

Claims:

1. A method of producing a population of haematopoietic progenitor cells (HPCs) comprising;
5 (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.
- 10 2. A method according to claim 1 wherein the haematopoietic induction medium comprises one or both of SCF and VEGF.
3. A method according to claim 2 wherein the haematopoietic induction medium comprises SCF and VEGF.
- 15 4. A method according to any one of claims 1 to 3 wherein 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.
- 20 5. A method according to claim 4 wherein the haematopoietic induction medium comprises VEGF, SCF, Thrombopoietin (TPO), Flt3 ligand (Flt3L), IGF-1, IL-3 and IL-6.
6. A method according to claim 4 wherein the haematopoietic induction medium comprises VEGF, SCF, Thrombopoietin (TPO), Flt3 ligand (Flt3L), IGF-1, IL-3, IL-6, and IL-7.
- 25 7. A method according to any one of claims 1 to 6 wherein the haematopoietic induction medium is devoid of one or more of BMP, FGF, SHH, EPO, angiotensin II and losartan.
- 30 8. A method according to any one of claims 1 to 3 wherein 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.
9. A method according to any one of claims 1 to 7 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.
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10. A method according to claim 9 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.
- 5 11. A method according to any one of claims 1 to 10 wherein the HECs display a CD34+ CD73- CXCR4- phenotype.
12. A method according to claim 11 wherein the HPCs display a CD34+ phenotype.
- 10 13. A method according to any one of the preceding claims wherein the HPCs comprise thymopoietic HPCs (tHPCs).
14. A method according to claim 13 wherein the tHPCs display a CD34+ CD7+phenotype
- 15 15. A method according to any one of the preceding claims wherein the HECs are cultured in the haematopoietic induction medium for 16-28 days to produce the HPCs.
16. 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).
- 20 17. A method according to claim 16 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).
- 25 18. A method according to claim 16 or claim 17 wherein the iPSCs are derived from T cells obtained from a donor individual.
- 30 19. A method according to claim 18 wherein the T cells obtained from the donor individual are specific for a target antigen.
20. A method according to claim 19 wherein the target antigen is a tumour antigen.
21. A method according to claim 19 or 20 wherein the T cells obtained from the donor individual are tumour-infiltrating lymphocytes (TILs).
- 35 22. A method according to any one of claims 17 to 21 wherein the iPSCs are differentiated into mesoderm cells by culturing the population of iPSCs under suitable conditions to promote mesodermal differentiation.

23. A method according to any one of claims 17 to 22 wherein the iPSCs are cultured sequentially in first, second and third mesoderm induction media to induce differentiation into mesoderm cells.
24. A method according to claim 23 wherein the first mesoderm induction medium stimulates SMAD2
5 and SMAD3 or SMAD2 and SMAD3 mediated signalling pathways.
25. A method according to claim 24 wherein the first mesoderm induction medium comprises activin.
26. A method according to claim 24 or claim 25 wherein the first mesoderm induction medium
10 consists of a chemically defined nutrient medium supplemented with one or more differentiation factors, wherein the one or more differentiation factors consist of activin.
27. A method according to any one of claims 23 to 26 wherein the second mesoderm induction medium (i) stimulates (a) SMAD1, SMAD2, SMAD3, SMAD5 and SMAD9 or (b) SMAD1, SMAD2,
15 SMAD3, SMAD5 and SMAD9 mediated signalling pathways and (ii) has fibroblast growth factor (FGF) activity.
28. A method according to claim 27 wherein the second mesoderm induction medium comprises
20 activin, BMP, and FGF.
29. A method according to claim 27 or claim 28 wherein the second mesoderm 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 activin, BMP, and FGF.
- 25 30. A method according to any one of claims 23 to 29 wherein 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 .
- 30 31. A method according to claim 30 wherein the third mesoderm induction medium comprises activin, BMP, FGF, and a GSK3 inhibitor.
32. A method according to claim 31 wherein the third mesoderm induction medium consists of a chemically defined nutrient medium supplemented with one or more differentiation factors, wherein the
35 one or more differentiation factors consist of activin, BMP, FGF, and a GSK3 inhibitor.
33. A method according to any one of claims 23 to 32 wherein the mesoderm cells display one or more of Brachyury, Goosecoid, Mixl1, KDR, FoxA2, GATA6 and PDGF α R .

34. A method according to any one of claims 23 to 33 wherein the mesoderm cells are differentiated into HECs by culturing the population of mesoderm cells under suitable conditions to promote haemogenic endothelial (HE) differentiation.
- 5 35. A method according to any one of claims 23 to 34 wherein the mesoderm cells are cultured in an HE induction medium to induce differentiation into HECs.
36. A method according to claim 35 wherein the HE induction medium (i) stimulates cKIT receptor (CD117) or cKIT receptor (CD117) mediated signalling pathways and (ii) stimulates VEGFR or VEGFR mediated signalling pathways.
- 10 37. A method according to claim 36 wherein the HE induction medium comprises SCF and VEGF.
38. A method according to claim 37 wherein the HE 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 VEGF.
- 15 39. A method according to any one of the preceding claims further comprising differentiating the population of HPCs into progenitor T cells.
- 20 40. A method according to claim 39 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.
41. A method according to claim 39 or 40 wherein the progenitor T cells have a CD5+CD7+ phenotype.
- 25 42. A method according to any one of claims 39 to 41 further comprising maturing the progenitor T cells to produce a population of double positive CD8+ CD4+ T cells.
- 30 43. A method according to claim 42 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.
44. A method according to any one of claims 42 to 43 comprising 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.
- 35 45. A method according to any one of claims 42 to 44 wherein the T cells specifically bind to cells expressing a target antigen.
- 40 46. A method according to claim 45 wherein the target antigen is a tumour antigen.

47. A method according to claim 46 wherein the T cells specifically bind to cancer cells expressing the tumour antigen.
- 5 48. A method according to any one of claims 16 to 47 wherein the iPSCs are derived from T cells obtained from a donor individual that are specific for the target antigen.
49. A method according to claim 48 wherein the T cells obtained from the donor individual are tumour-infiltrating lymphocytes (TILs).
- 10 50. A method according to any one of claims 1 to 44 wherein the method further comprises introducing heterologous nucleic acid encoding an antigen receptor into the iPSCs, HECs, HPCs, or progenitor T cells.
- 15 51. A method according to claim 50 wherein the heterologous nucleic acid encoding the antigen receptor is comprised in an expression vector.
52. A method according to claim 51 wherein the expression vector is a lentiviral vector or adeno-associated viral (AAV) vector.
- 20 53. A method according to claim 50 or 51 wherein the heterologous nucleic acid is incorporated into the genome of the iPSCs, HECs, HPCs, or progenitor T cells using a gene editing system.
54. A method according to claim 53 wherein the gene editing system is CRISPR/Cas9 or AAV.
- 25 55. A method according to any one of claims 50 to 54 wherein the antigen receptor is a TCR.
56. A method according to claim 55 wherein the TCR is an affinity enhanced TCR.
- 30 57. A method according to claim 54 or 55 wherein 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.
58. A method according to claim 57 wherein 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.
- 35 59. A method according to any one of claims 50 to 54 wherein the antigen receptor is a chimeric antigen receptor (CAR).
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60. A method according to claim 59 wherein the CAR binds specifically to a target antigen expressed by cells.
61. A method according to claim 60 wherein the CAR binds specifically to an MHC displaying a peptide fragment of a tumour antigen expressed by cancer cells.
- 5 62. A method according to any one of claims 50 to 54 wherein the antigen receptor is a NK cell receptor (NKCR).
63. A method according to claim 62 wherein the NKCR binds specifically to a target antigen
10 expressed by cells.
64. A method according to claim 63 wherein the NKCR binds specifically to an MHC displaying a peptide fragment of a tumour antigen expressed by cancer cells.
- 15 65. 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, and a differentiation factor that stimulates VEGFR or VEGFR mediated signalling pathways.
- 20 66. 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) 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
25 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.
67. A haematopoietic induction medium according to claim 66 wherein the differentiation factor that
30 stimulates MPL mediated signalling pathways is TPO.
68. A haematopoietic induction medium according to claim 66 or claim 67 wherein the differentiation factor that stimulates FLT3 mediated signalling pathways is FLT3L.
- 35 69. A haematopoietic induction medium according to any one of claims 66 to 68 wherein the differentiation factor that stimulates IGF1R mediated signalling pathways is IGF1.
70. A haematopoietic induction medium according to any one of claims 66 to 69 wherein the one or more differentiation factors that display interleukin activity are IL-3, IL-6 and optionally IL-7.
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71. A haematopoietic induction medium according to any one of claims 65 to 70 wherein the differentiation factor that stimulates cKit receptor (CD117) or cKit receptor (CD117) mediated signalling pathway is SCF.
- 5 72. A haematopoietic induction medium according to any one of claims 65 to 710 wherein the differentiation factor that stimulates VEGFR or VEGFR mediated signalling pathways is VEGF.
73. A population of HPCs produced by a method according to any one of claims 1 to 38, wherein the HPCs are thymocytic HPCs having the phenotype CD34+ CD7+.
- 10 74. Use of a haematopoietic induction medium according to any one of claims 65 to 7261 for the differentiation of mesoderm cells or HECs into HPCs.
75. A kit for use in the production of HPCs comprising a haematopoietic induction medium according to any one of claims 65 to 72.
- 15 76. A kit according to claim 75 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,.