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

obtained from step (d). 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 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).

[0007] In some examples, "obtaining" indicates the TILs employed in the method and/or process can be derived directly from the sample (including from a surgical resection, needle biopsy, core biopsy, small biopsy, or other sample) as part of the method and/or process steps. In some examples, "receiving" indicates the TILs employed in the method and/or process can be derived indirectly from the sample (including from a surgical resection, needle biopsy, core biopsy, small biopsy, or other sample) and then employed in the method and/or process, (for example, where step (a) begins will TILs that have already been derived from the sample by a separate process not included in part (a), such TILs could be refered to as "received").

[0008] In some examples, in step (b) the cell culture medium further comprises antigen-presenting cells (APCs), and wherein the number of APCs in the culture medium in step (c) is greater than the number of APCs in the culture medium in step (b).

[0009] In some examples in step (b) the cell culture medium further comprises antigen-presenting cells (APCs), and wherein the number of APCs in the culture medium in step (c) is equal to the number of APCs in the culture medium in step (b).

[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

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).

[0013] In some examples, in step (b) the cell culture medium further comprises antigen-presenting cells (APCs), and wherein the number of APCs in the culture medium in step (c) is greater than the number of APCs in the culture medium in step (b).

[0014] In some examples, in step (b) the cell culture medium further comprises antigen-presenting cells (APCs), and wherein the number of APCs in the culture medium in step (c) is the equal to the number of APCs in the culture medium in step (b).

[0015] In some examples, the PD-1 positive TILs are PD-1high TILS.

[0016] In some examples, 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.

[0017] In some examples, the monoclonal anti-PD-1 IgG4 antibody is nivolumab or variants, fragments, or conjugates thereof. In some examples, the the anti-IgG4 antibody is clone anti-human IgG4, Clone HP6023.

[0018] In some examples, 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.

[0019] In some examples, the ratio is selected from a range of from about 1.5:1 to about 10:1.

[0020] In some examples, the ratio is selected from a range of from about 2:1 to about 5:1.

[0021] In some examples, the ratio is selected from a range of from about 2:1 to about 3:1.

[0022] In some examples, the ratio is about 2:1.

[0023] In some examples, 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.

[0024] In some examples, 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.

[0025] In some examples, 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.

[0026] In some examples, about $2.5x10^8$ APCs are added to the priming first expansion and $5x10^8$ APCs are added to the rapid second expansion.

[0027] In some examples, 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.

[0028] In some examples, 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.

[0029] In some examples, 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 25:1.

[0030] In some examples, 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 20:1.

[0031] In some examples, 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 10:1.

[0032] In some examples, the second population of TILs is at least 50-fold greater in number than the first population of TILs.

[0033] In some examples, 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.

[0034] In some examples, 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.

[0035] In some examples, the plurality of separate containers comprises at least two separate containers.

[0036] In some examples, the plurality of separate containers comprises from two to twenty separate containers.

[0037] In some examples, the plurality of separate containers comprises from two to ten separate containers.

[0038] In some examples, the plurality of separate containers comprises from two to five separate containers.

[0039] In some examples, each of the separate containers comprises a first gas-permeable surface area.

[0040] In some examples, the multiple tumor fragments are distributed in a single container.

[0041] In some examples, the single container comprises a first gas-permeable surface area.

[0042] In some examples, 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.

[0043] In some examples, 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.

[0044] In some examples, 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.

[0045] In some examples, 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.

[0046] In some examples, 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.

[0047] In some examples, 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.

[0048] In some examples, 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.

[0049] In some examples, the second container is larger than the first container.

[0050] In some examples, 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.

[0051] In some examples, 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.

[0052] In some examples, 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.

[0053] In some examples, 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.

[0054] In some examples, 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 3.5 cell layers to about 4.5 cell layers.

[0055] In some examples, 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 4 cell layers.

[0056] In some examples, 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.

[0057] In some examples, each container comprises a first gas-permeable surface area.

[0058] In some examples, 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.

[0059] In some examples, in the step of the priming first expansion the APCs are layered onto the first gas-permeable surface area at an average thickness of from about 1.5 cell layers to about 2.5 cell layers.

[0060] In some examples, 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.

[0061] In some examples, 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.

[0062] In some examples, 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 3.5 cell layers to about 4.5 cell layers.

[0063] In some examples, 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 4 cell layers.

[0064] In some examples, 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.

[0065] In some examples, 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 1:1.2 to about 1:8.

[0066] In some examples, 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 raid second expansion is selected from the range of about 1:1.3 to about 1:7.

[0067] In some examples, 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 1:1.4 to about 1:6.

[0068] In some examples, 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 1:1.5 to about 1:5.

[0069] In some examples, 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 1:1.6 to about 1:4.

[0070] In some examples, 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 1:1.7 to about 1:3.5.

[0071] In some examples, 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 1:1.8 to about 1:3.

[0072] In some examples, 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 1:1.9 to about 1:2.5.

[0073] In some examples, 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.

[0074] In some examples, after 2 to 3 days in the step of the rapid second expansion, the cell culture medium is supplemented with additional IL-2.

[0075] In some examples, the method further comprises cryopreserving the harvested TIL population in the step of harvesting the therapeutic population of TILs using a cryopreservation process.

[0076] In some examples, the method further comprises the step of cryopreserving the infusion bag.

[0077] In some examples, the cryopreservation process is performed using a 1:1 ratio of harvested TIL population to cryopreservation media.

[0078] In some examples, the antigen-presenting cells are peripheral blood mononuclear cells (PBMCs).

[0079] In some examples, the PBMCs are irradiated and allogeneic.

[0080] In some examples, 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.

[0081] In some examples, 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.

[0082] In some examples, the antigen-presenting cells are artificial antigen-presenting cells.

[0083] In some examples, the harvesting in the step of harvesting the therapeutic population of TILs is performed using a membrane-based cell processing system.

[0084] In some examples, the harvesting in step (d) is performed using a LOVO cell processing system.

[0085] In some examples, 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.

[0086] In some examples, the multiple fragments comprise about 30 to about 60 fragments with a total volume of about 1300 mm^3 to about 1500 mm^3.

[0087] In some examples, the multiple fragments comprise about 50 fragments with a total volume of about 1350 mm^3.

[0088] In some examples, the multiple fragments comprise about 50 fragments with a total mass of about 1 gram to about 1.5 grams.

[0089] In some examples, the cell culture medium is provided in a container selected from the group consisting of a G-container and a Xuri cellbag.

[0090] In some examples, after 2 to 3 days in step (d), the cell culture medium is supplemented with additional IL-2.

[0091] In some examples, the IL-2 concentration is about 10,000 IU/mL to about 5,000 IU/mL.

[0092] In some examples, the IL-2 concentration is about 6,000 IU/mL.

[0093] In some examples, the infusion bag in the step of transferring the harvested therapeutic population of TILs to an infusion bag is a HypoThermosol-containing infusion bag.

[0094] In some examples, the cryopreservation media comprises dimethlysulfoxide (DMSO).

[0095] In some examples, the cryopreservation media comprises 7% to 10% DMSO.

[0096] In some examples, the first period in the step of the priming first expansion and the second period in the step of 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.

[0097] In some examples, the first period in the step of the priming first expansion is performed within a period of 5 days, 6 days, or 7 days.

[0098] In some examples, the second period in the step of the rapid second expansion is performed within a period of 7 days, 8 days, or 9 days.

[0099] In some examples, the first period in the step of the priming first expansion and the second period in the step of the rapid second expansion are each individually performed within a period of 7 days.

[00100] In some examples, the 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.

[00101] In some examples, the steps of the priming first expansion through the harvesting of the therapeutic population of TILs are performed within a period of about 15 days to about 16 days.

[00102] In some examples, the steps of the priming first expansion through the harvesting of the therapeutic population of TILs are performed within a period of about 14 days.

[00103] In some examples, the steps of the priming first expansion through the harvesting of the therapeutic population of TILs are performed within a period of about 15 days.

[00104] In some examples, the steps the priming first expansion through the harvesting of the therapeutic population of TILs are performed within a period of about 16 days.

[00105] In some examples, the method further comprises 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.

[00106] In some examples, 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.

[00107] In some examples, the number of TILs sufficient for a therapeutically effective dosage is from about 2.3×10^{10} to about 13.7×10^{10}.

[00108] In some examples, the third population of TILs in the step of the rapid second expansion provides for increased efficacy, increased interferon-gamma production, and/or increased polyclonality.

[00109] In some examples, the third population of TILs in the step of the rapid second expansion provides for at least a one-fold to five-fold or more interferon-gamma production as compared to TILs prepared by a process longer than 16 days.

[00110] In some examples, the effector T cells and/or central memory T cells obtained from the third population of TILs in the step of the rapid second expansion exhibit increased CD8 and CD28 expression relative to effector T cells and/or central memory T cells obtained from the second population of TILs in the step of the priming first expansion.

[00111] In some examples, the therapeutic population of TILs from the step of the harvesting of the therapeutic population of TILs are infused into a patient.

[00112] In some examples, the method further comprises the step of cryopreserving the infusion bag comprising the harvested TIL population in step (f) using a cryopreservation process.

[00113] In some examples, the cryopreservation process is performed using a 1:1 ratio of harvested TIL population to cryopreservation media.

[00114] In some examples, the antigen-presenting cells are peripheral blood mononuclear cells (PBMCs).

[00115] In some examples, the PBMCs are irradiated and allogeneic.

[00116] In some examples, the antigen-presenting cells are artificial antigen-presenting cells.

[00117] In some examples, the harvesting in step (e) is performed using a membrane-based cell processing system.

[00118] In some examples, the harvesting in step (e) is performed using a LOVO cell processing system.

[00119] In some examples, 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.

[00120] In some examples, the multiple fragments comprise about 30 to about 60 fragments with a total volume of about 1300 mm^3 to about 1500 mm^3.

[00121] In some examples, the multiple fragments comprise about 50 fragments with a total volume of about 1350 mm^3.

[00122] In some examples, the multiple fragments comprise about 50 fragments with a total mass of about 1 gram to about 1.5 grams.

[00123] In some examples, the cell culture medium is provided in a container selected from the group consisting of a G-container and a Xuri cellbag.

[00124] In some examples, the IL-2 concentration is about 10,000 IU/mL to about 5,000 IU/mL.

[00125] In some examples, the IL-2 concentration is about 6,000 IU/mL.

[00126] In some examples, the infusion bag in step (d) is a HypoThermosol-containing infusion bag.

[00127] In some examples, the cryopreservation media comprises dimethlysulfoxide (DMSO).

[00128] In some examples, the cryopreservation media comprises 7% to 10% DMSO.

[00129] In some examples, the first period in step (c) and the second period in step (c) are each individually performed within a period of 5 days, 6 days, or 7 days.

[00130] In some examples, the first period in step (c) is performed within a period of 5 days, 6 days, or 7 days.

[00131] In some examples, the second period in step (d) is performed within a period of 7 days, 8 days, or 9 days.

[00132] In some examples, the first period in step (c) and the second period in step (c) are each individually performed within a period of 7 days.

[00133] In some examples, steps (a) through (f) are performed within a period of about 14 days to about 16 days.

[00134] In some examples, steps (a) through (f) are performed within a period of about 15 days to about 16 days.

[00135] In some examples, steps (a) through (f) are performed within a period of about 14 days.

[00136] In some examples, steps (a) through (f) are performed within a period of about 15 days.

[00137] In some examples, steps (a) through (f) are performed within a period of about 16 days.

[00138] In some examples, steps (a) through (f) and cryopreservation are performed in 16 days or less.

[00139] In some examples, the therapeutic population of TILs harvested in step (f) comprises sufficient TILs for a therapeutically effective dosage of the TILs.

[00140] In some examples, the number of TILs sufficient for a therapeutically effective dosage is from about 2.3×10^{10} to about 13.7×10^{10}.

[00141] In some examples, the container in step (c) is larger than the container in step (b).

[00142] In some examples, the third population of TILs in step (d) provides for increased efficacy, increased interferon-gamma production, and/or increased polyclonality.

[00143] In some examples, the third population of TILs in step (d) provides for at least a one-fold to five-fold or more interferon-gamma production as compared to TILs prepared by a process longer than 16 days.

[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, 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;

(e) harvesting the therapeutic population of TILs obtained from step (c);
(f) transferring the harvested TIL population from step (d) to an infusion bag; and
(g) administering a therapeutically effective dosage of the TILs from step (e) to the subject.

[00147] In some examples, 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}.

[00148] In some examples, the PD-1 positive TILs are PD-1high TILS.

[00149] In some examples, 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.

[00150] In some examples, the monoclonal anti-PD-1 IgG4 antibody is nivolumab or variants, fragments, or conjugates thereof.

[00151] In some examples, the the anti-IgG4 antibody is clone anti-human IgG4, Clone HP6023.

[00152] In some examples, the antigen presenting cells (APCs) are PBMCs.

[00153] In some examples, prior to administering a therapeutically effective dosage of TIL cells in step (g), a non-myeloablative lymphodepletion regimen has been administered to the patient.

[00154] In some examples, the non-myeloablative lymphodepletion regimen comprises the steps of administration of cyclophosphamide at a dose of 60 mg/m^2/day for two days followed by administration of fludarabine at a dose of 25 mg/m^2/day for five days.

[00155] In some examples, the method further comprises the step of treating the patient with a high-dose IL-2 regimen starting on the day after administration of the TIL cells to the patient in step (g).

[00156] In some examples, the high-dose IL-2 regimen comprises 600,000 or 720,000 IU/kg administered as a 15-minute bolus intravenous infusion every eight hours until tolerance.

[00157] In some examples, the third population of TILs in step (c) provides for increased efficacy, increased interferon-gamma production, and/or increased polyclonality.

[00158] In some examples, the third population of TILs in step (d) provides for at least a one-fold to five-fold or more interferon-gamma production as compared to TILs prepared by a process longer than 16 days.

[00159] In some examples, the effector T cells and/or central memory T cells obtained from the third population of TILs in 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 in step (c).

[00160] In some examples, the cancer is selected from the group consisting of melanoma, ovarian cancer, cervical cancer, non-small-cell lung cancer (NSCLC), lung cancer, bladder cancer, breast cancer, cancer caused by human papilloma virus, head and neck cancer (including head and neck squamous cell carcinoma (HNSCC)), glioblastoma (including GBM), gastrointestinal cancer, renal cancer, and renal cell carcinoma.

[00161] In some examples, the cancer is selected from the group consisting of melanoma, HNSCC, cervical cancers, NSCLC, glioblastoma (including GBM), and gastrointestinal cancer.

[00162] In some examples, the cancer is melanoma.

[00163] In some examples, the cancer is HNSCC.

[00164] In some examples, the cancer is a cervical cancer.

[00165] In some examples, the cancer is NSCLC.

[00166] In some examples, the cancer is glioblastoma (including GBM).

[00167] In some examples, the cancer is gastrointestinal cancer.

[00168] In some examples, the cancer is a hypermutated cancer.

[00169] In some examples, the cancer is a pediatric hypermutated cancer.

[00170] In some examples, the container is a GREX-10.

[00171] In some examples, the closed container comprises a GREX-100.

[00172] In some examples, the closed container comprises a GREX-500.

[00173] In some examples, the subject has been previously treated with an anti-PD-1 antibody.

[00174] In some examples, the subject has not been previously treated with an anti-PD-1 antibody.

[00175] In some examples, in step (b) 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.

[00176] In some examples, 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.

[00177] In some examples, the anti-PD-1 antibody for use in the selection in step (b) 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.

[00178] In some examples, the anti-PD-1 antibody for use in the selection is EH12.2H7.

[00179] In some examples, the anti-PD-1 antibody for use in the selection in step (b) binds to a different epitope than nivolumab or pembrolizumab.

[00180] In some examples, the anti-PD-1 antibody for use in the selection in step (b) binds to the same epitope as EH12.2H7 or nivolumab.

[00181] In some examples, the anti-PD-1 antibody for use in the selection in step (b) is nivolumab.

[00182] In some examples, the subject has been previously treated with a first anti-PD-1 antibody, wherein in step (b) 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.

[00183] In some examples, the subject has been previously treated with a first anti-PD1 antibody, wherein in step (b) 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 blocked from binding to the first population of TILs by the first anti-PD-1 antibody insolubilized on the first population of TILs.

[00184] In some examples, the subject has been previously treated with a first anti-PD1 antibody, wherein in step (b) the PD-1 positive TILs are selected by performing the step of 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, 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, and then performing the step of isolating the first complex to obtain the PD-1 enriched TIL population.

[00185] In some examples, 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 wherein the step of isolating the first complex is performed by isolating the second complex.

[00186] In some examples, the second anti-PD-1 antibody comprises an Fc region, the subject has been previously treated with a first anti-PD1 antibody, wherein in step (b) the PD-1 positive TILs are selected by performing the step of 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, 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, and wherein after the step of forming 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 second anti-PD-1 antibody to form a second complex of the anti-Fc antibody and the first complex, and then performing the step of isolating the second complex to obtain the PD-1 enriched TIL population.

[00187] In some examples, the subject has been previously treated with a first anti-PD1 antibody, wherein in step (b) the PD-1 positive TILs are selected by performing the step of 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, wherein the second anti-PD-1 antibody is blocked from binding to the PD-1 positive TILs by the first anti-PD-1 antibody insolubilized on the first population of TILs, 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 obtaining the PD-1 enriched TIL population the method further comprises the step of contacting the first complex with an anti-Fc antibody that binds to 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.

[00188] In some examples, the PD-1 positive TILs are PD-1high TILS.

[00189] In some examples, the present disclosure provides a therapeutic population of tumor infiltrating lymphocytes (TILs) prepared from PD-1 positive cells selected from the tumor tissue of a patient, wherein the therapeutic population of TILs provides for increased efficacy and/or increased interferon-gamma production.

[00190] In some examples, the present disclosure provides a therapeutic population of tumor infiltrating lymphocytes (TILs) prepared from PD-1 positive cells selected from the tumor tissue of a patient, wherein the therapeutic population of TILs provides for increased efficacy and/or increased interferon-gamma production.

[00191] In some examples, the present disclosure provides a therapeutic population of tumor infiltrating lymphocytes (TILs) prepared from PD-1 positive cells selected from the tumor tissue of a patient, wherein the therapeutic population of TILs provides for increased increased interferon-gamma production.

[00192] In some examples, the present disclosure provides a therapeutic population of tumor infiltrating lymphocytes (TILs) prepared from PD-1 positive cells selected from the tumor tissue of a patient, wherein the therapeutic population of TILs provides for increased efficacy.

[00193] In some examples, the present disclosure provides a therapeutic population of tumor infiltrating lymphocytes (TILs) prepared from PD-1 positive cells selected from the tumor tissue of a patient, wherein the therapeutic population of TILs is capable of at least one-fold more interferon-gamma production as compared to TILs prepared by a process longer than 16 days.

[00194] In some examples, the present disclosure provides a therapeutic population of tumor infiltrating lymphocytes (TILs) prepared from PD-1 positive cells selected from the tumor tissue of a patient, wherein the therapeutic population of TILs is capable of at least one-fold more interferon-gamma production as compared to TILs prepared by a process longer than 16-22 days.

[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

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. The present disclosure also 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, 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.

[00202] In some examples, 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,

(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).

[00203] 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.

[00204] In some examples, the FACS gates are set-up after step (a).

[00205] In some examples, the PD-1 positive TILs are PD-1high TILs.

[00206] In some examples, at least 80% of the PD-1 enriched TIL population are PD-1 positive TILs.

[00207] In some examples, the third population of TILs comprises at least about 1 x 10^8 TILs in the container.

[00208] In some examples, the third population of TILs comprises at least about 1 x 10^9 TILs in the container.

[00209] In some examples, the number of PD-1 enriched TILs in the priming first expansion is from about 1×10^4 to about 1×10^6.

[00210] In some examples, the number of PD-1 enriched TILs in the priming first expansion is from about 5×10^4 to about 1×10^6.

[00211] In some examples, the number of PD-1 enriched TILs in the priming first expansion is from about 2×10^5 to about 1×10^6.

[00212] In some examples, the method further comprises the step of cyropreserving the first population of TILs from the tumor resected from the subject before performing step (a).

BRIEF DESCRIPTION OF THE DRAWINGS

[00213] Figure 1A-1B: A) Shows a comparison between the 2A process (approximately 22-day process) and an embodiment of the Gen 3 process for TIL manufacturing (approximately 14-days to 16-days process). **B)** Exemplary Process PD-1 Gen3 chart providing an overview of Steps A through F (approximately 14-days to 16-days process). **C)** Chart providing three exemplary Gen 3 processes

number of PD-1+ cells, in the PD-1-selected TIL, dropped substantially from 92.8% post-sort to an average of 27.1% post-REP. This is consistent with the data reported by others for melanoma and NSCLC TIL and suggests in vitro reinvigoration [10, 12].

[001900] To compare the extend of the in vitro expansion-induced PD-1 downregulation between PD-1-selected and unselected TIL, pre- and post-expansion percentages of PD-1+ TIL were assessed for both products. Results are shown in Figure 153.

[001901] The expression of PD-1 was significantly reduced in both the PD-1-selected TIL (average of 27.1%, ranging from 9.06 to 43.6) and unselected TIL products (average 10.6%, ranging from 4.93 to 29.3) relative to initial average PD-1 levels of 92.8% and 37.3%, respectively. Thus, the process of expanding the TIL equally affected both TIL preparations, with a >3-fold reduction in in the expression of PD-1.

Resident memory T cell markers in PD1-selected TIL

[001902] Integrins mediate the retention of lymphocytes in peripheral tissue. Some of these integrins are expressed on a subset of T cells known as resident memory T cells. These cells which strongly resemble effector memory T cells phenotypically, do not circulate and reside within tissues.

[001903] Several integrins such as αEβ7 (CD103), α1 β1(CD49a) are expressed on variable fractions of freshly isolated TIL [7, 8]. Along with CD39, a T cell surface molecule involved in the adenosine pathway and associated with an inhibitory signal, CD49 and CD103 have been identified on PD-1+ TIL that were shown to be tumor-reactive [2, 8, 10]. Furthermore, PD-1 and CD103 co-expression has been associated with a favorable clinical outcome in ovarian cancer [9].

[001904] To determine whether the PD1-selected TIL and unselected TIL products expressed markers associated with resident memory T cells, 13 tumors were analyzed for the expression for CD39, CD49a and CD103 expression. See, Figure 153.

[001905] No differences were observed in the percentages of CD49a+ and CD103+ cells in PD-1-selected TIL relative to unselected TIL, whereas CD39 expression was significantly higher in PD-1-selected TIL than unselected TIL. The difference could be related to higher levels of CD39 in unexpanded PD-1+ TIL, as suggested by the association of this marker with neoantigen specificity [6]. Overall, the 3 markers, were differentially expressed in the TIL products.

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 $13.7x10^{10}$.

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-l 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 I5-minute bolus intravenous infusion every eight hours until tolerance.

57. 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. The method according to any of claims 1-50, 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. The method according to any of claims 1-50 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. The method according to any of claims 1-50 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. The method according to any of claims 1-50 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. The method or the use according to claim 61, 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. The method or the use according to claim 62, 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. The method according to any of claims 1-50 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. The method of claim 1-50 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. The method or the use according to claim 65, 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. The method according to any of claims 1-50 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. The method according to any of claims 50 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. The method or the use according to claim 68, 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. 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. The method according to claim 70, 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. The method according to any one of claims 70 to 71, 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. The method according to any one of claims 70 to 72, 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 5x10^4 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.