CLAIMS

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

(a) obtaining T cells from a patient,

(b) activating the T cells with an anti-CD3 antibody and an anti-CD28 antibody,

(c) transducing the activated T cells with a lentiviral vector expressing a transgene at a volumetric concentration,

(d) expanding the transduced T cells,

wherein the volumetric concentration of the lentiviral vector is determined by

(i) obtaining primary T cells from at least one healthy individual;

(ii) activating T cells obtained in step (i) with an anti-CD3 antibody and an anti-CD28 antibody;

(iii) transducing the activated T cells of step (ii) with a transducing mixture comprising a cell concentration of 0.1×10^6 cells/ml to 1.0×10^8 cells/ml and the lentiviral vector at a plurality of volumetric concentrations;

(iv) expanding the transduced T cells of step (iii); and

(v) 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 integrated transgene without exceeding five copies of the integrated transgene in each of the expanded T cells from the at least one healthy individual

(e) transducing T cells obtained from the patient with the lentiviral vector at the identified volumetric concentration for the immunotherapy.

2. The method of claim 1, wherein the transgene expresses a T cell receptor (TCR).

3. The method of any one of claims 1-2, wherein the plurality of volumetric concentrations is selected from the group consisting of 0.01 µl to 1ml per 0.5 ml of a transduction mixture, 0.01 µl to 1 ml per 1.0 ml of a transduction mixture, 0.01 µl to 1 ml per 2.5 ml of transduction mixture, and 0.01 µl to 1 ml per 5 ml of transduction mixture.

4. The method of claim 3, wherein the transduction mixture comprises a cell concentration of from 0.1 x 10^6 cells/ml to 1.0 x 10^6 cells/ml, from 0.5 x 10^6 cells/ml to 1.0 x 10^6 cells/ml, from 1.0 x 10^6 cells/ml to 1.0 x 10^7 cells/ml, from 5.0 x 10^6 cells/ml to 1.0 x 10^7 cells/ml, from 1.0 x 10^7 cells/ml to 1.0 x 10^8 cells/ml, or from 5.0 x 10^7 cells/ml to 1.0 x 10^8 cells/ml.

5. The method of any one of claims 1 – **4**, 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 at least one individual.

6. The method of any one of claims 1 – 5, wherein the identifying comprises identifying the volumetric concentration that yields a maximum average copy number of integrated transgene in the expanded T cells from at least one individual.

7. The method of any one of claims 1-6, wherein the identified volumetric concentration is selected from the group consisting of 1 µl to 50 µl per 0.5 ml of a transduction mixture, 5 µl to 15 µl per 0.5 ml of a transduction mixture, and 8 µl to 12 µl per 0.5 ml of a transduction mixture.

8. The method of any one of claims 1-7, wherein the patient has cancer.

9. The method of claim 8, 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).

10. The method of any one of claims 1-9, wherein the T cells obtained from the patient and the at least one healthy individual are $CD8^+$ T cells.

11. Use of T cells transduced with a lentiviral vector at a volumetric concentration, in the manufacture of a medicament for the treatment of cancer,

wherein the T cells are obtained from a patient,

wherein the volumetric concentration is determined by

(a) obtaining primary 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,

(c) transducing the activated T cells with a transducing mixture comprising a cell concentration of 0.1×10^6 cells/ml to 1.0×10^8 cells/ml and a lentiviral vector expressing a transgene at a plurality of volumetric concentrations,

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

(e) identifying the volumetric concentration of the lentiviral vector 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 integrated transgene without exceeding five copies of the integrated transgene in each of the expanded T cells from the plurality of healthy donors, and

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

12. The use of claim 11, wherein the transgene expresses a T cell receptor (TCR).

13. The use of any one of claims 11 – 12, wherein the plurality of volumetric concentrations is selected from the group consisting of 0.01 µl to 1 ml per 0.5 ml of a transduction mixture, 0.01 µl to 1 ml per 1.0 ml of a transduction mixture, 0.01 µl to 1 ml per 2.5 ml of transduction mixture, and 0.01 µl to 1 ml per 5 ml of transduction mixture.

14. The use of claim 13, wherein the transduction mixture comprises a cell concentration of from 0.1 x 10^6 cells/ml to 1.0 x 10^6 cells/ml, from 0.5 x 10^6 cells/ml to 1.0 x 10^6 cells/ml, from 1.0 x 10^6 cells/ml to 1.0 x 10^7 cells/ml, from 5.0 x 10^6 cells/ml to 1.0 x 10^7 cells/ml, from 1.0 x 10^7 cells/ml to 1.0 x 10^8 cells/ml, or from 5.0 x 10^7 cells/ml to 1.0 x 10^8 cells/ml.

15. The use of any one of claims 11 – 14, 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 donors.

16. The use of any one of claims 11 – 15, wherein the identifying comprises identifying the volumetric concentration that yields a maximum average copy number of transgene in the expanded T cells from the plurality of donors.

17. The use of any one of claims 12 – 16, wherein the identified volumetric concentration is selected from the group consisting of 1 µl to 50 µl per 0.5 ml of a transduction mixture, 5 µl to 15 µl per 0.5 ml of a transduction mixture, and 8 µl to 12 µl per 0.5 ml of a transduction mixture.

18. The use of any one of claims 11 - 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).

19. The use of any one of claims 11 – 18, wherein the T cells obtained both from the plurality of healthy donors and the patient are $CD8^+$ T cells.