information in developing therapeutic TIL populations has yet to be fully realized. There is an urgent need to provide TIL manufacturing processes and therapies based on such processes that are appropriate for commercial scale manufacturing and regulatory approval for use in human patients at multiple clinical centers. The present invention meets this need by providing methods for preselecting TILs based on PD-1 expression in order to obtain TILs with enhanced tumor-specific killing capacity (*e.g.*, enhanced cytotoxicity).

SUMMARY

[0004] The present disclosure provides methods for expanding TILs and producing therapeutic populations of TILs, which includes a PD-1 status preselection step.

[0005] In one aspect, the present disclosure refers to a method for expanding tumor infiltrating lymphocytes (TILs) into a therapeutic population of TILs comprising: (a) obtaining and/or receiving a first population of TILs from a tumor resected from a subject by processing a tumor sample obtained from the subject into multiple tumor fragments; (b) selecting PD-1 positive TILs from the first population of TILs in step (a) to obtain a PD-1 enriched TIL population; (c) performing a priming first expansion by culturing the PD-1 enriched TIL population in a cell culture medium comprising IL-2, OKT-3, and antigen presenting cells (APCs) to produce a second population of TILs, wherein the priming first expansion is performed in a container comprising a first gas-permeable surface area, wherein the priming first expansion is performed for first period of ~~about~~ 1 to 7/8 days to obtain the second population of TILs, wherein the second population of TILs is greater in number than the first population of TILs; (d) performing a rapid second expansion by supplementing the cell culture medium of the second population of TILs with additional IL-2, OKT-3, and APCs, to produce a third population of TILs, wherein the number of APCs added in the rapid second expansion is at least twice the number of APCs added in step (c), wherein the rapid second expansion is performed for a second period of ~~about~~ 1 to 11 days to obtain the third population of TILs, wherein the third population of TILs is a therapeutic population of TILs, wherein the rapid second expansion is performed in a container comprising a second gas-permeable surface area; (e) harvesting the therapeutic population of TILs obtained from step (d); and (f) transferring the harvested TIL population from step (e) to an infusion bag. In some examples, the present disclosure provides a method for expanding tumor infiltrating lymphocytes (TILs) into a therapeutic population of TILs comprising:

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

(b) selecting PD-1 positive TILs from the first population of TILs in (a) to obtain a PD-1 enriched TIL population;

(c) performing a priming first expansion by culturing the PD-1 enriched TIL population in a cell culture medium comprising IL-2, OKT-3, and antigen presenting cells (APCs) to produce a second population of TILs, wherein the priming first expansion is performed in a container comprising a first gas-permeable surface area, wherein the priming first expansion is performed for first period of about 1 to 7/8 days to obtain the second population of TILs, wherein the second population of TILs is greater in number than the first population of TILs;

(d) performing a rapid second expansion by supplementing the cell culture medium of the second population of TILs with additional IL-2, OKT-3, and APCs, to produce a third population of TILs, wherein the number of APCs added in the rapid second expansion is at least twice the number of APCs added in step (b), wherein the rapid second expansion is performed for a second period of about 1 to 11 days to obtain the third population of TILs, wherein the third population of TILs is a therapeutic population of TILs, wherein the rapid second expansion is performed in a container comprising a second gas-permeable surface area;

(e) harvesting the therapeutic population of TILs obtained from step (d); and

(f) transferring the harvested TIL population from step (e) to an infusion bag.

[0006] 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 and/or receiving a first population of TILs from a tumor resected from a subject by processing a tumor sample obtained from the subject into multiple tumor fragments; b) selecting PD-1 positive TILs from the first population of TILs in step (a) to obtain a PD-1 enriched TIL population; c) performing a priming first expansion by culturing the PD-1 enriched TIL population in a cell culture medium comprising IL-2, OKT-3, and optionally comprising antigen presenting cells (APCs), to produce a second population of TILs, wherein the priming first expansion is performed for a first period of ~~about~~ 1 to 7/8 days to obtain the second population of TILs, wherein the second population of TILs is greater in number than the first population of TILs; d) performing a rapid second expansion by contacting the second population of TILs with a cell culture medium comprising IL-2, OKT-3, and APCs, to produce a third population of TILs, wherein the rapid second expansion is performed for a second period of ~~about~~ 1 to 11 days to obtain the third population of TILs, wherein the third population of TILs is a therapeutic population of TILs; and e) harvesting the therapeutic population of TILs

[0010] In some examples, the PD-1 positive TILs are PD-1high TILS.

[0011] In another aspect, the present disclosure provides a method for expanding tumor infiltrating lymphocytes (TILs) into a therapeutic population of TILs comprising:

(a) performing a priming first expansion by culturing a first population of TILs which have been selected to be PD-1 positive, said first population of TILs obtainable by processing a tumor sample from a subject by tumor digestion and selecting for the PD-1 positive TILs, in a cell culture medium comprising IL-2, OKT-3, and antigen presenting cells (APCs) to produce a second population of TILs, wherein the priming first expansion is performed in a container comprising a first gas-permeable surface area, wherein the priming first expansion is performed for first period of ~~about~~ 1 to 7/8 days to obtain the second population of TILs, wherein the second population of TILs is greater in number than the first population of TILs;

(b) performing a rapid second expansion by contacting the second population of TILs to a cell culture medium of the second population of TILs with additional IL-2, OKT-3, and APCs, to produce a third population of TILs, wherein the number of APCs in the rapid second expansion is at least twice the number of APCs in step (a), wherein the rapid second expansion is performed for a second period of ~~about~~ 1 to 11 days to obtain the third population of TILs, wherein the third population of TILs is a therapeutic population of TILs, wherein the rapid second expansion is performed in a container comprising a second gas-permeable surface area; and

(c) harvesting the therapeutic population of TILs obtained from step (b).

[0012] In another aspect, the present disclosure provides a method for expanding tumor infiltrating lymphocytes (TILs) into a therapeutic population of TILs comprising:

(a) performing a priming first expansion of TILs which have been selected to be PD-1 positive by culturing a first population of TILs in a cell culture medium comprising IL-2, OKT-3, and optionally comprising antigen presenting cells (APCs), to produce a second population of TILs, wherein the priming first expansion is performed for a first period of ~~about~~ 1 to 7/8 days to obtain the second population of TILs, wherein the second population of TILs is greater in number than the first population of TILs;

(b) performing a rapid second expansion by contacting the second population of TILs with a cell culture medium comprising IL-2, OKT-3, and APCs, to produce a third population of TILs, wherein the rapid second expansion is performed for a second period of ~~about~~ 1 to

[00144] In some examples, the effector T cells and/or central memory T cells obtained from the third population of TILs step (d) exhibit increased CD8 and CD28 expression relative to effector T cells and/or central memory T cells obtained from the second population of cells step (c).

[00145] In some examples, the TILs from step (f) are infused into a patient.

[00146] In another ~~aspect~~example, the present disclosure refers to the use of expanded tumor infiltrating lymphocytes (TILs) in the manufacture of a medicament for treating a subject with cancer, wherein the expanded TILs are to be administered to the subject, wherein the use comprising: (a) obtaining and/or receiving a first population of TILs from a tumor resected from a subject by processing a tumor sample obtained from the subject into multiple tumor fragments; (b) selecting PD-1 positive TILs from the first population of TILs in step (a) to obtain a PD-1 enriched TIL population; (c) performing a priming first expansion by culturing the PD-1 enriched TIL population in a cell culture medium comprising IL-2, OKT-3, and antigen presenting cells (APCs) to produce a second population of TILs, wherein the priming first expansion is performed in a container comprising a first gas-permeable surface area, wherein the priming first expansion is performed for about 1 to 7 days to obtain the second population of TILs; (d) performing a rapid second expansion by supplementing the cell culture medium of the second population of TILs with additional IL-2, OKT-3, and APCs, to produce a third population of TILs, wherein the number of APCs added to the rapid second expansion is at least twice the number of APCs added in step (c), wherein the rapid second expansion is performed for about 1 to 11 days to obtain the third population of TILs, wherein the third population of TILs is a therapeutic population of TILs, wherein the rapid second expansion is performed in a container comprising a second gas-permeable surface area; (e) harvesting the therapeutic population of TILs obtained from step (d); (f) transferring the harvested TIL population from step (e) to an infusion bag; and (g) a therapeutically effective dosage of the TILs from step (f) is to be administered to the subject. In some examples, the present disclosure provides a method for treating a subject with cancer, the method comprising administering expanded tumor infiltrating lymphocytes (TILs) comprising:

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

(b) selecting PD-1 positive TILs from the first population of TILs in (a) to obtain a PD-1 enriched TIL population;

(c) performing a priming first expansion by culturing the PD-1 enriched TIL population in a cell culture medium comprising IL-2, OKT-3, and antigen presenting cells (APCs) to produce a second population of TILs, wherein the priming first expansion is performed in a container comprising a first gas-permeable surface area, wherein the priming first expansion is performed for about 1 to 7 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;

(d) performing a rapid second expansion by supplementing the cell culture medium of the second population of TILs with additional IL-2, OKT-3, and APCs, to produce a third population of TILs, wherein the number of APCs added to the rapid second expansion is at least twice the number of APCs added in step (b), wherein the rapid second expansion is performed for about 1 to 11 days to obtain the third population of TILs, wherein the third population of TILs is a therapeutic population of TILs, wherein the rapid second expansion is performed in a container comprising a second gas-permeable surface area;

[00195] In some examples, selecting PD-1 positive TILs from the first population of TILs to obtain a PD-1 enriched TIL population comprises the selecting a population of TILs from a first population of TILs that are at least 11.27% to 74.4% PD-1 positive TILs.

[00196] In some examples, the selection of step comprises the steps of:

(i) exposing the first population of TILs and a population of PBMC to an excess of a monoclonal anti-PD-1 IgG4 antibody that binds to PD-1 through an N-terminal loop outside the IgV domain of PD-1,

(ii) adding an excess of an anti-IgG4 antibody conjugated to a fluorophore,

(iii) obtaining the PD-1 enriched TIL population based on the intensity of the fluorophore of the PD-1 positive TILs in the first population of TILs compared to the intensity in the population of PBMCs as performed by fluorescence-activated cell sorting (FACS).

[00197] In some examples, the intensity of the fluorophore in both the first population and the population of PBMCs is used to set up FACS gates for establishing low, medium, and high levels of intensity that correspond to PD-1 negative TILs, PD-1 intermediate TILs, and PD-1 positive TILs, respectively.

[00198] In some examples, the FACS gates are set-up after step (a).

[00199] In some examples, the PD-1 positive TILs are PD-1high TILs.

[00200] In some examples, at least 80% of the PD-1 enriched TIL population are PD-1 positive TILs.

[00201] In another aspect, the present disclosure refers to method for expanding tumor infiltrating lymphocytes (TILs) into a therapeutic population of TILs comprising: (a) obtaining and/or receiving a first population of TILs from a tumor resected from a subject by processing a tumor sample obtained from the subject into multiple tumor fragments; (b) selecting PD-1 positive TILs from the first population of TILs in step (a) to obtain a PD-1 enriched TIL population, wherein at least a range of 10% to 80% of the first population of TILs are PD-1 positive TILs; (c) performing a priming first expansion by culturing the PD-1 enriched TIL population in a cell culture medium comprising IL-2, OKT-3, and antigen presenting cells (APCs) to produce a second population of TILs, wherein the priming first expansion is performed in a container comprising a first gas-permeable surface area, wherein the priming first expansion is performed for first period of ~~about~~ 1 to 7/8 days to obtain the second population of TILs, wherein the second population of TILs is greater in number than the first population of TILs; (d) performing a rapid second expansion by supplementing the cell culture medium of the second population of TILs with additional IL-2, OKT-3, and APCs, to produce a third population of TILs, wherein the number of APCs added in the rapid second expansion is at least twice the number of APCs added in step (b), wherein the rapid second expansion is performed for a second period of ~~about~~ 1 to 11 days to obtain the third population of TILs, wherein the third population of TILs is a therapeutic population of TILs, wherein the rapid

WHAT IS CLAIMED IS:

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

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

(b) selecting PD-1 positive TILs from the first population of TILs in step (a) to obtain a PD-1 enriched TIL population;

(c) performing a priming first expansion by culturing the PD-1 enriched TIL population in a cell culture medium comprising IL-2, OKT-3, and antigen presenting cells (APCs) to produce a second population of TILs, wherein the priming first expansion is performed in a container comprising a first gas-permeable surface area, wherein the priming first expansion is performed for first period of ~~about~~ 1 to 7/8 days to obtain the second population of TILs, wherein the second population of TILs is greater in number than the first population of TILs;

(d) performing a rapid second expansion by supplementing the cell culture medium of the second population of TILs with additional IL-2, OKT-3, and APCs, to produce a third population of TILs, wherein the number of APCs added in the rapid second expansion is at least twice the number of APCs added in step (c), wherein the rapid second expansion is performed for a second period of ~~about~~ 1 to 11 days to obtain the third population of TILs, wherein the third population of TILs is a therapeutic population of TILs, wherein the rapid second expansion is performed in a container comprising a second gas-permeable surface area;

(e) harvesting the therapeutic population of TILs obtained from step (d); and

(f) transferring the harvested TIL population from step (e) to an infusion bag.

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

a) obtaining and/or receiving a first population of TILs from a tumor resected from a subject by processing a tumor sample obtained from the subject into multiple tumor fragments;

b) selecting PD-1 positive TILs from the first population of TILs in step (a) to obtain a PD-1 enriched TIL population;

c) performing a priming first expansion by culturing the PD-1 enriched TIL population in a cell culture medium comprising IL-2, OKT-3, and optionally comprising antigen presenting cells (APCs), to produce a second population of TILs, wherein the priming first expansion is performed for a first period of ~~about~~ 1 to 7/8 days to obtain the second population of TILs, wherein the second population of TILs is greater in number than the first population of TILs;

d) performing a rapid second expansion by contacting the second population of TILs with a cell culture medium comprising IL-2, OKT-3, and APCs, to produce a third population of TILs, wherein the rapid second expansion is performed for a second period of ~~about~~ 1 to 11 days to obtain the third population of TILs, wherein the third population of TILs is a therapeutic population of TILs; and

e) harvesting the therapeutic population of TILs obtained from step (d).

3. The method of claim 2, wherein in step (c) the cell culture medium further comprises antigen-presenting cells (APCs), and wherein the number of APCs in the culture medium in step (d) is greater than the number of APCs in the culture medium in step (c) or wherein in step (c) the cell culture medium further comprises antigen-presenting cells (APCs), and wherein the number of APCs in the culture medium in step (d) is equal to the number of APCs in the culture medium in step (c), wherein optionally said PD-1 positive TILs are PD-l high TILs.

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

(a) performing a priming first expansion by culturing a first population of TILs which have been selected to be PD-1 positive, said first population of TILs obtainable by processing a tumor sample from a subject by tumor digestion and selecting for the PD-1 positive TILs, in a cell culture medium comprising IL-2, OKT-3, and antigen presenting cells (APCs) to produce a second population of TILs, wherein the priming first expansion is performed in a container comprising a first gas-permeable surface area, wherein the priming first expansion is performed for first period of ~~about~~ 1 to 7/8 days to obtain the second population of TILs, wherein the second population of TILs is greater in number than the first population of TILs;

(b) performing a rapid second expansion by contacting the second population of TILs to a cell culture medium of the second population of TILs with additional IL-2, OKT-3, and APCs, to produce a third population of TILs, wherein the number of APCs in the rapid second expansion is at least twice the number of APCs in step (a), wherein the rapid second expansion is performed for a second period of ~~about~~ 1 to 11 days to obtain the third population of TILs, wherein the third population of TILs is a therapeutic population of TILs, wherein the rapid second expansion is performed in a container comprising a second gas-permeable surface area; and

(c) harvesting the therapeutic population of TILs obtained from step (b).

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

(a) performing a priming first expansion of TILs which have been selected to be PD-1 positive by culturing a first population of TILs in a cell culture medium comprising IL-2, OKT-3, and optionally comprising antigen presenting cells (APCs), to produce a second population of TILs, wherein the priming first expansion is performed for a first period of ~~about~~ 1 to 7/8 days to obtain the second population of TILs, wherein the second population of TILs is greater in number than the first population of TILs;

(b) performing a rapid second expansion by contacting the second population of TILs with a cell culture medium comprising IL-2, OKT-3, and APCs, to produce a third population of TILs, wherein the rapid second expansion is performed for a second period of ~~about~~ 1 to 11 days to obtain the third population of TILs, wherein the third population of TILs is a therapeutic population of TILs; and

(c) harvesting the therapeutic population of TILs obtained from step (b).

6. The method of claim 4, wherein in step (a) the cell culture medium further comprises antigen-presenting cells (APCs), and wherein the number of APCs in the culture medium in step (b) is greater than the number of APCs in the culture medium in step (a) or wherein in step (a) the cell culture medium further comprises antigen-presenting cells (APCs), and wherein the number of APCs in the culture medium in step (b) is the equal to the number of APCs in the culture medium in step (a), wherein optionally said PD-1 positive TILs are PD-l high TILs.

7. The method of claim 1 or 2 or 4 or 5, wherein the selection ofPD-1 positive TILs comprises the steps of (i) exposing the first population of TILs to an excess of a monoclonal anti-PD-1 IgG4 antibody that binds to PD-1 through an N-terminal loop outside the IgV domain of PD-1, (ii) adding an excess of an anti-IgG4 antibody conjugated to a fluorophore, and (iii) performing a flow-based cell sort based on the fluorophore to obtain a PD-1 enriched TIL population, wherein optionally the monoclonal anti-PD-1 IgG4 antibody is nivolumab or variants, fragments, or conjugates thereof, optionally wherein the anti-IgG4 antibody is clone anti-human IgG4, Clone HP6023.

8. The method of claim 1 or 2 or 4 or 5, wherein the ratio of the number of APCs in the rapid second expansion to the number of APCs in the priming first expansion is selected from a range of from ~~about~~ 1.5:1 to ~~about~~ 20:1, from ~~about~~ 1.5:1 to ~~about~~ 10:1, from ~~about~~ 2: 1 to ~~about~~ 5:1 or from ~~about~~ 2: 1 to ~~about~~ 3:1, wherein optionally wherein the ratio is ~~about~~ 2:1.

9. The method of claim 1 or 2 or 4 or 5, wherein the number of APCs in the priming first expansion is selected from the range of ~~about~~ $1x10^8$ APCs to ~~about~~ $3.5x10^8$ APCs, and wherein the number of APCs in the rapid second expansion is selected from the range of ~~about~~ $3.5x10^8$ APCs to ~~about~~ $1x10^9$ APCs

   , or wherein the number of APCs in the priming first expansion is selected from the range of ~~about~~ $1.5x10^8$ APCs to ~~about~~ $3x10^8$ APCs, and wherein the number of APCs in the rapid second expansion is selected from the range of ~~about~~ $4x10^8$ APCs to ~~about~~ $7.5x10^8$ APCs or wherein the number of APCs in the priming first expansion is selected from the range of ~~about~~ $2x10^8$APCs to ~~about~~ $2.5x10^8$ APCs, and wherein the number of APCs in the rapid second expansion is selected from the range of ~~about~~ $4.5x10^8$APCs to ~~about~~ $5.5x10^8$APCs, wherein optionally ~~about~~ $2.5x10^8$ APCs are added to the priming first expansion and $5x10^8$APCs are added to the rapid second expansion.

10. The method of any of claims 1-9, wherein the ratio of the number of TILs in the second population of TILs to the number of TILs in the first population of TILs is ~~about~~ 1.5:1 to ~~about~~ 100:1, wherein optionally the ratio of the number of TILs in the second population of TILs to the number of TILs in the first population of TILs is ~~about~~ 50:1, ~~about~~ 25:1, ~~about~~ 20:1 or ~~about~~ 10:1, optionally wherein the second population of TILs is at least 50- fold greater in number than the first population of TILs.

11. The method of any of claims 2-10, wherein the method comprises performing, after the step of harvesting the therapeutic population of TILs, the additional step of:

transferring the harvested therapeutic population of TILs to an infusion bag.

12. The method of any of claims 1-10, wherein the multiple tumor fragments are distributed into a plurality of separate containers, in each of which separate containers the second population of TILs is obtained from the first population of TILs in the step of the priming first expansion, and the third population of TILs is obtained from the second population of TILs in the step of the rapid second expansion, and wherein the therapeutic population of TILs obtained from the third population of TILs is collected from each of the plurality of containers and combined to yield the harvested TIL population, wherein optionally the plurality of separate containers comprises at least two separate containers or wherein the plurality of separate containers comprises from two to twenty separate containers, from two to ten separate containers or from two to five separate containers, wherein optionally each of the separate containers comprises a first gas-permeable surface area.

13. The method of any of claims 1-11, wherein the multiple tumor fragments are distributed in a single container, wherein optionally wherein the single container comprises a first gas-permeable surface area and/or wherein in the step of the priming first expansion the APCs are layered onto the first gas-permeable surface area at an average thickness of ~~about~~ 1.5 cell layers to ~~about~~ 2.5 cell layers.

14. The method of claim 12 or 13, wherein in the step of the priming first expansion the cell culture medium comprises antigen-presenting cells (APCs) and the APCs are layered onto the first gas-permeable surface area at an average thickness of ~~about~~ one cell layer to ~~about~~ three cell layers, wherein optionally in the step of the priming first expansion the APCs are layered onto the first gas-permeable surface area at an average thickness of ~~about~~ 2 cell layers.

15. The method of claim13 or 14, wherein in the step of the rapid second expansion the APCs are layered onto the first gas-permeable surface area at a thickness of ~~about~~ 3 cell layers to ~~about~~ 5 cell layers, wherein optionally in the step of the rapid second expansion the APCs are layered onto the first gas-permeable surface area at a thickness of ~~about~~ 3.5 cell layers to ~~about~~ 4.5 cell layers, optionally wherein in the step of the rapid second

expansion the APCs are layered onto the first gas-permeable surface area at a thickness of ~~about~~ 4 cell layers.

16. The method of any of claims 2-11, wherein in the step of the priming first expansion the priming first expansion is performed in a first container comprising a first gas-permeable surface area and in the step of the rapid second expansion the rapid second expansion is performed in a second container comprising a second gas-permeable surface area, wherein optionally the second container is larger than the first container.

17. The method of claim 15 or 16, wherein in the step of the priming first expansion the cell culture medium comprises antigen-presenting cells (APCs) and the APCs are layered onto the first gas-permeable surface area at an average thickness of ~~about~~ one cell layer to ~~about~~ three cell layers, wherein optionally in the step of the priming first expansion the APCs are layered onto the first gas-permeable surface area at an average thickness of ~~about~~ 1.5 cell layers to ~~about~~ 2.5 cell layers, optionally wherein in the step of the priming first expansion the APCs are layered onto the first gas-permeable surface area at an average thickness of ~~about~~ 2 cell layers.

18. The method of claim 16 or 17, wherein in the step of the rapid second expansion the APCs are layered onto the second gas-permeable surface area at an average thickness of ~~about~~ 3 cell layers to ~~about~~ 5 cell layers, ~~about~~ 3.5 cell layers to ~~about~~ 4.5 cell layers, or ~~about~~ 4 cell layers.

19. The method of any of claim 2-16, wherein for each container in which the priming first expansion is performed on a first population of TILs the rapid second expansion is performed in the same container on the second population of TILs produced from such first population of TILs, wherein optionally each container comprises a first gas-permeable surface area, optionally wherein

in the step of the priming first expansion the cell culture medium comprises antigen-presenting cells (APCs) and the APCs are layered onto the first gas-permeable surface area at an average thickness of from ~~about~~ one cell layer to ~~about~~ three cell layers, from ~~about~~ 1.5 cell layers to ~~about~~ 2.5 cell layers or ~~about~~ 2 cell layers.

20. The method of claim18 or 19, wherein in the step of the rapid second expansion the APCs are layered onto the first gas-permeable surface area at an average thickness of ~~about~~ 3 cell layers to ~~about~~ 5 cell layers, ~~about~~ 3.5 cell layers to ~~about~~ 4.5 cell layers or ~~about~~ 4 cell layers.

21. The method of any of claims 2-12, 15, 16 and 19, wherein for each container in which the priming first expansion is performed on a first population of TILs in the step of the priming first expansion the first container comprises a first surface area, the cell culture medium comprises antigen-presenting cells (APCs), and the APCs are layered onto the first gas-permeable surface area, and wherein the ratio of the average number of layers of APCs layered in the step of the priming first expansion to the average number of layers of APCs layered in the step of the rapid second expansion is selected from the range of ~~about~~ 1:1.1 to ~~about~~ 1:10, ~~about~~ 1: 1.2 to ~~about~~ 1:8, ~~about~~ 1:1.3 to ~~about~~ 1:7, ~~about~~ 1:1.4 to ~~about~~ 1:6, ~~about~~ 1:1.5 to ~~about~~ 1:5, ~~about~~ 1:1.6 to ~~about~~ 1:4, ~~about~~ 1:1.7 to ~~about~~ 1:3.5, ~~about~~ 1:1.8 to ~~about~~ 1:3 or ~~about~~ 1:1.9 to ~~about~~ 1:2.5, wherein optionally the ratio of the average number of layers of APCs layered in the step of the priming first expansion to the average number of layers of APCs layered in the step of the rapid second expansion is ~~about~~ 1:2.

22. The method of any of the preceding claims, wherein after 2 to 3 days in the step of the rapid second expansion, the cell culture medium is supplemented with additional IL-2.

23. The method according to any of the preceding claims, further comprising cryopreserving the harvested TIL population in the step of harvesting the therapeutic population of TILs using a cryopreservation process.

24. The method according to claim 1 or11, further comprising the step of cryopreserving the infusion bag, wherein optionally the cryopreservation process isperformed using a 1:1 ratio of harvested TIL population to cryopreservation media.

25. The method according to any of the preceding claims, wherein the antigen-presenting cells are peripheral blood mononuclear cells (PBMCs), wherein optionally the PBMCs are irradiated and allogeneic, optionally wherein the antigen-presenting cells are artificial antigen-presenting cells.

26. The method according to any of the preceding claims, wherein in the step of the priming first expansion the cell culture medium comprises peripheral blood mononuclear cells (PBMCs), and wherein the total number of PBMCs in the cell culture medium in the step of the priming first expansion is 2.5×10^8 and/or wherein in the step of the rapid second expansion the antigen-presenting cells (APCs) in the cell culture medium are peripheral blood mononuclear cells (PBMCs), and wherein the total number of

PBMCs added to the cell culture medium in the step of the rapid second expansion is 5×10^8.

27. The method according to any of the preceding claims, wherein the harvesting in the step of harvesting the therapeutic population of TILs is performed using a membrane-based cell processing system, wherein optionally the harvesting of the therapeutic population of TILs is performed using a LOVO cell processing system.

28. The method according to any of the preceding claims, wherein the multiple fragments comprise ~~about~~ 60 fragments per container in the step of the priming first expansion, wherein each fragment has a volume of ~~about~~ 27 mm^3 or wherein the multiple fragments comprise ~~about~~ 50 fragments with a total mass of ~~about~~ 1 gram to ~~about~~ 1.5 grams.

29. The method according to any of the preceding claims, 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, wherein optionally the multiple fragments comprise ~~about~~ 50 fragments with a total volume of ~~about~~ 1350 mm^3.

30. The method according to any of the preceding claims, wherein the cell culture medium is provided in a container selected from the group consisting of a G-container and a Xuri cellbag.

31. The method of claim to any of the preceding claims, wherein after 2 to 3 days in the second rapid expansion, the cell culture medium is supplemented with additional IL-2, wherein optionally the IL-2 concentration is ~~about~~ 10,000 IU/mL to ~~about~~ 5,000 IU/mL, optionally wherein the IL-2 concentration is ~~about~~ 6,000 IU/mL.

32. The method according to claim 1 or 11, wherein the infusion bag in the step of transferring the harvested therapeutic population of TILs to an infusion bag is a HypoThermosol-containing infusion bag.

33. The method according to claim23 or 24, wherein the cryopreservation media comprises dimethlysulfoxide (DMSO), wherein optionally the cryopreservation media comprises 7% to 10%DMSO.

34. The method according to any of the preceding claims, wherein the priming first expansion and the rapid second expansion are each individually performed within a period of 5 days, 6 days, 7 days, 8 days, 9 days, 10 days, or 11 days, wherein optionally the priming first expansion is performed within a period of 5 days, 6 days, or 7 days or the rapid

second expansion is performed within a period of 7 days, 8 days, or 9 days, optionally wherein the priming first expansion and the rapid second expansion are each individually performed within a period of 7 days.

35. The method according to any of claims 1-34, wherein steps of the priming first expansion through the harvesting of the therapeutic population of TILs are performed within a period of ~~about~~ 14 days to ~~about~~ 16 days or ~~about~~ 15 days to ~~about~~ 16 days, wherein optionally steps of the priming first expansion through the harvesting of the therapeutic population of TILs are performed within a period of ~~about~~ 14 days, ~~about~~ 15 days or ~~about~~ 16 days.

36. The method according to any of claims 1-34, further comprising the step of cryopreserving the harvested therapeutic population of TILs using a cryopreservation process, wherein steps of the priming first expansion through the harvesting of the therapeutic population of TILs and cryopreservation are performed in 16 days or less.

37. The method according to any one of claims 1 to 36, wherein the therapeutic population of TILs harvested in the step of harvesting of the therapeutic population of TILs comprises sufficient TILs for a therapeutically effective dosage of the TILs, wherein optionally the number of TILs sufficient for a therapeutically effective dosage is from ~~about~~ $2.3x10^{10}$ to ~~about~~ $13.7 x10^{10}$.

38. The method according to any of claims 1-4, further comprising the step of cryopreserving the infusion bag comprising the harvested TIL population using a cryopreservation process, wherein optionally the cryopreservation process is performed using a 1:1 ratio of harvested TIL population to cryopreservation media.

39. The method according to any of claims 1-4, wherein the antigen-presenting cells are peripheral blood mononuclear cells (PBMCs), wherein optionally the PBMCs are irradiated and allogeneic, optionally wherein the antigen-presenting cells are artificial antigen-presenting cells.

40. The method according to claim 1 or 2 or 4 or 5, wherein the harvesting of the therapeutic population of TILs is performed using a membrane-based cell processing system, wherein optionally the harvesting of the therapeutic population of TILs is performed using a LOVO cell processing system.

41. The method according to claim 1 or 2 or 4 or 5, wherein the multiple fragments comprise ~~about~~ 60 fragments per first gas-permeable surface area in step (c), wherein each

fragment has a volume of ~~about~~ 27 mm^3, wherein optionally the multiple fragments comprise ~~about~~ 30 to ~~about~~ 60 fragments with a total volume of ~~about~~ 1300 mm^3 to ~~about~~ 1500 mm^3, optionally wherein the multiple fragments comprise ~~about~~ 50 fragments with a total volume of ~~about~~ 1350 mm^3.

42. The method according to claim 1 or 2 or 4 or 5, wherein the multiple fragments comprise ~~about~~ 50 fragments with a total mass of ~~about~~ 1 gram to ~~about~~ 1.5 grams.

43. The method according to claim 1 or 2 or 4 or 5, wherein the cell culture medium is provided in a container selected from the group consisting of a G-container and a Xuri cellbag.

44. The method according to claim any of the preceding claims, wherein the IL-2 concentration is ~~about~~ 10,000 IU/mL to ~~about~~ 5,000 IU/mL, wherein optionally the IL-2 concentration is ~~about~~ 6,000 IU/mL.

45. The method according to claim 1, wherein the infusion bag in step (f) is a HypoThermosol-containing infusion bag, optionally wherein the method further comprises cryopreserving the infusion bag, wherein optionally the cryopreservation process is performed using a 1:1 ratio of harvested TIL population to cryopreservation media, optionally

, wherein the cryopreservation media comprises dimethlysulfoxide (DMSO), wherein optionally the cryopreservation media comprises 7% to 10% DMSO.

46. The method according to claim 1 or 2 or 4 or 5, wherein the priming first expansion and the rapid second expansion are each individually performed within a period of 5 days, 6 days, or 7 days, wherein optionally the priming first expansion is performed within a period of 5 days, 6 days, or 7 days or the rapid second expansion is performed within a period of 7 days, 8 days, or 9 days, optionally wherein the priming first expansion and the rapid second expansion are each individually performed within a period of 7 days.

47. The method according to claim 1 or 11, wherein the step of performing the first priming expansion through the step of transferring the harvested population of TILs are performed within a period of ~~about~~ 14 days to ~~about~~ 16 days, or ~~about~~ 15 days to ~~about~~ 16 days, wherein optionally the step of performing the first priming expansion through the step of transferring the harvested population of TILs are performed within a period of ~~about~~ 14 days, ~~about~~ 15 days or ~~about~~ 16 days, optionally wherein

the step of performing the first priming expansion through the step of transferring the harvested population of TILs and cryopreservation are performed in 16 days or less.

48. The method according to any one of claims 1 to 48, wherein the therapeutic population of TILs harvested comprises sufficient TILs for a therapeutically effective dosage of the TILs, optionally wherein the number of TILs sufficient for a therapeutically effective dosage is from ~~about~~ 2.3×10^{10} to ~~about~~ 13.7×10^{10}.

49. The method according to any one of claims 1 to 49, the container in the first priming expansion is larger than the container in the second rapid expansion.

50. The method according to any one of claims 1 to 50, wherein the effector T cells and/or central memory T cells obtained from the third population of TILs exhibit increased CD8 and CD28 expression relative to effector T cells and/or central memory T cells obtained from the second population of cells.

~~51. Use of expanded tumor infiltrating lymphocytes (TILs) in the manufacture of a medicament for treating a subject with cancer, wherein the expanded TILs are to be administered to the subject, wherein the usecomprising:~~

~~(a) obtaining and/or receiving a first population of TILs from a tumor resected from a subject by processing a tumor sample obtained from the subject into multiple tumor fragments;~~

~~(b) selecting PD-1 positive TILs from the first population of TILs in step (a) to obtain a PD-1 enriched TIL population;~~

~~(c) performing a priming first expansion by culturing the PD-1 enriched TIL population in a cell culture medium comprising IL-2, OKT-3, and antigen presenting cells (APCs) to produce a second population of TILs, wherein the priming first expansion is performed in a container comprising a first gas-permeable surface area, wherein the priming first expansion is performed for about 1 to 7 days to obtain the second population of TILs;~~

~~(d) performing a rapid second expansion by supplementing the cell culture medium of the second population of TILs with additional IL-2, OKT-3, and APCs, to produce a third population of TILs, wherein the number of APCs added to the rapid second expansion is at least twice the number of APCs added in step (c), wherein the rapid second expansion is performed for about 1 to 11 days to obtain the third population of TILs, wherein the third population of TILs is a therapeutic~~

population of TILs, wherein the rapid second expansion is performed in a container comprising a second gas permeable surface area;

(e) harvesting the therapeutic population of TILs obtained from step (d);

(f) transferring the harvested TIL population from step (e) to an infusion bag; and

(g) a therapeutically effective dosage of the TILs from step (f) is to be administered to the subject.

52. The use according to claim 51, wherein the number of TILs sufficient for administering a therapeutically effective dosage in step (g) is from about 2.3×10^{10} to about 13.7×10^{10}, wherein optionally said PD-1 positive TILs are PD-1 high TILs.

53. The use according to claim 51 or 52, wherein the selection of step (b) comprises the steps of (i) exposing the first population of TILs to an excess of a monoclonal anti-PD-1 IgG4 antibody that binds to PD-1 through an N-terminal loop outside the IgV domain of PD-1, (ii) adding an excess of an anti-IgG4 antibody conjugated to a fluorophore, and (iii) performing a flow-based cell sort based on the fluorophore to obtain a PD-1 enriched TIL population.

54. The use of claim 53, wherein the monoclonal anti-PD-1 IgG4 antibody is nivolumab or variants, fragments, or conjugates thereof or wherein the anti-IgG4 antibody is Clone HP6023, wherein optionally the antigen presenting cells (APCs) are PBMCs.

55. The use according to any of claims 51-54, wherein prior to administering a therapeutically effective dosage of TIL cells in step (g), a non-myeloablative lymphodepletion regimen has been administered to the patient, wherein optionally the non-myeloablative lymphodepletion regimen comprises the steps of administration of cyclophosphamide at a dose of 60 mg/m^2/day for two days followed by administration of fludarabine at a dose of 25 mg/m^2/day for five days.

56. The use according to any of claims 51-55, further comprising the step of treating the patient with a high-dose IL-2 regimen starting on the day after administration of the TIL cells to the patient in step (g), wherein optionally the high-dose IL-2 regimen comprises 600,000 or 720,000 IU/kg administered as a 15-minute bolus intravenous infusion every eight hours until tolerance.

57.51. The method according to any of claims 1-50, or the use according to any of claims 51-56, wherein the subject has cancer that 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)), glioblastoma (including GBM), gastrointestinal cancer, renal cancer, and renal cell carcinoma, wherein optionally the cancer is selected from the group consisting of melanoma, HNSCC, cervical cancers, NSCLC, glioblastoma (including GBM), and gastrointestinal cancer.

~~58.~~52. The method according to any of claims 1-~~50~~51, ~~or the use according to any of claims 51-57,~~ wherein the subject has cancer that is melanoma, HNSCC, cervical cancer, NSCLC, glioblastoma (including GBM) or gastrointestinal cancer, wherein optionally the cancer is hypermutated cancer, optionally wherein the cancer is a pediatric hypermutated cancer.

~~59.~~53. The method according to any of claims 1-5~~0~~2 ~~or 58~~, ~~or the use according to any of claims 51-58,~~ wherein the closed container is a GREX-10, a GREX-100 or a GREX-500.

~~60.~~54. The method according to any of claims 1-5~~0~~3 ~~or 58-59~~, ~~or the use according to any of claims 51-59,~~ wherein the subject has been previously treated with an anti-PD-1 antibody or wherein the subject has not been previously treated with an anti-PD-1 antibody.

~~61.~~55. The method according to any of claims 1-5~~0~~4 ~~or 58-60, or the use according to any of claims 51-60~~, wherein the PD-1 positive TILs are selected from the first population of TILs by performing the step of contacting the first population of TILs with an anti-PD-1 antibody to form a first complex of the anti-PD-1 antibody and TIL cells in the first population of TILs, and then performing the step of isolating the first complex to obtain the PD-1 enriched TIL population.

~~62.~~56. The method ~~or the use~~ according to claim ~~61~~55, wherein the anti-PD-1 antibody comprises an Fc region, wherein after the step of forming the first complexes and before the step of isolating the first complex the method further comprises the step of contacting the first complex with an anti-Fc antibody that binds to the Fc region of the anti-PD-1 antibody to form a second complex of the anti-Fc antibody and the first complex, and wherein the step of isolating the first complex is performed by isolating the second complex.

~~63.~~57. The method ~~or the use~~ according to claim ~~62~~56, wherein the anti-PD-1 antibody for use in the selection is selected from the group consisting of EH12.2H7, PD1.3.1, M1H4, nivolumab (BMS-936558, Bristol-Myers Squibb; Opdivo®), pembrolizumab

(lambrolizumab, MK03475 or MK-3475, Merck; Keytruda®), H12.1, PD1.3.1, NAT 105, humanized anti-PD-1 antibody JS001 (ShangHai JunShi), monoclonal anti-PD-1 antibody TSR-042 (Tesaro, Inc.), Pidilizumab (anti-PD-1 mAb CT-011, Medivation), anti-PD-1 monoclonal Antibody BGB-A317 (BeiGene), and/or anti-PD-1 antibody SHR-1210 (ShangHai HengRui), human monoclonal antibody REGN2810 (Regeneron), human monoclonal antibody MDX-1106 (Bristol-Myers Squibb), humanized anti-PD-1 IgG4 antibody PDR001 (Novartis), and RMP1-14 (rat IgG) - BioXcell cat# BP0146, wherein optionally the anti-PD-1 antibody for use in the selection of the PD-1 positive TILs is EH12.2H7 or nivolumab, optionally wherein the anti-PD-1 antibody for use in the selection of the PD-1 positive TILs binds to a different epitope than nivolumab or pembrolizumab or the anti-PD-1 antibody for use in the selection of the PD-1 positive TILs binds to the same epitope as EH12.2H7 or nivolumab.

64.58. The method according to any of claims 1-507 ~~or 58-63, or the use according to any of claims 51-63~~, wherein the subject has been previously treated with a first anti-PD1 antibody, wherein the PD-1 positive TILs are selected by contacting the first population of TILs with a second anti-PD-1 antibody, and wherein the second anti-PD-1 antibody is not blocked from binding to the first population of TILs by the first anti-PD-1 antibody insolubilized on the first population of TILs.

65.59. The method of claim 1-508 ~~or 58-64~~, ~~or the use according to any of claims 51-64,~~ wherein the subject has been previously treated with a first anti-PD1 antibody, wherein the PD-1 positive TILs are selected by contacting the first population of TILs with a second anti-PD-1 antibody to form a first complex of the second anti-PD-1 antibody and the first population of TILs and then performing the step of isolating the first complex to obtain the PD-1 enriched TIL population, and wherein the second anti-PD-1 antibody is blocked from binding to the first population of TILs by the first anti-PD-1 antibody insolubilized on the first population of TILs.

66.60. The method ~~or the use~~ according to ~~-~~ claim ~~65~~59, wherein the first anti-PD-1 antibody and the second anti-PD-1 antibody comprise an Fc region, wherein after the step of forming the first complex and before the step of isolating the first complex the method further comprises the step of contacting the first complex with an anti-Fc antibody that binds to the Fc region of the first anti-PD-1 antibody and the Fc region of the second anti-PD-1 antibody to form a second complex of the anti-Fc antibody and the first complex and contacting the first anti-PD-1 antibody insolubilized on the first population of TILs with

the anti-Fc antibody to form a third complex of the anti-Fc antibody and the first anti-PD-1 antibody insolubilized on the first population of TILs, and performing the step of isolating the second and third complexes to obtain the PD-1 enriched TIL population.

~~67.~~61. The method according to any of claims 1-~~56~~0 ~~or 58-66~~, ~~or the use according to any of claims 51-66,~~ wherein selecting PD-1 positive TILs from the first population of TILs to obtain a PD-1 enriched TIL population comprises the selecting a population of TILs from a first population of TILs that are at least 11.27% to 74.4% PD-1 positive TILs.

~~68.~~62. The method according to any of claims 50-61 ~~or 58-67~~, ~~or the use according to any of claims 51-66,~~ wherein the selection of step comprises the steps of:

(i) exposing the first population of TILs and a population of PBMC to an excess of a monoclonal anti-PD-1 IgG4 antibody that binds to PD-1 through an N-terminal loop outside the IgV domain of PD-1,

(ii) adding an excess of an anti-IgG4 antibody conjugated to a fluorophore, and

(iii) obtaining the PD-1 enriched TIL population based on the intensity of the fluorophore of the PD-1 positive TILs in the first population of TILs compared to the intensity in the population of PBMCs as performed by fluorescence-activated cell sorting (FACS).

~~69.~~63. The method ~~or the use~~ according to claim ~~68~~62, wherein the intensity of the fluorophore in both the first population and the population of PBMCs is used to set up FACS gates for establishing low, medium, and high levels of intensity that correspond to PD-1 negative TILs, PD-1 intermediate TILs, and PD-1 positive TILs, respectively, wherein optionally the FACS gates are set-up before the selection step and/or the PD-1 positive TILs are PD-1 high TILs, optionally wherein at least 80% of the PD-1 enriched TIL population are PD-1 positive TILs.

~~70.~~64. A method for expanding tumor infiltrating lymphocytes (TILs) into a therapeutic population of TILs comprising:

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

(b) selecting PD-1 positive TILs from the first population of TILs in step (a) to obtain a PD-1 enriched TIL population, wherein at least a range of 10% to 80% of the first population of TILs are PD-1 positive TILs;

(c) performing a priming first expansion by culturing the PD-1 enriched TIL population in a cell culture medium comprising IL-2, OKT-3, and antigen presenting cells (APCs) to produce a second population of TILs, wherein the priming first expansion is performed in a container comprising a first gas-permeable surface area, wherein the priming first expansion is performed for first period of ~~about~~ 1 to 7/8 days to obtain the second population of TILs, wherein the second population of TILs is greater in number than the first population of TILs;

(d) performing a rapid second expansion by supplementing the cell culture medium of the second population of TILs with additional IL-2, OKT-3, and APCs, to produce a third population of TILs, wherein the number of APCs added in the rapid second expansion is at least twice the number of APCs added in step (b), wherein the rapid second expansion is performed for a second period of ~~about~~ 1 to 11 days to obtain the third population of TILs, wherein the third population of TILs is a therapeutic population of TILs, wherein the rapid second expansion is performed in a container comprising a second gas-permeable surface area;

(e) harvesting the therapeutic population of TILs obtained from step (d); and

(f) transferring the harvested TIL population from step (e) to an infusion bag.

~~71.~~65. The method according to claim ~~70~~64, wherein the selection of step (b) comprises the steps of:

(i) exposing the first population of TILs and a population of PBMC to an excess of a monoclonal anti-PD-1 IgG4 antibody that binds to PD-1 through an N-terminal loop outside the IgV domain of PD-1,

(ii) adding an excess of an anti-IgG4 antibody conjugated to a fluorophore, and

(iii) obtaining the PD-1 enriched TIL population based on the intensity of the fluorophore of the PD-1 positive TILs in the first population of TILs compared to the intensity in the population of PBMCs as performed by fluorescence-activated cell sorting (FACS).

~~72.~~66. The method according to any one of claims ~~70~~ 64 to ~~71~~65, wherein the intensity of the fluorophore in both the first population and the population of PBMCs is used to set up FACS gates for establishing low, medium, and high levels of intensity that correspond to PD-1 negative TILs, PD-1 intermediate TILs, and PD-1 positive TILs, respectively,

wherein optionally the FACS gates are set-up after step (a) and/or the PD-1 positive TILs are PD-1 high TILs, optionally wherein at least 80% of the PD-1 enriched TIL population are PD-1 positive TILs.

~~73.~~67. The method according to any one of claims ~~70~~ 64 to ~~72~~66, wherein the third population of TILs comprises at least ~~about~~ 1 x 10^8 TILs in the container or wherein the third population of TILs comprises at least ~~about~~ 1 x 10^9 TILs in the container, wherein optionally the number of PD-1 enriched TILs in the priming first expansion is from ~~about~~ 1 x 10^4 to ~~about~~ 1x10^6, ~~about~~ 5x 10^4 to ~~about~~ 1x10^6 or 2x10^5 to ~~about~~ 1x10^6, optionally wherein the method further comprises the step of cryopreserving the first population of TILs from the tumor resected from the subject before performing the selection step.