effectiveness and scalability in manufacturing and more potent anti-cancer phenotypes of TIL preparations produced for treatment of human patients at multiple clinical centers. The present invention meets this need by providing a novel TIL expansion process which includes antigen-presenting feeder cells from the initiation of expansion, in order to prime the TILs for expansion, rather than a tradition pre-REP expansion step, thus allowing for a substantial reduction in overall time for the expansion process.

## SUMMARY

**[0004]** The present disclosure provides improved and/or shortened methods for expanding TILs and producing therapeutic populations of TILs.

**[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) that a first population of TILs is to be obtained and/or received from a tumor resected from a subject by processing a tumor sample obtained from the subject into multiple tumor fragments; (b) performing a priming first expansion by culturing the first population of 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; (c) 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; (d) harvesting the therapeutic population of TILs obtained from step (c); and (e) transferring the harvested TIL population from step (d) to an infusion bag. 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) performing a priming first expansion by culturing the first population of TILs in a cell culture medium comprising IL-2, optionally OKT-3, and optionally 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;

(c) 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;

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

(e) transferring the harvested TIL population from step (d) 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) that a first population of TILs is to be obtained and/or received from a tumor resected from a subject by processing a tumor sample obtained from the subject into multiple tumor fragments; (b) performing a priming first expansion by culturing the first population of TILs in a cell culture medium comprising IL-2, optionally 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; (c) 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 (d) harvesting the therapeutic population of TILs obtained from step (c). 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) performing a priming first expansion by culturing the first population of TILs in a cell culture medium comprising IL-2, optionally 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;

(c) 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

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

**[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 of the method, 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 another aspect, the present disclosure refers to 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, said first population of TILs obtainable by processing a tumor sample from a tumor resected from a subject into multiple tumor fragments, 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). 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, said first population of TILs obtainable by processing a tumor sample from a tumor resected from a subject into multiple tumor fragments, in a cell culture medium comprising IL-2, optionally OKT-3, and optionally 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).

**[0010]** In another aspect, the present disclosure alos 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 in a cell culture medium comprising IL-2, optionally 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).

**[0011]** In some examples of the method, 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 (c) is greater than the number of APCs in the culture medium in step (b).

**[0012]** 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.

**[0013]** 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 in a range of from about 1.5:1 to about 10:1.

**[0014]** 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 in a range of from about 2:1 to about 5:1.

**[0015]** 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 in a range of from about 2:1 to about 3:1.

**[0016]** 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 about 2:1.

**[0017]** In some examples, the number of APCs in the priming first expansion is selected from the range of about $1.0 \times 10^6$ APCs/cm$^2$ to about $4.5 \times 10^6$ APCs/cm$^2$, and wherein the number of APCs in the rapid second expansion is selected from the range of about $2.5 \times 10^6$ APCs/cm$^2$ to about $7.5 \times 10^6$ APCs/cm$^2$.

**[0018]** In some examples, the number of APCs in the priming first expansion is selected from the range of about $1.5 \times 10^6$ APCs/cm$^2$ to about $3.5 \times 10^6$ APCs/cm$^2$, and wherein the number of APCs in the rapid second expansion is selected from the range of about $3.5 \times 10^6$ APCs/cm$^2$ to about $6.0 \times 10^6$ APCs/cm$^2$.

**[0019]** In some examples, the number of APCs in the priming first expansion is selected from the range of about $2.0 \times 10^6$ APCs/cm$^2$ to about $3.0 \times 10^6$ APCs/cm$^2$, and wherein the number of APCs in the rapid second expansion is selected from the range of about $4.0 \times 10^6$ APCs/cm$^2$ to about $5.5 \times 10^6$ APCs/cm$^2$.

**[0020]** In some examples, the number of APCs in the priming first expansion is selected from the range of about $1 \times 10^8$ APCs to about $3.5 \times 10^8$ APCs, and wherein the number of APCs in the rapid second expansion is selected from the range of about $3.5 \times 10^8$ APCs to about $1 \times 10^9$ APCs.

**[0021]** In some examples, the number of APCs in the priming first expansion is selected from the range of about $1.5 \times 10^8$ APCs to about $3 \times 10^8$ APCs, and wherein the number of APCs in the rapid second expansion is selected from the range of about $4 \times 10^8$ APCs to about $7.5 \times 10^8$ APCs.

**[0022]** In some examples, the number of APCs in the priming first expansion is selected from the range of about $2 \times 10^8$ APCs to about $2.5 \times 10^8$ APCs, and wherein the number of APCs in the rapid second expansion is selected from the range of about $4.5 \times 10^8$ APCs to about $5.5 \times 10^8$ APCs.

**[0023]** In some examples, about $2.5 \times 10^8$ APCs are added to the priming first expansion and $5 \times 10^8$ APCs are added to the rapid second expansion.

**[0024]** 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.

**[0025]** 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.

**[0026]** 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.

**[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 20:1.

**[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 10:1.

**[0029]** In some examples, the second population of TILs is at least 50-fold greater in number than the first population of TILs.

**[0030]** 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.

**[0031]** 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.

**[0032]** In some examples, the plurality of separate containers comprises at least two separate containers.

**[0033]** In some examples, the plurality of separate containers comprises from two to twenty separate containers.

**[0034]** In some examples, the plurality of separate containers comprises from two to ten separate containers.

**[0035]** In some examples, the plurality of separate containers comprises from two to five separate containers.

**[0036]** In some examples, each of the separate containers comprises a first gas-permeable surface area.

**[0037]** In some examples, the multiple tumor fragments are distributed in a single container.

**[0038]** In some examples, the single container comprises a first gas-permeable surface area.

**[0039]** In some examples, 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.

**[0040]** In some examples, 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.

**[0041]** In some examples, 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.

**[0042]** In some examples, 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.

**[0043]** In some examples, 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.

**[0044]** In some examples, the rapid second expansion the APCs are layered onto the first gas-permeable surface area at a thickness of about 4 cell layers.

**[0045]** In some examples, 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.

**[0046]** In some examples, the second container is larger than the first container.

**[0047]** In some examples, 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.

**[0048]** In some examples, 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.

**[0049]** In some examples, 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.

**[0050]** In some examples, 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.

**[0051]** In some examples, 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.

**[0052]** In some examples, 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.

**[0053]** 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.

**[0054]** In some examples, each container comprises a first gas-permeable surface area.

**[0055]** In some examples, 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.

**[0056]** In some examples, 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.

**[0057]** In some examples, 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.

**[0058]** In some examples, 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.

**[0059]** In some examples, 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.

**[0060]** In some examples, 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.

**[0061]** In some examples, 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.

**[0062]** 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.

**[0063]** 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.

**[0064]** 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.

**[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 1:1.5 to about 1:5.

**[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 rapid second expansion is selected from the range of about 1:1.6 to about 1:4.

**[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 1:1.7 to about 1:3.5.

**[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 1:1.8 to about 1:3.

**[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 1:1.9 to about 1:2.5.

**[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 about 1:2.

**[0071]** 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.

**[0072]** 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.

**[0073]** In some examples, the method further comprises the step of cryopreserving the infusion bag.

**[0074]** In some examples, the cryopreservation process is performed using a 1:1 ratio of harvested TIL population to cryopreservation media.

**[0075]** In some examples, the antigen-presenting cells are peripheral blood mononuclear cells (PBMCs).

**[0076]** In some examples, the PBMCs are irradiated and allogeneic.

**[0077]** In some examples, the step of the priming first expansion the cell culture medium comprises peripheral blood mononuclear cells (PBMCs), and wherein the total number of PBMCs added to the cell culture medium in the step of the priming first expansion is about $2.5 \times 10^8$.

**[0078]** In some examples, 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 about $5 \times 10^8$.

**[0079]** In some examples, the e antigen-presenting cells are artificial antigen-presenting cells.

**[0080]** In some examples, the harvesting in the step of harvesting the therapeutic population of TILs is performed using a membrane-based cell processing system.

**[0081]** In some examples, the harvesting in step harvesting the therapeutic population of TILs is performed using a LOVO cell processing system.

**[0082]** 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 $mm^3$.

**[0083]** 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 $mm^3$.

**[0084]** In some examples, the multiple fragments comprise about 50 fragments with a total volume of about 1350 $mm^3$.

**[0085]** In some examples, the multiple fragments comprise about 50 fragments with a total mass of about 1 gram to about 1.5 grams.

**[0086]** 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.

**[0087]** In some examples, the IL-2 concentration is about 10,000 IU/mL to about 5,000 IU/mL.

**[0088]** In some examples, the IL-2 concentration is about 6,000 IU/mL.

**[0089]** 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.

**[0090]** In some examples, the cryopreservation media comprises dimethlysulfoxide (DMSO).

**[0091]** In some examples, the cryopreservation media comprises 7% to 10% DMSO.

**[0092]** 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, or 7 days.

**[0093]** 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.

**[0094]** 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.

**[0095]** 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.

**[0096]** 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.

**[0097]** 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.

**[0098]** 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.

**[0099]** 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.

**[00100]** 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.

**[00101]** 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.

**[00102]** 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.

**[00103]** In some examples, the number of TILs sufficient for a therapeutically effective dosage is from about $2.3\times10^{10}$ to about $13.7\times10^{10}$.

**[00104]** 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.

**[00105]** 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 18 days.

**[00106]** 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.

**[00107]** 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.

**[00108]** 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, the use comprising: (a) that a first population of TILs is to be obtained and/or received from a tumor resected from a subject by processing a tumor sample obtained from the subject into multiple tumor fragments; (b) performing a priming first expansion by culturing the first population of TILs in a cell culture medium comprising IL-2, optionally OKT-3, and optionally 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 7/8 days to obtain the second population of TILs; (c) 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; (d) harvesting the therapeutic population of TILs obtained from step (c); (e) transferring the harvested TIL population from step (d) to an infusion bag; and (f) that a therapeutically effective dosage of the TILs from step (e) is to be administered to the subject.

Ther present disclosure also 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) performing a priming first expansion by culturing the first population of TILs in a cell culture medium comprising IL-2, optionally OKT-3, and optionally 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 7/8 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;

(c) 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;

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

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

(f) administering a therapeutically effective dosage of the TILs from step (e) to the subject.

**[00109]** In some examples, the number of TILs sufficient for administering a therapeutically effective dosage in step (f) is from about $2.3\times10^{10}$ to about $13.7\times10^{10}$.

**[00110]** In some examples, the antigen presenting cells (APCs) are PBMCs.

**[00111]** In some examples, prior to administering a therapeutically effective dosage of TIL cells in step (f), a non-myeloablative lymphodepletion regimen has been administered to the patient.

**[00112]** 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.

**[00113]** 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 (f).

**[00114]** 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.

**[00115]** In some examples, the e third population of TILs in step (b) provides for increased efficacy, increased interferon-gamma production, and/or increased polyclonality.

**[00116]** In some examples, the third population of TILs in step (c) 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.

**[00117]** In some examples, the effector T cells and/or central memory T cells obtained from the third population of TILs in step (c) 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 (b).

**[00118]** In some examples, the cancer is a solid tumor.

**[00119]** 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.

**[00120]** In some examples, the cancer is selected from the group consisting of melanoma, HNSCC, cervical cancers, NSCLC, glioblastoma (including GBM), and gastrointestinal cancer.

**[00121]** In some examples, the cancer is melanoma.

**[00122]** In some examples, the cancer is HNSCC.

**[00123]** In some examples, the cancer is a cervical cancer.

**[00124]** In some examples, the cancer is NSCLC.

**[00125]** In some examples, the cancer is glioblastoma (including GBM).

**[00126]** In some examples, the cancer is gastrointestinal cancer.

**[00127]** In some examples, the cancer is a hypermutated cancer.

**[00128]** In some examples, the the cancer is a pediatric hypermutated cancer.

**[00129]** In some examples, the container is a closed container.

**[00130]** In some examples, the container is a G-container.

**[00131]** In some examples, the container is a GREX-10.

**[00132]** In some examples, the closed container comprises a GREX-100.

**[00133]** In some examples, the closed container comprises a GREX-500.

**[00134]** The presenting disclosure also provides a therapeutic population of tumor infiltrating lymphocytes (TILs) made by the method as disclosed herein.

**[00135]** The presenting disclosure also provides therapeutic population of tumor infiltrating lymphocytes (TILs) prepared from tumor tissue of a patient, wherein the therapeutic population of TILs provides for increased efficacy, increased interferon-gamma production, and/or increased polyclonality.

**[00136]** In some examples, the therapeutic population of TILs as disclosed herein provide for increased interferon-gamma production.

**[00137]** In some examples, the therapeutic population of TILs as disclosed herein provide for increased polyclonality.

**[00138]** In some examples, the therapeutic population of TILs as disclosed herein provide for increased efficacy.

**[00139]** In some examples, the therapeutic population of TILs as described herein is capable of at least one-fold more interferon-gamma production as compared to TILs prepared by a process longer than 16 days.

**[00140]** In some examples, the therapeutic population of TILs as described herein is capable of at least two-fold more interferon-gamma production as compared to TILs prepared by a process longer than 16 days.

**[00141]** In some examples, the therapeutic population of TILs as described herein is capable of at least three-fold more interferon-gamma production as compared to TILs prepared by a process longer than 16 days.

**[00142]** In some examples, the the present disclosure provides a therapeutic population of tumor infiltrating lymphocytes (TILs), 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 in which the first expansion of TILs is performed without any added antigen-presenting cells (APCs).

**[00143]** In some examples, the therapeutic population of TILs as described herein is capable of at least two-fold more interferon-gamma production as compared to TILs prepared by a process in which the first expansion of TILs is performed without any added APCs.

**[00144]** In some examples, the therapeutic population of TILs as described herein is capable of at least three-fold more interferon-gamma production as compared to TILs prepared by a process in which the first expansion of TILs is performed without any added APCs.

**[00145]** In some examples, the the present disclosure provides a therapeutic population of tumor infiltrating lymphocytes (TILs), 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 in which the first expansion of TILs is performed without any added OKT3.

**[00146]** In some examples, the therapeutic population of TILs as described herein is capable of at least two-fold more interferon-gamma production as compared to TILs prepared by a process in which the first expansion of TILs is performed without any added OKT3.

**[00147]** In some examples, the therapeutic population of TILs as described herein is capable of at least three-fold more interferon-gamma production as compared to TILs prepared by a process in which the first expansion of TILs is performed without any added OKT3.

**[00148]** In some examples, the the present disclosure provides a therapeutic population of tumor infiltrating lymphocytes (TILs), 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 in which the first expansion of TILs is performed with no added antigen-presenting cells (APCs) and no added OKT3.

**[00149]** In some examples, the therapeutic population of TILs as described herein is capable of at least two-fold more interferon-gamma production as compared to TILs prepared by a process in which the first expansion of TILs is performed with no added antigen-presenting cells (APCs) and no added OKT3.

**[00150]** In some examples, the therapeutic population of TILs as described herein is capable of at least three-fold more interferon-gamma production as compared to TILs prepared by a process in which the first expansion of TILs is performed with no added antigen-presenting cells (APCs) and no added OKT3.

**[00151]** In another aspect, the present disclosure also provides a tumor infiltrating lymphocyte (TIL) composition comprising the therapeutic population of TILs as described herein and a pharmaceutically acceptable carrier.

**[00152]** In another aspect, thepresent disclosure also provides a sterile infusion bag comprising the TIL composition as described herein.

**[00153]** In another aspect, the present disclosure also provides a cryopreserved preparation of the therapeutic population of TILs as described herein.

**[00154]** In another aspect, the present disclosure refers to a tumor infiltrating lymphocyte (TIL) composition comprising the therapeutic population of TILs as disclosed herein and a cryopreservation media, wherein optionally the cryopreservation media contains DMSO, optionally wherein the cryopreservation media contains 7-10% DMSO. The present disclosure also provides a tumor infiltrating lymphocyte (TIL) composition comprising the therapeutic population of TILs as described herein and a cryopreservation media.

**[00155]** In some examples, the the cryopreservation media contains DMSO.

**[00156]** In some examples, the cryopreservation media contains 7-10% DMSO.

**[00157]** In another aspect, the present disclosure also provides a cryopreserved preparation of the TIL composition as described herein.

**[00158]** In some examples, the tumor infiltrating lymphocyte (TIL) composition as described herein is for use as a medicament.

**[00159]** In some examples, the tumor infiltrating lymphocyte (TIL) composition as described herein is for use in the treatment of a cancer.

**[00160]** In some examples, the tumor infiltrating lymphocyte (TIL) composition as described herein is for use in the treatment of a solid tumor cancer.

**[00161]** In some examples, the tumor infiltrating lymphocyte (TIL) composition as described herein for use in treatment of a cancer selected from 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.

**[00162]** In some examples, the tumor infiltrating lymphocyte (TIL) composition as described herein is for use in treatment of a cancer selected from the group consisting of melanoma, HNSCC, cervical cancers, NSCLC, glioblastoma (including GBM), and gastrointestinal cancer.

**[00163]** In some examples, the TIL composition as described herein is for use in treatment of a cancer wherein cancer is melanoma.

**[00164]** In some examples, the TIL composition as described herein is for use in treatment of a cancer wherein cancer is HNSCC.

**[00165]** In some examples, the TIL composition as described herein is for use in treatment of a cancer wherein a cervical cancer.

**[00166]** In some examples, the TIL composition as described herein is for use in treatment of a cancer wherein the cancer is NSCLC.

**[00167]** In some examples, the TIL composition as described herein is for use in treatment of a cancer wherein the cancer is glioblastoma (including GBM).

**[00168]** In some examples, the TIL composition as described herein is for use in treatment of a cancer wherein the cancer is gastrointestinal cancer.

**[00169]** In some examples, the TIL composition as described herein is for use in treatment of a cancer wherein the cancer is a hypermutated cancer.

**[00170]** In some examples, the TIL composition as described herein is for use in treatment of a cancer wherein the cancer is a pediatric hypermutated cancer.

**[00171]** In another aspect, the preset disclosure refers to the use of the tumor infiltrating lymphocyte (TIL) composition as disclosed herein in the manufacture of a medicament for treating cancer in a subject, wherein the TIL composition is to be administered to the subject. In some examples, the present disclosure provides for the use of the tumor infiltrating lymphocyte (TIL) composition as dsecribed herein in a method of treating cancer in a subject comprising administering a therapeutically effective dosage of the TIL composition to the subject. In some examples, the cancer is a solid tumor. 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 In some examples, the cancer is selected from the group consisting of melanoma, HNSCC, cervical cancers, NSCLC, glioblastoma (including GBM), and gastrointestinal cancer. In some examples, the cancer is melanoma. In some examples, the cancer is HNSCC. In some examples, the cancer is a cervical cancer. In some examples, the cancer is NSCLC. In some examples, the cancer is glioblastoma (including GBM). In some

examples, the cancer is gastrointestinal cancer. In some examples, the cancer is a hypermutated cancer. In some examples, the cancer is a pediatric hypermutated cancer.

**[00172]** In some examples, the tumor infiltrating lymphocyte (TIL) composition as described herein is for use in a method of treating cancer in a subject comprising administering a therapeutically effective dosage of the TIL composition to the subject. In some examples, the cancer is a solid tumor. 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. In some examples, the cancer is selected from the group consisting of melanoma, HNSCC, cervical cancers, NSCLC, glioblastoma (including GBM), and gastrointestinal cancer.

**[00173]** The present disclosure also provides a method of treating cancer in a subject comprising administering to the subject a therapeutically effective dosage of the tumor infiltrating lymphocyte (TIL) composition as described herein.

**[00174]** In some examples, the cancer is a solid tumor.

**[00175]** 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.

**[00176]** In some examples, the cancer is selected from the group consisting of melanoma, HNSCC, cervical cancers, NSCLC, glioblastoma (including GBM), and gastrointestinal cancer. In some examples, the cancer is melanoma. In some examples, the cancer is HNSCC. In some examples, the cancer is a cervical cancer. In some examples, the cancer is NSCLC. In some examples, the cancer is glioblastoma (including GBM). In some embodiments, the cancer is gastrointestinal cancer. In some examples, the cancer is a hypermutated cancer. In some examples, the cancer is a pediatric hypermutated cancer.

**[00177]** In another aspect, the present disclosure refers to a method of expanding T cells comprising: (a) performing a priming first expansion of a first population of T cells which is to be obtained from a donor by culturing the first population of T cells to effect growth and to

prime an activation of the first population of T cells; (b) after the activation of the first population of T cells primed in step (a) begins to decay, performing a rapid second expansion of the first population of T cells by culturing the first population of T cells to effect growth and to boost the activation of the first population of T cells to obtain a second population of T cells; and (c) harvesting the second population of T cells. The present disclosure also provides a method of expanding T cells comprising:

(a) performing a priming first expansion of a first population of T cells obtained from a donor by culturing the first population of T cells to effect growth and to prime an activation of the first population of T cells;

(b) after the activation of the first population of T cells primed in step (a) begins to decay, performing a rapid second expansion of the first population of T cells by culturing the first population of T cells to effect growth and to boost the activation of the first population of T cells to obtain a second population of T cells; and

(c) harvesting the second population of T cells.

**[00177a]** In another aspect, the present disclosure refers to a method for expanding tumor infiltrating lymphocytes (TILs) into a therapeutic population of TILs comprising: (i) obtaining and/or receiving a first population of TILs from a tumor sample which is to be obtained from one or more small biopsies, core biopsies, or needle biopsies of a tumor in a subject by culturing the tumor sample in a first cell culture medium comprising IL-2 for about 3 days; (ii) performing a priming first expansion by culturing the first population of TILs in a second 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 7 or 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; (iii) performing a rapid second expansion by supplementing the second 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 (ii), wherein the rapid second expansion is performed for a second period of about 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; (iv) harvesting the therapeutic population of TILs obtained from step (iii); and (v) transferring the harvested TIL population from step (iv) to an infusion bag.

**[00177b]** In another aspect, the present disclosure refers to a method for expanding tumor infiltrating lymphocytes (TILs) into a therapeutic population of TILs comprising: (i) obtaining and/or receiving a first population of TILs from a tumor sample which is to be obtained from one or more small biopsies, core biopsies, or needle biopsies of a tumor in a subject by culturing the tumor sample in a first cell culture medium comprising IL-2 for about 3 days; (ii) performing a priming first expansion by culturing the first population of TILs in a second 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 for first period of about 7 or 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; (iii) performing a rapid second expansion by contacting the second population of TILs with a third 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 11 days to obtain the third population of TILs, wherein the third population of TILs is a therapeutic population of TILs; and (iv) harvesting the therapeutic population of TILs obtained from step (iii).

[00177c] In another aspect, the present disclosure refers to a method of expanding T cells comprising: (i) performing a priming first expansion of a first population of T cells from a tumor sample which is to be obtained from one or more small biopsies, core biopsies, or needle biopsies of a tumor in a donor by culturing the first population of T cells to effect growth and to prime an activation of the first population of T cells; (ii) after the activation of the first population of T cells primed in step (a) begins to decay, performing a rapid second expansion of the first population of T cells by culturing the first population of T cells to effect growth and to boost the activation of the first population of T cells to obtain a second population of T cells; and (iv) harvesting the second population of T cells.

**[00178]** In some examples, the priming first expansion of step (a) is performed during a period of up to 7 days.

**[00179]** In some examples, the rapid second expansion of step (b) is performed during a period of up to 11 days.

**[00180]** In some examples, the rapid second expansion of step (b) is performed during a period of up to 9 days.

**[00181]** In some examples, the priming first expansion of step (a) is performed during a period of 7 days and the rapid second expansion of step (b) is performed during a period of 9 days.

**[00182]** In some examples, the priming first expansion of step (a) is performed during a period of up to 8 days.

**[00183]** In some examples, the rapid second expansion of step (b) is performed during a period of up to 8 days.

**[00184]** In some examples, the priming first expansion of step (a) is performed during a period of 8 days and the rapid second expansion of step (b) is performed during a period of 8 days.

**[00185]** In some examples of the method, in step (a) the first population of T cells is cultured in a first culture medium comprising OKT-3 and IL-2.

**[00186]** In some examples, the first culture medium comprises OKT-3, IL-2 and antigen-presenting cells (APCs).

**[00187]** In some examples of the method, in step (b) the first population of T cells is cultured in a second culture medium comprising OKT-3, IL-2 and antigen-presenting cells (APCs).

**[00188]** In some examples of the method, in step (a) the first population of T cells is cultured in a first culture medium in a container comprising a first gas-permeable surface, wherein the first culture medium comprises optionally OKT-3, IL-2 and optionally a first population of antigen-presenting cells (APCs), wherein the first population of APCs is exogenous to the donor of the first population of T cells and the first population of APCs is layered onto the first gas-permeable surface, wherein in step (b) the first population of T cells is cultured in a second culture medium in the container, wherein the second culture medium comprises OKT-3, IL-2 and a second population of APCs, wherein the second population of APCs is exogenous to the donor of the first population of T cells and the second population of APCs is layered onto the first gas-permeable surface, and wherein the second population of APCs is greater than the first population of APCs.

**[00189]** In some examples, the ratio of the number of APCs in the second population of APCs to the number of APCs in the first population of APCs is about 2:1.

**[00190]** In some examples, the number of APCs in the first population of APCs is about 2.5 x $10^8$ and the number of APCs in the second population of APCs is about 5 x $10^8$.

**[00191]** In some examples of the method, in step (a) the first population of APCs is layered onto the first gas-permeable surface at an average thickness of 2 layers of APCs.

**[00192]** In some examples of the method, in step (b) the second population of APCs is layered onto the first gas-permeable surface at an average thickness selected from the range of 4 to 8 layers of APCs.

**[00193]** In some examples, the ratio of the average number of layers of APCs layered onto the first gas-permeable surface in step (b) to the average number of layers of APCs layered onto the first gas-permeable surface in step (a) is 2:1.

**[00194]** In some examples, the APCs are peripheral blood mononuclear cells (PBMCs).

**[00195]** In some examples, the APCs comprise PBMCs, wherein the PBMCs are irradiated and exogenous to the donor of the first population of T cells.

**[00196]** In some examples, the T cells are tumor infiltrating lymphocytes (TILs).

**[00197]** In some examples, the T cells are marrow infiltrating lymphocytes (MILs).

**[00198]** In some examples, the T cells are peripheral blood lymphocytes (PBLs).

**[00199]** In some examples, the cell culture medium is a defined medium and/or a serum free medium.

**[00200]** In some examples, the defined medium comprises (optionally recombinant) transferrin, (optionally recombinant) insulin, and (optionally recombinant) albumin.

**[00201]** In some examples, the serum-free or defined medium comprises a basal cell medium and a serum supplement and/or a serum replacement.

**[00202]** In some examples, the basal cell medium includes, but is not limited to CTS™ OpTmizer™ T-cell Expansion Basal Medium , CTS™ OpTmizer™ T-Cell Expansion SFM, CTS™ AIM-V Medium, CTS™ AIM-V SFM, LymphoONE™ T-Cell Expansion Xeno-Free Medium, Dulbecco's Modified Eagle's Medium (DMEM), Minimal Essential Medium (MEM), Basal Medium Eagle (BME), RPMI 1640, F-10, F-12, Minimal Essential Medium

(αMEM), Glasgow's Minimal Essential Medium (G-MEM), RPMI growth medium, and Iscove's Modified Dulbecco's Medium.

**[00203]** In some examples, the serum supplement or serum replacement is selected from the group consisting of CTS™ OpTmizer T-Cell Expansion Serum Supplement and CTS™ Immune Cell Serum Replacement.

**[00204]** In some examples, the cell culture medium comprises one or more albumins or albumin substitutes.

**[00205]** In some examples, the cell culture medium comprises one or more amino acids.

**[00206]** In some examples, the cell culture medium comprises one or more vitamins, one or more transferrins or transferrin substitutes.

**[00207]** In some examples, the cell culture medium comprises one or more antioxidants, one or more insulins or insulin substitutes.

**[00208]** In some examples, the cell culture medium comprises one or more collagen precursors, one or more antibiotics, and one or more trace elements. In

**[00209]** In some examples, the cell culture medium comprises albumin.

**[00210]** In some examples, the cell culture medium comprises albumin and one or more ingredients selected from the group consisting of glycine, L- histidine, L-isoleucine, L-methionine, L-phenylalanine, L-proline, L- hydroxyproline, L-serine, L-threonine, L-tryptophan, L-tyrosine, L-valine, thiamine, reduced glutathione, L-ascorbic acid-2-phosphate, iron saturated transferrin, insulin, and compounds containing the trace element moieties $Ag^{+}$, $Al^{3+}$, $Ba^{2+}$, $Cd^{2+}$, $Co^{2+}$, $Cr^{3}$", $Ge^{4+}$, $Se^{4+}$, Br, T, $Mn^{2+}$, P, $Si^{4+}$, $V^{5+}$, $Mo^{6+}$, $Ni^{2+}$, $Rb^{+}$, $Sn^{2+}$ and $Zr^{4+}$.

**[00211]** In some examples, the cell culture medium further comprises L-glutamine, sodium bicarbonate and/or 2-mercaptoethanol.

**[00212]** In some examples, the cell culture medium comprises a total serum replacement concentration (vol%) of from about 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 11%, 12%, 13%, 14%, 15%, 16%, 17%, 18%, 19%, or 20% by volume of the cell culture medium.

**[00213]** In some examples, the cell culture medium comprises a total serum replacement concentration of about 3%, about 5%, or about 10% of the total volume of the cell culture medium.

**[00214]** In some examples, the cell culture medium further comprises glutamine (i.e., GlutaMAX®) at a concentration of from about 0.1mM to about 10mM, 0.5mM to about 9mM, 1mM to about 8mM, 2mM to about 7mM, 3mM to about 6mM, or 4mM to about 5 mM.

**[00215]** In some examples, the cell culture medium further comprises glutamine (i.e., GlutaMAX®) at a concentration of about 2mM.

**[00216]** In some examples, the cell culture medium further comprises 2-mercaptoethanol at a concentration of from about 5mM to about 150mM, 10mM to about 140mM, 15mM to about 130mM, 20mM to about 120mM, 25mM to about 110mM, 30mM to about 100mM, 35mM to about 95mM, 40mM to about 90mM, 45mM to about 85mM, 50mM to about 80mM, 55mM to about 75mM, 60mM to about 70mM, or about 65mM.

**[00217]** In some examples, the cell culture medium further comprises 2-mercaptoethanol at a concentration of about 55mM.

**[00218]** In some examples, the cell culture medium comprises the defined media described in International PCT Publication No. WO/1998/030679.

**[00219]** In some examples, the cell culture medium comprises glycine in the range of from about 5-200 mg/L, L- histidine in the range of from about 5-250 mg/L, L-isoleucine in the range of from about 5-300 mg/L, L-methionine in the range of from about 5-200 mg/L, L-phenylalanine in the range of from about 5-400 mg/L, L-proline in the range of from about 1-1000 mg/L, L- hydroxyproline in the range of from about 1-45 mg/L, L-serine in the range of from about 1-250 mg/L, L-threonine in the range of from about 10-500 mg/L, L-tryptophan in the range of from about 2-110 mg/L, L-tyrosine in the range of from about 3-175 mg/L, L-valine in the range of from about 5-500 mg/L, thiamine in the range of from about 1-20 mg/L, reduced glutathione in the range of from about 1-20 mg/L, L-ascorbic acid-2-phosphate in the range of from about 1-200 mg/L, iron saturated transferrin in the range of from about 1-50 mg/L, insulin in the range of from about 1-100 mg/L, sodium selenite in the range of from about 0.000001-0.0001 mg/L, and/or albumin (e.g., AlbuMAX® I) in the range of from about 5000-50,000 mg/L.

**[00220]** In some examples, the cell culture medium comprises one or more of the non-trace element moiety ingredients in the defined medium are present in the concentration ranges listed in the column under the heading “Concentration Range in 1X Medium” in Table A provided herein.

**[00221]** In some examples, the osmolarity of the cell culture medium is between about 260 and 350 mOsmol.

**[00222]** In some examples, the cell culture medium further comprises about 3.7 g/L, or about 2.2 g/L sodium bicarbonate.

**[00223]** In some examples, the cell culture medium further comprises L-glutamine (final concentration of about 2 mM), one or more antibiotics, non-essential amino acids (NEAA; final concentration of about 100 μM), and/or 2-mercaptoethanol (final concentration of about 100 μM).

**[00224]** In some examples, the cell culture medium in the first and/or second gas permeable container lacks beta-mercaptoethanol (BME or βME; also known as 2-mercaptoethanol, CAS 60-24-2).

**[00225]** In some examples, the cell culture medium comprises CTS OpTmizer T-Cell Expansion SFM, 3% CTS Immune Cell Serum Replacement, 55mM BME, and optionally glutamine.

**[00226]** In some examples, the cell culture medium comprises CTS™OpTmizer™ T-Cell Expansion Basal Medium supplemented with CTS™ OpTmizer™ T-Cell Expansion Supplement (26mL/L), and 3% CTS™ Immune Cell SR, and 2 mM Glutamax, optionally further comprising 6,000 IU/mL of IL-2.

**[00227]** In some examples, the cell culture medium comprises CTS™OpTmizer™ T-Cell Expansion Basal Medium supplemented with CTS™ OpTmizer™ T-Cell Expansion Supplement (26mL/L), and 3% CTS™ Immune Cell SR, 2mM Glutamax, and optionaly further comprising 3,000 IU/mL of IL-2.

**[00228]** In another aspect, the present disclosure refers to a tumor infiltrating lymphocyte (TIL) or an expanded tumor infiltrating lymphocyte (TIL) composition comprising: i) a population of tumor infiltrating lymphocytes (TILs), and ii) defined medium or serum free medium optionally comprising (optionally recombinant) transferrin, (optionally recombinant) insulin, and (optionally recombinant) albumin. The present disclosure also provides a tumor infiltrating lymphocyte (TIL) composition comprising:

i) a therapeutic population of tumor infiltrating lymphocytes (TILs), and

ii) defined medium or serum free medium optionally comprising (optionally recombinant) transferrin, (optionally recombinant) insulin, and (optionally recombinant) albumin.

**[00229]** The present disclosure also provides an expanded tumor infiltrating lymphocyte (TIL) composition comprising:

i) a therapeutic population of tumor infiltrating lymphocytes (TILs), and

ii) defined medium or serum free medium optionally comprising (optionally recombinant) transferrin, (optionally recombinant) insulin, and (optionally recombinant) albumin.

**[00230]** In some examples, the defined medium or serum free medium comprises (optionally recombinant) transferrin, (optionally recombinant) insulin, and (optionally recombinant) albumin.

**[00231]** In some examples, the defined medium or serum free medium comprises a basal cell medium and a serum supplement and/or a serum replacement.

**[00232]** In some examples, the basal cell medium includes, but is not limited to CTS™ OpTmizer™ T-cell Expansion Basal Medium , CTS™ OpTmizer™ T-Cell Expansion SFM, CTS™ AIM-V Medium, CTS™ AIM-V SFM, LymphoONE™ T-Cell Expansion Xeno-Free Medium, Dulbecco's Modified Eagle's Medium (DMEM), Minimal Essential Medium (MEM), Basal Medium Eagle (BME), RPMI 1640, F-10, F-12, Minimal Essential Medium (αMEM), Glasgow's Minimal Essential Medium (G-MEM), RPMI growth medium, and Iscove's Modified Dulbecco's Medium.

**[00233]** In some examples, the serum supplement or serum replacement is selected from the group consisting of CTS™ OpTmizer T-Cell Expansion Serum Supplement and CTS™ Immune Cell Serum Replacement.

**[00234]** In some examples, the defined medium or serum free medium comprises one or more albumins or albumin substitutes.

**[00235]** In some examples, the defined medium or serum free medium comprises one or more amino acids.

**[00236]** In some examples, the defined medium or serum free medium comprises one or more vitamins, one or more transferrins or transferrin substitutes.

**[00237]** In some examples, the defined medium or serum free medium comprises one or more antioxidants, one or more insulins or insulin substitutes.

**[00238]** In some examples, the defined medium or serum free medium comprises one or more collagen precursors, one or more antibiotics, and one or more trace elements.

**[00239]** In some examples, the defined medium or serum free medium comprises albumin.

**[00240]** In some examples, the defined medium or serum free medium comprises albumin and one or more ingredients selected from the group consisting of glycine, L- histidine, L-isoleucine, L-methionine, L-phenylalanine, L-proline, L- hydroxyproline, L-serine, L-threonine, L-tryptophan, L-tyrosine, L-valine, thiamine, reduced glutathione, L-ascorbic acid-2-phosphate, iron saturated transferrin, insulin, and compounds containing the trace element moieties $Ag^{+}$, $Al^{3+}$, $Ba^{2+}$, $Cd^{2+}$, $Co^{2+}$, $Cr^{3"}$, $Ge^{4+}$, $Se^{4+}$, Br, T, $Mn^{2+}$, P, $Si^{4+}$, $V^{5+}$, $Mo^{6+}$, $Ni^{2+}$, $Rb^{+}$, $Sn^{2+}$ and $Zr^{4+}$.

**[00241]** In some examples, the defined medium or serum free medium further comprises L-glutamine, sodium bicarbonate and/or 2-mercaptoethanol.

**[00242]** In some examples, the defined medium or serum free medium comprises a total serum replacement concentration (vol%) of from about 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 11%, 12%, 13%, 14%, 15%, 16%, 17%, 18%, 19%, or 20% by volume of the cell culture medium.

**[00243]** In some examples, the defined medium or serum free medium comprises a total serum replacement concentration of about 3%, about 5%, or about 10% of the total volume of the cell culture medium.

**[00244]** In some examples, the defined medium or serum free medium further comprises glutamine (i.e., GlutaMAX®) at a concentration of from about 0.1mM to about 10mM, 0.5mM to about 9mM, 1mM to about 8mM, 2mM to about 7mM, 3mM to about 6mM, or 4mM to about 5 mM.

**[00245]** In some examples, the defined medium or serum free medium further comprises glutamine (i.e., GlutaMAX®) at a concentration of about 2mM.

**[00246]** In some examples, the defined medium or serum free medium further comprises 2-mercaptoethanol at a concentration of from about 5mM to about 150mM, 10mM to about 140mM, 15mM to about 130mM, 20mM to about 120mM, 25mM to about 110mM, 30mM to

about 100mM, 35mM to about 95mM, 40mM to about 90mM, 45mM to about 85mM, 50mM to about 80mM, 55mM to about 75mM, 60mM to about 70mM, or about 65mM.

**[00247]** In some examples, the defined medium or serum free medium further comprises 2-mercaptoethanol at a concentration of about 55mM.

**[00248]** In some examples, the defined medium or serum free medium comprises the defined media described in International PCT Publication No. WO/1998/030679.

**[00249]** In some examples, the defined medium or serum free medium comprises glycine in the range of from about 5-200 mg/L, L- histidine in the range of from about 5-250 mg/L, L-isoleucine in the range of from about 5-300 mg/L, L-methionine in the range of from about 5-200 mg/L, L-phenylalanine in the range of from about 5-400 mg/L, L-proline in the range of from about 1-1000 mg/L, L- hydroxyproline in the range of from about 1-45 mg/L, L-serine in the range of from about 1-250 mg/L, L-threonine in the range of from about 10-500 mg/L, L-tryptophan in the range of from about 2-110 mg/L, L-tyrosine in the range of from about 3-175 mg/L, L-valine in the range of from about 5-500 mg/L, thiamine in the range of from about 1-20 mg/L, reduced glutathione in the range of from about 1-20 mg/L, L-ascorbic acid-2-phosphate in the range of from about 1-200 mg/L, iron saturated transferrin in the range of from about 1-50 mg/L, insulin in the range of from about 1-100 mg/L, sodium selenite in the range of from about 0.000001-0.0001 mg/L, and/or albumin (e.g., AlbuMAX® I) in the range of from about 5000-50,000 mg/L.

**[00250]** In some examples, the defined medium or serum free medium comprises one or more of the non-trace element moiety ingredients in the defined medium are present in the concentration ranges listed in the column under the heading “Concentration Range in 1X Medium” in Table A provided herein.

**[00251]** In some examples, the osmolarity of the defined medium or serum free medium is between about 260 and 350 mOsmol.

**[00252]** In some examples, the defined medium or serum free medium further comprises about 3.7 g/L, or about 2.2 g/L sodium bicarbonate.

**[00253]** In some examples, the defined medium or serum free medium further comprises L-glutamine (final concentration of about 2 mM), one or more antibiotics, non-essential amino acids (NEAA; final concentration of about 100 μM), and/or 2-mercaptoethanol (final concentration of about 100 μM).

**[00254]** In some examples, the defined medium or serum free medium in the first and/or second gas permeable container lacks beta-mercaptoethanol (BME or βME; also known as 2-mercaptoethanol, CAS 60-24-2).

**[00255]** In some examples, the cell culture medium comprises CTS OpTmizer T-Cell Expansion SFM, 3% CTS Immune Cell Serum Replacement, 55mM BME, and optionally glutamine.

**[00256]** In some examples, the cell culture medium comprises CTS™OpTmizer™ T-Cell Expansion Basal Medium supplemented with CTS™ OpTmizer™ T-Cell Expansion Supplement (26mL/L), and 3% CTS™ Immune Cell SR, and 2 mM Glutamax, optionally further comprising 6,000 IU/mL of IL-2.

**[00257]** In some examples, the cell culture medium comprises CTS™OpTmizer™ T-Cell Expansion Basal Medium supplemented with CTS™ OpTmizer™ T-Cell Expansion Supplement (26mL/L), and 3% CTS™ Immune Cell SR, 2mM Glutamax, and optionaly further comprising 3,000 IU/mL of IL-2.

**[00258]** In some examples, the population of TILs is a therapeutic population of TILs.

**[00259]** In some examples, the therapeutic population of TILs exhibits a rise in serum IFN-γ, wherein the rise in IFN-γ is greater than 200 pg/ml, greater than 250 pg/ml, greater than 300 pg/ml, greater than 350 pg/ml, greater than 400 pg/ml, greater than 450 pg/ml, greater than 500 pg/ml, greater than 550 pg/ml, greater than 600 pg/ml, greater than 650 pg/ml, greater than 700 pg/ml, greater than 750 pg/ml, greater than 800 pg/ml, greater than 850 pg/ml, greater than 900 pg/ml, greater than 950 pg/ml, or greater than 1000 pg/ml.

## BRIEF DESCRIPTION OF THE DRAWINGS

**[00260] Figure 1A-1C: 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 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 with an overview of Steps A through F (approximately 14-days to 16-days process) for each of the three process variations.

**[00261] Figure 2:** Provides an experimental flow chart for comparability between GEN 2 (process 2A) versus GEN 3.

**[00262] Figure 3A-3C: A)** L4054 - Phenotypic characterization on TIL product on Gen 2 and Gen 3 process. **B)** L4055-Phenotypic characterization on TIL product on Gen 2 and Gen 3 process. **C)** M1085T-Phenotypic characterization on TIL product on Gen 2 and Gen 3 process.

**[00263] Figure 4A-4C**: **A)** L4054 – Memory markers analysis on TIL product from the Gen 2 and Gen 3 processes. **B)** L4055 – Memory markers analysis on TIL product from the Gen 2 and Gen 3 processes. **C)** M1085T- Memory markers analysis on TIL product from the Gen 2 and Gen 3 processes.

**[00264] Figure 5:** L4054 Activation and exhaustion markers **(A)** Gated on CD4+, **(B)** Gated on CD8+.

**[00265] Figure 6:** L4055 Activation and exhaustion markers **(A)** Gated on CD4+, **(B)** Gated on CD8+.

**[00266] Figure 7:** IFNγ production (pg/mL): (A) L4054, (B) L4055, and (C) M1085T for the Gen 2 and Gen 3 processes: Each bar represented here is mean + SEM for IFNγ levels of stimulated, unstimulated, and media control. Optical density measured at 450 nm.

**[00267] Figure 8:** ELISA analysis of IL-2 concentration in cell culture supernatant: **(A) L4054** and **(B) L4055**. Each bar represented here is mean + SEM for IL-2 levels on spent media. Optical density measured at 450 nm.

**WHAT IS CLAIMED IS:**

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

(a) that a first population of TILs is to be obtained and/or received from a tumor resected from a subject by processing a tumor sample obtained from the subject into multiple tumor fragments;

(b) performing a priming first expansion by culturing the first population of 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;

(c) 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;

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

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

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

(a) that a first population of TILs is to be obtained and/or received from a tumor resected from a subject by processing a tumor sample obtained from the subject into multiple tumor fragments;

(b) performing a priming first expansion by culturing the first population of TILs in a cell culture medium comprising IL-2, optionally 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;

(c) 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

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

3. The method of claim 2, wherein 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).

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, said first population of TILs obtainable by processing a tumor sample from a tumor resected from a subject into multiple tumor fragments, 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 by culturing a first population of TILs in a cell culture medium comprising IL-2, optionally 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 5, 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).

7. The method of claim 1 or 3 or 6, wherein the ratio of the number of APCs in the rapid second expansion to the number of APCs in the priming first expansion is in a range of from about 1.5:1 to about 20:1, from about 2:1 to about 5:1 or from about 2:1 to about 3:1, wherein optionally the ratio of the number of APCs in the rapid second expansion to the number of APCs in the priming first expansion is in a range of from about 1.5:1 to about 10:1, optionally wherein the ratio of the number of APCs in the rapid second expansion to the number of APCs in the priming first expansion is about 2:1.

8. The method of claim 1 or 3 or 6, wherein the number of APCs in the priming first expansion is in a range of about $1.0 \times 10^6$ APCs/$cm^2$ to about $4.5 \times 10^6$ APCs/$cm^2$, and wherein the number of APCs in the rapid second expansion is in a range of about $2.5 \times 10^6$ APCs/$cm^2$ to about $7.5 \times 10^6$ APCs/$cm^2$ or the number of APCs in the priming first expansion is in a range of about $1.5 \times 10^6$ APCs/$cm^2$ to about $3.5 \times 10^6$ APCs/$cm^2$, and wherein the number of APCs in the rapid second expansion is in a range of about $3.5 \times 10^6$ APCs/$cm^2$ to about $6.0 \times 10^6$ APCs/$cm^2$, wherein optionally the number of APCs in the priming first expansion is in a range of about $2.0 \times 10^6$ APCs/$cm^2$ to about $3.0 \times 10^6$

APCs/cm$^2$, and wherein the number of APCs in the rapid second expansion is in a range of about $4.0 \times 10^6$ APCs/cm$^2$ to about $5.5 \times 10^6$ APCs/cm$^2$ or the number of APCs in the priming first expansion is in a range of about $1 \times 10^8$ APCs to about $3.5 \times 10^8$ APCs, and wherein the number of APCs in the rapid second expansion is in a range of about $3.5 \times 10^8$ APCs to about $1 \times 10^9$ APCs, optionally wherein the number of APCs in the priming first expansion is in a range of about $1.5 \times 10^8$ APCs to about $3 \times 10^8$ APCs, and wherein the number of APCs in the rapid second expansion is in a the range of about $4 \times 10^8$ APCs to about $7.5 \times 10^8$ APCs or the number of APCs in the priming first expansion is in a range of about $2 \times 10^8$ APCs to about $2.5 \times 10^8$ APCs, and wherein the number of APCs in the rapid second expansion is in a range of about $4.5 \times 10^8$ APCs to about $5.5 \times 10^8$ APCs.

9. The method of claim 1 or 3 or 6, wherein about $2.5 \times 10^8$ APCs are added to the priming first expansion and $5 \times 10^8$ APCs are added to the rapid second expansion, wherein optionally the second population of TILs is at least 50-fold greater in number than the first population of TILs.

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.

11. The method of any of claims 2-6, 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 1-11, 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 and/or the plurality of separate containers comprises from two to twenty separate containers,

optionally wherein the plurality of separate containers comprises from two to ten separate containers or the plurality of separate containers comprises from two to five separate containers, and further optionally wherein 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 the single container comprises a first gas-permeable surface area, 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 1.5 cell layers to about 2.5 cell layers or 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.

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 of the single container or the first gas permeable surface area of each of the plurality of separate containers at an average thickness of about one cell layer to about three cell layers.

15. The method of claim 13 or 14, wherein in the step of the rapid second expansion the APCs are layered onto the first gas-permeable surface area of the single container or the first gas permeable surface area of each of the plurality of separate containers 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 of the single container or the first gas permeable surface area of each of the plurality of separate containers at a thickness of about 3.5 cell layers to about 4.5 cell layers or in the step of the rapid second expansion the APCs are layered onto the first gas-permeable surface area of the single container or the first gas permeable surface area of each of the plurality of separate containers 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 or 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, 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 1.5 cell layers to about 2.5 cell layers or 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.

17. The method of claim 16, 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, wherein optionally 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 or 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.

18. The method of any of claim 2-15, 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 and/or 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, 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 from about 1.5 cell layers to about 2.5 cell layers or 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.

19. The method of claim 18, 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, wherein optionally 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 or 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.

20. The method of any of claims 2-13, 16 and 18, 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 container comprises a first gas-permeable 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 in a range of about 1:1.1 to about 1:10, 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 in a range of 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, optionally 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 about 1:2.

21. 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 or wherein the method further comprises cryopreserving the harvested TIL population in the step of harvesting the therapeutic population of TILs using a cryopreservation process.

22. The method according to claim 1 or 11, further comprising the step of cryopreserving the infusion bag, wherein optionally the cryopreservation process is performed using a 1:1 ratio of harvested TIL population to cryopreservation media.

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

24. 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 added to the cell culture medium in the step of the priming first expansion is about $2.5 \times 10^8$ 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 about $5 \times 10^8$.

25. 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 in the harvesting step harvesting the therapeutic population of TILs is performed using a LOVO cell processing system.

26. 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 $mm^3$, wherein optionally the multiple fragments comprise about 30 to about 60 fragments with a total volume of about 1300 $mm^3$ to about 1500 $mm^3$ or the multiple fragments comprise about 50 fragments with a total volume of about 1350 $mm^3$, optionally wherein the multiple fragments comprise about 50 fragments with a total mass of about 1 gram to about 1.5 grams.

27. 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.

28. 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.

29. 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.

30. The method according to claim 22, wherein the cryopreservation media comprises dimethlysulfoxide (DMSO), wherein optionally the cryopreservation media comprises 7% to 10% DMSO.

31. 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, or 7 days, wherein optionally the priming first expansion is performed within a period of 5 days, 6 days, or 7 days and/or the rapid second expansion is performed within a period of 7 days, 8 days, or 9 days.

32. The method according to any of claims 1-30, wherein the priming first expansion and the rapid second expansion are each individually performed within a period of 7 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 to about 16 days, about 15 days to about 16 days or within a period of about 14 days, about 15 days or about 16 days, optionally wherein 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.

33. The method according to any one of claims 1 to 32, 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 $2.3\times10^{10}$ to about $13.7\times10^{10}$.

34. Use of expanded tumor infiltrating lymphocytes (TILs) in the manufacture of a medicament for treating a subject with cancer, the use comprising:

(a) that a first population of TILs is to be obtained and/or received from a tumor resected from a subject by processing a tumor sample obtained from the subject into multiple tumor fragments;

(b) performing a priming first expansion by culturing the first population of TILs in a cell culture medium comprising IL-2, optionally OKT-3, and optionally 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 7/8 days to obtain the second population of TILs;

(c) 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;

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

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

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

35. The use according to claim 34, wherein the number of TILs sufficient for administering a therapeutically effective dosage in step (f) is from about $2.3\times10^{10}$ to about $13.7\times10^{10}$ or wherein the antigen presenting cells (APCs) are PBMCs, wherein optionally prior to administering a therapeutically effective dosage of TIL cells in step (f), a non-myeloablative lymphodepletion regimen has been administered to the patient, optionally wherein 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.

36. The use according to claims 34 or 35, wherein 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 (f), wherein optionally the high-dose IL-2 regimen comprises 600,000 or 720,000 IU/kg administered as a 15-minute bolus intravenous infusion every eight hours until tolerance.

37. The use according to any one of claims 34-36, wherein the cancer is a solid tumor and/or 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, wherein optionally the cancer is selected from the group consisting of melanoma, HNSCC, cervical cancers, NSCLC, glioblastoma (including GBM), and gastrointestinal cancer.

38. The use according to any one of claims 34-37, wherein the cancer is a hypermutated cancer, wherein optionally the cancer is a pediatric hypermutated cancer.

39. The use according to any one of claims 34-38, wherein the container is a closed container, wherein optionally the container is a G-container or GREX-10, optionally wherein the closed container comprises a GREX-100 or GREX-500.

40. The therapeutic population of tumor infiltrating lymphocytes (TILs) made by the method of any of claims 1 to 33.

41. A tumor infiltrating lymphocyte (TIL) composition comprising the therapeutic population of TILs of claim 40 and a pharmaceutically acceptable carrier.

42. A sterile infusion bag comprising the TIL composition of claim 41.

43. A cryopreserved preparation of the therapeutic population of TILs of claim 40.

44. A tumor infiltrating lymphocyte (TIL) composition comprising the therapeutic population of TILs of claim 40 and a cryopreservation media, wherein optionally the cryopreservation media contains DMSO, optionally wherein the cryopreservation media contains 7-10% DMSO.

45. A cryopreserved preparation of the TIL composition of claims 41 or 44.

46. Use of the tumor infiltrating lymphocyte (TIL) composition of any of claims 41 or 44 in the manufacture of a medicament for treating cancer in a subject, wherein the TIL composition is to be administered to the subject.

47. The use of the TIL composition of claim 46, wherein the cancer is a solid tumor, wherein optionally 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, or selected from the group consisting of melanoma, HNSCC, cervical cancers, NSCLC, glioblastoma (including GBM), and gastrointestinal cancer.

48. The use of the TIL composition of claim 46, wherein the cancer is a hypermutated cancer, wherein optionally the cancer is a pediatric hypermutated cancer.

49. A method of expanding T cells comprising:

(a) performing a priming first expansion of a first population of T cells which is to be obtained from a donor by culturing the first population of T cells to effect growth and to prime an activation of the first population of T cells;

(b) after the activation of the first population of T cells primed in step (a) begins to decay, performing a rapid second expansion of the first population of T cells by culturing the first population of T cells to effect growth and to boost the activation of the first population of T cells to obtain a second population of T cells; and

(c) harvesting the second population of T cells.

50. The method of claim 49, wherein the priming first expansion of step (a) is performed during a period of up to 7 days or up to 8 days and/or the rapid second expansion of step (b) is performed during a period of up to 11 days, wherein optionally the rapid second expansion of step (b) is performed during a period of up to 9 days or up to 8 days.

51. The method of claim 49 or claim 50, wherein the priming first expansion of step (a) is performed during a period of 7 days and the rapid second expansion of step (b) is performed during a period of 9 days or wherein the priming first expansion of step (a) is performed during a period of 8 days and the rapid second expansion of step (b) is performed during a period of 8 days.

52. The method of any of claims 49-51, wherein in step (a) the first population of T cells is cultured in a first culture medium comprising OKT-3 and IL-2, wherein optionally the first culture medium comprises OKT-3, IL-2 and antigen-presenting cells (APCs).

53. The method of any of claims 49-51, wherein in step (b) the first population of T cells is cultured in a second culture medium comprising OKT-3, IL-2 and antigen-presenting cells (APCs), wherein optionally in step (a) the first population of T cells is cultured in a first culture medium in a container comprising a first gas-permeable surface, wherein the first culture medium comprises optionally OKT-3, IL-2 and optionally a first population of antigen-presenting cells (APCs), wherein the first population of APCs is exogenous to the donor of the first population of T cells and the first population of APCs is layered onto the first gas-permeable surface, wherein in step (b) the first population of T cells is cultured in a second culture medium in the container, wherein the second culture medium comprises OKT-3, IL-2 and a second population of APCs, wherein the second population of APCs is exogenous to the donor of the first population of T cells and the second population of APCs is layered onto the first gas-permeable surface, and wherein the second population of APCs is greater than the first population of APCs.

54. The method of claim 53, wherein the ratio of the number of APCs in the second population of APCs to the number of APCs in the first population of APCs is about 2:1 or the number of APCs in the first population of APCs is about $2.5 \times 10^8$ and the number of APCs in the second population of APCs is about $5 \times 10^8$, wherein optionally in step (a) the first population of APCs is layered onto the first gas-permeable surface at an average thickness of 2 layers of APCs and/or in step (b) the second population of

APCs is layered onto the first gas-permeable surface at an average thickness in a range of 4 to 8 layers of APCs.

55. The method of claim 53 or claim 54, wherein the ratio of the average number of layers of APCs layered onto the first gas-permeable surface in step (b) to the average number of layers of APCs layered onto the first gas-permeable surface in step (a) is 2:1, wherein optionally the APCs are peripheral blood mononuclear cells (PBMCs), optionally wherein APCs comprise PBMCs that are irradiated and exogenous to the donor of the first population of T cells.

56. The method of any of claims 49-55, wherein the T cells are tumor infiltrating lymphocytes (TILs), marrow infiltrating lymphocytes (MILs) or peripheral blood lymphocytes (PBLs).

57. The method of any of claims 1-33 or 49-56, wherein the cell culture medium is a defined medium and/or a serum free medium, wherein optionally the defined medium comprises (optionally recombinant) transferrin, (optionally recombinant) insulin, and (optionally recombinant) albumin or the serum-free or defined medium comprises a basal cell culture medium and a serum supplement and/or a serum replacement, optionally wherein the serum supplement or serum replacement is selected from the group consisting of CTS™ OpTmizer T-Cell Expansion Serum Supplement and CTS™ Immune Cell Serum Replacement or the basal cell culture medium is selected from the group consisting of CTS™ OpTmizer™ T-cell Expansion Basal Medium , CTS™ OpTmizer™ T-Cell Expansion SFM, CTS™ AIM-V Medium, CTS™ AIM-V SFM, LymphoONE™ T-Cell Expansion Xeno-Free Medium, Dulbecco's Modified Eagle's Medium (DMEM), Minimal Essential Medium (MEM), Basal Medium Eagle (BME), RPMI 1640, F-10, F-12, Minimal Essential Medium (αMEM), Glasgow's Minimal Essential Medium (G-MEM), RPMI growth medium, and Iscove's Modified Dulbecco's Medium.

58. The method of claim 57, wherein the cell culture medium comprises (a) one or more albumins or albumin substitutes; (b) one or more vitamins, one or more transferrins or transferrin substitutes; (c) one or more antioxidants, one or more insulins or insulin substitutes; and/or (d) one or more collagen precursors, one or more antibiotics, and one or more trace elements; wherein optionally the cell culture medium comprises one or more amino acids or the cell culture medium comprises albumin.

59. The method of claim 57 or claim 58, wherein the cell culture medium comprises albumin and one or more ingredients selected from the group consisting of glycine, L-histidine, L-isoleucine, L-methionine, L-phenylalanine, L-proline, L- hydroxyproline, L-serine, L-threonine, L-tryptophan, L-tyrosine, L-valine, thiamine, reduced glutathione, L-ascorbic acid-2-phosphate, iron saturated transferrin, insulin, and compounds containing the trace element moieties $Ag^{+}$, $Al^{3+}$, $Ba^{2+}$, $Cd^{2+}$, $Co^{2+}$, $Cr^{3+}$, $Ge^{4+}$, $Se^{4+}$, Br, T, $Mn^{2+}$, P, $Si^{4+}$, $V^{5+}$, $Mo^{6+}$, $Ni^{2+}$, $Rb^{+}$, $Sn^{2+}$ and $Zr^{4+}$, wherein optionally the cell culture medium further comprises L-glutamine, sodium bicarbonate and/or 2-mercaptoethanol.

60. The method of any of claims 57-59, wherein the cell culture medium comprises a total serum replacement concentration (vol%) of from about 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 11%, 12%, 13%, 14%, 15%, 16%, 17%, 18%, 19%, or 20% by volume of the cell culture medium, wherein optionally the cell culture medium comprises a total serum replacement concentration of about 3%, about 5%, or about 10% of the total volume of the cell culture medium.

61. The method of any of claims 57-60, wherein the cell culture medium further comprises glutamine (i.e., GlutaMAX®) at a concentration of from about 0.1mM to about 10mM, 0.5mM to about 9mM, 1mM to about 8mM, 2mM to about 7mM, 3mM to about 6mM, or 4mM to about 5 mM and/or the cell culture medium further comprises 2-mercaptoethanol at a concentration of from about 5mM to about 150mM, 10mM to about 140mM, 15mM to about 130mM, 20mM to about 120mM, 25mM to about 110mM, 30mM to about 100mM, 35mM to about 95mM, 40mM to about 90mM, 45mM to about 85mM, 50mM to about 80mM, 55mM to about 75mM, 60mM to about 70mM, or about 65mM, wherein optionally the cell culture medium further comprises glutamine (i.e., GlutaMAX®) at a concentration of about 2mM and/or the cell culture medium further comprises 2-mercaptoethanol at a concentration of about 55mM.

62. The method of any of claims 57-61, wherein the cell culture medium comprises glycine in the range of from about 5-200 mg/L, L- histidine in the range of from about 5-250 mg/L, L-isoleucine in the range of from about 5-300 mg/L, L-methionine in the range of from about 5-200 mg/L, L-phenylalanine in the range of from about 5-400 mg/L, L-proline in the range of from about 1-1000 mg/L, L- hydroxyproline in the range of from about 1-45 mg/L, L-serine in the range of from about 1-250 mg/L, L-threonine in the range of from about 10-500 mg/L, L-tryptophan in the range of from about 2-110 mg/L,

L-tyrosine in the range of from about 3-175 mg/L, L-valine in the range of from about 5-500 mg/L, thiamine in the range of from about 1-20 mg/L, reduced glutathione in the range of from about 1-20 mg/L, L-ascorbic acid-2-phosphate in the range of from about 1-200 mg/L, iron saturated transferrin in the range of from about 1-50 mg/L, insulin in the range of from about 1-100 mg/L, sodium selenite in the range of from about 0.000001-0.0001 mg/L, and/or albumin (e.g., AlbuMAX® I) in the range of from about 5000-50,000 mg/L, wherein optionally the cell culture medium comprises one or more of the non-trace element moiety ingredients in the concentration ranges listed in the column under the heading "Concentration Range in 1X Medium" in Table A provided herein and/or the osmolarity of the cell culture medium is between about 260 and 350 mOsmol, optionally wherein the cell culture medium further comprises about 3.7 g/L, or about 2.2 g/L sodium bicarbonate.

63. The method of any of claims 57-62, wherein the cell culture medium further comprises L-glutamine (final concentration of about 2 mM), one or more antibiotics, non-essential amino acids (NEAA; final concentration of about 100 μM), and/or 2-mercaptoethanol (final concentration of about 100 μM) and/or wherein the cell culture medium in the first and/or second gas permeable container lacks beta-mercaptoethanol (BME or βME; also known as 2-mercaptoethanol, CAS 60-24-2), wherein optionally the cell culture medium comprises CTS OpTmizer T-Cell Expansion SFM, 3% CTS Immune Cell Serum Replacement, 55mM BME, and optionally glutamine or the cell culture medium comprises CTS™OpTmizer™ T-Cell Expansion Basal Medium supplemented with CTS™ OpTmizer™ T-Cell Expansion Supplement (26mL/L), and 3% CTS™ Immune Cell SR, and 2 mM Glutamax, optionally further comprising 6,000 IU/mL of IL-2, optionally wherein the cell culture medium comprises CTS™OpTmizer™ T-Cell Expansion Basal Medium supplemented with CTS™ OpTmizer™ T-Cell Expansion Supplement (26mL/L), and 3% CTS™ Immune Cell SR, 2mM Glutamax, and optionally further comprising 3,000 IU/mL of IL-2.

64. The method of any of the claims 1-33 or 49-63, wherein the tumor sample or first population of T cells is to be obtained from one or more small biopsies, core biopsies, or needle biopsies of the tumor in the subject.

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

(i) obtaining and/or receiving a first population of TILs from a tumor sample which is to be obtained from one or more small biopsies, core biopsies, or needle biopsies of a tumor in a subject by culturing the tumor sample in a first cell culture medium comprising IL-2 for about 3 days;

(ii) performing a priming first expansion by culturing the first population of TILs in a second 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 7 or 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;

(iii) performing a rapid second expansion by supplementing the second 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 (ii), wherein the rapid second expansion is performed for a second period of about 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;

(iv) harvesting the therapeutic population of TILs obtained from step (iii); and

(v) transferring the harvested TIL population from step (iv) to an infusion bag.

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

(i) obtaining and/or receiving a first population of TILs from a tumor sample which is to be obtained from one or more small biopsies, core biopsies, or needle biopsies of a tumor in a subject by culturing the tumor sample in a first cell culture medium comprising IL-2 for about 3 days;

(ii) performing a priming first expansion by culturing the first population of TILs in a second 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 for first period of about 7 or 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;

(iii) performing a rapid second expansion by contacting the second population of TILs with a third 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 11 days to obtain the third population of TILs, wherein the third population of TILs is a therapeutic population of TILs; and

(iv) harvesting the therapeutic population of TILs obtained from step (iii).

67. The method of claim 65 or claim 66, wherein after day 5 of the second period the culture is split into 2 or more subcultures, and each subculture is supplemented with a fourth culture medium comprising IL-2 and cultured for about 6 days, wherein optionally after day 5 of the second period the culture is split into up to 5 subcultures and/or all steps in the method are completed in about 22 days.

68. A method of expanding T cells comprising:

(i) performing a priming first expansion of a first population of T cells from a tumor sample which is to be obtained from one or more small biopsies, core biopsies, or needle biopsies of a tumor in a donor by culturing the first population of T cells to effect growth and to prime an activation of the first population of T cells;

(ii) after the activation of the first population of T cells primed in step (a) begins to decay, performing a rapid second expansion of the first population of T cells by culturing the first population of T cells to effect growth and to boost the activation of the first population of T cells to obtain a second population of T cells; and

(iv) harvesting the second population of T cells.

69. The method of any of claims 65-68 wherein the tumor sample is to be obtained from a plurality of core biopsies, wherein optionally the plurality of core biopsies is selected from the group consisting of 2, 3, 4, 5, 6, 7, 8, 9 and 10 core biopsies.

70. A tumor infiltrating lymphocyte (TIL) or an expanded tumor infiltrating lymphocyte (TIL) composition comprising:

i) a population of tumor infiltrating lymphocytes (TILs), and

ii) defined medium or serum free medium optionally comprising (optionally recombinant) transferrin, (optionally recombinant) insulin, and (optionally recombinant) albumin.

71. The TIL or expanded TIL composition of claim 70, wherein the defined medium or serum free medium comprises (optionally recombinant) transferrin, (optionally recombinant) insulin, and (optionally recombinant) albumin, wherein optionally the defined medium or serum free medium comprises a basal cell medium and a serum supplement and/or a serum replacement.

72. The TIL or expanded TIL composition of claim 71, wherein the basal cell medium includes, but is not limited to CTS™ OpTmizer™ T-cell Expansion Basal Medium , CTS™ OpTmizer™ T-Cell Expansion SFM, CTS™ AIM-V Medium, CTS™ AIM-V SFM, LymphoONE™ T-Cell Expansion Xeno-Free Medium, Dulbecco's Modified Eagle's Medium (DMEM), Minimal Essential Medium (MEM), Basal Medium Eagle (BME), RPMI 1640, F-10, F-12, Minimal Essential Medium (αMEM), Glasgow's Minimal Essential Medium (G-MEM), RPMI growth medium, and Iscove's Modified Dulbecco's Medium, wherein optionally the serum supplement or serum replacement is selected from the group consisting of CTS™ OpTmizer T-Cell Expansion Serum Supplement and CTS™ Immune Cell Serum Replacement and/or wherein the defined medium or serum free medium comprises one or more albumins or albumin substitutes, one or more amino acids, one or more vitamins, one or more transferrins or transferrin substitutes, one or more antioxidants, one or more insulins or insulin substitutes, one or more collagen precursors, one or more antibiotics, and/or one or more trace elements, optionally wherein the defined medium or serum free medium comprises albumin.

73. The TIL or expanded TIL composition of any of claims 70-72, wherein the defined medium or serum free medium comprises albumin and one or more ingredients selected from the group consisting of glycine, L- histidine, L-isoleucine, L-methionine, L-phenylalanine, L-proline, L- hydroxyproline, L-serine, L-threonine, L-tryptophan, L-tyrosine, L-valine, thiamine, reduced glutathione, L-ascorbic acid-2-phosphate, iron saturated transferrin, insulin, and compounds containing the trace element moieties $Ag^{+}$, $Al^{3+}$, $Ba^{2+}$, $Cd^{2+}$, $Co^{2+}$, $Cr^{3+}$, $Ge^{4+}$, $Se^{4+}$, Br, T, $Mn^{2+}$, P, $Si^{4+}$, $V^{5+}$, $Mo^{6+}$, $Ni^{2+}$, $Rb^{+}$, $Sn^{2+}$ and $Zr^{4+}$, wherein optionally the defined medium or serum free medium further comprises L-glutamine, sodium bicarbonate and/or 2-mercaptoethanol and further optionally wherein the defined medium or serum free medium comprises a total serum

replacement concentration (vol%) of from about 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 11%, 12%, 13%, 14%, 15%, 16%, 17%, 18%, 19%, or 20% by volume of the cell culture medium, wherein optionally the defined medium or serum free medium comprises a total serum replacement concentration of about 3%, about 5%, or about 10% of the total volume of the cell culture medium.

74. The TIL or expanded TIL composition of any of claims 70-73, wherein the defined medium or serum free medium further comprises glutamine (i.e., GlutaMAX®) at a concentration of from about 0.1mM to about 10mM, 0.5mM to about 9mM, 1mM to about 8mM, 2mM to about 7mM, 3mM to about 6mM, or 4mM to about 5 mM, wherein optionally the defined medium or serum free medium further comprises glutamine (i.e., GlutaMAX®) at a concentration of about 2mM and further optionally wherein the defined medium or serum free medium further comprises 2-mercaptoethanol at a concentration of from about 5mM to about 150mM, 10mM to about 140mM, 15mM to about 130mM, 20mM to about 120mM, 25mM to about 110mM, 30mM to about 100mM, 35mM to about 95mM, 40mM to about 90mM, 45mM to about 85mM, 50mM to about 80mM, 55mM to about 75mM, 60mM to about 70mM, or about 65mM, wherein optionally the defined medium or serum free medium further comprises 2-mercaptoethanol at a concentration of about 55mM.

75. The TIL or expanded TIL composition of any of claims 70-74, wherein the defined medium or serum free medium comprises glycine in the range of from about 5-200 mg/L, L- histidine in the range of from about 5-250 mg/L, L-isoleucine in the range of from about 5-300 mg/L, L-methionine in the range of from about 5-200 mg/L, L-phenylalanine in the range of from about 5-400 mg/L, L-proline in the range of from about 1-1000 mg/L, L- hydroxyproline in the range of from about 1-45 mg/L, L-serine in the range of from about 1-250 mg/L, L-threonine in the range of from about 10-500 mg/L, L-tryptophan in the range of from about 2-110 mg/L, L-tyrosine in the range of from about 3-175 mg/L, L-valine in the range of from about 5-500 mg/L, thiamine in the range of from about 1-20 mg/L, reduced glutathione in the range of from about 1-20 mg/L, L-ascorbic acid-2-phosphate in the range of from about 1-200 mg/L, iron saturated transferrin in the range of from about 1-50 mg/L, insulin in the range of from about 1-100 mg/L, sodium selenite in the range of from about 0.000001-0.0001 mg/L, and/or albumin (e.g., AlbuMAX® I) in the range of from about 5000-50,000 mg/L, wherein optionally the defined medium or serum free medium comprises one or more of

the non-trace element moiety ingredients in the concentration ranges listed in the column under the heading "Concentration Range in 1X Medium" in Table A provided herein.

76. The TIL or expanded TIL composition of any of claims 70-75, wherein the osmolarity of the defined medium or serum free medium is between about 260 and 350 mOsmol and/or the defined medium or serum free medium further comprises about 3.7 g/L, or about 2.2 g/L sodium bicarbonate, wherein optionally the defined medium or serum free medium further comprises L-glutamine (final concentration of about 2 mM), one or more antibiotics, non-essential amino acids (NEAA; final concentration of about 100 μM), and/or 2-mercaptoethanol (final concentration of about 100 μM) and/or the defined medium or serum free medium in the first and/or second gas permeable container lacks beta-mercaptoethanol (BME or βME; also known as 2-mercaptoethanol, CAS 60-24-2).

77. The TIL or expanded TIL composition of any of claims 70-76, wherein the cell culture medium comprises CTS OpTmizer T-Cell Expansion SFM, 3% CTS Immune Cell Serum Replacement, 55mM BME, and optionally glutamine or the cell culture medium comprises CTS™OpTmizer™ T-Cell Expansion Basal Medium supplemented with CTS™ OpTmizer™ T-Cell Expansion Supplement (26mL/L), and 3% CTS™ Immune Cell SR, and 2 mM Glutamax, optionally further comprising 6,000 IU/mL of IL-2, wherein optionally the cell culture medium comprises CTS™OpTmizer™ T-Cell Expansion Basal Medium supplemented with CTS™ OpTmizer™ T-Cell Expansion Supplement (26mL/L), and 3% CTS™ Immune Cell SR, 2mM Glutamax, and optionally further comprising 3,000 IU/mL of IL-2.

78. The TIL or expanded TIL composition of any of claims 70-77, wherein the population of TILs is a therapeutic population of TILs, wherein optionally the therapeutic population of TILs exhibits a rise in serum IFN-γ, wherein the rise in IFN-γ is greater than 200 pg/ml, greater than 250 pg/ml, greater than 300 pg/ml, greater than 350 pg/ml, greater than 400 pg/ml, greater than 450 pg/ml, greater than 500 pg/ml, greater than 550 pg/ml, greater than 600 pg/ml, greater than 650 pg/ml, greater than 700 pg/ml, greater than 750 pg/ml, greater than 800 pg/ml, greater than 850 pg/ml, greater than 900 pg/ml, greater than 950 pg/ml, or greater than 1000 pg/ml.