

BRIEF SUMMARY OF THE INVENTION

[0004] The present ~~invention~~disclosure provides improved and/or shortened methods for expanding TILs and producing therapeutic populations of TILs.

[0004a] In one aspect, the present disclosure refers to 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, 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 for 3-14 days to obtain the second 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, optionally OKT-3, and antigen presenting cells (APCs), to produce a third population of TILs, wherein the second expansion is performed for 4 to 6 days to obtain the third 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) splitting the third population of TILs into a first plurality of five or less sub-populations of TILs, each sub-population comprising at least 1.0×10^9 TILs, and performing a third expansion of the first plurality of sub-populations of TILs by supplementing the cell culture medium of each sub-population of TILs with additional IL-2, optionally OKT-3, to produce a second plurality of sub-populations of TILs, wherein the second plurality of sub-populations of TILs comprise a therapeutic population of TILs, wherein the third expansion is performed for 5 to 7 days, wherein the third expansion for each sub-population is performed in a closed container providing a third 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.

[0004b] In another aspect, the present disclosure refers to a cryopreservation composition comprising the population of TILs obtained or obtainable by the method of any of the preceding claims, a cryoprotectant medium comprising dimethylsulfoxide (DMSO), and an electrolyte solution.

[0004c] In another aspect, the present disclosure refers to the cryopreservation composition as disclosed herein for use in therapy.

[0004d] In another aspect, the present disclosure refers to the use of the cryopreservation composition as disclosed herein in the manufacture of a medicament for treating cancer in a subject.

[0005] The present ~~invention~~disclosure provides 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 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 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, optionally 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.

[0006] In some ~~embodiment~~examples, the method further comprises the step of cryopreserving the infusion bag comprising the harvested TIL population in step (f) using a cryopreservation process.

[0007] In some ~~embodiment~~examples, the cryopreservation process is performed using a 1:1 ratio of harvested TIL population to cryopreservation media.

[0008] In some ~~embodiment~~examples, the antigen-presenting cells are peripheral blood mononuclear cells (PBMCs). In some ~~embodiment~~examples, the PBMCs are irradiated and allogeneic. In some ~~embodiment~~examples, the PBMCs are added to the cell culture on any of days 9 through 14 in step (d). In some ~~embodiment~~examples, the antigen-presenting cells are artificial antigen-presenting cells.

[0009] In some ~~embodiment~~examples, the harvesting in step (e) is performed using a membrane-based cell processing system.

[0010] In some ~~embodiment~~examples, the harvesting in step (e) is performed using a LOVO cell processing system.

[0011] In some ~~embodiment~~examples, the multiple fragments comprise about 4 to about 50 fragments, wherein each fragment has a volume of about 27 mm³.

[0012] In some ~~embodiment~~examples, the multiple fragments comprise about 30 to about 60 fragments with a total volume of about 1300 mm³ to about 1500 mm³.

[0013] In some ~~embodiment~~examples, the multiple fragments comprise about 50 fragments with a total volume of about 1350 mm³.

[0014] In some ~~embodiment~~examples, the multiple fragments comprise about 50 fragments with a total mass of about 1 gram to about 1.5 grams.

[0015] In some ~~embodiment~~examples, the cell culture medium is provided in a container selected from the group consisting of a G-container and a Xuri cellbag.

[0016] In some ~~embodiment~~examples, the cell culture medium in step (d) further comprises IL-15 and/or IL-21.

[0017] In some ~~embodiment~~examples, the the IL-2 concentration is about 10,000 IU/mL to about 5,000 IU/mL.

[0018] In some ~~embodiment~~examples, the IL-15 concentration is about 500 IU/mL to about 100 IU/mL.

[0019] In some ~~embodiment~~examples, the IL-21 concentration is about 20 IU/mL to about 0.5 IU/mL.

[0020] In some embodiment examples, the infusion bag in step (f) is a HypoThermosol-containing infusion bag.

[0021] In some embodiment examples, the cryopreservation media comprises dimethylsulfoxide (DMSO). In some embodiment examples, the cryopreservation media comprises 7% to 10% dimethylsulfoxide (DMSO).

[0022] In some embodiment 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.

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

[0024] In some embodiment examples, steps (a) through (f) are performed within a period of about 10 days to about 22 days.

[0025] In some embodiment examples, steps (a) through (f) are performed within a period of about 20 days to about 22 days.

[0026] In some embodiment examples, steps (a) through (f) are performed within a period of about 15 days to about 20 days.

[0027] In some embodiment examples, steps (a) through (f) are performed within a period of about 10 days to about 20 days.

[0028] In some embodiment examples, steps (a) through (f) are performed within a period of about 10 days to about 15 days.

[0029] In some embodiment examples, steps (a) through (f) are performed in 22 days or less.

[0030] In some embodiment examples, steps (a) through (f) are performed in 20 days or less.

[0031] In some embodiment examples, steps (a) through (f) are performed in 15 days or less.

[0032] In some embodiment examples, steps (a) through (f) are performed in 10 days or less.

[0033] In some embodiment examples, steps (a) through (f) and cryopreservation are performed in 22 days or less.

[0034] In some embodiment examples, the therapeutic population of TILs harvested in step (e) comprises sufficient TILs for a therapeutically effective dosage of the TILs.

[0035] In some embodiment examples, the number of TILs sufficient for a therapeutically effective dosage is from about 2.3×10^{10} to about 13.7×10^{10} .

[0036] In some ~~embodiment~~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.

[0037] In some ~~embodiment~~examples, the antigen-presenting cells are added to the TILs during the second period in step (d) without opening the system.

[0038] In some ~~embodiment~~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.

[0039] In some ~~embodiment~~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.

[0040] In some ~~embodiment~~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.

[0041] In some ~~embodiment~~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.

[0042] In some ~~embodiment~~examples, the risk of microbial contamination is reduced as compared to an open system.

[0043] In some ~~embodiment~~examples, the TILs from step (g) are infused into a patient.

[0044] In some ~~embodiment~~examples, the multiple fragments comprise about 4 fragments.

[0045] The present ~~invention~~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 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 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, optionally 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.

[0046] In some ~~embodiment~~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).

[0047] In some ~~embodiment~~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} .

[0048] In some ~~embodiment~~examples, the antigen presenting cells (APCs) are PBMCs.

[0049] In some ~~embodiment~~examples, the PBMCs are added to the cell culture on any of days 9 through 14 in step (d).

[0050] In some ~~embodiment~~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.

[0051] In some ~~embodiment~~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.

[0052] In some ~~embodiment~~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).

[0053] In some ~~embodiment~~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.

[0054] In some ~~embodiment~~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.

[0055] In some ~~embodiment~~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.

[0056] In some ~~embodiment~~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.

[0057] In some ~~embodiment~~examples, the cancer is selected from the group consisting of melanoma, HNSCC, cervical cancers, and NSCLC.

[0058] In some ~~embodiment~~examples, the cancer is melanoma.

[0059] In some ~~embodiment~~examples, the cancer is HNSCC.

[0060] In some ~~embodiment~~examples, the cancer is a cervical cancer.

[0061] In some ~~embodiment~~examples, the cancer is NSCLC.

[0062] The present ~~invention~~disclosure ~~also~~also provides 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 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 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, optionally 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.

[0063] In some ~~embodiment~~examples, the therapeutic population of TILs harvested in step (d) comprises sufficient TILs for a therapeutically effective dosage of the TILs.

[0064] In some ~~embodiment~~examples, the number of TILs sufficient for a therapeutically effective dosage is from about 2.3×10^{10} to about 13.7×10^{10} .

[0065] In some ~~embodiment~~examples, the method further comprises the step of cryopreserving the infusion bag comprising the harvested TIL population using a cryopreservation process.

[0066] In some ~~embodiment~~examples, the cryopreservation process is performed using a 1:1 ratio of harvested TIL population to cryopreservation media.

[0067] In some ~~embodiment~~examples, the antigen-presenting cells are peripheral blood mononuclear cells (PBMCs).

[0068] In some ~~embodiment~~examples, the PBMCs are irradiated and allogeneic.

[0069] 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).

[0070] In some ~~embodiment~~examples, the antigen-presenting cells are artificial antigen-presenting cells.

[0071] In some ~~embodiment~~examples, the harvesting in step (d) is performed using a LOVO cell processing system.

[0072] In some ~~embodiment~~examples, the multiple fragments comprise about 4 to about 50 fragments, wherein each fragment has a volume of about 27 mm^3 .

[0073] In some ~~embodiment~~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 .

[0074] In some ~~embodiment~~examples, the multiple fragments comprise about 50 fragments with a total volume of about 1350 mm^3 .

[0075] In some ~~embodiment~~examples, the multiple fragments comprise about 50 fragments with a total mass of about 1 gram to about 1.5 grams.

[0076] In some ~~embodiment~~examples, the multiple fragments comprise about 4 fragments.

[0077] In some ~~embodiment~~examples, the second cell culture medium is provided in a container selected from the group consisting of a G-container and a Xuri cellbag.

[0078] In some ~~embodiment~~examples, the infusion bag in step (e) is a HypoThermosol-containing infusion bag.

[0079] In some ~~embodiment~~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.

[0080] In some ~~embodiment~~examples, the first period in step (b) and the second period in step (c) are each individually performed within a period of 11 days.

[0081] In some ~~embodiment~~examples, steps (a) through (e) are performed within a period of about 10 days to about 22 days.

[0082] In some ~~embodiment~~examples, steps (a) through (e) are performed within a period of about 10 days to about 20 days.

[0083] In some ~~embodiment~~examples, steps (a) through (e) are performed within a period of about 10 days to about 15 days.

[0084] In some ~~embodiment~~examples, steps (a) through (e) are performed in 22 days or less.

[0085] In some ~~embodiment~~examples, steps (a) through (e) and cryopreservation are performed in 22 days or less.

[0086] In some ~~embodiment~~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.

[0087] In some ~~embodiment~~examples, the antigen-presenting cells are added to the TILs during the second period in step (c) without opening the system.

[0088] In some ~~embodiment~~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.

[0089] In some ~~embodiment~~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.

[0090] In some ~~embodiment~~examples, the risk of microbial contamination is reduced as compared to an open system.

[0091] In some ~~embodiment~~examples, the TILs from step (e) are infused into a patient.

[0092] In some ~~embodiment~~examples, the closed container comprises a single bioreactor.

[0093] In some ~~embodiment~~examples, the closed container comprises a G-REX-10.

[0094] In some ~~embodiment~~examples, the closed container comprises a G-REX -100.

[0095] In some ~~embodiment~~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.

[0096] In some ~~embodiment~~examples, the cell culture has a ratio of 2.5×10^9 APCs to 100×10^6 TILs.

[0097] In some ~~embodiment~~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.

[0098] In some ~~embodiment~~examples, the cell culture has ratio of 2.5×10^9 APCs to 100×10^6 TILs.

[0099] The present ~~invention~~disclosure ~~also~~also provides 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, optionally 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.

[00100] In some ~~embodiment~~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. In some

~~embodiment~~examples, the population of TILs is for use in the treatment of 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.

[00101] The present ~~invention~~disclosure provides 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, optionally OKT-3, and optionally a tumor necrosis factor receptor superfamily (TNFRSF) agonist, 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, optionally OKT-3, and optionally a tumor necrosis factor receptor superfamily (TNFRSF) agonist, 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.

[00102] The present ~~invention~~disclosure 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, optionally OKT-3, and optionally a tumor necrosis factor receptor superfamily (TNFRSF) agonist, 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, optionally OKT-3, and optionally a tumor necrosis factor receptor superfamily (TNFRSF) agonist, 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.

[00103] In some embodiments, the tumor necrosis factor receptor superfamily (TNFRSF) agonist is a 4-1BB antibody. In some ~~embodiment~~ examples, the TNFRSF agonist is a 4-1BB agonist, and the 4-1BB agonist is selected from the group consisting of urelumab, utomilumab, EU-101, a fusion protein, and fragments, derivatives, variants, biosimilars, and combinations thereof.

[00104] The present ~~invention~~ disclosure also provides a cryopreservation composition comprising a population of TILs as described above and herein, a cryoprotectant medium comprising dimethylsulfoxide (DMSO), and an electrolyte solution.

[00105] In some ~~embodiment~~ examples, the cryopreservation composition further comprises one or more stabilizers and one or more lymphocyte growth factors. In some ~~embodiment~~ examples, the one or more stabilizers comprise human serum albumin (HSA) and the one or more lymphocyte growth factors comprise IL-2. In some ~~embodiment~~ examples, the composition optionally comprises OKT-3.

[00106] In some ~~embodiment~~ examples, the DMSO and the electrolyte solution are present in a ratio of about 1.1:1 to about 1:1.1. In some ~~embodiment~~ examples, the DMSO and the electrolyte solution are present in a ratio of about 1:1.

[00107] In some ~~embodiment~~ examples, 100 mL of the cryopreservation composition comprises the population of TILs in an amount of about 1×10^6 to about 9×10^{14} , the cryoprotectant medium comprising DMSO in an amount of about 30 mL to about 70 mL, the electrolyte

solution in an amount of about 30 mL to about 70 mL, HSA in an amount of about 0.1 g to about 1.0 g, and IL-2 in an amount of about 0.001 mg to about 0.005 mg.

[00108] In some ~~embodiment~~examples, 100 mL of the cryopreservation composition comprises the population of TILs in an amount of about 1×10^7 to about 1×10^{11} ; the cryoprotectant medium in an amount of about 30 mL to about 70 mL, wherein the cryoprotectant medium comprises about 10% DMSO; the electrolyte solution in an amount of about 30 mL to about 70 mL; HSA in an amount of about 0.3 g to about 0.7 g; and IL-2 in an amount of about 0.001 mg to about 0.003 mg.

[00109] In some ~~embodiment~~examples, 100 mL of the cryopreservation composition consists essentially of the population of TILs in an amount of about 1×10^7 to about 1×10^{11} ; the cryoprotectant medium in an amount of about 30 mL to about 70 mL, wherein the cryoprotectant medium consists essentially of about 10% DMSO; the electrolyte solution in an amount of about 30 mL to about 70 mL; HSA in an amount of about 0.3 g to about 0.7 g; and IL-2 in an amount of about 0.001 mg to about 0.003 mg.

[00110] In some ~~embodiment~~examples, the composition is for use in treating a subject with cancer.

[00111] The present ~~invention~~disclosure also provides a cryopreservation composition comprising a population of TILs, a cryoprotectant medium comprising dimethylsulfoxide (DMSO), and an electrolyte solution, wherein 100 mL of the composition comprises the population of TILs in an amount of about 1×10^7 to about 1×10^{11} ; the cryoprotectant medium in an amount of about 30 mL to about 70 mL, wherein the cryoprotectant medium comprises about 10% DMSO; the electrolyte solution in an amount of about 30 mL to about 70 mL; HSA in an amount of about 0.3 g to about 0.7 g; and IL-2 in an amount of about 0.001 mg to about 0.003 mg.

[00112] The present ~~invention~~disclosure also provides a cryopreservation composition consisting essentially of a population of TILs, a cryoprotectant medium comprising dimethylsulfoxide (DMSO), and an electrolyte solution, wherein 100 mL of the composition consists essentially of the population of TILs in an amount of about 1×10^7 to about 1×10^{11} ; the cryoprotectant medium in an amount of about 30 mL to about 70 mL, wherein the cryoprotectant medium consists essentially of about 10% DMSO; the electrolyte solution in an amount of about 30 mL to about 70 mL; HSA in an amount of about 0.3 g to about 0.7 g; and IL-2 in an amount of about 0.001 mg to about 0.003 mg.

[00113] The present ~~invention~~disclosure also provides an infusion bag comprising a cryopreservation composition as described above and herein.

[00114] In some ~~embodiment~~examples, the infusion bag is a HypoThermosol-containing infusion bag.

[00115] The present ~~invention~~disclosure also provides a storage bag comprising a cryopreservation composition as described above and herein.

[00116] In some ~~embodiment~~examples, the storage bag of claim 54, wherein the storage bag is a CryoStore CS750 Freezing bag.

BRIEF DESCRIPTION OF THE DRAWINGS

[00117] **Figure 1:** Shows a diagram of an embodiment of process 2A, a 22-day process for TIL manufacturing.

[00118] **Figure 2:** Shows a comparison between the 1C process and an embodiment of the 2A process for TIL manufacturing.

[00119] **Figure 3:** Shows the 1C process timeline.

[00120] **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.

[00121] **Figure 5:** Shows the process of an embodiment of TIL therapy using process 2A for TIL manufacturing, including administration and co-therapy steps, for lower cell counts.

[00122] **Figure 6:** Shows a detailed schematic for an embodiment of the 2A process.

[00123] **Figure 7:** Shows characterization of TILs prepared using an embodiment of the 2A process by comparing interferon-gamma (IFN- γ) expression between fresh TILs and thawed TILs.

[00124] **Figure 8:** Shows characterization of TILs prepared using an embodiment of the 2A process by examining CD3 expression in fresh TILs versus thawed TILs.

[00125] **Figure 9:** Shows characterization of TILs prepared using an embodiment of the 2A process by examining recovery in fresh TILs versus thawed TILs.

WHAT IS CLAIMED IS:

1. 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, 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 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, optionally OKT-3, and antigen presenting cells (APCs), to produce a third population of TILs, wherein the second expansion is performed for ~~about 7-144~~ to 6 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) splitting the third population of TILs into a first plurality of five or less sub-populations of TILs, each sub-population comprising at least 1.0×10^9 TILs, and performing a third expansion of the first plurality of sub-populations of TILs by supplementing the cell culture medium of each sub-population of TILs with additional IL-2, optionally OKT-3, to produce a second plurality of sub-populations of TILs, wherein the second plurality of sub-populations of TILs comprise a therapeutic population of TILs, wherein the third expansion is performed for 5 to 7 days, wherein the third expansion for each sub-population is performed in a closed container providing a third gas-permeable surface area, and wherein the transition from step (c) to step (d) occurs without opening the system;
 - ~~(de)~~ harvesting the therapeutic population of TILs obtained from step ~~(ed)~~, wherein the transition from step ~~(ed)~~ to step ~~(de)~~ occurs without opening the system; and
 - ~~(ef)~~ transferring the harvested TIL population from step ~~(de)~~ to an infusion bag,

wherein the transfer from step (de) to (ef) occurs without opening the system.

2. The method according to claim 1, wherein the therapeutic population of TILs harvested in step (de) comprises sufficient TILs for a therapeutically effective dosage of the TILs.
3. The method according to claim 2, where the number of TILs sufficient for a therapeutically effective dosage is from ~~about~~ 2.3×10^{10} to ~~about~~ 13.7×10^{10} .
4. The method according to claim 3, further comprising the step of cryopreserving the infusion bag comprising the harvested TIL population using a cryopreservation process.
5. The method according to claim 4, wherein the cryopreservation process is performed using a 1:1 ratio of harvested TIL population to cryopreservation media.
6. The method according to claim 5, wherein the antigen-presenting cells are peripheral blood mononuclear cells (PBMCs).
7. The method according to claim 6, wherein the PBMCs are irradiated and allogeneic.
- ~~8. The method according to claim 7, wherein the PBMCs are added to the cell culture on any of days 9 through 14 in step (e).~~
- ~~9. The method according to claim 1, wherein the antigen-presenting cells are artificial antigen-presenting cells.~~
- ~~10. The method according to claim 1, wherein the harvesting in step (de) is performed using a LOVO cell processing system.~~
- ~~11. 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 .~~
- ~~12. 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 .~~
- ~~13. The method according to claim 2, wherein the multiple fragments comprise about 50 fragments with a total volume of about 1350 mm^3 .~~
- ~~14. 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.~~
- ~~15. The method according to claim 1, wherein the multiple fragments comprise about 4 fragments.~~

- ~~16. The method according to claim 1, wherein the second cell culture medium is provided in a container selected from the group consisting of a G container and a Xuri cellbag.~~
- ~~17.8.~~ The method according to claim 1, wherein the infusion bag in step (ef) is a hypothermic biopreservation HypoThermosol containing infusion bag.
- ~~18.9.~~ The method according to claim 1, wherein the first period in step (b) and is the second period in step (e) are each individually performed within a period of 10 days, 11 days, or 12 days.
- ~~19.10.~~ The method according to claim 1, wherein the first period in step (b) and the second period in step (e) are each individually is performed within a period of 11 days.
- ~~20.11.~~ The method according to claim 1, wherein steps (a) through (ef) are performed within a period of about 10 days to about 22 days.
- ~~21.12.~~ The method according to claim 1, wherein steps (a) through (ef) are performed within a period of about 10 days to about 20 days.
- ~~22.13.~~ The method according to claim 1, wherein steps (a) through (ef) are performed within a period of about 10 days to about 15 days.
- ~~23.14.~~ The method according to claim 1, wherein steps (a) through (ef) are performed in 22 days or less.
- ~~24.15.~~ The method according to claim 1, wherein steps (a) through (e) are performed in 22 days or less.
16. The method according to claim 1, wherein step (c) is performed for 108 hours to 132 hours.
17. The method according to claim 1, wherein step (c) is performed for 108 hours, 5 days or 132 hours.
18. The method according to claim 1, wherein step (c) and step (d) are performed within a period of 10 days to 12 days.
19. The method according to claim 1, wherein step (c) and step (d) are performed within a period of about 10 days, 11 days or 12 days.

~~25. The method according to any one of claims 1 to 24, 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.~~

~~26. The method according to any one of claims 1 to 25, wherein the antigen presenting cells are added to the TILs during the second period in step (c) without opening the system.~~

~~27-20.~~ The method according to any one of claims 1 to ~~26~~19, 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.

~~28-21.~~ The method according to any one of claims 1 to ~~27~~20, 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.

~~29-22.~~ The method according to any one of claims 1 to ~~28~~21, wherein the risk of microbial contamination is reduced as compared to an open system.

~~30. The method according to any one of claims 1 to 29, wherein the TILs from step (e) are infused into a patient.~~

~~31-23.~~ The method according to any of the preceding claims wherein ~~the~~each closed container comprises a single bioreactor.

24. The method according to claim 1, wherein the second population of TILs is at least 50-fold greater in number than the first population of TILs.

~~32. The method according to claim 31, wherein the closed container comprises a G REX-10.~~

~~33. The method according to claim 31, wherein the closed container comprises a G REX-100.~~

34. The method according to any one of claims 1 to 33, 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.

35. The method according to claim 34, wherein the cell culture has a ratio of 2.5×10^9 APCs to 100×10^6 TILs.

36. The method according to any one of claims 1 to 33, 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.

37. The method according to claim 36, wherein the cell culture has ratio of 2.5×10^9 APCs to 100×10^6 TILs.

38. 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, 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 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, optionally 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.
39. The population of TILs according to claim 38, wherein the method of producing the TILs further comprises one or more of the features recited in any one of claims 1 to 37.
- 40.25. A cryopreservation composition comprising the population of TILs obtained or obtainable according to by the method of any of the preceding claims claim 38, a cryoprotectant medium comprising dimethylsulfoxide (DMSO), and an electrolyte solution.
- 41.26. The cryopreservation composition according to claim 2540, further comprising one or more stabilizers and one or more lymphocyte growth factors.
- 42.27. The cryopreservation composition according to claim 2641, wherein the one or more stabilizers comprise human serum albumin (HSA) and the one or more lymphocyte growth factors comprise IL-2.
- 43.28. The cryopreservation composition according to claim 4227, wherein the composition optionally comprises OKT-3.
- 44.29. The cryopreservation composition according to claim 2540, wherein the cryoprotectant medium comprising DMSO and the electrolyte solution are present in a ratio of ~~about~~ 1.1:1 to ~~about~~ 1:1.1.
- 45.30. The cryopreservation composition according to claim 4429, wherein the cryoprotectant medium comprising DMSO and the electrolyte solution are present in a ratio of about 1:1.
- 46.31. The cryopreservation composition according to claim 4025, wherein 100 mL of the composition comprises the population of TILs in an amount of ~~about~~ 1×10^6 to ~~about~~ 9×10^{14} ; the cryoprotectant medium comprising DMSO in an amount of ~~about~~ 30 mL to ~~about~~ 70 mL, the electrolyte solution in an amount of ~~about~~ 30 mL to ~~about~~ 70 mL; HSA in an amount of ~~about~~ 0.1 g to ~~about~~ 1.0 g; and IL-2 in an amount of ~~about~~ 0.001 mg to ~~about~~ 0.005 mg.

~~47.32.~~ The cryopreservation composition according to claim ~~40~~25, wherein 100 mL of the composition comprises the population of TILs in an amount of ~~about~~ 1×10^7 to ~~about~~ 1×10^{11} ; the cryoprotectant medium in an amount of ~~about~~ 30 mL to ~~about~~ 70 mL, wherein the cryoprotectant medium comprises about 10% DMSO; the electrolyte solution in an amount of ~~about~~ 30 mL to ~~about~~ 70 mL; HSA in an amount of ~~about~~ 0.3 g to ~~about~~ 0.7 g; and IL-2 in an amount of ~~about~~ 0.001 mg to ~~about~~ 0.003 mg.

~~48.33.~~ The cryopreservation composition according to claim ~~40~~25, wherein 100 mL of the composition consists essentially of the population of TILs in an amount of ~~about~~ 1×10^7 to ~~about~~ 1×10^{11} ; the cryoprotectant medium in an amount of ~~about~~ 30 mL to ~~about~~ 70 mL, wherein the cryoprotectant medium consists essentially of about 10% DMSO; the electrolyte solution in an amount of ~~about~~ 30 mL to ~~about~~ 70 mL; HSA in an amount of ~~about~~ 0.3 g to ~~about~~ 0.7 g; and IL-2 in an amount of ~~about~~ 0.001 mg to ~~about~~ 0.003 mg.

~~34.~~ The cryopreservation composition according to claim ~~40~~25, ~~wherein the composition is for use in therapy treating a subject with cancer.~~

~~49.35.~~ Use of the cryopreservation composition according to claim 25 in the manufacture of a medicament for treating cancer in a subject.

~~50. A cryopreservation composition comprising a population of TILs, a cryoprotectant medium comprising dimethylsulfoxide (DMSO), and an electrolyte solution, wherein 100 mL of the composition comprises the population of TILs in an amount of about 1×10^7 to about 1×10^{11} ; the cryoprotectant medium in an amount of about 30 mL to about 70 mL, wherein the cryoprotectant medium comprises about 10% DMSO; the electrolyte solution in an amount of about 30 mL to about 70 mL; HSA in an amount of about 0.3 g to about 0.7 g; and IL 2 in an amount of about 0.001 mg to about 0.003 mg.~~

~~51. A cryopreservation composition consisting essentially of a population of TILs, a cryoprotectant medium comprising dimethylsulfoxide (DMSO), and an electrolyte solution, wherein 100 mL of the composition consists essentially of the population of TILs in an amount of about 1×10^7 to about 1×10^{11} ; the cryoprotectant medium in an amount of about 30 mL to about 70 mL, wherein the cryoprotectant medium consists essentially of about 10% DMSO; the electrolyte solution in an amount of about 30 mL to about 70 mL; HSA in an amount of about 0.3 g to about 0.7 g; and IL 2 in an amount of about 0.001 mg to about 0.003 mg.~~

~~52. An infusion bag comprising a cryopreservation composition according to any one of claims 40 to 51.~~

~~53. The infusion bag of claim 52, wherein the infusion bag is a HypoThermosol-containing infusion bag.~~

~~54. A storage bag comprising a cryopreservation composition according to any one of claims 40 to 51.~~

~~55. The storage bag of claim 54, wherein the storage bag is a CryoStore CS750 Freezing bag.~~