(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 one 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; 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 3-12 days to obtain the second population of TILs, wherein the transition from step (b) to step (c) occurs without opening the system;

(d) activating the second population of TILs using CD3 and CD28 activating beads or antibodies for 1 to 5 days;

(e) gene-editing at least a portion of the second population of TILs to obtain a third population of TILs; and

(~~d~~f) performing a second expansion by supplementing the cell culture medium of the ~~second~~ third population of TILs with additional IL-2, ~~optionally OKT-3,~~ and antigen presenting cells (APCs), to produce a ~~third~~ fourth population of TILs, wherein the second expansion is performed for 7-12 days to obtain the fourth ~~third~~ population of TILs, wherein the fourth ~~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~~g) harvesting the therapeutic population of TILs obtained from step (~~d~~f), wherein the transition from step (~~d~~f) to step (~~e~~g) occurs without opening the system; and

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

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

[0005b] 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 3-12 days to obtain the second population of TILs, wherein the transition from step (b) to step (c) occurs without opening the system;

(d) activating the second population of TILs using CD3 and CD28 activating beads or antibodies for 1 to 5 days;

(e) gene-editing at least a portion of the second population of TILs to obtain a third population of TILs; and

(~~d~~f) performing a second expansion by supplementing the cell culture medium of the third ~~second~~ population of TILs with additional IL-2, ~~optionally OKT-3,~~ and antigen presenting cells (APCs), to produce a ~~third~~ fourth population of TILs, wherein the second expansion is performed for 7-12 days to obtain the fourth ~~third~~ population of TILs, wherein the fourth ~~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~~g) harvesting the therapeutic population of TILs obtained from step (~~d~~f), wherein the transition from step (~~d~~f) to step (~~e~~g) occurs without opening the system; and

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

(~~g~~i) cryopreserving the infusion bag comprising the harvested TIL population from step (~~f~~h) using a cryopreservation process; and

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

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

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

[0005c] 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 2 to 5 days;

(d) ~~optioinally~~ adding OKT-3, to produce a second population of TILs, wherein the expansion is performed in a closed container providing a first gas-permeable surface area, wherein the expansion is performed for 1 to 3 days to obtain the second population of TILs, 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 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

[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.

[00102a] 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 3-12 days to obtain the second population of TILs, wherein the transition from step (a) to step (b) occurs without opening the system;

(c) activating the second population of TILs using CD3 and CD28 activating beads or antibodies for 1 to 5 days;

(d) gene-editing at least a portion of the second population of TILs to obtain a third population of TILs; and

(~~e~~e) performing a second expansion by supplementing the cell culture medium of the third ~~second~~ population of TILs with additional IL-2, ~~optionally OKT-3,~~ and antigen presenting cells (APCs), to produce a ~~third~~ fourth population of TILs, wherein the second expansion is performed for 7-12 days to obtain the fourth ~~third~~ population of TILs, wherein the fourth ~~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~~f) harvesting the therapeutic population of TILs obtained from step (~~e~~e), wherein the transition from step (~~e~~e) to step (~~d~~f) occurs without opening the system; and

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

~~(f) at any time during the method steps of (a)-(e), 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

[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.

~~**[00160a]** In another aspect, the present disclosure provides use of a population of expanded TILs in the manufacture of a medicament for treating, 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 3-12 days to obtain the second population of TILs, 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 7-12 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) 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 steps of (a)-(d), 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.

(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 2 to 5 days to produce a second population of TILs;

~~(d) optionally adding OKT-3, to produce a second population of TILs, wherein the expansion is performed in a closed container providing a first gas-permeable surface area, wherein the expansion is performed for 1 to 3 days to obtain the second population of TILs, wherein the transition from step (c) to step (d) occurs without opening the system;~~

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

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

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

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

(~~j~~i) 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

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.

[00184a] In another aspect, the present disclosure provides a use of a population of expanded TILs in the manufacture of a medicament for treating 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 2 to 5 days;

(d) optionally adding OKT-3, to produce a second population of TILs, wherein the expansion is performed in a closed container providing a first gas-permeable surface area, wherein the expansion is performed for 1 to 3 days to obtain the second population of TILs, 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 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.

[00186a] In another aspect, the present disclosure provides a cryopreservation composition comprising the population of expanded TILs as defined herein, 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 3-12 days to obtain the second population of TILs, wherein the transition from step (b) to step (c) occurs without opening the system;

(d) activating the second population of TILs using CD3 and CD28 activating beads or antibodies for 1 to 5 days;

(e) gene-editing at least a portion of the second population of TILs to obtain a third population of TILs; and

(~~d~~f) performing a second expansion by supplementing the cell culture medium of the ~~second~~ third population of TILs with additional IL-2, ~~optionally OKT-3,~~ and antigen presenting cells (APCs), to produce a ~~third~~ fourth population of TILs, wherein the second expansion is performed for 7-12 days to obtain the fourth ~~third~~ population of TILs, wherein the fourth ~~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~~g) harvesting the therapeutic population of TILs obtained from step (~~d~~f), wherein the transition from step (~~d~~f) to step (~~e~~g) occurs without opening the system; and

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

~~(g) at any time during the method steps of (a)-(d), 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~~h) using a cryopreservation process.

3. The method according to claim 1, wherein the antigen-presenting cells are peripheral blood mononuclear cells (PBMCs).

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

5. The method according to claim 3, wherein the PBMCs are added to the cell culture on any of days 9 through ~~14~~ 12 in step (~~d~~f).

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

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

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

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

10. The method according to claim 1, wherein the multiple fragments comprise about 50 fragments with a total volume of about 1350 mm^3.

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

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

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

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

15. The method according to claim ~~15~~14, wherein the IL-21 concentration is 20 IU/mL to 0.5 IU/mL.

16. The method according to claim 2, wherein the cryopreservation process is performed using cryopreservation media and wherein the cryopreservation media comprises dimethlysulfoxide (DMSO).

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

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

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

20. The method according to claim 1, wherein steps (a) through (~~f~~h) are performed within a period of 10 days to ~~22~~ 30 days.

21. The method according to claim 1, wherein steps (a) through (~~f~~h) are performed within a period of 20 days to ~~22~~ 28 days.

22. The method according to claim 1, wherein steps (a) through (~~f~~h) are performed within a period of 15 days to ~~20~~ 26 days.

23. The method according to claim 1, wherein steps (a) through (~~f~~h) are performed within a period of 10 days to ~~20~~ 26 days.

24. The method according to claim 1, wherein steps (a) through (~~f~~h) are performed within a period of 10 days to ~~15~~ 25 days.

25. The method according to claim 1, wherein steps (a) through (~~f~~h) are performed in ~~22~~ 30 days or less.

26. The method according to claim 1, wherein steps (a) through (~~f~~h) are performed in ~~20~~ 28 days or less.

27. The method according to claim 1, wherein steps (a) through (~~f~~h) are performed in ~~15~~ 26 days or less.

28. The method according to claim 1, wherein steps (a) through (~~f~~)h) are performed in ~~10~~ 25 days or less.

29. The method according to claim 2, wherein steps (a) through (~~f~~h) and cryopreservation are performed in ~~22~~ 25 days or less.

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

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

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

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

34. The method according to any one of claims 1 to 33, wherein the ~~third~~ fourth population of TILs in step (~~d~~f) 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.

35. The method according to any one of claims 1 to 34, wherein the fourth ~~third~~ population of TILs in step (~~d~~f) provides for at least a five-fold or more interferon-gamma production when administered to a subject.

36. The method according to any one of claims 1 to 35, wherein the fourth ~~third~~ population of TILs in step (~~d~~f) 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~~ third 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 third ~~second~~ population of cells.

37. The method according to any one of claims 1 to 36, wherein the effector T cells and/or central memory T cells obtained from the fourth ~~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 third ~~second~~ population of cells.

38. The method according to any one of claims 1 to 37, wherein the risk of microbial contamination is reduced as compared to an open system.

39. The method according to any one of claims 1 to 38, wherein the TILs from step (~~g~~h) are to be infused into a patient.

40. The method according to any one of claims 1 to 39, 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.

~~41. The method according to claim 40, 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.~~

~~42. The method according to claim 40, 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.~~

~~43. The method according to any of claims 1-42, wherein the gene editing is carried out on TILs from one or more of the first population, the second population, and the third population.~~

~~44. The method according to any of claims 1-42, wherein the gene editing is carried out on TILs from the first expansion, or TILs from the second expansion, or both.~~

~~45. The method according to any of claims 1-42, wherein the gene editing is carried out after the first expansion and before the second expansion.~~

~~46. The method according to any of claims 1-42, wherein the gene editing is carried out before step (c), before step (d), or before step (e).~~

~~47.~~41. The method according to any of claims 1-~~42~~40, 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~~.

~~48. The method according to any of claims 1-42, 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.~~

~~49.~~42. The method according to any of claims 1-~~42~~40, 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~~.

~~50.~~43. The method according to any of claims 1-~~49~~42, 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.

~~51.~~44. The method according to claim ~~50~~43, 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.

~~52.~~45. The method according to claim ~~50~~43, 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.

~~53.~~46. The method according to any of claims 1-~~49~~42, 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.

~~54.~~47. The method according to any of claims 1-~~53~~46, 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.

~~55.~~48. The method according to any of claims 1-~~53~~46, 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.

~~56.~~49. The method according to any of claims 1-~~53~~46, wherein the gene-editing comprises a CRISPR method.

~~57.~~50. The method according to claim ~~56~~49, wherein the CRISPR method is a CRISPR/Cas9 method.

~~58.~~51. The method according to any of claims 1-~~53~~46, wherein the gene-editing comprises a TALE method.

~~59.~~52. The method according to any of claims 1-~~53~~46, wherein the gene-editing comprises a zinc finger method.

~~60.~~53. 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 3-12 days to obtain the second population of TILs, wherein the transition from step (b) to step (c) occurs without opening the system;

(d) activating the second population of TILs using CD3 and CD28 activating beads or antibodies for 1 to 5 days;

(e) gene-editing at least a portion of the second population of TILs to obtain a third population of TILs; and

(~~d~~f) performing a second expansion by supplementing the cell culture medium of the third ~~second~~ population of TILs with additional IL-2, ~~optionally OKT-3,~~ and antigen presenting cells (APCs), to produce a ~~third~~ fourth population of TILs, wherein the second expansion is performed for 7-12 days to obtain the fourth ~~third~~ population of TILs, wherein the fourth ~~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~~g) harvesting the therapeutic population of TILs obtained from step (~~d~~f), wherein the transition from step (~~d~~f) to step (~~e~~g) occurs without opening the system; and

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

(~~g~~i) cryopreserving the infusion bag comprising the harvested TIL population from step (~~f~~h) using a cryopreservation process; and

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

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

~~61.~~54. The use according to claim ~~60~~53, wherein step (~~e~~g) is included, and wherein the therapeutic population of TILs harvested in step (~~e~~g) comprises sufficient TILs for administration of a therapeutically effective dosage of the TILs in step (~~h~~j).

~~62.~~55. The use according to claim ~~61~~54, wherein the number of TILs sufficient for administration of a therapeutically effective dosage in step (~~h~~j) is from 2.3×10^{10} to 13.7×10^{10}.

~~63.~~56. The use according to claim ~~62~~55, wherein the antigen presenting cells (APCs) are PBMCs.

~~64.~~57. The use according to claim ~~63~~56, wherein the PBMCs are added to the cell culture on any of days 9 through ~~14~~ 12 in step (~~d~~f).

~~65.~~58. The use according to any of claims ~~60~~ 53 to ~~64~~57, wherein prior to administration of a therapeutically effective dosage of TIL cells in step (~~h~~j), a non-myeloablative lymphodepletion regimen has been administered to the patient.

~~66.~~59. The use according to claim ~~65~~58, 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.

~~67.~~60. The use according to any of claims ~~60~~ 53 to ~~66~~59, 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~~j).

~~68.~~61. The use according to claim ~~67~~60, 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.

~~69.~~62. The use according to any of claims ~~60~~53-~~68~~61, wherein the fourth ~~third~~ population of TILs in step (~~d~~f) is a therapeutic population of TILs which comprises an increased subpopulation of effector T cells and/or central memory T cells relative to the third ~~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 third ~~second~~ population of cells.

~~70.~~63. The use according to any of claims ~~60~~53-~~69~~62, 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 third ~~second~~ population of cells.

~~71.~~64. The use according to any of claims ~~60~~53-~~70~~63, 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.

~~72.~~65. The use according to any of claims ~~60~~53-~~71~~64, wherein the cancer is selected from the group consisting of melanoma, HNSCC, cervical cancers, and NSCLC.

~~73.~~66. The use according to any of claims ~~60~~53-~~72~~65, wherein the cancer is melanoma.

~~74.~~67. The use according to any of claims ~~60~~53-~~73~~66, wherein the cancer is HNSCC.

~~75.~~68. The use according to any of claims ~~60~~53-~~74~~67, wherein the cancer is a cervical cancer.

~~76.~~69. The use according to any of claims ~~60~~53-~~75~~68, wherein the cancer is NSCLC.

~~77.~~70. The use according to any one of claims ~~60~~ 53 to ~~76~~69, 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.

~~78. The use according to claim 77, wherein the gene-editing is carried out after the 4-1BB agonist and/or the OX40 agonist is introduced into the cell culture medium.~~

~~79. The use according to claim 77, wherein the gene-editing is carried out before the 4-1BB agonist and/or the OX40 agonist is introduced into the cell culture medium.~~

~~80. The use according to any of claims 60-79, wherein the gene-editing is carried out on TILs from one or more of the first population, the second population, and the third population.~~

~~81. The use according to any of claims 60-79, wherein the gene-editing is carried out on TILs from the first expansion, or TILs from the second expansion, or both.~~

~~82. The use according to any of claims 60-79, wherein the gene-editing is carried out after the first expansion and before the second expansion.~~

~~83. The use according to any of claims 60-79, wherein the gene-editing is carried out before step (c), before step (d), or before step (e).~~

~~84.~~71. The use according to any of claims ~~60~~53-~~83~~70, 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~~.

~~85. The use according to any of claims 60-83, 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.~~

~~86.~~72. The use according to any of claims 53~~60~~-~~83~~70, 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~~.

~~87.~~73. The use according to any of claims ~~60~~53-72~~86~~, 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.

~~88.~~74. The use according to claim ~~87~~73, 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.

~~89.~~75. The use according to claim ~~87~~73, 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.

~~90.~~76. The use according to any of claims ~~60~~53-~~86~~72, 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.

~~91.~~77. The use according to any of claims ~~60~~53-~~90~~76, 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.

~~92.~~78. The use according to any of claims ~~60~~53-~~90~~76, 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.

~~93.~~79. The use according to any of claims ~~60~~53-~~90~~76, wherein the gene-editing comprises a CRISPR method.

~~94.~~80. The use according to claim ~~93~~79, wherein the CRISPR method is a CRISPR/Cas9 method.

~~95.~~81. The use according to any of claims ~~60~~53-~~90~~76, wherein the gene-editing comprises a TALE method.

~~96.~~82. The use according to any of claims ~~60~~53-~~90~~76, wherein the gene-editing comprises a zinc finger method.

~~97.~~83. A method for expanding tumor infiltrating lymphocytes (TILs) into a therapeutic population of TILs comprising:

(a) adding processed tumor fragments from a tumor resected from a patient into a closed system to obtain a first population of TILs;

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

population of TILs, wherein the first expansion is performed in a closed container providing a first gas-permeable surface area, wherein the first expansion is performed for 3-12 days to obtain the second population of TILs, wherein the transition from step (a) to step (b) occurs without opening the system;

(c) activating the second population of TILs using CD3 and CD28 activating beads or antibodies for 1 to 5 days;

(d) gene-editing at least a portion of the second population of TILs to obtain a third population of TILs; and

(~~e~~e) performing a second expansion by supplementing the cell culture medium of the third ~~second~~ population of TILs with additional IL-2, ~~optionally OKT-3,~~ and antigen presenting cells (APCs), to produce a ~~third~~ fourth population of TILs, wherein the second expansion is performed for 7-12 days to obtain the fourth ~~third~~ population of TILs, wherein the fourth ~~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~~f) harvesting the therapeutic population of TILs obtained from step (~~e~~e), wherein the transition from step (~~e~~e) to step (~~d~~f) occurs without opening the system; and

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

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

~~98.~~84. The method according to claim ~~97~~83, wherein the therapeutic population of TILs harvested in step (~~d~~f) comprises sufficient TILs for a therapeutically effective dosage of the TILs.

~~99.~~85. The method according to claim ~~98~~84, where the number of TILs sufficient for a therapeutically effective dosage is from 2.3×10^{10} to 13.7×10^{10}.

~~100.~~86. The method according to claim ~~99~~85, further comprising the step of cryopreserving the infusion bag comprising the harvested TIL population using a cryopreservation process.

~~101.~~87. The method according to claim ~~97~~83, wherein the antigen-presenting cells are peripheral blood mononuclear cells (PBMCs).

~~102.~~88. The method according to claim ~~101~~87, wherein the PBMCs are irradiated and allogeneic.

~~103.~~89. The method according to claim ~~102~~88, wherein the PBMCs are added to the cell culture on any of days 9 through ~~14~~ 12 in step (~~ee~~).

~~104.~~90. The method according to claim ~~97~~83, wherein the antigen-presenting cells are artificial antigen-presenting cells.

~~105.~~91. The method according to claim ~~97~~83, wherein the multiple fragments comprise 4 to 50 fragments, wherein each fragment has a volume of about 27 mm^3.

~~106.~~92. The method according to claim ~~97~~83, wherein the multiple fragments comprise 30 to 60 fragments with a total volume of 1300 mm^3 to 1500 mm^3.

~~107.~~93. The method according to claim ~~98~~83, wherein the multiple fragments comprise about 50 fragments with a total volume of about 1350 mm^3.

~~108.~~94. The method according to claim ~~97~~83, wherein the multiple fragments comprise about 50 fragments with a total mass of 1 gram to 1.5 grams.

~~109.~~95. The method according to claim ~~97~~83, wherein the multiple fragments comprise about 4 fragments.

~~110.~~96. The method according to claim ~~97~~83, wherein the first period in step (b) and the second period in step (~~ee~~) are each individually performed within a period of 10 days, 11 days, or 12 days.

~~111.~~97. The method according to claim ~~97~~83, wherein the first period in step (b) and the second period in step (~~ee~~) are each individually performed within a period of 11 days.

~~112.~~98. The method according to claim 83~~97~~, wherein steps (a) through (~~eg~~) are performed within a period of 10 days to ~~22~~ 30 days.

~~113.~~99. The method according to claim 83~~97~~, wherein steps (a) through (~~eg~~) are performed within a period of 10 days to ~~20~~ 28 days.

~~114.~~100. The method according to claim 83~~97~~, wherein steps (a) through (~~eg~~) are performed within a period of 10 days to ~~15~~ 26 days.

~~115.~~101. The method according to claim 8397, wherein steps (a) through (~~e~~g) are performed in ~~22~~ 25 days or less.

~~116.~~102. The method according to claim ~~100~~86, wherein steps (a) through (~~e~~g) and cryopreservation are performed in ~~22~~ 25 days or less.

~~117.~~103. The method according to any one of claims 8397 to ~~116~~102, 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.

~~118.~~104. The method according to any one of claims 8397 to ~~117~~103, wherein the antigen-presenting cells are added to the TILs during the second period in step (~~e~~e) without opening the system.

~~119.~~105. The method according to any one of claims 8397 to ~~118~~104, wherein the fourth ~~third~~ population of TILs in step (~~e~~e) is a therapeutic population of TILs which comprises an increased subpopulation of effector T cells and/or central memory T cells relative to the third ~~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 third ~~second~~ population of cells.

~~120.~~106. The method according to any one of claims 8397 to ~~119~~105, 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 third ~~second~~ population of cells.

~~121.~~107. The method according to any one of claims 8397 to ~~120~~106, wherein the risk of microbial contamination is reduced as compared to an open system.

~~122.~~108. The method according to any one of claims 8397 to ~~121~~107, wherein the TILs from step (~~e~~g) are to be infused into a patient.

~~123.~~109. The method according to any of claims 8397 to ~~122~~ 108 wherein the closed container comprises a single bioreactor.

~~124.~~110. The method according to any one of claims 8397 to ~~123~~109, wherein at step (~~ee~~) the antigen presenting cells (APCs) are added to the cell culture of the third ~~second~~ population of TILs at a APC:TIL ratio of 25:1 to 100:1.

~~125.~~111. The method according to claim ~~124~~110, wherein the cell culture has ratio of 2.5×10^9 APCs to 100×10^6 TILs.

~~126.~~112. The method according to any one of claims 8397-~~125~~111, 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.

~~127. The method according to claim 126, 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.~~

~~128. The method according to claim 126, 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.~~

~~129. The method according to any of claims 97-128, wherein the gene-editing is carried out on TILs from one or more of the first population, the second population, and the third population.~~

~~130. The method according to any of claims 97-128, wherein the gene-editing is carried out on TILs from the first expansion, or TILs from the second expansion, or both.~~

~~131. The method according to any of claims 97-128, wherein the gene-editing is carried out after the first expansion and before the second expansion.~~

~~132. The method according to any of claims 97-128, wherein the gene-editing is carried out before step (b), before step (c), or before step (d).~~

~~133.~~113. The method according to any of claims 8397-~~132~~112, 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~~.

~~134. The method according to any of claims 97-132, 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.~~

~~135.~~114. The method according to any of claims 8397-~~132~~112, 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~~.

~~136.~~115. The method according to any of claims 8397-~~135~~114, 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.

~~137.~~116. The method according to claim ~~136~~115, 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.

~~138.~~117. The method according to claim ~~136~~115, 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.

~~139.~~118. The method according to any of claims 8397-~~135~~114, 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.

~~140.~~119. The method according to any of claims 8397-~~139~~118, 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.

~~141.~~120. The method according to any of claims 8397-~~139~~118, 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.

~~142.~~121. The method according to any of claims 8397-~~139~~118, wherein the gene-editing comprises a CRISPR method.

~~143.~~122. The method according to claim ~~142~~121, wherein the CRISPR method is a CRISPR/Cas9 method.

~~144.~~123. The method according to any of claims 8~~397~~-~~139~~118, wherein the gene-editing comprises a TALE method.

~~145.~~124. The method according to any of claims 8~~397~~-~~139~~118, wherein the gene-editing comprises a zinc finger method.

~~146. Use of a population of expanded TILs in the manufacture of a medicament for treating 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 3-12 days to obtain the second population of TILs, 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 7-12 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) 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 steps of (a)-(d), gene-editing at least a portion of the TILs.

147. The use according to claim 146, 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.

148. The use according to claim 146, wherein the gene-editing is carried out on TILs from one or more of the first population, the second population, and the third population.

149. The use according to claim 146, wherein the gene-editing is carried out on TILs from the first expansion, or TILs from the second expansion, or both.

150. The use according to claim 146, wherein the gene-editing is carried out after the first expansion and before the second expansion.

151. The use according to claim 146, wherein the gene-editing is carried out before step (c), before step (d), or before step (e)

152. The use according to claim 146, 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.

153. The use according to claim 152, 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.

154. The use according to claim 152, 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.

155. The useaccording to claim 146, 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.~~

~~156. The use according to claim 146, 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.~~

~~157. The use according to claim 146, 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.~~

~~158.~~125. A cryopreservation composition comprising the population of expanded TILs ~~for use in the treatment of a subject with cancer according to~~as defined in claim ~~146~~53, a cryoprotectant medium comprising DMSO, and an electrolyte solution.

~~159.~~126. The cryopreservation composition of claim ~~158~~ 125 further comprising one or more stabilizers and one or more lymphocyte growth factors.

~~160.~~127. The cryopreservation composition of claim ~~159~~126, wherein the one or more stabilizers comprise Human Serum Albumin (HSA) and the one or more lymphocyte growth factors comprise IL-2.

~~161.~~128. The cryopreservation composition of claim ~~160~~127, wherein the cryoprotectant medium comprising DMSO and the electrolyte solution are present in a ratio of 1.1:1 to 1:1.1.

~~162.~~129. The cryopreservation composition of claim ~~160~~127, comprising the population of TILs in an amount of 1 X 10^6 to 9 X 10^{14}, the cryoprotectant medium comprising DMSO in an amount of 30 mL to 70 mL, the electrolyte solution in an amount of 30 mL to 70 mL, HSA in an amount of 0.1 g to 1.0 g, and IL-2 in an amount of 0.001 mg to 0.005 mg.

~~163.~~130. 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 2 to 5 days to produce a second population of TILs;

~~(d) optionally adding OKT-3, to produce a second population of TILs, wherein the expansion is performed in a closed container providing a first gas-permeable surface area, wherein the expansion is performed for 1 to 3 days to obtain the second population of TILs, wherein the transition from step (c) to step (d) occurs without opening the system;~~

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

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

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

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

(~~j~~i) 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.

~~164.~~131. The method according to claim ~~163~~ 130 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.

~~165.~~132. The method according to claim ~~163~~ 130 or claim ~~164~~131, 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.

~~166.~~133. The method according to claim ~~163~~ 130 or claim ~~164~~131, 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.

~~167.~~134. The method according to claim ~~163~~ 130 or claim ~~164~~131, 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.

~~168.~~135. The method according to claim ~~163~~ 130 or claim ~~164~~131, 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.

~~169.~~136. The method according to claim ~~163~~ 130 or claim ~~164~~131, 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.

~~170.~~137. The method according to claim ~~163~~ 130 or claim ~~164~~131, 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.

~~171.~~138. The method according to any of claims ~~163~~ 130 to ~~170~~137, wherein the electroporation step comprises the delivery of a Clustered Regularly Interspersed Short Palindromic Repeat (CRISPR) system.

~~172.~~139. The method according to any of claims ~~163~~130 to ~~170~~137, wherein the electroporation step comprises the delivery of a Transcription Activator-Like Effector (TALE) system.

~~173.~~140. The method according to any of claims ~~163~~130 to ~~170~~137, wherein the electroporation step comprises the delivery of a zinc finger system.

~~174.~~141. The method according to any of claims ~~163~~ 130 to ~~173~~140, wherein the dimethylsulfoxide-based cryopreservation medium comprises DMSO, and an electrolyte solution~~, optionally HSA, and optionally IL-2~~.

~~175.~~142. 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 2 to 5 days;

(d) ~~optionally~~ adding OKT-3, to produce a second population of TILs, wherein the expansion is performed in a closed container providing a first gas-permeable surface area, wherein the expansion is performed for 1 to 3 days to obtain the second population of TILs, 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 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.

~~176.~~143. The use according to claim ~~175~~142, 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.

~~177.~~144. The use according to claim ~~175~~142 or claim ~~176~~143, 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.

~~178.~~145. The use according to claim ~~175~~142 or claim ~~176~~143, 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.

~~179.~~146. The use according to claim ~~175~~142 or claim ~~176~~143, 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.

~~180.~~147. The use according to claim ~~175~~142 or claim ~~176~~143, 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.

~~181.~~148. The use according to claim ~~175~~142 or claim ~~176~~143, 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.

~~182.~~149. The use according to claim ~~175~~142 or claim ~~176~~143, 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.

~~183.~~150. The use according to any of claims ~~175~~142 to ~~182~~149, wherein the electroporation step comprises the delivery of a Clustered Regularly Interspersed Short Palindromic Repeat (CRISPR) system.

~~184.~~151. The use according to any of claims ~~175~~142 to ~~182~~149, wherein the electroporation step comprises the delivery of a Transcription Activator-Like Effector (TALE) system.

~~185.~~152. The use according to any of claims ~~175~~142 to ~~182~~149, wherein the electroporation step comprises the delivery of a zinc finger system.

~~186.~~153. The use according to any of claims ~~175~~ 142 to ~~182~~149, wherein the dimethylsulfoxide-based cryopreservation medium comprises DMSO, and an electrolyte solution~~, optionally HSA, and optionally IL-2~~.

187. Use of a population of expanded TILs in the manufacture of a medicament for treating 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 2 to 5 days;

(d) optionally adding OKT-3, to produce a second population of TILs, wherein the expansion is performed in a closed container providing a first gas-permeable surface area, wherein the expansion is performed for 1 to 3 days to obtain the second population of TILs, 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 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.

188. The use according to claim 187 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.

189. The useaccording to claim 187or claim 188, 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.

190. The useaccording to claim 187or claim 188, 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.

191. The useaccording to claim 187or claim 188, 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.

192. The useaccording to claim 187or claim 188, 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.

193. The useaccording to claim 187or claim 188, 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.~~

~~194. The useaccording to claim 187or claim 188, 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.~~

~~195. The useaccording to claim 187to 194, wherein the electroporation step comprises the delivery of a Clustered Regularly Interspersed Short Palindromic Repeat (CRISPR) system.~~

~~196. The useaccording to claim 187to 194, wherein the electroporation step comprises the delivery of a Transcription Activator-Like Effector (TALE) system.~~

~~197. The useaccording to claim 187to 194, wherein the electroporation step comprises the delivery of a zinc finger system.~~

~~198. The use according to claim 187to 197, wherein the dimethylsulfoxide-based cryopreservation medium comprises DMSO, an electrolyte solution, optionally HSA, and optionally IL-2.~~

~~199. The useaccording to claim 187to 197, wherein the electroporation step comprises a pulsed electroporation step.~~

~~200~~154. The method according to any of claims 1-~~53~~46, wherein the gene-editing comprises a CRISPR method, and the CRISPR method includes use of a high-fidelity Cas9.