

We claim

1. A method of producing pluripotent stem cell-derived natural killer (NK) cells, comprising:

(A) providing a bulk cell population comprising hematopoietic progenitor cells (HPC) (HP cell bulk) derived from pluripotent stem cells,

(B) culturing the HP cell bulk in CD4/CD8 induction media comprising a p38 inhibitor and SDF-1 to generate an intermediate heterogeneous cell population comprising CD4-/CD8- cells, CD4-/CD8+ cells, CD4+/CD8-, and CD4+/CD8+ cells (DP cell bulk), and

(C) culturing the DP cell bulk in an NK induction media to produce CD56+/CD3- NK cells;

wherein the method does not include a cell isolation step.

2. The method of claim 1, wherein step (A) comprises culturing pluripotent stem cells in HPC induction media to produce HP cell bulk, wherein the pluripotent stem cells are induced pluripotent stem cell (iPSC).

3. The method of claim 2, wherein the HPC induction media comprises at least one compound selected from bone morphogenetic protein-4 (BMP4) vascular endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF), ascorbic acid, Flt3 ligand (Flt3L), thrombopoietin (TPO) and TGF β inhibitor, wherein the HPC induction media comprises:

(a) BMP4 at a concentration of between 5 ng/mL to 500 ng/ml,

(b) bFGF at a concentration of between 5 ng/ml to 500 ng/ml,

(c) Flt3L at a concentration between 1 ng/ml to 100 ng/ml,

(d) TPO at a concentration of between 1 ng/ml to 200 ng/ml,

(e) VEGF at a concentration of between 5 ng/ml to 500 ng/ml, and/or

(f) ascorbic acid at a concentration of between 5 μ g/ml to 500 μ g/ml.

4. The method of claim 3, wherein the HPC induction media comprises:

(a) BMP4 at a concentration of 50 ng/ml,

- (b) bFGF at a concentration of 50 ng/ml,
 - (c) Flt3L at a concentration of 50 ng/ml,
 - (d) TPO at a concentration of 100 ng/ml,
 - (e) VEGF at a concentration of 50 ng/ml, and/or
 - (f) ascorbic acid at a concentration of 50 µg/ml.
5. The method of claim 1, wherein the CD4/CD8 induction media comprises at least one compound selected from the group consisting of ascorbic acid, stem cell factor (SCF), IL-7, Flt3L, and thrombopoietin (TPO).
6. The method of claim 4, wherein the CD4/CD8 induction media comprises:
- (a) ascorbic acid at a concentration between 5 µg/ml to 500 µg/ml,
 - (b) SCF at a concentration between 5 ng/ml to 100 ng/ml,
 - (c) IL-7 at a concentration between 1 ng/ml to 100 ng/ml,
 - (d) Flt3L at a concentration of between 1 ng/ml to 100 ng/ml,
 - (e) TPO at a concentration between 1 ng/ml to 200 ng/ml,
 - (f) p38 inhibitor at a concentration between 0.5 µM to 100 µM, and/or
 - (g) SDF-1 at a concentration between 10 ng/ml to 100 ng/ml.
7. The method of claim 6, wherein the CD4/CD8 induction media comprises:
- (a) ascorbic acid at a concentration of 50 µg/ml,
 - (b) SCF at a concentration of 50 ng/ml,
 - (c) IL-7 at a concentration of 50 ng/ml,
 - (d) Flt3L at a concentration of 50 ng/ml,
 - (e) TPO at a concentration of 100 ng/ml,
 - (f) p38 inhibitor at a concentration of 15 µM, and/or
 - (g) SDF-1 at a concentration of 30 nM.

8. The method of claim 1, wherein the NK induction media comprises at least one compound selected from the group consisting of a CD3 activator, IL-2 and IL7, wherein the NK induction media comprises:
- (a) IL-2 at a concentration of between 1 ng/ml to 100 ng/ml, and/or
 - (b) IL-7 at a concentration between 1 ng/ml to 100 ng/ml.
9. The method of claim 8, wherein the NK induction media comprises:
- (a) IL-2 at a concentration of 10 ng/ml; and/or
 - (b) IL-7 at a concentration of 10 ng/ml.
10. The method of claim 1, wherein each of the culturing step is performed at 3-6% oxygen.
11. The method of claim 1, wherein culturing pluripotent stem cells in the HPC induction media to obtain HP cell bulk lasts between 11 and 15 days.
12. The method of claim 1, wherein the iPSC are obtained from peripheral blood mononuclear cells.
13. The method of claim 1, wherein the CD56+/CD3- immune cells are genetically modified to express one or more chimeric antigen receptors (CAR), wherein the antigen is CD19.
14. A method of producing induced pluripotent stem cell (iPSC)-derived CD56+/CD3- natural killer (NK) cells comprising the following steps:
- (1) culturing iPSC in HPC induction media comprising at least one compound selected from vascular endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF), and ascorbic acid to obtain a heterogeneous population of cells comprising hematopoietic progenitor cells (HPC) (HP cell bulk);
 - (2) culturing the HP cell bulk obtained in (1) in CD4/CD8 induction media comprising a p38 inhibitor and SDF-1 to obtain an intermediate heterogeneous population of CD4-/CD8- cells, CD4-/CD8+ cells, CD4+/CD8-, and CD4+/CD8+ cells (DP cell bulk); and

(3) culturing the intermediate heterogeneous population of cells from (2) in NK induction media comprising at least one compound selected from the group consisting of a CD3 activator, IL-2 and IL-7 to obtain a population of cells comprising at least 50% CD56⁺/CD3⁻ NK cells without performing a cell isolation step.

15. Use of the NK cell population of claim 1 in the manufacture of a medicament for treating a cancer, wherein the cancer is a leukemia or a lymphoma.