

CLAIMS

1. A cell or a population thereof, in which

(i) the cell is an immune cell; an induced pluripotent cell (iPSC), a clonal iPSC, or an iPS cell line cell; or a derivative cell obtained from differentiating an iPSC;

5 (ii) the cell comprises a polynucleotide encoding a chimeric antigen receptor (CAR) comprising a binding domain;

(iii) the binding domain comprises a heavy chain variable region represented by the amino acid sequence of SEQ ID NO: 33; and

10 (iv) the binding domain comprises a light chain variable region represented by SEQ ID NO: 34.

2. A cell or population thereof according to Claim 1, in which 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 in which the CAR has at least one of the following characteristics:

15 (i) being T cell specific;

(ii) being NK cell specific;

(iii) comprising a scFV represented by the amino acid sequence of one of SEQ ID NOs: 35 or 36; or

20 (iv) 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, CD56, CIS, CBL-B, SOCS2, PD1, CTLA4, LAG3, TIM3, or TIGIT; and, optionally, in which the insertion knocks out expression of the gene in the locus.

3. A cell or population thereof according to Claim 1, in which the cell further comprises one or more of:

25 (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;
 - (v) an exogenous CD16;
 - (vi) a second chimeric antigen receptor (CAR) with a different targeting specificity;
 - (vii) a partial or full peptide of a cell surface expressed exogenous cytokine or a
5 receptor thereof;
 - (viii) 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
 - (ix) introduced or increased expression in at least one of HLA-E, 41BBL, CD3, CD4,
10 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. A cell or population thereof according to Claim 1, in which the derivative cell is an
15 immune effector 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
20 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,
- 25 in comparison to its native counterpart cell obtained from peripheral blood, umbilical cord blood, or any other donor tissues.

5. A cell or population thereof according to Claim 3, in which the exogenous CD16 comprises a high affinity non-cleavable CD16 (hnCD16).

6. A cell or population thereof according to Claim 5, in which the high affinity non-cleavable CD16 (hnCD16) comprises at least one of:

- 5 (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) signalling domain;
- 10 (f) a non-native (or non-CD16) stimulatory domain; and
- (g) transmembrane, signalling, and stimulatory domains that are not originated from CD16, and are originated from a same or different polypeptide.

7. A cell or population thereof according to Claim 6, in which

- 15 (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, 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, 20 ICOS, PD-1, LAG-3, 2B4, BTLA, DAP10, DAP12, CTLA-4, or NKG2D polypeptide;
- (c) the non-native signalling 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 25 stimulatory domain is derived from 2B4, and the non-native signalling domain is derived from CD3ζ.

8. A cell or population thereof according to Claim 3, in which the cell further comprises a second CAR, and in which the second CAR is:

- (i) T cell specific or NK cell specific;
- (ii) bi-specific antigen binding CAR;
- (iii) a switchable CAR;
- (iv) a dimerized CAR;
- 5 (v) a split CAR;
- (vi) a multi-chain CAR;
- (vii) an inducible CAR;
- (viii) a recombinant TCR;
- (ix) co-expressed with another CAR;
- 10 (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;
- 15 (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,
- 20 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, EGFIR, EGFR-VIII, ERBB folate-binding protein (FBP), fetal acetylcholine receptor (AChR), folate receptor-a, Ganglioside G2 (GD2), Ganglioside
- 25 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), Mucin 1 (Muc-1), Mucin 16 (Muc-16), Mesothelin
- 30 (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;

5 in which the second 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 second CAR insertion.

9. A cell or population thereof according to Claim 3, in which the cell comprises a partial or full peptide of a cell surface expressed exogenous cytokine and/or of a receptor thereof, in
10 which 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;

15 (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 ;

20 (vi) a fusion protein of IL15 and common receptor γ C, in which the common receptor γ C is native or modified; and

(vii) a homodimer of IL15R β ; in which any one of (i)-(vii) can be co-expressed with a CAR in separate constructs or in a bi-cistronic construct.

10. A cell or population thereof according to Claim 3, in which the cell is a derivative NK or
25 a derivative T cell, in which the derivative NK cell is capable of recruiting, and/or migrating T cells to tumor sites, and in which the derivative NK or the derivative T cell is capable of reducing tumor immunosuppression in the presence of one or more checkpoint inhibitors.

11. A cell or population thereof according to Claim 8 or 10, in which 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.

- 5 12. A cell or population thereof according to Claim 11, in which the checkpoint inhibitors comprise:
 - (a) one or more of atezolizumab, avelumab, durvalumab, ipilimumab, IPH4102, IPH43, IPH33, lirimumab, monalizumab, nivolumab or pembrolizumab; or
 - (b) at least one of atezolizumab, nivolumab, and pembrolizumab.
- 10 13. A cell or population thereof according to Claim 2, in which 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.
- 15 14. A cell or population thereof according to Claim 1, in which the cell comprises:
 - (i) one or more exogenous polynucleotides integrated in one safe harbour locus or a selected gene locus; or
 - (ii) more than two exogenous polynucleotides integrated in different safe harbour loci or two or more selected gene locus.
- 20 15. A cell or population thereof according to Claim 14, in which the safe harbour locus comprises at least one of AAVS1, CCR5, ROSA26, collagen, HTRP, H11, GAPDH, or RUNX1; and in which the selected gene locus is one of B2M, TAP1, TAP2, Tapasin, NLRC5, CIITA, RFXANK, RFX5, RFXAP, TCR, NKG2A, NKG2D, CD38, CD25, CD56, CIS, CBL-B, SOCS2, PD1, CTLA4, LAG3, TIM3, or TIGIT; and in which the integration of the
 - 25 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. A cell or population thereof according to Claim 15, in which the TCR locus is a constant region of TCR alpha or TCR beta.

17. A composition comprising a cell or population thereof according to any preceding claim.

18. A composition for therapeutic use comprising the immune cell according to any of Claims 1 to 16, and one or more therapeutic agents, in which the immune cell is an NK cell or a T cell.

19. A composition according to Claim 18, in which 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. A composition according to Claim 19, in which

(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 or pembrolizumab;

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

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

21. A composition according to Claim 19, in which 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 or elotuzumab; or

(c) daratumumab, and in which the immune cell comprises a CD38 knockout, and optionally an expression of hnCD16.

22. Use of a composition according to any of Claims 18 to 21 in the manufacture of a medicament for treatment of a hematological malignancy; a solid tumor; or a cancer.

23. A chimeric antigen receptor (CAR), in which:

(A) the CAR comprises a binding domain;

(B) the binding domain comprises heavy chain variable region represented by the amino acid sequence of SEQ ID NO: 33;

(C) the binding domain comprises a light chain variable region represented by SEQ ID NO: 34; and

(D) the CAR has at least one of the following characteristics:

(i) being T cell specific;

(ii) being NK cell specific;

(iii) reducing tumor cell surface shedding of MICA/B antigen;

(iv) increasing tumor cell surface MICA/B density;

(v) comprising a scFV represented by the amino acid sequence of SEQ ID NOs: 35 or 36; or

(vi) 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, CD56, CIS, CBL-B, SOCS2, PD1, CTLA4, LAG3, TIM3, or TIGIT; and optionally, in which the insertion knocks out expression of the gene in the locus.

24. A CAR according to Claim 23, in which the CAR is expressed in an iPSC or an effector cell, and in which the 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 a cell comprising a polynucleotide encoding a CAR according to Claim 23 or 24, in which:

- (a) the method comprises differentiating an iPSC to obtain the cell; and
- (b) the polynucleotide encoding the CAR is introduced into the iPSC before differentiation or is introduced to the cell after iPSC differentiation.

26. A method according to Claim 25, in which the 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;
- (v) a high affinity non-cleavable CD16 (hnCD16);
- (vi) a second chimeric antigen receptor (CAR) with different targeting specificity;
- (vii) a partial or full peptide of a cell surface expressed exogenous cytokine or a receptor thereof;
- (viii) 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
- (ix) 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. A method according to Claim 25, further comprising genomically engineering a clonal iPSC

5 (i) to knock out CD38;

(ii) to disrupt HLA-I and/or to disrupt HLA-II;

(iii) to knock out B2M and CIITA, or

(iv) to introduce expression of HLA-G or non-cleavable HLA-G, a high affinity non-cleavable CD16, a second CAR, and/or a partial or full peptide of a cell surface

10 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

15 (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.

20 28. A method according to Claim 27, in which the genomic engineering comprises targeted editing.

29. A method according to Claim 28, in which the targeted editing comprises deletion, insertion, or in/del.

30. CRISPR mediated editing of clonal iPSCs, in which the editing comprises a knock-in of a polynucleotide encoding a CAR according to Claim 23 or 24, in which the edited clonal
25 iPSCs comprise at least one of the genotypes listed in Table 1.

31. CRISPR mediated editing according to Claim 30, in which the editing of clonal iPSCs further comprises knocking out CD38.

32. CRISPR mediated editing according to Claim 30, in which the editing further comprises an insertion of the CAR or a second CAR at a TCR locus, and/or in which the CAR is driven

by an endogenous promoter of TCR, and/or in which the TCR is knocked out by the CAR insertion.

33. Use of a CAR according to Claim 23 or 24 in the manufacture of a medicament for improving treatment targeting tumor cell surface antigen MICA/B, in which (a) the medicament comprises effector cells comprising the CAR, and (b) the effector cells comprise NK cells or T cells.

34. Use according to Claim 33, in which the effector cells further comprise a CD38 knockout, a high affinity non-cleavable CD16, 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;
- (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. Use according to Claim 33, in which the effector cell 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 an effector cell without the CAR.