

CLAIMS

What is claimed is:

1. A cell or a population thereof, wherein (a) the cell comprises a polynucleotide encoding a recombinant cytokine signaling complex, and optionally a chimeric antigen receptor (CAR); (b) the cell is an induced pluripotent cell (iPSC), an eukaryotic cell, an animal cell, a human cell, an immune cell, a feeder cell, or a derivative cell differentiated from the iPSC; and (c) the cytokine signaling complex comprises (i) a full or partial cytokine and (ii) a full or partial IL7 receptor, IL2 receptor, IL4 receptor, IL9 receptor, IL21 receptor, or γ C receptor.
2. The cell or population thereof of claim 1, wherein the cytokine signaling complex is co-expressed with the CAR in separate constructs or in a bi-cistronic construct.
3. The cell or population thereof 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 comprises (i) a derivative CD34⁺ cell, a derivative hematopoietic stem and progenitor cell, a derivative hematopoietic multipotent progenitor cell, a derivative T cell progenitor, a derivative NK cell progenitor, a derivative T lineage cell, a derivative NKT lineage cell, a derivative NK lineage cell, or a derivative B lineage cell; or (ii) a derivative effector cell having one or more functional features that are not present in a counterpart primary T, NK, NKT, and/or B cell.
4. The cell or population thereof of any one of claims 1-3, wherein the cytokine signaling complex comprises a partial or full peptide of an IL7 and an IL7 receptor, and wherein the cytokine signaling complex:
 - (a) comprises at least one of:
 - (i) IL7 and IL7R α co-expressed by using a self-cleaving peptide;
 - (ii) a fusion protein of IL7 and IL7R α (IL7RF);
 - (iii) an IL7/IL7R α fusion protein with intracellular domain of IL7R α truncated or eliminated;
 - (iv) a fusion protein of IL7 and IL7R β ;

- (v) a fusion protein of IL7 and common receptor γ C, wherein the common receptor γ C is native or modified; and
 - (vi) a homodimer of IL7R β ,
- wherein any one of (i)-(vi) is optionally co-expressed with a CAR in separate constructs or in a bi-cistronic construct;
- and optionally,
- (b) is transiently expressed.
5. The cell or population thereof of any one of claims 1-4, 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, or knockout of one or both of CD58 and CD54;
 - (iv) an exogenous CD16 or a variant thereof;
 - (v) a chimeric fusion receptor (CFR);
 - (vi) a partial or full peptide of a cell surface expressed exogenous cytokine and/or a receptor thereof;
 - (vii) at least one of the genotypes listed in Table 1;
 - (viii) deletion or disruption of at least one of B2M, CIITA, TAP1, TAP2, Tapasin, NLRC5, RFXANK, RFX5, RFXAP, TCR, NKG2A, NKG2D, CD25, CD69, CD44, CD56, CIS, CBL-B, SOCS2, PD1, CTLA4, LAG3, TIM3, and TIGIT; or
 - (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 antibody or functional variant or fragment thereof, a checkpoint inhibitor, an engager, and surface triggering receptor for coupling with an agonist.
6. The cell or population thereof of claim 4, wherein the cell has therapeutic properties comprising one or more of:
- (i) increased cytotoxicity;
 - (ii) improved persistency and/or survival;

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

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

7. The cell or population thereof of claim 5, wherein the exogenous CD16 or a variant thereof comprises at least one of:

- (a) a high affinity non-cleavable CD16 (hnCD16);
- (b) F176V and S197P in ectodomain domain of CD16;
- (c) a full or partial ectodomain originated from CD64;
- (d) a non-native (or non-CD16) transmembrane domain;
- (e) a non-native (or non-CD16) intracellular domain;
- (f) a non-native (or non-CD16) signaling domain;
- (g) a non-native stimulatory domain; and
- (h) transmembrane, signaling, and stimulatory domains that are not originated from CD16, and are originated from a same or different polypeptide.

8. The cell of population thereof of claim 4, wherein the cell comprises a derivative effector cell differentiated from the iPSC, and further wherein the derivative effector cell comprises at least one of:

- (a) improved relative cell expansion;
- (b) increased percentage of CAR expression;
- (c) increased CD69 expression; and
- (d) decreased PD-1 expression,

in comparison to its counterpart cell without the cytokine signaling complex.

9. The cell or population thereof of any one of claims 1-4, wherein the CAR 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) an inactivation CAR;
 - (ix) co-expressed with a checkpoint inhibitor, optionally in separate constructs or in a bi-cistronic construct;
 - (x) specific to at least one of CD19, BCMA, CD20, CD22, CD38, CD123, HER2, CD52, EGFR, GD2, MICA/B, MSLN, VEGF-R2, PSMA and PDL1; and/or
 - (xi) 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; and optionally,

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

10. The cell or population thereof of claim 9, wherein the inactivation CAR targets an upregulated surface protein in activated recipient immune cells.

11. The cell or population thereof of claim 10, wherein the inactivation CAR comprises at least one of a CD38-CAR, a 4-1BB-CAR, an OX40-CAR, and a CD40L-CAR.

12. The cell or population thereof of claim 5, wherein the CFR comprises an ectodomain fused to a transmembrane domain, which is operatively connected to an endodomain, and wherein the ectodomain, transmembrane domain and the endodomain do not comprise any endoplasmic reticulum (ER) retention signals or endocytosis signals.

13. The cell or population thereof of claim 12, wherein the ectodomain of the CFR comprises a full or partial length of an extracellular portion of a signaling protein comprising at least one of CD3 ϵ , CD3 γ , CD3 δ , CD28, CD5, CD16, CD64, CD32, CD33, CD89, NKG2C, NKG2D, any functional variants, and a combination or a chimera thereof.

14. The cell or population thereof of claim 12, wherein the ectodomain of the CFR initiates signal transduction upon binding to a selected agonist comprising at least a binding domain that is specific to an extracellular portion of CD3, CD28, CD5, CD16, CD64, CD32, CD33, CD89, NKG2C, NKG2D, or any functional variants thereof; or wherein the selected agonist comprises a binding domain specific to at least one tumor antigen comprising 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, or ROR1.

15. The cell or population thereof of any one of claims 12-14, wherein the endodomain of the CFR comprises a cytotoxicity domain comprising at least a full length or a portion of CD3 ζ , 2B4, DAP10, DAP12, DNAM1, CD137 (4-1BB), IL21, IL7, IL12, IL15, NKp30, NKp44,

NKp46, NKG2C, or NKG2D polypeptide; and optionally wherein the endodomain further comprises one or more of:

(i) a co-stimulatory domain comprising a full length or a portion of CD2, CD27, CD28, CD40L, 4-1BB, OX40, ICOS, PD-1, LAG-3, 2B4, BTLA, DAP10, DAP12, CTLA-4, or NKG2D polypeptide, or any combination thereof;

(ii) a co-stimulatory domain comprising a full length or a portion of CD28, 4-1BB, CD27, CD40L, ICOS, CD2, or combinations thereof;

(iii) a persistency signaling domain comprising a full length or a portion of an endodomain of a cytokine receptor comprising IL7R, IL15R, IL18R, IL12R, IL23R, or combinations thereof; and/or

(iv) a full or a partial intracellular portion of a receptor tyrosine kinase (RTK), a tumor necrosis factor receptor (TNFR), an EGFR or a FAS receptor.

16. The cell or population thereof of claim 5, wherein the checkpoint inhibitor is an antagonist 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, and inhibitory KIR.

17. The cell or population thereof of any one of claims 1-4, wherein the cell comprises:

- (i) one or more exogenous polynucleotides integrated in a 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 loci.

18. The cell or population thereof of claim 17, wherein the safe harbor locus comprises at least one of AAVS1, CCR5, ROSA26, collagen, HTRP, H11, GAPDH, or RUNX1; or 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/or wherein the integration of the exogenous polynucleotides knocks out expression of the gene in the locus.

19. The cell or population thereof of claim 18, wherein the TCR locus is a constant region of TCR alpha and/or TCR beta.
20. The cell or population thereof of claim 3 or 4, wherein the derivative effector cell is of T lineage and is expanded in a culture medium that does not contain exogenous IL15.
21. The cell or population thereof of claim 20, wherein the culture medium includes one or both of exogenous IL2 and IL7.
22. The cell or population thereof of claim 20, wherein the culture medium does not contain either IL2 or IL7.
23. The cell or population thereof of any one of claims 20-22, wherein the derivative effector cell is CD69⁺, and/or PD1⁻ or PD1^{low}.
24. The cell or population thereof of any one of claims 20-22, wherein the derivative effector cell has increased antitumor function and/or persistency as compared to its counterpart cell without the cytokine signaling complex in the absence of cytokine support.
25. A method for improving T lineage cell expansion and/or tumor cell control and clearance, the method comprising introducing an IL7 cytokine signaling complex to the T lineage cell, thereby producing a T lineage cell having improved cell expansion and/or tumor cell control and clearance as compared to a counterpart cell without the cytokine signaling complex, wherein the T lineage cell optionally further comprises a chimeric antigen receptor (CAR).
26. The method of claim 25, wherein the step of introducing comprises (i) engineering an induced pluripotent cell (iPSC) to produce a genomically edited iPSC that comprises a polynucleotide encoding the IL7 cytokine signaling complex and optionally, a CAR; and (ii) differentiating the genomically edited iPSC to a derivative T lineage cell comprising the IL7 signaling complex.
27. The method of claim 26, wherein the genomically edited iPSC further comprises one or more edits resulting in:
- (i) CD38 knockout;
 - (ii) HLA-I deficiency and/or HLA-II deficiency;

- (iii) introduction of HLA-G or non-cleavable HLA-G, or knockout of one or both of CD58 and CD54;
- (iv) an exogenous CD16 or a variant thereof;
- (v) a chimeric fusion receptor (CFR);
- (vi) a partial or full peptide of a cell surface expressed exogenous cytokine and/or a receptor thereof;
- (vii) at least one of the genotypes listed in Table 1;
- (viii) deletion or disruption of at least one of B2M, CIITA, TAP1, TAP2, Tapasin, NLRC5, RFXANK, RFX5, RFXAP, TCR, NKG2A, NKG2D, CD25, CD69, CD44, CD56, CIS, CBL-B, SOCS2, PD1, CTLA4, LAG3, TIM3, and TIGIT; or
- (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 antibody or functional variant or fragment thereof, a checkpoint inhibitor, an engager, and surface triggering receptor for coupling with an agonist,

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

28. The method of claim 26, further comprising expanding the T lineage cell comprising the IL7 cytokine signaling complex in a culture medium that does not contain exogenous IL15.

29. The method of claim 28, wherein the culture medium includes one or both of exogenous IL2 and IL7.

30. The method of claim 28, wherein the culture medium does not contain either IL2 or IL7.

31. The method of any one of claims 28-30, wherein the T lineage cell is CD69⁺, and/or PD1⁻ or PD1^{low}.

32. The method of any one of claims 25-30, wherein the improved cell expansion and/or tumor cell control and clearance is *in vitro*

33. A composition comprising the cell or population thereof of any one of the claims 1-24.

34. A master cell bank (MCB) comprising the clonal iPSC of any one of the claims 1-24.

35. A composition comprising a derivative effector cell differentiated from the iPSC of any one of the claims 1-24, and one or more therapeutic agents.
36. The composition of claim 35, wherein the one or more therapeutic agents comprise a peptide, a cytokine, a checkpoint inhibitor, an antibody or functional variant or fragment thereof, 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).
37. The composition of claim 36, wherein:
- (a) the checkpoint inhibitor comprises:
 - (i) one or more antagonist 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, durvalumab, ipilimumab, IPH4102, IPH43, IPH33, lirimumab, monalizumab, nivolumab, pembrolizumab, and their derivatives or functional equivalents; or
 - (iii) at least one of atezolizumab, nivolumab, and pembrolizumab; or
 - (b) the therapeutic agents comprise one or more of venetoclax, azacitidine, and pomalidomide.
38. The composition of claim 36, wherein the antibody, or functional variant or fragment thereof comprises:
- (a) anti-CD20, anti-CD22, 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, ibritumomab, ocrelizumab, inotuzumab, moxetumomab, epratuzumab, trastuzumab, pertuzumab, alemtuzumab, cetuximab, 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 effector cell comprises a CD38 knockout, and optionally an expression of CD16 or a variant thereof.

39. The composition of claim 36, wherein the cytokine is IL2.

40. Use of the composition of any one of the claims 33 or 35-39 in the manufacture of a medicament for the treatment of an autoimmune disorder, a hematological malignancy, a solid tumor, cancer, or a viral infection.

41. The use of claim 40, wherein the subject does not need cytokine support during therapeutic treatment.

42. A method of manufacturing a derivative effector cell comprising a cytokine signaling complex and optionally, a CAR, wherein the method comprises: (i) differentiating a genetically engineered iPSC to the derivative effector cell, wherein the iPSC comprises a polynucleotide encoding the cytokine signaling complex and optionally the CAR; and (ii) expanding the derivative effector cell in a culture medium that does not contain exogenous IL15, and wherein the derivative effector cell has therapeutic properties comprising one or more of:

- (a) increased cytotoxicity;
- (b) improved persistency and/or survival;
- (c) enhanced ability in migrating, and/or activating or recruiting bystander immune cells, to tumor sites;
- (d) improved tumor penetration;
- (e) enhanced ability to reduce tumor immunosuppression;
- (f) improved ability in rescuing tumor antigen escape;
- (g) controlled apoptosis;
- (h) enhanced or acquired ADCC; and
- (i) ability to avoid fratricide,

in comparison to a cell cultured in a culture medium containing exogenous IL15.

43. The method of claim 42, wherein the cytokine signaling complex comprises an IL7 receptor fusion (IL7RF).

44. A method of producing a clonal master engineered iPSC line using CRISPR mediated editing of a clonal iPSC, wherein the editing comprises a knock-in of a polynucleotide encoding a cytokine signaling complex and optionally a CAR to the clonal iPSC, wherein the cytokine signaling complex comprises an IL7 receptor fusion (IL7RF), thereby producing producing the clonal master engineered iPSC line.

45. The composition of any one of claim 35-39 for use in therapy.