

CLAIMS

What is claimed is:

1. A composition comprising two or more synthetic cell populations, wherein the composition comprises:
 - (i) a first synthetic cell population comprising iPSC-derived NK cells, wherein the iPSC-derived NK cells comprise:
 - (a) an exogenous CD16 or a variant thereof; and
 - (b) one or both of (i) a first chimeric antigen receptor (CAR), and (ii) a partial or full length peptide of a cell surface expressed exogenous cytokine and/or a receptor thereof; and
 - (ii) a second synthetic cell population comprising iPSC-derived T cells, wherein the iPSC-derived T cells comprise: at least a second chimeric antigen receptor (CAR), and wherein the second CAR is expressed under control of an endogenous promoter of a TCR locus.
2. The composition of claim 1, wherein the exogenous CD16 or variant thereof is a high affinity non-cleavable exogenous CD16 (hnCD16); or wherein the exogenous CD16 or 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 non-native stimulatory domain; and
 - (g) transmembrane, signaling, and stimulatory domains that are not originated from CD16, and are originated from a same or different polypeptide.
3. The composition of claim 2, 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 CD3 ζ , 2B4, DAP10, DAP12, DNAM1, CD137 (4-1BB), 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 CD3 ζ .

4. The composition of claim 1, wherein the first CAR and the second CAR are the same or are different in targeting specificity, and wherein the first CAR or the second 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) co-expressed with another CAR;
- (ix) co-expressed with a partial or full length peptide of a cell surface expressed exogenous cytokine and/or a receptor thereof, optionally in separate constructs or in a bi-cistronic construct;
- (x) co-expressed with a checkpoint inhibitor, optionally in separate constructs or in a bi-cistronic construct;
- (xi) specific to CD19 or BCMA; and/or
- (xii) 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-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.

5. The composition of claim 1, wherein the partial or full length peptide of a cell surface expressed exogenous cytokine and/or a receptor thereof:

(a) comprises at least one of IL2, IL4, IL6, IL7, IL9, IL10, IL11, IL12, IL15, IL18, IL21, and/or respective receptors thereof; 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,

(c) is transiently expressed.

6. The composition of claim 1, wherein the iPSC-derived NK cells and/or the iPSC-derived T cells further comprise one or more of:

(i) HLA-I deficiency;

(ii) HLA-II deficiency;

(iii) introduced expression of HLA-G or non-cleavable HLA-G;

- (iv) at least one of lig^- , inR^+ , cs-CD3^+ , En^+ , and Ab^+ ; wherein
 - (1) lig^- is negative in an expressed alloantigen;
 - (2) inR^+ is positive in an expressed inactivation-CAR corresponding to the negative alloantigen;
 - (3) cs-CD3^+ is positive in cell surface expressed CD3;
 - (4) En^+ is positive in at least one expressed engager, wherein the engager comprises a bi-specific T cell engager (BiTE), or a tri-specific killer cell engager (TriKE); and
 - (5) Ab^+ is positive in at least one expressed antibody or checkpoint inhibitor;
- (v) deletion or reduced expression in at least one of B2M, CIITA, TAP1, TAP2, Tapasin, NLRC5, PD1, LAG3, TIM3, RFXANK, RFX5, RAG1, RFXAP, and any gene in the chromosome 6p21 region; and
- (vi) introduced or increased expression in at least one of HLA-E, HLA-G, 41BBL, CD4, CD8, CD16, CD47, CD113, CD131, CD137, CD80, PDL1, A_2AR , TCR, Fc receptor, and surface triggering receptor for coupling with bi- or multi- specific or universal engagers.

7. The composition of claim 6, wherein:

- (i) the alloantigen comprises CD40L, OX40, or 4-1BB;
- (ii) the inactivation-CAR comprises CD40L-CAR, OX40-CAR, or 4-1BB-CAR;
- (iii) the BiTE or the TriKE recognizes (a) an immune cell surface molecule comprising CD3, CD28, CD5, CD16, NKG2D, CD64, CD32, CD89, NKG2C, or a chimeric Fc receptor thereof; and (b) a tumor surface molecule 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;
- (iv) the BiTE comprises CD3-CD19, CD16-CD30, CD64-CD30, CD16-BCMA, CD64-BCMA, or CD3-CD33;
- (v) the TriKE comprises CD16-IL15-EPCAM, CD64-IL15-EPCAM, CD16-IL15-CD33, CD64-IL15-CD33, or NKG2C-IL15-CD33;
- (vi) the antibody comprises an anti-CD20, anti-HER2, anti-CD52, anti-EGFR, anti-CD123, anti-GD2, anti-PDL1, or anti-CD38 antibody; or

(vii) the checkpoint inhibitor comprises (a) an antagonist to a checkpoint molecule comprising PD-1, PDL-1, TIM-3, TIGIT, LAG-3, CTLA-4, 2B4, 4-1BB, 4-1BBL, A_{2A}R, BATE, BTLA, CD39, CD47, CD73, CD94, CD96, CD160, CD200, CD200R, CD274, CEACAM1, CSF-1R, Foxp1, GARP, HVEM, IDO, EDO, TDO, LAIR-1, MICA/B, NR4A2, MAFB, OCT-2, Rara (retinoic acid receptor alpha), TLR3, VISTA, NKG2A/HLA-E, or inhibitory KIR; (b) one of atezolizumab, avelumab, durvalumab, ipilimumab, IPH4102, IPH43, IPH33, lirimumab, monalizumab, nivolumab, pembrolizumab, and their derivatives or functional equivalents; or (c) one of atezolizumab, nivolumab, and pembrolizumab.

8. The composition of claim 1, wherein the iPSC-derived NK cells or the iPSC-derived T cells comprise:

- (i) one or more exogenous polynucleotides integrated in one desired integration site; or
- (ii) more than two exogenous polynucleotides integrated in different desired integration sites.

9. The composition of claim 8, wherein the desired integration site comprises at least one of 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, CD40L, CD44, CD54, CD56, CD58, CD69, CD71, OX40, 4-1BB, CIS, CBL-B, SOCS2, PD1, CTLA4, LAG3, TIM3, and TIGIT.

10. The composition of claim 8, wherein the desired integration site comprises TCR α or β constant region, CD25, CD38, CD40L, CD44, CD54, CD58, CD69, CD71, OX40 or 4-1BB; and optionally, wherein the TCR α or TCR β , CD25, CD38, CD40L, CD44, CD54, CD58, CD69, CD71, OX40 or 4-1BB is knocked out as a result of integrating said one or more exogenous polynucleotides at the respective integration site.

11. The composition of claim 1, wherein the iPSC-derived NK cells or the iPSC-derived T cells have 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, and
 - (viii) improved ability in rescuing tumor antigen escape,
- in comparison to its native counterpart cell obtained from peripheral blood, umbilical cord blood, or any other donor tissues.

12. The composition of claim 1, wherein the iPSC-derived NK cells or the iPSC-derived T cells comprise longer telomeres in comparison to their respective native counterpart cell obtained from peripheral blood, umbilical cord blood, or any other donor tissues.

13. The composition of claim 1, wherein the first synthetic cell population or the second synthetic cell population is modulated *ex vivo*.

14. The composition of claim 13, wherein the modulated first synthetic cell population comprising iPSC-derived NK cells comprises an increased number or ratio of type I NKT cells, and/or adaptive NK cells, as compared to the first synthetic cell population without being modulated; or wherein the second modulated synthetic cell population comprising iPSC-derived T cells comprises an increased number or ratio of naïve T cells, stem cell memory T cells, and/or central memory T cells, as compared to the second synthetic cell population without being modulated.

15. The composition of claim 1, wherein

- (i) the iPSC-derived NK cells and the iPSC-derived T cells are in a ratio ranging from 100:1 to 1:100;
- (ii) the composition further comprises one or more additional cell populations; or
- (iii) the composition further comprises one or more therapeutic agents.

16. The composition of claim 15, wherein the additional cell population comprises regulatory cells.

17. The composition of claim 16, wherein the regulatory cells are iPSC-derived immune regulatory cells or myeloid derived suppressor cells (MDSCs).
18. The composition of claim 15, wherein the one or more therapeutic agents comprise a peptide, a cytokine, a checkpoint inhibitor, a mitogen, a growth factor, a small RNA, a dsRNA (double stranded RNA), mononuclear blood cells, feeder cells, feeder cell components or replacement factors thereof, a vector comprising one or more polynucleic acids of interest, an antibody, a chemotherapeutic agent or a radioactive moiety, or an immunomodulatory drug (IMiD).
19. The composition of claim 18, wherein the checkpoint inhibitor comprises:
- (a) one or more antagonist checkpoint molecules comprising PD-1, PDL-1, TIM-3, TIGIT, LAG-3, CTLA-4, 2B4, 4-1BB, 4-1BBL, A_{2A}R, BATE, BTLA, CD39, CD47, CD73, CD94, CD96, CD160, CD200, CD200R, CD274, CEACAM1, CSF-1R, Foxp1, GARP, HVEM, IDO, EDO, TDO, LAIR-1, MICA/B, NR4A2, MAFB, OCT-2, Rara (retinoic acid receptor alpha), TLR3, VISTA, NKG2A/HLA-E, or inhibitory KIR;
 - (b) one or more of atezolizumab, avelumab, durvalumab, ipilimumab, IPH4102, IPH43, IPH33, lirimumab, monalizumab, nivolumab, pembrolizumab, and their derivatives or functional equivalents; or
 - (c) at least one of atezolizumab, nivolumab, and pembrolizumab.
20. The composition of claim 18, 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 retuximab, 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.
21. The composition of claim 1, wherein the first synthetic cell population and the second synthetic cell population are separate populations or are combined into a mixed population.

22. Therapeutic use of the composition of any one of the claims 1-21 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 method of improving tumor killing and/or clearance by a population of CAR-T cells comprising:
- providing a synthetic cell population comprising iPSC-derived NK cells to the population of CAR-T cells to obtain a combined cell population,
 - wherein the iPSC-derived NK cells comprise:
 - (a) an exogenous CD16 or a variant thereof; and
 - (b) one or both of (i) a first chimeric antigen receptor (CAR), and (ii) a partial or full length peptide of a cell surface expressed exogenous cytokine and/or a receptor thereof; and
 - wherein the first CAR of the iPSC-derived NK cells comprise a CAR targeting specificity that is same or different from that of the CAR-T cell.
24. The method of claim 23, wherein the combined cell population comprises cells having 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, and
 - (viii) improved ability in rescuing tumor antigen escape,
- in comparison to tumor killing and/or clearance by the population of CAR-T cells only without the combination of the iPSC-derived NK cells.
25. The method of claim 23, wherein the exogenous CD16 or variant thereof is a high affinity non-cleavable exogenous CD16 (hnCD16); or wherein the exogenous CD16 or 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 non-native stimulatory domain; and
 (g) transmembrane, signaling, and stimulatory domains that are not originated from CD16, and are originated from a same or different polypeptide.

26. The method of claim 25, wherein

(a) the non-native transmembrane domain is derived from CD3D, CD3E, CD3G, 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 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 CD3 ζ , 2B4, DAP10, DAP12, DNAM1, CD137 (4-1BB), 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 CD3 ζ .

27. The method of claim 23, wherein the partial or full length peptide of a cell surface expressed exogenous cytokine and/or a receptor thereof:

(a) comprises at least one of IL2, IL4, IL6, IL7, IL9, IL10, IL11, IL12, IL15, IL18, IL21, and/or respective receptors thereof; 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,
(c) is transiently expressed.

28. The method of claim 23, wherein the CAR-T cells are differentiated from an engineered iPSC, and/or wherein the CAR-T cells comprise a CAR having targeting specificity 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-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.

29. The method of claim 23, wherein the iPSC-derived NK cells and/or the CAR-T cells further comprise one or more of:

- (i) HLA-I deficiency;
- (ii) HLA-II deficiency;
- (iii) introduced expression of HLA-G or non-cleavable HLA-G;
- (iv) at least one of lig⁻, inR⁺, cs-CD3⁺, En⁺, and Ab⁺; wherein
 - (1) lig⁻ is negative in an expressed alloantigen;
 - (2) inR⁺ is positive in an expressed inactivation-CAR corresponding to the negative alloantigen;

- (3) cs-CD3⁺ is positive in cell surface expressed CD3;
 - (4) En⁺ is positive in at least one expressed engager, wherein the engager comprises a bi-specific T cell engager (BiTE), or a tri-specific killer cell engager (TriKE); and
 - (5) Ab⁺ is positive in at least one expressed antibody or checkpoint inhibitor;
 - (v) deletion or reduced expression in at least one of B2M, CIITA, TAP1, TAP2, Tapasin, NLRC5, PD1, LAG3, TIM3, RFXANK, RFX5, RAG1, RFXAP, and any gene in the chromosome 6p21 region; and
 - (vi) introduced or increased expression in at least one of HLA-E, HLA-G, 4-1BBL, CD4, CD8, CD16, CD47, CD113, CD131, CD137, CD80, PDL1, A_{2A}R, TCR, Fc receptor, and surface triggering receptor for coupling with bi- or multi- specific or universal engagers.
30. The method of claim 29, wherein
- (i) the alloantigen comprises CD40L, OX40, or 4-1BB;
 - (ii) the inactivation-CAR comprises CD40L-CAR, OX40-CAR, or 4-1BB-CAR;
 - (iii) the BiTE or the TriKE recognizes (a) an immune cell surface molecule comprising CD3, CD28, CD5, CD16, NKG2D, CD64, CD32, CD89, NKG2C, or a chimeric Fc receptor thereof; and (b) a tumor surface molecule 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;
 - (iv) the BiTE comprises CD3-CD19, CD16-CD30, CD64-CD30, CD16-BCMA, CD64-BCMA, or CD3-CD33;
 - (v) the TriKE comprises CD16-IL15-EPCAM, CD64-IL15-EPCAM, CD16-IL15-CD33, CD64-IL15-CD33, or NKG2C-IL15-CD33;
 - (vi) the antibody comprises an anti-CD20, anti-HER2, anti-CD52, anti-EGFR, anti-CD123, anti-GD2, anti-PDL1, or anti-CD38 antibody; or
 - (vii) the checkpoint inhibitor comprises (a) an antagonist to a checkpoint molecule comprising PD-1, PDL-1, TIM-3, TIGIT, LAG-3, CTLA-4, 2B4, 4-1BB, 4-1BBL, A_{2A}R, BATE, BTLA, CD39, CD47, CD73, CD94, CD96, CD160, CD200, CD200R, CD274, CEACAM1, CSF-1R, Foxp1, GARP, HVEM, IDO, EDO, TDO, LAIR-1, MICA/B, NR4A2, MAFB, OCT-2, Rara (retinoic acid receptor alpha), TLR3, VISTA, NKG2A/HLA-E, or inhibitory KIR; (b) one of

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

31. The method of claim 30, further comprising providing one or more therapeutic agents, wherein the one or more therapeutic agents comprise a peptide, a cytokine, a checkpoint inhibitor, an antibody, 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).

32. The method of claim 31, wherein the checkpoint inhibitor comprises:

(a) one or more antagonist checkpoint molecules comprising PD-1, PDL-1, TIM-3, TIGIT, LAG-3, CTLA-4, 2B4, 4-1BB, 4-1BBL, A_{2A}R, BATE, BTLA, CD39, CD47, CD73, CD94, CD96, CD160, CD200, CD200R, CD274, CEACAM1, CSF-1R, Foxp1, GARP, HVEM, IDO, EDO, TDO, LAIR-1, MICA/B, NR4A2, MAFB, OCT-2, Rara (retinoic acid receptor alpha), TLR3, VISTA, NKG2A/HLA-E, or inhibitory KIR;

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

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

33. The method of claim 31, 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 retuximab, 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.

34. A method of treating a subject using the composition of any one of claims 1-21, wherein the method comprises:

(I) administering the first synthetic cell population that comprises iPSC-derived NK cells to the subject, wherein the iPSC-derived NK cells comprise:

- (a) an exogenous CD16 or a variant thereof; and
- (b) one or both of (i) a first chimeric antigen receptor (CAR), and (ii) a partial or full length peptide of a cell surface expressed exogenous cytokine or a receptor thereof; and

(II) administering the second synthetic cell population that comprises iPSC-derived T cells to the subject, wherein the iPSC-derived T cells comprise: at least a second chimeric antigen receptor (CAR), wherein the second CAR is expressed under the control of an endogenous promoter of a TCR locus, and wherein the first CAR and the second CAR are same or different in targeting specificity.

35. The method of claim 34, wherein the subject has a condition comprising an autoimmune disorder; a hematological malignancy; a solid tumor; cancer, or a virus infection; and/or wherein the method provides enhanced improvement of the condition in comparison to using the first or the second synthetic cell population alone.

36. The method of claim 34, wherein the first synthetic cell population and the second synthetic cell population are administrated concurrently, or sequentially in any order.

37. The method of claim 34, further comprising:

administering one or more therapeutic agents; and/or administering an additional population of cells;

wherein the one or more therapeutic agents and/or the additional population of cells are administered concurrently or sequentially with either the first synthetic cell population or the second synthetic cell population.

38. The method of claim 37, wherein the additional cell population comprises regulatory cells.

39. The method of claim 38, wherein the regulatory cells are iPSC-derived immune regulatory cells or myeloid derived suppressor cells (MDSCs).

40. The method of claim 34, wherein the the first synthetic cell population and the second synthetic cell population are separate populations or are combined into a mixed population prior to administration to the subject.

41. The method of claim 37, wherein the one or more therapeutic agents comprise a peptide, a cytokine, a checkpoint inhibitor, a mitogen, a growth factor, a small RNA, a dsRNA (double stranded RNA), mononuclear blood cells, feeder cells, feeder cell components or replacement factors thereof, a vector comprising one or more polynucleic acids of interest, an antibody, a chemotherapeutic agent or a radioactive moiety, or an immunomodulatory drug (IMiD).

42. The method of claim 34, further comprising administering to the subject:

(i) a BiTE or a TriKE specific to (a) an immune cell surface molecule comprising CD3, CD28, CD5, CD16, NKG2D, CD64, CD32, CD89, NKG2C, or a chimeric Fc receptor thereof; and (b) a tumor surface molecule 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;

(ii) a BiTE comprising CD3-CD19, CD16-CD30, CD64-CD30, CD16-BCMA, CD64-BCMA, or CD3-CD33;

(iii) a TriKE comprising CD16-IL15-EPCAM, CD64-IL15-EPCAM, CD16-IL15-CD33, CD64-IL15-CD33, or NKG2C-IL15-CD33;

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

(v) a checkpoint inhibitor comprising (a) an antagonist to a checkpoint molecule 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; (b) one of atezolizumab, avelumab, durvalumab, ipilimumab, IPH4102, IPH43, IPH33, lirimumab, monalizumab, nivolumab, pembrolizumab, and their derivatives or functional equivalents; or (c) one of atezolizumab, nivolumab, and pembrolizumab.

43. A method of manufacturing the composition of claim 1, comprising:

(I) differentiating a first genetically engineered iPSC to obtain a first synthetic cell population comprising iPSC-derived NK cells, wherein the first iPSC comprises a polynucleotide encoding (a) an exogenous CD16 or a variant thereof; and (b) one or both of (i) a first chimeric antigen receptor (CAR), and (ii) a partial or full length peptide of a cell surface expressed exogenous cytokine and/or a receptor thereof, wherein the iPSC-derived NK cells comprise (a) and (b); and

(II) differentiating a second genetically engineered iPSC to obtain a second synthetic cell population comprising iPSC-derived T cells, wherein the second iPSC comprises a polynucleotide encoding at least a second chimeric antigen receptor (CAR), wherein the second CAR is expressed under the control of an endogenous promoter of a TCR locus, and wherein the iPSC-derived T cells comprise the second CAR,

thereby manufacturing the composition of claim 1.

44. The method of claim 43, wherein the exogenous CD16 or variant thereof is a high affinity non-cleavable exogenous CD16 (hnCD16); or wherein the exogenous CD16 or 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 non-native stimulatory domain; and
- (g) transmembrane, signaling, and stimulatory domains that are not originated from CD16, and are originated from a same or different polypeptide.

45. The method of claim 44, 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 CD3 ζ , 2B4, DAP10, DAP12, DNAM1, CD137 (4-1BB), 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 CD3 ζ .

46. The method of claim 43, wherein the first CAR and the second CAR are the same or are different in targeting specificity, and the first CAR or the second 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) co-expressed with another CAR;
- (ix) co-expressed with a partial or full length peptide of a cell surface expressed exogenous cytokine and/or a receptor thereof, optionally in separate constructs or in a bi-cistronic construct;
- (x) co-expressed with a checkpoint inhibitor, optionally in separate constructs or in a bi-cistronic construct;
- (xi) specific to CD19 or BCMA; and/or
- (xii) 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-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.

47. The method of claim 43, wherein the partial or full length peptide of a cell surface expressed exogenous cytokine and/or a receptor thereof:

(a) comprises at least one of IL2, IL4, IL6, IL7, IL9, IL10, IL11, IL12, IL15, IL18, IL21, and/or respective receptors thereof; 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,

(c) is transiently expressed.

48. The method of claim 43, wherein the first genetically engineered iPSC or the second genetically engineered iPSC further comprises one or more of:

(i) HLA-I deficiency;

(ii) HLA-II deficiency;

(iii) introduced expression of HLA-G or non-cleavable HLA-G;

(iv) at least one of lig⁻, inR⁺, cs-CD3⁺, En⁺, and Ab⁺; wherein

(1) lig⁻ is negative in an expressed alloantigen;

- (2) inR^+ is positive in an expressed inactivation-CAR corresponding to the negative alloantigen;
- (3) cs-CD3^+ is positive in cell surface expressed CD3;
- (4) En^+ is positive in at least one expressed engager, wherein the engager comprises a bi-specific T cell engager (BiTE), or a tri-specific killer cell engager (TriKE); and
- (5) Ab^+ is positive in at least one expressed antibody or checkpoint inhibitor;
- (v) deletion or reduced expression in at least one of B2M, CIITA, TAP1, TAP2, Tapasin, NLRC5, PD1, LAG3, TIM3, RFXANK, RFX5, RAG1, RFXAP, and any gene in the chromosome 6p21 region; and
- (vi) introduced or increased expression in at least one of HLA-E, HLA-G, 41BBL, CD4, CD8, CD16, CD47, CD113, CD131, CD137, CD80, PDL1, A_2AR , TCR, Fc receptor, and surface triggering receptor for coupling with bi- or multi- specific or universal engagers.

49. The method of claim 48, wherein

- (i) the alloantigen comprises CD40L, OX40, or 4-1BB;
- (ii) the inactivation-CAR comprises CD40L-CAR, OX40-CAR, or 4-1BB-CAR;
- (iii) the BiTE or the TriKE is specific to (a) an immune cell surface molecule comprising CD3, CD28, CD5, CD16, NKG2D, CD64, CD32, CD89, NKG2C, or a chimeric Fc receptor thereof; and (b) a tumor surface molecule 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;
- (iv) the BiTE comprises CD3-CD19, CD16-CD30, CD64-CD30, CD16-BCMA, CD64-BCMA, or CD3-CD33;
- (v) the TriKE comprises CD16-IL15-EPCAM, CD64-IL15-EPCAM, CD16-IL15-CD33, CD64-IL15-CD33, or NKG2C-IL15-CD33;
- (vi) the antibody comprises an anti-CD20, anti-HER2, anti-CD52, anti-EGFR, anti-CD123, anti-GD2, anti-PDL1, or anti-CD38 antibody; or
- (vii) the checkpoint inhibitor comprises (a) an antagonist to a checkpoint molecule comprising PD-1, PDL-1, TIM-3, TIGIT, LAG-3, CTLA-4, 2B4, 4-1BB, 4-1BBL, A_2AR , 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 of atezolizumab, avelumab, durvalumab, ipilimumab, IPH4102, IPH43, IPH33, lirimumab, monalizumab, nivolumab, pembrolizumab, and their derivatives or functional equivalents; or (c) one of atezolizumab, nivolumab, and pembrolizumab.

50. The method of claim 43, wherein the first genetically engineered iPSC or the second genetically engineered iPSC comprise:

- (i) one or more exogenous polynucleotides integrated in one desired integration site; or
- (ii) more than two exogenous polynucleotides integrated in different desired integration sites.

51. The method of claim 50, wherein the desired integration site comprises at least one of 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, CD40L, CD44, CD54, CD56, CD58, CD69, CD71, OX40, 4-1BB, CIS, CBL-B, SOCS2, PD1, CTLA4, LAG3, TIM3, and TIGIT.

52. The method of claim 50, wherein the desired integration site comprises TCR α or β constant region, CD25, CD38, CD40L, CD44, CD54, CD58, CD69, CD71, OX40 or 4-1BB; and optionally, wherein the TCR α or TCR β , CD25, CD38, CD40L, CD44, CD54, CD58, CD69, CD71, OX40 or 4-1BB is knocked out as a result of integrating said one or more exogenous polynucleotides at the respective integration site.

53. The method of claim 43, wherein the iPSC-derived NK cells or the iPSC-derived T cells have 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, and
(viii) improved ability in rescuing tumor antigen escape,
in comparison to its native counterpart cell obtained from peripheral blood, umbilical cord blood, or any other donor tissues.

54. The method of claim 43, wherein the first synthetic cell population or the second synthetic cell population is modulated *ex vivo*.

55. The method of claim 54, wherein the modulated first synthetic cell population comprising iPSC-derived NK cells comprises an increased number or ratio of type I NKT cells, and/or adaptive NK cells, as compared to the first synthetic cell population without being modulated; or wherein the second modulated synthetic cell population comprising iPSC-derived T cells comprises an increased number or ratio of naïve T cells, stem cell memory T cells, and/or central memory T cells, as compared to the second synthetic cell population without being modulated.

56. The method of claim 43, wherein:

- (i) the iPSC-derived NK cells and the iPSC-derived T cells are in a ratio ranging from 100:1 to 1:100;
- (ii) the method further comprises adding one or more additional cell populations to the produced first and second synthetic cell populations; or
- (iii) the method further comprises adding one or more therapeutic agents to the produced first and second synthetic cell populations.

57. The method of claim 56, wherein the one or more additional cell populations comprise regulatory cells.

58. The method of claim 57, wherein the regulatory cells are iPSC-derived immune regulatory cells or myeloid derived suppressor cells (MDSCs).

59. The method of claim 56, wherein the one or more therapeutic agents comprise a peptide, a cytokine, a checkpoint inhibitor, a mitogen, a growth factor, a small RNA, a dsRNA (double stranded RNA), mononuclear blood cells, feeder cells, feeder cell components or replacement

factors thereof, a vector comprising one or more polynucleic acids of interest, an antibody, a chemotherapeutic agent or a radioactive moiety, or an immunomodulatory drug (IMiD).

60. The method of claim 59, wherein the checkpoint inhibitor comprises:

(a) one or more antagonist checkpoint molecules comprising PD-1, PDL-1, TIM-3, TIGIT, LAG-3, CTLA-4, 2B4, 4-1BB, 4-1BBL, A_{2A}R, BATE, BTLA, CD39, CD47, CD73, CD94, CD96, CD160, CD200, CD200R, CD274, CEACAM1, CSF-1R, Foxp1, GARP, HVEM, IDO, EDO, TDO, LAIR-1, MICA/B, NR4A2, MAFB, OCT-2, Rara (retinoic acid receptor alpha), TLR3, VISTA, NKG2A/HLA-E, or inhibitory KIR;

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

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

61. The method of claim 59, 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 retuximab, 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.

62. The method of claim 43, further comprising combining the first synthetic cell population and the second synthetic cell population into a mixed population.