

CLAIMS

What is claimed is:

1. A method of producing T cells comprising
obtaining T cells from at least one individual,
activating the T cells,
expanding a first portion of the activated T cells over a period of time,
culturing the expanded T cells in the presence of at least one cytokine,
measuring a cytokine response in the cultured T cells,
identifying the period of time that yields a maximum cytokine response, and
expanding a second portion of the activated T cells for the period of time that yields
a maximum cytokine response.
2. The method of claim 1, further comprising freezing the expanded first portion of the
activated T cells prior to culturing.
3. The method of claim 2, further comprising thawing the frozen expanded first portion of
activated T cells prior to culturing.
4. The method of claim 3, further comprising resting the thawed expanded first portion of
the activated T cells prior to culturing.
5. The method of any one of claims 1 – 4, wherein the T cells are activated by a stimulator
comprising anti-CD3 antibody and an anti-CD28 antibody.
6. The method of any one of claims 1 – 5, wherein the period of time is from about 1 day to
about 15 days, from about 2 days to about 14 days, from about 3 days to about 13 days,
from about 3 days to about 12 days, from about 3 days to about 11 days, from about 3 days
to about 10 days, from about 3 days to about 9 days, from about 3 days to about 8 days,
from about 3 days to about 7 days, from about 3 days to about 6 days, from about 3 days to
about 5 days, from about 3 days to about 4 days, from about 4 days to about 6 days, or
from about 4 days to about 5 days after activation.

7. The method of any one of claims 1 - 6, wherein the at least one cytokine is selected from the group consisting of interleukin 2 (IL-2), interleukin 7 (IL-7), interleukin 15 (IL-15), and a combination thereof.

8. The method of claim 7, wherein the concentration of IL-2 is from about 10 U/ml to about 500 U/ml, from about 10 U/ml to about 450 U/ml, from about 10 U/ml to about 400 U/ml, from about 10 U/ml to about 350 U/ml, from about 10 U/ml to about 300 U/ml, from about 10 U/ml to about 250 U/ml, from about 10 U/ml to about 200 U/ml, from about 10 U/ml to about 150 U/ml, from about 10 U/ml to about 100 U/ml, from about 10 U/ml to about 50 U/ml, from about 20 U/ml to about 40 U/ml, from about 25 U/ml to about 35 U/ml, or from about 30 U/ml to about 35 U/ml.

9. The method of claim 7, wherein the concentration of IL-7 is from 0.1 ng/ml to 50 ng/ml, from 0.1 ng/ml to 45 ng/ml, from 0.1 ng/ml to 40 ng/ml, from 0.1 ng/ml to 35 ng/ml, from 0.1 ng/ml to 30 ng/ml, from 0.1 ng/ml to 25 ng/ml, from 0.1 ng/ml to 20 ng/ml, from 0.1 ng/ml to 15 ng/ml, from 0.1 ng/ml to 10 ng/ml, from 0.1 ng/ml to 5 ng/ml, from 0.1 ng/ml to 4 ng/ml, from 0.1 ng/ml to 3 ng/ml, from 0.1 ng/ml to 2 ng/ml, from 0.1 ng/ml to 1 ng/ml, or from 0.1 ng/ml to 0.5 ng/ml.

10. The method of any one of claim 7, wherein the concentration of IL-15 is from 0.1 ng/ml to 50 ng/ml, from 0.1 ng/ml to 45 ng/ml, from 0.1 ng/ml to 40 ng/ml, from 0.1 ng/ml to 35 ng/ml, from 0.1 ng/ml to 30 ng/ml, from 0.1 ng/ml to 25 ng/ml, from 0.1 ng/ml to 20 ng/ml, from 0.1 ng/ml to 15 ng/ml, from 0.1 ng/ml to 10 ng/ml, from 0.1 ng/ml to 5 ng/ml, from 0.1 ng/ml to 4 ng/ml, from 0.1 ng/ml to 3 ng/ml, from 0.1 ng/ml to 2 ng/ml, from 0.1 ng/ml to 1 ng/ml, or from 0.1 ng/ml to 0.5 ng/ml.

11. The method of any one of claims 1 – 10, wherein the cytokine response is selected from one or more of increased proliferation, reduced apoptosis, increased population of naïve T cells (T_N) and/or stem memory T cells (T_{scm})/T central memory (T_{cm}), and a combination thereof.

12. The method of claim 4, wherein the resting is carried out within a period of time from about 0.5 hour to about 48 hours, about 0.5 hour to about 36 hours, about 0.5 hour to about 24 hours, about 0.5 hour to about 18 hours, about 0.5 hour to about 12 hours, about 0.5 hour to about 6 hours, about 1 hour to about 6 hours, about 2 hours to about 5 hours, about 3 hours to about 5 hours, about 4 hours to 6 hours, about 1 hours to about 24 hours, about 2 to about 24 hours, about 12 to about 48 hours, about 0.5 hour to about 120 hours, about 0.5 hour to about 108 hours, about 0.5 hour to about 96 hours, about 0.5 hour to about 84 hours, about 0.5 hour to about 72 hours, or about 0.5 hour to about 60 hours.

13. The method of claim 5, wherein the anti-CD3 antibody and the anti-CD28 antibody each have a concentration of from about 0.1 µg/ml to about 10.0 µg/ml, about 0.1 µg/ml to about 8.0 µg/ml, about 0.1 µg/ml to about 6.0 µg/ml, about 0.1 µg/ml to about 4.0 µg/ml, about 0.1 µg/ml to about 2.0 µg/ml, about 0.1 µg/ml to about 1.0 µg/ml, about 0.1 µg/ml to about 0.8 µg/ml, about 0.1 µg/ml to about 0.6 µg/ml, about 0.1 µg/ml to about 0.5 µg/ml, about 0.1 µg/ml to about 0.25 µg/ml, about 0.2 µg/ml to about 0.5 µg/ml, about 0.2 µg/ml to about 0.3 µg/ml, about 0.3 µg/ml to about 0.5 µg/ml, about 0.3 µg/ml to about 0.4 µg/ml, or about 0.4 µg/ml to about 0.5 µg/ml.

14. The method of any one of claims 1 – 13, wherein the activation is carried out within a period of from about 1 hour to about 120 hours, about 1 hour to about 108 hours, about 1 hour to about 96 hours, about 1 hour to about 84 hours, about 1 hour to about 72 hours, about 1 hour to about 60 hours, about 1 hour to about 48 hours, about 1 hour to about 36 hours, about 1 hour to about 24 hours, about 2 hours to about 24 hours, about 4 hours to about 24 hours, about 6 hours to about 24 hours, about 8 hours to about 24 hours, about 10 hours to about 24 hours, about 12 hours to about 24 hours, about 12 hours to about 72 hours, about 24 hours to about 72 hours, about 6 hours to about 48 hours, about 24 hours to about 48 hours, about 6 hours to about 72 hours, or about 1 hours to about 12 hours.

15. The method of any one of claims 1 – 14, wherein the obtained T cell is a CD3⁺ CD8⁺ T cell.

16. The method of any one of claims 1 – 15, further comprising collecting the expanded second portion of the activated T cells for infusing into the at least one donor, patient, or individual.
17. A method of treating a patient having a cancer, comprising administering to the patient an effective amount of the collected expanded second portion of the activated T cells of any one of claims 1 – 16.
18. The method of claim 17, wherein the T cells are obtained from the patient.
19. The method of claim 17, wherein the T cells are obtained from the healthy donor.
20. The method of claim 17, wherein the cancer is selected from the group consisting of hepatocellular carcinoma (HCC), colorectal carcinoma (CRC), glioblastoma (GB), gastric cancer (GC), esophageal cancer, non-small cell lung cancer (NSCLC), pancreatic cancer (PC), renal cell carcinoma (RCC), benign prostate hyperplasia (BPH), prostate cancer (PCA), ovarian cancer (OC), melanoma, breast cancer, chronic lymphocytic leukemia (CLL), Merkel cell carcinoma (MCC), small cell lung cancer (SCLC), Non-Hodgkin lymphoma (NHL), acute myeloid leukemia (AML), gallbladder cancer and cholangiocarcinoma (GBC, CCC), urinary bladder cancer (UBC), acute lymphoblastic leukemia (ALL), and uterine cancer (UEC).
21. A pharmaceutical composition comprising the collected expanded second portion of the activated T cells of any one of claims 1 – 16 and a pharmaceutically acceptable carrier.
22. A method for increasing the growth of T cells comprising
obtaining T cells from at least one healthy donor, patient, or individual,
activating the T cells,
expanding the activated T cells for about 3 days to about 5 days after activation,

collecting the expanded T cells for infusing into the at least one healthy donor, patient, or individual,
wherein the growth of the T cells expanded for about 3 to about 5 days is greater than that of activated T cells expanded for about 7 days or more after activation.

23. The method of claim 22, wherein the T cells are expanded for about 4 days after activation and wherein the growth of the T cells is greater than that of activated T cells expanded for about 7 days or more after activation.

24. The method of any one of claims 22 – 23, wherein the expanded T cells are CD4+ and/or CD8+ T cells.

25. The method of any one of claims 22 – 24, wherein the T cells are activated by a stimulator comprising anti-CD3 antibody and an anti-CD28 antibody.

26. The method of any one of claims 22 – 25, wherein the T cells expanded for about 3 to about 5 days are used in adoptive immunotherapy in a patient in need of cancer treatment, wherein the cancer is selected from the group consisting of hepatocellular carcinoma (HCC), colorectal carcinoma (CRC), glioblastoma (GB), gastric cancer (GC), esophageal cancer, non-small cell lung cancer (NSCLC), pancreatic cancer (PC), renal cell carcinoma (RCC), benign prostate hyperplasia (BPH), prostate cancer (PCA), ovarian cancer (OC), melanoma, breast cancer, chronic lymphocytic leukemia (CLL), Merkel cell carcinoma (MCC), small cell lung cancer (SCLC), Non-Hodgkin lymphoma (NHL), acute myeloid leukemia (AML), gallbladder cancer and cholangiocarcinoma (GBC, CCC), urinary bladder cancer (UBC), acute lymphoblastic leukemia (ALL), and uterine cancer (UEC).

27. A method of decreasing cell death of T cells for use in adoptive immunotherapy comprising
obtaining T cells from at least one healthy donor, patient, or individual,
activating the T cells,
expanding the activated T cells for about 3 days to about 5 days after activation,

collecting the expanded T cells for infusing into the at least one healthy donor, patient, or individual,
wherein the cell death of the T cells expanded for about 3 to about 5 days is reduced relative to that of activated T cells expanded for about 7 days or more after activation.

28. The method of claim 27, wherein the expanded T cells are CD4+ and/or CD8+ T cells.

29. The method of any one of claims 27 – 28, wherein the number of expanded T cells exhibiting a naïve T cells (T_N) and/or stem memory T cells (T_{scm})/T central memory (T_{cm}) phenotype is more than that of the activated T cells expanded more than about 7 days after the activation.

30. A method for producing T cells with improved efficacy for adoptive immunotherapy comprising

obtaining T cells from at least one healthy donor, patient, or individual,
activating the T cells,
expanding the activated T cells for about 3 days to about 5 days after activation,
collecting the expanded T cells for infusing into the at least one healthy donor, patient, or individual,

wherein the efficacy for adoptive immunotherapy of the T cells expanded for about 3 to about 5 days is improved relative to activated T cells expanded for about 7 days or more after activation.

31. The method of claim 30, wherein the T cells are expanded for about 4 days after activation and wherein the efficacy for adoptive immunotherapy of the T cells is greater than that of activated T cells expanded for about 7 days or more after activation.

32. The method of any one of claims 30 – 31, wherein the expanded T cells are CD4+ and/or CD8+ T cells.

33. The method of any one of claims 30 – 32, wherein the expanded T cells exhibit a naïve T cells (T_N) and/or stem memory T cells (T_{scm})/T central memory (T_{cm}) phenotype.

34. The method of any one of claims 30 – 33, wherein the T cells are activated by a stimulator comprising anti-CD3 antibody and an anti-CD28 antibody.

35. The method of any one of claims 30 – 34, wherein the T cells expanded for about 3 to about 5 days are used in adoptive immunotherapy in a patient in need of cancer treatment, wherein the cancer is selected from the group consisting of hepatocellular carcinoma (HCC), colorectal carcinoma (CRC), glioblastoma (GB), gastric cancer (GC), esophageal cancer, non-small cell lung cancer (NSCLC), pancreatic cancer (PC), renal cell carcinoma (RCC), benign prostate hyperplasia (BPH), prostate cancer (PCA), ovarian cancer (OC), melanoma, breast cancer, chronic lymphocytic leukemia (CLL), Merkel cell carcinoma (MCC), small cell lung cancer (SCLC), Non-Hodgkin lymphoma (NHL), acute myeloid leukemia (AML), gallbladder cancer and cholangiocarcinoma (GBC, CCC), urinary bladder cancer (UBC), acute lymphoblastic leukemia (ALL), and uterine cancer (UEC).

36. The method of any one of claims 30 – 35, wherein the T cells expanded for about 3 to about 5 days exhibit increased proliferation and survival relative to activated T cells expanded for about 7 days or more after activation.

37. The method of any one of claims 1, 22, 27, and 30, further comprising transducing the activated T cells with a viral vector prior to the expanding, wherein the viral vector is a retroviral vector expressing a T cell receptor (TCR).

38. The method of claim 37, wherein the viral vector is a lentiviral vector expressing a T cell receptor (TCR).

39. A T cell population produced by a method of any one of claims 1, 22, 27, and 30.

40. A T cell population produced by a method of any one of claims 1 – 16.

41. The method of any one of claims 1-16, wherein the culturing is in the absence of an antigen-presenting cell.

42. The method of claim 41, wherein the culturing comprises seeding the expanded T cells at a density of from about 1,000 to about 1×10^6 cells/cm², from about 1,000 to about 500,000 cells/cm², from about 1,000 to about 250,000 cells/cm², from about 1,000 to about 200,000 cells/cm², from about 1,000 to about 150,000 cells/cm², from about 1,000 to about 100,000 cells/cm², from about 1,000 to about 50,000 cells/cm², from about 1,000 to about 10,000 cells/cm², or from about 1,000 to about 5,000 cells/cm².

43. The method of claim 41, wherein the culturing comprises seeding the expanded T cells at a density of from about 1,000 to about 200,000 cells/cm², from about 1,000 to about 150,000 cells/cm², or from about 1,000 to about 100,000 cells/cm².

44. A method for predicting in vivo persistence of T cells in a solid tumor, comprising
thawing cryopreserved T cells expanded for a plurality of expansion times,
resting the thawed T cells in the absence of a cytokine,
seeding the rested T cells,
culturing the seeded T cells for at least one cycle of time,
 wherein, at the beginning of the at least one cycle of time, one or more
 cytokines are added to the culture, and
 wherein, at the end of the at least one cycle of time, the added one or more
 cytokines are depleted,
sampling the cultured T cells at a plurality of time points during the at least one cycle
of time,
measuring a cytokine response of the sampled T cells,
identifying an expansion time of the sampled T cells exhibiting a maximum cytokine
response from the plurality of expansion times, and
formulating the T cells expanded for the identified expansion time into a composition
for treating the solid tumor.

45. The method of claim 44, wherein the T cells are expanded from about 1 day to about 15 days, from about 2 days to about 14 days, from about 3 days to about 13 days, from about 3 days to about 12 days, from about 3 days to about 11 days, from about 3 days to about 10 days, from about 3 days to about 9 days, from about 3 days to about 8 days, from about 3 days to about 7 days, from about 3 days to about 6 days, from about 3 days to about 5 days, from about 3 days to about 4 days, from about 4 days to about 6 days, or from about 4 days to about 5 days after activation.

46. The method of any one of claims 44-45, wherein the resting is carried out within a period of time from about 0.5 hour to about 48 hours, about 0.5 hour to about 36 hours, about 0.5 hour to about 24 hours, about 0.5 hour to about 18 hours, about 0.5 hour to about 12 hours, about 0.5 hour to about 6 hours, about 1 hour to about 6 hours, about 2 hours to about 5 hours, about 3 hours to about 5 hours, or about 1 hours to about 24 hours, about 2 to about 24 hours, about 12 to about 48 hours, about 0.5 hour to about 120 hours, about 0.5 hour to about 108 hours, about 0.5 hour to about 96 hours, about 0.5 hour to about 84 hours, about 0.5 hour to about 72 hours, or about 0.5 hour to about 60 hours.

47. The method of any one of claims 44-46, wherein the seeding is at a density of from about 1,000 to about 200,000 cells/cm², from about 1,000 to about 150,000 cells/cm², or from about 1,000 to about 100,000 cells/cm².

48. The method of any one of claims 44-47, wherein the one or more cytokine is selected from the group consisting of interleukin 2 (IL-2), interleukin 7 (IL-7), interleukin 15 (IL-15), and a combination thereof.

49. The method of any one of claims 44-48, wherein the one cycle of time is 1-10 days per cycle, 2-10 days per cycle, 3-10 days per cycle, 4-10 days per cycle, 5-10 days per cycle, 6-10 days per cycle, 7-10 days per cycle, 8-10 days per cycle, or 9-10 days per cycle,

50. The method of any one of claims 44-49, wherein the at least one cycle of time is 1 cycle of time, 2 cycles of time, 3 cycles of time, 4 cycles of time, 5 cycles of time, 6 cycles of time, 7 cycles of time, 8 cycles of time, 9 cycles of time, or 10 cycles of time.

51. The method of any one of claims 44-50, wherein the cytokine response is selected from one or more of increased proliferation, reduced apoptosis, increased population of naïve T cells (T_N) and/or stem memory T cells (T_{scm})/T central memory (T_{cm}), and a combination thereof.

52. The method of any one of claims 44-51, wherein the solid tumor is selected from the group consisting of hepatocellular carcinoma (HCC), colorectal carcinoma (CRC), glioblastoma (GB), gastric cancer (GC), esophageal cancer, non-small cell lung cancer (NSCLC), pancreatic cancer (PC), renal cell carcinoma (RCC), benign prostate hyperplasia (BPH), prostate cancer (PCA), ovarian cancer (OC), melanoma, breast cancer, Merkel cell carcinoma (MCC), small cell lung cancer (SCLC), gallbladder cancer and cholangiocarcinoma (GBC, CCC), urinary bladder cancer (UBC), and uterine cancer (UEC).