cell population comprising a third NeoTCR that binds a third neoantigen; wherein each NeoTCR is different from the others, and wherein each NeoTCR is derived from a patient.

In certain examples, the first, second, and/or third neoantigens are expressed by a single gene. In certain examples, the first, second, and/or third neoantigens are expressed by different genes. In certain examples, two of the first, second, and/or third neoantigens are expressed by a single gene. In certain examples, the first, second, and/or third NeoTCRs bind to a single major histocompatibility complex. In certain examples, the first, second, and/or third NeoTCRs bind to different major histocompatibility complexes. In certain examples, two of the first, second, and/or third NeoTCRs bind to a single major histocompatibility complex.

In certain examples, the composition comprises a pharmaceutically acceptable carrier. In certain examples, the composition comprises a cryopreservation agent. In certain examples, the composition comprises serum albumin. In certain examples, the composition comprises a crystalloid solution. In certain examples, the composition comprises Plasma-Lyte A, human serum album (HAS), and CryoStor® CS10.

The present disclosure provides a method of treating a cancer in a subject in need thereof, comprising administering a composition disclosed herein.

In certain examples, the composition comprises an amount of NeoTCR Cells of about 4 x 10^8 cells, 1.33 x 10^9 cells, or about 4 x 10^9 cells. In certain examples, the composition comprises an amount of NeoTCR cells greater than about 4 x 10^8 cells and less than about 1.33 x 10^9 cells, greater than about 1.33 x 10^9 cells and less than about 4 x 10^9 cells, or greater than about 4 x 10^9 cells. In certain examples, the composition comprises an amount of NeoTCR cells according to Table 4. In certain examples, the composition comprises an amount of NeoTCR cells according to Table 5. In certain examples, the composition is administered in a single dose. In certain examples, the composition is administered in multiple doses.

In certain examples, the method further comprises administering a combination agent. In certain examples, the combination agent is a cytokine, a PD-axis binding agent, a PD-1 binding agent, a PD-L1 binding agent, a PD-L2 binding agent, or a combination thereof. In certain examples, the cytokine is an IL-2 agent, an IL-7 agent, an IL-10 agent, an IL-12 agent, an IL-15 agent, an IL-18 agent, an IL-21 agent, or a combination thereof. In certain examples, the cytokine is an IL-2 agent. In certain examples, the cytokine is an IL-15 agent. In certain examples, the PD-axis binding agent comprises nivolumab, pembrolizumab, or atezolizumab.

In certain examples, the cancer is a liquid cancer or a solid cancer. In certain examples, the composition is administered following a pretreatment regime of fludarabine and cyclophosphamide.

The present disclosure provides a method of manufacturing a composition disclosed herein. The present disclosure also provides a kit for the administration of a composition disclosed herein.

The present disclosure provides a plurality of cells comprising a first modified cell comprising a first exogenous polynucleotide encoding a first NeoTCR binding a first antigen, wherein the first exogenous polynucleotide is integrated in an endogenous TRAC and/or TRBC locus of the first modified cell; a second modified cell comprising a second exogenous polynucleotide encoding a second NeoTCR binding a second antigen, wherein the second exogenous polynucleotide is integrated in an endogenous TRAC and/or TRBC locus of the second modified cell; a third modified cell comprising a third exogenous polynucleotide encoding a third NeoTCR binding a third antigen, wherein the third exogenous polynucleotide is integrated in an endogenous TRAC and/or TRBC locus of the third modified cell; or a combination thereof; and wherein the first, second and third NeoTCRs are patient derived and wherein the first, second and third modified cells are patient-derived primary cells.

In certain examples, the first, second, and third antigens are cancer antigens. In certain examples, the cancer antigens are neoantigens. In certain examples, the primary cells are lymphocytes. In certain examples, the primary cells are T cells, optionally wherein the T cells are: a) CD45RA+, CD62L+, CD28+, CD95-, CCR7+, and CD27+; b) CD45RA+, CD62L+, CD28+, CD95+, CD27+, CCR7+; or c) CD45RO+, CD62L+, CD28+, CD95+, CCR7+, CD27+, CD127+.

The present disclosure provides a composition comprising the plurality of cells disclosed herein. In certain examples, the composition further comprises a pharmaceutically acceptable excipient. In certain examples, the composition is administered to a patient in need thereof for the treatment of cancer.

In certain examples, the composition comprises a cryopreservation agent. In certain examples, the composition comprises serum albumin. In certain examples, the composition comprises a crystalloid solution. In certain examples, the composition comprises Plasma-Lyte A, human serum album (HAS), and CryoStor® CS10.

The present disclosure provides a method of treating a cancer in a subject in need thereof, the method comprising administering a plurality of cells, comprising: i) a first modified cell comprising a first exogenous polynucleotide encoding a first NeoTCR binding a first antigen, wherein the first exogenous polynucleotide is integrated in an endogenous TRAC

and/or TRBC locus of the first modified cell; ii) a second modified cell comprising a second exogenous polynucleotide encoding a second NeoTCR binding a second antigen, wherein the second exogenous polynucleotide is integrated in an endogenous TRAC and/or TRBC locus of the second modified cell; iii) a third modified cell comprising a third exogenous polynucleotide encoding a third NeoTCR binding a third antigen, wherein the third exogenous polynucleotide is integrated in an endogenous TRAC and/or TRBC locus of the third modified cell; or iv) a combination thereof; wherein the first, second and third NeoTCRs are patient derived and wherein the first, second and third modified cells are patient derived primary cells, thereby treating the cancer in the subject.

In certain examples, the first, second, and third antigens are cancer antigens. In certain examples, wherein the cancer antigens are neoantigens. In certain examples, the primary cells are lymphocytes. In certain examples, the primary cells are T cells, optionally wherein the T cells are: a) CD45RA+, CD62L+, CD28+, CD95-, CCR7+, and CD27+; b) CD45RA+, CD62L+, CD28+, CD95+, CD27+, CCR7+; or c) CD45RO+, CD62L+, CD28+, CD95+, CCR7+, CD27+, CD127+.

In certain examples, the method further comprises administering a combination agent. In certain examples, the combination agent is a cytokine, a PD-axis binding agent, a PD-1 binding agent, a PD-L1 binding agent, a PD-L2 binding agent, or a combination thereof. In certain examples, the cytokine is an IL-2 agent, an IL-7 agent, an IL-10 agent, an IL-12 agent, an IL-15 agent, an IL-18 agent, an IL-21 agent, or a combination thereof. In certain examples, the cytokine is an IL-2 agent. In certain examples, the cytokine is an IL-15 agent. In certain examples, the PD-axis binding agent comprises nivolumab, pembrolizumab, or atezolizumab. In certain examples, the cancer is a liquid cancer or a solid cancer.

The present disclosure provides a method of treating a cancer in a subject in need thereof, the method comprising: a) administering an effective amount of a modified cell comprising an exogenous polynucleotide encoding a NeoTCR binding a tumor antigen, wherein the exogenous polynucleotide is integrated in an endogenous TRAC and/or TRBC locus of the modified cell; and b) administering an effective amount of a combination agent; thereby treating the cancer in the subject.

In certain examples, the combination agent comprises a chemotherapeutic agent, an anti-hormonal agent, an endocrine therapeutic, a cytotoxic agent, a cytokine, a PD-axis binding agent, a PD-1 binding agent, a PD-L1 binding agent, a PD-L2 binding agent, or a combination thereof. In certain examples, the cytokine is an IL-2 agent, an IL-7 agent, an IL-10 agent, an IL-12 agent, an IL-15 agent, an IL-18 agent, an IL-21 agent, or a combination thereof. In certain

examples, the cytokine is an IL-2 agent. In certain examples, the cytokine is an IL-15 agent. In certain examples, the PD-axis binding agent comprises nivolumab, pembrolizumab, or atezolizumab. In certain examples, the cancer is a liquid cancer or a solid cancer.

The present disclosure provides a use of the composition as described herein in the manufacture of a medicament for treating a cancer in a subject in need thereof, wherein the composition is to be administered to the subject.The present disclosure provides a use of a plurality of cells in the manufacture of a medicamentfor treating a cancer in a subject in need thereof wherein the plurality of cells is to be administered to the subject, the plurality of cells, comprising:

i. a first modified cell comprising a first exogenous polynucleotide encoding a first NeoTCR binding a first antigen, wherein the first exogenous polynucleotide is integrated in an endogenous TRAC and/or TRBC locus of the first modified cell;

ii. a second modified cell comprising a second exogenous polynucleotide encoding a second NeoTCR binding a second antigen, wherein the second exogenous polynucleotide is integrated in an endogenous TRAC and/or TRBC locus of the second modified cell;

iii. a third modified cell comprising a third exogenous polynucleotide encoding a third NeoTCR binding a third antigen, wherein the third exogenous polynucleotide is integrated in an endogenous TRAC and/or TRBC locus of the third modified cell; or

iv. a combination thereof;

wherein the first, second and third NeoTCRs are patient derived and wherein the first, second and third modified cells are patient derived primary cells.

The present disclosure provides a use of

a. an effective amount of a modified cell comprising an exogenous polynucleotide encoding a NeoTCR binding a tumor antigen, wherein the exogenous polynucleotide is integrated in an endogenous TRAC and/or TRBC locus of the modified cell in the manufacture of a first medicament; and

b. an effective amount of a combination agent in the manufacture of a second medicament;

wherein the first and second medicaments are to be used in combination therapy to treat cancer in a subject in need thereof.

The present disclosure provides the composition as described herein, or the plurality of cells of as described herein, or an effective amount of a modified cell comprising an exogenous polynucleotide encoding a NeoTCR binding a tumor antigen, wherein the exogenous

polynucleotide is integrated in an endogenous TRAC and/or TRBC locus of the modified cell and an effective amount of a combination agent, for use in therapy.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows representative images obtained with time-lapse live microscopy (Days 0, 1, and 2) show antigen-specific cytotoxic activity and proliferation by neo12-TCR T cells (a representative NeoTCR Product) co-cultured with target T cells expressing cognate neo12 peptide-HLA (right column), but not when co-cultured with target T cells expressing an irrelevant peptide (left column). Neo12 is a NeoTCR that was identified through tumor and blood screening and later precision genome engineered into T cells and made into a NeoTCR Product. Tumor cells used in these experiments expressed a variant of green fluorescent protein (GFP or ZsGreen) in a stable and homogeneous manner. These cells exhibit high uniform green fluorescence that allows identification of the cells by fluorescent microscopy. To detect real time apoptosis, a highly-selective phosphatidylserine cyanine fluorescent dye (IncuCyte Annexin V in red) was added to the co-culture. Tumor cells not displaying the appropriate antigen continue to grow in the presence of neo12-TCR T cells (left column). In contrast, the majority of neo12 antigen displaying tumor cells are apoptotic or dead within 2 days (right column). T cells are not labeled in this experiment, but antigen-specific proliferation can be appreciated visually by increased numbers of T cells over the course of 2 days (right column). Raw microscopy and microarray scans of the cell samples (loaded onto the single-cell barcode chip) and protein secretion data were analyzed using Isoplexis image processing software to determine the locations of chambers containing single cells and subsequently extract their secretion readouts. Data from empty cell chambers were used to measure the background intensity levels for each analyzed protein. Single-cell readouts were then normalized using the background readouts to determine significant secretions and compare profiles across assays. Isoplexis proprietary software and the R statistical package were used for statistical data analysis. T cells used in this study were generated from a healthy donor. Edited T cells were incubated with target tumor cells at a ratio of 2:1 prior to loading onto the IsoPlexis platform for effector protein secretion profile analysis.

Figure 2 shows graphs showing the percentage of CD4 and CD8 T cells engineered to either express neo12 or F5 TCR, that secrete 2, 3, 4 or greater than- equal to 5 cytokines (shades of grey) when encountering cognate antigen. Cells were co-cultured with target T cells pulsed with no peptide, 10nM or 100nM specific peptide or with target T cells constitutively expressing

CLAIMS

1. A composition comprising:

    a. a first NeoTCR cell population comprising a first NeoTCR that binds a first neoantigen;

    b. a first NeoTCR cell population comprising a first NeoTCR that binds a first neoantigen, and a second NeoTCR cell population comprising a second NeoTCR that binds a second neoantigen; or

    c. a first NeoTCR cell population comprising a first NeoTCR that binds a first neoantigen, a second NeoTCR cell population comprising a second NeoTCR that binds a second neoantigen, and a third NeoTCR cell population comprising a third NeoTCR that binds a third neoantigen;

    wherein each NeoTCR is different from the others, and

    wherein each NeoTCR is derived from a patient.

2. The composition of claim 1, wherein the first, second, and/or third neoantigens are expressed by a single gene.

3. The composition of claim 1, wherein the first, second, and/or third neoantigens are expressed by different genes.

4. The composition of claim 1, wherein two of the first, second, and/or third neoantigens are expressed by a single gene.

5. The composition of any one of claims 1-4, wherein the first, second, and/or third NeoTCRs bind to a single major histocompatibility complex.

6. The composition of any one of claims 1-4, wherein the first, second, and/or third NeoTCRs bind to different major histocompatibility complexes.

7. The composition of any one of claims 1-4, wherein two of the first, second, and/or third NeoTCRs bind to a single major histocompatibility complex.

8. The compositions of any one of claims 1-7, wherein the composition comprises a pharmaceutically acceptable carrier.

9. The composition of any one of claims 1-8, wherein the composition comprises a cryopreservation agent.

10. The composition of any one of claims 1-9, wherein the composition comprises serum albumin.

11. The composition of any one of claims 1-10, wherein the composition comprises a crystalloid solution.

12. The composition of any one of claims 1-11, wherein the composition comprises Plasma-Lyte A, human serum album (HAS), and CryoStor® CS10.

13. Use of the composition of any one of claims 1-12 in the manufacture of a medicament for treating a cancer in a subject in need thereof, wherein the composition is to be administered to the subject.

14. The use of claim 13, wherein the composition comprises an amount of NeoTCR Cells of about 4×10^8 cells, 1.33×10^9 cells, or about 4×10^9 cells.

15. The use of claim 14, wherein the composition comprises an amount of NeoTCR cells greater than about 4×10^8 cells and less than about 1.33×10^9 cells, greater than about 1.33×10^9 cells and less than about 4×10^9 cells, or greater than about 4×10^9 cells.

16. The use of any one of claims 13-15, wherein the composition comprises an amount of NeoTCR cells according to Table 4.

17. The use of any one of claims 13-15, wherein the composition comprises an amount of NeoTCR cells according to Table 5.

18. The use of any one of claims 13-17, wherein the composition is to be administered in a single dose.

19. The use of any one of claims 13-17, wherein the composition is to be administered in multiple doses.

20. The use of any one of claims 13-19, wherein a combination agent is to be further administered.

21. The use of claim 20, wherein the combination agent is a cytokine, a PD-axis binding agent, a PD-1 binding agent, a PD-L1 binding agent, a PD-L2 binding agent, or a combination thereof.

22. The use of claim 21, wherein the cytokine is an IL-2 agent, an IL-7 agent, an IL-10 agent, an IL-12 agent, an IL-15 agent, an IL-18 agent, an IL-21 agent, or a combination thereof.
23. The use of claim 20 or 21, wherein the cytokine is an IL-2 agent.
24. The use of claim 20 or 21, wherein the cytokine is an IL-15 agent.
25. The use of any one of claims 21-24, wherein the PD-axis binding agent comprises nivolumab, pembrolizumab, or atezolizumab.
26. The use of any one of claims 13-25, wherein the cancer is a liquid cancer or a solid cancer.
27. The use of any one of claims 13-25, wherein the composition is to be administered following a pretreatment regime of fludarabine and cyclophosphamide.
28. A method of manufacturing the composition of any one of claims 1-12.
29. A kit for the administration of the composition of any one of claims 1-12.
30. A plurality of cells comprising:
    a. a first modified cell comprising a first exogenous polynucleotide encoding a first NeoTCR binding a first antigen, wherein the first exogenous polynucleotide is integrated in an endogenous TRAC and/or TRBC locus of the first modified cell;
    b. a second modified cell comprising a second exogenous polynucleotide encoding a second NeoTCR binding a second antigen, wherein the second exogenous polynucleotide is integrated in an endogenous TRAC and/or TRBC locus of the second modified cell;
    c. a third modified cell comprising a third exogenous polynucleotide encoding a third NeoTCR binding a third antigen, wherein the third exogenous polynucleotide is integrated in an endogenous TRAC and/or TRBC locus of the third modified cell; or
    d. a combination thereof; and
    wherein the first, second and third NeoTCRs are patient derived and wherein the first, second and third modified cells are patient-derived primary cells.
31. The plurality of cells of claim 30, wherein the first, second, and third antigens are cancer antigens.
32. The plurality of cells of claim 31, wherein the cancer antigens are neoantigens.
33. The plurality of cells of any one of claims 30-32, wherein the primary cells are lymphocytes.
34. The plurality of cells of any one of claims 30-33, wherein the primary cells are T cells, optionally wherein the T cells are:

a. CD45RA+, CD62L+, CD28+, CD95-, CCR7+, and CD27+;
b. CD45RA+, CD62L+, CD28+, CD95+, CD27+, CCR7+; or
c. CD45RO+, CD62L+, CD28+, CD95+, CCR7+, CD27+, CD127+.

35. A composition comprising the plurality of cells of any one of claims 30-34.

36. The composition of claim 35, further comprising a pharmaceutically acceptable excipient.

37. Use of the composition of claim 35 or 36 in the manufacture of a medicament for the treatment of cancer, wherein the composition is to be administered to a patient in need thereof.

38. The composition of claim 35 or 36 or the use of claim 37, wherein the composition comprises a cryopreservation agent.

39. The composition of claim 35 or 36 or the use of claim 37 or 38, wherein the composition comprises serum albumin.

40. The composition of claim 35 or 36 or the use of any one of claims 37-39, wherein the composition comprises a crystalloid solution.

41. The composition of claim 35 or 36 or the use of any one of claims 37-40, wherein the composition comprises Plasma-Lyte A, human serum album (HAS), and CryoStor® CS10.

42. Use of a plurality of cells in the manufacture of a medicament for treating a cancer in a subject in need thereof wherein the plurality of cells is to be administered to the subject,

a. the plurality of cells, comprising:
    i. a first modified cell comprising a first exogenous polynucleotide encoding a first NeoTCR binding a first antigen, wherein the first exogenous polynucleotide is integrated in an endogenous TRAC and/or TRBC locus of the first modified cell;
    ii. a second modified cell comprising a second exogenous polynucleotide encoding a second NeoTCR binding a second antigen, wherein the second exogenous polynucleotide is integrated in an endogenous TRAC and/or TRBC locus of the second modified cell;
    iii. a third modified cell comprising a third exogenous polynucleotide encoding a third NeoTCR binding a third antigen, wherein the third exogenous polynucleotide is integrated in an endogenous TRAC and/or TRBC locus of the third modified cell; or
    iv. a combination thereof;

wherein the first, second and third NeoTCRs are patient derived and wherein the first, second and third modified cells are patient derived primary cells.

43. The use of claim 42, wherein the first, second, and third antigens are cancer antigens.

44. The use of claim 43, wherein the cancer antigens are neoantigens.

45. The use of any one of claims 42-44, wherein the primary cells are lymphocytes.

46. The use of claim 45, wherein the primary cells are T cells, optionally wherein the T cells are:

    a. CD45RA+, CD62L+, CD28+, CD95-, CCR7+, and CD27+;

    b. CD45RA+, CD62L+, CD28+, CD95+, CD27+, CCR7+; or

    c. CD45RO+, CD62L+, CD28+, CD95+, CCR7+, CD27+, CD127+.

47. The use of any one of claims 42-46, wherein a combination agent is to be further administered.

48. The use of claim 47, wherein the combination agent is a cytokine, a PD-axis binding agent, a PD-1 binding agent, a PD-L1 binding agent, a PD-L2 binding agent, or a combination thereof.

49. The use of claim 48, wherein the cytokine is an IL-2 agent, an IL-7 agent, an IL-10 agent, an IL-12 agent, an IL-15 agent, an IL-18 agent, an IL-21 agent, or a combination thereof.

50. The use of claim 47 or 48, wherein the cytokine is an IL-2 agent.

51. The use of claim 47 or 48, wherein the cytokine is an IL-15 agent.

52. The use of any one of claims 47-51, wherein the PD-axis binding agent comprises nivolumab, pembrolizumab, or atezolizumab.

53. The use of any one of claims 42-52, wherein the cancer is a liquid cancer or a solid cancer.

54. Use of

    a. an effective amount of a modified cell comprising an exogenous polynucleotide encoding a NeoTCR binding a tumor antigen, wherein the exogenous polynucleotide is integrated in an endogenous TRAC and/or TRBC locus of the modified cell in the manufacture of a first medicament; and

    b. an effective amount of a combination agent in the manufacture of a second medicament;

    wherein the first and second medicaments are to be used in combination therapy to treat cancer in a subject in need thereof.

55. The use of claim 54, wherein the combination agent comprises a chemotherapeutic agent, an anti-hormonal agent, an endocrine therapeutic, a cytotoxic agent, a cytokine, a PD-axis binding agent, a PD-1 binding agent, a PD-L1 binding agent, a PD-L2 binding agent, or a combination thereof.

56. The use of claim 55, wherein the cytokine is an IL-2 agent, an IL-7 agent, an IL-10 agent, an IL-12 agent, an IL-15 agent, an IL-18 agent, an IL-21 agent, or a combination thereof.

57. The use of claim 55 or 56, wherein the cytokine is an IL-2 agent.

58. The use of claim 55 or 56, wherein the cytokine is an IL-15 agent.

59. The use of any one of claims 54-58, wherein the PD-axis binding agent comprises nivolumab, pembrolizumab, or atezolizumab.

60. The use of any one of claims 54-60, wherein the cancer is a liquid cancer or a solid cancer.

61. The composition of any one of claims 1-12 or 35-41, or the plurality of cells of any one of claims 30-34, or an effective amount of a modified cell comprising an exogenous polynucleotide encoding a NeoTCR binding a tumor antigen, wherein the exogenous polynucleotide is integrated in an endogenous TRAC and/or TRBC locus of the modified cell and an effective amount of a combination agent, for use in therapy.