CLAIMS

We claim:

1. A method of generating CD4-CD8+ T cells, comprising:
   - contacting a population of CD4+CD8+ cells expressing an antigen receptor with an antigen immobilized to a substrate, wherein the antigen binds to the antigen receptor.
2. The method of claim 1, wherein the CD4+CD8+ cells are derived in vitro from hematopoietic stem/progenitor cells.
3. The method of claim 2, wherein the hematopoietic stem/progenitor cells are derived in vitro from pluripotent stem cells.
4. The method of claim 2 or claim 3, wherein the CD4+CD8+ cells are derived according to a method comprising:
   - contacting a population of hematopoietic stem/progenitor cells with an immobilized Notch signalling ligand.
5. The method of claim 4, wherein the step of contacting the population of hematopoietic stem/progenitor cells with the immobilized Notch signalling ligand further comprises:
   - contacting the population of hematopoietic stem/progenitor cells with the 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.
6. The method of claim 5, 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.
7. The method of claim 5 or claim 6, further comprising a step of enriching the CD4+CD8+ cells for CD8α or CD8β.
8. The method of any one of claims 4-7, wherein the immobilized Notch signalling ligand is DL4.
9. The method of any one of claims 1-8, wherein the antigen is a CD19 polypeptide and the antigen receptor binds the CD19 polypeptide.
10. The method of claim 9, wherein the CD19 polypeptide is an engineered variant of CD19.
11. The method of claim 1, wherein the antigen is a polypeptide having the sequence of SEQ ID NO: 8.
12. The method of any one of claims 1-11, wherein the substrate is a particle.

13. The method of claim 12, wherein the particle is comprised of a material selected from a group consisting of polystyrene, iron oxide and gold.
14. The method of claim 13, wherein the particle is comprised of polystyrene and magnetizable iron oxides.
15. The method of any one of claims 12 to 14, wherein the antigen is covalently conjugated to the particle.
16. The method of any one of claims 1 to 15, wherein the step of contacting the population of CD4+CD8+ cells expressing the antigen receptor with the antigen coupled to the particle is conducted at a particle:cell ratio of 1:1.
17. A population of CD4-CD8+ T cells made according to the methods of any one of claims 1 to 16.
18. The population of claim 17, wherein the population of CD4-CD8+ T cells express a chimeric antigen receptor (CAR).
19. The population of claim 18, wherein the CAR is a CD19 CAR, and wherein the antigen is a CD19 polypeptide.
20. The population of any one of claims 17 to 19, wherein population of CD4-CD8+ T cells is derived in vitro from pluripotent stem cells.
21. The population of any one of claims 17 to 20, wherein the population of CD4-CD8+ T cells is TRAC-/- and/or CD3+.
22. The population of any one of claims 17 to 21, wherein the population of CD4-CD8+ T cells is greater than 50% CD8αα+.
23. A method of inducing cytotoxicity of a tumor cell comprising exposing the tumor cell to a population of CD4-CD8+ T cells, wherein the population of CD4-CD8+ T cells is made according to the method of any one of claims 1 to 16.
24. An effective amount of the CD4-CD8+ T cells for use in therapy, wherein the CD4-CD8+ T cells are generated by a method according to any one of claims 1 to 16.
25. A use of CD4-CD8+ T cells in the manufacture of a medicament for the treatment of a disease or condition, wherein the CD4-CD8+ T cells are generated by a method according to any one of claims 1 to 16.
26. A cell stimulatory particle, comprising a particle and a modified antigen immobilized on a surface of the particle, wherein the modified antigen comprises one or more amino acid

substitutions from a native antigen, and wherein the modified antigen has greater solubility and/or stability in vitro in comparison to the native antigen.

27. The cell stimulatory particle of claim 26, wherein

a) the modified antigen comprises an extracellular domain of an antigen;

b) the modified antigen is covalently conjugated to the particle;

c) the particle is comprised of polystyrene and magnetizable iron oxide;

d) the modified antigen is a CD19 antigen; and/or

e) the modified antigen is a polypeptide having the sequence of SEQ ID NO: 8.