

CLAIMS

1. A method of generating hematopoietic lineage cells with enhanced therapeutic properties, comprising:

(a) obtaining induced pluripotent stem cells (iPSCs) comprising one or more introduced genetic imprints, in which the one or more genetic imprints comprise (i) genomic insertion of a sequence encoding a receptor, or (ii) genomic insertion or deletion that deletes or reduces expression of a protein of an HLA complex; and

(b) directing differentiation of the iPSCs to hematopoietic lineage cells, in which directing differentiation comprises:

(i) contacting the iPSCs with a composition comprising a BMP receptor activator, and optionally bFGF, to obtain mesodermal cells; and

(ii) contacting the mesodermal cells with a composition comprising a BMP receptor activator, bFGF, and a GSK3 inhibitor, to obtain mesodermal cells having definitive hemogenic endothelium (HE) potential, in which the mesodermal cells having definitive hemogenic endothelium (HE) potential are capable of providing hematopoietic lineage cells;

in which the mesodermal cells and the mesodermal cells having definitive HE potential are obtained in steps (b)(i) and (b)(ii) without forming embryoid bodies;

in which the hematopoietic lineage cells comprise definitive hemogenic endothelium cells, hematopoietic stem and progenitor cells (HSC), hematopoietic multipotent progenitor (MPP) cells, pre-T cell progenitor cells, pre-NK cell progenitor cells, T cell progenitor cells, NK cell progenitor cells, T cells, NK cells, NKT cells, or B cells; and

in which the hematopoietic lineage cells retain the genetic imprints introduced to and comprised in the iPSCs.

2. A method according to Claim 1, in which:

(I) obtaining the iPSCs comprising the one or more introduced genetic imprints of step (a) comprises:

(a) introducing the one or more genetic imprints to the iPSC by genomic editing during or after reprogramming a non-pluripotent cell to the iPSC; or

- (b) introducing the one or more genetic imprints to the iPSC by
- (i) obtaining a source specific immune cell that is donor-, disease-, or treatment response- specific, in which the immune cell presents retainable therapeutic attributes, and in which the immune cell comprises the one or more genetic imprints; and
 - (ii) reprogramming the source specific immune cell to an iPSC, in which the iPSC comprises introduced genetic imprints and the retainable therapeutic attributes of the source specific immune cell; and optionally
 - (iii) introducing additional genetic imprints to the iPSC of step (ii) by genetic editing during or after reprogramming the source specific immune cell to the iPSC;

or,

(II) directing differentiation of the iPSCs to the hematopoietic lineage cells of step (b) further comprises:

- (a) contacting the mesodermal cells having definitive HE potential with a composition comprising bFGF and a ROCK inhibitor to obtain definitive HE cells, and optionally,
- (b) contacting the definitive HE cells with a composition comprising:
 - (i) a BMP receptor activator, and optionally a ROCK inhibitor, and one or more growth factors and cytokines selected from the group consisting of TPO, IL3, GMCSF, EPO, bFGF, VEGF, SCF, IL6, Flt3L and IL11 to obtain hematopoietic multipotent progenitor cells (MPP); or
 - (ii) one or more growth factors and cytokines selected from the group consisting of SCF, Flt3L, and IL7; and optionally one or more of a BMP receptor activator, a ROCK inhibitor, TPO, VEGF and bFGF to obtain pre-T cell progenitors, T cell progenitors, or T cells; or
 - (iii) one or more growth factors and cytokines selected from the group consisting of SCF, Flt3L, TPO, IL7 and IL15, and optionally one or more of a BMP receptor activator, a ROCK inhibitor, VEGF and bFGF to obtain pre-NK cell progenitors, NK cell progenitors, or NK cells;

or,

(III) prior to step (b), contacting the iPSCs with a composition comprising a MEK inhibitor, a GSK3 inhibitor, and a ROCK inhibitor, to seed and expand the iPSCs.

3. A method according to Claim 2, in which the genetic imprints comprise:

5 (a) one or more of: safety switch proteins, 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, or survival of the iPSCs or derivative cells thereof; or

10 (b) one or more of (i) deletion or reduced expression of B2M, TAP1, TAP2, Tapasin, NLRC5, PD1, LAG3, TIM3, RFXANK, CITTA, RFX5, or RFXAP; (ii) introduced or increased expression of HLA-E, HLA-G, HACD16, 41BBL, CD3, CD4, CD8, CD47, CD137, CD80, PDL1, A_{2A}R, CAR, TCR, or surface triggering receptors for bi-specific engagers;

15 in which, the therapeutic attributes of the source specific immune cell comprise one or more of (i) antigen targeting receptor expression; (ii) HLA presentation or lack thereof; (iii) resistance to tumor microenvironment; (iv) induction of bystander immune cells and immune modulations; (iv) improved on-target specificity with reduced off-tumor effect; (v) resistance to treatment such as chemotherapy; and (vi) improved homing, persistence, and cytotoxicity.

20 4. A method according to Claim 3, in which

(i) the surface triggering receptor is universal for the hematopoietic lineage cells comprising T, NK, NKT, macrophage, and neutrophils; or

25 (ii) the bi-specific engager is specific to the universal surface triggering receptor, and is specific to one or more tumor-specific antigen on the surface of a tumor cell.

5. A method according to Claim 4, in which:

(i) the universal surface triggering receptor comprises an anti-epitope and a co-stimulatory domain, and optionally the co-stimulatory domain comprises IL2; or

(ii) the universal surface triggering receptor comprises an anti-epitope that is specific to the bi-specific engager; or

(iii) the tumor-specific antigen comprises one or more of CD19, CD20, CD30, EGFR, HER2/ERBB2/neu, EPCAM, EphA2 and CEA.

5 6. Induced pluripotent stem cells (iPSCs) capable of differentiating into hematopoietic lineage cells through directed differentiation *in vitro* according to any preceding claim, in which (a) the iPSCs comprise the one or more genetic imprints, and (b) the one or more genetic imprints comprise genomic insertion or deletion that deletes or reduces expression of a protein of an HLA complex.

10 7. Induced pluripotent stem cells according to Claim 6, in which:

(a) the one or more genetic imprints comprise therapeutic attributes of a source specific immune cell comprising one or more of (i) antigen targeting receptor expression; (ii) HLA presentation or lack thereof; (iii) resistance to tumor microenvironment; (iv) induction of bystander immune cells and immune modulations;
15 (iv) improved on-target specificity with reduced off-tumor effect; (v) resistance to treatment such as chemotherapy; and (vi) improved homing, persistence, and cytotoxicity; or

(b) the hematopoietic lineage cells comprise definitive hemogenic endothelium cells, hematopoietic stem and progenitor cells (HSC), hematopoietic multipotent progenitor cell (MPP), pre-T cell progenitor cells, pre-NK cell progenitor cells, T cell progenitor cells, NK cell progenitor cells, T cells, NK cells, NKT cells, B cells, macrophage, or neutrophils.
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8. A method according to Claim 1, in which the hematopoietic lineage cells comprise:

(a) the T cells comprise T regulatory cells (Treg), central memory T cells (Tcm), stem cell memory (Tscm), or effector memory T cells (Tem); or
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(b) the NK cells comprise adaptive NK cells.

9. A composition comprising induced pluripotent stem cells according to Claim 6 or 7.

10. A composition according to Claim 9 for use in the manufacture of a medicament.

11. A composition for use according to Claim 10, in which the medicament is to be introduced to a subject suitable for adoptive cell therapy, 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.
- 5 12. A composition for use according to Claim 10 or 11, in which the medicament comprises a therapeutically sufficient number of T cells or T cell progenitors derived from the induced pluripotent stem cell (iPSC) by differentiation thereof.
13. A composition for use according to Claim 12, in which the subject
 - (i) is a candidate for bone marrow or stem cell transplantation, or the subject has
10 received chemotherapy or irradiation therapy;
 - (ii) has received bone marrow ablative or non-myelablative chemotherapy or radiation therapy;
 - (iii) has a hyperproliferative disorder or a cancer of hematopoietic system;
 - (iv) has a solid tumor; or
 - 15 (v) has a virus infection or a disease associated with virus infection;
 in which the administered T cell or T cell progenitors rejuvenate thymus and reconstitute T cells *in vivo*.
14. A method according to Claim 1, comprising
 - culturing the mesodermal cells having definitive hemogenic endothelium (HE) potential
20 in a medium comprising a GSK3 inhibitor to obtain definitive HE cells;
 - in which the obtained definitive HE cells
 - (i) are increased in numbers and percentage in cell population
 - (ii) have increased potency in differentiation; or
 - (iii) improved HE cellularity,
 - 25 as compared to culturing without the GSK3 inhibitor.
15. A method according to Claim 14, having at least one of the following characteristics:

(i) in which the medium further comprises a ROCK inhibitor, and one or more growth factors and cytokines selected from the group consisting of bFGF, VEGF, SCF, IGF, EPO, IL6, and IL11; and

(ii) in which the pluripotent stem cell pluripotent stem cells comprise naïve iPSCs.

5 16. A method according to Claim 2, in which reprogramming a source-specific immune cell comprises:

(a) isolating primary antigen specific T cells from a selected source that is donor-, disease-, or treatment response- specific; and

10 (b) reprogramming the primary antigen-specific T cells to obtain pluripotent stem cells; in which the iPSC comprises the introduced genetic imprints and the retainable therapeutic attributes of the source specific immune cell.

17. A method according to Claim 16, in which isolating the primary antigen specific T cells further comprises enriching the primary antigen specific T cells by:

15 (i) co-culturing the primary antigen specific T cells with (a) tumor cells expressing antigen(s) of interest; (b) non-transformed cells expressing antigen(s) of interest or (c) dendritic cells, thymic epithelial cell, endothelial cells or artificial antigen presenting cells, plasma particles or peptides expressing antigen(s) of interest, to enable the faster proliferation of antigen-specific T cells that recognize the cell-expressed antigen of interest; or

20 (ii) sorting the primary antigen specific T cells using T cell receptor-specific binding agents that are specific to antigen(s) of interest, thereby obtaining enriched primary antigen specific T cells recognizing antigen(s) of interest.

25 18. A method according to Claim 16, in which the genetic imprints comprise one or more of:

30 (I) safety switch proteins, signaling molecules, transcription factors, pharmaceutically active proteins and peptides, drug target candidates; cell surface proteins conveying a secondary or tertiary antigen specificity; or proteins promoting engraftment, trafficking, homing, viability, self-renewal, persistence, immune response regulation and modulation, or survival of the iPSCs or derivative cells thereof;

(II) deletion or reduced expression of B2M, TAP1, TAP2, Tapasin, NLRC5, PD1, LAG3, TIM3, RFXANK, CITTA, RFX5, or RFXAP; or

(III) introduced or increased expression of HLA-E, HLA-G, HACD16, 41BBL, CD3, CD4, CD8, CD47, CD137, CD80, PDL1, A_{2A}R, CAR, TCR, or surface triggering receptors for bi-specific engagers.

19. A method according to Claim 18, in which the surface triggering receptor has at least one of the following characteristics:

(i) being universal for hematopoietic lineage cells;

(ii) comprising an anti-epitope and a co-stimulatory domain, in which the anti-epitope is specific to the bi-specific engager; and optionally the co-stimulatory domain comprises IL2;

(iii) are for bi-specific engagers specific to one or more tumor-specific antigen on the surface of a tumor cell, in which the tumor-specific antigen optionally comprises one or more of CD19, CD20, CD30, EGFR, HER2/ERBB2/neu, EPCAM, EphA2 and CEA; and

(iv) in which the bi-specific engager is

(a) hematopoietic lineage cell type specific, and optionally in which the engager is specific to a surface receptor comprising CD3, CD16, CD64, or CD89; or

(b) hematopoietic lineage cell type independent, in which the hematopoietic lineage cells comprise a universal surface triggering receptor, and in which the engager is specific to the universal surface triggering receptor;

(c) specific to one or more tumor-specific antigen on the surface of a tumor cell, and optionally in which the tumor-specific antigen comprises one or more of CD19, CD20, CD30, EGFR, HER2/ERBB2/neu, EPCAM, EphA2 and CEA,

and in which the hematopoietic lineage cells comprise definitive hemogenic endothelium cells, hematopoietic stem and progenitor cells (HSC), hematopoietic multipotent progenitor cell (MPP), pre-T cell progenitor cells, pre-NK cell progenitor cells, T cell progenitor cells, NK cell progenitor cells, T cells, NK cells, NKT cells, B cells, macrophages, or neutrophils.

20. Induced pluripotent stem cell (iPSC) derived hematopoietic lineage cells generated using a method according to any of Claims 16 to 19, in which (a) the iPSCs comprise the one or more genetic imprints, and (b) the one or more genetic imprints comprise genomic insertion or deletion that deletes or reduces expression of a protein of an HLA complex.
- 5 21. A composition comprising iPSC-derived hematopoietic lineage cells according to Claim 20.
22. A pharmaceutical composition comprising iPSC-derived hematopoietic lineage cells according to Claim 20, and a pharmaceutically acceptable medium, and optionally one or more bi-specific engagers for coupling with surface receptors of hematopoietic lineage cells.
- 10 23. A pharmaceutical composition according to Claim 22 for use in a method of therapy, in which the composition is to be introduced to a subject suitable for adoptive cell therapy, 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 24. A method according to Claim 1, further comprising:
- contacting induced pluripotent stem cell-derived pre-T cell progenitors with a composition comprising one or more growth factors and cytokines selected from the group consisting of SCF, Flt3L, and IL7, to obtain induced pluripotent stem cell-derived T cell progenitors or T cells, in which the composition is free of one or more of VEGF, bFGF, TPO, BMP receptor activators and ROCK inhibitors;
- 20 in which the induced pluripotent stem cell-derived pre-T cell progenitors are obtained by contacting induced pluripotent stem cell-derived definitive hemogenic endothelium with a composition comprising a ROCK inhibitor, and one or more growth factors and cytokines selected from the group consisting of VEGF, bFGF, SCF, Flt3L, TPO, and
- 25 IL7, to allow cell differentiation and expansion;
- in which the induced pluripotent stem cell-derived definitive hemogenic endothelium are obtained by contacting the mesodermal cells having definitive HE potential with a composition comprising a ROCK inhibitor; one or more growth factors and cytokines selected from the group consisting of bFGF, VEGF, SCF, IL6 and IL11; and optionally,

one or more of a GSK3 inhibitor, IGF, and EPO, to allow cell differentiation and expansion; in which the composition is free of TGF β receptor/ALK inhibitor.

25. A method according to Claim 24, further comprising:

contacting induced pluripotent stem cells with a composition comprising a MEK inhibitor, a GSK3 inhibitor, and a ROCK inhibitor to seed and expand the induced pluripotent stem cells, in which the composition is free of TGF β receptor/ALK inhibitors.

26. A method according to Claim 1, further comprising:

contacting induced pluripotent stem cell-derived pre-NK cell progenitors with a composition comprising one or more growth factors and cytokines selected from the group consisting of SCF, Flt3L, IL3, IL7, and IL15, to obtain induced pluripotent stem cell-derived NK cell progenitors or NK cells, in which the composition is free of one or more of VEGF, bFGF, TPO, BMP receptor activators and ROCK inhibitors;

in which the induced pluripotent stem cell-derived pre-NK cell progenitors are obtained by contacting induced pluripotent stem cell-derived definitive hemogenic endothelium with a composition comprising a ROCK inhibitor; one or more growth factors and cytokines selected from the group consisting of VEGF, bFGF, SCF, Flt3L, TPO, IL3, IL7 and IL15; and optionally, a BMP receptor activator, to allow cell differentiation and expansion;

in which induced pluripotent stem cell-derived definitive hemogenic endothelium are obtained by contacting the mesodermal cells having definitive HE potential with a composition comprising a ROCK inhibitor; one or more growth factors and cytokines selected from the group consisting of bFGF, VEGF, SCF, IL6 and IL11; and optionally one or more of a GSK3 inhibitor, IGF and EPO, to allow cell differentiation and expansion, in which the composition is optionally free of TGF β receptor/ALK inhibitor.

27. A method according to Claim 26, further comprising one of more of the followings:

(i) subjecting seeded induced pluripotent stem cells, induced pluripotent stem cell-derived mesodermal cells, mesodermal cells with hemogenic endothelium potential, and/or definitive hemogenic endothelium under low oxygen tension between about 2% to about 10%;

(ii) contacting induced pluripotent stem cells with a composition comprising a MEK inhibitor, a GSK3 inhibitor, and a ROCK inhibitor, to seed and expand the cells, in which the composition is free of TGF β receptor/ALK inhibitors.

28. A method according to Claim 1, further comprising:

5 contacting the mesodermal cells having definitive HE potential with a composition comprising a ROCK inhibitor; one or more growth factors and cytokines selected from the group consisting of bFGF, VEGF, SCF, IL6 and IL11; and optionally one or more or a GSK3 inhibitor, IGF and EPO, to obtain induced pluripotent stem cell-derived definitive hemogenic endothelium, in which the composition is optionally free of TGF β receptor/ALK inhibitor.

29. A method according to Claim 1, further comprising:

15 contacting induced pluripotent stem cell-derived pre-HSC with a composition comprising a BMP receptor activator, and one or more growth factors and cytokines selected from the group consisting of TPO, IL3, GMCSF, EPO, bFGF, VEGF, SCF, IL6 and IL11, to obtain induced pluripotent stem cell-derived hematopoietic multipotent progenitors, in which the composition is free of ROCK inhibitor;

in which the induced pluripotent stem cell-derived pre-HSC are obtained by contacting induced pluripotent stem cell-derived definitive hemogenic endothelium with a composition comprising a BMP receptor activator, a ROCK inhibitor, and one or more growth factors and cytokines selected from the group consisting of TPO, IL3, GMCSF, EPO, bFGF, VEGF, SCF, IL6, Flt3L and IL11, to allow cell differentiation and expansion;

25 in which the induced pluripotent stem cell-derived definitive hemogenic endothelium are obtained by contacting the mesodermal cells having definitive HE potential with a composition comprising a ROCK inhibitor; one or more growth factors and cytokines selected from the group consisting of bFGF, VEGF, SCF, IGF, EPO, IL6, and IL11; and optionally, a GSK3 inhibitor; to allow cell differentiation and expansion, in which the composition is free of TGF β receptor/ALK inhibitor.

30. A method according to any of Claims 1 to 13, in which the cell populations, cell lines or clonal cells are selected from:

- (i) induced pluripotent stem cell-derived definitive hemogenic endothelium (iHE), in which the iHE cell line or clonal cells are CD34+, and at least one of CD43-, CD93-, CXCR4-, CD73-, and CXCR4-CD73-;
 - (ii) induced pluripotent stem cell-derived multipotent progenitor cells, in which the iMPP cells are CD34+CD45+;
 - (iii) induced pluripotent stem cell-derived T cell progenitors, in which the T cell progenitors are CD34+CD45+CD7+ or CD34-CD45+CD7+;
 - (iv) induced pluripotent stem cell-derived T cells, in which the T cells are CD45+CD3+CD4+ or CD45+CD3+CD8+;
 - (v) induced pluripotent stem cell derived NK cell progenitors, in which the NK cell progenitors are CD3-CD45+CD56+CD7+;
 - (vi) induced pluripotent stem cell-derived NK cells, in which the NK cells are CD3-CD45+CD56+, and optionally further defined by NKp46+, CD57+, and CD16+;
 - (vii) induced pluripotent stem cell-derived NKT, in which the NKT cells are CD45+V α 24J α 18+CD3+; and
 - (viii) induced pluripotent stem cell-derived B cells, in which the B cells are CD45+CD19+.
31. A composition comprising induced pluripotent stem cell-derived hematopoietic lineage cells obtained according to a method according to Claim 1 and one or more culture medium selected from the group consisting of:
- (i) CD34+ HE cells (iCD34), and one or more culture medium selected from iCD34-C, iMPP-A, iTC-A2, iTC-B2, iNK-A2, and iNK-B2;
 - (ii) definitive hemogenic endothelium (iHE), and one or more culture medium selected from iCD34-C, iMPP-A, iTC-A2, iTC-B2, iNK-A2, and iNK-B2;
 - (iii) definitive HSCs, and one or more culture medium selected from iMPP-A, iTC-A2, iTC-B2, iNK-A2, and iNK-B2;
 - (iv) multipotent progenitor cells (iMPP), and iMPP-A;
 - (v) T cell progenitors (ipro-T), and one or more culture medium selected from iTC-A2, and iTC-B2;

(vi) T cells (iTc), and iTc-B2;

(vii) NK cell progenitors (ipro-NK), and one or more culture medium selected from iNK-A2, and iNK-B2; and (viii) NK cells (iNK), and iNK-B2

in which iCD34-C comprises a ROCK inhibitor; one or more growth factors and cytokines selected from the group consisting of bFGF, VEGF, SCF, IGF, EPO, IL6, and IL11; and optionally, a GSK3 inhibitor;

in which iMPP-A comprises a BMP receptor activator, a ROCK inhibitor, and one or more growth factors and cytokines selected from the group consisting of TPO, IL3, GM-CSF, EPO, bFGF, VEGF, SCF, IL6, Flt3L and IL11;

in which iTc-A2 comprises a ROCK inhibitor; one or more growth factors and cytokines selected from the group consisting of VEGF, bFGF, SCF, Flt3L, TPO, and IL7; and optionally, a BMP receptor activator;

in which iTc-B2 comprises one or more growth factors and cytokines selected from the group consisting of SCF, Flt3L, and IL7;

in which iNK-A2 comprises a ROCK inhibitor; one or more growth factors and cytokines selected from the group consisting of VEGF, and bFGF SCF, Flt3L, TPO, IL3, IL7, IL15; and optionally, a BMP receptor activator,

in which iNK-B2 comprises one or more growth factors and cytokines selected from the group consisting of SCF, Flt3L, IL3, IL7 and IL15; and

in which the culture medium is a feeder-free culture medium.

32. A composition according to Claim 31, in which (i) iCD34-C is free of TGF β receptor/ALK inhibitor; in which iTc-A2 and iNK-A2 are free of BMP receptor activator; or in which iTc-B2 and iNK-B2 are free of one or more of VEGF, bFGF, BMP receptor activators, and ROCK inhibitors; or (ii) the induced pluripotent stem cells comprise naïve iPSCs, or iPSC having one or more genetic imprints retainable in hematopoietic lineage cells derived therefrom.