therapies based on such processes that are appropriate for commercial scale manufacturing and regulatory approval for use in human patients at multiple clinical centers. Moreover, there is a strong need for more effective TIL therapies that can increase a patient's response rate and response robustness.

BRIEF SUMMARY

[0004] The present disclosure provides methods for expanding TILs and producing therapeutic populations of TILs. According to exemplary examples, at least a portion of the therapeutic population of TILs are gene-edited to enhance their therapeutic effect.

[0005] In an aspect, the present 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;

(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) at any time during the method, gene-editing at least a portion of the TILs.

[0005a] In another aspect, the present disclosure provides use of expanded tumor infiltrating lymphocytes (TILs) in the manufacture of a medicament for the treatment of a subject with cancer, wherein the use comprises:

(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;

(h) wherein a therapeutically effective dosage of the third population of TILs from the

infusion bag in step (g) is to be administered to the patient; and

(i) at any time during the method steps (a)-(f), gene-editing at least a portion of the TILs.

[0005b] In yet another aspect, the present disclosure provides use of expanded tumor infiltrating lymphocytes (TILs) in the manufacture of a medicament for the treatment of a subject with cancer, wherein the use comprises:

(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 comprising OKT-3 and/or a 4-1BB agonist antibody for about 2 to 5 days;

(d) optioinally adding 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 1 to 3 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 (c) to step (d) occurs without opening the system;

(e) performing a sterile electroporation step on the second population of TILs, wherein the sterile electroporation step mediates the transfer of at least one gene editor;

(f) resting the second population of TILs for about 1 day;

(g) performing a second expansion by supplementing the cell culture medium of the second population of TILs with additional IL-2, optionally OKT-3 antibody, optionally an OX40 antibody, and antigen presenting cells (APCs), to produce a third population of TILs, wherein the second expansion is performed for about 7 to 11 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 (f) to step (g) occurs without opening the system;

(h) harvesting the therapeutic population of TILs obtained from step (g) to provide a harvested TIL population, wherein the transition from step (g) to step (h) occurs

without opening the system, wherein the harvested population of TILs is a therapeutic population of TILs;

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

(j) cryoharvesting the harvested TIL population using a dimethylsulfoxide-based cryopreservation medium; and

(k) wherein a therapeutically effective dosage of the harvested TIL population is to be administered from the infusion bag to the patient;

wherein the electroporation step comprises the delivery of a Clustered Regularly Interspersed Short Palindromic Repeat (CRISPR) system, a Transcription Activator-Like Effector (TALE) system, or a zinc finger system for inhibiting the expression of a molecule selected from the group consisting of PD-1, LAG-3, TIM-3, CTLA-4, TIGIT, CISH, TGFβR2, PRA, CBLB, BAFF (BR3), and combinations thereof.

[0006] 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.

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

[0008] 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.

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

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

[0011] In some examples, the multiple fragments comprise about 4 to about 50 fragments, wherein each fragment has a volume of about 27 mm^3.

[0012] 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.

[0013] In some examples, the multiple fragments comprise about 50 fragments with a total volume of about 1350 mm^3.

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

[0015] 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.

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

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

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

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

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

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

[0022] 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.

[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 11 days.

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

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

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

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

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

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

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

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

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

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

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

[0035] In some examples, the number of TILs sufficient for a therapeutically effective dosage is from about 2.3×1010 to about 13.7×1010.

[0036] 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.

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

[0038] 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.

[0039] 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.

[0040] 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.

[0041] 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.

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

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

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

[0045] In some examples, the cell culture medium further comprises a 4-1BB agonist and/or an OX40 agonist during the first expansion, the second expansion, or both.

[0046] In some examples, the gene-editing is carried out after the 4-1BB agonist and/or the OX40 agonist is introduced into the cell culture medium.

[0047] In some examples, the gene-editing is carried out before the 4-1BB agonist and/or the OX40 agonist is introduced into the cell culture medium.

[0048] In some examples, the gene-editing is carried out on TILs from one or more of the first population, the second population, and the third population.

[0049] In some examples, the gene-editing is carried out on TILs from the first expansion, or TILs from the second expansion, or both.

[0050] In some examples, the gene-editing is carried out after the first expansion and before the second expansion.

[0051] In some examples, the gene-editing is carried out before step (c), before step (d), or before step (e).

[0052] In some examples, the cell culture medium comprises OKT-3 during the first expansion and/or during the second expansion, and the gene-editing is carried out before the OKT-3 is introduced into the cell culture medium.

[0053] In some examples, the cell culture medium comprises OKT-3 during the first expansion and/or during the second expansion, and the gene-editing is carried out after the OKT-3 is introduced into the cell culture medium.

[0054] In some examples, the cell culture medium comprises OKT-3 beginning on the start day of the first expansion, and the gene-editing is carried out after the TILs have been exposed to the OKT-3.

[0055] In some examples, the gene-editing causes expression of one or more immune checkpoint genes to be silenced or reduced in at least a portion of the therapeutic population of TILs.

[0056] In some examples, the one or more immune checkpoint genes is/are selected from the group comprising PD-1, CTLA-4, LAG-3, HAVCR2 (TIM-3), Cish, TGFβ, PKA, CBL-B, PPP2CA, PPP2CB, PTPN6, PTPN22, PDCD1, BTLA, CD160, TIGIT, CD96, CRTAM, LAIR1, SIGLEC7, SIGLEC9, CD244, TNFRSF10B, TNFRSF10A, CASP8, CASP10, CASP3, CASP6, CASP7, FADD, FAS, SMAD2, SMAD3, SMAD4, SMAD10, SKI, SKIL, TGIF1, IL10RA, IL10RB, HMOX2, IL6R, IL6ST, EIF2AK4, CSK, PAG1, SIT1, FOXP3, PRDM1, BATF, GUCY1A2, GUCY1A3, GUCY1B2, and GUCY1B3.

[0057] In some examples, the one or more immune checkpoint genes is/are selected from the group comprising PD-1, CTLA-4, LAG-3, HAVCR2 (TIM-3), Cish, TGFβ, and PKA.

[0058] In some examples, the gene-editing causes expression of one or more immune checkpoint genes to be enhanced in at least a portion of the therapeutic population of TILs, the immune checkpoint gene(s) being selected from the group comprising CCR2, CCR4, CCR5, CXCR2, CXCR3, CX3CR1, IL-2, IL-4, IL-7, IL-10, IL-15, IL-21, the NOTCH 1/2 intracellular domain (ICD), and/or the NOTCH ligand mDLL1.

[0059] In some examples, the gene-editing comprises the use of a programmable nuclease that mediates the generation of a double-strand or single-strand break at said one or more immune checkpoint genes.

[0060] In some examples, the gene-editing comprises one or more methods selected from a CRISPR method, a TALE method, a zinc finger method, and a combination thereof.

[0061] In some examples, the gene-editing comprises a CRISPR method.

[0062] In some examples, the CRISPR method is a CRISPR/Cas9 method.

[0063] In some examples, the gene-editing comprises a TALE method.

[0064] In some examples, the gene-editing comprises a zinc finger method.

[0065] In another example, the present 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, 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;

(h) administering a therapeutically effective dosage of the third population of TILs from the infusion bag in step (g) to the patient; and

(i) at any time during the method steps (a)-(f), gene-editing at least a portion of the TILs.

[0066] 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).

[0067] In some examples, the number of TILs sufficient for administering a therapeutically effective dosage in step (h) is from about 2.3×1010 to about 13.7×1010.

[0068] In some examples, the antigen presenting cells (APCs) are PBMCs.

[0069] In some examples, the PBMCs are added to the cell culture on any of days 9 through 14 in step (d).

[0070] 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.

[0071] In some examples, the non-myeloablative lymphodepletion regimen comprises the steps of administration of cyclophosphamide at a dose of 60 mg/m^2/day for two days followed by administration of fludarabine at a dose of 25 mg/m^2/day for five days.

[0072] 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).

[0073] 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.

[0074] 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.

[0075] 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.

[0076] 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.

[0077] In some examples, the cancer is selected from the group consisting of melanoma, HNSCC, cervical cancers, and NSCLC.

[0078] In some examples, the cancer is melanoma.

[0079] In some examples, the cancer is HNSCC.

[0080] In some examples, the cancer is a cervical cancer.

[0081] In some examples, the cancer is NSCLC.

[0082] In some examples, the cell culture medium further comprises a 4-1BB agonist and/or an OX40 agonist during the first expansion, the second expansion, or both.

[0083] In some examples, the gene-editing is carried out after the 4-1BB agonist and/or the OX40 agonist is introduced into the cell culture medium.

[0084] In some examples, the gene-editing is carried out before the 4-1BB agonist and/or the OX40 agonist is introduced into the cell culture medium.

[0085] In some examples, the gene-editing is carried out on TILs from one or more of the first population, the second population, and the third population.

[0086] In some examples, the gene-editing is carried out on TILs from the first expansion, or TILs from the second expansion, or both.

[0087] In some examples, the gene-editing is carried out after the first expansion and before the second expansion.

[0088] In some examples, the gene-editing is carried out before step (c), before step (d), or before step (e).

[0089] In some examples, the cell culture medium comprises OKT-3 during the first expansion and/or during the second expansion, and the gene-editing is carried out before the OKT-3 is introduced into the cell culture medium.

[0090] In some examples, the cell culture medium comprises OKT-3 during the first expansion and/or during the second expansion, and the gene-editing is carried out after the OKT-3 is introduced into the cell culture medium.

[0091] In some examples, the cell culture medium comprises OKT-3 beginning on the start day of the first expansion, and the gene-editing is carried out after the TILs have been exposed to the OKT-3.

[0092] In some examples, the gene-editing causes expression of one or more immune checkpoint genes to be silenced or reduced in at least a portion of the therapeutic population of TILs.

[0093] In some examples, the one or more immune checkpoint genes is/are selected from the group comprising PD-1, CTLA-4, LAG-3, HAVCR2 (TIM-3), Cish, TGFβ, PKA, CBL-B, PPP2CA, PPP2CB, PTPN6, PTPN22, PDCD1, BTLA, CD160, TIGIT, CD96, CRTAM, LAIR1, SIGLEC7, SIGLEC9, CD244, TNFRSF10B, TNFRSF10A, CASP8, CASP10, CASP3, CASP6, CASP7, FADD, FAS, SMAD2, SMAD3, SMAD4, SMAD10, SKI, SKIL, TGIF1, IL10RA, IL10RB, HMOX2, IL6R, IL6ST, EIF2AK4, CSK, PAG1, SIT1, FOXP3, PRDM1, BATF, GUCY1A2, GUCY1A3, GUCY1B2, and GUCY1B3.

[0094] In some examples, the one or more immune checkpoint genes is/are selected from the group comprising PD-1, CTLA-4, LAG-3, HAVCR2 (TIM-3), Cish, TGFβ, and PKA.

[0095] In some examples, the gene-editing causes expression of one or more immune checkpoint genes to be enhanced in at least a portion of the therapeutic population of TILs, the immune checkpoint gene(s) being selected from the group comprising CCR2, CCR4, CCR5, CXCR2, CXCR3, CX3CR1, IL-2, IL-4, IL-7, IL-10, IL-15, IL-21, the NOTCH 1/2 intracellular domain (ICD), and/or the NOTCH ligand mDLL1.

[0096] In some examples, the gene-editing comprises the use of a programmable nuclease that mediates the generation of a double-strand or single-strand break at said one or more immune checkpoint genes.

[0097] In some examples, the gene-editing comprises one or more methods selected from a CRISPR method, a TALE method, a zinc finger method, and a combination thereof.

[0098] In some examples, the gene-editing comprises a CRISPR method.

[0099] In some examples, the CRISPR method is a CRISPR/Cas9 method.

[00100] In some examples, the gene-editing comprises a TALE method.

[00101] In some examples, the gene-editing comprises a zinc finger method.

[00102] In another aspect, the present disclosure provides 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 7-14 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;

(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; and

(f) at any time during the method, gene-editing at least a portion of the TILs.

[00103] In some examples, the therapeutic population of TILs harvested in step (d) comprises sufficient TILs for a therapeutically effective dosage of the TILs.

[00104] In some examples, the number of TILs sufficient for a therapeutically effective dosage is from about 2.3×1010 to about 13.7×1010.

[00105] In some examples, the method further comprises the step of cryopreserving the infusion bag comprising the harvested TIL population using a cryopreservation process.

[00106] In some examples, the cryopreservation process is performed using a 1:1 ratio of harvested TIL population to cryopreservation media.

[00107] In some examples, the antigen-presenting cells are peripheral blood mononuclear cells (PBMCs).

[00108] In some examples, the PBMCs are irradiated and allogeneic.

[00109] In some examples, the PBMCs are added to the cell culture on any of days 9 through 14 in step (c).

[00110] In some examples, the antigen-presenting cells are artificial antigen-presenting cells.

[00111] In some examples, the harvesting in step (d) is performed using a LOVO cell processing system.

[00112] In some examples, the multiple fragments comprise about 4 to about 50 fragments, wherein each fragment has a volume of about 27 mm^3.

[00113] 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.

[00114] In some examples, the multiple fragments comprise about 50 fragments with a total volume of about 1350 mm^3.

[00115] In some examples, the multiple fragments comprise about 50 fragments with a total mass of about 1 gram to about 1.5 grams.

[00116] In some examples, the multiple fragments comprise about 4 fragments.

[00117] 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.

[00118] In some examples, the infusion bag in step (e) is a HypoThermosol-containing infusion bag.

[00119] 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.

[00120] 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.

[00121] In some examples, steps (a) through (e) are performed within a period of about 10 days to about 22 days.

[00122] In some examples, steps (a) through (e) are performed within a period of about 10 days to about 20 days.

[00123] In some examples, steps (a) through (e) are performed within a period of about 10 days to about 15 days.

[00124] In some examples, steps (a) through (e) are performed in 22 days or less.

[00125] In some examples, steps (a) through (e) and cryopreservation are performed in 22 days or less.

[00126] 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.

[00127] In some examples, the antigen-presenting cells are added to the TILs during the second period in step (c) without opening the system.

[00128] 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.

[00129] 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.

[00130] In some examples, the risk of microbial contamination is reduced as compared to an open system.

[00131] In some examples, the TILs from step (e) are infused into a patient.

[00132] In some examples, the closed container comprises a single bioreactor.

[00133] In some examples, the closed container comprises a G-REX-10.

[00134] In some examples, the closed container comprises a G-REX -100.

[00135] 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.

[00136] In some examples, the cell culture has a ratio of 2.5×10^9 APCs to 100×10^6 TILs.

[00137] 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.

[00138] In some examples, the cell culture has ratio of 2.5×10^9 APCs to 100×10^6 TILs.

[00139] In some examples, the cell culture medium further comprises a 4-1BB agonist and/or an OX40 agonist during the first expansion, the second expansion, or both.

[00140] In some examples, the gene-editing is carried out after the 4-1BB agonist and/or the OX40 agonist is introduced into the cell culture medium.

[00141] In some examples, the gene-editing is carried out before the 4-1BB agonist and/or the OX40 agonist is introduced into the cell culture medium.

[00142] In some examples, the gene-editing is carried out on TILs from one or more of the first population, the second population, and the third population.

[00143] In some examples, the gene-editing is carried out on TILs from the first expansion, or TILs from the second expansion, or both.

[00144] In some examples, the gene-editing is carried out after the first expansion and before the second expansion.

[00145] In some examples, the gene-editing is carried out before step (b), before step (c), or before step (d).

[00146] In some examples, the cell culture medium comprises OKT-3 during the first expansion and/or during the second expansion, and the gene-editing is carried out before the OKT-3 is introduced into the cell culture medium.

[00147] In some examples, the cell culture medium comprises OKT-3 during the first expansion and/or during the second expansion, and the gene-editing is carried out after the OKT-3 is introduced into the cell culture medium.

[00148] In some examples, the cell culture medium comprises OKT-3 beginning on the start day of the first expansion, and the gene-editing is carried out after the TILs have been exposed to the OKT-3.

[00149] In some examples, the gene-editing causes expression of one or more immune checkpoint genes to be silenced or reduced in at least a portion of the therapeutic population of TILs.

[00150] In some examples, the one or more immune checkpoint genes is/are selected from the group comprising PD-1, CTLA-4, LAG-3, HAVCR2 (TIM-3), Cish, TGFβ, PKA, CBL-B, PPP2CA, PPP2CB, PTPN6, PTPN22, PDCD1, BTLA, CD160, TIGIT, CD96, CRTAM, LAIR1, SIGLEC7, SIGLEC9, CD244, TNFRSF10B, TNFRSF10A, CASP8, CASP10, CASP3, CASP6, CASP7, FADD, FAS, SMAD2, SMAD3, SMAD4, SMAD10, SKI, SKIL, TGIF1, IL10RA, IL10RB, HMOX2, IL6R, IL6ST, EIF2AK4, CSK, PAG1, SIT1, FOXP3, PRDM1, BATF, GUCY1A2, GUCY1A3, GUCY1B2, and GUCY1B3.

[00151] In some examples, the one or more immune checkpoint genes is/are selected from the group comprising PD-1, CTLA-4, LAG-3, HAVCR2 (TIM-3), Cish, TGFβ, and PKA.

[00152] In some examples, the gene-editing causes expression of one or more immune checkpoint genes to be enhanced in at least a portion of the therapeutic population of TILs, the immune checkpoint gene(s) being selected from the group comprising CCR2, CCR4, CCR5, CXCR2, CXCR3, CX3CR1, IL-2, IL-4, IL-7, IL-10, IL-15, IL-21, the NOTCH 1/2 intracellular domain (ICD), and/or the NOTCH ligand mDLL1.

[00153] In some examples, the gene-editing comprises the use of a programmable nuclease that mediates the generation of a double-strand or single-strand break at said one or more immune checkpoint genes.

[00154] In some examples, the gene-editing comprises one or more methods selected from a CRISPR method, a TALE method, a zinc finger method, and a combination thereof.

[00155] In some examples, the gene-editing comprises a CRISPR method.

[00156] In some examples, the CRISPR method is a CRISPR/Cas9 method.

[00157] In some examples, the gene-editing comprises a TALE method.

[00158] In some examples, the gene-editing comprises a zinc finger method.

[00159] In another example, the present disclosure provides a population of therapeutic TILs that have been expanded in accordance with any of the expansion methods described herein (e.g., for use in the treatment of a subject's cancer), wherein the population of therapeutic TILs has been permanently gene-edited.

[00160] In another aspect, the present disclosure 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, 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;

(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) at any time during the method, gene-editing at least a portion of the TILs.

[00161] In some examples, the above method further comprises one or more features recited in any of the methods and compositions described herein.

[00162] In some examples, the cell culture medium further comprises a 4-1BB agonist and/or an OX40 agonist during the first expansion, the second expansion, or both.

[00163] In some examples, the gene-editing is carried out after the 4-1BB agonist and/or the OX40 agonist is introduced into the cell culture medium.

[00164] In some examples, the gene-editing is carried out before the 4-1BB agonist and/or the OX40 agonist is introduced into the cell culture medium.

[00165] In some examples, the gene-editing is carried out on TILs from one or more of the first population, the second population, and the third population.

[00166] In some examples, the gene-editing is carried out on TILs from the first expansion, or TILs from the second expansion, or both.

[00167] In some examples, the gene-editing is carried out after the first expansion and before the second expansion.

[00168] In some examples, the gene-editing is carried out before step (c), before step (d), or before step (e).

[00169] In some examples, the cell culture medium comprises OKT-3 during the first expansion and/or during the second expansion, and the gene-editing is carried out before the OKT-3 is introduced into the cell culture medium.

[00170] In some examples, the cell culture medium comprises OKT-3 during the first expansion and/or during the second expansion, and the gene-editing is carried out after the OKT-3 is introduced into the cell culture medium.

[00171] In some examples, the cell culture medium comprises OKT-3 beginning on the start day of the first expansion, and the gene-editing is carried out after the TILs have been exposed to the OKT-3.

[00172] In some examples, the gene-editing causes expression of one or more immune checkpoint genes to be silenced or reduced in at least a portion of the therapeutic population of TILs.

[00173] In some examples, the one or more immune checkpoint genes is/are selected from the group comprising PD-1, CTLA-4, LAG-3, HAVCR2 (TIM-3), Cish, TGFβ, PKA, CBL-B, PPP2CA, PPP2CB, PTPN6, PTPN22, PDCD1, BTLA, CD160, TIGIT, CD96, CRTAM, LAIR1, SIGLEC7, SIGLEC9, CD244, TNFRSF10B, TNFRSF10A, CASP8, CASP10,

CASP3, CASP6, CASP7, FADD, FAS, SMAD2, SMAD3, SMAD4, SMAD10, SKI, SKIL, TGIF1, IL10RA, IL10RB, HMOX2, IL6R, IL6ST, EIF2AK4, CSK, PAG1, SIT1, FOXP3, PRDM1, BATF, GUCY1A2, GUCY1A3, GUCY1B2, and GUCY1B3.

[00174] In some examples, the one or more immune checkpoint genes is/are selected from the group comprising PD-1, CTLA-4, LAG-3, HAVCR2 (TIM-3), Cish, TGFβ, and PKA.

[00175] In some examples, the gene-editing causes expression of one or more immune checkpoint genes to be enhanced in at least a portion of the therapeutic population of TILs, the immune checkpoint gene(s) being selected from the group comprising CCR2, CCR4, CCR5, CXCR2, CXCR3, CX3CR1, IL-2, IL-4, IL-7, IL-10, IL-15, IL-21, the NOTCH 1/2 intracellular domain (ICD), and/or the NOTCH ligand mDLL1.

[00176] In some examples, the gene-editing comprises the use of a programmable nuclease that mediates the generation of a double-strand or single-strand break at said one or more immune checkpoint genes.

[00177] In some examples, the gene-editing comprises one or more methods selected from a CRISPR method, a TALE method, a zinc finger method, and a combination thereof.

[00178] In some examples, the gene-editing comprises a CRISPR method.

[00179] In some examples, the CRISPR method is a CRISPR/Cas9 method.

[00180] In some examples, the gene-editing comprises a TALE method.

[00181] In some examples, the gene-editing comprises a zinc finger method.

[00182] In another aspect, the present 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 comprising OKT-3 and/or a 4-1BB agonist antibody for about 2 to 5 days;

(d) optionally adding 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 1 to 3 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 (c) to step (d) occurs without opening the system;

(e) performing a sterile electroporation step on the second population of TILs, wherein the sterile electroporation step mediates the transfer of at least one gene editor;

(f) resting the second population of TILs for about 1 day;

(g) performing a second expansion by supplementing the cell culture medium of the second population of TILs with additional IL-2, optionally OKT-3 antibody, optionally an OX40 antibody, and antigen presenting cells (APCs), to produce a third population of TILs, wherein the second expansion is performed for about 7 to 11 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 (f) to step (g) occurs without opening the system;

(h) harvesting the therapeutic population of TILs obtained from step (g) to provide a harvested TIL population, wherein the transition from step (g) to step (h) occurs without opening the system, wherein the harvested population of TILs is a therapeutic population of TILs;

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

(j) cryopreserving the harvested TIL population using a dimethylsulfoxide-based cryopreservation medium,

wherein the electroporation step comprises the delivery of a Clustered Regularly Interspersed Short Palindromic Repeat (CRISPR) system, a Transcription Activator-Like Effector (TALE) system, or a zinc finger system for inhibiting the expression of a molecule selected from the group consisting of PD-1, LAG-3, TIM-3, CTLA-4, TIGIT, CISH, TGFβR2, PRA, CBLB, BAFF (BR3), and combinations thereof.

[00183] In another example, the present 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 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 comprising OKT-3 and/or a 4-1BB agonist antibody for about 2 to 5 days;

(d) optionally adding 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 1 to 3 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 (c) to step (d) occurs without opening the system;

(e) performing a sterile electroporation step on the second population of TILs, wherein the sterile electroporation step mediates the transfer of at least one gene editor;

(f) resting the second population of TILs for about 1 day;

(g) performing a second expansion by supplementing the cell culture medium of the second population of TILs with additional IL-2, optionally OKT-3 antibody, optionally an OX40 antibody, and antigen presenting cells (APCs), to produce a third population of TILs, wherein the second expansion is performed for about 7 to 11 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 (f) to step (g) occurs without opening the system;

(h) harvesting the therapeutic population of TILs obtained from step (g) to provide a harvested TIL population, wherein the transition from step (g) to step (h) occurs without opening the system, wherein the harvested population of TILs is a therapeutic population of TILs;

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

(j) cryopreserving the harvested TIL population using a dimethylsulfoxide-based cryopreservation medium; and

(k) administering a therapeutically effective dosage of the harvested TIL population from the infusion bag to the patient;

wherein the electroporation step comprises the delivery of a Clustered Regularly

Interspersed Short Palindromic Repeat (CRISPR) system, a Transcription Activator-Like Effector (TALE) system, or a zinc finger system for inhibiting the expression of a molecule selected from the group consisting of PD-1, LAG-3, TIM-3, CTLA-4, TIGIT, CISH, TGFβR2, PRA, CBLB, BAFF (BR3), and combinations thereof.

[00184] In another aspect, the present disclosure provides a population of expanded TILs for use in the treatment of a subject with cancer, wherein the population of expanded TILs is a harvested population of TILs obtainable by a method 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 comprising OKT-3 and/or a 4-1BB agonist antibody for about 2 to 5 days;

(d) optionally adding 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 1 to 3 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 (c) to step (d) occurs without opening the system;

(e) performing a sterile electroporation step on the second population of TILs, wherein the sterile electroporation step mediates the transfer of at least one gene editor;

(f) resting the second population of TILs for about 1 day;

(g) performing a second expansion by supplementing the cell culture medium of the second population of TILs with additional IL-2, optionally OKT-3 antibody, optionally an OX40 antibody, and antigen presenting cells (APCs), to produce a third population of TILs, wherein the second expansion is performed for about 7 to 11 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 (f) to step (g) occurs without opening the system;

(h) harvesting the therapeutic population of TILs obtained from step (g) to provide a harvested TIL population, wherein the transition from step (g) to step (h) occurs without opening the system, wherein the harvested population of TILs is a therapeutic population of TILs;

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

(j) cryopreserving the harvested TIL population using a dimethylsulfoxide-based cryopreservation medium,

wherein the electroporation step comprises the delivery of a Clustered Regularly Interspersed Short Palindromic Repeat (CRISPR) system, a Transcription Activator-Like Effector (TALE) system, or a zinc finger system for inhibiting the expression of a molecule selected from the group consisting of PD-1, LAG-3, TIM-3, CTLA-4, TIGIT, CISH, TGFβR2, PRA, CBLB, BAFF (BR3), and combinations thereof.

[00185] In some examples, the method comprises performing the first expansion by culturing the first population of TILs in a cell culture medium comprising IL-2, OKT-3 and a 4-1BB agonist antibody, wherein the OKT-3 and the 4-1BB agonist antibody are optionally present in the cell culture medium beginning on Day 0 or Day 1.

[00186] In another aspect, the present disclosure provides a cryopreservation composition comprising the population of TILs for use in the treatment of a subject with cancer, a cryoprotectant medium comprising DMSO, and an electrolyte solution.

[00187] In some examples, the cryopreservation composition may further comprise one or more stabilizers (e.g., HSA) and one or more lymphocyte growth factors (e.g., IL-2).

[00188] In some examples, the cryoprotectant medium comprising DMSO and the electrolyte solution are present in a ratio of about 1.1:1 to about 1:1.1.

[00189] In some examples, the cryopreservation composition comprises 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.

BRIEF DESCRIPTION OF THE DRAWINGS

[00190] **Figure 1:** Shows a diagram of an embodiment of process 2A, a 22-day process for TIL manufacturing.

[00191] **Figure 2:** Shows a comparison between the 1C process and an embodiment of the 2A process for TIL manufacturing.

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 tumor fragments into a closed system, wherein the tumor fragments are from a tumor resected from a patient;
    (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;
    (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) at any time during the method, gene-editing at least a portion of the TILs.

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 dimethlysulfoxide (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. The method according to any one of claims 1 to 44, wherein the cell culture medium further comprises a 4-1BB agonist and/or an OX40 agonist during the first expansion, the second expansion, or both.

46. The method according to claim 45, wherein and the gene-editing is carried out after the 4-1BB agonist and/or the OX40 agonist is introduced into the cell culture medium.

47. The method according to claim 45, wherein and the gene-editing is carried out before the 4-1BB agonist and/or the OX40 agonist is introduced into the cell culture medium.

48. The method according to any of claims 1-47, wherein the gene-editing is carried out on TILs from one or more of the first population, the second population, and the third population.

49. The method according to any of claims 1-47, wherein the gene-editing is carried out on TILs from the first expansion, or TILs from the second expansion, or both.

50. The method according to any of claims 1-47, wherein the gene-editing is carried out after the first expansion and before the second expansion.

51. The method according to any of claims 1-47, wherein the gene-editing is carried out before step (c), before step (d), or before step (e).

52. The method according to any of claims 1-47, wherein the cell culture medium comprises OKT-3 during the first expansion and/or during the second expansion, and the gene-editing is carried out before the OKT-3 is introduced into the cell culture medium.

53. The method according to any of claims 1-47, wherein the cell culture medium comprises OKT-3 during the first expansion and/or during the second expansion, and the gene-editing is carried out after the OKT-3 is introduced into the cell culture medium.

54. The method according to any of claims 1-47, wherein the cell culture medium comprises OKT-3 beginning on the start day of the first expansion, and the gene-editing is carried out after the TILs have been exposed to the OKT-3.

55. The method according to any of claims 1-54, wherein the gene-editing causes expression of one or more immune checkpoint genes to be silenced or reduced in at least a portion of the therapeutic population of TILs.

56. The method according to claim 55, wherein said one or more immune checkpoint genes is/are selected from the group comprising PD-1, CTLA-4, LAG-3, HAVCR2 (TIM-3), Cish, TGFβ, PKA, CBL-B, PPP2CA, PPP2CB, PTPN6, PTPN22, PDCD1, BTLA, CD160, TIGIT, CD96, CRTAM, LAIR1, SIGLEC7, SIGLEC9, CD244, TNFRSF10B, TNFRSF10A, CASP8, CASP10, CASP3, CASP6, CASP7, FADD, FAS, SMAD2, SMAD3, SMAD4, SMAD10, SKI, SKIL, TGIF1, IL10RA, IL10RB, HMOX2, IL6R, IL6ST, EIF2AK4, CSK, PAG1, SIT1, FOXP3, PRDM1, BATF, GUCY1A2, GUCY1A3, GUCY1B2, and GUCY1B3.

57. The method according to claim 55, wherein said one or more immune checkpoint genes is/are selected from the group comprising PD-1, CTLA-4, LAG-3, HAVCR2 (TIM-3), Cish, TGFβ, and PKA.

58. The method according to any of claims 1-54, wherein the gene-editing causes expression of one or more immune checkpoint genes to be enhanced in at least a portion of the therapeutic population of TILs, the immune checkpoint gene(s) being selected from the group comprising CCR2, CCR4, CCR5, CXCR2, CXCR3, CX3CR1, IL-2, IL-4, IL-7, IL-10, IL-15, IL-21, the NOTCH 1/2 intracellular domain (ICD), and/or the NOTCH ligand mDLL1.

59. The method according to any of claims 1-58, wherein the gene-editing comprises the use of a programmable nuclease that mediates the generation of a double-strand or single-strand break at said one or more immune checkpoint genes.

60. The method according to any of claims 1-58, wherein the gene-editing comprises one or more methods selected from a CRISPR method, a TALE method, a zinc finger method, and a combination thereof.

61. The method according to any of claims 1-58, wherein the gene-editing comprises a CRISPR method.

62. The method according to claim 61, wherein the CRISPR method is a CRISPR/Cas9 method.

63. The method according to any of claims 1-58, wherein the gene-editing comprises a TALE method.

64. The method according to any of claims 1-58, wherein the gene-editing comprises a zinc finger method.

65. Use of expanded tumor infiltrating lymphocytes (TILs) in the manufacture of a medicament for the treatment of a subject with cancer, wherein the use comprises:
    (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;

(h) wherein a therapeutically effective dosage of the third population of TILs from the infusion bag in step (g) is to be administered to the patient; and

(i) at any time during the method steps (a)-(f), gene-editing at least a portion of the TILs.

66. The use according to claim 65, wherein the therapeutic population of TILs harvested in step (e) comprises sufficient TILs for administration of a therapeutically effective dosage of the TILs in step (h).

67. The use according to claim 66, wherein the number of TILs sufficient for administration of a therapeutically effective dosage in step (h) is from about 2.3×10^{10} to about 13.7×10^{10}.

68. The use according to claim 67, wherein the antigen presenting cells (APCs) are PBMCs.

69. The use according to claim 68, wherein the PBMCs are added to the cell culture on any of days 9 through 14 in step (d).

70. The use according to any of claims 65 to 69, wherein prior to administration of a therapeutically effective dosage of TIL cells in step (h), a non-myeloablative lymphodepletion regimen has been administered to the patient.

71. The use according to claim 70, wherein the non-myeloablative lymphodepletion regimen comprises that cyclophosphamide is to be administered at a dose of 60 mg/m^2/day for two days followed by administration of fludarabine at a dose of 25 mg/m^2/day for five days.

72. The use according to any of claims 65 to 71, further comprising that the patient is to be treated with a high-dose IL-2 regimen starting on the day after the TIL cells have been administered to the patient in step (h).

73. The use according to claim 72, wherein the high-dose IL-2 regimen comprises that 600,000 or 720,000 IU/kg is to be administered as a 15-minute bolus intravenous infusion every eight hours until tolerance.

74. The use according to any of claims 65-73, 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.

75. The use according to any of claims 65-74, 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.

76. The use according to any of claims 65-75, 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.

77. The use according to any of claims 65-76, wherein the cancer is selected from the group consisting of melanoma, HNSCC, cervical cancers, and NSCLC.

78. The use according to any of claims 65-77, wherein the cancer is melanoma.

79. The use according to any of claims 65-78, wherein the cancer is HNSCC.

80. The use according to any of claims 65-79, wherein the cancer is a cervical cancer.

81. The use according to any of claims 65-80, wherein the cancer is NSCLC.

82. The use according to any one of claims 65 to 81, wherein the cell culture medium further comprises a 4-1BB agonist and/or an OX40 agonist during the first expansion, the second expansion, or both.

83. The use according to claim 82, wherein the gene-editing is carried out after the 4-1BB agonist and/or the OX40 agonist is introduced into the cell culture medium.

84. The use according to claim 82, wherein the gene-editing is carried out before the 4-1BB agonist and/or the OX40 agonist is introduced into the cell culture medium.

85. The use according to any of claims 65-84, wherein the gene-editing is carried out on TILs from one or more of the first population, the second population, and the third population.

86. The use according to any of claims 65-84, wherein the gene-editing is carried out on TILs from the first expansion, or TILs from the second expansion, or both.

87. The use according to any of claims 65-84, wherein the gene-editing is carried out after the first expansion and before the second expansion.

88. The use according to any of claims 65-84, wherein the gene-editing is carried out before step (c), before step (d), or before step (e).

89. The use according to any of claims 65-88, wherein the cell culture medium comprises OKT-3 during the first expansion and/or during the second expansion, and the gene-editing is carried out before the OKT-3 is introduced into the cell culture medium.

90. The use according to any of claims 65-88, wherein the cell culture medium comprises OKT-3 during the first expansion and/or during the second expansion, and the gene-editing is carried out after the OKT-3 is introduced into the cell culture medium.

91. The use according to any of claims 65-88, wherein the cell culture medium comprises OKT-3 beginning on the start day of the first expansion, and the gene-editing is carried out after the TILs have been exposed to the OKT-3.

92. The use according to any of claims 65-91, wherein the gene-editing causes expression of one or more immune checkpoint genes to be silenced or reduced in at least a portion of the therapeutic population of TILs.

93. The use according to claim 92, wherein said one or more immune checkpoint genes is/are selected from the group comprising PD-1, CTLA-4, LAG-3, HAVCR2 (TIM-3), Cish, TGFβ, PKA, CBL-B, PPP2CA, PPP2CB, PTPN6, PTPN22, PDCD1, BTLA, CD160, TIGIT, CD96, CRTAM, LAIR1, SIGLEC7, SIGLEC9, CD244, TNFRSF10B, TNFRSF10A, CASP8, CASP10, CASP3, CASP6, CASP7, FADD, FAS, SMAD2, SMAD3, SMAD4, SMAD10, SKI, SKIL, TGIF1, IL10RA, IL10RB, HMOX2, IL6R, IL6ST, EIF2AK4, CSK, PAG1, SIT1, FOXP3, PRDM1, BATF, GUCY1A2, GUCY1A3, GUCY1B2, and GUCY1B3.

94. The use according to claim 92, wherein said one or more immune checkpoint genes is/are selected from the group comprising PD-1, CTLA-4, LAG-3, HAVCR2 (TIM-3), Cish, TGFβ, and PKA.

95. The use according to any of claims 65-91, wherein the gene-editing causes expression of one or more immune checkpoint genes to be enhanced in at least a portion of the

therapeutic population of TILs, the immune checkpoint gene(s) being selected from the group comprising CCR2, CCR4, CCR5, CXCR2, CXCR3, CX3CR1, IL-2, IL-4, IL-7, IL-10, IL-15, IL-21, the NOTCH 1/2 intracellular domain (ICD), and/or the NOTCH ligand mDLL1.

96. The use according to any of claims 65-95, wherein the gene-editing comprises the use of a programmable nuclease that mediates the generation of a double-strand or single-strand break at said one or more immune checkpoint genes.

97. The use according to any of claims 65-95, wherein the gene-editing comprises one or more methods selected from a CRISPR method, a TALE method, a zinc finger method, and a combination thereof.

98. The use according to any of claims 65-95, wherein the gene-editing comprises a CRISPR method.

99. The use according to claim 98, wherein the CRISPR method is a CRISPR/Cas9 method.

100. The use according to any of claims 65-95, wherein the gene-editing comprises a TALE method.

101. The use according to any of claims 65-95, wherein the gene-editing comprises a zinc finger method.

102. 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 7-14 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;

(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; and

(f) at any time during the method, gene-editing at least a portion of the TILs.

103. The method according to claim 102, wherein the therapeutic population of TILs harvested in step (d) comprises sufficient TILs for a therapeutically effective dosage of the TILs.

104. The method according to claim 103, where the number of TILs sufficient for a therapeutically effective dosage is from about 2.3×10^{10} to about 13.7×10^{10}.

105. The method according to claim 104, further comprising the step of cryopreserving the infusion bag comprising the harvested TIL population using a cryopreservation process.

106. The method according to claim 105, wherein the cryopreservation process is performed using a 1:1 ratio of harvested TIL population to cryopreservation media.

107. The method according to claim 102, wherein the antigen-presenting cells are peripheral blood mononuclear cells (PBMCs).

108. The method according to claim 107, wherein the PBMCs are irradiated and allogeneic.

109. The method according to claim 108, wherein the PBMCs are added to the cell culture on any of days 9 through 14 in step (c).

110. The method according to claim 102, wherein the antigen-presenting cells are artificial antigen-presenting cells.

111. The method according to claim 102, wherein the harvesting in step (d) is performed using a LOVO cell processing system.

112. 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.

113. The method according to claim 102, 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.

114. The method according to claim 103, wherein the multiple fragments comprise about 50 fragments with a total volume of about 1350 mm^3.

115. The method according to claim 102, wherein the multiple fragments comprise about 50 fragments with a total mass of about 1 gram to about 1.5 grams.

116. The method according to claim 102, wherein the multiple fragments comprise about 4 fragments.

117. The method according to claim 102, wherein the second cell culture medium is provided in a container selected from the group consisting of a G-container and a Xuri cellbag.

118. The method according to claim 102, wherein the infusion bag in step (e) is a HypoThermosol-containing infusion bag.

119. The method according to claim 102, 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.

120. The method according to claim 102, wherein the first period in step (b) and the second period in step (c) are each individually performed within a period of 11 days.

121. The method according to claim 102, wherein steps (a) through (e) are performed within a period of about 10 days to about 22 days.

122. The method according to claim 102, wherein steps (a) through (e) are performed within a period of about 10 days to about 20 days.

123. The method according to claim 102, wherein steps (a) through (e) are performed within a period of about 10 days to about 15 days.

124. The method according to claim 102, wherein steps (a) through (e) are performed in 22 days or less.

125. The method according to claim 105, wherein steps (a) through (e) and cryopreservation are performed in 22 days or less.

126. The method according to any one of claims 102 to 125, 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.

127. The method according to any one of claims 102 to 126, wherein the antigen-presenting cells are added to the TILs during the second period in step (c) without opening the system.

128. The method according to any one of claims 102 to 127, 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.

129. The method according to any one of claims 102 to 128, 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.

130. The method according to any one of claims 102 to 129, wherein the risk of microbial contamination is reduced as compared to an open system.

131. The method according to any one of claims 102 to 130, wherein the TILs from step (e) are infused into a patient.

132. The method according to any of claims 102 to 131 wherein the closed container comprises a single bioreactor.

133. The method according to claim 132, wherein the closed container comprises a G-REX-10.

134. The method according to claim 132, wherein the closed container comprises a G-REX -100.

135. The method according to any one of claims 102 to 134, 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.

136. The method according to claim 135, wherein the cell culture has a ratio of 2.5×10^9 APCs to 100×10^6 TILs.

137. The method according to any one of claims 102 to 134, 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.

138. The method according to claim 137, wherein the cell culture has ratio of 2.5×10^9 APCs to 100×10^6 TILs.

139. The method according to any one of claims 102-138, wherein the cell culture medium further comprises a 4-1BB agonist and/or an OX40 agonist during the first expansion, the second expansion, or both.

140. The method according to claim 139, wherein and the gene-editing is carried out after the 4-1BB agonist and/or the OX40 agonist is introduced into the cell culture medium.

141. The method according to claim 139, wherein and the gene-editing is carried out before the 4-1BB agonist and/or the OX40 agonist is introduced into the cell culture medium.

142. The method according to any of claims 102-141, wherein the gene-editing is carried out on TILs from one or more of the first population, the second population, and the third population.

143. The method according to any of claims 102-141, wherein the gene-editing is carried out on TILs from the first expansion, or TILs from the second expansion, or both.

144. The method according to any of claims 102-141, wherein the gene-editing is carried out after the first expansion and before the second expansion.

145. The method according to any of claims 102-141, wherein the gene-editing is carried out before step (b), before step (c), or before step (d).

146. The method according to any of claims 102-145, wherein the cell culture medium comprises OKT-3 during the first expansion and/or during the second expansion, and the gene-editing is carried out before the OKT-3 is introduced into the cell culture medium.

147. The method according to any of claims 102-145, wherein the cell culture medium comprises OKT-3 during the first expansion and/or during the second expansion, and the gene-editing is carried out after the OKT-3 is introduced into the cell culture medium.

148. The method according to any of claims 102-145, wherein the cell culture medium comprises OKT-3 beginning on the start day of the first expansion, and the gene-editing is carried out after the TILs have been exposed to the OKT-3.

149. The method according to any of claims 102-148, wherein the gene-editing causes expression of one or more immune checkpoint genes to be silenced or reduced in at least a portion of the therapeutic population of TILs.

150. The method according to claim 149, wherein said one or more immune checkpoint genes is/are selected from the group comprising PD-1, CTLA-4, LAG-3, HAVCR2 (TIM-3), Cish, TGFβ, PKA, CBL-B, PPP2CA, PPP2CB, PTPN6, PTPN22, PDCD1, BTLA, CD160, TIGIT, CD96, CRTAM, LAIR1, SIGLEC7, SIGLEC9, CD244, TNFRSF10B, TNFRSF10A, CASP8, CASP10, CASP3, CASP6, CASP7, FADD, FAS, SMAD2, SMAD3, SMAD4, SMAD10, SKI, SKIL, TGIF1, IL10RA, IL10RB, HMOX2, IL6R, IL6ST, EIF2AK4, CSK, PAG1, SIT1, FOXP3, PRDM1, BATF, GUCY1A2, GUCY1A3, GUCY1B2, and GUCY1B3.

151. The method according to claim 149, wherein said one or more immune checkpoint genes is/are selected from the group comprising PD-1, CTLA-4, LAG-3, HAVCR2 (TIM-3), Cish, TGFβ, and PKA.

152. The method according to any of claims 102-148, wherein the gene-editing causes expression of one or more immune checkpoint genes to be enhanced in at least a portion of the therapeutic population of TILs, the immune checkpoint gene(s) being selected from the group comprising CCR2, CCR4, CCR5, CXCR2, CXCR3, CX3CR1, IL-2, IL-4, IL-7, IL-10, IL-15, IL-21, the NOTCH 1/2 intracellular domain (ICD), and/or the NOTCH ligand mDLL1.

153. The method according to any of claims 102-152, wherein the gene-editing comprises the use of a programmable nuclease that mediates the generation of a double-strand or single-strand break at said one or more immune checkpoint genes.

154. The method according to any of claims 102-152, wherein the gene-editing comprises one or more methods selected from a CRISPR method, a TALE method, a zinc finger method, and a combination thereof.

155. The method according to any of claims 102-152, wherein the gene-editing comprises a CRISPR method.

156. The method according to claim 155, wherein the CRISPR method is a CRISPR/Cas9 method.

157. The method according to any of claims 102-152, wherein the gene-editing comprises a TALE method.

158. The method according to any of claims 102-152, wherein the gene-editing comprises a zinc finger method.

159. 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;

(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) at any time during the method, gene-editing at least a portion of the TILs.

160. The population of TILs for use to treat a subject with cancer according to claim 159, wherein the method further comprises one or more of the features recited in any of claims 1 to 158.

161. The population of TILs for use to treat a subject with cancer according to claim 159, wherein the cell culture medium further comprises a 4-1BB agonist and/or an OX40 agonist during the first expansion, the second expansion, or both.

162. The population of TILs for use to treat a subject with cancer according to claim 159, wherein the gene-editing is carried out on TILs from one or more of the first population, the second population, and the third population.

163. The population of TILs for use to treat a subject with cancer according to claim 159, wherein the gene-editing is carried out on TILs from the first expansion, or TILs from the second expansion, or both.

164. The population of TILs for use to treat a subject with cancer according to claim 159, wherein the gene-editing is carried out after the first expansion and before the second expansion.

165. The population of TILs for use to treat a subject with cancer according to claim 159, wherein the gene-editing is carried out before step (c), before step (d), or before step (e)

166. The population of TILs for use to treat a subject with cancer according to claim 159, wherein the gene-editing causes expression of one or more immune checkpoint genes to be silenced or reduced in at least a portion of the population of expanded TILs.

167. The population of TILs for use to treat a subject with cancer according to claim 166, wherein said one or more immune checkpoint genes is/are selected from the group comprising PD-1, CTLA-4, LAG-3, HAVCR2 (TIM-3), Cish, TGFβ, PKA, CBL-B, PPP2CA, PPP2CB, PTPN6, PTPN22, PDCD1, BTLA, CD160, TIGIT, CD96, CRTAM, LAIR1, SIGLEC7, SIGLEC9, CD244, TNFRSF10B, TNFRSF10A, CASP8, CASP10, CASP3, CASP6, CASP7, FADD, FAS, SMAD2, SMAD3, SMAD4, SMAD10, SKI, SKIL, TGIF1, IL10RA, IL10RB, HMOX2, IL6R, IL6ST, EIF2AK4, CSK, PAG1, SIT1, FOXP3, PRDM1, BATF, GUCY1A2, GUCY1A3, GUCY1B2, and GUCY1B3.

168. The population of TILs for use to treat a subject with cancer according to claim 166, wherein said one or more immune checkpoint genes is/are selected from the group comprising PD-1, CTLA-4, LAG-3, HAVCR2 (TIM-3), Cish, TGFβ, and PKA.

169. The population of TILs for use to treat a subject with cancer according to claim 166, wherein the gene-editing causes expression of one or more immune checkpoint genes to be enhanced in at least a portion of the therapeutic population of TILs, the immune checkpoint gene(s) being selected from the group comprising CCR2, CCR4, CCR5, CXCR2, CXCR3, CX3CR1, IL-2, IL-4, IL-7, IL-10, IL-15, IL-21, the NOTCH 1/2 intracellular domain (ICD), and/or the NOTCH ligand mDLL1.

170. The population of TILs for use to treat a subject with cancer according to claim 159, wherein the gene-editing comprises the use of a programmable nuclease that mediates the generation of a double-strand or single-strand break at said one or more immune checkpoint genes.

171. The population of TILs for use to treat a subject with cancer according to claim 159, wherein the gene-editing comprises one or more methods selected from a CRISPR method, a TALE method, a zinc finger method, and a combination thereof.

172. A cryopreservation composition comprising the population of TILs for use in the treatmentof a subject with cancer according to claim 159, a cryoprotectant medium comprising DMSO, and an electrolyte solution.

173. The cryopreservation composition of claim 172 further comprising one or more stabilizers and one or more lymphocyte growth factors.

174. The cryopreservation composition of claim 173, wherein the one or more stabilizers comprise Human Serum Albumin (HSA) and the one or more lymphocyte growth factors comprise IL-2.

175. The cryopreservation composition of claim 174, 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.

176. The cryopreservation composition of claim 174 comprising the population of TILs in an amount of about 1 X 10^6 to about 9 X 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.

177. 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 comprising OKT-3 and/or a 4-1BB agonist antibody for about 2 to 5 days;

(d) optionally adding 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 1 to 3 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 (c) to step (d) occurs without opening the system;

(e) performing a sterile electroporation step on the second population of TILs, wherein the sterile electroporation step mediates the transfer of at least one gene editor;

(f) resting the second population of TILs for about 1 day;

(g) performing a second expansion by supplementing the cell culture medium of the second population of TILs with additional IL-2, optionally OKT-3 antibody, optionally an OX40 antibody, and antigen presenting cells (APCs), to produce a third population of TILs, wherein the second expansion is performed for about 7 to 11 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 (f) to step (g) occurs without opening the system;

(h) harvesting the therapeutic population of TILs obtained from step (g) to provide a harvested TIL population, wherein the transition from step (g) to step (h) occurs without opening the system, wherein the harvested population of TILs is a therapeutic population of TILs;

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

(j) cryopreserving the harvested TIL population using a dimethylsulfoxide-based cryopreservation medium,

wherein the electroporation step comprises the delivery of a Clustered Regularly Interspersed Short Palindromic Repeat (CRISPR) system, a Transcription Activator-Like Effector (TALE) system, or a zinc finger system for inhibiting the expression of a molecule selected from the group consisting of PD-1, LAG-3, TIM-3, CTLA-4, TIGIT, CISH, TGFβR2, PRA, CBLB, BAFF (BR3), and combinations thereof.

178. The method according to claim 177 comprising performing the first expansion by culturing the first population of TILs in a cell culture medium comprising IL-2, OKT-3 and a 4-1BB agonist antibody, wherein the OKT-3 and the 4-1BB agonist antibody are optionally present in the cell culture medium beginning on Day 0 or Day 1.

179. The method according to claim 177 or claim 178, wherein the electroporation step comprises the delivery of a Clustered Regularly Interspersed Short Palindromic Repeat (CRISPR) system, a Transcription Activator-Like Effector (TALE) system, or a zinc finger system for inhibiting the expression of a molecule selected from the group consisting of PD-1, LAG-3, TIM-3, CISH, and CBLB, and combinations thereof.

180. The method according to claim 177 or claim 178, wherein the electroporation step comprises the delivery of a Clustered Regularly Interspersed Short Palindromic Repeat (CRISPR) system, a Transcription Activator-Like Effector (TALE) system, or a zinc finger system for inhibiting the expression of PD-1.

181. The method according to claim 177 or claim 178, wherein the electroporation step comprises the delivery of a Clustered Regularly Interspersed Short Palindromic Repeat (CRISPR) system, a Transcription Activator-Like Effector (TALE) system, or a zinc finger system for inhibiting the expression of LAG-3.

182. The method according to claim 177 or claim 178, wherein the electroporation step comprises the delivery of a Clustered Regularly Interspersed Short Palindromic Repeat (CRISPR) system, a Transcription Activator-Like Effector (TALE) system, or a zinc finger system for inhibiting the expression of TIM-3.

183. The method according to claim 177 or claim 178, wherein the electroporation step comprises the delivery of a Clustered Regularly Interspersed Short Palindromic Repeat (CRISPR) system, a Transcription Activator-Like Effector (TALE) system, or a zinc finger system for inhibiting the expression of CISH.

184. The method according to claim 177 or claim 178, wherein the electroporation step comprises the delivery of a Clustered Regularly Interspersed Short Palindromic Repeat (CRISPR) system, a Transcription Activator-Like Effector (TALE) system, or a zinc finger system for inhibiting the expression of CBLB.

185. The method according to any of claims 177 to 184, wherein the electroporation step comprises the delivery of a Clustered Regularly Interspersed Short Palindromic Repeat (CRISPR) system.

186. The method according to any of claims 177 to 184, wherein the electroporation step comprises the delivery of a Transcription Activator-Like Effector (TALE) system.

187. The method according to any of claims 177 to 184, wherein the electroporation step comprises the delivery of a zinc finger system.

188. The method according to any of claims 177 to 187, wherein the dimethylsulfoxide-based cryopreservation medium comprises DMSO, an electrolyte solution, optionally HSA, and optionally IL-2.

189. Use of expanded tumor infiltrating lymphocytes (TILs) in the manufacture of a medicament for the treatment of a subject with cancer, wherein the use comprises:

(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 comprising OKT-3 and/or a 4-1BB agonist antibody for about 2 to 5 days;

(d) optionally adding 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 1 to 3 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 (c) to step (d) occurs without opening the system;

(e) performing a sterile electroporation step on the second population of TILs, wherein the sterile electroporation step mediates the transfer of at least one gene editor;

(f) resting the second population of TILs for about 1 day;

(g) performing a second expansion by supplementing the cell culture medium of the second population of TILs with additional IL-2, optionally OKT-3 antibody, optionally an OX40 antibody, and antigen presenting cells (APCs), to produce a third population of TILs, wherein the second expansion is performed for about 7 to 11 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 (f) to step (g) occurs without opening the system;

(h) harvesting the therapeutic population of TILs obtained from step (g) to provide a harvested TIL population, wherein the transition from step (g) to step (h) occurs without opening the system, wherein the harvested population of TILs is a therapeutic population of TILs;

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

(j) cryopreserving the harvested TIL population using a dimethylsulfoxide-based cryopreservation medium; and

(k) wherein a therapeutically effective dosage of the harvested TIL population from the infusion bag is to be administered to the patient;

wherein the electroporation step comprises the delivery of a Clustered Regularly Interspersed Short Palindromic Repeat (CRISPR) system, a Transcription Activator-Like Effector (TALE) system, or a zinc finger system for inhibiting the expression of a molecule selected from the group consisting of PD-1, LAG-3, TIM-3, CTLA-4, TIGIT, CISH, TGFβR2, PRA, CBLB, BAFF (BR3), and combinations thereof.

190. The use according to claim 189, comprising performing the first expansion by culturing the first population of TILs in a cell culture medium comprising IL-2, OKT-3 and a 4-1BB agonist antibody, wherein the OKT-3 and the 4-1BB agonist antibody are optionally present in the cell culture medium beginning on Day 0 or Day 1.

191. The use according to claim 189 or claim 190, wherein the electroporation step comprises the delivery of a Clustered Regularly Interspersed Short Palindromic Repeat (CRISPR) system, a Transcription Activator-Like Effector (TALE) system, or a zinc finger system for inhibiting the expression of a molecule selected from the group consisting of PD-1, LAG-3, TIM-3, CISH, and CBLB, and combinations thereof.

192. The use according to claim 189 or claim 190, wherein the electroporation step comprises the delivery of a Clustered Regularly Interspersed Short Palindromic Repeat (CRISPR) system, a Transcription Activator-Like Effector (TALE) system, or a zinc finger system for inhibiting the expression of PD-1.

193. The use according to claim 189 or claim 190, wherein the electroporation step comprises the delivery of a Clustered Regularly Interspersed Short Palindromic Repeat (CRISPR) system, a Transcription Activator-Like Effector (TALE) system, or a zinc finger system for inhibiting the expression of LAG-3.

194. The use according to claim 189 or claim 190, wherein the electroporation step comprises the delivery of a Clustered Regularly Interspersed Short Palindromic Repeat (CRISPR) system, a Transcription Activator-Like Effector (TALE) system, or a zinc finger system for inhibiting the expression of TIM-3.

195. The use according to claim 189 or claim 190, wherein the electroporation step comprises the delivery of a Clustered Regularly Interspersed Short Palindromic Repeat (CRISPR) system, a Transcription Activator-Like Effector (TALE) system, or a zinc finger system for inhibiting the expression of CISH.

196. The use according to claim 189 or claim 190, wherein the electroporation step comprises the delivery of a Clustered Regularly Interspersed Short Palindromic Repeat (CRISPR) system, a Transcription Activator-Like Effector (TALE) system, or a zinc finger system for inhibiting the expression of CBLB.

197. The use according to any of claims 189 to 196, wherein the electroporation step comprises the delivery of a Clustered Regularly Interspersed Short Palindromic Repeat (CRISPR) system.

198. The use according to any of claims 189 to 196, wherein the electroporation step comprises the delivery of a Transcription Activator-Like Effector (TALE) system.

199. The use according to any of claims 189 to 196, wherein the electroporation step comprises the delivery of a zinc finger system.

200. The use according to any of claims 189 to 196, wherein the dimethylsulfoxide-based cryopreservation medium comprises DMSO, an electrolyte solution, optionally HSA, and optionally IL-2.

201. A population of expanded TILs for use in the treatment of a subject with cancer, wherein the population of expanded TILs is a harvested population of TILs obtainable by a method 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 comprising OKT-3 and/or a 4-1BB agonist antibody for about 2 to 5 days;

(d) optionally adding 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 1 to 3 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 (c) to step (d) occurs without opening the system;

(e) performing a sterile electroporation step on the second population of TILs, wherein the sterile electroporation step mediates the transfer of at least one gene editor;

(f) resting the second population of TILs for about 1 day;

(g) performing a second expansion by supplementing the cell culture medium of the second population of TILs with additional IL-2, optionally OKT-3 antibody, optionally an OX40 antibody, and antigen presenting cells (APCs), to produce a third population of TILs, wherein the second expansion is performed for about 7 to 11 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 (f) to step (g) occurs without opening the system;

(h) harvesting the therapeutic population of TILs obtained from step (g) to provide a harvested TIL population, wherein the transition from step (g) to step (h) occurs without opening the system, wherein the harvested population of TILs is a therapeutic population of TILs;

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

(j) cryopreserving the harvested TIL population using a dimethylsulfoxide-based cryopreservation medium,

wherein the electroporation step comprises the delivery of a Clustered Regularly Interspersed Short Palindromic Repeat (CRISPR) system, a Transcription Activator-Like Effector (TALE) system, or a zinc finger system for inhibiting the expression of a molecule selected from the group consisting of PD-1, LAG-3, TIM-3, CTLA-4, TIGIT, CISH, TGFβR2, PRA, CBLB, BAFF (BR3), and combinations thereof.

202. The population of expanded TILs according to claim 201 comprising performing the first expansion by culturing the first population of TILs in a cell culture medium comprising IL-2, OKT-3 and a 4-1BB agonist antibody, wherein the OKT-3 and the 4-1BB agonist antibody are optionally present in the cell culture medium beginning on Day 0 or Day 1.

203. The population of expanded TILs according to claim 201 or claim 202, wherein the electroporation step comprises the delivery of a Clustered Regularly Interspersed Short Palindromic Repeat (CRISPR) system, a Transcription Activator-Like Effector (TALE) system, or a zinc finger system for inhibiting the expression of a molecule selected from the group consisting of PD-1, LAG-3, TIM-3, CISH, and CBLB, and combinations thereof.

204. The population of expanded TILs according to claim 201 or claim 202, wherein the electroporation step comprises the delivery of a Clustered Regularly Interspersed Short Palindromic Repeat (CRISPR) system, a Transcription Activator-Like Effector (TALE) system, or a zinc finger system for inhibiting the expression of PD-1.

205. The population of expanded TILs according to claim 201 or claim 202, wherein the electroporation step comprises the delivery of a Clustered Regularly Interspersed Short Palindromic Repeat (CRISPR) system, a Transcription Activator-Like Effector (TALE) system, or a zinc finger system for inhibiting the expression of LAG-3.

206. The population of expanded TILs according to claim 201 or claim 202, wherein the electroporation step comprises the delivery of a Clustered Regularly Interspersed Short Palindromic Repeat (CRISPR) system, a Transcription Activator-Like Effector (TALE) system, or a zinc finger system for inhibiting the expression of TIM-3.

207. The population of expanded TILs according to claim 201 or claim 202, wherein the electroporation step comprises the delivery of a Clustered Regularly Interspersed Short Palindromic Repeat (CRISPR) system, a Transcription Activator-Like Effector (TALE) system, or a zinc finger system for inhibiting the expression of CISH.

208. The population of expanded TILs according to claim 201 or claim 202, wherein the electroporation step comprises the delivery of a Clustered Regularly Interspersed Short Palindromic Repeat (CRISPR) system, a Transcription Activator-Like Effector (TALE) system, or a zinc finger system for inhibiting the expression of CBLB.

209. The population of expanded TILs according to claim 201 to 208, wherein the electroporation step comprises the delivery of a Clustered Regularly Interspersed Short Palindromic Repeat (CRISPR) system.

210. The population of expanded TILs according to claim 201 to 208, wherein the electroporation step comprises the delivery of a Transcription Activator-Like Effector (TALE) system.

211. The population of expanded TILs according to claim 201 to 208, wherein the electroporation step comprises the delivery of a zinc finger system.

212. The population of expanded TILs according to claim 201 to 211, wherein the dimethylsulfoxide-based cryopreservation medium comprises DMSO, an electrolyte solution, optionally HSA, and optionally IL-2.

213. The population of expanded TILs according to claim 201 to 211, wherein the electroporation step comprises a pulsed electroporation step.

214. The method according to any of claims 1-58, wherein the gene-editing comprises a CRISPR method, and the CRISPR method includes use of a high-fidelity Cas9.