CLAIMS

1. A method of manufacturing an immune cell or a population thereof, comprising:

(a) subjecting the immune cell to a small compound treatment comprising dexamethasone; and

(b) cryopreserving the immune cell subjected to the small compound treatment;

thereby obtaining an immune cell having enhanced post-thaw cytotoxicity as compared to a counterpart immune cell without the same small compound treatment; in which (i) the small compound treatment is during *in vitro* expansion of the immune cell, and (ii) the immune cell is a T cell or an NK cell.

2. A method according to Claim 1, in which the immune cell is a derivative effector immune cell differentiated from an induced pluripotent stem cell (iPSC).

3. A method according to Claim 2, in which the iPSC comprises at least one of the following edits:

(i) a first chimeric antigen receptor (CAR) having a first targeting specificity;

(ii) CD38 knockout;

(iii) HLA-I deficiency and/or HLA-II deficiency, in comparison to its native counterpart cell;

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

(v) a CD16 or a variant thereof;

(vi) a second CAR having a second targeting specificity;

(vii) a signaling complex comprising a partial or full peptide of a cell surface expressed exogenous cytokine and/or a receptor thereof;

(viii) at least one of the genotypes listed in Table 2;

(ix) deletion or reduced expression in at least one of B2M, CIITA, TAP1, TAP2, Tapasin, NLRC5, CIITA, RFXANK, RFX5, RFXAP, TCR α or β constant region, NKG2A, NKG2D, CD25, CD69, CD44, CD56, CIS, CBL-B, SOCS2, PD1, CTLA4, LAG3, TIM3, and TIGIT, in comparison to its native counterpart cell; or

(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 antibody or fragment thereof, or a checkpoint inhibitor; and in which the effector immune cell differentiated from the iPSC comprises the same one or more edits as the iPSC.

4. A method according to Claim 3, in which the first CAR comprises:

(i) an ectodomain comprising at least one antigen recognition region, a transmembrane domain, and an endodomain comprising at least one signaling domain; and in which the at least one signaling domain is originated from a cytoplasmic domain of a signal transducing protein specific to T and/or NK cell activation or functioning;

(ii) an antigen recognition domain that specifically binds an antigen associated with a disease, a pathogen, a liquid tumor, or a solid tumor; or

(iii) an antigen recognition domain that is specific to:

(a) any one of CD19, BCMA, CD20, CD22, CD38, CD123, HER2, CD52, EGFR, GD2, MICA/B, MSLN, VEGF-R2, PSMA and PDL1; or

(b) 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, EGFIR, 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-13Rα2), 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, TRBCI, TRBC2, vascular endothelial growth factor R2 (VEGF- R2), and Wilms tumor protein (WT-1).

5. A method according to Claim 3, in which the first CAR is comprised in a bi-cistronic construct co-expressing:

(1) a partial or full-length peptide of a cell surface expressed exogenous cytokine or a receptor thereof, in which the exogenous cytokine or receptor thereof comprises:

(a) at least one of IL2, IL4, IL6, IL7, IL9, IL10, IL11, IL12, IL15, IL18, IL21, or its respective receptor; or

(b) at least one of:

(i) co-expression of IL15 and IL15Rα by using a self-cleaving peptide;

(ii) a fusion protein of IL15 and IL15Rα;

(iii) an IL15/IL15Rα fusion protein with intracellular domain of IL15Rα truncated or eliminated;

(iv) a fusion protein of IL15 and membrane bound Sushi domain of IL15Rα;

(v) a fusion protein of IL15 and IL15Rβ;

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

(vii) a homodimer of IL15Rβ;

(2) an antibody or fragment thereof; or

(3) a checkpoint inhibitor.

6. A method according to Claim 1, further comprising treating the immune cell with the small compound treatment subsequent to cryopreservation of the immune cell.

7. A method according to Claim 1, in which the cryopreservation is free or substantially free of one or more small compounds of the treatment.

8. A method according to Claim 1, in which the enhanced post-thaw cytotoxicity comprises enhanced *in vivo* efficacy of immune cells thawed after cryogenic preservation, and

in which the post-thaw immune cells having the small compound treatment comprise at least one of the following characteristics:

(i) enhanced ability in tumor control, tumor clearance, and/or reducing tumor relapse;

(ii) improved tumor penetration; or

(iii) enhanced ability in migrating to bone marrow and/or to tumor sites,

as compared to post-thaw counterpart immune cells without the same small compound treatment.

9. A method according to Claim 1, in which the small compound treatment:

(i) is free or essentially free of cytokine IL7, optionally, in which the immune cell under the treatment is a T cell;

(ii) is free or essentially free of cytokine IL2 and/or cytokine IL15, optionally, in which the immune cell under the treatment is an NK cell;

(iii) is free or essentially free of cytokines;

(iv) is during immune cell expansion after differentiating the cell from iPSC; and/or

(v) lasts 1 to 12 days, or 3 to 6 days, prior to cryopreservation.

10. A method according to Claim 9, in which the dexamethasone is present at a concentration range of 10nM to 20μM.

11. A composition comprising (a) a cell or a population thereof, and (b) a cryopreservation medium, in which:

(i) the cell is an immune cell that has been subjected to a small compound treatment comprising dexamethasone in an amount effective to enhance post-thaw cytotoxicity as compared to a counterpart immune cell without the same small compound treatment;

(ii) the immune cell is a T cell or an NK cell; and

(iii) the composition is in cryopreservation.

12. A composition according to Claim 11, in which the immune cell is a derivative effector immune cell differentiated from an induced pluripotent stem cell (iPSC).

13. A composition according to Claim 12, in which the immune cell comprises at least one of the following edits:

(i) a first chimeric antigen receptor (CAR) having a first targeting specificity;

(ii) CD38 knockout;

(iii) HLA-I deficiency and/or HLA-II deficiency, in comparison to its native counterpart cell;

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

(v) a CD16 or a variant thereof;

(vi) a second CAR having a second targeting specificity;

(vii) a signaling complex comprising a partial or full peptide of a cell surface expressed exogenous cytokine and/or a receptor thereof;

(viii) at least one of the genotypes listed in Table 2;

(ix) deletion or reduced expression in at least one of B2M, CIITA, TAP1, TAP2, Tapasin, NLRC5, CIITA, RFXANK, RFX5, RFXAP, TCR α or β constant region, NKG2A, NKG2D, CD25, CD69, CD44, CD56, CIS, CBL-B, SOCS2, PD1, CTLA4, LAG3, TIM3, and TIGIT, in comparison to its native counterpart cell; or

(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 antibody or fragment thereof, or a checkpoint inhibitor; and in which the effector immune cell differentiated from the iPSC comprises the same one or more edits as the iPSC.

14. A composition according to Claim 13, in which the first and second CARs independently comprise:

(i) an ectodomain comprising at least one antigen recognition region, a transmembrane domain, and an endodomain comprising at least one signaling domain; and in which the at least one signaling domain is originated from a cytoplasmic domain of a signal transducing protein specific to T and/or NK cell activation or functioning;

(ii) an antigen recognition domain that specifically binds an antigen associated with a disease, a pathogen, a liquid tumor, or a solid tumor; or

(iii) an antigen recognition domain that is specific to:

(a) any one of CD19, BCMA, CD20, CD22, CD38, CD123, HER2, CD52, EGFR, GD2, MICA/B, MSLN, VEGF-R2, PSMA and PDL1; or

(b) 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, EGFIR, 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-13Rα2), 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, TRBCI, TRBC2, vascular endothelial growth factor R2 (VEGF-R2), and Wilms tumor protein (WT-1).

15. A composition according to Claim 13, in which the first CAR is comprised in a bi-cistronic construct co-expressing:

(1) a partial or full-length peptide of a cell surface expressed exogenous cytokine or a receptor thereof, in which the exogenous cytokine or receptor thereof comprises:

(a) at least one of IL2, IL4, IL6, IL7, IL9, IL10, IL11, IL12, IL15, IL18, IL21, or its respective receptor; or

(b) at least one of:

(i) co-expression of IL15 and IL15Rα by using a self-cleaving peptide;

(ii) a fusion protein of IL15 and IL15Rα;

(iii) an IL15/IL15Rα fusion protein with intracellular domain of IL15Rα truncated or eliminated;

(iv) a fusion protein of IL15 and membrane bound Sushi domain of IL15Rα;

(v) a fusion protein of IL15 and IL15Rβ;

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

(vii) a homodimer of IL15Rβ;

(2) an antibody or fragment thereof; or

(3) a checkpoint inhibitor.

16. A composition according to Claim 11, in which the dexamethasone is present at a concentration range of 10nM to 20μM.

17. A composition according to Claim 11, in which the enhanced post-thaw cytotoxicity comprises enhanced *in vivo* efficacy of immune cells thawed after cryogenic preservation, and further comprises at least one of the following characteristics:

(i) enhanced ability in tumor control, tumor clearance, and/or reducing tumor relapse;

(ii) improved tumor penetration; or

(iii) enhanced ability in migrating to bone marrow and/or to tumor sites,

as compared to post-thaw counterpart immune cells without the same small compound treatment.

18. A composition according to Claim 11, in which the small compound treatment:

(i) is free or essentially free of cytokine IL7, optionally, in which the immune cell under the treatment is a T cell;

(ii) is free or essentially free of cytokine IL2 and/or cytokine IL15, optionally, in which the immune cell under the treatment is an NK cell; or

(iii) is free or essentially free of cytokines.

19. A composition according to Claim 11, in which the immune cell comprises:

(i) up-regulation of one or more of SPOCK2, PTGDS, IL7R, LCNL1, RASGRP2, SMAP2, IL6ST, IL-7R, and IL2RA, as compared to a counterpart immune cell without the same small compound treatment; or

(ii) down-regulation of one or more of JCHAIN, KLF3, KLRB1, IGFBP4, NUCB2, CSF2RB, and CXCR6, as compared to a counterpart immune cell without the same small compound treatment.

20. A composition according to Claim 11, in which the small compound treatment:

(i) does not comprise cytokine IL7, and optionally, in which the immune cell is a T cell;

(ii) does not comprise cytokine IL2 or cytokine IL15, and optionally, in which the immune cell is an NK cell; or

(iii) is free or essentially free of cytokines.

21. A method of manufacturing an immune cell or a population thereof, in which the method comprises:

(a) differentiating a genetically engineered iPSC to obtain the immune cell, in which the iPSC comprises at least one of the following edits:

(i) a first chimeric antigen receptor (CAR) having a first targeting specificity;

(ii) CD38 knockout;

(iii) HLA-I deficiency and/or HLA-II deficiency, in comparison to its native counterpart cell;

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

(v) a CD16 or a variant thereof;

(vi) a second CAR having a second targeting specificity;

(vii) a signaling complex comprising a partial or full peptide of a cell surface expressed exogenous cytokine and/or a receptor thereof;

(viii) at least one of the genotypes listed in Table 2;

(ix) deletion or reduced expression in at least one of B2M, CIITA, TAP1, TAP2, Tapasin, NLRC5, CIITA, RFXANK, RFX5, RFXAP, TCR α or β constant region,

NKG2A, NKG2D, CD25, CD69, CD44, CD56, CIS, CBL-B, SOCS2, PD1, CTLA4, LAG3, TIM3, and TIGIT, in comparison to its native counterpart cell; or

(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 antibody or fragment thereof, or a checkpoint inhibitor; and

in which the immune cell differentiated from the iPSC comprises the same one or more edits as the iPSC;

(b) subjecting the immune cell to a small compound treatment comprising dexamethasone; and

(c) cryopreserving the immune cell subjected to the small compound treatment;

thereby obtaining an immune cell having enhanced post-thaw cytotoxicity as compared to a counterpart immune cell without the same small compound treatment; in which (i) the small compound treatment is during *in vitro* expansion of the immune cell, and (ii) the immune cell is a T cell or an NK cell.

22. A method according to Claim 21, further comprising genomically engineering a clonal iPSC to knock in a polynucleotide encoding the first CAR, and optionally:

(i) to knock out CD38;

(ii) to knock out B2M and CIITA; and/or

(iii) to introduce expression of HLA-G or non-cleavable HLA-G, a 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.

23. A composition according to any of Claims 11 to 20 for use in the manufacture of a medicament, in which the medicament comprises the immune cell and one or more therapeutic agents.

24. A composition according to Claim 23, in which 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).

25. A composition according to Claim 24, in which;

(i) 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, Foxpl, 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; or

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

26. A composition according to Claim 24, in which the antibody comprises:

(a) an anti-CD20, an anti-HER2, an anti-CD52, an anti-EGFR, an anti-CD123, an anti-GD2, an anti-PDL1, and/or an 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 in which the NK cell or T cell comprises a CD38 knockout, and optionally expression of CD16 or a variant thereof.

27. Use of a composition according to any of Claims 11 to 20 in the manufacture of a medicament for the treatment of an autoimmune disorder, a hematological malignancy, a solid tumor, cancer, or a virus infection.

28. A method according to Claim 21, further comprising:

(i) thawing the cryopreserved immune cell; and

(ii) formulating the thawed immune cell for administration to a subject.