CLAIMS

We claim:

1. A method of generating a population of CD4-CD8+ T cells, comprising:

    - contacting a population of hematopoietic stem/progenitor cells with an immobilized Notch signalling ligand at a first ligand concentration, thereby generating a population of progenitor T cells;

    - contacting the population of progenitor T cells with the immobilized Notch signalling ligand at a second ligand concentration, thereby generating a population of CD4+CD8+ cells, wherein the second ligand concentration is less than the first ligand concentration; and

    - contacting the population of CD4+CD8+ cells with a T cell activator, in the absence of the Notch signalling ligand, thereby generating a population of CD4-CD8+ T cells.

2. The method of claim 1, wherein the immobilized Notch signalling ligand is DL4.

3. The method of claim 2, wherein the step of contacting the population of hematopoietic stem/progenitor cells with the immobilized Notch signalling ligand at the first ligand concentration further comprises contacting the population of hematopoietic stem/progenitor cells with immobilized VCAM-1.

4. The method of one of claims 1 to 3, wherein the first ligand concentration is 3.15 $x10^{11}$ to 1.26 $x10^{12}$ molecules/mL and the second ligand concentration is 3.94 $x10^{10}$ to 6.31 $x10^{11}$ molecules/mL.

5. The method of any one of claims 1 to 4, further comprising:

    - contacting the population of CD4+CD8+ cells with the immobilized Notch signalling ligand at a third ligand concentration before contacting the population of CD4+CD8+ cells with a T cell activator, wherein the third ligand concentration is less than the second ligand concentration.

6. The method of claim 5, wherein the third ligand concentration is 3.94 $x10^{10}$ to 6.31 $x10^{11}$ molecules/mL.

7. The method of any one of claims 1 to 6, further comprising enriching the CD4+CD8+ cells for CD8α or CD8β.

8. The method of any one of claims 1 to 7, wherein the T cell activator is a CD3 stimulator and an integrin ligand.

9. The method of claim 8, wherein the CD3 stimulator is an anti-CD3/anti-CD28/anti-CD2 reagent, an anti-CD3 reagent, or a peptide-major histocompatibility complex (pMHC) tetramer, and the integrin ligand is laminin, ICAM, fibronectin, a fibronectin fragment, VCAM-1, or ICOS-L.

10. The method of claim 9, wherein the CD3 stimulator is an anti-CD3/anti-CD28/anti-CD2 reagent and the integrin ligand is a fibronectin fragment.

11. The method of any one of claims 1 to 7, wherein the T cell activator is a chimeric antigen receptor (CAR) activator.

12. The method of claim 11, wherein the CAR activator is an antigen immobilized on a substrate.

13. The method of claim 12, wherein the antigen is a CD19 antigen, and wherein the substrate is a particle.

14. The method of any one of claims 1 to 13, wherein the step of contacting the population of CD4+CD8+ cells with the T cell activator, in the absence of the Notch signalling ligand, is conducted in a cell culture medium comprising IL-7, IL-15, and IL-21.

15. The method of any one of claims 1 to 14, wherein the step of contacting the population of CD4+CD8+ cells with the T cell activator, in the absence of the Notch signalling ligand, is conducted in the absence of IL-2.

16. The method of any one of claims 1 to 15, further comprising:

- contacting the population of CD4-CD8+ T cells with a T cell activator and IL-7, IL-15, and IL-21.

17. The method of claim 16, wherein the T cell activator is a CD3 stimulator.

18. The method of claim 17, wherein the CD3 stimulator is an anti-CD3/anti-CD28/anti-CD2 reagent, an anti-CD3 reagent, or a peptide-major histocompatibility complex (pMHC) tetramer.

19. The method of any one of claims 1 to 18, further comprising increasing a cell density at the step of contacting the population of progenitor T cells with the immobilized Notch signalling ligand at the second ligand concentration, and/or increasing a cell density at the step of contacting the population of

CD4+CD8+ cells with the T cell activator and an integrin ligand in the absence of the Notch signalling ligand.

20. The method of any one of claims 1 to 19, wherein the hematopoietic stem/progenitor cells are derived from pluripotent stem cells.

21. The method of any one of claims 1 to 20, wherein the population of CD4-CD8+ T cells comprises a nucleic acid sequence encoding a CAR or an exogenous T cell receptor (TCR).

22. The method of any one of claims 1 to 21, wherein the population of CD4-CD8+ T cells is CD3+.

23. The method of any one of claims 1 to 21, wherein the population of CD4-CD8+ T cells is TRAC-/-.

24. The method of any one of claims 1 to 22, wherein the population of CD4-CD8+ T cells is enriched for CD8αβ+ cells.

25. The method of any one of claims 1 to 22, wherein the population of CD4-CD8+ T cells comprises a sub-population of TCRγδ+, CD49c+, and/or CD31+ cells.

26. A population of CD4-CD8+ T cells made according to the method of any one of claims 1 to 25.

27. The population of claim 26, wherein

a) the population of CD4-CD8+ T cells comprises a nucleic acid sequence encoding a CAR or an exogenous TCR;

b) the population of CD4-CD8+ T cells is CD3+; and/or

c) the population of CD4-CD8+ T cells is TRAC-/-.

28. A CD4-CD8+CD3+ TRAC-/- cell population derived in vitro from pluripotent stem cells, wherein

a) the cell population optionally comprises a nucleic acid sequence encoding (i) a CAR or an exogenous TCR; and/or (ii) a CAR or an exogenous TCR; and/or

b) the population of CD4-CD8+ T cells comprises a sub-population of TCRγδ+, CD49c+, and/or CD31+ cells.

29. A pharmaceutical composition comprising a population of CD4-CD8+ T cells and a pharmaceutically acceptable carrier, wherein the population of CD4-CD8+ T cells are CD3+ and TRAC-/-.

30. A use of a population of CD4-CD8+ T cells in the manufacture of a medicament for the treatment of a disease or condition, wherein the population of CD4-CD8+ T cells are generated by a method according to any one of claims 1 to 25.

31. The use of claim 30, wherein

a) the population of CD4-CD8+ T cells is CD3+ and/or TRAC-/-.; and/or

b) the disease or condition is a hematological malignancy, and wherein the CD4-CD8+ T cells comprise a nucleic acid sequence encoding a CAR or an exogenous T cell receptor (TCR).

32. A method of generating a population of CD4+CD8+ cells, comprising:

- contacting a population of hematopoietic stem/progenitor cells with an immobilized Notch signalling ligand at a first ligand concentration, thereby generating a population of progenitor T cells; and
- contacting the population of progenitor T cells with the immobilized Notch signalling ligand at a second ligand concentration, thereby generating a population of CD4+CD8+ cells, wherein the second ligand concentration is less than the first ligand concentration.

33. The method of claim 32, wherein the immobilized Notch signalling ligand is DL4.

34. The method of claim 32 or 33, wherein the first ligand concentration is 3.15 $x10^{11}$ to 1.26 $x10^{12}$ molecules/mL and the second ligand concentration is 3.94 $x10^{10}$ to 6.31 $x10^{11}$ molecules/mL.

35. The method of any one of claims 32 to 34, wherein the population of CD4+CD8+ cells are CD1a+CD28+ and ICOS+.

36. The method of any one of claims 32 to 35, wherein the population of CD4+CD8+ cells comprise a nucleic acid sequence encoding a CAR or an exogenous TCR.

37. A population of CD4+CD8+ cells made according to the method of any one of claims 32 to 36.

38. A population of CD4+CD8+ cells derived in vitro from pluripotent stem cells, wherein the CD4+CD8+ cells are CD1a+CD28+ and ICOS+.

39. A method of generating a population of CD4-CD8+ T cells, comprising:

- contacting a population of hematopoietic stem/progenitor cells with an immobilized Notch signalling ligand, thereby generating a population of progenitor T cells;

- contacting the population of progenitor T cells with a Notch signalling inhibitor and the immobilized Notch signalling ligand, thereby generating a population of CD4+CD8+ cells; and

- contacting the population of CD4+CD8+ cells with a T cell activator, in the absence of the Notch signalling ligand, thereby generating a population of CD4-CD8+ T cells.

40. A method of generating a population of CD4+CD8+ cells, comprising:

contacting a population of hematopoietic stem/progenitor cells with an immobilized Notch signalling ligand, thereby generating a population of progenitor T cells; and

contacting the population of progenitor T cells with a Notch signalling inhibitor and the immobilized Notch signalling ligand, thereby generating a population of CD4+CD8+ cells

41. The method of claim 39 or 40, wherein

a) the activity of the immobilized Notch signalling ligand is decreased in the step of contacting the population of progenitor T cells with the Notch signalling inhibitor and the immobilized Notch signalling ligand, in comparison to the activity of the immobilized Notch signalling ligand in the step of contacting the population of hematopoietic stem/progenitor cells with the immobilized Notch signalling ligand;

b) the Notch signalling inhibitor is a gamma-secretase inhibitor;

c) the Notch signalling inhibitor is a gamma-secretase inhibitor provided at a concentration of 0.1 to 1 micromolar; and/or

d) the immobilized Notch signalling ligand is provided at a first ligand concentration of 3.15 $x10^{11}$ to 1.26 $x10^{12}$ molecules/mL in the step of contacting the population of hematopoietic stem/progenitor cells with the immobilized Notch signalling ligand; and/or the immobilized Notch signalling ligand is provided at a second ligand concentration of 3.15 $x10^{11}$ to 2.52 $x10^{12}$ molecules/mL in the step of contacting the population of progenitor T cells with the Notch signalling inhibitor and the immobilized Notch signalling ligand.

42. Use of a population of CD4-CD8+ T cells in the manufacture of a medicament for the treatment of a disease or condition, wherein the population of CD4-CD8+ T cells are generated by the method according to any one of claims 39 to 41.

43. The use of claim 42, wherein the population of CD4-CD8+ T cells is CD3+ and/or TRAC-/-.