CLAIMS

1. A method of preparing a population of modulated immune cells for use in treating a subject in need thereof, in which:

the population of modulated immune cells comprises NK cells;

the method comprises contacting a population of immune cells with a sufficient amount of a composition comprising at least one agent for a time sufficient to obtain the population of modulated immune cells, in which the at least one agent comprises (a) a GSK3 inhibitor, and (b) one or more of IL-15, a MEK inhibitor, an mTOR inhibitor, and a STING agonist; and

the population of modulated immune cells has improved therapeutic potential in comparison to unmodulated immune cells.

2. A method according to Claim 1, in which

(a) the modulated immune cells comprise cells having

(i) improved proliferation, cytotoxicity, cytokine response, cytokine release, cell recall, and/or persistence;

(ii) improved cell expansion, maintenance, differentiation, dedifferentiation, and/or survival rate; and/or

(iii) increased number or ratio of one or more desired subpopulations of immune cells,

in comparison to immune cells not contacted with the at least one agent;

(b) the immune cells, prior to contacting with the at least one agent, have at least one of the following characteristics:

(i) comprising:

(1) CD57- NK cells; or

(2) CD57-NKG2C+ NK cells;

(ii) being isolated from or comprised in peripheral blood, bone marrow lymph node tissue, cord blood, thymus tissue, tissue from a site of infection, ascites, pleural effusion, spleen tissue, or tumors;

(iii) being isolated from

(1) a healthy subject;

(2) a subject having an autoimmune disease, a hematopoietic malignancy, a virus infection or a solid tumor;

(3) a subject previously administered with genetically modified immune cells; or

(4) a subject that is CMV seropositive;

(iv) being genomically engineered and comprise an insertion, a deletion, or a nucleic acid replacement;

(v) comprising at least one genetically modified modality;

(vi) being differentiated *in vitro* from stem cells, hematopoietic stem or progenitor cells, or progenitor cells; or

(vii) being trans-differentiated *in vitro* from non-pluripotent cells of hematopoietic or non-hematopoietic lineage;

(c) the composition comprising the at least one agent further comprises one or more additives; and/or

(d) the method further comprises one or both of:

(i) activating the immune cells prior to the step of contacting with a sufficient amount of the composition for modulation; and

(ii) depleting CD3 and CD19 cells from the immune cells prior to activation and modulation.

3. A method according to Claim 2, having at least one of the following characteristics:

(a) in which the stem cells comprise induced pluripotent stem cells (iPSCs) or embryonic stem cells (ESCs);

(b) in which the progenitor cell is a CD34+ hemogenic endothelium cell, a multipotent progenitor cell or a NK progenitor cell;

(c) in which the stem cell, hematopoietic stem or progenitor cell, or progenitor cell

(i) is genomically engineered and comprises an insertion, a deletion, or a nucleic acid replacement, or

(ii) comprises at least one genetically modified modality;

(d) in which the genetically modified modality of the immune cells comprises at least one of safety switch proteins, targeting modalities, receptors, signaling molecules, transcription factors, pharmaceutically active proteins and peptides, drug target candidates; or proteins promoting engraftment, trafficking, homing, viability, self-renewal, persistence, immune response regulation and modulation, and/or survival of the immune cells;

(e) in which the at least one genetically modified modality of the immune cells comprises one or more of:

(i) deletion or reduced expression of B2M, TAP1, TAP2, Tapasin, NLRC5, PD1, LAG3, TIM3, RFXANK, CIITA, RFX5, or RFXAP, and any gene in the chromosome 6p21 region; and

(ii) introduced or increased expression of HLA-E, HLA-G, HACD16, hnCD16, 41BBL, CD3, CD4, CD8, CD47, CD113, CD131, CD137, CD80, PDL1, A2AR, Fc receptor, or surface triggering receptors for coupling with bi- or multi- specific or universal engagers;

(f) in which the one or more additives comprise:

(i) at least one of peptides, antibodies, antibody fragments, cytokines, mitogens, growth factors, small RNAs, dsRNA, mononuclear blood cells, feeder cells, feeder cell components or replacement factors, vectors comprising one or more polynucleic acids of interest, chemotherapeutic agents or radioactive moieties, and immunomodulatory drugs (IMiDs);

(ii) at least one of stimulating cytokines comprising IL2, IL15, IL12, IL18 and IL21; or

(iii) one or more of a MEK inhibitor, rapamycin, and a STING agonist;

(g) in which the composition comprises:

(i) one or more of a TGFβ receptor inhibitor, a ROCK inhibitor, a MEK inhibitor, a PDK1 agonist, and rapamycin;

(ii) CHIR99021;

(iii) a GSK3 inhibitor and IL15;

(iv) a GSK3 inhibitor, IL15, and a STING agonist; or

(v) at least one organic solvent selected from the group consisting of dimethyl sulfoxide (DMSO), N,N-dimethylformamide (DMF), dimethoxyethane (DME), dimethylacetamide, ethanol and combinations thereof;

(h) in which the modulated immune cells or the one or more desired immune cell subpopulation having increased number or ratio upon contacting with the at least one agent, comprise:

(i) CD57+ NK cells, or adaptive NK cells;

(ii) adaptive NK cells comprising CD57+ and at least one of NKG2C+, low PLZF, low SYK, low FcεRγ, low EAT-2, low TIGIT, low PD1, low CD7, low CD161, high LILRB1, high CD45RO, and low CD45RA; or

(iii) CD57+ NK cells expressing hnCD16;

(i) in which the method further comprises isolating the one or more desired subpopulations from the modulated immune cells;

(j) in which the time sufficient is no less than 16 hours;

(k) in which the immune cells for modulation is in a feeder-free environment; or

(l) in which the modulated NK cells

(i) have increased expression in one or more of CD107a, NKG2C, NKG2D, CD16, KIR, CD2, NKp30, NKp44 and NKp46, in comparison to unmodulated NK cells, in which the expression thereof is increased by at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100% or above;

(ii) have increased expansion by at least 2 fold; and/or

(iii) have improved cytokine response comprising increased production of one or more cytokines comprising IFNγ and/or TNFα.

4. A method according to Claim 1, in which the method further comprises:

(a) obtaining the population of immune cells from

(i) a healthy subject;

(ii) a subject having an autoimmune disease, a hematopoietic malignancy, a virus infection or a solid tumor;

(iii) a subject previously administered with genetically modified immune cells; or

(iv) a subject that is CMV seropositive;

(b) depleting CD3 and CD19 cells from the population of immune cells; and

(c) contacting the population of immune cells resulting from step (b) with a sufficient amount of the composition comprising (a) a GSK3 inhibitor and (b) one or more of IL-15, a MEK inhibitor, an mTOR inhibitor and a STING agonist for a time sufficient to obtain the population of modulated NK cells having improved therapeutic potential for adoptive cell therapy in comparison to unmodulated NK cells,

in which prior to depleting CD3 and CD19 cells, the population of immune cells is not cultured with cytokine in the absence of GSK3 inhibitor; and

in which the modulated NK cells: have increased expression in one or more of CD107a, NKG2C, NKG2D, CD16, KIR, CD2, NKp30, NKp44 and NKp46, in comparison to unmodulated NK cells, in which the expression thereof is increased by at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100% or above; have increased expansion by at least 2 fold; and/or have improved cytokine response comprising increased production of one or more cytokines comprising IFNγ and/or TNFα.

5. Use of a composition comprising a population of immune cells, and two or more agents in the manufacture of a medicament, in which the two or more agents comprise (a) a GSK3 inhibitor, and (b) one or more of IL-15, a MEK inhibitor, an mTOR inhibitor, and a STING agonist, in which the immune cells comprise modulated NK cells, and in which the two or more agents are capable of modulating the immune cells to obtain modulated immune cells having improved therapeutic potential in comparison to the immune cells without being modulated by the two or more agents.

6. Use according to Claim 5, in which:

(a) the two or more agents are capable of modulating the immune cells

(i) to improve immune cell expansion, maintenance, differentiation, dedifferentiation, and/or survival rate;

(ii) to improve immune cell proliferation, cytotoxicity, persistence, cytokine response and secretion, and/or cell recall; and/or

(iii) to increase the number or ratio of a desired immune cell subpopulation; or

(b) the immune cells have at least one of the following characteristics:

(i) comprising:

(1) CD57- NK cells; or

(2) CD57-NKG2C+ NK cells;

(ii) being isolated from or comprised in peripheral blood, bone marrow lymph node tissue, cord blood, thymus tissue, tissue from a site of infection, ascites, pleural effusion, spleen tissue, or tumors;

(iii) being isolated from

(1) a healthy subject;

(2) a subject having an autoimmune disease, a hematopoietic malignancy, a virus infection or a solid tumor;

(3) a subject previously administered with genetically modified immune cells; or

(4) a subject that is CMV seropositive;

(iv) being genomically engineered and comprise an insertion, a deletion, or a nucleic acid replacement;

(v) comprising at least one genetically modified modality;

(vi) are differentiated *in vitro* from stem cells, hematopoietic stem or progenitor cells, or progenitor cells; or

(vii) are trans-differentiated *in vitro* from non-pluripotent cells of hematopoietic or non-hematopoietic lineage.

7. Use according to Claim 6,

(a) in which the modulated immune cells, or the desired immune cell subpopulation having increased number or ratio, comprise:

(i) CD57+ NK cells or adaptive NK cells;

(ii) adaptive NK cells comprising CD57+ and at least one of NKG2C+, low PLZF, low SYK, low FcεRγ, low EAT-2, low TIGIT, low PD1, low CD7, low CD161, high LILRB1, high CD45RO, and low CD45RA;

(iii) CD57+ NK cells expressing hnCD16; or

(iv) at least one genetically modified modality;

(b) in which the stem cells comprise induced pluripotent stem cells (iPSCs) or embryonic stem cells (ESCs);

(c) in which the progenitor cell is a CD34+ hemogenic endothelium cell, a multipotent progenitor cell or a NK progenitor cell;

(d) in which the stem cell, hematopoietic stem or progenitor cell, or progenitor cell

(i) is genomically engineered and comprises an insertion, a deletion, or a nucleic acid replacement, or

(ii) comprises at least one genetically modified modality;

(e) in which the genetically modified modality comprises at least one of safety switch proteins, targeting modalities, receptors, signaling molecules, transcription factors, pharmaceutically active proteins and peptides, drug target candidates; or proteins promoting engraftment, trafficking, homing, viability, self-renewal, persistence, immune response regulation and modulation, and/or survival of the immune cells;

(f) in which the at least one genetically modified modality comprises one or more of

(i) deletion or reduced expression of B2M, TAP1, TAP2, Tapasin, NLRC5, PD1, LAG3, TIM3, RFXANK, CIITA, RFX5, or RFXAP, and any gene in the chromosome 6p21 region; and

(ii) introduced or increased expression of HLA-E, HLA-G, HACD16, hnCD16, 41BBL, CD3, CD4, CD8, CD47, CD113, CD131, CD137, CD80, PDL1, A2AR, Fc receptor, or surface triggering receptors for coupling with bi- or multi- specific or universal engagers.

(g) in which the composition further comprises an additive, in which the additive comprises:

(i) at least one of peptides, antibodies, antibody fragments, cytokines, mitogens, growth factors, small RNAs, dsRNA, mononuclear blood cells, feeder cells, feeder cell components or replacement factors, vectors comprising one or more polynucleic acids of interest, chemotherapeutic agents or radioactive moieties, and immunomodulatory drugs (IMiDs);

(ii) at least one of stimulating cytokines comprising IL2, IL15, IL12, IL18 and IL21; or

(iii) one or more of a MEK inhibitor, rapamycin, and a STING agonist; or

(h) in which the composition comprises:

(i) one or more of a TGFβ receptor inhibitor, a ROCK inhibitor, a MEK inhibitor, a PDK1 agonist, and rapamycin;

(ii) a population of NK cells, a GSK3 inhibitor, and IL15;

(iii) a population of NK cells, a GSK3 inhibitor, IL15, and a STING agonist; or

(iv) at least one organic solvent selected from the group consisting of dimethyl sulfoxide (DMSO), N,N-dimethylformamide (DMF), dimethoxyethane (DME), dimethylacetamide, ethanol and combinations thereof.

8. A method according to any of Claims 1 to 4, in which the NK cells are differentiated *in vitro* from induced pluripotent stem cells.

9. A method according to any of Claims 1 to 4, in which the NK cells are adaptive NK cells.

10. A method according to any of Claims 1 to 4, in which the population of modulated immune cells comprises selectively expanded NK cells.

11. A method according to any of Claims 1 to 4, in which the NK cells have increased expression in one or more of CD107a, NKG2C, NKG2D, CD16, KIR, CD2, NKp30, NKp44 and NKp46 in comparison to unmodulated NK cells, in which the expression thereof is increased by at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100% or above.

12. A method according to any of Claims 1 to 4, in which the composition further comprises a therapeutically acceptable medium.

13. A method according to Claim 12, in which the composition further comprises one or more additional additives selected from the group consisting of peptides, cytokines, mitogens, growth factors, small RNAs, dsRNAs (double stranded RNAs), mononuclear blood cells, feeder cells, feeder cell components or replacement factors, vectors comprising one or more polynucleic acids of interest, antibodies, chemotherapeutic agents or radioactive moiety, and immunomodulatory drugs (IMiDs).

14. A method according to Claim 12 or 13, in which the use comprises a cell therapy that is autologous or allogeneic; and in which the subject has an autoimmune disorder, a hematological malignancy, a solid tumor, cancer, or an infection associated with HIV, RSV, EBV, CMV, adenovirus, or BK polyomavirus.

15. A method according to Claim 12, in which a therapeutically sufficient amount of the population of modulated immune cells is to be administered in combination with an antibody, a chemotherapeutic, or a radioactive treatment, in which the antibody, chemotherapeutic, or radioactive treatment is to be administered prior to, during or after administering the composition.

16. Use according to any of Claims 5, 6 or 7, in which the population of immune cells comprises CD57- NK cells or CD57-NKG2C+ NK cells.

17. Use according to Claim 16, in which the modulated NK cells have at least one of:

(a) increased gene expression in at least CD57;

(b) increased CD57+ cell subpopulation;

(c) increased adaptive NK cell subpopulation comprising CD57+ and at least one of NKG2C+, low PLZF, low SYK, low FcεRγ, low EAT-2, low TIGIT, low PD1, low CD7, low CD161, high LILRB1, high CD45RO, and low CD45RA;

(d) improved proliferation, cytotoxicity, cytokine response, cytokine release, cell recall, and/or persistence; and/or

(e) improved cell expansion, maintenance, differentiation, de-differentiation, and/or survival rate;

compared to an isolated population of immune cells comprising NK cells that has not been modulated.