

WHAT IS CLAIMED IS:

1. A method of making genetically modified T-cells, which have a chimeric antigen receptor, comprising:

separating or enriching a CD8⁺ expressing population of T-cells and/or a CD4⁺ expressing population of T-cells, such as T-cells that are derived from thymocytes or T-cells that are derived from engineered precursors, desirably iPS cells, from a mixed population of T-cells so as to generate a separated or enriched population of T-cells;

stimulating the separated or enriched population of T-cells so as to generate a stimulated population of CD8⁺ T-cells and/or CD4⁺ T-cells;

transducing the stimulated population of CD8⁺ T-cells and/or CD4⁺ T-cells with a vector, wherein the vector encodes a chimeric antigen receptor and a marker sequence, wherein said marker sequence encodes a cell surface selectable marker, so as to generate a transduced population of CD8⁺ T-cells and/or CD4⁺ T-cells;

contacting the transduced population of CD8⁺ T-cells and/or CD4⁺ T-cells with at least one cytokine, which can be provided exogenously to the T-cells, e.g., in addition to any cytokine that may be produced by the cells or present in media, such as for 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 days or for a period that is within a range defined by any two of the aforementioned time periods, so as to generate a transduced, cytokine-stimulated population of CD8⁺ T-cells and/or CD4⁺ T-cells;

enriching the transduced, cytokine-stimulated population of CD8⁺ T-cells and/or CD4⁺ T-cells by selection of the marker sequence so as to generate an enriched population of transduced, cytokine-stimulated CD8⁺ T-cells and/or CD4⁺ T-cells; and

propagating the enriched population of transduced, cytokine-stimulated CD8⁺ T-cells and/or CD4⁺ T-cells for at least one day, such as 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 days or for a period that is within a range defined by any two of the aforementioned time periods, so as to obtain said genetically modified T-cells, which have a chimeric antigen receptor.

2. The method of Claim 1, wherein the separating or enriching of the CD8⁺ population of T-cells and/or a CD4⁺ population of T-cells from a mixed population of T-

cells is performed by affinity selection for T-cells having an epitope present on CD8 and/or CD4.

3. The method of Claim 1 or 2, wherein the separating or enriching of the CD8+ population of T-cells and/or a CD4+ population of T-cells from a mixed population of T-cells is performed by flow cytometry.

4. The method of Claim 1 or 2, wherein the separating or enriching of the CD8+ population of T-cells and/or a CD4+ population of T-cells from a mixed population of T-cells is performed by immuno-magnetic selection.

5. The method of any one of Claims 1-4, wherein the genetically modified CD8+ T-cells and/or CD4+ T-cells comprise at least one receptor that promotes, induces, contributes to, or enhances engraftment fitness.

6. The method of Claim 5, wherein the at least one receptor is CD45 RA, CD45 RO, CCR7, CD25, CD127, CD57, CD137, CD27, CD28 and/or CD62L.

7. The method of Claim 5 or 6, wherein the at least one receptor is CD27, CD28 and/or CD62L.

8. The method of any one of Claims 1-7, wherein the stimulating of the isolated population of T-cells is performed by contacting the CD8+ and/or CD4+ T-cells with an antibody-bound support, such as a bead or particle.

9. The method of Claim 8, wherein the antibody-bound support comprises anti-TCR, anti-CD2, anti-CD3, anti-CD4 and/or anti-CD28 antibodies.

10. The method of Claim 8 or 9, wherein the antibody-bound support comprises anti-CD3 and/or anti-CD28 antibodies.

11. The method of any one of Claims 1-10, wherein the vector further comprises a first sequence encoding a leader sequence, a second sequence encoding a ligand binding domain, a third sequence encoding a signaling domain and a fourth sequence encoding a selectable marker.

12. The method of any one of Claims 1-11, wherein the vector further comprises a sequence encoding a spacer.

13. The method of Claim 12, wherein the spacer comprises an IgG4 hinge.

14. The method of any one of Claims 1-13, wherein the vector is a viral vector.

15. The method of Claim 14, wherein the viral vector is derived from simian virus 40, adenoviruses, adeno-associated virus (AAV), lentivirus, or retroviruses.

16. The method of Claim 14 or 15, wherein the viral vector is a recombinant adenovirus, adeno-associated virus, lentivirus or retrovirus vector.

17. The method of any one of Claims 14-16, wherein the viral vector is a lentivirus vector.

18. The method of any one of Claims 1-17, wherein the marker sequence encodes a truncated epidermal growth factor receptor (EGFRt).

19. The method of any one of Claims 1-18, wherein the at least one cytokine comprises GM-CSF, IL-7, IL-12, IL-15, IL-18, IL-2, and/or IL-21.

20. The method of any one of Claims 1-19, wherein the at least one cytokine comprises IL7, IL-15 and/or IL-21, which can be provided at 0.1ng/mL, 0.2ng/mL, 0.3ng/mL, 0.4ng/mL, 0.5ng/mL, 0.6ng/mL, 0.7ng/mL, 0.8ng/mL, 0.9ng/mL, or 1.0ng/mL or in an amount that is within a range defined by any two of the aforementioned amounts and/or at 10U/mL, 20U/mL, 30U/mL, 40U/mL, 50U/mL, 60U/mL, 70U/mL, 80U/mL, 90U/mL, or 100U/mL or in an amount that is within a range defined by any two of the aforementioned amounts.

21. The method of any one of Claims 1-19, wherein the at least one cytokine comprises IL-2, IL-15 and/or IL-21, wherein the amount of cytokine is provided at 0.5ng/mL and/or 50U/mL.

22. The method of any one of Claims 1-21, wherein the contacting is performed for 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 days or a period of time within a range defined by any two of these values.

23. The method of any one of Claims 1-20 or 22, wherein the method is performed with isolated, purified, enriched or separated CD4⁺ cells in the absence of, substantially depleted of, or enriched over CD8⁺ cells.

24. The method of any one of Claims 1-19, 21 or 22, wherein the method is performed with isolated, purified, enriched or separated CD8⁺ in the absence of, substantially depleted of, or enriched over CD4⁺ cells.

25. The method of any one of Claims 1-20, 22 or 23, wherein the CD4⁺ expressing T-cells are propagated for at least 1 day, such as 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15,

16, 17, 18, 19, or 20 days or for a period that is within a range defined by any two of the aforementioned time periods.

26. The method of any one of Claims 1-19, 21, 22 or 24, wherein the CD8⁺ T-cells are propagated at least 1 day, such as 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 days or for a period that is within a range defined by any two of the aforementioned time periods.

27. The method of any one of Claims 8-26, wherein the method further comprises removing the antibody-bound support, such as beads or particles.

28. The method of any one of Claims 1-27, wherein the ligand binding domain of the chimeric antigen receptor comprises an antibody, or a binding portion thereof.

29. The method of any one of Claims 1-28, wherein the ligand binding domain of the chimeric antigen receptor comprises a single chain variable fragment (scFv), or a binding portion thereof.

30. The method of any one of Claims 1-29, wherein the ligand binding domain of the chimeric antigen receptor comprises FMC63, or a binding portion thereof.

31. The method of any one of Claims 1-30, wherein the ligand binding domain of the chimeric antigen receptor is specific for CD19.

32. The method of any one of Claims 1-31, wherein the method further comprises cryopreserving the genetically modified CD8⁺ and/or CD4⁺ T-cells.

33. A population of genetically modified T-cells comprising:

a plurality of affinity selected CD8⁺ and/or CD4⁺ T-cells, absence of, substantially depleted of, or enriched over CD8⁻ and/or CD4⁻ T-cells, wherein said plurality of affinity selected CD8⁺ and/or CD4⁺ T-cells have stimulated CD2, CD3, CD4 and/or CD28 receptors, wherein said plurality of affinity selected CD8⁺ and/or CD4⁺ T-cells further comprise a gene encoding a chimeric antigen receptor and a cell surface selectable marker and, wherein said plurality of affinity selected CD8⁺ and/or CD4⁺ T-cells have been re-stimulated with at least one cytokine.

34. The population of genetically modified T-cells of Claim 33, wherein said plurality of affinity selected CD8⁺ and/or CD4⁺ T-cells further comprise at least one receptor that promotes, enhances, improves or contributes to engraftment fitness.

35. The population of genetically modified T-cells of Claim 33 or 34, wherein the at least one receptor that promotes, enhances, improves or contributes to engraftment fitness is CD45 RA, CD45 RO, CCR7, CD25, CD127, CD57, CD137, CD27, CD28 and/or CD62L.

36. The population of genetically modified T-cells of Claim 34 or 35, wherein the at least one receptor that promotes, enhances, improves or contributes to engraftment fitness is CD27, CD28 and/or CD62L.

37. The population of genetically modified T-cells of any one of Claims 33-36, wherein the plurality of affinity selected CD8⁺ and/or CD4⁺ T-cells further comprises a vector having a first sequence encoding a leader sequence, a second sequence encoding a ligand binding domain, a third sequence encoding a signaling domain and a fourth sequence encoding a selectable marker.

38. The population of genetically modified T-cells of Claim 37, wherein the vector further comprises a sequence encoding a spacer.

39. The population of genetically modified T-cells of Claim 38, wherein the spacer comprises an IgG4 hinge.

40. The population of genetically modified T-cells of any one of Claims 33-39, wherein the vector is a viral vector.

41. The population of genetically modified T-cells of Claim 40, wherein the viral vector is derived from simian virus 40, adenoviruses, adeno-associated virus (AAV), lentivirus, or retroviruses.

42. The population of genetically modified T-cells of Claim 40 or 41, wherein the viral vector is a recombinant adenovirus, adeno-associated virus, lentivirus or retrovirus vector.

43. The population of genetically modified T-cells of any one of Claims 40-42, wherein the viral vector is a lentivirus vector.

44. The population of genetically modified T-cells of any one of Claims 33-43, wherein the cell surface selectable marker encodes for a truncated epidermal growth factor receptor (EGFRt).

45. The population of genetically modified T-cells of any one of Claims 37-44, wherein the ligand binding domain comprises an antibody, or a binding portion thereof.

46. The population of genetically modified T-cells of any one of Claims 37-45, wherein the ligand binding domain comprises a single chain variable fragment (scFv), or a binding portion thereof.

47. The population of genetically modified T-cells of any one of Claims 37-46, wherein the ligand binding domain comprises FMC63, or a binding portion thereof.

48. The population of genetically modified T-cells of any one of Claims 37-47, wherein the ligand binding domain is specific for CD19.

49. The population of genetically modified T-cells of any one of Claims 33-48, wherein the population comprises isolated, purified, separated or enriched CD8+ T-cells in the absence of, substantially depleted of, or enriched over CD4+ T-cells.

50. The population of genetically modified T-cells of any one of Claims 33-48, wherein the population comprises isolated, purified, separated or enriched CD4+ T-cells in the absence of, substantially depleted of, or enriched over CD8+ T-cells.

51. A composition or product combination for human therapy, comprising:

a pharmaceutical excipient; and

at least one population of genetically modified T-cells according to any one or more of Claims 33-50.

52. The composition or product combination of Claim 51, wherein the composition or product combination comprises the population of genetically modified T-cells of Claim 49.

53. The composition or product combination of Claim 47, wherein the composition or product combination comprises the population of genetically modified T-cells of Claim 50.

54. The composition or product combination of Claim 47, wherein the composition or product combination comprises the population of genetically modified T-cells of Claim 49 and the population of genetically modified T-cells of Claim 50, mixed or co-administered in a 1:1 ratio.

55. A method of treating, inhibiting, or ameliorating a disease in a subject in need thereof comprising:

administering to the subject at least one composition or product combination of any one or more of Claims 51-54.

56. The method of claim 55, wherein the method comprises administering the composition or product combination of Claim 52.

57. The method of claim 55, wherein the method comprises administering the composition or product combination of claim 53.

58. The method of Claim 56, wherein the method further comprises administering the composition or product combination of Claim 53.

59. The method of Claim 57, wherein the method further comprises administering the composition or product combination of Claim 52.

60. The method of Claim 55, wherein the method comprises administering the composition or product combination of Claim 54, such as by an approach, wherein the CD8+ expressing T-cells are administered in advance of the CD4+ expressing T-cells, such as 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 60 minutes before the CD4+ T-cells are administered or a time period that is within a range defined by any two of the aforementioned times.

61. The method of any one or more of Claims 55-60, wherein the subject is identified or selected to receive an anti-cancer therapy.

62. The method of any one or more of Claims 55-61, further comprising measuring or evaluating an inhibition of a disease.

63. The method of any one or more of Claims 55-62, further comprising providing said subject an additional anti-cancer therapy before, during, or after administration of the composition or product combination of any one or more of claims 51-54.

64. The method of any one or more of Claims 55-63, wherein the composition or product combination of any one or more of claims 51-54 are administered to said subject by adoptive cell transfer.

65. The method of any one or more of Claims 55-64, wherein the composition or product combination of any one or more of claims 51-54 are administered to said subject after said subject has received another form of anti-cancer therapy.

66. The method of any one or more of Claims 55-65, wherein the composition or product combination of any one or more of Claims 51-54 are administered to said subject after said subject has received another form of anti-cancer therapy.

67. The method of any one or more of Claims 55-66, wherein the subject is suffering from leukemia.

68. The method of any one or more of Claims 55-67, wherein the subject has recurrent and/or chemotherapy refractory CD19+ childhood acute lymphoblastic leukemia (ALL).

69. The method of any one or more of Claims 55-68, wherein the subject has recurrent and/or chemotherapy refractory CD19+ acute lymphoblastic leukemia (ALL).

70. The method of any one or more of Claims 55-69, wherein the subject is suffering from an autoimmune disease.

71. The method of any one or more of Claims 55-70, wherein the subject is suffering from a post-HSCT relapse.

72. The method of claim 1, wherein the T cell is a precursor T cell.

73. The method of claim 72, wherein the precursor T cell is a hematopoietic stem cell (HSC)

CLAIMS:

1. A method of preparing an isolated population of genetically modified T-cells having an improved engraftment fitness, comprising:
 - (a) obtaining a population of T-cells comprising a chimeric antigen receptor (CAR), wherein the population of T-cells comprises CD4+ T-cells or CD8+ T-cells;
 - (b) contacting the population of T-cells with exogenous IL-2; and
 - (c) contacting the population of T-cells with an exogenous combination of IL-7 and IL-21, wherein step (b) is performed before step (c), so as to obtain said genetically modified T-cells having an improved engraftment fitness.
2. The method of claim 1, wherein step (a) further comprises separating a CD8+ population of T-cells or a CD4+ population of T-cells from a mixed population of T-cells by affinity selection for T-cells having an epitope present on CD8 or CD4.
3. The method of claim 2, wherein the separating is performed by flow cytometry.
4. The method of claim 2, wherein the separating is performed by immuno-magnetic selection.
5. The method of claim 1, wherein the population of genetically modified T-cells comprise at least one receptor that promotes, induces, contributes to, or enhances engraftment fitness selected from CD27, CD28, or CD62L.
6. The method of claim 1, wherein step (a) further comprises stimulating the population of T-cells by contacting the population of T-cells with an antibody-bound support comprising an antibody selected from an anti-TCR antibody, an anti-CD2 antibody, an anti-CD3 antibody, an anti-CD4 antibody, or an anti-CD28 antibody.
7. The method of claim 1, wherein step (a) further comprises transducing the population of T cells with a vector comprising a first sequence encoding a leader sequence, a

second sequence encoding a ligand binding domain, a third sequence encoding a signaling domain and a fourth sequence encoding a selectable marker.

8. The method of claim 7, wherein the vector is a viral vector.
9. The method of claim 8, wherein the viral vector is derived from a simian virus 40, an adenovirus, an adeno-associated virus (AAV), a lentivirus, or a retrovirus.
10. The method of claim 8, wherein the viral vector is a lentivirus vector.
11. The method of claim 1, wherein the IL-2, IL-7 or IL-21 comprises a recombinant cytokine.
12. The method of claim 1, wherein the IL-2, IL-7, or IL-21 is provided within a range from 0.1 ng/mL to 1.0 ng/mL, or within a range from 10 U/mL to 100 U/mL.
13. The method of claim 1, wherein the IL-2, IL-7, or IL-21 is provided at 0.5 ng/mL or 50 U/mL.
14. The method of claim 1, wherein the contacting is performed for a period within a range from 1 day to 20 days.
15. The method of claim 1, wherein the method is performed with isolated, purified, or separated CD4⁺ T-cells in the absence of or substantially depleted of CD8⁺ T-cells.
16. The method of claim 1, wherein the method is performed with isolated, purified, or separated CD8⁺ T-cells in the absence of or substantially depleted of CD4⁺ T-cells.
17. The method of claim 1, wherein the CD4⁺ T-cells are propagated for a period within a range from 1 day to 20 days.

18. The method of claim 1, wherein the CD8⁺ T-cells are propagated for a period within a range from 1 day to 20 days.
19. The method of claim 1, wherein the CAR comprises a ligand binding domain comprising FMC63 or an antigen binding portion thereof.
20. The method of claim 1, wherein the CAR comprises a ligand binding domain specific for CD19.
21. The method of claim 1, wherein the method further comprises cryopreserving the genetically modified T-cells.
22. The method of claim 1, wherein the population of T-cells comprises a precursor T cell.
23. The method of claim 22, wherein the precursor T cell is a hematopoietic stem cell.
24. A population of genetically modified T-cells having improved engraftment fitness, wherein the population is prepared according to the method of any one of claims 1-22, and wherein the population of T-cells comprise at least one receptor that promotes, enhances, improves or contributes to engraftment fitness.
25. A pharmaceutical composition for human therapy, comprising:
 - a pharmaceutical excipient; and
 - the population of genetically modified T-cells of claim 24.
26. The pharmaceutical composition of claim 25 comprising a population of genetically modified CD4⁺ T-cells and a population of genetically modified CD8⁺ T-cells mixed in a 1:1 ratio.
27. The pharmaceutical composition of claim 25 for use in treatment, inhibition or amelioration of a disorder in a subject.

28. The pharmaceutical composition of claim 27 adapted for administration to said subject by adoptive cell transfer.
29. The pharmaceutical composition of claim 27, wherein the disorder comprises a leukemia.
30. The pharmaceutical composition of claim 29, wherein the leukemia is a recurrent or chemotherapy refractory CD19+ childhood acute lymphoblastic leukemia (ALL).
31. The pharmaceutical composition of claim 27, wherein the disorder comprises an autoimmune disease.
32. The pharmaceutical composition of claim 27, wherein the disorder comprises a post-HSCT relapse.
33. An *in vitro* method of making an isolated population of genetically modified T-cells having an improved engraftment fitness, comprising:
 - (a) isolating a CD8+ expressing population of T-cells or a CD4+ expressing population of T-cells from a mixed population of isolated T-cells so as to generate an isolated population of T-cells;
 - (b) stimulating the isolated population of T-cells so as to generate a stimulated population of T-cells;
 - (c) transducing the stimulated population of T-cells with a vector encoding a chimeric antigen receptor (CAR), so as to generate a transduced population of T-cells;
 - (d) contacting the transduced population of T-cells with exogenous IL-2; and
 - (e) contacting the transduced population of T-cells with an exogenous combination of IL-7 and IL-21, wherein step (e) is performed subsequent to step (d).
34. The method of claim 33, wherein the transduced population of T-cells is contacted with the exogenous IL-2 for a period of 5 days.