

CLAIMS

1. A method for producing a composition of engineered cells, the method comprising:
 - (a) incubating, under stimulating conditions, an input composition comprising T cells enriched for CD4+ primary human T cells, said stimulating conditions comprising the presence of (i) 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 (ii) one or more cytokines, wherein at least one cytokine is or comprises recombinant human IL-2, thereby generating a stimulated composition; and
 - (b) introducing a recombinant receptor into the stimulated composition, thereby generating an engineered composition comprising engineered T cells.
2. The method of claim 1, wherein:
 - the input composition comprises greater than or greater than about 70%, greater than or greater than about 75%, greater than or greater than about 80%, greater than or greater than about 85%, greater than or greater than about 90%, greater than or greater than about 95% or greater than or greater than about 98% CD4+ primary human T cells; and/or
 - the input composition consists essentially of CD4+ primary human T cells.
3. The method of claim 1 or claim 2, wherein the concentration of recombinant IL-2 is from 10 IU/mL to 200 IU/mL, or from about 10 IU/mL to about 200 IU/mL.
4. The method of any of claims 1-3, wherein the one or more cytokines further comprises IL-7 and/or IL-15.
5. The method of claim 4, wherein:
 - the concentration of IL-7 is from 100 IU/mL to 1000 IU/mL or from about 100 IU/mL to about 1000 IU/mL; and/or
 - the concentration of IL-15 is from 1 IU/mL to 50 IU/mL or from about 1 IU/mL to about 50 IU/mL.
6. The method of any of claims 1-5, wherein the incubating is carried out in the presence of one or more antioxidants.

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

(a) incubating an input composition comprising T cells enriched for one or both of CD4+ and CD8+ primary human T cells, thereby generating a stimulated composition, wherein the incubating is carried out:

(1) under one or more stimulating conditions, said stimulating conditions comprising the presence of (i) 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 (ii) one or more cytokines; and/or

(2) in the presence of one or more antioxidant; and

(b) introducing a recombinant receptor into the stimulated composition, thereby generating an engineered composition comprising engineered T cells.

8. The method of claim 7, wherein:

the input composition comprises greater than about 70%, greater than or greater than about 75%, greater than or greater than about 80%, greater than or greater than about 85%, greater than or greater than about 90%, greater than or greater than about 95% or greater than or greater than about 98% CD4+ primary human T cells; and/or

the input composition consists essentially of CD4+ primary human T cells.

9. The method of claim 7 or claim 8, wherein the one or more cytokines are selected from recombinant IL-2, recombinant IL-7 and recombinant IL-15.

10. The method of any of claims 1-9, wherein the input composition is a first input composition enriched in CD4+ primary human T cells, the method further comprising:

(a) separately incubating, under stimulating conditions, a second input composition comprising T cells enriched for CD8+ primary human T cells, wherein:

said T cells enriched for CD8+ primary human T cells are isolated from the same biological sample as the T cells enriched for CD4+ primary human T cells; and

said stimulating conditions comprising the presence of (i) 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 (ii) one or more cytokines, thereby generating a second stimulated composition; and
(b) introducing a recombinant receptor into the second stimulated composition, thereby generating a second engineered composition comprising engineered T cells.

11. The method of claim 7 or claim 10, wherein:
the input composition and/or the second input composition comprises greater than about 70%, greater than or greater than about 75%, greater than or greater than about 80%, greater than or greater than about 85%, greater than or greater than about 90%, greater than or greater than about 95% or greater than or greater than about 98% CD8+ primary human T cells; and/or

the input composition and/or the second input composition consists essentially of CD8+ primary human T cells.

12. The method of any of claims 7, 10, and 11, wherein the one or more cytokines are selected from recombinant IL-2 and recombinant IL-15.

13. The method of claim 10, wherein the incubating of the input composition comprising T cells enriched for CD8+ primary human T cells is carried out in the presence of one or more antioxidants.

14. The method of any of claims 1-13, wherein the stimulatory reagent comprises a primary agent that specifically binds to a member of a TCR complex.

15. The method of claim 14, wherein the stimulatory reagent further comprises a secondary agent that specifically binds to a T cell costimulatory molecule.

16. The method of claim 14 or claim 15, wherein the primary and/or secondary agents comprise an antibody.

17. The method of any of claims 1-16, wherein the stimulatory reagent comprises an anti-CD3 antibody and an anti-CD28 antibody, or antigen-binding fragments thereof.

18. The method of any of claims 14-17, wherein the primary agent and/or secondary agent are present on the surface of a solid support.

19. The method of claim 18, wherein the solid support is or comprises a bead.

20. The method of claim 19, wherein the ratio of beads to cells is from 2:1 to 0.5:1; from about 2:1 to about 0.5:1; or at or at about 1:1.
21. The method of any of claims 6-20, wherein the one or more antioxidant comprises a sulfur containing antioxidant and/or a glutathione precursor.
22. The method of any of claims 6-21, wherein the one or more antioxidants comprise N-acetyl cysteine (NAC).
23. The method of claim 22, wherein the NAC is at a concentration of from 0.2 mg/mL to 2.0 mg/mL, or from about 0.2 mg/mL to about 2.0 mg/mL.
24. The method of any of claims 1-23, wherein the introducing comprises transducing cells of the stimulated composition and/or the second stimulated composition with a viral vector comprising a polynucleotide encoding the recombinant receptor.
25. The method of claim 24, wherein the viral vector is a retroviral vector.
26. The method of claim 24 or claim 25, wherein the viral vector is a lentiviral vector or gammaretroviral vector.
27. The method of any of claims 24-26, wherein the introducing is carried out in the presence of a transduction adjuvant.
28. The method of claim 27, wherein the transduction adjuvant is or comprises protamine sulfate; a fibronectin-derived transduction adjuvant; and/or RetroNectin.
29. The method of claim 28, wherein the transduction adjuvant is or comprises from 1 µg/ml to 50 µg/ml or from about 1 µg/ml to about 50 µg/ml protamine sulfate.
30. The method of any of claims 1-29, further comprising cultivating the engineered composition under conditions to promote proliferation or expansion of the engineered cells, thereby producing an output composition comprising the engineered T cells.
31. The method of claim 30, wherein the cultivating is carried out in the presence of one or more cytokines, wherein at least one cytokine is or comprises recombinant human IL-2.

32. The method of claim 31, wherein the one or more cytokines further comprises IL-7 and/or IL-15.

33. The method of any of claims 10-32, further comprising cultivating the second engineered composition under conditions to promote proliferation or expansion of the engineered cells, thereby producing a second output composition comprising the engineered T cells.

34. The method of claim 33, wherein the cultivating is carried out in the presence of one or more cytokines.

35. The method of claim 34, wherein the one or more cytokines comprises IL-2 and IL-15.

36. The method of any of claims 30-35, wherein the cultivating is carried out in the presence of a surfactant.

37. The method of claim 36, wherein the surfactant comprises a poloxamer

38. The method of claim 37, wherein the poloxamer is present at a concentration of from 0.5 $\mu\text{L/mL}$ to 5 $\mu\text{L/mL}$ or from about 0.5 $\mu\text{L/mL}$ to about 5 $\mu\text{L/mL}$.

39. The method of any of claims 30-38, wherein at least a portion of the cultivating is performed with continual mixing and/or perfusion.

40. The method of any of claims 30-39, wherein the stimulatory reagent is removed from the engineered composition and/or the second engineered composition prior to the cultivating.

41. The method of claim 40, wherein the stimulatory reagent is removed from 3 days to 6 days, from about 3 days to about 6 days, or at or at about 4 days after the initiation of the incubating.

42. A method for producing a composition of engineered cells, the method comprising cultivating, in the presence of one or more cytokines, an engineered cell composition comprising CD4⁺ primary human T cells comprising cells engineered with a recombinant receptor, wherein at least one cytokine is or comprises recombinant human IL-2;

wherein the method results in the proliferation or expansion of cells in the composition to produce an output composition comprising engineered CD4+ cells.

43. A method for producing a composition of engineered cells, the method comprising cultivating, in the presence of one or more cytokines, an engineered cell composition comprising one or both of CD4+ and CD8+ primary human T cells comprising cells engineered with a recombinant receptor, wherein the cultivating is carried out in the presence of a surfactant and/or at least a portion of the cultivating is performed with continual mixing and/or perfusion;

wherein the method results in the proliferation or expansion of cells in the composition to produce an output composition comprising engineered CD4+ and/or CD8+ T cells.

44. The method of any of claims 30-43, wherein the cultivating is performed at least until the output composition and/or the second output composition comprises a threshold number of T cells, threshold number of nucleated T cells, threshold number of viable T cells, threshold concentration of T cells, or threshold concentration of viable T cells.

45. The method of claim 44, wherein the cultivating is continued for at least one day after the threshold number of T cells, threshold number of nucleated T cells, threshold number of viable T cells, threshold concentration of T cells, or threshold concentration of viable T cells is reached.

46. The method of claim 44 or claim 45, wherein the threshold number of T cells, threshold number of nucleated T cells, threshold number of viable T cells, threshold concentration of T cells, or threshold concentration of viable T cells is at least 2-fold, at least 3-fold, at least 4-fold, at least 5-fold or more greater than the number or concentration, or viable number or concentration, of the engineered cell composition prior to the cultivation.

47. The method of any of claims 30-46, wherein the cultivating is performed for 2 days to 10 days inclusive, and/or the cultivating is performed until at least 9 days after the initiating of the incubating.

48. The method of any of claims 30-47, wherein subsequent to the cultivating, collecting cells of the output composition and/or the second output composition.

49. The method of claim 48, wherein the amount of time between initiation of the incubating and collecting cells of the output composition is from 7 days to 15 days or from about 7 days to about 15 days; from 9 days to 13 days or from about 9 days to about 13 days; or from 8 days to 13 days or from about 8 days to about 13 days.

50. The method of any of claims 30-49, further comprising formulating cells of the output composition and/or the second output composition for cryopreservation and/or administration to a subject.

51. The method of claim 50, wherein the cells of the output composition are formulated in the presence of a pharmaceutically acceptable excipient and/or a cryoprotectant.

52. The method of any of claims 1-41 and 44-51, further comprising isolating the CD4+ and/or the CD8+ T cells from a biological sample prior to the incubating.

53. The method of any of claims 1-52, 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.

54. The method of any of claims 1-53, wherein the recombinant receptor is or comprises a functional non-TCR antigen receptor, a TCR or antigen-binding fragment thereof, or a chimeric antigen receptor (CAR).

55. The method of any of claims 1-54, wherein the recombinant receptor is an anti-CD19 CAR.

56. The method of any of claims 44-55, wherein the output composition and/or the second 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.

57. The method of any of claims 1-56, wherein:
the method is performed in less than 21 days, inclusive; and/or
the method produces an output composition that is administered and/or is ready to be administered to a subject within a 95% confidence interval of less than 21 days, inclusive.

58. The method of any of claims 30-57, wherein during at least a portion of the cultivating, the cells are monitored for cell viability, concentration, density, number, or a combination thereof.

59. The method of claim 58, wherein the monitoring is carried out by an optical method, optionally microscopy, optionally differential digital holography microscopy (DDHM).

60. A composition comprising engineered cells produced by a method of any of claims 1-59.

61. The composition of claim 60, further comprising a pharmaceutically acceptable carrier and/or a cryoprotectant.

62. An article of manufacture, comprising the composition of claim 60 or claim 61, and instructions for administering the output composition to a subject.

63. An article of manufacture comprising a composition of engineered CD4+ T cells produced by the method of any of claims 1-59, a composition of engineered CD8+ T cells produced by the method of any of claims 7, 9, 10-41, and 43-59, and instructions for administering the engineered CD4+ T cells and the engineered CD8+ T cells to a subject.