

known as mononuclear cells (MNCs)), often from multiple donors, as feeder cells, as well as anti-CD3 antibody (OKT3) and high doses of IL-2. Dudley, *et al.*, *J. Immunother.* **2003**, 26, 332-42. TILs that have undergone an REP procedure have produced successful adoptive cell therapy following host immunosuppression in patients with melanoma. Current infusion acceptance parameters rely on readouts of the composition of TILs (*e.g.*, CD28, CD8, or CD4 positivity) and on fold expansion and viability of the REP product.

[0004] Current TIL manufacturing processes are limited by length, cost, sterility concerns, and other factors described herein such that the potential to commercialize such processes is severely limited, and for these and other reasons, at the present time no commercial process has become available. There is an urgent need to provide TIL manufacturing processes and therapies based on such processes that are appropriate for commercial scale manufacturing and regulatory approval for use in human patients at multiple clinical centers.

BRIEF SUMMARY

[0005] The present disclosure provides improved and/or shortened methods for expanding TILs and producing therapeutic populations of TILs.

[0006] In one aspect, the present disclosure refers to a method for expanding tumor infiltrating lymphocytes (TILs) into a 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 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 for about 3-14 days to obtain the second population of TILs, wherein the second population of 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) 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
- (f) 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.

[0007] In some examples, the method further comprises the step of cryopreserving the infusion bag comprising the harvested TIL population in step (f) using a cryopreservation process.

[0008] In some examples, the cryopreservation process is performed using a 1:1 ratio of harvested TIL population to cryopreservation media.

[0009] In some examples, the antigen-presenting cells are peripheral blood mononuclear cells (PBMCs). In some examples, the PBMCs are irradiated and allogeneic. In some examples, the PBMCs are added to the cell culture on any of days 9 through 14 in step (d). In some examples, the antigen-presenting cells are artificial antigen-presenting cells.

[0010] In some examples, the harvesting in step (e) is performed using a membrane-based cell processing system.

[0011] In some examples, the harvesting in step (e) is performed using a LOVO cell processing system.

[0012] In some examples, the multiple fragments comprise about 4 to about 50 fragments, wherein each fragment has a volume of about 27 mm^3 .

[0013] In some examples, the multiple fragments comprise about 30 to about 60 fragments with a total volume of about 1300 mm^3 to about 1500 mm^3 .

[0014] In some examples, the multiple fragments comprise about 50 fragments with a total volume of about 1350 mm^3 .

[0015] In some examples, the multiple fragments comprise about 50 fragments with a total mass of about 1 gram to about 1.5 grams.

[0016] In some examples, the cell culture medium is provided in a container selected from the group consisting of a G-container and a Xuri cellbag.

[0017] In some examples, the cell culture medium in step (d) further comprises IL-15 and/or IL-21.

[0018] In some examples, the the IL-2 concentration is about 10,000 IU/mL to about 5,000 IU/mL.

[0019] In some examples, the IL-15 concentration is about 500 IU/mL to about 100 IU/mL.

[0020] In some examples, the IL-21 concentration is about 20 IU/mL to about 0.5 IU/mL.

[0021] In some examples, the infusion bag in step (f) is a HypoThermosol-containing infusion bag.

[0022] In some examples, the cryopreservation media comprises dimethylsulfoxide (DMSO). In some examples, the cryopreservation media comprises 7% to 10% dimethylsulfoxide (DMSO).

[0023] In some examples, the first period in step (c) and the second period in step (e) are each individually performed within a period of 10 days, 11 days, or 12 days.

[0024] In some examples, the first period in step (c) and the second period in step (e) are each individually performed within a period of 11 days.

[0025] In some examples, steps (a) through (f) are performed within a period of about 10 days to about 22 days.

[0026] In some examples, steps (a) through (f) are performed within a period of about 20 days to about 22 days.

[0027] In some examples, steps (a) through (f) are performed within a period of about 15 days to about 20 days.

[0028] In some examples, steps (a) through (f) are performed within a period of about 10 days to about 20 days.

[0029] In some examples, steps (a) through (f) are performed within a period of about 10 days to about 15 days.

- [0030]** In some examples, steps (a) through (f) are performed in 22 days or less.
- [0031]** In some examples, steps (a) through (f) are performed in 20 days or less.
- [0032]** In some examples, steps (a) through (f) are performed in 15 days or less.
- [0033]** In some examples, steps (a) through (f) are performed in 10 days or less.
- [0034]** In some examples, steps (a) through (f) and cryopreservation are performed in 22 days or less.
- [0035]** In some examples, the therapeutic population of TILs harvested in step (e) comprises sufficient TILs for a therapeutically effective dosage of the TILs.
- [0036]** In some examples, the number of TILs sufficient for a therapeutically effective dosage is from about 2.3×10^{10} to about 13.7×10^{10} .
- [0037]** In some examples, 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.
- [0038]** In some examples, the antigen-presenting cells are added to the TILs during the second period in step (d) without opening the system.
- [0039]** In some examples, the third population of TILs in step (d) provides for increased efficacy, increased interferon-gamma production, increased polyclonality, increased average IP-10, and/or increased average MCP-1 when administered to a subject.
- [0040]** In some examples, the third population of TILs in step (d) provides for at least a five-fold or more interferon-gamma production when administered to a subject.
- [0041]** In some examples, the third population of TILs in step (d) is a therapeutic population of TILs which comprises an increased subpopulation of effector T cells and/or central memory T cells relative to the second population of TILs, wherein the effector T cells and/or central memory T cells in the therapeutic population of TILs exhibit one or more characteristics selected from the group consisting of expressing CD27+, expressing CD28+, longer telomeres, increased CD57 expression, and decreased CD56 expression relative to effector T cells, and/or central memory T cells obtained from the second population of cells.
- [0042]** In some examples, the effector T cells and/or central memory T cells obtained from the third population of TILs exhibit increased CD57 expression and decreased CD56

expression relative to effector T cells and/or central memory T cells obtained from the second population of cells.

[0043] In some examples, the risk of microbial contamination is reduced as compared to an open system.

[0044] In some examples, the TILs from step (g) are infused into a patient.

[0045] In some examples, the multiple fragments comprise about 4 fragments.

[0046] The present disclosure also provides a method for treating a subject with cancer, the method comprising administering expanded tumor infiltrating lymphocytes (TILs) comprising:

- (a) obtaining a first population of TILs from a tumor resected from a subject 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 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 for about 3-14 days to obtain the second population of TILs, wherein the second population of 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) 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
- (f) 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;

- (g) optionally cryopreserving the infusion bag comprising the harvested TIL population from step (f) using a cryopreservation process; and
- (h) administering a therapeutically effective dosage of the third population of TILs from the infusion bag in step (g) to the patient.

[0046a] In one 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 comprise a therapeutically effective dosage of a third 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 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 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 for about 3-14 days to obtain the second population of TILs, wherein the second population of 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) 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
- (f) 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
- (g) optionally cryopreserving the infusion bag comprising the harvested TIL population from step (f) using a cryopreservation process.

[0047] In some examples, the therapeutic population of TILs harvested in step (e) comprises sufficient TILs for administering a therapeutically effective dosage of the TILs in step (h).

[0048] In some examples, the number of TILs sufficient for administering a therapeutically effective dosage in step (h) is from about 2.3×10^{10} to about 13.7×10^{10} .

[0049] In some examples, the antigen presenting cells (APCs) are PBMCs.

[0050] In some examples, the PBMCs are added to the cell culture on any of days 9 through 14 in step (d).

[0051] In some examples, prior to administering a therapeutically effective dosage of TIL cells in step (h), a non-myeloablative lymphodepletion regimen has been administered to the patient.

[0052] In some examples, the non-myeloablative lymphodepletion regimen comprises the steps of administration of cyclophosphamide at a dose of 60 mg/m²/day for two days followed by administration of fludarabine at a dose of 25 mg/m²/day for five days.

[0053] In some examples, the method further comprises the step of treating the patient with a high-dose IL-2 regimen starting on the day after administration of the TIL cells to the patient in step (h).

[0054] In some examples, the high-dose IL-2 regimen comprises 600,000 or 720,000 IU/kg administered as a 15-minute bolus intravenous infusion every eight hours until tolerance.

[0055] In some examples, the third population of TILs in step (d) is a therapeutic population of TILs which comprises an increased subpopulation of effector T cells and/or central memory T cells relative to the second population of TILs, wherein the effector T cells and/or central memory T cells in the therapeutic population of TILs exhibit one or more characteristics selected from the group consisting of expressing CD27+, expressing CD28+, longer telomeres, increased CD57 expression, and decreased CD56 expression relative to effector T cells, and/or central memory T cells obtained from the second population of cells.

[0056] In some examples, the effector T cells and/or central memory T cells in the therapeutic population of TILs exhibit increased CD57 expression and decreased CD56 expression relative to effector T cells and/or central memory T cells obtained from the second population of cells.

[0057] In some examples, the cancer is selected from the group consisting of melanoma, ovarian cancer, cervical cancer, non-small-cell lung cancer (NSCLC), lung cancer, bladder cancer, breast cancer, cancer caused by human papilloma virus, head and neck cancer (including head and neck squamous cell carcinoma (HNSCC)), renal cancer, and renal cell carcinoma.

[0058] In some examples, the cancer is selected from the group consisting of melanoma, HNSCC, cervical cancers, and NSCLC.

[0059] In some examples, the cancer is melanoma.

[0060] In some examples, the cancer is HNSCC.

[0061] In some examples, the cancer is a cervical cancer.

[0062] In some examples, the cancer is NSCLC.

[0063] In one aspect, the present disclosure also refers to methods for expanding tumor infiltrating lymphocytes (TILs) into a therapeutic population of TILs comprising:

- (a) adding processed tumor fragments from a tumor resected from a patient into a closed system to obtain a first population of TILs;
- (b) 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, wherein the first expansion is performed in a closed container providing a first gas-permeable surface area, wherein the first expansion is performed for about 3-14 days to obtain the second population of TILs, wherein the second population of TILs is at least 50-fold greater in number than the first population of TILs, and wherein the transition from step (a) to step (b) occurs without opening the system;
- (c) 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 (b) to step (c) occurs without opening the system;

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

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

[0064] In some examples, the therapeutic population of TILs harvested in step (d) comprises sufficient TILs for a therapeutically effective dosage of the TILs.

[0065] In some examples, the number of TILs sufficient for a therapeutically effective dosage is from about 2.3×10^{10} to about 13.7×10^{10} .

[0066] In some examples, the method further comprises the step of cryopreserving the infusion bag comprising the harvested TIL population using a cryopreservation process.

[0067] In some examples, the cryopreservation process is performed using a 1:1 ratio of harvested TIL population to cryopreservation media.

[0068] In some examples, the antigen-presenting cells are peripheral blood mononuclear cells (PBMCs).

[0069] In some examples, the PBMCs are irradiated and allogeneic.

[0070] The method according to claim 68, wherein the PBMCs are added to the cell culture on any of days 9 through 14 in step (c).

[0071] In some examples, the antigen-presenting cells are artificial antigen-presenting cells.

[0072] In some examples, the harvesting in step (d) is performed using a LOVO cell processing system.

[0073] In some examples, the multiple fragments comprise about 4 to about 50 fragments, wherein each fragment has a volume of about 27 mm^3 .

[0074] In some examples, the multiple fragments comprise about 30 to about 60 fragments with a total volume of about 1300 mm^3 to about 1500 mm^3 .

[0075] In some examples, the multiple fragments comprise about 50 fragments with a total volume of about 1350 mm^3 .

- [0076]** In some examples, the multiple fragments comprise about 50 fragments with a total mass of about 1 gram to about 1.5 grams.
- [0077]** In some examples, the multiple fragments comprise about 4 fragments.
- [0078]** In some examples, the second cell culture medium is provided in a container selected from the group consisting of a G-container and a Xuri cellbag.
- [0079]** In some examples, the infusion bag in step (e) is a HypoThermosol-containing infusion bag.
- [0080]** In some examples, the first period in step (b) and the second period in step (c) are each individually performed within a period of 10 days, 11 days, or 12 days.
- [0081]** In some examples, the first period in step (b) and the second period in step (c) are each individually performed within a period of 11 days.
- [0082]** In some examples, steps (a) through (e) are performed within a period of about 10 days to about 22 days.
- [0083]** In some examples, steps (a) through (e) are performed within a period of about 10 days to about 20 days.
- [0084]** In some examples, steps (a) through (e) are performed within a period of about 10 days to about 15 days.
- [0085]** In some examples, steps (a) through (e) are performed in 22 days or less.
- [0086]** In some examples, steps (a) through (e) and cryopreservation are performed in 22 days or less.
- [0087]** In some examples, 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.
- [0088]** In some examples, the antigen-presenting cells are added to the TILs during the second period in step (c) without opening the system.
- [0089]** In some examples, the third population of TILs in step (d) is a therapeutic population of TILs which comprises an increased subpopulation of effector T cells and/or central memory T cells relative to the second population of TILs, wherein the effector T cells and/or central memory T cells obtained in the therapeutic population of TILs exhibit one or

more characteristics selected from the group consisting of expressing CD27+, expressing CD28+, longer telomeres, increased CD57 expression, and decreased CD56 expression relative to effector T cells, and/or central memory T cells obtained from the second population of cells.

[0090] In some examples, the effector T cells and/or central memory T cells obtained in the therapeutic population of TILs exhibit increased CD57 expression and decreased CD56 expression relative to effector T cells, and/or central memory T cells obtained from the second population of cells.

[0091] In some examples, the risk of microbial contamination is reduced as compared to an open system.

[0092] In some examples, the TILs from step (e) are infused into a patient.

[0093] In some examples, the closed container comprises a single bioreactor.

[0094] In some examples, the closed container comprises a G-REX-10.

[0095] In some examples the closed container comprises a G-REX -100.

[0096] In some examples, at step (d) the antigen presenting cells (APCs) are added to the cell culture of the second population of TILs at a APC:TIL ratio of 25:1 to 100:1.

[0097] In some examples, the cell culture has a ratio of 2.5×10^9 APCs to 100×10^6 TILs.

[0098] In some examples, at step (c) the antigen presenting cells (APCs) are added to the cell culture of the second population of TILs at a APC:TIL ratio of 25:1 to 100:1.

[0099] In some examples, the cell culture has ratio of 2.5×10^9 APCs to 100×10^6 TILs.

[00100] In one aspect, the present disclosure also refers to a population of expanded TILs for use in the treatment of a subject with cancer, wherein the population of expanded TILs is a third 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 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 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 for about 3-14 days to obtain the second population of TILs, wherein the second population of 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) 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
- (f) 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
- (g) optionally cryopreserving the infusion bag comprising the harvested TIL population from step (f) using a cryopreservation process.

[00101] 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.

BRIEF DESCRIPTION OF THE DRAWINGS

[00102] Figure 1: Shows a diagram of an embodiment of process 2A, a 22-day process for TIL manufacturing.

[00103] Figure 2: Shows a comparison between the 1C process and an embodiment of the 2A process for TIL manufacturing.

[00104] Figure 3: Shows the 1C process timeline.

[00105] Figure 4: Shows the process of an embodiment of TIL therapy using process 2A for TIL manufacturing, including administration and co-therapy steps, for higher cell counts.

WHAT IS CLAIMED IS:

1. A method for expanding tumor infiltrating lymphocytes (TILs) into a 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 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 for about 3-14 days to obtain the second population of TILs, wherein the second population of 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) 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
 - (f) 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.
2. The method according to claim 1, further comprising the step of cryopreserving the infusion bag comprising the harvested TIL population in step (f) using a cryopreservation process.
3. The method according to claim 1, wherein the cryopreservation process is performed using a 1:1 ratio of harvested TIL population to cryopreservation media.

4. The method according to claim 1, wherein the antigen-presenting cells are peripheral blood mononuclear cells (PBMCs).
5. The method according to claim 4, wherein the PBMCs are irradiated and allogeneic.
6. The method according to claim 4, wherein the PBMCs are added to the cell culture on any of days 9 through 14 in step (d).
7. The method according to claim 1, wherein the antigen-presenting cells are artificial antigen-presenting cells.
8. The method according to claim 1, wherein the harvesting in step (e) is performed using a membrane-based cell processing system.
9. The method according to claim 1, wherein the harvesting in step (e) is performed using a LOVO cell processing system.
10. The method according to claim 1, wherein the multiple fragments comprise about 4 to about 50 fragments, wherein each fragment has a volume of about 27 mm^3 .
11. The method according to claim 1, wherein the multiple fragments comprise about 30 to about 60 fragments with a total volume of about 1300 mm^3 to about 1500 mm^3 .
12. The method according to claim 9, wherein the multiple fragments comprise about 50 fragments with a total volume of about 1350 mm^3 .
13. The method according to claim 1, wherein the multiple fragments comprise about 50 fragments with a total mass of about 1 gram to about 1.5 grams.
14. The method according to claim 1, wherein the cell culture medium is provided in a container selected from the group consisting of a G-container and a Xuri cellbag.
15. The method according to claim 1, wherein the cell culture medium in step (d) further comprises IL-15 and/or IL-21.
16. The method according to claim any of the preceding claims, wherein the IL-2 concentration is about 10,000 IU/mL to about 5,000 IU/mL.
17. The method according to claim 15, wherein the IL-15 concentration is about 500 IU/mL to about 100 IU/mL.
18. The method according to claim 1, wherein the IL-21 concentration is about 20 IU/mL to about 0.5 IU/mL.

19. The method according to claim 1, wherein the infusion bag in step (f) is a HypoThermosol-containing infusion bag.
20. The method according to claim 3, wherein the cryopreservation media comprises dimethylsulfoxide (DMSO).
21. The method according to claim 17, wherein the wherein the cryopreservation media comprises 7% to 10% DMSO.
22. The method according to claim 1, wherein the first period in step (c) and the second period in step (e) are each individually performed within a period of 10 days, 11 days, or 12 days.
23. The method according to claim 1, wherein the first period in step (c) and the second period in step (e) are each individually performed within a period of 11 days.
24. The method according to claim 1, wherein steps (a) through (f) are performed within a period of about 10 days to about 22 days.
25. The method according to claim 1, wherein steps (a) through (f) are performed within a period of about 20 days to about 22 days.
26. The method according to claim 1, wherein steps (a) through (f) are performed within a period of about 15 days to about 20 days.
27. The method according to claim 1, wherein steps (a) through (f) are performed within a period of about 10 days to about 20 days.
28. The method according to claim 1, wherein steps (a) through (f) are performed within a period of about 10 days to about 15 days.
29. The method according to claim 1, wherein steps (a) through (f) are performed in 22 days or less.
30. The method according to claim 1, wherein steps (a) through (f) are performed in 20 days or less.
31. The method according to claim 1, wherein steps (a) through (f) are performed in 15 days or less.
32. The method according to claim 1, wherein steps (a) through (f) are performed in 10 days or less.

33. The method according to claim 2, wherein steps (a) through (f) and cryopreservation are performed in 22 days or less.
34. The method according to any one of claims 1 to 33, wherein the therapeutic population of TILs harvested in step (e) comprises sufficient TILs for a therapeutically effective dosage of the TILs.
35. The method according to claim 34, wherein the number of TILs sufficient for a therapeutically effective dosage is from about 2.3×10^{10} to about 13.7×10^{10} .
36. The method according to any one of claims 1 to 35, 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.
37. The method according to any one of claims 1 to 36, wherein the antigen-presenting cells are added to the TILs during the second period in step (d) without opening the system.
38. The method according to any one of claims 1 to 37, wherein the third population of TILs in step (d) provides for increased efficacy, increased interferon-gamma production, increased polyclonality, increased average IP-10, and/or increased average MCP-1 when administered to a subject.
39. The method according to any one of claims 1 to 38, wherein the third population of TILs in step (d) provides for at least a five-fold or more interferon-gamma production when administered to a subject.
40. The method according to any one of claims 1 to 39, wherein the third population of TILs in step (d) is a therapeutic population of TILs which comprises an increased subpopulation of effector T cells and/or central memory T cells relative to the second population of TILs, wherein the effector T cells and/or central memory T cells in the therapeutic population of TILs exhibit one or more characteristics selected from the group consisting of expressing CD27+, expressing CD28+, longer telomeres, increased CD57 expression, and decreased CD56 expression relative to effector T cells, and/or central memory T cells obtained from the second population of cells.
41. The method according to any one of claims 1 to 40, wherein the effector T cells and/or central memory T cells obtained from the third population of TILs exhibit increased

CD57 expression and decreased CD56 expression relative to effector T cells and/or central memory T cells obtained from the second population of cells.

42. The method according to any one of claims 1 to 41, wherein the risk of microbial contamination is reduced as compared to an open system.
43. The method according to any one of claims 1 to 42, wherein the TILs from step (g) are infused into a patient.
44. The method according to any one of claims 1 to 43, wherein the multiple fragments comprise about 4 fragments.
45. 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 comprise a therapeutically effective dosage of a third 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 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 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 for about 3-14 days to obtain the second population of TILs, wherein the second population of 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) 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
- (f) 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
- (g) optionally cryopreserving the infusion bag comprising the harvested TIL population from step (f) using a cryopreservation process.
46. The use according to claim 45, wherein the therapeutic population of TILs harvested in step (e) comprises sufficient TILs for administration of a therapeutically effective dosage of the TILs.
47. The use according to claim 46, wherein the number of TILs sufficient for administration of a therapeutically effective dosage is from about 2.3×10^{10} to about 13.7×10^{10} .
48. The use according to claim 47, wherein the antigen presenting cells (APCs) are PBMCs.
49. The use according to claim 48, wherein the PBMCs are added to the cell culture on any of days 9 through 14 in step (d).
50. The use according to any of claims 45 to 49, wherein prior to administration of a therapeutically effective dosage of TIL cells, a non-myeloablative lymphodepletion regimen has been administered to the patient.
51. The use according to claim 50, where the non-myeloablative lymphodepletion regimen comprises the steps of administration of cyclophosphamide at a dose of $60 \text{ mg/m}^2/\text{day}$ for two days followed by administration of fludarabine at a dose of $25 \text{ mg/m}^2/\text{day}$ for five days.
52. The use according to any of claims 45 to 51, wherein a high-dose IL-2 regimen is to be administered starting on the day after administration of the TIL cells to the patient.
53. The use according to claim 52, wherein the high-dose IL-2 regimen comprises administration of 600,000 or 720,000 IU/kg as a 15-minute bolus intravenous infusion every eight hours until tolerance.
54. The method according to any one of claims 1 to 44 or use according to any one of claims 45 to 53, wherein the third population of TILs in step (d) is a therapeutic population of TILs which comprises an increased subpopulation of effector T cells and/or central memory T cells relative to the second population of TILs, wherein the effector T cells and/or central memory T cells in the therapeutic population of TILs exhibit one or more characteristics selected from the group consisting of expressing CD27+, expressing

CD28+, longer telomeres, increased CD57 expression, and decreased CD56 expression relative to effector T cells, and/or central memory T cells obtained from the second population of cells.

55. The method according to any one of claims 1 to 44 or use according to any one of claims 45 to 54, wherein the effector T cells and/or central memory T cells in the therapeutic population of TILs exhibit increased CD57 expression and decreased CD56 expression relative to effector T cells and/or central memory T cells obtained from the second population of cells.
56. The method according to any one of claims 1 to 44 or use according to any one of claims 45 to 55, wherein the cancer is selected from the group consisting of melanoma, ovarian cancer, cervical cancer, non-small-cell lung cancer (NSCLC), lung cancer, bladder cancer, breast cancer, cancer caused by human papilloma virus, head and neck cancer (including head and neck squamous cell carcinoma (HNSCC)), renal cancer, and renal cell carcinoma.
57. The method according to any one of claims 1 to 44 or use according to any one of claims 45 to 56, wherein the cancer is selected from the group consisting of melanoma, HNSCC, cervical cancers, and NSCLC.
58. The method according to any one of claims 1 to 44 or use according to any one of claims 45 to 57, wherein the cancer is melanoma.
59. The method according to any one of claims 1 to 44 or use according to any one of claims 45 to 58, wherein the cancer is HNSCC.
60. The method according to any one of claims 1 to 44 or use according to any one of claims 45 to 59, wherein the cancer is a cervical cancer.
61. The method according to any one of claims 1 to 44 or use according to any one of claims 45 to 60, wherein the cancer is NSCLC.
62. A method for expanding tumor infiltrating lymphocytes (TILs) into a therapeutic population of TILs comprising:
 - (a) adding processed tumor fragments from a tumor resected from a patient into a closed system to obtain a first population of TILs;
 - (b) 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, wherein

- the first expansion is performed in a closed container providing a first gas-permeable surface area, wherein the first expansion is performed for about 3-14 days to obtain the second population of TILs, wherein the second population of TILs is at least 50-fold greater in number than the first population of TILs, and wherein the transition from step (a) to step (b) occurs without opening the system;
- (c) 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 (b) to step (c) occurs without opening the system;
- (d) harvesting the therapeutic population of TILs obtained from step (c), wherein the transition from step (c) to step (d) occurs without opening the system; and
- (e) transferring the harvested TIL population from step (d) to an infusion bag, wherein the transfer from step (d) to (e) occurs without opening the system.
63. The method according to claim 62, wherein the therapeutic population of TILs harvested in step (d) comprises sufficient TILs for a therapeutically effective dosage of the TILs.
64. The method according to claim 63, where the number of TILs sufficient for a therapeutically effective dosage is from about 2.3×10^{10} to about 13.7×10^{10} .
65. The method according to claim 64, further comprising the step of cryopreserving the infusion bag comprising the harvested TIL population using a cryopreservation process.
66. The method according to claim 65, wherein the cryopreservation process is performed using a 1:1 ratio of harvested TIL population to cryopreservation media.
67. The method according to claim 62, wherein the antigen-presenting cells are peripheral blood mononuclear cells (PBMCs).
68. The method according to claim 67, wherein the PBMCs are irradiated and allogeneic.
69. The method according to claim 68, wherein the PBMCs are added to the cell culture on any of days 9 through 14 in step (c).

70. The method according to claim 62, wherein the antigen-presenting cells are artificial antigen-presenting cells.
71. The method according to claim 62, wherein the harvesting in step (d) is performed using a LOVO cell processing system.
72. The method according to claim 62, wherein the multiple fragments comprise about 4 to about 50 fragments, wherein each fragment has a volume of about 27 mm^3 .
73. The method according to claim 62, wherein the multiple fragments comprise about 30 to about 60 fragments with a total volume of about 1300 mm^3 to about 1500 mm^3 .
74. The method according to claim 63, wherein the multiple fragments comprise about 50 fragments with a total volume of about 1350 mm^3 .
75. The method according to claim 62, wherein the multiple fragments comprise about 50 fragments with a total mass of about 1 gram to about 1.5 grams.
76. The method according to claim 62, wherein the multiple fragments comprise about 4 fragments.
77. The method according to claim 62, wherein the second cell culture medium is provided in a container selected from the group consisting of a G-container and a Xuri cellbag.
78. The method according to claim 62, wherein the infusion bag in step (e) is a HypoThermosol-containing infusion bag.
79. The method according to claim 62, wherein the first period in step (b) and the second period in step (c) are each individually performed within a period of 10 days, 11 days, or 12 days.
80. The method according to claim 62, wherein the first period in step (b) and the second period in step (c) are each individually performed within a period of 11 days.
81. The method according to claim 62, wherein steps (a) through (e) are performed within a period of about 10 days to about 22 days.
82. The method according to claim 62, wherein steps (a) through (e) are performed within a period of about 10 days to about 20 days.
83. The method according to claim 62, wherein steps (a) through (e) are performed within a period of about 10 days to about 15 days.

84. The method according to claim 62, wherein steps (a) through (e) are performed in 22 days or less.
85. The method according to claim 65, wherein steps (a) through (e) and cryopreservation are performed in 22 days or less.
86. The method according to any one of claims 62 to 85, 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.
87. The method according to any one of claims 62 to 86, wherein the antigen-presenting cells are added to the TILs during the second period in step (c) without opening the system.
88. The method according to any one of claims 62 to 87, wherein the third population of TILs in step (d) is a therapeutic population of TILs which comprises an increased subpopulation of effector T cells and/or central memory T cells relative to the second population of TILs, wherein the effector T cells and/or central memory T cells obtained in the therapeutic population of TILs exhibit one or more characteristics selected from the group consisting of expressing CD27+, expressing CD28+, longer telomeres, increased CD57 expression, and decreased CD56 expression relative to effector T cells, and/or central memory T cells obtained from the second population of cells.
89. The method according to any one of claims 62 to 88, wherein the effector T cells and/or central memory T cells obtained in the therapeutic population of TILs exhibit increased CD57 expression and decreased CD56 expression relative to effector T cells, and/or central memory T cells obtained from the second population of cells.
90. The method according to any one of claims 62 to 89, wherein the risk of microbial contamination is reduced as compared to an open system.
91. The method according to any one of claims 62 to 90, wherein the TILs from step (e) are to be infused into a patient.
92. The method according to any one of claims 1 to 44 and 62 to 91 or use according to any one of claims 45 to 61, wherein the closed container comprises a single bioreactor.
93. The method or use according to claim 92, wherein the closed container comprises a G-REX-10.

94. The method or use according to claim 92, wherein the closed container comprises a G-REX -100.
95. The method according to any one of claims 1 to 44 or use according to any one of claims 45 to 61, wherein at step (d) the antigen presenting cells (APCs) are added to the cell culture of the second population of TILs at a APC:TIL ratio of 25:1 to 100:1.
96. The method or use according to claim 95, wherein the cell culture has a ratio of 2.5×10^9 APCs to 100×10^6 TILs.
97. The method according to any one of claims 62 to 94, wherein at step (c) the antigen presenting cells (APCs) are added to the cell culture of the second population of TILs at a APC:TIL ratio of 25:1 to 100:1.
98. The method according to claim 97, wherein the cell culture has ratio of 2.5×10^9 APCs to 100×10^6 TILs.
99. A population of expanded TILs for use in the treatment of a subject with cancer, wherein the population of expanded TILs is a third 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 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 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 for about 3-14 days to obtain the second population of TILs, wherein the second population of 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) 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
- (f) 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
- (g) optionally cryopreserving the infusion bag comprising the harvested TIL population from step (f) using a cryopreservation process.

100. The population of TILs for use to treat a subject with cancer according to claim 99, wherein the method further comprises one or more of the features recited in any of claims 1 to 99.