

CLAIMS

What is claimed herein is:

1. A method comprising:
 - a) differentiating a population of pluripotent stem cells in aggregation media for a sufficient time to promote differentiation into a population of CD34⁺ hemogenic endothelium;
 - b) inhibiting a histone methyltransferase in the resultant population of CD34⁺ hemogenic endothelium; and
 - c) differentiating the resultant population of CD34⁺ hemogenic endothelium in a CD3⁺-T-cell differentiation media in the presence of a Notch ligand for a sufficient time to promote differentiation into a population of CD3⁺ T cells.
2. A method comprising:
 - a) differentiating a population of pluripotent stem cells in aggregation media for a sufficient time to promote differentiation into a population of CD34⁺ hemogenic endothelium;
 - b) inhibiting an epigenetic regulator in the resultant population of CD34⁺ hemogenic endothelium; and
 - c) differentiating the resultant population of CD34⁺ hemogenic endothelium in a CD3⁺-T-cell differentiation media in the presence of a Notch ligand for a sufficient time to promote differentiation into a population of CD3⁺ T cells.
3. A method comprising:
 - a) differentiating a population of pluripotent stem cells in aggregation media for a sufficient time to promote differentiation into a population of CD34⁺ hemogenic endothelium;
 - b) inhibiting G9a and/or GLP in the resultant population of CD34⁺ hemogenic endothelium; and
 - c) differentiating the resultant population of CD34⁺ hemogenic endothelium in a CD3⁺-T-cell differentiation media in the presence of a Notch ligand for a sufficient time to promote differentiation into a population of CD3⁺ T cells.
4. A method comprising:
 - a) differentiating a population of pluripotent stem cells in aggregation media for a sufficient time to promote differentiation into a population of CD34⁺ hemogenic endothelium; and

- b) differentiating the resultant population of CD34⁺ hemogenic endothelium in a CD3⁺-T-cell-differentiation media in the presence of a Notch ligand for a sufficient time to promote differentiation into a population of CD3⁺ T cells.
5. The method of any one of claims 1-4, wherein the Notch ligand is attached to a solid substrate.
 6. The method of any one of claims 1-5, wherein the Notch ligand is attached to a cell culture dish.
 7. The method of any one of claims 1-6, wherein the Notch ligand is not derived from a stromal cell.
 8. The method of any one of claims 1-7, wherein differentiating the hemogenic endothelium in the presence of a Notch ligand does not comprise co-culturing with a stromal cell expressing a Notch ligand.
 9. The method of any one of claims 1-8, wherein differentiating the hemogenic endothelium in the presence of a Notch ligand does not comprise co-culturing with OP9-DL1 cells or OP9-DL4 cells.
 10. The method of any one of claims 1-9, wherein the Notch ligand is selected from the group consisting of Delta-like-1 (DLL1), Delta-like-4 (DLL4), immobilized Delta1^{ext-IgG}, and immobilized Delta4^{ext-IgG}.
 11. The method of claim 10, wherein immobilized Delta1^{ext-IgG} consists of an extracellular domain of human Delta-like-1 fused to the Fc domain of human IgG1.
 12. The method of any one of claims 1-11, wherein the sufficient time to promote differentiation into a population of CD3⁺ T cells is at least 4 weeks.
 13. The method of any one of claims 1-12, wherein the CD3⁺-T-cell-differentiation media is serum-free.
 14. The method of any one of claims 1-13, wherein the CD3⁺-T-cell-differentiation media comprises FLT3 and IL7.
 15. The method of any one of claims 1-14, wherein the CD3⁺-T-cell-differentiation media comprises 15 ng/ml FLT3 and 25 ng/ml IL7.
 16. The method of any one of claims 1-15, wherein the CD3⁺-T-cell-differentiation media further comprises 5 ng/mL thrombopoietin (TPO) and/or 30 ng/ml SCF for at least the first 2 weeks of differentiating in the CD3⁺-T-cell-differentiation media.
 17. The method of any one of claims 1-16, wherein CD3⁺-T-cell-differentiation media comprising TPO promotes differentiation into a population of CD5⁺ CD7⁺ ProT cells.
 18. The method of any one of claims 1-4, wherein the population of CD3⁺ T cells comprises a population of CD4⁺CD8⁺ T cells.
 19. The method of claim 18, further comprising differentiating the population of CD4⁺CD8⁺ T cells in a single-positive-T-cell-differentiation media for a sufficient time to promote differentiation into a population of CD4⁺ cells and a population of CD8⁺ cells.

20. The method of claim 19, wherein the sufficient time to promote differentiation from the population of CD4⁺CD8⁺ T cells into a population of CD4⁺ T cells and a population of CD8⁺ cells is at least 1 week.
21. The method of claim 19, wherein the sufficient time to promote differentiation from the population of CD34⁺ hemogenic endothelium into a population of CD4⁺ T cells and a population of CD8⁺ cells is at least 5 weeks.
22. The method of claim 19, wherein the single-positive-T-cell-differentiation media comprises 10 ng/mL IL-15 and a T cell activator.
23. The method of claim 22, wherein the T cell activator comprises a 10ul/ml CD3/CD28 T cell activator.
24. The method of claim 22, wherein the T cell activator comprises one bead of CD3/CD28 T cell activator dynabeads per cell.
25. The method of any one of claims 18-24, further comprising, after at least 1 week, a step of CD4⁺ cell enrichment and/or CD8⁺ cell enrichment.
26. The method of any one of claims 1-4, wherein the population of pluripotent stem cells comprises induced pluripotent stem cells (iPS cells) or embryonic stem cells (ESC).
27. The method of claim 26, wherein the induced pluripotent stem cells are produced by introducing only reprogramming factors *OCT4*, *SOX2*, *KLF4* and optionally *c-MYC* or nanog and LIN28 into mature cells.
28. The method of claim 26, wherein the induced pluripotent stem cells are produced by introducing the reprogramming factors two or more times into the mature cells.
29. The method of any one of claims 1-4, wherein the population of pluripotent stem cells is differentiated into a population of CD34⁺ hemogenic endothelium using embryoid bodies or 2D adherent cultures.
30. The method of any one of claims 1-4, wherein the sufficient time to promote differentiation into a population of CD34⁺ hemogenic endothelium is at least 8 days.
31. The method of any one of claims 1-4, wherein the aggregation media comprises BMP4, SB-431542, CHIR99021, bFGF, VEGF, IL-6, IL-11, IGF-1, SCF, and EPO.
32. The method of any one of claims 29-31, wherein the aggregation media comprises 10 ng/ml BMP4, 6 mM SB-431542, 3 mM CHIR99021, 5 ng/ml bFGF, 15 ng/ml VEGF, 10 ng/ml IL-6, 5 ng/mL IL-11, 25 ng/mL IGF-1, 50 ng/mL SCF, and 2 U/ml EPO.
33. The method of any one of claims 29-32, further comprising selecting or isolating the resultant population of CD34⁺ hemogenic endothelium using expression of surface markers on the population of CD34⁺ hemogenic endothelium.
34. The method of any one of claims 29-33, wherein the population of CD34⁺ hemogenic endothelium is CD45 negative/low.

35. The method of any one of claims 29-34, wherein the population of CD34⁺ hemogenic endothelium is CD38 negative/low.
36. The method of any one of claims 1-4, further comprising the step of genetically modifying the resultant population of CD34⁺ hemogenic endothelium or the resultant population of CD3⁺ T cells.
37. The method of claim 36, wherein the genetic modification is editing an endogenous HLA, removing an endogenous TCR, and/or expressing a chimeric antigen receptor (CAR).
38. The method of claim 1, wherein the histone methyltransferase catalyzes the addition of methyl group to the histone 3 lysine residue 9 (H3K9) and/or histone 3 lysine residue 27 (H3K27).
39. The method of claim 1, wherein the histone methyltransferase H3K9 and/or H3K27 is inhibited by a small molecule inhibitor or a nucleic acid inhibitor.
40. The method of claim 39, wherein the histone methyltransferase H3K9 and/or H3K27 small molecule inhibitor is a heterorganic compound or an organometallic compound.
41. The method of claim 39, wherein the histone methyltransferase H3K9 and/or H3K27 small molecule inhibitor is selected from the group consisting of BIX-01294, UNC0638, E72, BRD4770, A-366, chaetocin, UNC0224, UNC0631, UNC0646, EPZ005687, EPZ-6438 (E7438), 3-deazaneplanocin A (DZNep), EII, GSK343, GSK126, and UNC1999.
42. The method of claim 39, wherein the nucleic acid inhibitor is a nucleic acid targeting the expression of histone methyltransferase.
43. The method of claim 39, wherein the nucleic acid inhibitor is a RNA interference inhibitor or agent.
44. The method of claim 39, wherein the nucleic acid inhibitor is a EZH1 specific nucleic acid that is selected from the group consisting of an aptamer that binds EZH1, a EZH1 specific RNA interference agent, and a vector encoding a EZH1 specific RNA interference agent, wherein the RNA interference agent comprises one or more of the nucleotide sequences selected from SEQ ID NO: 11-19.
45. The method of claim 2, wherein the epigenetic regulator is a DNA-methyltransferase (DNMT); a methyl-CpG-binding domain (MBD) protein; a DNA demethylase; a histone methyl transferase (HMT); a methyl-histone binding protein; a histone demethylase; a histone acetyl transferase (HAT); an acetyl-binding protein; or a histone deacetylase (HDAC).
46. The method of claim 45, wherein the inhibitor of an epigenetic regulator is selected from the group consisting of: UNC0224; MC1568; and CAY10591.
47. The method of any one of claims 45-46, wherein the inhibitor of an epigenetic regulator is provided at a concentration of at least 500 nM.
48. The method of any one of claims 45-46, wherein the sufficient time to promote differentiation from the population of CD34⁺ cells into a population of CD5⁺CD7⁺ proT cells is about 14 days.

49. The method of claim 3, wherein the G9a and/or GLP inhibitor is selected from the group consisting of: UNC0224; UNC0638; A366; BRD4770; BIX01294; UNC0642; UNC0631; UNC0646; UNC0321; E72; BIX-01338; BRD9539; Chaetocin; and DCG066.
50. The method of claim 49, wherein the G9a and/or GLP inhibitor is UNC0224.
51. The method of any one of claims 49-50, wherein the G9a and/or GLP inhibitor is provided at a concentration of 300 nM - 5uM.
52. The method of any one of claims 49-51, wherein the sufficient time to promote differentiation from the population of CD34⁺ cells into a population of CD5⁺CD7⁺ proT cells is about 14 days.
53. A method comprising:
- a) differentiating a population of pluripotent stem cells in aggregation media for a sufficient time to promote differentiation into a population of CD34⁺ hemogenic endothelium; and
 - b) differentiating the resultant population of CD34⁺ hemogenic endothelium in a CD3⁺-T-cell-differentiation media comprising 15 ng/ml FLT3 and 25 ng/ml IL7 in the presence of 10 µg/mL Notch ligand for at least 4 weeks to promote differentiation into a population of CD3⁺ T cells; wherein the CD3⁺-T-cell-differentiation media further comprises 5 ng/mL TPO and 30 ng/ml SCF for at least the first two weeks.
54. A method comprising:
- a) differentiating a population of pluripotent stem cells in aggregation media for a sufficient time to promote differentiation into a population of CD34⁺ hemogenic endothelium; and
 - b) differentiating the resultant population of CD34⁺ hemogenic endothelium in a CD3⁺-T-cell-differentiation media comprising 15 ng/ml FLT3 and 25 ng/ml IL7 in the presence of 10 µg/mL Notch ligand for at least 4 weeks to promote differentiation into a population of CD3⁺ T cells; wherein the CD3⁺-T-cell-differentiation media further comprises 5 ng/mL TPO, 30 ng/ml SCF, and a G9a/GLP inhibitor for at least the first two weeks.
55. The method of any one of claims 1-54, wherein the population of CD3⁺ T cells exhibits a gene expression profile that is most similar to alpha beta T cells.
56. The method of any one of claims 1-55, wherein the population of CD3⁺ T cells exhibits a gene expression profile that is at least 10%, 20%, 30%, 40% or more similar to alpha beta T cells.
57. The method of any one of claims 1-56, wherein the population of CD3⁺ T cells exhibits a gene expression profile with a Pearson's correlation coefficient compared to peripheral blood alpha beta T cells that is at least 0.85.

58. The method of any one of claims 1-57, wherein the population of CD3⁺ T cells exhibits a Productive Simpson Clonality value of about 0.025.
59. The method of any one of claims 1-58, wherein the population of CD3⁺ T cells exhibits a T cell receptor (TCR) complementarity-determining region (CDR) that is at least 3 nucleotides longer than an immune cell differentiated without inhibition of a methyltransferase or using stromal cells.
60. An immune cell produced by the method of any one of claims 1-59.
61. The immune cell of claim 60, wherein the immune cell exhibits a gene expression profile that is most similar to alpha beta T cells.
62. The immune cell of any one of claims 60-61, wherein the immune cell exhibits a gene expression profile that is at least 10%, 20%, 30%, 40% or more similar to alpha beta T cells.
63. The immune cell of any one of claims 60-62, wherein the immune cell exhibits a gene expression profile with a Pearson's correlation coefficient compared to peripheral blood alpha beta T cells that is at least 0.85.
64. The immune cell of any one of claims 60-63, wherein the immune cell exhibits a Productive Simpson Clonality value of about 0.025.
65. The immune cell of any one of claims 60-64, wherein the immune cell exhibits a T cell receptor (TCR) complementarity-determining region (CDR) that is at least 3 nucleotides longer than an immune cell differentiated without inhibition of methyltransferase, using stromal cells.
66. A composition comprising an immune cell of any one of claims 60-65 or population thereof.
67. The composition of claim 66, further comprising a pharmaceutically acceptable carrier.
68. A pharmaceutical composition comprising an immune cell of any one of claims 60-65 or population thereof, and a pharmaceutically acceptable carrier.
69. The pharmaceutical composition of claim 68 for use in cellular replacement therapy in a subject.
70. A method of cellular replacement therapy, the method comprising administering an immune cell of any one of claims 60-65 or population thereof, or a composition of claims 66-67, or a pharmaceutical composition of claims 68-69 to a recipient subject in need thereof.
71. The method of cellular replacement therapy of claim 70, wherein the recipient subject has undergone chemotherapy and/or irradiation.
72. The method of cellular replacement therapy of claim 70, wherein the recipient subject has deficiencies in immune function and/or lymphocyte reconstitution.
73. The method of cellular replacement therapy of any one of claims 70-72, wherein prior to transplanting, the immune cell or population thereof is treated *ex vivo* with prostaglandin E2 and/or antioxidant N-acetyl-L-cysteine (NAC) to promote subsequent engraftment in a recipient subject.

74. The method of cellular replacement therapy of any one of claims 70-73, wherein the immune cell or population thereof is autologous to the recipient subject.
75. The method of cellular replacement therapy of any one of claims 70-74, wherein the immune cell or population thereof is HLA type matched with the recipient subject.