

Culture Medium for Haematopoietic Induction

Field

This invention relates to culture media for supporting the differentiation of mammalian induced pluripotent stem cells (iPSCs) into Haematopoietic Progenitor Cells (HPCs).

5 Background

Immunotherapeutics are poised to transform the cancer treatment landscape with the promise of long-term survival (McDermott et al., Cancer Treat Rev. 2014 Oct; 40(9): 1056-64). There is a clear unmet medical need for new immunomodulatory drugs to expand patient population and range of tumor types. In addition, new agents are needed to enhance the magnitude and duration of anti-tumor responses. The development of these agents has been possible because of the in-depth understanding of the basic principles controlling T-cell immunity over the last two decades (Sharma and Allison, Cell. 2015 Apr 9; 161(2): 205-14). This typically requires tumor specific CD4+ and CD8+ T-cells recognising tumor-associated peptide antigens presented by MHC molecules. Different vaccination strategies and adoptive transfer of ex vivo expanded tumor infiltrated lymphocytes have in some cases demonstrated the ability of tumor specific T-cells to treat late stage cancer (Rosenberg et al., Nat Med. 2004 Sep; 10(9): 909-15).

However, current adoptive T cell therapies are limited by a lack of suitable patient and tumor-specific T cells and there is a need for therapeutically sufficient and functional antigen-specific T cells for effective use in immunotherapy.

The differentiation of induced pluripotent stem cells (iPSCs) into HPCs and T cells has been reported (Kennedy et al 2012 Cell Reports 2 6 1722-1735; Sturgeon et al 2014 Nat Biotechnol 32(6) 554-561; Ditadi et al (2015) Nat Cell Biol.;17(5):580-91). Typically this involves the use of embryoid body formation and/or serum, and, in later stages, the use of OP9-DLL4 stroma, for the end stage of T cell differentiation (also see Themeli et al (2013) Nat Biotechnol. 31(10):928-33; Vizcardo et al 2013 Cell Stem Cell. 2013 Jan 3;12(1):31-6.). Cells produced using media that contain feeder, stromal cells, serum or other undefined components are not suitable for clinical applications.

Summary

The present inventors have developed chemically defined haematopoietic induction media that support the differentiation of haemogenic endothelial cells (HECs) into HPCs capable of further differentiation into T cells. These media (referred to as SV, HEM7.2 and HEM7.3) may allow the isolation of HPCs at much later time points (d16-18), in a serum free and directed GMP compliant manner that permits onward culture towards the T cell lineage without the use of OP9-DI4 stroma, at a reduced costs relative to existing haematopoietic induction media. This may be useful for example in the production of clinical grade blood cells, such as T cells, for use in immunotherapy.

A first aspect of the disclosure provides a method of producing a population of haematopoietic progenitor cells (HPCs) comprising; (i) culturing a population of haemogenic endothelial cells (HECs) in a haematopoietic induction medium to produce a population of haematopoietic progenitor cells (HPCs),

wherein the haematopoietic induction medium (i) stimulates cKIT receptor (CD117) or cKIT receptor (CD117) mediated signalling pathways and/or (ii) stimulates VEGFR or VEGFR mediated signalling pathways.

5 A first example of the disclosure provides a method of producing a population of HPCs comprising (i) culturing a population of HECs in a haematopoietic induction medium to produce a population of HPCs, wherein the haematopoietic induction medium is a chemically defined medium that (a) stimulates cKIT receptor (CD117; KIT receptor tyrosine kinase) and/or cKIT receptor (CD117; KIT receptor tyrosine kinase) mediated signalling pathways and/or (b) stimulates VEGFR and/or VEGFR mediated signalling pathways.

Preferably, the haematopoietic induction medium both (a) stimulates cKIT receptor (CD117; KIT receptor tyrosine kinase) and/or cKIT receptor mediated signalling pathways and (b) stimulates VEGFR and/or VEGFR mediated signalling pathways.

15 The haematopoietic induction medium may comprise a differentiation factor that stimulates c-KIT receptor (CD117) or c-KIT receptor (CD117) mediated signalling pathways, such as SCF, and/or a differentiation factor that stimulates VEGFR or VEGFR mediated signalling pathways, preferably VEGFR2 or VEGFR2 mediated signalling pathways, such as VEGF-A.

20 Preferably, these are the only differentiation factors in the medium.

A preferred haematopoietic induction medium of the first example may comprise SCF and/or VEGF. For example, the haematopoietic induction medium may consist of a chemically defined nutrient medium supplemented with one or more differentiation factors, wherein the one or more differentiation factors consist of SCF and/or VEGF.

A second example of the disclosure provides a method of producing a population of HPCs comprising (i) culturing a population of HECs in a haematopoietic induction medium to produce a population of HPCs,

30 wherein the haematopoietic induction medium is a chemically defined medium that (a) stimulates cKIT receptor (CD117) and/or cKIT receptor (CD117) mediated signalling pathways, (b) stimulates VEGFR and/or VEGFR mediated signalling pathways, (c) stimulates MPL (CD110) and/or MPL (CD110) mediated signalling pathways (d) stimulates *FLT3* and/or *FLT3* mediated signalling pathways (e) stimulates *IGF1R* and/or *IGF1R* mediated signalling pathways and (f) displays interleukin (IL) activity.

A preferred haematopoietic induction medium of the second example may comprise VEGF, SCF, Thrombopoietin (TPO), Flt3 ligand (Flt3L), IL-3, IL-6, IGF-1 and optionally IL-7. For example, the haematopoietic induction medium may consist of a chemically defined nutrient medium supplemented with one or more differentiation factors, wherein the one or more differentiation factors consist of VEGF, SCF, Thrombopoietin (TPO), Flt3 ligand (Flt3L), IL-3, IL-6, IGF-1 and optionally IL-7.

The HPCs produced by the methods of the first and second examples may be further differentiated to produce T cells. For example, a method of the first example may further comprise;

- (ii) differentiating the population of HPCs into T cell progenitor cells; and
- 5 (iii) maturing the progenitor T cells to produce a population of double positive CD4+CD8+T cells.

A method of the first or second example may further comprise

- (iv) activating and expanding the double positive CD4+CD8+ T cells to produce a population of single positive CD8+ T cells or a population of single positive CD4+ T cells.

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In preferred examples, HECs for use in the methods of the first example may be produced by differentiating a population of iPSCs into HECs.

A second aspect of the disclosure provides a haematopoietic induction medium comprising

- 15 (i) a chemically defined nutrient medium supplemented with one or more differentiation factors, wherein the one or more differentiation factors consist of a differentiation factor that stimulates cKit receptor (CD117) or cKit receptor (CD117) mediated signalling pathways, and a differentiation factor that stimulates VEGFR or VEGFR mediated signalling pathways; or
- (ii) a chemically defined nutrient medium supplemented with one or more differentiation factors, wherein
- 20 the one or more differentiation factors consist of (a) a differentiation factor that stimulates cKIT receptor (CD117) mediated signalling pathways, (b) a differentiation factor that stimulates VEGFR mediated signalling pathways, (c) a differentiation factor that stimulates MPL (CD110) mediated signalling pathways (d) a differentiation factor that stimulates *FLT3* mediated signalling pathways (e) a differentiation factor that stimulates *IGF1R* mediated signalling pathways and (f) one or more differentiation factors that display
- 25 interleukin (IL) activity.

A third example of the disclosure provides a haematopoietic induction medium comprising a chemically defined nutrient medium supplemented with one or more differentiation factors, wherein the one or more differentiation factors consist of a differentiation factor that stimulates cKIT receptor (CD117) or cKIT receptor (CD117) mediated signalling pathways, such as SCF, and/or a differentiation factor that

30 stimulates VEGFR (optionally VEGFR2) or VEGFR (optionally VEGFR2) mediated signalling pathways, such as VEGF (optionally VEGF-A).

A fourth example of the disclosure provides a haematopoietic induction medium comprising a chemically defined nutrient medium supplemented with one or more differentiation factors, wherein the one or more

35 differentiation factors consist of a differentiation factor that stimulates cKIT receptor (CD117) and/or cKIT receptor (CD117) mediated signalling pathways, such as SCF; a differentiation factor that stimulates VEGFR and/or VEGFR mediated signalling pathways, such as VEGF; a differentiation factor that stimulates MPL (CD110) and/or MPL (CD110) mediated signalling pathways,

such as TPO; a differentiation factor that stimulates *FLT3* and/or *FLT3* mediated signalling pathways, such as Flt3L; a differentiation factor that stimulates *IGF1R* and/or *IGF1R* mediated signalling pathways, such as IGF-1, and; one or more differentiation factors that display interleukin (IL) activity, for example, one or more interleukins, such as IL-3, IL-6 and optionally IL-7.

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A third aspect of the disclosure provides a population of HPCs produced by a method according to the disclosure, wherein the HPCs are thymocytic HPCs having the phenotype CD34+ CD7+.

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A fourth aspect of the disclosure provides a use of a haematopoietic induction medium according to the disclosure for the differentiation of mesoderm cells or HECs into HPCs.

A fifth example of the disclosure provides the use of a haematopoietic induction medium of the third or fourth example in the differentiation of HECs into HPCs.

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A fifth aspect of the disclosure provides a kit for use in the production of HPCs comprising a haematopoietic induction medium according to the disclosure, optionally further comprising; a first mesoderm induction medium comprising activin, a second mesoderm induction medium comprising activin, BMP and FGF, and/or a third mesoderm induction medium comprising activin, BMP, FGF, and a GSK3 inhibitor.

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A sixth example of the disclosure provides a kit for the production of HPCs comprising a haematopoietic induction medium of the third or fourth example.

The kit may further comprise suitable media to support the differentiation of iPSCs into HECs. For example, the kit may further comprise;

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- a first mesoderm induction medium comprising activin,
- a second mesoderm induction medium comprising activin, BMP and FGF, and
- a third mesoderm induction medium comprising activin, BMP, FGF, and a GSK3 inhibitor..

These and other aspects and examples of the disclosure are described in more detail below.

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Brief Description of Figures

Figure 1 shows a schematic view of an example of a six-stage method for generating T cells from iPSCs.

Claims:

1. A method of producing a population of haematopoietic progenitor cells (HPCs) comprising;
 - (i) culturing a population of haemogenic endothelial cells (HECs) in a haematopoietic induction medium to produce a population of haematopoietic progenitor cells (HPCs), wherein the haematopoietic induction medium (i) stimulates cKIT receptor (CD117) or cKIT receptor (CD117) mediated signalling pathways and/or (ii) stimulates VEGFR or VEGFR mediated signalling pathways.
2. A method according to claim 1 wherein
 - (a) the haematopoietic induction medium comprises one or both of SCF and VEGF; and/or
 - (b) the haematopoietic induction medium (iii) stimulates MPL (CD110) or MPL (CD110) mediated signalling pathways; (iv) stimulates *FLT3* or *FLT3* mediated signalling pathways (v) stimulates *IGF1R* or *IGF1R* mediated signalling pathways and (vi) displays interleukin (IL) activity; and/or
 - (c) the haematopoietic induction medium comprises VEGF, SCF, Thrombopoietin (TPO), Flt3 ligand (Flt3L), IGF-1, IL-3 and IL-6; and/or
 - (d) the haematopoietic induction medium comprises VEGF, SCF, Thrombopoietin (TPO), Flt3 ligand (Flt3L), IGF-1, IL-3, IL-6, and IL-7; and/or
 - (e) the haematopoietic induction medium is devoid of one or more of BMP, FGF, SHH, EPO, angiotensin II and losartan; and/or
 - (f) the haematopoietic induction medium consists of a chemically defined nutrient medium supplemented with one or more differentiation factors, wherein the one or more differentiation factors consist of SCF and/or VEGF.
3. A method according to claim 1 or claim 2 wherein the haematopoietic induction medium consists of a chemically defined nutrient medium supplemented with one or more differentiation factors, wherein
 - (i) the one or more differentiation factors consist of VEGF, SCF, Thrombopoietin (TPO), Flt3 ligand (Flt3L), IGF-1, IL-3 and IL-6; or
 - (ii) the one or more differentiation factors consist of VEGF, SCF, Thrombopoietin (TPO), Flt3 ligand (Flt3L), IGF-1, IL-3, IL-6, and IL-7; optionally wherein the differentiation factors in the haematopoietic induction medium are
 - (i) VEGF, SCF, Thrombopoietin (TPO), Flt3 ligand (Flt3L), IGF-1, IL-3, IL-6, and IL-7; or
 - (ii) VEGF, SCF, Thrombopoietin (TPO), Flt3 ligand (Flt3L), IGF-1, IL-3, and IL-6.
4. A method according to any one of claims 1 to 3 wherein
 - (a) the HECs display a CD34⁺ CD73⁻ CXCR4⁻ phenotype; and/or
 - (b) the HPCs display a CD34⁺ phenotype; and/or
 - (c) the HPCs comprise thymopoietic HPCs (tHPCs), optionally wherein the tHPCs display a CD34⁺ CD7⁺ phenotype; and/or

(d) the HECs are cultured in the haematopoietic induction medium for 16-28 days to produce the HPCs.

5. A method according to any one of the preceding claims wherein the population of HECs is produced *in vitro* from induced pluripotent stem cells (iPSCs).
6. A method according to claim 5 wherein the method comprises
 - (i) differentiating a population of induced pluripotent stem cells (iPSCs) into mesoderm cells; and
 - (ii) differentiating the mesoderm cells to produce a population of haemogenic endothelial cells (HECs).
7. A method according to claim 5 or claim 6 wherein
 - (a) the iPSCs are derived from T cells obtained from a donor individual, optionally wherein the T cells obtained from the donor individual are specific for a target antigen, optionally a tumour antigen; optionally wherein the T cells obtained from the donor individual are tumour infiltrating lymphocytes (TILs); and/or
 - (b) the iPSCs are differentiated into mesoderm cells by culturing the population of iPSCs under suitable conditions to promote mesodermal differentiation.
8. A method according to claim 6 or claim 7 wherein the iPSCs are cultured sequentially in first, second and third mesoderm induction media to induce differentiation into mesoderm cells, optionally wherein
 - (a) the first mesoderm induction medium
 - (i) stimulates SMAD2 and SMAD3 or SMAD2 and SMAD3 mediated signalling pathways, and/or
 - (ii) comprises activin, and/or
 - (iii) consists of a chemically defined nutrient medium supplemented with one or more differentiation factors, wherein the one or more differentiation factors consist of activin; optionally wherein
 - (b) the second mesoderm induction medium
 - (i) stimulates (a) SMAD1, SMAD2, SMAD3, SMAD5 and SMAD9 or (b) SMAD1, SMAD2, SMAD3, SMAD5 and SMAD9 mediated signalling pathways and
 - (ii) has fibroblast growth factor (FGF) activity; and/or
 - (iii) comprises activin, BMP, and FGF; and/or
 - (iv) consists of a chemically defined nutrient medium supplemented with one or more differentiation factors, wherein the one or more differentiation factors consist of activin, BMP, and FGF; optionally wherein
 - (c) the third mesoderm induction medium
 - (i) stimulates (a) SMAD1, SMAD2, SMAD3, SMAD5 and SMAD9 or (b) SMAD1, SMAD2, SMAD3, SMAD5 and SMAD9 mediated signalling pathways
 - (ii) has fibroblast growth factor (FGF) activity and

- (iii) inhibits glycogen synthase kinase 3 β ; and/or
- (iv) comprises activin, BMP, FGF, and a GSK3 inhibitor; and/or
- (v) consists of a chemically defined nutrient medium supplemented with one or more differentiation factors, wherein the one or more differentiation factors consist of activin, BMP, FGF, and a GSK3 inhibitor.

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9. A method according to claim 8 wherein the mesoderm cells

(a) display one or more of Brachyury, Goosecoid, Mixl1, KDR, FoxA2, GATA6 and PDGF α R; and/or

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(b) are differentiated into HECs by culturing the population of mesoderm cells under suitable conditions to promote haemogenic endothelial (HE) differentiation; and/or

(c) are cultured in an HE induction medium to induce differentiation into HECs, optionally wherein the HE induction medium

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(i) stimulates cKIT receptor (CD117) or cKIT receptor (CD117) mediated signalling pathways and

(ii) stimulates VEGFR or VEGFR mediated signalling pathways, optionally wherein the HE induction medium comprises or consists of SCF and VEGF.

10. A method according to any one of the preceding claims further comprising differentiating the population of HPCs into progenitor T cells, optionally wherein the HPCs are differentiated by a method comprising culturing the population of HPCs in a lymphoid expansion medium to produce the progenitor T cells, optionally wherein the progenitor T cells have a CD5+CD7+ phenotype.

11. A method according to claim 10 further comprising maturing the progenitor T cells to produce a population of double positive CD8+ CD4+ T cells, optionally wherein the progenitor T cells are matured by a method comprising culturing the population of progenitor T cells in a T cell maturation medium to produce the double positive CD8+ CD4+ T cells, optionally wherein the method further comprises activating and expanding the double positive CD8+ CD4+ T cells to produce a population of T cells that have a CD8+ single positive phenotype or a CD4+ single positive phenotype.

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12. A method according to claim 11 wherein the T cells specifically bind to cells expressing a target antigen, optionally a tumour antigen, optionally wherein the T cells specifically bind to cancer cells expressing the tumour antigen.

13. A method according to any one of claims 9 to 12 wherein the iPSCs are derived from T cells obtained from a donor individual that are specific for the target antigen, optionally wherein the T cells obtained from the donor individual are tumour-infiltrating lymphocytes (TILs).

14. A method according to any one of claims 1 to 11 wherein the method further comprises introducing heterologous nucleic acid encoding an antigen receptor into the iPSCs, HECs, HPCs, or progenitor T cells.

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15. A method according to claim 14 wherein the heterologous nucleic acid encoding the antigen receptor is comprised in an expression vector, optionally wherein the expression vector is a lentiviral vector or adeno-associated viral (AAV) vector.
- 5 16. A method according to claim 14 or 15 wherein the heterologous nucleic acid is incorporated into the genome of the iPSCs, HECs, HPCs, or progenitor T cells using a gene editing system, optionally wherein the gene editing system is CRISPR/Cas9 or AAV.
- 10 17. A method according to any one of claims 14 to 16 wherein
- (a) the antigen receptor is a TCR, optionally an affinity enhanced TCR, optionally wherein
 - (i) the TCR binds specifically to an MHC displaying a peptide fragment of a target antigen expressed by cells or specifically binds to a target antigen or peptide thereof expressed by cells independently of MHC presentation, or
 - 15 (ii) the TCR binds specifically to an MHC displaying a peptide fragment of a tumour antigen expressed by cancer cells or binds specifically to a tumour antigen or peptide fragment thereof expressed by cancer cells independently of MHC presentation; or
 - (b) the antigen receptor is a chimeric antigen receptor (CAR), optionally wherein
 - (i) the CAR binds specifically to a target antigen expressed by cells, or
 - 20 (ii) the CAR binds specifically to an MHC displaying a peptide fragment of a tumour antigen expressed by cancer cells; or
 - (c) the antigen receptor is a NK cell receptor (NKCR), optionally wherein
 - (i) the NKCR binds specifically to a target antigen expressed by cells, or
 - 25 (ii) the NKCR binds specifically to an MHC displaying a peptide fragment of a tumour antigen expressed by cancer cells.
18. A haematopoietic induction medium comprising
- (i) a chemically defined nutrient medium supplemented with one or more differentiation factors, wherein the one or more differentiation factors consist of a differentiation factor that stimulates cKit
 - 30 receptor (CD117) or cKit receptor (CD117) mediated signalling pathways, and a differentiation factor that stimulates VEGFR or VEGFR mediated signalling pathways; or
 - (ii) a chemically defined nutrient medium supplemented with one or more differentiation factors, wherein the one or more differentiation factors consist of (a) a differentiation factor that stimulates cKIT
 - 35 mediated signalling pathways, (b) a differentiation factor that stimulates VEGFR mediated signalling pathways, (c) a differentiation factor that stimulates MPL (CD110) mediated signalling pathways (d) a differentiation factor that stimulates *FLT3* mediated signalling pathways (e) a differentiation factor that stimulates *IGF1R* mediated signalling pathways and (f) one or more differentiation factors that display interleukin (IL) activity.
- 40 19. A haematopoietic induction medium according to claim 18 (ii) wherein
- (a) the differentiation factor that stimulates MPL mediated signalling pathways is TPO; and/or

- (b) the differentiation factor that stimulates FLT3 mediated signalling pathways is FLT3L; and/or
 - (c) the differentiation factor that stimulates IGF1R mediated signalling pathways is IGF1; and/or
 - (d) the one or more differentiation factors that display interleukin activity are IL-3, IL-6 and optionally IL-7; and/or
 - 5 (e) the differentiation factor that stimulates cKit receptor (CD117) or cKit receptor (CD117) mediated signalling pathway is SCF; and/or
 - (f) the differentiation factor that stimulates VEGFR or VEGFR mediated signalling pathways is VEGF.
- 10 20. A population of HPCs produced by a method according to any one of claims 1 to 9, wherein the HPCs are thymocytic HPCs having the phenotype CD34+ CD7+.
21. Use of a haematopoietic induction medium according to claim 18 or claim 19 for the differentiation of mesoderm cells or HECs into HPCs.
- 15 22. A kit for use in the production of HPCs comprising a haematopoietic induction medium according to claim 18 or claim 19, optionally further comprising;
- a first mesoderm induction medium comprising activin,
 - a second mesoderm induction medium comprising activin, BMP and FGF, and/or
 - 20 a third mesoderm induction medium comprising activin, BMP, FGF, and a GSK3 inhibitor.