332-42. TILs that have undergone an REP procedure have produced successful adoptive cell therapy following host immunosuppression in patients with melanoma.

[0004] There is an urgent need to provide more potent or efficacious TIL manufacturing processes and therapies based on such processes that are appropriate for commercial scale manufacturing and regulatory approval for use in human patients at multiple clinical centers. The present invention meets this need by providing transient genetic alteration processes for reprogramming TILs in order to prepare therapeutic populations of TILs with increased therapeutic efficacy.

SUMMARY DISCLOSURE

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

[0006] The present disclosure provides a method for expanding tumor infiltrating lymphocytes (TILs) into a therapeutic population of TILs comprising: (i) obtaining a first population of TILs from a tumor resected from a patient; (ii) performing a first expansion by culturing the first population of TILs in a cell culture medium comprising IL-2, and optionally OKT-3, to produce a second population of TILs; (iii) performing a second expansion by supplementing the cell culture medium of the second population of TILs with additional IL-2, OKT-3, and antigen presenting cells (APCs), to produce a third population of TILs, wherein the third population of TILs is at least 100-fold greater in number than the second population of TILs, and wherein the second expansion is performed for at least 14 days in order to obtain the third population of TILs, wherein the third population of TILs is a therapeutic population of TILs; and (iv) exposing the second and/or third population of TILs to transcription factors (TFs) and/or other molecules capable of transiently altering protein expression, wherein the TFs and/or other molecules capable of transiently altering protein expression provide for altered expression of tumor antigens and/or an alteration in the number of tumor antigen-specific T cells in the therapeutic population of TILs.

[0006a] In one aspect, the present disclosure refers to a method for expanding tumor infiltrating lymphocytes (TILs) into a therapeutic population of TILs comprising:
(i) obtaining a first population of TILs from a tumor resected from a patient;

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

(iii) performing a second expansion by supplementing the cell culture medium of the second population of TILs with additional IL-2, OKT-3, and antigen presenting cells (APCs), to produce a third population of TILs, and wherein the second expansion is performed ~~for about 7-14~~within 12 days in order to obtain the third population of TILs, wherein the third population of TILs is a therapeutic population of TILs; and

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

[0007] In some examples, the method further comprises exposing the second and/or third population of TILs to transcription factors (TFs) and/or other molecules capable of transiently altering protein expression, wherein the TFs and/or other molecules capable of transiently altering protein expression provide for altered expression of tumor antigens and/or an alteration in the number of tumor antigen-specific T cells in the therapeutic population of TILs.

[0008a] In another aspect, the present disclosure refers to a method for expanding tumor infiltrating lymphocytes (TILs) into a therapeutic population of TILs comprising:

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

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

(c) performing a first expansion by culturing the first population of TILs in a cell culture medium comprising IL-2, 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~~within 12 days to obtain the second population of TILs, and wherein the transition from step (b) to step (c) occurs without opening the system;

(d) performing a second expansion by supplementing the cell culture medium of the second population of TILs with additional IL-2, OKT-3, and antigen presenting cells (APCs), to produce a third population of TILs, wherein the second expansion is performed ~~for about 7-14~~within 12 days to obtain the third population of TILs, wherein the third population of TILs is a therapeutic population of TILs, wherein the second expansion is performed in a closed container providing a second gas-permeable surface area, and wherein the transition from step (c) to step (d) occurs without opening the system;

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

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

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

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

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

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

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

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

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

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

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

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

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

(c) performing a first expansion by culturing the first population of TILs in a cell culture medium comprising IL-2, 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~~within 12 days to obtain the second population of TILs, and wherein the transition from step (b) to step (c) occurs without

opening the system;

(d) performing a second expansion by supplementing the cell culture medium of the second population of TILs with additional IL-2, OKT-3, and antigen presenting cells (APCs), to produce the third population of TILs, wherein the second expansion is performed ~~for about 7-14~~within 12 days to obtain the third population of TILs, wherein the third population of TILs is a therapeutic population of TILs, wherein the second expansion is performed in a closed container providing a second gas-permeable surface area, and wherein the transition from step (c) to step (d) occurs without opening the system;

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

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

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

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

[0096b] In another aspect, the present disclosure refers to use of a population of expanded TILs in the manufacture of a medicament for the treatment of a subject with cancer, wherein the population of expanded TILs is a 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~~within 12 days to obtain the second population of TILs, and wherein the transition from step (b) to step (c) occurs without opening the system;

(d) performing a second expansion by supplementing the cell culture medium of the

second population of TILs with additional IL-2, OKT-3, and antigen presenting cells (APCs), to produce the third population of TILs, wherein the second expansion is performed ~~for about 7-14~~within 12 days to obtain the third population of TILs, wherein the third population of TILs is a therapeutic population of TILs, wherein the second expansion is performed in a closed container providing a second gas-permeable surface area, and wherein the transition from step (c) to step (d) occurs without opening the system;

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

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

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

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

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

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

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

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

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

[00111] In some examples, the method further comprises the step of transducing the first population of TILs with an expression vector comprising a nucleic acid encoding a high-affinity T cell receptor.

[00112] In some examples, the step of transducing occurs before step (i).

[00113] In some examples, the method further comprises the step of transducing the first population of TILs with an expression vector comprising a nucleic acid encoding a chimeric antigen receptor (CAR) comprising a single chain variable fragment antibody fused with at least one endodomain of a T-cell signaling molecule.

[00114] In some examples, the step of transducing occurs before step (i).

[00115] In some examples, the TILs are assayed for viability.

[00116] In some examples, the TILs are assayed for viability after cryopreservation.

[00117] In some examples, the TILs are assayed for viability after cryopreservation and after step (iv).

[00118] In another ~~aspect~~example, the present disclosure refers to a method for expanding tumor infiltrating lymphocytes (TILs) into a therapeutic population of TILs comprising exposing TILs to transcription factors (TFs) and/or other molecules capable of transiently altering protein expression in order to generate a therapeutic population of TILs, wherein the TFs and/or other molecules capable of transiently altering protein expression provide for increased expression of tumor antigens and/or an increase in the number of tumor antigen-specific T cells in the therapeutic population of TILs.

[00119] In some examples, the transient altering of protein expression results in induction of protein expression.

[00120] In some examples, the transient altering of protein expression results in a reduction of protein expression.

[00121] In some examples, one or more sd-RNA(s) is employed to reduce the transient protein expression.

[00122] In another ~~aspect~~example, the present disclosure refers to a method for evaluating transcription factors (TFs) and/or other molecules capable of transiently altering protein expression, wherein the method comprises expanding tumor infiltrating lymphocytes (TILs) into a therapeutic population of TILs, exposing TILs to transcription factors (TFs) and/or other molecules

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

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

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

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

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

[00127a] In another aspect, the present disclosure refers to a method for expanding tumor infiltrating lymphocytes (TILs) into a 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 a 4-1BB agonist antibody for ~~about~~ 2 to 5 days;

(d) 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, 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 short interfering RNA or one messenger RNA;

(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 from step (h) 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.

[00127b] In another aspect, the present disclosure refers to a method for expanding tumor infiltrating lymphocytes (TILs) into a therapeutic population of TILs comprising:

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

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

(c) performing a first expansion by culturing the first population of TILs in a cell culture medium comprising IL-2 and optionally comprising a 4-1BB agonist antibody for ~~about~~ 2 to 5 days;

(d) 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, 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 short interfering RNA or one messenger RNA;

(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 from step (h) 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 sterile electroporation step comprises the delivery of a short interfering RNA 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.

[00127c] In another aspect, the present disclosure refers to a method for expanding tumor infiltrating lymphocytes (TILs) into a therapeutic population of TILs comprising:

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

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

(c) performing a first expansion by culturing the first population of TILs in a cell culture medium comprising IL-2 and optionally comprising a 4-1BB agonist antibody for ~~about~~ 2 to 5 days;

(d) contacting the first population of TILs with at least one sd-RNA, wherein the sd-RNA is added at a concentration of 0.1 μM sd-RNA/10,000 TILs/100 μL media, 0.5 μM sd-RNA/10,000 TILs /100 μL media, 0.75 μM sd-RNA/10,000 TILs /100 μL media, 1 μM sd-RNA/10,000 TILs /100 μL media, 1.25 μM sd-RNA/10,000 TILs /100 μL media, 1.5 μM sd-

RNA/10,000 TILs /100 µL media, 2 µM sd-RNA/10,000 TILs /100 µL media, 5 µM sd-RNA/10,000 TILs /100 µL media, or 10 µM sd-RNA/10,000 TILs/100 µL media, and wherein the sd-RNA is for inhibiting the expression of a molecule selected from the group consisting of PD-1, LAG-3, TIM-3, CISH, and CBLB, and combinations thereof;

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

(f) 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, and wherein the transitions from step (c) to step (f) occur without opening the system;

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

(h) 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 transitions from step (c) to step (h) occur without opening the system;

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

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

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

[00127d] In another aspect, the present disclosure refers to a method for expanding tumor infiltrating lymphocytes (TILs) into a therapeutic population of TILs comprising:

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

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

(c) performing a first expansion by culturing the first population of TILs in a cell culture medium comprising IL-2 and optionally comprising a 4-1BB agonist antibody for ~~about~~ 2 to 5 days;

(d) contacting the first population of TILs with at least one sd-RNA, wherein the sd-RNA is added at a concentration of 0.1 μM sd-RNA/10,000 TILs, 0.5 μM sd-RNA/10,000 TILs, 0.75 μM sd-RNA/10,000 TILs, 1 μM sd-RNA/10,000 TILs, 1.25 μM sd-RNA/10,000 TILs, 1.5 μM sd-RNA/10,000 TILs, 2 μM sd-RNA/10,000 TILs, 5 μM sd-RNA/10,000 TILs, or 10 μM sd-RNA/10,000 TILs, and wherein the sd-RNA is for inhibiting the expression of a molecule selected from the group consisting of PD-1, LAG-3, TIM-3, CISH, and CBLB, and combinations thereof;

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

(f) 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, and wherein the transitions from step (c) to step (f) occur without opening the system;

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

(h) 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 (g) to step (h) occurs without opening the system;

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

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

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

[00127e] In another aspect, the present disclosure refers to a method for expanding tumor infiltrating lymphocytes (TILs) into a therapeutic population of TILs comprising:

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

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

(c) performing a first expansion by culturing the first population of TILs in a cell culture medium comprising IL-2 and optionally comprising a 4-1BB agonist antibody for ~~about~~ 2 to 5 days;

(d) 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, and wherein the transition from step (c) to step (d) occurs without opening the system;

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

(f) 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 transitions from step (c) to step (f) occur without opening the system;

(g) contacting the second population of TILs during any of steps (d), (e), and/or (f) with at least one sd-RNA, wherein the sd-RNA is added at a concentration of 0.1 μM sd-RNA/10,000 TILs/100 μL media, 0.5 μM sd-RNA/10,000 TILs /100 μL media, 0.75 μM sd-RNA/10,000 TILs /100 μL media, 1 μM sd-RNA/10,000 TILs /100 μL media, 1.25 μM sd-RNA/10,000 TILs /100 μL media, 1.5 μM sd-RNA/10,000 TILs /100 μL media, 2 μM sd-RNA/10,000 TILs /100 μL media, 5 μM sd-RNA/10,000 TILs /100 μL media, or 10 μM sd-RNA/10,000 TILs/100 μL media, and wherein the sd-RNA is for inhibiting the expression of a molecule selected from the group consisting of PD-1, LAG-3, TIM-3, CISH, and CBLB, and combinations thereof;

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

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

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

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

[00127f] In another aspect, the present disclosure refers to a method for expanding tumor infiltrating lymphocytes (TILs) into a therapeutic population of TILs comprising:

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

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

(c) performing a first expansion by culturing the first population of TILs in a cell culture medium comprising IL-2 and optionally comprising a 4-1BB agonist antibody for ~~about~~ 2 to 5 days;

(d) 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, and wherein the transition from step (c) to step (d) occurs without opening the system;

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

(f) 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 (e) to step (f) occurs without opening the system;

(g) contacting the second population of TILs during any of steps (d), (e), and/or (f) with at least one sd-RNA, wherein the sd-RNA is added at a concentration of 0.1 μM sd-RNA/10,000 TILs, 0.5 μM sd-RNA/10,000 TILs, 0.75 μM sd-RNA/10,000 TILs, 1 μM sd-

WHAT IS CLAIMED IS:

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

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

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

(c) performing a first expansion by culturing the first population of TILs in a cell culture medium comprising IL-2, 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~~ within 12 days to obtain the second population of TILs, and wherein the transition from step (b) to step (c) occurs without opening the system;

(d) performing a second expansion by supplementing the cell culture medium of the second population of TILs with additional IL-2, OKT-3, and antigen presenting cells (APCs), to produce a third population of TILs, wherein the second expansion is performed within 12 ~~for about 7-14~~ days to obtain the third population of TILs, wherein the third population of TILs is a therapeutic population of TILs, wherein the second expansion is performed in a closed container providing a second gas-permeable surface area, and wherein the transition from step (c) to step (d) occurs without opening the system;

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

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

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

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

~~3. The method according to claim 2, wherein the cryopreservation process is performed using a 1:1 ratio of harvested TIL population to cryopreservation media.~~

~~4.~~3. The method according to claim ~~3~~2, wherein the antigen-presenting cells are peripheral blood mononuclear cells (PBMCs).

~~5.~~4. The method according to claim ~~4~~3, wherein the PBMCs are irradiated and allogeneic.

~~6.~~5. The method according to claim ~~4~~3, wherein the PBMCs are added to the cell culture on any of days 9 through 14 in step (d).

~~7.~~6. The method according to claim ~~4~~3, wherein the antigen-presenting cells are artificial antigen-presenting cells.

~~8.~~7. The method according to claim 1, wherein the harvesting in step (f~~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.~~8. 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.~~9. 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.~~10. The method according to claim ~~11~~9, wherein the multiple fragments comprise about 50 fragments with a total volume of about 1350 mm^3.

~~13.~~11. 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.~~12. The method according to claim 1, wherein the cell culture medium in step (d) further comprises IL-15 and/or IL-21.

~~16.~~13. The method according to any one of claims 1 to ~~15~~12, wherein the IL-2 concentration is ~~about~~ 10,000 IU/mL to ~~about~~ 5,000 IU/mL.

~~17.~~14. The method according to claim ~~15~~12, wherein the IL-15 concentration is ~~about~~ 500 IU/mL to ~~about~~ 100 IU/mL.

~~18.~~15. The method according to claim ~~15~~12, 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.~~16. The method according to claim ~~3~~2, wherein the cryopreservation ~~media~~ process comprises dimethlysulfoxide (DMSO).

~~21.~~17. The method according to claim ~~20~~16, wherein the wherein the cryopreservation ~~media~~ process comprises 7% to 10% DMSO.

~~22.~~18. The method according to claim 1, wherein the first period in step (c) and the second period in step (~~e~~d) are each individually performed within a period of 10 days, 11 days, or 12 days.

~~23.~~19. The method according to claim 1, wherein the first period in step (c) and the second period in step (~~e~~d) are each individually performed within a period of 11 days.

~~24.~~20. 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.~~21. 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.~~22. 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.~~23. 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.~~24. 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.~~25. The method according to claim 1, wherein steps (a) through (f) are performed in 22 days or less.

~~30.~~26. The method according to claim 1, wherein steps (a) through (f) are performed in 20 days or less.

~~31.~~27. The method according to claim 1, wherein steps (a) through (f) are performed in 15 days or less.

~~32.~~28. The method according to claim 1, wherein steps (a) through (f) are performed in 10 days or less.

~~33.~~29. The method according to claim ~~23~~, wherein steps (a) through (f) and cryopreservation are performed in 22 days or less.

~~34.~~30. The method according to any one of claims 1 to ~~33~~29, wherein the therapeutic population of TILs harvested in step (~~e~~f) comprises sufficient TILs for a therapeutically effective dosage of the TILs.

~~35.~~31. The method according to claim ~~34~~30, 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.~~32. The method according to any one of claims 1 to ~~35~~31, wherein steps (b) through (e) are performed in a single container, wherein performing steps (b) through (e) in a single container results in an increase in TIL yield per resected tumor as compared to performing steps (b) through (e) in more than one container.

~~37.~~33. The method according to any one of claims 1 to ~~36~~32, wherein the antigen-presenting cells are added to the TILs during the second period in step (d) without opening the system.

~~38.~~34. The method according to any one of claims 1 to ~~79~~33, 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.

~~39.~~35. The method according to any one of claims 1 to ~~38~~34, wherein the risk of microbial contamination is reduced as compared to an open system.

~~40.~~36. The method according to any one of claims 1 to ~~39~~35, wherein the TILs from step (f) or (g) are to be infused into a patient.

~~41.~~37. The method according to ~~any one of~~ claim~~s~~ 1 ~~to 40~~, wherein the multiple fragments comprise about 4 fragments.

~~42.~~38. Use of an expanded population of tumor infiltrating lymphocytes (TILs) in the manufacture of a medicament for treating a subject with cancer, wherein the population of TILs comprises a therapeutically effective dosage of a third population of TILs from an infusion bag in step (g) of a method comprising:

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

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

(c) performing a first expansion by culturing the first population of TILs in a cell culture medium comprising IL-2, and optionally OKT-3, to produce a second population of TILs, wherein the first expansion is performed in a closed container providing a first gas-permeable surface area, wherein the first expansion is performed within 12 ~~for about 3-14~~ days to obtain the second population of TILs, and wherein the transition from step (b) to step (c) occurs without opening the system;

(d) performing a second expansion by supplementing the cell culture medium of the second population of TILs with additional IL-2, OKT-3, and antigen presenting cells (APCs), to produce the third population of TILs, wherein the second expansion is performed within 12 ~~for about 7-14~~ days to obtain the third population of TILs, wherein the third population of TILs is a therapeutic population of TILs, wherein the second expansion is performed in a closed container providing a second gas-permeable surface area, and wherein the transition from step (c) to step (d) occurs without opening the system;

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

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

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

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

~~43.~~39. Use of a population of expanded TILs in the manufacture of a medicament for the treatment of a subject with cancer, wherein the population of expanded TILs is a 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 within 12 ~~for about 3-14~~ days to obtain the second population of TILs, and wherein the transition from step (b) to step (c) occurs without opening the system;

(d) performing a second expansion by supplementing the cell culture medium of the second population of TILs with additional IL-2, OKT-3, and antigen presenting cells (APCs), to produce the third population of TILs, wherein the second expansion is performed within 12 ~~for about 7-14~~ days to obtain the third population of TILs, wherein the third population of TILs is a therapeutic population of TILs, wherein the second expansion is performed in a closed container providing a second gas-permeable surface area, and wherein the transition from step (c) to step (d) occurs without opening the system;

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

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

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

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

~~44. The use according to claim 42 or 43, wherein the use further comprises one or more of the features recited in any of claims 1 to 41.~~

~~45. A method for expanding tumor infiltrating lymphocytes (TILs) into a therapeutic population of TILs comprising exposing TILs to transcription factors (TFs) and/or other molecules capable of transiently altering protein expression in order to generate a therapeutic population of TILs, wherein the TFs and/or other molecules capable of transiently altering protein expression provide for increased expression of tumor antigens and/or an increase in the number of tumor antigen-specific T cells in the therapeutic population of TILs.~~

~~46.~~40. The method according to any of claims 1-~~41~~37 ~~and 45~~, or the use of ~~any of claims 42-44~~claim 38 or 39, wherein the transient altering of protein expression results in induction of protein expression.

~~47.~~41. The method according to any of claims 1-~~41~~37 ~~and 45-46~~, or the use ~~of any of claims 42-44~~claim 38 or 39, wherein the transient altering of protein expression results in a reduction of protein expression.

~~48.~~42. The method or the use according to claim 41~~47~~, wherein one or more sd-RNA(s) is employed to reduce the transient protein expression.

~~49. A method for evaluating transcription factors (TFs) and/or other molecules capable of transiently altering protein expression, wherein the method comprises expanding tumor infiltrating lymphocytes (TILs) into a therapeutic population of TILs, exposing TILs to transcription factors (TFs) and/or other molecules capable of transiently altering protein expression in order to generate a therapeutic population of TILs, wherein the TFs and/or other molecules capable of transiently altering protein expression provide for altered expression of tumor antigens and/or an alteration in the number of tumor antigen-specific T cells in the therapeutic population of TILs.~~

~~50.~~43. The method according to any of claims 1-~~41~~37 and ~~45-49~~40-42, or the use of any of claims ~~42~~38-~~44~~42, wherein the transient altering of protein expression targets a gene selected from the group consisting of PD-1, TGFBR2, CBLB (CBL-B), CISH, CCRs (chimeric co-stimulatory receptors), IL-2, IL-12, IL-15, IL-21, NOTCH 1/2 ICD, TIM3, LAG3, TIGIT, TGFβ, CCR2, CCR4, CCR5, CXCR1, CXCR2, CSCR3, CCL2 (MCP-1), CCL3 (MIP-1α), CCL4 (MIP1-β), CCL5 (RANTES), CXCL1/CXCL8, CCL22, CCL17, CXCL1/CXCL8, VHL, CD44, PIK3CD, SOCS1, and cAMP protein kinase A (PKA).

~~51.~~44. 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 a 4-1BB agonist antibody for ~~about~~ 2 to 5 days;

(d) 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, 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 short interfering RNA or one messenger RNA;

(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 from step (h) 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.

~~52.~~45. The method of claim ~~51~~44, wherein the sterile electroporation step comprises the delivery of a short interfering RNA for inhibiting the expression of a molecule selected from the group consisting of PD-1, LAG-3, TIM-3, CTLA-4, TIGIT, CISH, TGFβR2, PKA, CBLB, BAFF (BR3), and combinations thereof.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 a 4-1BB agonist antibody for ~~about~~ 2 to 5 days;

(d) 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, and wherein the transition from step (c) to step (d) occurs without opening the system;

(e) performing a sterile electroporation step or a SQZ microfluidic membrane disruption step on the second population of TILs, wherein the sterile electroporation step or SQZ microfluidic membrane disruption step mediates the transfer of at least one short interfering RNA or one messenger RNA;

(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 from step (h) 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 short interfering RNA for inhibiting the expression of a molecule selected from the group consisting of PD-1, LAG-3, TIM-3, CTLA-4, TIGIT, CISH, TGFβR2, PKA, CBLB, BAFF (BR3), and combinations thereof, and further wherein an adhesion molecule selected from the group consisting of CCR2, CCR4, CCR5, CXCR2, CXCR3, CX3CR1, and combinations thereof is inserted by a gammaretroviral or lentiviral method into the first population of TILs, second population of TILs, or harvested population of TILs.

~~53.~~46. 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 a 4-1BB agonist antibody for ~~about~~ 2 to 5 days;

(d) 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, 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 short interfering RNA or one messenger RNA;

(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 from step (h) 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 sterile electroporation step comprises the delivery of a short interfering RNA 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.

~~54.~~47. 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 a 4-1BB agonist antibody for ~~about~~ 2 to 5 days;

(d) contacting the first population of TILs with at least one sd-RNA, wherein the sd-RNA is added at a concentration of 0.1 μM sd-RNA/10,000 TILs/100 μL media, 0.5 μM sd-RNA/10,000 TILs /100 μL media, 0.75 μM sd-RNA/10,000 TILs /100 μL media, 1 μM sd-RNA/10,000 TILs /100 μL media, 1.25 μM sd-RNA/10,000 TILs /100 μL media, 1.5 μM sd-

RNA/10,000 TILs /100 µL media, 2 µM sd-RNA/10,000 TILs /100 µL media, 5 µM sd-RNA/10,000 TILs /100 µL media, or 10 µM sd-RNA/10,000 TILs/100 µL media, and wherein the sd-RNA is for inhibiting the expression of a molecule selected from the group consisting of PD-1, LAG-3, TIM-3, CISH, and CBLB, and combinations thereof;

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

(f) 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, and wherein the transitions from step (c) to step (f) occur without opening the system;

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

(h) 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 transitions from step (c) to step (h) occur without opening the system;

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

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

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

~~55.~~48. 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 a 4-1BB agonist antibody for ~~about~~ 2 to 5 days;

(d) contacting the first population of TILs with at least one sd-RNA, wherein the sd-RNA is added at a concentration of 0.1 μM sd-RNA/10,000 TILs, 0.5 μM sd-RNA/10,000 TILs, 0.75 μM sd-RNA/10,000 TILs, 1 μM sd-RNA/10,000 TILs, 1.25 μM sd-RNA/10,000 TILs, 1.5 μM sd-RNA/10,000 TILs, 2 μM sd-RNA/10,000 TILs, 5 μM sd-RNA/10,000 TILs, or 10 μM sd-RNA/10,000 TILs, and wherein the sd-RNA is for inhibiting the expression of a molecule selected from the group consisting of PD-1, LAG-3, TIM-3, CISH, and CBLB, and combinations thereof;

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

(f) 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, and wherein the transitions from step (c) to step (f) occur without opening the system;

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

(h) 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 (g) to step (h) occurs without opening the system;

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

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

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

~~56.~~49. 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 a 4-1BB agonist antibody for ~~about~~ 2 to 5 days;

(d) 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, and wherein the transition from step (c) to step (d) occurs without opening the system;

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

(f) 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 transitions from step (c) to step (f) occur without opening the system;

(g) contacting the second population of TILs during any of steps (d), (e), and/or (f) with at least one sd-RNA, wherein the sd-RNA is added at a concentration of 0.1 μM sd-RNA/10,000 TILs/100 μL media, 0.5 μM sd-RNA/10,000 TILs /100 μL media, 0.75 μM sd-RNA/10,000 TILs /100 μL media, 1 μM sd-RNA/10,000 TILs /100 μL media, 1.25 μM sd-RNA/10,000 TILs /100 μL media, 1.5 μM sd-RNA/10,000 TILs /100 μL media, 2 μM sd-RNA/10,000 TILs /100 μL media, 5 μM sd-RNA/10,000 TILs /100 μL media, or 10 μM sd-

RNA/10,000 TILs/100 µL media, and wherein the sd-RNA is for inhibiting the expression of a molecule selected from the group consisting of PD-1, LAG-3, TIM-3, CISH, and CBLB, and combinations thereof;

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

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

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

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

~~57.~~50. 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 a 4-1BB agonist antibody for ~~about~~ 2 to 5 days;

(d) 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, and wherein the transition from step (c) to step (d) occurs without opening the system;

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

(f) 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 (e) to step (f) occurs without opening the system;

(g) contacting the second population of TILs during any of steps (d), (e), and/or (f) with at least one sd-RNA, wherein the sd-RNA is added at a concentration of 0.1 μM sd-RNA/10,000 TILs, 0.5 μM sd-RNA/10,000 TILs, 0.75 μM sd-RNA/10,000 TILs, 1 μM sd-RNA/10,000 TILs, 1.25 μM sd-RNA/10,000 TILs, 1.5 μM sd-RNA/10,000 TILs, 2 μM sd-RNA/10,000 TILs, 5 μM sd-RNA/10,000 TILs, or 10 μM sd-RNA/10,000 TILs, and wherein the sd-RNA is for inhibiting the expression of a molecule selected from the group consisting of PD-1, LAG-3, TIM-3, CISH, and CBLB, and combinations thereof;

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

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

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

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

~~58.~~51. The method according to any one of claims ~~54~~ 47 to ~~57~~50, wherein the sd-RNA is added to the first population of cells twice a day, once a day, every two days, every three days, every four days, every five days, every six days, or every seven days during the first expansion period.

~~59.~~52. The method according to any one of claims ~~54~~ 47 to ~~57~~50, wherein the sd-RNA is added to the second population of cells twice a day, once a day, every two days, every three days, every four days, every five days, every six days, or every seven days during the first expansion period.

60.53. The method according to any one of claims 54 47 to 5952, wherein two sd-RNAs are added for inhibiting the expression of two molecules selected from the group consisting of PD-1, LAG-3, TIM-3, CISH, and CBLB.

61.54. The method according to any one of claims 54 47 to 5952, wherein two sd-RNAs are added for inhibiting the expression of two molecules, wherein the two molecules are selected from the groups consisting of:

i. PD-1 and LAG-3,

ii. PD-1 and TIM-3,

iii. PD-1 and CISH,

iv. PD-1 and CBLB,

v. LAG-3 and TIM-3,

vi. LAG-3 and CISH,

vii. LAG-3 and CBLB,

viii. TIM-3 and CISH,

ix. TIM-3 and CBLB, and

x. CISH and CBLB.

62.55. The method according to any one of claims 54 47 to 6053, wherein more than two sd-RNAs are added for inhibiting the expression of more than two molecules selected from the group consisting of PD-1, LAG-3, TIM-3, CISH, and CBLB.

63.56. The method according to any one of claims 54 47 to 6255, wherein the expression of at least one molecule selected from the group consisting of PD-1, LAG-3, TIM-3, CISH, and CBLB is reduced by at least 80%, 85%, 90%, or 95% in the TILs contacted with the at least one sd-RNA.

64.57. The method according to any one of claims 54 48 to 6256, wherein the expression of at least one molecule selected from the group consisting of PD-1, LAG-3, TIM-3, CISH, and CBLB is reduced by at least 80%, 85%, 90%, or 95% for at least 12 hours, at least 24 hours, or at least 48 hours, in the TILs contacted with the at least one sd-RNA.

65.58. The method according to any one of claims 54 47 to 6255, wherein the sd-RNAs are prepared by a method comprising performing *in vitro* transcription from a linear double-

stranded DNA template obtained by Polymerase Chain Reaction (PCR) and suitable for *in vitro* transcription of the sd-RNA comprising from 5' to 3': an RNA polymerase promoter on the coding strand of the double-stranded DNA, a 5' untranslated region less than 3,000 nucleotides in length and effective for translation of the mRNA into a detectable polypeptide after transfection into a eukaryotic cell, an open reading frame that encodes the polypeptide, wherein the polypeptide is heterologous to the cell to be transfected and wherein the polypeptide is selected from the group consisting of a ligand or a receptor of an immune cell, a polypeptide that stimulates or inhibits a function of the immune system, and a polypeptide that inhibits the function of an oncogenic polypeptide, 3' untranslated region effective for translation of the mRNA into a detectable polypeptide after transfection into a eukaryotic cell, and a poly(A) stretch of 50-5,000 nucleotides on the coding strand of the double-stranded DNA, wherein the promoter is heterologous to the open reading frame, and wherein the DNA template is not contained within a DNA vector and terminates with the 3' end of the poly(A) stretch.

~~66.~~59. The method according to claim ~~65~~58, wherein the RNA polymerase promoter comprises a consensus binding sequence for an RNA polymerase selected from the group consisting of T7, T3 or SP6 RNA polymerase.

~~67.~~60. The method according to claim ~~65~~58, wherein the open reading frame encodes a polypeptide selected from the group consisting of PD-1, LAG-3, TIM-3, CISH, and CBLB.

~~68.~~61. The method according to claim ~~65~~58, wherein the linear double-stranded DNA template further comprises an internal ribosome entry site.

~~69.~~62. A method for expanding tumor infiltrating lymphocytes (TILs) into a therapeutic population of TILs comprising:

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

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

(iii) performing a second expansion by supplementing the cell culture medium of the second population of TILs with additional IL-2, OKT-3, and antigen presenting cells (APCs), to produce a third population of TILs, and wherein the second expansion is performed within 12 ~~for about 7-14~~ days in order to obtain the third population of

TILs, wherein the third population of TILs is a therapeutic population of TILs; and

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

~~70.~~63. The method according to claim ~~69~~62, wherein the method further comprises:

(v) performing an additional second expansion before or after step (iv) by supplementing the cell culture medium of the third population of TILs with additional IL-2, additional OKT-3, and additional APCs, wherein the additional second expansion is performed for at least 14 days to obtain a larger therapeutic population of TILs than obtained in step (iii), wherein the larger therapeutic population of TILs exhibits an alteration in the number of tumor antigen-specific T cells.

~~71.~~64. The method or use according to any of the preceding claims, wherein the second population of TILs is at least 50-fold greater in number than the first population of TILs.

~~72.~~65. The method or use according to any of the preceding claims, wherein the third population of TILs is at least 100-fold greater in number than the second population of TILs.