

CLAIMS

What is claimed is:

1. A cell or a population thereof, wherein
 - (i) the cell is (a) an immune cell; (b) an induced pluripotent cell (iPSC), a clonal iPSC, or an iPS cell line cell; or (c) a derivative cell obtained from differentiating the cell of (b); and
 - (ii) the cell comprises:
 - (1) a polynucleotide encoding a MICA/B-CAR (chimeric antigen receptor); or
 - (2) knockout in one or both of CD58 and CD54.
2. The cell or population thereof of claim 1, 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: 33;
 - (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: 34;
 - (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: 35 or 36;
 - (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, CD58, CD54, 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.

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

- (i) CD38 knockout;
- (ii) HLA-I deficiency and/or HLA-II deficiency
- (iii) B2M null or low, and optionally CIITA null or low, in comparison to its native counterpart cell;
- (iv) introduced expression of HLA-G or non-cleavable HLA-G, or knockout in one or both of CD58 and CD54;
- (v) a high affinity non-cleavable CD16 (hnCD16) or a variant thereof;
- (vi) a chimeric antigen receptor (CAR) with targeting specificity other than MICA/B;
- (vii) a partial or full peptide of a cell surface expressed exogenous cytokine or a receptor thereof;
- (viii) at least one of the genotypes listed in Table 1;
- (ix) deletion or reduced expression 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
- (x) introduced or increased expression in at least one of HLA-E, 41BBL, 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 cell or population thereof of claim 1, 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.

5. The cell or population thereof of claim 3, wherein the cell further comprises a high affinity non-cleavable CD16 (hnCD16) or a variant thereof.
6. The cell or population thereof of claim 5, 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.
7. The cell or population thereof of claim 6, wherein
 - (a) the non-native transmembrane domain is derived from CD3D, CD3E, CD3G, CD3ζ, 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 non-native 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 non-native signaling domain is derived from CD3ζ, 2B4, DAP10, DAP12, DNAM1, CD137 (41BB), IL21, IL7, IL12, IL15, NKp30, NKp44, NKp46, NKG2C, or NKG2D polypeptide; or
 - (d) the non-native transmembrane domain is derived from NKG2D, the non-native stimulatory domain is derived from 2B4, and the non-native signaling domain is derived from CD3ζ.
8. The cell or population thereof of claim 3, wherein the cell further comprises a second CAR, and wherein the CAR is:
 - (i) T cell specific or NK cell specific;
 - (ii) 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 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), CCRI, 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-a, Ganglioside G2 (GD2), Ganglioside G3 (GD3), human Epidermal Growth Factor Receptor 2 (HER-2), 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.

9. The cell or population thereof of claim 3, wherein the cell comprises a partial or full peptide of a cell surface expressed exogenous cytokine and/or of a receptor thereof, wherein the exogenous cytokine or receptor thereof

- (a) comprises 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 (i)-(vii) can be co-expressed with a CAR in separate constructs or in a bi-cistronic construct;
 and optionally, or
- (c) is transiently expressed.

10. The cell or population thereof 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.

11. The cell or population thereof of claim 8 or 10, 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, 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.

12. The cell or population thereof of claim 11, 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.

13. The cell or population thereof of claim 2, 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.

14. The cell or population thereof 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.
15. The cell or population thereof of claim 14, 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, CD58, CD54, CD56, 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.
16. The cell or population thereof of claim 15, wherein the TCR locus is a constant region of TCR alpha or TCR beta.
17. A composition comprising the cell or population thereof of any one of the claims 1-16.
18. A composition for therapeutic use comprising the derivative cell of any one of the claims 1-16, and one or more therapeutic agents.
19. The composition of claim 18, wherein the therapeutic agents comprise a peptide, a cytokine, a checkpoint inhibitor, a mitogen, a growth factor, a small RNA, a dsRNA (double stranded RNA), mononuclear blood cells, feeder cells, feeder cell components or replacement factors thereof, a vector comprising one or more polynucleic acids of interest, an antibody, a chemotherapeutic agent or a radioactive moiety, or an immunomodulatory drug (IMiD).
20. The composition of claim 19, wherein
- (1) the checkpoint inhibitor comprises:
 - (a) one or more antagonists to checkpoint molecules comprising PD-1, PDL-1, TIM-3, TIGIT, LAG-3, CTLA-4, 2B4, 4-1BB, 4-1BBL, 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;
 - (b) one or more of atezolizumab, avelumab, durvalumab, ipilimumab, IPH4102, IPH43, IPH33, lirimumab, monalizumab, nivolumab, pembrolizumab, and their derivatives or functional equivalents;
 - (c) at least one of atezolizumab, nivolumab, and pembrolizumab; or

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

21. The composition of claim 19, wherein the antibody comprises:

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

(b) one or more of rituximab, veltuzumab, ofatumumab, ublituximab, ocaratuzumab, obinutuzumab, trastuzumab, pertuzumab, alemtuzumab, certuximab, dinutuximab, avelumab, daratumumab, isatuximab, MOR202, 7G3, CSL362, elotuzumab, and their humanized or Fc modified variants or fragments and their functional equivalents and biosimilars; or

(c) daratumumab, and wherein the derivative hematopoietic cells comprise derivative NK cells or derivative T cells comprising a CD38 knockout, and optionally an expression of hnCD16 or a variant thereof.

22. Therapeutic use of the composition of any one of the claims 17-21 by introducing the composition to 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.

23. A chimeric antigen receptor (CAR) specific to tumor cell surface antigen MICA/B, 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) reducing tumor cell surface shedding of MICA/B antigen;
- (v) increasing tumor cell surface MICA/B density;
- (vi) comprising a scFV (single chain variable fragment) binding to the conserved $\alpha 3$ domain of MICA/B;
- (vii) 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: 33;
- (viii) 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: 34;
- (ix) 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: 35 or 36;
- (x) 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

(xi) 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, CD58, CD54, 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.

24. The CAR of claim 23, wherein the CAR is expressed in an iPSC or an effector cell, and wherein said effector cell has one or more following characteristics:

- (i) being a primary immune cell or being derived from the iPSC;
- (ii) preventing tumor antigen escape;
- (iii) overcoming tumor microenvironment suppression;
- (iv) enhancing effector cell activation and killing function compared to a corresponding effector cell lacking the CAR; and
- (v) capable of in vivo tumor progression control, tumor cell burden reduction, tumor clearance, and/or improving rate of survival of a subject carrying the tumor compared to a corresponding cell lacking the CAR.

25. A method of manufacturing the derivative cell comprising a polynucleotide encoding a MICA/B-CAR of claim 23 or 24, wherein the method comprises differentiating an iPSC to obtain the derivative cells, wherein the polynucleotide encoding a MICA/B-CAR is introduced into the iPSC before differentiation or is introduced to the derivative cells after iPSC differentiation.

26. The method of claim 25, wherein the iPSC and/or the derivative cell comprises one or more of:

- (i) CD38 knockout;
- (ii) HLA-I deficiency and/or HLA-II deficiency;
- (iii) B2M null or low, and optionally CIITA null or low, in comparison to its native counterpart cell;
- (iv) introduced expression of HLA-G or non-cleavable HLA-G, or knockout in one or both of CD58 and CD54;
- (v) a high affinity non-cleavable CD16 (hnCD16) or a variant thereof;
- (vi) a chimeric antigen receptor (CAR) with targeting specificity other than MICA/B;
- (vii) a partial or full peptide of a cell surface expressed exogenous cytokine or a receptor thereof;

- (viii) at least one of the genotypes listed in Table 1;
- (ix) deletion or reduced expression 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
- (x) introduced or increased expression in at least one of HLA-E, 41BBL, 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.

27. The method of manufacturing the derivative cells of claim 25, further comprising genomically engineering a clonal iPSC

- (i) to knock out CD38;
- (ii) to disrupt HLA-I and/or to disrupt HLA-II;
- (iii) to knock out B2M and CIITA, or to knock out one or both CD58 and CD54, or
- (iv) to introduce expression of HLA-G or non-cleavable HLA-G, a high affinity non-cleavable CD16 or a variant thereof, a second CAR, and/or a partial or full peptide of a cell surface expressed exogenous cytokine or a receptor thereof;
- (v) to delete or to reduce expression 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; or
- (vi) to introduce or increase expression in at least one of HLA-E, 41BBL, 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.

28. The method of manufacturing the derivative cell of claim 27, wherein the genomic engineering comprises targeted editing.

29. The method of manufacturing the derivative cell of claim 27, wherein the targeted editing comprises deletion, insertion, or in/del, and wherein the targeted editing is carried out by CRISPR, ZFN, TALEN, homing nuclease, homology recombination, or any other functional variation of these methods.

30. CRISPR mediated editing of clonal iPSCs, wherein the editing comprises a knock-in of a polynucleotide encoding a MICA/B CAR of claim 23 or 24; or knockout of one or both of CD58 and CD54, wherein the edited clonal iPSCs comprise at least one of the genotypes listed in Table 1.

31. The CRISPR mediated editing of claim 30, wherein the editing of clonal iPSCs further comprises knocking out CD38.

32. The CRISPR mediated editing of claim 30, wherein the editing further comprises an insertion of the MICA/B CAR or a second CAR at a TCR locus, and/or wherein the CAR is driven by an endogenous promoter of TCR, and/or wherein the TCR is knocked out by the CAR insertion.

33. A method of improving treatment targeting tumor cell surface antigen MICA/B comprising administering to a subject in need of the treatment effector cells comprising a MICA/B-CAR of claim 23 or 24.

34. The method of claim 33, wherein the effector cells comprise T cells, NK cells, derivative NK cells, or derivative T cells; wherein the cells further comprise a CD38 knockout, a high affinity non-cleavable CD16 or a variant thereof, and optionally comprise:

- (i) HLA-I deficiency and/or HLA-II deficiency;
- (ii) B2M and CIITA knockout;
- (iii) introduced expression of HLA-G or non-cleavable HLA-G, or knockout of one or both of CD58 and CD54;
- (iv) introduced expression of a second CAR, and/or a partial or full peptide of a cell surface expressed exogenous cytokine or a receptor thereof; and/or
- (v) at least one of the genotypes listed in Table 1.

35. The method of claim 33, wherein the method has one or more the following characteristics:

- (i) reducing tumor cell surface shedding of MICA/B antigen;
- (ii) increasing tumor cell surface MICA/B density;
- (iii) preventing tumor antigen escape;
- (iv) overcoming tumor microenvironment suppression;
- (v) enhancing effector cell activation and killing function; and
- (vi) capable of in vivo tumor progression control, tumor cell burden reduction, tumor clearance, and/or improving rate of survival;

as compared to treatment using effector cell without the MICA/B-CAR of claim 23 or 24.