

We claim

1. A method of producing pluripotent stem cell-derived 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 one or more culture medium to produce CD56+/CD3- cells,wherein the method does not include a cell isolation step.
2. The method of claim 1, wherein the method does not include a cell isolation step in steps (A) and (B).
3. The method of any one of claim 1 or 2, wherein the HP cell bulk in (A) comprises CD34+ cells, optionally wherein 20% or more of the cells in the HP cell bulk are CD34+ cells, optionally wherein between 20% and 90% of the HP cell bulk are CD34+ cells.
- ~~4. The method of claim 3, wherein 20% or more of the cells in the HP cell bulk are CD34+ cells.~~
- ~~5. The method of claim 4, wherein between 20% and 90% of the HP cell bulk are CD34+ cells.~~
- ~~46.~~ The method of any one of the preceding claims, wherein step (B) comprises
 - (i) culturing the HP cell bulk in CD4/CD8 induction media to generate an intermediate heterogeneous cell population comprises CD4-/CD8- cells, CD4-/CD8+ cells, CD4+/CD8-, and CD4+/CD8+ cells; and
 - (ii) culturing the intermediate heterogeneous cell population in NK induction media to produce the CD56+/CD3- cells.
- ~~57.~~ The method of any one of the preceding claims, wherein the method does not include a step of isolating CD4+/CD8+ cells.
- ~~68.~~ The method of any one of the preceding claims, 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).

~~9. The method of any one of the preceding claims, wherein the pluripotent stem cells are induced pluripotent stem cell (iPSC).~~

~~710.~~ The method of claim ~~68 or 9~~, 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, optionally wherein the HPC induction media comprises:-

~~11. The method of claim 10, wherein the HPC induction media comprises(a) BMP4 at a concentration of between 5 ng/mL to 500 ng/ml, optionally wherein the BMP4 is at a concentration of 50 ng/ml;-~~

~~12. The method of claim 11 wherein the BMP4 is at a concentration of 50 ng/ml.~~

~~13. The method of any one of claims 10-12, wherein the bulk cell medium comprises VEGF at a concentration of between 5 ng/ml to 500 ng/ml.~~

~~14. The method of claim 13, wherein the VEGF is at a concentration of about 50 ng/ml.~~

~~15. The method of any one of claims 10-14, wherein the HPC induction media comprises (b) bFGF at a concentration of between 5 ng/ml to 500 ng/ml, optionally wherein the bFGF is at a concentration of 50 ng/ml; -~~

~~16. The method of claim 15, wherein the bFGF is at a concentration of 50 ng/ml.~~

~~17. The method of any one of claims 10-16, wherein the bulk cell medium comprises ascorbic acid at a concentration of between 5 μ g/ml and 500 μ g/ml.~~

~~18. The method of claim 17, wherein the ascorbic acid is at a concentration of 50 μ g/ml.~~

~~19. The method of any one of claims 10-18, wherein the HPC induction media comprises (c) Flt3L at a concentration between 1 ng/ml to 100 ng/ml, optionally wherein the Flt3L is at a concentration of 50 ng/ml; and/or-~~

~~20. The method of claim 19, wherein the Flt3L is at a concentration of 50 ng/ml.~~

~~21. The method of any one of claims 10-20, wherein the HPC induction media comprises~~
(d) TPO at a concentration of between 1 ng/ml to 200 ng/ml, optionally wherein the TPO is at
a concentration of 100 ng/ml;-

wherein the bulk cell medium comprises:

(a) VEGF at a concentration of between 5 ng/ml to 500 ng/ml, optionally wherein the
VEGF is at a concentration of about 50 ng/ml; and

(b) ascorbic acid at a concentration of between 5 µg/ml and 500 µg/ml, optionally
wherein the ascorbic acid is at a concentration of 50 µg/ml.

~~22. The method of claim 21, wherein the TPO is at a concentration of 100 ng/ml.~~

823. The method of any one of claims 4-76-22, 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, thrombopoietin (TPO), p38 inhibitor and SDF-1.

924. The method of claim 823, wherein the CD4/CD8 induction media comprises:

(a) ascorbic acid at a concentration between 5 µg/ml to about 500 µg/ml, optionally
wherein the ascorbic acid is at a concentration of 50 µg/ml;-

~~25. The method of claim 24, wherein the ascorbic acid is at a concentration of 50 µg/ml.~~

~~26. The method of any one of claims 23-25, wherein the CD4/CD8 induction media~~
~~comprises (b) SCF at a concentration between 5 ng/ml to 100 ng/ml, optionally wherein the~~
~~SCF is at a concentration 50 ng/ml; -~~

~~27. The method of claim 26, wherein the SCF is at a concentration 50 ng/ml.~~

~~28. The method of any one of claims 23-27, wherein the CD4/CD8 induction media~~
~~comprises (c) IL-7 at a concentration between 1 ng/ml to 100 ng/ml, optionally, wherein the~~
~~IL-7 is at a concentration of 50 ng/ml;-~~

~~29. The method of claim 28, wherein the IL-7 is at a concentration of 50 ng/ml.~~

- ~~30. The method of any one of claims 23-29, wherein the CD4/CD8 induction media comprises (d) Flt3L at a concentration of between 1 ng/ml to 100 ng/ml, optionally wherein the Flt3L at a concentration of 50 ng/ml Flt3L;-~~
- ~~31. The method of claim 30, wherein the Flt3L at a concentration of 50 ng/ml Flt3L.~~
- ~~32. The method of any one of claims 23-31, wherein the CD4/CD8 induction media comprises (e) TPO at a concentration between 1 ng/ml to 200 ng/ml, optionally wherein the TPO is at a concentration of 100 ng/ml;-~~
- ~~33. The method of claim 32, wherein the TPO is at a concentration of 100 ng/ml.~~
- ~~34. The method of any one of claims 23-33, wherein the CD4/CD8 induction media comprises (f) p38 inhibitor at a concentration between 0.5 μ M to 100 μ M, optionally wherein the p38 inhibitor is SB203580, optionally wherein the SB203580 is at a concentration of 15 μ M; and/or-~~
- ~~35. The method of claim 34, wherein the p38 inhibitor is SB203580.~~
- ~~36. The method of claim 35, wherein the SB203580 is at a concentration of 15 μ M.~~
- ~~37. The method of any one of claims 23-36, wherein the CD4/CD8 induction media comprises (g) SDF-1inhibitor at a concentration between 10 ng/ml to about 100 ng/ml, optionally wherein the SDF-1inhibitor is at a concentration of 30 nM.-~~
- ~~38. The method of claim 37, wherein the SDF-1inhibitor is at a concentration of 30 nM.~~
1039. The method of any one of claims ~~46-938~~, wherein the NK induction media comprises at least one compound selected from the group consisting of a CD3 activator, IL-2 and IL7, optionally wherein the NK induction media comprises:-
- ~~40. The method of claim 39, wherein the NK induction media comprises(a) IL-2 at a concentration of between 1 ng/ml to 100 ng/ml, optionally wherein the IL-2 is at a concentration of 10 ng/ml; and/or-~~
- ~~41. The method of claim 40, wherein the IL-2 is at a concentration of 10 ng/ml.~~

~~42. The method of any one of claims 39-41, wherein the second induction medium comprises (b) IL-7 at a concentration between 1 ng/ml to 100 ng/ml, optionally wherein the IL-7 is at a concentration of 10 ng/ml.~~

~~43. The method of claim 42, wherein the IL-7 is at a concentration of 10 ng/ml.~~

~~1144.~~ The method of any one of the preceding claims, wherein each of the culturing step is performed at about 3-65% oxygen.

~~45. The method of any one of claims 1-43, wherein each of the culturing step is performed at greater than 14% oxygen.~~

~~46. The method of any one of claims 1-43, wherein each of the culturing step is performed at atmospheric oxygen.~~

~~47. The method of any one of claims 1-43, wherein each of the culturing step is performed at less than 5% oxygen.~~

~~48. The method of any one of claims 8-47, wherein culturing pluripotent stem cells in the bulk cell medium to obtain HP cell bulk lasts for greater than 10 days.~~

~~1249.~~ The method of any one of claims 6-1148, wherein culturing pluripotent stem cells in the HPC induction media~~bulk cell medium~~ to obtain HP cell bulk lasts between 11 and 15 days, optionally 14 days.

~~50. The method of claim 49, wherein culturing pluripotent stem cells in the bulk cell medium to obtain HP cell bulk lasts for 14 days.~~

~~1351.~~ The method of any one of claims 79-1250, wherein the iPSC are obtained from peripheral blood mononuclear cells.

~~1452.~~ The method of any one of the preceding claims, wherein at least about 50%, 55%, 60%, 75%, 80%, 85%, 90%, 95%, 97% or more of produced cells are CD56+/CD3- cells without a step of enrichment, optionally wherein less than about 25% of produced cells are CD3+ cells, optionally wherein the percentage of produced cells is determined by flow cytometry, or single-cell RNA sequencing (scRNAseq).

~~53.—The method of claim 52, wherein less than about 25% of produced cells are CD3+ cells.~~

~~54.—The method of claim 52 or 53, wherein the percentage of produced cells is determined by flow cytometry.~~

~~55.—The method of claim 52 or 53, wherein the percentage of produced cells is determined by single-cell RNA sequencing (scRNAseq).~~

1556. The method of any one of the preceding claims, further comprising a step of isolating CD56+/CD3- cells.

1657. The method of claim 1556, wherein CD56+/CD3- cells are isolated by fluorescence-activated cell sorting (FACS) or magnetic sorting.

1758. The method of any one of the preceding claims, wherein the CD56+/CD3- immune cells are (i) NK cells, and/or (ii) genetically modified to express one or more chimeric antigen receptors (CAR), optionally wherein the isolated CD56+/CD3- cells are genetically modified to express one or more chimeric antigen receptors (CAR), optionally wherein the antigen is CD19, optionally wherein the cells are further genetically modified to express IL-15R α /IL-15 complex.

~~59.—The method of any one of the preceding claims, wherein the CD56+/CD3- cells are genetically modified to express one or more chimeric antigen receptors (CAR).~~

~~60.—The method of claim 59, wherein the isolated CD56+/CD3- cells are genetically modified to express one or more chimeric antigen receptors (CAR).~~

~~61.—The method of claim 60, wherein the antigen is CD19.~~

~~62.—The method of any one of claims 59-61, wherein the cells are further genetically modified to express IL-15R α /IL-15 complex.~~

1863. A method of producing induced pluripotent stem cell (iPSC)-derived CD56+/CD3- immune 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 one or more of ascorbic acid, p38 inhibitor and SDF-1 to obtain an intermediate heterogeneous population of cells; 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.

1964. An NK cell population produced using a method of any one of the preceding claims.

2065. An unsorted cell population comprising pluripotent stem cell-derived CD56+/CD3- cells at a ratio of not less than 60% of total pluripotent stem cell-derived CD56+ immune cells.

2166. The unsorted cell population of claim 2065, wherein

(i) less than 5-25% of cells are CD3+ cells, an/or;

~~67.—The unsorted cell population of claims 65 or 66, wherein (ii) less than 5% of cells are monocytes or B cells,;~~

~~68.—The unsorted cell population of claims 65 or 66, wherein less than 5% of cells are B cells;~~

~~69.—The unsorted population of any one of claims 65-68, optionally wherein the percentage of cells is determined by flow cytometry or single-cell RNA sequencing (scRNAseq).~~

~~70.—The unsorted population of any one of claims 65-68, wherein the percentage of cells is determined by single-cell RNA sequencing (scRNAseq).~~

2271. Use of a NK cell population of claim 21 in the manufacture of a medicament for treating~~A method of treating~~ a subject in need of cell therapy, optionally comprising administering to the subject the NK cells of any one of the preceding claims.

~~72.—The method of claim 68, wherein the subject has a cancer, optionally;~~

73.—~~The method of claim 69,~~ wherein the cancer is leukemia or lymphoma.