Claims

1. A method for producing a composition of engineered cells, the method comprising:

(a) combining a composition of CD4+ T cells and a composition of CD8+ T cells at a ratio of between 2:1 and 1:2 CD4+ to CD8+ T cells, thereby generating an input composition;

(b) incubating the input composition under stimulating conditions, thereby generating a stimulated composition, wherein said stimulating conditions comprise the presence of a stimulatory reagent capable of activating one or more intracellular signaling domains of one or more components of a TCR complex and/or one or more intracellular signaling domains of one or more costimulatory molecules;

and wherein the input composition comprises at least 100 x 10^6 total CD4+ and CD8+ T cells at a concentration of less than 5 x 10^6 cells/mL.

2. The method of claim 1, wherein the CD4+ and CD8+ cells in the input composition are separately enriched or selected from a primary sample from a subject.

3. A method for producing a composition of engineered cells, the method comprising incubating an input composition under stimulating conditions, thereby generating a stimulated composition, wherein:

the input composition comprises a ratio of between 2:1 and 1:2 CD4+ to CD8+ T cells, and wherein the input composition comprises at least 100 x 10^6 total CD4+ and CD8+ T cells at a concentration of less than 5 x 10^6 cells/mL; and

the stimulating conditions comprise the presence of a stimulatory reagent capable of activating one or more intracellular signaling domains of one or more components of a TCR complex and/or one or more intracellular signaling domains of one or more costimulatory molecules.

4. The method of any of claims 1-3, wherein the incubation is performed in a serum free medium.

5. The methods of any of claims 1-4, wherein:

the input composition comprises at least 80%, at least 85%, at least 90%, or at least 95% cells that are CD4+ T cells and CD8+ T cells;

the input composition comprises between 100 x 10^6 and 500 x 10^6 total CD4+ and CD8+ T cells; and/or

the input composition comprises at or about 300 x 10^6 total CD4+ and CD8+ T cells.

6. The method of any of claims 1-5, wherein the total CD4+ and CD8+ T cells are viable cells.

7. The method of any of claims 1-6, wherein:

the input composition comprises a concentration of between 1 x 10^6 cells/mL and 5 x 10^6 cells/mL; and/or

the input composition comprises a concentration of or of about 3 x 10^6 cells/mL.

8. The method of any of claims 1-7, wherein:

the input composition comprises a ratio of between 1.5:1 and 1:1.5 CD4+ to CD8+ cells;

the input composition comprises a ratio of between 1.2:1 and 0.8:1 CD4+ to CD8+ cells; and/or

the input composition comprises a ratio of or of about 1:1 CD4+ to CD8+ cells.

9. The method of any of claims 1-8, further comprising:

introducing a recombinant receptor into cells from the stimulated composition thereby generating an engineered cell composition, wherein the introducing comprises contacting the cells of the stimulated composition with an agent comprising a polynucleotide encoding the recombinant receptor.

10. The method of claim 9, wherein the introducing comprises transducing the cells of the stimulated composition with a viral vector comprising a polynucleotide encoding the recombinant receptor.

11. The method of claim 9 or claim 10, wherein the introducing is performed in a serum free medium.

12. A method for producing a composition of engineered cells, the method comprising:

(a) incubating an input composition under stimulating conditions, thereby generating a stimulated composition, wherein:

the input composition comprises a ratio of between 2:1 and 1:2 CD4+ to CD8+ T cells and comprises at least 100 x 10^6 viable CD4+ and CD8+ T cells at a concentration of less than 5 x 10^6 cells/mL; and

the stimulating conditions comprise the presence of a stimulatory reagent capable of activating one or more intracellular signaling domains of one or more components of a TCR complex and/or one or more intracellular signaling domains of one or more costimulatory molecules; and

(b) introducing a recombinant receptor into cells of the stimulated composition, wherein the total number of cells for the introducing is less than 300 x 10^6 viable cells, thereby generating an engineered cell composition, wherein the introducing comprises contacting the cells of the stimulated composition with a viral vector comprising a polynucleotide encoding the recombinant receptor.

13. The method of any of claims 12, wherein the incubation and/or the introducing is performed in serum free media.

14. The method of any of claims 9-13, wherein:

the recombinant receptor is introduced into less than 300 x 10^6 cells from the stimulated composition;

the recombinant receptor is introduced into between 50 x 10^6 cells and 200 x 10^6 cells from the stimulated composition;

the recombinant receptor is introduced into at least about 100 x 10^6 cells and up to about 200 x 10^6 cells from the stimulated composition; and/or

the recombinant receptor is introduced into about 100 x 10^6 cells from the stimulated composition.

15. The method of any of claims 9-14, wherein:

during the introducing, the cells from the stimulated composition are cultured at a concentration of less than 3 x 10^6 cells/mL;

during the introducing, the cells from the stimulated composition are cultured at a concentration between 0.5 x 10^6 cells/mL and 2 x 10^6 cells/mL; and/or

during the introducing, the cells from the stimulated composition are cultured at a concentration of or of about 1 x 10^6 cells/mL.

16. The method of any of claims 9-15, wherein the cells from the stimulated composition are viable cells.

17. A method for producing a composition of engineered cells, the method comprising introducing a recombinant receptor into cells of a T cell composition, said T cell composition comprising a concentration of at least or about at least 1 x 10^6 viable cells per mL, wherein at least 80%, at least 85%, at least 90%, or at least 95% of the cells of the T cell composition are CD4+ T cells or CD8+ T cells.

18. The method of claim 17, wherein the concentration of the T cell composition is less than 5 x 10^6 viable cells per mL.

19. The method of claim 17 or claim 18, wherein:

the T cell composition comprises at least or at least about or about 100 x 10^6 viable cells, or wherein the T cell composition comprises at least about 100 x 10^6 viable cells and up to about 200 x 10^6 viable cells; and/or

the T cell composition comprises less than 300 x 10^6 viable cells.

20. The method of any of claims 9-19, wherein:

the introducing is initiated within 2 days after initiation of incubation under stimulating conditions and/or within 2 days after the CD4+ T cells and the CD8+ T cells of the input composition are combined;

the introducing is initiated within 36 hours after initiation of incubation under stimulating conditions and/or within 36 hours after the CD4+ T cells and the CD8+ T cells of the input composition are combined; and/or

the introducing is initiated within 30 hours after initiation of incubation under stimulating conditions and/or within 30 hours after the CD4+ T cells and the CD8+ T cells of the input composition are combined.

21. The method of any of claims 1-20, further comprising cultivating the engineered composition under conditions to promote proliferation and/or expansion of the engineered cells, thereby producing an output composition comprising the engineered T cells.

22. The method of claim 21, wherein the cultivating is performed in a serum free medium.

23. The methods of any of claims 1-16 and 20-22, wherein the incubation is performed in the presence of one or more cytokines, wherein the one or more cytokines are selected from recombinant IL-2, recombinant IL-7, and/or recombinant IL-15.

24. The method of claim 23, wherein:

the one or more cytokines comprise between 10 and 200 IU/mL recombinant IL-2; between 100 IU/mL and 1,000 IU/mL recombinant IL-7; and/or between 10 and 200 IU/mL recombinant IL-15; and/or

the one or more cytokines comprise between 10 and 200 IU/mL recombinant IL-2; between 100 IU/mL and 1,000 IU/mL recombinant IL-7; and between 10 and 200 IU/mL recombinant IL-15.

25. The method of any of claims 1-16 and 20-24, wherein the stimulatory reagent comprises a primary agent that specifically binds to a member of a TCR complex.

26. The method of claim 25, wherein the stimulatory reagent further comprises a secondary agent that specifically binds to a T cell costimulatory molecule.

27. The method of claim 25 or claim 26, wherein the primary agent is an anti-CD3 antibody or an antigen-binding fragment thereof and the secondary agent is an anti-CD28 antibody, or an antigen-binding fragment thereof.

28. The method of claim 26 or claim 27, wherein the primary agent and/or secondary agent are present on the surface of a bead.

29. The method of claim 28, wherein:

the ratio of beads to cells is less than 3:1;

the ratio of beads to cells is from or from about 2:1 to 0.5:1; and/or

the ratio of beads to cells is at or at about 1:1.

30. The method of claim 26 or claim 27, wherein the primary agent and secondary agent are reversibly bound on the surface of an oligomeric particle reagent comprising a plurality of streptavidin or streptavidin mutein molecules.

31. The methods of any of claims 1-30, wherein:

the input composition is incubated under stimulating conditions for less than 48 hours;

the input composition is incubated under stimulating conditions for between 12 hours and 36 hours, inclusive;

the input composition is incubated under stimulating conditions for between 18 hours and 30 hours, inclusive; and/or

the input composition is incubated under stimulating conditions for or for about 24 hours.

32. The methods of any of claims 9-16 and 20-31, wherein:

the contacting is carried out for less than 48 hours;

the contacting is carried out between 12 hours and 36 hours, inclusive;

the contacting is carried out for between 18 hours and 30 hours, inclusive; and/or

the contacting is performed for or for about 24 hours.

33. The method of any of claims 10-16 and 20-32, wherein the viral vector is a retroviral vector.

34. The method of any of claims 10-16 and 20-33, wherein the viral vector is a lentiviral vector or gammaretroviral vector.

35. The method of any of claims 9-16 and 20-34, wherein the contacting is carried out in the absence of a transduction adjuvant.

36. The methods of any of claims 9-35, wherein the introducing is performed in the presence of one or more cytokines, wherein the one or more cytokines are selected from recombinant IL-2, recombinant IL-7, and/or recombinant IL-15.

37. The method of claim 36, wherein:

the one or more cytokines comprise between 10 and 200 IU/mL recombinant IL-2; between 100 IU/mL and 1,000 IU/mL recombinant IL-7; and/or between 10 and 200 IU/mL recombinant IL-15; and/or

the one or more cytokines comprise between 10 and 200 IU/mL recombinant IL-2; between 100 IU/mL and 1,000 IU/mL recombinant IL-7; and between 10 and 200 IU/mL recombinant IL-15.

38. The method of any of claims 21-37, wherein at least a portion of the cultivating is performed with mixing and/or perfusion.

39. The method of claim 38, wherein:

at least a portion of the cultivating is performed with perfusion at a rate of, of about, or of least 500 mL/day, 600 mL/day, 700 mL/day, 750 mL/day, 800 mL/day, 900 mL/day, 1,000 mL/day, 1,200 mL/day, 1,400 mL/day, 1,500 mL/day, 1,600 mL/day, 1,800 mL/day, and/or 2,000 mL/day; and/or

at least a first portion of the cultivating is performed with a perfusion rate of, of about, or of least 500 mL/day, 750 mL/day, or 1,000 mL/day, and wherein at least a second portion of the cultivating is performed with a perfusion rate of, of about, or of at least 1,200 mL/day, 1,400 mL/day, or 1,500 mL/day.

40. The method of claim 38 or claim 39, wherein the perfusion is initiated when the cells reach a density of, of about, or of at least 0.4×10^6 cells, 0.5×10^6 cells, 0.6×10^6 cells, 0.8×10^6 cells, 1.0×10^6 cells, 1.2×10^6 cells, 1.4×10^6 cells, 1.6×10^6 cells, 1.8×10^6 cells, 2.0×10^6 cells, 2.2×10^6 cells, or 2.4×10^6 cells.

41. The method of any of claims 38-40, wherein the perfusion is initiated at a rate of or of about 750 mL/day when the cells reach a density of or of about 0.6×10^6 cells/mL.

42. The method of any of claims 38-41, wherein the perfusion is increased to a rate of or of about 1500 mL/day when the cells reach a density of or of about 2.0×10^6 cells/mL.

43. The method of any of claims 21-42, wherein the cultivating is performed in the presence of one or more cytokines, wherein the one or more cytokines are selected from recombinant IL-2, recombinant IL-7, and/or recombinant IL-15.

44. The method of claim 43, wherein:

the one or more cytokines comprise between 50 and 400 IU/mL recombinant IL-2; between 100 IU/mL and 2,000 IU/mL recombinant IL-7; and/or between 50 and 400 IU/mL recombinant IL-15; and/or

the one or more cytokines comprise between 50 and 400 IU/mL recombinant IL-2; between 100 IU/mL and 2,000 IU/mL recombinant IL-7; and between 50 and 400 IU/mL recombinant IL-15.

45. The method of any of claims 21-44, wherein:

the cultivating is initiated within 3 days after the initiation of the of the incubation under stimulating conditions and/or within 3 days after the CD4+ T cells and the CD8+ T cells of the input composition are combined;

the cultivating is initiated within 60 hours after the initiation of the of the incubation under stimulating conditions and/or within 60 hours after the CD4+ T cells and the CD8+ T cells of the input composition are combined; and/or

the cultivating is initiated within 48 hours after the initiation of the of the incubation under stimulating conditions and/or within 48 hours after the CD4+ T cells and the CD8+ T cells of the input composition are combined.

46. The method of any of claims 21-45, wherein the cultivating is performed at least until the composition comprises a threshold number of T cells, threshold number of viable T cells, threshold concentration of T cells, threshold concentration of viable T cells.

47. The method of claim 46, wherein the cultivating is continued for at least one day after the threshold number of T cells, threshold number of viable T cells, threshold concentration of T cells, threshold concentration of viable T cells is reached.

48. The method of claim 46 or claim 47, wherein:

the threshold number of T cells or threshold number of viable T cells is, is about, or is at least 2400 x 10^6 or 5500 x 10^6 total number of nucleated cells;

the viability of the total number of nucleated cells is about or at least about 75% or about or at least about 85%;

the threshold number of T cells or threshold number of viable T cells is, is about, or is at least 900 x 10^6, 1200 x 10^6, or 3500 x 10^6 total number of nucleated cells;

the threshold number of T cells is or is about 2400 x 10^6 of total number of nucleated cells with about or at least about 85% viability; and/or

the threshold number of T cells is or is about 5500 x 10^6 of total number of nucleated cells.

49. The method of any of claims 21-48, comprising collecting cells of the output composition.

50. The method of any of claims 21-49, comprising collecting cells of the output composition, wherein:

the cells of the output composition are collected at least 9 days after the initiation of the incubation under stimulating conditions; or

the cells of the output composition are collected at least 10 days after the initiation of the incubation under stimulating conditions.

51. The method of any of claims 21-50, wherein the cells of the output composition are formulated in the presence of a cryoprotectant for cyropreservation.

52. The method of claim 51, wherein the cryoprotectant comprises DMSO.

53. The method of any of claims 1-52, wherein the CD4+ and/or the CD8+ T cells have been isolated from a biological sample from a subject prior to the incubating.

54. The method of claim 53, wherein the subject is a human subject.

55. The method of claim 53 or claim 54, wherein the biological sample is or comprises a whole blood sample, a buffy coat sample, a peripheral blood mononuclear cell (PBMC) sample, an unfractionated T cell sample, a lymphocyte sample, a white blood cell sample, an apheresis product, or a leukapheresis product.

56. The method of any of claims 1-55, wherein the recombinant receptor is capable of binding to a target antigen that is associated with, specific to, and/or expressed on a cell or tissue of a disease, disorder or condition.

57. The method of claim 56, wherein:

the disease, disorder or condition is an infectious disease or disorder, an autoimmune disease, an inflammatory disease, or a tumor or a cancer; and/or

the target antigen is a tumor antigen.

58. The method of any of claims 1-57, wherein:

the recombinant receptor is or comprises a functional non-TCR antigen receptor or a TCR or antigen-binding fragment thereof;

the recombinant receptor is a chimeric antigen receptor (CAR); and/or

the recombinant receptor is an anti-BCMA CAR.

59. The method of any of claims 46-58, wherein:

the output composition comprising the threshold number or greater number of cells is produced among greater than or greater than about 85%, greater than or greater than about 90% or greater than or greater than about 95% of the iterations of the method; and/or

the methods produces output compositions exhibiting a threshold number of cells expressing the CAR in the output composition in at least about 80%, about 90%, about 95%, about 97%, about 99%, about 100%, or 100% of the human biological samples in which it is carried out among a plurality of different individual subjects.

60. The method of any of claims 21-59, wherein:

during at least a portion of the cultivating, the cells are monitored for cell viability, concentration, density, number, or a combination thereof; and

the monitoring is carried out until the cells reach a threshold number of T cells, a threshold number of viable T cells, a threshold concentration of T cells, or a threshold concentration of viable T cells.

61. The method of claim 60, wherein the monitoring is carried out by differential digital holography microscopy (DDHM).

62. The method of claim 60 or claim 61, wherein the monitoring and cultivation is carried out in a closed system.

63. The method of any one of claims 21-62, wherein:

A) at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or greater than 95% of the cells in the output composition are of a memory phenotype;

at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or greater than 95% of the cells in the output composition are of a central memory phenotype;

at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or greater than 95% of the cells in the output composition are CD27+, CD28+, CCR7+, CD45RA-, CD45RO+, CD62L+, CD3+, CD95+, granzyme B-, and/or CD127+; and/or

at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or greater than 95% of the cells in the output composition are CCR7+/CD45RA- or are CCR7+/CD45RO+; and/or

B) among a plurality of iterations of the method to produce a plurality of the output compositions:

i) the mean percentage of cells of a memory phenotype in the plurality of the output compositions is between about 40% and about 65%, between about 40% and about 45%, between about 45% and about 50%, between about 50% and about 55%, between about 55% and about 60%, or between about 60% and about 65%;

ii) the mean percentage of cells of a central memory phenotype in the plurality of the output compositions is between about 40% and about 65%, between about 40% and about 45%, between about 45% and about 50%, between about 50% and about 55%, between about 55% and about 60%, or between about 60% and about 65%;

iii) the mean percentage of cells that are CD27+, CD28+, CCR7+, CD45RA-, CD45RO+, CD62L+, CD3+, CD95+, granzyme B-, and/or CD127+ in the plurality of the output compositions is between about 40% and about 65%, between about 40% and about 45%, between about 45% and about 50%, between about 50% and about 55%, between about 55% and about 60%, or between about 60% and about 65%;

iv) the mean percentage of cells that are CCR7+/CD45RA- or CCR7+/CD45RO+ in the plurality of the output compositions is between about 40% and about 65%, between about 40% and about 45%, between about 45% and about 50%, between about 50% and about 55%, between about 55% and about 60%, or between about 60% and about 65%;

v) the mean percentage of central memory CD4+ T cells in the engineered CD4+ T cells, optionally CAR+CD4+ T cells, of the plurality of the output compositions is between about 40% and about 65%, between about 40% and about 45%, between about 45% and about 50%, between about 50% and about 55%, between about 55% and about 60%, or between about 60% and about 65%;

vi) the mean percentage of central memory CD8+ T cells in the engineered CD8+ T cells, optionally CAR+CD8+ T cells, of the plurality of the output compositions is between about 40% and about 65%, between about 40% and about 45%, between about 45% and about 50%, between about 50% and about 55%, between about 55% and about 60%, or between about 60% and about 65%; and/or

vii) the mean percentage of central memory T cells, optionally CD4+ central memory T cells and CD8+ central memory T cells, in the engineered T cells, optionally CAR+ T cells, of the plurality of the output compositions is between about 40% and about 65%, between about 40% and about 45%, between about 45% and about 50%, between about 50% and about 55%, between about 55% and about 60%, or between about 60% and about 65%.

64. A composition comprising engineered cells produced by a method of any of claims 1-63.

65. The composition of claim 64, wherein:

at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or greater than 95% of the cells in the composition are of a memory phenotype;

wherein at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or greater than 95% of the cells in the composition are of a central memory phenotype;

wherein at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or greater than 95% of the cells in the composition are CD27+, CD28+, CCR7+, CD45RA-, CD45RO+, CD62L+, CD3+, granzyme B-, and/or CD127+; and/or

wherein at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or greater than 95% of the cells in the output composition are CCR7+/CD45RA- or are CCR7+/CD45RO+.

66. The composition of claim 64 or claim 65, further comprising a pharmaceutically acceptable carrier and/or a cryoprotectant.

67. An article of manufacture, comprising the composition of any of claims 64-66, and instructions for administering the output composition to a subject, wherein the subject has a disease or condition, optionally wherein the recombinant receptor specifically recognizes or specifically bind to an antigen associated with, or expressed or present on cells of, the disease or condition.