CLAIMS

1. A method for in vitro production of hematopoietic lineage cells, comprising:
   (a) providing a plurality of first spheres comprising pluripotent stem cells (PSCs) in a first culture medium, wherein the first spheres have an average size of about 60-150 micrometers, about 70-120 micrometers or about 80-100 micrometers in diameter; wherein preferably the first spheres are generated from 3-dimensional (3D) sphere culturing while monitoring sphere size;
   (b) 3D sphere culturing the plurality of first spheres in a second culture medium to induce differentiation of the PSCs to generate a plurality of second spheres comprising hemogenic endothelial cells (HECs);
   (c) 3D sphere culturing the plurality of second spheres in a third culture medium to induce differentiation of the HECs to generate a plurality of third spheres comprising hematopoietic progenitor cells (HPCs);
   (d) permitting the HPCs to release from the plurality of third spheres to obtain a suspension of substantially single cells of HPCs; and
   (e) optionally, further differentiating the suspension of substantially single cells of HPCs into common erythroid/megakaryocytic progenitor cells, erythrocytes, megakaryocytes, platelets, common lymphoid progenitor cells, lymphoid lineage cells, lymphocytes (such as T lymphocytes), natural killer (NK) cells, common myeloid progenitor cells, common granulomonocytic progenitor cells, monocytes, macrophages, and/or dendritic cells.

2. A method for in vitro production of lymphoid lineage cells, comprising:
   (a) providing a plurality of first spheres comprising pluripotent stem cells (PSCs) in a first culture medium, wherein the first spheres have an average size of about 60-150 micrometers, about 70-120 micrometers or about 80-100 micrometers in diameter; wherein preferably the first spheres are generated from 3-dimensional (3D) sphere culturing while monitoring sphere size;
   (b) 3D sphere culturing the plurality of first spheres in a second culture medium to induce differentiation of the PSCs to generate a plurality of second spheres containing hemogenic endothelial cells (HECs);
   (c) enzymatically disassociating the plurality of second spheres to obtain a suspension of substantially single cells of HECs;
   (d) seeding the substantially single cells of HECs into a scaffold that mimics in vivo hematopoietic niche; and
   (e) culturing and differentiating, in the scaffold, the HECs into lymphoid lineage cells.

3. A method for in vitro production of lymphoid lineage cells, comprising:

(a) providing a plurality of first spheres comprising pluripotent stem cells (PSCs) in a first culture medium, wherein the first spheres have an average size of about 60-150 micrometers, about 70-120 micrometers or about 80-100 micrometers in diameter; wherein preferably the first spheres are generated from 3-dimensional (3D) sphere culturing while monitoring sphere size;

(b) 3D sphere culturing the plurality of first spheres in a second culture medium to induce differentiation of the PSCs to generate a plurality of second spheres containing hemogenic endothelial cells (HECs); and

(c) culturing and differentiating, in a scaffold-free third culture medium, the HECs in the second spheres into lymphoid lineage cells, while permitting the lymphoid lineage cells to release from the second spheres.

4. The method of any one of claims 1-3, wherein the PCSs are embryonic stem cells or induced pluripotent stem cells, preferably from human.

5. The method of any one of claims 1-3, wherein the PCSs are at least 95% positive for Oct-4 expression.

6. The method of any one of claims 1-3, wherein each 3D sphere culturing step comprises culturing in a spinner flask or stir-tank bioreactor, preferably under continuous agitation.

7. The method of any one of claims 1-3, wherein the first culture medium is a PSC culture medium supplemented with TGF-β of about 1-10 ng/mL, bFGF of about 10-500 ng/mL, and Y27632 of about 1-5 μM.

8. The method of claim 7, wherein the PSC culture medium is NutriStem®, mTeSR™1, mTeSR™2, TeSR™-E8™ or other culture medium suitable for 3D suspension culture.

9. The method of any one of claims 1-3, wherein the second culture medium is a PSC culture medium supplemented with BMP4, VEGF and bFGF, each preferably at a concentration of about 25 to about 50 ng/mL, and optionally supplemented with CHIR99012 and/or SB431542, each preferably at a concentration of about 1-10, about 2-5, or about 3 μM.

10. The method of claim 9, wherein the PSC culture medium is NutriStem®, mTeSR™1, mTeSR™2, TeSR™-E8™ or other culture medium suitable for 3D suspension culture.

11. The method of claim 9, wherein the second culture medium is supplemented with (i) BMP4, VEGF and bFGF for a first period of time (e.g., day 1 and day 2), (ii) BMP4, VEGF, bFGF and CHIR99012 for a second period of time (e.g., day 3), (iii) BMP4, VEGF, bFGF, CHIR99012 and SB431542 for a third period of time (e.g., day 4), (iv) BMP4, VEGF, bFGF, and SB431542 for a fourth period of time (e.g., day 5), and (v) BMP4, VEGF and bFGF for a fifth period of time (e.g., day 6).

12. The method of claim 9, wherein said culturing in the second culture medium is under hypoxia condition (about 5% oxygen) for the first period of time through the third period of time (e.g., day 1 through day 4), followed by normal oxygen concentration of about 20% for the fourth

period of time and the fifth period of time (e.g., day 5 and day 6).

13. The method of claim 1 or 3, wherein the third culture medium is a hematopoietic basal medium supplemented with one or more of TPO, SCF, Flt3L, IL-3, IL-6, IL-7, IL-15, SR1, sDLL-1, OSM and/or EPO.

14. The method of claim 13, wherein the hematopoietic basal medium is StemSpan™-ACF, PRIME-XV®, PromoCell® Hematopoietic Progenitor Expansion medium DXF and other culture system suitable for hematopoietic stem cell expansion.

15. The method of claim 1 or 2, wherein step (e) comprises culturing in a hematopoietic basal medium supplemented with one or more of TPO, SCF, Flt3L, IL-3, IL-6, IL-7, IL-15, SR1, sDLL-1, OSM and/or EPO.

16. The method of claim 15, wherein the hematopoietic basal medium is StemSpan™-ACF, PRIME-XV®, PromoCell® Hematopoietic Progenitor Expansion medium DXF and other culture medium suitable for lineage-specific expansion and maturation.

17. The method of claim 2 or 3, wherein the lymphoid lineage cells are T-cells, NK cells, dendritic cells and/or macrophages.

18. A composition for adoptive cell therapy, comprising a plurality of cells produced using the method of any one of claims 1-17, wherein preferably the cells have been engineered to express a chimeric antigen receptor, a T-cell receptor or other receptor for disease antigens for use in the treatment of cancer or other immune diseases, wherein more preferably the cells are T-cells, NK cells, dendritic cells and/or macrophages.

19. The composition for use ~~of~~ according to claim 18 in the manufacture of a medicament for the treatment of cancer or other immune diseases, wherein more preferably the cells are T-cells, NK cells, dendritic cells and/or macrophages.

20. Cells produced using the method of any one of claims 1-17 for use in the treatment of cancer or other immune diseases, wherein preferably the cells have been engineered to express a chimeric antigen receptor, a T-cell receptor or other receptor for disease antigens, wherein more preferably the cells are T-cells, NK cells, dendritic cells and/or macrophages.

21. The cells for use ~~of~~ according to claim 20 in the manufacture of a medicament for the treatment of cancer or other immune diseases, wherein preferably the cells have been engineered to express a chimeric antigen receptor, a T-cell receptor or other receptor for disease antigens, wherein more preferably the cells are T-cells, NK cells, dendritic cells and/or macrophages.