

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;

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

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

(ii) contacting the mesodermal cells with a composition comprising a BMP pathway activator, bFGF, and a WNT pathway activator, 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 mesodermal cells and mesodermal cells having definitive HE potential are obtained in steps (i) and (ii) without the step of forming embryoid bodies;

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, 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 iPSCs comprising one or more introduced genetic imprints of step (a) further comprises:

(a) Introducing one or more genetic imprints to an iPSC by genomic editing during or after reprogramming a non-pluripotent cell to iPSC, in which the genetic imprint comprises one or more genetically modified modalities obtained through genomic insertion, deletion or substitution in the genome of the iPSC; or

(b) Introducing one or more genetic imprints to 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

5 (ii) reprogramming the source specific immune cell to iPSC, in which the iPSC comprises introduced genetic imprints associable with the retainable therapeutic attributes; and optionally

(iii) introducing additional genetic imprints to iPSC of step (ii) by genetic editing during or after reprogramming the source specific immune cell to
10 iPSC;

or,

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

15 (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:

20 (i) a BMP 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

25 (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 activator, a ROCK inhibitor, TPO, VEGF and bFGF to obtain pre-T cell progenitors, T cell progenitors, and/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 activator, a ROCK inhibitor, VEGF and bFGF to obtain pre-NK cell progenitors, NK cell progenitors, and/or NK cells;

30 or,

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

3. A method according to Claim 2, in which the genetically modified modalities comprise:

5 (a) one or more 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 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, A₂A_R, CAR, TCR, or surface triggering receptors for bi- or multi-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; (v) improved on-target specificity with reduced off-tumor effect; (vi) resistance to treatment such as chemotherapy; and (vi) improved homing,
20 persistence, and cytotoxicity.

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- or multi- 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

30 (ii) the anti-epitope is specific to the bi- or multi- specific engager; or

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

6. Hematopoietic lineage cells having enhanced therapeutic properties, comprising genetic imprints comprised in pluripotent stem cells from which the hematopoietic lineage cells are derived through directed differentiation in vitro according to any of Claims 1 to 5, in which the genetic imprints of the pluripotent stem cells comprise:

(a) one or more genetically modified modalities obtained through genomic insertion, deletion or substitution in the genome of the pluripotent cells during or after reprogramming a non-pluripotent cell to iPSC; or

(b) one or more retainable therapeutic attributes of a source specific immune cell that is donor-, disease-, or treatment response- specific, and in which the pluripotent cells are reprogrammed from the source specific immune cell.

7. Hematopoietic lineage cells according to Claim 6, in which:

(a) 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; 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.

8. Hematopoietic lineage cells according to Claim 7, in which

(a) the T cells comprise T regulatory cells (Treg), central memory T cells (Tcm), stem cell memory (Tscm), and/or effector memory T cells (Tem); or

(b) the NK cells comprise adaptive NK cells.

9. A composition comprising the hematopoietic lineage cells according to any of Claims 6 to 8.

10. A pharmaceutical composition comprising one or more of the hematopoietic lineage cells according to any of Claims 6 to 8 and a pharmaceutically acceptable medium.

11. A pharmaceutical composition according to Claim 9 or 10 for use in a method of medical treatment, 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.

12. A therapeutically sufficient number of T cells or T cell progenitors derived from induced pluripotent stem cell (iPSC) differentiation according to any of Claims 1 to 5 for use in a method of treating a subject in need of cell therapy.

13. A therapeutically sufficient number of T cells or T cell progenitors according to Claim 12, in which the subject

(i) is a candidate for bone marrow or stem cell transplantation, or the subject has received chemotherapy or irradiation therapy;

(ii) has received bone marrow ablative or non-myeolablative chemotherapy or radiation therapy;

(iii) has a hyperproliferative disorder or a cancer of hematopoietic system;

(iv) has a solid tumor; or

(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. An antibody composition comprising one or more antibodies specific to at least one markers selected from CCR10, CD164, CD95, CD144, CD166, Lymphotoxin β Receptor, CD252, CD55, CD40, CD46, CD340, CD119, CD106, CD66a/c/e, CD49d, CD45RB, DLL4, CD107a, CD116, CD324, CD123, CD49f, CD200, CD71, CD172a, CD21, CD184, CD263, CD221, Notch 4, MSC, CD97, CD319, CD69, CD338, Podoplanin, CD111, CD304, CD326, CD257, CD100, CD32, CD253, CD79b, CD33, CD83, GARP, CD183, CD357, CD31, CD165, CD102, CD146, CD49c, CD13, CD58, Integrin $\alpha 9\beta 1$, CD51, CD10, CD202b, CD141, CD49a, CD9, CD201, CD47, CD262, CD109, CD39, CD317, CD143, integrin $\beta 5$,

CD105, CD155, SSEA-4 and CD 93; in which the markers are for identifying definitive hemogenic endothelium.

15. An antibody composition according to Claim 14, in which the composition comprises an antibody specific to CD93; in which the cells conjugated with the antibody specific to CD93 have one or more of CD34+, CD43-, CD73-, CXCR4-, and CD73-CXCR4- phenotype.

16. A method of identifying definitive hemogenic endothelium in pluripotent stem cell differentiation using an antibody composition according to Claim 14 or 15, comprising

- (i) obtaining a cell population undergoing differentiation;
- (ii) introducing to the cell population an antibody specific to CD93; and
- (iii) identifying the cell population having CD93 expression level less than 1%.

17. A method according to Claim 16, in which step (i) further comprises

isolating CD34+ cells from the cell population undergoing differentiation; or
isolating CD34+CD43- cells from the cell population undergoing differentiation.

18. A method of generating pluripotent stem cell derived definitive hemogenic endothelium, comprising

culturing pluripotent stem cell derived mesodermal cells having definitive hemogenic endothelium (HE) potential in a medium comprising a Wnt pathway agonist 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; and/or
- (iii) improved HE cellularity,

as compared to culturing without the Wnt pathway activator.

19. A method according to Claim 18, 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 (a) iPSCs; (b) naïve iPSCs; and/or iPSCs having one or more genetic imprints retainable after differentiation

20. A method of generating antigen-specific induced pluripotent stem cells (iPSC) and
5 derivative hematopoietic lineage cells comprising:

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

(b) reprogramming the primary antigen-specific T cells to obtain pluripotent stem cells; and

10 (c) directing differentiation of the pluripotent stem cells of step (b) to hematopoietic lineage cells, in which directing differentiation comprises:

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

15 (ii) contacting the mesodermal cells with a composition comprising a BMP pathway activator, bFGF, and a WNT pathway activator, to obtain mesodermal cells having definitive hemogenic endothelium (HE) potential;

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

21. A method according to Claim 20, in which isolating the primary antigen specific T cells
20 further comprises enriching the primary antigen specific T cells by:

(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
25 proliferation of antigen-specific T cells that recognize the cell-expressed antigen of interest; or

(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
30 interest; in which, optionally, the primary antigen specific T cells or enriched primary

antigen specific T cells are further contacted by transcription factors or small molecules to rejuvenate the cells.

22. A method according to Claim 20, in which step (b) further comprises:

introducing one or more genetic imprints to the pluripotent stem cell by genetic editing during or after reprogramming, in which the genetic imprint comprises one or more genetically modified modalities obtained through genomic insertion, deletion or substitution in the genome of the pluripotent stem cell.

23. A method according to Claim 22, in which the genetically modified modalities comprise one or more of:

(I) safety switch proteins, targeting modalities, receptors, 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, and/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- or multi- specific engagers.

24. A method according to Claim 23, 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- or multi- specific engager; and optionally the co-stimulatory domain comprises IL2;

(iii) are for bi- or multi-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- or multi- 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

5 (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,

10 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.

15 25. Antigen-specific induced pluripotent stem cells (iPSC) or derivative hematopoietic lineage cells generated using a method according to any of Claims 20 to 24.

26. A composition comprising antigen-specific induced pluripotent stem cells (iPSC) or derivative hematopoietic lineage cells according to Claim 25.

20 27. A pharmaceutical composition comprising induced pluripotent stem cells (iPSC) or derivative hematopoietic lineage cells according to Claim 25, and a pharmaceutically acceptable medium, and optionally one or more bi- or multi- specific engagers for coupling with surface receptors of hematopoietic lineage cells.

28. A pharmaceutical composition according to Claim 27 for use in a method of therapy, in which the composition is to be introduced to a subject suitable for adoptive cell therapy, in
25 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.

29. A method for determining the clonality of induced pluripotent stem cells and derivative cells thereof, comprising

- (a) reprogramming a mature source T or B cell to obtain induced pluripotent stem cells (iPSCs); and optionally,
- (b) directing differentiation of the pluripotent stem cells of step (a) to hematopoietic lineage cells;
- 5 and
- (c) detecting the presence, in the iPSCs or the hematopoietic lineage cells, of a specific V(D)J recombination that is same as the one comprised in the mature source T or B cell for generating the iPSC and hematopoietic lineage cells;

and optionally further comprising:

- 10 (i) obtaining a mature source T or B cell for reprogramming and determining V(D)J recombination comprised in immunoglobulins (Ig) or T cell receptors (TCR) that is specific to the mature source T or B cell; and/or
- (ii) isolating iPSCs or hematopoietic lineage cells comprising the same V(D)J recombination as that of the mature source T or B cell from which they are derived.

- 15 30. A method of tracking an adoptive cell *in vivo*, comprising

obtaining a sample of blood, tissue, or tumor biopsy from a subject receiving adoptive cells for therapy, in which the adoptive cells are derived from pluripotent stem cells reprogrammed from a mature source T or B cell, and in which the mature source T or B cell comprises a specific V(D)J recombination;

- 20 isolating effector cells in the sample;

determining the V(D)J recombination in the effector cells;

in which the presence of a same V(D)J recombination as that of the mature source T or B cell is indicative of adoptive cell homing, persistence and/or expansion.

- 31. A method for directing differentiation of pluripotent stem cells to hematopoietic lineage
- 25 cells, comprising: (i) contacting the pluripotent stem cells with a composition comprising a BMP pathway activator, and optionally bFGF, to obtain mesodermal cells; and (ii) contacting the mesodermal cells with a composition comprising a BMP pathway activator, bFGF, and a WNT pathway activator, 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 comprising 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, or B cells; and in which mesodermal cells and mesodermal cells having definitive HE potential are obtained in steps (i) and (ii) without the step of forming embryoid bodies.

32. A method according to Claim 31, further comprising: (iii) contacting the mesodermal cells having definitive HE potential with a composition comprising bFGF and a ROCK inhibitor, and optionally one or more of a Wnt pathway activator, IGF and EPO to obtain definitive HE cells; in which the pluripotent stem cells, the mesodermal cells, and/or the mesodermal cells having definitive hemogenic endothelium potential are optionally subject to low oxygen tension between about 2% to about 10%; and in which prior to step (i), optionally contacting the pluripotent stem cells with a composition comprising a MEK inhibitor, a GSK3 inhibitor, and a ROCK inhibitor, to seed and expand the cells.

33. A method according to Claim 32, further comprising one of the followings:

(a) contacting the definitive HE cells with a composition comprising a BMP 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);

(b) contacting the definitive HE cells with a composition comprising one or more growth factors and cytokines selected from the group consisting of SCF, Flt3L, TPO, and IL7; and optionally one or more of a BMP activator, a ROCK inhibitor, VEGF and bFGF to obtain pre-T cell progenitors, T cell progenitors, and/or T cells;

(c) contacting the definitive HE cells with a composition comprising one or more growth factors and cytokines selected from the group consisting of SCF, Flt3L, TPO, IL3, IL7 and IL15, and optionally one or more of a BMP activator, a ROCK inhibitor, VEGF and bFGF to obtain pre-NK cell progenitors, NK cell progenitors, and/or NK cells.

34. A method according to Claim 31, in which:

(i) the pluripotent stem cell differentiation to hematopoietic lineage cells is free of, or essentially free of, TGF β receptor/ALK inhibitors;

(ii) the pluripotent stem cell differentiation to hematopoietic lineage cells is under feeder-free condition;

5 (iii) the pluripotent stem cell differentiation to hematopoietic lineage cells is under stromal-free condition;

(iv) the pluripotent stem cell comprises induced pluripotent stem cell (iPSC), naïve iPSCs, and/or iPSCs having one or more genetic imprints retainable in the hematopoietic lineage cells differentiated therefrom;

10 (v) the WNT pathway activator is a GSK3 inhibitor; optionally in which the GSK3 inhibitor is CHIR99021;

(vi) the BMP pathway activator is BMP4.

35. A method for generating pluripotent stem cell-derived T lineage cells comprising:

15 contacting 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 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 activators and ROCK inhibitors;

20 in which the pluripotent stem cell-derived pre-T cell progenitors are obtained by contacting 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 IL7, to allow cell differentiation and expansion;

25 in which the pluripotent stem cell-derived definitive hemogenic endothelium are obtained by contacting pluripotent stem cell-derived 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 Wnt pathway activator, IGF, and EPO, to allow cell differentiation and expansion; in which the composition is free of TGF β receptor/ALK inhibitor;

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in which the pluripotent stem cell-derived mesodermal cells having definitive HE potential are obtained by contacting pluripotent stem cell-derived mesodermal cells with a composition comprising a BMP activator, bFGF, and a GSK3 inhibitor to allow cell differentiation and expansion, in which the composition is free of TGF β receptor/ALK inhibitor, and

in which the pluripotent stem cell-derived mesodermal cells are obtained by contacting pluripotent stem cells with a composition comprising a BMP activator, and optionally bFGF, to allow cell differentiation and expansion.

36. A method according to Claim 35, further comprising:

contacting pluripotent stem cells with a composition comprising a MEK inhibitor, a GSK3 inhibitor, and a ROCK inhibitor to seed and expand the pluripotent stem cells, in which the composition is free of TGF β receptor/ALK inhibitors; and optionally in which: (a) generating pluripotent stem cell-derived T lineage cells is (i) void of the step of generating embryoid bodies; (ii) under monolayer culturing; (iii) under feeder-free condition; and/or (iv) under stromal-free condition; (b) the pluripotent stem cells comprise iPSCs, naïve iPSCs, and/or iPSCs having one or more genetic imprints retainable in the T lineage cells derived therefrom.

37. A method for generating pluripotent stem cell-derived NK lineage cells comprising:

contacting 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 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 activators and ROCK inhibitors;

in which the pluripotent stem cell-derived pre-NK cell progenitors are obtained by contacting 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 activator, to allow cell differentiation and expansion;

in which pluripotent stem cell-derived definitive hemogenic endothelium are obtained by contacting pluripotent stem cell-derived 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 Wnt pathway activator, IGF and EPO, to allow cell differentiation and expansion, in which the composition is optionally free of TGF β receptor/ALK inhibitor;

5 in which the pluripotent stem cell-derived mesodermal cells having definitive HE potential are obtained by contacting pluripotent stem cell-derived mesodermal cells with a composition comprising a BMP activator, bFGF, and a GSK3 inhibitor, to allow cell differentiation and expansion, in which the composition is optionally free of TGF β receptor/ALK inhibitor; and

10 in which the pluripotent stem cell-derived mesodermal cells are obtained by contacting pluripotent stem cells with a composition comprising a BMP activator, and optionally bFGF, to allow cell differentiation and expansion.

38. A method according to Claim 37, further comprising one of more of the followings:

15 (i) subjecting seeded pluripotent stem cells, 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 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; and optionally in which

20 (a) the generation of pluripotent stem cell-derived NK lineage cells is (1) void of generation of embryoid bodies; (2) under monolayer culturing; (3) under feeder-free condition; and/or (4) under stromal-free condition; or

(b) the pluripotent stem cells comprise iPSCs, naïve iPSCs, and/or iPSC having one or more genetic imprints retainable in the NK lineage cells derived therefrom.

25 39. A method for generating pluripotent stem cell-derived definitive hemogenic endothelium, comprising:

contacting pluripotent stem cell-derived 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 Wnt pathway activator, IGF and EPO, to obtain

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pluripotent stem cell-derived definitive hemogenic endothelium, in which the composition is optionally free of TGF β receptor/ALK inhibitor;

in which the pluripotent stem cell-derived mesodermal cells having definitive HE potential are obtained by contacting pluripotent stem cell-derived mesodermal cells with a composition comprising a BMP activator, bFGF, and a GSK3 inhibitor, to allow cell differentiation and expansion, in which the composition is optionally free of TGF β receptor/ALK inhibitor; and

in which the pluripotent stem cell-derived mesodermal cells are obtained by contacting pluripotent stem cells with a composition comprising a BMP activator, and optionally bFGF, to allow cell differentiation and expansion.

40. A method according to Claim 39, further comprising:

contacting pluripotent stem cells with a composition comprising a MEK inhibitor, a GSK3 inhibitor, and a ROCK inhibitor, to seed and expand the pluripotent stem cells, in which the composition is free of TGF β receptor/ALK inhibitors; and/or subjecting seeded pluripotent stem cells, pluripotent stem cell-derived mesodermal cells, mesodermal cells having definitive hemogenic endothelium potential, and/or definitive hemogenic endothelium under low oxygen tension between about 2% to about 10%; and optionally in which: (a) the generation of pluripotent stem cell-derived definitive hemogenic endothelium is (i) void of the step of generating embryoid bodies; (ii) under monolayer culturing; (iii) under feeder-free condition; and/or (iv) under stromal-free condition; (b) the pluripotent stem cells comprise iPSCs, naïve iPSCs, and/or iPSC having one or more genetic imprints retainable in the definitive hemogenic endothelium derived therefrom.

41. A method for generating pluripotent stem cell-derived hematopoietic multipotent progenitors, comprising:

contacting pluripotent stem cell-derived pre-HSC with a composition comprising a BMP 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 pluripotent stem cell-derived hematopoietic multipotent progenitors, in which the composition is free of ROCK inhibitor;

in which the pluripotent stem cell-derived pre-HSC are obtained by contacting pluripotent stem cell-derived definitive hemogenic endothelium with a composition comprising a BMP 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;

in which the pluripotent stem cell-derived definitive hemogenic endothelium are obtained by contacting pluripotent stem cell-derived 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 Wnt pathway activator; to allow cell differentiation and expansion, in which the composition is free of TGF β receptor/ALK inhibitor;

in which the pluripotent stem cell-derived mesodermal cells having definitive HE potential are obtained by contacting pluripotent stem cell-derived mesodermal cells with a composition comprising a BMP activator, bFGF, and a GSK3 inhibitor, to allow cell differentiation and expansion, in which the composition is free of TGF β receptor/ALK inhibitor;

in which the pluripotent stem cell-derived mesodermal cells are obtained by contacting pluripotent stem cell-derived mesodermal cells with a composition comprising a BMP activator, bFGF, and a GSK3 inhibitor, to allow cell differentiation and expansion, in which the composition is free of TGF β receptor/ALK inhibitor; and

in which contacting pluripotent stem cell-derived mesodermal cells are obtained by contacting pluripotent stem cells with a composition comprising a BMP activator, and optionally bFGF, to allow cell differentiation and expansion.

42. A method according to Claim 41, further comprising:

contacting pluripotent stem cells with a composition comprising a MEK inhibitor, a GSK3 inhibitor, and a ROCK inhibitor, to seed and expand the pluripotent stem cells, in which the composition is free of TGF β receptor/ALK inhibitors; and/or subjecting seeded pluripotent stem cells, pluripotent stem cell-derived mesodermal cells, mesodermal cells having definitive hemogenic endothelium potential, and/or definitive hemogenic endothelium under low oxygen tension between about 2% to about 10%;

optionally in which (a) the generation of the pluripotent stem cell-derived hematopoietic multipotent progenitors is (i) void of the step of generating embryoid bodies; (ii) under monolayer culturing; (iii) under feeder-free condition; and/or (iv) under stromal-free condition; and/or (b) the pluripotent stem cells comprise iPSCs, naïve iPSCs, and/or iPSC having one or more genetic imprints retainable in the multipotent progenitors derived therefrom.

43. A method of promoting adoptive cell therapy using a composition comprising one or more of cell populations, cell lines or clonal cells generated using a methods according to any of Claims 1 to 13, in which the cell populations, cell lines or clonal cells are selected from:

(i) 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) pluripotent stem cell-derived multipotent progenitor cells, in which the iMPP cells are CD34+CD45+;

(iii) pluripotent stem cell-derived T cell progenitors, in which the T cell progenitors are CD34+CD45+CD7+ or CD34-CD45+CD7+;

(iv) pluripotent stem cell-derived T cells, in which the T cells are CD45+CD3+CD4+ or CD45+CD3+CD8+;

(v) pluripotent stem cell derived NK cell progenitors, in which the NK cell progenitors are CD3-CD45+CD56+CD7+;

(vi) 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) pluripotent stem cell-derived NKT, in which the NKT cells are CD45+V α 24J α 18+CD3+; and

(viii) pluripotent stem cell-derived B cells, in which the B cells are CD45+CD19+.

44. A method according to Claim 43, in which the cell populations, cell lines or clonal cells generated using a method according to any of Claims 1 to 13 comprise genetic imprints comprised in the pluripotent cells from which the cell populations, cell lines or clonal cells are derived.

45. A composition comprising:

- (a) a culture medium, optionally, free of TGFβ receptor/ALK inhibitors; and
- (b) one or more cell populations generated from a method according to any of Claims 1 to 13:

- 5 (i) pluripotent stem cell-derived definitive hemogenic endothelium (iHE), in which the iHE cells are CD34+, and at least one of CD43-, CD93-, CXCR4-, CD73-, and CXCR4-CD73-;
- (ii) pluripotent stem cell-derived definitive HSCs, in which the iHSC is CD34+CD45+;
- 10 (iii) pluripotent stem cell-derived multipotent progenitor cells, in which the iMPP cells are CD34+CD45+;
- (iv) pluripotent stem cell-derived T cell progenitors, in which the T cell progenitors are CD34+CD45+CD7+ or CD34-CD45+CD7+;
- (v) pluripotent stem cell-derived T cells, in which the T cells are CD45+CD3+CD4+ or CD45+CD3+CD8+;
- 15 (vi) pluripotent stem cell-derived NK cell progenitors, in which the NK cell progenitors are CD45+CD56+CD7+; and
- (vii) pluripotent stem cell-derived NK cells, in which the NK cells are CD3-CD45+CD56+, and optionally further defined by NKp46+, CD57+, and CD16+;
- (viii) pluripotent stem cell-derived NKT cells, in which the NKT cells are
20 CD45+Vα24Jα18+CD3+; and
- (ix) pluripotent stem cell-derived B cells, in which the B cells are CD45+CD19+.

46. A composition according to Claim 45, in which one or more cell populations generated from a method according to any of Claims 1 to 13 comprise genetic imprints comprised in the
25 pluripotent cells from which the one or more cell populations are derived from.

47. A composition comprising pluripotent stem cell-derived hematopoietic lineage cells 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;

5 (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;

10 (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 Wnt pathway activator;

15 in which iMPP-A comprises a BMP 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;

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 activator;

20 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 activator, and

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

48. A composition according to Claim 47, in which (i) iCD34-C is free of TGF β receptor/ALK inhibitor; in which iTC-A2 and iNK-A2 are free of BMP activator; and/or in which iTC-B2 and iNK-B2 are free of one or more of VEGF, bFGF, BMP activators, and
30 ROCK inhibitors; and/or (ii) the pluripotent stem cells comprise iPSCs, naïve iPSCs, and/or

iPSC having one or more genetic imprints retainable in hematopoietic lineage cells derived therefrom.

49. A pharmaceutical composition comprising the pluripotent cell derived hematopoietic lineage cells made by a method according to any of Claims 31 to 42 and a pharmaceutically
5 acceptable medium.

50. A pharmaceutical composition according to Claim 49 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
10 polyomavirus.