CLAIMS

What is claimed is:

1. A method of transducing a T cell, comprising
thawing frozen peripheral blood mononuclear cells (PBMC),
resting the thawed PBMC,
activating the T cell in the rested PBMC with an anti-CD3 antibody and an anti-CD28 antibody,
transducing the activated T cell with a viral vector,
expanding the transduced T cell, and
obtaining the expanded T cell.

2. The method of claim 1, wherein the activating step comprises immobilizing the T cell in the rested PBMC with the anti-CD3 antibody and the anti-CD28 antibody on a solid phase support.

3. The method of any one of claims 1 – 2, wherein the resting step 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.

4. The method of any one of claims 1-3, wherein the anti-CD3 antibody and the anti-CD28 antibody each have a concentration of no more than about 0.1 µg/ml, no more than about 0.2 µg/ml, no more than about 0.3 µg/ml, no more than about 0.4 µg/ml, no more than about 0.5 µg/ml, no more than about 0.6 µg/ml, no more than about 0.7 µg/ml, no more than about 0.8 µg/ml, no more than about 0.9 µg/ml, no more than about 1.0 µg/ml, no more than about 2.0 µg/ml, no more than about 4.0 µg/ml, no more than about 6.0 µg/ml, no more than about 8.0 µg/ml, or no more than about 10.0 µg/ml.

5. The method of any one of claims 1-3, 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.

6. The method of any one of claims 1-5, wherein the activation is carried out within a period of no more than about 1 hour, about 2 hours, 3 about hours, 4 about hours, 5 about hours, 6 about hours, 7 about hours, 8 about hours, 9 about hours, 10 about hours, 11 about hours, 12 about hours, 14 about hours, 16 about hours, 18 about hours, 20 about hours, about 22 hours, about 24 hours, about 26 hours, about 28 hours, about 30 hours, about 36 hours, about 48 hours, about 60 hours, about 72 hours, about 84 hours, about 96 hours, about 108 hours, or about 120 hours.

7. The method of any one of claims 1-5, 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.

8. The method of any one of claims 1-7, wherein the solid phase is a surface of a bead, a plate, a flask, or a bag.

9. The method of claim 8, wherein the plate is a 6-well, 12-well, or 24-well plate.

10. The method of claim 8, wherein the flask has a seeding surface area of about 25 cm^2 to about 75 cm^2, about 25 cm^2 to about 100 cm^2, about 25 cm^2 to about 150 cm^2, or about 50 cm^2 to about 1720 cm^2.

11. The method of claim 8, wherein the bag has a volume of from about 5 ml to about 100 liters, about 100 ml to about 100 liters, about 150 ml to about 100 liters, about 200 ml to about 100 liters, about 250 ml to about 100 liters, about 500 ml to about 100 liters, about 1 liter to about 100 liters, about 1 liter to about 75 liters, about 1 liter to about 50 liters, about 1 liter to about 25 liters, about 1 liter to about 20 liters, about 1 liter to about 15 liters, about 1 liter to about 10 liters, about 1 liter to about 5 liters, about 1 liter to about 2.5 liters, or about 1 liter to about 2 liters.

12. The method of any one of claims 1-11, wherein the resting is carried out in the presence of at least one cytokine.

13. The method of claim 12, wherein the at least one cytokine comprises interleukin 7 (IL-7) and/or interleukin 15 (IL-15).

14. The method of claim 13, wherein the concentration of IL-7 is no more than about 1 ng/ml, no more than about 2 ng/ml, no more than about 3 ng/ml, no more than about 4 ng/ml, no more than about 5 ng/ml, no more than about 6 ng/ml, no more than about 7 ng/ml, no more than about 8 ng/ml, no more than about 9 ng/ml, no more than about 10 ng/ml, no more than about 11 ng/ml, no more than about 12 ng/ml, no more than about 13 ng/ml, no more than about 14 ng/ml, no more than about 15 ng/ml, no more than about 16 ng/ml, no more than about 17 ng/ml, no more than about 18 ng/ml, no more than about 19 ng/ml, no more than about 20 ng/ml, no more than about 25 ng/ml, no more than about 30 ng/ml, no more than about 35 ng/ml, no more than about 40 ng/ml, no more than about 45 ng/ml, no more than about 50 ng/ml, no more than about 60 ng/ml, no more than about 70 ng/ml, no more than about 80 ng/ml, no more than about 90 ng/ml, or no more than about 100 ng/ml.

15. The method of claim 13, wherein the concentration of IL-7 is from about 1 ng/ml to 90 ng/ml, about 1 ng/ml to 80 ng/ml, about 1 ng/ml to 70 ng/ml, about 1 ng/ml to 60 ng/ml, about 1 ng/ml to 50 ng/ml, about 1 ng/ml to 40 ng/ml, about 1 ng/ml to 30 ng/ml, about 1 ng/ml to 20 ng/ml, about 1 ng/ml to 15 ng/ml, about 1 ng/ml to 10 ng/ml, about 2 ng/ml to 10 ng/ml, about 4 ng/ml to 10 ng/ml, about 6 ng/ml to 10 ng/ml, or about 5 ng/ml to 10 ng/ml.

16. The method of any one of claims 13-15, wherein the concentration of IL-15 is no more than about 5 ng/ml, no more than about 10 ng/ml, no more than about 15 ng/ml, no more than about 20 ng/ml, no more than about 25 ng/ml, no more than about 30 ng/ml, no more than about 35 ng/ml, no more than about 40 ng/ml, no more than about 45 ng/ml, no more than about 50 ng/ml, no more than about 60 ng/ml, no more than about 70 ng/ml, no more than about 80 ng/ml, no more than about 90 ng/ml, no more than about 100 ng/ml, no more than about 110 ng/ml, no more than about 120 ng/ml, no more than about 130 ng/ml, no more than about 140 ng/ml, no more than about 150 ng/ml, 200 ng/ml, 250 ng/ml, 300 ng/ml, 350 ng/ml, 400 ng/ml, 450 ng/ml, or 500 ng/ml.

17. The method of anyone of claims 13-15, wherein the concentration of IL-15 is from about 5 ng/ml to 500 ng/ml, about 5 ng/ml to 400 ng/ml, about 5 ng/ml to 300 ng/ml, about 5 ng/ml to 200 ng/ml, about 5 ng/ml to 150 ng/ml, about 5 ng/ml to 100 ng/ml, about 10 ng/ml to 100 ng/ml, about 20 ng/ml to 100 ng/ml, about 30 ng/ml to 100 ng/ml, about 40 ng/ml to 100 ng/ml, about 50 ng/ml to 100 ng/ml, about 60 ng/ml to 100 ng/ml, about 70 ng/ml to 100 ng/ml, about 80 ng/ml to 100 ng/ml, about 90 ng/ml to 100 ng/ml, about 10 ng/ml to 50 ng/ml, about 20 ng/ml to 50 ng/ml, about 30 ng/ml to 50 ng/ml, or about 40 ng/ml to 50 ng/ml.

18. The method of any one of claims 1-16, wherein the transducing is carried out within a period of no more than about 1 hour, no more than about 2 hours, no more than about 3 hours, no more than about 4 hours, no more than about 5 hours, no more than about 6 hours, no more than about 7 hours, no more than about 8 hours, no more than about 9 hours, no more than about 10 hours, no more than about 11 hours, no more than about 12 hours, no more than about 14 hours, no more than about 16 hours, no more than about 18

hours, no more than about 20 hours, no more than about 22 hours, no more than about 24 hours, no more than about 26 hours, no more than about 28 hours, no more than about 30 hours, no more than about 36 hours, no more than about 42 hours, no more than about 48 hours, no more than about 54 hours, no more than about 60 hours, no more than about 66 hours, no more than about 72 hours, no more than about 84 hours, no more than about 96 hours, no more than about 108 hours, or no more than about 120 hours.

19. The method of any one of claims 1-16, wherein the transducing is carried out within a period of from about 1 hour to 120 hours, about 1 hour to 108 hours, about 1 hour to 96 hours, about 1 hour to 72 hours, about 1 hour to 48 hours, about 1 hour to 36 hours, about 1 hour to 24 hours, about 2 hour to 24 hours, about 4 hour to 24 hours, about 6 hour to 24 hours, about 8 hour to 24 hours, about 10 hour to 24 hours, about 12 hour to 24 hours, about 14 hour to 24 hours, about 16 hour to 24 hours, about 18 hour to 24 hours, about 20 hour to 24 hours, or about 22 hour to 24 hours.

20. The method of any one of claims 1-19, wherein the viral vector is a retroviral vector expressing a T cell receptor (TCR).

21. The method of any one of claims 1-20, wherein the viral vector is a lentiviral vector expressing a TCR.

22. The method of any one of claims 1-21, wherein the transducing is carried out in the presence of at least one cytokine.

23. The method of claim 22, wherein the at least one cytokine comprises interleukin 7 (IL-7) and/or interleukin 15 (IL-15).

24. The method of claim 23, wherein the concentration of IL-7 is no more than about 1 ng/ml, no more than about 2 ng/ml, no more than about 3 ng/ml, no more than about 4 ng/ml, no more than about 5 ng/ml, no more than about 6 ng/ml, no more than about 7 ng/ml, no more than about 8 ng/ml, no more than about 9 ng/ml, no more than about 10 ng/ml, no more than about 11 ng/ml, no more than about 12 ng/ml, no more than about 13

ng/ml, no more than about 14 ng/ml, no more than about 15 ng/ml, no more than about 16 ng/ml, no more than about 17 ng/ml, no more than about 18 ng/ml, no more than about 19 ng/ml, no more than about 20 ng/ml, no more than about 25 ng/ml, no more than about 30 ng/ml, no more than about 35 ng/ml, no more than about 40 ng/ml, no more than about 45 ng/ml, no more than about 50 ng/ml, no more than about 60 ng/ml, no more than about 70 ng/ml, no more than about 80 ng/ml, no more than about 90 ng/ml, or no more than about 100 ng/ml.

25. The method of claim 23, wherein the concentration of IL-7 is from about 1 ng/ml to 100 ng/ml, about 1 ng/ml to 90 ng/ml, about 1 ng/ml to 80 ng/ml, about 1 ng/ml to 70 ng/ml, about 1 ng/ml to 60 ng/ml, about 1 ng/ml to 50 ng/ml, about 1 ng/ml to 40 ng/ml, about 1 ng/ml to 30 ng/ml, about 1 ng/ml to 20 ng/ml, about 1 ng/ml to 15 ng/ml, about 1 ng/ml to 10 ng/ml, about 2 ng/ml to 10 ng/ml, about 4 ng/ml to 10 ng/ml, about 6 ng/ml to 10 ng/ml, about 8 ng/ml to 10 ng/ml, or about 9 ng/ml to 10 ng/ml.

26. The method of any one of claims 23-25, wherein the concentration of IL-15 is no more than about 5 ng/ml, no more than about 10 ng/ml, no more than about 15 ng/ml, no more than about 20 ng/ml, no more than about 25 ng/ml, no more than about 30 ng/ml, no more than about 35 ng/ml, no more than about 40 ng/ml, no more than about 45 ng/ml, no more than about 50 ng/ml, no more than about 60 ng/ml, no more than about 70 ng/ml, no more than about 80 ng/ml, no more than about 90 ng/ml, no more than about 100 ng/ml, no more than about 110 ng/ml, no more than about 120 ng/ml, no more than about 130 ng/ml, no more than about 140 ng/ml, no more than about 150 ng/ml, 200 ng/ml, 250 ng/ml, 300 ng/ml, 350 ng/ml, 400 ng/ml, 450 ng/ml, or 500 ng/ml.

27. The method of any one of claim 23-25, wherein the concentration of IL-15 is from about 5 ng/ml to 500 ng/ml, about 5 ng/ml to 400 ng/ml, about 5 ng/ml to 300 ng/ml, about 5 ng/ml to 200 ng/ml, about 5 ng/ml to 150 ng/ml, about 5 ng/ml to 100 ng/ml, about 10 ng/ml to 100 ng/ml, about 20 ng/ml to 100 ng/ml, about 30 ng/ml to 100 ng/ml, about 40 ng/ml to 100 ng/ml, about 50 ng/ml to 100 ng/ml, about 60 ng/ml to 100 ng/ml, about 70 ng/ml to 100 ng/ml, about 80 ng/ml to 100 ng/ml, about 90 ng/ml to 100 ng/ml, about 10 ng/ml to 50

ng/ml, about 20 ng/ml to 50 ng/ml, about 30 ng/ml to 50 ng/ml, or about 40 ng/ml to 50 ng/ml.

28. The method of any one of claims 1-27, wherein the expanding is carried out in the presence of at least one cytokine.

29. The method of claim 28, wherein the at least one cytokine comprises interleukin 7 (IL-7) and/or interleukin 15 (IL-15).

30. The method of claim 29, wherein the concentration of IL-7 is no more than about 1 ng/ml, no more than about 2 ng/ml, no more than about 3 ng/ml, no more than about 4 ng/ml, no more than about 5 ng/ml, no more than about 6 ng/ml, no more than about 7 ng/ml, no more than about 8 ng/ml, no more than about 9 ng/ml, no more than about 10 ng/ml, no more than about 11 ng/ml, no more than about 12 ng/ml, no more than about 13 ng/ml, no more than about 14 ng/ml, no more than about 15 ng/ml, no more than about 16 ng/ml, no more than about 17 ng/ml, no more than about 18 ng/ml, no more than about 19 ng/ml, no more than about 20 ng/ml, no more than about 25 ng/ml, no more than about 30 ng/ml, no more than about 35 ng/ml, no more than about 40 ng/ml, no more than about 45 ng/ml, no more than about 50 ng/ml, no more than about 60 ng/ml, no more than about 70 ng/ml, no more than about 80 ng/ml, no more than about 90 ng/ml, or no more than about 100 ng/ml.

31. The method of claim 29, wherein the concentration of IL-7 is from about 1 ng/ml to 100 ng/ml, about 1 ng/ml to 90 ng/ml, about 1 ng/ml to 80 ng/ml, about 1 ng/ml to 70 ng/ml, about 1 ng/ml to 60 ng/ml, about 1 ng/ml to 50 ng/ml, about 1 ng/ml to 40 ng/ml, about 1 ng/ml to 30 ng/ml, about 1 ng/ml to 20 ng/ml, about 1 ng/ml to 15 ng/ml, or about 1 ng/ml to 10 ng/ml.

32. The method of any one of claims 29-31, wherein the concentration of IL-15 is no more than about 5 ng/ml, 10 ng/ml, 15 ng/ml, 20 ng/ml, 25 ng/ml, 30 ng/ml, 35 ng/ml, 40 ng/ml, 45 ng/ml, 50 ng/ml, 60 ng/ml, 70 ng/ml, 80 ng/ml, 90 ng/ml, 100 ng/ml, 110 ng/ml, 120

ng/ml, 130 ng/ml, 140 ng/ml, 150 ng/ml, 200 ng/ml, 250 ng/ml, 300 ng/ml, 350 ng/ml, 400 ng/ml, 450 ng/ml, or 500 ng/ml.

33. The method of any one of claim 29-31, wherein the concentration of IL-15 is from about 5 ng/ml to 500 ng/ml, about 5 ng/ml to 400 ng/ml, about 5 ng/ml to 300 ng/ml, about 5 ng/ml to 200 ng/ml, about 5 ng/ml to 150 ng/ml, about 5 ng/ml to 100 ng/ml, about 10 ng/ml to 100 ng/ml, about 20 ng/ml to 100 ng/ml, about 30 ng/ml to 100 ng/ml, about 40 ng/ml to 100 ng/ml, about 50 ng/ml to 100 ng/ml, about 60 ng/ml to 100 ng/ml, about 70 ng/ml to 100 ng/ml, about 80 ng/ml to 100 ng/ml, about 90 ng/ml to 100 ng/ml, about 10 ng/ml to 50 ng/ml, about 20 ng/ml to 50 ng/ml, about 30 ng/ml to 50 ng/ml, or about 40 ng/ml to 50 ng/ml.

34. The method of any one of claims 1-33, wherein the expanding is carried out within a period of no more than about 1 day, no more than about 2 days, no more than about 3 days, no more than about 4 days, no more than about 5 days, no more than about 6 days, no more than about 7 days, no more than about 8 days, no more than about 9 days, no more than about 10 days, no more than about 15 days, no more than about 20 days, no more than about 25 days, or no more than about 30 days.

35. The method of any one of claims 1-33, wherein the expanding is carried out within a period of from about 1 day to about 30 days, about 1 day to about 25 days, about 1 day to about 20 days, about 1 day to about 15 days, about 1 day to about 10 days, about 2 days to about 10 days, about 3 days to about 10 days, about 4 days to about 10 days, about 5 days to about 10 days, about 6 days to about 10 days, about 7 days to about 10 days, about 8 days to about 10 days, or about 9 days to about 10 days.

36. The method of any one of claims 1-35, wherein the number of the obtained T cell is at least about 1×10^9, may be at least about 2×10^9, may be at least about 3×10^9, may be at least about 4×10^9, may be at least about 5×10^9, may be at least about 6×10^9, may be at least about 7×10^9, may be at least about 8×10^9, may be at least about 9×10^9, may be at least about 1×10^{10}, may be at least about 5×10^{10}, may be at least about 1×10^{11}, may be

at least about 5 x 10^{11}, may be at least about 1 x 10^{12}, may be at least about 5 x 10^{12} or may be at least about 1 x 10^{13} cells.

37. The method of any one of claims 1-35, wherein the number of the obtained T cell is from about 1 x 10^{9} to about 1 x 10^{13}, about 1 x 10^{9} to about 5 x 10^{12}, about 1 x 10^{9} to about 1 x 10^{12}, about 1 x 10^{9} to about 5 x 10^{11}, about 1 x 10^{9} to about 1 x 10^{11}, about 1 x 10^{9} to about 5 x 10^{10}, about 1 x 10^{9} to about 1 x 10^{10}, about 2 x 10^{9} to about 1 x 10^{10}, about 3 x 10^{9} to about 1 x 10^{10}, about 4 x 10^{9} to about 1 x 10^{10}, about 5 x 10^{9} to about 1 x 10^{10}, about 6 x 10^{9} to about 1 x 10^{10}, about 7 x 10^{9} to about 1 x 10^{10}, about 8 x 10^{9} to about 1 x 10^{10}, or about 9 x 10^{9} to about 1 x 10^{10} cells.

38. The method of any one of claims 1-37, wherein the obtained T cell is a $CD3^{+}$ $CD8^{+}$ T cell.

39. A method of treating a patient having a cancer, comprising administering to the patient an effective amount of the obtained T cell produced by the method of any one of claims 1-38.

40. The method of claim 39, wherein the PBMC is obtained from the patient.

41. A genetically transduced T cell produced by the method of any one of claims 1-38.

42. A pharmaceutical composition comprising the genetically transduced T cell of claim 41 and a pharmaceutically acceptable carrier.

43. A method of preparing a T cell population, comprising
thawing frozen peripheral blood mononuclear cells (PBMC),
resting the thawed PBMC,
activating the T cell in the rested PBMC with an anti-CD3 antibody and an anti-CD28 antibody immobilized on a solid phase,
expanding the activated T cell, and
obtaining the T cell population comprising the expanded T cell.

44. The method of claim 43, wherein the resting is carried out within a period of no more than about 1 hour, about 2 hours, 3 about hours, 4 about hours, 5 about hours, 6 about hours, 7 about hours, 8 about hours, 9 about hours, 10 about hours, 11 about hours, 12 about hours, 14 about hours, 16 about hours, 18 about hours, 20 about hours, about 22 hours, about 24 hours, about 26 hours, about 28 hours, about 30 hours, about 36 hours, about 48 hours, about 60 hours, about 72 hours, about 84 hours, about 96 hours, about 108 hours, or about 120 hours.

45. The method of claim 43, wherein the resting 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.

46. The method of any one of claims 43-45, wherein the anti-CD3 antibody and the anti-CD28 antibody each have a concentration of no more than about 0.1 µg/ml, no more than about 0.2 µg/ml, no more than about 0.3 µg/ml, no more than about 0.4 µg/ml, no more than about 0.5 µg/ml, no more than about 0.6 µg/ml, no more than about 0.7 µg/ml, no more than about 0.8 µg/ml, no more than about 0.9 µg/ml, no more than about 1.0 µg/ml, no more than about 2.0 µg/ml, no more than about 4.0 µg/ml, no more than about 6.0 µg/ml, no more than about 8.0 µg/ml, or no more than about 10.0 µg/ml.

47. The method of any one of claims 43-45, 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, or about 0.2 µg/ml to about 0.5 µg/ml.

48. The method of any one of claims 43-47, wherein the activation is carried out within a period of no more than about 1 hour, no more than about 2 hours, no more than about 3 hours, no more than about 4 hours, no more than about 5 hours, no more than about 6 hours, no more than about 7 hours, no more than about 8 hours, no more than about 9 hours, no more than about 10 hours, no more than about 11 hours, no more than about 12 hours, no more than about 14 hours, no more than about 16 hours, no more than about 18 hours, no more than about 20 hours, no more than about 22 hours, no more than about 24 hours, no more than about 26 hours, no more than about 28 hours, no more than about 30 hours, no more than about 36 hours, no more than about 42 hours, no more than about 48 hours, no more than about 54 hours, no more than about 60 hours, no more than about 66 hours, no more than about 72 hours, no more than about 84 hours, no more than about 96 hours, no more than about 108 hours, or no more than about 120 hours.

49. The method of any one of claims 43-47, 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 14 hours to about 24 hours, about 16 hours to about 24 hours, about 18 hours to about 24 hours, about 20 hours to about 24 hours, about 22 hours to about 24 hours, or about 23 hours to about 24 hours.

50. The method of any one of claims 43-49, wherein the solid phase is a surface of a bead, a plate, a flask, or a bag.

51. The method of claim 50, wherein the plate is a 6-well, 12-well, or 24-well plate.

52. The method of claim 50, wherein the flask has a seeding surface area of about 25 cm^2 to about 75 cm^2, about 25 cm^2 to about 100 cm^2, about 25 cm^2 to about 150 cm^2, or about 50 cm^2 to about 1720 cm^2.

53. The method of claim 50, wherein the bag has a volume of from about 50 ml to about 100 liters, about 100 ml to about 100 liters, about 150 ml to about 100 liters, about 200 ml to about 100 liters, about 250 ml to about 100 liters, about 500 ml to about 100 liters, about 1 liter to about 100 liters, about 1 liter to about 75 liters, about 1 liter to about 50 liters, about 1 liter to about 25 liters, about 1 liter to about 20 liters, about 1 liter to about 15 liters, about 1 liter to about 10 liters, about 1 liter to about 5 liters, about 1 liter to about 2.5 liters, or about 1 liter to about 2 liters.

54. The method of any one of claims 43-53, wherein the activation is carried out in the presence of at least one cytokine.

55. The method of claim 54, wherein the at least one cytokine comprises interleukin 7 (IL-7) and/or interleukin 15 (IL-15).

56. The method of claim 55, wherein the concentration of IL-7 is no more than about 1 ng/ml, no more than about 2 ng/ml, no more than about 3 ng/ml, no more than about 4 ng/ml, no more than about 5 ng/ml, no more than about 6 ng/ml, no more than about 7 ng/ml, no more than about 8 ng/ml, no more than about 9 ng/ml, no more than about 10 ng/ml, no more than about 11 ng/ml, no more than about 12 ng/ml, no more than about 13 ng/ml, no more than about 14 ng/ml, no more than about 15 ng/ml, no more than about 16 ng/ml, no more than about 17 ng/ml, no more than about 18 ng/ml, no more than about 19 ng/ml, no more than about 20 ng/ml, no more than about 25 ng/ml, no more than about 30 ng/ml, no more than about 35 ng/ml, no more than about 40 ng/ml, no more than about 45 ng/ml, no more than about 50 ng/ml, no more than about 60 ng/ml, no more than about 70 ng/ml, no more than about 80 ng/ml, no more than about 90 ng/ml, or no more than about 100 ng/ml.

57. The method of claim 55, wherein the concentration of IL-7 is from about 1 ng/ml to 100 ng/ml, about 1 ng/ml to 90 ng/ml, about 1 ng/ml to 80 ng/ml, about 1 ng/ml to 70 ng/ml, about 1 ng/ml to 60 ng/ml, about 1 ng/ml to 50 ng/ml, about 1 ng/ml to 40 ng/ml, about 1 ng/ml to 30 ng/ml, about 1 ng/ml to 20 ng/ml, about 1 ng/ml to 15 ng/ml, about 1 ng/ml to 10 ng/ml, about 2 ng/ml to 10 ng/ml, about 4 ng/ml to 10 ng/ml, about 6 ng/ml to 10 ng/ml, or about 5 ng/ml to 10 ng/ml.

58. The method of any one of claims 55-57, wherein the concentration of IL-15 is no more than about 5 ng/ml, no more than about 10 ng/ml, no more than about 15 ng/ml, no more than about 20 ng/ml, no more than about 25 ng/ml, no more than about 30 ng/ml, no more than about 35 ng/ml, no more than about 40 ng/ml, no more than about 45 ng/ml, no more than about 50 ng/ml, no more than about 60 ng/ml, no more than about 70 ng/ml, no more than about 80 ng/ml, no more than about 90 ng/ml, no more than about 100 ng/ml, no more than about 110 ng/ml, no more than about 120 ng/ml, no more than about 130 ng/ml, no more than about 140 ng/ml, or no more than about 500 ng/ml.

59. The method of anyone of claims 55-57, wherein the concentration of IL-15 is from about 5 ng/ml to 500 ng/ml, about 5 ng/ml to 400 ng/ml, about 5 ng/ml to 300 ng/ml, about 5 ng/ml to 200 ng/ml, about 5 ng/ml to 150 ng/ml, about 5 ng/ml to 100 ng/ml, about 10 ng/ml to 100 ng/ml, about 20 ng/ml to 100 ng/ml, about 30 ng/ml to 100 ng/ml, about 40 ng/ml to 100 ng/ml, about 50 ng/ml to 100 ng/ml, about 60 ng/ml to 100 ng/ml, about 70 ng/ml to 100 ng/ml, about 80 ng/ml to 100 ng/ml, about 90 ng/ml to 100 ng/ml, about 1 ng/ml to 50 ng/ml, about 5 ng/ml to 50 ng/ml, about 10 ng/ml to 50 ng/ml, or about 20 ng/ml to 50 ng/ml.

60. The method of any one of claims 43-59, further comprising transducing the activated T cell with a viral vector.

61. The method of claim 62, wherein the viral vector is a retroviral vector expressing a T cell receptor (TCR).

62. The method of claim 60 or 61, wherein the viral vector is a lentiviral vector expressing a TCR.

63. The method of any one of claims 60-62, wherein the transducing is carried out in the presence of at least one cytokine.

64. The method of claim 63, wherein the at least one cytokine comprises interleukin 7 (IL-7) and/or interleukin 15 (IL-15).

65. The method of claim 64, wherein the concentration of IL-7 is no more than about 1 ng/ml, no more than about 2 ng/ml, no more than about 3 ng/ml, no more than about 4 ng/ml, no more than about 5 ng/ml, no more than about 6 ng/ml, no more than about 7 ng/ml, no more than about 8 ng/ml, no more than about 9 ng/ml, no more than about 10 ng/ml, no more than about 11 ng/ml, no more than about 12 ng/ml, no more than about 13 ng/ml, no more than about 14 ng/ml, no more than about 15 ng/ml, no more than about 16 ng/ml, no more than about 17 ng/ml, no more than about 18 ng/ml, no more than about 19 ng/ml, no more than about 20 ng/ml, no more than about 25 ng/ml, no more than about 30 ng/ml, no more than about 35 ng/ml, no more than about 40 ng/ml, no more than about 45 ng/ml, no more than about 50 ng/ml, no more than about 60 ng/ml, no more than about 70 ng/ml, no more than about 80 ng/ml, no more than about 90 ng/ml, or no more than about 100 ng/ml.

66. The method of claim 64, wherein the concentration of IL-7 is from about 1 ng/ml to 100 ng/ml, about 1 ng/ml to 90 ng/ml, about 1 ng/ml to 80 ng/ml, about 1 ng/ml to 70 ng/ml, about 1 ng/ml to 60 ng/ml, about 1 ng/ml to 50 ng/ml, about 1 ng/ml to 40 ng/ml, about 1 ng/ml to 30 ng/ml, about 1 ng/ml to 20 ng/ml, about 1 ng/ml to 15 ng/ml, or about 1 ng/ml to 10 ng/ml.

67. The method of any one of claims 64-66, wherein the concentration of IL-15 is no more than about 5 ng/ml, no more than about 10 ng/ml, no more than about 15 ng/ml, no more than about 20 ng/ml, no more than about 25 ng/ml, no more than about 30 ng/ml, no more than about 35 ng/ml, no more than about 40 ng/ml, no more than about 45 ng/ml, no more

than about 50 ng/ml, no more than about 60 ng/ml, no more than about 70 ng/ml, no more than about 80 ng/ml, no more than about 90 ng/ml, no more than about 100 ng/ml, no more than about 110 ng/ml, no more than about 120 ng/ml, no more than about 130 ng/ml, no more than about 140 ng/ml, no more than about 150 ng/ml, no more than about 200 ng/ml, no more than about 250 ng/ml, no more than about 300 ng/ml, no more than about 350 ng/ml, no more than about 400 ng/ml, no more than about 450 ng/ml, or no more than about 500 ng/ml.

68. The method of anyone of claims 64-66, wherein the concentration of IL-15 is from about 5 ng/ml to 500 ng/ml, about 5 ng/ml to 400 ng/ml, about 5 ng/ml to 300 ng/ml, about 5 ng/ml to 200 ng/ml, about 5 ng/ml to 150 ng/ml, about 5 ng/ml to 100 ng/ml, about 10 ng/ml to 100 ng/ml, about 20 ng/ml to 100 ng/ml, about 30 ng/ml to 100 ng/ml, about 40 ng/ml to 100 ng/ml, or about 50 ng/ml to 100 ng/ml..

69. A T cell population prepared by the method of any one of claims 43-68.

70. The method of any one of claims 1-38 and 43-68, wherein the expanding is carried out in the presence of at least one cytokine selected from the group consisting of IL-2, IL-7, IL-12, IL-15, and IL-21.

71. The method of claim 70, wherein the at least one cytokine comprises IL-2.

72. The method of claim 70, wherein the at least one cytokine comprises IL-7 and IL-15.

73. A method of preparing a T cell population, comprising

thawing peripheral blood mononuclear cells (PBMC),

resting the thawed PBMC for about 4 to about 6 hours,

activating the T cell in the rested PBMC with an anti-CD3 antibody and an anti-CD28 antibody,

transducing the activated T cell with a viral vector,

expanding the transduced T cell, and

obtaining the expanded T cell.

74. The method of claim 73, wherein the obtained T cells are expanded from the group consisting of about 2 fold to 5 fold, about 5 fold to about 10 fold, about 5 fold to about 15 fold, about 5 fold to about 20 fold, about 5 fold to about 25 fold, about 25 fold to about 50 fold, about 50 fold to about 60 fold, about 60 fold to about 100 fold, about 65 fold to about 90 fold, about 70 fold to about 85 fold, and about 67-fold to about 88-fold.

75. The method of claim 73, wherein the fold expansion of obtained T cells with a resting time of about 4 to about 6 hours is at least about 1.5 fold more than that of obtained T cells prepared under identical conditions with a resting time of about 16 to about 20 hours.

76. The method of claim 73, wherein the number of the obtained T cells is selected from the group consisting of from about 2 x 10^9 to about 5 x 10^9, about 5 x 10^9 to about 10 x 10^9, about 10 x 10^9 to about 15 x 10^9, about 5 x 10^9 to about 35 x 10^9, about 5 x 10^9 to about 30 x 10^9, about 10 x 10^9 to about 30 x 10^9, about 15 x 10^9 to about 20 x 10^9, about 20 x 10^9 to about 35 x 10^9, about 24 x 10^9 to about 33 x 10^9, and about 24.8 x 10^9 to about 32.2 x 10^9.

77. The method of claim 73, wherein number of obtained T cells with a resting time of about 4 hours to about 6 hours is about 1.5 to about 2.0 greater than that of the obtained T cells prepared under identical conditions with a resting time of about 16 hours to 20 hours.

78. The method of any one of claims 1-38, 43-68, and 70-77, wherein the thawing, the resting, the activating, the transducing, the expanding, and the obtaining are performed in a closed system.

79. The method of claim 78, wherein the closed system is CliniMACS Prodigy™, WAVE (XURI™) Bioreactor, WAVE (XURI™) Bioreactor in combination with BioSafe Sepax™ II, G-Rex/GatheRex™ closed system, or G-Rex/GatheRex™ closed system in combination with BioSafe Sepax™ II.

80. A method of preparing a T cell population, comprising

obtaining fresh peripheral blood mononuclear cells (PBMC),

activating the T cell in the fresh PBMC with an anti-CD3 antibody and an anti-CD28 antibody,

transducing the activated T cell with a viral vector,

expanding the transduced T cell, and

harvesting the expanded T cell.

81. The method of claim 80, wherein the expanding is performed for from about 1 day to 2 days, from about 1 day to 3 days, from about 1 day to about 4 days, from about 1 day to about 5 days, from about 1 day to 6 days, from about 1 day to 7 days, from about 1 day to 8 days, from about 1 day to 9 days, from about 1 day to 10 days, from about 2 days to 3 days, from about 2 days to 4 days, from about 2 days to 5 days, from about 2 days to 6 days, from about 2 days to 7 days, from about 2 days to 8 days, from about 2 days to 9 days, from about 2 days to 10 days, from about 3 days to 4 days, from about 3 days to 5 days, from about 3 days to 6 days, from about 3 days to 7 days, from about 3 days to 8 days, from about 3 days to 9 days, from about 3 days to 10 days, from about 4 days to 5 days, from about 4 days to 6 days, from about 4 days to 7 days, from about 4 days to 8 days, from about 4 days to 9 days, from about 4 days to 10 days, from about 5 days to 6 days, from about 5 days to 7 days, from about 5 days to 8 days, from about 5 days to 9 days, or from about 5 days to 10 days.

82. The method of claim 80, wherein the harvesting is performed after the activating within from about 4 days to about 12 days, from about 4 days to about 11 days, from about 4 days to about 10 days, from about 4 days to about 9 days, from about 4 days to about 8 days, from about 4 days to about 7 days, from about 4 days to about 6 days, from about 4 days to about 5 days, from about 5 days to about 12 days, from about 5 days to about 11 days, from about 5 days to about 10 days, from about 5 days to about 9 days, from about 5 days to about 8 days, from about 5 days to about 7 days, or from about 5 days to about 6 days.

83. The method of any one of claims 80-82, wherein the number of the harvested T cells is selected from the group consisting of from about 2 x 10^9 to about 5 x 10^9, about 5 x 10^9 to about 10 x 10^9, about 10 x 10^9 to about 15 x 10^9, about 5 x 10^9 to about 35 x 10^9, about 5 x 10^9 to about 30 x 10^9, about 10 x 10^9 to about 30 x 10^9, about 15 x 10^9 to about 20 x 10^9,

about 20 x 10^9 to about 35 x 10^9, about 24 x 10^9 to about 33 x 10^9, and about 24.8 x 10^9 to about 32.2 x 10^9.

84. The method of any one of claims 80-83, wherein the activating, the transducing, the expanding, and the harvesting are performed in a closed system.

85. The method of claim 84, wherein the closed system is CliniMACS Prodigy™, WAVE (XURI™) Bioreactor, WAVE (XURI™) Bioreactor in combination with BioSafe Sepax™ II, G-Rex/GatheRex™ closed system, or G-Rex/GatheRex™ closed system in combination with BioSafe Sepax™ II.

86. The method of any one of claims 80-85, wherein the fresh PBMC is not obtained by thawing a cryopreserved PBMC.

87. The method of any one of claims 80-86, wherein the obtaining and the activating are performed for no more than 1 day.

88. The method of any one of claims 80-87, wherein the expanding is performed for more than 1 day.