CLAIMS

1. A method of modulating a population of immune cells, comprising:

contacting the immune cells with a sufficient amount of a composition comprising at least one agent selected from the group consisting of compounds listed in Table 1, and derivatives and analogues thereof, for a time sufficient to obtain a population of modulated immune cells having 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 have

(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) T cells, NKT cells, and/or NK cells;

(2) NK cells;

(3) CD57- NK cells; or

(4) 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:

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

(ii) depleting CD3 and CD19 cells from the immune cells for 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, a T cell progenitor cell, a NK progenitor cell, or a NKT 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; or

(g) in which the composition comprises:

(i) one or more of a GSK3 inhibitor, a TGFβ receptor inhibitor, a ROCK inhibitor, a MEK inhibitor, a PDK1 agonist, and rapamycin;

(ii) a GSK3 inhibitor;

(iii) CHIR99021;

(iv) a GSK3 inhibitor and IL15;

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

(vi) 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 one or more agents, comprise:

(i) naïve T cells, stem cell memory T cells, central memory T cells, type I NKT cells, 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;

in which the modulated immune cells or the one or more desired immune cell subpopulation optionally comprise at least one genetically modified modality;

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

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

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

(m) in which the modulated immune cells comprise NK cells, 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 of modulating a population of NK cells, comprising:

(a) obtaining a 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 (ii) with a sufficient amount of a composition comprising a GSK3 inhibitor for a time sufficient to obtain a 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 NK 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. A composition comprising a population of immune cells, and one or more agents selected from the group consisting of compounds listed in Table 1, and derivatives and analogues thereof, in which the one or more agents is capable of modulating the immune cells to obtain modulated immune cells having improve therapeutic potential in comparison to the immune cells without being modulated by the one or more agents.

6. A composition according to Claim 5, in which

(a) the one or more agents is 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;

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

(i) comprising:

(1) T cells, NKT cells, and/or NK cells;

(2) CD57- NK cells; or

(3) 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. A composition according to Claim 6,

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

(i) naïve T cells, stem cell memory T cells, or central memory T cells, type I NKT cells, 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, a T cell progenitor cell, a NK progenitor cell, or a NKT 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 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 GSK3 inhibitor, a TGFβ receptor inhibitor, a ROCK inhibitor, a MEK inhibitor, a PDK1 agonist, and rapamycin;

(ii) a GSK3 inhibitor;

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

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

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

(vi) 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 of making a therapeutic composition comprising modulated immune cells comprising T, NK or NKT cells for cell therapies according to any of Claims 1 to 4.

9. A population of modulated immune cells made by a method according to any of Claims 1 to 4.

10. A population of modulated NK cells made by a method according to any of Claims 1 to 4.

11. A population of modulated adaptive NK cells made by a method according to any of Claims 1 to 4.

12. A population of modulated immune cells comprising selectively expanded NK cells made by a method according to any of Claims 1 to 4.

13. A population of modulated NK cells having 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.

14. A composition comprising modulated immune cells obtained from a method according to any of Claims 1 to 4.

15. A composition comprising modulated cells according to any of Claims 9 to 13 and a therapeutically acceptable medium.

16. A composition according to Claim 15, further comprising 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).

17. A composition according to Claim 15 or 16 for use in a method of treating a subject in need of an adoptive cell therapy, in which the cell therapy 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.

18. A composition according to Claim 15 for use in a method of treating a subject, in which a therapeutically sufficient amount of the composition 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.

19. Use of a mixture for manufacturing of a therapeutic composition for cell therapies according to a method according to any of Claims 1 to 4, in which the mixture comprises:

(a) an isolated population of immune cells, and

(b) a composition comprising a GSK3 inhibitor,

in which the GSK3 inhibitor is capable of modulating the cells of (a).

20. Use according to Claim 19, in which the isolated population of immune cells comprise NK cells.

21. Use according to Claim 20, in which, upon modulation, the isolated population of immune cells comprises NK cells having 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.