CLAIMS

What is claimed is:

1. A method of transducing T cells for immunotherapy, comprising

(a) obtaining T cells from at least one individual,

(b) activating the T cells with an anti-CD3 antibody and an anti-CD28 antibody, transducing the activated T cells with a viral vector at a plurality of volumetric concentrations,

(c) expanding the transduced T cells,

(d) measuring a quantity of the expanded T cells that express the transgene and/or a copy number of integrated transgene in each of the expanded T cells at the plurality of volumetric concentrations,

(e) identifying the volumetric concentration that yields a maximum average of the quantity of the expanded T cells that express the transgene and/or a maximum average of the copy number of the integrated transgene without exceeding five copies of the integrated transgene in each of the expanded T cells from the at least one individual, and

(f) transducing T cells obtained from the at least one individual with the viral vector at the identified volumetric concentration for the immunotherapy.

2. The method of claim 1, wherein the viral vector is a retroviral vector expressing a T cell receptor (TCR).

3. The method of claim 1 or 2, wherein the viral vector is a lentiviral vector expressing a TCR.

4. The method of any one of claims 1-3, wherein the plurality of volumetric concentrations is selected from the group consisting of about 0.01 µl to about 1ml per 0.5 ml of a transduction mixture, about 0.01 µl to about 1 ml per 1.0 ml of a transduction mixture, about 0.01 µl to about 1 ml per 2.5 ml of transduction mixture, and about 0.01 µl to about 1 ml per 5 ml of transduction mixture.

5. The method of claim 4, wherein the transduction mixture comprises a cell concentration of from about 0.1 x 10^6 cells/ml to about 1.0 x 10^6 cells/ml, from about 0.5 x 10^6 cells/ml to about 1.0 x 10^6 cells/ml, from about 1.0 x 10^6 cells/ml to about 1.0 x 10^7 cells/ml, from about 5.0 x 10^6 cells/ml to about 1.0 x 10^7 cells/ml, from about 1.0 x 10^7 cells/ml to about 1.0 x 10^8 cells/ml, or from about 5.0 x 10^7 cells/ml to about 1.0 x 10^8 cells/ml.

6. The method of any one of claims 1 – 5, wherein the T cells are obtained from a plurality of healthy individuals.

7. The method of any one of claims 1 – 6 comprising expanding the transduced T cells.

8. The method of any one of claims 1 – 7, wherein the identifying the volumetric concentration that yields a maximum average of the quantity of the transduced T cells that express the transgene in the expanded T cells from the at least one individual.

9. The method of any one of claims 1 – 8, wherein the identifying the volumetric concentration that yields a maximum average copy number of integrated transgene in the expanded T cells from at least one individual.

10. The method of any one of claims 1-9, wherein the identified volumetric concentration is selected from the group consisting of about 1 µl to about 50 µl per 0.5 ml of a transduction mixture, about 5 µl to about 15 µl per 0.5 ml of a transduction mixture, and about 8 µl to about 12 µl per 0.5 ml of a transduction mixture.

11. The method of any one of claims 1-10, wherein the individual to be treated is a cancer patient.

12. The method of claim 11, 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).

13. The method of any one of claims 1-12, wherein the T cells obtained from the at least one donor, patient, or individual are $CD8^+$ T cells.

14. A method of treating a patient, comprising administering to the patient T cells transduced with a viral vector at a volumetric concentration,

wherein the T cells are obtained from the patient,

wherein the volumetric concentration is determined by

(a) obtaining T cells from a plurality of healthy donors,

(b) activating the T cells obtained from step (a) with an anti-CD3 antibody and an anti-CD28 antibody, transducing the activated T cells with a viral vector at a plurality of volumetric concentrations,

(c) expanding the transduced T cells obtained from step (b),

(d) measuring a quantity of the expanded T cells that express the transgene and/or a copy number of integrated transgene in each of the expanded T cells obtained from step (c) at the plurality of volumetric concentrations,

(e) identifying the volumetric concentration that yields a maximum average of the quantity of the expanded T cells that express the transgene and/or a maximum average of the copy number of the integrated transgene without exceeding five copies of integrated transgene measured by step (d), and

(f) transducing the T cells obtained from the patient with the viral vector at the volumetric concentration identified by step (e).

15. The method of claim 14, wherein the viral vector is a retroviral vector expressing a T cell receptor (TCR).

16. The method of claims 14 or 15, wherein the viral vector is a lentiviral vector expressing a TCR.

17. The method of any one of claims 14 – 16, wherein the plurality of volumetric concentrations is selected from the group consisting of about 0.01 µl to about 1 ml per 0.5 ml of a transduction mixture, about 0.01 µl to about 1 ml per 1.0 ml of a transduction mixture, about 0.01 µl to about 1 ml per 2.5 ml of transduction mixture, and about 0.01 µl to about 1 ml per 5 ml of transduction mixture.

18. The method of claim 17, wherein the transduction mixture comprises a cell concentration of from about 0.1 x 10^6 cells/ml to about 1.0 x 10^6 cells/ml, from about 0.5 x 10^6 cells/ml to about 1.0 x 10^6 cells/ml, from about 1.0 x 10^6 cells/ml to about 1.0 x 10^7 cells/ml, from about 5.0 x 10^6 cells/ml to about 1.0 x 10^7 cells/ml, from about 1.0 x 10^7 cells/ml to about 1.0 x 10^8 cells/ml, or from about 5.0 x 10^7 cells/ml to about 1.0 x 10^8 cells/ml.

19. The method of any one of claims 14 – 18, wherein the identifying the volumetric concentration that yields a maximum average of the quantity of the transduced T cells that express the transgene in the expanded T cells from the plurality of donors.

20. The method of any one of claims 14 – 19, wherein the identifying the volumetric concentration that yields a maximum average copy number of integrated transgene in the expanded T cells from the plurality of donors.

21. The method of any one of claims 15 – 20, wherein the identified volumetric concentration is selected from the group consisting of about 1 µl to about 50 µl per 0.5 ml of a transduction mixture, about 5 µl to about 15 µl per 0.5 ml of a transduction mixture, and about 8 µl to about 12 µl per 0.5 ml of a transduction mixture.

22. The method of any one of claims 14 – 21, wherein the patient has cancer.

23. The method of claim 22, 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).

24. The method of any one of claims 14 – 23, wherein the T cells obtained both from the plurality of healthy donors and the patient are $CD8^+$ T cells.

25. T cells transduced with a viral vector at a volumetric concentration for immunotherapy,
wherein the T cells are obtained from a patient,
wherein the volumetric concentration is determined by
(a) obtaining T cells from a plurality of healthy donors,
(b) activating the T cells obtained from step (a) with an anti-CD3 antibody and an anti-CD28 antibody, transducing the activated T cells with a viral vector at a plurality of volumetric concentrations,
(c) expanding the transduced T cells obtained from step (b),
(d) measuring a quantity of the expanded T cells that express the transgene and/or a copy number of integrated transgene in each of the expanded T cells obtained from step (c) at the plurality of volumetric concentrations,
(e) identifying the volumetric concentration that yields a maximum average of the quantity of the expanded T cells that express the transgene and/or a maximum average of the copy number of the integrated transgene

without exceeding five copies of integrated transgene measured by step (d), and

(f) transducing the T cells obtained from the patient with the viral vector at the volumetric concentration identified by step (e).

26. The T cells of claim 25, wherein the viral vector is a retroviral vector expressing a T cell receptor (TCR).

27. The T cells of claims 25 or 26, wherein the viral vector is a lentiviral vector expressing a TCR.

28. The T cells of any one of claims 25 – 27, wherein the plurality of volumetric concentrations is selected from the group consisting of about 0.01 µl to about 1 ml per 0.5 ml of a transduction mixture, about 0.01 µl to about 1 ml per 1.0 ml of a transduction mixture, about 0.01 µl to about 1 ml per 2.5 ml of transduction mixture, and about 0.01 µl to about 1 ml per 5 ml of transduction mixture.

29. The method of claim 28, wherein the transduction mixture comprises a cell concentration of from about 0.1 x 10^6 cells/ml to about 1.0 x 10^6 cells/ml, from about 0.5 x 10^6 cells/ml to about 1.0 x 10^6 cells/ml, from about 1.0 x 10^6 cells/ml to about 1.0 x 10^7 cells/ml, from about 5.0 x 10^6 cells/ml to about 1.0 x 10^7 cells/ml, from about 1.0 x 10^7 cells/ml to about 1.0 x 10^8 cells/ml, or from about 5.0 x 10^7 cells/ml to about 1.0 x 10^8 cells/ml.

30. The T cells of any one of claims 25 – 29, wherein the identifying comprises identifying the volumetric concentration that yields a maximum average of the quantity of the transduced T cells that express the transgene in the expanded T cells from the plurality of healthy donors.

31. The T cells of any one of claims 25 – 29, wherein the identifying comprises identifying the volumetric concentration that yields a maximum average copy number of integrated transgene in the expanded T cells from the plurality of healthy donors.

32. The T cells of any one of claims 25 – 31, wherein the identified volumetric concentration is selected from the group consisting of about 1 µl to about 50 µl per 0.5 ml of a transduction mixture, about 5 µl to about 15 µl per 0.5 ml of a transduction mixture, and about 8 µl to about 12 µl per 0.5 ml of a transduction mixture.

33. The T cells of any one of claims 25 – 32, wherein the patient has cancer.

34. The T cells of claim 33, 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).

35. A population of T cells produced by the method of any one of claims 1 – 13.

36. A population of T cells produced by the method of any one of claims 14 – 24.

37. The T cells of claim 25, wherein the expanding is in the presence of IL-7, IL-10, IL-12, IL-15, and IL-21, provided that the expanding is not in the presence of IL-2 alone, IL-7 alone, a combination of IL-2, IL-7, and IL-15, or a combination of IL-2 and IL-7.

38. The T cells of claim 37, wherein the transduced T cells exhibit a phenotype of CD45RA+CCR7+ or CD45RA+CCR7+CD62L+ or CD45RO-CCR7+ or CD45RO-CCR7+CD62L+

39. The T cells of claim 37, wherein the transduced T cells exhibit a phenotype of CD28+CD27+.