CLAIMS

1. A method for differentiating a population of stem cells into a population of hematopoietic progenitors, comprising:

(i) culturing the population of stem cells in a 2D culture system for a period of time sufficient to form stem cell aggregates;

(ii) passaging the stem cell aggregates from the 2D culture system to a 3D suspension culture system;

(iii) contacting the stem cell aggregates in the 3D suspension culture system with a differentiation media comprising a bone morphogenetic protein (BMP) pathway activator, a fibroblast growth factor (FGF), a vascular endothelial growth factor (VEGF) and a PI3K inhibitor, for a period of time sufficient to differentiate the population of stem cells into the population of hematopoietic progenitors.

2. The method of claim 1, wherein the population of hematopoietic progenitors comprises CD34+/CD43+/CD45+ cells.

3. The method of claim 1 or claim 2, wherein the stem cells are progenitor cells.

4. The method of any one of claims 1 to 3, wherein the 3D suspension culture has a volume of between 50-50,000 ml.

5. The method of any one of claims 1 to 4, wherein the 3D suspension culture is agitated, optionally wherein the 3D suspension culture is agitated at a rate of between 10 revolutions per minute (RPM) to 100 RPM.

6. The method of any one of claims 1 to 5, wherein the BMP pathway activator is BMP4, the FGF is FGF2, and/or the VEGF is VEGF-165.

7. The method of any one of claims 1 to 6, wherein the differentiation media comprises:

(a) Rho-associated coiled coil forming protein serine/threonine kinase (ROCK) inhibitor, optionally wherein the ROCK inhibitor is Y27632;
(b) stem cell factor (SCF);
(c) thrombopoietin (TPO); and
(d) low-density lipoprotein (LDL).

8. The method of any one of claims 1 to 7, wherein:
(iii) comprises contacting the population of stem cell aggregates with the differentiation media for 1-5 days.

9. The method of claim 7, wherein the differentiation media comprises 1-50 ng/mL BMP, 1-50 ng/mL FGF, 5-100 ng/mL VEGF, 0.1-20 uM ROCK inhibitor, 1-200 ng/mL SCF, 1-100 ng/mL TPO, and 1-50 ug/mL LDL, or any combination thereof.

10. The method of any one of claims 1 to 9, wherein the progenitor cells or stem cells are induced pluripotent stem cells (iPSCs) or human embryonic stem cells (hESCs).

11. The method of any one of claims 1 to 10, wherein the differentiation media is serum free and/or xenogeneic-free.

12. A method of generating a population of NK cells, comprising:

(a) culturing a population of stem cells in a 2D culture system for a period of time sufficient to form stem cell aggregates;

(b) passaging the stem cell aggregates from the 2D culture system to a 3D suspension culture system;

(c) contacting the stem cell aggregates in the 3D suspension culture system with a first media comprising a BMP pathway activator, an FGF, a VEGF, and optionally an inhibitor of ROCK, for a period of time sufficient to generate embryoid bodies;

(d) contacting the embryoid bodies with a first differentiation media comprising a BMP pathway activator, a FGF, VEGF, and a PI3K inhibitor, for a period of time sufficient to generate a population of hematopoietic progenitors;

(e) contacting the population of hematopoietic progenitors with a second differentiation media, for a period of time sufficient to generate the population of NK cells.

13. The method of claim 12, wherein the second differentiation media comprising SCF, IL-7, IL-12, IL-15, FLT3L, a pyrimido-[4,5-b]-indole derivative, and an AhR inhibitor, optionally wherein the pyrimido-[4,5-b]-indole derivative is UM729 and the AhR inhibitor is SR1.

14. The method of any one of claims 12 to 13, wherein the BMP pathway activator is BMP4, the FGF is FGF2, the VEGF is VEGF-165, and the inhibitor of ROCK is Y27632.

15. The method of any one of claims 12 to 14, wherein each media of steps (b)-(e) is serum free.

16. The method of any one of claims 12 to 15, wherein the method is xenogenic-free.

17. The method of any one of claims 12 to 16, wherein the stem cells are induced pluripotent stem cells (iPSCs) or human embryonic stem cells (hESCs).

18. The method of any one of claims 1 to 17, comprising:

(a) genetically engineering a population of stem cells to express a synthetic cytokine receptor for a non-physiological ligand,

wherein the cytokine receptor comprises:

a synthetic gamma chain polypeptide comprising a first dimerization domain, a first transmembrane domain, and an interleukin-2 receptor subunit gamma (IL-2RG) intracellular domain, and

a synthetic beta chain polypeptide comprising a second dimerization domain, a second transmembrane domain, and an intracellular domain;

(b) culturing the population of stem cells in a 2D culture system for a period of time sufficient to form stem cell aggregates;

(c) passaging the stem cell aggregates from the 2D culture system to a 3D suspension culture system; and

(d) contacting the stem cell aggregates in the 3D suspension culture system with a differentiation media for a period of time sufficient to generate hematopoietic progenitors.

19. The method of claim 18, wherein the intracellular domain of the synthetic beta chain polypeptide is selected from an interleukin-2 receptor subunit beta (IL-2RB) intracellular domain, an interleukin-7 receptor subunit beta (IL-7RB) intracellular domain, and/or an interleukin-21 receptor subunit beta (IL-21RB) intracellular domain.

20. The method of claim 18 or 19, comprising engineering the population of stem cells to be resistant to rapamycin, optionally wherein engineering the population of stem cells to be resistant to rapamycin comprises knocking out a FKBP12 gene.

21. The method of any one of claims 18 to 20, wherein the second differentiation media comprises SCF, IL-7, IL-12, IL-15, FLT3L, a pyrimido-[4,5-b]-indole derivative, and an AhR inhibitor, optionally wherein the pyrimido-[4,5-b]-indole derivative is UM729 and the AhR inhibitor is SR1.

22. The method of any one of claims 18 to 21, wherein the second differentiation media comprises 1-100 ng/mL SCF, 1-50 ng/mL IL-7, 1-100 ng/mL IL-12, 1-100 ng/mL IL-15, 1-100 ng/mL FLT3L, 0.1-10 uM pyrimido-[4,5-b]-indole derivative, 0.1-10 uM AhR antagonist, and any combination thereof, optionally wherein the pyrimido-[4,5-b]-indole derivative is UM729 and the AhR inhibitor is SR1.

23. The method of any one of claims 18 to 22, wherein the period of time of step (b) is 2-8 days, the period of time of step (c) is 1-5 days, the period of time of step (d) is 3-15 days, and the period of time of step (e) is 11-25 days.

24. The method of any one of claims 18 to 23, wherein steps (a)-(e) occur within 40-50 days.

25. The method of any one of claims 18 to 24, wherein the stem cells are induced pluripotent stem cells (iPSCs) or human embryonic stem cells (hESCs).

26. The method of any one of claims 18 to 25, wherein the population of hematopoietic progenitors comprises about 50% to about 100% CD34+/CD43+/CD45+ cells.

27. The method of any one of claims 18 to 26, wherein the population of NK cells comprises about 60% to about 100% CD43+/CD45+/CD56+/LFA1+ cells.

28. The method of any one of claims 18 to 27, comprising expanding the population of NK cells, wherein the population of NK cells expands about 1,000 to about 10,000 fold.

29. The method of any one of claims 18 to 28, wherein the population of hematopoietic progenitors is genetically engineered or edited.

30. The method of any one of claims 18 to 29, wherein the population of NK cells is genetically engineered or edited.

31. A population of cells comprising hematopoietic progenitors produced by the method of any one of claims 1 to 11.

32. A population of cells comprising NK cells produced by the method of any one of claims 12 to 30.

33. A pharmaceutical composition comprising the cell population of any one of claims 31 to 32.

34. A method of generating a population of hematopoietic progenitors, comprising:

(a) genetically engineering a population of stem cells to express a synthetic cytokine receptor for a non-physiological ligand,

wherein the cytokine receptor comprises:

a synthetic gamma chain polypeptide comprising a first dimerization domain, a first transmembrane domain, and an interleukin-2 receptor subunit gamma (IL-2RG) intracellular domain, and

a synthetic beta chain polypeptide comprising a second dimerization domain, a second transmembrane domain, and an intracellular domain;

(b) culturing the population of stem cells in a 2D culture system for a period of time sufficient to form stem cell aggregates;

(c) passaging the stem cell aggregates from the 2D culture system to a 3D suspension culture system; and

(d) contacting the stem cell aggregates in the 3D suspension culture system with a differentiation media for a period of time sufficient to generate hematopoietic progenitors.

35. A method of generating a population of NK cells, comprising:

(a) genetically engineering a population of stem cells to express a synthetic cytokine receptor for a non-physiological ligand,

wherein the cytokine receptor comprises:

a synthetic gamma chain polypeptide comprising a first dimerization domain, a first transmembrane domain, and an interleukin-2 receptor subunit gamma (IL-2RG) intracellular domain, and

a synthetic beta chain polypeptide comprising a second dimerization domain, a second transmembrane domain, and an intracellular domain;

(b) culturing the population of stem cells in a 2D culture system for a period of time sufficient to form stem cell aggregates;

(c) passaging the stem cell aggregates from the 2D culture system to a 3D suspension culture system;

(d) contacting the stem cell aggregates in the 3D suspension culture system with a first differentiation media for a period of time sufficient to generate hematopoietic progenitors; and

(e) contacting the population of hematopoietic progenitors with a second differentiation media, for a period of time sufficient to generate the population of NK cells.

36. The method of claims 34 or 35, wherein the intracellular domain of the synthetic beta chain polypeptide is selected from an interleukin-2 receptor subunit beta (IL-2RB) intracellular domain, an interleukin-7 receptor subunit beta (IL-7RB) intracellular domain, and/or an interleukin-21 receptor subunit beta (IL-21RB) intracellular domain.

37. The method of any one of any one of claims 34 to 36, comprising engineering the population of stem cells to be resistant to rapamycin, optionally wherein engineering the population of stem cells to be resistant to rapamycin comprises knocking out a FKBP12 gene.

38. A population of cells produced by the method of any one of any one of claims 34 to 37.

39. A pharmaceutical composition comprising the cell population of claim 38.

40. A method of treating a cancer in a subject, comprising administering to the subject an effective amount of the population of cells of claim 31 or 39, or the pharmaceutical composition of claim 33.

41. A kit comprising the population of cells of claim 31 or 39 and instructions for administering the cell population to a subject in need thereof.