[0008a] In another aspect, the present disclosure refers to a method for expanding tumor infiltrating lymphocytes (TILs) into a modified therapeutic population of TILs comprising:
(a) obtaining a first population of TILs from a tumor resected from a patient by processing a tumor sample obtained from the patient into multiple tumor fragments;
(b) adding the tumor fragments into a closed system;
(c) performing a first expansion by culturing the first population of TILs in a cell culture medium comprising IL-2, and optionally OKT-3, to produce a second population of TILs, wherein the first expansion is performed in a closed container providing a first gas-permeable surface area, wherein the first expansion is performed within 12 days to obtain the second population of TILs, and wherein the transition from step (b) to step (c) occurs without opening the system;
(d) performing a second expansion by supplementing the cell culture medium of the second population of TILs with additional IL-2, OKT-3, and antigen presenting cells (APCs), to produce a third population of TILs, wherein the second expansion is performed within 12 days to obtain the third population of TILs, wherein the third population of TILs is a therapeutic population of TILs, wherein the second expansion is performed in a closed container providing a second gas-permeable surface area, and wherein the transition from step (c) to step (d) occurs without opening the system;
(e) exposing the second and/or third population of TILs to transcription factors (TFs) and/or other molecules capable of transiently altering protein expression, wherein the other molecules are selected from one or more sd-RNAs and one or more mRNAs and combinations thereof,wherein the TFs and/or other molecules capable of transiently altering protein expression provide for altered expression of tumor antigens and/or an alteration in the number of tumor antigen-specific T cells in the therapeutic population of TILs to produce a modified therapeutic population of TILs, wherein the transient alteration of protein expression targets a gene selected from the group consisting of PD-1, CTLA-4, TIGIT, LAG-3, TIM-3, CISH, CBLB and combinations thereof;
(f) harvesting the therapeutic population of TILs obtained from step (d), wherein the transition from step (e) to step (f) occurs without opening the system; and
(g) transferring the harvested TIL population from step (f) to an infusion bag, wherein the transfer from step (f) to (g) occurs without opening the system.

[0009] In some examples, the method further comprises performing an additional second expansion before or after step (iv) by supplementing the cell culture medium of the third population of TILs with additional IL-2, additional OKT-3, and additional APCs, wherein the

TILs is at least 50-fold greater in number than the first population of TILs, and wherein the transition from step (b) to step (c) occurs without opening the system;

(d) performing a second expansion by supplementing the cell culture medium of the second population of TILs with additional IL-2, OKT-3, and antigen presenting cells (APCs), to produce a third population of TILs, wherein the second expansion is performed for about 7-14 days to obtain the third population of TILs, wherein the third population of TILs is a therapeutic population of TILs, wherein the second expansion is performed in a closed container providing a second gas-permeable surface area, and wherein the transition from step (c) to step (d) occurs without opening the system;

(e) exposing the second and/or third population of TILs to transcription factors (TFs) and/or other molecules capable of transiently altering protein expression, wherein the TFs and/or other molecules capable of transiently altering protein expression provide for increased expression of tumor antigens and/or an increase in the number of tumor antigen-specific T cells in the therapeutic population of TILs;

(f) harvesting the therapeutic population of TILs obtained from step (d), wherein the transition from step (d) to step (e) occurs without opening the system; and

(g) transferring the harvested TIL population from step (e) to an infusion bag, wherein the transfer from step (e) to (f) occurs without opening the system; and

(h) optionally cryopreserving the infusion bag comprising the harvested TIL population from step (f) using a cryopreservation process.

[0096a] In another aspect, the present disclosure refers to use of an expanded population of tumor infiltrating lymphocytes (TILs) in the manufacture of a medicament for treating a subject with cancer, wherein the population of TILs comprises a therapeutically effective dosage of a modified therapeutic population of TILs from an infusion bag in step (g) of a method comprising:

(a) obtaining a first population of TILs from a tumor resected from a subject by processing a tumor sample obtained from the subject into multiple tumor fragments;
(b) adding the tumor fragments into a closed system;
(c) performing a first expansion by culturing the first population of TILs in a cell culture medium comprising IL-2, and optionally OKT-3, to produce a second population of TILs, wherein the first expansion is performed in a closed container providing a first gas-permeable surface area, wherein the first expansion is performed within 12 days to obtain the second population of TILs, and wherein the transition from step (b) to step (c) occurs without

opening the system;
(d) performing a second expansion by supplementing the cell culture medium of the second population of TILs with additional IL-2, OKT-3, and antigen presenting cells (APCs), to produce the third population of TILs, wherein the second expansion is performed within 12 days to obtain the third population of TILs, wherein the third population of TILs is a therapeutic population of TILs, wherein the second expansion is performed in a closed container providing a second gas-permeable surface area, and wherein the transition from step (c) to step (d) occurs without opening the system;
(e) exposing the second and/or third population of TILs to transcription factors (TFs) and/or other molecules capable of transiently altering protein expression, wherein the other molecules are selected from one or more sd-RNAs and one or more mRNAs and combinations thereof,wherein the TFs and/or other molecules capable of transiently altering protein expression provide for increased expression of tumor antigens and/or an increase in the number of tumor antigen-specific T cells in the therapeutic population of TILs to produce a modified therapeutic population of TILs, wherein the transient alteration of protein expression targets a gene selected from the group consisting of PD-1, CTLA-4, TIGIT, LAG-3, TIM-3, CISH, CBLB and combinations thereof;
(f) harvesting the modified therapeutic population of TILs obtained from step (e), wherein the transition from step (e) to step (f) occurs without opening the system; and
(g) transferring the harvested TIL population from step (f) to the infusion bag, wherein the transfer from step (f) to (g) occurs without opening the system; and

(h) optionally cryopreserving the infusion bag comprising the harvested TIL population from step (f) using a cryopreservation process.

[0096b] In another aspect, the present disclosure refers to use of a population of expanded TILs in the manufacture of a medicament for the treatment of a subject with cancer, wherein the population of expanded TILs is a modified therapeutic population of TILs obtainable by a method comprising:
(a) obtaining a first population of TILs from a tumor resected from a subject by processing a tumor sample obtained from the subject into multiple tumor fragments;
(b) adding the tumor fragments into a closed system;
(c) performing a first expansion by culturing the first population of TILs in a cell culture medium comprising IL-2, and optionally OKT-3, to produce a second population of TILs, wherein the first expansion is performed in a closed container providing a first gas-permeable surface area, wherein the first expansion is performed within 12 days to obtain the second population of TILs, and wherein the transition from step (b) to step (c) occurs without opening the system;
(d) performing a second expansion by supplementing the cell culture medium of the

second population of TILs with additional IL-2, OKT-3, and antigen presenting cells (APCs), to produce the third population of TILs, wherein the second expansion is performed within 12 days to obtain the third population of TILs, wherein the third population of TILs is a therapeutic population of TILs, wherein the second expansion is performed in a closed container providing a second gas-permeable surface area, and wherein the transition from step (c) to step (d) occurs without opening the system;

(e) exposing the second and/or third population of TILs to transcription factors (TFs) and/or other molecules capable of transiently altering protein expression, wherein the other molecules are selected from one or more sd-RNAs and one or more mRNAs and combinations thereof, wherein the TFs and/or other molecules capable of transiently altering protein expression provide for increased expression of tumor antigens and/or an increase in the number of tumor antigen-specific T cells in the therapeutic population of TILs to produce a modified therapeutic population of TILs, wherein the transient alteration of protein expression targets a gene selected from the group consisting of PD-1, CTLA-4, TIGIT, LAG-3, TIM-3, CISH, CBLB and combinations thereof;

(f) harvesting the modified therapeutic population of TILs obtained from step (e), wherein the transitions from step (d) to step (e) and from step (e) step (f) occur without opening the system; and

(g) transferring the harvested TIL population from step (f) to an infusion bag, wherein the transfer from step (f) to (g) occurs without opening the system; and

(h) optionally cryopreserving the infusion bag comprising the harvested TIL population from step (f) using a cryopreservation process.

[0097] In some examples, the population of TILs is for use to treat a subject with cancer according the methods described above and herein, wherein the method further comprises one or more of the features recited above and herein.

[0098] The present disclosure also provides assay methods for determining TIL viability. The present disclosure provides methods for assaying TILs for viability by expanding tumor infiltrating lymphocytes (TILs) into a larger population of TILs comprising:

(i) obtaining a first population of TILs which has been previously expanded;

(ii) performing a first expansion by culturing the first population of TILs in a cell culture medium comprising IL-2 to produce a second population of TILs; and

(iii) performing a second expansion by supplementing the cell culture medium of the second population of TILs with additional IL-2, OKT-3, and antigen presenting cells

RNA/10,000 TILs, 1.25 μM sd-RNA/10,000 TILs, 1.5 μM sd-RNA/10,000 TILs, 2 μM sd-RNA/10,000 TILs, 5 μM sd-RNA/10,000 TILs, or 10 μM sd-RNA/10,000 TILs, and wherein the sd-RNA is for inhibiting the expression of a molecule selected from the group consisting of PD-1, LAG-3, TIM-3, CISH, and CBLB, and combinations thereof;

(h) optionally performing a sterile electroporation step on the second population of TILs, wherein the sterile electroporation step mediates the transfer of the at least one sd-RNA;

(i) harvesting the therapeutic population of TILs obtained from steps (g) or (h) to provide a harvested TIL population, wherein the transitions from step (e) to step (h) occur without opening the system, wherein the harvested population of TILs is a therapeutic population of TILs;

(j) transferring the harvested TIL population from step (i) to an infusion bag, wherein the transfer from step (h) to (i) occurs without opening the system; and

(k) cryopreserving the harvested TIL population using a dimethylsulfoxide-based cryopreservation medium.

[00127a] In another aspect, the present disclosure refers to a method for expanding tumor infiltrating lymphocytes (TILs) into a modified therapeutic population of TILs comprising: (i) obtaining a first population of TILs from a tumor resected from a patient; (ii) performing a first expansion by culturing the first population of TILs in a cell culture medium comprising IL-2, and optionally OKT-3, to produce a second population of TILs; (iii) performing a second expansion by supplementing the cell culture medium of the second population of TILs with additional IL-2, OKT-3, and antigen presenting cells (APCs), to produce a third population of TILs, and wherein the second expansion is performed within 12 days in order to obtain the third population of TILs, wherein the third population of TILs is a therapeutic population of TILs; and (iv) exposing the second and/or third population of TILs to transcription factors (TFs) and/or other molecules capable of transiently altering protein expression, wherein the other molecules are selected from one or more sd-RNAs and one or more mRNAs and combinations thereof, wherein the TFs and/or other molecules capable of transiently altering protein expression provide for altered expression of tumor antigens and/or an alteration in the number of tumor antigen-specific T cells in the therapeutic population of TILs to produce a modified therapeutic population of TILs, wherein the transient alteration of protein expression targets a gene selected from the group consisting of PD-1, CTLA-4, TIGIT, LAG-3, TIM-3, CISH, CBLB and combinations thereof.

WHAT IS CLAIMED IS:

1. A method for expanding tumor infiltrating lymphocytes (TILs) into a modified therapeutic population of TILs comprising:
    (a) obtaining a first population of TILs from a tumor resected from a patient by processing a tumor sample obtained from the patient into multiple tumor fragments;
    (b) adding the tumor fragments into a closed system;
    (c) performing a first expansion by culturing the first population of TILs in a cell culture medium comprising IL-2, and optionally OKT-3, to produce a second population of TILs, wherein the first expansion is performed in a closed container providing a first gas-permeable surface area, wherein the first expansion is performed within 12 days to obtain the second population of TILs, and wherein the transition from step (b) to step (c) occurs without opening the system;
    (d) performing a second expansion by supplementing the cell culture medium of the second population of TILs with additional IL-2, OKT-3, and antigen presenting cells (APCs), to produce a third population of TILs, wherein the second expansion is performed within 12 days to obtain the third population of TILs, wherein the third population of TILs is a therapeutic population of TILs, wherein the second expansion is performed in a closed container providing a second gas-permeable surface area, and wherein the transition from step (c) to step (d) occurs without opening the system;
    (e) exposing the second and/or third population of TILs to transcription factors (TFs) and/or other molecules capable of transiently altering protein expression, wherein the other molecules are selected from one or more sd-RNAs and one or more mRNAs and combinations thereof, wherein the TFs and/or other molecules capable of transiently altering protein expression provide for altered expression of tumor antigens and/or an alteration in the number of tumor antigen-specific T cells in the therapeutic population of TILs to produce a modified therapeutic population of TILs, wherein the transient alteration of protein expression targets a gene selected from the group consisting of PD-1, CTLA-4, TIGIT, LAG-3, TIM-3, CISH, CBLB and combinations thereof;

(f) harvesting the modified therapeutic population of TILs obtained from step (e), wherein the transition from step (e) to step (f) occurs without opening the system; and

(g) transferring the harvested TIL population from step (f) to an infusion bag, wherein the transfer from step (f) to (g) occurs without opening the system.

2. The method according to claim 1, further comprising the step of cryopreserving the infusion bag of step (g) comprising the harvested TIL population of step (f) using a cryopreservation process.

3. The method according to claim 2, wherein the antigen-presenting cells are peripheral blood mononuclear cells (PBMCs).

4. The method according to claim 3, wherein the PBMCs are irradiated and allogeneic.

5. The method according to claim 3, wherein the PBMCs are added to the cell culture on any of days 9 through 12 in step (d).

6. The method according to claim 1, wherein the antigen-presenting cells are artificial antigen-presenting cells.

7. The method according to claim 1, wherein the harvesting in step (f) is performed using a membrane-based cell processing system.

8. The method according to claim 1, wherein the multiple fragments comprise 4 to 50 fragments, wherein each fragment has a volume of about 27 mm^3.

9. The method according to claim 1, wherein the multiple fragments comprise 30 to 60 fragments with a total volume of 1300 mm^3 to 1500 mm^3.

10. The method according to claim 9, wherein the multiple fragments comprise about 50 fragments with a total volume of about 1350 mm^3.

11. The method according to claim 1, wherein the multiple fragments comprise about 50 fragments with a total mass of 1 gram to 1.5 grams.

12. The method according to claim 1, wherein the cell culture medium in step (d) further comprises IL-15 and/or IL-21.

13. The method according to any one of claims 1 to 12, wherein the IL-2 concentration is 5,000 IU/mL to 10,000 IU/mL.

14. The method according to claim 12, wherein the IL-15 concentration is 100 IU/mL to 500 IU/mL.

15. The method according to claim 12, wherein the IL-21 concentration is 0.5 IU/mL to 20 IU/mL.

16. The method according to claim 2, wherein the cryopreservation process comprises dimethlysulfoxide (DMSO).

17. The method according to claim 16, wherein the cryopreservation process comprises 7% to 10% DMSO.

18. The method according to claim 1, wherein the first period in step (c) and the second period in step (d) are each individually performed within a period of 10 days, 11 days, or 12 days.

19. The method according to claim 1, wherein the first period in step (c) and the second period in step (d) are each individually performed within a period of 11 days.

20. The method according to claim 1, wherein steps (a) through (f) are performed within a period of 10 days to 22 days.

21. The method according to claim 1, wherein steps (a) through (f) are performed within a period of 20 days to 22 days.

22. The method according to claim 1, wherein steps (a) through (f) are performed within a period of 15 days to 20 days.

23. The method according to claim 1, wherein steps (a) through (f) are performed within a period of 10 days to 20 days.

24. The method according to claim 1, wherein steps (a) through (f) are performed within a period of 10 days to 15 days.

25. The method according to claim 1, wherein steps (a) through (f) are performed in 22 days or less.

26. The method according to claim 1, wherein steps (a) through (f) are performed in 20 days or less.

27. The method according to claim 1, wherein steps (a) through (f) are performed in 15 days or less.

28. The method according to claim 1, wherein steps (a) through (f) are performed in 10 days or less.

29. The method according to claim 2, wherein steps (a) through (f) and cryopreservation are performed in 22 days or less.

30. The method according to any one of claims 1 to 29, wherein the modified therapeutic population of TILs harvested in step (f) comprises sufficient TILs for a therapeutically effective dosage of the TILs.

31. The method according to claim 30, wherein the number of TILs sufficient for a therapeutically effective dosage is from 2.3×10^{10} to 13.7×10^{10}.

32. The method according to any one of claims 1 to 31, wherein steps (b) through (e) are performed in a single container, wherein performing steps (b) through (e) in a single container results in an increase in TIL yield per resected tumor as compared to performing steps (b) through (e) in more than one container.

33. The method according to any one of claims 1 to 32, wherein the antigen-presenting cells are added to the TILs during the second expansion in step (d) without opening the system.

34. The method according to any one of claims 1 to 33, wherein the risk of microbial contamination is reduced as compared to an open system.

35. The method according to any one of claims 1 to 34, wherein the TILs from step (f) or (g) are to be infused into a patient.

36. The method according to claim 1, wherein the multiple fragments comprise about 4 fragments.

37. Use of an expanded population of tumor infiltrating lymphocytes (TILs) in the manufacture of a medicament for treating a subject with cancer, wherein the population of TILs comprises a therapeutically effective dosage of a modified therapeutic population of TILs from an infusion bag in step (g) of a method comprising:

    (a) obtaining a first population of TILs from a tumor resected from a subject by processing a tumor sample obtained from the subject into multiple tumor fragments;

    (b) adding the tumor fragments into a closed system;

    (c) performing a first expansion by culturing the first population of TILs in a cell culture medium comprising IL-2, and optionally OKT-3, to produce a second

population of TILs, wherein the first expansion is performed in a closed container providing a first gas-permeable surface area, wherein the first expansion is performed within 12 days to obtain the second population of TILs, and wherein the transition from step (b) to step (c) occurs without opening the system;

(d) performing a second expansion by supplementing the cell culture medium of the second population of TILs with additional IL-2, OKT-3, and antigen presenting cells (APCs), to produce the third population of TILs, wherein the second expansion is performed within 12 days to obtain the third population of TILs, wherein the third population of TILs is a therapeutic population of TILs, wherein the second expansion is performed in a closed container providing a second gas-permeable surface area, and wherein the transition from step (c) to step (d) occurs without opening the system;

(e) exposing the second and/or third population of TILs to transcription factors (TFs) and/or other molecules capable of transiently altering protein expression, wherein the other molecules are selected from one or more sd-RNAs and one or more mRNAs and combinations thereof, wherein the TFs and/or other molecules capable of transiently altering protein expression provide for altered expression of tumor antigens and/or an alteration in the number of tumor antigen-specific T cells in the therapeutic population of TILs to produce a modified therapeutic population of TILs, wherein the transient alteration of protein expression targets a gene selected from the group consisting of PD-1, CTLA-4, TIGIT, LAG-3, TIM-3, CISH, CBLB and combinations thereof;

(f) harvesting the modified therapeutic population of TILs obtained from step (e), wherein the transition from step (e) to step (f) occurs without opening the system; and

(g) transferring the harvested TIL population from step (f) to the infusion bag, wherein the transfer from step (f) to (g) occurs without opening the system; and

(h) optionally cryopreserving the infusion bag comprising the harvested TIL population from step (f) using a cryopreservation process.

38. Use of a population of expanded TILs in the manufacture of a medicament for the treatment of a subject with cancer, wherein the population of expanded TILs is a modified therapeutic population of TILs obtainable by a method comprising:

(a) obtaining a first population of TILs from a tumor resected from a subject by

processing a tumor sample obtained from the subject into multiple tumor fragments;

(b) adding the tumor fragments into a closed system;

(c) performing a first expansion by culturing the first population of TILs in a cell culture medium comprising IL-2, and optionally OKT-3, to produce a second population of TILs, wherein the first expansion is performed in a closed container providing a first gas-permeable surface area, wherein the first expansion is performed within 12 days to obtain the second population of TILs, and wherein the transition from step (b) to step (c) occurs without opening the system;

(d) performing a second expansion by supplementing the cell culture medium of the second population of TILs with additional IL-2, OKT-3, and antigen presenting cells (APCs), to produce the third population of TILs, wherein the second expansion is performed within 12 days to obtain the third population of TILs, wherein the third population of TILs is a therapeutic population of TILs, wherein the second expansion is performed in a closed container providing a second gas-permeable surface area, and wherein the transition from step (c) to step (d) occurs without opening the system;

(e) exposing the second and/or third population of TILs to transcription factors (TFs) and/or other molecules capable of transiently altering protein expression, wherein the other molecules are selected from one or more sd-RNAs and one or more mRNAs and combinations thereof, wherein the TFs and/or other molecules capable of transiently altering protein expression provide for altered expression of tumor antigens and/or an alteration in the number of tumor antigen-specific T cells in the therapeutic population of TILs to produce a modified therapeutic population of TILs, wherein the transient alteration of protein expression targets a gene selected from the group consisting of PD-1, CTLA-4, TIGIT, LAG-3, TIM-3, CISH, CBLB and combinations thereof;

(f) harvesting the modified therapeutic population of TILs obtained from step (e), wherein the transitions from step (d) to step (e) and from step (e) to step (f) occur without opening the system; and

(g) transferring the harvested TIL population from step (f) to an infusion bag, wherein the transfer from step (f) to (g) occurs without opening the system; and

(h) optionally cryopreserving the infusion bag comprising the harvested TIL population from step (f) using a cryopreservation process.

39. The method according to any of claims 1-36, or the use of claim 37 or 38, wherein the transient altering of protein expression results in induction of protein expression.

40. The method according to any of claims 1-36, or the use of claim 37 or 38, wherein the transient altering of protein expression results in a reduction of protein expression.

41. The method or the use according to claim 40, wherein one or more sd-RNA(s) is employed to reduce the transient protein expression.

42. A method for expanding tumor infiltrating lymphocytes (TILs) into a modified therapeutic population of TILs comprising:

(i) obtaining a first population of TILs from a tumor resected from a patient;

(ii) performing a first expansion by culturing the first population of TILs in a cell culture medium comprising IL-2, and optionally OKT-3, to produce a second population of TILs;

(iii) performing a second expansion by supplementing the cell culture medium of the second population of TILs with additional IL-2, OKT-3, and antigen presenting cells (APCs), to produce a third population of TILs, and wherein the second expansion is performed within 12 days in order to obtain the third population of TILs, wherein the third population of TILs is a therapeutic population of TILs; and

(iv) exposing the second and/or third population of TILs to transcription factors (TFs) and/or other molecules capable of transiently altering protein expression, wherein the other molecules are selected from one or more sd-RNAs and one or more mRNAs and combinations thereof, wherein the TFs and/or other molecules capable of transiently altering protein expression provide for altered expression of tumor antigens and/or an alteration in the number of tumor antigen-specific T cells in the therapeutic population of TILs to produce a modified therapeutic population of TILs, wherein the transient alteration of protein expression targets a gene selected from the group consisting of PD-1, CTLA-4, TIGIT, LAG-3, TIM-3, CISH, CBLB and combinations thereof;

43. The method or use according to any of the preceding claims, wherein the second population of TILs is at least 50-fold greater in number than the first population of TILs.

44. The method or use according to any of the preceding claims, wherein the third population of TILs is at least 100-fold greater in number than the second population of TILs.