enables IL7 signaling. In some embodiments, the cytokine signaling complex comprised in the iPSC comprising a MICA/B tumor antigen targeting specificity describe herein, or derivative cells thereof, enables IL10 signaling. In some embodiments, the cytokine signaling complex comprised in the iPSC a MICA/B tumor antigen targeting specificity described herein, or derivative cells thereof, enables IL15 signaling. Said iPSC comprising a MICA/B tumor antigen targeting specificity and IL, and its derivative cells of the above embodiments, 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 overexpress a tumor associated protein and 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. For example, effector cells overexpressing a homing related chemokine receptor have improved cell trafficking which can be further enhanced when a matching chemokine expressed by the tumor is upregulated by a tumor sensitizing composition through tumor cell preconditioning. Therefore, in various embodiments, the effector cells or iPSCs are genetically engineered to further comprise, among other editings as contemplated and described herein, an exogenous CXCR2 or a variant thereof. A non-limiting example of the amino acid sequence of human CXCR2 useful for such editing is one registered as UniProtKB No: P25025. In some embodiments, the variant of CXCR2 comprises a CXCR2

isoform, such as those registered as UniProtKB Nos: Q6LCZ7, C9JW47, C9JG19, C9J1J7, and C9J2F9.

[000201] Also provided is an iPSC comprising a polynucleotide encoding a MICA/B tumor antigen targeting specificity described herein, wherein the cell additionally comprises one or more of HLA-I and/or HLA-II deficiency (e.g., a B2M knockout and/or a CIITA knockout), TCR^{neg}, CD38 negative, a second CAR, IL, an engager, a checkpoint inhibitor, an antibody and/or other attributes provided in this application, and further optionally, one of HLA-G overexpression, CD58 knockout and CD54 knockout, and wherein the iPSC is capable of directed differentiation to produce functional derivative hematopoietic cells. In a further embodiment, the HLA-I and/or HLA-II deficient iPSC comprising a MICA/B tumor antigen targeting specificity described herein, and its derivative effector cells, the cells are also CD38 negative, and can be used with an anti-CD38 antibody to induce ADCC without causing effector cell elimination, thereby increasing the persistence and/or survival of the iPSC and its effector cell. In some embodiments, the effector cell has increased persistence and/or survival *in vivo*.

[000202] As such, the present application provides iPSCs and their functional derivative hematopoietic cells, which comprise any one of the following genotypes in Table 1. “CAR^(2nd)”, as provided in Table 1 of this application, stands for a CAR having a targeting specificity different from MICA/B-CAR, and the non-limiting examples thereof include a CAR targeting at least one of CD19, BCMA, CD20, CD22, CD123, HER2, CD52, EGFR, GD2, MSLN, VEGF-R2, PSMA and PDL1. In a particular embodiment, the CAR^(2nd) has targeting specificity for BCMA. “IL”, as provided in Table 1, stands for a cytokine signaling complex providing signaling of any one or more of IL2, IL4, IL6, IL7, IL9, IL10, IL11, IL12, IL15, IL18, and IL21, depending on which specific cytokine/receptor expression is selected. Further, “IL” also encompasses the IL15Δ embodiment, which is detailed above as a truncated fusion protein of IL15 and IL15Rα but without an intracellular domain. Further, when iPSCs and their functional derivative hematopoietic cells have a genotype comprising both CAR (MICA/B-CAR and/or a second CAR) and IL, the CAR and IL may be comprised in a bi-cistronic expression cassette comprising a 2A sequence. As comparison, in some other embodiments, CAR and IL are in separate expression cassettes comprised in iPSCs and their functional derivative hematopoietic cells. In one particular embodiment, comprised in the iPSCs and their functional derivative effector cells expressing both CAR and IL, is IL15, wherein the IL15 construct is comprised in an expression cassette with, or separate from, the CAR.

Table 1: Applicable Exemplary Genotypes of the Cells Provided:

MICA/B-CAR or MICA/B-TCR	CD38 ^{-/-}	CD16 ^{exo}	CAR ^(2nd)	IL	B2M ^{-/-} CIITA ^{-/-}	HLA-G or (CD58 ^{-/-} w/ or w/o CD54 ^{-/-})	Genotype
✓							1. MICA/B-CAR
✓							2. MICA/B-TCR
✓	✓						3. MICA/B-CAR CD38 ^{-/-}
✓		✓					4. MICA/B-TCR CD38 ^{-/-}
✓							5. MICA/B-CAR CD16 ^{exo}
✓			✓				6. MICA/B-TVCR CD16 ^{exo}
✓							7. MICA/B-CAR CAR ^(2nd)
✓				✓			8. MICA/B-TCR CAR ^(2nd)
✓							9. MICA/B-CAR IL
✓					✓		10. MICA/B-TCR IL
✓							11. MICA/B-CAR B2M ^{-/-} CIITA ^{-/-}
✓					✓		12. MICA/B-TCR B2M ^{-/-} CIITA ^{-/-}
✓					✓	✓	13. MICA/B-CAR B2M ^{-/-} CIITA ^{-/-} CD58 ^{-/-}
✓							14. MICA/B-TCR B2M ^{-/-} CIITA ^{-/-} CD58 ^{-/-}
✓							15. MICA/B-CAR B2M ^{-/-} CIITA ^{-/-} CD54 ^{-/-}
✓							16. MICA/B-TCR B2M ^{-/-} CIITA ^{-/-} CD54 ^{-/-}
✓							17. MICA/B-CAR B2M ^{-/-} CIITA ^{-/-} CD58 ^{-/-} CD54 ^{-/-}
✓							18. MICA/B-TCR B2M ^{-/-} CIITA ^{-/-} CD58 ^{-/-} CD54 ^{-/-}
✓							19. MICA/B-CAR B2M ^{-/-} CIITA ^{-/-} HLA-G
✓							20. MICA/B-TCR B2M ^{-/-} CIITA ^{-/-} HLA-G
✓	✓	✓					21. MICA/B-CAR CD38 ^{-/-} CD16 ^{exo}
✓	✓		✓				22. MICA/B-TCR CD38 ^{-/-} CD16 ^{exo}
✓							23. MICA/B-CAR CD38 ^{-/-} CAR ^(2nd)
✓				✓			24. MICA/B-TCR CD38 ^{-/-} CAR ^(2nd)
✓							25. MICA/B-CAR CD38 ^{-/-} IL
✓							26. MICA/B-TCR CD38 ^{-/-} IL
✓	✓				✓		27. MICA/B-CAR CD38 ^{-/-} B2M ^{-/-} CIITA ^{-/-}

✓	✓								28. MICA/B-TCR CD38 ^{-/-} B2M ^{-/-} CIITA ^{-/-}
							✓	✓	29. MICA/B-CAR CD38 ^{-/-} B2M ^{-/-} CIITA ^{-/-} CD58 ^{-/-}
									30. MICA/B-CAR CD38 ^{-/-} B2M ^{-/-} CIITA ^{-/-} CD54 ^{-/-}
									31. MICA/B-CAR CD38 ^{-/-} B2M ^{-/-} CIITA ^{-/-} CD58 ^{-/-} CD54 ^{-/-}
									32. MICA/B-CAR CD38 ^{-/-} B2M ^{-/-} CIITA ^{-/-} HLA-G
									33. MICA/B-TCR CD38 ^{-/-} B2M ^{-/-} CIITA ^{-/-} CD58 ^{-/-}
									34. MICA/B-TCR CD38 ^{-/-} B2M ^{-/-} CIITA ^{-/-} CD54 ^{-/-}
									35. MICA/B-TCR CD38 ^{-/-} B2M ^{-/-} CIITA ^{-/-} CD58 ^{-/-} CD54 ^{-/-}
									36. MICA/B-TCR CD38 ^{-/-} B2M ^{-/-} CIITA ^{-/-} HLA-G
✓		✓							37. MICA/B-CAR CD16 ^{exo} CAR ^(2nd)
									38. MICA/B-TCR CD16 ^{exo} CAR ^(2nd)
✓		✓				✓			39. MICA/B-CAR CD16 ^{exo} IL
									40. MICA/B-TCR CD16 ^{exo} IL
✓		✓					✓		41. MICA/B-CAR CD16 ^{exo} B2M ^{-/-} CIITA ^{-/-}
									42. MICA/B-TCR CD16 ^{exo} B2M ^{-/-} CIITA ^{-/-}
✓		✓					✓	✓	43. MICA/B-CAR CD16 ^{exo} B2M ^{-/-} CIITA ^{-/-} CD58 ^{-/-}
									44. MICA/B-CAR CD16 ^{exo} B2M ^{-/-} CIITA ^{-/-} CD54 ^{-/-}
									45. MICA/B-CAR CD16 ^{exo} B2M ^{-/-} CIITA ^{-/-} CD58 ^{-/-} CD54 ^{-/-}
									46. MICA/B-CAR CD16 ^{exo} B2M ^{-/-} CIITA ^{-/-} HLA-G
									47. MICA/B-TCR CD16 ^{exo} B2M ^{-/-} CIITA ^{-/-} CD58 ^{-/-}
									48. MICA/B-TCR CD16 ^{exo} B2M ^{-/-} CIITA ^{-/-} CD54 ^{-/-}
									49. MICA/B-TCR CD16 ^{exo} B2M ^{-/-} CIITA ^{-/-} CD58 ^{-/-} CD54 ^{-/-}
									50. MICA/B-TCR CD16 ^{exo} B2M ^{-/-} CIITA ^{-/-} HLA-G
✓			✓				✓		51. MICA/B-CAR CAR ^(2nd) IL
									52. MICA/B-TCR CAR ^(2nd) IL
✓			✓					✓	53. MICA/B-CAR CAR ^(2nd) B2M ^{-/-} CIITA ^{-/-}
									54. MICA/B-TCR CAR ^(2nd) B2M ^{-/-} CIITA ^{-/-}
✓			✓					✓	55. MICA/B-CAR CAR ^(2nd) B2M ^{-/-} CIITA ^{-/-} CD58 ^{-/-}
									56. MICA/B-CAR CAR ^(2nd) B2M ^{-/-} CIITA ^{-/-} CD54 ^{-/-}
									57. MICA/B-CAR CAR ^(2nd) B2M ^{-/-} CIITA ^{-/-} CD58 ^{-/-} CD54 ^{-/-}
									58. MICA/B-CAR CAR ^(2nd) B2M ^{-/-} CIITA ^{-/-} HLA-G
									59. MICA/B-TCR CAR ^(2nd) B2M ^{-/-} CIITA ^{-/-} CD58 ^{-/-}

									60. MICA/B-TCR CAR ^(2nd) B2M ^{-/-} CIITA ^{-/-} CD54 ^{-/-}
✓							✓	✓	61. MICA/B-TCR CAR ^(2nd) B2M ^{-/-} CIITA ^{-/-} CD58 ^{-/-} CD54 ^{-/-}
									62. MICA/B-TCR CAR ^(2nd) B2M ^{-/-} CIITA ^{-/-} HLA-G
✓							✓	✓	63. MICA/B-CAR IL B2M ^{-/-} CIITA ^{-/-}
							✓	✓	64. MICA/B-TCR IL B2M ^{-/-} CIITA ^{-/-}
								✓	65. MICA/B-CAR IL B2M ^{-/-} CIITA ^{-/-} CD58 ^{-/-}
									66. MICA/B-CAR IL B2M ^{-/-} CIITA ^{-/-} CD54 ^{-/-}
									67. MICA/B-CAR IL B2M ^{-/-} CIITA ^{-/-} CD58 ^{-/-} CD54 ^{-/-}
									68. MICA/B-CAR IL B2M ^{-/-} CIITA ^{-/-} HLA-G
									69. MICA/B-TCR IL B2M ^{-/-} CIITA ^{-/-} CD58 ^{-/-}
									70. MICA/B-TCR IL B2M ^{-/-} CIITA ^{-/-} CD54 ^{-/-}
									71. MICA/B-TCR IL B2M ^{-/-} CIITA ^{-/-} CD58 ^{-/-} CD54 ^{-/-}
✓	✓	✓	✓	✓	✓	✓			72. MICA/B-TCR IL B2M ^{-/-} CIITA ^{-/-} HLA-G
✓									73. MICA/B-CAR CD38 ^{-/-} CD16 ^{exo} CAR ^(2nd)
✓							✓		74. MICA/B-TCR CD38 ^{-/-} CD16 ^{exo} CAR ^(2nd)
									75. MICA/B-CAR CD38 ^{-/-} CD16 ^{exo} IL
✓	✓	✓	✓	✓	✓	✓		✓	76. MICA/B-TCR CD38 ^{-/-} CD16 ^{exo} IL
									77. MICA/B-CAR CD16 ^{exo} B2M ^{-/-} CIITA ^{-/-}
									78. MICA/B-TCR CD16 ^{exo} B2M ^{-/-} CIITA ^{-/-}
✓	✓	✓	✓	✓	✓	✓		✓	79. MICA/B-CAR CD38 ^{-/-} CD16 ^{exo} B2M ^{-/-} CIITA ^{-/-} CD58 ^{-/-}
									80. MICA/B-CAR CD38 ^{-/-} CD16 ^{exo} B2M ^{-/-} CIITA ^{-/-} CD54 ^{-/-}
									81. MICA/B-CAR CD38 ^{-/-} CD16 ^{exo} B2M ^{-/-} CIITA ^{-/-} CD58 ^{-/-} CD54 ^{-/-}
									82. MICA/B-CAR CD38 ^{-/-} CD16 ^{exo} B2M ^{-/-} CIITA ^{-/-} HLA-G
									83. MICA/B-TCR CD38 ^{-/-} CD16 ^{exo} B2M ^{-/-} CIITA ^{-/-} CD58 ^{-/-}
									84. MICA/B-TCR CD38 ^{-/-} CD16 ^{exo} B2M ^{-/-} CIITA ^{-/-} CD54 ^{-/-}
									85. MICA/B-TCR CD38 ^{-/-} CD16 ^{exo} B2M ^{-/-} CIITA ^{-/-} CD58 ^{-/-} CD54 ^{-/-}
✓	✓	✓	✓	✓	✓	✓	✓	✓	86. MICA/B-TCR CD38 ^{-/-} CD16 ^{exo} B2M ^{-/-} CIITA ^{-/-} HLA-G
									87. MICA/B-CAR CD38 ^{-/-} CAR ^(2nd) IL
									88. MICA/B-TCR CD38 ^{-/-} CAR ^(2nd) IL
✓	✓	✓	✓	✓	✓	✓		✓	89. MICA/B-CAR CD38 ^{-/-} CAR ^(2nd) IL B2M ^{-/-} CIITA ^{-/-}
									90. MICA/B-TCR CD38 ^{-/-} CAR ^(2nd) IL B2M ^{-/-} CIITA ^{-/-}
✓	✓	✓	✓	✓	✓	✓		✓	91. MICA/B-CAR CD38 ^{-/-} CAR ^(2nd) IL B2M ^{-/-} CIITA ^{-/-} CD58 ^{-/-}

							92. MICA/B-CAR CD38 ^{-/-} CAR ^(2nd) IL B2M ^{-/-} CIITA ^{-/-} CD54 ^{-/-}
							93. MICA/B-CAR CD38 ^{-/-} CAR ^(2nd) IL B2M ^{-/-} CIITA ^{-/-} CD58 ^{-/-} CD54 ^{-/-}
							94. MICA/B-CAR CD38 ^{-/-} CAR ^(2nd) IL B2M ^{-/-} CIITA ^{-/-} HLA-G
							95. MICA/B-TCR CD38 ^{-/-} CAR ^(2nd) IL B2M ^{-/-} CIITA ^{-/-} CD58 ^{-/-}
							96. MICA/B-TCR CD38 ^{-/-} CAR ^(2nd) IL B2M ^{-/-} CIITA ^{-/-} CD54 ^{-/-}
							97. MICA/B-TCR CD38 ^{-/-} CAR ^(2nd) IL B2M ^{-/-} CIITA ^{-/-} CD58 ^{-/-} CD54 ^{-/-}
							98. MICA/B-TCR CD38 ^{-/-} CAR ^(2nd) IL B2M ^{-/-} CIITA ^{-/-} HLA-G
✓	✓	✓	✓	✓	✓	✓	99. MICA/B-CAR CD38 ^{-/-} IL B2M ^{-/-} CIITA ^{-/-}
							100. MICA/B-TCR CD38 ^{-/-} IL B2M ^{-/-} CIITA ^{-/-}
✓	✓	✓	✓	✓	✓	✓	101. MICA/B-CAR CD38 ^{-/-} IL B2M ^{-/-} CIITA ^{-/-} CD58 ^{-/-}
							102. MICA/B-CAR CD38 ^{-/-} IL B2M ^{-/-} CIITA ^{-/-} CD54 ^{-/-}
							103. MICA/B-CAR CD38 ^{-/-} IL B2M ^{-/-} CIITA ^{-/-} CD58 ^{-/-} CD54 ^{-/-}
							104. MICA/B-CAR CD38 ^{-/-} IL B2M ^{-/-} CIITA ^{-/-} HLA-G
							105. MICA/B-TCR CD38 ^{-/-} IL B2M ^{-/-} CIITA ^{-/-} CD58 ^{-/-}
							106. MICA/B-TCR CD38 ^{-/-} IL B2M ^{-/-} CIITA ^{-/-} CD54 ^{-/-}
							107. MICA/B-TCR CD38 ^{-/-} IL B2M ^{-/-} CIITA ^{-/-} CD58 ^{-/-} CD54 ^{-/-}
							108. MICA/B-TCR CD38 ^{-/-} IL B2M ^{-/-} CIITA ^{-/-} HLA-G
✓	✓	✓	✓	✓	✓	✓	109. MICA/B-CAR CD16 ^{exo} CAR ^(2nd) IL
							110. MICA/B-TCR CD16 ^{exo} CAR ^(2nd) IL
✓	✓	✓	✓	✓	✓	✓	111. MICA/B-CAR CD16 ^{exo} CAR ^(2nd) B2M ^{-/-} CIITA ^{-/-}
							112. MICA/B-TCR CD16 ^{exo} CAR ^(2nd) B2M ^{-/-} CIITA ^{-/-}
✓	✓	✓	✓	✓	✓	✓	113. MICA/B-CAR CD16 ^{exo} CAR ^(2nd) B2M ^{-/-} CIITA ^{-/-} CD58 ^{-/-}
							114. MICA/B-CAR CD16 ^{exo} CAR ^(2nd) B2M ^{-/-} CIITA ^{-/-} CD54 ^{-/-}
							115. MICA/B-CAR CD16 ^{exo} CAR ^(2nd) B2M ^{-/-} CIITA ^{-/-} CD58 ^{-/-} CD54 ^{-/-}
							116. MICA/B-CAR CD16 ^{exo} CAR ^(2nd) B2M ^{-/-} CIITA ^{-/-} HLA-G
							117. MICA/B-TCR CD16 ^{exo} CAR ^(2nd) B2M ^{-/-} CIITA ^{-/-} CD58 ^{-/-}
							118. MICA/B-TCR CD16 ^{exo} CAR ^(2nd) B2M ^{-/-} CIITA ^{-/-} CD54 ^{-/-}
							119. MICA/B-TCR CD16 ^{exo} CAR ^(2nd) B2M ^{-/-} CIITA ^{-/-} CD58 ^{-/-} CD54 ^{-/-}
							120. MICA/B-TCR CD16 ^{exo} CAR ^(2nd) B2M ^{-/-} CIITA ^{-/-} HLA-G
✓	✓	✓	✓	✓	✓	✓	121. MICA/B-CAR CD16 ^{exo} IL B2M ^{-/-} CIITA ^{-/-}
							122. MICA/B-TCR CD16 ^{exo} IL B2M ^{-/-} CIITA ^{-/-}
✓	✓	✓	✓	✓	✓	✓	123. MICA/B-CAR CD16 ^{exo} IL B2M ^{-/-} CIITA ^{-/-} CD58 ^{-/-}

									124. MICA/B-CAR CD16 ^{exo} IL B2M ^{-/-} CIITA ^{-/-} CD54 ^{-/-}
									125. MICA/B-CAR CD16 ^{exo} IL B2M ^{-/-} CIITA ^{-/-} CD58 ^{-/-} CD54 ^{-/-}
									126. MICA/B-CAR CD16 ^{exo} IL B2M ^{-/-} CIITA ^{-/-} HLA-G
									127. MICA/B-TCR CD16 ^{exo} IL B2M ^{-/-} CIITA ^{-/-} CD58 ^{-/-}
									128. MICA/B-TCR CD16 ^{exo} IL B2M ^{-/-} CIITA ^{-/-} CD54 ^{-/-}
									129. MICA/B-TCR CD16 ^{exo} IL B2M ^{-/-} CIITA ^{-/-} CD58 ^{-/-} CD54 ^{-/-}
									130. MICA/B-TCR CD16 ^{exo} IL B2M ^{-/-} CIITA ^{-/-} HLA-G
✓			✓			✓	✓		131. MICA/B-CAR CAR ^(2nd) CD16 ^{exo} IL B2M ^{-/-} CIITA ^{-/-}
									132. MICA/B-TCR CAR ^(2nd) CD16 ^{exo} IL B2M ^{-/-} CIITA ^{-/-}
✓			✓			✓	✓	✓	133. MICA/B-CAR CAR ^(2nd) CD16 ^{exo} IL B2M ^{-/-} CIITA ^{-/-} CD58 ^{-/-}
									134. MICA/B-CAR CAR ^(2nd) CD16 ^{exo} IL B2M ^{-/-} CIITA ^{-/-} CD54 ^{-/-}
									135. MICA/B-CAR CAR ^(2nd) CD16 ^{exo} IL B2M ^{-/-} CIITA ^{-/-} CD58 ^{-/-} CD54 ^{-/-}
									136. MICA/B-CAR CAR ^(2nd) CD16 ^{exo} IL B2M ^{-/-} CIITA ^{-/-} HLA-G
									137. MICA/B-TCR CAR ^(2nd) CD16 ^{exo} IL B2M ^{-/-} CIITA ^{-/-} CD58 ^{-/-}
									138. MICA/B-TCR CAR ^(2nd) CD16 ^{exo} IL B2M ^{-/-} CIITA ^{-/-} CD54 ^{-/-}
									139. MICA/B-TCR CAR ^(2nd) CD16 ^{exo} IL B2M ^{-/-} CIITA ^{-/-} CD58 ^{-/-} CD54 ^{-/-}
									140. MICA/B-TCR CAR ^(2nd) CD16 ^{exo} IL B2M ^{-/-} CIITA ^{-/-} HLA-G
✓	✓	✓	✓	✓	✓	✓	✓		141. MICA/B-CAR CD38 ^{-/-} CD16 ^{exo} CAR ^(2nd) IL
									142. MICA/B-TCR CD38 ^{-/-} CD16 ^{exo} CAR ^(2nd) IL
✓	✓	✓	✓	✓	✓	✓	✓		143. MICA/B-CAR CD38 ^{-/-} CD16 ^{exo} CAR ^(2nd) B2M ^{-/-} CIITA ^{-/-}
									144. MICA/B-TCR CD38 ^{-/-} CD16 ^{exo} CAR ^(2nd) B2M ^{-/-} CIITA ^{-/-}
✓	✓	✓	✓	✓	✓	✓	✓	✓	145. MICA/B-CAR CD38 ^{-/-} CD16 ^{exo} CAR ^(2nd) B2M ^{-/-} CIITA ^{-/-} CD58 ^{-/-}
									146. MICA/B-CAR CD38 ^{-/-} CD16 ^{exo} CAR ^(2nd) B2M ^{-/-} CIITA ^{-/-} CD54 ^{-/-}
									147. MICA/B-CAR CD38 ^{-/-} CD16 ^{exo} CAR ^(2nd) B2M ^{-/-} CIITA ^{-/-} CD58 ^{-/-} CD54 ^{-/-}
									148. MICA/B-CAR CD38 ^{-/-} CD16 ^{exo} CAR ^(2nd) B2M ^{-/-} CIITA ^{-/-} HLA-G
									149. MICA/B-TCR CD38 ^{-/-} CD16 ^{exo} CAR ^(2nd) B2M ^{-/-} CIITA ^{-/-} CD58 ^{-/-}
									150. MICA/B-TCR CD38 ^{-/-} CD16 ^{exo} CAR ^(2nd) B2M ^{-/-} CIITA ^{-/-} CD54 ^{-/-}
									151. MICA/B-TCR CD38 ^{-/-} CD16 ^{exo} CAR ^(2nd) B2M ^{-/-} CIITA ^{-/-} CD58 ^{-/-} CD54 ^{-/-}
									152. MICA/B-TCR CD38 ^{-/-} CD16 ^{exo} CAR ^(2nd) B2M ^{-/-} CIITA ^{-/-} HLA-G
✓	✓	✓	✓	✓	✓	✓	✓		153. MICA/B-CAR CD38 ^{-/-} CD16 ^{exo} IL B2M ^{-/-} CIITA ^{-/-}
									154. MICA/B-TCR CD38 ^{-/-} CD16 ^{exo} IL B2M ^{-/-} CIITA ^{-/-}
✓	✓	✓	✓	✓	✓	✓	✓	✓	155. MICA/B-CAR CD38 ^{-/-} CD16 ^{exo} IL B2M ^{-/-} CIITA ^{-/-} CD58 ^{-/-}

								156. MICA/B-CAR CD38 ^{-/-} CD16 ^{exo} IL B2M ^{-/-} CIITA ^{-/-} CD54 ^{-/-}
								157. MICA/B-CAR CD38 ^{-/-} CD16 ^{exo} IL B2M ^{-/-} CIITA ^{-/-} CD58 ^{-/-} CD54 ^{-/-}
								158. MICA/B-CAR CD38 ^{-/-} CD16 ^{exo} IL B2M ^{-/-} CIITA ^{-/-} HLA-G
								159. MICA/B-TCR CD38 ^{-/-} CD16 ^{exo} IL B2M ^{-/-} CIITA ^{-/-} CD58 ^{-/-}
								160. MICA/B-TCR CD38 ^{-/-} CD16 ^{exo} IL B2M ^{-/-} CIITA ^{-/-} CD54 ^{-/-}
								161. MICA/B-TCR CD38 ^{-/-} CD16 ^{exo} IL B2M ^{-/-} CIITA ^{-/-} CD58 ^{-/-} CD54 ^{-/-}
								162. MICA/B-TCR CD38 ^{-/-} CD16 ^{exo} IL B2M ^{-/-} CIITA ^{-/-} HLA-G
✓	✓	✓	✓	✓	✓	✓	✓	163. MICA/B-CAR CD38 ^{-/-} CAR ^(2nd) IL B2M ^{-/-} CIITA ^{-/-}
								164. MICA/B-TCR CD38 ^{-/-} CAR ^(2nd) IL B2M ^{-/-} CIITA ^{-/-}
✓	✓	✓	✓	✓	✓	✓	✓	165. MICA/B-CAR CD38 ^{-/-} CAR ^(2nd) IL B2M ^{-/-} CIITA ^{-/-} CD58 ^{-/-}
								166. MICA/B-CAR CD38 ^{-/-} CAR ^(2nd) IL B2M ^{-/-} CIITA ^{-/-} CD54 ^{-/-}
								167. MICA/B-CAR CD38 ^{-/-} CAR ^(2nd) IL B2M ^{-/-} CIITA ^{-/-} CD58 ^{-/-} CD54 ^{-/-}
								168. MICA/B-CAR CD38 ^{-/-} CAR ^(2nd) IL B2M ^{-/-} CIITA ^{-/-} HLA-G
								169. MICA/B-TCR CD38 ^{-/-} CAR ^(2nd) IL B2M ^{-/-} CIITA ^{-/-} CD58 ^{-/-}
								170. MICA/B-TCR CD38 ^{-/-} CAR ^(2nd) IL B2M ^{-/-} CIITA ^{-/-} CD54 ^{-/-}
								171. MICA/B-TCR CD38 ^{-/-} CAR ^(2nd) IL B2M ^{-/-} CIITA ^{-/-} CD58 ^{-/-} CD54 ^{-/-}
								172. MICA/B-TCR CD38 ^{-/-} CAR ^(2nd) IL B2M ^{-/-} CIITA ^{-/-} HLA-G
✓		✓	✓	✓	✓	✓	✓	173. MICA/B-CAR CD16 ^{exo} CAR ^(2nd) IL B2M ^{-/-} CIITA ^{-/-}
								174. MICA/B-TCR CD16 ^{exo} CAR ^(2nd) IL B2M ^{-/-} CIITA ^{-/-}
✓		✓	✓	✓	✓	✓	✓	175. MICA/B-CAR CD16 ^{exo} CAR ^(2nd) IL B2M ^{-/-} CIITA ^{-/-} CD58 ^{-/-}
								176. MICA/B-CAR CD16 ^{exo} CAR ^(2nd) IL B2M ^{-/-} CIITA ^{-/-} CD54 ^{-/-}
								177. MICA/B-CAR CD16 ^{exo} CAR ^(2nd) IL B2M ^{-/-} CIITA ^{-/-} CD58 ^{-/-} CD54 ^{-/-}
								178. MICA/B-CAR CD16 ^{exo} CAR ^(2nd) IL B2M ^{-/-} CIITA ^{-/-} HLA-G
								179. MICA/B-TCR CD16 ^{exo} CAR ^(2nd) IL B2M ^{-/-} CIITA ^{-/-} CD58 ^{-/-}
								180. MICA/B-TCR CD16 ^{exo} CAR ^(2nd) IL B2M ^{-/-} CIITA ^{-/-} CD54 ^{-/-}
								181. MICA/B-TCR CD16 ^{exo} CAR ^(2nd) IL B2M ^{-/-} CIITA ^{-/-} CD58 ^{-/-} CD54 ^{-/-}
								182. MICA/B-TCR CD16 ^{exo} CAR ^(2nd) IL B2M ^{-/-} CIITA ^{-/-} HLA-G
✓	✓	✓	✓	✓	✓	✓	✓	183. MICA/B-CAR CD38 ^{-/-} CD16 ^{exo} CAR ^(2nd) IL B2M ^{-/-} CIITA ^{-/-}
								184. MICA/B-TCR CD38 ^{-/-} CD16 ^{exo} CAR ^(2nd) IL B2M ^{-/-} CIITA ^{-/-}
✓	✓	✓	✓	✓	✓	✓	✓	185. MICA/B-CAR CD38 ^{-/-} CD16 ^{exo} CAR ^(2nd) IL B2M ^{-/-} CIITA ^{-/-} CD58 ^{-/-}
								186. MICA/B-CAR CD38 ^{-/-} CD16 ^{exo} CAR ^(2nd) IL B2M ^{-/-} CIITA ^{-/-} CD54 ^{-/-}

187. MICA/B-CAR CD38 ^{-/-} CD16 ^{exo} CAR ^(2nd) IL B2M ^{-/-} CIITA ^{-/-} CD58 ^{-/-} CD54 ^{-/-}						
188. MICA/B-CAR CD38 ^{-/-} CD16 ^{exo} CAR ^(2nd) IL B2M ^{-/-} CIITA ^{-/-} HLA-G						
189. MICA/B-TCR CD38 ^{-/-} CD16 ^{exo} CAR ^(2nd) IL B2M ^{-/-} CIITA ^{-/-} CD58 ^{-/-}						
190. MICA/B-TCR CD38 ^{-/-} CD16 ^{exo} CAR ^(2nd) IL B2M ^{-/-} CIITA ^{-/-} CD54 ^{-/-}						
191. MICA/B-TCR CD38 ^{-/-} CD16 ^{exo} CAR ^(2nd) IL B2M ^{-/-} CIITA ^{-/-} CD58 ^{-/-} CD54 ^{-/-}						
192. MICA/B-TCR CD38 ^{-/-} CD16 ^{exo} CAR ^(2nd) IL B2M ^{-/-} CIITA ^{-/-} HLA-G						

7. *Additional modifications*

[000203] 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 1. In some embodiments, the iPSC, and its derivative effector cells comprising any one of the genotypes in Table 1 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.

[000204] Engagers are fusion proteins consisting of two or more single-chain variable fragments (scFvs) of different antibodies, with at least one scFv binds to an effector cell surface molecule, and at least another to a tumor cell via a tumor 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.

[000205] In some embodiments, the engager is used in combination with a population of the effector cells described herein by concurrent or consecutive administration thereof, 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 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 above, 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.

[000206] 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, EGFR ν III, EPCAM, FLT-3, FOLR1, FOLR3, GD2, gpA33, HER2, HM1.24, LGR5, MSLN, MCSP, MICA/B, PSMA, PAMA, P-cadherin, ROR1. In one embodiment, the bi-specific antibody is 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.

[000207] 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 may also originate from other cytokines including, but not limited to, IL2, IL4, IL6, IL7, IL9, IL10, IL11, IL12, IL18, and IL21.

[000208] 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 1, 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.

8. *Antibodies for immunotherapy*

[000209] In some embodiments, in addition to the genomically engineered effector cells 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 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.

[000210] As discussed above, the shedding of MICA/B on tumor cells results in increased soluble MICA/B, which may enable tumor evasion/escape and inhibit immunosurveillance thereof. As provided in the present specification, an antibody that binds to a MIC alpha 3-domain (e.g., a MICA or MICB alpha 3-domain) may be used to prevent surface MICA/B shedding observed in many human and murine tumor cell lines, resulting in an increase in MICA/B cell surface density, reduced soluble shedding of MICA/B, enhanced detection capability and/or enhanced NK and/or T cell mediated tumor killing. In some embodiments, the antibody targeting a MIC alpha 3-domain (e.g., a MICA or MICB alpha 3-domain) is used in combination with a population of the effector cells described herein by concurrent or consecutive administration thereof to a subject in need of treatment. 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.

[000211] Thus, the genomically engineered effector cells as provided herein may be used in a combination therapy that includes a MICA/B antibody that binds to a cell surface MICA/B antigen and/or one or more additional therapeutic agents comprising an antibody, or an antibody fragment that targets a different antigen associated with a condition, a disease, or an indication. In some embodiments, the MICA/B antibody that binds to a MIC alpha 3-domain of a cell surface MICA/B antigen. In some embodiments, the additional therapeutic antibody is a monoclonal antibody. In some embodiments, the additional therapeutic antibody is a humanized antibody, a humanized monoclonal antibody, or a chimeric antibody. In some embodiments, the additional therapeutic antibody, or antibody fragment, specifically binds to a viral antigen. In

other embodiments, the additional therapeutic 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 additional therapeutic antibodies suitable for combinational treatment as an additional therapeutic agent to the administered iPSC-derived effector cells, either alone or in combination with an antibody that binds to a MIC alpha 3-domain (e.g., a MICA or MICB alpha 3-domain), 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 (certuximab, amivantamab), 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 antibody (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.

[000212] In some embodiments, the iPSC-derived effector cells comprise hematopoietic lineage cells comprising a genotype listed in Table 1. In some embodiments, the iPSC-derived effector cells comprise NK cells comprising a genotype listed in Table 1. In some embodiments, the iPSC-derived effector cells comprise T cells comprising a genotype listed in Table 1.

[000213] In some embodiments of a combination useful for treating liquid or solid tumors, the combination comprises one or more preselected monoclonal antibodies and iPSC-derived NK or T cells comprising MICA/B tumor antigen targeting specificity, wherein the cells optionally comprise one or more polynucleotides encoding a MICA/B-CAR, an exogenous TCR targeting a tumor MICA/B peptide, a CD16 or a variant thereof that mediates ADCC when in combination with an anti-MICA/B antibody, or any combination thereof. In some embodiments of a combination useful for treating liquid or solid tumors, the combination comprises an anti-MICA/B monoclonal antibody and iPSC-derived NK or T cells comprising MICA/B tumor antigen targeting specificity described herein. In some embodiments of a treatment combination

comprising a MICA/B monoclonal antibody and iPSC-derived NK or T cells described herein, the MICA/B monoclonal antibody is expressed in a population of NK cells comprising a MICA/B tumor antigen targeting specificity described herein, wherein the cells further comprise a polynucleotide encoding the MICA/B monoclonal antibody. In some embodiments, the MICA/B monoclonal antibody is one of 7C6, 6F11 and 1C2. In some embodiments of a combination useful for treating liquid or solid tumors, the combination comprises iPSC-derived NK or T cells comprising MICA/B tumor antigen targeting specificity described herein, the cells further comprise CD38 knockout, and one or more anti-CD38 antibodies. In one embodiment, the anti-CD38 antibodies comprise daratumumab, isatuximab, and MOR202. In one embodiment, the combination comprises iPSC-derived NK cells comprising MICA/B tumor antigen targeting specificity described herein, wherein the cells further comprise CD38 knockout, and daratumumab. In some further embodiments, the iPSC-derived NK cells comprised in the combination with daratumumab further comprise a IL15 and/or a second CAR targeting CD38 or one of CD19, BCMA, CD20, CD22, CD123, HER2, CD52, EGFR, GD2, MSLN, VEGF-R2, PSMA and PDL1; wherein the IL15 is co- or separately expressed with the CAR; and IL15 is in any one of the forms discussed above. In some particular embodiments, IL15 is co- or separately expressed with the CAR.

[000214] In some embodiments, the combination comprises iPSC-derived NK cells comprising MICA/B tumor antigen targeting specificity described herein; and one or more of an anti-HER2 antibody (e.g., trastuzumab, pertuzumab), an anti-EGFR antibody (e.g., cetuximab, amivantamab), or an anti-PDL1 antibody (e.g., avelumab). In some further embodiments, the iPSC-derived NK cells comprised in the combination with any of the anti-HER2 antibodies, anti-EGFR antibodies or anti-PDL1 antibodies, comprise MICA/B tumor antigen targeting specificity described herein, and optionally one or more of CD38 knockout, IL15, and a second CAR targeting CD38 or one of CD19, BCMA, CD20, CD22, CD123, HER2, CD52, EGFR, GD2, MSLN, VEGF-R2, PSMA and PDL1; wherein the IL15 is co- or separately expressed with the CAR; and IL15 is in any one of the forms discussed above. In some particular embodiments, IL15 is co- or separately expressed with the CAR. In some embodiments of a combination useful for treating liquid or solid tumors, the combination comprises iPSC-derived NK or T cells comprising MICA/B tumor antigen targeting specificity described herein, wherein the cells optionally comprise one or more of TCR^{neg}, CD38 knockout, HLA-I and/or HLA-II deficiency, and a cytokine signaling complex; and a therapeutic antibody as described above.

9. Checkpoint inhibitors

[000215] 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 some embodiments, the checkpoint inhibitor is used in combination with a population of the effector cells 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 described herein comprise at least one checkpoint inhibitor to target at least one checkpoint molecule; wherein the derivative cells comprise MICA/B tumor antigen targeting specificity and optionally one or more additional attributes listed in Table 1.

[000216] In some embodiments, the exogenous polynucleotide sequence encoding the checkpoint inhibitor, or a fragment thereof is co-expressed with a CAR, either in separate constructs or in a bi-cistronic construct. In some further embodiments, the sequence encoding the checkpoint inhibitor 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 are 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 one embodiment of the combination therapy,

the derivative effector cells are NK lineage cells, as described above. In another embodiment of the combination therapy, the derivative effector cells are T lineage cells, as described above.

[000217] 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 (Pdcd1, 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).

[000218] 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.

[000219] 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.

[000220] In some embodiments, the checkpoint inhibitor is co-expressed with a CAR and inhibits at least one of the following 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 a CAR in a derivative cell having a genotype listed in Table 1 is selected from the group comprising 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, 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.

[000221] In some other embodiments of the combination therapy comprising the derivative cells provided herein and at least one antibody inhibiting a checkpoint molecule, said antibody is not produced by, or in, the derivative cells and is additionally administered before, with, or after the administering of the derivative cells comprising MICA/B tumor antigen targeting specificity described herein and optionally one or more additional attributes listed in Table 1. In some embodiments, the administering of one, two, three or more checkpoint inhibitors in a combination therapy with the provided derivative NK or T cells are simultaneous or sequential. In one embodiment of the combination treatment comprising derived NK cells or T cells comprising MICA/B tumor antigen targeting specificity described herein and optionally one or more additional genomic edits resulting in a genotype listed in Table 1, 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 comprising MICA/B tumor antigen targeting specificity described herein and optionally one or more additional genomic edits resulting in a genotype listed in Table 1, 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 comprising MICA/B tumor antigen targeting specificity described herein and optionally one or more additional genomic edits resulting in a genotype listed in Table 1, 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 comprising MICA/B tumor antigen targeting specificity described herein and optionally one or more additional genomic edits resulting in a genotype listed in Table 1, the checkpoint inhibitor included in the treatment is pembrolizumab, or its humanized or Fc modified variant, fragment or its functional equivalent or biosimilar.

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

[000222] 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.

[000223] 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.

[000224] 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.

[000225] Inserting one or more transgenes at a selected position in a gene locus of interest (GOI) to knock-out the gene at the same time can be achieved. Gene loci suitable for simultaneous knock-in and knockout (KI/KO) include, but are not limited to, B2M, TAP1, TAP2, tapasin, NLRC5, CIITA, RFXANK, RFX5, RFXAP, TCR α or β constant region, NKG2A, NKG2D, CD38, CD25, CD69, CD71, CD44, CD58, CD54, CD56, CIS, CBL-B, SOCS2, PD1, CTLA4, LAG3, TIM3, and TIGIT. 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 in 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, 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.

[000226] 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.

[000227] 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.

[000228] 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 Patent Application No. 2011/0145940, which is herein incorporated by reference. The most recognized example of a TALEN in the art is a fusion polypeptide of the FokI nuclease to a TAL effector DNA binding domain.

[000229] 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.

[000230] 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.

[000231] 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.

[000232] 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.

[000233] 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 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.

[000234] 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.

[000235] Promising sites for targeted integration include, but are not limited to, safe harbor loci, or 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 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 integration 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 precise 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.

[000236] 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. Additionally, 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. Further, collagen and HTRP gene loci may also be used as safe harbor for targeted integration. However, validation of each selected site has been shown to be necessary 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.

[000237] For targeted in/dels, 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 immune response regulation and modulation. In some other embodiments, the endogenous gene comprising a targeted in/del is associated with targeting modality, receptors, signaling molecules, transcription factors, drug target candidates, immune response regulation and modulation, or proteins suppressing engraftment, trafficking, homing, viability, self-renewal, persistence, and/or survival of stem cells and/or progenitor cells, and the derived cells therefrom.

[000238] As such, another aspect of the present invention provides a method of targeted integration in a selected locus including genome safe harbor or a preselected locus known or proven to be safe and well-regulated for continuous or temporal gene expression such as the B2M, TAP1, TAP2, Tapasin, TRAC, or CD38 locus as provided herein. In one embodiment, the genome safe harbor for the method of targeted integration comprises one or more desired integration site comprising AAVS1, CCR5, ROSA26, collagen, HTRP, H11, beta-2 microglobulin, CD38, GAPDH, TCR or RUNX1, or other loci meeting the criteria of a genome safe harbor. In one embodiment, the method of targeted integration in a cell comprising introducing a construct comprising one or more exogenous polynucleotides to the cell, and introducing a construct comprising a pair of homologous arm 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, 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, NKG2A, NKG2D, CD38, CD25, CD69, CD71, CD44, CD58, CD54, CD56, CIS, CBL-B, SOCS2, PD1, CTLA4, LAG3, TIM3, or TIGIT.

[000239] 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, 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, 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, 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, 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.

[000240] Further, as provided herein, the above method for targeted integration in a safe harbor 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 safety switch protein. Suitable suicide gene systems for induced cell death include, but not limited to Caspase 9 (or caspase 3 or 7) and AP1903; thymidine kinase (TK) and ganciclovir (GCV); cytosine deaminase (CD) and 5-fluorocytosine (5-FC). Additionally, some suicide gene systems are cell

type specific, for example, the genetic modification of T lymphocytes with the B-cell molecule CD20 allows their elimination upon administration of mAb Rituximab. Further, modified EGFR containing epitope recognized by cetuximab can be used to deplete genetically engineered cells when the cells are exposed to cetuximab. As such, one aspect of the invention provides a method of targeted integration of one or more suicide genes encoding safety switch proteins selected from caspase 9 (caspase 3 or 7), thymidine kinase, cytosine deaminase, modified EGFR, and B cell CD20.

[000241] 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.

[000242] 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 AAVS1 locus in the genome of a cell. In one embodiment, at least one integrated polynucleotide is driven by the endogenous AAVS1 promoter. In another embodiment, the method described herein is used for targeted integration at ROSA26 locus in the genome of a cell. In one embodiment, at least one integrated polynucleotide is driven by the endogenous ROSA26 promoter. In still another embodiment, the method described herein is used for targeted integration at H11 locus in the genome of a cell. In one embodiment, at least one integrated polynucleotide is driven by the endogenous H11 promoter. In another embodiment, the method described herein is used for targeted integration at collagen locus in the genome of a cell. In one embodiment, at least one integrated polynucleotide is driven by the endogenous collagen promoter. In still another embodiment, the method described herein is used for targeted integration at HTRP locus in the genome of a cell. In one embodiment, at least one integrated polynucleotide is driven by the endogenous HTRP promoter. Theoretically, only correct insertions at the desired location would enable gene expression of an exogenous gene driven by an endogenous promoter.

[000243] 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 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.

[000244] 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, pXT1, pRc/CMV, pRc/RSV, pcDNA1/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. 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 1.

III. Method of Obtaining and Maintaining Genome-engineered iPSCs

[000245] The present invention also provides a method of obtaining and maintaining genome-engineered iPSCs comprising one or more targeted edits (e.g., multiplex genomic 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 site. The targeted editing introduces into the genome iPSC, and derivative cells therefrom, 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 effector 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.

[000246] 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;

provided genomic stability without the need for culture cleaning or selection; and are readily to give rise to all three somatic lineages, *in vitro* differentiation via embryoid bodies or monolayer (without formation of embryoid bodies); and *in vivo* differentiation by teratoma formation. See, for example, International Pub. No. WO2015/134652, the disclosure of which is incorporated herein by reference.

[000247] 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.

[000248] 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 integrations 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 integration 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.

[000249] 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.

[000250] 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.

[000251] 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 comprising MICA/B tumor antigen targeting specificity described herein and optionally one or more additional genomic edits resulting in 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 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. 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.

[000252] 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 particular embodiments, the one or more polynucleotides encoding at least one reprogramming factor are introduced by a lentiviral vector. In some embodiments, the one or more polynucleotides 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.

[000253] 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 modality, 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 process 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 hiPSC with multiple modalities integrated to one or more selected target sites.

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

[000254] A further aspect of the present invention provides a method of *in vivo* differentiation of genome-engineered iPSCs by teratoma formation, wherein the differentiated cells derived *in vivo* from the genome-engineered iPSCs retain the intact and functional targeted edits including targeted integration(s) and/or in/dels at the desired site(s). In some embodiments, the differentiated cells derived *in vivo* from the genome-engineered iPSCs via teratoma formation comprise one or more inducible suicide genes integrated at one or more desired sites comprising AAVS1, CCR5, ROSA26, collagen, HTRP H11, beta-2 microglobulin, CD38, GAPDH, TCR or RUNX1, or other loci meeting the criteria of a genome safe harbor. In some other embodiments, the differentiated cells derived *in vivo* from the genome-engineered iPSCs via teratoma formation comprise polynucleotides encoding targeting modalities, or encoding proteins promoting trafficking, homing, viability, self-renewal, persistence, and/or survival of stem cells and/or progenitor cells. In some embodiments, the differentiated cells derived *in vivo* from the genome-engineered iPSCs via teratoma formation comprising one or more inducible suicide genes further comprise one or more in/dels in endogenous genes associated with immune response regulation and mediation. In some embodiments, the in/del is comprised in one or more endogenous checkpoint genes. In some embodiments, the in/del is comprised in one or more endogenous T cell receptor genes. In some embodiments, the in/del is comprised in one or more endogenous MHC class I suppressor genes. In some embodiments, the in/del is comprised in one or more endogenous genes associated with the major histocompatibility complex. In some embodiments, the in/del is comprised in one or more endogenous genes including, but not limited to, AAVS1, CCR5, ROSA26, collagen, HTRP, H11, 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, and TIGIT.

[000255] 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).

[000256] 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.

[000257] 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.

[000258] 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.

[000259] 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.

[000260] 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.

[000261] 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.

[000262] 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⁻.

[000263] 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⁺.

[000264] 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⁺.

[000265] In some embodiments, the genome-engineered iPSC-derived cells obtained from the above methods comprise one or more inducible suicide gene integrated at one or more desired integration sites comprising AAVS1, CCR5, ROSA26, collagen, HTRP, H11, 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, 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.

[000266] 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 Exogenous Functional Modalities Differentiated from Genetically Engineered iPSCs

[000267] 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 MICA/B tumor antigen targeting specificity via: a MICA/B-CAR, a CD16 or a variant thereof that mediates ADCC when in combination with an anti-MICA/B antibody, an exogenous TCR

targeting a tumor MICA/B peptide, or any combination thereof, and optionally further comprise one or more additional targeted genetic edits that result in a genotype such as that listed in Table 1, 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.

[000268] 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).

[000269] 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.

[000270] 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.

[000271] 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.

[000272] 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 MICA/B tumor antigen targeting specificity, wherein the cells optionally comprise a MICA/B-CAR, a CD16 or a variant thereof that mediates ADCC when in combination with an anti-MICA/B antibody, an exogenous TCR targeting a tumor MICA/B peptide, or any combination thereof, and optionally one or more additional targeted genetic edits that result in a genotype listed in Table 1. In some other embodiments of the composition, the genetically modified iPSC and the derivative cells thereof comprising MICA/B tumor antigen targeting specificity described herein further comprise additional genetically modified modalities comprising (1) disruption of one or more 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 and TIGIT; and/or (2) introduction of one or more 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.

[000273] 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 a combination of at least two of the following: (i) expression of one or more antigen targeting receptors; (ii) modified HLA; (iii) resistance to tumor microenvironment; (iv) recruitment of bystander immune cells and immune modulations; (v) improved on-target

specificity with reduced off-tumor effect; and (vi) improved homing, persistence, cytotoxicity, or antigen escape rescue.

[000274] In some embodiments of the composition, the iPSC-derived hematopoietic cells comprising MICA/B tumor antigen targeting specificity described herein and optionally one or more additional targeted genetic edits that result in a genotype listed in Table 1, express at least one cytokine and/or its receptor comprising IL2, IL4, IL6, IL7, IL9, IL10, IL11, IL12, IL15, IL18, or IL21, or any modified protein thereof, and express at least a MICA/B-CAR a CD16 or a variant thereof that mediates ADCC when in combination with an anti-MICA/B antibody, an exogenous TCR targeting a tumor MICA/B peptide, or any combination thereof. In some embodiments of the composition, the cells express at least one cytokine and/or its receptor comprising IL7 or IL15. 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, 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.

[000275] Additionally, the present application makes possible a combined therapeutic approach by providing rationally designed effector cells capable of synergizing with a tumor sensitizing procedure that upregulates MICA/B to augment effector cell tumor site homing, trafficking and retention, which contributes to increased effector cell cytotoxicity and persistency. As presented herein, MICA and MICB are pan tumor associated stress antigens, such that when the tumor cells are exposed to a sensitizing/stressing agent, the tumor cells have upregulated tumor cell surface MICA and MICB expression. The upregulation of the stress protein MICA/B on the tumor cell upon sensitizing also mitigates or avoids the loss of MICA/B tumor antigen by shedding (MICA/B tumor cell surface expression is dynamic and is continually being shed at different rates across different tumor lines). This aspect of the application therefore provides a method comprising exposing a MICA/B expressing tumor cell to a sensitizing agent to elevate surface MICA/B levels, thereby enhancing the therapeutic index of effector cells comprising tumor MICA/B antigen targeting specificity, including, but not limited to, via a MICA/B-CAR, a MICA/B targeting TCR, or via a MICA/B antibody mediated ADCC capability of the effector cells.

[000276] Tumor sensitization may be utilized to overcome tumor resistance by tweaking 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 drug, such as the allogeneic effector cell with desired engineered therapeutic attributes as described herein, thereby enhancing the efficacy of the therapeutic effector cells targeting the tumor. Without being bound by theory, exemplary sensitizing agents useful in the compositions and methods of the present invention may be radiation therapy, radiopharmaceuticals, or chemotherapeutic agents. Thus, the above-discussed compositions of the invention may further comprise a sensitizing agent, as described herein. In various embodiments, the sensitizing agent increases tumor cell surface expression of MICA/B.

[000277] 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).

[000278] 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 80 Gy. 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 particular embodiments, the amount of sensitizing agent is about 25.0 Gy.

[000279] 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.

[000280] 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, thiotepe, 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, 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, thiotepe, topotecan, toremifene, tositumomab, trastuzumab, tretinoin, uracil mustard, valrubicin, vinorelbine, and zoledronate. Other suitable chemo- 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.

[000281] Further, the present application provides various embodiments of a method of treating a subject having a cancer or tumor, such as a solid cancer or tumor, wherein the method comprises a step of sensitizing the cancer or tumor cell in the subject to increase or enhance surface MICA/B expression, as compared to MICA/B surface expression prior to such

contacting/exposure. In some embodiments, following sensitization of the cancer or tumor cell to increase or enhance surface MICA/B expression, an effector cell or population thereof, as described above, is given/administered to the subject, wherein the effector cell comprises MICA/B tumor antigen targeting specificity via a MICA/B-CAR, a CD16 or a variant thereof that mediates ADCC when in combination with an anti-MICA/B antibody, an exogenous TCR targeting a MICA/B peptide, or any combination thereof. In some other embodiments, one or more therapeutic agents as provided herein are further administered for combinational treatment with the effector cell or population thereof. In some embodiments, the one or more therapeutic agents comprise an antibody, an engager, or a checkpoint inhibitor, which may be provided prior to, concurrently with, or after administering the effector cells.

[000282] A variety of diseases may be ameliorated by introducing the derivative effector cells, compositions, and/or methods of the invention to a subject suitable for adoptive cell therapy. In some embodiments, the iPSC-derived hematopoietic cells, compositions, or methods 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 methods 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. In various embodiments, such therapeutic use may further comprise sensitizing a tumor cell prior to introducing the therapeutic composition comprising the effector cells and optionally one more therapeutic agents to a subject.

[000283] 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, cancer of the brain, prostate, breast, lung, colon, uterus, skin, liver, bone, pancreas, ovary, testes, bladder, kidney, head, neck, stomach, cervix, rectum, larynx, and esophagus. 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.

[000284] The treatment using the derived effector 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 particular 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.

[000285] 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.

[000286] 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 a combinational therapy can also 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.

[000287] In some embodiments of a combinational cell therapy, the therapeutic combination comprises the iPSC-derived hematopoietic lineage 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, veltuzumab, ofatumumab, ublituximab, ocaratuzumab, obinutuzumab), anti-HER2 antibodies (e.g., trastuzumab, pertuzumab), anti-CD52 antibodies (e.g., alemtuzumab), anti-EGFR antibodies (e.g., cetuximab, amivantamab), 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), anti-MICA/B antibodies (7C6, 6F11, 1C2), and their humanized or Fc modified variants or fragments or their functional equivalents or biosimilars. The present invention provides therapeutic compositions comprising the iPSC-derived effector cells comprising MICA/B tumor antigen targeting specificity described herein, wherein the cells optionally comprise one or more

additional genomic editings that result in a phenotype listed in Table 1, and an additional therapeutic agent that is an antibody, or an antibody fragment, as described above.

[000288] 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 are provided above.

[000289] 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).

[000290] 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.

[000291] The combination therapies comprising the derivative effector cells 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.

[000292] 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.

[000293] 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, thiotepe, busulfan, carmustine, lomustine, semustine), antimetabolites (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), 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-fluorouracil, fludarabine, flutamide, fulvestrant, gefitinib, gemcitabine, goserelin, hydroxyurea, ibritumomab, idarubicin, ifosfamide, imatinib, interferon alpha (2a, 2b), irinotecan, letrozole, leucovorin, leuprolide, levamisole, meclorethamine, 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](https://www.fda.gov/cder/cancer/druglistframe.htm)), both as updated from time to time.

[000294] 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.

[000295] 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).

[000296] 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.

[000297] 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.

[000298] The invention also provides, in part, the use of a pharmaceutically acceptable cell culture medium in particular compositions and/or cultures of embodiments of the present invention. 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 particular 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.

[000299] 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. For example, in some embodiments, the iPSC-derived hematopoietic lineage cells can have at least 50%, 60%, 70%, 80%, 90%, 95%, 98%, or 99% NK cells. For example, in some embodiments, the iPSC-derived hematopoietic lineage cells can have at least 50%, 60%, 70%, 80%, 90%, 95%, 98%, or 99% T 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 iPSC-derived hematopoietic lineage cells have about 95% to about 100% NK cells. In some embodiments, the iPSC-derived hematopoietic lineage cells have about 95% to about 100% T cells. 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. In some embodiments, the present invention provides therapeutic compositions having purified NK cells, such as a composition having an isolated population of about 95% NK cells to treat a subject in need of the cell therapy. In some embodiments, the present invention provides therapeutic compositions having purified T cells, such as a composition having an isolated population of about 95% T cells to treat a subject in need of the cell therapy.

[000300] 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 a MICA/B tumor antigen targeting specificity, wherein the cells optionally comprise a MICA/B-CAR, a CD16 or a variant thereof that mediates ADCC when in combination with an anti-MICA/B antibody, an exogenous TCR targeting a MICA/B peptide, or any combination thereof, and wherein the cells optionally comprise one or more additional genomic editings resulting in a genotype listed in Table 1. In some embodiments, the present application provides a method of treating a subject having a cancer or tumor by first sensitizing a cancer or tumor cell in the subject to increase or enhance surface expression of MICA/B, as compared MICA/B 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 MICA/B tumor antigen targeting specificity described herein, and wherein the cells optionally comprise one or more additional genomic editings resulting in a genotype listed in Table 1. 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.

[000301] 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 MICA/B tumor antigen targeting specificity described herein, and wherein the cells optionally comprise one or more additional genomic editings resulting in a genotype listed in Table 1; and administering 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, wherein the sensitizing agent comprises radiation therapy, radiopharmaceuticals, or chemotherapeutic agents as provided herein.

[000302] 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.

[000303] 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.

[000304] 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.

[000305] 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.

[000306] 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.

[000307] 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.

[000308] 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.

[000309] The compositions comprising a population of derived hematopoietic lineage cells 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.

[000310] 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.

[000311] 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

[000312] The following examples are offered by way of illustration and not by way of limitation.

EXAMPLE 1 – Materials and Methods

[000313] To effectively select and test suicide systems under the control of various promoters in combination with different safe harbor loci integration strategies, a proprietary hiPSC platform of the applicant was used, which enables single cell passaging and high-throughput, 96-well plate-based flow cytometry sorting, to allow for the derivation of clonal hiPSCs with single or multiple genetic modulations.

[000314] ***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) for 3–5 min at 37°C. 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, transferred tissue culture plates previously coated with Matrigel and fed every 2–3 days with FMM. Cell cultures were maintained in a humidified incubator set at 37°C and 5–10% CO₂.

[000315] ***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 2.5µg ZFN-L, 2.5µg ZFN-R and 5µg donor construct, for AAVS1 targeted insertion. For CRISPR mediated genome editing, 2 million iPSCs were transfected with a mixture of 5µg ROSA26-gRNA/Cas9 and 5µg donor construct, for ROSA26 targeted insertion. Transfection was done using 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.

[000316] ***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 containing Hanks' Balanced Salt Solution (MediaTech), 4% fetal bovine serum (Invitrogen), 1x penicillin/streptomycin (Mediatech) and 10 mM Hepes (Mediatech); 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 μ M Thiazovivn for flow cytometry sorting. Flow cytometry sorting was performed on FACS Aria II (BD Biosciences). 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 μ L Accutase. The dissociated colony is transferred to another well of a 96-well plate previously coated with 5x 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 and serves as a master cell bank.. Flow cytometry analysis was performed on Guava EasyCyte 8 HT (Millipore) and analyzed using Flowjo (FlowJo, LLC).

EXAMPLE 2 – Immune Cells Expressing MICA/B-CAR Demonstrate Pan Tumor Reactivity

[000317] The Cancer Cell Line Encyclopedia (CCLE) public database has a list of about 1600 cancer lines, of which 1083 (~65%) are either MICA or MICB positive. MICA, MICB and several other tumor co-antigen mRNA expression levels across a select collection of candidate tumor lines are shown in FIG. 1A, which indicates targetability of such cancers/tumors including, but not limited to, melanoma, cervical cancer, colorectal cancer, breast cancer, ovarian cancer and osteosarcoma, in addition to liquid tumors expressing MICA/B, by the methods and compositions provided herein. Surface protein expression of MICA/B, epidermal growth factor receptor (EGFR), HER2 and PD-L1 was further determined by flow cytometry across the selection of representative solid tumor lines, and the number of surface antigen molecules was calculated through comparing relative fluorescence intensity to reference latex beads containing a specified number of PE molecules per bead (FIG. 1B). Finally, treatment naïve patient tumor surgical sections were evaluated for MICA/B and EGFR expression using established immunohistochemical (IHC) methods. Positive MICA/B expression was observed at different staining densities across all three tumors. In contrast EGFR demonstrated distinct staining

patterns determined by tumor indication (FIG. 1C). The observed antigen heterogeneity and multiple antigen presence within the same tumor highlights an opportunity for multi-antigen targeting to maximize tumor cytotoxicity.

[000318] To test reactivity of iPSC-derived NK cells expressing a MICA/B-CAR comprising the binding domain of 7C6 antibody (MICA/B-CAR iNK), the cells were incubated with a broad collection of MICA and/or MICB positive tumor lines at a 2:1 E:T ratio at 37°C in the presence of a CD107ab antibody. After 4 hrs, the MICA/B-CAR iNK cells were surfaced stained with CD56 and CD16, to distinguish the cells from residual tumor cells, and the % IFN γ , TNF α and degranulating (CD107ab⁺) cells were quantified, showing that MICA/B-CAR iNK cells are reactive to MICA/B expressing tumor cells, as measured by cytokine production and degranulation, and as compared to CAR negative iNK cells (FIG. 2).

[000319] In a separate experiment, iPSC-derived NK cells comprising a MICA/B-targeting CAR, wherein the antigen recognition region is a scFV that specifically binds to the conserved $\alpha 3$ domain of MICA and MICB (referred to as “MICA/B-CAR” herein) were prepared and cryopreserved. A transgene profile (FIG. 3A) of the MICA/B-CAR iNK cells, as compared to unmodified iNK, expanded peripheral blood (PB) NK and NK cells contained within a peripheral blood derived mononuclear cell (PBMC) fraction, shows that the MICA/B-CAR iNK cells further comprise exogenous CD16, CD38 knockout, and IL15RF. All NK cells have the CD45⁺CD56⁺CD3⁻ profile. MICA/B-specific cytokine release in response to a 4-hour co-incubation with indicated high, medium, and low natively expressing MICA/B tumor cell lines at a 1:1 E:T ratio was measured by Meso-Scale Discover (MSD) (FIG. 3B).

[000320] Tumor growth inhibition of the MICA/B-CAR iNK cells was evaluated *in vivo* in a lung cancer xenograft model. 5 x 10⁵ lung adenocarcinoma (Calu3; low MICA/B expressing) tumor cells were injected intravenously into NSG mice on Day 0. At Day 3, mice received a first intravenous dose of 1 x 10⁷ thawed MICA/B-CAR iNK cells or 2 x 10⁶ primary T cells expressing a HER2-CAR, followed by a second equivalent dose of the same cells at Day 7 and a third dose at Day 10 (FIG. 4A). Tumor growth was evaluated during the course of treatment (FIG. 4B) and bioluminescent imaging (BLI) measurements were performed weekly (FIG. 4C).

EXAMPLE 3 – Immune Cells Expressing MICA/B-CAR Exhibits Enhanced Effector Function in the Presence of ADCC-Competent Antibodies

[000321] To test whether the pan-tumor reactivity of MICA/B-CAR iNK cells can be further extended in combination with ADCC, MICA/B-CAR iNK cells further expressing hnCD16 were incubated at a 2:1 E:T ratio with 1 x 10⁵ CaSki cells with or without Avelumab (an anti-PD-L1 antibody), Cetuximab (an anti-EGFR antibody), or Trastuzumab (an anti-HER2 antibody) at

about 1 µg/mL for 4 hrs, with the supernatant concentrations of IFN γ and TNF α quantified using the MSD platform (FIG. 5). As compared to the CAR-negative iNK cells which show minimal natural cytotoxicity against MICA/B positive tumor cell lines (refer to FIG. 2) and can only perform cytotoxicity in the context of ADCC, the tumor cell reactivity of MICA/B-CAR iNK cells was specific to the tumor line that expresses surface MICA or MICB and can be enhanced/extended effectively by ADCC-competent monoclonal antibodies (mAbs).

[000322] Additional and multiple representative resistant MICA/B⁺ tumor cell lines were tested by incubating with control NK cells (CAR-negative iNK cells) and MICA/B-CAR⁺ NK cells at a 3:1 effector:target (E:T) ratio with or without Cetuximab (an anti-EGFR antibody), Trastuzumab (an anti-HER2 antibody) or Avelumab (an anti-PD-L1 antibody). Tumor cytotoxicity was measured using xCELLigenceTM and/or IncuCyte® cell assay platforms. The direct and monoclonal antibody mediated ADCC (antibody dependent cellular cytotoxicity) shown in FIGs. 6A-6B demonstrate the multi-antigen targeting capability of MICA/B-CAR CD16⁺ iNK cells, and therefore limit any tumor escape through loss of MICA/B.

[000323] Further, wild type (WT) and MICA/B knock out (KO) cells of the triple negative breast cancer (TNBC) line, MDA-MB-231, were co-cultured with MICA/B-CAR iNK cells at a 3:1 E:T ratio with or without 5 µg/mL Avelumab. Tumor cytotoxicity was measured using xCELLigenceTM and/or IncuCyte® cell assay platforms. The direct cytotoxicity and ADCC was evaluated via the detection of differentially expressed fluorescent proteins and presented as a percentage of remaining tumor cells relative to tumor alone conditions (FIG. 7).

[000324] An EGFR exon 20 insertion mutant of the NCI-H1975 non-small cell lung cancer (NSCLC) line (FIG. 8A) and lung adenocarcinoma Calu3 cells (FIG. 8B) were incubated with MICA/B-CAR iNK cells at a 3:1 E:T ratio with and without equimolar concentrations of anti-EGFR/c-MET (Amivantamab, an IgG1 bispecific antibody targeting EGFR and MET, and exhibiting ADCC capability through Fc), anti-EGFR (Cetuximab), anti-PD-L1 (Avelumab) or anti-HER2 (Trastuzumab) mAbs and percent cytolysis or the percentage of remaining tumor cells relative to tumor alone conditions was measured over time. As shown in FIGs. 7, 8A and 8B, MICA/B-CAR iNK cell anti-tumor activity and antigen recognition is enhanced in combination with tumor targeting monoclonal antibodies.

[000325] For MICA/B-CAR effector cell *in vivo* function characterization, B16/F10 melanoma cells were engineered to express surface detectable human MICA protein and injected intravenously (i.v.) at a dose of 1.0×10^4 cells per NSG mouse. On day 3 post tumor implantation, 2×10^6 pooled primary T cells or 1×10^7 iNK cells, both engineered to express a MICA/B-CAR, were injected i.v. into mice containing wild type B16/F10 (MICA⁺) or B16/F10

MICA positive (MICA⁺) metastatic tumors. After 11 days, the number of lung B16/F10 metastatic (met) tumors were enumerated using a low magnification microscope (FIG. 9A), and both the MICA/B-CAR containing T and iNK cells reduced tumor burden *in vivo*. MICA/B-CAR iNK significantly reduced the number of lung (FIG. 9B) and liver (not shown) metastases compared to CAR negative iNK control cells in a murine metastatic melanoma model using B16-F10 cells engineered to overexpress human MICA.

[000326] In a separate *in vivo* characterization assay illustrated in FIG. 10A tumor growth inhibition related to multi-dosing of effector cells was evaluated in a human lung adenocarcinoma xenograft model. 5×10^5 lung adenocarcinoma (Calu3; low MICA/B expressing) tumor cells were injected intravenously into NSG mice on Day 0. At Day 3, mice received a first intravenous dose of 1×10^7 3MICA/B-CAR iNK cells alone, or in combination with trastuzumab (an anti-HER2 antibody commercially known as HerceptinTM), followed by a second equivalent dose of the same cells at Day 7 and a third dose at Day 10. Tumor growth was evaluated during the course of treatment and bioluminescent imaging (BLI) measurements were performed weekly (FIG. 10B). As shown, the combinatorial therapy (i.e., MICA/B-CAR iNK cells in combination with trastuzumab) demonstrate significant tumor growth inhibition (TGI) compared to HerceptinTM alone. As such, MICA/B-CAR iNK cells demonstrate *in vivo* functionality as well as enhanced *in vivo* functionality in the context of anti-HER2 (trastuzumab) ADCC.

EXAMPLE 4 – Immune Cells Expressing Dual CARs Demonstrate Increased Functionality

[000327] Resistance to targeted cell therapy can arise from antigen loss and clonal heterogeneity within the tumor. A dual CAR approach targeting two Tumor Associated Antigens (TAA) was tested, and demonstrated an effective combination targeting B cell maturation antigen (BCMA) and MICA/B. In this example, primary T cells were transduced with lentiviruses having a BCMA-CAR, MICA/B-CAR, or a combination thereof to generate single CAR- and dual CAR- T cells. Transduced cells were enriched on reporter genes located downstream of each CAR, as shown in FIG. 11. When used in combination with a therapeutic antibody, such as anti-CD38, the resulting adoptive cell therapy targets three TAAs for a complete therapeutic approach against malignant cells, such as multiple myeloma, that shed or downregulate tumor antigens to evade treatment.

[000328] To assess avidity and functional responses of the dual CAR-T cells, Nalm6-BCMA-MICA cells were incubated with the indicated CAR-T sample and exposed to a ramp of acoustic force ranging from 0 to 1000 pN using the Lumicks z-Movi®, which makes use of

acoustic waves to exert well-controlled forces to hundreds of individual cells in parallel and measure the interactions between single cells and their targets with high throughput. When the effector cells are being subjected to the force ramp corresponding to forces ranging from 0 to 1000 pN, the unbound and weakly-bound cells lift off from their targets. The higher the percentage of the bound cells, the higher the avidity and functional responses the effector cells have. The percentage of bound cells over this ramp is shown in FIG. 12A, and the dual CAR-T cells demonstrated higher avidity than CAR-T cells expressing each individual CAR.

[000329] To assess cytotoxicity of the dual CAR-T cells, Nalm6-BCMA-MICA target cells were incubated with the indicated CAR-T cell samples shown in FIG. 12B at a 1:8 E:T ratio. Co-cultures were monitored for target cell killing over 36 hours using the IncuCyte® cell assay platform. As shown, the dual CAR-T cells demonstrated increased cytotoxicity over CAR-T cells expressing each individual CAR.

[000330] To test reactivity, MICA/B-CAR T cells, BCMA-CAR T cells, or dual-CAR T cells were incubated with the indicated target lines at a 1:1 E:T ratio for 4 hours in the presence of protein transport inhibitors. At the end of the assay, cells were collected and surface stained, and the % TNF α and degranulating (CD107a⁺) cells were quantified, showing that the dual CAR-T cells demonstrate increased reactivity to MICA/B expressing tumor cells, BCMA expressing tumor cells, and tumor cells expressing both antigens, as measured by cytokine production and degranulation and compared to CAR-T cells expressing each individual CAR (FIG. 13).

[000331] A panel of multiple myeloma tumor lines was then screened for expression of tumor antigens BCMA (blue) and MICA/B (purple) by flow cytometry. As shown in FIG. 14, variable expression of each antigen was observed across several lines, and all lines were positive for at least one antigen. In a separate experiment, NLR-labeled H929 multiple myeloma tumor cells and RPMI8226 multiple myeloma tumor cells were cultured with indicated CAR-T cells, as shown in FIG. 15, and monitored for cell killing over 48 hours using the IncuCyte® assay platform. As shown, the dual CAR-T cells demonstrated greater cytotoxicity to the multiple myeloma tumor cell lines, as compared to CAR-T cells expressing each individual CAR, with corresponding area under the curve values shown in the right panel. Each of the multiple myeloma tumor lines from FIG. 14 or Nalm6-BCMA cells were co-cultured with untransduced or BCMA/MICA dual CAR-T cells for 4 hours at varying E:T ratios, before being stained for caspase 3/7 and analyzed by flow cytometry. As shown in FIG. 16, the untransduced cells demonstrated no specific cytotoxicity against the target cells, while the dual CAR-T cells recognized and killed the tumor cells of several multiple myeloma tumor lines. Calculated EC50 values for best-fit curves are also shown.

[000332] In a separate *in vivo* assay, NSG mice were injected intravenously with luciferized MM1S tumor cells and were treated two days later with vehicle only, untransduced effector cells or BCMA/MICA dual CAR-T cells. As shown in FIG. 17, tumor growth was monitored for over 16 days via total flux of luminescence and the BCMA/MICA dual CAR-T cells demonstrated significant tumor growth inhibition (TGI) compared to vehicle only or untransduced effector cells. As such, the dual CAR-T cells demonstrate enhanced *in vivo* functionality.

EXAMPLE 4 – Sensitizing a Tumor Cell Augments MICA/B Expression

[000333] An array of cancer cell lines was exposed to 0 to 25Gy x-ray radiation (± 25 Gy) to explore the possibility of sensitization of a MICA/B expressing tumor cell. After irradiation, the cells were immediately placed in culture with a MICA/B $\alpha 3$ domain binding antibody at about 15 μ g/mL to prevent cell surface MICA/B shedding and to enhance detection capability, or in culture with a control mAb, for about 36 hours at 37°C. Following incubation, cells were removed from culture using TrypLE™, to minimize any surface antigen loss, and cell surface MICA/B was detected using the 6D4 pan MICA/B binding mAb. As shown in FIG. 18A, surface MICA/B expression is stabilized by the alpha-3 domain targeting mAb, and applying a stressing agent, including irradiation, elevates surface MICA/B expression across different solid tumor cell lines. The MICA/B fold upregulation per condition ($\pm$ irradiation and $\pm$ MICA/B $\alpha 3$ domain binding antibody) was also calculated by normalizing to 0 Gy alone standard culture conditions per cell line, showing sensitizing tumor cells using, for example, irradiation, augments MICA/B expression on tumor cell surface (FIG. 18B).

[000334] To test whether tumor sensitizing would enhance tumor sensitivity to effector cells expressing MICA/B CAR, the array of tumor cell lines were plated 1×10^4 per well with or without 25Gy exposure. Approximately 18 hours later, iPSC-derived NK cells expressing a MICA/B-CAR (MICA/B-CAR iNK), or MICA/B-CAR negative PBNK cells with two-week expansion using 4-1BBL⁺ IL21⁺ K562 cells, were added at a 3:1 E:T ratio to the tumor cell line cells and incubated for an additional 24 hrs to measure the percent tumor cell cytotoxicity using the xCELLigence™ RTCA system. As shown in FIG. 19, the tumor pre-conditioned with a sensitizing agent can augment tumor cell surface MICA/B levels, which translates into enhanced tumor sensitivity to MICA/B-CAR iNK cells. This enhanced sensitivity is MICA/B:MICA/B-CAR specific as the enhanced sensitivity is not observed when MICA/B-CAR negative NK cells were used (PBNK cells). These data suggest that MICA/B surface levels somewhat correlated with MICA/B CAR iNK reactivity levels. As such, the capability of MICA/B-CAR effector cells to synergize with sensitization (e.g., irradiation) of a cancer or tumor cell, optionally in

combination with an MICA/B antibody that binds to the MIC $\alpha 3$ domain of MICA/B antigen to increase expression and/or reduce shedding, can increase the therapeutic index of an off-the-shelf product of effector cells, including iPSC-derived NK or T cells, which are further enhanced with ADCC mechanism through an exogenous CD16 expression in these effector cells.

[000335] 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.

[000336] 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.

[000337] 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 composition comprising a cell or population thereof, wherein the cell is an immune cell, an induced pluripotent cell (iPSC) or a derivative cell obtained from differentiating the iPSC; and wherein the cell comprises a MICA/B tumor antigen targeting specificity; wherein the cell optionally comprises one or more exogenous polynucleotides encoding a MICA/B-CAR (chimeric antigen receptor), a CD16 or a variant thereof that mediates ADCC when in combination with an anti-MICA/B antibody, an exogenous TCR targeting a MICA/B tumor antigen, or any combination thereof; and wherein:

- (i) the cell or population thereof further comprises a CAR having a tumor antigen targeting specificity other than MICA/B, or
- (ii) the composition further comprises a sensitizing agent, wherein the sensitizing agent increases tumor cell surface levels of MICA/B.

2. The composition of claim 1, wherein 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 wherein the derivative cell is a hematopoietic cell, and comprises longer telomeres in comparison to its native counterpart cell obtained from peripheral blood, umbilical cord blood, or any other donor tissues; or wherein the MICA/B-CAR has at least one of the following characteristics:

- (i) being T cell specific;
- (ii) being NK cell specific;
- (iii) binding to surface MICA/B;
- (iv) comprising a scFV (single chain variable fragment) binding to the conserved $\alpha 3$ domain of MICA/B;
- (v) comprising a heavy chain variable region represented by an amino acid sequence that is of at least about 99%, 98%, 96%, 95%, 90%, 85%, or 80% identity to SEQ ID NO: 1;
- (vi) comprising a light chain variable region represented by an amino acid sequence that is of at least about 99%, 98%, 96%, 95%, 90%, 85%, or 80% identity to SEQ ID NO: 2;
- (vii) comprising a scFV represented by an amino acid sequence that is of at least about 99%, 98%, 96%, 95%, 90%, 85%, or 80% identity to SEQ ID NOs: 3 or 4;
- (viii) comprising a heavy chain variable region of a MICA/B binding scFV functionally linked to a first constant region of a T cell receptor (TCR), and a light chain variable region of a MICA/B binding scFV functionally linked to a second constant region of a T cell receptor (TCR); and

(ix) being inserted at one of the gene loci: B2M, TAP1, TAP2, Tapasin, NLRC5, CIITA, RFXANK, RFX5, RFXAP, TCR α or β constant region, NKG2A, NKG2D, CD38, CD25, CD44, CD58, CD54, CD56, CD69, CD71, CIS, CBL-B, SOCS2, PD1, CTLA4, LAG3, TIM3, or TIGIT; and, optionally, wherein the insertion knocks out expression of the gene in the locus.

3. The composition of claim 1 or 2, wherein the cell further comprises one or more of:

- (i) CD38 knockout;
- (ii) HLA-I deficiency and/or HLA-II deficiency;
- (iii) introduction of HLA-G or non-cleavable HLA-G;
- (iv) an exogenous CD16 or a variant thereof;
- (v) a chimeric antigen receptor (CAR) with targeting specificity other than MICA/B;
- (vi) a cytokine signaling complex comprising a partial or full peptide of a cell surface expressed exogenous cytokine or a receptor thereof;
- (vii) at least one of the genotypes listed in Table 1;
- (viii) disruption of at least one of B2M, TAP1, TAP2, Tapasin, NLRC5, CIITA, RFXANK, RFX5, RFXAP, TCR α or β constant region, NKG2A, NKG2D, CD56, CIS, CBL-B, SOCS2, PD1, CTLA4, LAG3, TIM3, and TIGIT, in comparison to its native counterpart cell; and
- (ix) introduction of at least one of IILA-E, 4-1BBL, CD3, CD4, CD8, CD16, CD47, CD113, CD131, CD137, CD80, PDL1, A_{2A}R, antigen-specific TCR, Fc receptor, an engager, and surface triggering receptor for coupling with bi- or multi- specific or universal engagers, in comparison to its native counterpart cell.

4. The composition of claim 3, wherein the exogenous CD16 comprises a high affinity non-cleavable CD16 (hnCD16) or a variant thereof.

5. The composition of claim 4, wherein the high affinity non-cleavable CD16 (hnCD16) or a variant thereof comprises at least one of:

- (a) F176V and S197P in ectodomain domain of CD16;
- (b) a full or partial ectodomain originated from CD64;
- (c) a non-native (or non-CD16) transmembrane domain;
- (d) a non-native (or non-CD16) intracellular domain;
- (e) a non-native (or non-CD16) signaling domain;
- (f) a stimulatory domain; and

- (g) transmembrane, signaling, and stimulatory domains that are not originated from CD16, and are originated from a same or different polypeptide.
6. The composition of claim 1, wherein the sensitizing agent comprises at least one of external beam radiation, brachytherapy, a radiopharmaceutical and a chemotherapeutic agent.
7. The composition of claim 6, wherein the sensitizing agent comprises:
- (i) at least one of 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; or
 - (ii) paclitaxel.
8. The composition of claim 1 or 6, further comprising one or more therapeutic agents.
9. The composition of claim 8, wherein the one or more therapeutic agents comprise a peptide, a cytokine, a checkpoint inhibitor, an engager, an antibody, an antibody conjugate, 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, or an immunomodulatory drug (IMiD).
10. The composition of claim 9, wherein:
- (a) the checkpoint inhibitor comprises:
 - (i) one or more antagonists checkpoint molecules comprising PD-1, PDL-1, TIM-3, TIGIT, LAG-3, CTLA-4, 2B4, 4-1BB, 4-1BBL, A₂A_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;
 - (ii) one or more of atezolizumab, avelumab, cetuximab, durvalumab, ipilimumab, IPH4102, IPH43, IPH33, lirimumab, monalizumab, nivolumab, pembrolizumab, trastuzumab, pertuzumab, and their derivatives or functional equivalents;
 - (iii) at least one of atezolizumab, nivolumab, and pembrolizumab; or
 - (b) the therapeutic agents comprise one or more of venetoclax, azacitidine, and pomalidomide.
11. The composition of claim 1 or 9, wherein the antibody comprises:

- (a) an MICA/B antibody that binds to a MIC alpha 3-domain of a cell surface MICA/B antigen;
- (b) an antibody triggering antibody-dependent cellular cytotoxicity (ADCC);
- (c) an anti-CD20, an anti-CD22, an anti-HER2, an anti-CD52, an anti-EGFR, an anti-CD123, an anti-GD2, an anti-PDL1, and/or an anti-CD38 antibody; and/or
- (d) one or more of rituximab, velutuzumab, ofatumumab, ublituximab, ocaratuzumab, obinutuzumab, ibritumomab, ocrelizumab, inotuzumab, moxetumomab, epratuzumab, trastuzumab, pertuzumab, alemtuzumab, cetuximab, amivantamab, dinutuximab, avelumab, daratumumab, isatuximab, MOR202, 7G3, CSL362, elotuzumab, and their humanized or Fc modified variants or fragments and their functional equivalents and biosimilars.

12. The composition of any one of claims 1-11, further comprising a multi-specific engager.

13. The composition of claim 12, wherein the multi-specific engager is at least one of: (i) a T cell engager; (ii) an NK cell engager; (iii) a bi-specific T cell engager (BiTE); (iv) a bi-specific killer cell engager (BiKE); (v) a tri-specific killer cell engager (TriKE); (vi) a MICA/B engager; or (vii) a CD16 engager.

14. The composition of any one of claims 1-13, wherein the cell is a derivative NK or a derivative T cell, and has at least one of the following characteristics comprising:

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

in comparison to its native counterpart cell obtained from peripheral blood, umbilical cord blood, or any other donor tissues.

15. The composition of claim 5, wherein:

- (a) the transmembrane domain is derived from CD3 δ , CD3 ϵ , CD3 γ , CD3 ζ , CD4, CD8, CD8a, CD8b, CD27, CD28, CD40, CD84, CD166, 4-1BB, OX40, ICOS, ICAM-1, CTLA-4, PD-1, LAG-3, 2B4, BTLA, CD16, IL7, IL12, IL15, KIR2DL4, KIR2DS1, NKp30, NKp44, NKp46, NKG2C, NKG2D, or T cell receptor (TCR) polypeptide;
- (b) the stimulatory domain is derived from CD27, CD28, 4-1BB, OX40, ICOS, PD-1, LAG-3, 2B4, BTLA, DAP10, DAP12, CTLA-4, or NKG2D polypeptide;
- (c) the signaling domain is derived from CD3 ζ , 2B4, DAP10, DAP12, DNAM1, CD137 (4-1BB), IL21, IL7, IL12, IL15, NKp30, NKp44, NKp46, NKG2C, or NKG2D polypeptide; or
- (d) the transmembrane domain is derived from NKG2D, the non-native stimulatory domain is derived from 2B4, and the non-native signaling domain is derived from CD3 ζ .

16. The composition of claim 1, wherein the CAR having a tumor antigen targeting specificity other than MICA/B is:

- (i) T cell specific or NK cell specific;
- (ii) a bi-specific antigen binding CAR;
- (iii) a switchable CAR;
- (iv) a dimerized CAR;
- (v) a split CAR;
- (vi) a multi-chain CAR;
- (vii) an inducible CAR;
- (viii) a recombinant TCR;
- (ix) co-expressed with another CAR;
- (x) co-expressed with a cytokine signaling complex comprising a partial or full peptide of a cell surface expressed exogenous cytokine or a receptor thereof, optionally in separate constructs or in a bi-cistronic construct;
- (xi) co-expressed with a checkpoint inhibitor, optionally in separate constructs or in a bi-cistronic construct;
- (xii) specific to at least one of CD19, BCMA, CD20, CD22, CD38, CD123, HER2, CD52, EGFR, GD2, MSLN, VEGF-R2, PSMA and PDL1; and/or
- (xiii) specific to any one of ADGRE2, 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, CD99, CD123, CD133, CD138, CDS, CLEC12A, an antigen of a cytomegalovirus (CMV) infected cell, epithelial glycoprotein2 (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), 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), Lewis A (CA19.9), Lewis Y (LeY), L1 cell adhesion molecule (L1-CAM), LILRB2, melanoma antigen family A1 (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;

wherein the CAR of any one of (i) to (xiii) is optionally inserted at TRAC locus, and/or is driven by an endogenous promoter of TCR, and/or the TCR is knocked out by the CAR insertion.

17. The composition of claim 16, wherein the the CAR having a tumor antigen targeting specificity other than MICA/B is specific to BCMA.

18. The composition of claim 3, wherein the cytokine signaling complex:

- (a) comprises a partial or full peptide of at least one of IL2, IL4, IL6, IL7, IL9, IL10, IL11, IL12, IL15, IL18, IL21, and its respective receptor; or
- (b) comprises at least one of:
 - (i) co-expression of IL15 and IL15R α by using a self-cleaving peptide;
 - (ii) a fusion protein of IL15 and IL15R α ;
 - (iii) an IL15/IL15R α fusion protein with intracellular domain of IL15R α truncated;
 - (iv) a fusion protein of IL15 and membrane bound Sushi domain of IL15R α ;
 - (v) a fusion protein of IL15 and IL15R β ;
 - (vi) a fusion protein of IL15 and common receptor γ C, wherein the common receptor γ C is native or modified; and
 - (vii) a homodimer of IL15R β ; wherein any one of (b)(i)-(vii) can be co-expressed with a CAR in separate constructs or in a bi-cistronic construct; or
- (c) comprises at least one of:

- (i) a fusion protein of IL7 and IL7R α ;
 - (ii) a fusion protein of IL7 and common receptor γ C, wherein the common receptor γ C is native or modified; and
 - (iii) a homodimer of IL7R β , wherein any one of (c)(i)-(iii) is optionally co-expressed with a CAR in separate constructs or in a bi-cistronic expression cassette;
- and optionally,
- (d) is transiently expressed.

19. The composition of claim 3, wherein the cell is a derivative NK or a derivative T cell, wherein the derivative NK cell is capable of recruiting, and/or migrating T cells to tumor sites, and wherein the derivative NK or the derivative T cell is capable of reducing tumor immunosuppression in the presence of one or more checkpoint inhibitors.

20. The composition of claim 19, wherein the checkpoint inhibitors are antagonists to one or more 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.

21. The composition of claim 20, wherein the checkpoint inhibitors comprise:

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

22. The composition of claim 3, wherein 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 cell, a derivative NKT cell, a derivative NK cell, or a derivative B cell.

23. The composition of claim 1, wherein the cell comprises:

- (i) one or more exogenous polynucleotides integrated in one safe harbor locus or a selected gene locus; or

(ii) more than two exogenous polynucleotides integrated in different safe harbor loci or two or more selected gene locus.

24. The composition of claim 23, wherein the safe harbor locus comprises at least one of AAVS1, CCR5, ROSA26, collagen, HTRP, H11, GAPDH, or RUNX1; and wherein the selected gene locus is one of B2M, TAP1, TAP2, Tapasin, NLRC5, CIITA, RFXANK, RFX5, RFXAP, TCR, NKG2A, NKG2D, CD38, CD25, CD44, CD58, CD54, CD56, CD69, CD71, CIS, CBL-B, SOCS2, PD1, CTLA4, LAG3, TIM3, or TIGIT; and wherein the integration of the exogenous polynucleotide optionally knocks out expression of the gene in the locus, and optionally the exogenous polynucleotide expresses under an endogenous promoter at the gene locus.

25. The composition of claim 24, wherein the TCR locus is a constant region of TCR alpha or TCR beta.

26. Therapeutic use of the composition of any one of claims 1-25 by introducing the composition into a subject suitable for adoptive cell therapy, wherein the subject has an autoimmune disorder; a hematological malignancy; a solid tumor; cancer, or a virus infection.

27. A method of treating a disease or a condition comprising administering to a subject in need thereof the composition of any one of claims 1-7, 16 or 17.

28. The method of claim of 27, wherein the method further comprises administering one or more therapeutic agents to the subject.

29. A method of treating a subject comprising:

(a) administering a sensitizing agent to the subject to precondition tumor cells in the subject; and

(b) administering a cell or population thereof to the subject following preconditioning the tumor cells, wherein the cell is an immune cell or a derivative cell obtained from differentiating an iPSC; wherein the cell comprises a MICA/B tumor antigen targeting specificity; and wherein the cell optionally comprises one or more exogenous polynucleotides encoding a MICA/B-CAR (chimeric antigen receptor), a CD16 or a variant thereof that mediates ADCC when in combination with an anti-MICA/B antibody, an exogenous TCR targeting a MICA/B tumor antigen, or any combination thereof,

wherein the subject has an autoimmune disorder; a hematological malignancy; a solid tumor; cancer, or a virus infection.

30. The method of claim 29, wherein the sensitizing agent increases MICA/B surface expression and/or MICA/B density on the tumor cell upon contact therewith.
31. The method of claim 29, wherein the sensitizing agent comprises at least one of a chemotherapeutic agent, external beam radiation, brachytherapy, and a radiopharmaceutical.
32. The method of claim 31, wherein the sensitizing agent comprises:
- (i) at least one of x-ray radiation, gamma radiation, photon radiation, proton radiation, and neutron radiation; or
 - (ii) at least one of 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; or
 - (iii) paclitaxel.
33. The method of claim 31, further comprising administering an antibody that binds to a MIC alpha 3-domain of MICA/B tumor cell surface antigen prior to or during administering the cell or population thereof.
34. The method of claim 31, wherein the derivative cell is a hematopoietic cell, and comprises longer telomeres in comparison to its native counterpart cell obtained from peripheral blood, umbilical cord blood, or any other donor tissues; or wherein the MICA/B-CAR has at least one of the following characteristics:
- (i) being T cell specific;
 - (ii) being NK cell specific;
 - (iii) binding to surface MICA/B;
 - (iv) comprising a scFV (single chain variable fragment) binding to the conserved $\alpha 3$ domain of MICA/B;
 - (v) comprising a heavy chain variable region represented by an amino acid sequence that is of at least about 99%, 98%, 96%, 95%, 90%, 85%, or 80% identity to SEQ ID NO: 1;
 - (vi) comprising a light chain variable region represented by an amino acid sequence that is of at least about 99%, 98%, 96%, 95%, 90%, 85%, or 80% identity to SEQ ID NO: 2;
 - (vii) comprising a scFV represented by an amino acid sequence that is of at least about 99%, 98%, 96%, 95%, 90%, 85%, or 80% identity to SEQ ID NOs: 3 or 4;

(viii) comprising a heavy chain variable region of a MICA/B binding scFV functionally linked to a first constant region of a T cell receptor (TCR), and a light chain variable region of a MICA/B binding scFV functionally linked to a second constant region of a T cell receptor (TCR); and

(ix) being inserted at one of the gene loci: B2M, TAP1, TAP2, Tapasin, NLRC5, CIITA, RFXANK, RFX5, RFXAP, TCR α or β constant region, NKG2A, NKG2D, CD38, CD25, CD44, CD58, CD54, CD56, CD69, CD71, CIS, CBL-B, SOCS2, PD1, CTLA4, LAG3, TIM3, or TIGIT; and, optionally, wherein the insertion knocks out expression of the gene in the locus.

35. The method of any one of claims 29-34, wherein the cell further comprises one or more of:

- (i) CD38 knockout;
- (ii) HLA-I deficiency and/or HLA-II deficiency;
- (iii) introduction of HLA-G or non-cleavable HLA-G;
- (iv) an exogenous CD16 or a variant thereof;
- (v) a chimeric antigen receptor (CAR) with targeting specificity other than MICA/B;
- (vi) a cytokine signaling complex comprising a partial or full peptide of a cell surface expressed exogenous cytokine or a receptor thereof;
- (vii) at least one of the genotypes listed in Table 1;
- (viii) disruption in at least one of TAP1, TAP2, Tapasin, NLRC5, CIITA, RFXANK, RFX5, RFXAP, TCR α or β constant region, NKG2A, NKG2D, CD56, CIS, CBL-B, SOCS2, PD1, CTLA4, LAG3, TIM3, and TIGIT, in comparison to its native counterpart cell; and
- (ix) introduction of at least one of HLA-E, 4-1BBL, CD3, CD4, CD8, CD16, CD47, CD113, CD131, CD137, CD80, PDL1, A_{2A}R, antigen-specific TCR, Fc receptor, an engager, and surface triggering receptor for coupling with bi- or multi- specific or universal engagers, in comparison to its native counterpart cell.

36. The method of claim 35, wherein the exogenous CD16 comprises a high affinity non-cleavable CD16 (hnCD16) or a variant thereof.

37. The method of claim 36, wherein the high affinity non-cleavable CD16 (hnCD16) or a variant thereof comprises at least one of:

- (a) F176V and S197P in ectodomain domain of CD16;
- (b) a full or partial ectodomain originated from CD64;

- (c) a non-native (or non-CD16) transmembrane domain;
- (d) a non-native (or non-CD16) intracellular domain;
- (e) a non-native (or non-CD16) signaling domain;
- (f) a stimulatory domain; and
- (g) transmembrane, signaling, and stimulatory domains that are not originated from CD16, and are originated from a same or different polypeptide.

38. The method of claim 35, wherein the chimeric antigen receptor (CAR) of (v) with targeting specificity other than MICA/B is:

- (i) T cell specific or NK cell specific;
- (ii) a bi-specific antigen binding CAR;
- (iii) a switchable CAR;
- (iv) a dimerized CAR;
- (v) a split CAR;
- (vi) a multi-chain CAR;
- (vii) an inducible CAR;
- (viii) a recombinant TCR;
- (ix) co-expressed with another CAR;
- (x) co-expressed with a cytokine signaling complex comprising a partial or full peptide of a cell surface expressed exogenous cytokine or a receptor thereof, optionally in separate constructs or in a bi-cistronic construct;
- (xi) co-expressed with a checkpoint inhibitor, optionally in separate constructs or in a bi-cistronic construct;
- (xii) specific to at least one of CD19, BCMA, CD20, CD22, CD38, CD123, HER2, CD52, EGFR, GD2, MSLN, VEGF-R2, PSMA and PDL1; and/or
- (xiii) specific to any one of ADGRE2, 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, 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), 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), 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;

wherein the CAR of any one of (i) to (xiii) is optionally inserted at TRAC locus, and/or is driven by an endogenous promoter of TCR, and/or the TCR is knocked out by the CAR insertion.

39. The method of claim 38, wherein the CAR is specific to BCMA.

40. The method of claim 29, further comprising administering one or more therapeutic agents.

41. The method of claim 40, wherein the one or more therapeutic agents comprise a peptide, a cytokine, a checkpoint inhibitor, an engager, an antibody, an antibody conjugate, 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, a chemotherapeutic agent or a radioactive moiety, or an immunomodulatory drug (IMiD).

42. The method of claim 41, wherein:

(a) the checkpoint inhibitor comprises:

(i) one or more antagonists 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;

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

(iii) at least one of atezolizumab, nivolumab, and pembrolizumab; or

(b) the therapeutic agents comprise one or more of venetoclax, azacitidine, and pomalidomide.

43. The method of claim 41, wherein the antibody comprises:

- (a) an MICA/B antibody that binds to a MIC alpha 3-domain of a cell surface MICA/B antigen;
- (b) an antibody triggering antibody-dependent cellular cytotoxicity (ADCC);
- (c) an anti-CD20, an anti-CD22, an anti-HER2, an anti-CD52, an anti-EGFR, an anti-CD123, an anti-GD2, an anti-PDL1, and/or an anti-CD38 antibody; and/or
- (d) one or more of rituximab, velutuzumab, ofatumumab, ublituximab, ocaratuzumab, obinutuzumab, ibritumomab, ocrelizumab, inotuzumab, moxetumomab, epratuzumab, trastuzumab, pertuzumab, alemtuzumab, cetuximab, amivantamab, dinutuximab, avelumab, daratumumab, isatuximab, MOR202, 7G3, CSL362, elotuzumab, and their humanized or Fc modified variants or fragments and their functional equivalents and biosimilars.

44. The method of any one of claims 29-43, further comprising administering a multi-specific engager.

45. The method of claim 44, wherein the multi-specific engager is at least one of: (i) a T cell engager; (ii) an NK cell engager; (iii) a bi-specific T cell engager (BiTE); (iv) a b-specific killer cell engager (BiKE); (v) a tri-specific killer cell engager (TriKE); (vi) a MICA/B engager; or (vii) a CD16 engager.

46. A method of treating a subject comprising:

administering a cell or population thereof to the subject, wherein the cell is an immune cell or a derivative cell obtained from differentiating an iPSC, wherein the cell:

- (a) comprises a MICA/B tumor antigen targeting specificity; and
- (b) comprises an exogenous polynucleotide encoding a CAR having specificity to a tumor antigen other than MICA/B;

and optionally wherein the MICA/B tumor antigen targeting specificity comprises one or more exogenous polynucleotides encoding: (i) a MICA/B-CAR (chimeric antigen receptor); (ii) a CD16 or a variant thereof that mediates ADCC when in combination with an anti-MICA/B antibody; (iii) an exogenous TCR targeting a MICA/B tumor antigen; or (iv) any combination thereof; and

wherein the subject has an autoimmune disorder; a hematological malignancy; a solid tumor; cancer, or a virus infection.

47. The method of claim 46, wherein the CAR of (b) is:

- (i) T cell specific or NK cell specific;
- (ii) a bi-specific antigen binding CAR;
- (iii) a switchable CAR;
- (iv) a dimerized CAR;
- (v) a split CAR;
- (vi) a multi-chain CAR;
- (vii) an inducible CAR;
- (viii) a recombinant TCR;
- (ix) co-expressed with another CAR;
- (x) co-expressed with a cytokine signaling complex comprising a partial or full peptide of a cell surface expressed exogenous cytokine or a receptor thereof, optionally in separate constructs or in a bi-cistronic construct;
- (xi) co-expressed with a checkpoint inhibitor, optionally in separate constructs or in a bi-cistronic construct;
- (xii) specific to at least one of CD19, BCMA, CD20, CD22, CD38, CD123, HER2, CD52, EGFR, GD2, MSLN, VEGF-R2, PSMA and PDL1; and/or
- (xiii) specific to any one of ADGRE2, 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, 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), 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), 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; wherein the CAR of any one of (i) to (xiii) is optionally inserted at TRAC locus, and/or is driven by an endogenous promoter of TCR, and/or the TCR is knocked out by the CAR insertion.

48. The method of claim 47, wherein the CAR of (b) is specific to BCMA.

49. The method of any one of claims 46-48, further comprising administering a sensitizing agent to the subject to precondition tumor cells in the subject.

50. The method of claim 49, wherein the sensitizing agent increases MICA/B surface expression and/or MICA/B density on the tumor cell upon contact therewith.

51. The method of claim 49, wherein the sensitizing agent comprises at least one of a chemotherapeutic agent, external beam radiation, brachytherapy, and a radiopharmaceutical.

52. The method of claim 51, wherein the sensitizing agent comprises:

- (i) at least one of x-ray radiation, gamma radiation, photon radiation, proton radiation, and neutron radiation; or
- (ii) at least one of 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; or
- (iii) paclitaxel.

53. The method of claim 51, further comprising administering an antibody that binds to a MIC alpha 3-domain of MICA/B tumor cell surface antigen prior to or during administering the cell or population thereof.

54. The method of claim 49, wherein the derivative cell is a hematopoietic cell, and comprises longer telomeres in comparison to its native counterpart cell obtained from peripheral blood, umbilical cord blood, or any other donor tissues; or wherein the MICA/B-CAR has at least one of the following characteristics:

- (i) being T cell specific;
- (ii) being NK cell specific;
- (iii) binding to surface MICA/B;

- (iv) comprising a scFV (single chain variable fragment) binding to the conserved $\alpha 3$ domain of MICA/B;
- (v) comprising a heavy chain variable region represented by an amino acid sequence that is of at least about 99%, 98%, 96%, 95%, 90%, 85%, or 80% identity to SEQ ID NO: 1;
- (vi) comprising a light chain variable region represented by an amino acid sequence that is of at least about 99%, 98%, 96%, 95%, 90%, 85%, or 80% identity to SEQ ID NO: 2;
- (vii) comprising a scFV represented by an amino acid sequence that is of at least about 99%, 98%, 96%, 95%, 90%, 85%, or 80% identity to SEQ ID NOs: 3 or 4;
- (viii) comprising a heavy chain variable region of a MICA/B binding scFV functionally linked to a first constant region of a T cell receptor (TCR), and a light chain variable region of a MICA/B binding scFV functionally linked to a second constant region of a T cell receptor (TCR); and
- (ix) being inserted at one of the gene loci: B2M, TAP1, TAP2, Tapasin, NLRC5, CIITA, RFXANK, RFX5, RFXAP, TCR α or β constant region, NKG2A, NKG2D, CD38, CD25, CD44, CD58, CD54, CD56, CD69, CD71, CIS, CBL-B, SOCS2, PD1, CTLA4, LAG3, TIM3, or TIGIT; and, optionally, wherein the insertion knocks out expression of the gene in the locus.

55. The method of any one of claims 46-54, wherein the cell further comprises one or more of:

- (i) CD38 knockout;
- (ii) HLA-I deficiency and/or HLA-II deficiency;
- (iii) introduction of HLA-G or non-cleavable HLA-G;
- (iv) an exogenous CD16 or a variant thereof;
- (v) a cytokine signaling complex comprising a partial or full peptide of a cell surface expressed exogenous cytokine or a receptor thereof;
- (vi) at least one of the genotypes listed in Table 1;
- (vii) disruption in at least one of TAP1, TAP2, Tapasin, NLRC5, CIITA, RFXANK, RFX5, RFXAP, TCR α or β constant region, NKG2A, NKG2D, CD56, CIS, CBL-B, SOCS2, PD1, CTLA4, LAG3, TIM3, and TIGIT, in comparison to its native counterpart cell; and
- (viii) introduction of at least one of HLA-E, 4-1BBL, CD3, CD4, CD8, CD16, CD47, CD113, CD131, CD137, CD80, PDL1, A_{2A}R, antigen-specific TCR, Fc receptor, an engager, and surface triggering receptor for coupling with bi- or multi- specific or universal engagers, in comparison to its native counterpart cell.

56. The method of claim 55, wherein the exogenous CD16 comprises a high affinity non-cleavable CD16 (hnCD16) or a variant thereof.
57. The method of claim 56, wherein the high affinity non-cleavable CD16 (hnCD16) or a variant thereof comprises at least one of:
- (a) F176V and S197P in ectodomain domain of CD16;
 - (b) a full or partial ectodomain originated from CD64;
 - (c) a non-native (or non-CD16) transmembrane domain;
 - (d) a non-native (or non-CD16) intracellular domain;
 - (e) a non-native (or non-CD16) signaling domain;
 - (f) a stimulatory domain; and
 - (g) transmembrane, signaling, and stimulatory domains that are not originated from CD16, and are originated from a same or different polypeptide.
58. The method of claim 46, further comprising administering one or more therapeutic agents.
59. The method of claim 58, wherein the one or more therapeutic agents comprise a peptide, a cytokine, a checkpoint inhibitor, an engager, an antibody, an antibody conjugate, 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, a chemotherapeutic agent or a radioactive moiety, or an immunomodulatory drug (IMiD).
60. The method of claim 59, wherein:
- (a) the checkpoint inhibitor comprises:
 - (i) one or more antagonists 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;
 - (ii) one or more of atezolizumab, avelumab, cetuximab, durvalumab, ipilimumab, IPH4102, IPH43, IPH33, lirimumab, monalizumab, nivolumab, pembrolizumab, trastuzumab, pertuzumab, and their derivatives or functional equivalents;
 - (iii) at least one of atezolizumab, nivolumab, and pembrolizumab; or

(b) the therapeutic agents comprise one or more of venetoclax, azacitidine, and pomalidomide.

61. The method of claim 59, wherein the antibody comprises:

- (a) an MICA/B antibody that binds to a MIC alpha 3-domain of a cell surface MICA/B antigen;
- (b) an antibody triggering antibody-dependent cellular cytotoxicity (ADCC);
- (c) an anti-CD20, an anti-CD22, an anti-HER2, an anti-CD52, an anti-EGFR, an anti-CD123, an anti-GD2, an anti-PDL1, and/or an anti-CD38 antibody; and/or
- (d) one or more of rituximab, velutuzumab, ofatumumab, ublituximab, ocaratuzumab, obinutuzumab, ibritumomab, ocrelizumab, inotuzumab, moxetumomab, epratuzumab, trastuzumab, pertuzumab, alemtuzumab, cetuximab, amivantamab, dinutuximab, avelumab, daratumumab, isatuximab, MOR202, 7G3, CSL362, elotuzumab, and their humanized or Fc modified variants or fragments and their functional equivalents and biosimilars.

62. The method of any one of claims 46-61, further comprising administering a multi-specific engager.

63. The method of claim 62, wherein the multi-specific engager is at least one of: (i) a T cell engager; (ii) an NK cell engager; (iii) a bi-specific T cell engager (BiTE); (iv) a b-specific killer cell engager (BiKE); (v) a tri-specific killer cell engager (TriKE); (vi) a MICA/B engager; or (vii) a CD16 engager.

64. A method of enhancing tumor surface MICA/B expression and/or density comprising contacting a tumor cell with a sensitizing agent, thereby increasing surface MICA/B expression as compared to MICA/B expression prior to contact with the sensitizing agent.

65. The method of claim 64, wherein the sensitizing agent comprises at least one of external beam radiation, brachytherapy, a radiopharmaceutical, and a chemotherapeutic agent.

66. The method of claim 65, wherein the sensitizing agent comprises:

- (i) at least one of x-ray radiation, gamma radiation, photon radiation, proton radiation, and neutron radiation; or
- (ii) at least one of 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; or

(iii) paclitaxel.

67. The method of claim 64, further comprising contacting the tumor cell with an antibody that binds to a MIC alpha 3-domain of tumor cell surface MICA/B antigen.

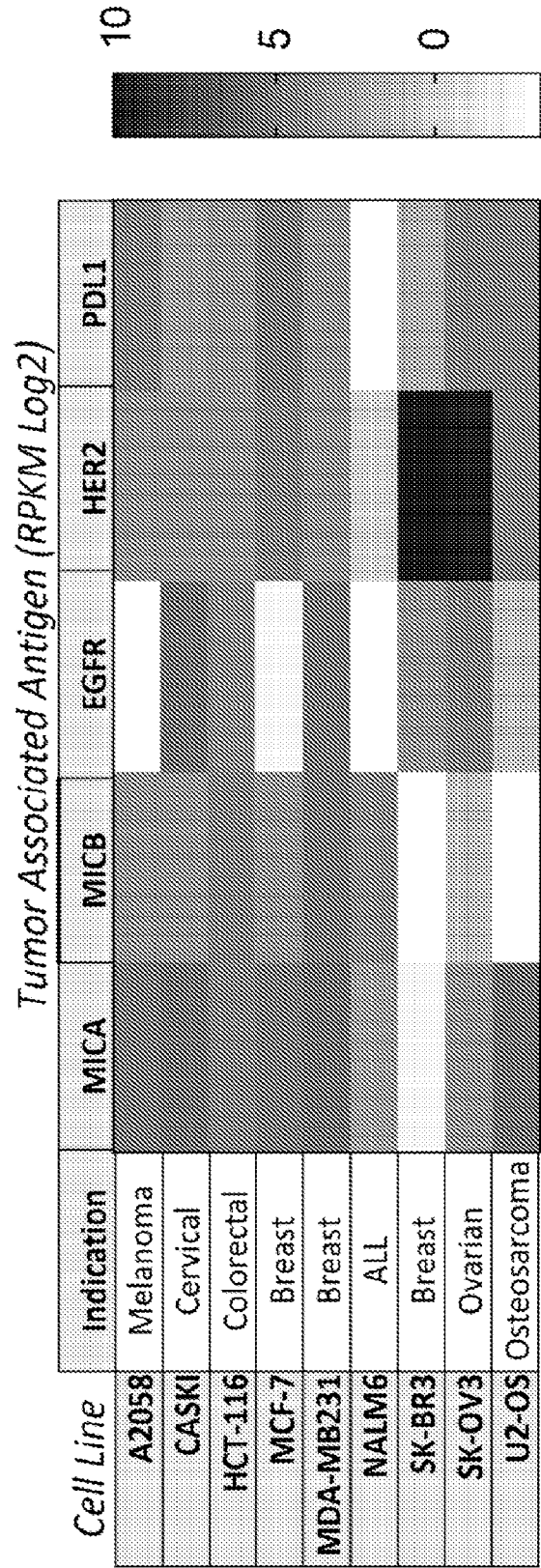


FIG. 1A

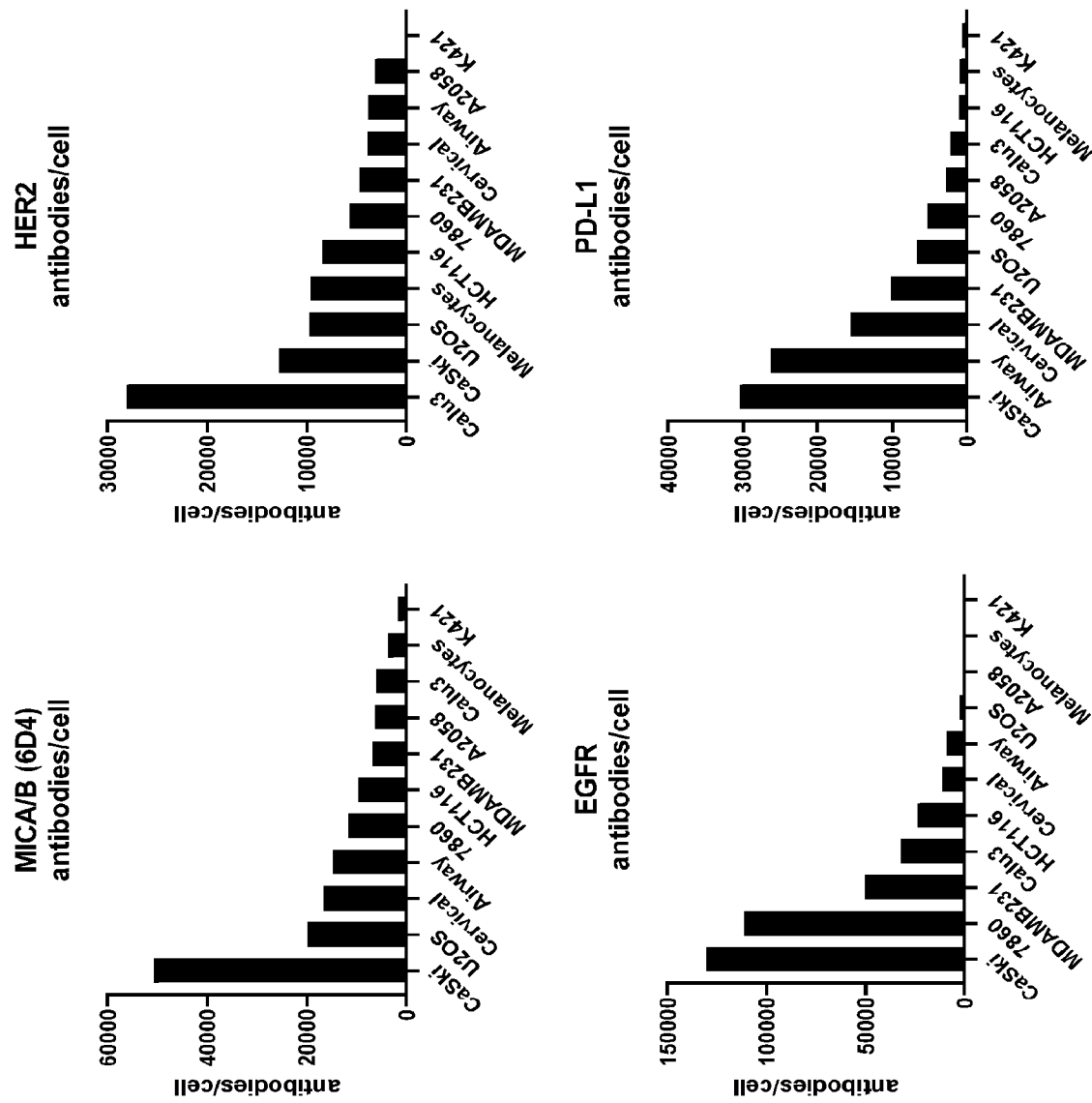


FIG. 1B

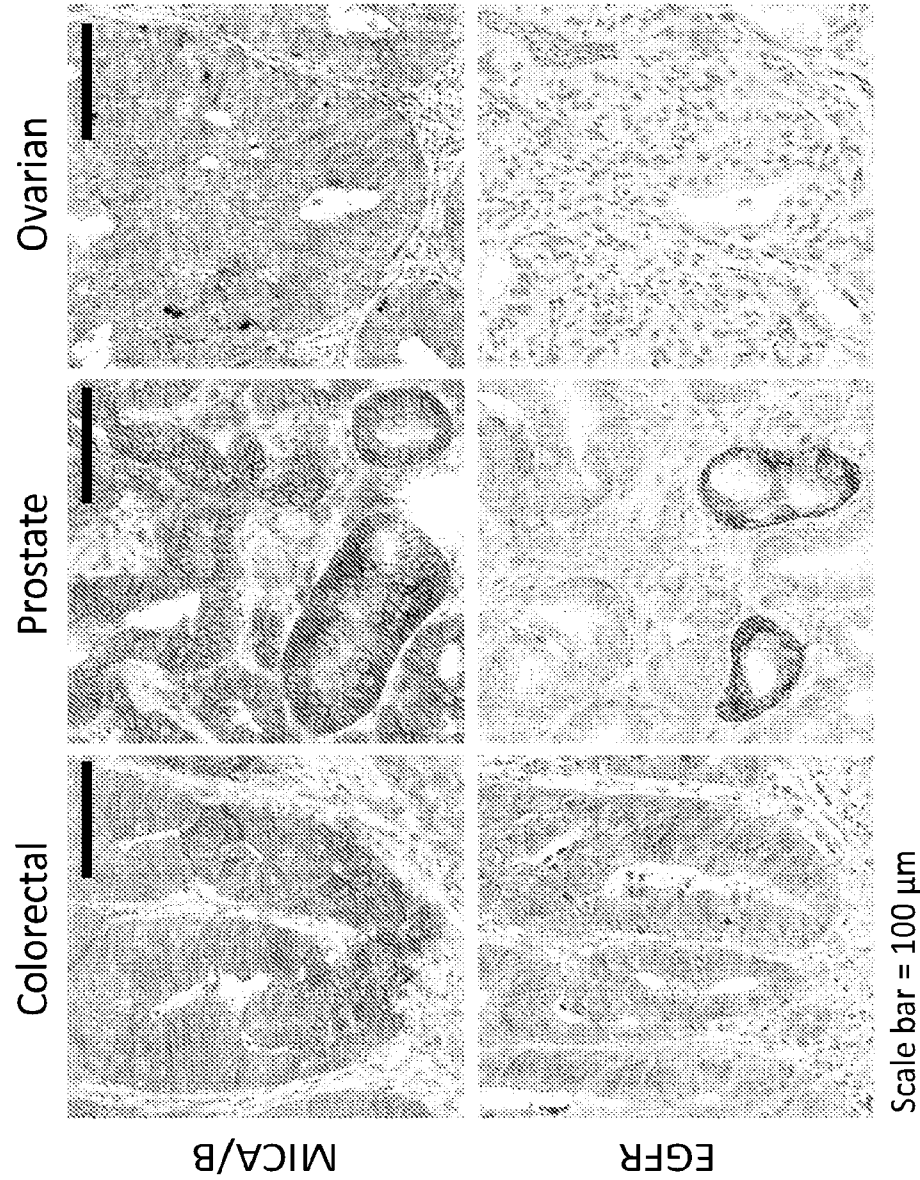


FIG. 1C

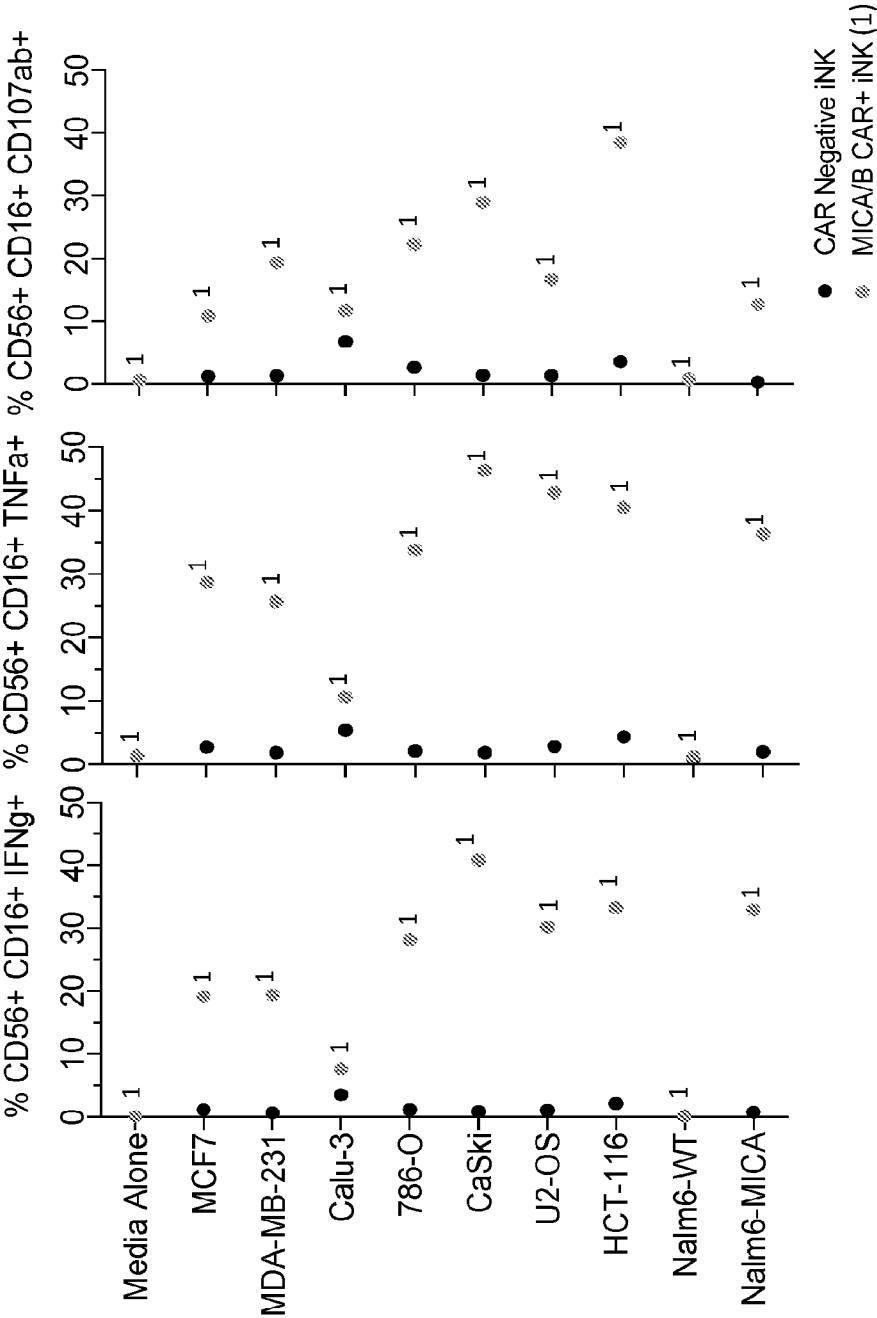


FIG. 2

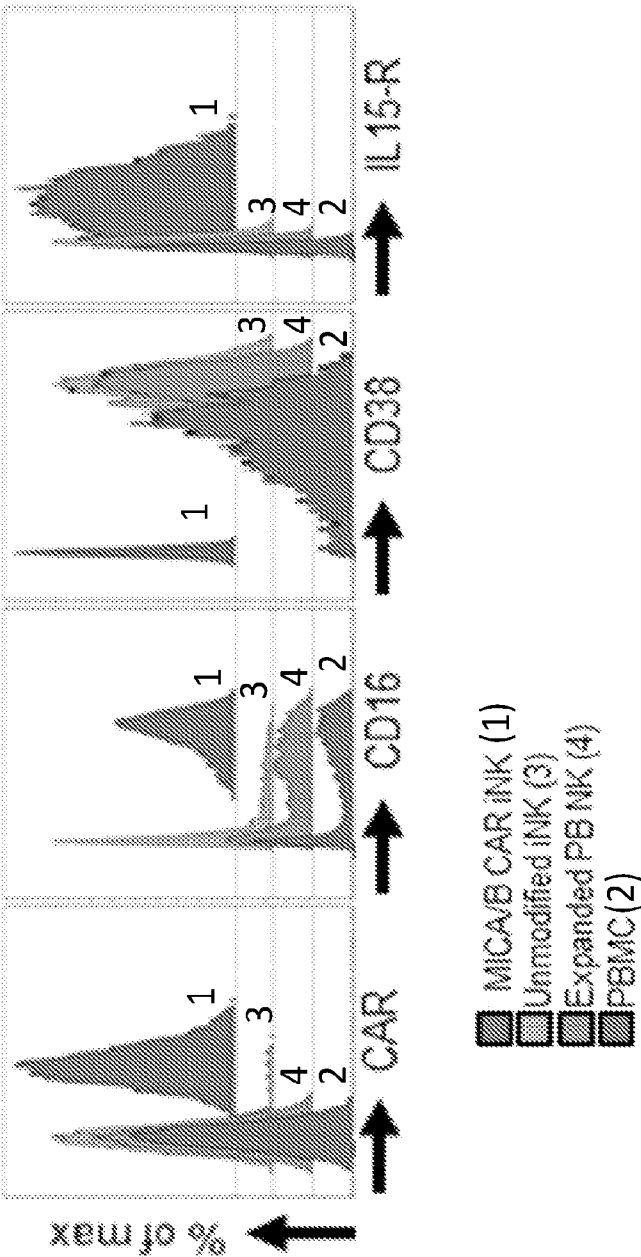
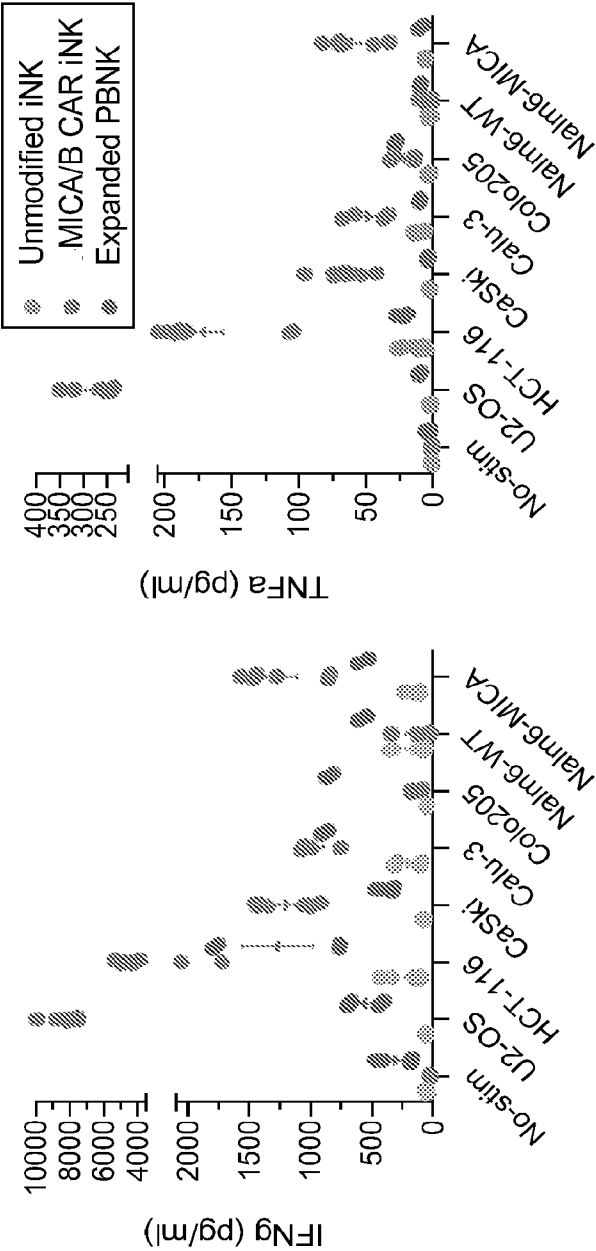


FIG. 3A

FIG. 3B



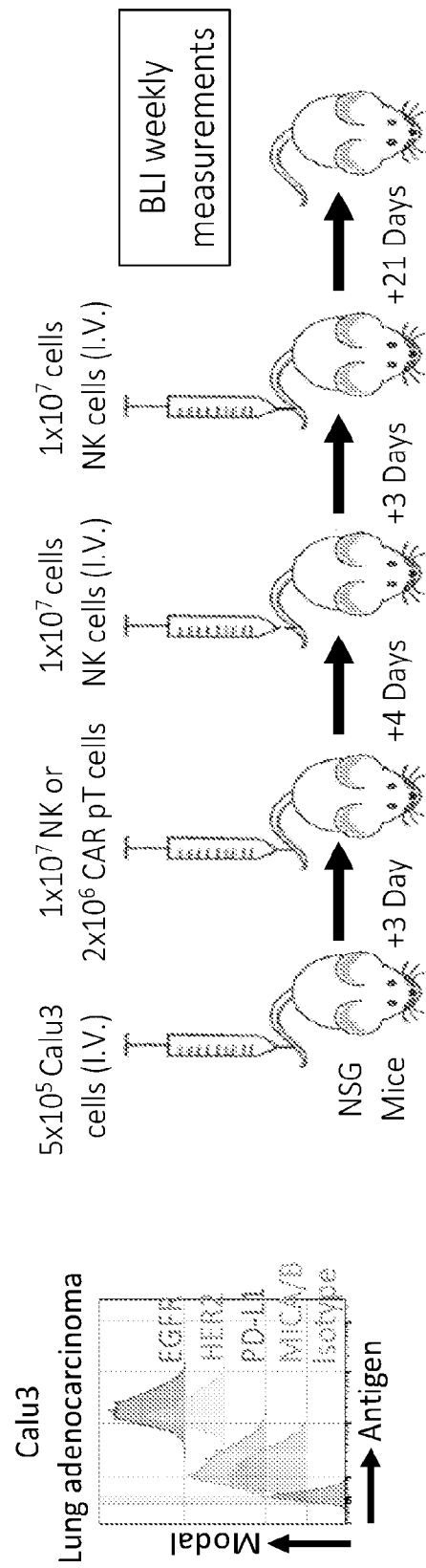
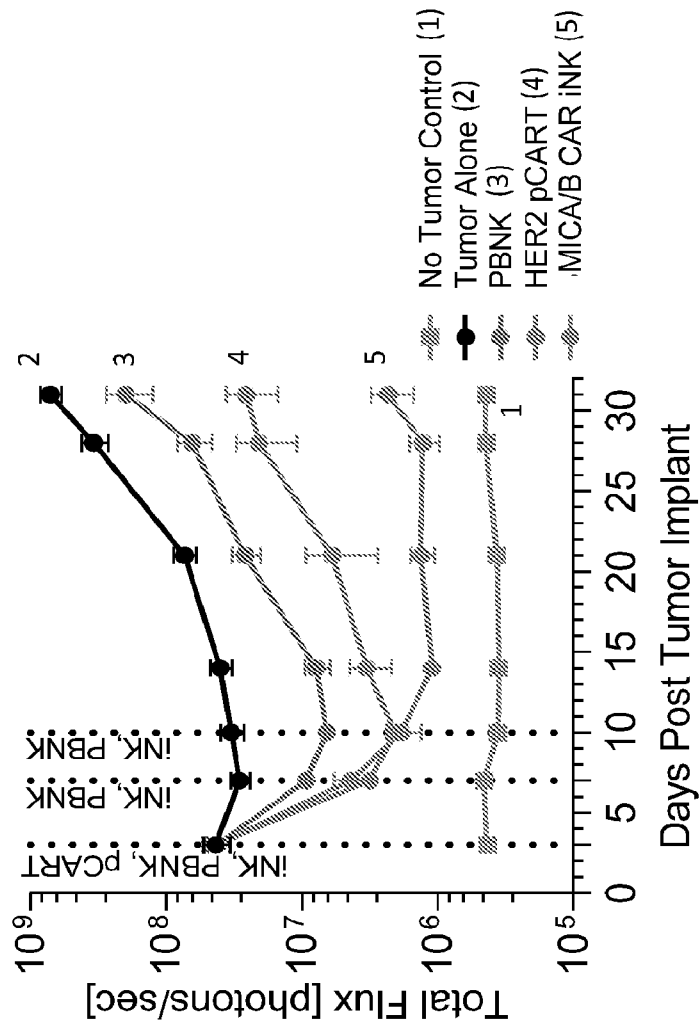


FIG. 4A

FIG. 4B



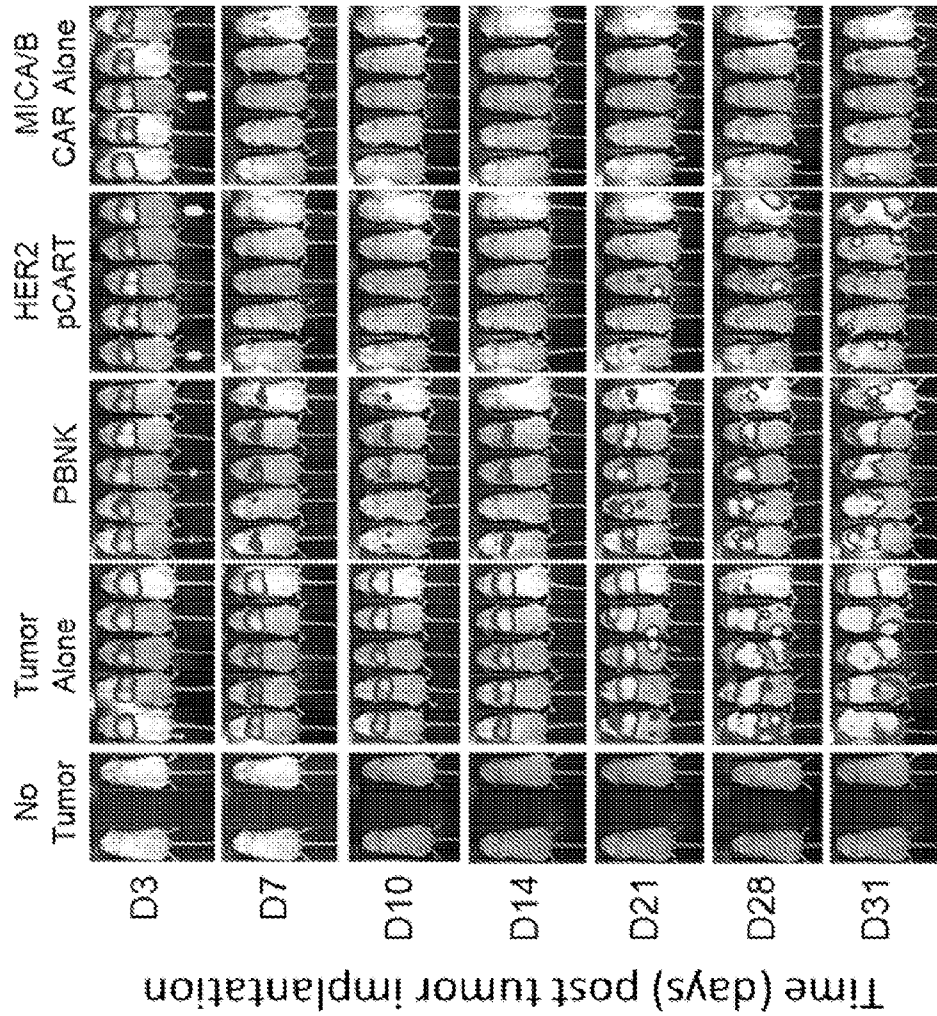


FIG. 4C

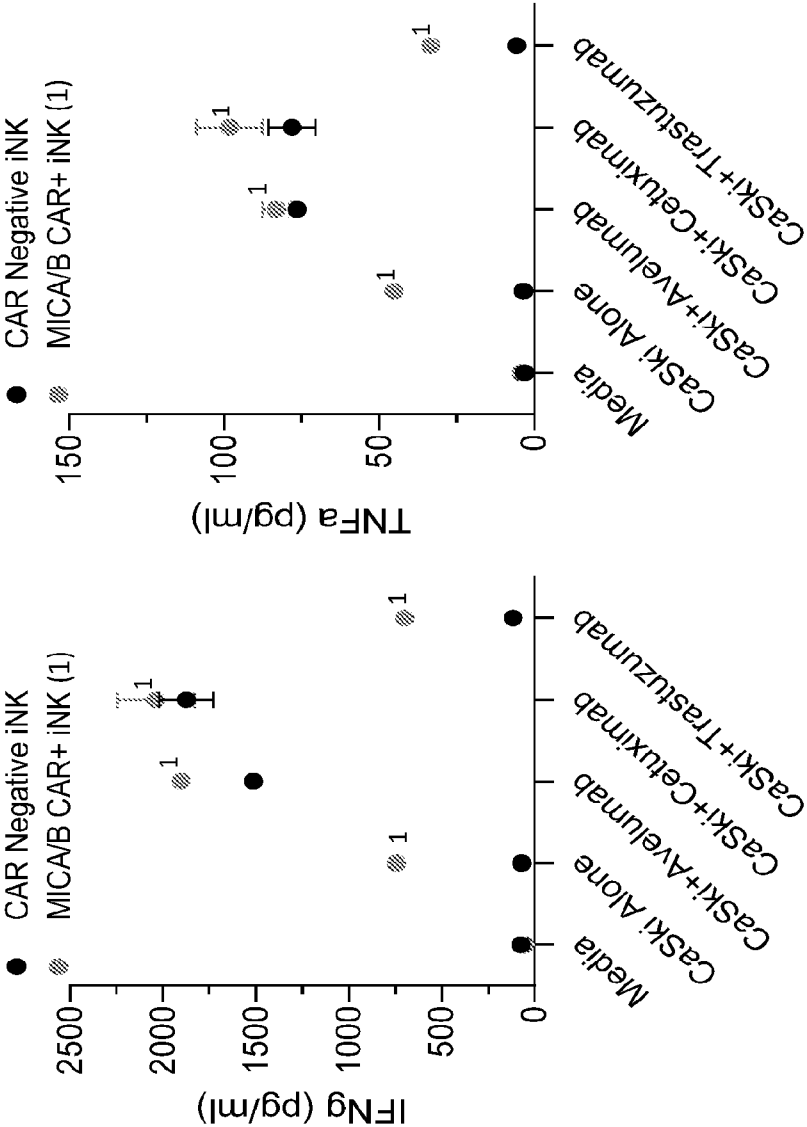
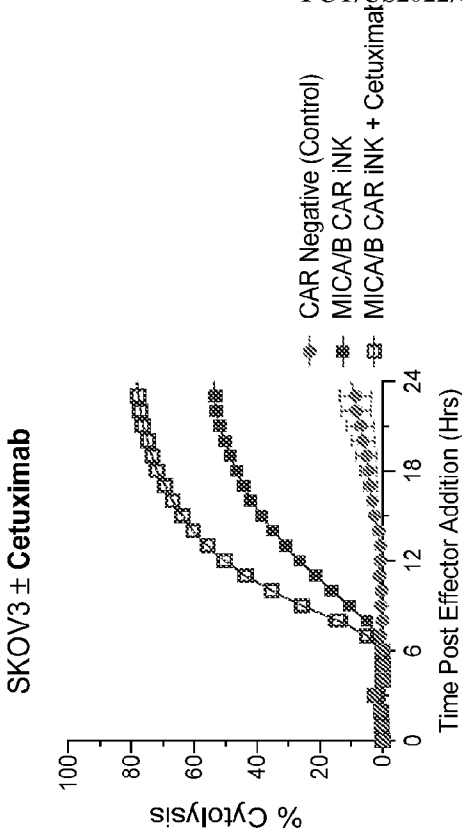
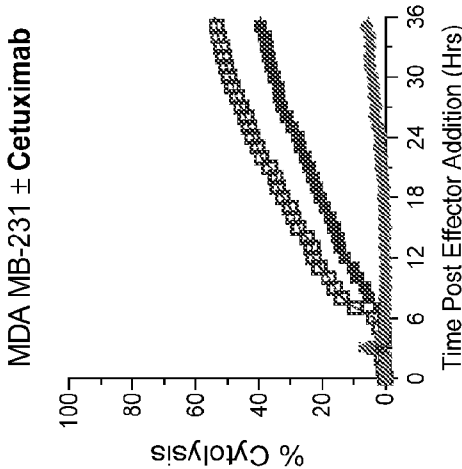
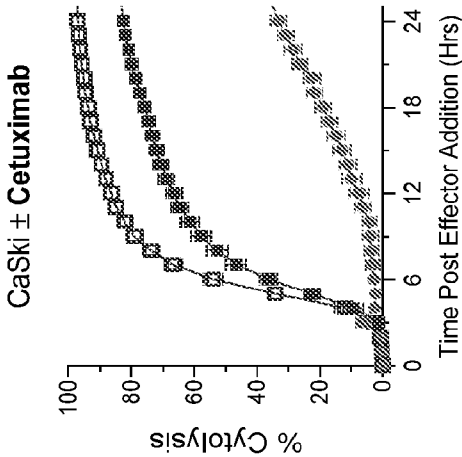
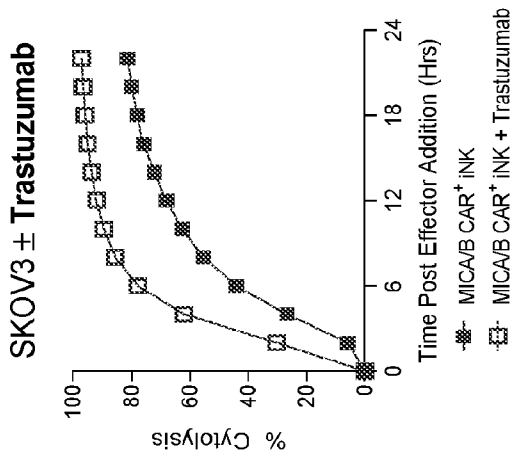


FIG. 5

FIG. 6A



- Combination mAb Therapy
1. Cetuximab (αEGFR)
 2. Trastuzumab (αHer2)
 3. Avelumab (αPD-L1)

FIG. 6B

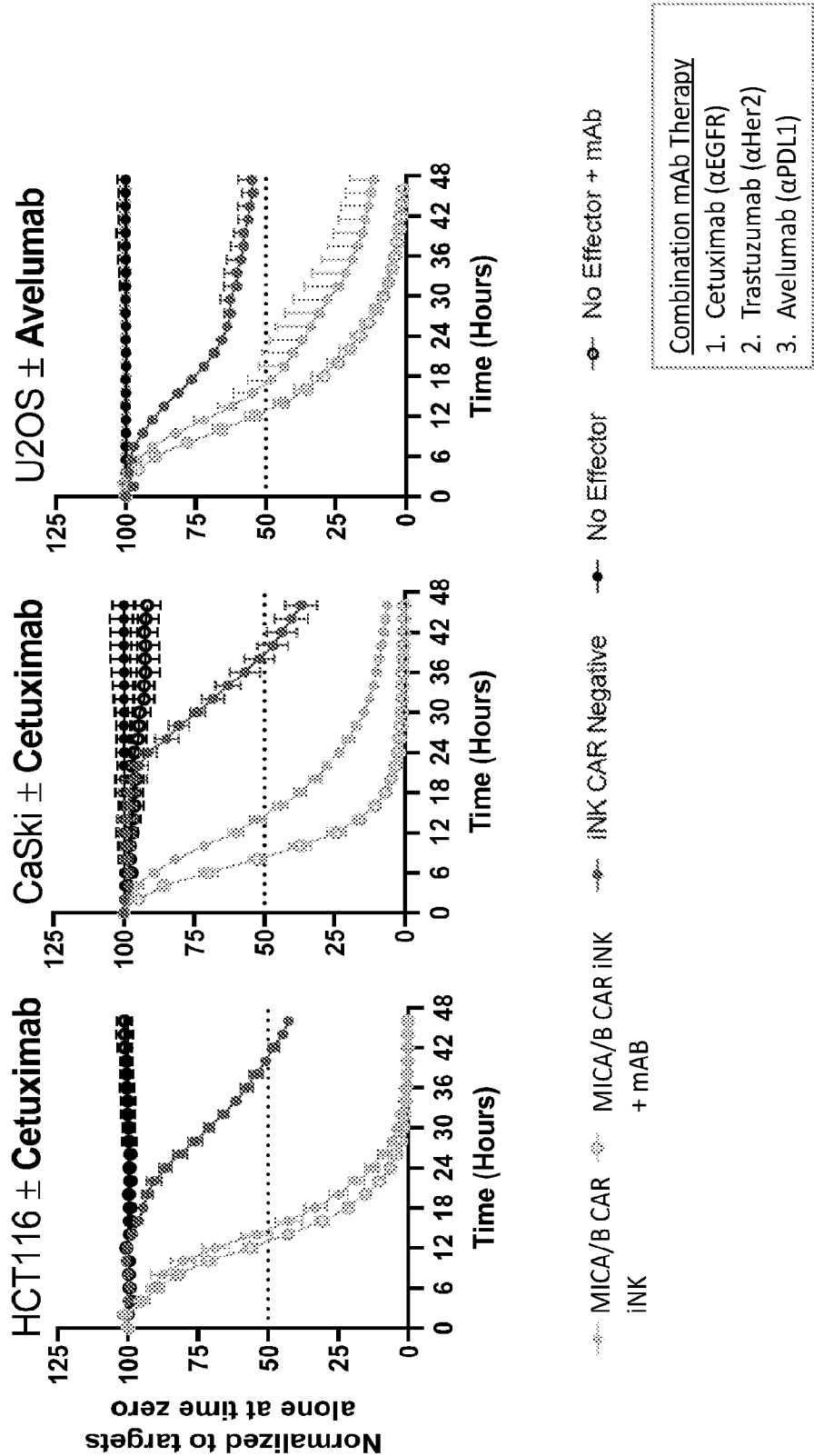
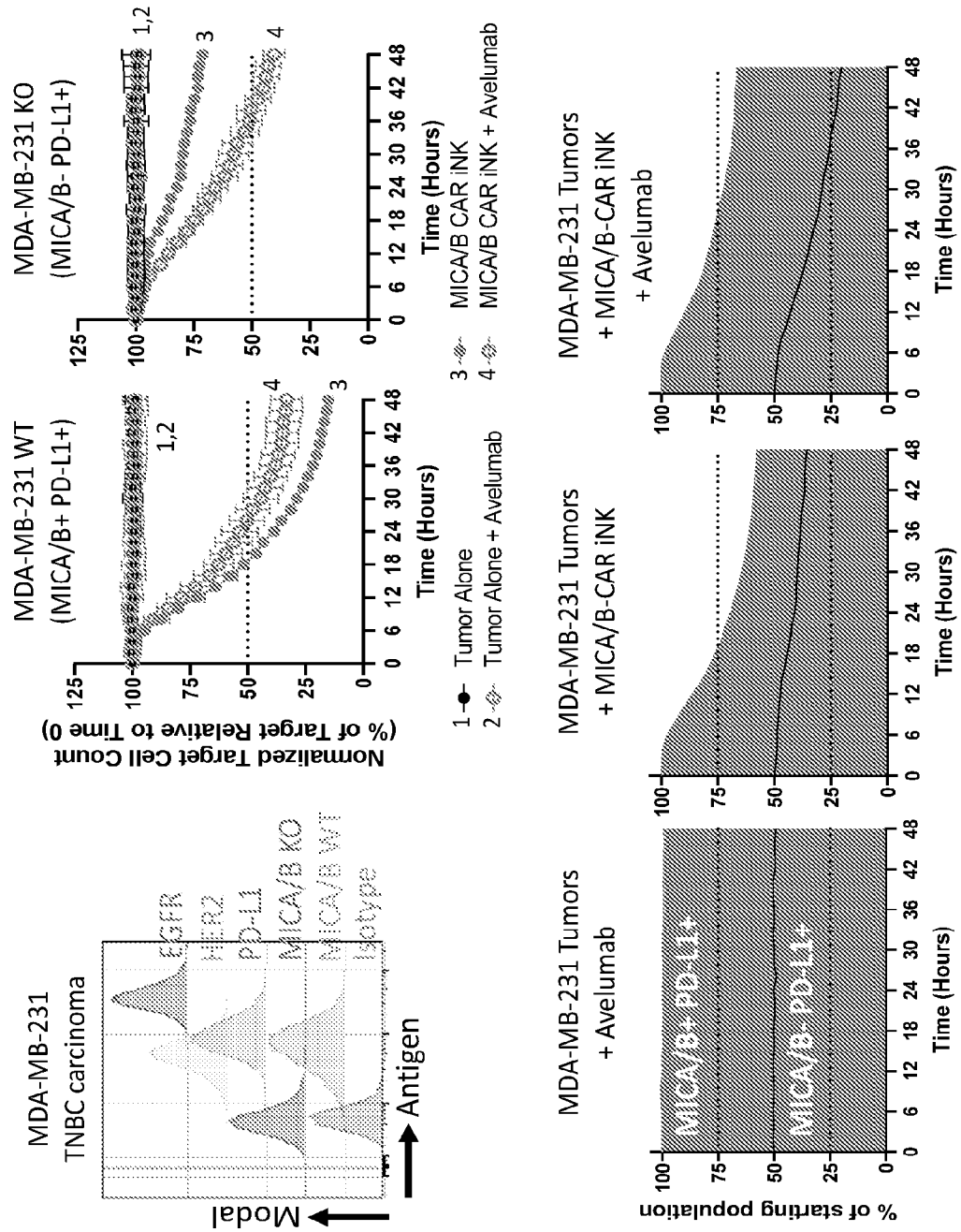


FIG. 7



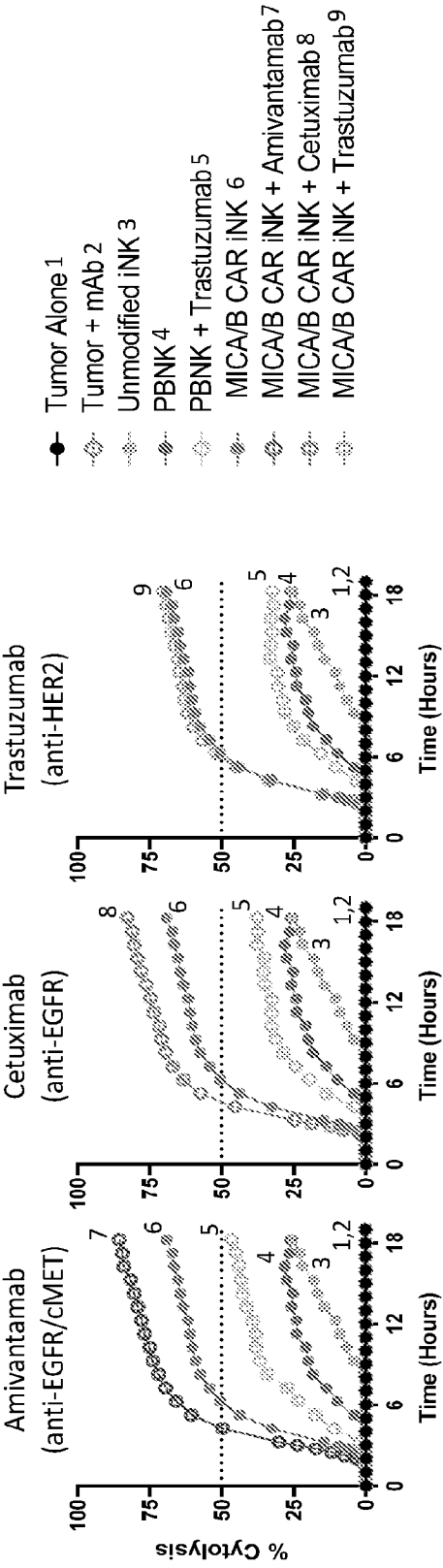
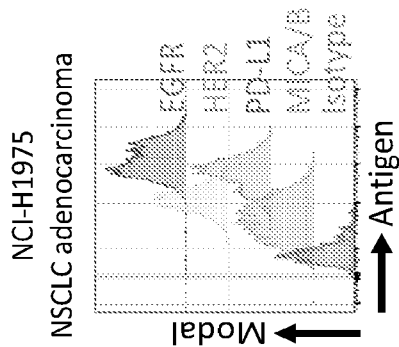


FIG. 8A

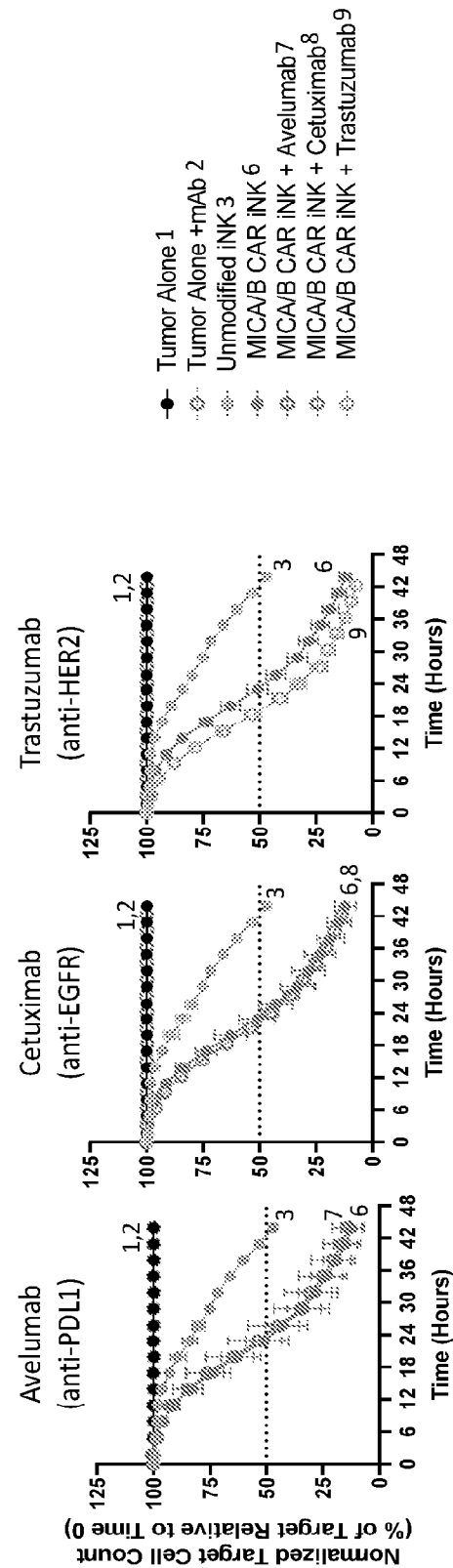
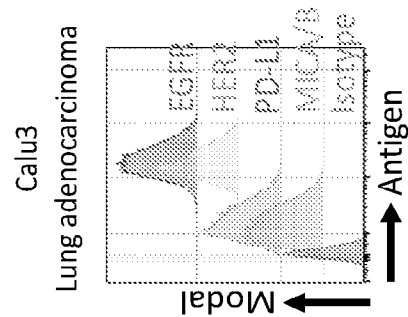


FIG. 8B

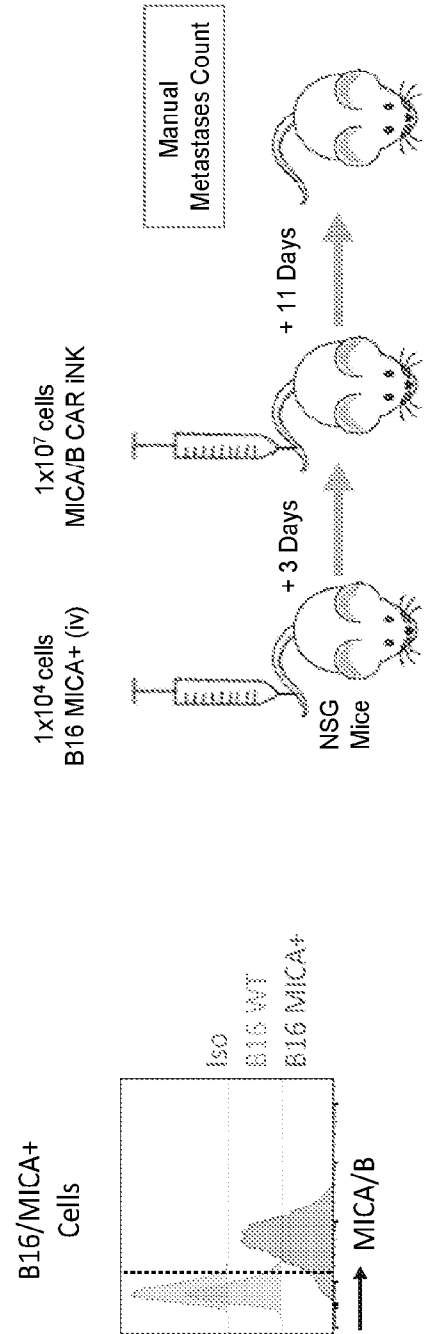


FIG. 9A

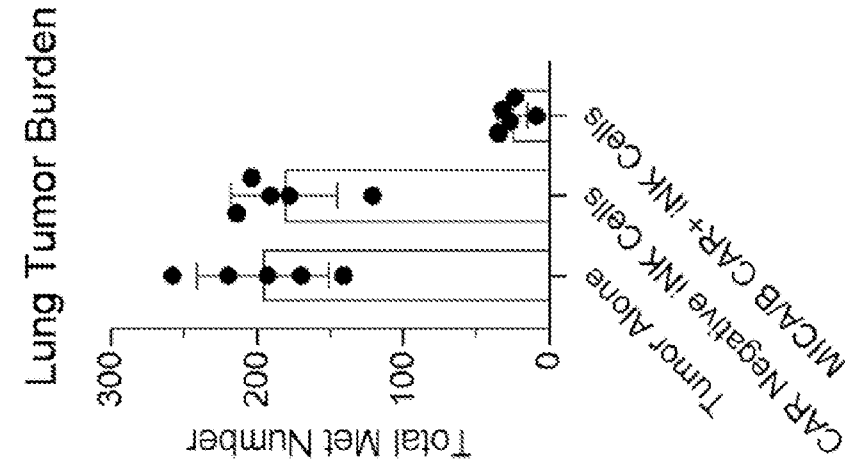
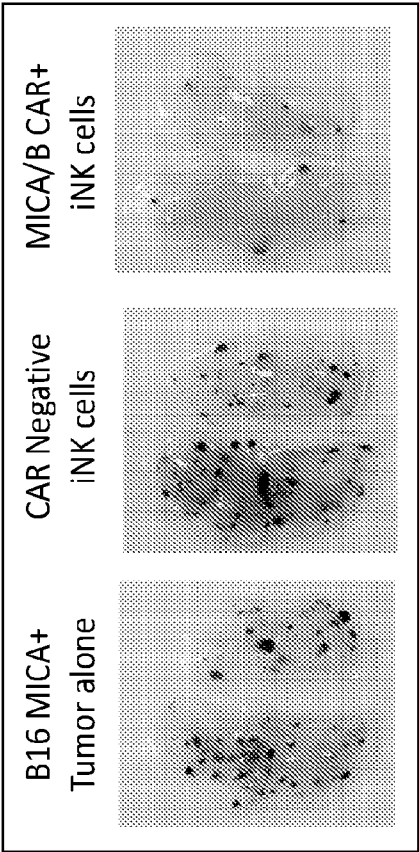
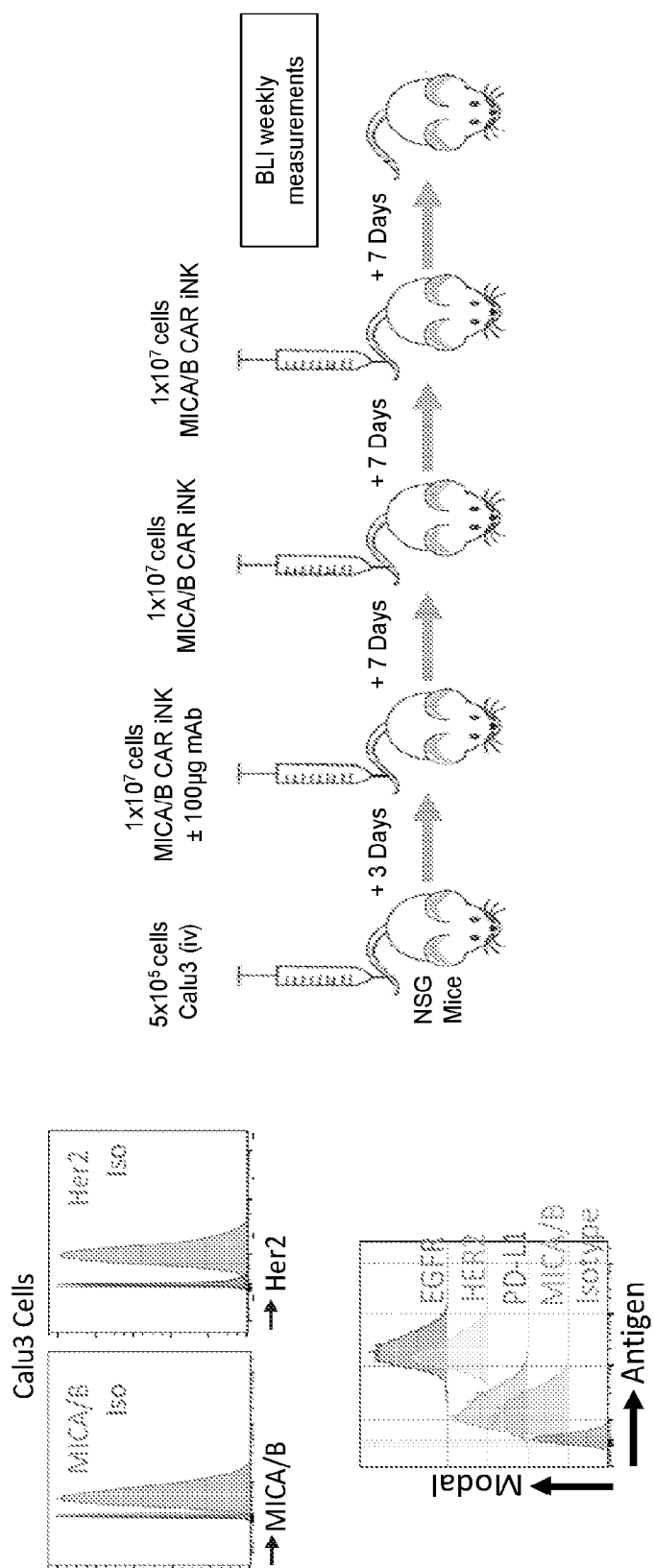


FIG. 9B





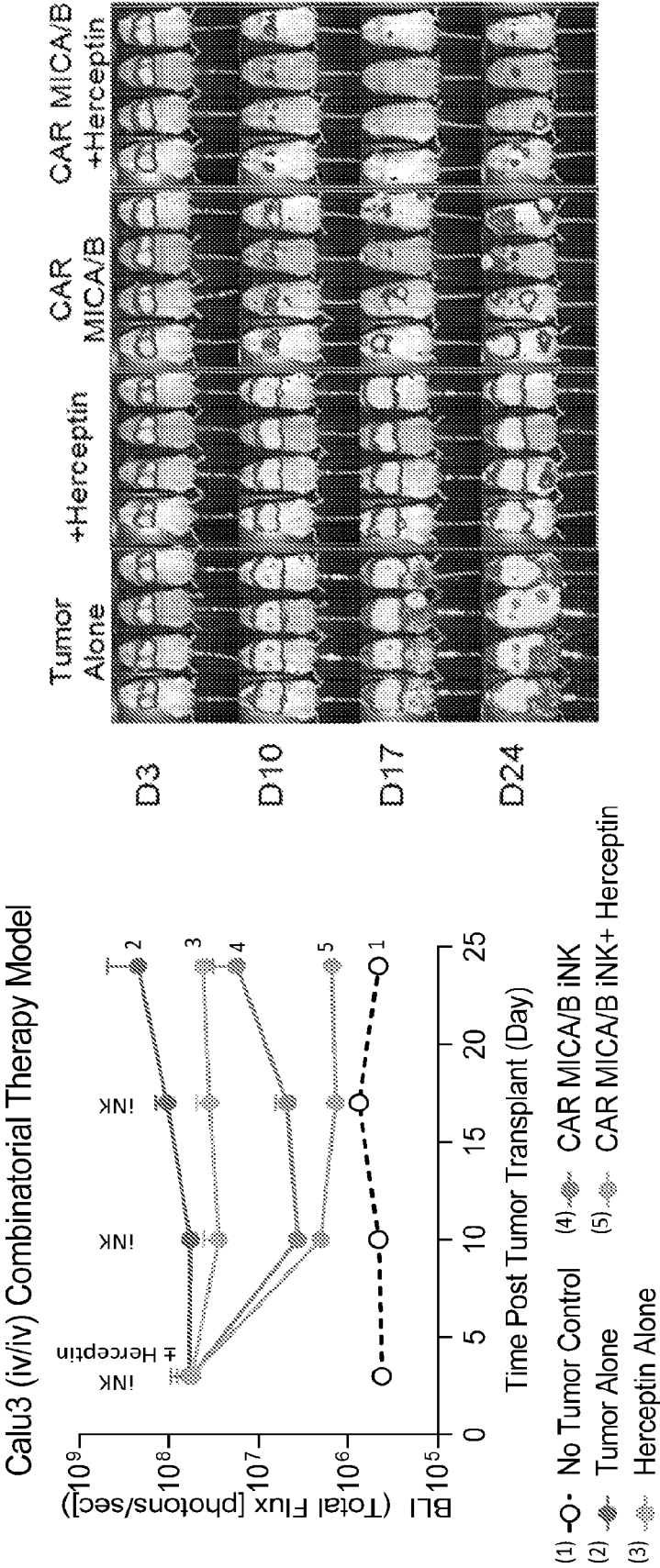


FIG. 10B

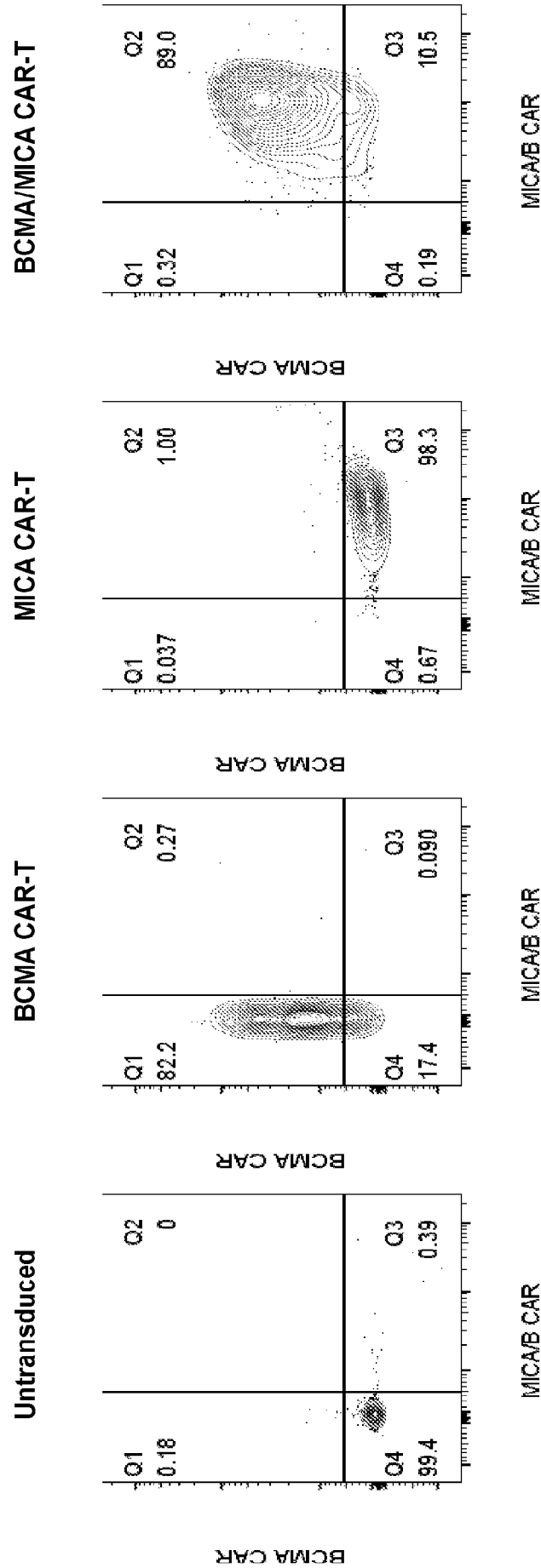


FIG. 11

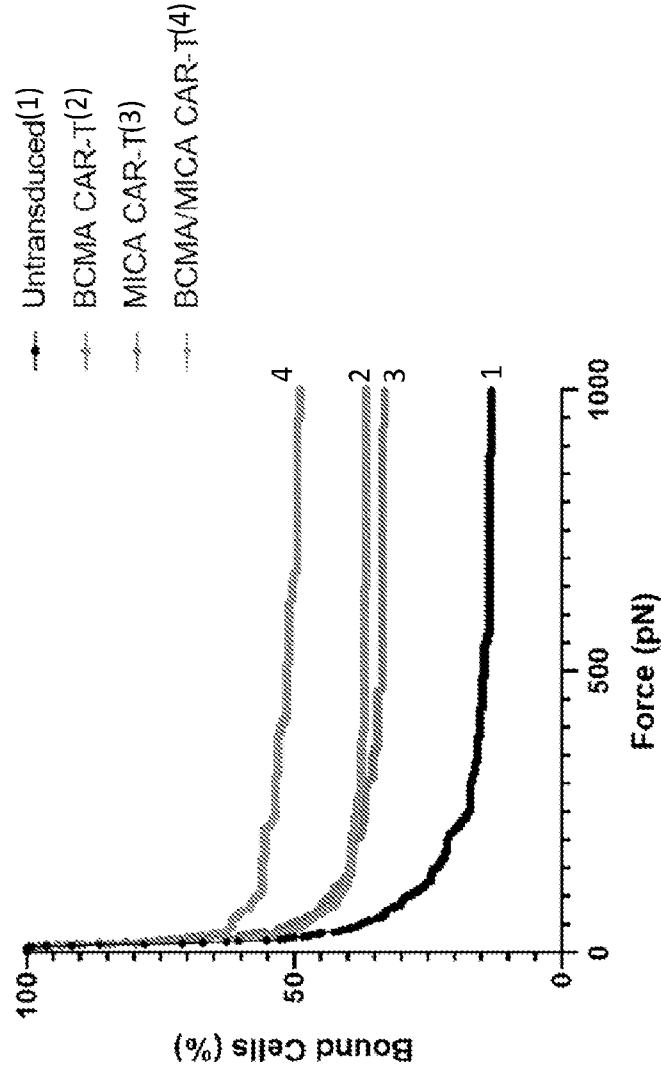


FIG. 12A

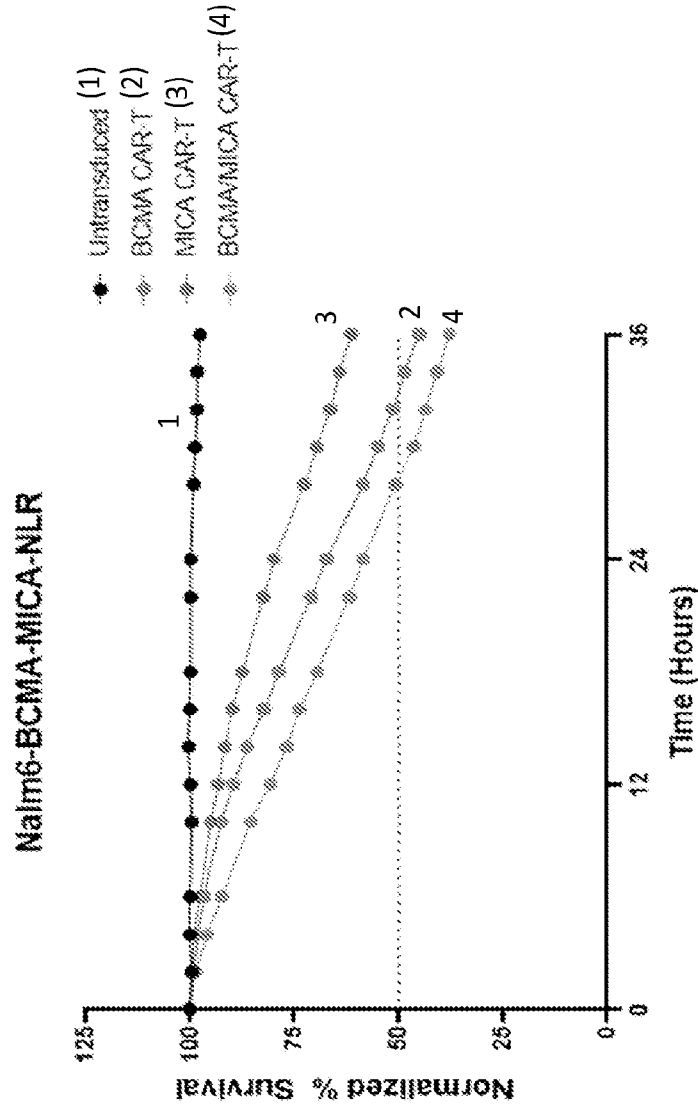


FIG. 12B

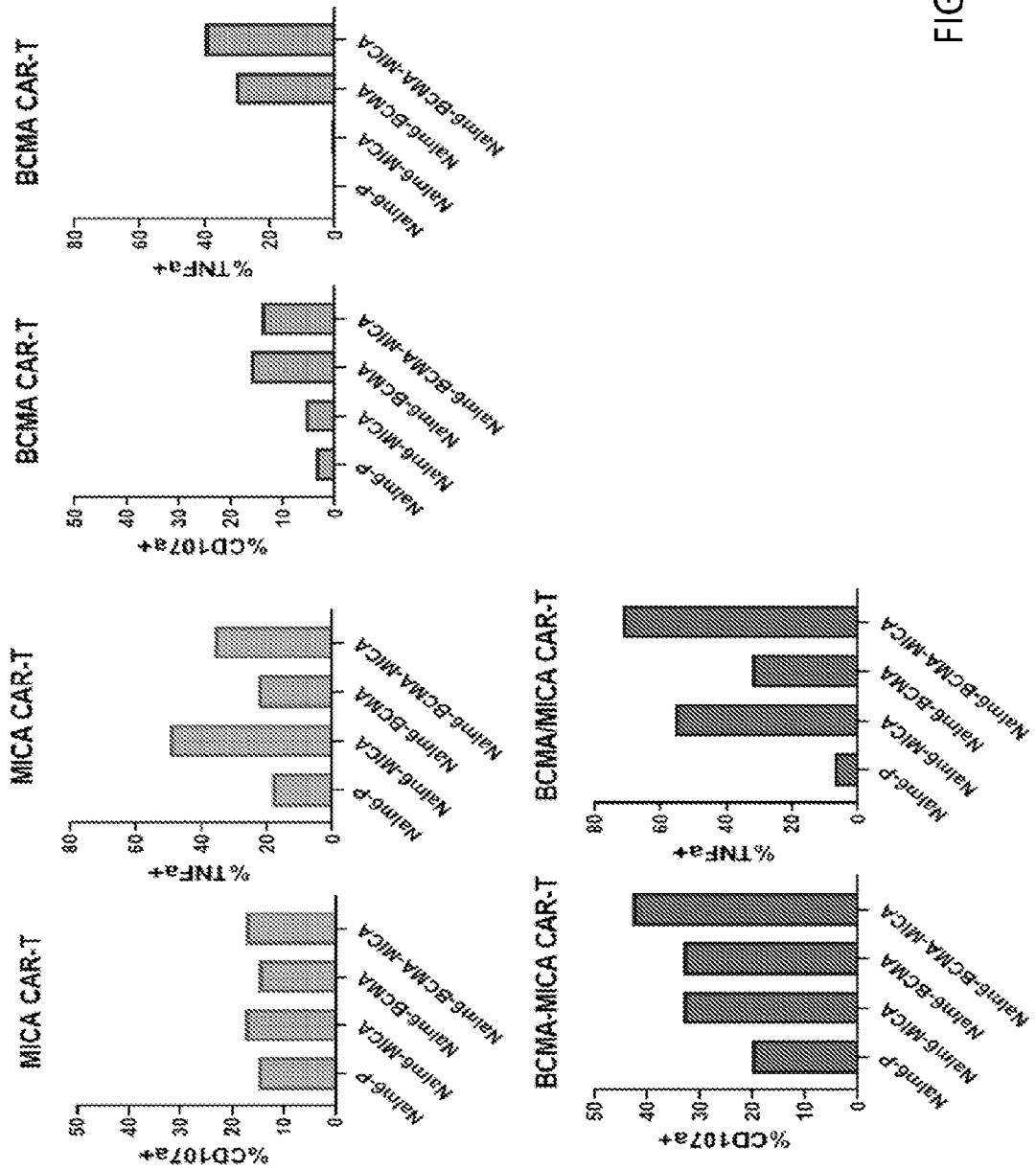


FIG. 13

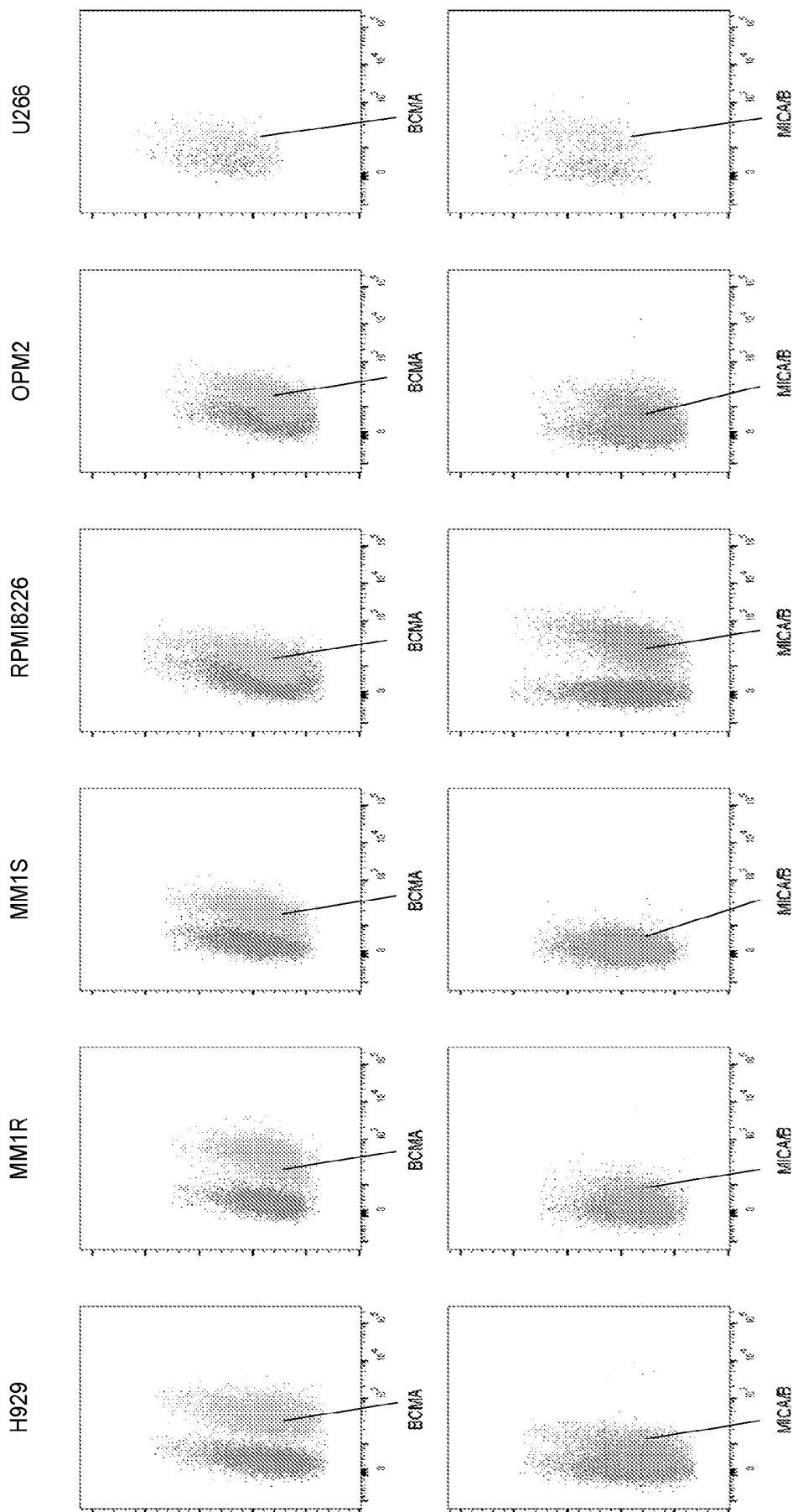
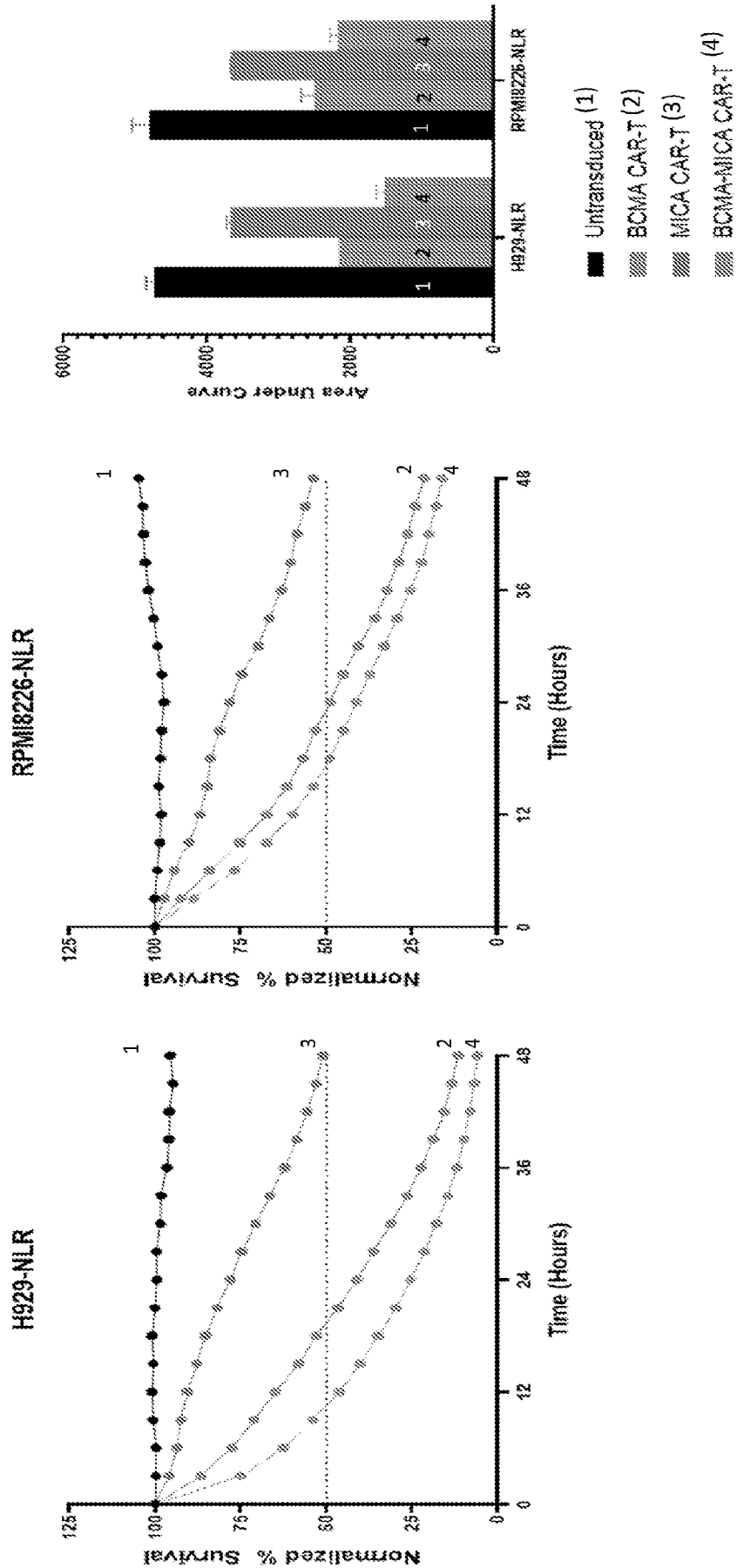
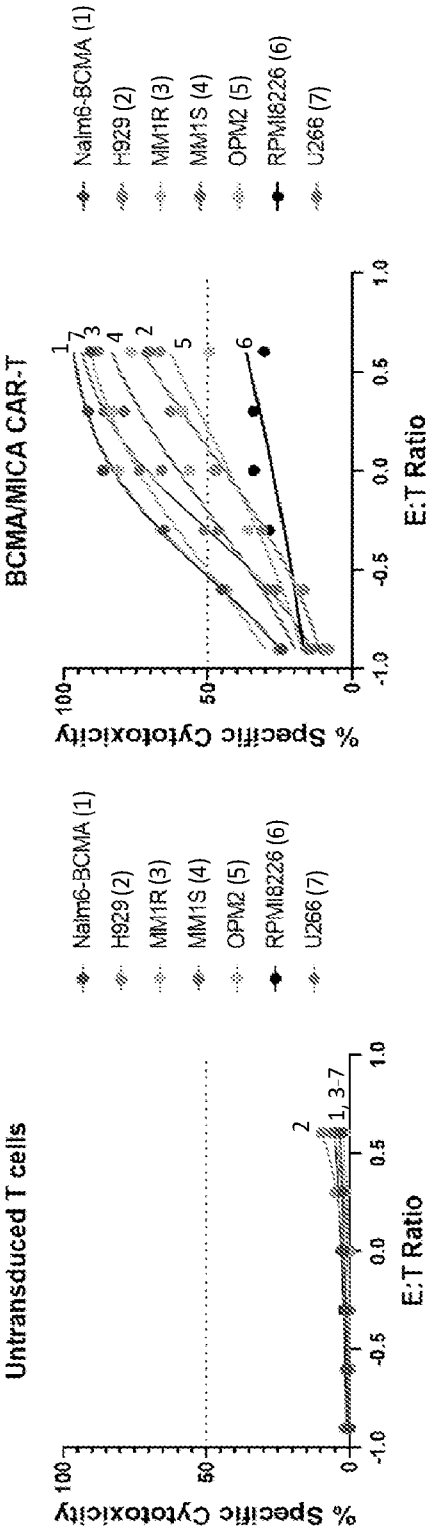


FIG. 14

FIG. 15

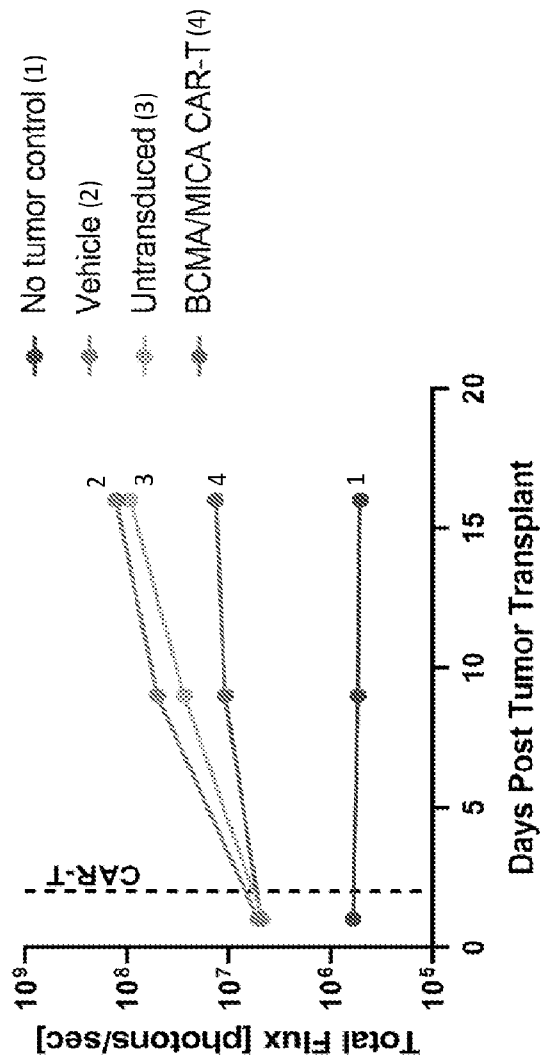




T cell	H929	MM1R	MM1S	OPM2	RPMI8226	U266
Untransduced	27.1	53.4	24.2	129.5	1160.0	1302.0
BCMA/MICA CART	1.3	0.3	0.6	1.6	23.1	0.5

FIG. 16

FIG. 17



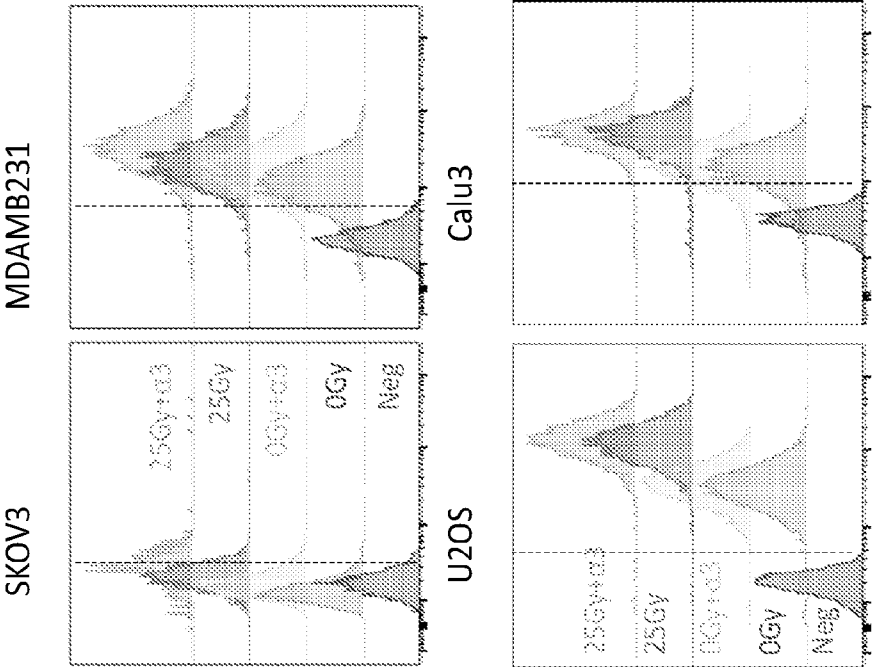


FIG. 18A

Tumor Cell Line	Fold Change over 0Gy Baseline		
	0Gy+α3	25Gy	25Gy+α3
U2OS	1.09	3.74	4.26
SKOV	1.27	1.54	1.98
MDAMB231	1.58	1.86	2.81
Calu3	1.01	2.13	2.23

FIG. 18B

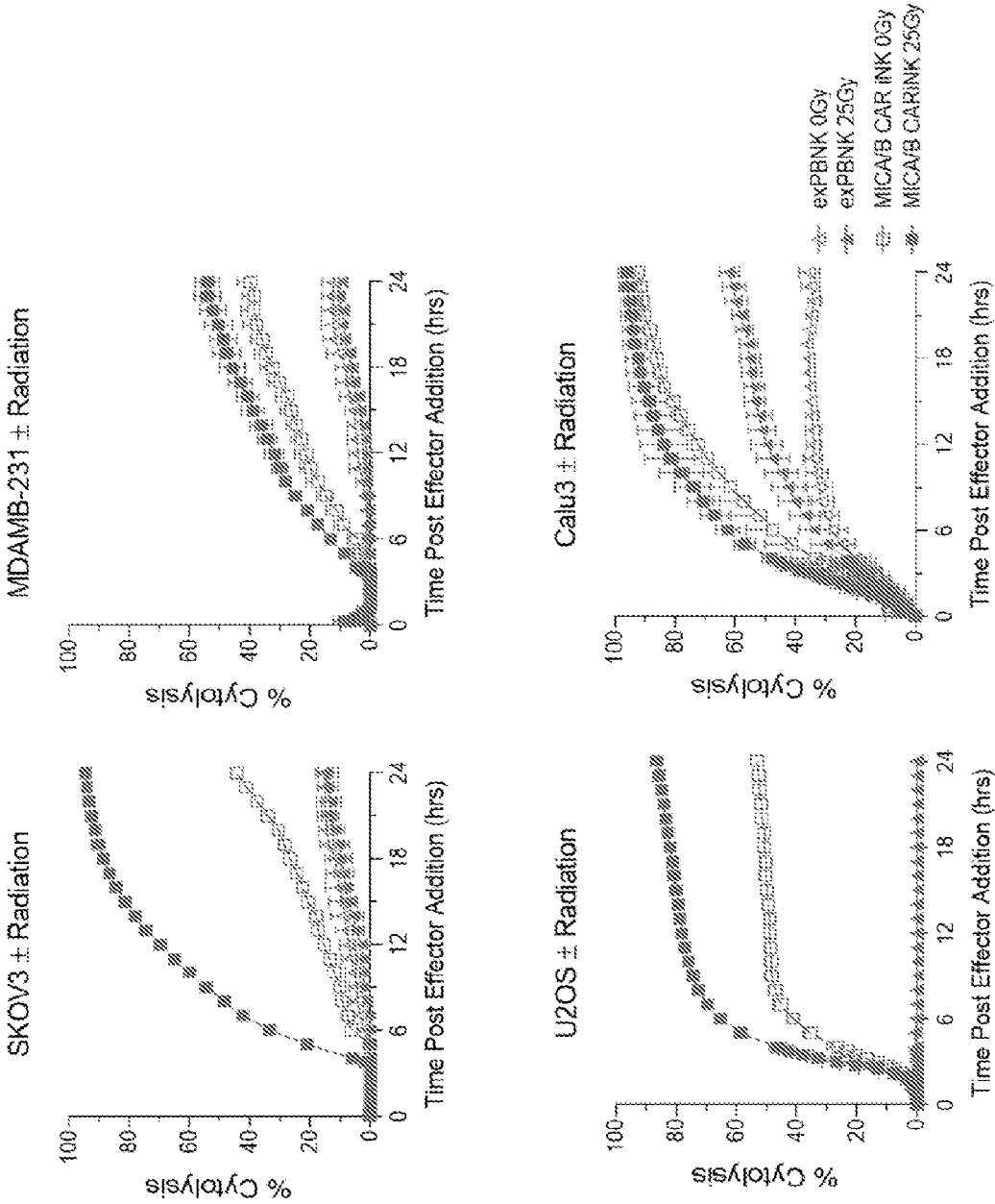


FIG. 19

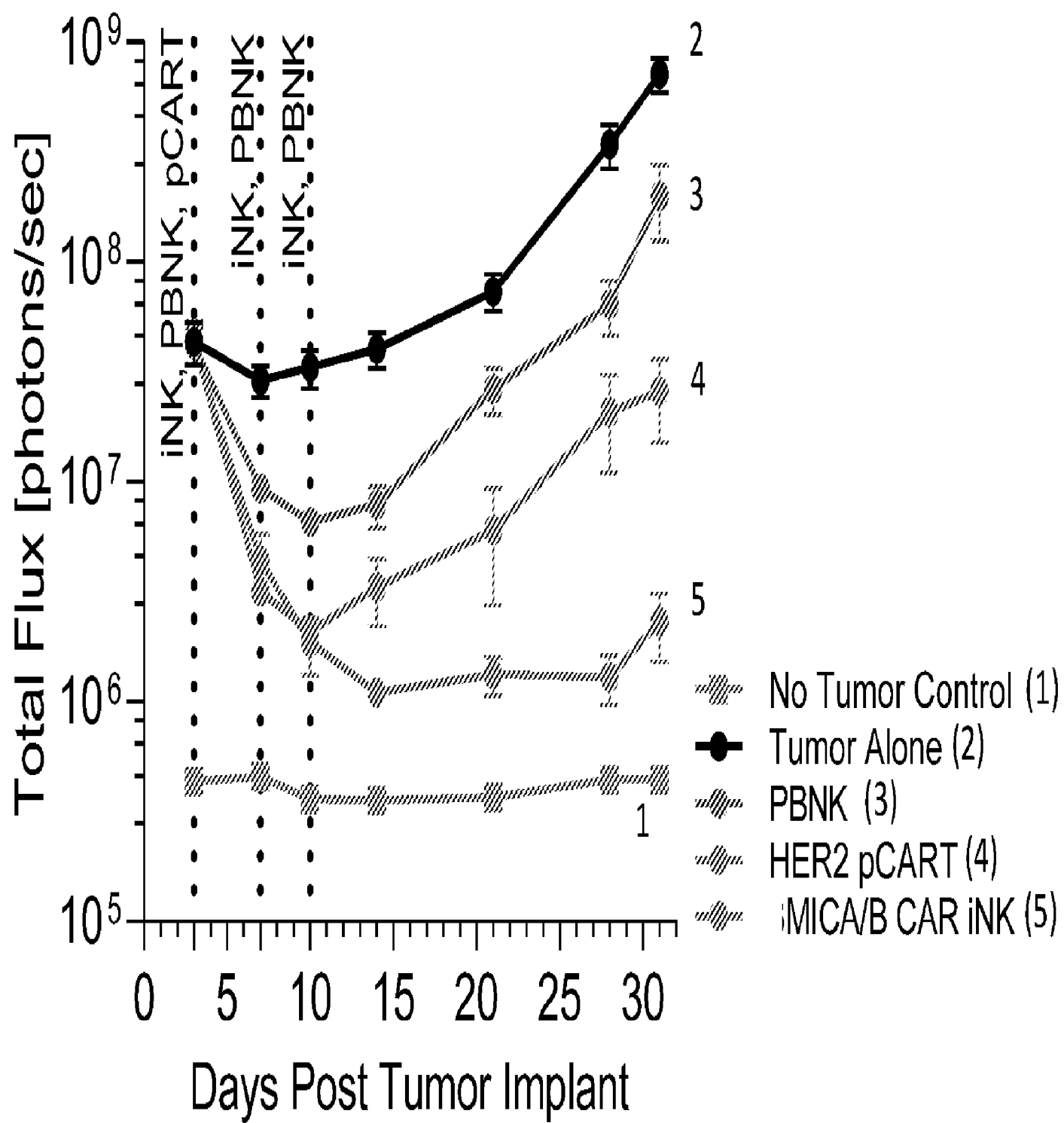


FIG. 4B